THE UNIVERSITY LIBRARY

PROTECTION OF AUTHOR'S COPYRIGHT

This copy has been supplied by the Library of the University of Otago on the understanding that the following conditions will be observed:

1. To comply with s56 of the Copyright Act 1994 [NZ], this thesis copy must only be used for the purposes of research or private study.

2. The author's permission must be obtained before any material in the thesis is reproduced, unless such reproduction falls within the fair dealing guidelines of the Copyright Act 1994. Due acknowledgement must be made to the author in any citation.

3. No further copies may be made without the permission of the Librarian of the University of Otago.

August 2010

Endophytic Phaeophyceae from New Zealand

Svenja Heesch

A thesis submitted for the degree of
Doctor of Philosophy
at the University of Otago,
Dunedin, New Zealand

October 2005

"... a phenomenon in which dissimilar organisms live together, or symbiosis..."

(HEINRICH ANTON DE BARY 1879)

ABSTRACT

The aims of this study were to find endophytic brown algae in marine macroalgae from New Zealand, isolate them into culture and identify them using morphological as well as molecular markers, to study the prevalence of pigmented endophytes in a representative host-endophyte relationship, and to reveal the ultrastructure of the interface between the obligate parasite *Herpodiscus durvillaeae* (LINDAUER) SOUTH and its host *Durvillaea antarctica* (CHAMISSO) HARRIOT.

Three species of pigmented endophytic Phaeophyceae were isolated from New Zealand macrophytes. They were distinguished based on morphological characters in culture, in combination with their distribution among different host species and symptoms associated with the infection of hosts. ITS1 nrDNA sequences confirmed the identity of two of the species as *Laminariocolax macrocystis* (PETERS) PETERS in BURKHARDT & PETERS and *Microspongium tenuissimum* (HAUCK) PETERS. A new genus and species, *Xiphophorocolax aotearoae* gen. et sp. ined., is suggested for the third group of endophytic Phaeophyceae. Three genetic varieties of *L. macrocystis* as well as two varieties each of *M. tenuissimum* and *X. aotearoae* were present among the isolates. *L. macrocystis* and *X. aotearoae* constitute new records for the marine flora of the New Zealand archipelago, on genus and species level. The red algal endophyte *Mikrosyphar pachymeniae* LINDAUER previously described from New Zealand is possibly synonymous with *Microspongium tenuissimum.*

The prevalence of infection by *Laminariocolax macrocystis* was investigated in three populations of *Macrocystis pyrifera* along the Otago coast. Two of the populations situated inside and at the entrance of Otago Harbour showed high infection rates (average between 95 and 100%), while an offshore population was less infected (average of 35%).

The phylogenetic affinities of the parasitic brown alga *Herpodiscus durvillaeae*, an obligate endophyte of *Durvillaea antarctica* (Fucales, Phaeophyceae) in New Zealand, were investigated. Analyses combined nuclear encoded ribosomal and plastid encoded RuBisCO genes. Results from parsimony, distance and likelihood methods suggest a placement of this species within the order Sphacelariales. Even though *H. durvillaeae* shows a reduced morphology, molecular data were supported by two morphological features characteristic for the Sphacelariales: the putative presence of apical cells and the transistory blackening of the cell wall with 'Eau de Javelle'.

Ultrastructural sections showed evidence for a symplastic contact between the cells of the parasite *H. durvillaeae* and its host *D. antarctica.* Within the host cortex, parasite cells attack

the fields of plasmodesmata connecting host cells. In these areas, parasite cells squeeze between the host cells and form secondary plasmodesmata connecting the primary plasmodesmata of the host cells with the cytoplasma of the parasite cell.

Moreover, despite being described as lacking pigments, *H. durvillaeae* possesses a *rbc*L gene, and its plastids show red autofluorescence in UV light, suggesting the presence of a possibly reduced, but functional photosynthetic apparatus. Vestigial walls between developing spores in the 'secondary unilocular sporangia' of *H. durvillaeae* confirm the identity of these sporangia as plurilocular gametangia, derived from reduced gametophytes which were entirely transformed into gametangia.

CONTENTS

I LIST OF ABBREVIATIONS

18S	region of nrDNA coding for the SSU rRNA
26S	region of nrDNA coding for the 5'-end of the LSU rRNA
°C	degree Celsius
ANOVA	analysis of variance
bp	base pair
CIA	chloroform-isoamyl alcohol
cm	centimetre
comb.	combinatio
cont.	continued
D	distance
DC	disease category
dd H_2O	double-destilled water
DNA	deoxyribonucleic acid
dNTP	deoxy nucleotide triphosphate
ddNTP	dideoxy nucleotide triphosphate
ed./ eds.	editor, editors
EDTA	ethylene diamine tetra acetic acid disodium salt
e. g.	for example
emend.	corrected
et al.	*et alii* (and others)
F	test value of an ANOVA
fam.	family
g	gramme
g	gravitational force
gen.	genus
h	hour
H_0	null hypothesis
i. e.	id est (that means)
incl.	including
ined.	ineditum (not published yet)

I./Is.	Island or Islands, respectively
kg	kilogramme
ITS	internal transcribed spacer between coding regions of the nrDNA
L	litre
LSU	large subunit of ribosome, consisting of 26S+5.8S and 5S rRNAs + proteins
LSU gene	region of the nrDNA coding for the 26S and 5.8S rRNA
M	molar
m	metre
mA	milliampere
mg	milligramme
min	minute
ML	maximum likelihood
mL	millilitre
mM	millimolar
mm	millimetre
mmol	millimol
MOPS	3-(N-morpholino) propane sulfonic acid
MP	maximum parsimony
n/a	not applicable
n. d.	not determined, no data (in tables according to legend)
ng	nanogramme
NJ	neighbour-joining
no.	number
nov.	novum
nrDNA	nuclear encoded cistron for ribosomal RNA genes
p	probability
PAR	photosynthetic active radiation
PCR	polymerase chain reaction
PE	Provasoli's enrichment
PES	Provasoli's enriched seawater
PFD	photon flux density
pH	potentium hydrogeni

pmol	picomol
PSU	practical salinity unit
*rbc*L	large subunit of RuBisCO
RNA	ribonucleic acid
RNAse	ribonucleic acidase
rpm	rounds per minute
RT	room temperature, circa 20 °C
RuBisCO	ribulose-1,5-bisphosphate-carboxylase/oxygenase
s	second
S	Svedberg unit (sedimentation coefficient)
SCUBA	self-contained underwater breathing apparatus
SEM	scanning electron microscopy
s. l.	*sensu lato* (in a wide sense)
sp./spp.	species (singular, plural)
ssp.	sub-species
s. s.	*sensu stricto* (in a narrow sense)
SSU	small subunit of ribosome, consisting of 18S rRNA + proteins
SSU gene	region of the nrDNA coding for the 18S rRNA
TE	Tris-EDTA
TAE	Tris-acetate-EDTA
TEM	transmission electron microscopy
ti/tv	transition/transversion ratio
UV	ultraviolet
µg	microgramme
µL	microlitre
µm	micrometer
µmol	micromol
V	Volt
var.	variatio
vs.	versus

II LIST OF FIGURES

Chapter 1: Introduction

Chapter 2: Materials and methods

Chapter 3: Pigmented endophytes - results and discussion

Chapter 4: *Herpodiscus durvillaeae* - results and discussion

Appendices

Appendix B: Morphological data of pigmented endophytes

Appendix C: Field data

III LIST OF PHOTOGRAPHIC PLATES

Chapter 3: Pigmented endophytes

Chapter 4: *Herpodiscus durvillaeae*

IV LIST OF TABLES

Chapter 2: Materials and methods

Chapter 3: Pigmented endophytes - results and discussion

Chapter 4: *Herpodiscus durvillaeae* - results and discussion

Appendix B: Morphological data of pigmented endophytes

Appendix C: Field data

Appendix D: Molecular data

Distance matrices

Appendix E: TEM protocols

1 INTRODUCTION

1.1 Endophytic algae: definitions

As part of marine benthic communities, macroalgae form the habitat for a variety of other organisms, ranging from bacteria and protozoans to crustaceans, molluscs and vertebrates. Macroalgae may, for example, serve as food source, but also provide hiding places or hatching grounds for juvenile fish (DAYTON 1985; SCHIEL & FOSTER 1986; HURD *et al.* 2004). They also form a habitat for smaller algae, which live as epiphytes on the surface of macroalgae or which grow inside algal thalli. The latter are termed endophytes, i. e. "organisms living within a host plant" (greek: éndon = inside; phytón = plant; WOMERSLEY 1987).

Parasites, in contrast to endophytes, are defined as organisms which benefit to the detriment of their host organism. Usually, this means a physiological dependance, i. e. parasitic algae are unpigmented and thus, as heterotrophic organisms, rely at least to some extent on their host for nutrition, especially carbohydrates (GOFF 1983; CORREA 1994, 1997). Endophytic algae may but need not be parasitic, while not all parasites live inside the tissue of their host. Nevertheless, both endophytes and parasites live with their hosts in a close relationship, a symbiosis. Nowadays, the latter term is often used to describe a relationship in which both partners benefit from each other, as the opposite to parasitism. However, in this context, 'symbiosis' will be used *sensu* DE BARY (1879), meaning "...a phenomenon in which dissimilar organisms live together..." (DE BARY 1879, cited in PARACER & AHMADJIAN 2000; GOFF 1983; CORREA 1994).

Whether parasitic or not, the presence of an endophyte may have a negative effect on its host organism. In this case, the endophyte acts as a pathogen causing a disease, which is defined as "... the abnormal, injurious and continuous interference with physiological activities of the host" or "...as disturbance of the normal appearance and function of a plant" (ANDREWS 1979a, page 429, and 1979b, page 448; CORREA 1994). A disease caused by another organism, such as an endophyte, is called an 'infectious disease', in contrast to physiological diseases which are caused by abiotic factors such as UV light, high or low temperature, or by dehydration (GÄUMANN 1951; ANDREWS 1976). In the present study, the terms 'infection' and 'infectious disease' are used synonymously, even though, in a strict sense, the infection only describes the stage from entering of the host by the pathogen until a stable host-pathogen relationship is established (GÄUMANN 1951).

1.2 Pigmented endophytic Phaeophyceae

Endophytic representatives are found among all major macroalgal groups. In the red algae, most of the endophytic species are strictly parasitic, while in the green algae only pigmented endophytes are known (LÜNING 1985). In the brown algae, a single parasitic endophyte is described, the New Zealand endemic *Herpodiscus durvillaeae* (LINDAUER) SOUTH (SOUTH 1974). All other known brown endophytes are pigmented.

Pigmented endophytic Phaeophyceae have been known since the 19th century (e. g. DERBÈS & SOLIER 1851; PRINGSHEIM 1863; KNY 1873; SAUVAGEAU 1892; ROSENVINGE 1893; KUCKUCK 1894), however, their classification has always been difficult due to their simple, possibly reduced, morphologies. The possession of plastids with pyrenoids and a filamentous thallus structure place most of them within the Ectocarpales *s. l.* (BURKHARDT & PETERS 1998). However, additional distinguishing features are usually limited, thus creating problems for a further classification in sub-ordinal taxa.

DERBÈS & SOLIER were the first to propose the genus *Streblonema* DERBÈS & SOLIER (CASTAGNE 1851) to accommodate an endophyte they found in *Liebmannia leveillei* J. AGARDH at Marseille. The type species of this genus, *S. volubilis*, was formally described by PRINGSHEIM (1863). However, the introduction of a special genus for endophytes did not help to solve the taxonomic problems, as most of the brown endophyte species possess uniseriate plurilocular sporangia, while *S. volubilis* PRINGSHEIM has pluriseriate plurilocular sporangia. Despite this discrepancy (and in some cases probably due to a lack of accompanying morphological studies) pigmented brown endophytes have been, and still are, conveniently referred to as *Streblonema* sp. (e. g. SETCHELL & GARDNER 1925; ANDREWS 1977; GOFF 1983; APT 1988a; LEIN *et al.* 1991), even though a number of other genera have been described in which endophytic brown algae may be accommodated. These include *Cylindrocarpus* CROUAN & CROUAN, *Entonema* REINSCH, *Phycocelis* STROEMFELDT, *Mikrosyphar* KUCKUCK, *Myrionema* GREVILLE, *Gononema* KUCKUCK & SKOTTSBERG, *Pilocladus* KUCKUCK emend. KORNMANN, *Onslowia* SEARLE, *Verosphaela* HENRY, and most recently *Laminarionema* KAWAI & TOKUYAMA, *Ascoseirophila* PETERS and *Austrofilum* PETERS. ZINOVA even created the family Streblonemataceae to accomodate endophyte genera (ZINOVA 1953, cited in BURKHARDT & PETERS 1998).

Onslowia endophytica SEARLES and *Verosphacela ebrachia* HENRY are separated from all other endophytes by their propagules and the lack of pyrenoids, which in combination with apical growth and partially biseriate filaments suggest a close relationship to members of the order

Sphacelariales (SEARLES & LEISTER 1980; HENRY 1987b). Characters distinguishing the remaining genera include the morphology of sporangia, cell sizes, and the presence vs. absence of certain features such as erect filaments or basal pseudoparenchyma. However, little is known about the phenotypic plasticity of endophytic species. Even though pigmented endophytes may be cultivated in absence of their host, in seawater with inorganic nutrients, they still may not reveal many characters in culture. Moreover, some existing distinctions, e. g. the presence of Phaeophycean hairs, have been shown to depend on environmental conditions (PEDERSEN 1984) and thus their absence does not allow a reliable classification.

Moreover, PEDERSEN (1984) emphasized the similarity of the morphology of brown endophytes with descriptions of microthalli of free living macroalgae, and therefore suggested that endophytes are stages in the life history of macroalgae. 'Streblonematoid' stages have indeed been discovered in many cultures of members of the Ectocarpales *s. l.*, for example, in *Scytosiphon* sp. (LOISEAUX 1970). PEDERSEN's suggestion was supported by the recent discovery of gametophytes of a member of the Desmarestiales and of some Laminariales growing endophytically in red macroalgae (MOE & SILVA 1989; GARBARY *et al.* 1999). However, algae belonging to these orders lack pyrenoids (DE REVIERS & ROUSSEAU 1999) and thus are distinguished from most of the endophyte taxa mentioned above. Moreover, culture studies revealed complete life cycles, including sexual reproduction, in two endophyte species, *Streblonema macrocystis* PETERS, an endophyte from *Macrocystis pyrifera* (L.) C. AGARDH of Chile, and *Laminarionema elsbetiae* KAWAI & TOKUYAMA, an endophyte in *Laminaria* spp. from North West Europe and Japan. These endophytes have hetermorphic life histories with slightly different gametophytes and sporophytes, which are both microscopic, confirming these taxa as distinct entities (PETERS 1991; KAWAI & TOKUYAMA 1995; PETERS & ELLERTSDOTTIR 1996).

The advances in molecular techniques in recent years have provided taxonomists with genetic markers which are fast and reliable tools to identify and classify organisms based on characters independent from environmental conditions. Two gene regions, whose sequences are most widely used to solve taxonomic and phylogenetic affiliations in the Phaeophyceae are the nuclear encoded ribosomal DNA (nrDNA) and the plastid-encoded RuBisCO genes (DE REVIERS & ROUSSEAU 1999; ROUSSEAU *et al.* 2001; DRAISMA *et al.* 2001, 2003).

The nrDNA codes for the three ribosomal RNA molecules which, together with proteins, form the two sub-units of ribosomes. In the Phaeophyceae, the small sub-unit (SSU) contains an 18S rRNA molecule, and the large sub-unit (LSU) the 5S rRNA and a 28S rRNA molecule. The latter consists of linked 26S + 5.8S rRNA molecules, however, for convenience,

in this context the term 'LSU' refers to the 26S rRNA. Within the nuclear DNA, the genes coding for 18S, 5.8S and 26S rRNA are located together in an operon (Figure 2.7). This operon exists in multiple copies in tandem repeats, which undergo concerted evolution resulting in the homogenisation of all gene copies (e. g. ARNHEIM *et al.* 1980, cited in SOLTIS & KUZOFF 1993; DOVER 1982, cited in BHATTACHARYA 1997; BALDWIN 1992). In most eukaryotes, the gene coding for the 5S RNA is found elsewhere in the nuclear DNA, however, in the brown alga *Scytosiphon lomentaria* (LYNGBYE) LINK, it is located downstream from the 26S coding region, and is considered to be linked to the nrDNA repeating unit (KAWAI *et al.* 1995, 1997).

Ribosomes play a key role in protein assemblage in eukaryotic cells, thus the coding regions of the nrDNA are highly conserved among all eukaryotes. Generally, the SSU region is more conserved than the LSU region and is thus used to separate kingdoms, phyla and orders (HWANG & KIM 1999), however, the level may depend on the phylogenetic age of a group. For example, in phylogenetically old groups such as the Rhodophyta, SSU sequences may even be used in some genera to separate species (e. g. in *Porphyra*, Bangiales; BROOM *et al.* 1999, 2002), while in other red algae they are too conservative to separate taxa at the family or ordinal level (HARPER & SAUNDERS 2001). The Phaeophyceae, in contrast to the red algae, is a comparatively young group: most taxa of this class are considered to have evolved within a comparatively short time, during the so-called 'crown radiation of the brown algae' (DE REVIERS & ROUSSEAU 1999; DRAISMA *et al.* 2003). Thus, among the brown algae, the taxonomic value of the coding regions of the nrDNA is limited mainly to separate taxa at the ordinal level or above (SAUNDERS & DRUEHL 1992; SAUNDERS & KRAFT 1995; TAN & DRUEHL 1994). However, variable regions, e. g. the D1 and D2 regions in the LSU gene, may allow a separation of taxa also below the ordinal level (ROUSSEAU *et al.* 1997).

The internal transcribed spacer (ITS) regions, separating the three conservative coding regions of the nrDNA (Figure 2.7), are spliced during the further processing of the rDNA. Thus the ITS sequences are under less functional constraint and evolve much faster than the coding rDNA regions (BALDWIN 1992; GOFF *et al.* 1994). Generally, due to their comparatively high variability, ITS sequences may only be alignable between closely related taxa, but not between members of different families (DRAISMA *et al.* 2002). Thus they are used to solve phylogenetic questions on the genus and species level, or to solve biogeographical questions on the population level, e. g. in higher plants and macroalgae (BALDWIN 1992; BAKKER *et al.* 1992, 1995; SOLTIS & KUZOFF 1993; VAN OPPEN *et al.* 1993; GOFF *et al.* 1994; STACHE-CRAIN *et al.* 1997; PILLMANN *et al.* 1997; BLOMSTER *et al.* 1998). In the Ectocarpales, taxa that are not

alignable over most of the ITS1 (separating 18S and 5.8S genes) are considered to belong to different genera (PETERS 2003).

DRAISMA *et al.* (2001) listed molecular phylogenetic studies since 1993, which included at least three or more phaeophycean orders. Most of these studies were based on partial nrDNA alone (e. g. DE REVIERS & ROUSSEAU 1999; ROUSSEAU *et al.* 2001) and failed to resolve relationships among brown algal orders within the 'crown'. In their own study, DRAISMA *et al.* (2001) combined partial nrDNA with *rbc*L sequences in a data set with a comparatively high number of brown algae and outgroup taxa. Their results, in the same way as others with similar combined data sets (e. g. PETERS & RAMIREZ 2001) showed an improved phylogenetic signal and thus emphazised the benefits of combining genetic information from the nucleus with those from other compartments, i. e. the plastids or the mitochondria.

The *rbc*L gene is part of the plastom, encoding for the large sub-unit of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (RuBisCO), which is the most important enzyme by weight in photosynthetic organisms (MOHR & SCHOPFER 1992). In red and brown algae, the genes coding for the large and the small (*rbc*S) RuBisCO sub-units are located in an operon and are separated by the RuBisCO spacer (Figure 2.8). The *rbc*L and *rbc*S genes, in contrast to the nrDNA genes, are protein encoding regions, i. e. sequences comprise base pair triplets coding for amino acids. Among the three positions of each codon, the first and the second are under more functional constraint than the third, as substitutions in the latter may be silent (not resulting in an amino acid change; VIS & ENTWISTLE 2000). Mutational saturation in the third codon position may affect the phylogenetic signal of a data set, however, recent phylogenetic studies have shown that in the Phaeophyceae, third codon positions in the *rbc*L gene either do not have a negative influence (DRAISMA *et al.* 2001), or may even increase the phylogenetic signal (SIEMER *et al.* 1998). In these studies, a combination of *rbc*L and RuBisCO spacer sequences has been successfully employed to reveal phylogenetic affiliations within the Ectocarpales *s. l.* and in the Sphacelariales (SIEMER *et al.* 1998; DRAISMA *et al.* 2001), while other studies use only the more variable RuBisCO spacer region to distinguish taxa at the family level (e. g. in the Alariaceae, Laminariales; YOON & BOO 1999) or at the species level (e. g. in the red algal genus *Porphyra*; BRODIE *et al.* 1996).

Among the pigmented endophytic brown algae, however, the spacer regions of the nrDNA gene have been shown to be the most useful sequences to delineate taxa (BURKHARDT & PETERS 1998; PETERS & BURKHARDT 1998; PETERS 2003). ITS1 sequences revealed, for example, the taxonomic positions of the *Macrocystis* endophyte from Chile and some other

species formerly accomodated in the genus *Streblonema:* *S. macrocystis* and *S. aecidioides* (ROSENVINGE) FOSLIE in DE TONI, an endophyte from *Laminaria* spp. of Northwest Europe, together with another kelp endophyte of South Africa, form a group of closely related taxa with the type species of the genus *Laminariocolax* KYLIN, *L. tomentosoides* (FARLOW) KYLIN (BURKHARDT & PETERS 1998), while two endophytes isolated from red algae, *S. tenuissimum* HAUCK and *S. radians* HOWE represent a sister clade to *Laminariocolax* and are accomodated in the genus *Microspongium* REINKE (PETERS 2003). Both *Laminariocolax* and *Microspongium* species share their closest free-living relative, *Chordaria* sp., while they are not closely related to the kelp endophyte *Laminarionema elsbetiae* (BURKHARDT & PETERS 1998; PETERS & BURKHARDT 1998). This result together with the even more distant taxonomic positions of *Onslowia endophytica* and *Verosphacela ebrachia* showed that endophytism has evolved separately several times in the brown algae (BURKHARDT & PETERS 1998; PETERS & BURKHARDT 1998; DRAISMA & PRUD'HOMME VAN REINE 2001; DRAISMA *et al.* 2002; PETERS 2003).

1.3 Endophytic Phaeophyceae in New Zealand

Endophytic Phaeophyceae are known from temperate coasts of Pacific and Atlantic, for example on the Northern Hemisphere from Japan, North America, Greenland and a number of European coasts (e. g. ROSENVINGE 1893; SETCHELL & GARDNER 1925; KYLIN 1947; TAYLOR 1957; ABBOTT & HOLLENBERG 1976; YOSHIDA & AKIYAMA 1979; SOUTH & TITTLEY 1986; APT 1988a, 1988b; KAWAI & TOKUYAMA 1995; BURKHART & PETERS 1998; PETERS & BURKHARDT 1998). In the Southern Hemisphere, endophytes have been reported from South Africa, Chile, the Antarctic Peninsula, Australia and New Zealand (LINDAUER 1947, 1960; WOMERSLEY 1987; PETERS 1991, 2003; BURKHART & PETERS 1998; E. BURKHARDT, personal communication).

New Zealand is located in the South West Pacific on a major active transcurrent fault between the Pacific plate and the Australian-Indian plate (SUGGATE 1978). It is an archipelago comprising three main islands, North Is., South Is. and Stewart Is., plus over 600 additional islands and islets. These stretch over 13 degree latitudes, from the subtropical Kermadec Islands to the subantarctic Campbell Islands (MOLLOY & DINGWALL 1990, cited in NELSON 1994). Since the separation of New Zealand from Gondwanaland ca. 80 million years ago (CHESHIRE *et al.* 1995), a diverse flora and fauna has evolved on land and in the sea, comprising many endemic species (FLEMING 1978). For the marine flora, more than 600 macroalgal species are

recorded (ADAMS 1994). However, among these are only two species of endophytic brown algae: *Herpodiscus durvillaeae* (LINDAUER) SOUTH is an obligate parasite of the 'Southern bull kelp' *Durvillaea antarctica* (CHAMISSO) HARIOT, while the pigmented *Mikrosyphar pachymeniae* LINDAUER is hosted by the red alga *Pachymenia lusoria* (GREVILLE) J. AGARDH (as *P. himanthophora* J. AGARDH). Both endophytes are endemic to New Zealand (LINDAUER 1947, 1960; SOUTH 1974).

It is surprising that endophytes have not been reported from other macroalgae, even though algal communities along New Zealand's coasts contain a number of species that may be potential hosts. The marine flora of New Zealand shares many elements with other Southern Hemisphere floras (HOMMERSAND 1986; NELSON 1994). HOMMERSAND proposed that over 60% of red algal species of the west coast of southern Africa have closest relationships with species from South America, the Antarctic Peninsula and temperate waters of Australia and New Zealand. In South Africa and Chile, large kelps and red algae are known to host endophytic brown algae, such as *Macrocystis pyrifera*, *Ecklonia maxima* (OSBECK) PAPENFUSS and *Aeodes* sp. (PETERS 1991; BURKHARDT & PETERS 1998; E. BURKHARDT, personal communication). The same species, or close relatives thereof, occur also in New Zealand.

Despite their wide distribution, infections by endophytic Phaeophyceae have been quantified only in few kelp populations from the Northern Hemisphere. In Europe and Canada, prevalences of infection by endophytic Phaeophyceae are generally high: in *Nereocystis luetkeana* (MERTENS) POSTELS & RUPRECHT from British Columbia, up to 90% of the thalli are infected (ANDREWS 1977), while in *Laminaria* spp. from Norway and Germany, infection rates range from 75-100% (LEIN *et al.* 1991; PETERS & SCHAFFELKE 1996; ELLERTSDÓTTIR & PETERS 1997). Host algae may show a variety of symptoms associated with an infection by pigmented brown endophytes, such as dark spots, lesions, galls and morphological changes. Endophytes may even influence the mortality and thus the population structure of their hosts: in *Laminaria saccharina* (L.) LAMOUROUX for example, endophytes are known to cause severe distortions which may lead to the death of the infected host thallus: When the stipe contains endophytes, it becomes brittle and breaks during heavy wave action, thus the phylloid is lost (ELLERTSDÓTTIR & PETERS 1997; PETERS & SCHAFFELKE 1996). Moreover, the presence of endophytic algae may reduce the commercial value of industrially cultivated and/or harvested algae, e. g. in japanese *Undaria pinnatifida* (HARVEY) SURINGAR which is used for human consumption (YOSHIDA & AKIYAMA 1979).

In New Zealand, mariculture is a growing business, with the marine flora including a number of native or introduced macroalgae which are, or in future may be, of economical value (SCHIEL & NELSON 1990; ZEMKE-WHITE *et al.* 1999; HURD *et al.* 2004). Brown and red algae are of special interest, as their cell walls contain phycocolloids such as alginates, carrageenans and agars, which serve as important basic materials for many industrial products (LÜNING 1985; MCHUGH 2003).

In New Zealand, seaweeds are mostly harvested from natural populations, and those which are grown in mariculture are mainly used as food for farmed marine animals (ZEMKE-WHITE *et al.* 1999; HURD *et al.* 2004). Among the wild harvested algae are the large Phaeophytes *Durvillaea antarctica*, which hosts the parasite *Herpodiscus durvillaeae*, and *Macrocystis pyrifera*, which is a potential host for pigmented endophytic brown algae. Due to their high alginate contents, both species may be of potential interest for commercial harvest and cultivation. Studies on their diseases as part of the ecology of these commercially interesting macroalgae are required for the sustainable management of natural and cultured populations (CORREA & CRAIGIE 1991).

Endophytes may not only affect the yield of commercially interesting algae. They also constitute a part of marine algal diversity in New Zealand, which scientists are only just beginning to understand (HURD *et al.* 2004). The present thesis provides base-line data on the infection of New Zealand macrocalgae by endophytes and thus adds to the ongoing research on macroalgal diversity.

1.4 The parasite *Herpodiscus durvillaeae*

Taxonomic affiliations

Taxonomic placements have been problematical not only for the pigmented endophytic brown algae, but also for *Herpodiscus durvillaeae*. This monospecific parasite is only found as an obligate epi-endophyte growing on the surface and inside the tissue of *Durvillaea antarctica*. Even though the host species has a circum-antarctic distribution (HAY 1978; CHESHIRE *et al.* 1995), *Herpodiscus* is confined to host populations in New Zealand (LINDAUER *et al.* 1961). The thallus of *Herpodiscus* is filamentous, consisting of a perennial endophytic portion and seasonal epiphytic filaments emerging from the internal hyphae. The external phase of the parasite only occurs in autumn and winter, forming characteristic circular, red-brown patches with a velvet-like texture on the host surface (Plate 4.1, Figure C-F). Reproductive structures are limited to this emergent part, with unilocular sporangia sitting terminally on short erect filaments between longer

vegetative filaments (Plate 4.1, Figure G). The cells of *Herpodiscus* are described as being unpigmented, thus a parasitic life style is assumed (SOUTH 1974; PETERS 1990). In fact, as no other unpigmented brown alga has been discovered so far, *H. durvillaeae* is the only known parasitic brown alga worldwide (LÜNING 1985).

Its parasitic life style makes *H. durvillaeae* unique, but it also generates some problems for studying its morphology: attempts to cultivate this species have been unsuccessful to date (E. C. HENRY, personal communication; unpublished results). No direct information is therefore available on some morphological characters such as growth patterns and the formation of filaments. Additionally, the perennial part of the thallus is literally hidden within the tissue of its host *Durvillaea antarctica*: the apparent lack of phaeophycean pigments in the *Herpodiscus* cells makes its internal filaments difficult to distinguish from the likewise colourless internal host cells (SOUTH 1974). Moreover, as a result of its parasitic life style, *Herpodiscus* is likely to have a reduced and/or specialized morphology (PETERS 1990). Consequently, classifying *H. durvillaeae* has always been tentative (LINDAUER 1947, 1949; SOUTH 1974; JOHN & LAWSON 1974; PETERS 1990).

Originally, the parasite was assigned to the genus *Herponema* J. AGARDH (LINDAUER 1947) and thus placed in the family Ralfsiaceae FARLOW (formerly Chordariales SETCHELL & GARDNER 1925; now either Ectocarpales *s. l.*; NELSON 1982; WOMERSLEY 1987; or Ralfsiales *nomen nudum*; SILVA *et al.* 1996, cited in DE REVIERS & ROUSSEAU 1999). The Ralfsiaceae comprises crustose, usually epilithic brown algae, that are constructed of a discoid base, from which short errect, coherent filaments arise (FLETCHER 1978; WOMERSLEY 1987). LINDAUER later transferred the parasite within the Ralfsiaceae to the genus *Hapalospongidion* SAUNDERS and described it as the new species *H. durvilleae* LINDAUER 1949.

In 1974, JOHN & LAWSON moved the two *Hapalospongidion* species from New Zealand (including the parasite) to a new genus *Basispora*, based on discrepancies in the plastid morphology: while members of *Basispora* have several small plastids per cell, the type species of the genus *Hapalospongidion*, *H. gelatinosum* as well as other members of the Ralfsiaceae usually have a single or few plate-like chloroplasts – this would also exclude the genus *Basispora* from the order Ralfsiales *sensu* NAKAMURA (JOHN & LAWSON 1974; NELSON 1982). However, JOHN & LAWSON made the remark that the parasitic life style and the basal penetrating filaments of *B. durvillaeae* comb. nov. differed markedly from the other two *Basispora* species which are epilithic and crustose.

SOUTH (1974) studied the morphology of the parasite in detail. He showed that the spores released from the unilocular sporangia settle in and on the sporangium, or on nearby filaments, and turn completely into so-called secondary unilocular sporangia, as he did not see cell walls between the four developing spores. SOUTH (1974) erected the monotypic *Herpodiscus* gen. nov. to accommodate the parasite as *H. durvilleae* (LINDAUER) comb nov.. He found some resemblance of the secondary unilocular sporangia of *Herpodiscus* to unilocular sporangia of some *Elachista* species (FRITSCH 1945) and also considered the growth pattern of the parasite to be less similar to members of the Ralfsiaceae than to members of the Elachistaceae KJELLMAN (either Chordariales; LEE *et al.* 2002; or Ectocarpales *sensu lato*; ROUSSEAU & DE REVIERS 1999; PETERS & RAMIREZ 2001). Consequently, SOUTH (1974) included the genus *Herpodiscus* in the Elachistaceae, where it is still officially placed.

The Elachistaceae are small pulvinate to tufted epiphytes. Their thalli consist of long unbranched assimilatory filaments and shorter branched filaments which either form a pseudoparenchymatous, cushion-like medulla, or are free, with rhizoids sometimes penetrating into the host surface (FLETCHER 1987; WOMERSLEY 1987). Superficially, the morphology of *Herpodiscus* resembles that of some members of the Elachistaceae without cushion-like medulla, such as *Leptonematella* sp. or *Neoleptonema yongpilii* E.-Y. LEE & I. K. LEE (FLETCHER 1987; WOMERSLEY 1987; LEE *et al.* 2002). For example, branching in *Herpodiscus* only occurs in the internal filaments or near the base of the external filaments just below the host surface, thus its erect filaments appear similar to the unbranched assimilators of the Elachistaceae (SOUTH 1974). Additionally, unilocular and plurilocular sporangia of *Leptonematella* and *Neoleptonema* are positioned at a distinct height on the branched filaments above the host surface, forming a row, like the primary unilocular sporangia of *Herpodiscus* do.

However, PETERS (1990) pointed out that the unilocular sporangia in Elachistaceae are borne laterally, while in *Herpodiscus* they have a terminal position (Figure 1.1, 1), a feature unknown for the Elachistaceae. Additionally, the Elachistaceae are reported to have a partially pseudoparenchymatous thallus (CLAYTON 1981), while in *Herpodiscus* there is no evidence for either external or internal filaments being compacted in any way (SOUTH 1974; PETERS 1990). Plastid morphology distinguishes the parasite from its proposed relatives: plastids in Elachistaceae, as in other Chordariales/Ectocarpales *s. l.*, are usually equipped with pedunculated pyrenoids (WOMERSLEY 1987; LEE *et al.* 2002), while pyrenoids appear to be absent in cells of *Herpodiscus* (SOUTH 1974; PETERS 1990).

The peculiar secondary unilocular sporangia of *Herpodiscus* do not resemble any structure in the other Elachistaceae. Therefore SOUTH (1974) emphasized that the inclusion of *Herpodiscus* in the Elachistaceae should only be tentative and concluded that the placement of the parasite within the brown algae would continue to be problematical until its life-history and mode of reproduction were revealed.

The missing detail of the life history of *Herpodiscus durvillaeae* was discovered by PETERS (1990): he found that the zooids developing in SOUTH's secondary unilocular sporangia are actually gametes, which after release display isogamous plasmogamy. Although PETERS, like SOUTH (1974), failed to see cell walls between the zooids, he concluded that the secondary unilocular sporangia are in fact gametophytes which are completely transformed into gametangia, and proposed that *Herpodiscus* has a heteromorphic life cycle, with a macroscopic sporophyte and a strongly reduced gametophyte (Figure 1.1,1; PETERS 1990).

This life history clearly separates *Herpodiscus* from both the Ralfsiaceae and Elachistaceae (PETERS 1990): in the Ralfsiaceae, gametophytes and sporophytes are either isomorphic or the gametophyte is the macroscopic generation. In the Elachistaceae, sexuality and terminal unilocular sporangia are unknown. Additionally, in Chordariales (or Chordariaceae, Ectocarpales *s. l.*) other than Elachistaceae, where sexuality is known, gametophytes are microscopic but they are not reduced to the degree observed in *Herpodiscus* (PETERS 1987, 1990). Pigmented endophytic brown algae classified in the Ectocarpales *s. l.*, such as *Laminariocolax* species or *Laminarionema elsbetiae*, have isomorphic or only slightly heteromorphic life histories (PETERS 1991; KAWAI & TOKUYAMA 1995; PETERS & ELLERTSDÓTTIR 1996).

The life history of *Herpodiscus durvillaeae* rather resembles the life history of *Syringoderma floridana* HENRY, a member of the Syringodermatales (Figure 1.1, 2), which has a macroscopic sporophyte as well as a reduced gametophyte settling on the sporophyte and turning completely into a gametangium (HENRY 1984). Consequently, HENRY (E. C. HENRY, personal communication; PETERS 1990) suggested *Herpodiscus* should be placed in the neighbourhood of the Syringodermatales. The morphology of the *Herpodiscus* sporophyte, however, is very different from the sporophytes of the Syringodermatales, which are fan-shaped and composed of appressed filaments. The thallus of *Herpodiscus* is formed by single filaments, which in the external phase, like a lawn, rise separately from the prostrate internal phase (SOUTH 1974). PETERS (1990) concluded that the morphology of *Herpodiscus durvillaeae* is too distinct from *Syringoderma* species for a close relationship.

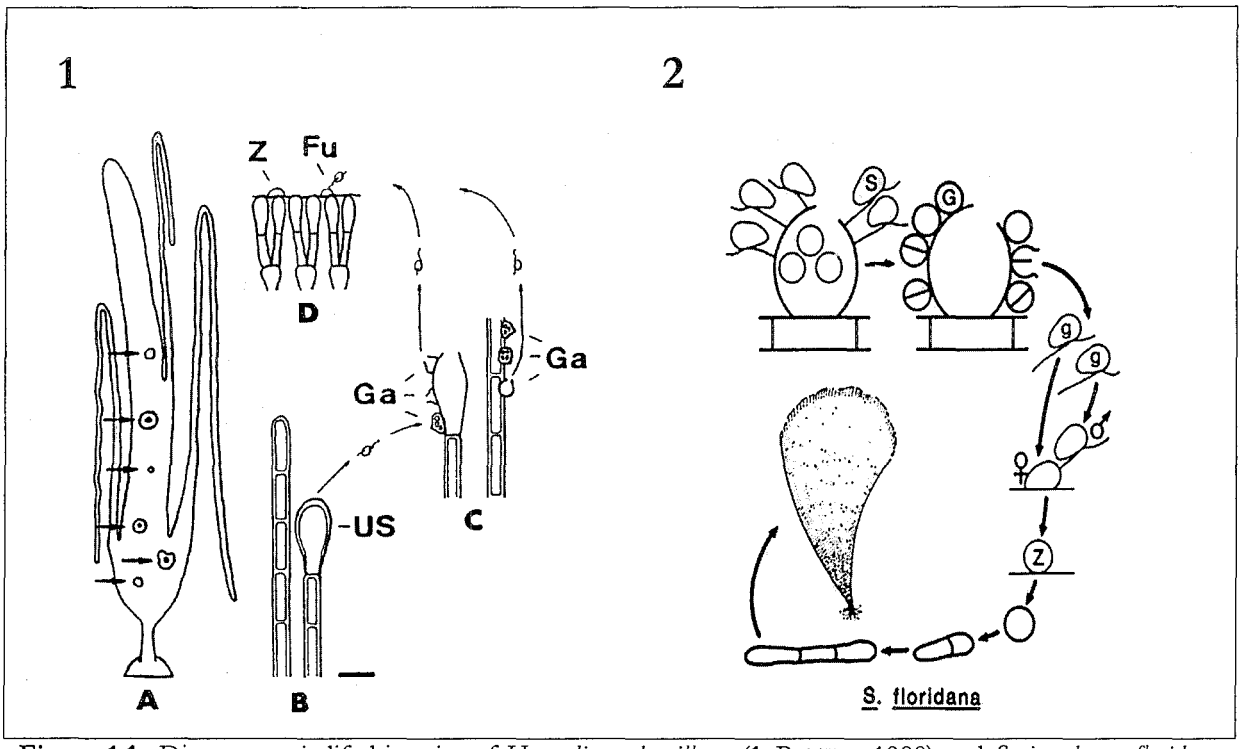


Figure 1.1: Diagrammatic life histories of *Herpodiscus durvillaeae* (**1**; PETERS 1990) and *Syringoderma floridana* (**2**; HENRY 1984). A to D: stages in the life cycle of *H. durvillaeae* on its host *Durvillaea antarctica*; US: unilocular sporangium; GA and G, respectively: gametophyte; S: spore; g: gamete; Fu: isogamous fusion; Z: zygote.

But where else are relatives of the parasite to be found? In the Rhodophyceae, for a comparison, 85% of all parasites are closely related to their hosts: they belong to the same order or even family as the host species and are therefore referred to as "adelphoparasites" (adelphós: greek for "brother"), in contrast to "alloparasites" (állos: greek for "different"), which appear to be not related to their hosts. Adelphoparasites are assumed to have evolved from stages of the life cycle of their host which germinated on the thallus of the parent and became more and more dependent of their host, while alloparasites might have evolved from obligate epiphytes (EVANS *et al.* 1978; GOFF 1982; GOFF & ZUCCARELLO 1994; GOFF *et al.* 1996). A recent molecular systematic study, however, presents evidence that some alloparasites may have evolved from their hosts as well, but are much older taxa, compared to the newly evolved adelphoparasites (ZUCCARELLO *et al.* 2004).

The only other brown alga considered to be at least partially parasitic, *Notheia anomala* HARVEY & BAILEY, a species endemic in Australasia, is indeed related to its obligate hosts, *Hormosira banksii* (TURNER) DECAISNE and *Xiphophora chondrophylla* (R. BROWN ex TURNER) MONTAGNE ex HARVEY, which both belong to the Fucales. Morphological and molecular studies place *N. anomala* in its own family (Notheiaceae SCHMIDT), either in its own order

(Notheiales WOMERSLEY 1987) or at the base of the Fucales (GIBSON & CLAYTON 1987; SAUNDERS & KRAFT 1995; ADAMS 1994; DE REVIERS & ROUSSEAU 1999).

The host of *Herpodiscus durvillaeae*, *Durvillaea antarctica*, has a similar position as *Notheia anomala*, being placed in a separate family (Durvillaeaceae (OLTMANNS) DE TONI) either in its own order (Durvillaeales PETROV; WOMERSLEY 1987; ADAMS 1994) or within the Fucales (DE REVIERS & ROUSSEAU 1999). But unlike *N. anomala* and its hosts, *H. durvillaeae* and *D. antarctica* are not closely related: even though the genus *Durvillaea* is characterised by a largely haplostichous construction, in contrast to other Fucales, its thallus is nevertheless compact and highly differentiated (WOMERSLEY 1987). This morphology, in addition to a diplontic oogamous life history, appears to clearly separate *D. antarctica* from the filamentous parasite *H. durvillaeae* with its diplo-haplontic isogamous life history.

The question remains, of where *H. durvillaeae* is to be placed within the Phaeophyceae, as the current accommodation of *Herpodiscus* in the Elachistaceae can no longer be justified (PETERS 1990). Considering the scarcity of morphological characters in parasitic organisms, the systematic position of *H. durvillaeae* should be investigated using molecular markers.

Ultrastructure of *Herpodiscus*

Not only do the unresolved taxonomic affiliations of *Herpodiscus durvillaeae* require attention, but also certain aspects of its morphology and physiology. Studies of *Herpodiscus* have focused mainly on the morphology of the external phase, due to the difficulties in observing the internal phase in the absence of any discriminating stains (LINDAUER *et al.* 1961; SOUTH 1974; PETERS 1990).

Herpodiscus is a parasite (LINDAUER *et al.* 1961; SOUTH 1974; PETERS 1990): its external cells are poorly pigmented and contain only a few grey plastids, while plastids are described to be absent from the internal phase. Due to this apparent lack of pigments, *Herpodiscus* is assumed to be unable to use photosynthetic energy to synthesize its carbohydrates. As an obligate heterotrophic endophyte, it ultimately relies on assimilates provided by its host *Durvillaea antarctica*. But how does *Herpodiscus* gain nutrition from *Durvillaea*, i. e. how are assimilates transferred from the host to the parasite?

The perennial phase of the parasite is situated within the unpigmented inner tissue of the thallus of *Durvillaea*, which, like other large algae, is differentiated into a photosynthetically active layer of pigmented cells enclosing an inactive internal tissue (HAY 1994). Thus any putative transfer of metabolites to the parasite has to be preceeded by transportation within the thallus of

the host. This transfer of assimilates, from the metabolic 'sources' (i. e. regions where more carbohydrates are produced, or mobilised, than are metabolized, such as photosynthetically active tissue or cotyledons in the Streptophyta) to the metabolic 'sinks' (i. e. regions where more carbohydrates are metabolized than produced, or where they are stored, such as the colourless internal tissue of algae and storage tissue; MOHR & SCHOPFER 1992) is well studied in higher plants. Carbohydrates are transferred along a decreasing gradient of assimilate concentrations from the source to the sink, either via the symplast, the continuum of the cytoplasm of all cells of the plant or algal thallus linked by cytoplasmatic connections, or via the apoplast, outside of this continuum (KLEINIG & SITTE 1992). Accordingly, direct cytoplasmatic contacts, i. e. plasmodesmata, between the cells of the source and of the sink are a requirement for a symplastic transfer.

In higher plants, the symplastic transfer of assimilates over great distances within the plant is facilitated by the perforated sieve plates in the phloem which represent fields of specialized plasmodesmata between adjacent sieve tube cells (KLEINIG & SITTE 1992). Similar cell connections are found in certain brown algal orders (VAN DEN HOEK *et al.* 1995), suggesting an effective translocation of assimilates between parts of their thalli. The so-called 'trumpet hyphae' in the medulla of members of the Laminariales, for example, display plates with pores which, in old filaments, are plugged by callose, similar to the sieve plates of higher plants (SCHMITZ & SRIVASTAVA 1974). The thalli of the Fucales such as *Durvillaea* sp. do not possess a specialized tissue for the translocation of assimilates, however, the concentration of plasmodesmata in fields very much ressembles the sieve plates of higher plant phloem and of Laminarialean trumpet hyphae (CLAYTON *et al.* 1987; VAN DEN HOEK *et al.* 1995).

For an apoplastic transfer, in contrast to the symplastic transfer, assimilates must cross at least two membranes, those of the source and the sink cells, in additional to the extracellular apoplast. Membrane-bound proteins may be involved in the transfer of substances through the membranes, allowing the transit to be selective (KLEINIG & SITTE 1992).

Regardless of the path assimilates take inside a plant or an algal thallus, not only may its own tissue act as a metabolic sink, but also other organisms, i. e. mutualistic and parasitic symbionts (FARRAR 1995; HARRISON 1999). The holoparasitic phanerogam *Cuscuta reflexa* ROXB. (Convolvulaceae), for example, is estimated to take as much as 80% of the photosynthate of its host *Lupinus albus* L. (JESCHKE *et al.* 1994), while the biomass of biotrophic fungi infecting leaves may add up to 50% of the leaf dry weight (HALL & WILLIAMS 2000), demonstrating the importance biotrophic symbionts may have as sinks for higher plant assimilates.

In most symbioses between biotrophic fungi and higher plants, the parasites scavenge substances from the apoplast of the host (SMITH & SMITH 1990; LUCAS 1998). Hyphae of downy mildew and rust fungi, for example, are suggested to grow towards high concentrations of sugar in the host apoplast (JACOBS 1990). Upon reaching the vascular tissue, they insert haustoria (organs of nutrient absorption) into the host cells, presumably to intercept assimilates from the host's phloem ('transfer intercept strategy'; SPENCER-PHILLIPS 1997). The haustoria do not enter the cytoplasm of the host, though, but remain outside the host membrane and thus the host symplast. Therefore, as no direct plasmatic connections exist at the interface between the cells of the two symbionts, assimilates have to be transferred from the host to the parasite via the apoplast, i. e. through the host cell membrane, the extrahaustorial matrix, the haustorial wall and the fungal plasma membrane (SPENCER-PHILLIPS 1997; LUCAS 1998).

Symbiotic interfaces have also been studied in macroalgal hosts and their obligate epiphytes and parasites. For example, ultrastructural studies on the symbiosis of *Notheia anomala* and *Hormosira banksii* (HALLAM *et al.* 1980) have shown that direct cytoplasmatic connections are absent between the endophytic tissue of *Notheia* and its host. However, *Notheia* does not grow well in culture without extracts from *Hormosira* (HALLAM *et al.* 1980). Therefore, even though *Notheia* is fully pigmented, this species appears to be at least partially parasitic. Accordingly, assimilates or any other substances putatively required by *Notheia* may be transferred via the apoplast, even though the existance of a transfer of substances between both symbionts has yet to be verified.

An apoplastic transfer may also be assumed for the symbiosis of the epiphyte *Polysiphonia lanosa* (L.) TANDY (Rhodophyta) with its obligate basiphyte *Ascophyllum nodosum* (L.) LE JOLIS (Phaeophyceae, Fucales): a translocation of metabolites from the host to the epiphyte has been demonstrated using radioactive markers (CITHAREL 1972), even though the epiphyte does not seem to depend on host assimilates alone (HARLIN & CRAIGIE 1975). Again, cytoplasmatic connections appear to be absent from the interface between both partners, even though, upon penetration of the host tissue, single host cells may be enclosed and digested by the epiphyte rhizoid (RAWLENCE 1972).

Green algae endophytic in red algae usually grow in the interstices of their hosts without cytoplasmatic contact between both symbionts, but they can still cause deteriorations in their host thalli (e. g. Correa *et al.* 1994). However, occasionally green endophytes have also been observed to directly penetrate the cells of their hosts and destroy them, for example, *Acrochaete operculata* CORREA & NIELSEN infecting *Chondrus crispus* STACKHOUSE (CORREA & MCLACHLAN

1994). Nevertheless, all known endophytic green algae are pigmented (LÜNING 1985) and can usually be cultivated in absence of their hosts (CORREA & MCLACHLAN 1991; Correa *et al.* 1988, 1994; DEL CAMPO *et al.* 1998). Therefore, until a biotrophic or necrotrophic relationship has been demonstrated between both symbionts, these endophytes should be regarded as pathogens rather than parasites of their hosts (CORREA 1994).

Parasitic red algae only infect other Rhodophyceae, following a strategy different from other endophytic algae or biotrophic fungi. Ultrastructural studies have revealed that especially in adelphoparasites (but to a lesser degree also in alloparasites), parasite cells fuse with host cells. By transfering their nuclei into the host cells, they transform them into a heterokaryotic syncytium within the host tissue (GOFF & COLEMAN 1984, 1985, 1995; GOFF & ZUCCARELLO 1994). I. e., the parasite gains access to the assimilates of its host by becoming part of the host symplast. Striking similarities of the behaviour of adelphoparasites with that of carposporophytes which develop on the female gametophyte of the host and depend on nutrients from it, have led to the suggestion that the parasites evolved from stages of the life history of their host taxa (GOFF & ZUCCARELLO 1994). A transfer of carbohydrates from red algal hosts to their parasites has, for example, been demonstrated for the alloparasite *Harveyella mirabilis* (REINSCH) REINKE infecting *Odonthalia floccosa* (ESPER) FALKENBERG (GOFF 1979) and *Rhodomela confervoides* (HUDSON) SILVA (KREMER 1983), but also for some adelphoparasites (GOFF 1982).

The variety of approaches of known parasites and putative biotropic symbionts to access metabolites of their hosts leads back to the question of how the parasite *Herpodiscus durvillaeae* gains nutrition from *Durvillaea antarctica.* From light microscopic studies, SOUTH concluded that "Circumstantial evidence of penetration of host cells by *H. durvillaea* has been obtained, but requires substantiation." (SOUTH 1974, page 458). However, he did not provide any details with this statement, nor has any further investigation followed. Considering the limitations of light microscopic observations on the internal phase of *Herpodiscus*, ultrastructural investigations using transmission electron microscopy are needed to clarify the nature of the interface between host and parasite. Presence vs absence of cytoplasmatic connections between *Herpodiscus* and *Durvillaea* may indicate whether assimilates are transferred to the parasite via the apoplast or the symplast of the host.

Ultrastructural studies are moreover required to clarify whether vestigial walls are formed during the development of the gametophytes of *H. durvillaeae* (thus confirming the identity of the sporangia as gametophytes-turned gametangia at the ultrastructural level; PETERS 1990), or

whether the lack of walls observed under the light microscope by earlier authors (SOUTH 1974; PETERS 1990) is also evident in the electron microscope. Taxonomically, the level of reduction in the gametophytes is only of limited value, e. g. in distinguishing *Herpodiscus* from members of the Elachistaceae and Ralfsiaceae. However, an absence of internal walls in the gametangia would separate the parasite from some phaeophycean taxa with reduced gametophytes, which may be relatives, such as the Syringodermatales (HENRY 1984; PETERS 1990), while gametangial walls are also absent in groups that are unlikely to be related to *Herpodiscus*, for example in some members of the Fucales (MCCULLY 1968).

1.5 Objectives

In the present study, four aspects of endophytic brown algae from New Zealand were investigated. The objectives were to reveal the diversity of pigmented endophytic brown algae from New Zealand and to study their prevalence in a representative host-endophyte symbiosis. Moreover, the taxonomic affinities of the endemic parasite *Herpodiscus durvillaeae* and the ultrastructure of the interface between this obligate parasite and its host *Durvillaea antarctica* were studied.

Macroalgal communities around New Zealand were examined for the presence of pigmented endophytic brown algae. The prevalence of infection of the kelp *Macrocystis pyrifera* by pigmented endophytic brown algae was studied in three host populations along the Otago coast during the year 2000. To identify the pigmented endophytes present in *M. pyrifera* as well as in other macroalgae, endophytes were isolated from their hosts and cultivated as unialgal strains in the laboratory. Morphological data were combined with molecular markers to address the following questions:

- Which species of pigmented endophytic Phaeophyceae are present in New Zealand?
- What is the relationship of the pigmented endophytes from New Zealand to species from other parts of the world?
- What is the prevalence of infection by endophytic brown algae in the representative host species *Macrocystis pyrifera*?

Another aim of the study was to reveal the taxonomic affinities of the parasitic brown alga *H. durvillaeae* based on molecular markers. By combining results inferred from DNA sequence comparisons with morphological observations, the following questions were investigated:

- What is the natural phylogenetic position of *Herpodiscus durvillaeae*?
- Is the systematic position inferred from DNA sequences matched by morphology and other characters of the parasite's closest relatives?

Finally, ultrastructural studies on *H. durvillaeae* and its host *D. antarctica*, accompanied by observations in the field and by light microscopy, focused on these questions:

- What is the nature of the interface between *Herpodiscus durvillaeae* and *Durvillaea antarctica*, regarding a putative transfer of assimilates from the host to the parasite?
- Are vestigial walls present in the 'secondary unilocular sporangia' of *Herpodiscus durvillaeae*, confirming their identity as gametophyte-turned gametangia, or is their apparent absence verified at the ultrastructural level?

Chapter 2 focuses on the study sites and macroalgae investigated, and on the methods employed to study the various aspects of the endophyte biology. Results for the two groups of endophytes, i. e. for the pigmented endophytes and for the parasite *Herpodiscus durvillaeae*, are presented and discussed separately: the taxonomy of the pigmented endophytes from New Zealand based on morphological and molecular observations as well as the base-line study on the infection by pigmented endophytes of *Macrocystis pyrifera* are presented in Chapter 3. Chapter 4 describes the molecular and morphological taxonomy and the ultrastructure of the parasite *Herpodiscus durvillaeae*. Chapter 5 presents aspects of the endophyte biology which need to be addressed in future studies.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Collection sites

From June 1997 until October 2000, algal communities at various sites around New Zealand (Figure 2.1) were visited and examined for the presence of endophytic brown algae. Sites on the southern South Island, primarily in the Otago region around Dunedin, were frequently visited, while sites outside this area were sampled only on single occasions. The latter include the Doubtful Sound system in Fiordland, the southern part of the North Island around Wellington, and the Chatham Islands.

2.1.1.1 Otago Region

The hydrography of the south-eastern coast of the South Island is mainly influenced by the Southland Current. This current, derived from the Tasman Sea, carries relatively warm, high salinity water along the coast in north-eastern direction. Its waters are modified by freshwater input from the Clutha and Taieri Rivers, which also add large sediment loads to the water (JILLET 1969; HAWKE 1989; MURDOCH *et al.* 1990).

The Otago Harbour near Dunedin (Figure 2.2) is a drowned volcanic valley (GRAY 1991). In the late Miocene, around 10-13 million years ago, the Dunedin Volcano errupted and left behind the Otago Peninsula as the main outcrop, consisting of igneous rocks (WATTERS 1978a). Two islands, Goat Island and Quarantine Island, separate the Upper Harbour basin from the Lower Harbour basin. The Harbour is fed by the Southland Current, with an additional input of freshwater to the Upper Harbour by the Leith stream resulting in a slightly depressed salinity compared to the marine water outside the Harbour (29-34 PSU in the Upper Harbour, 33-34 PSU in the Lower Harbour; JILLET 1969; SMITH 1991; GROVE & PROBERT 1999). Mean surface temperatures range from 6.4 °C to 16 °C (ROPER & JILLET 1981).

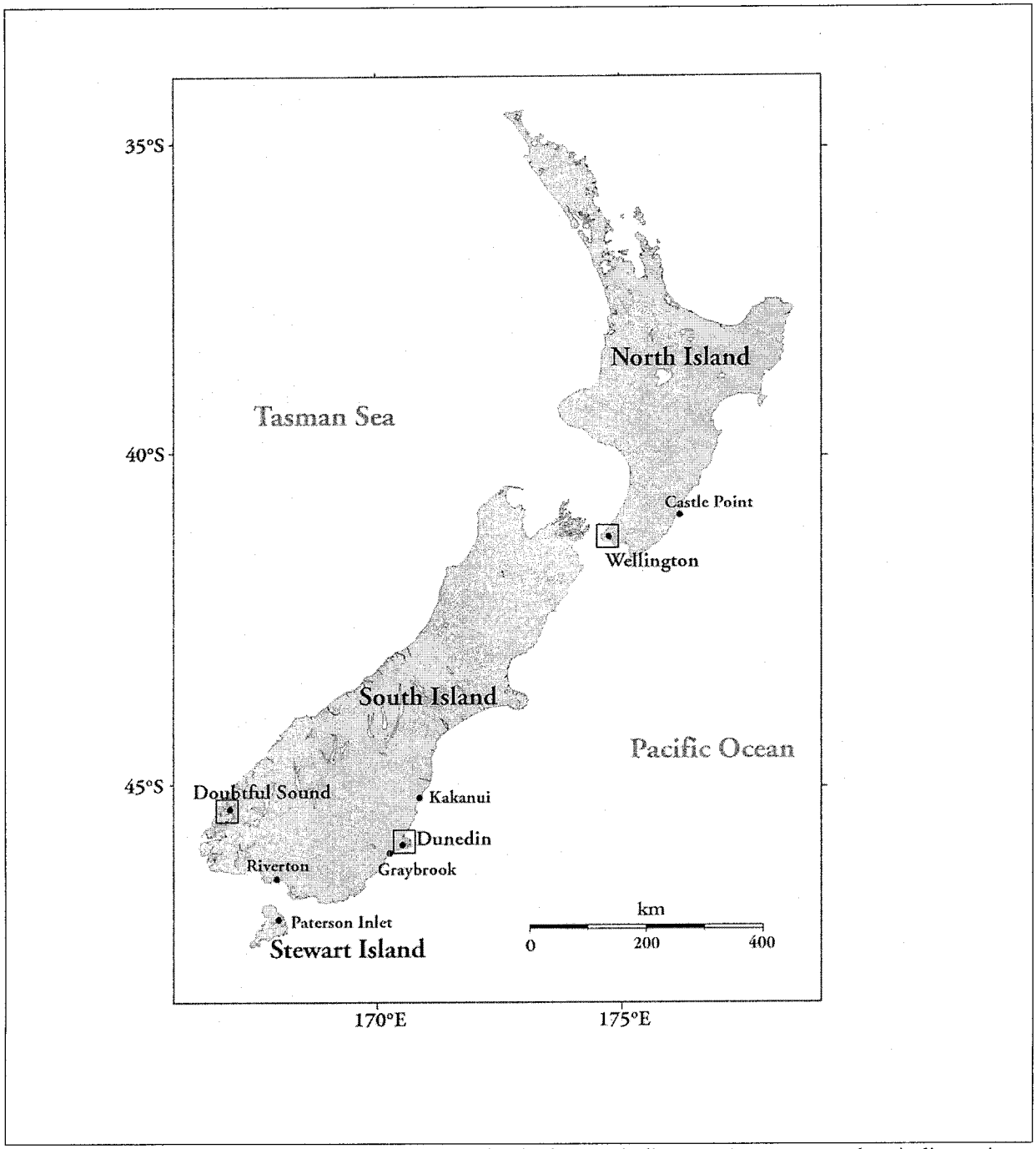


Figure 2.1: Map of the main islands of New Zealand. Arrows indicate major currents; dots indicate sites visited during the course of this study from 1997-2001. Details of locations marked with small frames are shown in Figures 2.2-2.5. The Chatham Islands (Figure 2.5) are located outside the range of this map, around 860 km east of South Island.

Otago Harbour forms a shallow inlet with an aproximate total length of 23 km, a width between 2 and 4 km and an average depth of 4.5 m. The entrance at Taiaroa Head is 0.4 km wide (RAINER 1981; COURNANE 1991; GROVE & PROBERT 1999). At low tide (mean sping tide at Dunedin: 1.8 m; SMITH 1991), extensive sediment flats are exposed, covering nearly 30 % of the surface of the Harbour (HEATH 1974). These flats are fed by the sediments carried in the Southland Current. In sheltered bays such as at Quarantine Point, subtidal sediments are fine and muddy, with a high content of organic detritus, while the exposed banks display sandy sediments. Modifications of the Otago Harbour that began in 1862 also included the construction of a mole of circa 1 km length at Aramoana to keep the entrance of the Harbour free from sediment deposits (SMITH 1991; LAUDER 1991). A habitat structure comparable with the Otago Harbour is found in the two smaller inlets on the Otago Peninsula, Papanui Inlet and Hoopers Inlet (ROPER & JILLET 1981).

On the sediment flats inside Otago Harbour, the seagrass *Zostera novazelandica* SETCHELL forms extensive meadows (FYFE *et al.* 1999). Macroalgae are mainly found on hard substrates, on some stretches of rocky shore (e. g. near Portobello), on shells and boulders in the soft sediments (e. g. at Quarantine Point), on the artificial walls along the fringe of the Harbour (e. g. at Wellers Rock) and at Aramoana Mole. The subtidal algal communities of Otago Harbour include conspicuous inhabitants such as the Laminarialean kelps *Macrocystis pyrifera* (at Aramoana Mole; Plate 3.1, Figure B) and *Ecklonia radiata* (C. AGARDH) J. AGARDH, and increasingly, the recently introduced species *Undaria pinnatifida* (HAY 1990a, 1990b; STUART 1998), but also a variety of smaller Phaeophyceae, Rhodophyceae (for example *Lenormandia chauvinii* HARVEY in the subtidal or *Pachymenia lusoria* in the intertidal) and Chlorophyceae (such as *Ulva* spp., incl. *Enteromorpha* spp.). Members of the Fucales, e. g. *Hormosira banksii* and *Cystophora* species, are limited to intertidal rocky shores (NAYLOR 1954; BATHAM 1956, 1958; MORTON & MILLER 1968; RAINER 1981; JANKE 1989; PROBERT 1991).

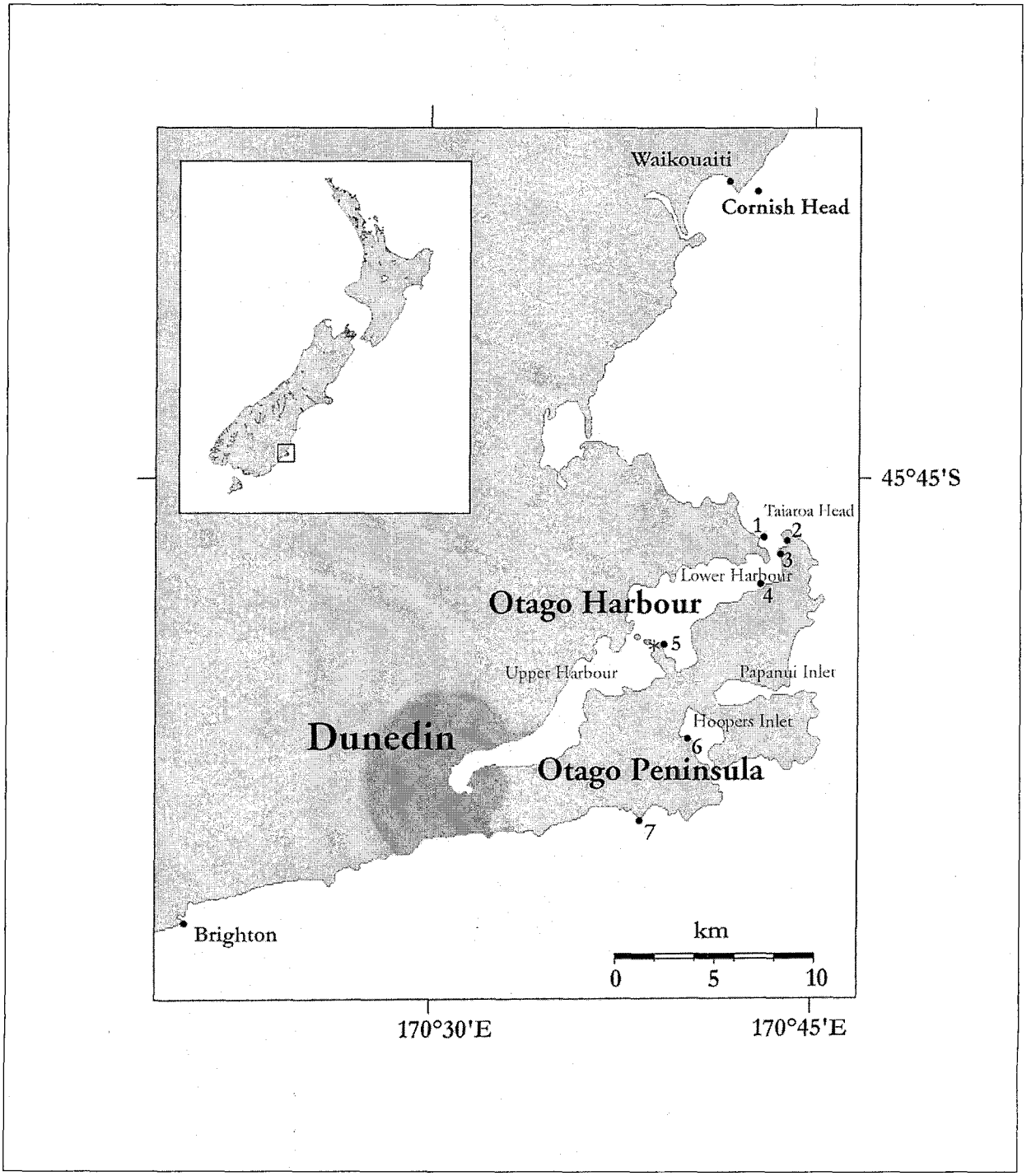


Figure 2.2: Map of the Greater Dunedin area, South Island. Dots indicate sites along the Otago coastline visited during this study from 1997-2001. Numbers relate to following sites: 1: Aramoana mole; 2: Pilots Beach; 3: Harington Point; 4: Wellers Rock; 5: Quarantine Point; 6: Hoopers Inlet; 7: Seal Point; *: Portobello Marine Laboratory. The insert shows the location (small frame) within New Zealand.

Outside the Harbour, along the Otago coast and southwards, long sandy beaches alternate with exposed rocky shores. Brighton Beach presents an example of a very wave-exposed intertidal rock platform, displaying a macrophyte zonation typical for south New Zealand rocky shores (MORTON & MILLER 1968): the horizontal zones are dominated by large brown algae of the order Fucales (including Durvillaeales) and Laminariales. *Durvillaea* species in particular form conspicuous belts from the lower intertidal (*D. antarctica*) to the upper subtidal (*D. willana* LINDAUER 1949). The zone above the *Durvillaea* belt is occupied by smaller Fucales such as *Xiphophora gladiata* (LABILLARDIERE) MONTAGNE and *Hormosira banksii*, while the upper most intertidal zone is inhabited by the red algae *Stictosiphonia arbuscula* (HARVEY) KING et PUTTOCK, together with *Porphyra* spp., *Apophlaea lyallii* HOOK. f. et HARVEY and the brown alga *Scytothamnus australis* (J. AGARDH) HOOK. f. et HARVEY.

In the lower intertidal and upper subtidal, a variety of smaller Rhodophyta is found, including *Gigartina* spp. as well as the foliose *Pachymenia lusoria,* which forms a band among the *Durvillaea* holdfasts. *Grateloupia intestinalis* (HOOK. f. et HARVEY) SETCHELL ex PARKINSON, in contrast, prefers more sheltered sites. Other conspicuous algae include green algae such as *Caulerpa brownii* (C. AGARDH) ENDLICHER, *Ulva* spp. or the brown alga *Splachnidium rugosum* (L.) GREVILLE.

In contrast to the Fucales, kelps of the order Laminariales are restricted to the subtidal. *Lessonia variegata* for example, grows below *D. willana* on exposed coasts, while the larger thalli of the giant kelp *Macrocystis pyrifera* form extensive forests in deeper water offshore, such as the kelp bed off Cornish Head (Plate 3.1, Figure A; FYFE *et al.* 1999). *M. pyrifera* grows in calmer bays, but smaller specimens (up to a few metres long) can also survive in large rockpools at Brighton Beach (Plate 3.1, Figure D). *Ecklonia radiata* tolerates more wave exposure than *M. pyrifera,* but is also found growing among populations of *Macrocystis*, e. g. at Cornish Head (MORTON & MILLER 1968; ADAMS 1994; author, personal observation).

NAYLOR (1954) listed a total of 223 marine algae species for the Dunedin District. This area comprises the coast stretching from Brighton up to Puketeraki (45° 39' S; 170° 39' E). Most sites visited during the course of this study are situated in the Otago region near Dunedin, along the coastlines of the Otago Peninsula (Seal Point, Hoopers Inlet) and the Otago Harbour (Aramoana mole, Pilots Beach, Harington Point, Wellers Rock, Quarantine Point; Figure 2.2). Other sites (Figure 2.1) included Brighton, Graybrook and Riverton/Southland south of the Otago Peninsula. Sites sampled north of the Otago Harbour entrance were Waikouaiti, the offshore kelp bed off Cornish Head close to Waikouaiti, and Kakanui Beach.

2.1.1.2 Doubtful Sound

The south-western coast of the South Island, Fiordland, is characterized by several fiords and fiord systems. Shaped by glaciers some 20 000 years ago, the fiords are drowned valleys consisting of narrow deep basins, open to the Tasman Sea via shallow entrance sills and surrounded by steep, high-rising mountains (HEATH 1985). Doubtful Sound is one of the largest fiords, with an approximate length of 40 km, an average width of 1.2 km, a maximum depth of 421 m, and an entrance sill at a depth of 100 m (STANTON & PICKARD 1981; BOYLE *et al.* 2001; KIM *et al.*, in preparation). It is connected to two other sounds, Thompson and Bradshaw. Together, they form the Doubtful Sound system (Figure 2.3).

The marine environment inside the fiords is characterized by steep vertical gradients of salinity and light (GRANGE *et al.* 1981; GRANGE & SINGLETON 1988; BOYLE *et al.* 2001). The fiords are fed with high salinity marine water by the Tasman Current, which moves in an eastern direction along the Subtropical Convergence (HEATH 1985). High annual rainfall throughout Fiordland (average in Doubtful Sound: 465 mm $month^{-1}$) causes heavy loads of freshwater to run down into the fiords from the surrounding mountains. Doubtful Sound receives an additional anthropogenic freshwater inflow from the discharge of the Manapouri hydroelectric power station at Deep Cove, which is several times higher than the natural catchment runoff. Thus, a steep gradient is created between a quasi-permanent warm, low-salinity layer at the surface (5-10 PSU, 9-17 °C) above a cold seawater layer with oceanic salinity values (up to 35 PSU, 12-13 °C), separated by a halocline. The low-salinity layer in Doubtful Sound reaches an average depth of circa 9 m at the innermost site decreasing towards the entrance of the fiord (LAMARE 1998; GIBBS *et al.* 2000; GIBBS 2001; MILLER 2003). An additional thermocline between 50-75 m varies depending on seasons (PEAKE *et al.* 2001).

Additional to creating a brackish surface layer, the catchment runoff carries large amounts of tannins from the surrounding forests into the fiords. These tannins cause a yellow colouration of the surface layer that reduces the amount and quality of light available to submerged algae (GRANGE *et al.* 1981; GRANGE & SINGLETON 1988). Light conditions in the photic zone are further influenced by the shading from the surrounding steep mountains, i. e. the amount of light reaching the algae can vary extremely, depending on the geographic orientation of a site (BATHAM 1965; BOYLE *et al.* 2001).

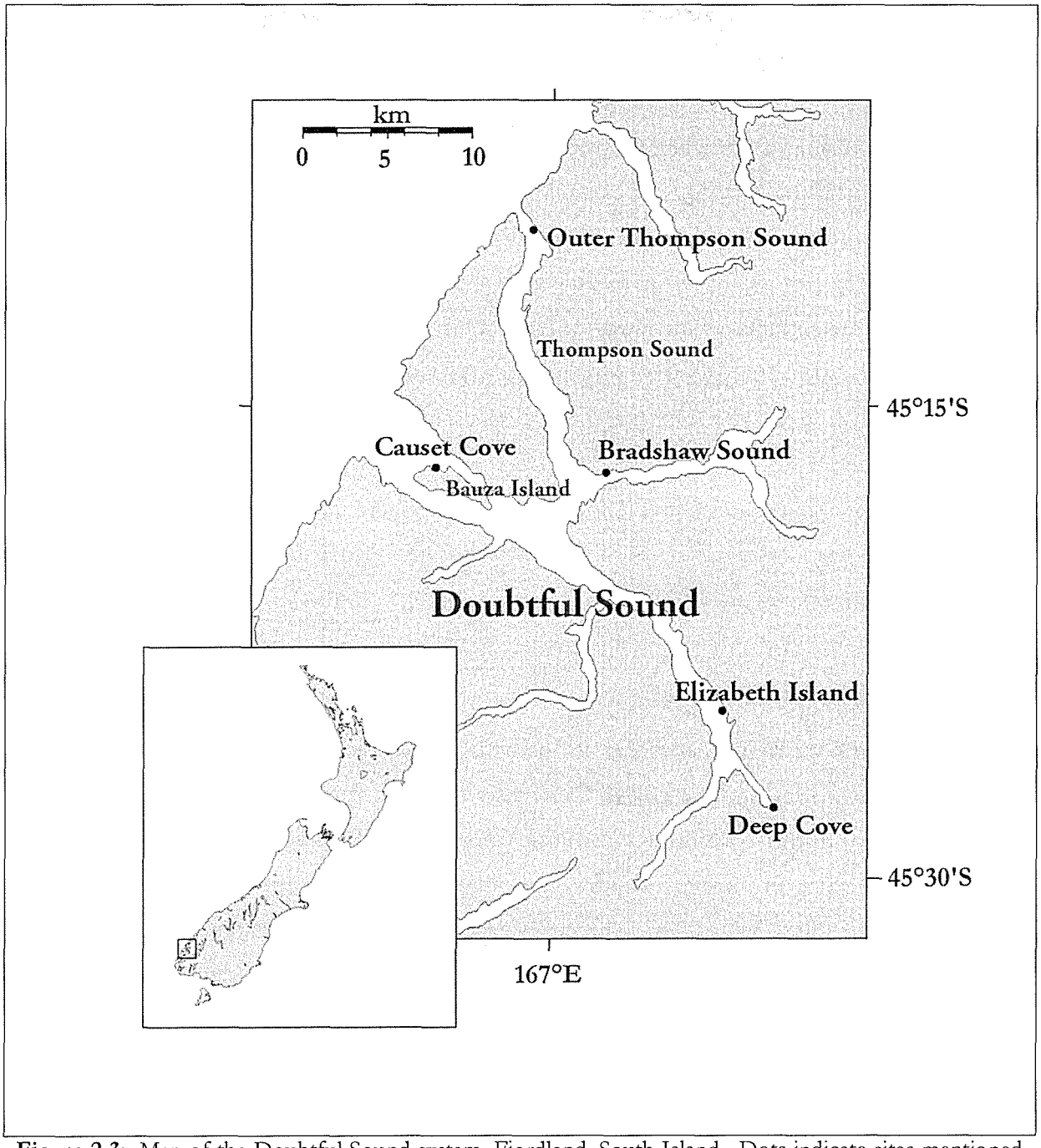


Figure 2.3: Map of the Doubtful Sound system, Fiordland, South Island. Dots indicate sites mentioned in this section (S. M. MILLER, personal communication). The insert shows the location (small frame) within New Zealand.

Most shores within the Doubtful Sound system consist of steep rocky walls, with a tidal range in Doubtful Sound between 1 and 2 m (BOYLE *et al.* 2001). During a survey in October 2000, around 130 intertidal and subtidal algae species were recorded from the Doubtful Sound system (W. A. NELSON, personal communication). The shores within Doubtful Sound are comparatively sheltered from wind and waves, therefore *Durvillaea* species are restricted to the exposed shores near the entrance (for example the western and southern coast of Bauza Island) and outside the fiord. Inside the sound, large kelps are mainly represented by *Ecklonia radiata*, while *Macrocystis pyrifera* only occurs at a moderately sheltered site on the northern coast of Bauza Island, close to the entrance of the fiord (BATHAM 1965; MORTON & MILLER 1968; HAY 1990a; BOYLE *et al.* 2001; MILLER 2003; S. M. MILLER, personal communication).

Macroalgae from the following sites were examined for the presence of endophytic Phaeophyceae during the expedition in 2000: Deep Cove, Elisabeth Island, Causet Cove (Bauza Island), Bradshaw Sound and Outer Thompson Sound (Figure 2.3). Additional specimens of *Ecklonia radiata* were collected by S. M. MILLER at Causet Cove (May 1999) and at Outer Thompson Sound (November 1999).

2.1.1.3 Wellington

The area around Wellington including its harbour, Port Nicholson (Figure 2.4), is mainly influenced by two currents, the colder Canterbury Current from the south and the warmer D'Urville Current from the west (BRODIE 1960; ADAMS 1972). Wellington is situated on a major active fault stretching in northeast-southwestern direction, between the Australian-Indian and the Pacific plates (SUGGATE 1978). The coastline around Wellington comprises rocky shores alternating with sandy beaches. Sites inside Wellington Harbour are moderately sheltered from wind and waves; the mean tidal range is 1.4 m (ADAMS 1972).

ADAMS (1972) listed a total of 370 species of macroalgae from the Wellington area (stretching from Kapiti Island on the West Coast to Mataikona on the East Coast). Large brown algae that are abundant include Laminariales such as *Macrocystis pyrifera, Ecklonia radiata* and *Lessonia variegata*, as well as Fucalean genera such as *Durvillaea, Xiphophora* and *Marginariella* (ADAMS 1972). Castle Point, a promontory with limestone reefs and sandy beaches on the East Coast (Figure 2.1), marks the northern most boundary of several large seaweed species in New Zealand waters, such as *Macrocystis pyrifera* and *Marginariella* spp. (HAY 1990a; ADAMS 1994).

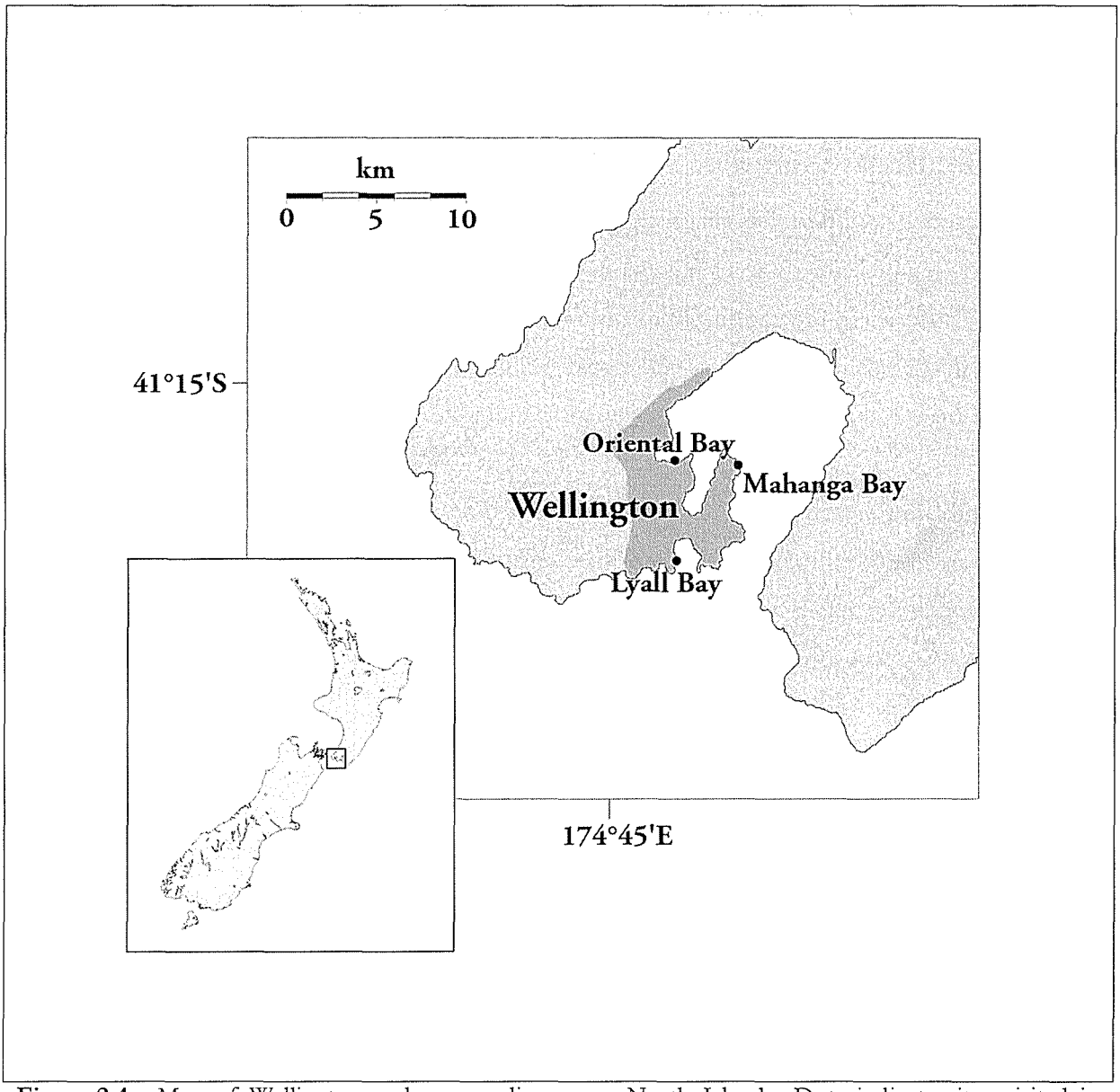


Figure 2.4: Map of Wellington and surrounding areas, North Island. Dots indicate sites visited in March/April 1998. The insert shows the location (small frame) within New Zealand.

The field trip to the Wellington area in March/April 1998 concentrated on sites inside and outside Port Nicholson and included Oriental Bay, Mahanga Bay and Arthur's Nose at Lyall Bay (Figure 2.4). Additionally, a visit was paid to Castle Point and Mataikona on the eastern coast.

2.1.1.4 Chatham Island

Chatham Island belongs to the Chatham Islands archipelago situated around 860 km east of South Island (Figure 2.5). The Chathams were separated from mainland New Zealand some 70 million years ago and are connected to the South Island by the Chatham Rise, an underwater ridge with banks reaching up to 50 m below sea level (HAY *et al.* 1970; NELSON 1994).

The marine life around the Chathams is mainly influenced by an oceanic convergence, which is fed by warm waters from the subtropical East Cape Current and by cold waters from the Southland and Canterbury Currents. Depending on the season, the Subtropical Convergence lies either north or south of the Chatham Rise, creating a mild climate with mean monthly sea temperatures not exceeding 9-18 °C (GARNER 1959; HEATH 1985; SCHIEL 1996). Due to these environmental conditions, marine communities include warm-temperate northern species as well as cold-temperate southern species (KNOX 1954). Additionally, the long time of separation, together with the distance to the main islands of New Zealand, led to the evolution of several endemic species in the terrestrial as well as in the marine environment (WARDLE 1991; NELSON *et al.* 1991; ATKINSON 1996). The 235 species recorded for the marine flora of the Chatham Islands include seven endemic macroalgal species (NELSON *et al.* 1991; SCHIEL 1996).

Chatham Island is a flat island, with the peaks of some extinct volcanoes raising above the plain in the north (WATTERS 1978b). They give testimony to the active geological history of the Chathams, as do, for example, the basalt columns at Ohira Bay (CAMPBELL 1996). Along the coast line of Chatham Island, long streches of sandy beaches at sheltered sites (e. g. Petre Bay, Waitangi West) alternate with rocky promontories. Tidal amplitudes are small compared to mainland New Zealand, e. g. 0.4-0.6 m at Waitangi (NELSON *et al.* 1991).

The rocky shores of Chatham Island show a macroalgal zonation similar to beaches on mainland New Zealand. *Durvillaea* species, especially *D. antarctica*, are the predominant macroalgae in the intertidal and upper subtidal of exposed coasts. *D. willana*, however, is replaced by the endemic *D. chathamensis* HAY 1979 (NELSON *et al.* 1991). Other endemic brown algae include the subtidal *Lessonia tholiformis* HAY 1989 (Laminariales) and *Landsburgia myricifolia* J. AGARDH (Fucales).

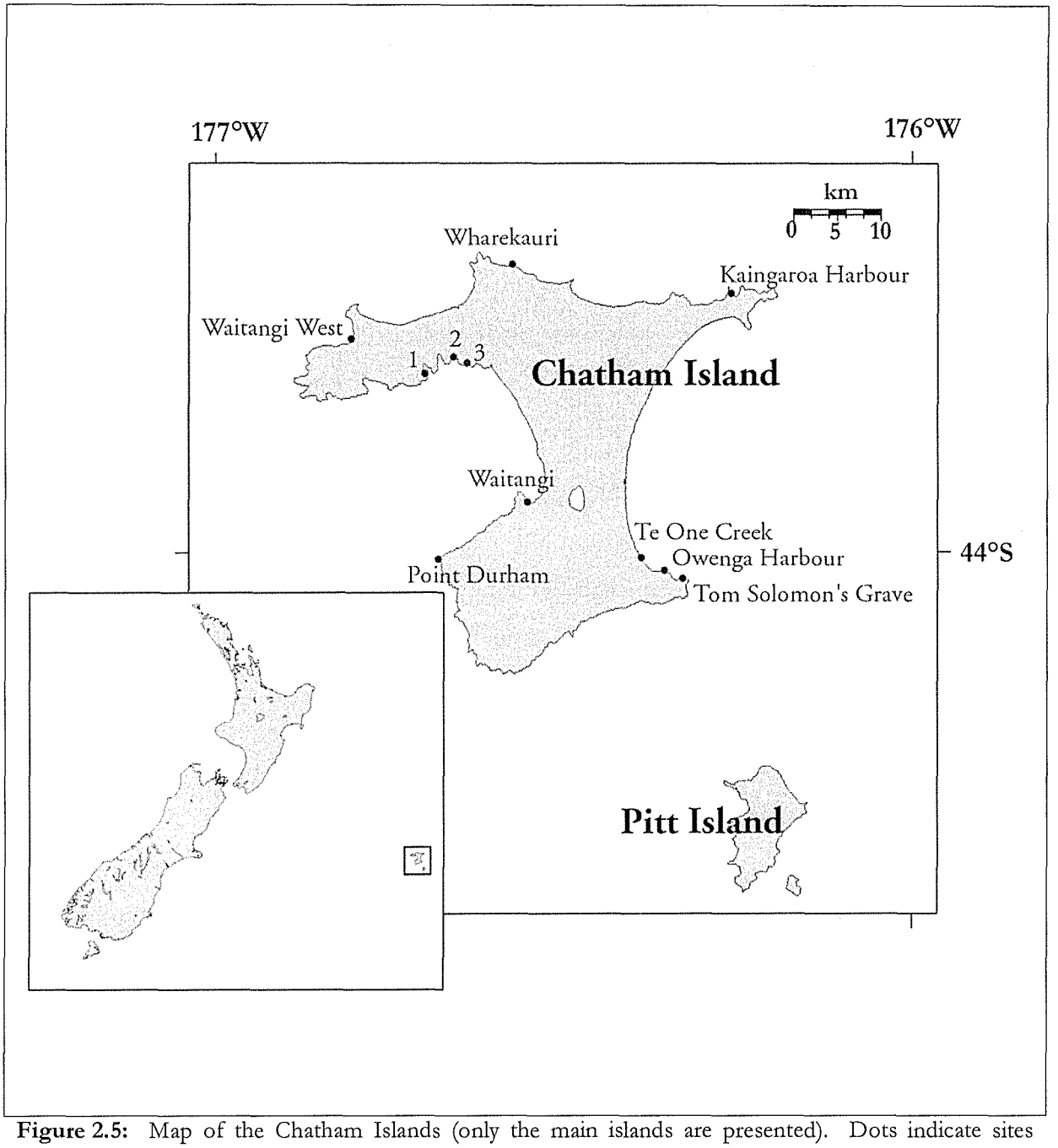


Figure 2.5: Map of the Chatham Islands (only the main islands are presented). Dots indicate sites visited in May 1999. Numbers relate to following sites: 1: Port Hutt; 2: Wangatete Inlet; 3: Ohira Bay. The insert shows the position of the Chatham Islands archipelago (small frame) in relation to mainland New Zealand.

Offshore, in the deeper water of sheltered sites, *Macrocystis pyrifera* forms extensive kelp forests, similar to those on the coast of mainland New Zealand (SCHIEL *et al.* 1995; SCHIEL 1996). *Ecklonia radiata*, however, is conspicuously absent from the Chatham Islands. Even though it was recorded in late 19th and early 20th century, respectively (REINBOLD 1899; LEMMERMANN 1906), it has not been found since (NELSON *et al.* 1991).

During a field trip in May 1999, several sites were visited on Chatham Island (Figure 2.5). These included Point Durham, Waitangi, Ohira Bay, Wangatete Inlet, Port Hutt, Waitangi West, Wharekauri (with a well developed platform reef), Kaingaroa Harbour, and three sites around Owenga in the south east (Te One Creek, Owenga Harbour, "Tom Solomon's Grave").

2.1.2 Host algae

Macrophytes examined for endophytic Phaeophyceae included all major macroalgal groups, the Phaeophyceae (especially large seaweeds of the orders Laminariales and Fucales, including Durvillaeaceae), as well as Rhodophyta and Chlorophyta. The following sections present short desciptions of the biology of the eleven macroalgae found to be hosts to endophytic brown algae in New Zealand (Table 2.1). Photographs of selected host algae are included in the photographic plates in sections 3.1 and 4.1.2

2.1.2.1 Laminariales

Members of the order Laminariales have a diplontic-haplontic heteromorphic life cycle. A macroscopic, morphologically complex diploid sporophyte generation alternates with a microscopic, haploid gametophyte generation of filamentous thalli. The sporophytes are sub-divided into phylloids (blades), cauloids (stipes) and a basal system of rhizoids (holdfast) anchoring the thallus to the substrate (VAN DEN HOEK *et al.* 1995). The parenchymatic tissue of the sporophyte is differentiated into a meristoderm at the surface, a thick and solid cortex and, as the inner-most tissue, a less firmly constructed medulla. Plastids are mainly located in the meristoderm and the outer cortex, while the inner cortex and the medulla are almost unpigmented. Large mucilage ducts (cavities) may run through the cortex. Due to the trumpet hyphae, which, analogous to the sieve tubes of higher plants, conduct assimilates to distribute them through the thallus, the Laminariales is considered to be one of the most highly differentiated algal orders (VAN DEN HOEK *et al.* 1995).

Table 2.1: Macroalgae from New Zealand, from which endophytic Phaeophyceae were isolated. Systematics after ADAMS 1994; WOMERSLEY 1987; ROUSSEAU & DE REVIERS 1999; L. RUSSELL, personal communication. *: W. A. NELSON, personal communication.

division/class	order	family	species
Heterokontophyta/ Phaeophyceae	Laminariales	Lessoniaceae	*Macrocystis pyrifera* (LINNAEUS) C. AGARDH
			Lessonia tholiformis C. H. HAY
		Alariaceae	*Ecklonia radiata* (C. AGARDH) J. AGARDH
			Undaria pinnatifida (HARVEY) SURINGAR
	Fucales	Fucaceae	*Xiphophora gladiata* (LABILLARDIERE) MONTAGNE *ssp. novae-zelandia* RICE
		Seirococcaceae	*Marginariella urvilliana* (A. RICHARD) TANDY
		Durvillaeaceae	*Durvillaea antarctica* (CHAMISSO) HARIOT
			Durvillaea willana LINDAUER
Rhodophyta/ Rhodophyceae	Cryptonemiales	Halymeniaceae	*Pachymenia lusoria* (GREVILLE) J. AGARDH
			Grateloupia intestinalis (HOOK. f. et HARVEY) SETCHELL ex PARKINSON
		Kallymeniaceae	undescribed species*

Reproductive tissue is formed in specific areas of the sporophyte thallus, in so-called sori, on the surface either of the phylloids or of specialized sporophylls. Sori consist of unilocular sporangia, containing meiospores, and paraphyses. The meiospores are motile, settle on suitable substrate and, upon germination, grow into the microscopic filamentous gametophytes (VAN DEN HOEK *et al.* 1995).

The gametophytes are usually dioecious, with the larger female and the smaller male thalli having similar morphologies among species. The female gametangia produce eggs which on release excrete pheromones that trigger the discharge of the male gametangia and attract the motile spermatozoids to the eggs (MAIER 1995). The fertilized eggs (zygotes), which often stick to the end of oogonia, develop into the macroscopic sporophytes. Growth in the sporophyte is initiated from an intercalary meristematic zone that is located either sub-apically or at the base of the phylloids (VAN DEN HOEK *et al.* 1995).

For New Zealand, eight species of Laminariales are recorded. Apart from the single representatives of the genera *Macrocystis* (*M. pyrifera*) and *Undaria* (*U. pinnatifida*), these comprise four species of *Lessonia* and two species of *Ecklonia* (ADAMS 1994).

2.1.2.1.1 *Macrocystis pyrifera*

The sporophyte thallus of *Macrocystis pyrifera* consists of a number of fronds arising from a single holdfast. Along each cauloid, phylloids are regularly attached via hollow, air-filled pneumatocysts (bladders), which keep the frond upright, with the apical parts floating on the water surface (Section 2.2.2, Figure 2.6; Plate 3.1, Figure D). Growth occurs with a sub-apical meristem located near the tip of the fronds, at the base of the youngest phylloid (apical scimitar). Thalli are perennial and can survive for a few to several years (LEVRING *et al.* 1969; LOBBAN 1978; DAYTON *et al.* 1984; SCHIEL & FOSTER 1986), but when the apical part with the meristem is removed, fronds start decaying and become senescent. Sori are located on specialized sporophylls at the base of each frond (for a review on *Macrocystis* biology see NORTH 1971, 1994).

M. pyrifera is found on temperate Pacific shores of both the Southern and the Northern Hemisphere, forming extended offshore kelp forests (NORTH 1994). In New Zealand, *M. pyrifera* is distributed on all main islands as well as the Chatham Is. and the subantarctic islands, except The Snares. It occurs along the eastern coast of the South and up to Castle Point on the southern North Island (ADAMS 1972, 1994; HAY *et al.* 1985; HAY 1990a). The distribution in New Zealand is mainly influenced by average surface temperatures, as *Macrocystis* sporophytes do not survive for long in areas with water temperatures exceeding 22°C for short periods of time. At greater water temperatures, thalli deteriorate (HAY 1990a).

M. pyrifera is the largest alga known, with individuals reported to grow up to a length of 50-70 m in kelp forests along the Californian coast, USA (LEVRING *et al.* 1969), thus earning it its common name "Giant Kelp". Its fronds can grow very fast, reaching elongation rates of up to 10% or 30 cm per day, respectively (NORTH 1971; WILSON *et al.* 1977, cited in LÜNING 1985). The length of the New Zealand thalli range from fronds as short as 1 m (sometimes found in sheltered tidal pools along the coast, e. g. Katiki Beach, Otago: Plate 3.1, Figure D) up to a maximal length of 20 m in offshore beds (such as Cornish Head, Otago – Plate 3.1, Figure A; ADAMS 1994).

2.1.2.1.2 *Lessonia tholiformis*

Four *Lessonia* species are recorded for the New Zealand archipelago, of which only *L. variegata* J. AGARDH is widespread on the main islands of New Zealand (ADAMS 1994). Two species (*L. brevifolia* J. AGARDH and *L. adamsiae* C. H. HAY; HAY 1987) are endemic to the subantarctic islands, while *L. tholiformis* is restricted to the Chatham Islands (HAY 1989; ADAMS 1994).

Lessonia species are usually limited to the subtidal of rocky shores and are only exposed at extremly low tides (e. g. *L. tholiformis*; Plate 3.5, Figure B).

The thallus of the perennial *Lessonia* sporophyte is comparatively tough and does not float. The rhizoids (haptera) forming the holdfast are either fused or not, depending on the species. Several short, dichotomously branched cauloids arise from the holdfast, topped by phylloids which soon split and form strap-like blades. The fronds grow by an intercalary meristem at the base of the phylloids. The sori are located on the phylloids (EDDING *et al.* 1994).

L. tholiformis is distinguished from the other New Zealand species by its solid holdfast consisting of fused haptera. Its cauloids are rigid and terete at the base, in contrast, for example, to *L. variegata* which has rather flexible, flattened cauloids. Additionally, the phylloids of *L. tholiformis* are rugose when young and smooth when mature, while the other New Zealand *Lessonia* species dispay either only smooth (*L. variegata* and *L. brevifolia*) or only corrugated blades (*L. adamsiae*), irrespectively of their age (HAY 1989).

L. tholiformis reaches a height of 1.5 m. On the Chatham Islands, it dominates the subtidal algal communities at many localities by forming dense kelp forests. It is reported from waters up to 13 m deep (HAY 1989; NELSON *et al.* 1991).

2.1.2.1.3 *Ecklonia radiata*

Ecklonia radiata is widely distributed in the Southern hemisphere. The morphology of the sporophyte is variable, leading to a number of different synonyms for this species (BOLTON & ANDERSON 1994). A single stipe with an unsplit phylloid arises from the rhizoidal holdfast (Plate 3.2, Figure E). The phylloid can be rather spiny. Secondary blades along the margin of the primary blade are formed by the intercalary meristem and develop while the primary blade grows. Sori are located on the distal part of the phylloid (ADAMS 1994; BOLTON & ANDERSON 1994).

In the New Zealand archipelago, *Ecklonia radiata* is widespread around nearly all islands, apart from the Chathams and some subantarctic islands. It forms dense stands in the subtidal, especially in water depths of 10-17 m, but has also been observed in 60 m depth at Three King Islands. Thalli can reach thallus lengths of 1 m or more (CHOAT & SCHIEL 1982; ADAMS 1994).

A second *Ecklonia* species, *E. brevipes* J. AGARDH, is reported from a few sites around New Zealand. Whether it represents a true distinct species or is a modification of *E. radiata* adapted unfavourable environmental conditions, it requires further studies (ADAMS 1994).

2.1.2.1.4 *Undaria pinnatifida*

As for *Ecklonia radiata*, the rhizoidal holdfast of *Undaria pinnatifida* gives rise to a single stipe with a single phylloid. The phylloid itself is deeply lobed and has a conspicuous midrib. Thalli grow with an intercalar meristem at the base of the phylloid. In contrast to *E. radiata*, the sori of *U. pinnatifida* are located on specialized sporophylls fringing the base of the stipe like a collar of frills (ADAMS 1994; ANONYMOUS 1999). The sporophyte of *U. pinnatifida* grows to a length of 1-2 m (HAY 1990b).

U. pinnatifida is an annual species native to the northwest Pacific. In Japan and Korea, it is traditionally cultivated as "Wakame" for human consumption, with a high commercial value (YOSHIDA & AKIYAMA 1979). It was accidentally introduced to New Zealand, presumably in the early 1980s. The first official record in New Zealand is from Oriental Bay/Wellington Harbour in 1987 (HAY & LUCKENS 1987). *U. pinnatifida* is a highly invasive species, which was distributed along the New Zealand coasts by ships, carrying the fouling sporophytes and gametophytes on their hull from harbour to harbour. Since its introduction to New Zealand, *U. pinnatifida* is spreading along the coast lines of North and South Island. It is now found at many sites along the East coast, from Gisborne in the North Island to Paterson Inlet on Stewart Island, mainly in harbours, but also on adjacent beaches, such as Moeraki, North Otago (ANONYMOUS 1999; FORREST *et al.* 2000).

2.1.2.2 Fucales

The Fucales have a life cycle similar to that of higher plants: the gametophyte generation is almost completely reduced and the sporophytes directly release eggs and sperm. Apart from one species (*Notheia anomala*; GIBSON & CLAYTON 1987), the reproduction is oogamous, i. e. female reproductive cells are comparatively large and non-motile (VAN DEN HOEK *et al.* 1995).

Gametangia develop in cavities (conceptacles) on the thallus, which are usually concentrated in specific areas (receptacles), often at the tips of the fronds. Eggs and sperm are either produced in the same (= bisexual) conceptacle or in different (= unisexual) ones, which either develop on the same thallus (monoecious) or on two separate thalli (dioecious; WOMERSLEY 1987). The three walls surrounding the female gametes and some vestigial cells in the oogonia of some Fucalean species are thought to be remnants of the reduced gametophyte and gametangia (CLAYTON 1984). In some species, reproduction is synchronized by the tidal cycle, with gametes being released at high tides (CLAYTON 1981). The non-motile eggs attract the male gametes with genus-specific pheromones (MÜLLER 1989; MAIER 1995).

Fucales have thalli that are quite tough, thus enabling them to grow in the intertidal and survive wave action as well as periods of dessiccation (DRING 1992). Some species display hollow bladders (vesicles), which enable the thallus to float (VAN DEN HOEK *et al.* 1995). Like in the Laminariales, the tissue of the Fucalean thallus is differentiated into a surface layer containing highly pigmented cells and a more or less unpigmented cortex and medulla, the latter forming the innermost tissue (CLAYTON 1981).

The thalli of most Fucalean genera (Fucales *s. s.*, e. g. members of the Fucaceae and Seirococcaceae; ROUSSEAU & DE REVIERS 1999) grow with apical cells and are parenchymatous (CLAYTON 1981). *Durvillaea* species (Fucales *s. l.*, Durvillaeaceae; ROUSSEAU & DE REVIERS 1999), however, have a pseudo-parenchymatous, haplostichous thallus construction, growing with a meristoderm covering the entire thallus surface (HAY 1994). This character, among others such as the development of conceptacles and embryos, led to the placement of the genus *Durvillaea* by most authors into a separate order, the Durvillaeales PETROV. (WOMERSLEY 1987), before molecular systematics gave support to the present classification (ROUSSEAU & DE REVIERS 1999).

In New Zealand, the order Fucales *s. l.* is represented by nine genera with 29 species. Apart from the genera presented in the following section, these include *Sargassum*, *Cystophora*, the Australasian *Notheia* and *Hormosira* as well as the New Zealand endemic *Landsburgia* and *Carpophyllum* (ADAMS 1994).

2.1.2.2.1 *Xiphophora gladiata*

In New Zealand, the Australasian endemic species *Xiphophora gladiata* is represented by *X. gladiata* ssp. *novae-zelandiae*. Its perennial thallus consists of several fronds, which arise from a small, solid holdfast, and each of whom can be ≥ 50 centimeters long and up to 1 cm wide (Plate 3.5; Figure A). The fronds are compressed, fan-shaped and frequently sub-dichotomously branched (ADAMS 1994; WOMERSLEY 1987). *X. gladiata* is monoecious and has unisexual conceptacles.

The thalli of *X. gladiata* grow in the lower intertidal of exposed rocky shores, but are also found subtidally. The species occurs on all main islands of the New Zealand archipelago, including the sub-antarctic islands and the Chatham Islands, but mostly in the south, while the second *Xiphophora* species known from New Zealand, *X. chondrophylla*, is restricted to the far north (ADAMS 1994).

2.1.2.2.2 *Marginariella urvilliana*

The genus *Marginariella* is endemic to New Zealand. Two of the three species, *M. boryana* (A. RICHARD) TANDY and *M. urvilliana*, are distributed from Castle Point to the south of New Zealand as well as on the Chatham, Snares and Auckland Islands. A putative third species found on sub-antarctic islands has not been further identified (ADAMS 1994).

Both *M. urvilliana* and *M. boryana* form perennial thalli up to 2 m long. They consist of a single flat stem that arises from a holdfast and carries several flat fronds on the margin. The fronds are usually 1-3 cm wide and have alternately toothed margins. The receptacles develop on the inner side of the lower fronds. Both species are mainly distinguished by their vesicles, which are round in *M. urvilliana* and oval in *M. boryana.* They usually grow in the subtidal of exposed coasts, with *M. urvilliana* extending into more sheltered areas (ADAMS 1994).

2.1.2.2.3 *Durvillaea antarctica* and *D. willana*

The perennial thallus of *Durvillaea* species consists of a solid holdfast giving rise to a single terete cauloid and a broad, flattened phylloid (HAY 1994). The holdfasts of closely growing specimens can coalesce in such way that they appear as a single holdfast supporting several stipes. *D. willana*, in contrast to all other *Durvillaea* species, develops secondary blades on its cauloid (Plate 3.5, Figure D).

The shape of the *Durvillaea* phylloid depends on the habitat. In *D. antarctica*, for example, to withstand the wave forces at very exposed sites, its phylloid is split into a mass of thin stripes (so-called 'thonged' form of *D. antarctica*), while phylloids in more sheltered sites are rather split into large lobes ('cape' form; Plate 4.1, Figure A; HAY 1994). *D. antarctica* is furthermore distinguished from all other *Durvillaea* species by its "honeycomb tissue", hollow, air-filled cavities in the medulla, which enable the phylloid to float. Due to its buoyancy, *D. antarctica* has a circum-antarctic distribution (HAY 1978; CHESHIRE *et al.* 1995), while the other, solid-bladed species are restricted to certain islands, e. g. *D. willana* to the main islands of New Zealand and *D. chathamensis* to the Chatham and Antipodes Islands (HAY 1979; ADAMS 1994).

D. antarctica is one of the largest seaweeds worldwide. Individuals on South Island, New Zealand, may reach 10 m length and a freshweight of 100 kg (C. L. HURD, personal communication), while specimens on the Chathams are recorded to grow up to 20 m long (SCHIEL 1996). On very wave-exposed rocky shores of New Zealand, *D. antarctica* forms dense stands and dominates the whole intertidal. *D. willana* occupies the zone below *D. antarctica* in the lower intertidal and upper subtidal (HAY 1994).

2.1.2.3 Rhodophyta

The three red host algae, *Pachymenia lusoria, Grateloupia intestinalis* and a so far undescribed species tentatively placed in the family Kallymeniaceae, belong to the order Cryptonemiales (ADAMS 1994; L. RUSSELL, personal communication). Their thalli are pseudoparenchymatous, with a multiaxial construction composed of branched filaments growing by apical cells.

All three species have a triphasic life-history with isomorphic gametophytes and tetrasporophytes (WOMERSLEY 1994). The free-living gametophytes are either dioecious or monoecious. Both female and male gametes are non-motile. The male spermatia are released, while the eggs remain in the oogonium on the gametophyte. The spermatium is caught by the trichogyne, a cell protuberance on the oogonium, and the male nucleus is led to the egg. After fertilisation, the oogonium develops into the carposporophyte, a diploid tissue which remains on the haploid gametophyte and physiologically depends on it. Via mitosis, the carposporophyte produces diploid carpospores. Upon release these germinate and grow into the second independent generation, the diploid tetrasporophyte. Meiosis occurs on the tetrasporophyte, leading to the formation of tetrasporangia. The haploid tetraspores germinate into the gametophytes (VAN DEN HOEK *et al.* 1995).

2.1.2.3.1 *Pachymenia lusoria*

The thallus of *Pachymenia lusoria* consists of a discoid holdfast giving rise to several foliose and irregularly lobed fronds (Plate 3.4, Figure A; ADAMS 1994; L. RUSSELL, personal communication). At the thallus surface, the cells are arranged in dense layers while the filaments in the innermost parts form a loose net, giving the thallus the appearance of having a strongly pigmented outer cortex and a less pigmented medulla. The gametophytes are dioecious (ADAMS 1994; WOMERSLEY 1994). The thick outer cortex of *P. lusoria* makes its thallus cartilaginous and very robust.

P. lusoria is found in a distinct band in the intertidal of exposed rocky coasts throughout New Zealand, often growing in clumps of coalescent individuals between the holdfasts of *Durvillaea antarctica* (ADAMS 1994). Apart from this species, two other *Pachymenia* species are currently described from New Zealand. *P. laciniata* J. AGARDH, like *P. lusoria*, is a foliose erect species, occuring at the intertidal–sublittoral fringe on rocky shores. *P. crassa* LINDAUER, a prostrate species from the subtidal, has so far only been found on the Three King Islands (ADAMS 1994; L. RUSSELL, personal communication).

2.1.2.3.2 *Grateloupia intestinalis*

The dioecious gametophytes and the tetrasporophyte of *G. intestinalis* consist of a small discoid holdfast that gives rise to an erect branched tube. Its outer cortex comprises small and well pigmented cells which are densely packed, while the inner cortex consists of stellate cells forming a loose net. The medulla consists of a more or less hollow central cavity filled with mucilage. The thallus is soft and slippery and can reach a length of 50 cm.

Grateloupia intestinalis is found throughout New Zealand. It grows on rocks in the intertidal or in rockpools in sheltered sites, but it can also withstand some sand cover (ADAMS 1994; WOMERSLEY 1994).

2.1.2.3.3 Undescribed species

The specimen hosting brown endophytes was collected on a subtidal rocky substrate at Causet Cove, Doubtful Sound in 15 m depth (S. M. MILLER, personal communication). It had an erect foliose thallus which was irregularly lobed and had an internal structure similar to the other red algal hosts. It was tentatively placed in the family Kallymeniaceae (W. A. NELSON, personal communication). Details of its biology and its geographical distribution are unknown to date. Even though the systematic placement was only tentative, for convenience this alga is treated as a true member of the Kallymeniaceae throughout the following sections.

2.1.3 Collection of endophytes

2.1.3.1 Pigmented endophytes

For the isolation of pigmented endophytic Phaeophyceae (section 2.2.1.1), natural populations of macroalgae were visited and examined. During the qualitative collection of potential host thalli, preference was given to specimens with an overall unhealthy appearance, including obvious macroscopical aberrations such as distortions, galls, discolourations or rough surface areas. Throughout this study, the term 'gall' is used in a broad sense, i. e. for all types of abnormal protuberances from the host surface, regardless of the degree of differentiation of host tissues involved in their formation. This definition also includes any amorphous, undifferentiated tumours of unlimited growth.

Host algae were collected either at low tide in the intertidal or by SCUBA divers in the subtidal. Whole thalli or pieces of collected macroalgae were either wrapped in seawater-soaked newspaper or put into seawater-filled plastic bags, placed into a chill bin on ice, and were transported directly to the laboratory within a few hours.

2.1.3.2 *Herpodiscus durvillaeae*

Samples of *Durvillaea antarctica* infected with *Herpodiscus durvillaeae* were collected in the intertidal at Brighton Beach, or St. Kilda Beach in South Dunedin, on the day of preparation. *D. antarctica* is a desiccation-resistant alga of the lower intertidal. However, to reduce any deterioration of the material prior to processing, thallus pieces were kept moist and cool by wrapping them into seawater-soaked newspaper and transporting them back to the laboratory in a chill bin. At the laboratory, the wrapped samples were stored in a refrigerator (at 4 °C).

For DNA extractions (section 2.2.3.1), field material of *Durvillaea antarctica* infected with *Herpodiscus durvillaeae* was collected in September 1997 and in June 2000. Thallus pieces taken from three host individuals were brought back to the laboratory and were macroscopically examined for the presence of epiphytes. From each *Durvillaea* individual, small fragments (2-5 cm^2) with patches of *Herpodiscus durvillaeae* without any macroscopically visible epiphytes were chosen. The thallus fragments were rinsed twice with sterile seawater and freeze-dried for 3 to 6 days. Material from the *Herpodiscus* patches was then harvested by scraping filaments from the surface with a sterile razor blade. The material was transferred into sterilized (autoclaved) Eppendorf tubes. The tubes were stored in plastic bags filled with dessicated silica gel at RT until DNA extraction.

To classify possible contaminants prior to extracting DNA, material from the *Herpodiscus* patches collected in June 2000 (containing parasite filaments and putative epiphytes) was removed before freeze-drying and was cultivated: from each of the three patches of *H. durvillaeae*, surface material was scraped off with a sterilized razor blade, and inoculated into ten separate sterile petri dishes (polysterole, 10 mL; NUNC, Denmark) filled with sterile, PROVASOLI enriched seawater (after STARR & ZEIKUS 1993). Germanium dioxide was added to the cultures to suppress the growth of diatoms (section 2.2.1.2.1). The total of 30 petri dishes were placed into a growth cabinet and cultivated in the culture conditions described in section 2.2.1.2. After three to four weeks, the petri dishes were examined for the presence of epiphytic algae. Representatives of all brown epiphyte types found were sub-isolated by pipetting into separate petri dishes and cultivated until morphological details such as the type of sporangia, shape and

number of chloroplasts, and presence/absence of pyrenoids allowed their classification and enough material for DNA extractions could be harvested.

Samples of *D. antarctica* infected with *H. durvillaeae* collected for electron microscopy studies (section 2.2.4) were treated in the same way as samples for DNA extractions (section 2.2.3.1). Immersing samples of *D. antarctica* into the fixation medium directly on the beach did not appear to improve the quality of fixation, therefore the thalli were transported back to the laboratory before further processing.

2.2 Methods

2.2.1 Morphology of pigmented endophytes

2.2.1.1 Isolation

In the laboratory, potential host algae were macro- and microscopically examined for the presence of endophytes (see section 2.2.1.3.1 for specifications of the microscope used). Pigmented endophytic brown algae were isolated from their hosts under a dissecting microscope (Olympus SZ-ST, Japan, magnification 0.67–4x, eyepiece GSWH 10x). The interior parts of infected hosts, outer and inner cortex, were cut into small fragments (cubes of circa 0.5 mm length) with a sterilized razor blade (see section 2.2.1.2.3 for sterile conditions). To avoid contamination with epiphytes, the thallus fragments were mechanically cleaned of any surface material by removing the outer cell layers with the razor blade and then washing vigorously in sterile seawater several times. Contaminations with the brown alga *Herpodiscus durvillaeae* (i. e. in the case of endophyte isolate No. 24) were not problematical as this parasite does not grow in culture.

The tissue fragments containing endophyte filaments were placed into sterile polysterene petri dishes (NUNC, Denmark) containing culture medium. The petri dishes were then placed into the culture conditions described below (section 2.2.1.2). After four to eight weeks in culture, depending on the isolate, endophyte filaments grew out of the host tissue fragments and produced sporangia. The released spores settled down on all available surfaces and grew to young thalli which were sub-isolated by pipetting into clonal cultures.

Shortened names used to refer to isolates were of the following principle: After the letter "E" ("endophyte of") came an abridgement of the host species name (e. g. "Ma" for *Macrocystis pyrifera*, or "Dwil" for *Durvillaea willana*). The abbreviation for the collection site (e. g.

"BB" for Brighton Beach, or "Waki" for Waikouaiti) was followed by the month and year of collection (e. g. "10/00" for October of the year 2000).

2.2.1.2 Cultivation

2.2.1.2.1 Culture medium

All cultures were uni-algal and non-axenic. They were cultivated in sterilized natural surface seawater (30-35 PSU) enriched with nutrients. The seawater was collected from the Otago coast, either from Cornish Head, Brighton Beach or Harington Point (Otago Harbour). The surface seawater used for cultivation in Germany originated from the Skagerrak, North Sea, north of Denmark.

The water was filtered through glass microfibre filters (GF/C, Whatman, England; pore size 45 µm) in a glass microanalysis filter holder assembly (Advantec, USA), filled into 1 L glass bottles (Schott, Germany) and sterilized in an autoclave (TOMY autoclave SS-325, Alphatech, Auckland) at 1.2 bar, 121 °C for 30 minutes. PROVASOLI's enrichment (after STARR & ZEIKUS 1993, see Appendix A 1.1) was used as a source of nutrients, metals and vitamins. Twenty mL of enrichment were added to 1 L of seawater to make up the culture medium (PES, "PROVASOLI-enriched seawater"). In some cultures, half of the recommended amount of PROVASOLI's enrichment, 10 mL per litre culture medium, was added to control an excessive growth of bacteria developing in TRIS buffer. The enrichment was sterilized separately prior to addition to the seawater to avoid a reaction between the contents of both solutions (precipitation of metals in seawater) while autoclaving.

The culture medium was changed every one to three weeks in the endophyte cultures, and every one to two months in the kelp gametophyte cultures. To suppress the growth of diatoms in new cultures, GeO_2 (6 mg/L) was added to all fresh cultures and at the first medium changes (LEWIN 1966; see Appendix A 1.2).

2.2.1.2.2 Light and temperature conditions

At the Botany Department of the University of Otago, Dunedin, the cultivation was done in two different growth cabinets (Table 2.2).

Table 2.2: Culture conditions in growth cabinets, Botany Department, University of Otago.

growth cabinet	artificial light source	temperature	daylength	photon flux density [µmol photons m^{-2} s^{-1}]
Contherm Phytotron Climate Simulator	Phillips TLD 36W/33 cool white	10 ± 1 °C	short: 10:14 hours light:darkness	25
		15 ± 1 °C	long: 16:8 hours light:darkness	25
Sanyo growth cabinet	(as above)	12 ± 0.5 °C	neutral: 12:12 hours light: darkness	40-60

Cultures isolated in Wellington in March/April 1998 were maintained for 1-5 days in a growth cabinet in the laboratory of Dr. W. NELSON at the Museum of New Zealand Te Papa Tongarewa (temperature 10 °C; photoperiod: 10 hours light, 14 hours darkness (short day); fluorescence light; further details unknown). From June 1998 until February 1999, all cultures isolated in New Zealand during 1997 and 1998 were maintained in a walk-in growth cabinet at the Marine Science Institute, Christian-Albrechts-University, Kiel/Germany, at the available settings (temperature 14 °C; photoperiod: 10 hours light, 14 hours darkness (short day); light source: fluorescence light, Osram, colour 21, 20-40 µmol photons m^{-2} s^{-1}). The cultures isolated on Chatham Island in May 1999 were kept in a household refrigerator (temperature: 4 °C; no illumination) until transporting them back to Dunedin. For transportation from one laboratory to another, the cultures were kept in a chill bin with frozen cooling elements or ice.

2.2.1.2.3 Sterile conditions

New endophyte cultures were either placed in sterile polysterene growth chambers (brand unknown) or sterilized Eppendorf tubes. All other cultures were cultivated in sterile non-aerated petri dishes (10 mL polysterole petri dishes, NUNC, Denmark), or in 300 mL glass jars, or in 1 L preserving glasses ('Agee jars') with one half of a glass petri dish serving as a lid. Culture vessels were sealed with Parafilm®. All glassware and heatproof plasticware that came into contact with the cultures had been sterilized by autoclaving (121 °C, 1.5 bar for 15 minutes) prior to use. Heat sensitive plasticware and all surfaces (table tops and metalware) were sterilized with 70% ethanol.

To enhance the growth of cultures in the large culture vessels, these were stirred with airbubbles using pasteur glass pipettes connected to an aquarium pump via silicon tubes and

sterile filters (cellulose-nitrate-filter; Sartorius; 0.2 µm pore size). The glass pipettes and tubes were sterilized by autoclaving (121 °C, 1.5 bar for 15 minutes) prior to use.

The cultures isolated in New Zealand between June 1997 and June 1998 were taken to Germany in June 1998. They were reimported to New Zealand in March 1999 under the Biosecurity Act 1993, permit no. 35744 (Ministry of Agriculture and Fisheries 19.8.98), and were treated as potentially bio-hazardous to the environment. The following precautions were taken to avoid a contamination of the New Zealand flora: Live cultures were only handled in especially secured ('PC2') laboratories. Culture vessels were labeled with biohazard stickers and were kept in closed containers away from non-biohazardardous cultures. All material that was in contact with the cultures was sterilized after use by autoclaving (121 °C, 1.5 bar for 15 minutes) or wiped with 70% ethanol (surfaces such as table tops and metalware). All waste (solid and liquid) was autoclaved before disposal.

2.2.1.3 Morphological classification

2.2.1.3.1 Light microscopy

Microscopic observations were made using an Olympus CH2 laboratory microscope, fitted with the following objectives: EA x4/0.10, EA x10/0.25, EA x40/0.65 and EA x100/0.1.25 Oil. Cells were measured using a scaled eyepiece (x10, Olympus) and an ocular micrometer.

Light microscope photographs were taken either using a Leitz Research Microscope equipped with bright field (Marine Science Institute, Kiel, Germany) or using an Olympus AX70 Research Microscope equipped with bright field and epi-fluorescence (South Campus Electron Microscopy Unit, Anatomy and Physiology Department of the Medical School, University of Otago, Dunedin, New Zealand). Specimens were examined using the following objectives: UplanFl x4/0.13, UplanFl x10/0.30 Ph1, UplanFl x20/0.50 Ph1, UplanFl x40/0.75, PlanApo x60/1.40 Oil and PlanApo x100/1.40 Oil (for details on the photographic documentation see section 3.2.5).

2.2.1.3.2 Morphological data collected

For a gross classification of the endophyte isolates, the following morphological characters were recorded: type of filaments (uni-/pluriseriate), cell size, plastid shape and number per cell, presence/absence of pyrenoids, upright filaments and true phaeophycean hairs (with or without basal sheath), type and size of reproductive structures (uni-/plurilocular sporangia) and type of germination (with/without narrow germination tube, with/without emptying of embryospore).

For statistical comparisons of the cell and sporangia sizes, ten cells and ten sporangia were measured as replicates for each isolate. Cells and sporangia were chosen haphazardly among several thalli. Data were combined for species and subspecies of endophytes confirmed by DNA analyses. To compare the data among groups, the numbers of replicates were reduced to the minimal number occuring in any of the groups using random numbers. Statistical analyses including post-hoc tests (Tukey-Kramer; SACHS 1984) were performed using the program SuperANOVA (ABACUS Concepts Inc.). Significance levels were set at $p = 0.05$ (for further details on the statistics see section 2.2.2).

2.2.2 Endophyte prevalence in *Macrocystis pyrifera*

2.2.2.1 Sampling

The prevalence of infections by pigmented endophytic brown algae in *Macrocystis pyrifera* was determined in three populations along the Otago coast. The sampling was designed to test the null-hypothesis of no difference in prevalence or severity of infection among sites and seasons. During the year 2000, *Macrocystis* thalli were collected from Quarantine Point in the lower Otago Harbour, from Aramoana mole at the Harbour entrance and from an offshore kelp bed northeast of Cornish Head, south of Pleasant River (Figure 2.2; photographs of the sites in Plate 3.1, Figures A-C).

The *Macrocystis* population at Quarantine Point grows in a sheltered bay on the eastern side of the promontory that separates the upper from the lower Otago Harbour, outside the main shipping channel. The thalli were collected in circa 2 m depth (below mean low water) close to an intertidal mudbed. Along the Aramoana Mole, *Macrocystis* thalli occur in 1-5 m depth, and thalli were collected at the lower distribution limit. In contrast to Quarantine Point, this population is exposed to strong tidal currents, even though the site is partly sheltered from wind and waves by the promontory of Taiaroa Head on the other side of the Harbour entrance. The *Macrocystis* population sampled off Cornish Head comprises the southern most part of a large off-shore kelp bed stretching over ca. 300 ha northeast of Waikouaiti (FYFE *et al.* 1999), with thalli growing in 10-12 m water depth. This site is fully exposed to wind and waves from south-eastern directions.

At the two Harbour sites, thalli were collected every three months, in January (summer), April (autumn), July (winter) and November (spring). The population off Cornish Head was sampled twice, in January and July, as collections were not possible in autumn 2000 due to

continuing bad weather conditions and in spring 2000 due to a substantial decline of the population during the preceding winter. Additionally, *Macrocystis* thalli were collected at the same three sites, prior to the field study in 2000, on the follwing dates: at Aramoana: 03.12.1997 (15 thalli), 05.03.1998 (ten thalli), 05.10.1999 (seven thalli); at Quarantine Point: 11.10.1999 (five thalli); at Cornish Head: 07.10.1999 (five thalli).

During the study in 2000, at each sampling date nine entire *Macrocystis* thalli (i. e. three subsamples which each consisted of three thalli that were in 2-3 m distance from one-another) were haphazardly collected from each site by SCUBA divers. Thalli were transported back to the laboratory and stored in plastic bags until further processing took place (usually the day following collection). The bags were kept in the shadow outside the laboratory, as cooling facilities were not available for the large amount of material collected.

For each thallus, its overall length (cm), fresh weight (kg), size of the holdfast (length*width*height) as well as the frond composition, i. e. the number and age group of fronds, were recorded. Age groups were determined as follows: according to the length of its longest fronds (henceforth called canopy fonds), each thallus was divided into three equal parts (base, middle, and top). Canopy fronds were those reaching the top. Sub-canopy fronds were those reaching between one third and two thirds of the overall thallus length. Juvenile fronds attained less than one third of the thallus length. Fronds which had lost their apical scimitar were considered senescent, irrespective of their length.

Depending on the presence of endophytes and the severity of the symptoms associated with the infection, each thallus was classified into one of four arbitrary disease categories (DC, Table 2.3; after PETERS & SCHAFFELKE 1996), relative to the highest DC found in any of its fronds. The entire thallus was first macroscopically examined for the presence or absence of infection symptoms. Parts displaying symptoms were hand-sectioned, and the sections were viewed under a compound microscope (for details see section 2.2.1.3.1).

Observations on specimens collected before 2000 suggested that the infection of New Zealand *Macrocystis pyrifera* with brown endophytes was mainly located in the stipes. Additionally, cauloids persisted longer on the thallus than the phylloids. For example, most senescent fronds consisted only of the cauloid, with a few bladders still attached, while the phylloids had already decayed. Therefore, to classify thalli without visible disease symptoms, examinations concentrated on the cauloids of the fronds.

Table 2.3: Arbitrary disease categories for infection with endophytic brown algae (after PETERS & SCHAFFELKE 1996).

disease category	description
0	healthy, no endophytes detected under the microscope
1	endophytes detected by microscopy, but no macroscopic symptoms associated with the infection
2	endophytes present; thallus displays moderate macroscopic symptoms (such as dark patches, warts or galls, but no thallus deformations)
3	endophytes present; thallus displays severe macroscopic symptoms (morphological changes, such as cauloid distortions or a stunted appearance)

Each unsymptomatic thallus was sampled as follows (Figure 2.6): from each basal, mid and top part of every frond, three cauloid pieces 4-5 cm long were sampled by using a random number table, giving nine pieces for a canopy frond. The pieces were transferred to small plastic bags and were stored at –20 °C until further processing. For microscopic examination, three thin sections were cut by hand with a razor blade from every cauloid piece while still frozen. The sections were then examined under a compound microscope (10x and 40x magnification; for details see section 2.2.1.3), and the presence or absence of endophyte filaments was recorded. Thus, depending on the frond length, between three (juvenile and short senescent fronds) and up to 27 (canopy fronds) sections were examined for each frond. If at least one of the sections examined displayed endophyte filaments, the frond was considered infected, and thus the thallus was classified in DC 1.

Some individuals with heavy infection symptoms in the cauloids also possessed affected pneumatocysts (e. g. in a thallus from Aramoana Mole, collected in November 2000; Plate 3.1, Figure F), while the vegetative phylloids rarely showed galls (e. g. on a thallus from Pilots Beach, collected in June 1997). However, no macroscopic infection symptoms (including dark patches) were observed on any of the sporophylls examined during this study. To confirm this observation by microscopy, additional thalli were collected at Harington Point (27.04.1999: nine thalli) and at Aramoana Mole (01.02.2001: ten thalli) and surveyed for the presence of endophytes in phylloids and sporophylls.

From every thallus collected at Harington Point in 1999, three fronds and a sporophyll were haphazardly chosen. From each frond, three pieces of cauloid with attached pneumatocyst and phylloid were taken. Three sections were hand-cut from each of these organs as well as from the sporophyll, and were examined for the presence of endophytic Phaeophyceae.

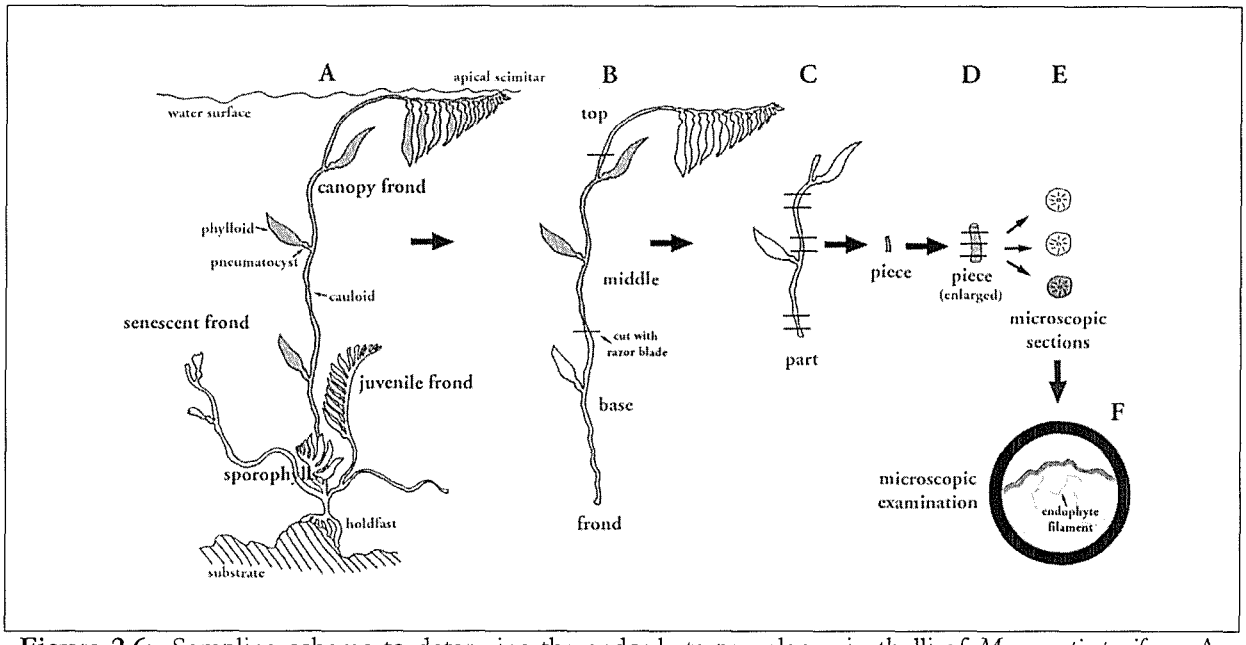


Figure 2.6: Sampling scheme to determine the endophyte prevalence in thalli of *Macrocystis pyrifera*. A: Thallus of *M. pyrifera* in its habitat, with terms mentioned in the text. B-F: Steps of processing, for example, of a piece (D) of the middle part (C) of a canopy frond (B). Single sections (E) of the piece were examined under a compound microscope (F). The part of the thallus cut off and further processed is highlighted (dark grey). Figures are not scale.

From each thallus collected at Aramoana Mole in February 2001, a sporophyll and two mature vegetative fronds were sampled, one appearing healthy (DC 0 or 1) and the other one showing moderate macroscopic infection symptoms (DC 2). Three cross sections were taken from each sporophyll. From each frond (healthy or infected), three cauloid pieces with attached bladder and phylloid were sectioned. All sections were examined for endophyte filaments under a compound microscope.

2.2.2.2 Statistical analyses

To test the H_0 of no difference in the prevalence or severity of infection with *L. macrocystis* among host populations or seasons sampled, analyses of variance (ANOVAs) or Chi-square tests were performed, using the programs SuperANOVA and StatView SE+Graphics™ (both Abacus Concepts Inc., Berkeley, California, USA), respectively, on a Power Macintosh G3 computer. Dependent variables were continuous (e. g. length or fresh weight of thalli) or discrete (e. g. number of infected thalli). The latter were transformed into continuous variables, with the prevalence of infection expressed as percentage of infected thalli in sub-samples, or the severity of infection expressed as average disease category of a sub-sample, respectively.

Three conditions have to be met to allow an ANOVA (REUSCH 1995): independent and randomised sampling, normal distribution of the data, and homogeneity of variances. Thalli were not sampled randomly, but haphazardly, whereas sampling within each thallus was based on random numbers. Biological data rarely show a normal distribution, however, the test value *F* of the ANOVA is moderately robust against a violation of normal distribution if the variances are homogeneous, and if the sampling design is balanced (REUSCH 1995). The sampling design was balanced by excluding the Cornish Head population from analyses comparing all four seasons, and by excluding spring and autumn data from analyses comparing all three sites. Data were subjected to nested ANOVAs to test whether the three sub-samples (of three thalli) for every site and season contributed to the residual variance. In cases where these were markedly insignificant ($p > 0.2$) for the sites as well as for the seasons (i. e. the variances within the sub-samples were not significantly larger than the variances among the sub-samples), the nine thalli could be regarded as true replicates (UNDERWOOD 1981, cited in REUSCH 1995). Post-hoc tests after TUKEY-KRAMER were performed in SuperANOVA, to identify any levels of factors contributing to signifcant differences (REUSCH 1995).

Homogeneities of variances were tested after COCHRAN (according to SACHS 1984). In cases where variances were heterogenous even after transformation (e. g. Arcsine-square-root transformed percentage values), data were tested for significant differences using Chi-square tests (SACHS 1984). No variances could, for example, be calculated for the prevalences of infection when all thalli were infected in sub-samples (i. e. at a prevalence of 100%). For the assessment of these data, especially in comparisons of prevalences of infection among the three sites, the disease category was included as a factor, to enhance differences by accentuating the severity of infections.

2.2.3 Molecular systematics

2.2.3.1 DNA extraction

The DNA of cultures isolated in 1997 and 1998 was extracted in a laboratory in the Institute for Marine Science in Kiel, Germany. All DNA extractions from cultures isolated after February 1999 were performed in a laboratory at the Biochemistry Department of the University of Otago, New Zealand.

2.2.3.1.1 Extraction after VAN OPPEN *et al.* (1993)

DNA from freeze-dried material of *Herpodiscus durvillaeae* collected at Brighton Beach in September 1997 was extracted following a modified method after VAN OPPEN *et al.* (1993).

All instruments and reaction vessels that came in contact with the alga material were sterilised prior to use to avoid a contamination with alien DNA. Heat stable glass- and plasticware (such as Eppendorf tubes and extraction tubes) and sea sand for grinding were autoclaved (121 °C, 1.5 bar, 20 min), volumetric glassware was heat sterilised (5 hours at 150 °C), mortars and pestels were wiped with 70% ethanol.

Extraction buffer was freshly prepared on the day of the extraction. For one sample, it contained 0.3975 mL NaCl (4 M; Merck, Germany), 0.237 mL EDTA (0.5 M; Merck, Germany), 0.3945 mL Tris-HCl (1 M, pH 8; Boehringer, Merck, both Germany), 6.471 mL dd H_2O and 0.4875 mg dithiotreitol (Bio-Rad, USA).

To break down cell walls, 5-20 mg of dried material were ground with sea sand and liquid nitrogen to a fine powder, using a pre-frozen (at –20 °C) porcelain mortar and pestle. The powder was transferred into a 50 mL sterilised extraction tube ("Oak Ridge" tubes, Nalgene, USA), and 7.5 mL extraction buffer, 100 µL mercaptoethanol solution (Merck, Germany) and 300 µL Triton-X-100 (Boehringer, Germany) were added. After vortexing the mixture for 1 minute, 2.25 mL phenol (Tris-EDTA saturated; Merck, Germany), 2.25 mL chloroform (Merck, Germany) and 1 mL isoamylalcohol were added one after another, with gentle mixing after every addition. Centrifugation at 1800 *g* (table-top centrifuge, Hettich Universal 16A) separated the contents into four layers.

The water phase on top containing the DNA was transferred into a new tube; the other phases (denatured proteins, pigments in chloroform, sand) were discarded. To remove more protein from the water phase, an equal amount of CIA (chloroform-isoamylalcohol in a ratio of 24:1) was added, and the mixture was centrifuged. To precipitate polysaccharides, the water phase was transferred to a new tube, and 1/10 volume of potassium acetate (5 M; Merck, Germany), 1/4 volume of 100% ethanol (Merck, Germany) and one volume of CIA were added, taking the new resulting volume in account for each following step and mixing gently after each addition. After centrifuging (1800 *g*) and transferring the water phase again, the DNA was precipitated by adding 1/10 volume of sodium acetate (4 M; Merck, Germany) and the same volume ice cold (-20 °C) isopropanol (Merck, Germany), and leaving the samples at –20 °C overnight.

On the following day, the sample was centrifuged with 20,000 *g* for 20 minutes at 4 °C (Heraeus, Cryofuge 20-3). The supernatant was discarded. The DNA pellet was washed three times with 0.5 mL 70% ice cold (-20 °C) ethanol to remove salts and isopropanol, vacuum dried in an exsiccator for 10 minutes and then resuspended in 0.5 mL dd H_2O.

To digest RNA, 55 µL RNAse (0.087 units μL^{-1}; RNAse A, Qiagen, Germany) were added and the sample was then incubated for 30 minutes at 37 °C. Thereafter, contaminations were removed by filtering the sample through a Qiagen 100 column (Qiagen, Germany): to prepare the column, it was equilibrated three times with equilibration buffer (Table 2.4). The DNA solution was mixed with loading buffer and dd H_2O, and then loaded onto the column. After washing the column two times with wash buffer, the DNA was eluted with two elution buffers of different pHs (pH 7.5 and 8), into a reaction tube filled with 10 ml ice-cold (-20 °C) isopropanol.

All buffers used in this cleaning step contained MOPS (3-(N-morpholino) propane sulfonic acid; Sigma, Germany). For 1 M MOPS solution, 20.93 g of MOPS were solved in ca 70 mL dd H_2O. The pH was adjusted with NaOH, and the solution was made up to 100 mL with dd H_2O.

Table 2.4: Solutions for cleaning DNA with Qiagen 100 columns. All volumes are in mL. *: volume includes DNA solution.

	NaCl	MOPS pH 7.0	MOPS pH 7.5	MOPS pH 8.0	Ethanol	dd H_2O	total volume
equilibration buffer	0.47	0.15	-	-	0.375	1.53	2.5
loading solution (buffer with DNA and water)	0.55	0.15	-	-	-	2.3*	3
wash buffer	2.5	0.5	-	-	1.5	5.5	10
elution buffer 1 (pH 7.5)	0.94	-	0.125	-	0.375	1.06	2.5
elution buffer 2 (pH 8.0)	0.94	-	-	0.125	0.375	1.06	2.5

After precipitating the DNA over night at –20 °C, the sample was centrifuged at 20,000 *g* for 20 minutes at 4 °C. The supernatant was removed, and the pellet was carefully rinsed once with 0.5 mL ice-cold (-20 °C) 70% ethanol. After removing the ethanol, the pellet was vacuum-dried in a desiccator for 10 minutes and then resuspended in 100 µL TE buffer (0.1% 1 M Tris, 0.02% 0.5 M EDTA; pH 8; Merck, Germany). The dissolved DNA was transferred into an Eppendorf tube and an aliquot was diluted with TE buffer to reach the working concentration of 1:10 and 1:100 for subsequent amplifications. DNA solutions were stored at –20 °C.

The success of the extraction was controlled on a submerged horizontal agarose gel (1% SeaKem® LE agarose in Tris-acetate-EDTA (TAE) buffer) stained with 0.015% ethidium

bromide (Bio-Rad, USA). Five µL of undiluted DNA sample were mixed with 2 µL loading dye (Orange-G + Ficoll; Merck, Germany) and loaded into a well of the gel. Gel apparatus and power Pack were supplied by Biometra, Germany. A 20 mL gel (20 mL TAE buffer, 0.2 g agarose, 3 µL ethidium bromide solution (10 mg mL^{-1})) was run at 50 V (40 mA) for 20 minutes. After the run, the gel was placed on top of a UV transilluminator (Biometra, Germany), and a photograph was taken with a direct screen instant camera (Polaroid, USA) to document the results.

2.2.3.1.2 Extraction with Qiagen Plant DNAeasy Kit

Freeze-dried DNA from *Herpodiscus durvillaeae* collected at Brighton Beach in June 2000, and DNA from living culture material of endophytic brown algae were extracted using the Qiagen DNeasy Plant Mini Kit (Qiagen, Germany). All centrifugation steps were performed using a table-top centifuge (Eppendorf 5415C or SIGMA 1-15 laboratory centrifuge).

The culture medium was removed by filtration through a sterilised (autoclaved) glass fibre filter (GF/C; Whatman, England), washing the filter and any adhering alga material two times with Millipore® water and pat-drying the filter. Between 10 and 60 mg of the fresh algal material or 20 mg of the dried *H. durvillaeae* material were then ground in liquid nitrogen in a porcelain mortar and pestle (sterilised with 70% ethanol and pre-frozen at –20 °C). DNA was extracted from the resulting fine powder with the Qiagen kit, following the manufacturer's instructions (ANONYMOUS 1997). As marine algae produce large amounts of precipitates after adding the precipitation buffer (AP2, step 4), samples were always centrifuged for 5 minutes at 14,000 rpm before removing contaminants with the QIAshredder columns.

After the extraction, the resulting DNA solutions were diluted with TE buffer (buffer AE of the Qiagen kit) to reach the working concentration of 1:10 and 1:100 for subsequent amplifications. DNA solutions were stored at –20 °C.

The success of the extractions was monitored on 1% agarose gels as described in section 2.2.3.1.1. At the Biochemistry Department, University of Otago, the gel was run on a Bio-Rad MiniSUB cell GT, powered by a Bio-Rad Power Pack 300. The samples loaded onto the gel each contained 1 µl DNA solution and 4 µl loading dye (10-20 mg mL^{-1} bromophenol blue). Ethidium bromide to stain the DNA was added to the TAE buffer running the cell, instead of adding it to the gel itself. A 150 mL gel (150 mL TAE buffer, 1.5 g agarose) was run at 120 V for 40 minutes. For photographic documentation, the gels were exposed to UV light, and the images were captured by an imaging system linked to a personal computer.

2.2.3.1.3 Extraction after P. LOCKHART (personal communication)

High molecular weight DNA was obtained from fresh *Herpodiscus* samples collected in May 2001 using a modified recipe for CTAB extraction (P. LOCKHART, personal communication). The procedure excluded any pelleting step to avoid shearing of the DNA.

Around 20 mg of fresh material of *Herpodiscus* were filled into a sterilised Eppendorf tube. Four hundred µL CTAB buffer (without mercaptoethanol; RT; Table 2.5) were added. To break open cell walls, the sample was first cooled by dipping the tube into liquid nitrogen. Then the tissue was ground with a sterilised glass Pasteur pipette. The sample was placed into a waterbath at 65 °C, and while extracting, was flipped and mixed several times. After 10 to 15 minutes in the waterbath, an equal amount of chloroform (400 µL, at room temperature) was added, the tube flipped to mix and left at room temperature for 10 minutes.

A short spin in a table-top centrifuge separated cell debris from the liquid. The supernatant was transferred into a new sterilised Eppendorf tube (pre-cooled on ice). For this transfer, the tip of the pipette was cut off to avoid shearing of the DNA.

Table 2.5: CTAB extraction buffer for DNA extraction (without mercaptoethanol, modified after P. LOCKHART, personal communication).

ingredient	amount
2% CTAB (hexadecyltrimethyl-ammonium-bromide)	40 mg
1% PVP (polyvinylpolypyrrolidone)	20 mg
NaCl	163,6 mg
0.02 M EDTA	11,6 mg
0.1 M Tris-HCl pH 8	24,2 mg
dd H_2O	2 mL

An equal amount of 100% isopropanol (room temperature) was added to the mixture. The tube was carefully inverted once and then left to stand on ice for 10 to 15 minutes. This step resulted in the DNA forming a fluffy buoyant mass in the middle of the tube. The precipitated DNA was then washed by transferring it into 1 mL of 80% ethanol (at room temperature) and carefully turning. After repeating this step once or twice, depending on the amount of pigments carried over, most ethanol was removed, and the sample was air-dried at room temperature. The dried DNA was resuspended in 20 µL TE buffer and diluted (1:10 and 1:100) for subsequent PCR reactions. DNA solutions were stored at –20 °C.

2.2.3.2 Polymerase chain reaction

The oligonucleotide primers used for amplification (Table 2.6) were designed for brown algae in general or were specific for brown endophytes (PETERS & BURKHARDT 1998; BURKHARDT & PETERS 1998; A. F. PETERS, personal communication), or were general primers for eukaryotes (SAUNDERS & KRAFT 1994; BROOM *et al.* 1999). Apart from G04 and J04, all oligonucleotide primers were manufactured by Tib Molbiol, Berlin, Germany. The primers G04 and J04 were supplied by Life Technologies (Auckland, New Zealand). The dry primers were first solved in sterilised water (dd H_2O or Millipore® water), in the volume stated by the manufacturer (dependant on the amount of primer) to obtain a stock solution with a concentration of 100 pmol μL^{-1}, then the stock solutions were diluted to a working solution with a concentration of 20 pmol μL^{-1}.

Table 2.6: Oligonucleotide primers used in the present study. References: [1]: A. F. PETERS, unpublished; [2]: PETERS & BURKHARDT 1998; [3]: PETERS & RAMIREZ 2001; [4]: SAUNDERS & KRAFT 1994; [5]: BROOM *et al.* 1999.

primer	sequence (5'-3')	direction	opposite primer	region	specific for
L'colax2(F)[1]	AGTAACGTCTTACAGCATTG	forward	5.8S1(R)	ITS1	*Laminariocolax* sp.
Streblo2(F)[1]	AAACTGCGCGAAAGAATCTC	forward	5.8S2(R)	ITS1	*Microspongium* sp.
AFP2(F)[2]	AGCTCTTTCTTGATTCTATG	forward	5.8S1(R)	ITS1	brown algae
			LSU-115(R)	18S-26S	brown algae
5.8S1(R)[2]	TGATGATTCACTGGATTCTG	reverse	L'colax2(F)	ITS1	brown algae
			AFP2(F)	ITS1	brown algae
5.8S2(R)[3]	CAACAGACAATACGACAAGC	reverse	Streblo2(F)	ITS1	brown algae
LSU-16(F)[3]	CCGATCAAGCAAGAGGACC	forward	LSU-115(R)	26S	brown algae
LSU-115(R)[3]	CTCTCCAGACTACAATTCGG	reverse	LSU-16(F)	26S	brown algae
			AFP2(F)	26S-18S	brown algae
LSU1046(R)[3]	TGGCCCACTAGCAACCTTC	reverse	LSU-16(F)	26S	brown algae
G04[4]	CAGAGGTGAAATTCTTGGAT	forward	J04	18S	eukaryotes
J04[5]	AAACCTTGTTACGACTTCTCC	reverse	G04	18S	eukaryotes
rbcL95F[3]	ATGGGATATTGGGATGCTGA	forward	rbcL1087SR	*rbc*L	plastids
rbcL1087SR[3]	CCATATCAAAGAATAAACCTTC	reverse	rbcL95F	*rbc*L	plastids

The oligonucleotide primers for amplifying nuclear encoded ribosomal DNA are positioned on various sites on the gene (Figure 2.7). A test with the genus-specific forward primers for endophytic brown algae, L'colax2(F) and Streblo2(F), allowed a tentative classification of the isolates. The 3' end of the 18S subunit gene and the whole ITS1 region were amplified with the primer pair AFP2(F)-5.8S1(R) specific for brown algae. The primer pair AFP2(F)-LSU115(R) was used to amplify the whole ITS region (end of 18S-ITS1-5.8S-ITS2-start of 26S).

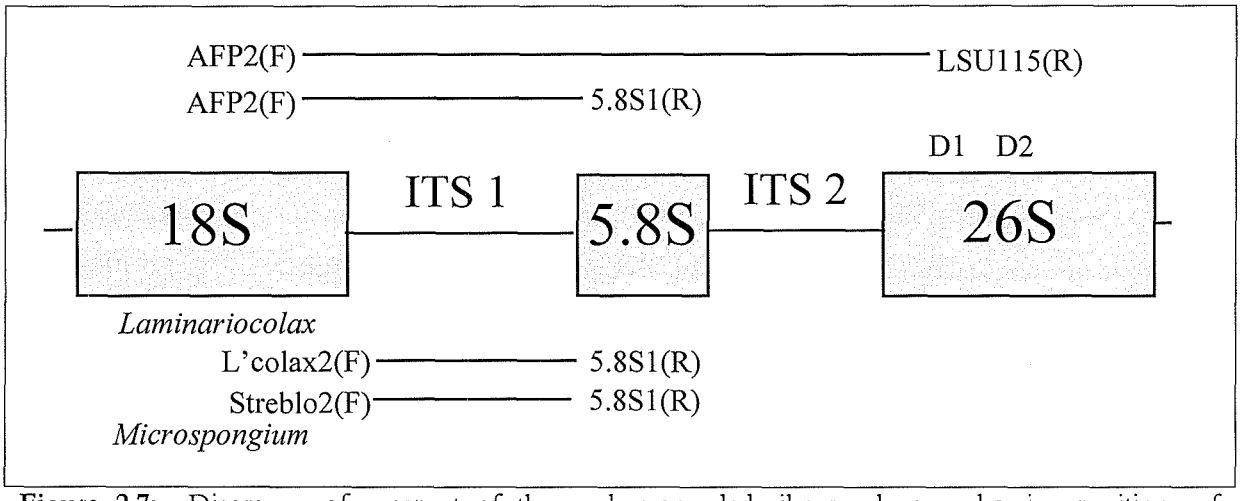


Figure 2.7: Diagramm of a repeat of the nuclear-encoded ribosomal gene showing positions of oligonucleotide primers and regions amplified with them. ITS: internal transcibed spacer; D1 and D2: variable domains within the 26S (ROUSSEAU *et al.* 1997); (F). forward primer; (R): reverse primer. Lengths are not proportional.

Within the 5'-end of the 26S, there are two regions with a relatively high variability in brown algae, D1 and D2 (ROUSSEAU *et al.* 1997), which were amplified using the primer pair LSU16(F)-LSU1046(R). The gene for the large subunit of the chloroplast encoded ribulose-1,5-bisphosphate carboxylase/ oxygenase (RuBisCO), *rbc*L, was amplified with the primer pair rbcL95F and rbcL1087SR (Figure 2.8).

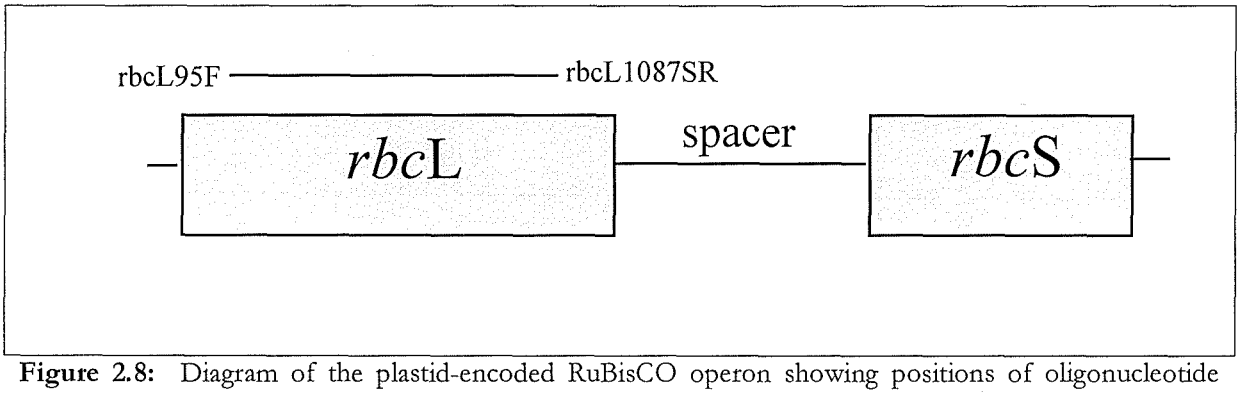


Figure 2.8: Diagram of the plastid-encoded RuBisCO operon showing positions of oligonucleotide primers used for amplifying the partial *rbc*L. *rbc*L: large subunit coding gene; *rbc*S: small subunit coding gene. Lengths are not proportional.

A change of equipment (e. g. thermocycler) and providers for ingredients in the laboratory at the University of Otago, compared to the laboratory in Germany, made adjustments to the recipes for the PCR master mixture and the reaction mixture necessary. The final recipe for the master mixture already contained all water. Compared to the initially used master mixture, the

magnesium concentration was reduced to 2.5 mM and the primer concentration was raised to 1 pmol μL^{-1} for each primer (Table 2.7).

The enzyme, recombinant *Taq* DNA polymerase (1 U μL^{-1}), the PCR buffer (10x: 750 mM Tris-HCl, 200 mM $(NH_4)_2SO_4$, 0.1% Tween® 20), magnesium chloride (25 mM $MgCl_2$) and desoxynucleotides were purchased from MBI Fermentas, Germany. The dried dNTPs were solved in dd H_2O or Millipore® water to a stock concentration of 100 mM. To obtain a working solution containing all dNTPs in a concentration of 200 µM each (= 10x dNTPs), 20 µL of each dNTP stock solution were mixed together with 920 µL dd H_2O or Millipore® water. All chemicals and solutions were kept at –20 °C.

Table 2.7: Master mixtures for polymerase chain reactions. [1]: dd H_2O; [2]: Millipore® water; [3]: not determined, as primers varied in their molecular weigths depending on composition and length.

	initial recipe		final recipe	
ingredient	amount per 10 µL reaction	final concentration in reaction	amount per 10 µL reaction	final concentration in reaction
sterilised H_20	0.8 µL[1]		5.25 µL[2]	
$MgCl_2$	1.6 µL	4 mM	1 µL	2.5 mM
10x buffer	1 µL	1x	1 µL	1x
10x dNTPs	1 µL	20 µM for each dNTP	1 µL	20 µM for each dNTP
forward primer	0.2 µL (50 ng μL^{-1})	n. d.[3]	0.5 µL (20 pmol μL^{-1})	1 pmol μL^{-1}
reverse primer	0.2 µL (50 ng μL^{-1})	n. d.[3]	0.5 µL (20 pmol μL^{-1})	1 pmol μL^{-1}
Taq polymerase (1U μl^{-1})	0.4 µL	0.04 U μL^{-1}	0.25 µL	0.025 U μL^{-1}

Diagnostic PCRs (Table 2.8) had a reaction volume of 10 or 20 µL (initial/final recipe, respectively). Preparative PCR reactions for subsequent sequencing had a volume of 20-40 µL reaction volume for the automatic sequencing or 100 µL for the manual sequence reaction.

Every PCR run included a negative control containing only the master mix to detect any DNA contamination of the reaction mixture. Positive controls were included after the first successful PCRs; these consisted of DNA that was previously successfully amplified with the respective primer pair.

Table 2.8: Reaction mixtures for diagnostic polymerase chain reactions. For preparative PCR reactions a 40 or 100 µL reaction mix was used. DNA was added in a 1:10 or 1:100 dilution. *: dd H_2O.

ingredient	initial recipe	final recipe
Master mix	10 µL	19 µL
H_2O	8 µL*	(already contained in Master mix)
DNA	2 µL	1 µL
total volume	20 µL	20 µL

The PCR protocol was as follows (Table 2.9): an initial denaturation of the double stranded DNA at 94 °C was followed by 32 to 35 cycles of denaturation, annealing of the primers at 55 °C, and extension of the complementary DNA by the DNA polymerase at 72 °C. After an additional final extension, the samples were kept on a cold block at the "stop" temperature before removing them to a household refrigerator (4 °C).

Table 2.9: PCR protocols. Temperatures and duration of steps during PCR.

		steps during one cycle						
	initial denaturation	1. denaturation	2. annealing	3. extension	no. of cycles (step 1-3)	final extension	cooling down	stop
initial protocol	94 °C 3 min	94 °C 1 min	55 °C 0.5 min	72 °C 1.5 min	32	72 °C 3.5 min	15 °C 1.5 min	4 °C
final protocol	94 °C 3 min	94 °C 1 min	55 °C 1 min	72 °C 1.5 min	32 or 35	72 °C 3.5 min		6 °C

Initially, PCRs were performed on a thermocycler (PTC 100, MJ Research, USA) using the program stated in Table 2.9. From March 1998, a robocycler (Robocycler Gradient 96, Stratagene Corporation, La Jolla, California) was employed. The success of amplifications was monitored on 1% agarose gels as described in section 2.2.3.1. The lengths of the gene fragments were compared to a DNA marker (0.5 µg μL^{-1}, GeneRuler™ 100 bp DNA Ladder plus, MBI Fermentas, Germany).

2.2.3.3 Purification of PCR products

To remove reaction ingredients such as primers, enzyme and dNTPs, the PCR products were purified before subsequent sequencing reactions. Initially, the QIAquick Purification Kit (Qiagen, Germany) was used following the instructions of the manufacturer. The purified DNA was resuspended in 55 µL dd H_2O, enough for five sequence reactions.

From March 1998 onwards, PCR products were purified by a polyethylene glycol precipitation (J. E. BROOM, personal communication): the PCR product was transferred to a sterile Eppendorf tube. An equal amount of polyethylene glycol-NaCl solution containing 20%

polyethylene glycol (PEG 8000) and 2.5 M NaCl was added. After gently mixing the contents, the mixture was incubated for 15 minutes at 37 °C to precipitate the DNA. The precipitate was spun down at 14,000 rpm (25 minutes), and the supernatant was removed. The DNA pellet was washed by adding 200 µL 75% ice-cold (-20 °C) ethanol, followed by another centrifugation for 15 minutes at 14,000 rpm. The supernatant was discarded. After repeating this washing step once, the pellet was air dried in a drying oven at 30 °C for at least an hour.

The dried DNA pellet was resuspended in 20 µL sterilised Millipore® water. The concentration of the DNA in the solution was estimated by applying 1 µL of the DNA solution to an 1% agarose gel (see section 2.2.3.1) and comparing the brightness of the band with the known concentration of a DNA marker (BioMarker Low) containing 10 ng DNA μL^{-1}. Cleaned PCR products were stored at –20 °C.

2.2.3.4 Sequencing

Sequences were obtained with the Dideoxy Chain Termination method after SANGER *et al.* (1977): in addition to deoxy nucleotide triphosphates (dNTPs), dideoxy nucleotide triphosphates (ddNTPs) are present in the reaction mix. The second deoxy group in the ddNTP prevents a reaction at the 3'-end of the newly forming DNA, thus in cases when the polymerase appends a ddNTP instead of a dNTP to the DNA, the chain is terminated.

After synthesis of the DNA, fragments are separated electrophoretically on a polyacrylamide gel, which can discriminate between fragments with length differences down to one base pair. Radioactive or fluorescence labeling of the DNA enables the visualisation of the DNA. The resulting band pattern on the gel is translated directly into the DNA sequence

2.2.3.4.1 Manual sequencing

Initially, sequences were obtained manually by using radioactively labeled ^{35}S-dATP in the sequencing reaction. ^{35}S emits β radiation, with a half-life of 87.4 to 90 days. For visualisation, X-ray film (Kodak X-OMAT) was exposed to dried gels. All work with radioactive material was carried out in the isotope laboratory of the Marine Science Institute in Kiel, Germany.

The entire sequencing process consisted of four steps: the sequencing reaction, the production of the polyacrylamide gel, the loading and running of the gel and the exposure of photographic film to the radioactively labeled gel. Sequences were read by eye from the developed films.

Sequence reaction

The ^{T7}Sequencing Kit of Pharmacia, Germany, was used for the sequencing reaction. For each reaction, two solutions were prepared: the "annealing mix" contained 2 µL annealing buffer (supplied in the kit), 2 µL primer (120 ng μL^{-1}), and 10 µL template DNA. The "labeling mix" contained 2.5 µL enzyme dilution buffer, 1.5 µL labeling mix A, 1.0 µL of the radioactively labeled ^{35}S-dATP (Hartmann, Germany; 1:1 diluted with dd H_2O), and 0.5 µL T-7 DNA polymerase. Apart from the ^{35}S-dATP, all ingredients of the labeling mix were part of the kit. The primers used were different from the PCR primers and annealed to conservative sequences within the PCR products. For the control reaction, a separate annealing mix with 2 µL universal primer, 3 µL template DNA from the kit and 7 µL dd H_2O was prepared. For each reaction, 2.5 µL of each of the four ddNTPs was pipetted into a separate, differently coloured Eppendorf tube and pre-heated to 37 °C in a heating block.

In the first step of each reaction, the annealing mix was heated up to 95 °C for 3 minutes to denaturate the double stranded DNA. The solution was then snap-cooled in ice-water for 30 seconds and kept on ice for the addition of 5.5 µL labeling mix. The contents were mixed, spun down, and 4.5 µL of this mixture were pipetted into each of the coloured tubes containing the ddNTPs. After 5 seconds of centrifugation, the reaction tubes were placed into a heating block at 37 °C. The reaction was incubated for 5 minutes before addition of 3.5 µL stop solution (from the kit) terminated the enzyme activity. The reactions were stored at –20 °C till the gel run.

Preparation of the polyacrylamide gel

The DNA fragments resulting from the sequence reaction were separated in an 8% polyacrylamide 'top gel' sitting on top of a 'bottom gel' which contained sucrose to slow down the fragments' movements. Before preparing the gel solutions, the gel sandwich (Life Technologies, England) was assembled: two glass plates (38 x 38 cm) were washed with ethanol, siliconised with Sigmacote (Merck, Germany) and washed again with ethanol. Two plastic spacers cleaned with ethanol were placed between the glass plates, holding them 0.4 mm apart. The sandwich of glass plates and plastic spacers was sealed on three sides with tape and fixed into a rubber holder.

Acryl stock solution was prepared from Acrylamide/Bis 19:1 crystals (Bio-Rad, USA), by adding 23.7 mL dd H_2O to 15 g crystals. Both gels were freshly prepared from stock solutions (Table 2.10) on the day of use. The stock solutions, 55 mL for the top gel and 5 mL for the

bottom gel, were degased in a vacuum prior to use. Then tetramethylene diamine (TEMED; Sigma, Germany) and ammonium persulfate (25 mg APS in 100 µL dd H_2O) were added to each solution: 44 µL TEMED and 88 µL APS for the top gel and 1.7 µL TEMED and 8.3 µL APS for the bottom gel.

Table 2.10: Stock solutions for the sequencing gels. The solutions were stored at 4 °C.

ingredient	top gel stock	bottom gel stock
acryl stock solution	15 mL	15 mL
urea	42 g	42 g
buffer (10 x Tris-boric acid- EDTA)	10 mL	50 mL
sucrose	-	10 g
bromphenol blue dye (10-20 mg mL^{-1} stock solution)	-	1–2 mL
	add to 100 mL with dd H_2O	add to 100 mL with dd H_2O

To seal the bottom of the gel, 5 mL of the top gel solution were mixed with the bottom gel solution and filled into the gel chamber first, before adding the top gel solution. To obtain a smooth upper end of the gel, a shark-tooth comb was placed on top of the gel with the straight side down. Metal clamps were added to the sandwich to allow the gel to polymerise in an evenly thickness. Wet sponges were added to the top of the gel to keep it moist while polymerising over night.

Gel loading and run

After removing the metal clamps, the wet sponge, the rubber holder and the tape, the polymerized gel, sandwiched between the two glass plates, was positioned into the bottom trough of an electrophoresis apparatus (Model S2, Life Technologies, England). To create a contact between electrodes and gel, around 400 mL of 1x TBE buffer were filled into both troughs. The shark-tooth comb was turned upside down and placed on top of the gel. The resulting wells were washed with buffer to remove any urea. To test the quality of the gel, every second well was filled with stop solution (from the ^{T7}Sequencing kit) diluted 1:3 with TBE buffer, and the gel was run at 1.9 V (circa 70 mA) for about 30 to 45 minutes, resulting in a gel temperature of 50 °C.

Prior to applying the DNA samples, these were denatured for 3 minutes at 85 °C to separate the two strands. After washing the gel wells again with buffer to remove any urea, 1.6 µL sample were pipetted into each well. After pipetting 6x4 samples, the gel was run for

10 minutes to reduce the diffusion of the samples intoadjacents wells, before pipetting the next samples.

After applying a maximum of 20x4 samples, the gel was run at the same settings as the test run, i. e. 1.9 V. The running time, two to five hours, depended on the length of the DNA fragments to be separated.

After the run, the gel was taken out of the electrophoresis and briefly cooled under running tap water. Then the top glass plate was removed, and the gel was fixed in a bath of 10% acetic acid, 10% methanol and 80% deionisised water for 10 minutes. After carefully levelling out its surface and removing air bubbles, the gel was transferred onto a sheet of chromatographic paper and vacuum-dried at 90 °C for around two hours.

Autoradiography

The dried gel was placed onto a sheet of X-ray film into a metal case. The film was exposed to the radioactivity of the DNA in the gel for about a week. The film was then developed in an X-ray developer solution (Kodak LX 24, 1:5 diluted) for 10 minutes, rinsed in water, fixed for 10 minutes in X-ray fixation solution (Kodak AL 4), rinsed again and air-dried.

2.2.3.4.2 Automatic sequencing

From March 1998 onwards, the DNA samples were sequenced automatically using fluorescence labeled dNTPs. Around 5 to 6 ng DNA per 100 bp of the amplification product were used in the sequencing reaction. In 10 µL (13 µL) reactions, 4 µL (5.5 µL) of a pre-mix (containing *Taq* polymerase, dNTPS, fluorescence labeled ddNTPS, buffer and $MgCl_2$; supplied by the Centre for Gene Research CGR, Department of Microbiology, University of Otago), 0.5 µL of sequencing primer (10 pmol) and 5.5 µL (7 µL) of DNA + water (sterilised Millipore® water) were gently mixed together. In the amplification protocol for the sequencing, the denaturation at 96 °C for 30 seconds was followed by an annealing at 50 °C for 15 seconds and an elongation at 60 °C for 4 minutes. Each cycle was repeated 30 times.

After the cycle sequencing reaction, the products were cleaned with an ethanol + salt precipitation: 1/10 volume of the sequence reaction of 3 M sodium acetate (1.0 µL or 1.3 µL, respectively) and 2-2.5 times the volume of 95% ethanol (25 µL or 32.5 µL, respectively) were added to the reaction mix. After gently mixing the solution, it was incubated for 30 minutes at RT. The precipitated DNA was spun down at 14,000 rpm for 30 minutes. The pellets were washed twice by adding 100 µL 75% ice-cold (-20 °C) ethanol and centrifugation at 14,000 rpm

for 15 min. After the last washing step, the pellets were spun at 12,000 rpm for another 5 minutes, and all ethanol was removed. The pellets were dried at 30 °C in a drying oven for at least an hour. The dried products were resuspended, electrophoresed, and the sequence data were automatically read by an ABI 377 DNA Sequencer (Perkin Elmer Applied Biosystems Foster City, California) at the CGR, Department of Microbiology, University of Otago. The *rbc*L sequences of *Herpodiscus durvillaeae* were commercially sequenced on an ABI PRISM 3700 (MWG-Biotech, Munich, Germany).

2.2.3.5 Data analyses

All data analyses were performed using Apple Macintosh computers (I-Mac or Power Macintosh G3).

2.2.3.5.1 Alignment of sequences

Reading of sequences

Bands from manually produced sequences were read from the film material by eye (on a transluminent table) and were directly written into the text editor of PAUP 4.0b.10 (SWOFFORD 2002). Automatically produced sequences were checked in the sequence chromatograms, viewed in the program SEQED (ABI) and then transferred into the PAUP text editor.

Published sequences

Published sequences of endophytic and free-living brown algae, and of Xanthophyceae were obtained from the EMBL/GenBank/DDBJ Nucleotide Sequence database (www.ncbi.nlm.nih.gov/Web/GenBank) or via personal communication (A. F. PETERS). GenBank accession numbers are provided in Appendix D, Table D 1.1.

Outgroups

The Xanthophyceae are considered to be one of the sister taxa to the brown algae and are commonly used as an outgroup for phylogenetic analyses of conserved, protein-coding genes, such as the subunits of ribosomal and the RuBisCO genes (e. g. DAUGBJERG & ANDERSEN 1997; POTTER *et al.* 1997; SAUNDERS *et al.* 1997). The xanthophycean species *Tribonema aequale* PASCHER was selected as an outgroup for the *Herpodiscus* alignments.

Within the alignment analysing the phylogenetic relationships of *Herpodiscus durvillaeae* with other Sphacelariales, six phaeophyceaen species were referred to the outgroup (following

DRAISMA *et al.* 2002): *Dictyota dichotoma* (HUDSON) J. V. LAMOURROUX, *D. cervicornis* KÜTZING (both Dictyotales), *Syringoderma phinneyi* E. C. HENRY & D. G. MÜLLER (Syringodermatales), *Onslowia endophytica* SEARLES in SEARLES & LEISTER, *Verosphacela ebrachia* HENRY (both Onslowiaceae; DRAISMA & PRUD'HOMME VAN REINE 2001) and *Choristocarpus tenellus* (KÜTZING) ZANARDINI (Choristocarpaceae). Onslowiaceae and Choristocarpaceae are considered either Sphacelariales *sensu lato* or *incertae sedis*, depending on authors (WOMERSLEY 1987; DRAISMA & PRUD'HOMME VAN REINE 2001; DRAISMA *et al.* 2001).

For the phylogenetic analysis of the ITS1 region of the pigmented endophytes from New Zealand, the type species of the family Chordariaceae (Chordariales or Ectocarpales *sensu lato*; PETERS & RAMIREZ 2001), *Chordaria flagelliformis* (O. F. MÜLLER) C. AGARDH, was chosen as an outgroup. This species is not as closely related to the endophytes as for example *Dictyosiphon foeniculaceus* (HUDSON) GREVILLE and *Ascoseirophila violodora* PETERS (BURKHARDT & PETERS 1998; PETERS 2003), but in contrast to the latter, *C. flagelliformis* has a very short ITS1 sequence (291 bp) and thus is alignable with the endophytes over most of this region. Usually, only taxa belonging to the same species or genus can be aligned over most of the highly variable ITS1 region, while taxa of different genera cannot (PETERS 2003).

Aligning

Sequences were either pre-aligned with the program CLUSTAL W (THOMPSON *et al.* 1994), then checked by eye and manually adjusted using HOMED (STOCKWELL & PETERSEN 1987) or were directly edited in the text editor of PAUP 4.0b.10 (SWOFFORD 2002). Complementary strands were aligned manually comparing the chromatograms, or automatically using AutoAssembler (ABI, version 2.0) before aligning in HOMED.

2.2.3.5.2 Phylogenetic analyses

Analyses of molecular data were performed with PAUP version 4.0b10 (SWOFFORD 2002). Data were analysed with heuristic search methods, using maximum parsimony (MP) or maximum likelihood (ML) as optimality criteria. Distance trees were also constructed using the neighbour-joining (NJ) tree building algorithm.

Maximum parsimony analyses were performed by stepwise random sequence addition (10 replicates) followed by branch swapping using the tree-bisection-reconnection (TBR) branch swapping algorithm. All nucleotides were unordered and weighted equally. Ambiguous nucleotides and gaps were treated as missing data (with the exception of one modified data set,

see below). Maximum likelihood analyses employed a HKY85 model of sequence evolution with empirical base frequencies and transversions weighted 2:1 over transitions. Sequence addition was randomly (10 replicates). MaxTrees were set to "automatically increase by 100" for MP and ML analyses. A Kimura-2-parameter model was used to calculate mean distance values as a base for neighbour-joining analyses (KIMURA 1980). Saturation of alignments was tested using transition/transversion (*ti/tv*) ratios, with saturation indicated by values ≤ 1 (HOLMQUIST 1983; BAKKER *et al.* 1995).

Confidence of branching points was estimated by bootstrapping (1000 replicates in parsimony and neighbour-joining analyses and 100 replicates in likelihood analyses). Decay indices of parsimony trees were produced "manually" by re-running MP analyses and computing strict consensus from all trees within six additional steps (score + 6; MORGAN 1997), filtered down to two additional steps. From these consensus trees, the numbers of additional steps necessary to let nodes collapse were determined.

The congruence of combined datasets (RuBisCO and ribosomal subunit genes) was tested with the partition homogeneity test (PHT; FARRIS *et al.* 1995; CUNNINGHAM 1997) in PAUP. The optimality criterion was set to maximum parsimony (1000 replications and simple addition of taxa), with uninformative positions deleted before analysis. Data sets are considered to be congruent if $p > 0.05$, i. e. combining the data sets improves the phylogenetic accuracy (FARRIS *et al.* 1995; CUNNINGHAM 1997).

Differences between sequences of isolates in all three groups of pigmented endophytes were mainly due to indels, rather than to single nucleotide substitutions. In analyses treating deletions as missing data, these do not contribute to the resulting phylogenetic tree. To accommodate these differences, additional MP analyses were run with a modified data set: Within every indel comprised of two or more missing nucleotides, all but one positions were recoded to question marks ("?" = missing data). In cases where sequences showed various differences in length of the indel, the number of gaps within was arranged accordingly. The program was then set to "treat deletions as fifth base" before analysis, thus counting every indel (rather than every missing nucleotide) as a single evolutionary event (J. E. BROOM, personal communication; BROOM *et al.* 2002; PILLMANN *et al.* 1997).

Several isolates of pigmented endophytes had identical or very similar ITS1 sequences, resulting in long computing times and poor resolution of the phylogenetic trees. To avoid these problems, data sets were reduced, and analyses were run with single representatives of clades of identical taxa (SERRAO *et al.* 1999).

2.2.4 Ultrastructure of *Herpodiscus durvillaeae*

All preparations and electron microscopic study of the ultrastructure of the infection of *Durvillaea antarctica* with *Herpodiscus durvillaeae* were performed in the laboratory of the South Campus Electron Microscopy Unit, Anatomy and Physiology Department of the Medical School, University of Otago, Dunedin, New Zealand. *Durvillaea antarctica* infected with *Herpodiscus durvillaeae* was collected either at Brighton Beach or at St. Kilda. Thalli were wrapped in seawater-soaked newspaper, placed in a chill bin, and immediately transported back to the laboratory. Collection data of specimens sampled for electron microscopy are included in Table E 1 (Appendix E).

2.2.4.1 Light microscopy

Hand-sectioned fresh material of *Durvillaea antarctica* infected with *Herpodiscus durvillaeae* and freeze-dried material of *Herpodiscus durvillaeae* (prepared for DNA extractions; see section 2.2.3.1) were transferred into filtered seawater and examined with an Olympus AX70 Research Microscope (for details on the microscope see section 2.2.1.3.1). For the autofluorescence microscopy, hand-cut sections of field collected material were exposed to the ultraviolet light emitted by a Xenon lamp (source unknown). A yellow FITC (fluorescein isothiocyanate) filter was used to limit the range of wavelengths to ultraviolet light (excitation maximum at 490 nm, emission from 500-550 nm). Excitation maxima of autofluorescent objects were not recorded. Details on the photographic documentation are compiled in section 2.2.5.

Fresh material of *Durvillaea antarctica* with patches of *Herpodiscus durvillaeae* and Ectocarpalean epiphytes (collected at St. Kilda Beach, Dunedin, on 15.03.2001) and freeze-dried material of *H. durvillaeae* were exposed to 'Eau de Javelle' (circa 5% aqueous sodium hypochlorite solution, DE REVIERS & ROUSSEAU 1999) and immediately observed with the light microscope. Cells of members of the order Sphacelariales, in contrast to all other brown algae, show a transistory blackening when exposed to 'Eau de Javelle' (REINKE 1890; MIGULA 1909). Therefore, as a positive control for the reaction, fresh material of *Halopteris* sp. (Sphacelariales, Stypocaulaceae, collected at St. Clair Beach, Dunedin, on 15.03.2001) was treated in a similar way. 'Eau de Javelle' was freshly prepared from common household bleach (brand "White Magic", containing 42 g/L sodium hypochlorite) by 1:10 dilution with Millipore® water (final concentration of sodium hypochlorite: 4.2 g/L).

2.2.4.2 Scanning electron microscopy

Specimen preparation for scanning electron microscopy (SEM) followed a procedure after MAIER & WOLFF (S. WOLFF, personal communication). Exact recipes for the solutions are compiled in Appendix A 3.1.

2.2.4.2.1 Fixation

The whole preparation took place in a fume hood. All media (i. e. fixative and wash buffer) were made fresh on the day of preparation from stock solutions kept at 4°C. The fixative contained 4% glutardialdehyde in a 0.1 M sodium cacodylate buffer with salts added to reduce osmotic stress (146 mM NaCl, 3.4 mM KCl, 17 mM $CaCl_2$). Solutions were transferred to small glass vials. Depending on the desired fixation temperature, solutions were allowed to reach room temperature or were pre-cooled in the refrigerator.

Pieces of the *Durvillaea* thallus (collected at Brighton Beach on 09.09.1997, 22.09.1997 and on 25.08.1999) were transferred to a petri dish containing filtered seawater. Sections of the thallus surface (1 mm thick) were cut with a razor blade and chopped into smaller fragments. These were then immersed into the fixative. Vials with fixative and samples were placed in a rotator (TAAB Laboratories, UK), thus samples were constantly agitated during the fixation. Samples were fixed over-night (17-19h) at either at RT or at 4°C. On the following day, the samples were washed with pre-cooled wash buffer (six times for 10 minutes each). During the washing process, cooled solutions reached room temperature.

2.2.4.2.2 Dehydration

Samples were dehydrated in an increasing ethanol gradient, i. e. 25%, 50%, two times 70%, 85%, 95%, two times 100%, 100% (extra dry ethanol). Each step took 15 minutes. In some cases, samples were kept in 70 % ethanol till the preparation was continued.

2.2.4.2.3 Critical point drying and coating

Samples were dried in a Critical Point Dryer (Balzer CPD 030), by exchanging the ethanol with liquid CO_2 (three times) and then, at its critical point, transforming the liquid carbon dioxide to gas without passing the phase boundary. The dry samples were mounted on mushroom-shaped stubs using colloidal silver paste (EMS cat. 12640; Washington, USA) and carbon tape (STR Tape, Shinto Paint Co. Ltd.). Stubs were transferred to a sputter unit (BioRad SEM Coating

System) and were coated with gold/palladium in an argon atmosphere for 120 seconds, resulting in a 60 to 80 nm thick coating. Stubs with samples were removed from the sputter unit and were transferred to a tied-closed box with silica gel to keep the samples dry and free from dust.

2.2.4.2.4 Electron microscopy

Samples were viewed in a scanning electron microscope (Cambridge Stereo Scan 360).

2.2.4.3 Transmission electron microscopy

Specimens of *Durvillaea antarctica* infected with *Herpodiscus durvillaea* were prepared for transmission electron microscopy (TEM) using chemical fixation techniques as well as cryofixation combined with cryosubstitution: Samples were fixed, dehydrated and embedded in resin. The resin was hardened and sectioned. Sections were stained to enhance the contrast, and were examined in the electron beam of a TEM.

The preparation followed standard protocols adapted for brown algae (e. g. MAIER & WOLFF, personal communication; CLAYTON & ASHBURNER 1994; SCHOENWALDER & CLAYTON 1998a, 1998b). Procedures were tested and modified to enhance the quality of the samples, especially of the inner tissue of the host, as cellular contacts between host and parasite were found to be concentrated in this area. Exact recipes are given in Appendix A 3.2, protocols for all samples processed are compiled in Appendix E.

2.2.4.3.1 Chemical fixation

Fixatives contained aldehydes to link proteins, either glutardialdehyde or a mixture of glutardialdehyde and paraformaldehyde (after KARNOVSKY 1965). Paraformaldehyde penetrates tissue faster than glutardialdehyde but provides a less stable fixation of proteins, thus a combination of both was used.

The media were buffered with sodium cacodylate in a pH range of 7.2 to 7.5 (according to the respective recipe). Seawater or a solution of chloride salts (either NaCl and $CaCl_2$, or a combination of NaCl, KCl and $CaCl_2$) were added to all media to reduce any osmotic shock to the tissue, i. e. in order to reduce shrinking or swelling of the cells and their compartments. The osmolarity of the media was monitored using a osmometer (5500 Vapor Pressure Osmometer, WesCor). Caffeine was added to fixatives and wash buffer solutions to stabilize physodes which are a common feature of brown alga cells (CLAYTON & BEAKES 1983). Membranes and other

lipid containing tissue were fixed in a separate process by exposure to the heavy metal osmium (as osmium tetroxide). An additional step of 'en bloc' staining with uranyl acetate was used to enhance the contrast in the sections (STEMPAK & WARD 1964).

Initial protocol

Various combinations of conditions and concentrations of chemicals were tried to improve the fixation of the tissue. In the initial recipe after MAIER & WOLFF, Universität Konstanz, Germany (S. WOLFF, personal communication), the samples were fixed in a solution containing 2.5% glutardialdehyde in 70% seawater, buffered with 0.05M sodium cacodylate at pH 7.4-7.5, either at RT or at 4°C. Caffeine (0.2%) was only added to the fixative but not to the washing buffer. Also, fixatives were tried containing 4% glutardialdehyde and 4% paraformaldehyde (KARNOVSKY 1965), or in which the amount of seawater was reduced or was replaced by sodium salts.

Final protocol

Better results were obtained with a fixative containing 2% glutardialdehyde, 1% paraformaldehyde, and 1% caffeine in cacodylate buffer (0.1M sodium cacodylate, 2% NaCl, 0.1% $CaCl_2$, pH 7.2; modified KARNOVSKY mixture after CLAYTON & ASHBURNER 1994 and SCHOENWAELDER & CLAYTON 1998a, 1998b). Pieces of tissue were immersed into the fixative solution and were cut into small cubes (300 to 500 µm length) with razor blades. The tissue was transferred to vials containing fresh fixative. Samples were fixed over-night, at 4°C or at RT. A long fixation time was found to enhance the quality of the fixation in the centre of the cubes. To aid the penetration, the tissue blocks were constantly agitated in the fixative by moving the vials on a rotator.

After fixation, the tissue blocks were rinsed three times (each 15 minutes) in cacodylate buffer with 1% caffeine and were then post-fixed for two hours with 1% osmiumtetroxid (in cacodylate buffer without caffeine) at RT, before rinsing the samples in three steps (1: 100% cacodylate buffer (without caffeine); 2: 50% buffer/50% dd H_2O; 3: 100% dd H_2O; each step 15 minutes). 'En bloc' staining in 1% uranyl acetate in dd H_2O for one hour, which was part of the initial protocol, was omitted from later preparations to reduce the high contrast in the samples (M. N. CLAYTON, personal communication).

Microwaving

Microwaving was tried as a means to enhance the quality of fixation (by achieving a faster penetration of the chemicals into the tissue; BOON & KOK 1992). A laboratory microwave oven (A20, Electron Microscopy Science, maximal power: 780 Watt) was pre-warmed with 500 mL of dd H_2O in a 2 L glass beaker (two minutes on 100%). The samples were placed into fresh fixative, in a glass petri dish with a liquid crystal thermometer in the bottom to observe the development of the temperature of the fixative. The petri dish was placed in the middle of the microwave. A 250 mL glass beaker filled with dd H_2O was placed in the back of the microwave to avoid any overheating of the samples. The samples were heated with microwave zaps of eight seconds length (at 100%), until the temperature of the fixative reached a maximum of 30 °C.

2.2.4.3.2 Dehydration

To embed the samples into resin, first all water had to be removed from the tissue by replacing it with a medium mixable with the resins, such as ethanol. Dehydration took place at RT.

Initially, a standard procedure was employed which was used in the host laboratory for animal and human tissue. Samples were dehydrated in a steep gradient from of 100% dd H_2O, 25% ethanol, 50%, 75%, 85%, 90%, 95% up to three times 100% ethanol (each step taking 15 minutes). This gradient was found to be insufficient to completely dehydrate the tissue of *Durvillaea antarctica.*

Alternative protocols were adopted which contained more and longer steps of dehydration, with each step taking between 12 minutes and 2.5 hour, depending on the protocol. Best results were obtained with a gradient starting at 1% ethanol in dd H_2O, going up in small steps (3% ethanol, 6%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%) and finishing with three times 100% ethanol (each step taking 20 minutes). An automatic tissue processor (Lynx, Biomedical Corporation Europe Ltd.) was employed to provide reproducible results. Tissue blocks were transferred into porous Teflon baskets and immersed into the first solution. By moving the baskets up and down in the solution, the samples were continuously stirred. At set times, the tissue processor automatically moved the baskets into the next solution.

2.2.4.3.3 Infiltration and embedding

The dehydrated tissue was slowly infiltrated with resin. The resin was then cured to provide blocks which could be sectioned with an ultra microtome.

Resins

One of the problems encoutered with preparing *Durvillaea antarctica* for TEM was a poor dehydration and subsequent poor infiltration of the tissue, as resins tend to be hydrophobic. Three resins with different hydrophobic properties were tested: the low-viscosity epoxy resins "SPURR's" (SPURR 1969) and "Quetol 651" (KUSHIDA 1974) as well as the acrylic resin "LR White" (London Resin Company; NEWMAN *et al.* 1983). SPURR's is not mixable with water at all, while Quetol is considered to be more tolerant to small amounts of water in the tissue (R. LANDER, personal communication). LR White is mixable with water up to 12% (ROLAND & VIAN 1991), but has the disadvantage of being less stable in the electron beam of the TEM than epoxy resins (DYKSTRA 1993).

The epoxy resins were prepared on the day of use from four stock solutions provided by the supplier (Appendix A, A 2.2.2). Stock solutions were stored at RT, with the exception of Quetol 651 which was kept at 4°C. The resin base was mixed with the hardener and the flexibilizer and stirred for 1 min. After adding the accelerator, the resin was stirred for another 5 minutes. SPURR's resin was used in the standard grade (SPURR 1969) and was degased with a vacuum pump prior to use.

LR White resin was prepared by adding a catalyst (provided by the supplier) and stirring the mixture for 24 hours on a shaker table at RT. The solution was stored at 4 °C and could be used straight from the bottle. Even though LR White is mixable with water to a certain degree, the resin was allowed to reach RT before use to avoid an excess of water precipitation on the cold resin surface. All chemicals needed for the preparation of the resins were supplied by Pro Sci Tech, Australia.

Infiltration

Quetol and LR White resins are mixable with ethanol, while SPURR's is not. Therefore before infiltration with SPURR's, ethanol had to be replaced with a hydrophobic solvent, propylene oxide (1,2-epoxypropan; 100%, two times for 20 min).

The recipe used initially (after MAIER & WOLFF, S. WOLFF, personal communication) included an infiltration gradient from 100% solvent, 25% resin in solvent, 50%, 75% and three times 100% resin. Infiltration times were 4 to 16 hours, except at 75% (over night). This gradient was found to be insufficient to completely infiltrate the tissue blocks of *D. antarctica.* Therefore infiltration times at the 100% resin step were increased (up to 14 days, with daily change of resin). Additionally, a slower infiltration gradient was adopted (M. N. CLAYTON,

personal communication). The gradient was divided in similar steps as the dehydration (100% ethanol, 1% resin in ethanol, 3%, 6%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, three to five times 100% resin). Each step took between 2 and 12 h (see Appendix F). Infiltration was either done by hand, with samples constantly rotating in glass vials on a rotator and 12 hour to daily change of freshly prepared resin, or by employing the automatic tissue processor.

Microwaving

Microwaving was tried as means to enhance the quality of infiltration by speeding up the penetration by resin molecules. In a procedure similar to microwave-enhanced fixation (section 2.2.4.3.1), the vials, containing the samples in 100% fresh resin, were placed into the pre-heated laboratory microwave oven. The samples were heated up with four to five 8-second-microwave zaps (at 100% power), to avoid temperatures in the resin exceeding 25 °C.

Embedding

Before embedding, the samples were immersed in freshly prepared resin for another four hours. Epoxy resin samples were embedded either in clean silicon rubber moulds or in Beem® capsules. Epoxy resins were polymerized in an embedding oven (TAAB) at 60 ± 1 °C for 48 to 60 hours. LR White does not polymerize in the presence of oxygen, therefore the LR White samples were embedded in gelatine capsules placed in Eppendorf tubes. The resin was polymerized on a heat plate at 55–60 °C for a maximum of 24 hours.

2.2.4.3.4 Cryopreparation

Cryofixation and cryosubstitution were tested as an alternative to standard chemical fixation. Samples were physically preserved, i. e. they were suddenly exposed to very low temperatures to immobilize cellular components, by freezing any water contained in the sample without the formation of large ice crystals. The ice was then subsequently replaced by a substitution medium containing fixatives to chemically stabilize structures, before thawing the sample.

Durvillaea antarctica infected with *Herpodiscus durvillaeae* were collected at Brighton Beach on three occasions (20.04.2000, 21.06.2000 and 04.07.2000). In June and July 2000, cryofixation/cryosubstitution was used simultanously with chemical fixation protocols. The cryopreparation followed standard protocols (GALWAY *et al.* 1995; R. LANDER, personal communication). For the cryofixation, an automatic unit was used (Reichert KF80). Small

hand-sectioned blocks of the host tissue (<0.3 mm) were plunge-frozen in liquid propane at -180°C, and were then transferred to liquid nitrogen (-80 °C) in an automatic freeze-substitution unit (Reichert AFS). The substitution medium was prepared from three stock solutions (HUMBEL *et al.* 1983; R. LANDER, personal communication). For stock solution 1 (9% glutardialdehyde in methanol), 5 mL of 70% glutardialdehyde were mixed with 33 mL methanol. Stock solution 2 contained 3% OsO_4 in methanol, made up from 1 g OsO_4 dissolved in 33 mL methanol. Stock solution 3 contained 1.5% uranyl acetate in methanol, for which 0.5 g uranyl acetate was dissolved in 33 mL methanol. All stock solutions were cooled to -20° C prior to use. Then 10 mL of each stock solution 1, 2 and 3 were thoroughly mixed and cooled to the desired temperature in an automatic freeze substitution unit (AFS). Caffeine was omitted from the substitution medium as it was found to precipitate in methanol at -20°C.

The substitution medium, containing 3% glutardialdehyde, 1% OsO_4 and 0.5% uranyl acetate in 100% methanol, was pre-cooled to –80 °C in the AFS. Samples were tranferred from the liquid nitrogen to the substitution medium, where they were held at –80 °C for 306 hours. The medium was replaced with pre-cooled fixative once a day.

After six days, the temperature was slowly raised (10°C per hour) and held at -20°C for 48 hours. During this time the samples were rinsed twice with 100% methanol. After another temperature rise (5°C per hour), the samples were held at 10°C for 48 hours before raising the temperature to RT and removing the samples from the AFS. Samples were infiltrated in a methanol/resin gradient (Quetol; Appendix E) using the Lynx tissue processor (see section 2.2.4.3.2). The blocks were cured at 60°C for 48 hours.

2.2.4.3.5 Sectioning

Polymerized and cooled resin blocks with embedded tissue were roughly trimmed with a razorblade. Semi-thin sections (200 to 300 nm thick) were cut from the block face with a glass knife mounted on an ultramicrotome (Leica-Reichert-Jung Ultracut E, Vienna, Austria). Glass knifes with an angle of 45° were prepared from glass strips with a Reichert-Jung knife maker (Leica UK Ltd., England). Semi-thin sections were heat-mounted onto glass slides (10 to 30 seconds at 60°C) and stained with Methylen Blue-Azure-II stain (Appendix A, A 2.2.3) for 1-6 minutes at 60°C (until the drop of stain started to dry at the margins). Slides were then rinsed in water and dried at 60°C. The stained semi-thin sections were examined with a standard light microscope to select areas of interest for ultra-thin sections, before re-trimming the block for ultramicrotomy.

Ultra-thin sections of silvery colour (70-80 nm thick) were cut with a diamond knife (Microstar, 2mm, 45°) mounted on the ultramicrotome. Sections were collected from the water-filled trough of the diamond knife on single slot copper grids (slot 1000*2000) and air-dried. Prior to use, grids were coated with a support film: Grids were washed in acetone to remove any dust and fatty residues, and were then air-dried. Cleaned glass slides were immersed in a 0.6% solution of Formvar (polyvinyl formal resin) in chloroform for 30 seconds, and were then air-dried for one minute. By cutting around the edges of the glass slide, breathing onto the slide and immersing it into dd H_2O, the Formvar film was lifted from the glass slide and floated on the water surface. The clean copper grids were placed onto the film. The film with adhering grids was then attached to a glass slide coated with Parafilm®. Coated grids were stored away from dust in a petridish at 4 °C.

2.2.4.3.6 Ultrastaining

Sections were stained in an automatic stainer (Ultrostainer 2168, Carlsberg System, LKB Bromma). The program contained the following steps: washing (6 minutes 5 seconds at RT), staining in uranyl acetate (30 minutes at 40°C), washing (4 minutes 30 seconds at RT), staining in lead citrate (WATSON 1958; 1 minute 20 seconds at 20°C), washing (5 minutes at RT). Standard solutions for uranyl acetate and lead citrate were provided by the manufacturer. After staining, the grids were carefully dried with filter paper and stored in grid boxes.

2.2.4.3.7 Electron microscopy

The stained sections were examined using either a Philips EM 410 transmission electron microscope or an Akashi EM 002A transmission electron microscope, at 80 and 100 kV respectively.

2.2.5 Photographic documentation

Photographs of the collection sites and of host plants in the field were taken with an automatic camera (Riva 115 mm, Minolta, Japan) on print film (Kodak Gold 200, Australia). Light microscopical photographs were taken on slide film (Tungsten 64 or Agfa RSX II Professional daylight slide film, ISO 100, Germany). Slides and colour print positives were developed by professional laboratories (slides: University Photographic Services, Otago University; print positives: K-mart, Dunedin). Photographs from the transmission electron microscope were

taken on sheet or 35 mm print negative films (Agfa or Kodak black and white film). Negatives were developed in standard developer and fixative (Agfa, Germany) and air-dried. Pictures taken in the scanning electron microscope were digitalised directly.

Slides and print negatives were scanned with a slide scanner (Microtek ScanMaker 35t plus), print positives were scanned with flat-bed scanners (Microtek ScanMaker E6 or Umax Astra 2000P). Scanners were operated using ScanWizard™ Microtek 3.0.7 or Vista Scan (Umax Data Systems, Inc.), respectively. Digitalised photographs were processed and assembled to photographic plates using Adobe Photoshop® version 5.0.

3 PIGMENTED ENDOPHYTES – RESULTS AND DISCUSSION

3.1 Results

3.1.1 Morphology

3.1.1.1 Host algae and their infection symptoms

Most host algae colonized by endophytic Phaeophyceae displayed a variety of symptoms associated with the infection. These ranged from dark spots, warts and galls up to massive distortions of the thallus. Occasionally, endophytes were found without causing any macroscopically visible symptoms.

If present, endophyte cells were easily detectable under the microscope, due to the large size of their plastids, especially if they grew in the inner host tissue. In all infected host algae, pigmented endophytic Phaeophyceae were found invading the intercellular space of the host tissue. No endophyte cells were observed to penetrate host cells. Furthermore, endophyte filaments were not associated with necrotic tissue in any of the examined host algae.

Pigmented endophytic brown algae were found in eleven macrophyte species, eight members of the Phaeophyceae, and three members of the Rhodophyta (Table 2.1). The collection data of the strains of pigmented endophytes isolated from New Zealand macrophytes are compiled in Table 3.1.

All but one of the Laminariales species examined for the presence of pigmented endophytes were infected. However, no endophytes were found in sporophytes of *Lessonia variegata* J. AGARDH from Castle Point, North Island, and from Seal Point, Otago Peninsula. Members of the Fucales which were examined but did not display any endophyte infection included *Marginariella boryana*, *Hormosira banksii*, *Carpophyllum maschalocarpum* (TURNER) GREVILLE, and *Cystophora scalaris* J. AGARDH. Other Phaeophyceae that were examined, but also did not contain endophytes, were *Scytothamnus australis*, *Splachnidium rugosum* (both Scytothamnales) and *Adenocystis utricularis* (BORY) SKOTTSBERG (Ectocarpales).

Potential red algal hosts which upon examination proved to be uninfected by endophytes included mainly large, broad-bladed species such as *Sarcothalia lanceata* (J. AGARDH) HOMMERSAND. No infections were found in any member of the Chlorophyta that were

examined for the presence of brown endophytes (e. g. *Codium fragile* (SURINGAR) HARRIOT, *Ulva* spp. incl. *Enteromorpha* spp.).

3.1.1.1.1 Laminarialean hosts

Macrocystis pyrifera

Macrocystis pyrifera was frequently colonized by endophytic brown algae. All populations from around New Zealand, that were examined for their presence, contained infected specimens. A total of 14 strains of endophytic brown algae were isolated from *M. pyrifera* (Table 3.1, isolates no. 1-6 and no. 11-18).

Microscopic cross-sections through infected host tissue showed that endophyte filaments grew mainly in the cortex, often in a radial direction. In heavily infected host specimens, they were also encountered in the medulla. Often, endophyte filaments crossed the cavities formed by the mucilage ducts. Filaments grew in the intercellular space criss-crossing the tissue, following the outlines of the host cells.

Macroscopically, most infected *M. pyrifera* thalli displayed symptoms of the infection visible to the naked eye, such as spots or galls (Plate 3.1, Figure E). Infected areas, including the galls, usually had a darker pigmentation than the surrounding healthy tissue. Additionally, their surface was rough and uneven, in contrast to the smooth surface of unifected host tissue.

The endophyte-associated galls in *M. pyrifera* were irregularly shaped. They ranged from small conical-shaped warts, hardly visible to the naked eye, to larger, slightly raised areas, or to conspicuous tumour-like outgrowths of the host surface (e. g. Plate 3.1, Figure F). The latter galls reached diameters up to 5-6 mm, while the raised areas sometimes stretched over several centimetres, with a width of 3-5 mm.

In cross-sections, the galls appeared to have resulted from abnormal proliferations (hyperplasia) of the cortex cells into unorganised cell clumps rather than from an abnormal growth (hypertrophy) of the cells of the involved tissue. Transverse sections through some galls, however, revealed that the protuberances on the host surface were not only associated with a hyperplasia of the cells alone, but also with large cavities in the inner cortex tissue underneath (Plate 3.2, Figure A). These cavities were situated in the same tissue layer as the mucilage ducts and appeared to have evolved from an enlargement of the latter.

Not all infections with endophytic Phaeophyceae resulted in macroscopically detectable symptoms. During the field study conducted on three *Macrocystis* populations of the Otago region (section 2.2.2), single endophyte filaments were often found in cauloid sections that

macroscopically appeared to be healthy. On the other hand, distorted *M. pyrifera* thalli were frequently found, which were not associated with any endophytic Phaophyceae. However, while distortions signaling a heavy infection with endophytes were always accompanied by a rough, uneven surface and dark pigmentation, the crippled fronds without endophytes never displayed the surface typical for the endophyte-associated galls.

In *M. pyrifera*, galls were mainly found on the cauloids. In heavily infected thalli, they sometimes grew in rows on the stipe, following the spiral arrangement of the pneumatocysts and phylloids along the cauloid due to nutation (LOBBAN 1978; Plate 3.1, Figure F). If the cauloids were heavily infected, sometimes the neighbouring pneumatocysts and, for example in a thallus from Aramoana (collected 15.10.1997), also the phylloids displayed galls. A few times, fronds were infected close to the apex. Most endophytes, however, appeared to grow in the basal parts of senescent and mature fronds.

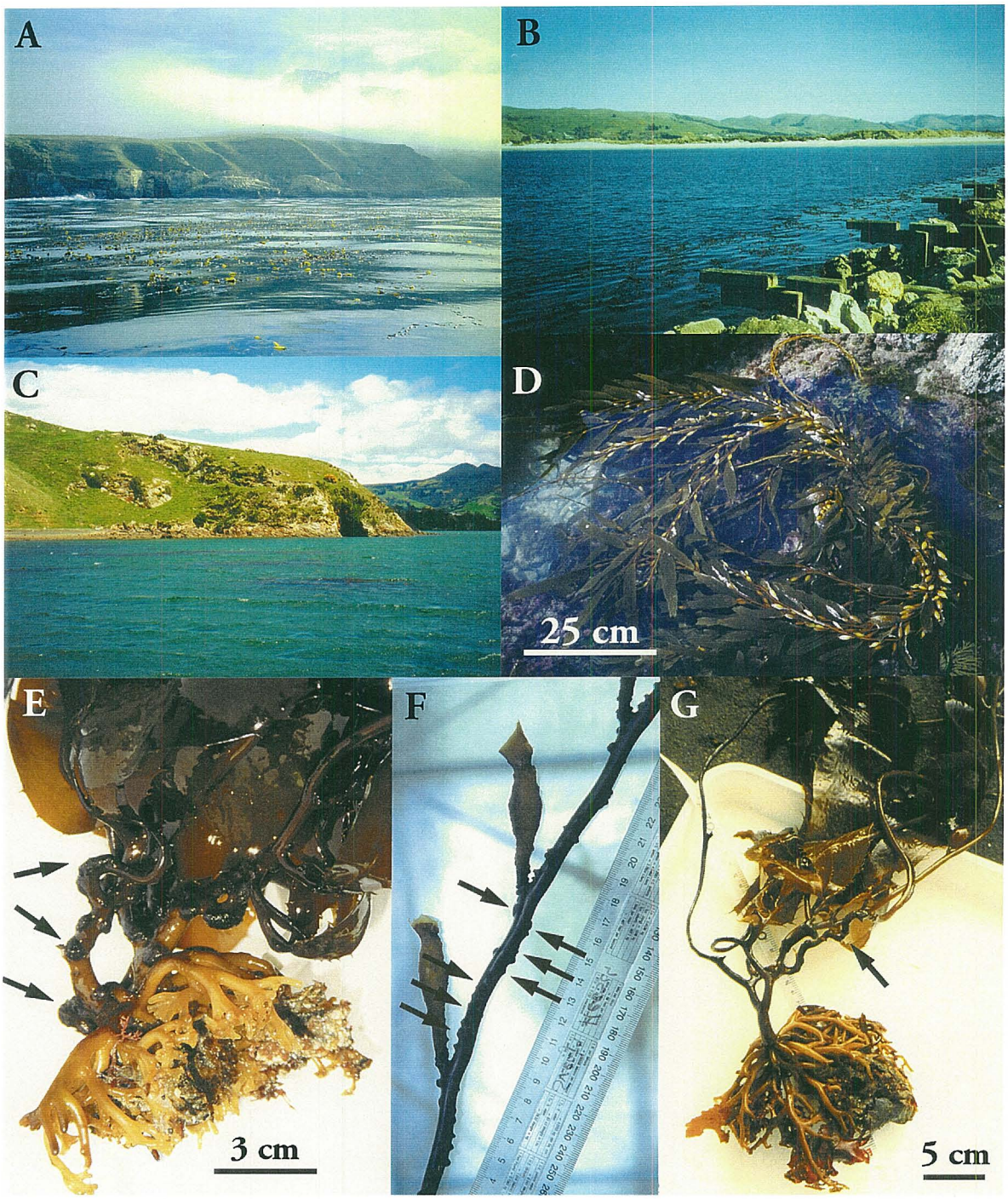
In addition to the specimens used for the isolation of endophytes (Table 3.1), two thalli of *M. pyrifera* were macroscopically examined for endophyte infections: one collected at Paterson Inlet, Stewart Island, in spring 1997 (Source: M. STUART) and the other from Kakanui near Oamaru (January 1998). Both specimens had galls with a rough surface typical of infections with endophytic brown algae. In the Stewart Island thallus, the infection was confirmed by light microscopy. No endophyte culture could be isolated from this specimen, though, as it had been salt-preserved prior to examination (M. STUART, personal communication).

Lessonia tholiformis

Two specimens of *Lessonia tholiformis* from Chatham Island were infected with endophytic brown algae. In both cases, the endophytes were found in the cauloid of the infected thallus. The specimen from Wharekauri had a large, conspicuous gall associated with endophytes (Plate 3.5, Figure C): it resembled a button, was solid, had a diameter of 18 mm and a height of 4 mm. It was slightly more darkly pigmented than the surrounding tissue, and had a rough surface. The other host thallus, from Te One Creek near Owenga, displayed dark brown stripes along the cauloid, with a rough surface similar to that of the gall from the specimen from Wharekauri. Endophyte strains were isolated from both infected host thalli (Table 3.1, isolates no. 28 and 29).

Plate 3.1: The endophyte *Laminariocolax macrocystis* in the field.

A-C: Populations of the host *Macrocystis pyrifera* examined during the field study in 2000. **A**: Offshore kelp bed at Cornish Head, Otago. **B**: Population along Aramoana Mole, Otago Harbour (background: Aramoana Spit and Otago Peninsula). **C**: Population at Quarantine Point, Otago Harbour. **D**: Specimen in rockpool at Brighton Beach, Otago. **E-G**: Host species *Macrocystis pyrifera*. Symptoms associated with endophyte infections. **E**: Basal part of *M. pyrifera* with galls (arrows; Aramoana; 05.03.1998; isolate no. 1). **F**: Row of galls (arrows) following the spiral arrangement of pneumatocysts along the *Macrocystis* cauloid. Phylloids are decayed (Aramoana; 06.11.2000). **G**: Base of host thallus with distorted stipe of disease category 3 (arrow; Aramoana, 05.03.1998).


A
B
C
D
25 cm
E
3 cm
F
G
5 cm

Ecklonia radiata

Thalli of *Ecklonia radiata*, like those of *Macrocystis pyrifera*, were frequently colonized by endophytic brown algae. Five strains of endophytic brown algae were isolated from this host species (Table 3.1, isolates no. 7-10 and no. 26).

Infected specimens of *Ecklonia radiata* did not show uniform symptoms. In the thalli from Karitane and from the two sites in Fiordland, which all had infections in the cauloid, the presence of endophytic brown algae was only indicated by a dark patch, which, in the case of the Fiordland specimens, had a slightly rough surface. The infections in the two specimens from Waikouaiti/South Island and from Wellington/North Island, however, were associated with conspicuous, conical-shaped galls. Similar to the endophyte galls of *Macrocystis pyrifera*, these had a rough surface, and were of a dark brown pigmentation.

The infected *E. radiata* from Waikouaiti displayed a single gall on its phylloid, with a length of ca. 1 cm and a height of ca. 5 mm (Plate 3.2, Figure G). Apart from the gall, it appeared to be healthy. In the specimen from Lyall Bay/Wellington, in contrast, the whole phylloid was covered with dark spots and a large number of galls ranging from 1 to 7 mm in length (Plate 3.2, Figure F). Dark spots and stripes were also found on its cauloid. Overall, the thallus of this heavily infected specimen had a stunted appearance and reduced pigmentation, compared to a healthy specimen collected on the same day from the same location (Plate 3.2, Figure E). It was not determined whether the *E. radiata* galls were solid, or hollow like some of the galls found on *Macrocystis pyrifera*.

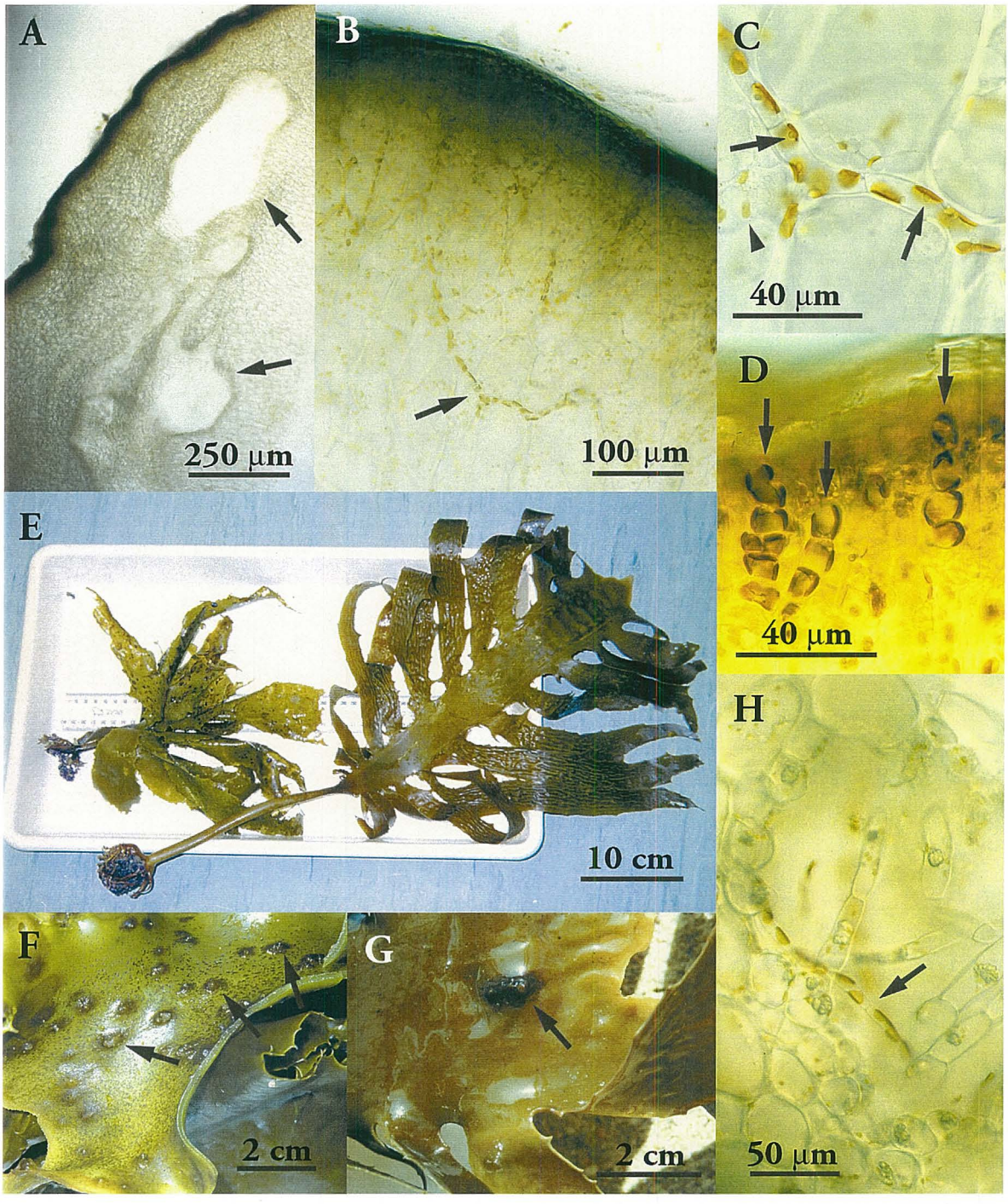
Undaria pinnatifida

In *Undaria pinnatifida*, endophytic brown algae were only found once, in the meristem of a thallus collected in Oriental Bay/Wellington Harbour in April 1998 (Table 3.1, isolate no. 25). The infected area did not display any macroscopic infection symptoms: neither were any galls visible, nor did the surface show any changes in smoothness or pigmentation.

No infection was detected in any specimens from the Otago Harbour, where *U. pinnatifida* was infrequently collected and examined for the presence of brown endophytes from July 1997 till the beginning of 2001. The number of thalli examined was not quantified.

Plate 3.2: The endophyte *Laminariocolax macrocystis* in the field (continued).

A-D: Host species *Macrocystis pyrifera*. Microscopic sections of endophyte galls. **A**: Transverse section through a gall displaying internal cavities (arrows; Aramoana, 06.11.2000). **B**: Radial section through gall with endophyte filaments (arrow). **C**: Detail with endophyte filament creeping in the host interstice. Endophyte cells contain several large, discoid plastids with pyrenoids (arrows), in contrast to host cells with small plastids lacking pyrenoids (arrowhead). **D**: Developing endophyte sporangia (arrows) close to the host surface (B-D: Quarantine Point, 25.07.2000). **E-H**: Host species *Ecklonia radiata*. Symptoms associated with endophyte infections. **E**: Infected (left) and non-infected (right) specimen (E-F: Arthur's Nose, Lyall Bay, Wellington, 30.03.1998; isolate no. 7). **F**: Detail of the infected specimen with phylloid galls (arrows). **G**: Phylloid gall (arrow; G-H: Waikouaiti/Otago; 20.10.2000; isolate no. 9). **H**: Microscopic section through the same gall displaying an endophyte filament (arrow) inside a mucilage duct.


A
250 µm
B
100 µm
C
40 µm
D
40 µm
E
10 cm
F
2 cm
G
2 cm
H
50 µm

3.1.1.1.2 Fucalean hosts

Xiphophora gladiata* ssp. *novae-zelandiae

Two strains of endophytic brown algae were isolated from *Xiphophora gladiata*, both from the same population from Brighton Beach/Otago, but from subsequent years (Table 3.1, isolates no. 30 and 31). Both infected specimens of *X. gladiata* displayed galls on the base of their cauloids close to the holdfast (Plate 3.5, Figure A), but apart from the galls they appeared healthy. The galls were 4 and 5 mm in diameter and 3 and 5 mm high, respectively. Their surface was rough and had the same dark pigmentation as the surrounding host tissue. It was not determined whether the galls were hollow or solid. Apart from the galls, no other infection symptoms (such as morphological changes) were visible.

Marginariella urvilliana

The specimen of *Marginariella urvilliana* hosting an endophytic brown alga was found at Wharekauri on Chatham Island. Large numbers of endophyte filaments criss-crossed the cortex of the infected cauloid displaying some brown spots, but no warts or galls. Apart from this affected area, the thallus appeared healthy. A single endophyte strain was isolated from this host (Table 3.1, isolate no. 32).

Durvillaea antarctica

The infection of a *Durvillaea antarctica* specimen from Brighton Beach/Otago with a pigmented endophyte was discovered underneath a fertile patch of the epi-endophytic parasite *Herpodiscus durvillaeae*. No macroscopic symptoms indicating the presence of the pigmented endophyte were observed – if any were present, they were possibly obscured by the much more conspicuous symptoms of the *Herpodiscus* infection. The pigmented endophyte was isolated from underneath the parasite patch (Table 3.1, isolate no. 33).

Durvillaea willana

Durvillaea willana displayed an infection symptom different from all other macroalgae hosting pigmented endophytic brown algae. In this host species, the surface of the part of the phylloid where the endophytes were present appeared to be less pigmented than the surrounding tissue (Plate 3.5, Figure E). The pale patch had a diameter of circa 7 cm. Even though macroscopically the surface seemed to be rough and possibly damaged by grazers, microscopic

sections did not reveal any cellular damage or necrotic tissue. Instead, the cells at the surface including their cuticle appeared to be intact (Plate 3.5, Figure F).

The infected specimen was found at Brighton Beach, at the same site as the infected *D. antarctica*. The endophytic brown alga from *Durvillaea willana* was isolated as strain no. 34 (Table 3.1).

3.1.1.1.3 Rhodophycean hosts

All three red algal hosts, *Pachymenia lusoria, Grateloupia intestinalis* and the undescribed species of the Kallymeniaceae, showed similar infection symptoms. They did not display any morphological changes, such as galls, or warts, as did the Phaeophyceae hosting brown endophytes. Instead, infections of the rhodophycean hosts with endophytic brown algae only became conspicuous as dark brown, round patches or brown colouration, when the host thalli were held against a bright background or a light source. No obvious surface alterations were associated with the endophyte patches.

In *P. lusoria* and *G. intestinalis*, the endophytes were mainly located in the distal parts of the host phylloids. Their thalli reached diameters of up to 5 mm in *P. lusoria* (Plate 3.4, Figure A), while the ones in *G. intestinalis* were smaller (2-3 mm). In the member of the Kallymeniaceae, endophytes were distributed over the entire phylloid causing a brown colouration.

In all three host algae, the endophytes grew transversely through the whole host thallus, but were most conspicuous in the internal parts where the host appeared less pigmented. In case of *G. intestinalis*, many endophyte filaments were found inside the mucilage-filled central cavity of the tubular thallus.

Five strains of endophytic brown algae were isolated from *Pachymenia lusoria* (Table 3.1, isolates no. 19-22 and no. 27). The other two red algal hosts, *Grateloupia intestinalis* and the undescribed species, however, were each collected only once during the course of this study (at Graybrook/Otago and at Causet Cove, Doubtful Sound/Fjordland, respectively). Therefore only a single endophyte strain resulted from each of these two species (*G. intestinalis*: isolate no. 23; undescribed species: isolate no. 24; both Table 3.1). In none of the red algae species was it determined whether the endophytes were isolated from host gameto- or sporophytes.

Table 3.1: Collection details of isolated endophytic brown algae. [1]: according to section 3.1.3; [2]: collectors (SH: S. HEESCH; CH: CH. HEPBURN; GK: G. KNIGHT; SM: S. M. MILLER; KN: K. NEILL; JP: J. PHILLIPS; MS & JF: M. STUART & J. FYFE); [3]: underneath a patch of *Herpodiscus durvillaeae*; *: taxon ined..

no.	species[1]	variety (group)[1]	isolate (abbreviation)	host species	location	coordinates	date	collected by [2]	part of the host infected	symptoms of infection
1	*Laminariocolax macrocystis*	*radiatae** (group L_1)	EMa A 3/98	*Macrocystis pyrifera*	Aramoana Mole, Otago Harbour	45°46'41"S 170°42'20"E	06.03.1998	MS & JF	cauloid	gall
2			EMa HP 5/98	*Macrocystis pyrifera*	Harington Point, Otago Harbour	45°47'15"S 170°43'5"E	16.05.1998	CH	cauloid	gall
3			EMa HP 4/99	*Macrocystis pyrifera*	Harington Point, Otago Harbour	45°47'15"S 170°43'5"E	27.04.1999	CH	cauloid	gall
4			EMa SP 5/98	*Macrocystis pyrifera*	Seal Point, Otago Peninsula	45°54'19"S 170°37'41"E	21.05.1998	CH	cauloid	gall
5			EMa WW 5/99	*Macrocystis pyrifera*	Waitangi West, Chatham Island (drift)	43°47'0"S 176°48'37"W	17.05.1999	SH	cauloid	gall
6			EMa HI 10/99	*Macrocystis pyrifera*	Hoopers Inlet, Otago Peninsula	45°52'9"S 170°39'36"E	28.10.1999	SH	cauloid	dark patch
7			EEck W 3/98	*Ecklonia radiata*	Lyall Bay, Wellington	41°20'45"S 174°47'45"E	30.03.1998	SH	phylloid	gall
8			EEck DS 5/99	*Ecklonia radiata*	Causet Cove, Doubtful Sound, Fiordland	45°16'55"S 160°53'52"E	24.05.1999	SM	cauloid	dark patch
9			EEck Waki 10/00	*Ecklonia radiata*	Waikouaiti, Otago	45°37'7"S 170°41'18"E	20.10.2000	CH	phylloid	gall
10			EEck OTS 11/99	*Ecklonia radiata*	Outer Thompson Sound, Fiordland	45°09'29"S 166°59'06"E	15.11.1999	SM	cauloid	dark patch
11		*macrocystis* (group L_2)	EMa A 10/97	*Macrocystis pyrifera*	Aramoana Mole, Otago Harbour	45°46'41"S 170°42'20"E	15.10.1997	CH	cauloid	gall
12			EMa QP 7/97	*Macrocystis pyrifera*	Quarantine Point, Otago Harbour	45°49'41"S 170°38'21"E	28.07.1997	CH	cauloid	gall
13		*novae-zelandiae** (group L_3)	EMa PB 6/97	*Macrocystis pyrifera*	Pilots Beach, Otago Harbour (drift)	45°46'47"S 170°43'16"E	19.06.1997	SH	cauloid	gall
14			EMa BB 10/97	*Macrocystis pyrifera*	Brighton Beach, Otago	45°57'11"S 170°20'9"E	01.10.1997	SH	cauloid	gall
15			EMa CH 5/98	*Macrocystis pyrifera*	Cornish Head, Otago	45°37'16"S 170°41'52"E	21.05.1998	MS & JF	cauloid	gall
16			EMa W 3/98	*Macrocystis pyrifera*	Mahanga Bay, Wellington	41°17'49"S 174°50'12"E	30.03.1998	SH	cauloid	gall
17			EMa OW 5/99	*Macrocystis pyrifera*	Owenga Harbour, Chatham Island	44°1'28"S 176°22'7"W	15.05.1999	SH	cauloid	gall
18			EMa WR 4/00	*Macrocystis pyrifera*	Wellers Rock, Otago Harbour	45°47'58"S 170°42'33"E	17.04.2000	CH	cauloid	gall
19	*Microspongium tenuissimum*	*tenuissimum* (group M_1)	EPa A 10/97	*Pachymenia lusoria*	Aramoana Mole, Otago Harbour	45°46'41"S 170°42'20"E	15.10.1997	SH	phylloid	brown patch
20			EPa Riv 9/99	*Pachymenia lusoria*	Riverton, Southland	46°22'42"S 168°1'44"E	02.09.1999	SH	phylloid	brown patch
21			EPa BB 4/99	*Pachymenia lusoria*	Brighton Beach, Otago	45°57'11"S 170°20'9"E	26.04.1999	SH	phylloid	brown patch
22			EPa BS 10/00	*Pachymenia lusoria*	Bradshaw Sound, Fiordland	46°17'29"S 167°1'50"E	03.10.2000	KN	phylloid	brown patch
23			EGra BB 2/98	*Grateloupia intestinalis*	Graybrook, Otago	45°58'41"S 170°16'53"E	11.02.1998	SH	phylloid	brown patch
24			EKal CC 10/00	(Fam. Kallymeniaceae)	Causet Cove, Doubtful Sound, Fiordland	46°17'47"S 166°53'51"E	03.10.2000	JP	phylloid	brown area
25			EUpi W 4/98	*Undaria pinnatifida*	Oriental Bay, Wellington	41°17'52"S 174°47'39"E	01.04.1998	SH	cauloid	no symptoms
26			EEck Kari 5/99	*Ecklonia radiata*	Cornish Head, Otago	45°37'16"S 170°41'52"E	24.05.1999	SH	cauloid	brown patch
27		*radians* (group M_2)	EPa OW 5/99	*Pachymenia lusoria*	Tom Solomon's Grave, Owenga, Chatham Island	44°1'28"S 176°22'7"W	15.05.1999	SH	phylloid	brown patch
28	*Xiphophorocolax aotearoae**	*aotearoae** (group X_1)	ELes OW 5/99	*Lessonia tholiformis*	Te One Creek, Owenga, Chatham Is.	44°1'28"S 176°22'7"W	15.05.1999	GK	cauloid	brown stripes
29			ELes Wk 5/99	*Lessonia tholiformis*	Wharekauri, Chatham Island	43°40'44"S 176°34'27"W	16.05.1999	SH	cauloid	gall
30			EXi BB 11/97	*Xiphophora gladiata*	Brighton Beach, Otago	45°57'11"S 170°20'9"E	15.11.1997	SH	cauloid	gall
31			EXi BB 8/99	*Xiphophora gladiata*	Brighton Beach, Otago	45°57'11"S 170°20'9"E	10.08.1999	SH	cauloid	gall
32			EMu Wk 5/99	*Marginariella urvilliana*	Wharekauri, Chatham Island	43°40'44"S 176°34'27"W	16.05.1999	SH	cauloid	brown spot
33			EDan BB 4/99	*Durvillaea antarctica*	Brighton Beach, Otago	45°57'11"S 170°20'9"E	21.04.1999	SH	phylloid	no symptoms[3]
34		*willanae** (group X_2)	EDwil BB 8/00	*Durvillaea willana*	Brighton Beach, Otago	45°57'11"S 170°20'9"E	04.08.2000	SH	phylloid	pale patch, no gall

3.1.1.2 Isolated endophytes and their morphologies

A total of 34 strains of endophytic Phaeophyceae were isolated from New Zealand macroalgae. Collection data for all isolates are compiled in Table 3.1, characteristics in Table 3.2. Appendix B includes cell and sporangia sizes (Table B 1.1), the results of statistical comparisons of cell and sporangia sizes (ANOVA Tables, Tables B 1.2-1.6), as well as distribution maps (Figures B 2.1 - B 2.3).

The morphological characters of the isolated endophytes allowed their separation into three groups. According to DNA analyses (section 3.1.3) they represented three different species: *Laminariocolax macrocystis* (PETERS) PETERS in BURKHARDT & PETERS 1998, *Microspongium tenuissimum* (HAUCK) PETERS 2003 and a so-far undescribed taxon, for which the new genus and species *Xiphophorocolax aotearoae* gen. et sp. ined. will be proposed.

3.1.1.2.1 *Laminariocolax macrocystis*

All of the isolates from *Macrocystis pyrifera* and all but one of the *Ecklonia radiata* strains (Table 3.1, isolates no. 1-19) were classified as *Laminariocolax macrocystis*. Their morphological features are documented in Plate 3.3.

The *L. macrocystis* isolates had microscopic thalli which were constructed from uniseriate, irregularly branched filaments (Plate 3.3, Figure A). The overall appearance of the thalli varied, from prostrate, compact agglomerates to rather loose tufts, however, these growth forms were not distinguishable by cell and sporangia sizes. Erect filaments were observed in some isolates (no. 1-3, 5 and 10). Additionally, several isolates (no. 1, 2, 7-9, 13, 15-17) displayed true phaeophycean hairs without basal sheaths (Plate 3.3, Figure C). In field material, however, neither filaments rising over the host surface nor hairs were observed.

In culture, cells were round to cylindrical, with lengths of 18.1 ± 7.5 µm and widths of 7 ± 2.3 µm (S.D., n = 180; Table B 1.1). Usually, most cells of a thallus displayed several (in some isolates up to nine or 10) plastids per cell (Table 3.2). Occasionally, single cells had only two to three plastids. Plastids were discoid and had stalked pyrenoids (Plate 3.3, Figure D).

Table 3.2: Morphological data and ITS1 lengths of isolated endophytic brown algae. [1]: according to section 3.1.3; [2]: ± standard deviation, number of cells or sporangia measured in brackets; [3]: type 1 with germination tube and emptying of embryospore, type 2 without these characters; [4]: complete ITS sequence not known; [5]: rarely slightly lobed plastids; *: taxon ined.; + : present; - : absent; n/a: not applicable; n. d.: not determined; n. o.: not observed. No unilocular sporangia were observed in any of the isolates.

no.	species[1]	variety (group)[1]	isolate (abbreviation)	thallus organisation	uprights	true hairs		cell size	plastids			plurilocular sporangia		germination type[3]	length of ITS1 [bp]
					present ?	present ?	basal sheath	length x width [µm][2]	no. per cell	shape	pyrenoid	type	length x width [µm], loculi per sp.[2]		
1	*Laminariocolax macrocystis*	*radiatae** (group L_1)	EMa A 3/98	uniseriate filaments	+	+	-	21±4 x 9±2 (n=10)	several (2-6)	discoid	+	uniseriate, simple	120±34 x 7±1, 33±7 loculi (n=10)	1	679
2			EMa HP 5/98	uniseriate filaments	+	+	-	14±5 x 8±3 (n=10)	several (4-9)	discoid[5]	+	uniseriate, simple	44±24 x 7±1, 14±8 loculi (n=10)	n. d.	678
3			EMa HP 4/99	uniseriate filaments	+	n. o.	n/a	10±3 x 7±1 (n=10)	several (2-5)	discoid	+	uniseriate, simple	43±6 x 8±1, 14±4 loculi (n=10)	n. d.	678
4			EMa SP 5/98	uniseriate filaments	n. o.	n. o.	n/a	21±10 x 9±2 (n=10)	several (2-9)	discoid[5]	+	uniseriate, simple	81±15 x 7±1, 20±6 loculi (n=10)	1	679
5			EMa WW 5/99	uniseriate filaments	+	n. o.	n/a	19±5 x 7±2 (n=10)	several (2-5)	discoid	+	uniseriate, simple	79±22 x 8±1, 26±8 loculi (n=10)	1	679
6			EMa HI 10/99	uniseriate filaments	n. o.	n. o.	n/a	14±6 x 8±3 (n=10)	several (4-7)	discoid	+	uniseriate, simple	40±15 x 7±1, 14±5 loculi (n=10)	n. d.	678
7			EEck W 3/98	uniseriate filaments	n. o.	+	n. d.	21±7 x 8±3 (n=10)	several (-10)	discoid	+	uniseriate, simple	n. d.	n. d.	678
8			EEck DS 5/99	uniseriate filaments	n. o.	+	n. d.	20±10 x 6±2 (n=10)	several (2-4)	discoid	+	uniseriate, simple	n. d.	n. d.	n. d.[4]
9			EEck Waki 10/00	uniseriate filaments	n. o.	+	-	25±10 x 8±2 (n=10)	several (2-5)	discoid	+	uniseriate, simple	n. d.	1	684
10			EEck OTS 11/99	uniseriate filaments	+	n. o.	n/a	14±6 x 5±1 (n=10)	several (2-5)	discoid	+	uniseriate, simple	59±23 x 5±1, 18±6 loculi (n=10)	n. d.	677
11		*macrocystis* (group L_2)	EMa A 10/97	uniseriate filaments	n. o.	n. o.	n/a	13±5 x 7±1 (n=10)	several (2-8)	discoid[5]	+	uniseriate, simple	58±20 x 8±1, 18±6 loculi (n=10)	1	507
12			EMa QP 7/97	uniseriate filaments	n. o.	+	n. d.	23±7 x 6±1 (n=10)	several (3-6)	discoid	+	uniseriate, simple	64±15 x 7±1, 17±4 loculi (n=10)	1	511
13		*novae-zelandiae** (group L_3)	EMa PB 6/97	uniseriate filaments	n. o.	+	-	19±6 x 6±1 (n=10)	several (2-5)	discoid	+	uniseriate, simple	64±11 x 6±1, 22±4 loculi (n=10)	1, 2	n. d.[4]
14			EMa BB 10/97	uniseriate filaments	n. o.	+	-	16±5 x 6±2 (n=10)	several (3-6)	discoid	+	uniseriate, simple	25±7 x 7±1, 9±2 loculi (n=10)	1	479
15			EMa CH 5/98	uniseriate filaments	n. o.	+	n. d.	18±4 x 9±2 (n=10)	several (2-5)	discoid	+	uniseriate, simple	56±23 x 8±2, 14±8 loculi (n=10)	1	481
16			EMa W 3/98	uniseriate filaments	n. o.	+	-	16±3 x 4±1 (n=10)	several (n. d.)	discoid	+	uniseriate, simple	n. d.	1	476
17			EMa OW 5/99	uniseriate filaments	n. o.	+	-	16±3 x 6±1 (n=10)	several (n. d.)	discoid	+	uniseriate, simple	43±11x 6±1, 14±5 loculi (n=10)	1	481
18			EMa WR 4/00	uniseriate filaments	n. o.	n. o.	n/a	28±9 x 7±1 (n=10)	several (2-5)	discoid	+	uniseriate, simple	23±10 x 7±1 (n=10)	1	478

Table 3.2 (continued): Morphological data and ITS1 lengths of isolated endophytic brown algae. [1]: according to section 3.1.3; [2]: ± standard deviation, number of cells or sporangia measured in brackets; [3]: type 1 with germination tube and emptying of embryospore, type 2 without these characters; [4]: complete ITS sequence not known; *: taxon ined.; + : present; - : absent; n/a: not applicable; n. d.: not determined; n. o.: not observed. No unilocular sporangia were observed in any of the isolates.

no.	species[1]	variety (group)[1]	isolate (abbreviation)	thallus organisation	uprights	true hairs		cell size	plastids			plurilocular sporangia		germination type[3]	length of ITS1 [bp]
					present ?	present ?	basal sheath	length x width [µm] [2]	no. per cell	shape	pyrenoid	type	length x width [µm], loculi per sp. [2]		
19	*Microspongium tenuissimum*	*tenuissimum* (group M_1)	EPa A 10/97	uniseriate filaments, partly pectinate	n. o.	+	n. d.	9±2 x 5±1 (n=10)	n. d.	discoid	n. d.	uniseriate, pectinate	n. d.	n. d.	288
20			EPa Riv 9/99	uniseriate filaments, partly pectinate	n. o.	+	n. d.	7±4 x 5±1 (n=10)	1-2	discoid	+	uniseriate, pectinate	24±5 x 5±1, 6±1 loculi (n=10)	n. d.	288
21			EPa BB 4/99	uniseriate filaments, partly pectinate	+	n. o.	n/a	6±2 x 4±1 (n=10)	2 (1-3)	discoid	+	uniseriate, pectinate	16±5 x 4±1, 6±1 loculi (n=10)	2	288
22			EPa BS 10/00	uniseriate filaments, partly pectinate	+	+	n. d.	5±2 x 3±1 (n=10)	1-2	discoid	+	uniseriate, pectinate	16±5 x 4±1, 5±2 loculi (n=10)	2	288
23			EGra BB 2/98	uniseriate filaments	+	+	n. d.	17±3 x 5±2 (n=10)	1-2	discoid	+	n. d.	n. d.	2	288
24			EKal CC 10/00	uniseriate filaments, partly pectinate	+	+	n. d.	8±3 x 5±1 (n=10)	2	discoid	+	uniseriate, pectinate + simple	41±11 x 4±1, 14±4 loculi (n=10)	2	288
25			EUpi W 4/98	uniseriate filaments	+	+	n. d.	22±7 x 7±2 (n=20)	2	discoid	+	uniseriate, simple	70±24 x 6±1, 18±7 loculi (n=10)	n. d.	288
26			EEck Kari 5/99	uniseriate filaments	+	+	+	18±4 x 4±1 (n=10)	1-2	discoid	+	uniseriate, simple	56±14 x 4±0.5, 20±4 loculi (n=10)	n. d.	288
27		*radians* (group M_2)	EPa OW 5/99	uniseriate filaments; prostrate pseudo-parenchymatous base	+	n. o.	n/a	12±4 x 4±1 (n=10)	2-3	discoid	+	uniseriate, simple	31±6 x 5±1, 8±2 loculi (n=10)	n. d.	258
28	*Xiphophoreolax aotearoae**	*aotearoae** (group X_1)	ELes OW 5/99	uniseriate filaments	-	n. o.	n/a	9±3 x 8±1 (n=10)	2	discoid	+	n. d.	n. d.	n. d.	582
29			ELes Wk 5/99	uniseriate filaments	-	n. o.	n/a	11±2 x 5±1 (n=10)	2-3	discoid	+	n. d.	n. d.	2	583
30			EXi BB 11/97	uniseriate filaments	n. o.	n. o.	n/a	7±2 x 6±1 (n=10)	1-2	discoid	+	n. d.	n. d.	n. d.	n. d.[4]
31			EXi BB 8/99	uniseriate filaments	-	n. o.	n/a	7±3 x 5±1 (n=10)	1-2	discoid	+	uniseriate, simple	n. d.	n. d.	581
32			EMu Wk 5/99	uniseriate filaments	-	+	+	10±3 x 5±1 (n=10)	1-2	discoid	+	uniseriate, simple	38±8 x 6±1, 14±3 loculi (n=10)	n. d.	583
33			EDan BB 4/99	uniseriate filaments	-	n. o.	n/a	9±3 x 5±1 (n=10)	2	discoid	+	uniseriate, simple	18±9 x 6±1, 6±3 loculi (n=10)	2	583
34		*willanae** (group X_2)	EDwil BB 8/00	uniseriate filaments	+	n. o.	n/a	7±2 x 6±1 (n=10)	1-2	discoid	+	uniseriate, simple	n. d.	2	553

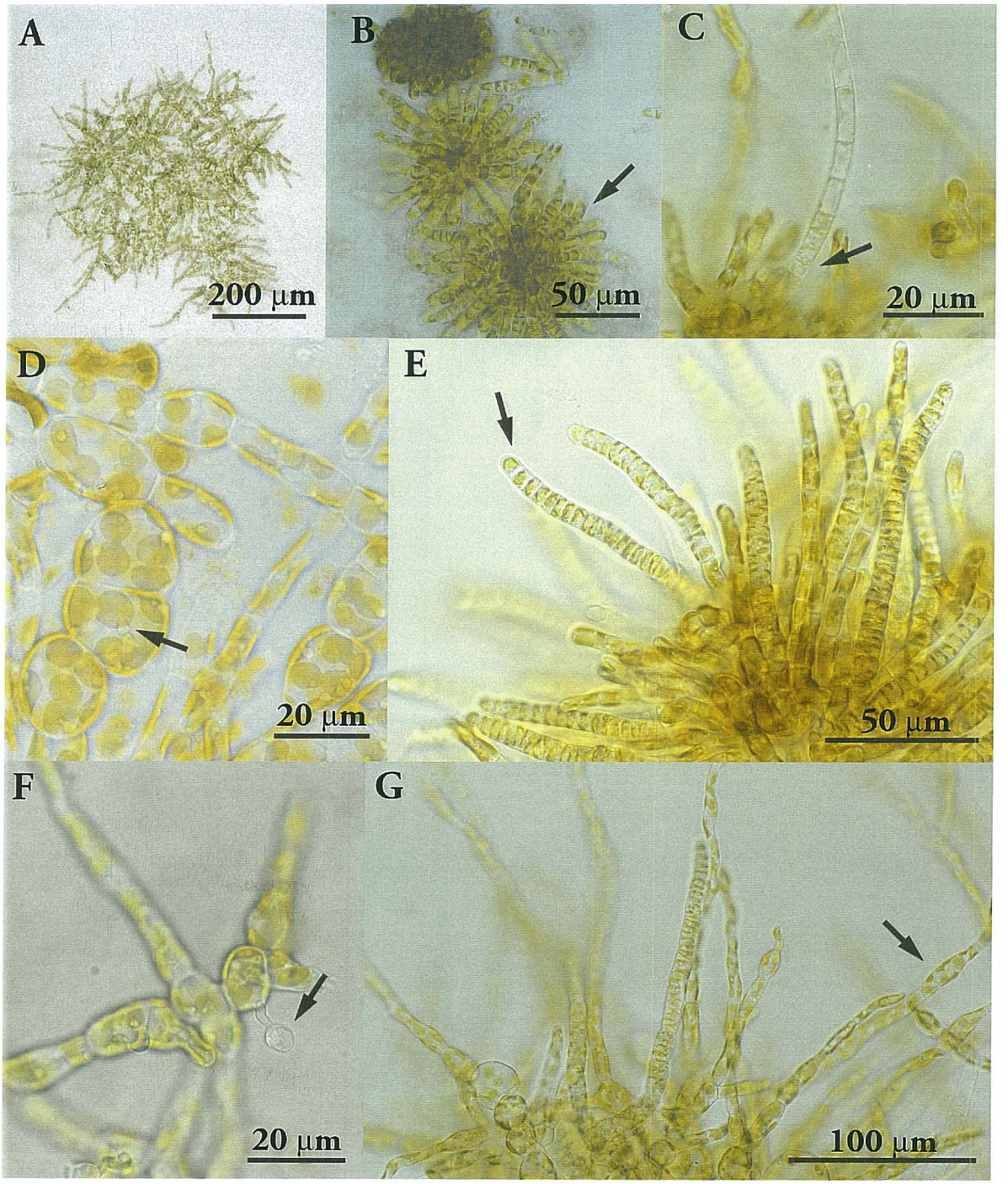
Most *L. macrocystis* isolates showed a direct life cycle in culture, reproducing via plurilocular sporangia (Plate 3.3, Figure E). The sporangia were always uniseriate, 57.2 ± 30.5 µm long, 6.9 ± 1.2 µm wide (n = 125) and had 19 ± 9 loculi (S.D., n = 116; Table B 1.1). Mitospores were released from the plurilocular sporangia through an apical pore. In all but one isolate, there was no evidence of whether gametophytes and/or sporophytes were present in the cultures, as no unilocular sporangia were observed in any of the isolates. Spores usually settled and germinated without fusing with other spores.

Upon germination, the so-called embryospore usually formed a narrow germ tube (circa 2 µm diameter) with a swelling developing at the tip. The contents of the embryospore then migrated into this swelling. A cell wall developed separating it from the germ tube, and thus turned it into the first cell of the thallus, leaving the empty embryospore and germ tube behind (germination type 1: Table 3.2; micrograph: Plate 3.3, Figure F). Alternatively, spores could germinate without the narrow germ tube and emptying of the embryospore. This germination type (type 2) was, for example, observed in isolate no. 13, early after the start of the culture.

DNA sequence analyses (section 3.1.3.3.2) revealed the presence of three genetically different groups among the *Laminariocolax* isolates: group L_1 (isolates no. 1-10), group L_2 (isolates no. 11 and 12) and group L_3 (isolates no. 13-18). These, however, could not be sufficiently separated by morphological characters. Cell and sporangia sizes, for example, were not significantly different among the groups (Tukey-Kramer test; n = 10; Table B 1.3). The only character separating groups in culture was the formation of uprights, which were observed in some of the isolates of group L_1 (Plate 3.3, Figure G), but appeared to be absent in group L_2 and L_3. The latter two groups did not show morphological differences from each other under the set culture conditions.

Plate 3.3: The endophyte *Laminariocolax macrocystis* in culture (micrographs).

A: Example for a loose, fluffy habit (isolate no. 13). **B**: Young thalli showing compact agglomerate-like habit with short uniseriate plurilocular sporangia (arrows; isolate no. 3). **C**: Phaeophyceaen hair without sheath, with basal meristem (arrow; isolate no. 17). **D**: Cells with several plastids, some in the process of division, displaying stalked pyrenoids (arrow; isolate no. 12). **E**: Long uniseriate plurilocular sporangia (arrow; isolate no. 17). **F**: Young thallus displaying empty embryospore (arrow) with narrow germination tube (isolate no. 12). **G**: Thallus of *L. macrocystis* var. *eckloniae* (group L_1) with erect filaments (arrow) and uniseriate plurilocular sporangia (isolate no. 2).


A
200 µm
B
50 µm
C
20 µm
D
20 µm
E
50 µm
F
20 µm
G
100 µm

3.1.1.2.2 *Microspongium tenuissimum*

A single isolate from *Ecklonia radiata* (from Karitane; Table 3.1, isolate no. 26) together with the endophyte from *Undaria pinnatifida* (isolate no. 25) and all strains isolated from red algal hosts (isolates no. 19-24, no. 27) fell into the second group identified as *Microspongium tenuissimum*. Their morphology is documented in Plate 3.4.

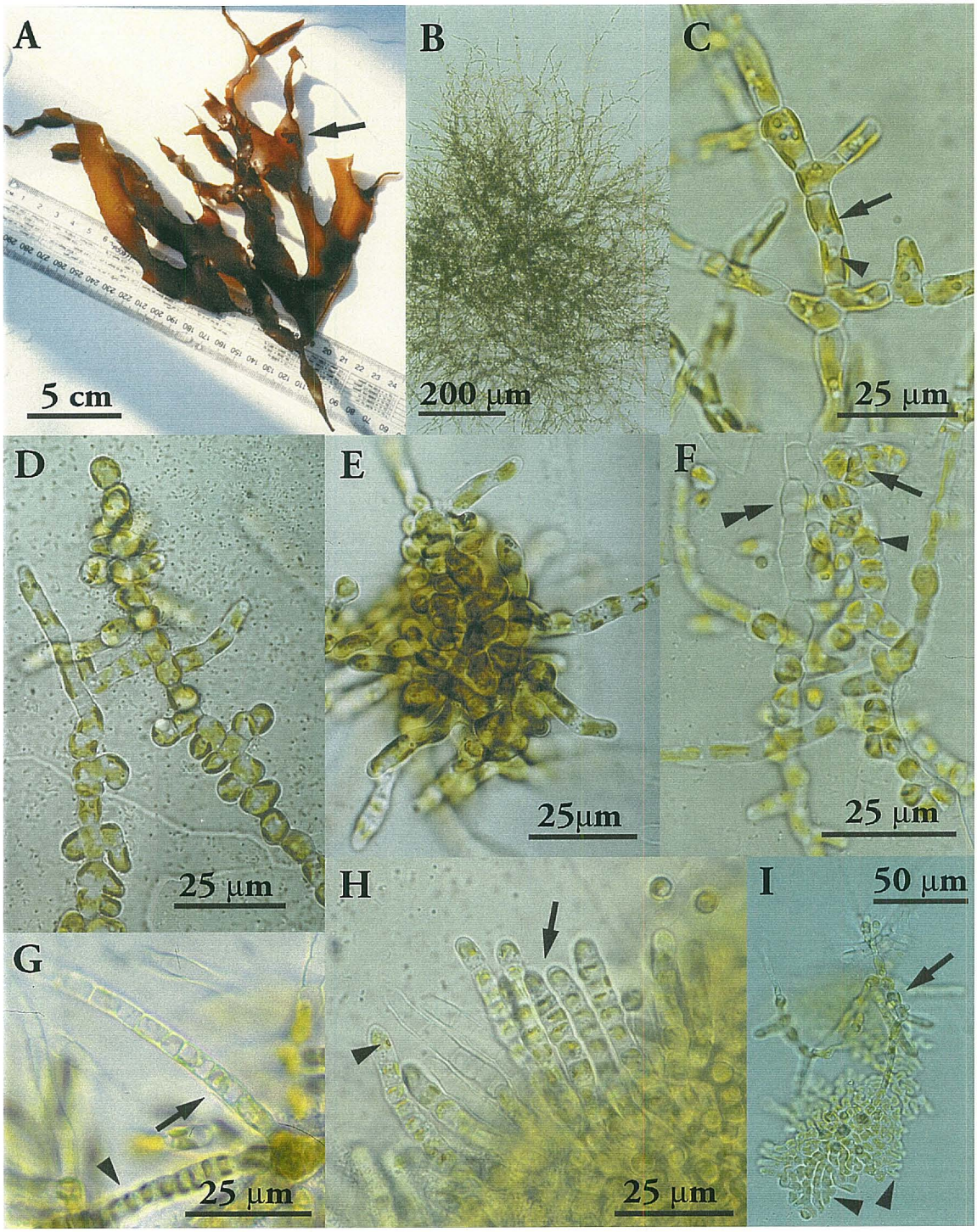
As in the former species, the thallus of *M. tenuissimum* was microscopic and consisted of uniseriate filaments. Also, two different types of thalli appeared in the cultures, fluffy loose ones and compact prostrate ones. In the prostrate thalli (type 1, e. g. observed in isolates no. 20-22), along the filaments, short laterals often developed from every cell to one side (or even in two oppositing directions), giving the thallus a pectinate appearance (Plate 3.4, Figure D). The loose thalli (type 2, e. g. observed in isolates no. 24-26), on the other hand, usually displayed simple, irregularly branched filaments (Plate 3.4, Figures B and C). Hairs with sheaths (Plate 3.4, Figure G) and uniseriate erect filaments were present in most isolates. In the endophyte isolated from *Pachymenia lusoria* on Chatham Island (isolate no. 27), the uprights arose from a pseudoparenchymatic disc constructed from a single layer of closely lying filaments. Cells in the disc had irregular contours, with some of the cells at the margin being y-shaped (Plate 3.4, Figure I).

With the data of all isolates combined, *M. tenuissimum* cells reached an average length of 11.6 ± 6.5 µm and width of 4.6 ± 1.3 µm (S.D., n = 90; Table B 1.1), and thus were significantly smaller than those of the *L. macrocystis* isolates (Tukey-Kramer test; n = 70; Table B 1.2). The length to width ratio, however, was of the same range in both species (2.9 ± 1.5 in *L. macrocystis*, 2.6 ± 1.5 in *M. tenuissimum*; S.D.; Table B 1.2). Between the two thallus types of *M. tenuissimum*, cells of type 1 were significantly smaller than those of type 2 (Tukey-Kramer test; n = 10; Table B 1.5). Plastids in *M. tenuissimum* were discoid with pyrenoids, with usually only two plastids present in each cell (Plate 3.4, Figure C), in contrast to the *L. macrocystis* isolates. Occasionally, *M. tenuissimum* cells displayed one or three plastids.

All *M. tenuissimum* isolates showed a direct life cycle. Only uniseriate plurilocular sporangia were observed in culture (average length: 35 ± 21.2; width: 4.4 ± 0.7; no. of loculi: 11 ± 7; S.D.; n between 65 and 70; Table B 1.1). On the prostrate thalli (including isolate no. 27), sporangia were comparatively short (Plate 3.4, Figure H), with the erect filaments producing apical sporangia, while pectinate filaments had sporangia which also involved the short branches (semi-intercalary sporangia; Plate 3.4, Figure F). The loose thalli, however, only displayed apical sporangia which were similar to those found in the *L. macrocystis* isolates. They

Plate 3.4: The endophyte *Microspongium tenuissimum* in the field and in culture.

A: Specimen of the host *Pachymenia lusoria* displaying round brown patches associated with *M. tenuissimum* (arrow; collected at Brighton Beach, Otago, 26.04.1999; isolate no. 21).
B-H: Micrographs of culture material. **B**: Habit of a fluff-like growing thallus (isolate no. 25). **C**: Detail of fluff-like growing thallus. Cells contain 1-2 plastids (arrow) with a pyrenoid (arrowhead; isolate no. 25). **D**: Young thallus with pectinate appearance (isolate no. 24). **E**: Young thallus showing agglomerate-like growth (isolate no. 20). **F**: Young thallus with a pectinate filament that turned into a plurilocular sporangium. The irregularly shaped loculi give the sporangium a partly biseriate appearance (arrow). Unreleased mature spores display eyespots (arrowhead). The double arrowhead points to an emptied sporangium (isolate no. 21). **G**: Thallus displaying a phaeophycean hair with a sheath at the base (arrow) and part of a long plurilocular sporangium (arrowhead; isolate no. 26). **H**: Thallus with short plurilocular sporangia (arrow) showing regularly shaped loculi. Unreleased mature spores display eyespots (arrowhead; isolate no. 27). **I**: An erect filament (arrow) arises above a prostrate base in a thallus of *M. tenuissimum* var. *radians* (group M_2, isolate no. 27). The margin of the base displays y-shaped cells (arrowheads).


A
5 cm
B
200 µm
C
25 µm
D
25 µm
E
25µm
F
25 µm
G
25 µm
H
25 µm
I
50 µm

had many more loculi and thus were significantly longer than the apical sporangia of the prostrate thalli (Tukey-Kramer test; n = 30; Table B 1.5). Whether the two thallus types with their different plurilocular sporangia represented the two generations of *M. tenuissimum* in culture was not determined as neither fusions of spores nor unilocular sporangia were observed in any of the isolates. Spores from any of the sporangia germinated without a particularly narrow germ tube or evacuation of the embryospore.

DNA sequence analyses (section 3.1.3.3.3) revealed the presence of two genetically different groups, group M_1 (isolates no. 19-26) and group M_2 (isolate no. 27). In culture, these could only be separated by the pseudoparenchymatic discs which were frequently formed by isolate no. 27 but were not observed in any of the isolates of group M_1. The absence of phaeophycean hairs in cultures of isolate no. 27, on the other hand, could not be used as a distinguishing character, as hairs were also missing in an isolate of group M_1 (isolate no. 21). Additionally, average cell and sporangia sizes were not significantly different between the two sub-species (Tukey-Kramer test; n = 10; Table B 1.4). Cell sizes of isolate no. 27 took a medium position between the two thallus types present in group M_1. However, cells of the prostrate base were not included in the data set.

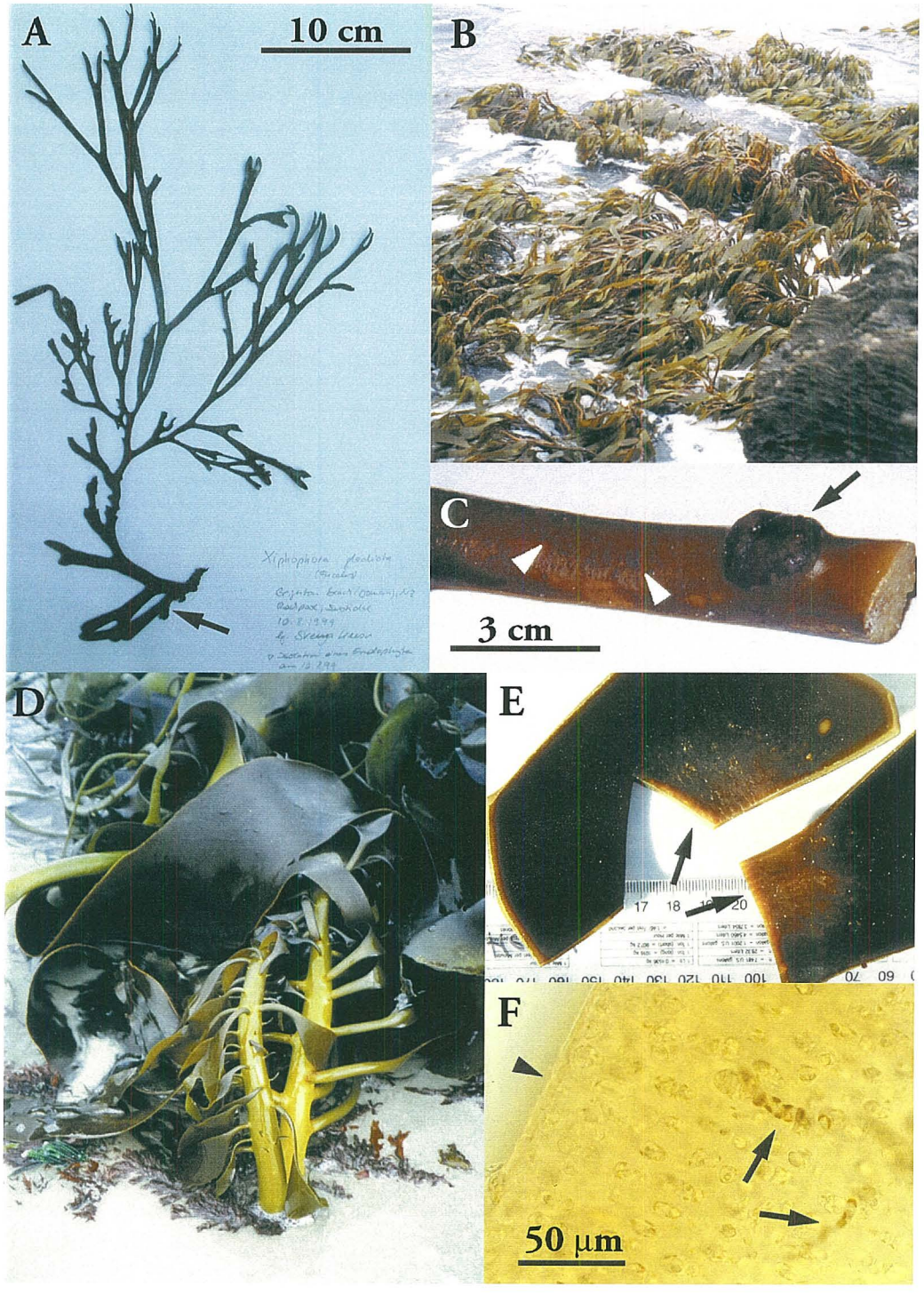
3.1.1.2.3 *Xiphophorocolax aotearoae* sp. et gen. ined.

The last group comprised seven strains, the two isolates from *Lessonia tholiformis* and all isolates from members of the Fucales s. l. (Table 3.1, isolates no. 28-34). Morphologies of these isolates classified as *Xiphophorocolax aotearoae* gen. et sp. ined. are documented in Plate 3.6.

The *Xiphophorocolax* isolates had microscopic thalli consisting of uniseriate filaments, like in the other two endophyte species. Pectinate filaments with short laterals from every other cell to one side formed a (pigmented) prostrate base which was compact but not pseudoparenchymatous like the basal disc in *Microspongium tenuissimum radians*. From this base, short, irregularly branched filaments arose giving the thallus the appearance of a dense, globular-shaped tuft (Plate 3.6, Figure A). A single strain, the endophyte from *Marginariella urvilliana*, displayed phaeophycean hairs, which were 54.8 ± 16.4 µm long and 3 ± 0.5 µm wide (S.D., n = 10; data not shown) and had inconspicuous basal sheaths (Plate 3.6, Figure B). Erect filaments were only observed in the isolate from *Durvillaea willana* (isolate no. 34; Plate 3.6, Figure E).

Plate 3.5: The endophyte *Xiphophorocolax aotearoae* gen. et sp. ined. in the field.

A: Host species *Xiphophora gladiata* ssp. *novae-zelandiae.* Herbarium specimen displaying a gall (arrow) associated with endophytes (collected at Brighton Beach, Otago, 10.08.1999; isolate no. 31). **B-C**: Host species *Lessonia tholiformis.* **B**: Host population at spring low tide near Kaingaroa, Chatham, May 1999. **C**: Gall (arrow) and dark spots (arrowheads) on cauloid associated with *X. aotearoae* (collected at Wharekauri, Chatham, 16.05.1999; isolate no. 29). **D-F**: Host species *Durvillaea willana.* **D**: Habit of the host (Brighton Beach, Otago, January 1999). **E**: Pale patch on the host lamina associated with *X. aotearoae.* **F**: Micrograph of endophyte filaments (arrows) inside the host tissue. The cuticle at the host surface appears to be intact (arrowhead; E-F: collected at Brighton Beach, 04.08.2000; isolate no. 34).


A
10 cm
B
C
3 cm
D
E
F
50 µm

Like the cells of the *M. tenuissimum* isolates, cells of *X. aotearoae* were comparatively small. Each cell displayed two (1-3) discoid plastids with pyrenoids (Plate 3.6, Figure C). With lengths of 8.3 ± 2.9 µm and widths of 5.8 ± 1.3 µm (S.D., n = 70; Table B 1.1), the *X. aotearoae* cells were slightly shorter but wider than the *M. tenuissimum* cells. Even though these differences between the two species were not significant, they resulted in a significantly smaller length to width ratio in *X. aotearoae* cells (1.5 ± 0.7) than in all *M. tenuissimum* cells combined (Tukey-Kramer test; n = 70; Table B 1.1). The *X. aotearoae* ratio, however, was in the same range as the ratio of *M. tenuissimum tenuissimum* cells of thallus type 1.

Xiphophorocolax cultures reproduced in a direct life cycle, i. e. spores developed into new tufts with a morphology similar to the parent thallus. When a thallus became fertile, the distal parts of filaments turned into short, uniseriate plurilocular sporangia (Plate 3.6, Figure F). Sometimes entire sections of a filament were involved and thus developed into a branched sporangium. In two isolates, sporangia reached 27.6 ± 13.5 µm in length, 5.5 ± 0.6 µm in width and consisted of 10 ± 5 loculi (S.D., n = 20; Table B 1.1).

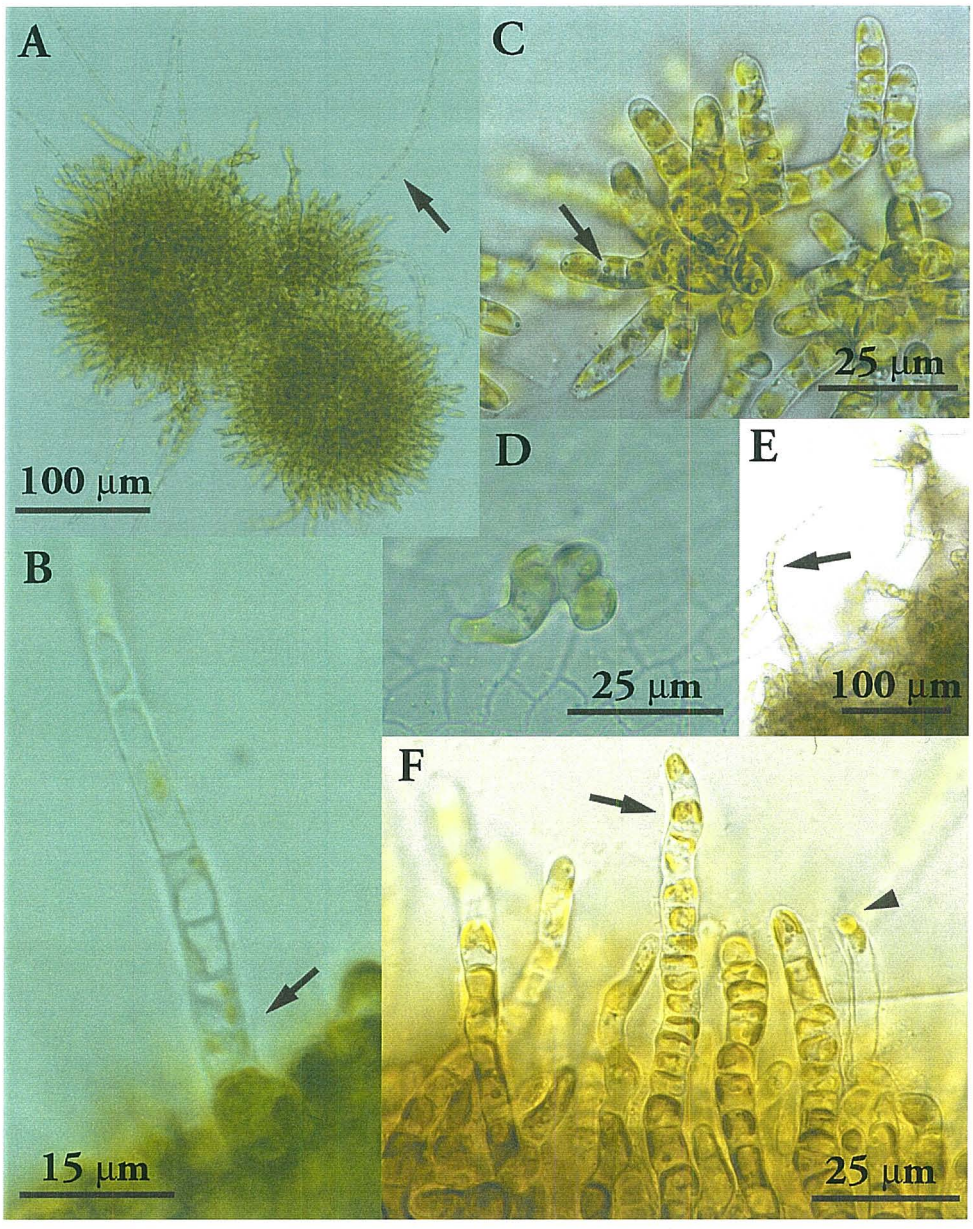
Spore release was observed in the endophyte isolated from *Marginariella urvilliana* (isolate no. 32). Spores left the plurilocular sporangia via an apical pore (Plate 3.6, Figure F). They displayed the morphology typical for brown algal swarmers, were motile, had an eyespot and showed positive phototaxis. They settled on every available surface of the culture vessel and germinated unipolarly, without the narrow germination tube or evacuation of the embryospore observed in most *Laminariocolax* isolates (Plate 3.6, Figure D). The generations present in the cultures were not determined as no fusion of spores was observed nor did unilocular sporangia appear in any of the *X. aotearoae* isolates.

Under the set culture conditions, *X. aotearoae* showed the slowest growth and/or reproduction rates among the three pigmented endophyte species found in New Zealand. In the *X. aotearoae* isolates, it took a few months to produce the amount of algal material necessary for DNA extractions (10-60 mg), compared to a few weeks in the isolates of *L. macrocystis* and *M. tenuissimum* (section 2.2.3.1.2). This observation, however, was not quantified.

DNA sequence analyses (section 3.1.3.3.4) revealed the presence of two genetically different groups, group X_1 (isolates no. 28-33) and group X_2 (isolate no. 34). Morphologically, these were distinguishable by the presence of uprights in isolate no. 34 which were not observed in group X_1 isolates. Cell sizes, however, did not significantly differ between the groups (Tukey-Kramer test; n = 10; Table B 1.6). The size of plurilocular sporangia was not recorded for isolate no. 34.

Plate 3.6: The endophyte *Xiphophorocolax aotearoae* gen. et sp. ined. in culture (micrographs).

A: Thalli showing a compact, agglomerate-like habit, with phaeophyceaen hairs (arrow; isolate 32). **B**: Base of a phaeophyceaen hair with small cells forming the growth zone and a small sheath (arrow; isolate 32). **C**: Young thalli showing compact growth, with cells displaying 1-2 plastids with pyrenoids (arrow; isolate 31). **D**: Germling (5-celled) on a small piece of glass, without an embryospore and a narrow germination tube (isolate 29). **E**: Thallus of *X. aotearoae* var. *willanae* (group X_2) with a developing branched erect filament (arrow; isolate 34). **F**: Uniseriate plurilocular sporangium (arrow). In another sporangium, the last spore is being released through an apical pore (arrowhead; isolate 32).


A
100 µm
B
15 µm
C
25 µm
D
25 µm
E
100 µm
F
25 µm

3.1.2 Endophyte prevalence in *Macrocystis pyrifera*

Endophytic brown algae were found in all three populations of *Macrocystis pyrifera* studied during 2000. Table 3.3 lists the results of all examinations, including those of additional collections during 1997, 1998 and 1999. The data for all thalli examined and ANOVA Tables are compiled in Appendix D 2.

The *Macrocystis* populations at Aramoana Mole and Quarantine Point showed high prevalences of infection: more than 95% of the thalli collected at both sites between 1997 and 2001 hosted endophytic Phaeophyceae. At Aramoana, all thalli examined during 2000 were infected (Table 3.3). Of these, around 78% displayed moderate disease symptoms (DC 2), another 14% had fronds with distortions (DC 3). Only once, during a preliminary collection in autumn 1998, was a thallus found in Aramoana without any macro- or microscopic signs of infection (DC 0). The season in which thalli were collected in 2000 did not have any impact on the severity of symptoms ($\chi^2 = 0.008$, $p = 0.9998$). However, thalli collected in winter were longer than thalli from summer or autumn (ANOVA: $F = 6.259$, $p = 0.0171$), even though their fresh weight did not differ from those of the thalli collected in other seasons (χ^2 test: $p > 0.05$). Summer thalli, on the other hand, had significantly more canopy fronds than thalli from the following winter (ANOVA: $F = 4.163$, $p = 0.0474$). The overall number of fronds, or the number of senescent fronds, did not change among seasons.

At Quarantine Point, more than 80% of the thalli from 2000 showed moderate infection symptoms (DC 2) and around 6% were severily affected by endophytes (DC 3). Only one of the 36 thalli collected during 2000 was uninfected (DC 0), which resulted in the 90% prevalence of infection in the summer thalli (Table 3.3). There were no seasonal differences detectable in the frond composition or the length of the thalli collected at Quarantine Point (ANOVAs or χ^2 tests, respectively: $p > 0.05$).

The populations from Quarantine Point and Aramoana did not show significant differences in the prevalence of infection (four seasons; $\chi^2 = 0.007$, $p = 0.9329$) or the severity of symptoms (four seasons; ANOVA: $F = 1.565$, $p = 0.2289$) from each other, while they displayed significantly higher prevalences of infection (two seasons; ANOVA: $F = 18.5$, $p = 0.0002$) and higher disease categories (two seasons; ANOVA: $F = 29.913$, $p = 0.0001$) than the off-shore population at Cornish Head. Here, only 35% (39% in 2000) of the collected thalli hosted endophytes, and of all 24 thalli collected at Cornish Head between 1999 and 2000, only two (8.7%) displayed macroscopic infection symptoms (DC 2), and none of the thalli examined

fell into DC 3. Thalli from the off-shore population were significantly longer than those collected in the Harbour (ANOVA: length: $F = 49.172$, $p = 0.0001$), and thalli from Aramoana were longer than those from Quarantine Point (ANOVA: $F = 85.212$, $p = 0.0001$). Frond numbers did not differ among the three populations (ANOVA: $F = 3.414$, $p = 0.067$).

Table 3.3: Prevalence of endophyte infection in populations of *Macrocystis pyrifera* along the Otago coast. Data included in the main field study in 2000 are shaded, data summarizing collections from a single site are set in bold. DC: disease category (see section 2.2.2 for details on arbitrary disease categories).

site	date	season	no. of thalli examined	infected thalli [%]	disease category [%]			
					DC 0	DC 1	DC 2	DC 3
Aramoana Mole	03.12.1997	summer	15	100	0	40	53.3	6.7
	05.03.1998	autumn	10	90	10	10	70	10
	05.10.1999	spring	7	100	0	14.3	85.7	0
	19.01.2000	summer	9	100	0	22.2	44.4	33.3
	12.04.2000	autumn	9	100	0	0	100	0
	19.07.2000	winter	9	100	0	0	100	0
	06.11.2000	spring	9	100	0	11.1	66.7	22.2
	year 2000		**36**	**100**	**0**	**8.3**	**77.8**	**13.9**
	01.02.2001	summer	10	100	0	0	100	0
	1997-2001		**78**	**98.7**	**1.3**	**14.1**	**75.6**	**9**
Quarantine Point	11.10.1999	spring	5	80	20	0	60	20
	19.01.2000	summer	9	89.9	11.1	33.3	55.6	0
	12.04.2000	autumn	9	100	0	0	100	0
	25.07.2000	winter	9	100	0	0	77.8	22.2
	01.11.2000	spring	9	100	0	11.1	88.9	0
	year 2000		**36**	**97.2**	**2.8**	**11.1**	**80.6**	**5.6**
	1999-2000		**41**	**95.1**	**4.9**	**9.8**	**78**	**7.3**
Cornish Head	07.10.1999	spring	5	20	80	20	0	0
	14.01.2000	summer	9	55.6	44.4	33.3	22.2	0
	25.07.2000	winter	9	22.2	77.8	22.2	0	0
	year 2000		**18**	**38.9**	**61.1**	**27.8**	**11.1**	**0**
	1999-2000		**23**	**34.8**	**65.2**	**26.1**	**8.7**	**0**
Harington Point	27.04.1999	autumn	9	77.8	22.2	33.3	44.4	0

At Cornish Head, season appeared to have some influence on the thallus size and the infection in *Macrocystis*, although differences were not significant, possibly due to the low number and small size of the samples. For example, the prevalence of infection appeared to be 33.3% lower in the thalli collected in winter 2000, compared to the thalli from summer, and the average disease category was reduced from DC 0.77 to DC 0.22, as none of the winter thalli displayed macroscopic symptoms. By combining prevalences on infection with the factor disease category, winter thalli collected at Cornish Head showed significantly less severe infections than the Harbour thalli (two seasons; $\chi^2 = 11.066$, $p = 0.004$), while in summer no difference was

detectable among the three sites (two seasons; $\chi^2 = 2.094$, $p = 0.351$). Also, the average winter thallus at Cornish Head appeared to possess less canopy fronds but slightly more senescent fronds (i. e. fronds without an apical scimitar). Furthermore, the winter thalli showed greater overall lengths but less weight than thalli from the previous summer. However, only the fresh weight (but none of the other variables; ANOVAs or χ^2 tests, respectively: $p > 0.05$) was significantly different between seasons (ANOVA: $F = 13.391$, $p = 0.0216$).

Phylloids and sporophylls of *Macrocystis pyrifera* were systematically checked for endophytes on two occasions: of the nine thalli collected at Harington Point in April 1999, seven were infected with endophytes. In one of these thalli, endophytes were found in a pneumatocyst neighbouring an infected cauloid section, but not in the phylloid. However, no infections were detected in any of the other pneumatocysts, phylloids or sporophylls examined. The thalli from Harington Point appeared to be less infected than the other Harbour populations, as less than 80% tested positive for endophytes. However, only nine thalli were examined in total.

In the infected thalli collected at Aramoana in February 2001, none of the sporophylls examined hosted endophytes. Of the infected fronds, one displayed an infection in the stipe and the attached pneumatocyst as well as the phylloid, but none of the other nine fronds had infected bladders or blades. In the fronds without macroscopic infection symptoms, endophytes were found once in a cauloid and once in a pneumatocyst (attached to a healthy stipe), but none were observed in any of the phylloids.

3.1.3 Molecular systematics

The highly variable ITS1 region and adjacent coding SSU and 5.8S regions of the nrDNA gene were used to resolve the phylogenetic affinities of the pigmented endophytic Phaeophyceae isolated in New Zealand. The boundaries between regions within the nrDNA gene were set as follows: between SSU and ITS1 at the pattern "gatcattaCCGAA" (after position 2216 of the sequence of *Scytosiphon lomentaria*; KAWAI *et al.* 1995), and between ITS1 and the 5.8S unit at the pattern "CGTTGTAAaactttcag" (after position 2709 of the *S. lomentaria* sequence; KAWAI *et al.* 1995). Thus in the alignment (Appendix D, D 3.1), the ITS1 region comprised the positions 562-1452.

The region from the end of the SSU to the beginning of the 5.8S unit was sequenced in two to three parts, depending on the length of the sequence. The primer AFP2(F) started at the

3' end of the small subunit gene (after position 1656 of the sequence of *S. lomentaria*; KAWAI *et al.* 1995). The partial SSU and complete ITS 1 were sequenced for most, the partial 26S region was sequenced only for a selected isolate (isolate no. 29). Alignments are presented in Appendix D (D 3.1 and D 3.3), sequence statistics for all isolates in Appendix D 2 (Tables D 2.1 and D 2.2). The distance matrices (absolute and Kimura-2-parameter distances) are presented in Appendix D 4 (Tables D 4.1.1-D 4.1.6).

3.1.3.1 General results

According to their ITS1 sequences, the 34 strains of endophytic brown algae isolated from New Zealand formed three clades. The isolates within each clade had nearly identical ITS1 sequences. Comparisons with published sequences showed that two of these clades comprised brown endophytes of the genera *Laminariocolax* (18 isolates) and *Microspongium* (nine isolates), of the order Ectocarpales *s. l.* (ROUSSEAU & DE REVIERS 1999; PETERS & RAMIREZ 2001). The third clade contained seven isolates with ITS1 sequences that were markedly different from those of the other isolates. A BLAST search in GenBank (20.03.2003) revealed that the ITS1 as well as SSU and partial LSU sequences of this clade were different from all other sequences submitted up to that date, thus *Xiphophorocolax* gen. ined. and *X. aotearoae* sp. ined. will be proposed to accommodate endophytes of the third clade.

3.1.3.2 Alignment properties

3.1.3.2.1 All endophytes

Differences between the three clades did not allow an unambigous alignment of sequences over the entire length of the ITS1 region. Only the beginning and the end of ITS1 with adjacent coding regions (partial SSU and 5.8S unit) could be aligned for all isolates, over a length of 186 bp (positions 559-648 and 1257-1353 of the alignment; Appendix D 3.1). Within these alignable regions, most members of the three clades had identical sequences, thus only a few representatives of each clade were included in the phylogenetic analyses comprising a total of 15 taxa. Parsimony analyses were run twice, with gaps either treated as missing or as a fifth base. Average transition/transversion ratios were $ti/tv = 1.00874$ including the outgroup and $ti/tv = 1.02575$ without the outgroup indicating saturation was not reached. The average base composition was A: 0.26553, C: 0.26002, G: 0.26389, and T: 0.20996.

3.1.3.2.2 *Laminariocolax* clade

Most *Laminariocolax* isolates had very similar ITS1 sequences and, due to some indels, differed mainly in the length of the region (Figure 3.1). Therefore, affinities within the clade were resolved using the re-coded alignment, and parsimony analyses were run twice, with gaps either treated as missing or as fifth base. To avoid long computing times and unresolved branches due to the presence of identical sequences, only three sequences of New Zealand isolates each representing ITS1 regions of different lengths (endophytes of *M. pyrifera* from Aramoana, Quarantine Point and Brighton Beach, i. e. isolates no. 1, 12 and 14) were among the seven taxa analysed.

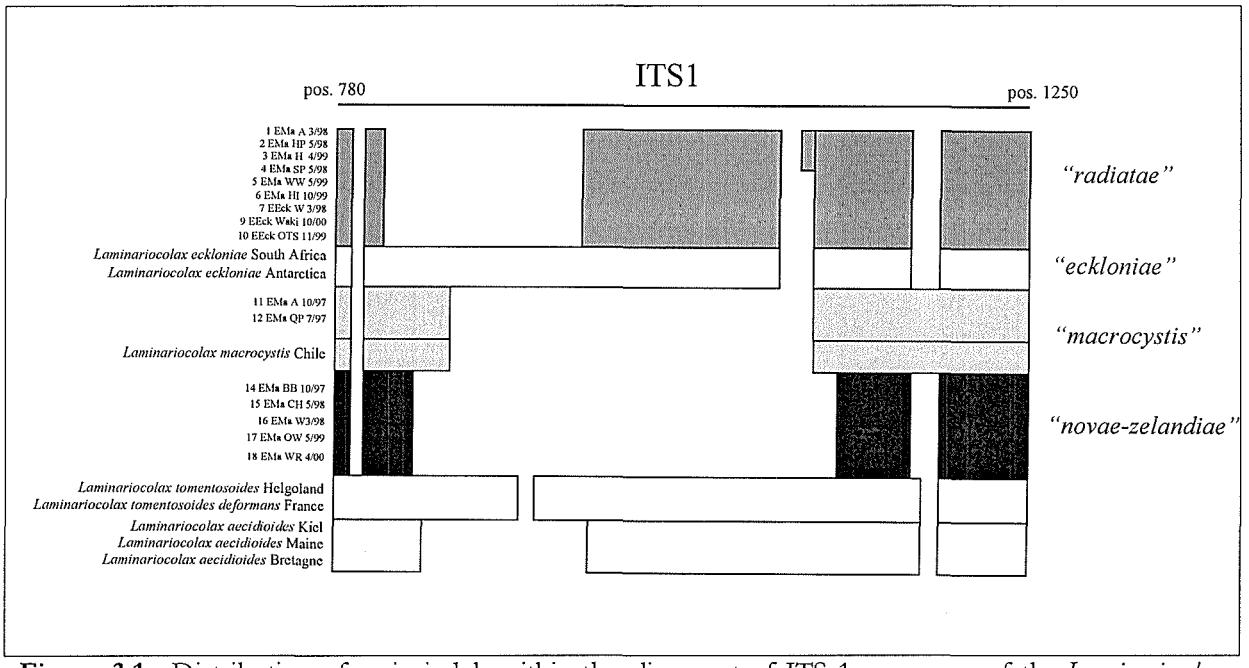


Figure 3.1: Distribution of main indels within the alignment of ITS 1 sequences of the *Laminariocolax* clade. Presented are positions 780-1250 of the 18S-ITS1-5.8S alignment of *Laminariocolax* species compared to *Laminariocolax* isolates from New Zealand (isolates no. 1-18, excl. no. 8 and 13). Boxed parts symbolise regions present in the ITS1 sequence. Identical or nearly identical sequences are hatched in the same way. Distances are not to scale.

To enable analyses of almost the complete ITS1 region, the outgroup species *Chordaria flagelliformis* was excluded from the data set. The parts of the alignment analysed comprised 795 bp (positions 559-1353 of the alignment; Appendix D 3.1). The average transition/transversion ratio of $ti/tv = 1.368$ indicate that saturation was not reached. The average base composition was A: 0.23202, C: 0.25223, G: 0.28039, and T: 0.23536.

3.1.3.2.3 *Microspongium* clade

For the analyses of the *Microspongium* clade, the re-coded ITS 1 alignment was reduced to six taxa, with three endophytes of *Pachymenia lusoria* from Aramoana/Otago, Brighton Beach/Otago and Owenga/Chatham Island (isolates no. 19, 21 and 27) representing the New Zealand isolates. To allow almost the complete ITS1 region to be analysed, outgroup species were again omitted from the data set. MP analyses were run twice, with gaps either treated as missing or as fifth base. The alignment comprised 276 bp (positions 559-691 and 1257-1400 of the alignment; Appendix D 3.1), with an average transition/transversion ratio of *ti/tv* = 1.10462 indicating that saturation was not reached. The average base composition was A: 0.26252, C: 0.28827, G: 0.26252, and T: 0.18670.

3.1.3.2.4 *Xiphophorocolax* clade

Internal Transcribed Spacer 1

For the analyses of this clade, the data set was reduced to the six *Xiphophorocolax* isolates, as the outgroup species *Chordaria flagelliformis* as well as other close relatives, for example *Dictyosiphon foeniculaceus*, were unalignable over the whole ITS1 region. MP analyses were again run twice, with gaps treated either as missing or as fifth base. The analysed region of the re-coded alignment had a length of 596 bp comprising the complete ITS1 region (positions 559-962 and 1257-1448 of the alignment; Appendix D 3.1), with an average transition/transversion ratio of *ti/tv* = 0.33308 indicating saturation of the alignment. The average base composition was A: 0.21634, C: 0.29340, G: 0.27304 and T: 0.21722.

Coding regions of the nrDNA and *rbc*L

To resolve the phylogenetic affinities of *Xiphophorocolax* gen. ined. within the brown algae, partial 26S sequences from a representative isolate of the *Xiphophorocolax* clade (isolate no. 29) as well as partial 26S and *rbc*L sequences of additional ectocarpalean taxa were included in a re-run of the second combined *Herpodiscus* data set (*rbc*L + LSU; see section 4.1.1.2.3; alignment in Appendix D 3.4). This analysis included *rbc*L data to stabilize branches between Ectocarpalean taxa, even though *rbc*L sequences were not determined for *Xiphophorocolax aotearoa*e. Omitting the 26S sequence of *X. aotearoa*e prior to the analysis did not contradict the results inferred from the combined data set (results not shown).

The data set comprised a total of 31 species (see distance matrix for taxa included; Appendix D, D 4.1.5 and D 4.1.6) over a length of 1866 bp (positions 1-1255 of the *rbc*L

alignment (alignment in Appendix D, D 3.4) and positions 42-653 of the 26S alignment (alignment in Appendix D, D 3.4). Average transition/transversion ratio of all included sequences was *ti/tv* = 1.07520 (including outgroup) and *ti/tv* = 1.10305 (without outgroup) indicated that saturation was not reached. The average base composition was A: 0.27312, C: 0.17954, G: 0.25089, and T: 0.29646.

3.1.3.3 Phylogenetic analyses

The properties of all alignments and the statistics for the most parsimonious trees are summarized in Table 3.4. In the following sections, "MP_m" will refer to maximum parsimony based on gaps treated as missing and "MP_{5th}" to maximum parsimony based on gaps treated as fifth base. Treating gaps as fifth base instead of as missing increased the number of informative characters in all MP analyses at least by 30%.

Table 3.4: Summary of properties of the endophyte alignments and of tree statistics for parsimony analyses. CI: consistency index; RI: retention index; RC: rescaled consistency index.

analysed gene regions	Internal Transcribed Spacer 1								*rbc*L and partial 26S
endophytes included	representatives of all endophyte clades		*Laminariocolax* clade		*Microspongium* clade		*Xiphophorocolax* clade		*Xiphophorocolax aotearoae* sp. ined.
gaps treated as	missing	fifth base	missing	fifth base	missing	fifth base	missing	fifth base	missing
No. of taxa/isolates	15	15	7	7	6	6	6	6	31
Length of alignment	187	187	795	795	276	276	596	596	1866
Variable positions	63 (33.7%)	82 (43.9%)	50 (6.3%)	92 (11.6%)	22 (8.0%)	33 (12.0%)	6 (1.0%)	11 (1.8%)	801 (42.9%)
Informative sites	45 (24.1%)	59 (31.6%)	21 (2.6%)	33 (4.2%)	2 (0.7%)	5 (1.8%)	2 (0.3%)	3 (0.5%)	554 (29.7%)
No. of most parsimonious trees	44	35	5	1	2	3	1	1	8
No. of trees within one step of MP trees	1100	184	15	8	19	11	6	11	20
Length of most parsimonious trees [steps]	87	125	53	103	23	35	6	5	2939
CI	0.920	0.896	0.981	0.942	1.0	0.971	1.0	1.0	0.426
RI	0.943	0.925	0.953	0.842	1.0	0.880	1.0	1.0	0.414
RC	0.867	0.829	0.937	0.793	1.0	0.777	1.0	1.0	0.198
Tree topology	not shown	Figure 3.2	not shown	Figure 3.4	not shown	Figure 3.5	not shown	Figure 3.6	Figure 3.7

3.1.3.3.1 All endophytes

Maximum parsimony analyses lead to 44 MP_m trees and 35 MP_{5th} trees, with a length of 87 and 125 steps, respectively. Treating gaps as fifth base resulted in a slightly better resolution of the relationships of the joined taxa.

In the MP_{5th} trees (one presented in Figure 3.2), the three clades of endophytes were strongly supported, with the *Microspongium* and the *Xiphophorocolax* clades both receiving 100%

bootstrap support (same in the MP_m tree). With 92% (MP_m: 90%) bootstrap support, the *Laminariocolax* clade was less stable: this node collapsed after additionally two steps in the MP analysis, while the other two clades persisted with a decay index of five.

The 35 MP_{5th} trees (and 44 MP_m trees, respectively) differed regarding the arrangement of nodes within the *Laminariocolax* and *Microspongium* clades: in the MP_{5th} trees, there was only moderate to low support for two branches in the *Laminariocolax* clade, one connecting *L. eckloniae* PETERS in BURKHARDT & PETERS 1998 and *L. macrocystis* (71%), the other one grouping the three New Zealand isolates (62%). In the MP_m analysis, only the node including all these five taxa received some support (62%). However, all branches within the *Laminariocolax* clade collapsed within one further step in both MP analyses. In the *Microspongium* clade, similarly unresolved affinities were observed: only one node was supported in the MP_{5th} tree, relating *M. radians* to the endophyte from *Pachymenia lusoria* from Owenga/Chatham Island (isolate no. 27; 61%), while in the MP_m tree a relationship of the isolate from *Pachymenia lusoria* from Aramona/Otago (isolate no. 19) with *M. tenuissimum* had low support (57%). However, in both analyses, these branches collapsed after one further step.

Both distance and maximum likelihood analyses resulted in the same over-all tree topology as the MP analyses, with all three clades receiving high bootstrap support (98–100%), except for the *Laminariocolax* clade in the ML tree which had only moderate support (76%). Affinities within the clades were again not well resolved: In the ML tree, for example, only two other nodes received a bootstrap support above 50%: *Microspongium alariae* was basal to the other *Microspongium* taxa, which formed a well supported clade (90%), while the separation of the southern hemisphere *Laminariocolax* taxa from the northern hemisphere species (*L. tomentosoides* and *L. aecidioides*) had only low support (60%).

In the neighbour-joining tree, however, these two *Laminariocolax* species formed a moderately supported clade (81%), while relationships between the other taxa were unresolved - New Zealand isolates were separated from *L. eckloniae* and *L. macrocystis* in only 53% of all most parsimonious trees. In the *Microspongium* clade, the endophyte from *Pachymenia lusoria* from Aramoana/Otago (isolate no. 19) formed a well supported clade (97%) with *M. tenuissimum*, similar to the MP_m consensus tree. The remaining taxa were resolved in the following order: [[[*M. alariae* (bootstrap support 60%)] isolate no. 27 (59%)] *M. radians*].

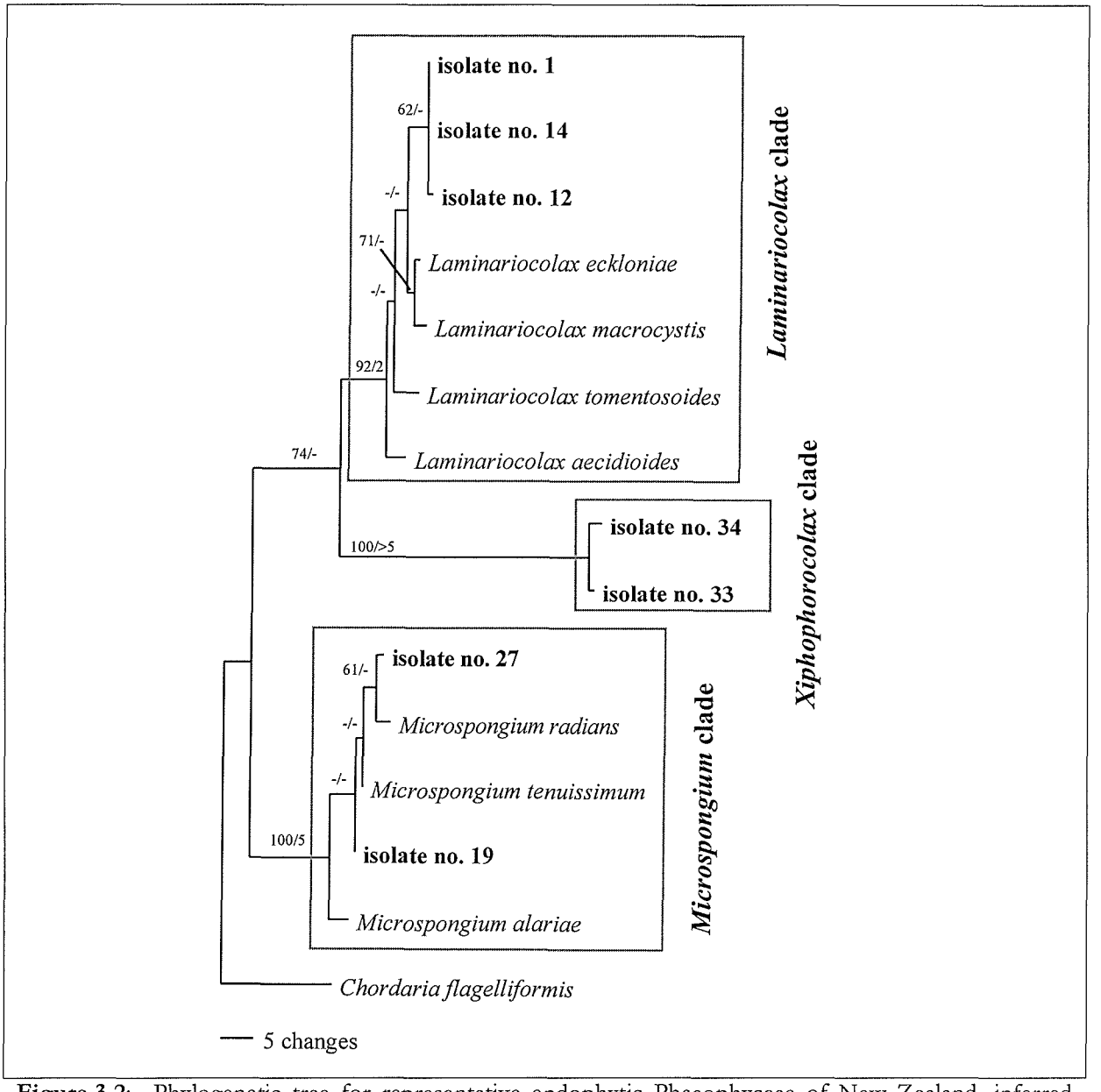


Figure 3.2: Phylogenetic tree for representative endophytic Phaeophyceae of New Zealand, inferred from ITS1 and adjacent partial 18S and 5.8S sequences. One of 35 most parsimonious trees in phylogram style, with gaps treated as fifth base (MP_{5th}). Numbers indicate bootstrap values (left) and decay indices (right). Dashes indicate that branches received a bootstrap support of 50% or less, or collapsed within one further step. Isolates from New Zealand are set in bold.

3.1.3.3.2 *Laminariocolax* clade

The genus-specific primer L'colax2(F) could be used as a fast method of identifying whether isolates belonged to the *Laminariocolax* clade or not. However, for sequence analyses, the ITS1 was amplified with the the forward primer AFP2(F) rather than with L'colax2(F) as a second site

was found in the ITS 1 of some of the isolates nearly identical with the primer site (1st site: positions 698-720, 2nd site: positions 970-988 of Alignment D 3.1, Appendix D).

All *Laminariocolax* isolates from New Zealand had almost identical ITS1 sequences. However, three different groups were distinguishable, varying markedly in the size of their ITS1 region (Table 3.5) due to a large indel (Figure 3.3). By the length of their ITS1 sequence, all isolates of the *Laminariocolax* clade could be identified as belonging to one of these three groups.

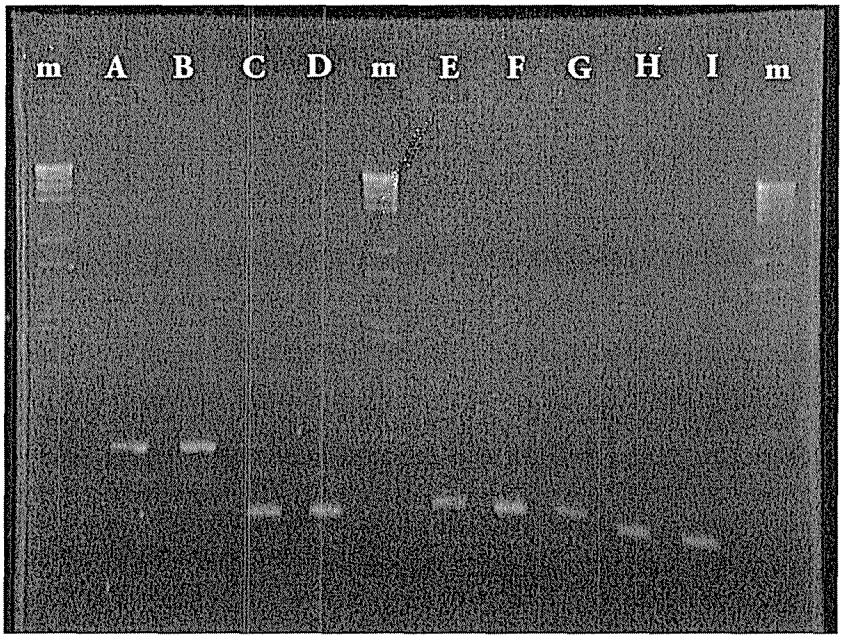


Figure 3.3: Size differences in the ITS1 region of varieties of *Laminariocolax macrocystis*: results of *L. macrocystis* from Chile (PETERS 1991; BURKHARDT & PETERS 1998) compared to representatives of the *L. macrocystis* isolates from New Zealand. Letters above lanes refer to the following groups and isolates, respectively: A-B: group L_1 (A: isolate no. 7; B: isolate no. 4); C-D, H-I: group L_3 (C: isolate no. 15; D: isolate no. 13; H: isolate no. 16; I: isolate no. 14); E: *L. macrocystis* from Chile; F-G: group L_2 (F: isolate no. 12; G: isolate no. 11); m: marker. Sequences were amplified with the primer pair L'colax2F-5.8S1R (specifications of the gel: 2% agarose gel (GEA, KEM Agarose, FUC, Rockland, USA); running time: 280 minutes at 36 mA). The "smiling" of the gel is an artifact.

The first group L_1 comprised isolates with an ITS1 region of a length of ca. 680 bp. Isolates belonging to this group were endophytes from *Ecklonia radiata* from Wellington (isolate no. 7), Doubtful Sound (Causet Cove: isolate no. 8; Outer Thompson Sound: isolate no. 10) and Waikouaiti/Otago (isolate no. 9), as well as endophytes from *Macrocystis pyrifera* from Waitangi West/Chatham Island (isolate no. 5), and from around the Otago Peninsula (Aramoana: isolate no. 1; Seal Point: isolate no. 4; Harington Point: isolates no. 2and 3; Hoopers Inlet: isolate no. 6).

Apart from a large indel (positions 800-933; Appendix D, Alignment D 3.1) and some additional smaller indels, all group L_1 sequences were very similar to the ITS1 sequence of *Laminariocolax eckloniae* from Antarctica (and South Africa), which is 789 bp in length. The ITS1 regions of the Northern Hemisphere species *L. tomentosoides* and *L. aecidioides*, in contrast, were of a similar length as the group L_1 isolates, but had different sequences.

Two isolates, endophytes from *Macrocystis pyrifera* from Aramoana (isolate no. 11) and from Quarantine Point (isolate no. 12), both Otago Harbour, had an almost identical ITS1 region with an even larger indel (positions 828-1157) than group L_1 isolates: The ITS1 regions from these isolates were ca. 174 bp shorter than those of most members of L_1 (507 bp and 511 bp, respectively; group L_2) and thus were of almost the same length as the ITS1 of *Laminariocolax macrocystis* from Chile. The two strains isolated from Aramoana mole from two consecutive years belonged to two different groups: the ITS1 of isolate no. 1 had a length of 679 bp, in contrast to isolate no. 11 with 507 bp. Apart from this length difference, the sequences were almost identical.

Table 3.5: Comparison of lengths of ITS1 region (excluding indels) of described endophytic brown algae with different clades of pigmented endophytes from New Zealand (shaded background). For a detailed list: see Table 3.2. For GenBank accession number of described species: see Appendix D, Table D 1.1.

genus	species or isolate group (present study), respectively	number of bp in the ITS1 region [bp]
Laminariocolax	*L. eckloniae*	789
	L. aecidioides	679
	L. tomentosoides	674
	L. macrocystis	503
	group L_1	677-684
	group L_2	507-511
	group L_3	476-481
Microspongium	*M. tenuissimum*	284
	M. radians	263
	M. alariae	304
	group M_1	288
	group M_2	258
Xiphophorocolax gen. nov.	group X_1	581-583
	group X_2	553

Isolates of group L_3 had the largest indel (positions 811-1163) and thus the shortest ITS1 sequences, between 476 and 481 bp. Endophytes belonging to this group were all isolates from *Macrocystis pyrifera*, from the Otago Coast (Pilots Beach: isolate no. 13; Brighton Beach: isolate

no. 14; off Cornish Head: isolate no. 15; Wellers Rock: isolate no. 18), from Wellington (isolate no. 16) and from Owenga, Chatham Island (isolate no. 17).

With only one isolate from each group included in the phylogenetic analyses, five MP_m trees (53 steps long) and a single MP_{5th} tree (score 103, Figure 3.4) were inferred from the re-coded data set. Two main clades were recovered in the maximum parsimony trees as well as neighbour-joining and maximum likelihood trees (not shown): the first comprised all Southern Hemisphere taxa and formed a sister clade to the second, which consisted of the two Northern Hemisphere species *L. tomentosoides* and *L. aecidioides*. The separation of both clades had a bootstrap support of 100% in the MP_{5th} and MP_m trees as well as the neighbour-joining and ML tree.

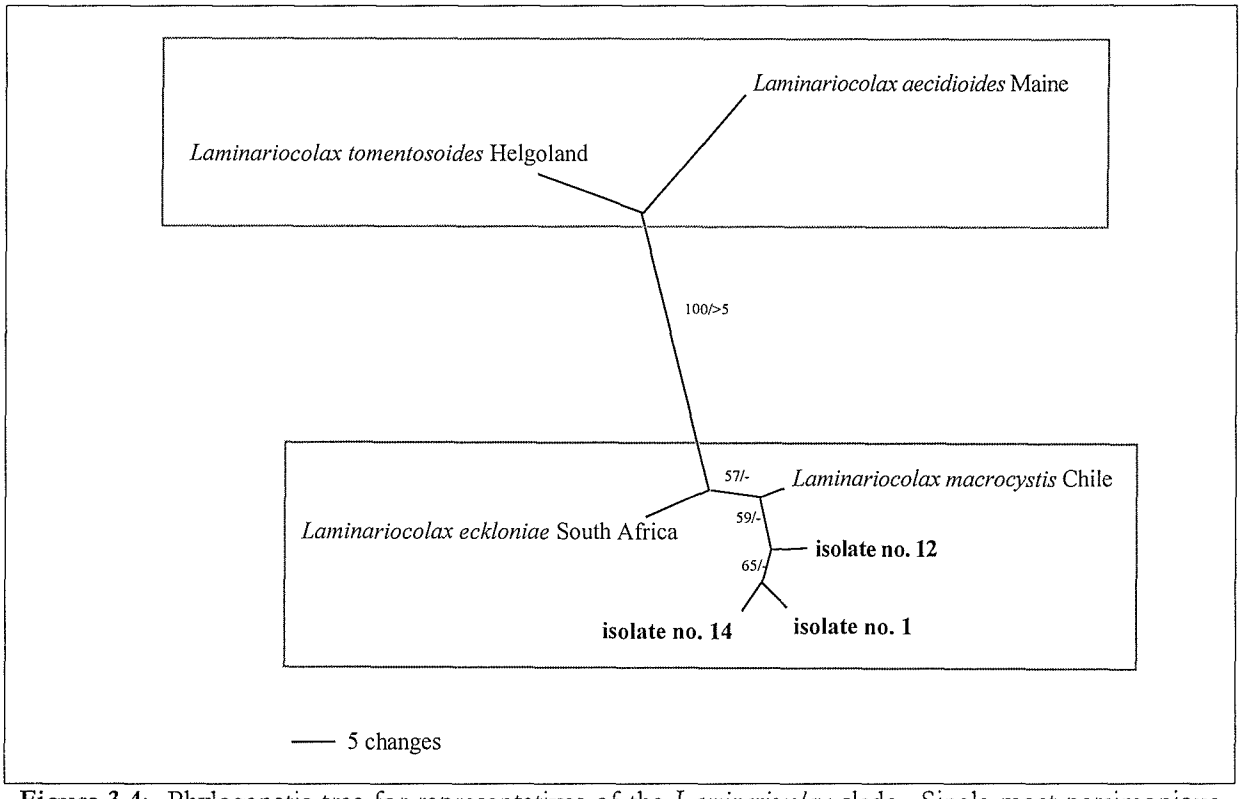


Figure 3.4: Phylogenetic tree for representatives of the *Laminariocolax* clade. Single most parsimonious tree in phylogram style (unrooted) inferred from ITS1 sequences, with gaps treated as fifth base. Numbers indicate bootstrap values (left) and decay indices (right). Dashes indicate that branches received a bootstrap support of 50% or less, or collapsed within one further step. Top box: Northern Hemisphere taxa; basal box: Southern Hemisphere taxa with New Zealand isolates (bold).

However, the affinities within the Southern Hemisphere clade were not completely resolved in any of the analyses. *L. eckloniae* was resolved as basal to the rest of this clade, but this node was either not well supported (MP_{5th}: 57%; D: 75%) or collapsed in the bootstrap tree (MP_m, ML

analyses). The close relationship of the endophyte from *Macrocystis pyrifera* from Quarantine Point/Otago Harbour (isolate no. 12, group L_2) with *L. macrocystis* received good support in most analyses (MP_m: 84%, NJ: 86%, ML: 85%), although this node did not appear in the MP_{5th} tree.

In conclusion, apart from the indels, sequences of the New Zealand isolates and other Southern Hemisphere species were very similar. Therefore, even with the gaps included in the analysis as fifth base, the differences in the lengths of the ITS1 region between the three groups of isolates did not lead to a marked separation in the phylogenetic trees.

3.1.3.3.3 *Microspongium* clade

This clade comprised isolates from New Zealand grouping with *Microspongium* species from both the Northern and Southern Hemisphere. As in the *Laminariocolax* clade, all New Zealand *Microspongium* isolates displayed nearly identical ITS1 sequences separated mainly by small indels.

Most isolates had an ITS1 region comprised of 288 bp that was almost identical to that of *Microspongium tenuissimum* (HAUCK) PETERS 2003. These strains belonged to group M_1 (Table 3.5): the endophytes isolates from the kelps *Undaria pinnatifida* from Wellington (isolate no. 25) and *Ecklonia radiata* from Karitane (isolate no. 26) as well as strains whose hosts were the red algae *Pachymenia lusoria* (from Aramoana/Otago: isolate no. 19; Riverton/Southland: isolate no. 20; Brighton Beach/Otago: isolate no. 21; Bradshaw Sound/Fiordland: isolate no. 22), *Grateloupia intestinalis* (from Greybrook: isolate no. 23) and an undesribed species of the family Kallymeniaceae (from Causet Cove, Doubtful Sound: isolate no. 24). The sequences of the isolates no. 19, 23 and 25 were identical over the whole length of the ITS1, as were isolates no. 20 and 22, or were isolates no. 24 and 26.

The last isolate of this clade, an endophyte from *Pachymenia lusoria* from Owenga/Chatham Island (isolate no. 27) was distinctly different from all other isolates and was therefore given its own designation M_2 (Table 3.5): it had an indel of 29 bp similar to that separating *Microspongium radians* (HOWE) PETERS 2003 from *M. tenuissimum* (positions 636-667; Appendix D, Alignment D 3.1) The sequence of *Microspongium alariae* (PEDERSEN) PETERS 2003 was slightly different from both *M. tenuissimum* and *M. radians*. No sequences absolutely identical to that of *M. alariae* were found among the isolates from New Zealand, even though this species shared some identical positions exclusively with the isolates no. 20-22.

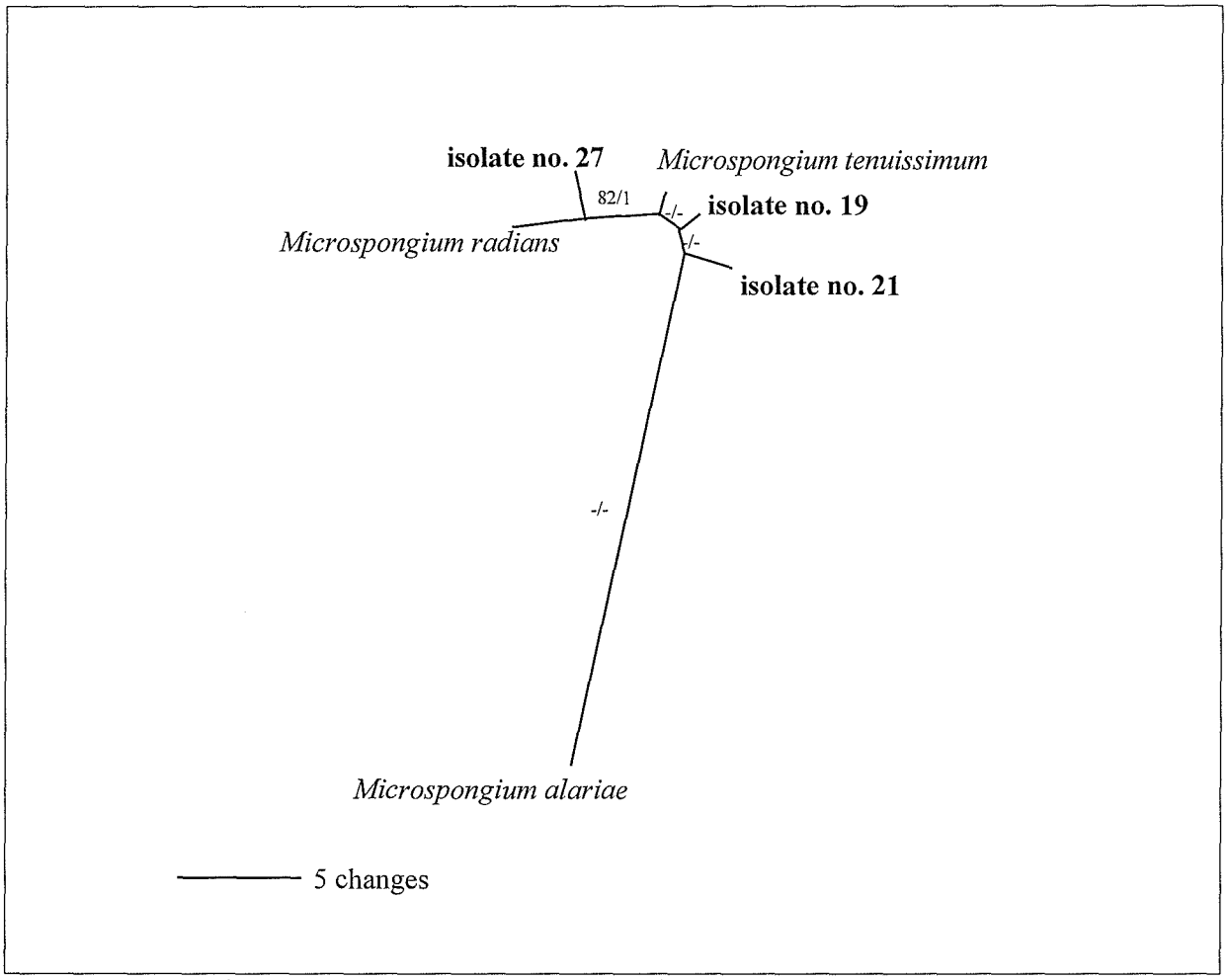


Figure 3.5: Phylogenetic tree for representatives of the *Microspongium* clade. One of 11 most parsimonious trees in phylogram style (unrooted) inferred from ITS1 sequences, with gaps treated as fifth base. Numbers indicate bootstrap values (left) and decay indices (right). Dashes indicate that branches received a bootstrap support of 50% or less, or collapsed within one further step. New Zealand isolates are set in bold.

Representatives of both groups (isolates no. 19, 21 and 27) were included in the phylogenetic analyses. Two MP_m and three $MP5_{th}$ trees (one shown in Figure 3.5) were inferred from the data set, with a length of 23 and 35 steps, respectively. Trees resulting from both MP analyses as well as the neighbour-joining and ML trees (not shown) had similar over-all topologies, with *Microspongium alariae* opposing a little resolved clade comprised of the rest of the taxa (*M. tenuissimum, M. radians* and the New Zealand isolates). In the MP_{5th} tree, only the grouping of *M. radians* with the isolate no. 27 was well supported (82%; MP_m tree: 55%), but within the next two steps, even this node collapsed. Overall, the resolution of all phylogenetic trees was poor, due to the very similar sequences of the taxa.

3.1.3.3.4 *Xiphophorocolax* clade

Internal Transcribed Spacer 1

The *Xiphophorocolax* clade comprised seven isolates, which all had almost identical ITS1 sequences. In six of the isolates, the ITS1 had a length of ca. 583 bp. Isolates belonging to this group (group X_1; Table 3.5) were the two endophytes of *Xiphophora gladiata* from Brighton Beach, Otago (isolate no. 30 (not included in the analyses) and no. 31), the three isolates from Chatham Island, from *Lessonia tholiformis* (isolates no. 28 and 29) and from *Marginariella urvilliana* (isolate no. 32) as well as the endophyte of *Durvillaea antarctica* from Brighton Beach/Otago (isolate no. 33). The latter differed from the other isolates at three positions (576, 631 and 1442; Alignment D 3.1 in Appendix D).

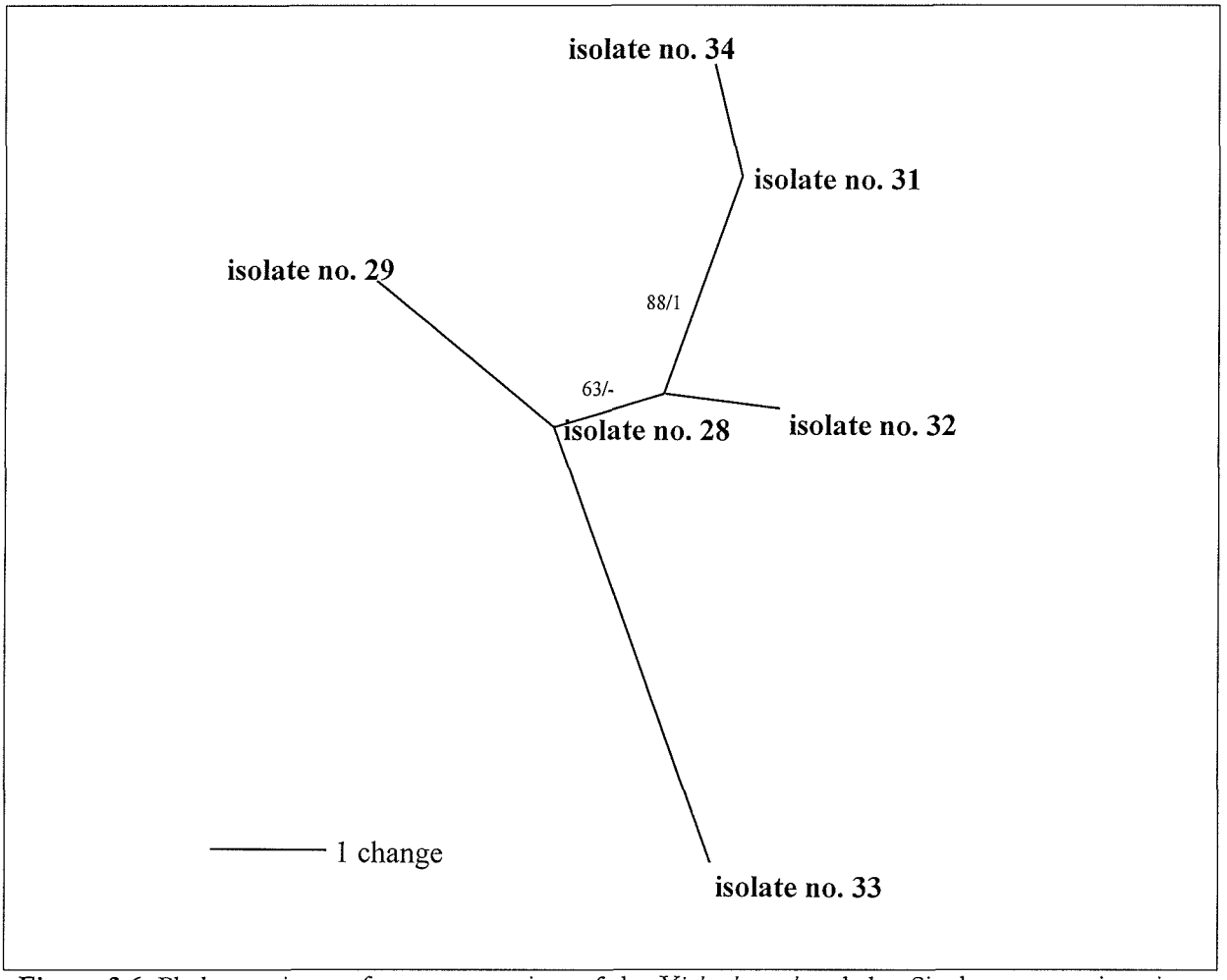


Figure 3.6: Phylogenetic tree for representatives of the *Xiphophorocolax* clade. Single most parsimonious tree in phylogram style (unrooted) inferred from ITS1 sequences, with gaps treated as fifth base. Numbers indicate bootstrap values (left) and decay indices (right). The dash indicates that the branch received a bootstrap support of 50% or less, or collapsed within one further step. All isolates are from New Zealand.

The ITS1 sequence of the endophyte of *Durvillaea willana* from Brighton Beach (isolate no. 34, group X_2; Table 3.5) was slightly shorter than all other sequences, with an indel comprising a total of 29 bp (positions 624-652; Alignment D 3.1 in Appendix D) unique to this isolate as well as a gap and a base change shared with the isolate(s) of *Xiphophora gladiata* (isolate no. 30 and 31, at positions 734 and 612 respectively).

With only three (MP_{5th}) and two (MP_m) informative sites (Table 3.4) available, due to the very similar sequences included in the data set, the resolution in both single MP trees (with scores of 11 and six steps, respectively) was very poor. The unrooted MP trees (one presented in Figure 3.6) had over-all topologies identical to the neighbour-joining and ML trees (not shown): A relationship of the endophyte of *Durvillaea willana* (isolate no. 34) with the isolate from *Xiphophora gladiata* from 1999 (isolate no. 31) had some support in all trees (MP_{5th}: 88% ; MP_m: 64%; NJ: 70%; ML: 61%), as well as the branch separating these two isolates and the endophyte of *Marginariella urvilliana* (isolate no. 32) from the other three isolates (MP_{5th}: 63% ; MP_m: 63%; NJ: 77%; ML: 63%). Distances were the largest between the endophyte isolated from *Durvillaea antarctica* (isolate no. 33) versus the rest of the *Xiphophorocolax* isolates, thus in all trees, this strain stood the furthest away from all others.

Coding regions of nrDNA and *rbc*L

The parsimony analysis of the combined *rbc*L +26S sequences including *Xiphophorocolax aotearoae* sp. ined. led to eight MP trees with a length of 2939 steps each (one of them presented in Figure 3.7). A partition homogeneity test revealed the two data sets to be congruent ($p = 0.474$; FARRIS *et al.* 1995; CUNNINGHAM 1997). Regarding the arrangement of the Phaeophycean orders in the base and the crown, the over-all topologies of MP consensus, neighbour-joining and ML trees were similar to those of the trees inferred from the 2nd combined data set in section 4.1.1.3.3.

Within the Ectocarpales, three clades were recovered. *Ectocarpus siliculosus* (DILLWYN) LYNGB. (Ectocarpaceae) formed a moderately supported clade (MP: 76%; NJ: 66%; ML: 76%) with *Scytosiphon lomentaria* (Scytosiphonaceae). The Adenocystacecae with *Adenocystis utricularis* (BORY) SKOTTSBERG and *Caepidium antarcticum* J. AGARDH was highly supported (MP: 99%; NJ: 92%; ML: 100%), as was the Chordariaceae (MP: 99%; ML: 98%; moderate support in NJ: 81%). *Pylaiella littoralis* (L.) KJELLM. as a member of the Acinetosporaceae took a position between Chordariaceae and the other two clades (all family affiliations according to PETERS & RAMIREZ 2001).

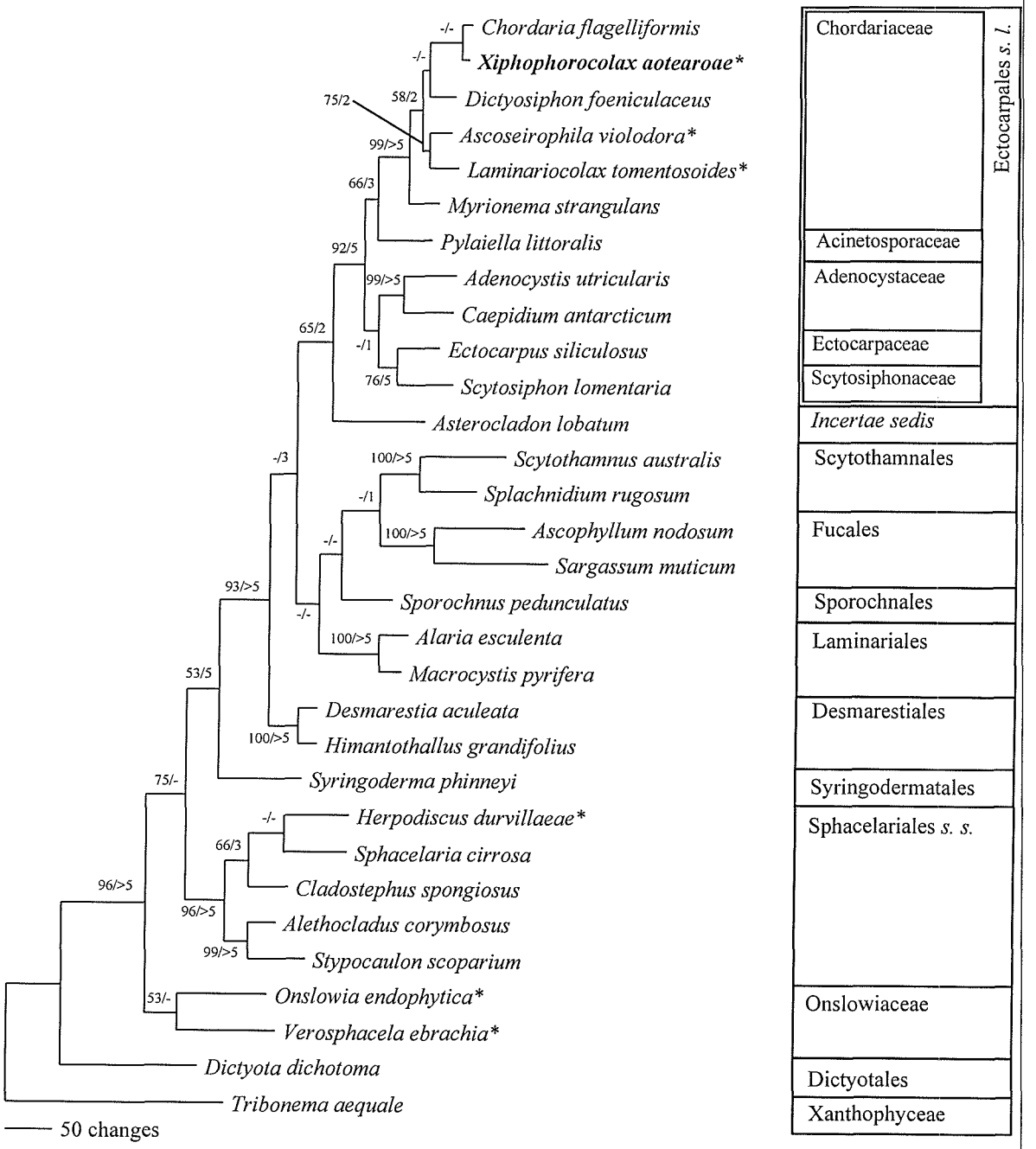


Figure 3.7: Phylogenetic tree for the Phaeophyceae including *Xiphophorocolax aotearoae*, based on combined *rbc*L and 26S data. One of eight most parsimonious trees in phylogram style. Numbers indicate bootstrap values (left) and decay indices (right). Dashes indicate that branches received a bootstrap support of 50% or less, or collapsed within one further step; an asterix indicates endophytic or epi-endophytic taxa. Family affiliations within the Ectocarpales *s. l.* are according to PETERS & RAMIREZ (2001).

The endophyte *Xiphophorocolax aotearoae* sp. ined. unambigously clustered with the various members of the Chordariaceae. However, apart from the moderately supported grouping of *Laminariocolax* with *Ascoseirophila* (MP: 75%; NJ: 80%; ML: 80%), branches within this clade did not receive any bootstrap support of ≥ 50%. Thus affinities between *Xiphophorocolax* and any of the other endopyhtic genera included, *Laminariocolax*, *Microspongium* or *Ascoseirophila*, or relationships with free-living members of the Chordariaceae remained unresolved.

3.2 Discussion

Pigmented endophytic Phaeophyceae were frequent among macroalgae of the New Zealand archipelago. Their distribution among host species and the specific symptoms associated with their presence, combined with the morphological characters that the isolated endophytes displayed in culture, allowed a gross classification into three groups. By DNA sequence comparisons with known endophytes from other parts of the world, these groups were identified as *Laminariocolax macrocystis* (PETERS) PETERS in BURKHARDT & PETERS 1998, *Microspongium tenuissimum* (HAUCK) PETERS 2003, and a previously unknown genus and species, *Xiphophorocolax aotearoae* gen. et sp. ined.. All three taxa constitute new records for the marine flora of the New Zealand archipelago, at genus and species level.

3.2.1 Quality of data

In small and cryptic organisms such as the endophytic brown algae, there is always an imminent danger of contamination of isolates and subsequent cultures and thus invalidation of results. However, the results discussed in the following sections are based not on single observations, but on repeated isolations of all three endophyte species minimising the problem of contamination. Thus the consistent morphological data and nearly identical DNA sequences among isolates confirm the status of each of the three groups. Differences within nearly identical ITS1 sequences among various isolates belonging to one species in the range of 4-5 base pairs (Appendix D, Tables D 4.1.2-4) may be PCR artifacts, as single and not pooled PCR reactions were used for the sequencing.

3.2.2 *Laminariocolax macrocystis*

3.2.2.1 The genus *Laminariocolax* KYLIN

All strains isolated from *Macrocystis pyrifera* as well as some of the isolates from *Ecklonia radiata* (isolates no. 1-19) belonged to the species *Laminariocolax macrocystis*. The genus *Laminariocolax* KYLIN is known from temperate coasts worldwide (BURKHARDT & PETERS 1998). Its members mainly infect kelps, such as *Laminaria* spp., *Undaria pinnatifida*, *Hedophyllum sessile* (ARESCHOUG) SETCHELL, *Ecklonia maxima* and *Macrocystis pyrifera*, but have also been reported from a member of the Desmarestiales, *Himanthothallus grandifolius* (GEPP) ZINOVA from Antarctica as well as from red algae, such as *Palmaria palmata* (L.) KUNTZE and *Grateloupia doryphora* (MONTAGNE) HOWE (SETCHELL & GARDNER 1922; YOSHIDA & AKIYAMA 1979; PETERS 1991; BURKHARDT & PETERS 1998; VILLALARD-BOHNSACK & HARLIN 2001; PETERS 2003).

The genus *Laminariocolax* was erected by KYLIN to accommodate a northern hemisphere species originally described by FARLOW (1889, cited in KYLIN 1947) as *Ectocarpus tomentosoides*. *L. tomentosoides* (FARLOW) KYLIN is characterised by erect filaments which are up to 1 cm long, entwined and carry short laterals. Its plurilocular sporangia are uniseriate, while unilocs have not been observed so far. *L. tomentosoides* is an epi-endophyte, with most of its thallus arising over the host surface, while *L. aecidioides* (ROSENVINGE) PETERS in BURKHARDT & PETERS 1998, another species of the northern hemisphere, grows endophytically, with only the fertile parts penetrating the host surface. In *Laminaria saccharina* (L.) LAMOUROUX, the endophyte forms characteristic lesions similar to the fruiting bodies of some pathogenic fungi, so-called aecidia. However, these aecidium-like structures are not observed in all infected host specimens. Erect filaments are absent in this species, however, unlike the type species, it forms phaeophycean hairs (ROSENVINGE 1893; PETERS 1991).

From the southern hemisphere, only the two species *Laminariocolax macrocystis* and *L. eckloniae* PETERS are known (PETERS 1991; BURKHARDT & PETERS 1998). *L. macrocystis*, an endophyte growing in *Macrocystis pyrifera* from Chile, South America, was originally described as *Streblonema macrocystis* PETERS. Molecular systematic studies (BURKHARDT & PETERS 1998) revealed this endophyte to be closely related to *L. tomentosoides* and *L. aecidioides*. Moreover, the uniseriate plurilocular sporangia found in *S. macrocystis* disagreed with the pluriseriate and branched sporangia described for the type species of the genus *Streblonema*, *S. volubilis* (PRINGSHEIM 1863). As the structure of the sporangia is considered a conservative trait, this species was moved to the genus *Laminariocolax* (BURKHARDT & PETERS 1998).

Additionally, PETERS described another endophyte, *L. eckloniae* PETERS in BURKHARDT & PETERS 1998, isolated from *Ecklonia maxima* (OSBECK) PAPENFUSS in South Africa. According to molecular systematics, this species is closely related to *L. macrocystis*. Both species share nearly identical ITS1 sequences, which differ mainly in their length, due to a large indel (positions 828-1157 of Alignment D 3.1, Appendix D). The ITS1 of *L. macrocystis* is much shorter, comprising only 501 bp, compared to 789 bp in *L. eckloniae*.

Morphologically, *L. eckloniae* and *L. macrocystis* are also similar, apart from a character used to distinguish the two species: In contrast to *L. macrocystis*, South African *L. eckloniae* failed to form true phaeophycean hairs in culture (BURKHARDT & PETERS 1998; PETERS 1991). The absence vs. presence of hairs in algae may constitute a good character but may in some cases depend on the nutritional status of cultures. In red algae, for example, hairs are considered to be produced in nutrient-poor environment as a means to increase the absorptive surface area of the thallus (KAIN & NORTON 1990). Also for brown algae, the formation of hairs can be stimulated by cultivation without sufficient nutrient supply (PEDERSEN 1981a). In the holotype material of *L. tomentosoides*, true hairs are absent, but they have been observed in the sub-species, *L. tomentosoides deformans* (PETERS 2003). Indeed, PETERS recently found hairs in another *L. eckloniae* strain, which had grown endophytically in Antarctic *Himantothallus grandifolius* (GEPP) ZINOVA, a member of the Desmarestiales (PETERS 2003). Thus the only morphological character to distinguish South African *L. eckloniae* from Chilean *L. macrocystis* is problematical. And even though both species are separated geographically and are distinct in their ITS1 sequences, the New Zealand isolates literally fill these gaps, geographically as well as genetically.

3.2.2.2 *L. macrocystis* in New Zealand

The ITS1 sequences of the New Zealand *Laminariocolax* isolates were nearly identical to those of *L. macrocystis* and *L. eckloniae*, but included three length variations of the large indel leading to their separation into three groups (groups L_1-L_3), which were classified accordingly: The strains of group L_1 (isolates no. 1-10) presented ITS1 sequences with a length between *L. eckloniae* and *L. macrocystis* sequences (677-679 bp). These strains are referred to as *'radiatae'* isolates, as this group contained all the *Laminariocolax* strains isolated from *Ecklonia radiata*, apart from some isolates from *Macrocystis pyrifera*. The two isolates from *Macrocystis* populations from the Otago Harbour of group L_2 (isolates no. 11 and 12) shared identical ITS1 sequences including the length of the large indel with *L. macrocystis* and are henceforth referred to as *'macrocystis'* isolates. The third group of isolates (isolates no. 13-18, group L_3), found only in *M. pyrifera*, had an even

shorter ITS1 sequence (circa 481 bp) than *L. macrocystis* and the *'macrocystis'* isolates. To acknowledge the fact that this short sequence has so far only been observed in strains from New Zealand, members of group L_3 are referred to as *'novae-zelandiae'* isolates.

Morphologically, the New Zealand *Laminariocolax* isolates agreed well with the descriptions given for *L. macrocystis* and *L. eckloniae*, respectively (PETERS 1991; BURKHARDT & PETERS 1998). For example, the average cell and sporangia sizes measured in the isolates of all three groups did not show significant differences, but were all in the range of sizes given for *L. macrocystis* sporophytes (PETERS 1991). *L. macrocystis* is known to have slightly heteromorphic generations, with gametophytes displaying smaller cells, but longer plurilocular sporangia, compared to the sporophyte (PETERS 1991). In one of the strains (isolate no. 1; Table 3.2), however, sporangia were of the length given for gametophytes, while the vegetative cells were even wider than described for sporophyte cells. Whether this culture or any of the others contained sporophytes or gametophytes, could not be determined from the morphological data, as unilocular sporangia were not observed in any of the strains, either. PETERS (1991), who also did not find any unilocular sporangia in his sporophyte cultures from Chile, suggested that their formation might depend on host-endophyte interactions. Erect filaments, which are known from both sporo- and gametophytes (PETERS 1991), appeared only in the strains of the *'radiatae'* group, while phaeophycean hairs were displayed by isolates of all three groups.

3.2.2.3 Taxonomic consequences

With the New Zealand isolates 'filling the gaps', a separation between *L. macrocystis* and *L. eckloniae* based on molecular genetics alone seems futile. Indeed, when indels are excluded from phylogenetic analyses, both taxa have more than 99% of positions of the entire ITS1 in common. Levels of inter- and intra-specific variations of ITS (i. e. ITS1+ITS2) sequences vary among and even within eukaryote groups, therefore they cannot be used uniformly for all organisms to define species boundaries. In the Compositae, for example, ITS sequence divergences between species show values from 0.4% up to 15-18%, depending on the respective genus (BALDWIN 1992). In red algae, 0-4% of intra-specific and 0.35-31% of inter-specific variations have been recorded (GOFF *et al.* 1994; VAN OPPEN *et al.* 1995; CHOPIN *et al.* 1996), while in green algae, sequences divergences even within species range from 0.5% to 21%, with the latter observed in inter-oceanic populations of *Cladophora albida* (HUDSON) KÜTZING (VAN OPPEN *et al.* 1993; BAKKER *et al.* 1992). Other groups, e. g. fungi also show wide ranges of intra-specific variations: In the oomycete *Phytophthora*, variations in the ITS can be low or

undetectable within species, while in the ascomycete *Fusarium sambucinum*, intra-specific divergence reach values of up to 15% (LEE & TAYLOR 1991; O'DONNELL 1992).

In the brown algae, intra-specific variations in the ITS are similarly variable. The species complex *Desmarestia viridis/willii*, for example, shows very low sequences divergences, i. e. a single base change in ITS2 (VAN OPPEN *et al.* 1993), while in *Macrocystis pyrifera* variations in ITS1 can reach up to 6.8% between individuals from geographically different populations, and up to 4.6% were observed between clones from the same individual (COYER *et al.* 2001). In *Ectocarpus* species, populations from sites around the world display not only considerable sequence variations, but also different genome sizes and biochemical characters, resulting in interfertility barriers. With distinguishing morphological characters lacking, these genetically distinct races are nevertheless regarded as belonging to the same taxonomic species, i. e. indels are not recognized as species-specific, but as intra-specific variations (STACHE-CRAIN *et al.* 1997).

Consequently, *L. macrocystis* and *L. eckloniae*, including the New Zealand isolates, should be regarded as a single taxonomic species, under the name *Laminariocolax macrocystis* PETERS in BURKHARDT & PETERS 1998, as *Streblonema macrocystis* PETERS 1991 has priority over *L. eckloniae* PETERS in BURKHARDT & PETERS 1998. Whether they form a biological species as well, i. e. whether they share a gene pool common enough to produce fertile off-spring (MAYR 2000), would have to be tested in cross-breeding experiments between sexually reproducing strains of all entities.

With three genetically different groups of *L. macrocystis* present, New Zealand superficially appears to be a "hot-spot" of endophyte evolution. However, within New Zealand, no geographical distribution pattern was detectable among the three groups of isolates. Moreover, the most small-scaled variations were observed in the Otago region, in an area that was also most frequently visited (see distribution map, Appendix B, Figure B 2.1). At the entrance of Otago Harbour, for example, members of all three groups were detected within a few kilometers: On the southern shore of the harbour, at Harington Point, *'radiatae'* was isolated twice, while the host population at Wellers Rock, southwest of Harington Point, hosted *'novae-zelandiae'*. Another *'novae-zelandiae'* strain was found in a drifting *Macrocystis* specimen at Pilots Beach, east of Harington Point. Finally, at the Aramoana Mole, both *'macrocystis'* and *'radiatae'* strains were isolated from the same *Macrocystis* population within five months. Thus, the detection of this high level of intra-specific variation among *L. macrocystis* populations within the New Zealand archipelago is most likely the result of repeated isolations and sequence comparisons.

As members of the different groups of New Zealand isolates were neither morphologically, geographically nor temporally separated from each other, all entities should therefore be regarded as cryptic genetic varieties within the *L. macrocystis* complex, rather than as sub-species (SCHUBERT & WAGNER 1993). Thus, the members of groups L_1 and L_3 would be distinguished from Chilean *L. macrocystis* (including the *'macrocystis'* isolates, group L_2) as *L. macrocystis* var. *radiatae* var. ined. (group L_1) and *L. macrocystis* var. *novae-zelandiae* var. ined. (group L_3). Consequently, this complex should also include *L. eckloniae* as another genetic variety, *L. macrocystis* var. *eckloniae* comb. ined..

3.2.2.4 Biogeographical considerations

In contrast to New Zealand, only single isolates of *L. macrocystis* were sequenced from Chile and South Africa. Additional sampling in these areas may reveal more genetic variation as well, i. e. show whether the varieties observed in New Zealand are also present. This may provide further information about the origin of the genetic variations, whether they are specific to New Zealand as by-product of a speciation-in-progress, or whether they are the result of gene flow and hybridisation among populations from the whole Southern Hemisphere.

Members of the genus *Macrocystis* have a wide distribution around the Southern Hemisphere (cited in RICKER 1987; cited in NORTH 1994; COYER *et al.* 2001). Like other large buoyant brown algae, they are used by a variety of epiphytic organisms not only as a habitat, but also as means of transport. So-called 'kelp rafts', floating masses of detached brown algae travelling in open ocean currents, are considered a source for long-distance dispersal not only for the brown algae themself, but also for the associated epiphytes such as red algae and many invertebrates (HOMMERSAND 1986; EDGAR 1987; HELMUTH *et al.* 1994; HOBDAY 2000a, 2000b). Similarly, infected host thalli may allow the distribution and thus gene flow for endophytic algae such as *L. macrocystis*.

In the genus *Macrocystis*, four species are currently recognized (NORTH 1994). However, a lack of genetic diversity among populations from various sites around the Southern Hemisphere suggests the presence of only a single species, *Macrocystis pyrifera* (COYER *et al.* 2001). Moreover, identical ITS1 sequences in populations from Chile, South Africa, Marion Island and New Zealand suggest that this species has been distributed quite recently, or that a gene flow may still be ongoing between South America, South Africa and the southeastern Pacific (COYER *et al.* 2001).

The West Wind Drift, carrying Sub-antarctic Surface Water eastward along the Antarctic Convergence, is considered to be one of the main pathways for long-distance dispersal of seaweeds in the Southern Ocean (HOMMERSAND 1986; JOHN *et al.* 1994; SMITH 2002). Within its path lay numerous islands, which are populated by *M. pyrifera*, such as the Falkland Is., Gough Is., Îles Crozet, Îles Kerguelen or Macquarie Is., including the subantarctic islands south and southeast of New Zealand, Auckland Is., Campbell Is., Antipodes Is. and Bounty Is.. *Macrocystis* populations are moreover found south of the Antarctic Convergence, on South Georgia and South Sandwich Is., as well as north of the Sub-tropical Convergence, on Tristan da Cunha and St. Paul (HAY *et al.* 1985; RICKER 1987; cited in NORTH 1994). A survey on these putative host populations for *L. macrocystis* may provide information about a tentative gene flow between endophyte populations in the Southern Ocean. It is noteworthy, that *Macrocystis* thalli from Cape Town, South Africa, share identical ITS1 sequences with populations from sites within the West Wind Drift, even though, generally, the marine flora of the western coast of South Africa is considered somewhat distinct from most other marine floras of the Southern Ocean, due to its separation by the Subtropical Convergence (HOMMERSAND 1986; NELSON 1994; HOMMERSAND & FREDERICQ 2002). Nevertheless, Sub-antarctic Surface Water may occasionally reach the western coast of South Africa (DIETRICH *et al.* 1975). New Zealand is also located north of this front, but here the Subtropical Convergence (as the Southland Front) surrounds the South Island relatively close to the coast following the continental shelf, where waters carried in the Southland Current may be mixed with the Sub-antarctic Surface Water (HEATH 1985). Thus, kelp rafts transported in the West Wind Drift may reach the coasts of Stewart Island and South Island.

Although South Africa and Antarctica seem to be geographically separated by the two circumantarctic convergences, the *L macrocystis* isolates from both regions share nearly identical ITS1 sequences indicating the presence of some genetic flow between them, probably via natural dispersal mechanisms, such as drift, or by man. Furthermore, their long ITS1 sequences distinguish them from isolates from Chile or New Zealand, suggesting that the origin of Southern Hemisphere *Laminariocolax* may be found in South Africa or Antarctica. However, additional isolates from other Southern Hemisphere populations will be required to clarify this question.

Additional sampling in search for cryptic genetic varieties of *L. macrocystis* should also include other potential host species to provide information about the range of possible hosts of *L. macrocystis*. In Antarctica, for example, it was found in a member of the Desmarestiales. In

South Africa, in contrast, *L. macrocystis* has so far only been isolated from *Ecklonia maxima*, but not from *M. pyrifera* (BURKHARDT & PETERS 1998; PETERS 2003; A. F. PETERS, personal communication), even though it is possible that *Macrocystis* populations in South Africa are infected as well. In New Zealand, *L. macrocystis* has only been observed in the two kelp species *M. pyrifera* and *E. radiata*. While *M. pyrifera* hosted all three varieties of *L. macrocystis*, only var. *radiatae* was isolated from *E. radiata*. Whether this is the result of some level of host specifity among the varieties, or whether this is due to insufficient numbers of isolates from *Ecklonia*, requires further sampling to answer.

3.2.2.5 Infection symptoms associated with *L. macrocystis*

Host populations in Chile, South Africa and New Zealand showed differences in the infection by *L. macrocystis*, especially in the expression of macroscopic infection symptoms. The presence of conspicuous protuberances from the host surface, so-called galls, associated with the endophytes, separate New Zealand *L. macrocystis* from strains isolated in Chile and South Africa.

At the type locality of *L. macrocystis* in Chile, the thalli of *Macrocystis pyrifera* display infections mainly in their sporophylls, but also in the basal parts of the stipe, representing different habitats occupied by the heteromorphic generations of *L. macrocystis* in the field: The gametophyte grows epi-endophytically on the basal part of the host, while the sporophyte grows endophytically in the host sporophylls (PETERS 1991). Here, the endophyte can be detected macroscopically by dark patches which may include the whole lamina and the adjacent stipes. Infected cauloids can be twisted and, like the sporophylls, appear less flexible, with a rough surface. However, conspicuous protuberances from the host surface were not observed in Chilean *Macrocystis* infected with *L. macrocystis* (PETERS 1991). In South African *Ecklonia maxima*, *L. macrocystis* var. *eckloniae*, occurs mainly in the lamina and is associated with dark spots, but no galls were observed (BURKHARDT & PETERS 1998).

In New Zealand, while in some individuals of *M. pyrifera* hosting *L. macrocystis* an infection could only be detected microscopically, most thalli showed macroscopically visible symptoms, i. e. at least a rough dark surface in the infected area, similar to Chilean *Macrocystis* hosting *L. macrocystis*. However, the New Zealand thalli frequently displayed conspicuous galls and distortions. Moreover, these galls were almost always restricted to the cauloids of *M. pyrifera*, while infections were rarely observed on phylloids including pneumatocysts and never in sporophylls. In *E. radiata* from New Zealand, the presence of the endophytes was often

associated with morphological alterations comparable to those in *Macrocystis*, e. g. rough, dark surfaces, and in severely affected specimens, galls and thallus distortions.

Additionally, most New Zealand isolates appeared to reproduce asexually: Upon microscopic examination of field material prior to isolation, plurilocular sporangia were observed, but not unilocular ones. Moreover, in the field, endophytes lacked epiphytic or projecting filaments, which are characteristic for Chilean gametophytes.

Even though they are absent in South American and South African host thalli, galls similar to those observed on *M. pyrifera* and *E. radiata* from New Zealand are also reported from kelps infected with pigmented endophytic Phaeophyceae growing along the North American west coast: *M. integrifolia* BORY, *Nereocystis luetkeana* and *Laminaria* spp. (ANDREWS 1976, 1977; APT 1988a, 1988b). In *Nereocystis*, protrusions from the surface of the cauloid form ridges running spirally around the stipe (APT 1988a, 1988b). A similar spiral arrangement of galls following nutation of the cauloid was detected in some *M. pyrifera* individuals from New Zealand (for example, see Plate 3.1, Figure F), suggesting that the infection took place in the apical part of the developing frond before nutation and internode elongation started.

The endophyte associated with the galls in *Nereocystis* was classified as *Streblonema* sp., but information regarding the structure of its plurilocular sporangia (i. e. pluriseriate vs. uniseriate) are lacking. The phylogenetic relationship of this endophyte is not known, i. e. whether it belongs to the genus *Streblonema*, or is more closely related to *L. macrocystis*. In re-infection studies, the endophyte was identified as the causal agent of the gall formation: In bialgal cultures with endophytes, host sporophytes developed galls similar to those observed in the field. Endophytes isolated from these galls had a morphology similar to those isolated from the field, thus KOCH's postulates for proving causality in diseases (ANDREWS 1977; ANDREWS & GOFF 1985) were fulfilled (APT 1988a).

Until recently, it was unknown how the endophyte infection spreads between host individuals. Vegetative endophyte filaments were suggested as the infective agent, possibly entering the host thallus via wounds (APT 1988a; ROUND 1991). However, SEM studies on bialgal cultures of the kelp *Laminaria saccharina* with its natural endophytes *Laminariocolax aecidioides* or *Laminarionema elsbetiae* showed that in these endophyte species, zoids from plurilocular sporangia act as the infection agents. Upon germination, germination tubes developing from settled spores penetrate the intact surface of their host thallus, apparently using an enzymatic rather than a mechanical penetration mechanism (HEESCH & PETERS 1999). The biochemistry of this initial stage of development of the host-endophyte relationship has been

studied in the association of *Laminaria digitata* with *Laminariocolax tomentosoides*, where a complex cascade of action vs. reaction between host and endophyte is observed (KÜPPER *et al.* 2001, 2002): normally, degradation products of the host cell wall (oligo-guluronates), which may be released during a putative enzymatic digestion by an attacking pathogen, act as elicitors that trigger an active defense reaction in *Laminaria*, i. e. the host surface cells release activated oxygen species cytotoxic to pathogens. *Laminariocolax tomentosoides*, however, is capable of quenching this oxidative burst and this may be the reason why it can successfully infect its host. Elicitation with synthetic oligo-guluronates, on the other hand, leads to some kind of immunity in the host alga against *Laminariocolax* building up slowly and lasting up to eight days. Apparently, only surface cells of the host are involved in the oxidative burst, as the first line of defence in an endophyte attack (KÜPPER *et al.* 2002).

However, most of what happens, once the endophyte has successfully entered the host, is unknown, i. e. the further development of the symbiosis on a cellular and sub-cellular level. On the surface, the host cells are able to recognize endophyte spores, so is a similar "communication" (i. e. a system of chemical action and reaction) established between both species with the endophyte inside? And what leads to the formation of galls in the host tissue, i. e. how do the endophytes manage to disrupt ordered cell divisions within the host tissue?

So far only the morphology of the galls caused by endophytic Phaeophyceae has been studied. In *Nereocystis*, meristoderm and cortex of the cauloid are involved in the formation of galls, which are the result of hyperplasia (proliferation) rather than hypertrophy (enlargement) of the cells (APT 1988a, 1988b). A similar process is assumed for the galls displayed by *Macrocystis* from New Zealand. Large cavities, such as those associated with galls in *Macrocystis*, were not observed in *Nereocystis* galls, though. In *M. pyrifera*, these cavities possibly have developed from enlarged mucilage ducts. However, the way they were formed remains unclear.

In higher plants, bacteria or virus infections may lead to a transfer of genetic material (i. e. plasmids) from the pathogen into host cells, resulting in amorphous, undifferentiated heterokaryotic tissue with unlimited growth, i. e. tumours (BEIDERBECK 1977). In contrast, plant galls in a strict sense comprise localised anomalies consisting of differentiated tissue with limited growth, which may develop under the influence of substances acting as growth regulators, e. g. phytohormone-like substances released by pathogens such as insect larvae using the gall as a sheltered habitat (STÖCKER & DIETRICH 1986). Galls in marine macroalgae are associated with a variety of organisms, e. g. fungi, nematodes, copepods or other algae, but can also be caused by bacteria or pollution (AGUILERA *et al.* 1988; review in APT 1988b). In some

red algae parasitised by adelphoparasites, callus-like growth has been observed, e. g. in *Sarcodiotheca gaudichaudii* (ABBOTT & HOLLENBERG) GABIELSON infected by *Gardneriella tuberifera* KYLIN (GOFF & ZUCCARELLO 1994). Host-parasite relationships are well studied in the red algae (e. g. GOFF 1982; GOFF & COLEMAN 1984, 1985, 1995; GOFF & ZUCCARELLO 1994; GOFF *et al.* 1994, 1996). Most parasitic red algae transfer genetic material into cells of their host via specialized plasmodesmata, so-called secondary pit connections. The transformed host cells may grow into masses of heterokaryotic cells forming the parasite thallus (GOFF & COLEMAN 1984, 1985, 1995; GOFF & ZUCCARELLO 1994). *Gardneriella* moreover induces the host cells to proliferate and form a callus prior to the transfer of genetic material (GOFF & ZUCCARELLO 1994). Likewise, callus growth has been observed in a related species, *Agardhiella subulata*, treated with substances regulating the growth in higher plants (BRADLEY & CHENEY 1990), suggesting that the parasite *Gardneriella* may also release substances acting as growth regulators.

In the pigmented endophytic brown algae, a transfer of genetic material into the host cells resulting in heterokaryotic gall tissue seems unlikely, as neither secondary plasmodesmata nor any other means of invasion into host cells, e. g. haustoria, have been observed between endophytes and host cells, even though secondary infections with potentially tumour-causing agents such as bacteria and viruses cannot be ruled out. Instead, it appears to be more likely that, similar to higher plants or in the *Gardneriella-Sarcodiotheca* symbiosis, in infections by endophytic brown algae the formation of galls may be triggered by the presence of phytohormone-like substances. Substances acting as phytohormones are not only known stimulate reactions in red algae (BRADLEY & CHENEY 1990), but also in Phaeophyceae. For example, cytokinin-like substances have been reported to enhance growth in *M. pyrifera* (DE NYS *et al.* 1990, 1991).

Such substances could be released by the endophytes, however, a putative increase of hormone-like substances in the host tissue could also be a reaction of the host itself, e. g. as an attempt to encapsulate and thus isolate the intruder. Nevertheless, the physiology of the symbiosis between pigmented brown alge and their hosts has not been studied beyond the first stage of infection, and it is not known whether any substances acting as growth regulators are involved. As the presence of endophytes is not always associated with galls in the host, their formation may, for example, depend on the number of endophyte filaments involved.

3.2.2.6 Prevalence of infection in *M. pyrifera* from New Zealand

Laminariocolax macrocystis was the only endophyte isolated from *Macrocystis pyrifera* during the present study. This endophyte appears to be wide-spread within the New Zealand archipelago, as all populations of *Macrocystis* examined for the presence of endophytes contained infected host specimens. In the three populations on the Otago coast quantitatively examined for infections with *L. macrocystis*, endophytes were present throughout the year. In the two populations inside and at the entrance of Otago Harbour, Quarantine Point and Aramoana, prevalences were high, with 95-99% (97-100% in 2000) of all thalli collected hosting endophytes. In the off-shore population at Cornish Head, in contrast, only one third of all thalli examined hosted endophytes. The values found in the Harbour populations are consistent with high infection rates observed in kelp populations from other parts of the world, e. g. British Columbia or North West Europe (ANDREWS 1977; LEIN *et al.* 1991; PETERS & SCHAFFELKE 1996; ELLERTSDÓTTIR & PETERS 1997). In *M. pyrifera* from Chile, the frequency of infection with *L. macrocystis* was estimated from the time required to find specimens with macroscopic symptoms, distinguishing among a 'rare', 'frequent' or 'very frequent' occurrence of endophytes (PETERS 1991). However, further quantitative data from the Southern Hemisphere are lacking, not only from Chile, but also from *L. macrocystis* infecting South African *Ecklonia maxima*.

In Chile, infection symptoms of *M. pyrifera* such as dark patches on the sporophylls were present throughout the year, but were most frequently found in autumn, winter and early spring (PETERS 1991). Similarly, ELLERTSDÓTTIR & PETERS (1997) found a weak seasonality in the infection rates of *Laminaria* spp. from the German Bight, with a drop in late spring. In the New Zealand populations, in contrast, seasonal influences on the prevalence of infection or the severity of symptoms, if detectable, were statistically insignificant. However, the study of ELLERTSDÓTTIR & PETERS (1997) had three times the number of thalli examined than in the present study. In the present study, a higher number of samples would be required to statistically support any apparent seasonal differences at the three sites. Larger sub-samples would also be preferable to avoid the factor 'sub-sample' becoming significant in nested ANOVAs (as was observed in some analyses in the present study; see Appendix C 2). However, larger collections are difficult to process because of the size and complexity of the *Macrocystis* thalli. Also, there was a possible danger of eradicating smaller populations such as the one at Quarantine Point by a destructive sampling design. A selective sampling of single fronds was not feasible either, as the fronds of single and coalescing *Macrocystis* thalli are usually heavily entangled with each other and thus are difficult to separate under water.

The *Macrocystis* thalli in the off-shore population at Cornish Head were significantly less infected than the populations inside the Harbour. However, these differences in the prevalence of infection and severity of symptoms are difficult to relate to specific environmental conditions, as many abiotic factors, such as temperature, salinity, wave-exposure, water depth and turbidity, naturally co-varied at the three study sites. *M. pyrifera* is adaptable to a variety of conditions, e. g. it grows in clear and turbid waters, along open coasts as well as in sheltered harbours (HAY 1990a). However, sub-optimal environmental conditions may reduce the health of a species and thus may be responsible for a greater susceptibility to infections and, subsequently, more severe infection symptoms. *Macrocystis* sporophytes, for example, appear to be adversly affected by elevated water temperatures (HAY 1990a). Within the shallow Otago Harbour, water exchange is slow (SMITH 1991), and thus temperature and salinity may show a greater mean monthly range than outside the Harbour (ANONYMOUS 1991). Near the two islands separating the two parts of the Harbour, temperature changes are the steepest. In the Upper Otago Harbour, the long flushing time (27.5 tidal cycles, i. e. circa 14 days; QUINN 1978, cited in PROBERT 1991) and the additional fresh water input by the Leith stream result in a stratified water column in summer with reduced salinity and elevated temperatures at the surface (ANONYMOUS 1991). Based on these observations, *Macrocystis* populations inside the Upper Harbour are considered to grow under sub-optimal conditions as they are closer to their upper temperature limit and are exposed to a reduced salinity and high loads of sediments in the water, compared to open-coast populations (J. FYFE, personal communication).

The water inside the Lower Harbour, in contrast, has only a short residence time of approximately 1.2 tidal cycles (14-15 hours; QUINN 1978, cited in PROBERT 1991), and is completely mixed (ANONYMOUS 1991). Accordingly, temperature and salinity of the Lower Harbour are similar to values measured outside the Harbour (RAINER 1981), although, at its far end, at Quarantine Point, the surface water may show a slightly depressed salinity and a greater range of sea surface temperatures (Appendix C 3, Table C 1 and Figure C 3.1, respectively). Thus it is possible that the temperature and salinity at Quarantine Point adversly affected the health of *Macrocystis*, compared to the other sites.

In contrast to small differences in temperature and salinity, the wave-exposure and water depth differed markedly among the three populations. The Quarantine Point site is wave-sheltered and shallow. Low water velocities and the close intertidal mudbeds result in thalli that were often covered by a layer of fine sediments. At Aramoana, in contrast, the thalli grow in water up to 5 m deep, and experience tidal currents and some wave action. The Cornish Head

site is fully wave-exposed, and the *Macrocystis* population grows in 10-12 m depth. At the populations at Aramoana and at Cornish Head, the strong currents and wave action appeared to prevent any sedimentation on the *Macrocystis* phylloids. A slower flow may also explain the higher number of bryozoan colonies settling on the phylloids at Quarantine Point, compared to the thalli from Aramoana and Cornish Head (observation not quantified). However, this apparently did not influence the settlement of endophyte spores because the thalli at Aramoana had endophyte infection rates similar to Quarantine Point thalli.

Because of the exposure at Cornish Head, the canopy cover of the *Macrocystis* population is extremely variable over time, as fronds may break in heavy wave action and storm events, especially in autumn and winter, and drift to the nearby shores, where they form piles of decaying biomass (FYFE *et al.* 1999). In spring 2000, for example, the population had suffered substantial loss in population density, which prevented more sampling in the area. Such a periodical decline appears to be part of a natural cycle of renewal in this off-shore population (FYFE *et al.* 1999). In Californian kelp beds, a periodic break-down of populations is speculated to be due to self-fertilization and subsequent inbreeding depression which may result in the senescence of populations (RAIMONDI *et al.* 2004). However, it is possible that infection by endophytic brown algae may also be involved.

The thalli collected from Cornish Head in winter 2000 weighed significantly less than the thalli from the preceeding summer. There was also a non-significant trend of winter thalli being longer and containing less canopy fronds but more senescent fronds than the thalli collected in summer. However, larger numbers of samples will be required for statistical support. Nevertheless, if these trends were supported by statistics, it would indicate that the winter thalli may have grown in length, but may have lost some apical scimitars. Considering that infection symptoms such as galls are persistent but that the winter thalli still seemed to be less infected, infected fronds or upper parts of these fronds may have been removed to a greater extent than non-infected ones. Galls on the stipes possibly change the biomechanic and hydrodymamic properties of the *Macrocystis* fronds, so that they may become more susceptible to drag. This idea is supported by observations in other algae: in *Laminaria* spp., for example, the presence of endophytes appears to reduce the flexibility of the fronds, thus infected thalli are considered to be less capable of withstanding strong wave action, compared to healthy thalli (LEIN *et al.* 1991; SCHAFFELKE *et al.* 1996; ELLERTSDÓTTIR & PETERS 1997). Likewise, in the red alga *Mazzaella laminarioides* (BORY) FREDERICQ, fronds infected with green algal endophytes appear to be selectively removed by storms (CORREA & SANCHEZ 1996; CORREA *et al.* 1997). *Laminaria*

populations from Helgoland, German Bight, nevertheless showed higher percentages of infection and more severe disease symptoms at the more wave-exposed site, in contrast to *Macrocystis* in New Zealand. However, population parameters, e. g. host densities, may have differed between the sites investigated on Helgoland (ELLERTSDÓTTIR & PETERS 1997).

The presence of endophytes may have a similar effect on the flexibility of *Macrocystis* thalli, but the consequences could differ from other host species. I. e., a brittle stipe may have a much more severe impact on the survival in single-phylloid thalli such as *Laminaria* spp., compared to the multi-frond thalli of *Macrocystis*. In *Macrocystis*, the thallus may replace lost fronds, as long as the holdfast and the frond initials persist. In *Laminaria*, the meristem is located at the base of the phylloid, and thus, once the cauloid breaks, the phylloid including the meristem is ripped off and the thallus cannot regenerate. In *Macrocystis*, endophytes may reduce the life span of single fronds. Thus, a putative regular seasonal removal of infected fronds in the exposed Cornish Head may reduce the overall prevalence of infection, in comparison to more sheltered populations such as those in Otago Harbour.

Alternatively, the same environmental factors that adversely affect the host species, may have a different effect on the endophytes, for example elevated water temperatures. Observations of *Laminaria* species along the German coast show that despite constant rates of infections with endophytic brown algae, the associated symptoms are more severe in summer and/or in thalli growing in shallower water (SCHAFFELKE *et al.* 1996; ELLERTSDÓTTIR & PETERS 1997). Moreover, in laboratory experiments, the respective endophytes, *Laminariocolax aecidioides* and *L. tomentosoides*, show maximum growth under summer conditions, suggesting that their growth may be temperature limited in winter and light limited in greater water depths (HEESCH 1996). Their Laminarialean hosts, in contrast, display maximum growth in winter, due to endogenous circannual rhythms (LÜNING 1993; SCHAFFELKE & LÜNING 1994). Thus the deeper subtidal, where endophyte growth is light limited, may provide a refuge from endophyte disease (HEESCH 1996; HEESCH & PETERS 1996). Likewise, the high prevalences of infection and severities of symptoms observed in thalli from Quarantine Point and Aramoana may have reflected better environmental conditions for the growth of *Laminariocolax macrocystis* at the Harbour sites, in comparison to Cornish Head.

Similar to the *Laminaria* spp. at Helgoland, a decline of the growth rate was observed in *Macrocystis* from Otago Harbour during summer (BROWN *et al.* 1997). However, not all other Southern Hemisphere populations show a seasonal growth pattern (reviewed in BROWN *et al.* 1997), thus there is no evidence to suggest that the growth rhythm of *Macrocystis pyrifera* could be

endogenous rather than driven by abiotic factors. Endogenous circannual growth rhythms with a lag phase in summer have been reported from kelps belonging to the Laminariaceae and Alariaceae, e. g. *Laminaria digitata*, *L. hyperborea* and *Pterygophora californica* (LÜNING 1991, 1993; SCHAFFELKE & LÜNING 1994). However, members of the Lessoniaceae have so far not been examined for the presence of endogenous growth rhythms.

BROWN *et al.* (1997) suggested that, in accordance with studies on Californian *Macrocystis* populations (NORTH & ZIMMERMAN 1984; ZIMMERMAN & KREMER 1986), the growth of the thalli in Otago Harbour may be limited by the low nutrient concentrations accompanying the elevated water temperatures in summer, instead of by the temperatures themselves. However, their generalisation on "Seasonal growth in the giant kelp *Macrocystis pyrifera* in New Zealand" (BROWN *et al.* 1997) seems problematical without any comparative data from off-shore populations supporting their observations.

In short, elevated temperatures and reduced salinities may affect the host thalli inside the Harbour, while the offshore population appeared to be mainly affected by the wave-exposure. The differences of environmental conditions for Harbour and off-shore populations are reflected by the morphologies of *Macrocystis* fronds, which differ between sites inside (i. e. at Aquarium Point, near Quarantine Point) and outside the Otago Harbour (KAIN 1982). They may also explain the variation in the prevalence of infection and severity of symptoms between the Harbour populations and the off-shore population. Future studies may show whether the morphology of thalli growing at Aramoana is closer to that of thalli from off-shore or from Harbour populations. According to infection rates and severity of symptoms, both the Harbour populations had more in common with each other than with the off-shore population.

To explain the differences in the infection rates, not only abiotic but also biotic factors must be taken into account which may have had some influence on the infection, such as the population density and the distribution of age groups, or the mortality and the biomass production. Finally, all three populations hosted different genetic varieties of *L. macrocystis*, which may show differences in their success to infect *Macrocystis* and to cause infection symptoms: at Cornish Head, *L. macrocystis* var. *novae-zelandiae* was isolated, while at Quarantine Point, *Macrocystis* hosted *L. macrocystis* var. *macrocystis*. The latter variety was also found in Aramoana, together with *L. macrocystis* var. *radiatae*.

In conclusion, this study presents the first quantitative observation on endophytic brown algae in New Zealand. By recording the prevalence of infection and severity of symptoms, it provides base-line information on the infection of *Macrocystis* by *L. macrocystis*. However, in order

to relate these results to single biotic or abiotic factors, or to interactions of factors, further studies will be needed that focus on epidemiological aspects and therefore include the quantification of a range of environmental conditions as well as the population genetics of the host populations. Also, additional host populations in other inlets and in the open ocean need to be studied, to verify the differences observed among the Otago Harbour and the Cornish Head populations. Populations of *Ecklonia radiata* which grow in various environmental conditions and are infected by *Laminariocolax macrocystis* should also be examined quantitatively, to evaluate the effect of the factor 'host species'.

The present study emphasizes the importance of including populations from various habitats to allow for some generalization. Likewise, BROWN and co-workers admit at the end of their discussion in which they generalize the growth pattern of New Zealand *Macrocystis* based on data from a single population inside the Otago Harbour: "It would be of interest to determine whether the pattern of seasonal growth in adjacent open-coast populations differ from that described here." (BROWN *et al.* 1997, pages 423-424).

3.2.3 *Microspongium tenuissimum*

3.2.3.1 The genus *Microspongium* REINKE

All New Zealand endophytes isolated from red algae as well as two isolates from kelps belonged to the species *Microspongium tenuissimum.* This endophyte was orginally described as *Streblonema tenuissimum* by HAUCK (1885) who discovered it in *Nemalion helminthoides* (VELLEY) BATTERS from the Mediterranean Sea. By studying the molecular systematics of the so-called 'simple' brown algae using *rbc*L sequences, SIEMER (1998) found *S. tenuissimum* to be closely related to the type species of the genus *Microspongium* REINKE, *M. gelatinosum* REINKE, rather than to the type species of the genus *Streblonema*, *S. volubilis* PRINGSHEIM. Moreover, similar to the *Laminariocolax* species, plurilocular sporangia in *S. tenuissmum* are uniseriate, unlike the pluriseriate sporangia in *S. volubilis.* Therefore PETERS (2003) removed *S. tenuissimum* as well as the closely related species *S. radians* HOWE from the genus *Streblonema*, and placed them, together with another endophyte (*Gononema alariae* PEDERSEN), in the genus *Microspongium.*

This genus was erected by REINKE (1889) to accommodate two epiphytes he described from the western Baltic Sea: *Microspongium gelatinosum*, growing on *Fucus* spp., and *M. globosum*, growing on *Zostera marina* L. and on some filamentous green and red algae. Their thallus is characterised by a prostrate pseudoparenchymatous base from which erect filaments arise

(REINKE 1888, 1889). The status of the genus as a separate taxon has been the subject of some controversies as the morphology of the prostrate base is similar to that of the microscopic sporophyte, the so-called "*Microspongium*" stage, of *Scytosiphon lomentaria* (LYNGBYE) LINK. Some authors (e. g. LUND 1966; MCLACHLAN *et al.* 1971; KOGAME 1998; PARENTE *et al.* 2003) therefore treat *Microspongium gelatinosum* as the microstage of *Scytosiphon.* There are, however, some morphological differences between both taxa: the number of plastids per cell, the number of cell layers in the prostrate base, and the presence of plurilocular sporangia in thalli of *Microspongium* spp., which are supposed to be absent in the microscopic sporophyte of *Scytosiphon lomentaria* (REINKE 1888; KRISTIANSEN & PEDERSEN 1979; VAN DEN HOEK *et al.* 1995). Nowadays, it is generally accepted that REINKE's material, collected in two consecutive years from different substrata, was actually heterogenous (KYLIN 1947; KRISTIANSEN & PEDERSEN 1979; FLETCHER 1987; SIEMER 1998; A. F. PETERS, personal communication). Accordingly, the material from 1888 (with 1-4 plastids per cell, only plurilocular sporangia and a single stromatic layer; REINKE 1889, Plate 7) represents the true *M. gelatinosum*, while the material from 1889 (REINKE 1889, Plate 8, characterised by a single parietal plastid per cell, the presence of unilocular sporangia and two stromatic layers) was most likely the sporophyte of *Scytosiphon lomentaria.* In this study, to avoid any confusion with the latter, the genus is referred to as *Microsponguim* REINKE sensu KRISTIANSEN & PEDERSEN 1979.

3.2.3.2 *M. tenuissimum* in New Zealand

In the New Zealand isolates, most strains of the *Microspongium* clade (isolates no. 19-26, group M_1) had ITS1 sequences which were nearly identical to that of *M. tenuissimum* isolated from *Polysiphonia elongata* (HUDS.) SPENG. from the Baltic Sea (as *Streblonema* sp.; later identified as *S. tenuissimum*; BURKHARDT & PETERS 1998; PETERS 2003). The isolates of group M_1 were classified accordingly and are henceforth referred to as '*tenuissimum*' isolates.

The average size of the vegetative cells of the '*tenuissimum*' isolates was within the range for Mediterranean *M. tenuissimum*, although at the lower end of the range given by HAUCK (1885). HAUCK's observations, however, were based on field material and therefore some discrepancies are to be expected compared to data from cultures. For example, in the New Zealand isolates, the average width of the plurilocular sporangia was smaller than that of the sporangia of the type material. Some of the isolates, however, displayed slightly larger sporangia, e. g. cultures of the endophyte from *Undaria pinnatifida* (isolate no. 25; Appendix B, Table B 1.1). There is no information in HAUCK (1885) regarding the length of the plurilocular sporangia or the number

of loculi observed in the type material. He also did not provide a figure from which this information could be derived. A figure of field material of *M. tenuissimum* (as *Phycocelis tenuissima* (HAUCK) KUCKUCK comb. nov.) was provided by KUCKUCK (†1954: page 105), with the endophyte displaying mostly short sporangia with a few loculi (and only a single longer sporangium), similar to the short sporangia observed in the New Zealand isolates. An example of different sized sporangia in field collected vs. cultured material is *Laminariocolax macrocystis*. In field material of gametophytes, sporangia appear to be relatively short, compared to those from cultured thalli with various lengths (as *Streblonema macrocystis*; PETERS 1991).

3.2.3.3 Morphological variations observed in culture

Two morphologically different growth types were observed in the '*tenuissimum*' isolates from New Zealand. The compact growth type encountered in some cultures is different from the filamentous habit of HAUCK's type specimen. However, the species description was based on endophytically growing material, not on thalli grown in unialgal cultures. The morphologically different growth types observed in the '*tenuissimum*' isolates from New Zealand are most likely the result of phenotypic plasticity due to environmental (i. e. culture) conditions, rather than heteroblasty or heteromorphic generations.

A heteromorphic life cycle is known from some endophytic brown algae, e. g. *Laminariocolax macrocystis* (PETERS 1991) or *Laminarionema elsbetiae* (PETERS & ELLERTSDÓTTIR 1996). For example, in a sporophyte culture of *Laminariocolax aecidioides*, from Helgoland, German Bight, displaying both unilocular and plurilocular sporangia, spores germinated in two ways (with or without forming a narrow germtube and evacuation of the embryospore), resulting in two growth types (fluffy open vs. compact ones) with differences in their attachment to the surface they grew on. They were suggested to represent slightly dimorphic gametophytes and sporophytes, with different habitats (endophytes vs. epiphytes), respectively (HEESCH 1996). In the New Zealand '*tenuissimum*' cultures, however, only plurilocular sporangia were observed, suggesting that all cultures had the same ploidy and reproduced only directly via mitospores.

Variations in growth forms are not limited to thalli of different levels of ploidy. They can also be displayed by spores which originate from a single source (e. g. a plurilocular sporangium). Heteroblasty means that spores of the same sporangium type of one parent thallus behave differently from the beginning, upon germination. This phenomenon has been observed in various members of the Ectocarpales *s. l.*, for example, in the Myrionemataceae (LOISEAUX 1968). In cultures of *Cladosiphon zosterae* (J. AG.) KYLIN, a member of the Chordariaceae

(LOCKHART 1979), spores produced asexually in plurilocular sporangia could germinate in two ways, similar to *L. aecidioides* (HEESCH 1996), and grow into filamentous or disc-shaped thalli, respectively. The variations were linked to different nitrogen sources in the culture medium or the presence of certain bacteria (LOCKHART 1979). However, a single germination type was observed in the *'tenuissimum'* cultures, thus heteroblasty is an unlikely explanation of the growth types.

The habit of small brown algae can be highly dependent on environmental conditions such as the nutritional status, as shown in cultures of a related species, *M. alariae* (PEDERSEN) PETERS, an endophyte known e. g. from *Fucus* spp. in the Baltic Sea (PETERS 2003). The thalli of *M. alariae* display a habit similar to the compact growth form found in some 'tenuissimum' isolates. They consist of "uniseriate, branched filaments which may form pseudo-parenchymatous masses of rounded cells in the central part of the horizontal system" (as *Gononema alariae*; PEDERSEN 1981a, page 264; PETERS 2003). But *M. alariae* also forms long filaments, whose apical parts can turn into long uniseriate plurilocular sporangia. Under certain conditions (i. e. nutrient deficiency), the thalli even develop long filaments with pectinate laterals. In the New Zealand *'tenuissimum'* isolates, strains differed morphologically even when measured on the same day, i. e. they would have been cultivated in similar vessels for the same period of time and under the same conditions prior to measuring. Some cultures, however, were rather crowded, possibly resulting in local nutrient deficiencies.

Also, the substrata upon which individual thalli grew could have influenced their habit. Surface energy effects by the substratum have, for example, been discussed as another reason to cause heteroblasty (FLETCHER *et al.* 1985). The example of the epi-endophyte *Mikrosyphar polysiphoniae* KUCKUCK (1895a) shows how morphology can depend on the substratum: When growing in unialgal cultures, with no firm attachment to the abiotic substrate, this species forms 'lumpy' thalli, while conspicuous pseudoparenchymatous discs appear when the endophytes grow on the thalli of their host in mixed cultures with *Polysiphonia stricta* (DILLWYN) GREVILLE (as *P. urceolata* (LIGHTF. ex DILLWYN) GREVILLE; PEDERSEN 1984). Spores of the *'tenuissimum'* isolates from New Zealand settled on all available surfaces within the culture vessels and thus grew on diverse materials, such as polysterene (in Petri dishes) or glass (either in glass vessels or on glass fragments floating in the culture medium as a result of the reaction of seawater with the surface of the Schott® bottles during autoclaving), or floated freely in the culture medium. Spores also settled on each other, resulting in lumps of possibly coalescing individuals, which might have influenced their thallus morpholgy as well.

Discs similar to the ones observed by PEDERSEN (1984) in *Mikrosyphar polysiphoniae* were lacking in the cultures of the *'tenuissimum'* isolates from New Zealand, even though a basal pseudoparenchyma is one of the characteristics of the genus *Microspongium* (REINKE 1889). Neither HAUCK (1885) nor later authors dealing with this species mention such a basal disc (e. g. KUCKUCK emend. KORNMANN 1954; PANKOW 1990; BURKHARDT & PETERS 1998). Even PETERS who proposed the new combination *M. tenuissimum* did not observe this character, but instead based his decision on molecular data alone (PETERS 2003; A. F. PETERS, personal communication). Furthermore, although PEDERSEN (1981a) referred to the agglomerates of rounded cells in the centre of the thalli of *M. alariae* (as *Gononema alariae*), similar to the compact forms of the *'tenuissimum'* isolates, as 'pseudoparenchymatous', they are different from the pseudoparenchyma displayed by epiphytic *Microspongium*. According to REINKE (1889), epiphytic thalli of *Microspongium* form a prostrate disc constructed of a monostromatic layer of closely lying cells of irregular shapes.

However, a basal layer agreeing with the description by REINKE (1889) was observed in one *Microspongium* strain from New Zealand, the endophyte of *Pachymenia lusoria* from Owenga, Chatham Island (isolate no. 27; compare present study, Plate 3.4, Figure I, with REINKE 1889, Plate 7, Figures 4-6). In contrast to *M. gelatinosum* and *M. globosum*, in which many erect filaments form a dense carpet (REINKE 1888, 1889), isolate no. 27 only displayed a few erect filaments rising above the basal disc (Plate 3.4, Figure I). The ITS1 sequence of isolate no. 27 was similar to those of the *'tenuissimum'* isolates but differed in a small indel of 27 bp that is missing in the endophyte from Owenga. An identical ITS1 sequence, with the same indel missing, had previously been found in *M. radians* (as *S. radians* HOWE) isolated from *Grateloupia doryphora* (MONTAGNE) HOWE from Chile (BURKHARDT & PETERS 1998). Therefore the New Zealand isolate no. 27 was classified accordingly and is henceforth referred to as the *'radians'* isolate.

HOWE (1914) described *Streblonema radians* as an endophyte of *Grateloupia* sp. from Peru. He separated it from *M. tenuissimum* (HAUCK) PETERS (as *Streblonema tenuissimum* HAUCK) based on its "very short, reduced, sometimes branched, plurilocular sporangia" which he considered "different from the somewhat thicker 'fadenförmig' (filamentous) sporangia" of *M. tenuissimum* (HOWE 1914, p. 47). In the *'radians'* isolate from Chatham Island, the cell sizes and the width of the plurilocular sporangia were in agreement with the sizes given by HOWE for the type material from Peru, even though at the minimum level. The length of the sporangia of the isolate from New Zealand, however, exeeded those measured in HOWE's specimens: The Peruvian sporangia were 11-15 µm long and had 1-6 (usually 3-5) loculi (HOWE 1914), while in the New Zealand

isolate, they were 31 ± 6 µm long, with 8 ± 2 loculi and thus were in the range of the sporangia measured in the *'tenuissimum'* isolates. As HAUCK (1885) did not measure the length of the sporangia he observed in *M. tenuissimum*, HOWE (1914) could only use the size and shape of the plurilocular sporangia – which he states were "inferred 'from HAUCK's brief description' " (HOWE 1914, page 47) – to separate his endophyte from HAUCK's species. In fact, in the figure given by KUCKUCK of *M. tenuissimum* (as *Phycocelis tenuissima* (HAUCK) KUCKUCK, KUCKUCK †1954, page 105, Figure 1), the sporangia (apart from the single long one) are similar to the ones in HOWE's figure of *M. radians* (1914, plate 11, Figure 8). Moreover, there were no significant differences in average cell and sporangium sizes between the *'radians'* isolate and the *'tenuissimum'* cultures.

The prostrate disc observed in the New Zealand *'radians'* isolate seems to appear only under culture conditions - it is not mentioned by HOWE (1914), who, like HAUCK (1885), studied only material from the field. A similar discrepancy between field and culture material was reported for another *Microspongium* species, *M. immersum* (LEVRING) PEDERSEN 1984, also a former member of the genus *Streblonema.* From this red algal endophyte originally only field material with simple uniseriate filaments was described (LEVRING 1937). In cultures of this species, however, PEDERSEN (1984) observed conspicuous pseudoparenchymatous discs. As heterotrichous thalli are unknown in the genus *Streblonema*, he moved the species to the genus *Microspongium.*

The discs in the New Zealand *'radians'* isolate were similar to those described for *M. immersum.* Indeed both taxa appear to be closely related, if not synonymous. The only distiguishing feature is the presence of true phaeophycean hairs in *M. immersum*, while they appeared to be absent in the New Zealand *'radians'* isolate. However, as discussed above, the formation of hairs often depends on the nutritional status of a culture, and hairs have moreover been observed in Peruvian *M. radians* (HOWE 1914). And even though the cells of *M. immersum* are smaller than those of Peruvian *M. radians*, they are in the range of those measured in the *'radians'* isolate from New Zealand, while the size of sporangia in *M. immersum* take a medium position between *M. radians* from Peru and the *'radians'* isolate (HOWE 1914; LEVRING 1937; PEDERSEN 1984; this study).

The question remains as to why a basal pseudoparenchyma is absent in the *'tenuissimum'* isolates from New Zealand. Are they genetically incapable of forming a 'proper *Microspongium* disc', or were their culture requirements just not met, i. e. is this a case of phenotypic plasticity? Or could the presence vs. absence of a basal disc be a result of heteroblasty or even of

heteromorphic generations, i. e. a sign of epiphytism in one of the two putative generations? Even though unilocular sporangia or different germination types were not observed in any of the *Microspongium* isolates, cultures were not under constant surveillance. To address these questions, further observations will be required, including a determination of the ploidy status of the isolates in question.

3.2.3.4 Taxonomic consequences

Whatever the cause of the absence of the basal discs in the *'tenuissimum'* isolates may be, both *'tenuissimum'* and *'radians'* isolates are more or less in accordance with the measures given for the type material of *M. tenuissimum* and moreover share nearly identical ITS1 sequences. Following the argument in the case of *Laminariocolax macrocystis* and *L. eckloniae*, a separation of both taxa on the species level does not seem justified, and both entities of *Microspongium* should instead be merged under the older name *Microspongium tenuissimum* (HAUCK) PETERS 2003. Any differences have to be regarded as variations within the general morphological capabilities of this species. The *'radians'* strain was the only isolate obtained from a red algal host from Chatham Island, while it was not found on mainland New Zealand. Thus *'tenuissimum'* and *'radians'* may be geographically separated in New Zealand. However, in southern Chile and in South Africa, both entities co-occur (PETERS 2003). When taxa are not geographically separated, though, then a single morphological difference such as the presence vs. absence of the basal pseudoparenchyma is not sufficient enough to distinguish between taxa on the sub-species level (SCHUBERT & WAGNER 1993). Therefore, *M. radians* should be ranked as a taxonomic variety of *M. tenuissimum*, i. e. as *M. tenuissimum* var. *radians* comb. ined..

3.2.3.5 *M. tenuissimum* vs. *Mikrosyphar pachymeniae*

In DNA sequence comparisons endophytes isolated from red algae in New Zealand were similar to *Microspongium* isolates from other parts of the world. But what is their relationship to *Mikrosyphar pachymeniae*, another red algal endophyte from New Zealand? *M. pachymeniae,* the only pigmented endophytic brown alga known from New Zealand prior to this study, was originally described as an endophyte from *Pachymenia lusoria* found in Russell, Bay of Islands, North Island (LINDAUER 1960). According to ADAMS (1994), it is found throughout New Zealand's main islands (North Is., South Is. and Stewart Is.), but has not been observed on Chatham Is. (NELSON *et al.* 1991).

During the present study, however, the only endophyte isolated from specimens of *Pachymenia lusoria* (and other red algae) was *Microspongium tenuissimum*. It was frequently found among host populations in the south of South Island and on Chatham Island. However, no sites further north than Wellington were visited during this study. Two different scenarios could explain these observations: First, both endophyte species could be present in New Zealand, with LINDAUER'S *Mikrosyphar pachymeniae* in the north and *Microspongium tenuissimum* in the south and on Chatham Island. This would mean that until now the (southern) populations of *Microspongium tenuissimum* have apparently been mistaken for *Mikrosyphar pachymeniae*. Secondly, *Mikrosyphar pachymeniae* could be synonymous with *Microspongium tenuissimum* (HAUCK) PETERS 2003 and could occur throughout New Zealand.

The genus *Mikrosyphar*, with the type species, *Mikrosyphar zosterae* KUCKUCK (1895a), an endophyte from *Zostera marina* L. from the Baltic Sea, was erected by KUCKUCK to accommodate three epi-endophytes he found on seagrass and red algae (KUCKUCK 1895a, 1897a, b). The genus is charaterised by plurilocular sporangia that develop directly from cells of short vegetative filaments, thus the sporangia are very short, only displaying one to few loculi. The thalli consist of uniseriate irregularly branched creeping filaments which sometimes can form a pseudoparenchymatous disc (KUCKUCK 1895a; KUCKUCK, in contrast to other early phycologists such as HAUCK (1885) and HOWE (1914), gained many of his descriptions from observing cultures). The cells of *Mikrosyphar* contain 1-3 plastids. At this point KUCKUCK (1897a) remarks on the similarity, for example, to *Microspongium gelatinosum*, even though, unlike in *Microspongium*, he did not see any pyrenoids occuring in the plastids of *Mikrosyphar*. However, the main difference between *Microspongium* and *Mikrosyphar* seems to be the absence of erect filaments in the latter (KUCKUCK 1895a, 1897a, b; REINKE 1888, 1889).

Considering the description of the genus by KUCKUCK, LINDAUER'S placement of his endophyte in *Mikrosyphar* seems problematical. For example, there is some inconsistency in LINDAUER's original description regarding the plurilocular sporangia of *M. pachymeniae* (LINDAUER 1960): In the text he states that he did not observe any in the material he examined. The figure accompanying the description, however, shows plurilocular sporangia, which have an unusual shape and are moreover pluriseriate (LINDAUER 1960, page 165 vs. page 166, Figure 3.2 - see present study, Figure 3.8 B). Thus, they are very different from the typically uniseriate plurilocular sporangia described for the genus *Mikrosyphar* (KUCKUCK 1897a; PEDERSEN 1984; present study, Figure 3.8 A).

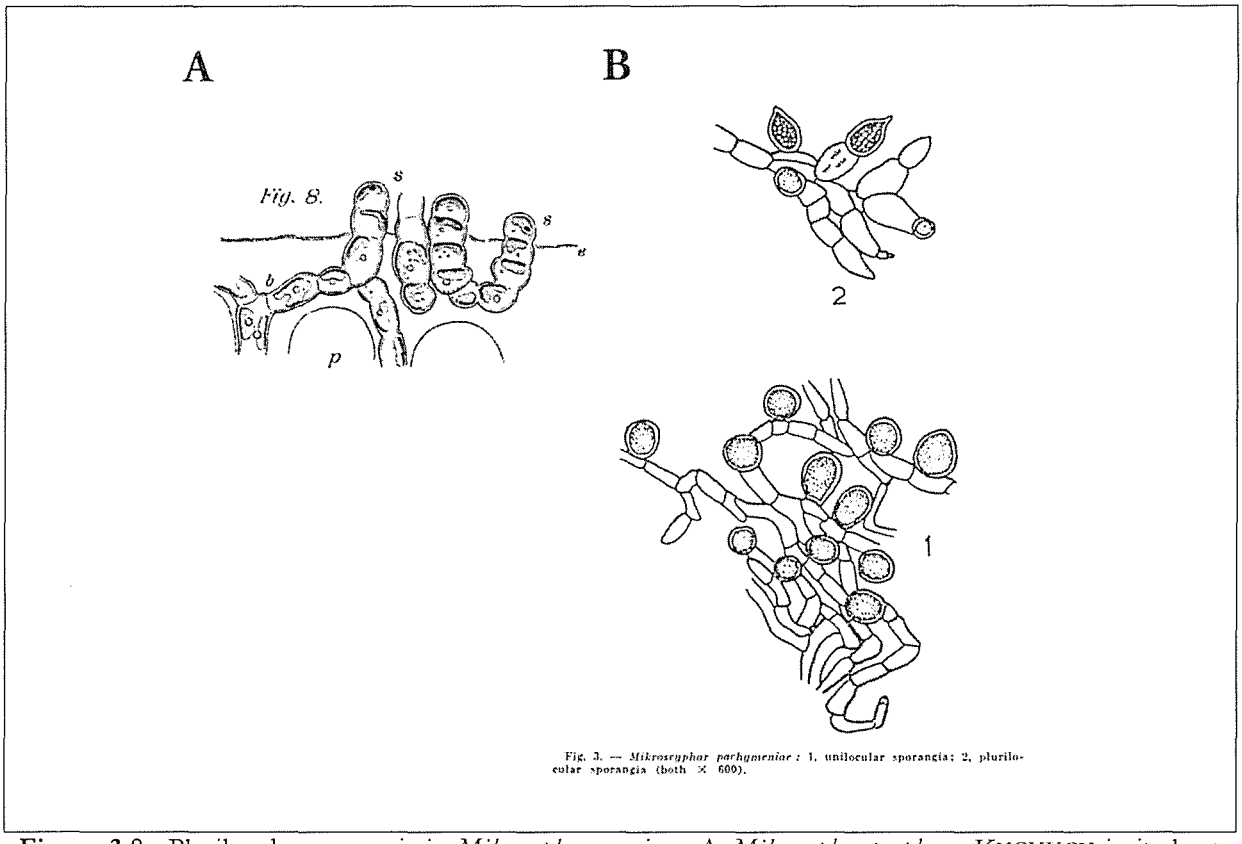


Figure 3.8: Plurilocular sporangia in *Mikrosyphar* species. A: *Mikrosyphar porphyrae* KUCKUCK in its host *Porphyra* sp.; e: cuticle of the *Porphyra* tissue; p: cell of *Porphyra*; s: mature (plurilocular) sporangium of the endophyte (one of them emptied); b: sterile endophyte cell; figure from KUCKUCK 1897a (Plate IX, Figure 8). B: *Mikrosyphar pachymeniae* LINDAUER; figure from LINDAUER 1960 accompanying the original description (page 166). Lengths are not to scales.

Additionally, the question arises, if the filaments of *M. pachymeniae*, which LINDAUER described as "somewhat loosely to densely compact" (LINDAUER 1960, page 165) could form a pseudoparenchyma, another typical feature of the genus *Mikrosyphar* (KUCKUCK 1895a). For example, samples of *M. pachymeniae* taken from the type specimen, a thallus of *Pachymenia lusoria* (as *P. himanthophora* J. AGARDH), show an endophyte thallus consisting of filaments which are not compact (personal observation). However, LINDAUER appears to have studied only field material, and his specimen was, moreover, a sporophyte. This could account for some differences to the descriptions of other, only asexuelly reproducing, *Mikrosyphar* species (KUCKUCK 1895a, 1897a, b), e. g. the absence of the pseudoparenchymtous filaments. Still, because of the enigmatic plurilocular sporangia, the placement of LINDAUER's endophyte in the genus *Mikrosyphar* requires confirmation.

As morphological comparisons cannot give satisfactory answers regarding the relationship of LINDAUER's endophyte to other endophytic Phaeophyceae, molecular systematics

will be required to investigate the relationship of *M. pachymeniae* to other members of the genus *Mikrosyphar* and to *Microspongium tenuissimum*. According to molecular analyses, the genera *Microspongium* and *Mikrosyphar* both belong to the Ectocarpales *s. l.*, but are not closely related to each other. Moreover, the genus *Mikrosyphar* appears to be polyphyletic (SIEMER 1998).

DNA sequence comparisons would require genetic material of LINDAUER's endophyte, which ideally originates from the type specimen or at least from a specimen from the type locality. DNA from herbarium specimens has been successfully used in molecular studies before (e. g. BRUNS *et al.* 1990; MAYES *et al.* 1992; GOFF & MOON 1993; BRODIE *et al.* 1996). Unfortunately, LINDAUER seems to have stained the type specimen of *Mikrosyphar pachymeniae* and any other herbarium material available (personal observation), therefore, it seems unlikely that intact DNA suitable for genetic analyses could be extracted from these specimens. Furthermore, using new material from the type locality of *M. pachymeniae* is not an option, as the host alga *Pachymenia lusoria* was not present at the locality in a recent survey of *Pachymenia* in the North Island (L. RUSSELL, personal communication). Therefore, at present testing the molecular systematics of *M. pachymeniae* seems hardly possible.

Future collections from the North Island might reveal whether indeed two separate species of endophytes are present in New Zealand red algae. Meanwhile, it has to be considered that all brown endophytes isolated from red algae during this study have been without any doubt identified as *Microspongium tenuissimum*. Moreover, apart from its problematical plurilocular sporangia, *Mikrosyphar pachymeniae* shares the host species and a similar morphology with *Microspongium tenuissimum*. Therefore, it is possible that LINDAUER's endophyte is synonymous with *M. tenuissimum* after all. The valid name of this taxon would be *Microspongium tenuissimum* (HAUCK) PETERS 2003, as *Streblonema tenuissimum* HAUCK (HAUCK 1885) would have priority over *Mikrosyphar pachymeniae* LINDAUER (LINDAUER 1960).

3.2.3.6 *M. tenuissimum* and its host algae

M. tenuissimum was originally described as an endophyte of *Nemalion helminthoides* from the Mediterranean Sea (HAUCK 1885) and has since been found in red algae not only from other European coasts (for example the Baltic Sea), but also other continents, e. g. in *Aeodes orbitosa* (SUHR) SCHMITZ from South Africa and in *Grateloupia* spp. from Chile and the Canary Islands (KYLIN 1944; PANKOW 1990; BURKHARDT & PETERS 1998; PETERS 2003). In New Zealand, *M. tenuissimum* also mainly infects red algae. However, the isolates from the kelps *Undaria pinnatifida* and *Ecklonia radiata* show that this species is also capable of infecting brown algae,

confirming a statement by PETERS (2003, page 298): "Endophytes of the genus *Microspongium* are found in hosts from different algal phyla". *M. alariae* (PEDERSEN) PETERS 2003, for example, is mainly found in large brown algae, in members of the Laminariales such as *Alaria esculenta* (L.) GREVILLE and *Laminaria saccharina* (L.) LAMOUROUX, or in Fucales such as *Fucus vesiculosus* L. from the Eastern Baltic Sea (JAASUND 1963; PEDERSEN 1981a; PETERS 2003).

Whether *M. tenuissimum* is capable of infecting other kelps or members of the Fucales, is not known. However, the infections of macroalgae with this endophyte are much less severe, and thus less conspicuous than those with *Laminariocolax macrocystis*. Especially in the infected kelps, no warts, galls, or morphological changes were detected in association with the presence of *M. tenuissimum*. Instead, similar to the red algal hosts, they displayed dark circular patches which might easily be obscured by the pigments of a phaeophycean host.

Studies on endophytic Phaeophyceae in red algae have so far concentrated on taxonomic descriptions of the endophytes, but further information about the association is lacking, possibly because infections are less severe and conspicuous than, for example, those of commercially important Rhodophyceae by green algal endophytes (e. g. CORREA *et al.* 1987, 1988; CORREA & MCLACHLAN 1991, 1992, 1994; CRAIGIE & CORREA 1996). Quantitative data of infections of red algae by endophytic brown algae are scarce (e. g. CORREA *et al.* 1987). New Zealand, however, would provide ideal conditions for an epidemiological field study, for example, on the host-endophyte system *Pachymenia lusoria*/*M. tenuissimum*: The endophyte appears to be widespread in populations of its host, which are found on all islands of New Zealand (ADAMS 1994) and are easily accessible at low tide. Additionally, an infection of the host with the endophyte is easy to recognize in the field.

Apart from the prevalence and the severity of an infection with *M. tenuissimum*, such an epidemiological study would also need to address possible differences in the susceptibility to an infection between host generations, i. e. gametophytes and tetrasporophytes, and thus the effects on the population structure of the host (ANDREWS 1979; CORREA & SANCHEZ 1996). In Chilean populations of the red alga *Chondrus crispus*, for example, gametophytes appeared to be less susceptible to an invasion by the endophytic green alga *Acrochaete operculata* than sporophytes. It was suggested that cell wall components, e. g. different forms of carrageenan found in gametophytes and sporophytes, might influence the capability of the endophyte to develop inside the host (CORREA & MCLACHLAN 1991). However, in European *Chondrus* populations, infection levels were found to be similar in both gametophytes and sporophytes (J. BRODIE,

personal communication). In *Pachymenia lusoria*, the polysaccharides at least of females and tetrasporophytes are identical (MILLER *et al.* 1997).

3.2.4 *Xiphophorocolax aotearoae* gen. et sp. ined.

3.2.4.1 The genus *Xiphophorocolax* gen. ined.

In addition to *Laminariocolax macrocystis* and *Microspongium tenuissimum*, a third group of endophytes was isolated from New Zealand macrophytes comprising the strains from the kelp *Lessonia tholiformis* and from members of the Fucales *s. l.*. For these endophytes, due to their unique ITS1 sequences, the new genus and species *Xiphophorocolax aotearoae* gen. et sp. ined. will be proposed. The simple filamentous thallus structure of *X. aotearoae* and its plastids possessing pyrenoids place this species in the Ectocarpales *s. l.* (ROUSSEAU & DE REVIERS 1999). Comparisons of nrDNA sequences moreover revealed that within the order Ectocarpales, *X. aotearoae,* like *L. macrocystis* and *M. tenuissimum*, belongs to the family Chordariaceae (sensu PETERS & RAMIREZ 2001). Its closest free living-relative among the taxa included appear to be *Chordaria flagelliformis* and *Dictyosiphon foeniculaceus*, however, relationships within the family were not sufficiently resolved. Non-alignable large sections or indels of ITS1 sequences indicate that *X. aotearoae* is separated on the generic level not only from *C. flagelliformis*, *D. foeniculaceus* and the other New Zealand endophytes *L. macrocystis* and *M. tenuissimum*, but also from other endophytic brown algae, such as *Laminarionema elsbetiae* KAWAI & TOKUYAMA, *Ascoseirophila violodora* PETERS, and *Austrofilum incommodum* (SKOTTSBERG) PETERS (data not presented).

A separation of *Xiphophorocolax* from other endophytes based on morphological characters is not that easy, as *X. aotearoae* shares its simple morphology with many endophyte genera described. However, even though only a limited number of characters are available to distinguish taxa, the difficulty to align ITS1 sequences with those of other endophytes agrees with certain morphological differences: *Xiphophorocolax* is, for example, distinguished from *Laminariocolax* and *Laminarionema* by average cell sizes or the number of plastids. In *Xiphophorocolax*, three discoid plastids was the maximum observed in any cell, while the other two genera possess either several discoid plastids (*Laminarionema*: KAWAI & TOKUYAMA 1995; some *Laminariocolax* species: e. g. this study, Plate 3.3, Figure D) or two band-shaped ones (*Laminariocolax tomentosoides*: KYLIN 1947). Additionally, *Laminarionema* is characterized by the presence of micro- and macrosporangia, a feature unique to this endophyte (KAWAI & TOKUYAMA 1995). Morphologically, *X. aotearoae* is very similar to *Microspongium tenuissimum*,

however, both species are distinguished by a combination of characters, such as average cell sizes in culture, speed of growth and/or reproduction, respectively, and host range. The latter also separates *Xiphophorocolax* from the genera *Ascoseirophila* and *Austrofilum*, which grow in *Ascoseira mirabilis* or *Adenocystis* and similar species, respectively (PETERS 2003). However, both *Ascoseirophila* and *Austrofilum* share a simple morphology with *Xiphophorocolax*, and thus have more in common with the New Zealand species than some other Phaeophycean genera from which endophytes have been described: Plurilocular sporangia, for example, are uniseriate in *Xiphophorocolax*, in contrast to the pluriseriate plurilocular sporangia of *Streblonema* (DERBES et SOLIER in CASTAGNE 1851). Moreover, *Xiphophorocolax* has erect filaments, which are absent in *Streblonema*, as well as in other endophyte genera such as *Phycocelis*, *Myrionema* and *Entonema* (REINSCH 1875; PEDERSEN 1981a). The genus *Pilocladus* has been described with short erect filaments, however, its cells contain several plastids each (KUCKUCK emend. KORNMANN 1954), similar to *Laminariocolax* and *Laminarionema*, but different from *Xiphophorocolax*.

Xiphophorocolax is moreover distinguished from the genera *Cylindrocarpus* and *Gononema*: Epi-endophytic *Cylindrocarpus* species display thalli with densely pigmented assimilators and a less pigmented medulla (CROUAN & CROUAN 1851; KUCKUCK 1899; SKOTTSBERG 1921; FLETCHER 1987), while in *Gononema pectinatum*, a colourless basal pseudoparenchyma is present (PEDERSEN 1981a). In *Xiphophorocolax*, neither a less pigmented medulla nor basal pseudoparenchyma have been observed so far, the latter a feature separating the new genus also from *Microspongium* and *Mikrosyphar* (KUCKUCK 1895a). *Mikrosyphar* is moreover different from *Xiphophorocolax* because of its typical short, few-celled plurilocular sporangia.

3.2.4.2 Morphology vs. molecular systematics in *Xiphophorocolax*

All *Xiphophorocolax* isolates were nearly identical in their ITS1 sequences, but there were slight morphological differences in two of the strains. For example, true phaeophycean hairs were only observed in cultures of the isolate from *Marginariella urvilliana* (isolate no. 32), while the endophyte from *Durvillaea willana* (isolate no. 34) was the only one to display erect filaments in culture. Isolate no. 34 could be distinguished from the others by a small indel of 29 bp missing at positions 624-652 in the ITS1, while isolate no. 32 did not display any marked differences to the other strains, i. e. isolates no. 28-31. The greatest genetic dissimilarities, however, were found in the isolate from *D. antarctica* (isolate no. 33); these may have been at least partially PCR artifacts, as the morphology of isolate no. 33 was nevertheless in accordance with the other strains.

Phaeophycean hairs, such as found in the endophyte from *Marginariella*, can be attributed to phenotypic plasticity, as they may be produced in response to nutrient deficiency in this culture (see section 3.2.2; PEDERSEN 1981a). The presence vs. absence of erect filaments in culture, however, is regarded as sufficient to distinguish between taxa on the level of sub-species, e. g. in genetically identical strains in *Laminariocolax tomentosoides* from Helgoland, German Bight, and Brittany, France (PETERS 2003). These strains were geographically separated, though, while in the New Zealand *Xiphophorocolax*, isolate no. 34 originated from the same site, Brighton Beach, as the endophyte from *D. antarctica* and the two isolates from *Xiphophora gladiata* (isolates no. 30 and 31). Thus, the endophyte from *D. willana* was neither temporally nor geographically separated from the other strains, and, following the argument for *Laminariocolax macrocystis* and *Microspongium tenuissimum*, should be treated as a cryptic genetic variety of *X. aotearoae* rather than a sub-species, i. e. *X. aotearoae* var. *willanae* var. ined..

3.2.4.3 Host-endophyte relationships in infections by *X. aotearoae*

Xiphophorocolax appears to occupy a niche different from that of the other two New Zealand endophytes, even though all three endophyte species infect members of the Laminariales. *X. aotearoae* has been isolated from *Lessonia tholiformis*, but also from members of the order Fucales. Cross-infection experiments would be useful to determine whether *X. aotearoae, Laminariocolax macrocystis* and *Microspongium tenuissimum* would generally be capable of infecting each others hosts, and if so, whether the apparent limitation of *X. aotearoae* to its hosts might be due to a competition between the endophyte species. Compared to the other two species, *X. aotearoae* grows and/or reproduces more slowly, thus it could be less successful in infecting fast growing host species such as *Macrocystis pyrifera*. *L. macrocystis* and *M. tenuissimum*, on the other hand, may be less successful in attacking the macroalgae hosting *X. aotearoae*. Interestingly, all five host species of *X. aotearoae*, regardless of their taxonomic position, possess perennial thalli which are conspicuously tough and leathery allowing them to grow at exposed coasts. Moreover, some members of the Fucales are known to regularly slough their epidermis. This renewal of the surface is suggested to be a means to successfully remove most epiphytes in perennial algae such as *Ascophyllum nodosum* (L.) LE JOLIS (FILION-MYKLEBUST& NORTON 1981) or *Halydris siliquosa* (L.) LYNGB. (MOSS 1982). Shedding of the surface has also been observed in *Durvillaea* species (CLAYTON 1990b; HAY 1994; present study) and *Lessonia* spp. from Chile (MARTINEZ & CORREA 1993). However, similar information regarding other members of the Laminariales is lacking. *Macrocystis* fronds are less persistent than *Lessonia* fronds: they only

survive for up to nine months (NORTH 1994), thus surface shedding may not be necessary in *Macrocystis*. Whether the surface shedding contributes to a possible formation of niches among the New Zealand endophytes, remains to be resolved. If *X. aotearoae* was the only endophyte capable of infecting its host species in New Zealand, its slow growth/reproduction rates could be the result of a lack in competition. On the other hand, the host range of *X. aotearoae* could be due to its slow growth/reproduction rates, as a niche to avoid competition with faster growing endophyte species. Thus, re-infection studies may also provide information about the way *X. aotearoae* attacks its host algae and possibly avoids being sloughed off.

Most likely, spores are involved in carrying the *Xiphophorocolax* infection from host to host, as was shown for *Laminariocolax aecidioides* and *Laminarionema elsbetiae*. Both of these endophyte species do not require openings in the host surface to enter their host: their spores settle on the intact surface and upon germination penetrate the host surface with a narrow germ tube through which the content of the embryospore migrates into the first cell developing inside the host (HEESCH & PETERS 1999). Spores with narrow germ tubes or emptying of the embryospore were not observed in *X. aotearoae* cultures, though, thus it is not known if its infection mechanism is similar to the one, for example, of *Laminariocolax* spp.. However, the absence of specialised narrow germ tubes in culture does not necessary imply that *X. aotearoae* is incapable of forming them. The formation of germ tubes could, for example, depend on the availability of a suitable substrate, such as the surface of a potential host (see discussion on heteroblasty in section 3.2.2). Or germ tubes could be limited to spores of a certain stage in the life cycle. In *Xiphophorocolax,* only a direct development was observed, i. e. neither unilocular sporangia nor zoid fusions appeared in the culture conditions examined. It is not known whether this species possess sexuality at all and which generation was present in the cultures. In *L. macrocystis*, where sexual reproduction was observed in field material, unilocular sporangia were not formed in culture, possibly because their formation depends on host presence (PETERS 1991). Similarly, sexual reproduction in *X. aotearoae* could be dependent on the presence of a potential host thallus.

On the other hand, spores may also be able to penetrate the host surface without a specialised narrow germ tube, or *Xiphophorocolax* may require preformed openings in the host surface, similar, for example, to the red alga *Harveyella mirabilis*. Spores of this parasite depend on wounds caused e. g. by herbivores to enter the host *Odonthalia floccosa* (GOFF & COLE 1976a, 1976b). In both infected *Durvillaea* specimens, the thallus surface in the region of the *Xiphophorocolax* infection indeed showed some kind of damage. In *D. antarctica, X. aotearoae* was

found underneath a large patch of *Herpodiscus durvillaeae* whose fertile epiphytic filaments seriously disturb the integrity of the host surface. The size of the parasite patch in contrast to the presence of only few filaments of *X. aotearoae* suggests that *Herpodiscus* might have been the primary infectant, followed by *Xiphophorocolax*, which could have used the disturbance of the host surface by the parasite as a port of entry.

The endophyte from *Durvillaea willana*, on the other hand, was detected in a pale patch, that superficially looked like grazing marks left by herbivores, even though microscopically the cuticle on the surface appeared to be largely intact, and necrotic tissue was absent. However, the host cells were unusually pale, i. e. they appeared to have lost their pigmentation. The question is whether this was caused by the presence of *X. aotearoae,* or by other pathogens, e. g. bacteria or fungi, which either could have provided a way for the endophytic brown alga to enter, or may have infected the host following *Xiphophorocolax*. In the red alga *Chondrus crispus*, for example, the primary damage to the host is caused by the green endophyte *Acrochaete* sp., followed by a secondary bacterial infection leading to the deterioration of the host tissue (CORREA & CRAIGIE 1991).

Generally, the pale patch in *D. willana* appears to be unusual for an infection with endophytic Phaeophyceae. Similar to the host algae of *Laminariocolax macrocystis*, most of the macroalgae infected by *X. aotearoae*, i. e. *Lessonia tholiformis*, *Xiphophora gladiata* and *Marginariella urvilliana*, displayed galls and or at least dark patches associated with the infection, whereas in *Durvillaea antarctica*, any macroscopic symptoms associated with *Xiphophorocolax* may have been obscured by the much more conspicuous *Herpodiscus* infection.

3.2.4.4 Distribution of *X. aotearoae*

Xiphophorocolax aotearoae has so far only been found in New Zeland, in host species of which most are endemic to New Zealand or Australasia, i. e. *Lessonia tholiformis*, *Marginariella urvilliana*, *Durvillaea willana* and *Xiphophora gladiata.* Moreover, these macroalgae carry host-specific epiphytes which are also restricted to New Zealand waters (LINDAUER *et al.* 1961; ADAMS 1994): *Lessonia tholiformis*, for example, is the basiphyte of the endemic red alga *Pyrophyllon cameronii* (W. A. NELSON) W. A. NELSON (NELSON 1993; NELSON *et al.* 2003), while the brown epiphyte *Herponema maculaeformis* (J. AGARDH) LAING has so far only been found on *Xiphophora gladiata* in New Zealand. Another *Herponema* species, *H. hormosirae* LINDAUER & CHAPMAN, grows only on *Hormosira banksii*, which as a member of the Fucales may also be a potential host species for *Xiphophorocolax*. Other epiphytes have wider host ranges, but are still restricted to New Zealand,

e. g. *Hecatonema stewartensis* CHAPMAN, an epiphyte of *Marginariella urvilliana*, also grows on members of the Laminariales (ADAMS 1994). Another *Pyrophyllon* species, *P. subtumens* (J. AGARDH) NELSON *et al.* 2003 is only found on the stipes of New Zealand *Durvillaea* spp. (*D. antarctica, D. willana* and *D. chathamensis;* NELSON *et al.* 2003). *Xiphophorocolax* has so far not been observed in *D. chathamensis*, however, considering the lack of uniform infection symptoms in the other two *Durvillaea* species, an infection may have been overlooked. This also applies to other *Lessonia* spp. and to the remaining members of the Fucales, i. e. not only *Hormosira banksii*, but also *Xiphophora chondrophylla* as well as *Cystophora*, *Landsburgia*, *Carpophyllum* and *Sargassum* species.

The apparent limitation of *Xiphophorocolax* to mostly endemic host species suggests that the endophyte may be endemic as well. Indeed, most of the host species do not float, thus its chance for a long-distance distribution appears to be small. *D. antarctica* is the only one of the host species of *Xiphophorocolax* with a buoyant thallus, which is frequently observed rafting off-shore (SMITH 2002). The wide distribution of *D. antarctica* comprising most of the Southern Hemisphere is considered to be a result of the buoyant thallus (HAY 1978; CHESHIRE *et al.* 1995) and may also be a means of distributing *Xiphophorocolax*. However, the other endophytic brown alga occuring in *D. antarctica*, namely its specific parasite *Herpodiscus durvillaeae*, has not been distributed with its host, but is endemic to the New Zealand archipelago. Infections of *Durvillaea* spp. by Plasmodiophorales, on the other hand, are not only known from host populations in Australia and New Zealand (*Durvillaea potatorum* and *D. antarctica*, respectively), but also from Chilean *D. antarctica* (AGUILERA *et al.* 1988). It has not been examined so far, though, whether the myxomycetes from both sides of the Pacific are closely related or even identical, which may be a clue for a recent gene flow between myxomycete and host populations, respectively.

3.2.4.5 Putative relatives of *X. aotearoae*

Assuming that *Xiphophorocolax* is endemic, which brown algae in New Zealand would be its closest relatives? *X. aotearoae* appears to be more closely related to free-living genera than to other endophytes, including those present in New Zealand. Recent molecular systematic studies revealed that within the Phaeophyceae, endophytism must have evolved several times, e. g. in *Laminarionema elsbetiae* and *Onslowia endophytica* (PETERS & BURKHARDT 1998; DRAISMA & PRUD'HOMME VAN REINE 2001; DRAISMA *et al.* 2001). Moreover, the close relationship of endophytic brown algae with epiphytic species show that the endophytes are most likely derived from epiphytes. For example, the genus *Microspongium* comprises both epiphytic and endophytic

species (e. g. *M. gelatinosum* vs. *M. tenuissimum*), while the type species of the genus *Laminariocolax*, *L. tomentosoides*, is an epi-endophyte (BURKHARDT & PETERS 1998). Likewise, the closest relatives of *Xiphophorocolax* may be found among epiphytic brown algae. *Xiphophorocolax* displays a relatively simple thallus structure, which may represent a reduced morphology. However, it could also be a stage in the life cycle of another, possibly epiphytic alga, such as the species growing on its host algae, i. e. the endemic *Herponema* and *Hecatonema* spp..

The genus *Hecatonema* SAUVAGEAU with its type species *H. maculans* (COLLINS) SAUVAGEAU is characterised by a disc-shaped prostrate base, however, culture studies have shown that the presence vs. absence of this base may be influenced by the substratum (i. e. heteroblasty; PEDERSEN 1984). Similarly, in the genus *Microspongium*, a prostrate base is characteristic for the epiphytic members, but appears to be absent in the endophytic *M. tenuissimum* var. *tenuissmum* (present study). In *Hecatonema*, plurilocular sporangia are nevertheless pluriseriate (KYLIN 1947), thus excluding this genus from a close relationship to *Xiphophorocolax*.

The genus *Herponema* J. AGARDH is also described to have monostromatic basal layers, such as in *H. maculaeformis*, a species, which, moreover, has pluriseriate plurilocular sporangia (LINDAUER 1949). However, the type species of the genus, *H. velutinum* (R. K. GREVILLE) J. G. AGARDH, lacks a distinct basal layer which led LINDAUER (1949) to question its legitimacy as type species. Instead, *H. velutinum* forms vertical filaments which penetrate its basiphyte, and thus it has been termed 'parasitic' (LINDAUER 1949). Prostrate bases are moreover absent in two other species, *H. hormosirae* from New Zealand and *H. desmarestiae* (GRAN) CARDINAL from Europe (as *Feldmannia desmarestiae* (GRAN) KYLIN, KYLIN 1947; NIELSEN *et al.* 1995). The latter, like *H. velutinum*, grows partly endophytic in its basiphyte, *Desmarestia viridis* (O. F. MÜLLER) J. V. LAMOUROUX. Plurilocular sporangia have not been observed in any of these three species thus their structure is unknown (KYLIN 1947; LINDAUER 1949; LINDAUER *et al.* 1961). Also, no information exists about the number of plastids in their cells.

Based on morphological characters, both *Herponema* and *Hecatonema* have been placed in various families, including the Myrionemataceae (Chordariales) and the Punctariaceae (Dictyosiphonales; LINDAUER *et al.* 1961; WOMERSLEY 1987; FLETCHER 1987). The taxonomic positions of the New Zealand species are still unresolved (ADAMS 1994). According to recent molecular systematic studies, brown algae with stalked pyrenoids, i. e. members of the Ectocarpales, Chordariales, Scytosiphonales, Punctariales and Dictyosiphonales, are closely related to each other, but are distant from all other brown algae and thus should be merged in an

extended order Ectocarpales (TAN & DRUEHL 1993; ROUSSEAU & DE REVIERS 1999). Within the Ectocarpales *s. l.*, five families can be distinguished, of which one, the extended Chordariaceae, includes former members of the Dictyosiphonales, e. g. the former families Myriotrichaceae, Punctariaceae, Striariaceae, but also the type species of the former Myrionemataceae, *Myrionema strangulans* GREVILLE (PETERS & RAMIREZ 2001). Accordingly, *Herponema* and *Hecatonema* would be included in the same family as *Xiphophorocolax*, the Chordariaceae. However, SIEMER (1998) showed that, according to *rbc*L sequences, the genus *Hecatonema* is polyphyletic, possibly due to mis-identifications, as some members of the former Punctariaceae (Chordariaceae *s. l.*) have heteromorphic life cycles with *Hecatonema*-like microstages (PEDERSEN 1984). One of the isolates included in her analyses, tentatively called *H. maculans* (COLLINS) SAUVAGEAU, had nearly identical sequences with *Punctaria tenuissima* (J. AGARDH) GREVILLE, while the other isolate, *Hecatonema* sp. from Leigh, North Island, New Zealand, was not closely related to members of the Chordariaceae *s. l.* (SIEMER 1998). Whether this is generally true for members of the genus *Hecatonema* in New Zealand, and thus what their relationship is to epiphyte species from other parts of the world or even to *X. aotearoae*, remains to be investigated. Neither type material of *H. maculans* nor other isolates from the type locality have been studied with molecular genetic methods. Similarly, no *Herponema* species has been subjected to phylogenetic studies.

So far *X. aotearoae* has been isolated mainly from single host specimens showing conspicuous macroscopic symptoms, however, the endophyte may be further distributed among its various host species. Most host individuals were collected at their upper distribution limits, i. e. in the lower intertidal (e. g. *Xiphophora gladiata* and *Durvillaea antarctica*) or the upper subtidal zone (e. g. *Lessonia tholiformis* and *D. willana*), but the endophyte may also occur in host specimens growing in greater water depths. Further investigations studying the epidemics of infections by *Xiphophorocolax* may reveal not only the prevalence in natural host populations, but also an influence of the endophyte infection on the population structures of its host species. Furthermore, this may also shed some light on whether the presence of *Xiphophorocolax* in *D. antarctica* already infected by *Herpodiscus* was a single event or is a common feature.

3.2.5 Conclusions

The present study emphazises the importance of combining different approaches, in order to reliably classify pigmented endophytic brown algae. Field observations, i. e. of host species and

associated symptoms, may provide preliminary information about the identity of an endophyte. To distinguish entities, however, microscopic observations are needed in order to record basic features of the endophyte morphology, even though it may be restricted by the physical confinement within the host tissue.

Accompanying culture studies may reveal more characters to allow a gross classification of the endophytes, and also determine whether or not the endophyte is a stage in the life history of another macroscopic alga. However, observations in cultures have their limitations, especially in groups such as the Phaeophycean endophytes with simple morphologies and a high phenotypic plasticity. Consequently, PEDERSEN states: "The results obtained by the study of unialgal cultures must preferably be compared with observations from nature, because results from culture studies can always be refuted as being products of artificial conditions" (PEDERSEN 1981b, page 208).

Genetic markers, in contrast, are independent of environmental conditions and moreover contain very detailed information, thus DNA sequence comparisons appear to be the most accurate way to reliably identify taxa on all levels. However, based on the detail gained from DNA sequences it may be tempting to separate entities that are otherwise not distinguishable. In fact, one of the problems arising from the comparison of morphological and molecular characters of the pigmented endophytes found in New Zealand was the question of species boundaries. I. e., on what taxonomic level should entities be recognized, if they are distinguished only by molecular markers within a comparatively variable gene region, but which are neither sufficiently separable by their morphology, host specifity nor associated infection symptoms, and which are, moreover, not isolated in space or time? Does, for example, the genotypic plasticity discovered in the Southern Hemisphere *Laminariocolax* complex justify a separation at species level?

The position of species as the central taxonomic entities in biological studies has triggered many discussions among biologists. The most widely considered concepts are Biological, Phylogenetic and Evolutionary Species Concepts (CRACRAFT 2000). Biological species concepts such as *sensu* MAYR (2000) or the Hennigian Species Concept (MEIER & WILLMANN 2000) are based on the reproductive isolation of populations or groups of populations delimiting a species from another. However, these concepts cannot, *per definitionem*, be applied to asexual organisms and thus cause problems in classifying groups in which sexuality may be rare or lacking, such as the endophytic brown algae.

Phylogenetic Species Concepts (e. g. *sensu* WHEELER & PLATNIK 2000 or *sensu* MISHLER & THERIOT 2000) as well as the Evolutinary Species Concept (WILEY & MAYDEN 2000), on the other hand, emphasize common lineages of descendancy as the most important criterion, but with different approaches on how to apply this criterion to delimit species, e. g. by shared morphological characters, monophyly and/or apomorphy. Some of these concepts (i. e. those *sensu* MISHLER & THERIOT 2000 or WILEY & MAYDEN 2000) are criticised (by defenders of biologial species concepts) for not using objective criteria (such as the ability vs. inability to interbreed). Instead they use subjective and thus potentially artificial delimitations to define species boundaries, and by this, essentially return to the out-of-date Morphological or Typological Species Concepts. These state that entities should be regarded as separate species, as long as they are sufficiently morphologically different, or "a species is what a competent taxonomist says it is" (REGAN 1926, cited in MEIER & WILLMANN 2000, page 36). The Phylogenetic Species Concept *sensu* WHEELER & PLATNICK avoids this by considering species as "diagnosable by a unique combination of character states" (NIXON & WHEELER 1990, cited in MEIER & WILLMANN 2000), however, this may result in exploding numbers of species, when, for example, any genetic mutations are considered unique character states. Accordingly, in applying this concept to the endophytes from New Zealand, every variety would have to be considered as a separate species. This would make generalizations problematical, for example, regarding the prevalences of infection in *Macrocystis* populations, which contain more than one *Laminariocolax* 'species'.

Consequently, as no agreement on a single species concept exists among biologists, decisions have to be made on a case to case basis. Which species concept is applicable, may depend on the aims of a study, upon what level groups of organisms need to be united, in order to allow generalizations without having to study every single sub-unit or even every individual of a group. Objectives may, for example, be to record the number of different entities of a certain group in order to describe the diversity in a certain area, or to describe an ecological niche organisms occupy in their environment.

For the purposes of the present study, the Phylogenetic Species Concept *sensu* MISHLER & THERIOT, using monophyly and apomorphies as criteria to distinguish species (MISHLER & THERIOT 2000), was considered to provide the most practicable approach, to deal for example with ecological questions, even though the proposed species boundaries are subjective and thus may be artificial. In order to apply this broader species concept, results based on molecular markers need to be put into perspective; to do so, field and culture observations should

accompany molecular systematic studies and vice versa, in an extension of PEDERSEN's statement (PEDERSEN 1981b).

Thus, based on the present study, some assumptions can be made for macroscopic observations in the field, to aid the identification of endophytic brown algal species in New Zealand: *Macrocystis pyrifera* is most likely infected by *Laminariocolax macrocystis*, while members of the Fucales as well as *Lessonia tholiformis* typically host *Xiphophorocolax aotearoae*. Additional microscopic observations in *Ecklonia radiata* (i. e. recording the number of plastids per endophyte cell) may reveal whether a specimen is infected by *L. macrocystis* or *Microspongium tenuissimum*. However, even though *M. tenuissimum* was the only endophyte isolated from red algal hosts during the present study, this cannot be generalizised, until the taxonomic status of *Mikrosyphar pachymeniae* has been revealed. Moreover, all three endophyte species have been shown to infect hosts belonging to different orders or even phyla (PETERS 2003; present study). Thus, further generalizations should be avoided, until host preferences have been confirmed in cross-infection studies.

Regardless of the taxonomical level at which they are recognized, endophytic brown algae displayed a high diversity in New Zealand. Moreover, they were frequently encountered in all macroalgal communities examined. Most host species are widely distributed among New Zealands islands, thus endophytic brown algae are expected to occur throughout the whole New Zealand archipelago. New Zealand's sub-antarctic islands, for example, share their marine flora with the south of South Island (FINERAN 1969; HAY *et al.* 1985; HAY 1987), therefore, the endophytes are likely to be found there as well. On the other side, the geographical range of *D. antarctica* includes the Three King Islands, while *Ecklonia radiata* is even found on the Kermadec Islands (NELSON & ADAMS 1984; ADAMS & NELSON 1985), thus providing a potential habitat for *Laminariocolax macrocystis* and *Microspongium tenuissimum* in the most northern part of New Zealand. Here, the marine flora shows a slightly different species composition, which includes more subtropical taxa (ADAMS & NELSON 1985; NELSON & ADAMS 1987; ADAMS 1994), among which additional endophyte species or varieties may be discovered.

Furthermore, the New Zealand kelp species which have not, or only on single occasions, been examined, may also to a certain degree be infected by pigmented endophytic brown algae. In case of *Lessonia variegata*, only some drift material from Castle Point (North Island) and a few specimens from a rock pool at Seal Point (Otago Peninsula) was examined, but these did not show any macro- or microscopical signs of infection. Specimens of *Ecklonia brevipes*, *L. adamsiae* and *L. brevifolia* were not available for examination.

Interestingly, the introduced kelp *Undaria pinnatifida* was only infected by *M. tenuissimum* and may not be susceptible to the New Zealand kelp endophyte *L. macrocystis*. *U. pinnatifida* is a highly invasive species and thus is considered a threat to native kelp communities (ANONYMOUS 1999). In Japan and in Europe, it is frequently attacked and severely affected by another *Laminariocolax* species, *L. aecidioides* (YOSHIDA & AKIYAMA 1979; VEIGA *et al.* 1997). Cross-infection studies may reveal whether *L. macrocystis* is capable of infecting *Undaria* at all. Moreover, in New Zealand *U. pinnatifida* is an annual species, in contrast to the perennial native kelps. As pigmented endophytic brown algae only invade the interstices of their host tissue but not the cells, endophytes cannot infect the filamentous kelp gametophytes, but only the sporophytes. Accordingly, the longevity of the *Macrocystis* and *Ecklonia* sporophytes may allow the endophytes to firmly establish themselves in the host, while in the opportunistic *Undaria*, sporophytes only persist for a few months (HAY & VILLOUTA 1993) and thus may give the endophytes less opportunities to settle and to severily affect their health. Therefore, even if *Laminariocolax macrocystis* was able to infect *Undaria*, it seems unlikely that the endophyte may serve as a means to control this invasive species.

4 *HERPODISCUS DURVILLAEAE* – RESULTS AND DISCUSSION

4.1 Results

4.1.1 Molecular systematics

4.1.1.1 Sequence statistics

Sequence statistics of *Herpodiscus durvillaeae* are compiled in Table 4.1. GC contents for amplified partial genes were: 18S: 51.60%; ITS1: 53.33%; 26S: 57.98%; *rbc*L: 37.51%.

Table 4.1: Statistics for amplified parts of nuclear encoded rDNA and of plastid encoded RuBisCO DNA sequences of *Herpodiscus durvillaeae*.

gene		RuBisCO gene	nuclear encoded ribosomal gene		
		partial *rbc*L	partial 18S	ITS 1	partial 26S
length [bp]		981	533	300	595
base frequencies [%]	A	29.867	21.764	23.860	23.529
	C	15.189	23.265	25.965	25.714
	G	22.324	28.330	27.368	32.269
	T	32.620	26.642	22.807	18.487

4.1.1.2 Alignment properties

Subunits of nrDNA and RuBisCO genes were analysed separately and in combined data sets. Alignments for *rbc*L, 18S and 26S of nrDNA are given in Appendix D (D 3.2-D 3.4), the properties of the alignments (separate and combined data sets) are summarized in Table 4.2. Pairwise comparisons of distances of included sequences for *rbc*L and nrDNA subunits (absolute and Kimura-2-parameter distances) are provided in Appendix D (D 4.2).

As far as possible, alignments comprised representatives of all Phaeophycean orders currently recognized. At the time of writing, no *rbc*L sequences were available for the Ascoseirales, Ralfsiales and Cutleriales, and for some other brown algae included in the nrDNA analyses. Some species of doubtful phylogentic affinities within the Phaeophyceae were

excluded from the analyses, such as *Bachelotia antillarum* (GRUNOW) GERLOFF and *Halosiphon tomentosum* (LYNGB.) JAASUND.

Even though *Choristocarpus tenellus* appeared to be related to *Herpodiscus durvillaeae* when analysing nrDNA data (section 4.1.1.3), this species was excluded from the analyses combining nuclear and plastid encoded genes: partition homogeneity tests revealed that including *C. tenellus* led to nrDNA and *rbc*L data being incongruent. Removing this species from the combined data sets on the other hand resulted in p-values well above 0.05 (Table 4.2), meaning data sets were no longer significantly different (FARRIS *et al.* 1995).

Neither of the species omitted from the data sets were identical with or were closely related to *Herpodiscus durvillaeae*. A BLAST search on the 20.3.2003 confirmed that sequences of *H. durvillaeae* were not identical to any other sequences submitted up to that date.

4.1.1.2.1 18S nrDNA

The alignment of the ribosomal small subunit gene (Appendix D, D 3.2) had a length of 543 base pairs. The partial sequences comprised positions 1285-1818 of the 3'-end of the complete 18S sequence of *Scytosiphon lomentaria* (length: 1818 base pairs; KAWAI *et al.* 1995). Thirty six phaeophacean taxa and the outgroup species *Tribonema aequale* were aligned. For several of these sequences, some positions at one or both ends were unknown. Before analysing, the data set was therefore reduced to 268 base pairs (positions 1494-1754 of KAWAI's *S. lomentaria* sequence) to avoid too many ambiguities.

The average transition/transversion ratio including the outgroup was $ti/tv = 1.42937$, without outgroup $ti/tv = 1.46181$. These values indicate that saturation was not reached as values were well above $ti/tv = 1$ (HOLMQUIST 1983; BAKKER *et al.* 1995). The average base composition for this alignment was: A: 0.22002, C: 0.24936, G: 0.25681, and T: 0.27381.

4.1.1.2.2 26S nrDNA

With sequences from 39 Phaeophyceae plus outgroup species aligned, this alignment of partial 26S sequences (Appendix D, D 3.3) contained the highest number of species. It was 648 base pairs in length, comprising positions 66-612 of the 5'-end of the complete 26S sequence of *Scytosiphon lomentaria* (length: 4245 base pairs; KAWAI *et al.* 1995). This included the variable D1 and D2 regions (D1: positions 45-216 and D2: positions 381-629 of the alignment; ROUSSEAU *et al.* 1997). The alignment was not completely unambiguous. Some ambiguities were due to

problems to align the outgroup species, *Tribonema aequale*, e. g. leading to some gaps at the 3'-end of the alignment (for example positions 580-617 of the alignment).

Average transition/transversion ratios of $ti/tv = 0.96$ (including outgroup) and $ti/tv = 0.98$ (without outgroup) indicated some degree of saturation. Average base composition was A: 0.22369, C: 0.23433, G: 0.32650, and T: 0.21548.

4.1.1.2.3 *rbc*L gene (Phaeophyceae)

With a length of 1255 base pairs, the partial alignment of the *rbc*L (Appendix D, D 3.4) comprised ca. 85.5% of the complete Phaeophycean RuBisCO large subunit gene sequence (1467 bp; SIEMER *et al.* 1998; DRAISMA *et al.* 2002). Compared to nrDNA, fewer *rbc*L sequences were available for brown algae. Only 27 taxa were therefore joined in this alignment, including 26 Phaeophyceae and *T. aequale* as the outgroup.

Some ambiguities were due to a few sequences not covering the whole length of the alignment: Regions of unidentified bases started in *Herpodiscus durvillaeae* from position 865 of the alignment onwards, in *Alaria esculenta* (L.) GREVILLE from position 1018 onwards, in *Desmarestia aculeata* (L.) LAMOUROUX from position 1159 onwards, in *Asterocladon lobatum* MÜLLER *et al.* from position 1220 onwards and in *Tribonema aequale* from position 1241 onwards. Average transition/transversion ratios of $ti/tv = 0.98$ (including outgroup) and $ti/tv = 1.0$ (without outgroup) indicate some saturation for this alignment. The average base composition was: A: 0.29741, C: 0.15510, G: 0.21807, and T: 0.32942.

4.1.1.2.4 *rbc*L gene (Sphacelariales only)

This *rbc*L alignment was based on the previous one (Appendix D, D 3.4), but with a modified taxon sampling, closely following DRAISMA *et al.* (2002): For analysing the affinities of *Herpodiscus durvillaeae* within the Sphacelariales, taxa other than Sphacelariales *sensu lato* were reduced to two *Dictyota* species. Instead, 15 additional species of Sphacelariales were included.

Three of the 27 sequences included contained regions of unidentified bases: *Sphacelaria radicans* (DILLWYN) C. AGARDH (positions 478-1065), *Herpodiscus durvillaeae* (from position 865 onwards) and *Halopteris filicina* (GRATELOUP) KÜTZING (no. 1; first 15 positions). These unidentified characters caused some ambiguities during the phylogenetic analyses.

Even though the *rbc*L alignment showed some saturation when representatives of the whole class of Phaeophyceae were included, no saturation was found aligning the Sphacelariales: the average transition/transversion ratio was $ti/tv = 1.24$ including the outgroup and $ti/tv = 1.52$

for the ingroup only. The average base composition was: A: 0.29936, C: 0.15039, G: 0.21677, and T: 0.33347.

4.1.1.2.5 Internal transcribed spacer 1 of nrDNA

ITS1 sequences of *Herpodiscus durvillaeae* and of species of the order Sphacelariales available in GenBank were unalignable over most positions. This made any phylogenetic analyses of the ITS1 gene for this group impossible.

4.1.1.3 Phylogenetic analyses

The properties of all alignments and the statistics for the most parsimonious trees are summarized in Table 4.2.

Table 4.2: Summary of properties of the *Herpodiscus* alignments and combinations of them, and of tree statistics for parsimony analyses. CI: consistency index; RI: retention index; RC: rescaled consistency index; n/a: not applicable; n. d.: not determined; *: inferred from 100 replicates.

gene	18S	26S	*rbc*L	1st combined data set 18S + 26S	2nd combined data set 26S + *rbc*L	3rd combined data set 18S + 26S + *rbc*L	Sphacelariales only *rbc*L
No. of taxa	37	40	27	37	26	24	27
Length of alignment	268	647	1255	915	1902	2170	1255
p-value (PHT)	n/a	n/a	n/a	0.66*	0.113	0.323	n/a
Variable positions	84 (31.3%)	309 (47.8%)	533 (42.5%)	392 (42.8%)	786 (41.3%)	858 (39.5%)	484 (38.6%)
Informative sites	59 (22.0%)	202 (31.2%)	397 (31.6%)	258 (28.2%)	531 (27.9%)	560 (25.8%)	320 (25.5%)
ti/*tv* ratio with/ without outgroup	1.42937/ 1.46181	0.96920/ 0.99563	0.98416/ 1.00489	0.99411/ 1.01217	0.96942/ 0.99312	1.00431/ 1.03178	1.23519/ 1.52000
No. of most parsimonious trees	17874	2	4	4	8	3	1
No. of trees within one step of MP trees	n. d.	51	18	23	22	7	37
Length of most parsimonious trees [steps]	237	1178	1948	1388	2697	2668	1362
CI	0.570	0.458	0.402	0.483	0.446	0.485	0.486
RI	0.664	0.537	0.425	0.542	0.442	0.465	0.519
RC	0.378	0.246	0.171	0.262	0.197	0.225	0.252
Tree topology	not shown	Figure 4.1	Figure 4.3	Figure 4.2	Figure 4.4	Figure 4.5	Figure 4.6

4.1.1.3.1 18S nrDNA

The partial sequences of the 18S gene proved to be very conservative. Only a comparatively small percentage of informative sites among the joined brown algal taxa, around 22%, was found, resulting in long computing times and poor resolution of phylogenetic trees.

Maximum parsimony analyses led to 17874 equally parsimonious trees. In the strict consensus of MP trees (not shown), only three monophyletic clusters were recovered, Ectocarpales *s. l.*, Dictyotales and a subset of Fucales, consisting of three species (*Ascophyllum nodosum* (L.) LEJOLIS, *Fucus vesiculosus* L., *Xiphophora chondrophylla* (R. BROWN *ex* TURNER)

MONTAGNE *ex* HARVEY) clustering together. The other Fucales (*sensu* DE REVIERS & ROUSSEAU 1999), i. e. *Sargassum muticum* (YENDO) FENSHOLT, *Notheia anomala* and *Durvillaea antarctica*, were not separated from the rest of the brown algae. None of the other Phaeophycean orders were recovered under MP analyses.

The three clusters were also represented in the neighbour-joining tree (not shown), with branches receiving high bootstrap support (Ectocarpales *s. l.*: 99%; Dictyotales: 90%; subset of Fucales: 99%; 700 replicates). Additionally, in this analysis, other groups such as the Laminariales (together with *Sporochnuns pedunculatus* (HUDSON) C. AGARDH) and the Scytothamnales were also recovered, although these groups received only low to moderate support (Scytothamnales: 55%; Laminariales + *Sporochnus*: 73%).

Herpodiscus durvillaeae clustered with the Sphacelariales *s. s.*, *Syringoderma phinneyi* HENRY & MÜLLER and *Onslowia endophytica*, but this clade was also only moderately supported (65%). Apart from poorly supported Stypocaulaceae (comprising *Alethocladus corymbosus* (DICKIE) SAUVAGEAU and *Stypocaulon scoparium* (L.) KÜTZING; 54%), relationships were not further resolved within this group.

No bootstrap analyses were performed on the 18S data set using maximum parsimony as optimality criterion - the large size of the data set in combination with the low number of informative characters did not allow a sufficient number of replicates without the computer running out of memory. For the same reason, maximum likelihood analyses were not performed either. Overall, the 18S data set was not informative enough to effectively resolve relationships within the Phaeophyceae. For analyses, it had to be combined with 26S, or 26S and *rbc*L sequence data.

4.1.1.3.2 26S nrDNA

Two equally parsimonious trees were inferred from the 26S data set, with a length of 1178 steps (Figure 4.1). In both trees, Dictyotales was the most basal group. This monophyletic order had high bootstrap support, but the branch separating it from the other brown algae was only moderately supported. Above this clade, two species branched off, *Onslowia endophytica* and *Verosphacela ebrachia* (Onslowiaceae, Sphacelariales *s. l.* or *Incertae sedis*, depending on authors; HENRY 1987a; DRAISMA *et al.* 2002). The two MP trees differed in their positions; in one tree, these two species grouped together, while in the other tree, they stood in hierachial order (in which case *O. endophytica* took the upper position). While the Onslowiaceae had moderate bootstrap support (68%), this branch collapsed at one further step in decay analysis.

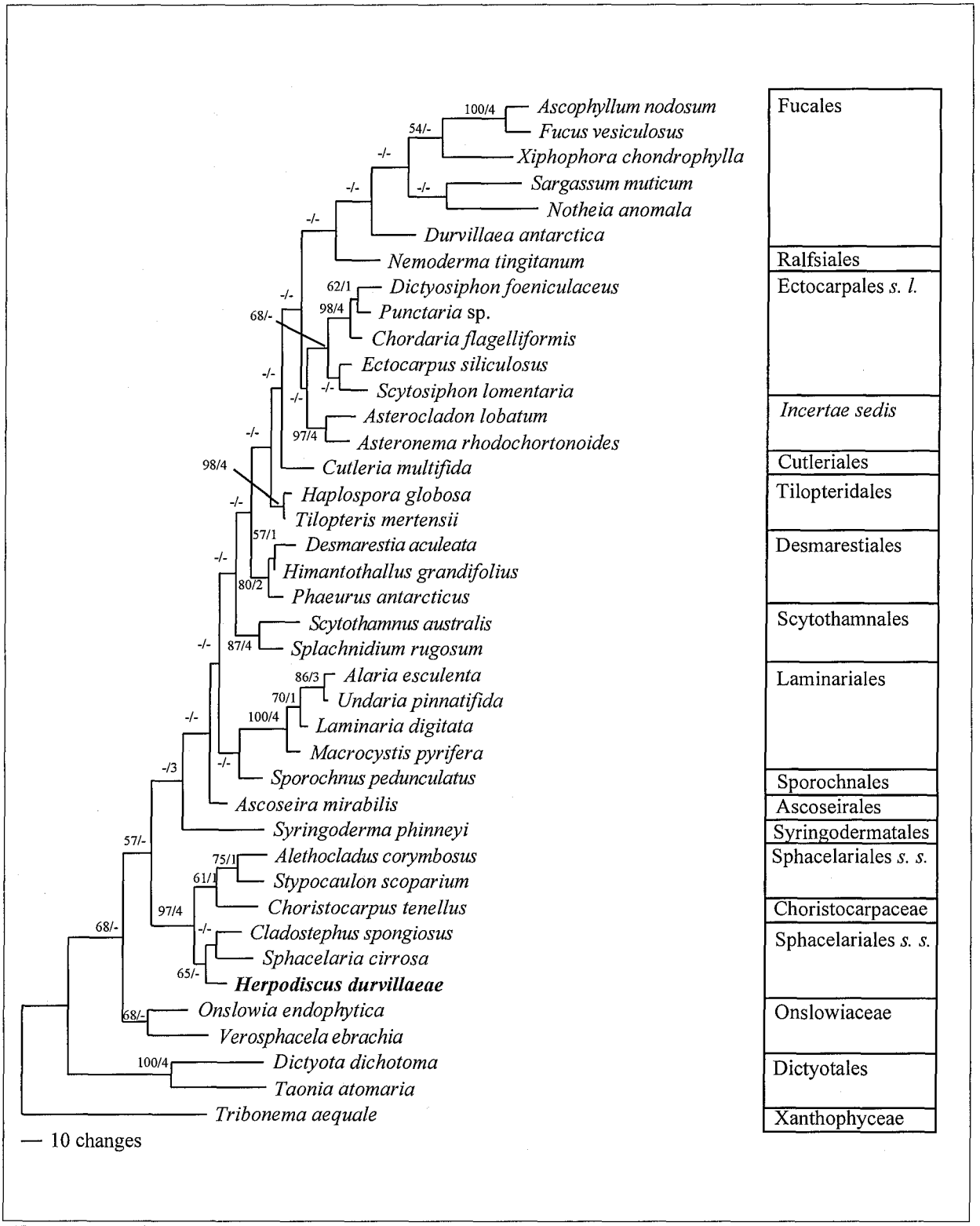


Figure 4.1: Phylogenetic tree for the Phaeophyceae inferred from 26S sequences. One of two most parsimonious trees in phylogram style. Numbers indicate bootstrap values (left) and decay indices (right). Dashes indicate that branches received a bootstrap support of 50% or less, or collapsed within one further step.

Herpodiscus durvillaeae clustered with the Sphacelariales in a well supported monophyletic clade (97%), that branched off above Onslowiaceae. Within this clade, *H. durvillaeae* formed a group together with *Cladostephus spongiosus* (HUDSON) C. AGARDH and *Sphacelaria cirrosa* (ROTH) C. AGARDH. *Choristocarpus tenellus* (Sphacelariales *s. l.* or *Incertae sedis*, depending on authors) stood at the base of the Stypocaulaceae. Branches separating these two groups had only a moderate bootstrap support of 61% and 65 %, respectively.

Above Sphacelariales, most taxa clustered in monophyletic clades representing the currently recognized Phaeophycean orders, e. g., Laminariales, Scytothamnales, Desmarestiales, Tilopteridales, and Ectocarpales *s. l.*. The brown algal orders themselves were well supported, the relationships between the clades did not have any support, though: the inner topology of the MP tree collapsed during bootstrap analysis.

Within the Fucales, there was only poor support (54%) for a clade of three species, *Ascophyllum nodosum*, *Fucus vesiculosus* and *Xiphophora chondrophylla*, while the positions of *Sargassum muticum*, *Notheia anomala* and *Durvillaea antarctica* on the base of this clade were not supported at all. *Syringoderma phinneyi* and *Ascoseira mirabilis* SKOTTSBERG as single representatives of their orders (Syringodermatales and Ascoseirales, respectively) stood between Sphacelariales and Laminariales, while *Nemoderma tingitanum* SCHOUSBOE *in* BORNET (Ralfsiales) grouped with the Fucales. Sporochnales formed a sister taxon to the Laminariales, but positions of both *Sporochnus pedunculatus* and *N. tingitanum* had bootstrap values of less than 50%.

Neighbour-joining and maximum likelihood trees showed a topology similar to the MP tree, with Dictyotales as first diverging branch, followed by Onslowiaceae and Sphacelariales *s. s.* (including *Choristocarpus tenellus*) and, in the 'crown', the rest of Phaeophyceae. While these branches were moderately supported in the NJ tree, all branches between monophyletic orders in the ML tree collapsed during bootstrapping, at the base as well as within the crown.

In the crown of the NJ tree, in comparison, branches were more strongly supported than in the MP trees. For example, Sporochnales as a sister taxon to the Laminariales, or *Durvillaea antarctica*, *Sargassum muticum*, and *Notheia anomala* belonging to the Fucales (positioned at the base of the crown) had some moderate support. But most branches in the crown still collapsed in bootstrap analysis.

Despite differences between MP, distance and ML trees (i. e. in the topology of the crown), with all three methods *Herpodiscus durvillaeae* unambigously clustered with the Sphacelariales *s. s.*. In all three analyses, this order was monophyletic and had strong bootstrap support (97% in MP tree, 98% in NJ tree, 96% in ML tree).

First combined data set: 18S + 26S

By combining the18S and 26S alignments, the number of joined taxa had to be reduced to 37 as 18S sequences were not available for *Tilopteris mertensii* (TURNER in SMITH) KÜTZING, *Verosphacela ebrachia* and *Undaria pinnatifida*. A partition homogeneity test before maximum parsimony analysis revealed 18S and 26S data to be congruent (p = 0.66; 100 replicates).

Four equally parsimonious trees with a length of 1388 steps were inferred from the combined data set (one tree shown in Figure 4.2). Differences between MP trees lay in the branching order of Ascoseirales, Scytothamnales and Laminariales + Sporochnales within the crown. Apart from this, the analysis led to a similar tree topology as analysing 26S sequences alone, but with stabilised branches.

In contrast, the Sphacelariales *s. s.* (incl. *Choristocarpus tenellus*) received less, but still strong bootstrap support. By adding 18S sequences to the analysis, this order, together with the Syringodermatales, was separated from the 'crown' group of Phaeophyceae and placed with *Onslowia endophytica* near to the base of the tree, above Dictyotales.

Within the crown, branches received slightly higher bootstrap and decay values compared to 26S analyses alone. An inclusion of *Durvillaea antartica*, *Notheia anomala* and *Sargassum muticum* into the Fucales was moderately supported, as was the grouping of Ectocarpales with *Asterocladon* + *Asteronema* and Laminariales with Sporochnales. However, support was still too low to stabilize branches between orders, which collapsed during bootstrap analysis.

The maximum likelihood tree was basically the same as the MP trees, apart from the relative arrangement of orders within the crown. As for parsimony analyses, branches collapsed during bootstrap analysis (e. g. positions of *Ascoseira mirabilis* or Cutleriales), leveling out differences between trees. In ML as well as MP trees, Dictyotales were the first diverging brown algae. But in contrast to the parsimony trees, in the ML tree this branch received no bootstrap support at all, leaving the relationships between Dictyotales, *Onslowia endophytica* and Sphacelariales *s. s.* (including *Choristocarpus tenellus*) unresolved.

In the neighbour-joining tree, monophyletic orders received slightly higher bootstrap support as in MP and ML analyses, but again, relationships between the orders within the crown were not resolved: all but one branch collapsed during bootstrap analysis. Only the Fucales were separated, but this branch had only low support (56%).

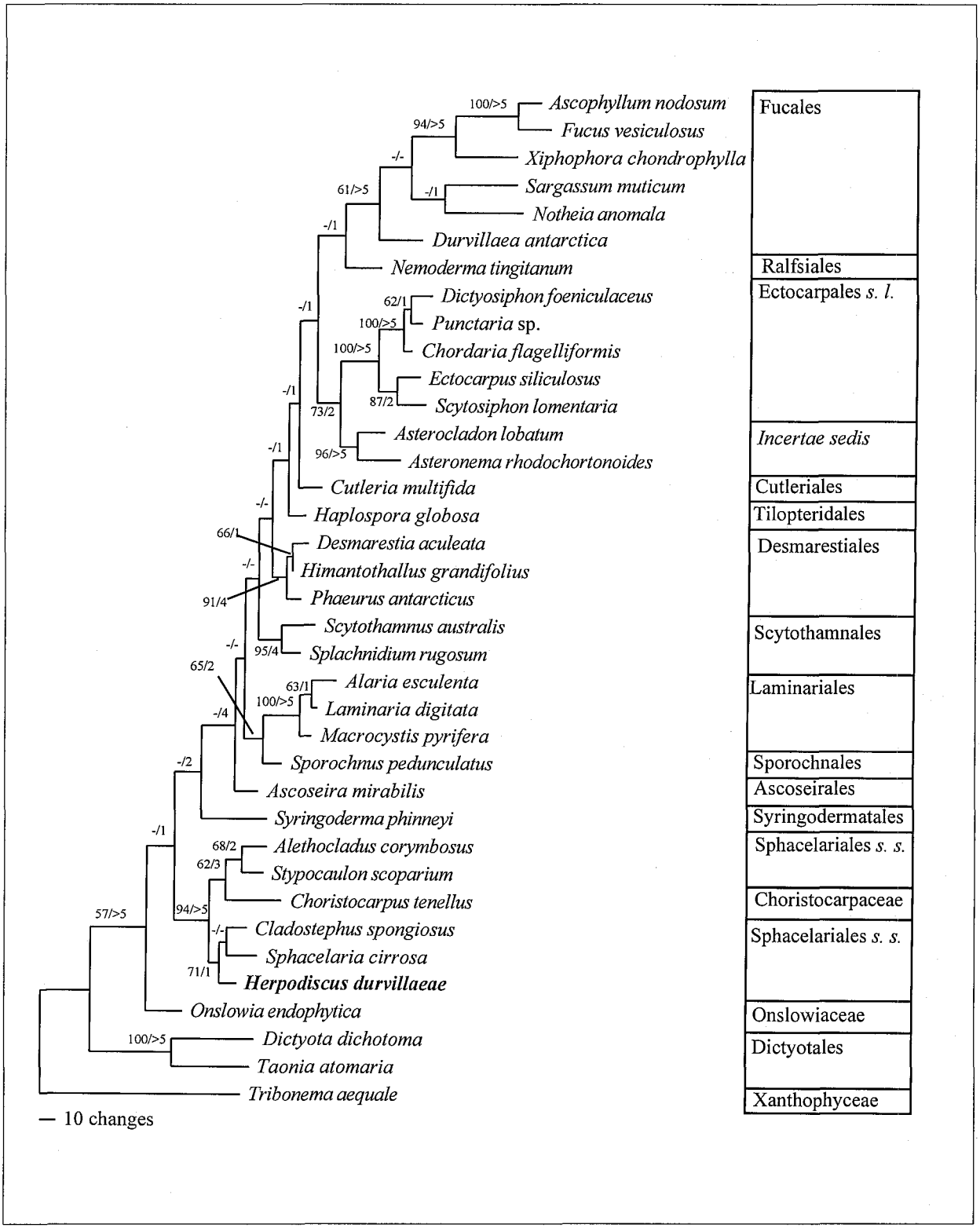


Figure 4.2: Phylogenetic tree for the Phaeophyceae inferred from 18S + 26S sequences. One of four most parsimonious trees in phylogram style. Numbers indicate bootstrap values (left) and decay indices (right). Dashes indicate that branches received a bootstrap support of 50% or less, or collapsed within one further step.

In all three analyses, the Sphacelariales were stronly supported (MP: 94%; ML: 83%; NJ: 98%). These included *Choristocarpus tenellus* as well as *Herpodiscus durvillaeae*, which clustered with *Cladostephus spongiosus* and *Sphacelaria cirrosa.*

4.1.1.3.3 *rbc*L gene (Phaeophyceae)

Maximum parsimony analysis of the *rbc*L alignment led to four MP trees of a length of 1948 steps (of which two are presented in Figure 4.3). In contrast to the nrDNA analyses, the most basic brown alga was *Choristocarpus tenellus* (Sphacelariales *s. l.* or *incertae sedis*).

The family Onslowiaceae (Sphacelariales *s. l.* or *incertae sedis*), positioned above *Dictyota dichotoma*, was moderately supported, but like the Sphacelariales *s. s.* and *Syringoderma phinneyi*, branches did not receive significant bootstrap support. They collapsed within one or two further steps.

Within the crown, a weakly supported clade was formed by *Asterocladon lobatum* with the well supported orders Ectocarpales *s. l.* and Laminariales. Fucales grouped with *Tilopteris mertensii* (Tilopteridales), while the positions of the monophyletic Desmarestiales and Scytothamnales were not recovered in the bootstrap analysis. The position of *Sporochnus scoparius* HARVEY (either between Desmarestiales and Fucales or above the latter) was not resolved either, causing some of the variation among the four MP trees.

Like in nrDNA analyses, *Herpodiscus durvillaeae* clustered with Sphacelariales *s. s.*. Within this order, *Alethocladus corymbosus* and *Stypocaulon scoparium* formed a well supported clade, but the exact positions of *H. durvillaeae* and *Cladostephus spongiosus* were not resolved (Figure 4.3: tree A and B, respectively). All together, Sphacelariales *s. s.* only had low bootstrap support (56%), when analysing the *rbc*L data alone.

Differences between maximum parsimony, maximum likelihood and neighbour-joining trees were mainly located within the crown, regarding the branching order of monophyletic groups such as Desmarestiales, Fucales etc.. As most branches within the crown collapsed during bootstrapping with all three methods, all differences but two vanished: in contrast to NJ and ML trees, in the MP tree the Laminariales were incorporated in a cluster with Ectocarpales *s. l.* and *Asterocladon lobatum.* Moreover, while in likelihood and parsimony trees, Onslowiaceae, Sphacelariales *s. s.* and *Syringoderma phinneyi* diverged in hierachial order, in the NJ tree they clustered together, but without bootstrap support.

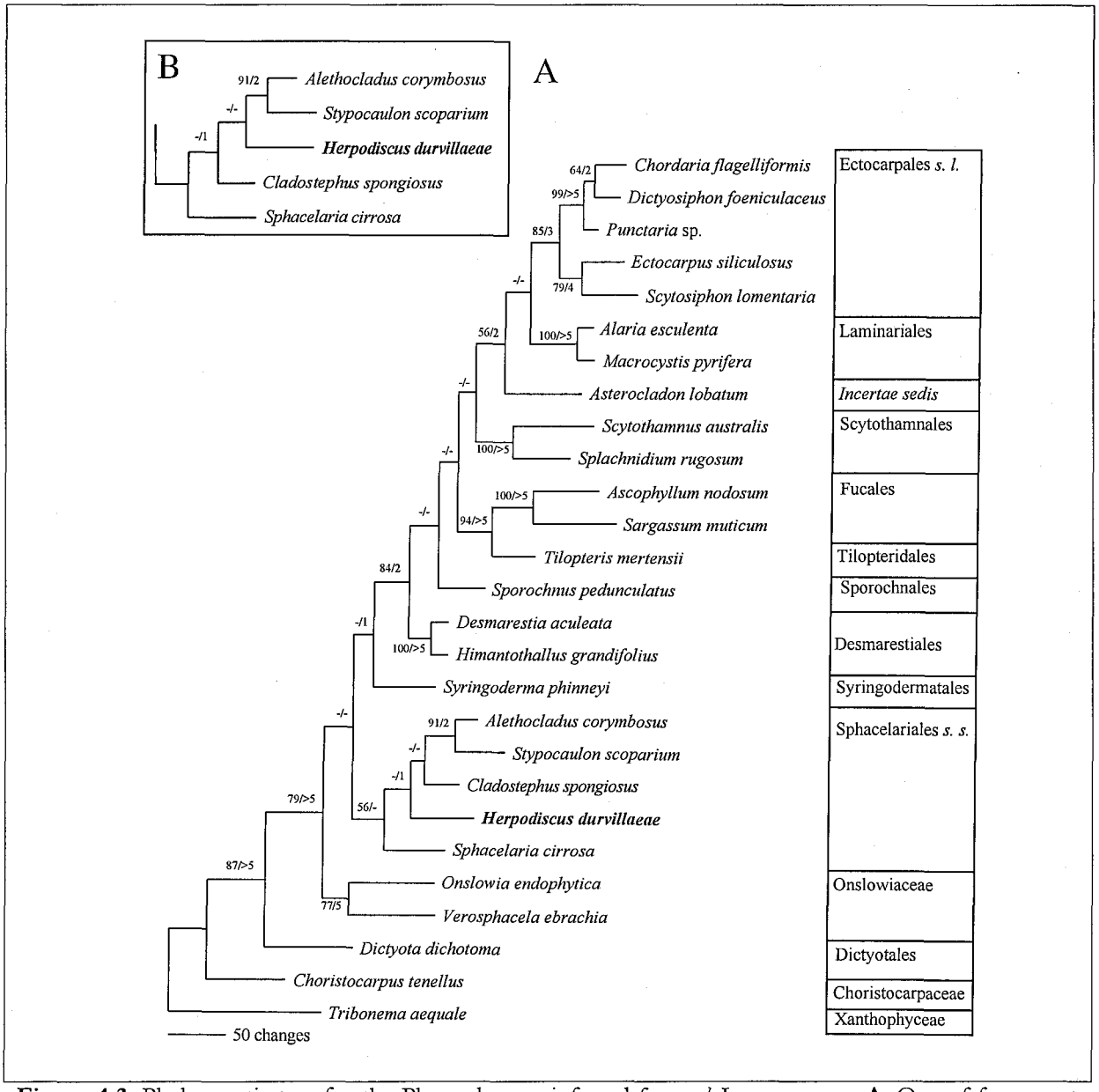


Figure 4.3: Phylogenetic tree for the Phaeophyceae inferred from *rbc*L sequences. **A**: One of four most parsimonious trees shown in phylogram style. **B**: Showing different arrangement within Sphacelariales *s. s.* in two of the four MP trees. Numbers indicate bootstrap values (left) and decay indices (right). Dashes indicate that branches received a bootstrap support of 50% or less, or collapsed within one further step.

Sphacelariales *s. s.* (including *Herpodiscus durvillaeae*) themselves received a higher support in likelihood (83%) and distance analyses (78%) than in parsimony analyses. Apart from the strongly supported Stypocaulaceae however, relationships within the Sphacelariales were not resolved in distance and likelihood trees, either.

Second combined data set: 26S + *rbc*L

For the combined data set, the number of joined taxa was reduced, as *rbc*L sequences were not available for all species of the 26S alignment. Additionally, *Choristocarpus tenellus* was removed prior to analysis (section 4.1.1.2): a partition homogeneity test revealed 26S and *rbc*L data including this species to be significantly different ($p = 0.001$), while without *C. tenellus*, the PHT resulted in $p = 0.113$, meaning data sets were congruent (FARRIS *et al.* 1995; CUNNINGHAM 1997). The combined data set therefore only included 26 taxa.

Over all, the tree topology resulting from the combined data set was very similar to the maximum parsimony trees of the *rbc*L alignment, as this gene contributed almost twice as many informative sites as the 26S gene. Differences between separate and combined analyses were reflected in the eight most parsimonious trees (2697 steps) inferred from the combined data set (one of them shown in Figure 4.4). The differences mainly concerned the exact arrangement of phaeophyceaen orders within the crown: Ectocarpales + *Asterocladon lobatum* and Laminariales either formed a monophyletic cluster, that was positioned on top of the tree, or were separated, with Ectocarpales branching off just above Desmarestiales. Scytothamnales + *Sporochnus* sp., and Fucales + *Tilopteris mertensii* were either sister taxa or stood in hierachial order.

Other branches in the crown which also existed in the *rbc*L tree were strengthened by the addition of 26S data. But, concerning the phylogenetic affinities between orders, most nodes still did not receive any bootstrap support and collapsed within the next step, as in the analyses above.

In the base of the MP trees, there was less support for the Onslowiaceae in the combined tree (57%) than in separate analyses. Sphacelariales *s. s.*, on the other hand received a much higher support due to the addition of 26S data (97%), than in *rbc*L analyses alone (56%). Within the Sphacelariales, only Stypocaulaceae were well supported (99%), while relationships within the second cluster consisting of *Herpodiscus durvillaeae*, *Sphacelaria cirrosa* and *Cladostephus spongiosus* were again not resolved.

Like in the separate 26S tree, the first brown alga to branch off was *Dictyota dichotoma*. When *Choristocarpus tenellus* was included into the combined data set, despite incongruence, this species took the position of the first diverging alga above the outgroup species, but the node separating it from the other Phaeaophyceae was only poorly supported (51%; results not presented).

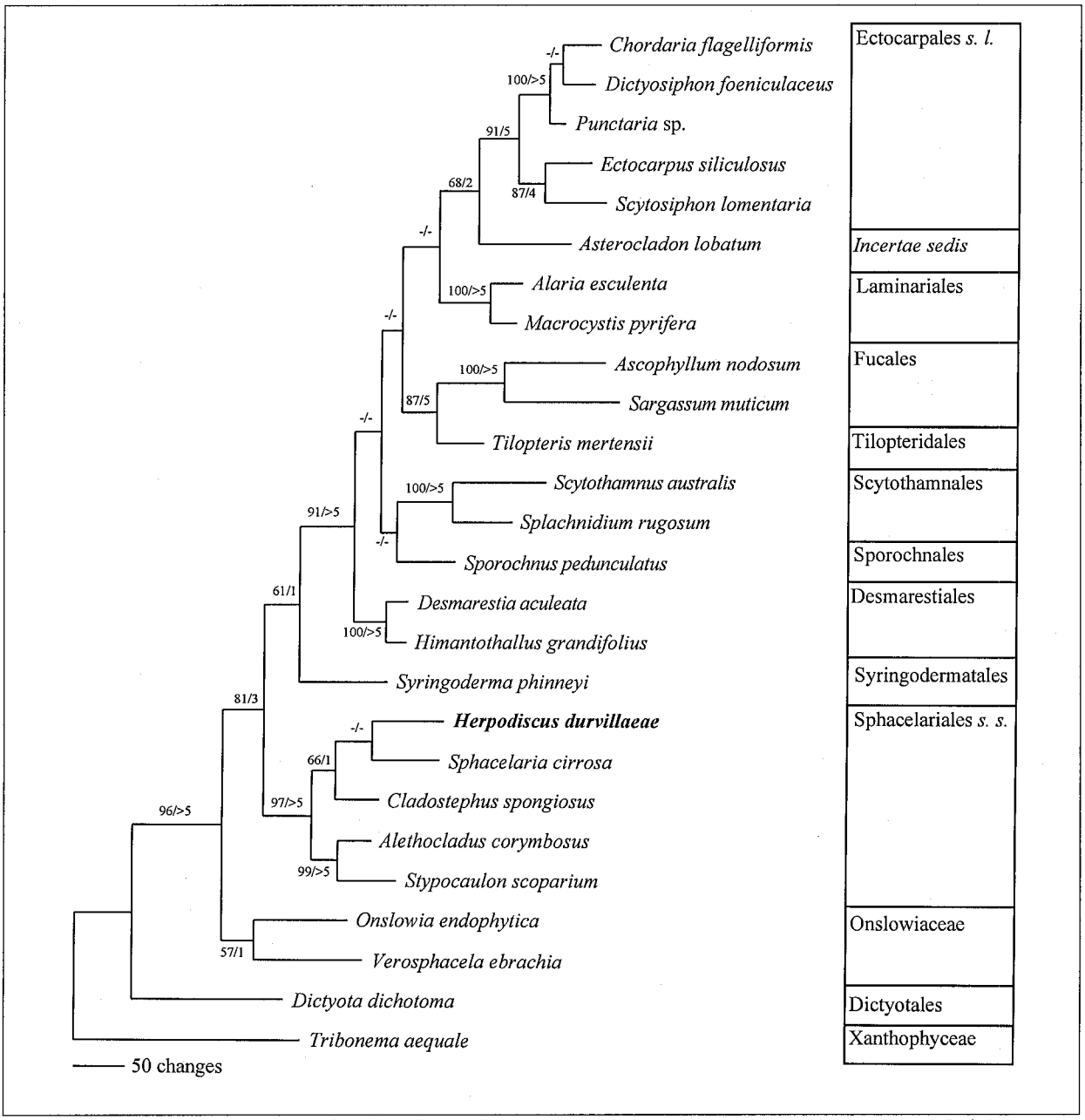


Figure 4.4: Phylogenetic tree for the Phaeophyceae combining *rbc*L and 26S data. One of eight most parsimonious trees in phylogram style Numbers indicate bootstrap values (left) and decay indices (right). Dashes indicate that branches received a bootstrap support of 50% or less, or collapsed within one further step.

The topology of the maximum likelihood tree was identical to the one of the MP tree presented in Figure 4.4, but with slightly better support for relationships: within the crown, for example, the branch separating Ectocarpales + *Asterocladon* and Laminariales from the other orders and the cluster comprising Scytothamnales + *Sporochnus* both still existed after boostrapping. These nodes were only poorly supported (55%), though.

The NJ tree presented a different arrangement of the highly supported monophyletic orders within the crown, but again, most differences disappeared after bootstrapping as branches collapsed, e. g. affinities of *Sporochnus* and *Tilopteris* were not resolved. Only the cluster containing the Ectocapales, *Asterocladon* and Laminariales had moderate support (70%). In the base, the NJ tree was similar to MP and ML trees, apart from *Syringoderma phinneyi*, which grouped, moderately supported (68%), with the Sphacelariales *s. s.*.

Like in separate analyses of nrDNA and *rbc*L data sets, *Herpodiscus durvillaeae* was unambiguously part of the Sphacelariales *s. s.*, which formed a highly supported cluster (97% in the MP tree, 99% in the ML tree, and 100% in the NJ tree). Within this order, Stypocaulaceae again were well supported in all three trees, while relationships between the other three species were only resolved in the distance tree. Here, *Sphacelaria* was the first species to branch off, while *Herpodiscus* + *Cladostephus* formed a well supported clade (82%), opposite to Stypocaulaceae (100%).

Third combined data set: 18S + 26S + *rbc*L

This combined data set contained only 24 taxa as SSU sequences were not available for *Verosphacela ebrachia* and *Tilopteris mertensii*. Like in the second combined data set, partition homogeneity tests revealed the three data sets to be significantly different ($p = 0.001$) if *Choristocarpus tenellus* was included in the analyses. Again, a removal of this species markedly improved the congruence between data sets ($p = 0.323$).

The maximum parsimony analysis resulted in three equally parsimonious trees with a length of 2668 steps. They differed only in the topology within the crown: positions of the Fucales and Scytothamnales were exchanged, and the Ectocarpales *s. l.* branched off either at the base of the crown or above Desmarestiales (shown in Figure 4.5).

The topology of the crown was also the only difference to the MP trees inferred from the second data set: based on the third data set, Laminariales and Sporochnales together with Scytothamnales were positioned on top of the tree, while Ectocarpales *s. l.* branched off before the Fucales. But again, branches separating orders in the crown collapsed during bootstrap analyses, resulting in similar bootstrap consensus trees inferred from either the second or the third combined data set. Bootstrap values were of a similar order.

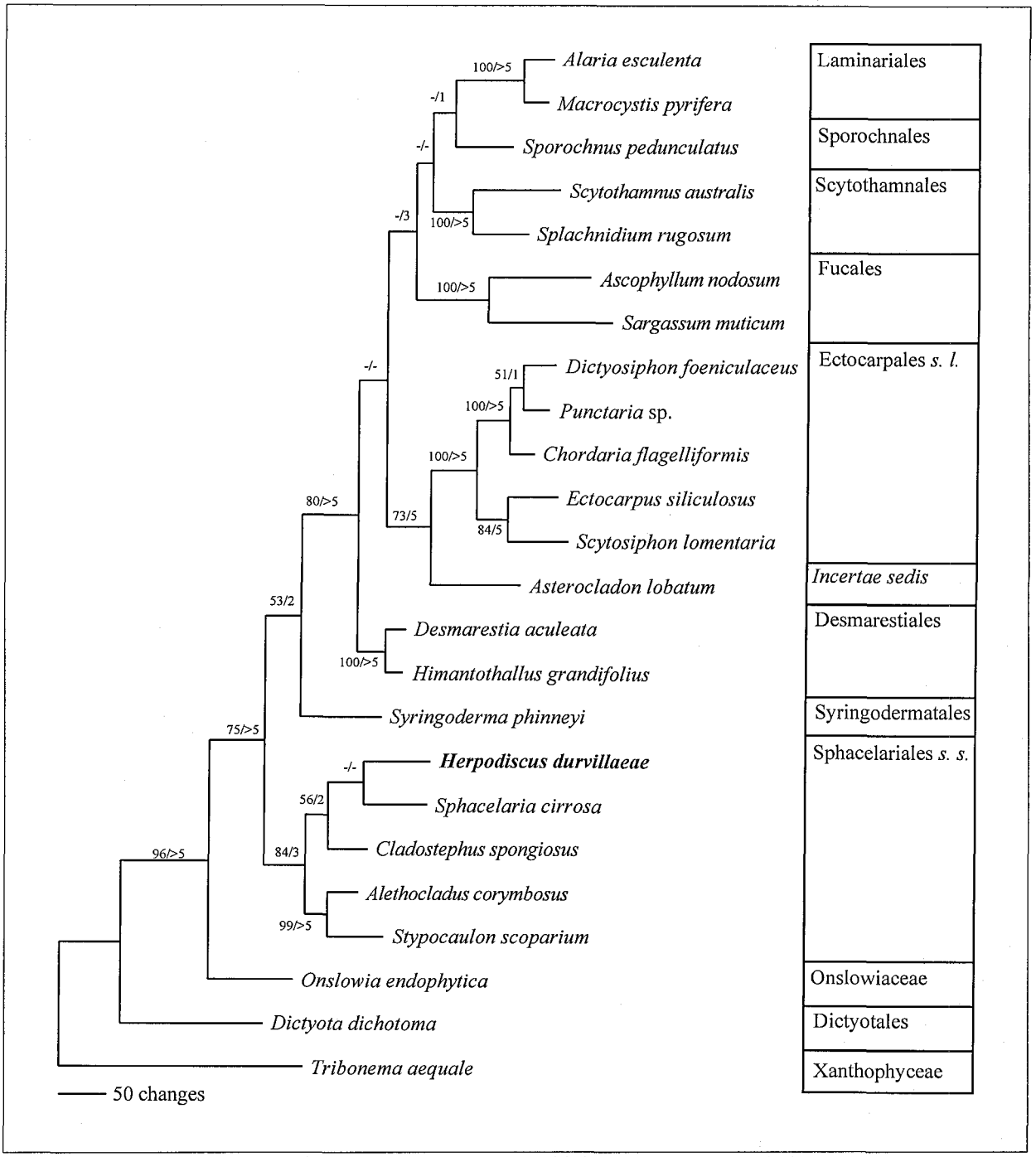


Figure 4.5: Phylogenetic tree for the Phaeophyceae combining *rbc*L and nrDNA data. One of three most parsimonious trees in phylogram style. Numbers indicate bootstrap values (left) and decay indices (right). Dashes indicate that branches received a bootstrap support of 50% or less, or collapsed within one further step.

The ML tree inferred from the third combined data set was similar to the one based on the second data set, but the relationships within the crown were less supported, e. g. regarding the clusters of Scytothamnales with *Sporochnus*, or Laminariales with Ectocarpales *s. l.* + *Asterocladon*. In the NJ tree based on the third data set, this last node collapsed during bootstrapping as well,

in contrast to the tree inferred from the second data set. Additionally, the NJ trees differed in the position of the Fucales, which, according to the third combined data set, stood at the base of the crown. This separation of the Fucales from the other orders of the crown received strong support (95%).

In the base of the NJ tree, omitting *Verosphacela ebrachia* from the data set led to inclusion of *Onslowia endophytica* in a moderately supported cluster (68%) with the Sphacelariales *s. s.* and *Syringoderma phinneyi*. Within the Sphacelariales cluster, adding 18S sequences to the combined 26S and *rbc*L data did not markedly improve the resolution of relationships. Like in the other data sets, there was strong support for Stypocaulaceae, and again, *Herpodiscus durvillaeae* was part of the Sphacelariales *s. s.*. But the close affinities of the parasite were not resolved: in the most parsimonious and maximum likelihood trees, it clustered with *Sphacelaria cirrosa*, but this branch did not have any bootstrap support in both consensus trees nor did it exist within the next step of the MP analysis. In distance analyses, on the other hand, *H. durvillaeae* grouped in a moderately supported cluster (83%) with *Cladostephus spongiosus*.

4.1.1.3.4 *rbc*L gene (Sphacelariales)

In prior analyses including 26S and *rbc*L sequences (sections 4.1.1.3.2 and 4.1.1.3.3), *Herpodiscus durvillaeae* was revealed to be part of a well supported monophyletic group, the order Sphacelariales *s. s.*. In these analyses including representatives of all brown algal orders, however, the inner topology of the Sphacelariales was not resolved. Additional analyses were therefore run with a *rbc*L data set comprising an ingroup of 21 species of Sphacelariales *s. s.* including *H. durvillaeae*, and an outgroup consisting of six species, which, like the Sphacelariales, were positioned at the base of the Phaeophyceaen tree: *Dictyota dichotoma*, *D. cervicornis* (both Dictyotales), *Syringoderma phinneyi* (Syringodermatales) *Onslowia endophytica*, *Verosphacela ebrachia* (both Onslowiaceae) and *Choristocarpus tenellus* (*incertae sedis*).

A single most parsimonious tree was inferred from the data set, with a length of 1362 steps (Figure 4.6), while omitting *Choristocarpus tenellus* from the data set led to six MP trees (length: 1238 steps; not shown). Bootstrap consensus trees from both analyses (with or without *C. tenellus*) showed identical topologies, and bootstrap values were of the same order.

Over all, the topology of the MP tree (including *C. tenellus*) was very similar to the NJ tree. Within the outgroup, *C. tenellus* took the position of the earliest diverging taxon, followed by the well supported Dictyotales (*Dictyota dichotoma* and *D. cervicornis*, 99%). *Syringoderma phinneyi* clustered with Onslowiaceae, but this relationship did not have any bootstrap support, in

contrast to the distance tree, where it was moderately supported (60%). In the ML tree, *S. phinneyi* took a position between the other outgroup members and the ingroup. Apart from this branch, the bases of MP, ML and NJ trees were identical.

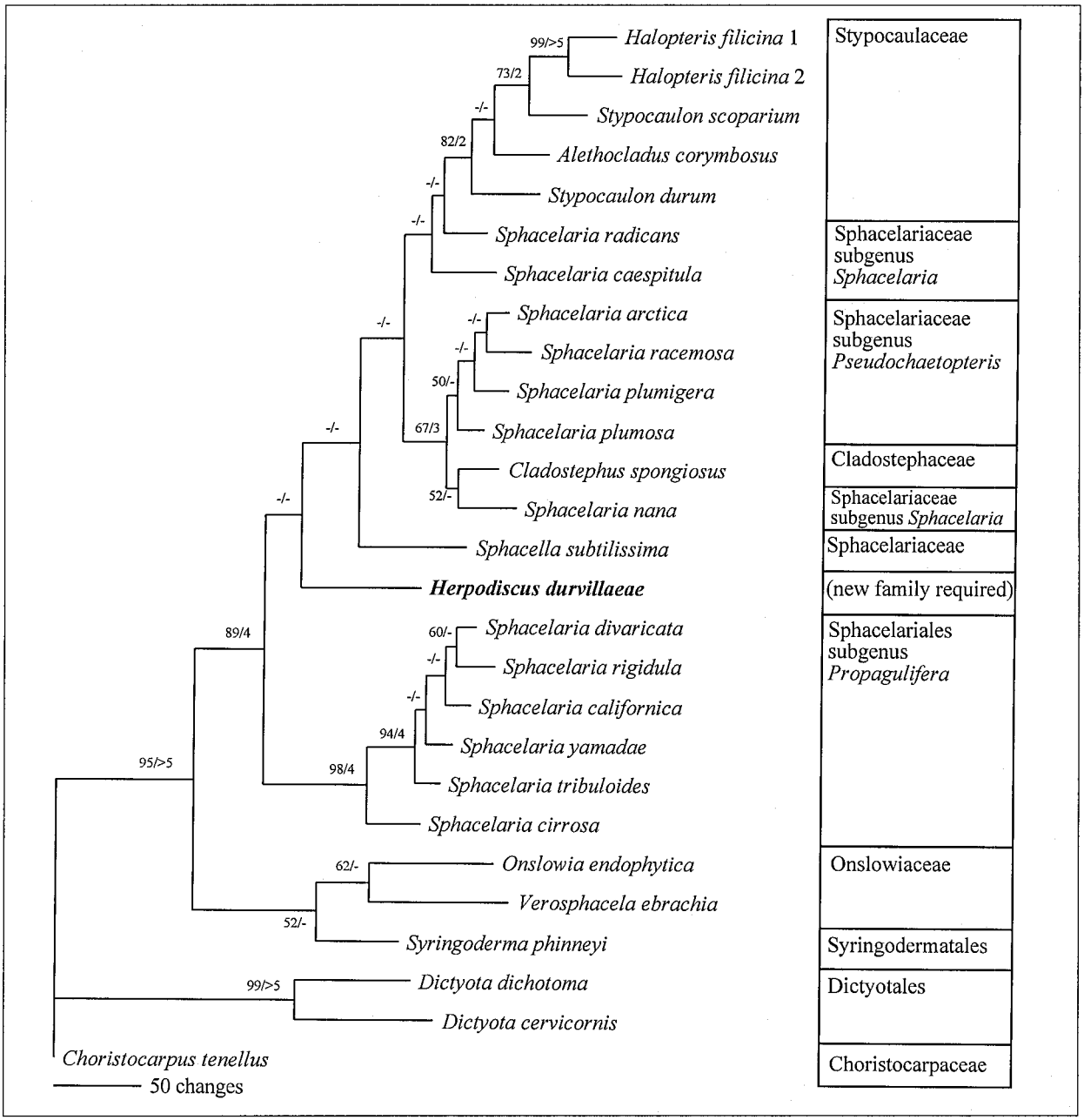


Figure 4.6: Phylogenetic tree for the Sphacelariales inferred from *rbc*L sequences. Single most parsimonious tree in phylogram style. Numbers indicate bootstrap values (left) and decay indices (right). Dashes indicate that branches received bootstrap support of 50% or less, or collapsed within one further step. Systematic affinities after DRAISMA *et al.* 2002.

Within the ingroup of Sphacelariales *s. s.*, three monophyletic clusters were recovered which appeared in the distance and the maximum likelihood tree as well: *Sphacelaria* species of the sub-genus *Propagulifera* formed a well supported group, which branched off first. Another sub-genus

of *Sphacelaria*, *Pseudochaetopteris*, grouped with *Cladostephus spongiosus* and *Sphacelaria nana* (of sub-genus *Sphacelaria*). This cluster had only moderate bootstrap support, though. Two other species of the sub-genus *Sphacelaria*, *S. radicans* and *S. caespitula*, grouped with members of the family Stypocaulaceae which were positioned on top of the tree. This relationship was not supported at all in the MP tree as branches collapsed during the bootstrap analysis. In the ML tree this cluster was the first to branch off in the ingroup, but the branch collapsed during bootstrapping as well. In the distance tree, in contrast, the relationship between Stypocaulaceae and the two *Sphacelaria* species was well supported (89%).

The phylogenetic affinities of the last two species, *Herpodiscus durvillaeae* and *Sphacella subtilissima* REINKE were not completely resolved with any of the three methods: in the MP and the NJ tree, *H. durvillaeae* took a position between the *Propagulifera-* and the *Pseudochaetopteris* + *Cladostephus*-cluster, but the branch collapsed after a further step or during bootstrapping. In both trees, *S. subtilissima* branched off just above *H. durvillaeae*. In the MP tree this branch was not supported, while in the NJ tree, the relationship to the *Pseudochaetopteris* + *Cladostephus* clade had some support (65%), as distances (absolute and Kimura-2-parameter values; Appendix D, Table D 4.2.4) of *H. durvillaeae* and *S. subtilissima* to *Sphacelaria arctica* HARVEY, *S. racemosa* GREVILLE and *S. plumosa* LYNGBYE were slightly lower than to all other Sphacelariales.

In the maximum likelihood tree, both *H. durvillaeae* and *S. subtilissima* stood above this clade and instead clustered with *Sphacelaria* species of the sub-genus *Propagulifera*, which formed the top of the tree. But again, this relationship did not have any bootstrap support.

4.1.2 Ultrastructure

4.1.2.1 Ultrastructure of *Durvillaea antarctica*

Longitudinal sections through the host thallus showed the typical haplostichous structure of the meristoderm, while cells in the cortex formed a net-like parenchyma of interwoven hyphae (Plate 4.2, Figure A; Plate 4.5, Figure A), indicated by the occasional presence of the product of longitudinal cell divisions (Plate 4.7, Figure C). The *Durvillaea* cells had massive cell walls, up to 3.5 µm wide (Plate 4.2, Figure C), and/or were surrounded by large amounts of intercellular matrix, forming a solid tissue without any cavities between cells. Plasmodesmata connecting two

cells were concentrated in one plane, in a 'field of plasmodesmata' (Plate 4.2, Figure B). In this area, the cell walls were the thinnest (circa 90 nm). Plasmodesmata had a diameter of circa 50 nm and lacked a core.

The most prominent feature of the host cells was the presence of large numbers of physodes (e. g. Plate 4.2, Figure A). These vesicles, which are characteristic for brown algal cells, contain polyphenolics visible in the TEM as electron-dense material. Other cellular compartments and organelles observed included nuclei, plastids, mitochondria and, occasionally, dictyosomes (Plate 4.2, Figure D). In the plastids, thylakoids were arranged in stacks of three (Plate 4.2, Figure E) typical for the lamellae of brown algal cells (DODGE 1973). The thallus surface was enclosed by a cuticle consisting of the outer-most layers of the surface cell walls (Plate 4.2, Figure A).

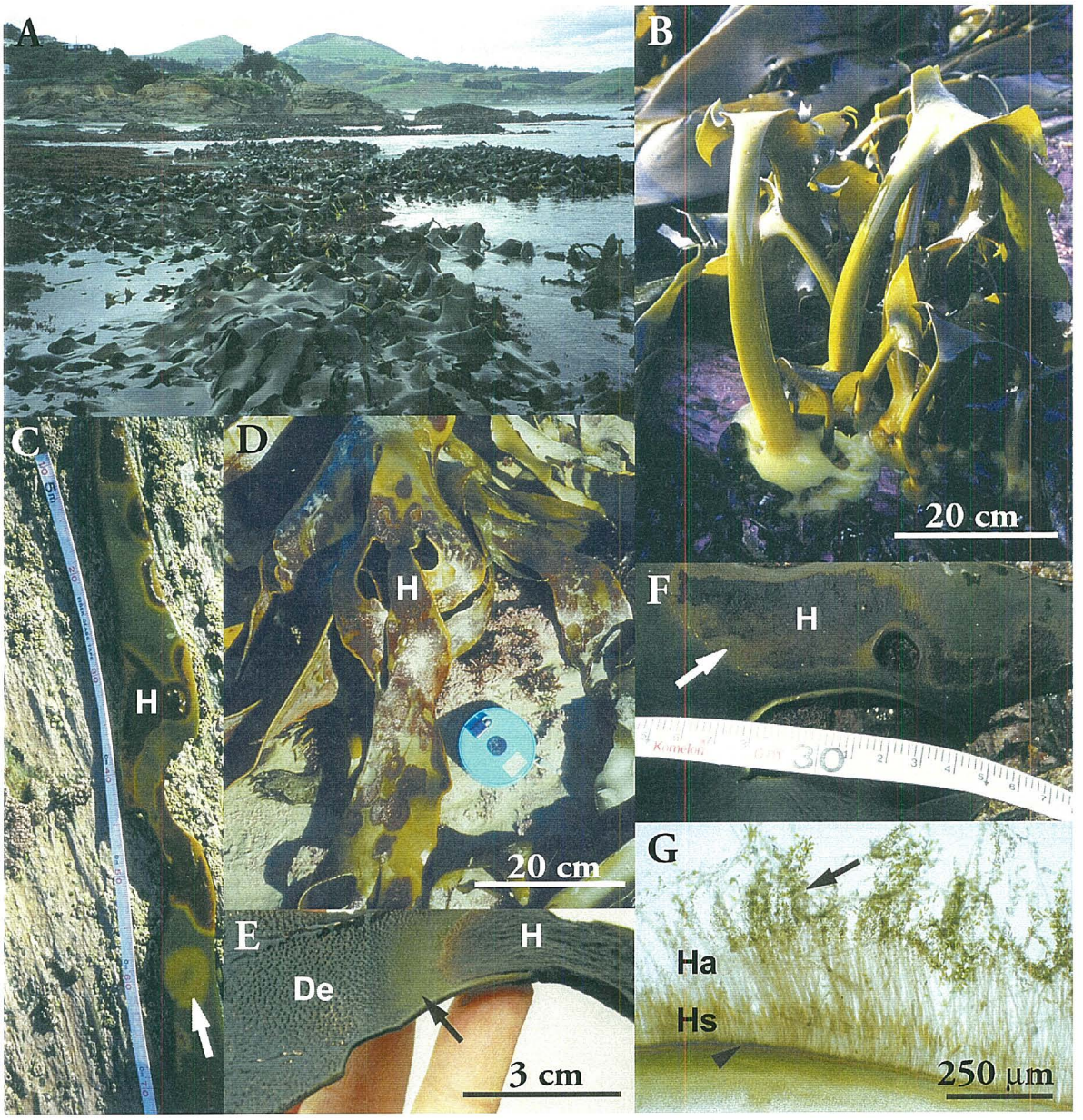
In late winter and early spring, the *Durvillaea* thallus shed its surface, however this process became macroscopically evident only in those areas displaying external patches of *Herpodiscus durvillaeae* (Plate 4.1, Figure F). In healthy areas of the host, only the cuticle was sloughed off in large flakes, with no surface cells attached (Plate 4.3, Figure A). In infected areas, the basal parts of the parasite filaments appeared to be embedded in a dense layer consisting of host surface cells (Plate 4.3, Figures B and D), from which the unbranched external filaments arose. This whole layer was shed, i. e. all external parts of *Herpodiscus* were removed together with the outer-most cells of the host surface (Plate 4.3, Figures B and D). The base of the peeled layer as well as the cleared host surface displayed a brown-reddish colouring (Plate 4.3, Figure C). This was possibly due to the accumulation of electron-dense material, putative polyphenolics, not only inside but also outside the cell membranes, e. g. as deposits on the cell walls (Plate 4.3, Figure D). Moreover, a yellowish autofluorescence in UV light was observed underneath the external *Herpodiscus* patches, prior to the surface renewal, and on the undersurface of the shedded flakes (results not shown).

4.1.2.2 Ultrastructure of *Herpodiscus durvillaeae*

Differences between the seasonal external and the perennial internal cells of the heterotrichous thallus of *Herpodiscus durvillaeae* were not only noticable macroscopically, but also on the ultrastructural level.

Plate 4.1: *Herpodiscus durvillaeae* in the field.

A: Sheltered population of the host *Durvillaea antarctica* at low tide (Brighton Beach, Otago, October 2000). **B**: Habit of *D. antarctica* (Brighton Beach, June 1999). **C**: Infected phylloid at the beginning of winter showing parasite patches with external filaments (H). Infected areas display a yellow margin. This margin is also apparent in areas where parasite filaments have not yet penetrated the host surface (arrow; Brighton Beach, April 1998). **D**: Heavily infected specimen with parasite patches (H) covering large proportions of the phylloid (Brighton Beach, August 1997). **E**: Phylloid of a fertile female of *D. antarctica.* Oogonia are released over its whole surface (De), but not in the area of the parasite patch (H) and the margin surrounding it (arrow; Brighton Beach, June 1998). **F**: Infected host phylloid in late winter showing reduced parasite patches. Areas where external parasite filaments have disappeared are lighter in colour (arrow; Brighton Beach, September 1997). **G**: Light micrograph of a section through a *Herpodiscus* patch showing the external thallus of the parasite. Above the host surface (arrowhead), a row of long assimilatory filaments (Ha) and a row of short filaments (Hs) arise, the former densely covered with microalgae (arrow), the latter carrying terminal unilocular sporangia (fresh material, Brighton Beach, July 2000).


A
B
20 cm
C
H
D
H
20 cm
E
H
De
3 cm
F
H
G
Ha
Hs
250 µm

4.1.2.2.1 External phase

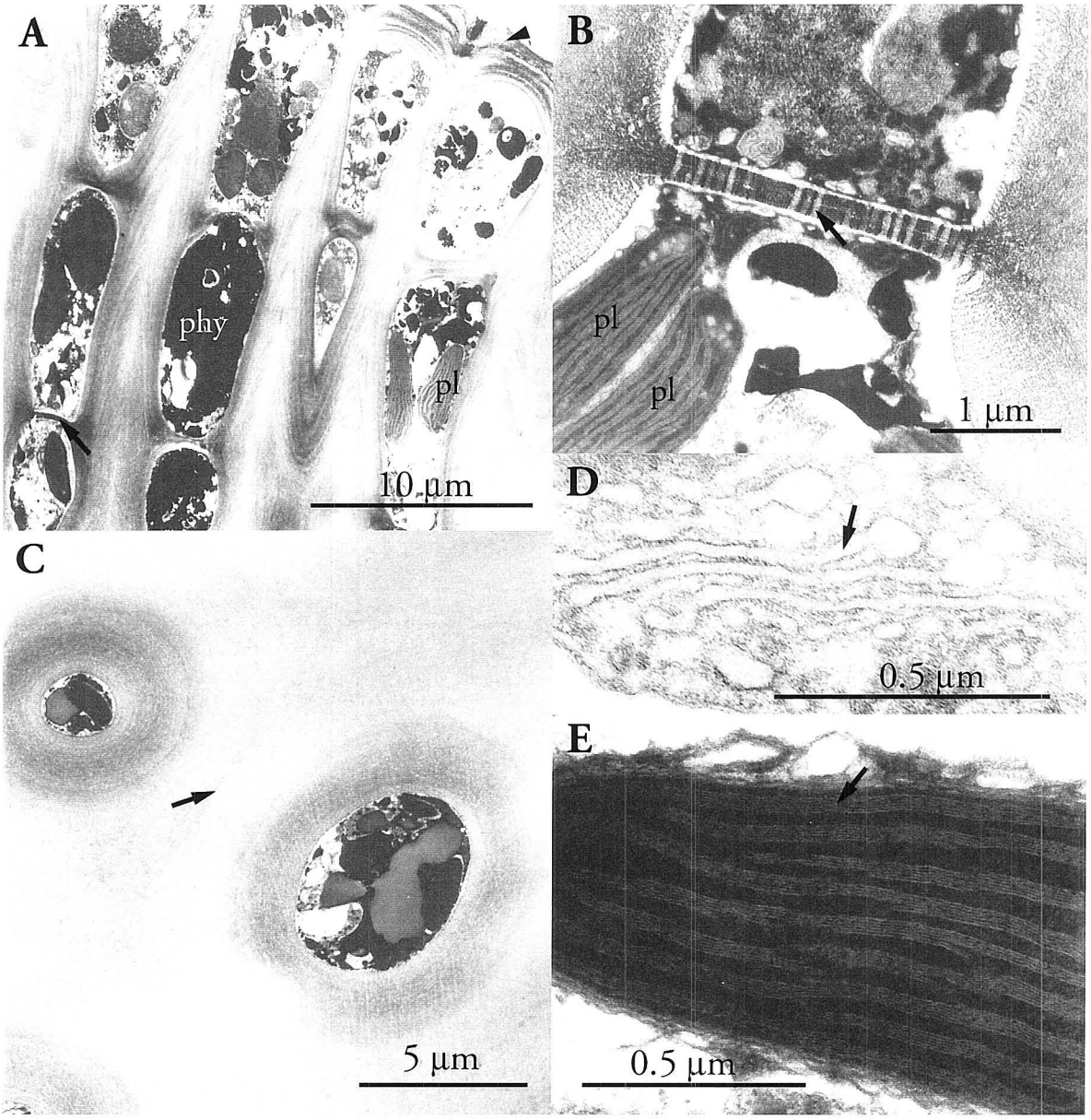
The external phase of the parasite (Plate 4.1, Figure G) was present at the Otago coast from end of March until early October (Plate 4.1, Figures C-F), when the host thalli had completely renewed their surfaces. In March, i. e. at the beginning of the growth season of the parasite, infected areas were macroscopically recognizable on the host surface due to an apparently reduced pigmentation of the host tissue (Plate 4.1, Figure C). In these areas, parasite cells occurred directly at the surface between host cells (Plate 4.3, Figure E). The cytoplasma and organelles of these parasite cells appeared to be concentrated in the apical portion, suggesting that they were apical cells.

External filaments were free, i. e. they did not adhere to each other, apart from their base, and were unbranched. Vegetative filaments were up to 15 µm wide and 800 µm long, and consisted of regular, cylindrical cells. Apart from the usual cellular compartments such as nuclei, dictyosomes and mitochondria, the external cells also displayed physodes. The physodes of the parasite were smaller (ca. 1-1.5 µm diameter) and less numerous, and appeared less dense than those of the host (Plate 4.5, Figures A and A'). Cell walls were layered, sometimes with a more electron-dense layer on the inside and a less dense layer on the outside (Plate 4.4, Figure E). Additionally, the cell walls often carried irregular deposits of electron-dense material on their inside and among the cell wall layers. Cells of the filament were connected with plasmodesmata (Plate 4.4, Figure E), however, these appeared to be less numerous and more unevenly distributed at the site of contact between two cells, compared to the fields of plasmodesmata of *Durvillaea.*

External filaments were teminated by elongated cells, which often had swollen tips and displayed central large nuclei (Plate 4.6, Figure G). Some vegetative filaments displayed terminal cells without a swollen tip which in the light microscope were of a slightly darker brown colour than subsequent cells (Plate 4.6, Figure A). In UV light, these cells exibited a bright yellowish autofluorescence (Plate 4.6, Figure A') which seemed to be located in the cytoplasm and comprised the whole cell lumen. They did not seem to contain any cell organelles, such as the large nuclei observed in the non-fluorescing terminal cells. Instead, they were filled with an accumulation of material, which in TEM sections (Plate 4.6, Figure B) appeared more electron-dense than the cytoplasma of normal parasite cells and which was apparently not enclosed by membranes.

Plate 4.2: *Durvillaea antarctica*: Ultrastructure of the host. The protocol numbers in this and the following plates relate to TEM protocols listed in Table 1 (Appendix E), if not stated otherwise. Additional details on the fixation are given for protocols, that included varying conditions. A-E: Transmission electron micrographs (phy: physode; pl: plastid).

A: Longitudinal section showing the haplostichous construction of the meristoderm and outer cortex. Cells contain large numbers of electron-dense physodes and are connected to each other via 'fields of plasmodesmata' (arrow). The surface is formed by a cuticle (arrowhead) consisting of the outer-most layers of the walls of surface cells. The shrinkage of the cell contents and the extraction of cell wall materials are artifacts (Brighton Beach, 08.03.1998; protocol no. 3; fixation at 4°C). **B**: Two cells of the outer cortex are connected via plasmodesmata (arrow), which are arranged in a field (Brighton Beach, 07.04.1998; protocol no. 4; microwave enhanced fixation). **C**: Section parallel to the surface showing the dimensions of the cell walls of cells of the outer cortex (arrow: border between two cells; Brighton Beach, 03.08.1998; protocol no. 3; fixation at RT). **D**: Cross section through a dictyosome with four cisternae (arrow; Brighton Beach, 13.12.2000; protocol no. 12/2, fixation for 24 hours). **E**: Cross section through a plastid showing numerous lamellae (including the girdle lamella; arrow), each consisting of stacks of three thylakoids. Their 'negative staining' is an artifact. The number of membranes surrounding the plastid was not determined (Brighton Beach, 07.04.1998; protocol no. 4; microwave enhanced fixation).


A
phy
pl
10 µm
B
pl
pl
1 µm
C
5 µm
D
0.5 µm
E
0.5 µm

The autofluorescing terminal cells were separated from the subterminal cell by a thick vaulted wall, which was not transversed by plasmodesmata. It appeared to have been formed by the subterminal cell, and adhered to the sidewall as an additional layer (Plate 4.6, Figure B).

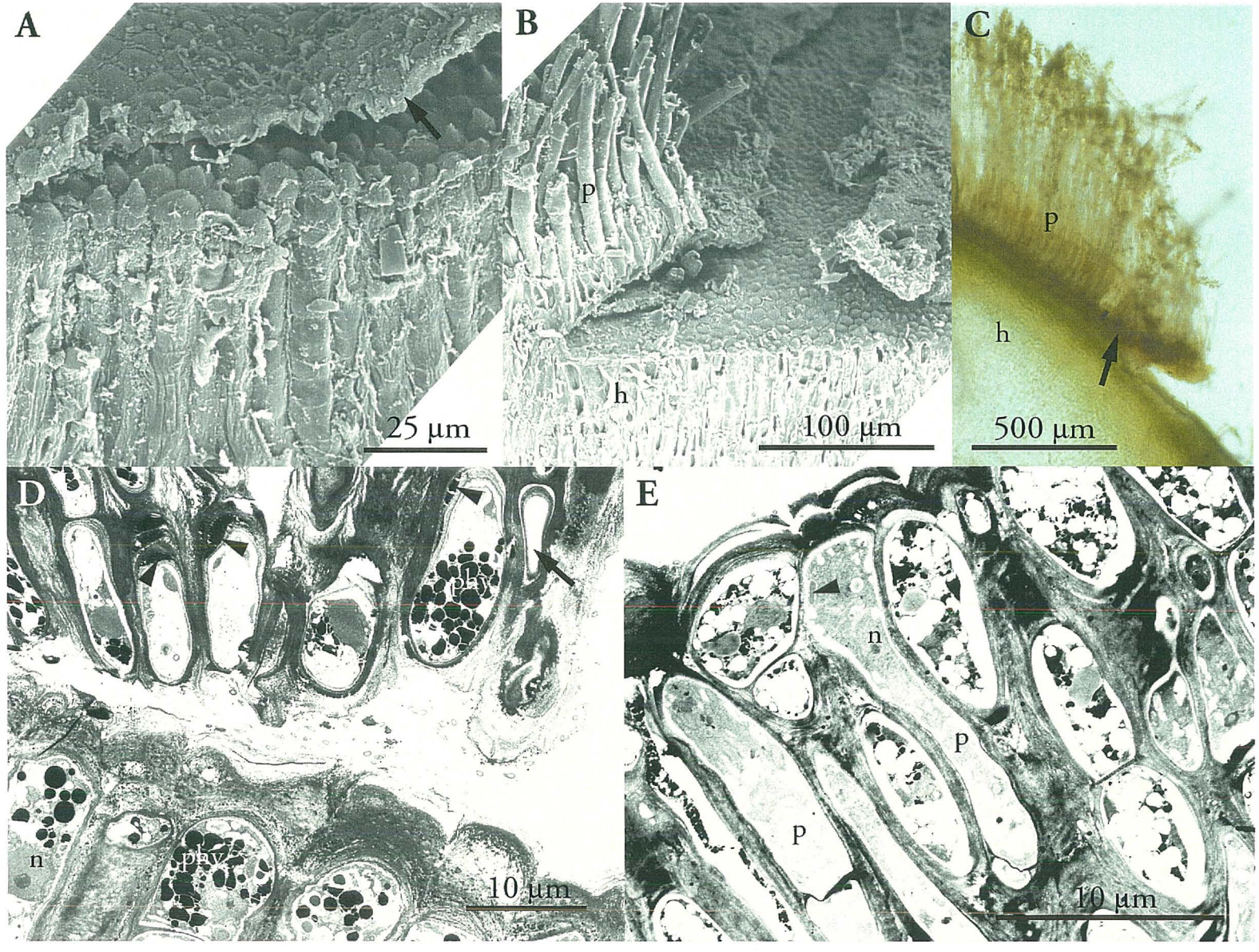
A similarly strong, but rather greenish autofluorescence was observed in some unilocular sporangia (Plate 4.6, Figure E) comprising the whole content of these sporangia, while other sporangia displayed only small greenish dots (Plate 4.6, Figure E) or no greenish autofluorescence at all (Plate 4.6, Figure C'). Apart from occasionally autofluorescing settled spores (result not shown), all other *Herpodiscus* cell walls showed only a weak autofluorescence in their cell walls.

Furthermore, in nearly all cells of the external parasite filaments including some unilocular sporangia and attached gametophytes, numerous small organelles were observed which showed a weak red autofluorescence similar to the characteristic autofluorescence of chlorophyll (Plate 4.6, Figures A', C'-D'). The identity of these organelles as plastids was confirmed by TEM. The plastids were discoid and had a diameter of ca. 1.5-2 µm. Their stroma contained a girdle lamella and few to several thylakoids, which appeared to be single or arranged in lamellae consisting of two thylakoids (vegetative cell: Plate 4.5, Figure A"; gametophyte: Plate 4.5, Figure F). No pyrenoids were associated with the plastids. In sporangia displaying a greenish autofluorescence, plastid autofluorescence was obscured.

Meiospores settled and developed into four-celled gametophytes either inside the unilocular sporangium (Plate 4.5, Figure D) or outside the sporangium on neighbouring filaments. Gametophytes completely transformed into plurilocular gametangia, with one gamete developing in each of the four loculi. Gametes displayed nuclei, plastids and mitochondria (Plate 4.5, Figure E), as well as dictyosomes (not shown). Loculi were separated from each other by thin cell walls which were at least 80 nm wide (Plate 4.5, Figures E and F). The walls became visible in the light microscope during treatment with 'Eau de Javelle' (see below; Plate 4.6, Figure G). The cell wall of the gametophyte-turned-gametangium was ca. 0.3 µm wide. It appeared to consist of two dark fibrillous layers, of which the inner layer formed the loculus enclosing the developing zoid (Plate 4.5, Figure F). After gametes were released, only the outside wall of the gametangium remained, while the walls separating the loculi inside were gone (results not presented).

Plate 4.3: *Durvillaea antarctica*: Shedding of the host surface.
A-B: Scanning electron micrographs; C: Light micrograph; D-E: Transmission electron micrographs (h: host tissue; n: nucleus; p: parasite filaments or cells, respectively; phy: physodes).

A: Surface shedding in an uninfected area. The cuticle is sloughed off in flakes (arrow), with no surface cells attached (Brighton Beach, 09.09.1997). **B-D**: Surface of an infected host specimen. **B**: External *Herpodiscus* filaments are shed together with host surface cells (Brighton Beach, 09.09.1997). **C**: A dense layer consisting of the base of the parasite filaments is sloughed off together with host surface cells. The area of incision shows a brown-reddish colouring (arrow; Brighton Beach, 23.08.2000). **D**: Longitudinal section through the host surface, with a layer just peeling off. This layer consists mainly of host cells containing physodes and single parasite cells, distinguishable by their smaller width (arrow). Some cells show deposits of electron-dense material outside their tonoplasts (arrowheads; Brighton Beach, 14.09.1999; protocol no. 8, LR White resin). **E**: Longitudinal section through the meristoderm at the end of the host's growth season showing two putative apical cells of *Herpodiscus* (p) among host cells at the *Durvillaea* surface. Their cell contents (including a reduced plastid, arrowhead) seem to be concentrated at the apical part. The shrinkage of the host cells and the loss of most of the physodes are artifacts (Brighton Beach, 08.03.2000; protocol no. 11/3).


A
25 µm
B
P
h
100 µm
C
P
h
500 µm
D
phv
phv
n
10 µm
E
n
P
P
10 µm

Test with 'Eau de Javelle'

Tissue of the host *Durvillaea antarctica* (fresh and freeze-dried) as well as field and culture material of ectocarpalean epiphytes isolated from *Herpodiscus* patches were rapidly and completely bleached by 'Eau de Javelle', without changing their colour. External filaments of *H. durvillaeae* and material from *Halopteris* sp, in contrast, showed the characteristic transitory blue-black colouring of all cell walls when exposed to 'Eau de Javelle' (Plate 4.6, Figures F-H), before being completely bleached. There was no difference noticeable in reactions of fresh versus freeze-dried material of *Herpodiscus*. The reaction was also observed in gametophytes settled on the external filaments (Plate 4.6, Figure F)

In the external filaments of *Herpodiscus*, some terminal cells still displayed a slightly blueish 'colour' for another couple of seconds (Plate 4.6, Figure H) while adjacent cells might have bleached completely. This 'colour' seemed to be located in the cytoplasm or the innermost layer of the cell wall. In some terminal cells it was evident as a blue band just below the tip of the cell (Plate 4.6, Figure G). Attempts to observe the reaction in the internal filaments of *Herpodiscus* were unsuccessful. Parasite filaments were apparently too rare and unevenly spread within the host tissue to locate them within the short time frame before the chlorine bleached the whole tissue.

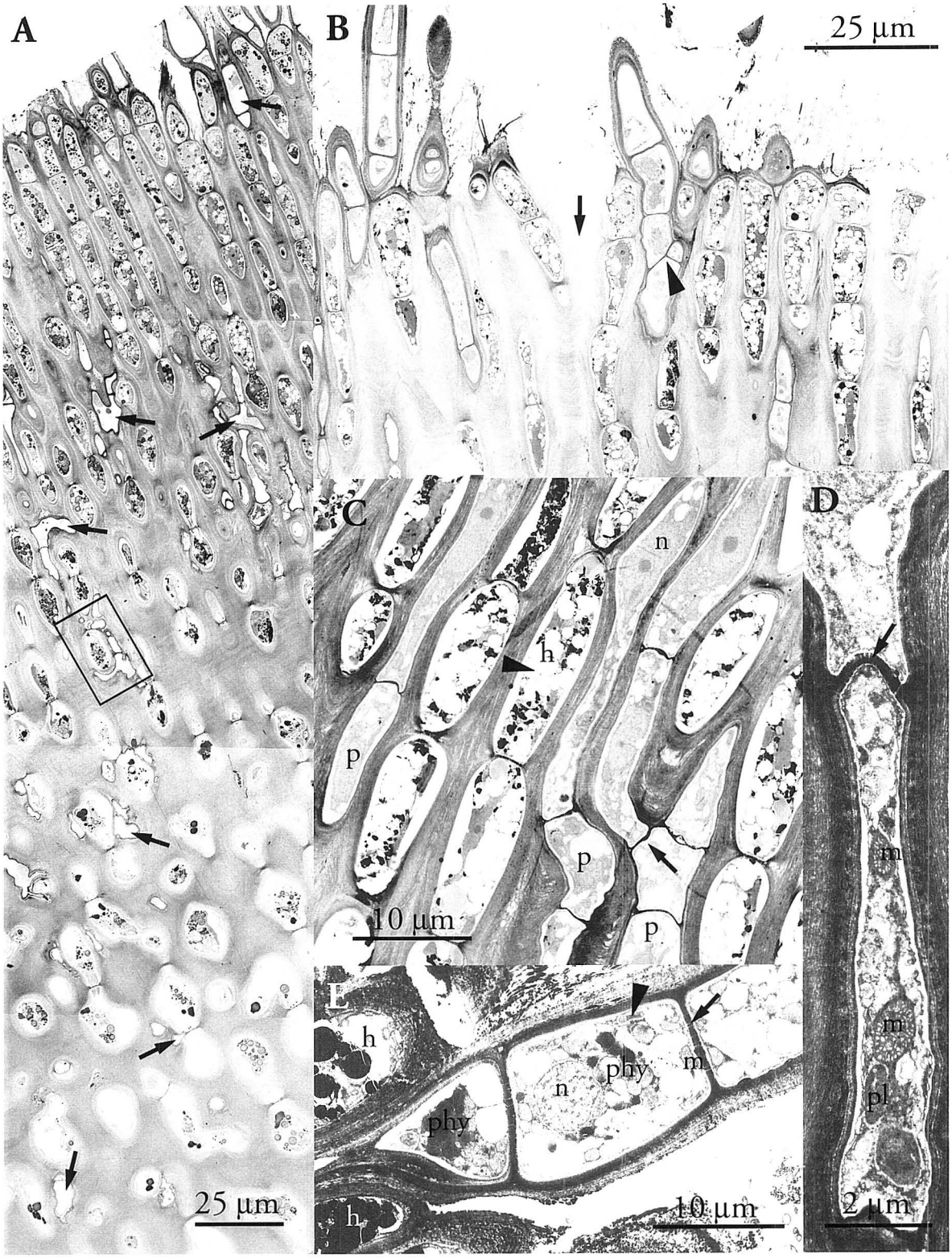
4.1.2.2.2 Internal phase

Internal filaments of the parasite grew in close proximity to the host cells, inside their cell walls or in the intercellular matrix, but did not appear to push the host cells aside by their presence. For example, the arrangment columns of host cells in the outer cortex seemed not to be altered in infected tissue, compared to healthy *Durvillaea.*

In contrast to the external cells with their regular cylindrical outlines, the parasite cells of the internal phase were of variable shape, i. e. their length and width seemed to depend on the tissue the cells grew in. Cells in the meristoderm, for example, were rather long and thin, with a cylindrical form approaching that of the external cells, but narrower (up to 10 µm; Plate 4.4, Figures C and D). The parasite cells growing in the host cortex, in contrast, did not show a specific form at all: Their shapes were highly irregular. The parasite cells were thus easily recognizable within the rather ordered structure of the host meristoderm and cortex (e. g. Plate 4.4, Figure A; Plate 4.7, Figures C and D).

Plate 4.4: *Herpodiscus durvillaeae*: Filaments at the host surface and inside the host.
A-E: Transmission electron micrographs (h: host cell; n: nucleus; m: mitochondria; p: parasite cell; pl: plastid).

A: Longitudinal section through an infected thallus of *Durvillaea antarctica*. Numerous parasite cells (e. g. arrows) grow among host cells, distinguished from the latter by their irregular shapes. Cells of the host show a haplostichous arrangements in the meristoderm and outer cortex, and a loose, net-like structure embedded in intercellular matrix in the cortex (Brighton Beach, 29.08.2000; protocol no. 14/2; detail in Plate 4.7, Figure B). **B**: Longitudinal section through the base of a parasite patch. The integrity of the host surface is severed within the parasite patch (arrow). Parasite filaments show acroblastic branching (arrowhead; Brighton Beach, 04.07.2000; protocol no. 13/2). **C-D**: Longitudinal sections through the meristoderm and outer cortex of the host, respectively, showing internal parasite filaments. **C**: Close to the host surface, three parasite filaments are within the cell walls and/or intercellular matrix of the host, without any direct contact to each other or to the host cells. One filament shows 'simple' branching (arrow). Parasite cells display large interphase nuclei with nucleoli. The host cells contain densely packed holes where physode contents have been lost during preparation (arrowhead; Brighton Beach, 24.2.2000; protocol no. 10/4, fast dehydration). **D**: An internal parasite cell showing some mitochondria and possibly reduced plastids, but no physodes. A field of plasmodesmata connects this cell to another one (arrow; St. Kilda, 04.08.1999; protocol no. 6). **E**: Detail of the base of a parasite filament among host meristoderm cells. Parasite cells display plasmodesmata (arrow), a nucleus, reduced plastids, mitochondria and some small physodes. Cell walls show two layers, a darker inner and a lighter outer one (St. Kilda, 04.08.1999; protocol no. 6).


A
B
25 µm
C
n
h
p
p
p
10 µm
D
m
m
pl
2 µm
E
h
phy
n
phy
m
h
10 µm
25 µm

This made the parasite cells also more conspicuous in the light microscope (Plate 4.7, Figure A and Plate 4.8, Figure A). Here, parasite cells were otherwise difficult to observe due to their lack of conspicuous cellular markers, e. g. pigmentation.

Internal parasite cells were not only distinguishable from the host cells by their irregular shapes, but also by their cell walls which often appeared to be more electron-dense than the surrounding host matrix (e. g. Plate 4.7, Figure B). Additionally, parasite cells in the cortex were often slightly retracted from the host matrix (possibly an artifact of the preparation), thus a thin halo seemed to surround the cells, further enhancing the contrast (Plate 4.8, Figure D). Often, the intercellular matrix of the host close to the parasite cells appeared to be more affected by extraction during the preparation of samples than other areas (Plate 4.8, Figure D), possibly indicating digestion of host intercellular material by the parasite.

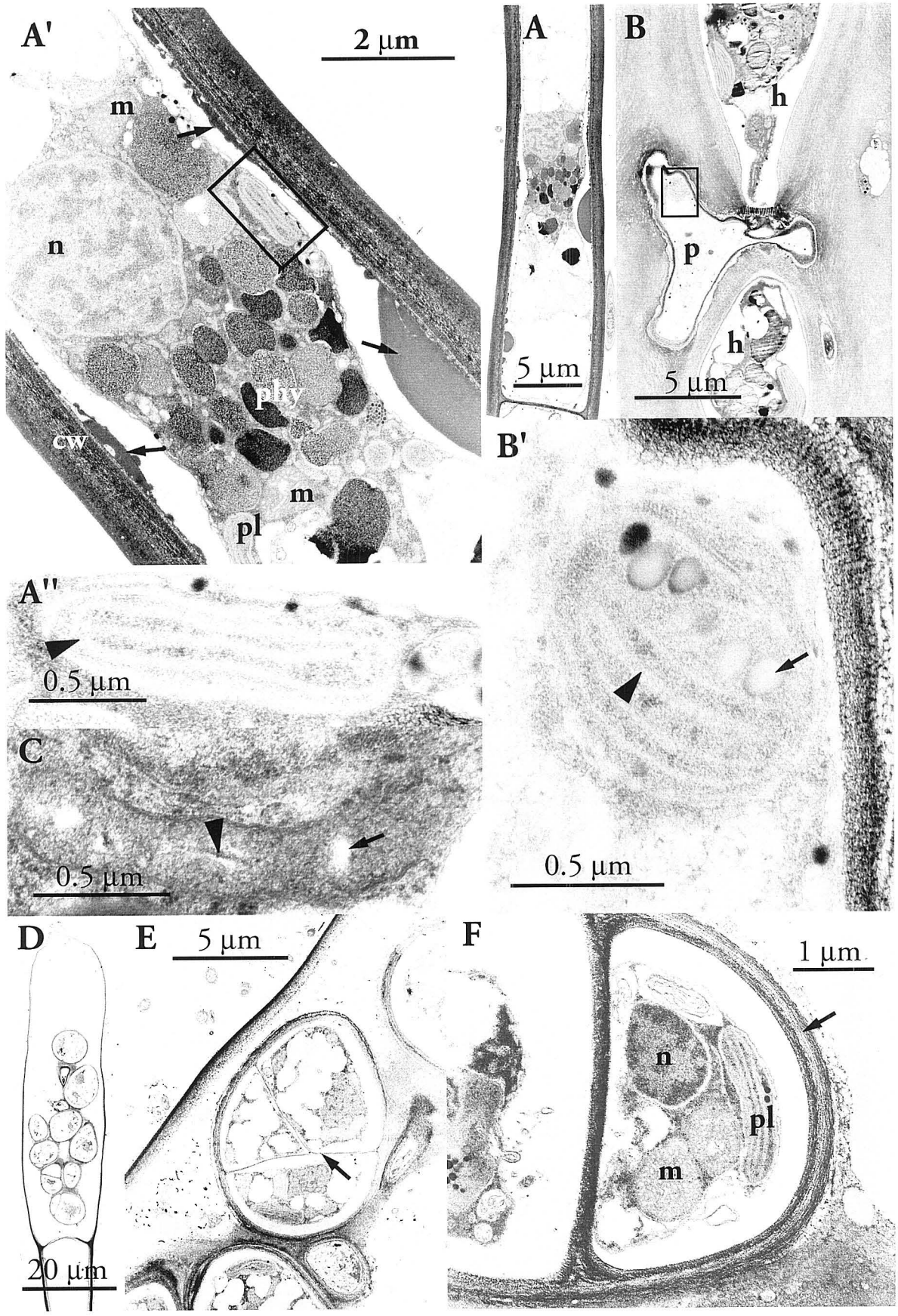
Parasite cells growing in the inner-most cortex did not display many organelles. For example, physodes appeared to be lacking in most of them (e. g. Plate 4.4, Figures C and D). Not once were they observed in cells growing in the host cortex (e. g. Plate 4.7, Figures B-D), however, this could be an artifact due to insufficient preparation. Occasionally, physodes were present in cells close to the host surface, which were markedly smaller than the host physodes (results not shown).

Mitochondria were frequently present in parasite cells of the outer host cortex (e. g. Plate 4.4, Figure D). Sometimes plastids were also observed. These were of a similar size to those of the external cells, and also displayed few thylakoids which were single or appeared to be assembled to lamellae in stacks of two. The number of membranes surrounding the plastids was not determined. Also a girdle lamella, if present, could not be distinguished from other thylacoid stacks, due to the insufficient fixation of membranes. Occasionally, the plastid stroma contained opaque inclusions, possibly storage products (Plate 4.5, Figure B').

Moreover, organelles were observed which may represent reduced plastids. They had a dumb-bell shape, and were ca. 1.5-2 μm long and 0.3-1 μm wide (Plate 4.3, Figure E; Plate 4.5, Figure C). However, apart from a membranous structure in their middle section, they did not show any sign of thylakoids. Electron-transparent regions at each end might have been genophores containing the plastom (Plate 4.5, Figure C), but this may also have been a preparation artifact. No plastid autofluorescence was observed in any internal parasite cells, however, it may have been obscured by the stronger chlorophyll autofluorescence of the host plastids surrounding them.

Plate 4.5: *Herpodiscus durvillaeae*: Plastids and gametophytes.
A-F: Transmission electron micrographs (cw: cell wall; h: host cell; m: mitochondria; n: nucleus; p: parasite cell; phy: physode; pl: plastid).

A-A'': Longitudinal section through a cell of an external filament (Brighton Beach, 29.08.2000; protocol no. 14/4). **A**: Overview. **A'**: Detail of A, showing the nucleus, plastids, physodes and mitochondria. The nucleus displays slightly condensed chromatin. Cell walls carry deposits of electron dense material on the inside (arrows): **A''**: Detail of A', displaying a plastid with a few 'negatively stained' thylakoids and a girdle lamella (arrowhead). **B-B'**: An internal parasite cell between two host cells (Brighton Beach, 29.08.2000; protocol no. 14/2). **B**: Overview. **B'**: Detail of B, showing a plastid with a few 'negatively stained' thylakoids and round globular inclusions (arrow), possibly storage products. Thylakoids are single or arranged in stacks of two (arrowhead). **C**: Detail of a parasite cell growing in the inner cortex of the host, showing a possibly reduced dumbbell-shaped plastid with electron-transparent regions at each end, possibly the genophore (arrow). The structure in the middle (arrowhead) may represent a reduced thylakoid. The membranes are dissolved at each end, possibly a preparation artifact (Brighton Beach, 08.03.2000; protocol no. 11/2, no microwaving). **D**: Longitudinal section through a unilocular sporangium with several gametophytes which develop inside the sporangium (Brighton Beach, 04.07.2000; protocol no. 13/2). **E**: Detail of gametophytes developing inside a unilocular sporangium. Cell walls between loculi are comparatively thin (arrow; Brighton Beach, 04.07.2000; protocol no. 13/3). **F**: Detail of a gametophyte developing inside a unilocular sporangium. In the cell on the right, the nucleus, two mitochondria and a plastid are visible. The outer cell wall consists of two dark layers (arrow), with the inner layer also enclosing the developing zoid. The shrinkage of the cell content is an artifact (Brighton Beach, 04.07.2000; protocol no. 13/2).


A'
2 µm
m
n
phy
cw
m
pl
A
5 µm
B
h
p
h'
5 µm
B'
0.5 µm
A''
0.5 µm
C
0.5 µm
D
20 µm
E
5 µm
F
1 µm
n
pl
m

Like the host cells, internal parasite cells were connected to each other via numerous plasmodesmata assembled in fields (Plate 4.4, Figure D). The fields of plasmodesmata between internal parasite cells appeared to be more pronounced than those between external cells. Branching of the *Herpodiscus* thallus was restricted to the internal phase. Most filaments showed an irregular branching pattern (Plate 4.4, Figure C). On a single occasion, an acroblastic branching mode was observed in cells at the host surface (Plate 4.4, Figure B).

4.1.2.2.3 Parasite/host interaction

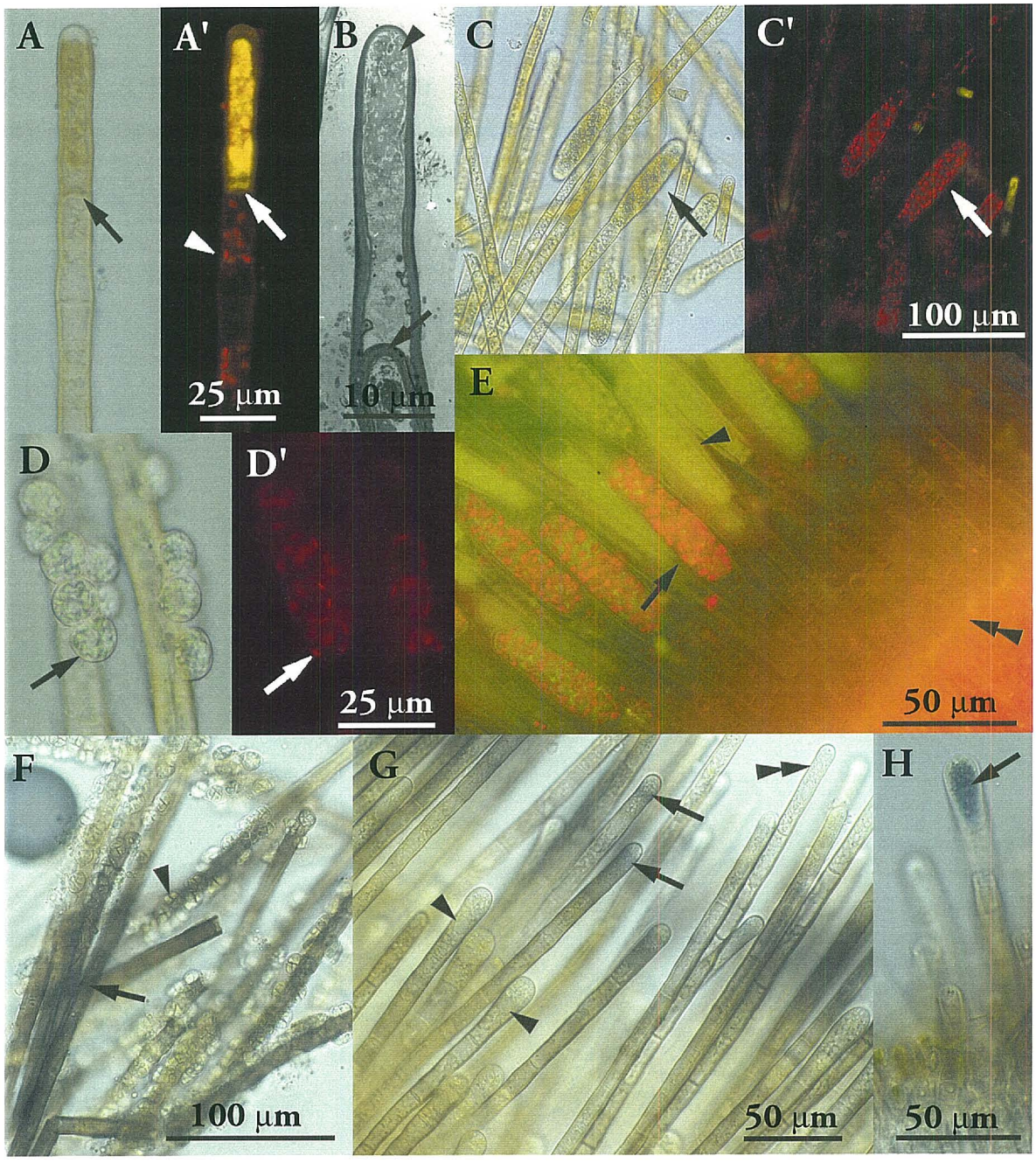
In the meristoderm, long thin parasite cells grew among the rows of host cells, without close contact between both species (Plate 4.4, Figures C and D). In the inner cortex, in contrast, irregular-shaped parasite cells were often observed close to the fields of plasmodesmata between host cells (Plate 4.7, Figure A), i. e. they seemed to attack the host cells especially at the fields of plasmodesmata.

Sections displayed various levels of contact to the host cells. Serial sections showed, for example, a parasite cell which appeared to 'throttle' the host cell in one section and displayed a dumb-bell shape lying over the connection of the host cells in the next section (Plate 4.7, Figures C' and D'), indicating that this parasite cell laid like a collar around the field of plasmodesmata between the host cells. Other parasite cells, by forming claw-like protrusions, encircled host cells (Plate 4.7, Figures A and B) and thus were in direct contact with at least one of the fields of plasmodesmata (Plate 4.7, Figure B).

In the light microscope, some *Herpodiscus* cells formed a conical-shaped protrusion pointing at the field of plasmodesmata between two *Durvillaea* cells (Plate 4.8, Figure A). In TEM sections, a similarly shaped parasite cell appeared to have squeezed between the host cell walls within the fields of plasmodesmata (Plate 4.8, Figures B and B'). In this way, the host cells were separated somewhere within one cell wall, thus revealing the fibrillous structure of the walls (Plate 4.8, Figures B' and C'). Host cells appeared to be mechanically forced apart (Plate 4.8, Figure C), exposing the fibrillous cell wall material to the apoplastic space between the two walls (Plate 4.8, Figure C'). The wall of the parasite cell squeezing between the host cells displayed many small perforations, presumably plasmodesmata (Plate 4.8, Figure B'), which at that stage did not show any connection to the host plasmodesmata.

Plate 4.6: *Herpodiscus durvillaeae*: Epi-fluorescence and reaction with 'Eau de Javelle'.
A, C-G: Light micrographs: B: Transmission electron micrograph.

A, C-D: Micrographs of parasite filaments showing the same object in bright field optics (left) and in UV light (right). **A**: Terminal cell of a vegetative external filament of *H. durvillaeae*, displaying a yellowish autofluorescence in UV light. This cell is separated from the adjacent cell by a vaulted wall (arrow). The cell below contains red autofluorescing plastids (arrowhead). B: Terminal cell, displaying a vaulted wall without plasmodesmata (arrow) and an accumulation of electron-dense material (arrowhead), but no obvious cell compartments (Brighton Beach, 04.07.2000; protocol no. 13/3). **C**: Vegetative filaments and unilocular sporangia (arrow) containing red autofluorescing plastids. **D**: Spores, settled on an assimilatory filament, each displaying several small red autofluorescing plastids (A, C-D: fresh material; Brighton Beach, Otago, May 2001). **E**: Section through a fertile parasite patch (displaying many unilocular sporangia) in UV light, with the host surface in the lower right corner (double arrowhead). The sporangia are filled either with a greenish-yellow autofluorescencing material (arrowhead), or with spores containing red autofluorescing plastids (arrow; fresh material, Brighton Beach, June 2000). **F-H**: Reaction of *H. durvillaeae* with 'Eau de Javelle'. **F**: Immediately after contact with the bleach, external filaments show the typical black-brown colouring (arrow). Gametangia-turned gametophytes display coloured internal walls (arrowhead). **G**: Front of bleach moving through the tips of external filaments. Some terminal cells are completely bleached (double arrohead), while others display blue bands of colouring just below their apical hemisphere (arrows). Terminal cells show swollen tips and contain large nuclei (arrowheads). **H**: Parasite filaments on the host surface: surrounding cells are already bleached, while a terminal cell (arrow) shows a blueish 'colour' for a few more seconds (F: dried material, scraped from the surface of a parasite patch, Brighton Beach, June 2000; G-H: fresh material, Brighton Beach, March 2001).


A
A'
B
C
C'
25 µm
10 µm
100 µm
D
D'
E
25 µm
50 µm
F
G
H
100 µm
50 µm
50 µm

A direct continuity of the *Herpodiscus* plasmodesmata with those of the *Durvillaea* cell, however, was observed in another section (Plate 4.8, Figure D'), thus presenting evidence for a symplastic connection between host and parasite. Direct cellular contacts between both species seemed to be limited to the fields of plasmodesmata. Invasions of the lumen of host cells, for example by haustoria, were not observed. The interconnecting plasmodesmata lacked cores on both the parasite's and the host's side.

4.2 Discussion

4.2.1 Molecular systematics

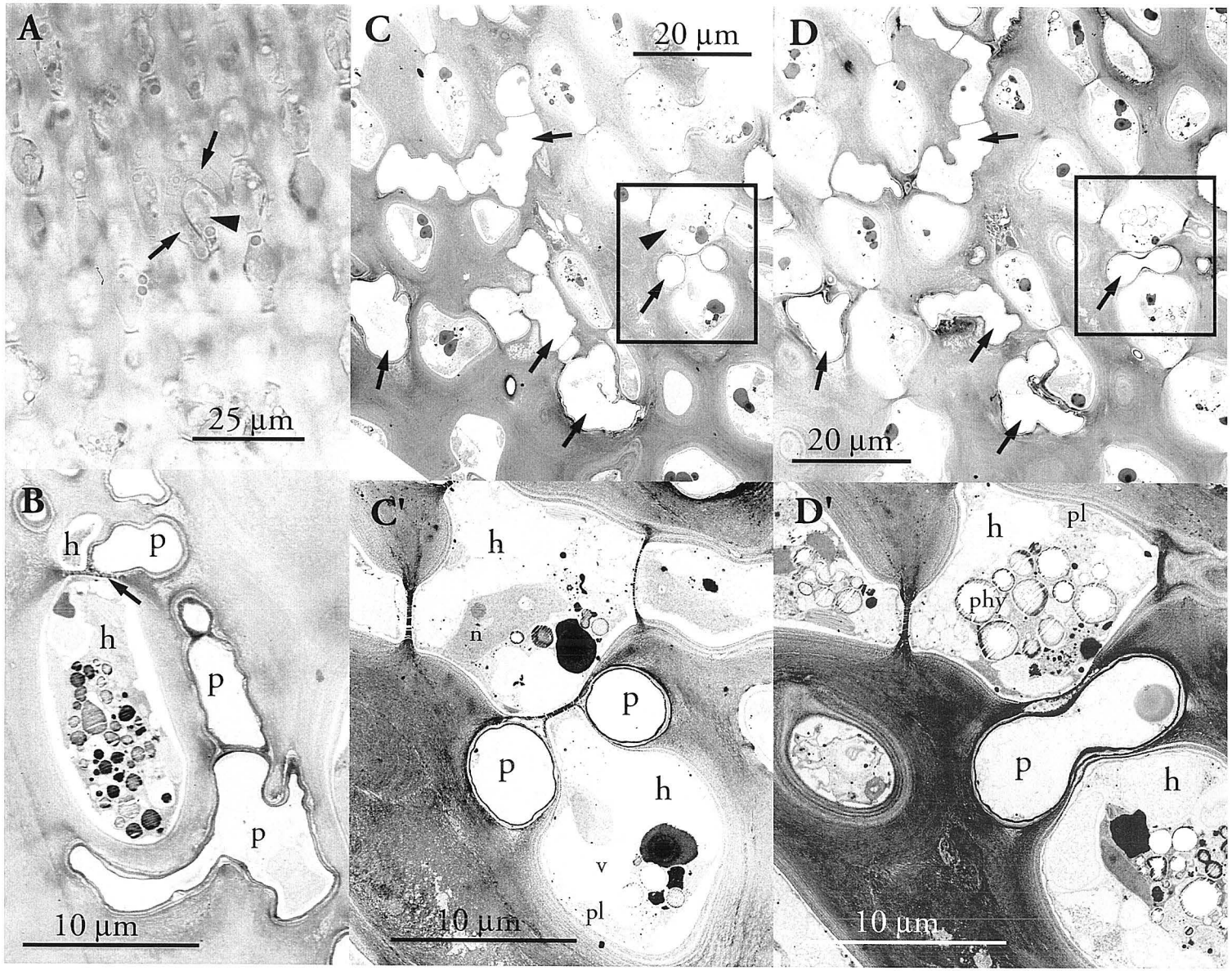
4.2.1.1 Quality of data

The phylogenetic trees presented in this study were in general accordance with trees published by other authors (e. g. DE REVIERS & ROUSSEAU 1999; ROUSSEAU *et al.* 2001; DRAISMA *et al.* 2001, 2003). Based on similar data sets but individual alignments, the addition of sequences of *Herpodiscus durvillaeae* did not alter the topologies of trees, but supported the phylogeny of the Phaeophyceae currently recognized (DE REVIERS & ROUSSEAU 1999; ROUSSEAU *et al.* 2001; DRAISMA *et al.* 2001, 2003).

The present study combined genes from separate compartments within the cell (i. e. *rbc*L and nrDNA), in order to improve the validity of the results. Moreover, representatives from some smaller Phaeophycean orders such as Tilopteridales, Sporochnales and Cutleriales were included in the data sets. Combining sequences from different genes stabilized the nodes within clusters, however, the branches between clusters still collapsed during bootstrap analyses, thus leveling out most differences between trees obtained with Maximum Parsimony or Maximum Likelihood methods or inferred from distance analyses. Therefore, the analyses failed to resolve relationships between monophyletic orders within the crown of the Phaeophycean tree, as did the other studies before (DE REVIERS & ROUSSEAU 1999; ROUSSEAU *et al.* 2001; DRAISMA *et al.* 2001, 2003).

Plate 4.7: *Herpodiscus durvillaeae:* The parasite/host interaction.

A: Light micrograph; B-D': Transmission electron micrographs (h: host cell; n: nucleus; p: parasite cell; phy: physodes; pl: plastid; v: vacuole).
A: Longitudinal section through the outer cortex of *D. antarctica*, showing parasite cells (arrows) surrounding a host cell (arrowhead) in a claw-like manner (Brighton Beach, 17.03.2001). **B**: Longitudinal section displaying a similar situation to A. The upper parasite cell is in direct contact with the field of plasmodesmata between the (attacked) host cell and the adjacent host cell (arrow; Brighton Beach, 29.08.2000; protocol no. 14/2; overview in Plate 4.4 Figure A). **C, D**: Serial sections from the inner cortex of the host, with numerous parasite cells present (e. g. arrows). Connections of a host cell to four other host cells (arrowhead in C) indicate the presence of intercalary longitudinal cell divisions in the cortex of *D. antarctica* (Brighton Beach, 29.08.2000; protocol no. 14/4). **C', D'**: Details of C and D, respectively, showing two layers of the same parasite cell. This cell appears to surround the field of plasmodesmata between two host cells like a collar (resulting in the 'dumb-bell' shape in D'). Host cells show a nucleus, plastids and large vacuoles. The physodes in D' are only partially fixed (artifact).


A
25 µm
B
h
p
h
p
p
10 µm
C
20 µm
C'
h
n
p
p
h
v
pl
10 µm
D
20 µm
D'
h
pl
phy
p
h
10 µm

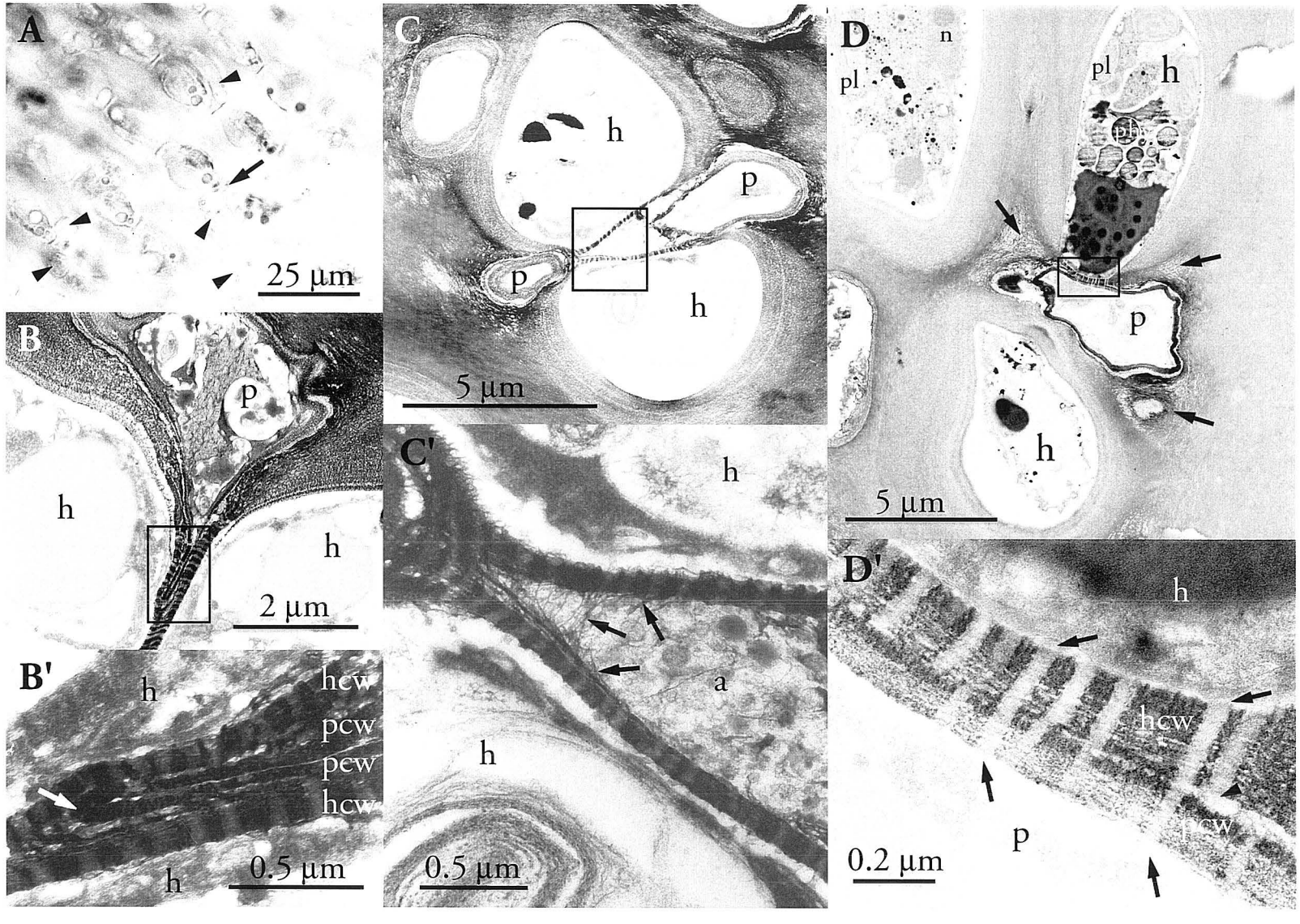
In contrast to the affinities within the crown, the relationships below the crown were well resolved in combined data sets: Dictyotales, Sphacelariales and Syringodermatales, together with *Choristocarpus tenellus* and Onslowiaceae, formed the base of the Phaeophycean tree, with Syringodermatales being the closest to the 'higher Phaeophyceae' and *C. tenellus* apparently being the most basal brown alga. Some authors even propose the Sphacelariales, Dictyotales and Syringodermatales to be closely related, as the 'SDS' group, as all three share apical growth, even though they do not form a monophyletic group (SAUNDERS & KRAFT 1995, cited by DE REVIERS & ROUSSEAU 1999).

DRAISMA *et al.* (2002) analysed *rbc*L sequences of all these taxa included in the base of the Phaeophyceaen tree, in order to reveal the phylogeny within the Sphacelariales. In the present study, *Herpodiscus* sequences were added to a *rbc*L data set comprising essentially the same taxon sampling as DRAISMA *et al.* (2002). The addition did not change the overall clustering of taxa within the Sphacelariales, and among the more basal brown algae, indicating that the *Herpodiscus* sequence was in accordance with the findings by DRAISMA and co-workers.

Generally, the inclusion of sequences of *Herpodiscus durvillaeae* did not affect the homogeneity between data sets in analyses of combined *rbc*L and nrDNA data, in contrast to *Choristocarpus tenellus* sequences. Originally, *C. tenellus* was considered to have an intermediate position between Ectocarpaceae and Sphacelariales or Tilopteridales (KUCKUCK 1895b; OLTMANNS 1922). It was later placed in the Sphacelariales, taking into account the apical growth and the presence of propagules, even though the latter lack the small lenticular apical cells found in propagules of Sphacelariales *s. s.* (PRUD'HOMME VAN REINE 1993). KJELLMAN (1891) created its own family, Choristocarpaceae, which also included *Discosporangium mesarthrocarpum* (MENEGHINI) HAUCK. However, neither *C. tenellus* nor *D. mesarthrocarpum* show the transistory blackening with Eau de Javelle (WOMERSLEY 1987), which is characteristic of the order Sphacelariales MIGULA 1909 (REINKE 1890; MIGULA 1909). The Choristocarpaceae are therefore sometimesreferred to as Sphacelariales *s. l.*, but their placement remains doubtful (PRUD'HOMME VAN REINE 1982, 1993; DE REVIERS & ROUSSEAU 1999).

Plate 4.8: *Herpodiscus durvillaeae:* The parasite/host interaction (continued).
A: Light micrograph; B-D': Transmission electron micrographs (a: apoplast; h: host cell; hcw: host cell wall; n: nucleus; p: parasite cell; pcw: parasite cell wall; phy: physode; pl: plastid).

A: Longitudinal section through infected *D. antarctica.* Parasite cells (e. g. arrowheads) grow in close proximity to host cells. A parasite cell seems to point at the field of plasmodesmata between two host cells (arrow; Brighton Beach, 17.03.2001). **B**: Section though an infected host cortex showing a similarly 'pointing' parasite cell between two host cells. The parasite cell has squeezed halfway though the field of plasmodesmata connecting the host cells. **B'**: Detail. The host cell walls seem to be slightly forced apart by the parasite cell (end indicated by the arrow). The parasite cell wall shows perforations, possibly small plasmodesmata. Cell contents of both host and parasite are not well preserved (B-B': Brighton Beach, 22.11.1999; protocol no. 9/2, microwave enhanced fixation). **C**: Section though an infected host cortex showing a similar situation to that in B. In the field of plasmodesmata, the host cell walls are widely forced apart. The parasite cell appears to be retracted, leaving a large apoplastic space between the two host cells. **C'**: Detail of the apoplastic space (a). Fibrillar material (arrows) of the host cell walls stretch through the opening. The myelin figure in one of the host cells is an artifact (C-C': Brighton Beach, 14.09.1999; protocol no. 8, LR White resin). **D**: Section through infected host cortex. A parasite cell has direct contact with the field of plasmodesmata of a host cell. The nature of the dark (i. e. electron-dense) material in the upper host cell in D is not known. The intercellular matrix surrounding the parasite cell shows signs of extraction (arrows). **D'**: Detail of the field of plasmodesmata at the interface of host and parasite. Some plasmodesmata of the host stop at the parasite cell wall, and may form a median cavity (arrowhead), while others continue on the parasite side. Thin strands of cytoplasma seem to stretch through these 'secondary plasmodesmata' (arrows), apparently forming a symplastic bridge between the host and the parasite (D-D': Brighton Beach, 29.08.2000; protocol no. 14/2).


A
25 µm
B
p
h
h
2 µm
B'
h
hcw
pcw
pcw
hcw
h
0.5 µm
C
h
p
p
h
5 µm
C'
h
a
h
0.5 µm
D
n
pl
pl
h
p
h
5 µm
D'
h
hcw
p
pcw
0.2 µm

DRAISMA *et al.* (2001) were the first to present molecular data for *Choristocarpus tenellus*. In trees based on nrDNA data, this species appears as part of a well supported cluster otherwise comprised of Sphacelariales *s. s.*, while Dictyotales form the earliest divergence in the Phaeophyceae. In analyses of plastid-encoded *rbc*L sequences, in contrast, *C. tenellus* takes the position of the most basal brown alga, below Dictyotales. This basal position of *C. tenellus* is also retrieved in combined nrDNA and *rbc*L data sets, presumably because the *rbc*L data contribute many more informative sites to the analyses as the nrDNA data. However, in partition homogeneity tests, DRAISMA *et al.* (2001) found their data sets to be incongruent. They nevertheless proposed *Choristocarpus tenellus* to be the most basal of all brown alga and therefore separated the Choristocarpaceae from the order Sphacelariales.

The present study, based on a similar combined data set as DRAISMA *et al.* (2001) but with a slightly different taxon sampling, reached similar conclusions. This study further revealed that the incongruence between nuclear encoded ribosomal and plastid encoded RuBisCo genes, detected by DRAISMA and co-authors, was indeed due to the differences in the position of *Choristocarpus tenellus*. Thus, excluding *C. tenellus* from separate and combined data sets markedly improved the stability of branches. In these analyses the Dictyotales took the position of the most basal brown alga, and the Sphacelariales *s. s.* were well separated from the other brown algae.

In a recent molecular systematic study, BURROWES *et al.* (2003) presented a different 26S sequence which has confirmed *Choristocarpus* as the most basal brown alga. Therefore, the 26S sequence, which was published by DRAISMA and co-workers (2001) and was also included in the nrDNA analyses of the present study, appears to have been mis-attributed to this species.

4.2.1.2 Phylogenetic affinities of *Herpodiscus durvillaeae*

The present study is the first to investigate the phylogenetic relationships of the only described fully parasitic brown alga worldwide, the New Zealand endemic *Herpodiscus durvillaeae*. The analyses of the molecular data revealed that *H. durvillaeae* is neither closely related to the Ralfsiaceae (Ralfsiales nomen nudum, formerly Chordariales; SILVA *et al.* 1996, cited in DE REVIERS & ROUSSEAU 1999), where it was originally placed (LINDAUER 1949; JOHN & LAWSON 1974), nor to members of the family Elachistaceae (or Chordariaceae respectively, Ectocarpales *sensu lato*, formerly Chordariales; ROUSSEAU & DE REVIERS 1999; PETERS & RAMIREZ 2001), in which *H. durvillaeae* is currently placed (SOUTH 1974), and which includes other endophytic genera, such as *Laminariocolax* and *Microspongium* (BURKHARDT & PETERS 1998;

PETERS 2003). *H. durvillaeae* is also not closely related to the order Fucales, which comprises its host, *Durvillaea antarctica* and the other brown alga known to be at least partially parasitic, *Notheia anomala* (formerly Durvillaeales and Notheiales, respectively; DE REVIERS & ROUSSEAU 1999). Instead, *Herpodiscus durvillaeae* unambigously clustered with members of the order Sphacelariales MIGULA 1909.

These results inferred from molecular data confirm the findings of PETERS (1990), that the morphology and life history of *Herpodiscus durvillaeae* are too distinct for a close relationship with the brown algal orders and families it was associated with by various authors before (LINDAUER 1949; JOHN & LAWSON 1974; SOUTH 1974; E. C. HENRY, personal communication, and in PETERS 1990). But these results, however, lead to the question if the placement of the parasite in the Sphacelariales would also be justified given the morphological features characteristic for this order.

4.2.1.2.1 Placement of *Herpodiscus durvillaeae* in the order Sphacelariales

Reaction with 'Eau de Javelle'

Two main features characterise the order Sphacelariales: the growth with prominent apical cells and a positive reaction with 'Eau de Javelle' (MIGULA 1909). While apical growth is shared by several other Phaeophycean orders, such as the Syringodermatales, Dictyotales, Scytothamnales, Cutleriales and Fucales (HENRY 1984; VAN DEN HOEK *et al.* 1995; DE REVIERS & ROUSSEAU 1999), the positive reaction with 'Eau de Javelle' is a feature that distinguishes members of the Sphacelariales from all other brown algae: treatment with clorine bleach ('Eau de Javelle': 5% aqueous solution of sodium hypochlorite) leads to a characteristic transitory blackening of the cell walls (REINKE 1890; MIGULA 1909; PRUD'HOMME VAN REINE 1982; DE REVIERS & ROUSSEAU 1999). Members of the families Choristocarpacecae and Onslowiaceae (DRAISMA & PRUD'HOMME VAN REINE 2001), for example, are commonly placed in the Sphacelariales *s. l.* (HENRY 1987a, 1987b; WOMERSLEY 1987), but their systematic position has always been doubtful, as they, unlike the other members of the Sphacelariales, do not show the positive reaction with 'Eau de Javelle' (PRUD'HOMME VAN REINE 1982, 1993). Recent molecular systematic studies support an exclusion of both families from the Sphacelariales *s. s.* (DRAISMA *et al.*, 2001; DRAISMA & PRUD'HOMME VAN REINE, 2001; present study). In *Herpodiscus durvillaeae*, however, a positive reaction with 'Eau de Javelle' was observed: when treated with bleach, external filaments always showed the typical black-brown "colouring" (Plate 4.6, Figures F-H).

Even though the phenomenon of the transitory blackening of Sphacelariales cells treated with bleach has been known since the 19th century (REINKE 1890), the underlying chemistry is still unknown (W. PRUD'HOMME VAN REINE, personal communication). The stained substance is thought to be localised in the cell walls, in the middle lamella (REINKE 1890). After contact with the bleach, cells at first turn dark brown to black, then they bleach and thus become colourless. In *Herpodiscus durvillaeae*, even the usual brownish colour of the cell walls described by SOUTH (1974) disappeared after treatment with the bleach (Plate 4.6, Figure F).

The positive reaction of *Herpodiscus durvillaeae* with 'Eau de Javelle' presents a strong argument against a possible contamination of the samples used for phylogenetic analyses with epiphytic Sphacelariales. Additionally, three independant samples (spring 1997, winter 1998, autumn 2001) gave identical DNA sequences. Test runs of PCR products of *Herpodiscus* samples on agarose gels confirmed their purity, as only single bands were visible in the gels. Moreover, phaeophyceaen epiphytes that were commonly found growing on *Herpodicus* patches showed a negative reaction with 'Eau de Javelle' (results not presented), as did the tissue of the host, *Durvillaea antarctica*. An epiphytic brown alga isolated from a *Herpodiscus* patch, from which some of the extracted DNA originated (section 2.2.3.1), was not related to the parasite, but instead proved to be a member of the Ectocarpales *s. l.* (results not shown).

Evidence for apical growth

Only indirect evidence could be found for growth by apical cells in *Herpodiscus durvillaeae*, due to difficulties observing growth patterns in this obligate parasite. Filaments of *Herpodiscus* are uniseriate, with a single cell terminating each unbranched filament in the external phase. The evidence for apical growth was based on the strong reaction of terminal cells of the external filaments with 'Eau de Javelle', and on ultrastructural observations of the internal phase.

In young parasite patches, the external filaments displayed prominent, elongated terminal cells, which showed a stronger reaction with 'Eau de Javelle', compared to other cells. Apparently, the substance reacting with the bleach occured at elevated levels in these cells. All cells of the parasite reacted with 'Eau de Javelle', therefore the substance appears to be a constant part of the cell wall. Accordingly, a higher concentration may indicate the new formation of cell walls, suggesting that the terminal cells are actively growing apical cells.

The cell walls of Sphacelariales are multi-layered (KARYOPHYLLIS *et al.* 2000). In the apical cells of *Sphacelaria rigidula* KÜTZING, the cell wall is thinnest at the apical hemisphere, i. e. in the area where the cell grows. The deposition of additional layers of wall material appears to

be limited to the area just below the apical hemisphere where the cell wall reaches its final width (KARYOPHYLLIS *et al.* 2000). Similarly, in the putative apical cells of *Herpodiscus* treated with 'Eau de Javelle', a blue band was observed just below the apical hemisphere, which persisted slightly longer than the 'colouring' of the rest of the cell. This blue band possibly indicated the site of fresh deposition of wall material, i. e. the site of growth.

In the internal phase of *Herpodiscus*, observations of terminal cells were difficult, due to the endophytic nature of the parasite. However, cells that were observed with TEM close to the host surface in March 2000, at the beginning of the external season of the parasite, showed a concentration of compartments and cell plasma in their apical portions. A similar polarization was observed in apical cells of *Sphacelaria tribuloides* MENEGH. (KATSAROS *et al.* 1983), suggesting that the cells of *Herpodiscus* were also apical cells, about to break through the surface and to develop into external filaments.

Based on the above findings, it is likely that apical growth also occures in those parts of endophytic phase that are deeply immersed in the host tissue. Apical growth appears to be more suitable to penetrate the very though thallus of *Durvillaea antarctica* than intercalary growth. TEM studies (section 4.1.2.4) present evidence that, presumably to get photosynthetic products from its host, parasite filaments squeeze between plasmodesmata of connected host cells and physically force these apart. Likewise, LUCAS assumes for parasitic fungi: "The apical mode of growth of most fungi is the key to the success of these organisms ... as ... parasites." (LUCAS 1998, page 30).

4.2.1.2.2 Placement of *Herpodiscus durvillaeae* within the Sphacelariales

The positive reaction with 'Eau de Javelle' and the (tentative) presence of apical cells as well as its DNA sequences identify *Herpodiscus durvillaeae* as a true member of the order Sphacelariales *sensu* MIGULA 1909 (Sphacelariales *s. s.*; DRAISMA *et al.* 2002). This raises the question, where the parasite is to be placed within the order, in which family in particular.

Molecular classification of *Herpodiscus durvillaeae*

Within the Sphacelariales *s. s.*, three families are currently recognized, based on morphological characters: the Sphacelariaceae, Stypocaulaceae and Cladostephaceae (OLTMANNS 1922; PRUD'HOMME VAN REINE 1982, 1993). A recent molecular study, however, revealed that the systematics within this order are in need of revision: by using partial RuBisCO gene sequences (*rbc*L + spacer), DRAISMA *et al.* (2002) showed that the family Sphacelariaceae is paraphyletic,

with the monotypic Cladostephaceae embedded within. Only the Stypocaulaceae forms a well supported clade. Not all five genera of the Stypocaulaceae were represented in the study, though, as none of the three *Phloiocaulon* species or *Ptilopogon botrycladus* (HOOKER & HARVEY) REINKE 1890 have been sequenced so far. Furthermore, by comparing their molecular results with morphological data, DRAISMA and co-workers (2002) found that most diagnostic characters separating families and genera within the Sphacelariales are not very useful, due to high percentages of homoplasy, phenotypic plasticity and/or polymorphisms of these characters.

To accommodate for these findings, DRAISMA *et al.* (2002) suggest different options of rearranging families and genera within the Sphacelariales: the most radical option would be to merge all genera of the order into a single genus, which would be either *Cladostephus* (as this name is older and according to the International Code of Botanical Nomenclature (GREUTER 2000) would have priority) or *Sphacelaria* (as this name is more widely known and could be conserved). The other options are based on keeping the families Stypocaulaceae and Sphacelariaceae (including Cladostephaceae). These could be comprised of a single genus each. Or, in the most divisive option, the currently recognized genera could be kept, in which case new genera would have to be created in the Sphacelariaceae for the various clusters within the paraphyletic genus *Sphacelaria.*

None of the options presented was given preference by the authors, though, as all of them would require some adjustments (DRAISMA *et al.* 2002). For example, to retain Stypocaulaceae and Sphacelariaceae would not be without problems, as the type species of the genus *Sphacelaria*, *S. reticulata* LYNGBYE in HORNEMANN, is possibly extinct, and its putatively closest relative, *S. radicans* (PRUD'HOMME VAN REINE 1982) clusters with the Stypocaulaceae rather than other *Sphacelaria* species. It has to be noted, though, that the branch separating *S. radicans* and the Stypocaulaceae from the other Sphacelariales does not have any bootstrap support (DRAISMA *et al.* 2002).

DRAISMA *et al.* (2002) found that including the *rbc*L spacer region into the analyses did not significantly influence the resulting phylogenetic trees. The present study, with a taxon sampling closely following DRAISMA and co-workers, but based on *rbc*L sequences only, indeed led to comparable results. However, the addition of sequences of *Herpodiscus durvillaeae* to the data set resulted in a slightly different phylogenetic tree, producing rather more confusion than clarifying the picture: like in the trees of DRAISMA *et al.*, Stypocaulaceae as well as the *Sphacelaria*-subgenera *Propagulifera* and *Pseudochaetopteris* (incl. *Cladostephus*) were well supported clades, but in the ingroup of Sphacelariales *s. s.*, not Stypocaulaceae, but members of the *Propagulifera* were the

first to branch off. *Sphacelaria radicans* and *S. caespitula* together took a basal position to the Stypocaulaceae on top of the tree, but these branches did not receive bootstrap support. *Herpodiscus durvillaeae*, as well as *Sphacella subtilissima*, stood on an unsupported position between the *Propagulifera* and *Pseudochaetopteris* clusters, thus, from a molecular systematic point of view, leaving the affinities of the parasite within the order unresolved.

Morphological classification of *Herpodiscus durvillaeae*

Ignoring the confusion within the order caused by the paraphyly of Sphacelariaceae for a moment and trying to classify *Herpodiscus durvillaeae* based on morphological characters instead bears its own problems. The families of the Sphacelariales (incl. the paraphyletic Sphacelariaceae) are mainly separated by two features, their different branching patterns and the presence or absence of secondary growth in the segments (PRUD'HOMME VAN REINE 1982). The branching mode was difficult to observe in *Herpodiscus*, as the only branched part of the parasite is the internal phase, which is hidden within the tissue of its host. Internal filaments showed mostly irregular branching modes, but at a single occasion, also an acroblastic branching pattern was observed. Evidence for the presence of secondary growth in the segments of *Herpodiscus* was sparse, as most cells in the external filaments had the same length as the apical cells. Occasionally, shorter cells were observed, or single intercalary cells at the beginning of the mitosis. However, whether they indicated rare events of secondary segment formation, or whether secondary segments were formed regularly in the external filaments and most of them had secondarily grown, could not be determined.

Moreover, external filament cells of *Herpodiscus* do not enlarge in diameter, which suggests a leptocaulous (i. e. no further enlargement of segments) rather than an auxocaulous (i. e. segments can enlarge in either length and/or diameter) growth pattern (PRUD'HOMME VAN REINE 1993). The difference in diameter noticeable between internal and external filament cells (section 4.1.2.2) was presumably caused by the pressure put on internal cells by adjacent host cells, due to the tight structure of the host cortex. Auxocaulous growth, however, is characteristic for the Cladostephaceae, as well as for some members of the Stypocaulaceae.

Another feature, that distinguishes, for example, members of the Stypocaulaceae from all other Sphacelariales (including *Herpodiscus*) is the presence of axillary zoidangia and their either aniso- or oogamous reproduction (DRAISMA *et al.* 2002; WOMERSLEY 1987). Additionally, Cladostephaceae and Stypocaulaceae are clearly separated from Sphacelariaceae - and *Herpodiscus* - by the strong cortication of the former two. Sphacelariaceae display only basal cortication, if

any at all (PRUD'HOMME VAN REINE 1982), while *Herpodiscus* is uncorticated. One has to keep in mind, though, that in a parasite like *Herpodiscus*, a feature such as a lack in cortication could also be the result of a reduction of the thallus in adaptation to the parasitic and/or endophytic life style rather than a character inherited from a free-living ancestor.

Nevertheless, based on these observations and the currently used morphological classification, *Herpodiscus* would have to be placed into the neighbourhood of the Sphacelariaceae – under the assumption that this family was monophyletic and therefore its description was still valid. A comparison of distances between DNA sequences (Appendix D, Table D 4.2.4) indeed shows that *Herpodiscus* appears to be most closely related to some *Sphacelaria* species of the *Propagulifera*-cluster, while an even closer distance of *Herpodiscus* to *S. radicans* is most likely due to the high number of unidentified bases at the 3'-end of both sequences.

The family Sphacelariaceae furthermore includes the monotypic *Sphacella subtilissima* REINKE 1890, which in the maximum parsimony and maximum likelihood trees, took a similar unresolved position between the *Propagulifera-* and the *Pseudochaetopteris*-clusters as *Herpodiscus.* And even though *Sphacella* and *Herpodiscus* are no more closely related to each other than to the average member of the Sphacelariales, judged from their distances, both species apparently share some morphological characters which separate them not only from the other members of the Sphacelariacae, but also from all other Sphacelariales (Table 4.3).

Absence of longitudinal walls

Sphacella, for example, only rarely forms longitudinal walls, which are also absent in *Herpodiscus.* The thalli of *Herpodiscus* and *Sphacella* therefore appear to be less complex, in contrast to the other members of the Sphacelariales with their at least partially parenchymatic thalli (WOMERSLEY 1987).

DRAISMA *et al.* (2002) pointed out that this lack of longitudinal walls is the only character that really separates the genus *Sphacella* from *Sphacelaria*, as the absence of some other features in the former genus are a consequence of it, e. g. the absence of a cortex or a hypacroblastic branching mode. They concluded that these differences were therefore not a good basis for the recognition of *Sphacella* as a separate genus, as, in their phylogenetic trees, *S. subtilissima* is nested within the genus *Sphacelaria.*

In the MP and ML trees of the present study, both *Herpodiscus* and *Sphacella,* i. e. both representatives lacking longitudinal walls, were separated from the monophyletic clades containing *Sphacelaria* species. However, these results did not receive any bootstrap support, and

were moreover sensitive to the number of taxa included in the analyses, as omitting other species from the data set (e. g. *Choristocarpus tenellus*) led again to a grouping of *Sphacella* with *Sphacelaria* species (results not shown).

Table 4.3: Comparison of the morphology of *Herpodiscus durvillaeae* and of members of families of the Sphacelariales *s. s.* currently recognized. References: PRUD'HOMME VAN REINE 1982, 1993; WOMERSLEY 1987; PETERS 1990; present study. *: based on indirect evidence; n/a: not applicable; n. d.: no data.

	Sphacelariaceae		**Stypocaulaceae**	**Cladostephaceae**	
genera, species	*Sphacelaria* sp.	*Sphacella subtilissima*	*Alethocladus corymbosus*, *Halopteris* sp., *Phloiocaulon* sp., *Ptilopogon botrycladus*, *Stypocaulon* sp.	*Cladostephus spongiosus*	***Herpodiscus durvillaeae***
growth modus	leptocaulous	leptocaulous	leptocaulous or auxocaulous	auxocaulous	possibly leptocaulous
subdivision of subapical cells	present	not regularly present	present	present	present (possibly not regularly)
secondary growth of segments	absent	absent	present	present	n. d. (possibly only enlargement in cell length)
longitudinal walls	present	absent (rarely present)	present	present	absent
thallus structure	polystichous	haplostichous	polystichous	polystichous	haplostichous
cortication	only basal rhizoidal	absent	present (heavily corticated)	present (heavily corticated)	absent
propagules	present, with 2-4 lobes, or absent	absent	absent	absent	absent
formation of laterals	hypacroblastic, irregularly, radial or distichous	hypacroblastic, irregularly	mostly acroblastic, irregularly, radial or distichous	(simultaneous occurence of several different branching modes) main axis: hypacroblastic, laterals: acroblastic	irregular, rarely acroblastic (branching only present in the internal phase)
phaeophycean hairs	present	absent	in axillary clusters or absent	present	absent
life history	isomorphic or slightly heteromorphic	n. d.	isomorphic	isomorphic	heteromorphic with very reduced gametophyte
reproduction	iso- or anisogamous	n. d.	aniso- or oogamous	isogamous	isogamous
position of unilocular sporangia	lateral	terminal or lateral	axillary	lateral	terminal
position of plurilocular sporangia	lateral	n. d.	axillary or n/a	lateral	n/a (gametophyte completely turns into a four-celled gametangium)
natural habitat	epilithic, epiphytic, epi-endophytic	obligate epi-endophytic	usually epilithic	epilithic (rarely epiphytic)	obligate epi-endophytic

To further investigate whether a separation of *Sphacella* from *Sphacelaria* would be justified on molecular grounds or not, and what the affinities to *Herpodiscus* are, sequences of other 'simple' Sphacelariaceae should be included in the phylogentic analyses, such as *Sphacelaria pulvinata* HOOKER & HARVEY, an epiphyte of *Carpophyllum maschalocarpum* (TURNER) GREVILLE endemic in New Zealand (ADAMS 1994). REINKE (1891) considered this species to be morphologically the least complex form of *Sphacelaria* and thus very similar to *Sphacella subtilissima.* Its erect

filaments are strictly uniseriate, only in the upper parts pluriseriate, and are rarely branched (REINKE 1891). So far, no molecular data are available from this species.

Absence of secondary growth of segments

Another feature present in all Sphacelariales except *Sphacella* is the formation of secondary segments by the subdivision of subapical cells. In *Sphacella*, secondary transverse walls are not completely lacking, as proposed by SAUVAGEAU (cited in PRUD'HOMME VAN REINE 1982), but are also not regularly observed (PRUD'HOMME VAN REINE 1982). On the other hand, what is completely absent in *Sphacella*, like in all other members of the Sphacelariaceae, is the secondary growth of segments, i. e. *Sphacella* cells are supposed to remain the same size they were at the time of the cell division. Thus, an occasional presence of transverse cell walls is indicated by cells half the length of usual ones.

A similar pattern is assumed for the external filaments of *Herpodiscus*, suggesting that the parasite possibly shares these characters with *Sphacella* as well, i. e. an only occasional formation of secondary segments in combination with a lack in secondary growth. No data are available regarding the internal hyphae of *Herpodiscus*, though. If present at all, a secondary growth of segments might be restricted to the endophytic part of the parasite thallus.

In conclusion, *Herpodiscus durvillaeae* shares some features with *Sphacella subtilissima*. Nevertheless, there are two characters that still distinguish *H. durvillaeae* from *Sphacella* as well as all other members of the Sphacelariales: its parasitic life style and its heteromorphic life history.

Parasitism

The presence of a partially endophytic thallus, such as in *Herpodiscus*, is not an unusual feature for the Sphacelariaceae, in contrast to the Stypocaulaceae and Cladostephaceae which usually grow epilithically (WOMERSLEY 1987). REINKE (1891) presented *Sphacelaria* species with basal parts anchored in the tissue of their phaeophycean hosts, e. g. *S. bornetii* HARIOT on *Cystophora subfarcinata* (MERTENS) J. AGARDH. He even used the presence or absence of this character as a criterion to distinguish between groups of *Sphacelaria* species by calling taxa with an endophytic base 'Sphacelariae parasiticae', in contrast to 'Sphacelariae autonomae' without (REINKE 1890), thus using the terms 'endophytic' and 'parasitic' as synonyms.

SAUVAGEAU considered this separation to be artificial, as he found the formation of endophytic filaments to be dependent on the substratum, rather than indicating a physiologcial dependance from the host (SAUVAGEAU 1900, cited in PRUD'HOMME VAN REINE 1982). But

despite these observations, other authors continued to use the terms "endophytic" and "parasitic" synonymously for algae which are at least partially embedded into host tissue, possibly assuming that this includes a dependance on photosynthetic products from the host as well (e. g. OLTMANNS 1922). In the partly endophytic *S. bipinnata* (KÜTZING) SAUVAGEAU, however, no physiologcial dependance from its host *Halidrys siliquosa* (L.) LYNGBYE could be detected (GOODBAND 1973). Moreover, some European *Sphacelaria* species with endophytic base, such as *S. caespitula* LYNGBYE, *S. rigidula* KÜTZING or *S. cirrosa* (ROTH) C. AGARDH, are indeed capable of growing either epiphytically or epilithically (PRUD'HOMME VAN REINE 1982).

Sphacella subtilissima, on the other hand, has been found only as an epi-endophyte growing on some members of the Sporochnales on the Balearic Islands (Mediterranean) and in Southern Australia. It forms an extensive endophytic base, which can consist either of a parenchymatic disc up to several cell layers thick, or of filaments which deeply penetrate into the host tissue (REINKE 1891; PRUD'HOMME VAN REINE 1982; WOMERSLEY 1987). But despite being considered an obligate epi-endophyte in the field, this species is nevertheless pigmented and apparently can be cultivated without its host (D. G. MÜLLER, personal communication; DRAISMA *et al.* 2001).

In *Herpodiscus durvillaeae*, in contrast, there is evidence for genuine parasitism, in a physiological sense: apart from being an obligate epi-endophyte of its host *Durvillaea antarctica*, it is nearly unpigmented and so far has not been successfully cultivated under laboratory conditions, either with or without its host (SOUTH 1974; PETERS 1990; E. C. HENRY personal communication). And even though no study has proven the existence of a transport of assimilates from the host to the parasite, there is indirect evidence for this as the presence of a symplastic contact between the parasite and its host *Durvillaea antarctica* was demonstrated in ultrastructual studies (section 4.1.2.4). Nevertheless, as *Herpodiscus* is the only known obligately parasitic brown alga, physiological dependence from a host, to whatever degree, is not a useful character for classifying this species, neither within the Phaeophyceae nor the Sphacelariales.

Heteromorphic life history

Members of the Sphacelariales usually have a diplohaplontic life history with isomorphic or only slightly heteromorphic generations, even though, in some species sexuality might be reduced or is unknown (CLAYTON 1981; PRUD'HOMME VAN REINE 1982, 1993; WOMERSLEY 1987; DRAISMA *et al.* 2002). *Herpodiscus durvillaeae* has a diplohaplontic life history as well, but in

contrast to the other Sphacelariales, its sporophyte and gametophyte are heteromorphic, with the gametophyte and gametangia being extremely reduced (PETERS 1990; section 4.2.2.2).

In the Phaeophyceae, the life history - isomorphic versus heteromorphic - is generally considered a character with a strong taxonomic signal: together with other features, such as thallus structure and sexual reproduction, it is traditionally used to characterise orders (CLAYTON 1981, 1990a; VAN DEN HOEK *et al.* 1995; GRAHAM & WILCOX 2000). This character has led to the suggestion to place *Herpodiscus* in close neighbourhood of the order Syringodermales (E. C. HENRY, personal communication, and in PETERS 1990). However, since the advent of molecular studies, systematics in the Phaeophyceae are in revision resulting not only in the creation of new orders, such as the Scytothamnales (PETERS & CLAYTON 1998), but also in the merging of formerly well defined orders, thus re-evaluating the taxonomic value of certain morphological characters.

An example is the order Ectocarpales: it has been controversially discussed by various authors, based on morphological characters, whether this order should be recognized either in a narrow or rather in a broad sense, with suggestions differing which groups other than Ectocarpales *s. s.* should be included (e. g. FRITSCH 1945; RUSSELL & FLETCHER 1975; CLAYTON 1981, 1990a; WOMERSLEY 1987; VAN DEN HOEK *et al.* 1995; review in ROUSSEAU & DE REVIERS 1999). Molecular genetic studies have presented evidence that the Ectocarpales *sensu stricto* are closely related to the Chordariales, Dictyosiphonales and Scytosiphonales (TAN & DRUEHL 1993; DRUEHL *et al.* 1997). Supported by morphological characters, such as the presence of pedunculate pyrenoids, which are absent in all other Phaeophyceae, these four orders were proposed to be merged to the Ectocarpales *sensu lato* (SIEMER *et al.* 1998; ROUSSEAU & DE REVIERS 1999; DE REVIERS & ROUSSEAU 1999). As a result, the Ectocarpales *s. l.* comprises taxa with various diplohaplontic life histories, the difference between generations being one of the characters used to separate the five families within the Ectocarpales *s. l.* proposed by PETERS & RAMIREZ (2001). The Ectocarpales thus show that life-history patterns may evolve rapidly within the brown algae.

In the Sphacelariales, the original description does not mention the life history of its members (MIGULA 1909), rendering the inclusion of *Herpodiscus* in this order unproblematical, and instead emphazising the importance of biochemical characters for taxonomic purposes, such as the positive reaction with 'Eau de Javelle'. Still its life history with an extremely reduced gametophyte separates *Herpodiscus durvillaeae* from families with isomorphic generations, not only, as argued before (PETERS 1990), from the Ralfsiaceae and Chordariaceae, but also from the

various families of the Sphacelariales. Consequently, to accommodate *Herpodiscus durvillaeae* within this order, it should be placed in its own monotypic family, the Herpodiscaceae fam. ined..

Taxonomic consequences

This proposal requires the most divisive option presented by DRAISMA *et al.* (2002) of rearranging taxa within the Sphacelariales *s. s.*: by keeping the Stypocaulaceae with its genera, the subgenera of *Sphacelaria* will have to be split into different families, as the Stypocaulaceae would otherwise be embedded within a paraphyletic Sphacelariaceae. A new family will have to be erected, for example, for the *Propagulifera* cluster (with *Propagulifera* gen. ined.). Cladostephaceae will include *Cladostephus* as well as some new genera for former *Sphacelaria* species, e. g. for the *Pseudochaetopteris* cluster or *Sphacelaria nana.* Sphacelariaceae with the single genus *Sphacelaria* will only comprise *S. radicans* and *S. caespitula.*

The position of *Sphacella subtilissima*, however, remains doubtful. As its affinities proved to be sensitive to the number of sequences included in the analysis (results not presented), more sequences of *Sphacelaria* species as well as sequences of Australian isolates of *Sphacella* should be added to the data set. This might improve the stability of its position separate from the various groups comprised of former *Sphacelaria* species and might resolve its relationship to *Herpodiscus durvillaeae.*

The closest relatives of *Herpodiscus* may be expected within the Sphacelariales of New Zealand. However, until the phylogenetic affinities of *Herpodiscus* with *Sphacella* and other members of the Sphacelariales are fully discovered, other questions will remain unsolved: is the simple morphology of *Herpodiscus* and *Sphacella* ancient or the result of reduction, i. e. are these two species to be placed at the base of the Sphacelariales, or are they derived from a (possibly common) heterotrichous ancestor with typical Sphacelarialean morphology? Additionally, the position of the Sphacelariales at the base of the Phaeophycean tree below the crown radiation suggests this order to be rather old. So where is the origin of the Sphacelariales to be found, north or south of the equator?

Answers to all these questions will require the inclusion of Southern Hemisphere taxa into future phylogenetic analyses, especially of species from the Australasian region. Following Europe, New Zealand and Australia are considered to be the main centres of distribution of the Sphacelariales (PRUD'HOMME VAN REINE 1982): WOMERSLEY (1987) recorded a total of 25 species for Australia: *Sphacella subtilissima*, 15 species of *Sphacelaria* (both genera family

Sphacelariaceae), six species of *Halopteris* (of which two species were moved to Stypocaulon; DRAISMA *et al.* 2002), two species of *Phloiocaulon* (family Stypocaulaceae) and the monospecific *Cladostephus spongiosus* (family Cladostephaceae). The only Sphacelarialean genera not present in the Australian flora belong to the Stypocaulaceae: *Alethocladus* (which is restricted to antarctic and sub-antarctic regions; PRUD'HOMME VAN REINE 1993) and *Ptilopogon.*

The genus *Ptilopogon*, however, is found in New Zeland. For the New Zealand marine flora, ADAMS (1994) lists a total of 17 species of Sphacelariales: apart from *Ptilopogon botryocladus*, there are eight species of *Halopteris*, and *Cladostephus spongiosus* as well as seven species of *Sphacelaria.* Only two of the *Sphacelaria* species are cosmopolitan, *S. cirrosa* and *S. tribuloides.* One of the other five occurs only in New Zealand and South Australia, the other four are endemic to New Zealand.

Thus all current and tentatively new families are represented in Australasia (PRUD'HOMME VAN REINE 1993); Herpodiscaceae fam. ined. is even endemic in New Zealand. However, apart from the present study, the phylogenetic affinities of members of the Sphacelariales of New Zealand have not been subject to any research. For example, none of New Zealand's four endemic *Sphacelaria* species has been part of genetic investigations. In general, there is no comprehensive study on Southern Hemisphere Sphacelariales (DRAISMA *et al.* 2002).

Therefore, future molecular systematic studies in the order Sphacelariales should not only generally include more species, but should especially focus on representatives from the whole Southern Hemisphere. A detailed (morphological and molecular genetic) study on New Zealand and Australian species may solve the phylogenetic affinites of *Herpodiscus durvillaeae* to other members of the Sphacelariales, e. g. to Australian *Sphacella subtilissima.* Additionally, by including species from South Africa and South America, some interesting new concepts may arise regarding the phylogenetic affinities within the whole order and its geographical origin.

Furthermore, it should be clarified whether *Herpodiscus durvillaeae* represents a genetically uniform taxon, or possibly comprises a complex of cryptic entities. For this, DNA samples from specimens covering the whole geographical range of the parasite in New Zealand need to be analysed, which may also reveal the age of this parasite species, and thus the probable age of the symbiosis of *Herpodiscus* and *Durvillaea.*

The genus *Durvillaea* is thought to have evolved in the Southern Hemisphere, with currently four species being distributed in Australasia, South America and the subantarctic islands (CHESHIRE *et al.* 1995). Studies on the biogeography of the species in Australasia

suggests that the genus *Durvillaea* was established before the separation of Australia and New Zealand from Gondwanaland around 80 million years ago (CHESHIRE *et al.* 1995). At the same time, around 70-80 million years ago, the Chatham Islands were split from mainland New Zealand (cited in NELSON 1994), thus an endemic *Durvillaea* species could evolve, *D. chathamensis* HAY 1979, as a sister species of *D. antarctica.*

The parasite *Herpodiscus durvillaeae* mainly infects *Durvillaea antarctica*, and has only rarely been observed on *D. willana* (E. C. HENRY, personal communication), suggesting that the host-parasite relationship may have evolved after the speciation of the genus *Durvillaea* took place. Moreover, only host populations on mainland New Zealand are infected - the parasite is absent from the Chatham Islands, in contrast to its host (NELSON *et al.*, 1991; author, personal observation) - thus *Herpodiscus* possibly evolved well after the establishment of the circum-Antarctic distribution of *Durvillaea antarctica.*

Comparisons of the genetic diversity of populations of the parasite and of the host may reveal whether the symbiosis between *Herpodiscus* and *Durvillaea* is another case to support the 'Red Queen hypothesis' (BELL 1982). This hypothesis suggests that sexuality is maintained to reach a high level of genetic diversity in a host population, and so prevent fast-evolving parasites adapting themselves to host genotypes by optimizing their infection mechanisms (BELL 1982). Similar to its host *Durvillaea antarctica*, but in contrast to the pigmented endophytic brown algae, *Herpodiscus durvillaeae* shows regular sexual reproduction. Its meiospores do not swim far, but settle nearby, on neighbouring sporophyte filaments or even remain in the sporangium, where they develop into very reduced and short-lived gametophytes. The fate of the gametes has not been observed, but they may be distributed further away from their parent thallus and, upon spore fusion and zygote germination, infect their host. In this way, the parasite may ensure that only genetically recombinant offspring act as means to carry the infection to other host individuals. In his famous book 'Through the Looking Glass', LEWIS CARROLL lets the Red Queen say to Alice: "Now, *here*, you see, it takes all the running *you* can do, to keep in the same place." (CARROLL *et al.* 2000, page 174). Evidence for a genetic 'race of arms' between *Durvillaea* and *Herpodiscus*, may be obtained, for example, using AFLP (Amplified Fragment Length Polymorphism) which gives reliable results at a high resolution to test for genetic variation within populations (P. LOCKHART, personal communication; DE BRUIN *et al.* 2004).

4.2.2 Ultrastructure

4.2.2.1 Methods

The sample preparation for light and scanning electron microscopy of *Durvillaea antarctica* infected with *Herpodiscus durvillaeae* readily produced suitable sections. Problems were, however, frequently encountered with the preparation of specimens for transmission electron microscopy. Large brown algae are notorious for causing difficulties in preparations for TEM (HALLAM & LUFF 1988). *Durvillaea antarctica* was no exception. This species has very thick cell walls and large amounts of intercellular material which give the thallus a leathery appearance and help it survive in the surf of exposed coasts. However, this also slows down the penetration by chemicals necessary to immobilize cellular structures. Most of the common artifacts mentioned by HALLAM & LUFF (1988) were also encountered in the present study, including the shrinkage and extraction of cell contents as well as wall materials, the loss of physodes, negatively contrasted membranes e. g. of thylakoids, and the presence of myelin figures. Additionally, ice crystal damage was evident in samples subjected to cryofixation and cryosubstitution (GALWAY *et al.* 1995).

Tissue samples had to be comparatively large, in order to find areas of interest, as cellular connections between parasite and host cells appeared to occur only in the cortex and were, moreover, unevenly spread over sections. These large samples consisted mostly of extracellular material. Together with mucopolysaccharides exuded by the *Durvillaea* tissue acting as an additional barrier, the incomplete penetration by chemicals resulted in a poor fixation and dehydration, and subsequent poor infiltration with hydrophobic resins, producing brittle blocs with holes in the middle of sections.

The present study did not manage to optimize the preparation of tissue of *Durvillaea antartica* for TEM. Due to persistent problems with the infiltration, for example, the effects of different concentrations of aldehydes, cacodylate buffer and salt solutions were not determined. However, comparatively good results were achieved with a chemical fixation using a modified KARNOVSKY mixture (after CLAYTON & ASHBURNER 1994 and SCHOENWAELDER & CLAYTON 1998a, 1998b), which took place at low temperatures overnight. For *D. antartica*, longer times of fixation were required so that chemicals could reach the areas of interest, even though other studies show that a fixation for longer than four hours may lead to deterioration of the tissue, e. g. in *Xiphophora* (S. LUFF, unpublished script).

A marked improvement of dehydration and infiltration was observed when longer steps were used in combination with gentle gradients of solvents. The step length, however, proved to be critical because prolonged contact with solvents in high concentrations of solvents could result in extraction of cell contents and wall material. The problem of extraction was solved by using Quetol resin, which is less hydrophobic than SPURR's resin, and thus the replacement of ethanol before infiltration with the highly volatile intermedium propylenoxide could be omitted from the protocol.

The shrinkage of cell contents in preparations of *Durvillaea* sp. is a common problem (HALLAM & LUFF 1988). It may have been caused by high osmolarities of fixatives and wash buffers. Alternatively, shrinkage could have occured during dehydration and infiltration (as suggested by HALLAM & LUFF 1988). The 'negative image' of thylakoids in plastids was due to a fixation of the plastid plasma and an extraction of the thylakoid membrane material which appears to be a common artifact of the chemical fixation (SCHMID 2003).

The addition of caffeine to fixation media and wash-buffers was essential for stabilizing physodes. Omitting this substance from the solutions inevitably resulted in the partial or complete loss of physode contents. This was especially evident in cryofixed/cryosubstituted material, as the caffeine precipitated in methanol at -20°C. However, even if physodes were sufficiently stabilized during chemical fixation, they were still sometimes lost during sectioning, due to being harder than the surrounding tissue. In this case, smooth round holes were left in the cells. Often, cutting marks were visible especially in the physodes.

Microwave-enhanced fixation and infiltration did not seem to influence the quality of the preparation. However, this method was only employed in the beginning of the present study, thus any differences might have been obscured by other artifacts caused by insufficient fixation and infiltration. Physically immobilizing structures by cryofixation and subsequent cryosubstitution, on the other hand, had a markedly negative effect on the samples. With this method, only the outer-most 10-20 µm of a sample can be sufficiently fixed without causing damage to cellular structures by the formation of ice crystals (GALWAY *et al.* 1995). Thus cryofixation only allows the preservation of very small samples. The *Durvillaea* cells showed ice crystal damage, however, this was expected to happen because of the large size of the samples. But even the external filaments of *Herpodiscus* displayed signs of slight ice crystal damage, indicating that the procedure was not sufficiently optimized for these samples (results not presented). High-pressure freezing, a technique to immobilize samples up to 200 µm thick (GALWAY *et al.* 1995; TIEDEMANN *et al.* 1997), was not available in the host laboratory or

elsewhere in New Zealand (staff of South Campus Electron Microscopy Unit, personal communication).

4.2.2.2 Ultrastructure of *Herpodiscus durvillaeae*

The present study is the first to investigate the ultrastructure of *Herpodiscus durvillaeae* and of its interactions with its obligate host *Durvillaea antarctica.*

4.2.2.2.1 The interface between *Herpodiscus* and *Durvillaea*

At the interface between *Herpodiscus* and *D. antarctica*, no evidence was found for parasite cells entering host cells as proposed by SOUTH (1974). Also, neither intracellular phases such as haustoria nor fusions of parasite cells with host cells were observed. The former are a common feature, for example, of biotrophic fungi infecting higher plants (SMITH & SMITH 1990) and the latter typical for infections of red algae by Rhodophycean parasites (GOFF & ZUCCARELLO 1994). The interaction between *Herpodiscus* and *Durvillaea* cells also did not show similarities to the *Notheia/Hormosira* symbiosis, in which the lack of cytoplasmatic connections between epiphyte and host suggest an apoplastic transfer of assimilates. Instead, ultrastructural evidence has been found for a unique symplastic contact between the cells of *Herpodiscus* and *D. antarctica*, which, however, in some aspects ressembles the interaction of the holoparasitic phanerogam genus *Cuscuta* L. (Convolvulaceae) with their higher plant hosts.

Members of the genus *Cuscuta* (commonly called 'dodder') parasitize a large range of higher plants (KUIJT 1969). Like biotrophic fungi, they attack the vascular tissue of their hosts, in order to intercept the transfer of assimilates and thus to form a metabolic sink (BIRSCHWILKS 2003). However, in contrast to the fungi, *Cuscuta* may form symplastic contacts to its hosts (DÖRR 1969, 1990; BIRSCHWILKS 2003). *Cuscuta* species insert multicellular haustoria into the tissue of their hosts. From the tips of these haustoria, inter- and intracellular 'search hyphae' grow in all directions, apparently in search for the hosts' vascular tissue. As soon as a 'search hypha' comes into contact with a sieve element of the host phloem, it differentiates into an 'absorption hypha' (DÖRR 1969, 1972): The parasite cell forms protusions, which like the fingers of a hand, surround the sieve element. Moreover, its nucleolus, tonoplast, ribosomes and dictyosomes degenerate, giving the 'absorption hypha' the appearance of a sieve element itself. At the same time, the part of the plasmamembrane that is in direct contact with the host sieve element forms protuberances to enlarge the surface area, thus the 'absorption hypha' also shows characters of a transfer cell (DÖRR 1972). In *Cuscuta odorata* parasitising on *Pelargonium zonale*,

symplastic connections were observed between search hyphae and host cells (DÖRR 1969, 1990). BIRSCHWILKS (2003) verified the presence of plasmodesmata between the contact hyphae of *Cuscuta reflexa* ROXB. and cells of the tissue surrounding the sieve elements of its host, e. g. *Vicia faba* L.. The plasmodesmata at the interface between another holoparasitc phanerogam species, *Orobranche crenata* FORSK., and its host *Vicia narbonensis* L. may even develop into sieve pores (DÖRR & KOLLMANN 1995).

Analogous to these higher plant parasites, *Herpodiscus durvillaeae* appeared to interfere with the transfer of assimilates within the thallus of *D. antarctica*, by forming direct cytoplasmatic connections to host cells. Interactions were observed anywhere within the cortex, as the *Durvillaea* thallus lacks a differentiated transport tissue (CLAYTON 1990b). However, the fields of plasmodesmata appeared to be the prime target, presumably because they represent the sites of transfer of substances between adjacent cells. Moreover, here the host cell walls are the thinnest. Therefore, parasite cells may have been attracted to them, possibly by increasing concentrations of substances leaking into the apoplast. Likewise, biotrophic fungi (JACOBS 1990; SPENCER-PHILLIPS 1997) or *Cuscuta* cells (DÖRR 1972; BIRSCHWILKS 2003) appear to grow towards the phloem of their phanerogam hosts.

In a way resembling the behaviour of the 'absorption hyphae' of *Cuscuta* species, *Herpodiscus* filaments surrounded the host fields of plasmodesmata like 'single fingers' or 'hooks'. However, the 'fingers' of the higher plant parasite cell cover the length of the host sieve tube and form cytoplasmatic connections to the host anywhere. *Herpodiscus*, in contrast, squeezed between the host cells within the field of plasmodesmata and established a symplastic contact by forming plasmodesmata connecting to the plasmodesmata of the host cells.

In *Cuscuta*, the growth inside the host is suggested to be a combination of mechanical and enzymatic processes (KUIJT 1969). That enzymes are invoved, is indicated by a high activity of enzymes in the affected tissue and the presence of partly dissolved cell walls (NAGAR *et al.* 1984; SRIVASTAVA *et al.* 1994). Likewise, the extracellular matrix of *Durvillaea* often appeared to be loosened around *Herpodiscus* cells. Moreover, host cells did not appear to be relocated by the presence of the parasite cells, suggesting an enzymatic mode of growth for the parasite. However, a putative presence of cell wall degrading enzymes in internal cells of *Herpodiscus* requires verification.

Entering the interstices of the fields of plasmodesmata between host cells, on the other hand, and forcing these apart seems to be a mainly mechanical process, by which the fibrillar structure of the host cell walls may be exposed and the cytoplasmatic bridges between host cells

may rupture. It is not clear, though, how this is accomplished. For example, do the TEM sections in Plates 4.7 and 4.8 showing attacks on the *Durvillaea* fields of plasmodesmata display a succession of the development of the cellular contact between parasite and host cells, or do they represent sections through different parts or even different modes of attacks? Three-dimensional pictures based on serial sections documenting single attacks may provide further information not only about the anatomy of the interface between both species, but also about their development.

The plasmodesmata, by which the *Herpodiscus* cells were connected to their host cells, developed independently of cytokinesis, connecting non-sibling cells. Therefore they should be termed 'secondary plasmodesmata', in contrast to 'primary plasmodesmata' which are the result of cytokinesis (EHLERS & KOLLMANN 2001) and thus connect sibling cells. During cytokinesis in higher plants, primary plasmodesmata are formed along strands of the endoplasmatic reticulum (ER) trapped in the developing cell plate. Later, the constricted ER cisternae become the cores (desmotubuli) of the plasmodesmata (EHLERS & KOLLMANN 2001).

The plasmodesmata of brown algae, in contrast, are simple plasma membrane-lined tubes which are considered to lack a core (LA CLAIR 1981; LUCAS *et al.* 1993). Their formation, however, is not well understood (LUCAS *et al.* 1993). In *Cutleria cylindrica* OKAMURA, the cell wall between separating cells is formed by centripetal infurrowing of the plasmalemma. Precursors of plasmodesmata can be observed as plasmalemma-lined channels within this nascent furrow. ER cisternae seem not to be involved in their formation (LA CLAIR 1981). However, a central core of electron-dense material or even desmotubuli are reported from plasmodesmata of *Sphacelaria tribuloides* MENEGH. (GALATIS *et al.* 1977) or from sieve plates of *Laminaria groenlandica* ROSENV. (SCHMITZ & SRIVASTAVA 1974, according to MARCHANT 1976), respectively. In contrast, the plasmodesmata between of *Durvillaea* cells, or between cells of *Herpodiscus* and *Durvillaea*, appeared to have a simple structure lacking a core, similar to those described from *Cutleria*. However, the absence of constricted ER cisternae may have been a preparation artifact.

Secondary plasmodesmata may occur between cells of the same organism, cells of different organisms belonging to the same species and between cells of organisms belonging to different species (review in EHLERS & KOLLMANN 2001). The latter, interspecific secondary plasmodesmata are known from parasites and also from heterografts (KOLLMANN & GLOCKMANN 1985, 1990, 1991; KOLLMANN *et al.* 1985). Whether inter- or intraspecific, the formation of secondary plasmodesmata in higher plants follows a similar scheme: At the interfacing cell walls, ER cisternae of both involved cells, e. g. of both stock and scion of graft

unions, become closely associated with the plasmalemma. Upon further loosening of the cell wall material, the membranes of both cells come into contact and fuse, thus producing a single ER strand transversing the wall (EHLERS & KOLLMANN 2001). Thinning of the cell walls, possibly by cell wall lysis (JONES 1976), appears to be a pre-requisite of this process. Moreover, some coordination and thus communication between the cells is required, in order to exactly match their ER cisternae. Both partners form so-called 'half plasmodesmata'. Half plasmodesmata that display dead endings halfway through the interfacing cell wall are considered the result of mismatches between both cells. Usually, secondary plasmodesmata are branched as continuous strands of ER are involved in their formation (EHLERS & KOLLMANN 2001).

In graft unions, phytohormones leaking out of the wounded vascular tissue may induce a dedifferentiation of cells of the stock and the scion (KOLLMANN & GLOCKMANN 1990), thus these may be at the same stage of development and develop secondary plasmodesmata synchronously. Most continuous secondary plasmodesmata are, moreover, found in areas where tissues of stock and scion match, i. e. where both are of the same type of tissue (KOLLMANN *et al.* 1985). Parasites, on the other hand, face the problem of having to form secondary plasmodesmata to already differentiated host cells of non-matching tissue. Nevertheless, even in the symbioses between *Cuscuta* and its hosts, like in other higher plant parasites, both partners seem to be involved in the formation of the secondary plasmodesmata (EHLERS & KOLLMANN 2001; BIRSCHWILKS 2003). In the *Herpodiscus/Durvillaea* symbiosis, in contrast, the host plasmodesmata already existed, therefore only the parasite cell was required to form new, outer-wall plasmodesmata on its side of the interface. These secondary 'half plasmodesmata' may then have fused with the primary 'half plasmodesmata' of the host.

Primary symplastic connections that turn into secondary ones are also known from parasitic red algae and their hosts, however, the 'secondary plasmodesmata' of *Herpodiscus* are different from them. In multicellular Rhodophyta, cells are in contact via so-called 'pit connections', complex structures derived from plasmodesmata which are plugged by electron-dense material consisting of polysaccharides and proteins. The structure of the plugs is used as a character to distinguish taxonomic groups (VAN DEN HOEK *et al.* 1995). Similar to plasmodesmata, primary pit connections connect sibling cells derived from a cell division, while secondary pit connections are those found between non-sibling cells. Most of the latter are the result of cell fusions which are a common feature of red algal cells. For example, in parasitic red algae, upon cell fusion between the host and the parasite cell, former primary pit connections between the parasite cell and its sibling cells become secondary pit connections between the

sibling cells and the newly formed heterokaryotic cell. In this way, a cytoplasmatic continuity is reached between the host and the parasite tissue, allowing the symplastic transport of assimilates to the parasite (GOFF & COLEMAN 1985; GOFF & ZUCCARELLO 1994).

Secondary pit connections in red algae may also be formed directly between adjacent host and parasite cells: interspecific secondary pit connections to which both cells have contributed may display differently structured pit plugs on each side, according to the origin of the involved membranes (GOFF 1982). Such 'true secondarily formed' secondary pit connections have, for example, been reported from the interface of the alloparasite *Holmsella pachyderma* (REINSCH) STURCH and its host *Gracilaria verrucosa* (HUDS.) PAPENFUSS (QUIRK & WETHERBEE 1980).

Regardless of how secondary pit connections are formed, the term is generally restricted to the complex secondary cell connections established between red algal cells (G. ZUCCARELLO, personal communication). Therefore, the term 'pit fields' given by HALLAM & LUFF (1988) for the fields of plasmodesmata between *Durvillaea* cells should be avoided. Otherwise, the 'secondary plasmodesmata' between *Herpodiscus* and *Durvillaea* would have to be termed 'secondary pit connections', despite their simple structure.

The presence of symplastic connections between *Herpodiscus* and *Durvillaea*, however simple they may appear, raises a number of questions. For example, how are these 'secondary plasmodesmata' formed? Putatively, the internal filaments, like those of the external phase, grow with apical cells. These, like in other Sphacelariales (KARYOPHYLLIS *et al.* 2000), may have a very thin cell wall at their tip. This tip may come into close contact with the host cell wall at the fields of plasmodesmata. However, what happens after a contact with the host cell wall is established remains to be investigated. How, for example, do *Herpodiscus* cells actually manage to develop channels through the parasite cell wall that exactly match those of the host plasmodesmata? Like in the secondary plasmodesmata of higher plants, this process would require some coordination by the parasite, i. e. the parasite would need to somehow locate the host plasmodesmata. Moreover, the parasite half plasmodesmata appeared to be straight and simple, and not branched like the secondary plasmodesmata in higher plants, leading back to the question of how simple brown algal plasmodesmata are generally formed (LUCAS *et al.* 1993), in cases where the ER appears to not be involved in this process. SCHMITZ & SRIVASTAVA (1974) found evidence for secondarily formed plasmodesmata in neighbouring cells of *Laminaria groenlandica*, which they considered to be formed by enzymatic digestion of the cell walls. Accordingly, *Herpodiscus* may

also be able to form its secondary half-plasmodesmata by digestion of the cell wall, provided, cell wall degrading enzymes were indeed present.

In addition to the morphological questions, a number of physiological questions are also raised. For example, the putative transfer of substances to the parasite requires confirmation. Physiological studies using trace markers may, for example, reveal the nature and the direction of a metabolite transport between host and parasite. In *Cuscuta reflexa*, the presence of a directed symplastic transport from the host phloem to the parasite phloem was demonstrated by fluorescence markers (BIRSCHWILKS 2003) which show a similar distribution as assimilates, such as jellyfish green fluorescent protein (GFP; IMLAU *et al.* 1999) or carboxyfluorescin (CF; WRIGHT & OPARKA 1996).

Also, it is not clear what else *Herpodiscus* may gain from its host, apart from the proposed assimilates. For example, where do the polyphenolics in the *Herpodiscus* physodes originate from? And how does the putative drainage of metabolites affects the host cells? Host cells, for example may show signs of senescence, while parasite cells involved in the interface may indicate high metabolic activity by elevated numbers of mitochondria, dictyosomes and ribosomes (DÖRR 1969). However, in the present study cells of *Durvillaea* and *Herpodiscus* were not preserved well enough to show marked alterations between attacked and unattacked host cells, or between attacking and 'normal' internal parasite cells, respectively.

Last but not least, the metabolic sink which *Herpodiscus* represents for its host needs to be quantified, i. e. the amount of assimilates or other metabolites drained from the host should be estimated in order to assess the impact the infection may have on the host. However, for a calculation of the net uptake of *Herpodiscus*, the assimilation of the parasite itself also has to be taken into account, because the present study showed that the plastids of the parasite may be less reduced than previously thought (PETERS 1990).

4.2.2.2.2 Plastids in *Herpodiscus durvillaeae*

The plastids of *Herpodiscus* were relatively small, but showed characteristic features of brown algal plastids, e. g. a girdle lamella and thylakoids. The latter, however, were scarce and were not assembled in stacks of three which is typical of brown algal lamellae (DODGE 1973; see also Plate 4.2, Figure E). Instead, lamellae were in stacks of two, or thylakoids were single, indicating some reduction in the plastids. Moreover, some plastids seemed to be even more reduced: they were dumbbell-shaped and did not show any developed thylakoids, but nevertheless displayed electron-transparent areas at each end, which may represent the genophore (BISALPUTRA &

BISALPUTRA 1969). Fixation artifacts cannot be ruled out as cause for these oddly structured plastids. However, similar small plastids with only few or no lamellae were also observed in apical cells of *Sphacelaria tribuloides* (KATSAROS *et al.* 1983). The plastids in non-dividing cells of Sphacelariales, in contrast, are larger and display numerous thylakoids (BISALPUTRA & BISALPUTRA 1969; KATSAROS *et al.* 1983). Likewise, holoparasitic angiosperms such as *Cuscuta* sp. have plastids which may be reduced in size and contain only few thylakoids, compared to those of non-parasitic species (BIRSCHWILKS 2003). The dumbbell-shaped plastids moreover ressembled the shape of vestigial plastids of apicomplexan parasites such as *Toxoplasma gondii* NICOLLE & MANCEAUX. Here, the dumbbell shape is suggested to mark a stage in the plastid division prior to cytokinesis (MCFADDEN *et al.* 1997). Whether this is also true for *Herpodiscus* plastids remains to be investigated. Generally, there was no pattern evident for the distribution of dumbbell-shaped plastids or plastids displaying thylakoids, as both types occured in external as well as internal cells. In the internal phase, plastids with thylakoids were even observed in parasite cells attacking *Durvillaea* cells.

The plastids of *Herpodiscus* may to some degree be reduced, however, in UV light the plastids of external parasite cells displayed a weak red autofluorescence similar to the characteristic autofluorescence of chlorophyll. This was surprising, as *Herpodiscus* was described as having grey plastids lacking pigments (PETERS 1990). Additionally, *Herpodiscus durvillaeae* apparently possesses an intact *rbc*L gene: even though not all positions have been determined, the 5'-end sequenced in the present study did not display any indels or major base changes, thus making the *rbc*L sequence of *Herpodiscus* unambiguously alignable to those of the other Phaeophycean sequences.

Intact RuBisCO subunit genes may be present in non-photosynthetic species: a functional *rbc*L gene was, for example, observed in the heterotrophic euglenoid flagellate *Astasia longa* (GOCKEL & HACHTEL 2000). In parasitic higher plants, in contrast, the plastid genome is usually reduced, compared to non-parasitic relatives: as the parasite relies on photosynthetic products from its host, the genes coding for photosynthetic enzymes are under relaxed functional constraint. In some species of the genus *Orobanche*, the *rbc*L gene has degenerated to a pseudogene, while some *Cuscuta* species have retained a potentially functioning gene (WOLFE & DE PAMPHILIS 1997; HIBBERD *et al.* 1998). The *Orobanche* species are holoparasites completely lacking chlorophyll, thus the presence of a potentially functioning *rbc*L gene in some of these parasitic plants as well as in *Astasia longa* may reflect a relatively recent loss of photosynthetic abilities, or the gene might serve some other, so far unknown, function (WOLFE & DEPAMPHILIS

1997). The latter is also assumed for apicomplexan parasites such as *Toxoplasma gondii* or *Plasmodium falciparum* WELCH, the pathogen causing Malaria, which contain plastid genes on a circular 35kb DNA (MCFADDEN *et al.* 1997).

In *Herpodiscus*, the apparently intact *rbc*L gene in combination with autofluorescence that indicates the presence of chlorophyll suggests the presence of a functional RuBisCO, in contrast to the parasites mentioned above, and a low level of photoautotrophic carbon fixation. PETERS (1990) stated that the term 'assimilator' for the vegetative external filaments suggested by SOUTH (1974) should be avoided, due to the lack of evidence for photosynthetic activity; however, the results of the present study confirm that this term is appropriate. Even though the 'assimilators' may not produce enough assimilates to support the parasite, they nevertheless seem to be able to photosynthetize.

Furthermore, the round opaque inclusions observed in some *Herpodiscus* plastids may represent storage products, possibly the harvest from the parasite's own photosynthetic activity. However, higher plant parasites are known to store any carbohydrate taken from the host, which is not immediately used, in their plastids, transforming them into amyloplasts (SINGH *et al.* 1968; BARCKHAUS & WEINERT 1974; cited in BIRSCHWILKS 2003). Therefore, the origin of the putative storage products in the plastids of *Herpodiscus* needs to be clarified, particularly, as Phaeophycean accessory pigments such as fucoxanthin appear to be absent. Pigment extracts to prove their presence or absence, however, will be difficult to obtain from the parasite, as material from the field will most likely be contaminated with fully pigmented epiphytes such as pennate diatoms and other brown algae.

In higher plant physiology, those parasites which are still able to photosynthetize to a certain degree, and may partly support themselves, are considered hemiparasites, while those which totally depend on their hosts for assimilates and nutrients are termed holoparasites (SCHUBERT & WAGNER 1993). *Herpodiscus* as an obligate endophyte, which apparently has only reduced photosynthetic abilities and cannot be cultivated without its host, seems to belong to the holoparasites. However, the inability of a parasite to grow in absence of its host could also be due to inappropriate culture conditions. As GOFF states: "...the term obligate is also a measure of our culturing abilities." (GOFF 1982, page 363). Accordingly, further attempts to cultivate *Herpodiscus* may reveal whether this parasite could live saprophytically on some medium containing, for example, carbohydrates, or whether it depends on more from its host than just assimilates, e. g. a structural habitat.

4.2.2.2.3 Gametophytes of *Herpodiscus durvillaeae*

The gametophytes and the gametes of *Herpodiscus*, like its vegetative cells, appear to possess 'functioning' plastids with thylakoids and chlorophyll autofluorescence. Moreover, some gametophytes showed a slight green colouring that was evident even in bright field light. Thus, the gametophytes also appear to be less reduced than PETERS (1990) suggested and may rely on their own photosynthesis for the short duration of their existance. Eyespots, however, were not observed in the developing gametes, thus confirming PETERS' observations. Eyespots are also lacking in meiospores of *Halopteris filicina* (KATSAROS & GALATIS 1986), but have, for example, been observed in gametes of *Sphacelaria furcigera* KÜTZING (VAN DEN HOEK & FLINTERMAN 1968).

The presence of vestigial walls between the developing spores in the 'secondary unilocular sporangia' of *Herpodiscus* (SOUTH 1974) confirms, on the ultrastructural level, their identity as reduced gametophytes transforming completely into plurilocular gametangia, as suggested by PETERS (1990). The cell walls between loculi in the gametophyte-turned gametangia also showed the transistory blackening with 'Eau de Javelle' and became visible even under the light microscope.

The outer cell wall of the gametophyte-turned gametangium consisted of two layers. In some members of the Fucales, the presence of three distinct layers in the cell walls of the oogonia are considered remnants of the cell walls of the unilocular sporangium, the very reduced gametophyte and the gametangium (CLAYTON 1984). Accordingly, in the *Herpodiscus* gametophyte, the outer layer may be a remnant of the zooid that was transformed into the gametophyte without germinating. The inner layer, which, moreover, encloses the gametes and thus forms the loculi, may represent the wall of the plurilocular gametangium.

No remnants of the inner walls separating the loculi were observed in emptied gametangia, however. In other brown algae, for example, *Sphacelaria furcigera*, *Ectocarpus siliculosus* or *Xiphophorocolax aotearoae*, regardless of whether their plurilocular sporangia are uni- or pluriseriate, spores are released through an apical pore that belongs to a row of underlying loculi. Accordingly, the walls in the middle and at the apex have to disintegrate before spores are liberated through the pore (VAN DEN HOEK & FLINTERMAN 1968; VAN DEN HOEK 1995; present study, Plate 3.6, Figure F). In *Herpodiscus*, gametes are also liberated through a single pore and are, moreover, not enclosed in a walled tetrad (PETERS 1990). Therefore the inner walls must have completely disintegrated prior to gamete release.

Generally, the reduced *Herpodiscus* gametophytes ressemble those of *Syringoderma* species (E. C. HENRY, personal communication) which show a succession of reduction (HENRY 1984): *S. phinneyi* still features a free living gametophyte generation, while in *S. floridana*, meiospores are released which upon settling, directly turn into two-celled gametophytes releasing two gametes (see Figure 1.1, 2). In *S. abyssicola* (SETCHELL & GARDNER) LEVRING, the meiospores do not leave the unilocular sporangia, but develop inside. Gametes are released from the unilocular sporangium in tetrads, still surrounded by the gametangium wall. *Herpodiscus* meiospores released from the unilocular sporangia settled on filaments nearby, like the spores of *S. floridana*, or even remained inside the sporangium, like those of *S. abyssicola* (HENRY 1984). Thus, gametes released from the gametophytes of *Herpodiscus*, which developed inside the unilocular sporangium, may superficially appear to have been directly released from that unilocular sporangium.

4.2.2.2.4 Autofluorescence of unilocular sporangia and terminal cells

Developing unilocular sporangia and some terminal cells exibited a bright autofluorescence, while the other cells of the filaments showed only a weak autofluorescence. In higher plants, autofluorescence in UV light is used as an indicator for phenolic compounds (SMITH & O'BRIEN 1979; PETERSON 1991). In some brown algal species, e. g. *Hormosira banksii* (Fucales, Phaeophyceae), eggs and zygotes exibit autofluorescence, due to the presence of phenolics (SCHOENWAELDER & CLAYTON 1999). In the Fucales, this phenomenon seems to be species-specific: for example in *Durvillaea potatorum* eggs and zygotes do not autofluoresce (except for pigments; CLAYTON & ASHBURNER 1994), while in *Durvillaea antarctica*, the eggs show a greenish autofluorescence, which is different from the red colour usually exibited by pigmented cells (HEESCH, unpublished data).

Phenolics are a common component of brown algal cells. They are concentrated in small, membrane-bound organelles known as physodes (e. g. RAGAN 1976; PELLEGRINI 1980), and are considered to be part of the defense against herbivores (HAY & FENICAL 1988; VAN ALTENA & STEINBERG 1992). Furthermore, physodes play an important role in the formation of cell walls, for example in zygotes of the Fucales (SCHOENWAELDER & CLAYTON 1998a, 1998b). By the physodes releasing their contents into the developing wall, the phenolics eventually become part of the cell wall. Their function could probably be to cross-link other components such as alginates and cellulose, and thus to stabilize the walls (SCHOENWAELDER & CLAYTON 1999), as phenolics do in higher plant cells (SMITH & O'BRIEN 1979).

As for the Fucales, the autofluorescence of *Herpodiscus durvillaeae* may also be due to phenolics concentrated in the growing cells, which are involved in the formation of cell walls. In the unilocular sporangia, for example, the presence of a greenish autofluorescence seemed to indicate certain stages of spore formation: In some developing sporangia, the autofluorescence comprised the whole lumen, while in others it was reduced to small bodies within the developing spores, or it had completely vanished. In *Halopteris filicina*, the centre of unilocular sporangia is filled with storage bodies, while multiplied nuclei, organelles and physodes are concentrated at the periphery of the sporangium, before they are distributed among the developing spores (KATSAROS & GALATIS 1986). Likewise, organelles or vesicles putatively containing the autofluorescent substance may first be concentrated in the periphery of the *Herpodiscus* sporangia. Upon spore formation, their contents may either be included in the developing spores, or they may be metabolized, e. g. during wall formation. No greenish autofluorescence was observed in the cytoplasm of settled spores and gametophytes, however, the walls of the former occasionally displayed a slight autofluorescence, possibly at the site of the attachment.

In contrast, the autofluorescence observed in some terminal cells of external filaments most likely did not indicate growth, but appeared to be due to large accumulations of material, possibly polyphenolics. These were not enclosed in physodes, but formed a more or less compact matrix filling most of the lumen of the terminal cells, while organelles and the plasmalemma seemed to be reduced or absent. Moreover, the autofluorescing areas were separated from the adjacent cells by vaulted and enforced cell walls without plasmodesmata, suggesting that the subterminal cells had retracted from the terminal cells. The latter possibly served as disposal sites for these substances which were either of no further use to the parasite, or which originated from the host and could not be metabolised by *Herpodiscus*. *Cuscuta* species, for example, do not only receive assimilates from their hosts, but also other substances which are not beneficial to the parasite or may even be toxic, such as alkaloids, glycosides or herbicides (review in BIRSCHWILKS 2003).

However, these putative deposits of polyphenolics could also serve another purpose: they may, for example, deter herbivores from the external parasite filaments. Indeed, the *Herpodiscus* patches seemed to be largely untouched by grazers such as herbivore molluscs which are otherwise frequently observed on macroalgae displaying 'lawns' of epiphyte growth.

Future studies focusing on the chemical composition of contents of the cytoplasm and cell walls in *Herpodiscus durvillaeae* may clarified whether indeed phenolics or another class of substances are the cause for the autofluorescence of the terminal cells and unilocular sporangia

of *Herpodiscus*, and possibly other Sphacelariales as well. Examining the excitation spectrum could, for example, be used for a first characterisation of the substance(s) in question (fluorescence spectroscopy; JAKUBKE & JESCHKEIT 1987). Furthermore, these studies may identify the substance causing the 'Eau de Javelle' reaction and study its function in the Sphacelariales cells, e. g. regarding its possible involvement in the growth of cells and formation of cell walls.

4.2.2.2.5 Shedding of the *Durvillaea* surface

Surface shedding in perennial algae as a means to remove epiphytes has been reported from a number of large brown algae, such as *Durvillaea potatorum* (LABILLARDIÈRE) ARESCH. (CLAYTON 1990b), *Ascophyllum nodosum* (FILION-MYKLEBUST& NORTON 1981), *Halydris siliquosa* (L.) LYNGB. (MOSS 1982) and *Lessonia* spp. (MARTINEZ & CORREA 1993). In these macrophytes, the sloughing of the surface is described to be a continuous process, in which either outer wall layers or even the outer-most cell layer are regularly removed. Likewise, healthy *Durvillaea antarctica* shed only the outer-most layers of the surface cell walls. An accumulation, presumably of polyphenolics, was observed to preceed this process. It was evident in UV light as an autofluorescent layer displaying a yellowish autofluorescence similar to the terminal cells. In healthy *Durvillaea*, this autofluorescent layer was very thin and appeared on the surface, while in areas infected with *Herpodiscus*, it was situated inside the host tissue underneath the parasite patch. Here, the larger width of the autofluorescent layer and its brown-reddish colour displayed in bright field light may indicate the formation of necrotic host tissue at the sites where cell layers were later disconnected.

In *Durvillaea antarctic*a, in contrast to the other brown algae, shedding of the surface seemed to follow a seasonal pattern. In areas infected by *Herpodiscus*, it became macroscopically evident in late winter and early spring, when the emergent phase of *Herpodiscus durvillaeae* disappeared with the sloughed surface. According to SOUTH (1974), the erosion of the external phase of *Herpodiscus* in spring follows the completion of the reproductive process of the parasite, i. e. as a natural part of its annual growth cycle. This would mean, that the shedding of the host is hindered by the presence of the parasite and occurs in the infected areas only when the external *Herpodiscus* filaments themselves start to be eroded. However, it seems more likely that the erosion of the external phase of *Herpodiscus* in spring may instead be dictated by the start of the annual growth cycle of its host. I. e., the shedding of *D. antarctica* may be restricted to its growth season from spring until autumn, in order to allow an unhindered release of gametes

during the reproductive winter months. An occasional peeling of the surface of (healthy) *D. antarctica* during summer has indeed been observed by HAY (1994), however, in this case, the complete surface meristoderm comprising 4-5 cell layers is sloughed off, possibly to get rid of old conceptacles. With the meristoderm gone, growth is assumed by the underlying cortex cells becoming meristematic again (HAY 1994).

While the presence of the parasite may not hinder the shedding of the host surface, it certainly hinders the release of the host gametes during winter (SOUTH 1974; present study). This was not only observed for the external filaments, but also for the internal phase, whose extension became visible as a rim around the external patches in which no gametes were released (Plate 4.1, Figure E). SOUTH reports that eggs and sperm from underneath parasite patches are released only after the external parasite filaments are eroded in spring (SOUTH 1974). Accordingly, heavily parasitised specimens (such as the one displayed in Plate 4.1, Figure D) may release fewer gametes or reproduce later; thus the infection may affect the reproductive success of these host individuals. Moreover, considering that up to half of the *Durvillaea* adults may be infected by *Herpodiscus* (HEESCH, unpublished data), also the genetic structure and diversity of the host population may be influenced by the presence of the parasite. Epidemiological studies are needed to reveal the impact of the infection by *Herpodiscus durvillaeae* on the physiological perfomance, reproduction and mortality of its host *Durvillaea antarctica.*

4.2.3 Conclusions

Based on the observations of SOUTH (1974), of PETERS (1990) and from the present study, the infection of *Durvillaea antarctica* by *Herpodiscus durvillaeae* is proposed to develop in the following sequence: During winter, the infection may be carried to a healthy host specimen via gametes. After an isogamous cell fusion on the host surface, the zygote may, upon germination, enter the host by penetrating its surface and thus start the endophytic phase of the parasite.

In spring, all external parts of the parasite are removed when the host sheds its surface. Thus, during the growth season of the host in summer, the parasite thallus is limited to the internal phase which may establish contact with the host cells. In the cortex of the host, the endophytic filaments grow towards the host plasmodesmata, putatively with apical cells and by enzymatic digestion of the host intercellular matrix, and possibly following a gradient of substances leaking into the apoplast. Single filaments surround the fields of plasmodesmata, squeeze inbetween and mechanically force the host cell walls apart. Once the tip of the parasite cell wall comes into contact with the host cell wall, it may form secondary half-plasmodesmata

matching those of the host. As soon as a symplastic contact is established, the parasite cell may function for sink metabolites transferred from the host cells and by this may sustain the development of the parasite thallus.

Once the host meristoderm ceases growth in autumn, parasite filaments growing among host meristoderm cells may emerge from the host surface and develop into the external phase, by forming assimilators and unilocular sporangia. Meiospores released from the latter settle on nearby filaments and first transform into gametophytes and then into gametangia. The gametes released from these may carry the infection to other host thalli, thus closing the infection circle.

Many aspects of this infection cycle are still hypothetical. For example, nothing is known about the conditions under which *Herpodiscus* infects *D. antarctica*, and flourishes in its host. For example, is the infection process influenced by abiotic factors such as temperature, and exposure at low tides, or is it dependent on wounds suggested for other endophytic brown algae (ROUND 1991). Clarifying the way *Herpodiscus* infects *D. antarctica* may also shed some light on the apparent host specifity of this parasite.

Finally, the question remains as to why *Herpodiscus* is confined to host populations from New Zealand. As an obligate parasite, the geographical distribution of *Herpodiscus* is closely linked to that of its host *Durvillaea antarctica.* Therefore, the restriction to New Zealand host populations is surprising as the parasite could well be distributed along with its floating host. In contrast, the other parasite of *D. antarctica*, a plasmodiophoral fungus, occurs in host populations from Chile, and also in New Zealand (AGUILERA *et al.* 1988; personal observation). Considering that *Herpodiscus* is also absent from host populations on the Chatham Islands, which are only 800 km away from mainland New Zealand and provide a similar marine environment, specific environmental conditions, which may be only met in New Zealand but not in Chile, may be ruled out as a selective factor. The parasite may, on the other hand, require a healthy and attached host thallus in order to complete its reproduction cycle and thus its infection cycle. However, once a *Durvillaea* thallus is detached and floats, it is constantly submerged, and its surface may start to desintegrate, which may in turn adversely affect the reproductive external parasite phase. Or the parasite possibly requires a tidal cue to reproduce. Further studies on the interaction between host and parasite may shed some light on the conditions, which are to be met for the infection cycle to be completed, and thus may help to explain the limitation of *Herpodiscus* to *D. antarctica* populations in New Zealand.

5 Perspectives

Despite their small size, endophytes may have considerable influence on the life of some of the largest macroalgae known worldwide. However, even though endophytic brown algae appear to be a common feature of marine algal communities along temperate coasts worldwide, the interactions of the endophytes with their macroalgal hosts are not well understood. Two areas in particular lack fundamental knowledge: the epidemiology of endophyte-related infectious diseases and the interaction of endophytes and hosts on a cellular and sub-cellular level.

Following terrestrial plant pathology, CORREA (1997) proposed three main stages of studying infectious diseases in macroalgae: 1. the description of the causing agent and the symptoms associated with the disease, 2. the demonstration of causality by fulfilling KOCH's postulates, and 3. the assessment of the ecological importance of the disease. The epidemiology of diseases related to the presence of endophytic algae have been studied in detail in Rhodophyceae infected by green endophytes (e. g. CORREA *et al.* 1987, 1988, 1994, 1997; CORREA & MCLACHLAN 1991, 1992, 1994; CORREA & SANCHEZ 1996). Studies on diseases associated with endophytic Phaeophyceae, in contrast, are scarce: few studies provide quantitative data on these symbioses (LEIN *et al.* 1991; PETERS 1991; PETERS & SCHAFFELKE 1996; ELLERTSDÓTTIR & PETERS 1997; present study), while causality of an infectious disease in macroalgae infected by brown algal endophytes has been strictly demonstrated only once (APT 1988a).

Epidemiological studies should not only record the prevalence and severity of infections within temporal and spatial patterns, but also need to comprise data, for example, on generations and/or age groups mostly susceptible to a given disease, or the thallus parts mostly affected, i. e. vegetative or fertile parts (CORREA & SANCHEZ 1996). An assessment of how endophytes affect the overall performance, reproductive success and mortality of host individuals may allow the prediction of any potential influence of the endophytes on the host population and, subsequently, on the structure of the whole macroalgal community, including the associated fauna.

Also, genetics of host and endophyte populations need to be studied in detail, as spatial patchiness of infection in a given population may not only be due to variations in the microenvironment affecting the health of the host individuals, but could also be caused by a varation in susceptibilty (SORCI *et al.* 1997). The presence of more than one potential host species within a community may, moreover, influence competition among these species if they

show different susceptibilities to an endophyte species. However, even within the population of a single host species differences in susceptibility or resistance may lead to the selection of certain haplotypes, i. e., by driving the structure of host populations, endophyte diseases may change the genetic diversity in a given host population. On the other hand, the ability to infect may differ among endophyte strains. Therefore, similar to parasitic relationships (SORCI *et al.* 1997), information on the spatial genetic structures of both host and endophyte populations have to form an important part of epidemiological studies. They may allow insight into the possible mechanisms of how endophytes affect the overall performance of host individuals.

However, the influence of genes on the varying susceptibility may be fully understood only when the factors involved in cell-cell recognition between host and endophyte have been identified. This leads to the second research area which is of interest in studying endophyte symbioses: the cellular and sub-cellular aspects of host/endophyte interactions.

Recent studies (WEINBERGER *et al.* 1999; KÜPPER *et al.* 2001, 2002; review in BOUARAB *et al.* 2001) have provided first insights in the biochemical processes at the surface of the host, i. e. the host/endophyte recognition and subsequent signal transduction. The release of activated oxygen species observed in some members of the Phaeophyceae and of the Rhodophyceae when challenged with degradation products of their own cell wall polysaccharides appears to be similar to the reaction of higher plant cells (KÜPPER *et al.* 2001, 2002; BOUARAB *et al.* 1999). However, the biochemistry behind it has yet to be identified for algal cells, such as proteins recognizing elicitors on the host surface as well as enzymes and transmitters involved in subsequent signal transduction. More detailed studies on the pathways of signal transduction within the host cell may also reveal how the kelp epi-endophyte *Laminariocolax tomentosoides* is capable of quenching the response of its host *Laminaria digitata* or how the host, on the other hand, manages to build up an induced resistance against the endophyte (KÜPPER *et al.* 2000, 2002). Future studies need to focus in particular on the processes beyond the point of entrance, as nothing is known, apart from the findings of the present study regarding *Herpodiscus durvillaeae*, of the fate of endophytes once they have successfully entered the host. I. e. how do host and endophyte cells interact in the phase from the penetration of the host surface by the endophyte to the stage when the symbiosis, may it be parasitic, pathogenic or just endophytic, is fully established? Studying the biochemical interactions between the two symbionts within the host tissue may shed light on the mechanisms leading to twisting of cauloids or the formation of galls in kelps infected by endophyte genera such as *Laminariocolax* and *Xiphophorocolax*. Such studies may provide information about the processes involved in growth or tissue development of the

host algae themselves.

The present study, as the first to focus on endophytic Phaeophyceae in New Zealand, provides some answers to basic questions such as: How diverse are brown endophytes in New Zealand? What is their identity? How abundant are they? And what do they do inside their host algae? However, this is only the beginning of unveiling their biology.

6 REFERENCES

ABBOTT, I. A. & HOLLENBERG, G. J. (1976): Marine Algae of California. Stanford University Press, Stanford, California, 827 pages.

ADAMS, N. M. (1972): The Marine Algae of the Wellington Area. Records of the Dominion Museum 8 (4): 43-98.

ADAMS, N. M. (1994): Seaweeds of New Zealand. Canterbury University Press, Christchurch, 360 pages.

ADAMS, N. M. & NELSON, W. A. (1985): Marine algae of the Three King Islands. National Museum of New Zealand, Miscellaneous Series 13: 1-29.

AGUILERA, M., RIVERA, P. J. & WESTERMEIER, R. (1988): The presence of Plasmodiophorales in plants of *Durvillaea antarctica* (CHAM.) HARIOT (Phaeophyta, Durvillaeaceae) in southern Chile. Gayana, Botanica 45 (1-4): 337-343.

ANDREWS, J. H. (1976): The pathology of marine algae. Biological Review 51: 211-253.

ANDREWS, J. H. (1977): Observations on the pathology of seaweeds in the Pacific Northwest. Canadian Journal of Botany 55: 1019-1027.

ANDREWS, J. H. (1979a): Pathology of seaweeds: Current status and future prospects. Introduction. Experientia 35(4): 429.

ANDREWS, J. H. (1979b): Pathology of seaweeds: Current status and future prospects. Conclusion: The seaweed pathosystem. Experientia 35(4): 448-450

ANDREWS, J. H. & GOFF, L. J. (1985): Pathology. In: LITTLER, M. M. & LITTLER, D. S. (ed.): Handbook of Phycological Methods: Ecological Field Methods: Macroalgae. Cambridge University Press, Cambridge, pp. 573-591.

ANONYMOUS (1991): Summary: Key Features and Issues of the Physical Systems and Ecosystems of the Otago Harbour. In: Report of the Ecosystems and Physical Systems Working Group, Otago Regional Council and Dunedin City Council Joint Discussion Series No. 2, Dunedin, pp. 1-8.

ANONYMOUS (1997): DNeasy Plant Mini Handbook (8/1997) for DNA isolation from plant tissue. Qiagen, Germany, 15 pages.

ANONYMOUS (1999): *Undaria* the alien Seaweed that could become the "Gorse of the Sea". Brochure by Department of Conservation and Ministery of Fisheries, New Zealand, 6 pages.

APT, K. E. (1988a): Etiology and development of hyperplasia induced by *Streblonema* sp. (Phaeophyta) on members of the *Laminariales* (Phaeophyta). Journal of Phycology 24: 28-34.

APT, K. E. (1988b): Galls and tumor-like growth on marine macroalgae. Diseases of Aquatic Organisms 4: 211-217.

ARNHEIM, N., KRYSTAL, M., SCHMICKEL, R., WILSON, G., RYDER, O. & ZIMMER, E. (1980): Molecular evidence for genetic exchanges among ribosomal genes on nonhomologous chromosomes in man and apes. Proceedings of the National Academy of Science USA 77: 7323-7327.

ATKINSON, I. (1996): Major Habitats. In: The Chatham Islands. Heritage and Conservation. Department of Conservation and Canterbury University Press, Christchurch, New Zealand, pp. 49-61.

BAKKER, F. T., OLSEN, J. L., STAM, W. T. & VAN DEN HOEK, C. (1992): Nuclear ribosomal DNA internal transcribed spacer regions (ITS1 and ITS2) define discrete biogeographic groups in *Cladophora albida* (Chlorophyta). Journal of Phycology 28: 839-845.

BAKKER, F. T., OLSEN, J. L. & STAM, W. T. (1995): Global phylogeography in the cosmopolitan species *Cladophora vagabunda* (Chlorophyta) based on nuclear rDNA ITS sequences. European Journal of Phycology 30: 197-208.

BALDWIN, B. G. (1992): Phylogenetic Utility of the Internal Transcribed Spacers of Nuclear Ribosomal DNA in Plants: An Example from the Compositae. Molecular Phylogenetics and Evolution 1: 3-16.

BARCKHAUS, R. H. & WEINERT, H. (1974): A parastitic plant (*Cuscuta odorata*) on *Pelargonium*: Light and electron microscopy studies. Mikrokosmos 63: 238-248.

BATHAM, E. J. (1956): Ecology of Southern New Zealand Sheltered Rocky Shore. Transactions of the Royal Society of New Zealand 84 (2): 447-465.

BATHAM, E. J. (1958): Ecology of Southern New Zealand Exposed Rocky Shore at Little Papanui, Otago Peninsula. Transactions of the Royal Society of New Zealand 85 (4): 647-658.

BATHAM, E. J. (1965): Rocky shore ecology of a southern New Zealand fiord. Transactions of the Royal Society of New Zealand, Zoology 6 (21): 215-227.

BEIDERBECK, R. (1977): Pflanzentumoren. Verlag Eugen Ulmer Stuttgart, 216 pages.

BELL, G. (1982): The Masterpiece of Natur: The Evolution and Genetics of Sexuality. University of California Press, Berkeley, 635 pp.

BHATTACHARYA, D. (1997): An introduction to algal phylogeny and phylogenetic methods. Plant Systematics and Evolution (Suppl.) 11: 1-11.

BIRSCHWILKS, M. (2003): Untersuchungen zum Stofftransport zwischen dem phanerogamen Holoparasiten *Cuscuta* spec. und seinen Wirtspflanzen. Ph.D. thesis, University Halle-Wittenburg, Germany, 125 pages.

BISALPUTRA, T. & BISALPUTRA, A. (1969): The ultrastructure of chloroplasts of a brown alga *Sphacelaria* sp. I. Plastid DNA configuration, the chloroplast genophore. Journal of Ultrastucture Research 29: 151-170.

BLOMSTER, J., MAGGS, C. A. & STANHOPE, M. J. (1998): Molecular and morphological analysis of *Enteromorpha intestinalis* and *E. compressa* (Chlorophyta) in the British Isles. Journal of Phycology 34: 319-340.

BOLTON, J. J. & ANDERSON, R. J. (1994): *Ecklonia.* In: AKATSUKA, I. (ed.): Biology of Economic Algae. SPB Academic Publishing, The Hague, Netherlands, pp. 385-406.

BOON, M. E. & KOK, L. R (1992): Microwave Cookbook for Microscopists. 3rd edition, Coulomb Press, Leyden, The Netherlands, 449 pages.

BOUARAB, K., POTIN, P., CORREA, J. & KLOAREG , B. (1999): Sulfated Oligosaccharides Mediate the Interaction between a Marine Red Alga and Its Green Algal Pathogenic Endophyte. Plant Cell 11: 1635-1650.

BOUARAB, K., POTIN, P., KLOAREG , B. & CORREA, J. A. (2001): Ecological and biochemical aspects in algal infectious diseases. Cahiers du Biologie Marine 42: 41-100.

BOYLE, M. C., JILLETT, J. B. & MLADENOV, P. V. (2001): Intertidal communities in Doubtful Sound, New Zealand: changes over time. New Zealand Journal of Marine and Freshwater Research 35: 663-673

BRADLEY, P. M. & CHENEY, D. P. (1990): Some effects of plant growth regulators on tissue cultures of the marine red alga *Agardhiella subulata* (Gigartinales, Rhodophyta). Hydrobiologia 204/205: 253-260.

BRODIE, J., HAYES, P. K., BARKER, G. L. & IRVINE, L. M. (1996): Molecular and morphological characters distinguishing two *Porphyra* species (Rhodophyta: Bangiophycidae). European Journal of Phycology 31:303-308.

BRODIE, J. W. (1960): Coastal surface currents around New Zealand. New Zealand Journal of Geology and Geophysics 3: 235-252.

BROOM, J. E., JONES, W. A., HILL, D. F., KNIGHT, G. A. & NELSON, W. A. (1999): Species recognition in New Zealand *Porphyra* using 18S rDNA sequencing. Journal of Applied Phycology 11: 421-428.

BROOM, J. E., NELSON, W. A., YARISH, C., JONES, W. A., AGUILAR ROSAS, R. & AGUILAR ROSAS, L. E. (2002): A reassessment of the taxonomic status of *Porphyra suborbiculata*, *Porphyra carolinensis* and *Porphyra lilliputiana* (Bangiales, Rhodophyta) based on molecular and morphological data. European Journal of Phycology 37: 227-235.

BROWN, M. T., NYMAN, M. A., KEOGH, J. A. & CHIN, N. K. M. (1997): Seasonal growth in the giant kelp *Macrocystis pyrifera* in New Zealand. Marine Biology 129: 417-424.

BRUNS, T. D., FOGEL, R. & TAYLOR, J. W. (1990): Amplification and sequencing of DNA from fungal herbarium specimens. Mycologia 82(2): 175-184.

BURKHARDT, E. & PETERS, A. F. (1998): Molecular evidence from nrDNA ITS sequences that *Laminariocolax* (Phaeophyceae, Ectocarpales sensu lato) is a worldwide clade of closely related kelp endophytes. Journal of Phycology 34: 682-691.

BURROWES, R., ROUSSEAU, F., MÜLLER, D. G. & DE REVIERS, B. (2003): Taxonomic placement of *Microzonia* (Phaeophyceae) in the Syringodermatales based on the *rbc*L and 28S nrDNA sequences. Cryptogamie, Algologie 24 (1): 63-73.

CAMPBELL, H. (1996): Geology. In: The Chatham Islands. Heritage and Conservation. Department of Conservation and Canterbury University Press, Christchurch, New Zealand, pp. 34-48.

CARROLL, L., GARDNER, M (ed.) & TENNIEL, J. (illust., 2000): LEWIS CARROLL: The Annotated Alice. The definitive edition. Rev. ed., ALLEN LANE, The Penguin Press, 384 pages.

CHESHIRE, A. C., CONRAN, J. G. & HALLAM, N. D. (1995): A cladistic analysis of the evolution and biogeogrgaphy of *Durvillaea* (Phaeophyta). Journal of Phycology 31: 644-655.

CHOAT, J. H. & SCHIEL, D. R. (1982): Patterns of distribution and abundance of large brown algae and invertebrate herbivores in subtidal regions of northern New Zealand. Journal of Experimental Marine Biology and Ecology 60: 129-162.

CHOPIN, T., BIRD, C. J., MURPHY, C. A., OSBORNE, J. A., PATWARY, M. V. & FLOC'H, J.-Y. (1996): A molecular investigation of polymorphism in the North Atlantic red alga *Chondrus crispus* (Gigartinales). Phycological Research 44: 69-80.

CITHAREL, J. (1972): Contridution à l'étude du métabolisme azoté des Algues marines. Utilisation métabolique d'acide glutamique - ^{14}C par *Ascophyllum nodosum* (LINNE) LE JOLIS et *Polysiphonia lanosa* (LINNE) TANDY. Botanica Marina 15: 157-161.

CLAYTON, M. N. (1981): Phaeophyta. In: CLAYTON, M. N. & KING, R. J. (eds.): Marine Botany: An Australasian Perspective. Longman Cheshire, Melbourne, 468 pages.

CLAYTON, M. N. (1984): Evolution of the Phaophyceae with particular reference to the Fucales. Progress in Phycological Research 3: 11-46.

CLAYTON, M. N. (1990a): 35. Phylum Phaeophyta. In: MARGULIS, L., CORLISS, J. O., MELKONIAN, M., CHAPMAN, D. J. (EDS.) & MCKHANN, H. I. (editorial coordinator): Handbook of protoctista. Boston, Jones & Bartlett Publ.: 698-714.

CLAYTON, M. N. (1990b): Phaeophyta. In: CLAYTON, M. N. & KING, R. J. (eds.): Biology of Marine Plants. Longman Cheshire, Melbourne: 149-182.

CLAYTON, M. N. & BEAKES, G. W. (1983): Effects of fixatives in the ultrastructure of physodes in vegetative cells of *Scytosiphon lomentaria* (Scytosiphonaceae, Phaeophyta). Journal of Phycology 19: 4-16.

CLAYTON, M. N. & ASHBURNER, C. M. (1994): Secretion of phenolic bodies following fertilisation in *Durvillaea potatorum* (Durvillaeales, Phaeophyta). European Journal of Phycology 29: 1-9.

CLAYTON, M. N., HALLAM, N. D. & SHANKLY, C. M. (1987): The seasonal pattern of conceptacle development and gamete maturation in *Durvillaea potatorum* (Durvillaeales, Phaeophyta). Phycologia 26: 35-45.

CORREA J. A. (1994) Infections by pigmented algal endophytes: misuse of concepts and terminology. Revista Chilena de Historia Natural 67: 4-8.

CORREA, J. A. (1997): Infectious diseases of marine algae: current knowledge and approaches. Progress in Phycological Research 12: 149-180.

CORREA, J. A., NIELSEN, R., GRUND, D. W. & MCLACHLAN, J. (1987): Endophytic algae of Irish moss (*Chondrus crispus* STACKH.). Hydrobiologia 151/152: 223-228.

CORREA, J. A., NIELSEN, R. & GRUND, D. W. (1988): Endophytic algae of *Chondrus crispus* (Rhodophyta). II. *Acrochaete heteroclada* sp. nov., *A. operculata* sp. nov. and *Phaeophila dendroides* (Chlorophyta). Journal of Phycology 24: 528-539.

CORREA, J. A. & CRAIGIE, J. S. (1991): Algal Pathology. In: REINA, G. G. & PEDERSEN, M. (eds.): Seaweed Cellular Biotechnology, Physiology and Intensive Cultivation. Cost-48, Universidad de Las Palmas de Gran Canaria: 67-82.

CORREA, J. A. & MCLACHLAN, J. L. (1991): Endophytic algae of *Chondrus crispus* (Rhodophyta). III. Hostspecifity. Journal of Phycology 27: 448-459.

CORREA, J. A. & MCLACHLAN, J. L. (1992): Endophytic algae of *Chondrus crispus* (Rhodophyta). IV. Effects on the host following infections by *Acrochaete operculata* and *A. heteroclada* (Chlorophyta). Marine Ecology Progress Series. 81: 73-87.

CORREA, J. A. & MCLACHLAN, J. L. (1994): Endophytic algae of *Chondrus crispus* (Rhodophyta). V. Fine structure of the infection by *Acrochaete operculata* (Chlorophyta). European Journal of Phycology 29: 33-47.

CORREA, J. A., FLORES, V. & GARRIDO, J. (1994): Green patch disease in *Iridaea laminarioides* (Rhodophyta) caused by *Endophyton* sp. (Chlorophyta). Diseases of Aquatic Organisms 19: 203-213.

CORREA, J. A. & SANCHEZ, P. A. (1996): Ecological Aspects of algal infectious diseases. Hydrobiologia 326/327: 89-95.

CORREA, J. A., BUSCHMANN, A., RETAMALES. C. & BELTRÁN, J. (1997): Infectious diseases of *Mazaella laminarioides* (Rhodophyta): Changes in infection prevalence and disease expression associated with latitude, season and within-site location. Journal of Phycology 33: 344-352.

COURNANE, S. (1991): Sedimentology of Otago Harbour. In: Report of the Ecosystems and Physical Systems Working Group, Otago Regional Council and Dunedin City Council Joint Discussion Series No. 2, Dunedin, pp.57-78.

COYER, J. A., SMITH, G. J. & ANDERSEN, R. A. (2001): Evolution of *Macrocystis* spp. (Phaeophyceae) as determined by ITS1 and ITS2 sequences. Journal of Phycology 37: 574-585.

CRACRAFT, J. (2000): Species Concepts in Theoretical and Applied Biology: A Systematic Debate with Consequences. In: WHEELER, Q. D. & MEIER, R. (eds.): Species Concepts and Phylogentic Theory. A Debate. Columbia University Press, New York: 3-14.

CRAIGIE, J. S. & CORREA, J. A. (1996): Etiology of infectious diseses in cultivated *Chondrus crispus* (Gigartinales, Rhodophyta). Hydrobiologia 326/327: 97-104.

CROUAN, H. M. & CROUAN, P. L. (1851): Etudes microscopiques sur quelques algues nouvelles ou peu connues constituant un genre nouveau. Annals du Science Naturelle de Botanique ser. 3/4, 15: 359-366 + 2 plates.

CUNNINGHAM, C. W. (1997): Can three incongruence tests predict when data should be combined? Molecular Biology and Evolution 14: 733-740.

224

DAUGBJERG, N. & ANDERSEN, R. A. (1997): A molecular phylogeny of the heterokont algae based on analyses of chloroplast-encoded *rbc*L sequence data. Journal of Phycology 33: 1031-1041.

DAYTON, P. K., CURRIE, V., GERRODETTE, T., KELLER, B. D., ROSENTHAL, R. & VEN TRESCA, D. (1984): Patch dynamics and stability of come California kelp communities. Ecological Monographs 54 (3): 253-289.

DAYTON, P. K. (1985): Ecology of kelp communities. Annual Review of Ecology and Systematics 16: 215-245.

DE BARY, H. A. (1879): Die Erscheinung der Symbiose. Verlag Karl J. Trübner, Strassburg.

DE BRUN, A., IBELINGS, B. W., RIJKEBOER, M., BREHM, M. & VAN DONK, E. (2004): Genetic variation in *Asterionella formosa* (Bacillariophyceae): Is it linked to frequent epidemics of host-specific parasitic fungi? Journal of Phycology 40: 823-830.

DEL CAMPO, E., GARCÍA-REINA, G. & CORREA, J. A. (1998): Degradative disease in *Ulva rigida* (Chlorophyceae) associated with *Acrochaete geniculata* (Chlorophyceae). Journal of Phycology 34 (1): 160-166.

DE NYS, R., JAMESON, P. E., CHIN, N., BROWN, M. T. & SANDERSON, K. J. (1990): The cytokinins as endogenous growth regulators in *Macrocystis pyrifera* (L.) C. AGARDH (Phaeophyceae). Botanica Marina 33 (6): 467-475.

DE NYS, R., JAMESON, P. E. & BROWN, M. T. (1991): The influence of cytokinins on the growth of *Macrocystis pyrifera*. Botanica Marina 34 (6): 465-467.

DERBÈS, A. & SOLIER, A. J. J. (1851): Algues. In: CASTAGNE, J. L. M. (ed.): Supplément au catalogue des plantes qui croissent naturellement aux environs de Marseille. Nicot & Pardignon, Aix: 93-121.

DE REVIERS, B. & ROUSSEAU, F. (1999): Towards a new classification of the brown algae. Progress in Phycological Research 13: 107-201.

DIETRICH, G., KALLE, K., KRAUS, W. & SIEDLER, G. (1975): Allgemeine Meereskunde. 3rd edition, Gebrüder Bornträger, Stuttgart, Germany, 593 pages + 8 plates.

DODGE, J. D. (1973): The Fine Structure of Algal Cells. Academic Press, London, New York, 261 pages.

DÖRR, I. (1969): Feinstruktur intrazellulär wachsender *Cuscuta*-Hyphen. Protoplasma 67: 123–137.

DÖRR, I. (1972): Der Anschluß der *Cuscuta*-Hyphen an die Siebröhren ihrer Wirtspflanzen. Protoplasma 75: 167–184.

DÖRR, I. (1990): Sieve elements in haustoria of parasitic angiosperms. In: BEHNKE H. D. & SJOLUND, R. D. (eds): Sieve elements - comparative structure, induction and development. Berlin, Heidelberg, New York: Springer-Verlag: 239–256.

DÖRR, I. & KOLLMANN, R. (1995): Symplasmic Sieve Element Continuity between *Orobanche* and its Host. Botanica Acta 108: 47–55.

DOVER, G. A. (1982): Molecular drive: a cohesive mode of species evolution. Nature 299: 111-117.

DRAISMA, S. G. A. & PRUD'HOMME VAN REINE, W. F. (2001): Onslowiaceae fam. nov. (Phaeophyceae). Journal of Phycology 37: 647-649.

DRAISMA, S. G. A., PRUD'HOMME VAN REINE, W. F., STAM, W. T. & OLSEN, J. L. (2001): A reassessment of phylogenetic relationships within the Phaeophyceae based on RUBISCO large subunit and ribosomal DNA sequences. Journal of Phycology 37: 586-603.

DRAISMA, S. G. A., OLSEN, J. L., STAM, W. T. & PRUD'HOMME VAN REINE, W. F. (2002): Phylogenetic relationships within the Sphacelariales (Phaeophyceae): *rbc*L, RUBISCO spacer and morphology. European Journal of Phycology 38: 385-401.

DRAISMA, S. G. A., PETERS, A. F. & FLETCHER, R. L. (2003): Evolution and taxonomy in the Phaeophyceae: effects of the molecular age on brown algal systematics. European Journal of Phycology 39: 87-102.

DRING, M. J. (1992): The Biology of Marine Plants. Cambridge University Press, 199 pages.

DRUEHL, L. D., MAYES, C., TAN, I. H & SAUNDERS, G. W. (1997): Molecular and morphological phylogenies of kelp and associated brown algae. Plant Systematics and Evolution [Suppl.] 11: 221-235.

DYKSTRA, M. J. (1993): A Manual of Applied Techniques for Biological Electron Microscopy. Plenum Press, New York and London, 257 pp.

EDDING, M. E., FONCK, E. A. & MACCHIAVELLO, J. E. (1994): *Lessonia.* In: AKATSUKA, I. (ed.): Biology of Economic Algae. SPB Academic Publishing bv, The Hague, Netherlands, pp. 407-446.

EDGAR, G. J. (1987): Dispersal of faunal and floral propagules associated with drifting *Macrocystis pyrifera* plants. Marine Biology 95: 599-610.

EHLERS, K, & KOLLMANN, R. (2001): Primary and secondary plasmodesmata: structure, origin and functioning. Protoplasma 216: 1-30.

ELLERTSDÓTTIR, E. & PETERS, A. F. (1997): High incidence of infection by endophytic brown algae in natural populations of *Laminaria* spp. (Phaeophyceae). Marine Ecology Progress Series 146: 135-143.

EVANS, L. V., CALLOW, J. A. & CALLOW, M. E. (1978): Parasitic Red Algae: An Appraisal. In: IRVINE, D. E. G. & PRICE, J. H. (eds.): Modern approaches to the Taxonomy of Red and Brown Algae. Academic Press, London: 87-109.

FARLOW, W. G. (1889): On some new or imperfectly known algae of the United States I. Bull. Torrey Bot. Club. 16: 7-12.

FARRAR, J. F. (1995): Just another sink? Sources of assimilate for foliar pathogens. Aspects of Applied Biology 42: 81-89.

FARRIS, J. S., KÄLLERSJÖ, M., KLUGE, A. G. & BULT, C. (1995): Testing significance of incongruence. Cladistics 10: 315-319.

FILION-MYKLEBUST, C. & NORTON, T. A. (1981): Epidermis shedding in the brown seaweed *Ascophyllum nodosum* (L.) LE JOLIS, and its ecological significance. Marine Biology Letters 2: 45-51.

FLEMING, C. A. (1978): [The New Zealand Environment] Flora and Fauna history. In: SUGGATE, R. P., STEVENS, G. R. & TE PUNGA, M. T. (eds.): The Geology of New Zealand. Government Printer, Wellington, New Zealand, VolumeI: 48-52.

FLETCHER, R. L. (1978): Studies on the family Ralfsiaceae (Phaeophyta) around the British Isles. In: IRVINE, D. E. G. & PRICE, J. H. (eds.): Modern approaches to the Taxonomy of Red and Brown Algae. Academic Press, London: 371-398.

FLETCHER, R. L. (1987): Seaweeds of the British Isles. Volume 3, Part 1 Fucophyceae (Phaeophyceae). The Natural History Museum, London, 359 pages.

FLETCHER, R. L., BAIER, R. E. & FORNALIK, M. S. (1985): The effects of surface energy on the development of some marine macroalgae. British Phycological Journal 20: 184-185.

FINERAN, B. A. (1969): The Flora of the Snares Islands, New Zealand. Transactions of the Royal Society of New Zealand, Botany, 3 (17): 237-270.

FORREST, B. M., BROWN, S. N., TAYLOR, M. D., HURD, C. L. & HAY, C. H. (2000): The role of natural dispersal mechanisms in the spread of *Undaria pinnatifida* (Laminariales, Phaeophyceae). Phycologia 39 (6): 547-553.

FRITSCH, F. E. (1945): Structure and Reproduction of the Algae. Vol 2. Cambridge University Press, 939 pages.

FYFE, J., ISRAEL, S. A., CHONG, A., ISMAIL, N., HURD, C. L. & PROBERT, P. K. (1999): Mapping Marine Habitats in Otago, Southern New Zealand. Geocarto International 14 (3): 15-26.

GÄUMANN, E. (1951): Pflanzliche Infektionslehre. 2nd edition, published by Birkhäuser, Basel, 611 pages.

GARBARY, D. J., KIM, K. Y., KLINGER, T. & DUGGINS, D. (1999): Red algae as hosts for endophytic kelp gametophytes. Marine Biology 135: 35-40.

GARNER, D. M. (1959): The sub-tropical convergence in New Zealand surface waters. New Zealand Journal of Geology and Geophysics 2: 315-337.

GALATIS, B. D., KATSAROS, C. & MITRAKOS, K. (1977): Fine structure of vegetative cells of *Sphacelaria tribuloides* Menegh. (Phaeophyceae, Sphacelariales) with special reference to some unusual proliferations of the plasmalemma. Phycologia 16 (2): 139-151.

GALWAY, M. E., HECKMANN, J. W., HYDE, G. J. & FOWKE, L. C. (1995): Advances in high-pressure and plunge-freeze fixation. In: GALBRAITH, D. W., BOHNERT, H. J. & BOURQUE, D. P. (eds.): Methods in Cell Biology. Vol. 49, Academic Press, New York: 3-19.

GIBBS, M. T. (2001): Aspects of the structure and variability of the low-salinity layer in Doubtful Sound, a New Zealand fiord. New Zealand Journal of Marine and Freshwater Research 35: 59-72

GIBBS, M. T., BOWMAN, M. J. & DIETRICH, D. E. (2000): Maintenance of near-surface stratification in Doubtful Sound, a New Zealand fjord. Estuarine, Coastal and Shelf Science 51: 683-704.

GIBSON, G. & CLAYTON, M. N. (1987): Sexual reproduction, early development and branching in *Notheia anomala* (Phaeophyta) and its classification in the Fucales. Phycologia 26 (3): 363-373.

GOCKEL, G. & HACHTEL, W. (2000): Complete Gene Map of the Plastid Genome of the Nonphotosynthetic Euglenoid Flagelatte *Astasia longa*. Protist 151 (4): 347-351.

GOFF, L. J. (1979): The biology of *Harveyella mirabilis* (Cryptonemiales, Rhodophyceae) VI. Translocation of photoassimilated ^{14}C. Journal of Phycology 15: 82-87.

GOFF, L. J. (1982): The biology of parasitic red algae. Progress in Phycological Research 1: 289-369.

GOFF, L. J. (1983): Marine algal interactions: epibiosis, endobiosis, parasitism and disease. In: TSENG, C. K. (ed.): Proceedings of the Joint China-U.S. Phycology Symposium. Science Press, Beijing: 221-274.

GOFF, L. J. & COLE, K. (1976a): The biology of *Harveyella mirabilis* (Cryptonemiales, Rhodophyceae) III. Spore germination and subsequent development within the host *Odonthalia floccosa* (Ceramiales, Rhodophyceae). Canadian Journal of Botany 54: 268-280.

GOFF, L. J. & COLE, K. (1976b): The biology of *Harveyella mirabilis* (Cryptonemiales, Rhodophyceae) IV. Life history and phenology. Canadian Journal of Botany 54: 281-292.

GOFF, L. J. & COLEMAN, A. W. (1984): Transfer of nuclei from a parasite to its host. Proceedings of the National Academy of Science 81: 5420-5424.

GOFF, L. J. & COLEMAN, A. W. (1985): The role of secondary pit connections in red algal parasitism. Journal of Phycology 21, 483-508.

GOFF, L. J. & COLEMAN, A. W. (1995): Fate of parasite and host organelle DNA during cellular transformation of red algae by their parasites. The Plant Cell 7: 1899-1911.

GOFF, L. J. & MOON, D. A. (1993): PCR amplification of nuclear and plastid genes from algal herbarium specimens and algal spores. Journal of Phycology 29: 381-384.

GOFF, L. J. & ZUCCARELLO, G. (1994): The evolution of parasitism in red algae: cellular interactions of adelphoparasites and their hosts. Journal of Phycology 30: 695-720.

GOFF, L. J., MOON, D. A. & COLEMAN, A. W. (1994): Molecular delineation of species and species relationships in the red algal agarophytes *Gracilariopsis* and *Gracilaria* (Gracilariales). Journal of Phycology 30: 521-537.

GOFF, L. J., MOON, D. A., NYVALL, P., STACHE, B., MANGIN, K. & ZUCCARELLO, G. (1996): The evolution of parasitism in red algae: molecular comparisons of adelphoparasites and their hosts. Journal of Phycology 32: 297-312.

GOODBAND, S. J. (1973): Observations on the development of endophytic filaments of *Sphacelaria bipinnata* (KÜTZ.) SAUV. on *Halidrys siliquosa* (L.) LYNGB. British Journal of Phycology 8: 175-179.

GRAHAM, L. E. & WILCOX, L. W. (2000): Algae. Prentice Hall, Sydney, 640 pages.

GRANGE, K. R. & SINGLETON, R. J. (1988): Population structure of black coral, *Antipathes aperta*, in the southern fiords of New Zealand. New Zealand Journal of Zoology 15: 481-489.

GRANGE, K. R., SINGLETON, R. J., RICHARDSON, J. R., HILL, P. J. & MAIN, W. D. (1981): Shallow rock-wall biological associations of some southern fiords of New Zealand. New Zealand Journal of Zoology 8: 209-227.

GRAY, F. (1991): Geology of the Otago Harbour Catchment Area. In: Report of the Ecosystems and Physical Systems Working Group, Otago Regional Council and Dunedin City Council Joint Discussion Series No. 2, Dunedin, pp. 40-56.

GREUTER, W. *et al.*, eds. (2000): International Code of Botanical Nomenclature (St. Louis Code). http://www.bgbm.org/iapt/nomenclature/code/SaintLouis/0000St.Luistitle.htm

GROVE, S. L. & PROBERT, P. K. (1999): Sediment macrobenthos of upper Otago Harbour, New Zealand. New Zealand Journal of Marine and Freshwater Research 33: 469-480.

HALL, J. L. & WILLIAMS, L. E. (2000): Assimilate transport and partitioning in fungal biotrophic interactions. Australian Journal of Plant Physiology 27: 549-560.

HALLAM, N. D. & LUFF, S. E. (1988): The Fixation and Infiltration of Larger Brown Algae (Phaeophyta) for Electron Microscopy. British Phycological Journal 23: 337-346.

HALLAM, N. D., CLAYTON, M. N. & PARISH, D. (1980): Studies on the association between *Notheia anomala* and *Hormosira banksii* (Phaeophyta). Australian Journal of Botany 28: 239-248.

HARLIN, M. M. & CRAIGIE, J. S. (1975): The distribution of photosynthate in *Ascophyllum nodosum* as it relates to epiphytic *Polysiphonia lanosa.* Journal of Phycology 11 (Supplement): 113-119.

HARPER, J. T. & SAUNDERS, G. W. (2001): Molecular systematics of the Florideophyceae (Rhodophyta) using nuclear large and small subunit rDNA sequence data. Journal of Phycology 37: 1073-1082.

HARRISON, M. J. (1999): Biotrophic interfaces and nutrient transport in plant/fungal symbioses. Journal of Experimental Botany 50: 1013-1022.

HAUCK, F. (1885): Die Meeresalgen Deutschlands und Oesterreichs. In: RABENHORST, L. (ed.): Kryptogamenflora von Deutschland, Oesterreich und der Schweiz. Kummer, Leipzig, 575 pages.

HAWKE, D. J. (1989): Hydrology and near-surface nutrient distribution along the South Otago continental shelf, New Zealand, in summer and winter 1986. New Zealand Journal of Marine and Freshwater Research 23: 411-420.

HAY, C. H. (1978): A phytogeographical account of the southern bull kelp seaweeds *Durvillaea* spp. BORY 1826 (Durvilleales PETROV 1965). Proceedings of the International Symposium for Biogeography and Evolution in the Southern Hemisphere Auckland, New Zealand 2: 443-453.

230

HAY, C. H. (1979): Nomenclature and taxonomy within the genus *Durvillaea* BORY (Phaeophyceae: Durvilleales PETROV.). Phycologia 18: 191-202.

HAY, C. H. (1987): *Lessonia adamsiae* sp. nov. (Phaeophyta, Laminariales) from the Snares Islands, New Zealand. New Zealand Journal of Botany 25: 295-308.

HAY, C. H. (1989): *Lessonia tholiformis* sp. nov. (Phaeophyta, Laminariales) from the Chatham Islands, New Zealand. New Zealand Journal of Botany 27: 461-469.

HAY, C. H. (1990a): The distribution of *Macrocystis* (Phaeophyta: Laminariales) as a biological indicator of cool surface temperature, with special reference to New Zealand waters. Journal of the Royal Society of New Zealand 20 (4): 313-336.

HAY, C. H. (1990b): The Dispersal of Sporophytes of *Undaria pinnatifida* by Coastal Shipping in New Zealand, and Implications for Further Dispersal on *Undaria* in France. British phycological Journal 25: 301-313.

HAY, C. H. (1994): *Durvillaea* (Bory). In: AKATSUKA, I. (ed.): Biology of Economic Algae. SPB Academic Publishing bv, The Hague, Netherlands, pp. 353-384.

HAY, C. H & LUCKENS, P. A. (1987): The Asian kelp *Undaria pinnatifida* (Phaeophyta: Laminariales) found in a New Zealand harbour. New Zealand Journal of Botany 25: 329-332.

HAY, C. H. & VILLOUTA, E. (1993): Seasonality of the adventive Asian kelp *Undaria pinnatifda* in New Zealand. Botanica Marina 36: 461-476.

HAY, C. H., ADAMS, N. M. & PARSONS, M. J. (1985): The marine algae of the subantarctic islands of New Zealand. National Museum of New Zealand, Miscellanous Series 11: 1-70.

HAY, M. E. & FENICAL, W. (1988): Marine plant-herbivore interactions: the ecology of chemical defense. Annual Review of Ecology and Systematics 19: 111-145.

HAY, R. F., MUTCH, A. R. & WATTERS, W. A. (1970): Geology of the Chatham Islands. New Zealand Geological Survey Bulletin 83: 1-86.

HEATH, R. A. (1974): Frictional influence on sea level oscillations in Otago Harbour. New Zealand Journal of Marine and Freshwater Research 8: 415-424.

HEATH, R. A. (1985): A review of the physical oceanography of the seas around New Zealand – 1982. New Zealand Journal of Marine and Freshwater Research 19: 79-124.

HEESCH, S. (1996): Untersuchungen zur Infektion von Laminarien durch endophytische Braunalgen. Unpublished Diploma thesis, Universität Kiel, Germany, 74 pages.

HEESCH, S. & PETERS, A. F. (1996): SEM and growth studies on infection and ecophysiology of microscopic brown algal endophytes of *Laminaria*. 1st European Phycological Congress, Cologne, Germany. Cambridge University Press, Cambridge, UK: 33.

HEESCH, S. & PETERS, A. F. (1999): Scanning electron microscopy observation of host entry by two brown algae endophytic in *Laminaria saccharina* (Laminariales, Phaeophyceae). Phycological Research 47: 1-5.

HELMUTH, B., VEIT, R. R. & HOLBERTON, R. (1994): Long-distance dispersal of a subantarctic brooding bivalve (*Gaimardia trapesina*) by kelp-rafting. Marine Biology 120: 421-426.

HENRY, E. C. (1984): Syringodermatales ord. nov. and *Syringoderma floridana* sp. nov. (Phaeophyceae). Phycologia 23 (4): 419-426.

HENRY, E. C. (1987a): The life history of *Onslowia endophytica* (Sphacelariales, Phaeophyceae) in culture. Phycologia 26 (2): 175-181.

HENRY, E. C. (1987b): Morphology and life history of *Onslowia bahamensis* sp. nov. and *Verosphacela ebrachia* gen. et. sp. nov., with a reassessment of the Choristocarpaceae (Sphacelariales, Phaeophyceae) in culture. Phycologia 26 (2): 182-191.

HIBBERD, J. M., BUNGARD, R. A., PRESS, W. D., SCHOLES, J. D. & QUICK, W. P. (1998): Localization of photosynthetic metabolism in the parasitic angiosperm *Cuscuta reflexa*. Planta 205: 506-513.

HOBDAY, A. J. (2000a): Abundance and dispersal of drifting kelp *Macrocystis pyrifera* rafts in the Southern California Bight. Marine Ecology Progress Series 195: 101-116.

HOBDAY, A. J. (2000b): Persistence and transport of fauna on drifting kelp (*Macrocystis pyrifera* (L.) C. AGARDH) rafts in the Southern California Bight. Journal of Experimental Marine Biology and Ecology 253 (1): 75-96.

HOLMQUIST, R. (1983): Transitions and Transversions in Evolutionary Descent: An Approach to Understanding. Journal of Molecular Evolution 9: 134-144.

HOMMERSAND, M. H. (1986): The Biogeography of the South African Marine Red algae: A Model. Botanica Marina 29: 257-270.

HOMMERSAND, M. H. & FREDERICQ, S. (2002): Biogeography of the red seaweeds of the South African west coast: a molecular approach. Proceedings of the XVIIth International Seaweed Symposium, Oxford University Press (in press).

HOWE, M. A. (1914): The marine algae of Peru. Memoirs of the Torrey Botanical Club 15: 1-184, 66 pls.

HUMBEL, B., MARTI, T. & MÜLLER, M. (1983): Improved structural preservation by combining freeze-substitution and low-temperature embedding. Beiträge zur elektronenmikroskopischen Direktabbildung von Oberflächen 16: 585-594.

HURD, C. L., NELSON, W. A., FALSHAW, R. & NEILL, K. F. (2004): History, current status and future of marine macroalgal research in New Zealand: Taxonomy, ecology, physiology and human uses. Phycological Research 52 (2): 80-106.

HWANG, U.-W & KIM, W. (1999): General properties and phylogenetic utilities of nuclear ribosomal DNA and mitochondrial DNA commonly used in moelcular systematics. Korean Journal of Parasitology 37 (4): 215-228.

IMLAU, A., TRUERNIT, E. & SAUER, N. (1999): Cell-to-cell and long distance trafficking of the green fluorescent protein in the phloem and symplastic unloading of the protein into sink tissue. The Plant Cell 11: 309-322.

JAASUND, E. (1963): Beiträge zur Systematik der Norwegischen Braunalgen. Botanica Marina 5: 1-8.

JACOBS, TH. (1990): Directional growth of wheat leaf rust infection structures after penetration and formation of the substomatal vesicle. Mycological Research 94: 122-124.

JAKUBKE, H.-D. & JESCHKEIT, H. (1987, eds.): Fachlexikon ABC Chemie, Band I. Verlag Harri Deutsch, Thun and Frankfurt/Main, GDR, 632 pages.

JANKE, K. (1989): Lebensgemeinschaften und ihre Besiedlungsstrukturen in der Gezeitenzone felsiger Meeresküsten. Die Bedeutung biologischer Wechselwirkungen für die Entstehung und Erhaltung der Biozönose im Nordost-Felswatt von Helgoland. Ph.D. thesis, Christian-Albrechts-University, Kiel, 119 pages.

JESCHKE, W. D., RÄTH, N., BÄUMEL, P., CZYGAN, F. C. & PROKSCH, P. (1994): Modelling the flow and partitioning of carbon and nitrogen in the holoparasite *Cuscuta reflexa* ROXB. and its host *Lupinus albus* L. II. Flows between host and parasite and within the parasitized host. Journal of Experimental Botany 45: 801-812.

JILLET, J. B. (1969): Seasonal hydrology of waters off the Otago Peninsula, south-eastern New Zealand. New Zealand Journal of Marine and Freshwater Research 3: 349-375.

JOHN, D. M. & LAWSON, G. W. (1974): *Basispora*, a new genus of the Ralfsiaceae. British Phycological Journal 9: 285-290.

JOHN, D. M., TITTLEY, I., LAWSON, G. W. & PUGH, P. J. A. (1994): Distribution of Seaweed Floras in the Southern Ocean. Botanica Marina 37: 235-239.

JONES, M. G. K. (1976): The origin and development of plasmodesmata. In: GUNNING, B. E. S. & ROBARDS, A. W. (eds.): Intercellular Communication in Plants: Studies on Plasmodesmata. Springer-Verlag, Heidelberg - New York: 81-105.

KAIN, J. M (1982): Morphology and growth of the giant kelp *Macrocystis pyrifera* in New Zealand and California. Marine Biology 67: 143-157.

KAIN, J. M. & NORTON, T. A. (1990): Marine Ecology. In: COLE, K. M. & SHEATH, R. G. (eds.): Biology of the red algae. Cambridge University Press: 377-422.

KARNOVSKY, M. J. (1965): A Formaldehyde-Glutaraldehyde Fixative of High Osmolarity For Use in Electron Microscopy. The Journal of Cell Biology 27: 137A-138A.

KARYOPHYLLIS, D., KATSAROS, C. & GALATIS, B. (2000): F-Actin involvement in apical cell morphologenesis of *Sphacelaria rigidula* (Phaeophyceae): mutual alignment between cortical actin filaments and cellulose microfibrils. European Journal of Phycology 35 (2): 195-203.

KATSAROS, C. & GALATIS, B. (1986): Ultrastructural studies on zoosporogenesis of *Halopteris filicina* (Sphacelariales, Phaeophyta). Phycologia 25: 358-370.

KATSAROS, C., GALATIS, B. & MITRAKOS, K. (1983): Fine structural studies on the interphase and dividing apical cells of *Sphacelaria tribuloides* (Phaeophyta). Journal of Phycology 19: 16-30.

KAWAI, H. & TOKUYAMA, M. (1995): *Laminarionema elsbetiae* gen. et sp. nov. (Ectocarpales, Phaeophyceae), a new endophyte in *Laminaria* sporophytes. Phycological Research 43: 185-190.

KAWAI, H., MUTO, H., FUJII, T. & KATO, A. (1995): A linked 5S rRNA gene in *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyceae). Journal of Phycology 31: 306-311.

KAWAI, H., NAKAYAMA, T., INOUYE, I. & KATO, A. (1997): Linkage of 5S ribosomal DNA to other rDNAs in the chromophytic algae and related taxa. Journal of Phycology 33: 505-511.

KIMURA, M. (1980): A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111-120.

KJELLMAN, F. R. (1891): Choristocarpaceae. In: ENGLER, A. & PRANTL, A. (eds.): Die natürlichen Pflanzenfamilien. Ed. 1, 1(2), Wilhelm Engelmann Verlag, Leipzig: 190-191.

KLEINIG, H. & SITTE, P. (1992): Zellbiologie. 3rd edition, Gustav Fischer Verlag, Stuttgart, 591 pages.

KNOX, G. A. (1954): The intertidal flora and fauna of the Chatham Islands. Nature 174: 871-874.

KNY, L. (1873): Ueber einige parasitische Algen. Sitzungsbericht der Gesellschaft der naturforschenden Freunde zu Berlin, 19.11.1872. Botanische Zeitung, Leipzig, 31:139-144.

KOGAME, K. (1998): A taxonomic study of Japanese *Scytosiphon* (Scytosiphonales, Phaeophyceae) including two new species. Phycological Research 46: 39-56.

KOLLMANN, R. & GLOCKMANN, Ch. (1985): Studies on graft unions. I. Plasmodesmata between cells belonging to different unrelated taxa. Protoplasma 124: 224-235.

KOLLMANN, R. & GLOCKMANN, Ch. (1990): Sieve elements of graft unions. In: BEHNKE, H. D. & SJOLUND, R. D. (eds.): Sieve elements – comparative structure, induction and development. Springer, Berlin: 219-237.

KOLLMANN, R. & GLOCKMANN, Ch. (1991): Studies on graft unions III. On the mechanism of secondary formation of plasmodesmata at the graft interface. Protoplasma 165: 71-85.

KOLLMANN, R., YANG, S. & GLOCKMANN, Ch. (1985): Studies on graft unions. II. Continuous and half plasmodesmata in different regions of the graft interface. Protoplasma 126: 19-29.

KREMER, B. P. (1983): Carbon economy and nutrition of the alloparasitic red alga *Harveyella mirabilis*. Marine Biology 76: 231-239.

KRISTIANSEN, A. & PEDERSEN, P. M. (1979): Studies of the life history and seasonal variation of *Scytosiphon lomentaria* (Fucophyceae, Scytosiphonales) in Denmark. Botanisk Tidsskrift 74: 31-56.

KUCKUCK, P. (1894): Bemerkungen zur marinen Algenvegetation von Helgoland. I. Wissenschaftliche Meeresuntersuchungen, Biologische Anstalt Helgoland, 1: 223-263

KUCKUCK, P. (1895a): Ueber einige neue Phaeosporeen der westlichen Ostsee. Botanische Zeitung 53: 175-188.

KUCKUCK, P. (1895b): Ueber die Schwärmsporenbildung bei den Tilopterideen und über *Choristocarpus tenellus* (KÜTZ.) ZANARD. Jahrb. Wiss. Bot. 28: 290-322.

KUCKUCK, P. (1897a): Beiträge zur Kenntnis der Meeresalgen, 1-4. Wissenschaftliche Meeresuntersuchungen, N. F., 2: 325-371.

KUCKUCK, P. (1897b): Bemerkungen zur marinen Algenvegetation von Helgoland. II. Wissenschaftliche Meeresuntersuchungen, N. F., 2: 373-400.

KUCKUCK, P. (1899): Beiträge zur Kenntnis der Meeresalgen. Wissenschaftliche Meeresuntersuchungen, Biologische Anstalt Helgoland, 3: 12-83.

KUCKUCK, P. emend. KORNMANN P. (1954): Ectocarpaceen-Studien II. Helgoländer Wissenschaftliche Meeresuntersuchungen 5(1): 103-117.

KÜPPER, F. C., KLOAREG, B., GUERN, J. & POTIN, P. (2001): Oligoguluronates elicit an oxidative burst in the brown algal kelp *Laminaria digitata*. Plant Physiology 125: 278-291.

KÜPPER, F. C., MÜLLER, D. G., PETERS, A. F., KLOAREG, B. & POTIN, P. (2002): Oligoalginate recognition and oxidative burst play a key role in natural and induced resistance of the sporophyte of Laminariales. Journal of Chemical Ecology 28: 2057-2081.

KUIJT, J. (1969): The Biology of Parasitic Flowering Plants. University of California Press, Berkeley/Los Angeles, California, 246 pages.

KUSHIDA, H. (1974): A new method for embedding with a low viscosity epoxy resin "Quetol 651". Journal of Electron Microsc. 23: 197.

KYLIN, H. (1944): Die Rhodophyceen der schwedischen Westküste. Lunds Univ. Arsskr. N. F., Avd. 2, 40, Nr. 2: 1-104.

KYLIN, H. (1947): Die Phaeophyceen der schwedischen Westküste. Lunds Univ. Arsskr. N. F., Avd. 2, 43, Nr. 2: 1-99.

LA CLAIR, J. W. II (1981): Occurence of Plasmodesmata during Infurowing in a Brown Alga. Biology of the Cell 40: 139-142.

LAMARE, M. D. (1998): Origin and transport of the larvae of the sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea) in a New Zealand fiord. Marine Ecology Progress Series 174: 107-121.

LAUDER, G. (1991): Coastal Inlet and Shoreline Processes. In: Report of the Ecosystems and Physical Systems Working Group, Otago Regional Council and Dunedin City Council Joint Discussion Series No. 2, Dunedin, pp. 87-103.

LEE, E.-Y., PEDERSEN, P. M. & LEE, I. K. (2002): *Neoleptonema yongpilii* E.-Y. LEE & I. K. LEE, gen. et sp. nov. (Phaeophyceae), based on morphological characters and RuBisCo spacer sequences. European Journal of Phycology 37: 237-245.

LEE, S. B. & TAYLOR, J. W. (1991): Phylogeny of five fungal-like protoctistan *Phytophthora* species inferred from the internal transcribed spacers of ribosomal DNA. Molecular Biology and Evolution 9: 636-653.

236

LEIN, T. E., SJØTUN, K. & WAKILI, S. (1991): Mass-occurence of a brown filamentous endophyte in the Lamina of the kelp *Laminaria hyperborea* (GUNNERUS) FOSLIE along the southwestern coast of Norway. Sarsia 76: 187-193.

LEMMERMANN, E. (1906): Die Algenflora der Chatham Islands. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 38: 343-382, 2 pls.

LEVRING, T. (1937): Zur Kenntnis der Algenflora der Norwegischen Westküste. Lunds Universitets Arsskrift. N.F. Avd. 2 Bd. 33 (8): 36-77.

LEVRING, T., HOPPE, H. A. & SCHMID, O. J. (1969): Marine Algae. A Survey of Research and Utilization. Cram, De Gruyter & Co., Hamburg, 421 pages.

LEWIN, J. (1966): Silicon Metabolism in Diatoms. V. Germanium Dioxide, a Specific Inhibitor of Diatom Growth. Phycologia 6 (1): 1-12.

LINDAUER, V. W. (1947): An annotated List of the Brown Seaweeds, Phaeophyceae, of New Zealand. Transactions of the Royal Society of New Zealand 76 (4): 542-566.

LINDAUER, V. W. (1949): Notes on marine algae of New Zealand. I. Pacific Science 3: 340-352.

LINDAUER, V. W. (1960): New species of Phaeophyceae from New Zealand. Revue algologique 5(3): 161-172.

LINDAUER, V. W., CHAPMAN, V. J. & AITKEN, M. (1961): The marine algae of New Zealand. II. Phaeophyceae. Nova Hedwigia 3: 129-350.

LOBBAN, C. S. (1978): The growth and death of the *Macrocystis* sporophyte (Phaeophyceae, Laminariales). Phycologia 17 (2): 196-212.

LOCKHART, J. C. (1979): Factors determining various forms in *Cladosiphon zosterae* (Phaeophyceae). American Journal of Botany 66(7): 836-844.

LOISEAUX, S. (1968): Sur les phénomènes d'hétéroblastie et de dimorphisme chez les phéophycées. Revue Générale de Botanique 75: 229-244.

LOISEAUX, S. (1970): *Streblonema anomalum* S. et G. and *Corponema sporangiiferum* S. et G. stages in the life history of a minute *Scytosiphon*. Phycologia 8: 11-15.

LUCAS, J. A. (1998): Plant Pathology and Plant Pathogens. 3rd edition, Blackwell Science, Oxford, 274 pages.

LUCAS, W. J. , DING, B. & VAN DER SCHOOT, C. (1993): Plasmodesmata and the supracellular nature of plants. New Phytologist 125: 435-476.

LÜNING, K. (1985): Meeresbotanik. Verbreitung, Ökophysiologie und Nutzung der marinen Makroalgen. Georg Thieme Verlag, Stuttgart, Germany, 375 pages.

LÜNING, K. (1991): Circannual growth rhythm in a brown alga, *Pterygophora californica*. Botanica Acta 104: 157-162.

LÜNING, K. (1993): Environmental and internal control of seasonal growth in seaweeds. Hydrobiologia 260/261: 1-14.

LUND, S. (1966): On a sporangia-bearing microthallus of *Scytosiphon lomentaria* from nature. Phycologia 6: 67-78.

MAIER, I. (1995): Brown algal pheromones. Progress in Phycological Research 11: 51-102.

MARCHANT, H. J. (1976): Plasmodesmata in algae and fungi. In: GUNNING, B. E. S. & ROBARDS, A. W. (eds.): Intercellular Communication in Plants: Studies on Plasmodesmata. Springer-Verlag, Heidelberg - New York: 59-80.

MARTINEZ, E. & CORREA, J. (1993): Sorus-specific epiphytism affecting the kelps *Lessonia nigrescens* and *L. trabeculata*. Marine Ecology Progress Series 96: 83-92.

MAYES, C., SAUNDERS, G. W., TAN, I. H. & DRUEHL, L. D. (1992): DNA extraction methods for kelp (Laminariales) tissue. Journal of Phycology 28: 712-716.

MAYR, E. (2000): The Biological Species Concept. In: WHEELER, Q. D. & MEIER, R. (eds.): Species Concepts and Phylogentic Theory. A Debate. Columbia University Press, New York: 17-29.

MCCULLY, M. E. (1968): Histological Studies on the Genus *Fucus*. II. Histology of the Reproductive Tissues. Protoplasma 66: 205-230.

MCFADDEN, G. I., WALLER, R. F., REITH, M. E.& LANG-UNNASCH, N. (1997): Plastids in apicomplexan parasites. Plant Systematics and Evolution [Suppl.] 11: 261-287.

MCHUGH, D. J. (2003): A guide to the seaweed industry. FAO Fisheries Technical Paper 441: 1-105.

MCLACHLAN, J., CHEN, L. C.-M., & EDELSTEIN, T. (1971): The life history of *Microspongium* sp. Phycologia 10: 83-87.

MEIER, R. & WILLMANN, R. (2000): The Henningian Species Concept. In: WHEELER, Q. D. & MEIER, R. (eds.): Species Concepts and Phylogentic Theory. A Debate. Columbia University Press, New York: 30-43.

MIGULA, W. (1909): Kryptogamen-Flora von Deutschland, Deutsch-Österreich und der Schweiz. Band II. Algen. 2. Teil: Rhodophyceae, Phaeophyceae, Characeae. Verlag Friedrich von Zezschwitz, Gera, 383 pages.

MILLER, I. J., FALSHAW, R., FURNEAUX, R. H. & HEMMINGSON, J. A. (1997): Variations in the Constituent Sugars of the Polysaccharides from New Zealand Species of *Pachymenia* (Halimeniaceae). Botanica Marina 40: 119-127.

MILLER, S. M. (2003): Ecophysiology of *Ecklonia radiata* (Alariaceae: Laminariales) in Doubtful Sound, Fiordland. Ph.D. thesis, University of Otago, 260 pages.

MISHLER, B. D. & THERIOT, E. C. (2000): The Phylogenetic Species Concept (*sensu* Mishler and Theriot): Monophyly, Apomorphy and Phylogenetic Species Concepts. In: WHEELER, Q. D. & MEIER, R. (eds.): Species Concepts and Phylogentic Theory. A Debate. Columbia University Press, New York: 44-54.

MOE, R. L. & SILVA, P. C. (1989): *Desmarestia antarctica* (Desmarestiales, Phaephyceae), a new ligulate Antarctic species with an endophytic gametophyte. Plant Systematic Evolution 164: 273-283.

MOHR, H. & SCHOPFER, P. (1992): Pflanzenphysiologie. 4th edition, Springer Verlag, Berlin, 659 pages.

MOLLOY, L. F. & DINGWALL, P. R. (1990): World heritage values of New Zealand Islands. In: TOWNS, D. R., DAUGHERTY, C. H. & ATKINSON, I. A. E. (eds.): Ecological Restoration of New Zealand Islands. Conservation Sciences Publication No. 2, Department of Conservation, Wellington: 194-206.

MORGAN, D. R. (1997): Decay analysis of large sets of phylogenetic data. Taxon 46: 509-517.

MORTON, J. & MILLER, M. (1968): The New Zealand Sea Shore. Collins, London, 638 pages.

MOSS, B. L. (1982): The control of epiphytes on *Halidrys siliquosa* (L.) LYNGBY. (Phaeophyta, Cystoseiraceae). Phycologia 21: 185-191.

MÜLLER, D. (1989): The role of pheromones in sexual reproduction of brown algae. In: COLEMAN, A. W., GOFF, L. J. & STEIN-TAYLOR, J. R (eds.): Algae as Experimental Systems. Plant Biology Vol. 7, Alan R.. Liss, Inc: 201-213.

MURDOCH, R. C., PROCTOR, R., JILLET, J. B. & ZELDIS, J. R. (1990): Evidence for an eddy over the continental shelf in the downstream lee of Otago Peninsula, New Zealand. Estuarine, Coastal and Shelf Science 30: 489-507.

NAGAR, R. SINGH, M. & SANWAL, G. G. (1984): Cell wall degrading enzymes in *Cuscuta reflexa* and its hosts. Journal of Experimental Botany 35: 1104-1112.

NAYLOR, M. (1954): A Check List of the Marine Algae of the Dunedin District. Transactions of the Royal Society of New Zealand 82 (3): 645-663.

NELSON, W. A. (1982): A critical review of the Ralfsiales, Ralfsiaceae and the taxonomic position of *Analipus japonicus* (Harv.) Wynne (Phaeophyta). British Phycological Journal 17: 311-320.

NELSON, W. A. (1993): Epiphytic Species of *Porphyra* (Bangiales, Rhodophyta) from New Zealand. Botanica Marina 36: 525-534.

NELSON, W. A. (1994): Distribution of Macroalgae in New Zealand – An Archipelago in Space and Time. Botanica Marina 37: 221-233.

NELSON, W. A. & ADAMS, N. M. (1984): Marine Algae of the Kermadec Islands. National Museum of New Zealand, Miscellaneous Series 10: 1-29.

NELSON, W. A. & ADAMS, N. M. (1987): Marine Algae of the Bay of Islands Area. National Museum of New Zealand, Miscellaneous Series 16: 1-47.

NELSON, W. A., ADAMS, N. M. & HAY, C. H. (1991): Marine Algae of the Chatham Islands. National Museum of New Zealand, Miscellaneous Series 23: 1-58.

NELSON, W. A., BROOM, J. E. & FARR, T. J. (2003): *Pyrophyllon* and *Chlidophyllon* (Erythropeltidales, Rhodophyta), two new genera for obligate epiphytic species previously placed in *Porphyra*, and a discussion of the orders Erythropeltidales and Bangiales. Phycologia 42: 308-315.

NEWMAN, G. R., JASANI, B., WILLIAMS, E. R. (1983): A simple post-embedding system for the rapid demonstration of tissue antigens under the electron microscope. Histochemical Journal 15: 543-555.

NIELSEN, R., KRISTIANSEN, A., MATHIESEN, L. & MATHIESEN H. (1995): Distributional index of the benthic macroalgae of the Baltic Sea area. Acta Botanica Fennica 155: 1-51.

NIXON, K. C. & WHEELER, Q. D. (1990): Am amplification of the phylogenetic species concept. Cladistics 6: 211-223.

NORTH, W. J. (1971): Growth of the Mature Giant Kelp. In: NORTH, W. J (ed.): The Biology of Giant Kelp Beds (*Macrocystis*) in California. Nova Hedwigia 32: 123-168.

NORTH, W. J. (1994): Review of *Macrocystis* Biology. In: AKATSUKA, I. (ed.): Biology of Economic Algae. SPB Academic Publishing bv, The Hague, Netherlands, pp. 447-527.

NORTH, W. J. & ZIMMERMAN, R. C. (1984): Influences of macronutrients and water temperatures on summertime survival of *Macrocystis pyrifera* canopies. Hydrobiologia 116/117: 419-424.

240

O'DONNELL, K. (1992): Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). Curr. Genet. 22: 213-220.

OLTMANNS, F. (1922): Morphologie und Biologie der Algen. Zweiter Band: Phaeophyceae – Rhodophyceae. 2nd edition. Fischer, Jena, Germany, 439 pages.

PANKOW, H. (1990): Ostsee-Algenflora. Gustav Fischer-Verlag, Jena, 648 pages.

PARACER, S. & AHMADJIAN, V. (2000): Symbiosis – An Introduction to Biological Associations. 2nd edition, Oxford University Press, 291 pages.

PARENTE, M. I., NETO, A. I. & FLETCHER, R. L. (2003): Morphology and life history of *Scytosiphon lomentaria* (Scytosiphonaceae, Phaeophycecae) from the Azores. Journal of Phycology 39: 353-359.

PEAKE, B. M., WALLS, D. J. & GIBBS, M. T. (2001): Spatial variations in the levels of nutrients, chlorophyll *a*, and dissolved oxygen in summer and winter in Doubtful Sound, Fiordland. New Zealand Journal of Marine and Freshwater Research 35: 681-694.

PEDERSEN, P. M. (1981a): The life histories in culture of the brown algae *Gononema alariae* sp. nov. and *G. aecidioides* comb. nov. from Greenland. Nordic Journal of Botany 1: 263-270.

PEDERSEN, P. M. (1981b): Phaeophyta: Life histories. In: LOBBAN, C. S. & WYNNE, M. J. (eds.): The biology of seaweeds. Blackwell Scientific Publications, Oxford: 194-217.

PEDERSEN, P. M. (1984): Studies on primitive brown algae (Fucophyceae). Opera Botanica 74: 1-76.

PELLEGRINI, L. (1980): Cytological studies on physodes in the vegetative cells of *Cystoseira stricta* SAUVAGEAU (Phaeophyta, Fucales). Journal of Cell Science 41:209-231.

PETERS, A. F. (1987): Reproduction and sexuality in the Chordariales (Phaeophyceae). A review of culture studies. Progress in Phycological Research 5: 223-263.

PETERS, A. F. (1990): Taxonomic implications of gamete fusions in the parasitic brown alga *Herpodiscus durvilleae*. Canadian Journal of Botany Vol. 68: 1398-1401.

PETERS, A. F. (1991): Field and culture studies of *Streblonema macrocystis* sp. nov. (Ectocarpales, Phaeophyceae) from Chile, a sexual endophyte of giant kelp. Phycologia 30 (4): 365-377.

PETERS, A. F. (2003): Molecular identification, taxonomy and distribution of brown algal endophytes, with emphasis on species from Antarctica. In: CHAPMAN, A. R. O., ANDERSON, R. J., VREELAND, V. & DAVISON, I. R. (eds.): Proceedings of the 17th International Seaweed Symposium. Oxford University Press, New York: 293-302

PETERS, A. F. & BURKHARDT, E. (1998): Systematic position of the kelp endophyte *Laminarionema elsbetiae* (Ectocarpales *sensu lato* (Phaeophyceae) inferred from nuclear ribosomal DNA sequences. Phycologia 37 (2): 114-120.

PETERS, A. F. & CLAYTON, M. N. (1998): Molecular and morphological investigations of three brown algal genera with stellate plastids: evidence for Scytothamnales ord. nov. (Phaeophyceae). Phycologia 37 (2): 106-113.

PETERS, A. F. & ELLERTSDÓTTIR, E. (1996): New record of the kelp endophyte *Laminarionema elsbetiae* (Phaeophyceae, Ectocarpales) at Helgoland and its life history in culture. Nova Hedwigia 62: 341-349.

PETERS, A. F. & RAMIREZ, M. E. (2001): Molecular phylogeny of small brown algae, with special reference to the systematic position of *Caepidium antarcticum* (Adenocystaceae, Ectocarpales). Cryptogamie, Algologie 22 (2): 187-200.

PETERS, A. F. & SCHAFFELKE, B. (1996): *Streblonema* (Ectocarpales, Phaeophyceae) infection in the kelp *Laminaria saccharina* (Laminariales, Phaeophyceae) in the Western Baltic. Hydrobiologia 326/327: 111-116.

PETERSON, R. L. (1991): Histochemistry of Ectomycorrhiza. Methods in Microbiology 23: 107-120.

PILLMANN, A., WOOLCOTT, G. W., OLSEN. J. L., STAM, W. T. & KING, R. J. (1997): Inter- and intraspecific genetic variation in *Caulerpa* (Chlorophyta) based on nuclear rDNA ITS sequences. European Jorunal of Phycology 32: 379-386.

POTTER, D., SAUNDERS, G. W. & ANDERSEN R. A. (1997): Phylogenetic relationships of the Rhaphidophyceae and Xanthophyceae as inferred from nucleotide sequences of the 18S ribosomal RNA gene. American Journal of Botany 84: 966-972.

PRINGSHEIM, N. (1863): Beiträge zur Morphologie der Meeresalgen. Abhandlungen der Akademie der Wissenschaften, Berlin 1862: 1-37.

PROBERT, P. K. (1991): Marine Biology and Ecology of Otago Harbour (excluding seabirds and marine mammals). In: Report of the Ecosystems and Physical Systems Working Group, Otago Regional Council and Dunedin City Council Joint Discussion Series No. 2, Dunedin, pp. 104-128.

PRUD'HOMME VAN REINE, W. F. (1982): A taxonomic revision of the European Sphacelariales. Leiden Botanical Series 6: 1-293.

PRUD'HOMME VAN REINE, W. F. (1993): Sphacelariales (Phaeophyceae) of the world, a new synthesis. Korean Journal of Phycology 8: 145-160.

QUINN, J. M. (1978): The hydrology and plankton of Otago Harbour. Unpublished BSc (Hons.) thesis, Otago University, 87 pages.

QUIRK, H. M. & WETHERBEE, R. (1980): Structural studies on the host parasite association between the red algae *Gracilaria* and *Holmsella.* Micron 11: 511-512.

RAGAN, M. (1976): Physodes and the phenolic compounds of brown algae. Composition and significance of physodes in vivo. Botanica Marina 19:145-154.

RAIMONDI, P. T., REED, D. C., GAYLORD, B. & WASHBURN, L. (2004): Effects of self-fertilization in the giant kelp *Macrocystis pyrifera.* Ecology 85 (2): 3267-3276.

RAINER, S. F. (1981): Soft-bottom Benthic Communities in Otago Harbour and Blueskin Bay, New Zealand. New Zealand Oceanographic Institute Memoir 80: 1-38.

RAUTENBERG, E. (1960): Zur Morphologie und Ökologie einiger epiphytischer und epi-endophytischer Algen. Botanica Marina 2: 133-148.

RAWLENCE, D. J. (1972): An ultrastructural study of the relationship between rhizoids of *Polysiphonia lanosa* (L.) TANDY (Rhodophyceae) and tissue of *Ascophyllum nodosum* (L.) LE JOLIS (Phaeophyceae). Phycologia 11: 279-290.

REGAN, C. T. (1926): Organic evolution. Report of the British Association for the Advancement of science 1925: 75-86.

REINBOLD, TH. (1899): Ergebnisse einer Reise nach dem Pacific (Prof. Dr. SCHAUINSLAND 1896-1897). Meeresalgen. Abhandlungen herausgegeben vom Naturwissenschaft-lichen Verein zu Bremen 16: 287-302.

REINKE, J. (1888): Die braunen Algen (Fucaceen und Phaeosporeen) der Kieler Bucht. Berichte der Deutschen Botanischen Gesellschaft 6: 14-20.

REINKE, J. (1889): Atlas deutscher Meeresalgen. Erstes Heft. Tafel 1 bis 25. Kommission zur wissenschaftlichen Untersuchung der deutschen Meere. Berlin.

REINKE, J. (1890): Uebersicht der bisher bekannten Sphacelariaceen. Berichte der Deutschen Botanischen Gesellschaft 8: 201-215.

REINKE, J. (1891): Beiträge zur vergleichenden Anatomie und Morphologie der Sphacelariaceen. Bibliotheca Botanica 23: 1-40.

REINSCH, P. F. (1875): Contributiones ad algologiam et fungologiam. Weigel, Leipzig: 1-103, 131 plates.

REUSCH, T. B. H. (1995): Planung und Auswertung von ökologischen Experimenten. Praktikumsskript, Zusatzveranstaltung zum biologisch-meereskundlichen Groß-praktikum 1994/95. Unpublished script, Kiel, Germany, 107 pages.

RICKER, R. W. (1987): Taxonomy and Biogeography of Macquarie Island Seaweeds. British Museum (Natural History), London, 344 pages.

ROLAND, J. C. & VIAN, B. (1991): General Preparation and Staining of Thin Sections. In: HALL, J. L. & HAWES, C. (eds.): Electron Microscopy of Plants Cells. Academic Press, London, 466 pages.

ROPER, D. S. & JILLET, J. B. (1981): Seasonal occurrence and distribution of flatfish (Pisces: Pleuronectiformes) in inlets and shallow water along the Otago coast. New Zealand Journal of Marine and Freshwater Research 15: 1-13.

ROSENVINGE, L. K. (1893): Grønlands Havalger. Meddr. om Grønland: 765-981.

ROUND, F. E. (1991): Epibiotic and endobiotic associations between chromophyte algae and their hosts. In: REISSER, W. (ed.): Algae and Symbiosis: Plants, animals, fungi, viruses, interaction explored. Biopress, Bristol: 593-617.

ROUSSEAU, F., LECLERC, M. C. & DE REVIERS, B. (1997): Molecular phylogeny of the European Fucales (Phaeophyceae) based on large-subunit rDNA sequence comparisons. Phycologia 36 (6): 438-446.

ROUSSEAU, F. & DE REVIERS, B. (1999): Circumscription of the order Ectocarpales (Phaeophyceae): bibliographical synthesis and molecular evidence. Cryptogamie: Algologie 20: 5-18.

ROUSSEAU, F., BURROWES, R., PETERS, A. F., KUHLENKAMP, R. & DE REVIERS, B. (2001): A comprehensive phylogeny of the Phaeophyceae based on nrDNA sequences resolves the earliest divergences. Life Sciences 324: 305-319.

RUSSELL, G. & FLETCHER, R. L. (1975): A numerical taxonomic study of the British Phaeophyta. Journal of the Marine Biological Association of the U. K. 55: 763-783.

SACHS, L. (1984): Angewandte Statistik. Anwendung statistischer Methoden. 6th edition, Springer, Berlin, 552 pages.

SANGER, F., NICKLEN, S. & COULSON, A. R. (1977): DNA sequencing with chain terminating inhibitors. Proceedings of the National Academy of Science USA 74: 5463-5467.

SANTELICES, B. (1989): Algas marinas de Chile. Ediciones Universidad Católica de Chile, Santiago, 399 pages.

244

SAUNDERS, G. W. & DRUEHL, L. D. (1992): Nucleotide sequences of the small-subunit ribosomal RNA genes from selected Laminariales (Phaeophyta): Implications for kelp evolution. Journal of Phycology 28: 544-549.

SAUNDERS, G. W. & KRAFT, G. T. (1994): Small-subunit rRNA gene sequences from representatives of selected families of the Gigartinales and Rhodymeniales (Rhodophyta). 1. Evidence for the Plocarmiales ord. nov. Canadian Journal of Botany 72: 1250-1263.

SAUNDERS, G. W. & KRAFT, G. T. (1995): The phylogenetic affinities of *Notheia anomala* (Fucales, Phaeophyceae) as determined from partial small-subunit rRNA gene sequences. Phycologia 34: 383-389.

SAUNDERS, G. W., POTTER, D. & ANDERSEN, R. A. (1997): Phylogenetic affinities of the Sarcinochrysidales and Chrysomeridales (Heterokonta) based on analysis of molecular and combined data. Journal of Phycology 33: 310-318.

SAUVAGEAU, M. C. (1892): Sur quelques Algues Phéosporées Parasites. Journal de Botanique 6th Année (1): 1-105.

SCHAFFELKE, B.& LÜNING, K. (1994): A circannual rhythm controls seasonal growth in the kelps *Laminaria hyperborea* and *Laminaria digitata* from Helgoland (North Sea). European Journal of Phycology 29: 49-56.

SCHAFFELKE, B., PETERS, A. F. & REUSCH, T. B. H. (1996): Factors influencing depth distribution of soft bottom inhabiting *Laminaria saccharina* (L.) LAMOUR. in Kiel Bay, Western Baltic. Hydrobiologia 326/327: 117-123.

SCHIEL, D. R. (1996): Marine Life. In: Department of Conservation (ed.): The Chatham Islands. Heritage and Conservation. Canterbury University Press, Christchurch, New Zealand, pp. 62-73.

SCHIEL, D. R. & FOSTER, M. S. (1986): The structure of subtidal algal stands in temperate waters. Oceanogr. Mar. Biol. Ann. Rev. 24: 265-307.

SCHIEL, D. R. & NELSON, W. A. (1990): The harvesting of macroalgae in New Zealand. In: LINDSTROM, S. C. & GABRIELSON, P. W. (eds.): Proceedings of the Thirteenth International Seaweed Symposium, Hydrobiologia 204/205: 25-33.

SCHIEL, D. R., ANDREW, N. L & FOSTER, M. S. (1995): The structure of subtidal algal and invertebrate assemblages at the Chatham Islands, New Zealand. Marine Biology 123: 355-367.

SCHMID, A.-M. M. (2003): Endobacteria in the diatom *Pinnularia* (Bacillariophyceae). I. "Scattered ct-nucleoids" explained: DAPI-DNA complexes stem from exoplastidial bacteria boring into the chloroplasts. Journal of Phycology 39: 122-138.

SCHMITZ, K. & SRIVASTAVA, L. M. (1974): Fine structure and development of sieve tubes in *Laminaria groenlandica* ROSENV. Cytologie 10: 66-87.

SCHOENWAELDER, M. E. A. & CLAYTON, M. N. (1998a): Secretion of phenolic substances into the zygote wall and cell plate in embryos of *Hormosira* and *Acrocarpia* (Fucales, Phaeophyceae). Journal of Phycology 34: 969-980.

SCHOENWAELDER, M. E. A. & CLAYTON, M. N. (1998b): The secretion of phenolic compounds following fertilization in *Acrocarpia paniculata* (Fucales, Phaeophyta). Phycologia 37 (1): 40-46.

SCHOENWAELDER, M. E. A. & CLAYTON, M. N. (1999): The presence of phenolic compounds in isolated cell walls of brown algae. Phycologia 38 (3): 161-166.

SCHUBERT, R. & WAGNER, G. (1993): Botanisches Wörterbuch. Pflanzennamen und botanische Fachwörter. 11th edition, Eugen Ulmer Verlag, Stuttgart, 645 pages.

SEARLES, R. B. & LEISTER, G. L. (1980): North Carolina marine algae. IX. *Onslowia endophytica* gen. et sp. nov. (Phaeophyta, Sphacelariales) and notes on other new records for North Carolina. Journal of Phycology 16: 35-40.

SERRAO, E. A., ALICE, L. A. & BRAWLEY, S. A. (1999): Evolution of the Fucaceae (Phaeophyceae) inferrred from nrDNA-ITS. Journal of Phycology 35: 382-394.

SETCHELL, W. A. & GARDNER, N. L. (1922): Phycological contributions II to VI. University of California Publications in Botany Vol. 7, No. 11: 333-426.

SETCHELL, W. A. & GARDNER, N. L. (1925): The marine algae of the Pacific coast of North America. Part III: Melanophyceae. University of California Publications in Botany Vol. 8: 383-898.

SIEMER, B. L. (1998): Molecular phylogenetic studies in the simple brown algae – Ectocarpales, Chordariales, Dictyosiphonales, Scytosiphonales and Tilopteridales. Ph.D. thesis, University of Copenhagen, 138 pages.

SIEMER, B. L., STAM, W. T., OLSEN, J. L. & PEDERSEN, P. M. (1998): Phylogenetic relationships of the brown algal orders Ectocarpales, Chordariales, Dictyosiphonales, and Tilopteridales (Phaeophyceae) based on RUBISCO large subunit and spacer sequences. Journal of Phycology 34: 1038-1048.

SILVA, P. C. & DE REVIERS, B. (2000): Ordinal names in the Phaeophyceae. Cryptogamie, Algologie 21: 49-58.

SILVA, P. C. , BASSON, P. W. & MOE, R. L. (1996): Catalogue of the Benthic Marine Algae of the Indian Ocean. University of California Publications in Botany 79. University of California Press, 1259 pages.

SINGH, M., SINGH, D. V., MISRA, P.C., TEWARI, K, K, & KRISHNAN, P. S. (1968): Biochemical aspects of parasitism by the angiosperm parasites: starch accumulation. Physiol. Plant. 21: 525-538.

SKOTTSBERG, C. (1921): Botanische Ergebnisse der schwedischen Expedition nach Patagonien und dem Feuerlande 1907-1909. VIII. Marine Algen. 1. Phaeophyceae. Kungl. Svenska Vetenskapsakademiens Handlingar 61 (11): 1-56.

SMITH, L. (1991): Harbour Hydraulics and Related Processes. In: Report of the Ecosystems and Physical Systems Working Group, Otago Regional Council and Dunedin City Council Joint Discussion Series No. 2, Dunedin, pp. 81-86.

SMITH, M. M & O'BRIEN, T. P. (1979): Distribution of Autoflourescence and Esterase and Peroxidase Activities in the Epidermis of Wheat Roots. Australian Journal of Plant Physiology 6: 201-219.

SMITH, S. D. (2002): Kelp rafts in the Southern Ocean. Global Ecology and Biogeography 11 (1): 67-69.

SMITH, S. E. & SMITH, F. A. (1990): Structure and function of the interfaces in biotrophic symbioses as they relate to nutrient transport. New Phytologist 114: 1-38.

SOLTIS, P. & KUZOFF, R. K. (1993): ITS sequence variation within and among populations of *Lomatium grayi* and *L. laevigatum* (Umbelliferae). Molecular Phylogenetics and Evolution 2: 166-170.

SORCI, G., MØLLER, A. P. & BOULINIER, T. (1997): Genetics of host-parasite interactions. Trends in Ecology and Evolution 12: 196-200.

SOUTH, G. R. (1974): *Herpodiscus* gen. nov. and *Herpodiscus durvilleae* (LINDAUER) comb. nov., a parasite of *Durvillea antarctica* (CHAMISSO) HARIOT endemic to New Zealand. Journal of the Royal Society of New Zealand Vol. 4 (4): 455-461.

SOUTH, G. R. & TITTLEY, I. (1986): A checklist and distributional index of the benthic marine algae of the North Atlantic Ocean. Huntsman Marine Laboratory and British Museum (Natural History), St. Andrews and London: 1-76.

SPENCER-PHILLIPS, P. T. N. (1997): Function of haustoria in epiphytic and endophytic infections. Advances in Botanical Research 24: 309-333.

SPURR, A. R. (1969): A Low-Viscosity Epoxy Resin Embedding Medium for Electron Microscopy. Journal for Ultrastructure Research 26: 31-43.

SRIVASTAVA, S., NIGHOJKAR, A. & KUMAR, A. (1994): Multiple forms of pectin methylesterase from *Cuscuta reflexa* filaments. Phytochemistry 37 (5): 1233-1236.

STACHE-CRAIN, B., MÜLLER, D. G. & GOFF, L. J. (1997): Molecular systematics of *Ectocarpus* and *Kuckuckia* (Ectocarpales, Phaeophyceae) inferred from phylogenetic analysis of nuclear- and plastid-encoded DNA sequences. Journal of Phycology 33: 152-168.

STANTON, B. R. & PICKARD, G. L. (1981): Physical Oceanography of the New Zealand Fiords. New Zealand Oceanographic Institute Memoir 88: 1-37.

STARR, R. C. & ZEIKUS, J. A. (1993): UTEX – The culture collection of algae at the University of Texas at Austin 1993 List of cultures. Journal of Phycology 29 (supplement): 1-106.

STEMPAK, J. G. & WARD, R. T. (1964): An improved staining method for electron microscopy. Journal of Cell Biology 22: 697-701.

STÖCKER, F. W. & DIETRICH, G., eds. (1986): Fachlexikon ABC Biologie. Verlag Harri Deutsch, Thun and Frankfurt/Main, GDR, 1013 pages + 48 plates.

STOCKWELL, P. A. & PETERSEN, G. B. (1987): HOMED: a homologous sequence editor. Comp. Appl. Bioscience 3: 37-43.

STUART, M. D. (1998): The seasonal ecophysiology of *Undaria pinnatifida* (HARVEY) SURINGAR in Otago Harbour. Ph.D. thesis, University of Otago, 1993 pages.

SUGGATE, R. P. (1978): [The New Zealand Environment] Physiography. In: SUGGATE, R. P., STEVENS, G. R. & TE PUNGA, M. T. (eds.): The Geology of New Zealand. Government Printer, Wellington, New Zealand, VolumeI: 10-17.

SWOFFORD, D. L. (2002): PAUP 4.0 b10: Phylogenetic analysis using parsimony, version 4. Sinauer Associates, Sunderland, Massachusetts.

TAN, I. H. & DRUEHL, L. D. (1993): Phylogeny of the Northeast Pacific brown algal (Phaeophycean) orders as inferred from18S rRNA gene sequences. Hydrobiologia 260/261: 699-704.

TAN, I. H. & DRUEHL, L. D. (1994): A molecular analysis of *Analipus* and *Ralfsia* (Phaeophyceae) suggests the order Ectocarpales is polyphyletic. Journal of Phycology 30: 721-729.

248

TAYLOR, W. R. (1957): Marine Algae of the Northeastern Coast of North America. 2nd revised edition, University of Michigan Press, 421 pages.

THOMPSON, J. D., HIGGINS, D. G. & GIBSON, T. J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673-4680.

TIEDEMANN, J., HOHENBERG, H. & KOLLMANN, R. (1997): High-pressure freezing of plant cells cultures in cellulose microcapillaries. Journal of Microscopy 189: 163-171.

UNDERWOOD, A. J. (1981): Techniques of analysis of variance in experimental marine biology and ecology. Oceanography and Marine Biology: An Annual Review 19: 513-605.

VAN ALTENA, I. A. & STEINBERG, P. D. (1992): Are differences in the responses between North American and Australasian marine herbivores to phlorotannins due to differences in phlorotannin structure? Biochemical Systematics and Ecology 6: 493-499.

VAN DEN HOEK, C. & FLINTERMAN, A. (1968): The life history of *Sphacelaria furcigera*. Blumea 16: 195-242.

VAN DEN HOEK. C., MANN, D. G. & JAHNS, H. M. (1995): Algae. An introduction to phycology. Cambridge University Press, Cambridge, United Kingdom, 627 pages.

VAN OPPEN, M. J. H.., OLSEN, J. L., STAM, W. T., VAN DEN HOEK, C. & WIENCKE, C. (1993): Arctic-antarctic disjunctions in the benthic seaweeds *Acrosiphonia arcta* (Chlorophyta) and *Desmarestia viridis/willii* (Phaeophyta) are of recent origin. Marine Biology 115: 381-386.

VAN OPPEN, M. J. H., DRAISMA, S. G. A., OLSEN, J. L. & STAM, W. T. (1995): Multiple trans-Arctic passages in the red alga *Phycodrys rubens*: evidence from nuclear rDNA ITS sequences. Marine Biology 123: 179-188.

VEIGA, A. J., CREMADES, J. & BÁRBARA, I. (1997): *Gononema aecidioides* (Ectocarpaceae), un nuevo feófito para la peninsula Ibérica. Anales Jardín Botanico de Madrid, 55 (1): 155-156.

VILLALARD-BOHNSACK, M. & HARLIN, M. M. (2001): *Grateloupia doryphora* (Halymeniaceae, Rhodophyta) in Rhode Island waters (USA): geographical expansion, morphological variations and associated algae. Phycologia 40: 372-380.

VIS, M. L. & ENTWISLE, T. J. (2000): Insights into the phylogeny of the Batrachospemales (Rhodophyta) from *rbc*L sequence data of Australian taxa. Journal of Phycology 36: 1175-1182.

WARDLE, P. (1991): Vegetation of New Zealand. Cambridge University Press, Melbourne, 672 pages.

WATSON, M. L. (1958): Staining of tissue sections for electron microscopy with heavy metals. Journal of Biophysical and Biochemical Cytolology 4 (4): 475-485.

WATTERS, W. A. (1978a): [The late mobile phase: Igeneous Activity] Miocene. In: SUGGATE, R. P., STEVENS, G. R. & TE PUNGA, M. T. (eds.): The Geology of New Zealand. Government Printer, Wellington, New Zealand, Volume II: 637-644.

WATTERS, W. A. (1978b): [The late mobile phase: Igeneous Activity] Pliocene. In: SUGGATE, R. P., STEVENS, G. R. & TE PUNGA, M. T. (eds.): The Geology of New Zealand. Government Printer, Wellington, New Zealand, Volume II: 644-647.

WEINBERGER, F., FRIEDLANDER, M. & HOPPE, H.-G. (1999): Oligoagars elicit an oxidative burst in *Gracilaria conferta* (Rhodophyta). Journal of Phycology 35: 747-755.

WHEELER, Q. D. & PLATNICK, N. I. (2000): The Phylogenetic Species Concept (*sensu* Wheeler and Platnick). In: WHEELER, Q. D. & MEIER, R. (eds.): Species Concepts and Phylogentic Theory. A Debate. Columbia University Press, New York: 55-69.

WILEY, E. O. & MAYDEN, R. L. (2000): The Evolutionary Species Concept. In: WHEELER, Q. D. & MEIER, R. (eds.): Species Concepts and Phylogentic Theory. A Debate. Columbia University Press, New York: 70-89.

WILSON, K. C., HAAKER, P. L. & HANAN, D. A. (1977): Kelp restoration in southern California. In: KRAUSS, R. W. (ed.): The marine plant biomass of the Pacific northwest coast. Oregon State University Press: 183-202.

WOLFE, A. D. & DEPAMPHILIS, C. W. (1997): Alternate paths of evolution for the photosynthetic gene *rbc*L in four nonphotosynthetic species of *Orobanche*. Plant Molecular Biology 33: 965-977.

WOMERSLEY, H. B. S. (1987): The marine benthic flora of southern Australia Part II. South Australian Government Printing Division, Adelaide, 484 pages.

WOMERSLEY, H. B. S. (1994): The marine benthic flora of southern Australia. Rhodophyta - Part IIIA. Bangiophyceae and Floridophyceae (Acrochaetiales, Nemaliales, Gelidiales, Hildenbrandiales and Gigartinales *sensu lato*). South Australian Government Printing Division, Adelaide, 508 pages.

WRIGHT, K. M. & OPAKA, K. J. (1996): The fluorescent probe HPTS as a phloem-mobile symplastic tracer: an evaluation using confocal laser scanning microscopy. Journal of Experimental Botany 47: 439-445.

YOON, H. S. & BOO, S. M. (1999): Phylogeny of Alariaceae (Phaeophyta) with special reference to *Undaria* based on sequences of the RuBisCo spacer region. Hydrobiologia 398/399: 47-55.

YOSHIDA, T. & AKIYAMA, K. (1979): *Streblonema* (Phaeophyceae) infection in the frond of cultivated *Undaria* (Phaeophyceae). In: JENSEN, A. & STEIN, J. R. (eds.): Proceedings of the Ninth International Seaweed Symposium. Science Press, Princeton: 219-223.

ZEMKE-WHITE, W. L., BREMNER, G. & HURD, C. L. (1999): The status of commercial algal utilization in New Zealand. Hydrobiologia 398/399: 487-494.

ZIMMERMAN, R. C. & KREMER, J. N. (1986): In situ growth and chemical copmposition of the giant kelp *Macrocystis pyrifera*: response to temporal change in ambient nutrient availability. Marine Ecology Progress Series 27: 277-285.

ZINOVA, A. D. (1953): Determination of the Brown Algae of the Northern Seas of the USSR. Izdatel'stvo Akademii Nauk SSSR. Moskva, Leningrad, 224 pages. [in Russian]

ZUCCARELLO, G., MOON, D. & GOFF, L. J. (2004): A phylogenetic study of parasitic genera placed in the family Choreocolacaceae (Rhodophyta). Journal of Phycology 40: 937-945.

ACKNOWLEDGEMENTS

This thesis would not exist without the support and help of a lot of great people, to whom I am greately indebted.

First of all I would like to thank my supervisors Assoc. Prof. Dr. Catriona L. Hurd, Department of Botany, Otago University, and Assoc. Prof. Dr. Akira F. Peters, Station Biologique, Roscoff, France, for their constant support and encouragement, and lots of fruitful discussions, mostly over long distances - without you I would have given up. This may also be the place to thank you, Akira, for introducing me to these tiny but great creatures, and for teaching me so much about brown algae and molecular systematics.

I especially thank Prof. em. Dr. Klaus Müller, then Botany Department, Christian-Albrechts-Universität zu Kiel, Germany, whose support of my application for the DAAD scholarship allowed me to come to New Zealand in the first place.

A big "Thank You!" goes, of course, to all staff and students from the Botany Department, Otago University, especially to MaryAnne Miller, Trish Fleming, Stewart Bell, Vickey Clark, and the late Rob Daly, who made things running smoothly in the background, thus allowing me to concentrate on my work, and to Assoc. Prof. Dr. Paul Guy, Botany Department, Otago University, for his valuable comments.

Many former and current students and collegues helped with field work, were great pals in the lab - despite differing opinions about music - and were always there for a chat, encouragement and suggestions, so a lot of thanks go to Drs. Sheryl Miller, Chris Hepburn, Lisa Russell, Abi Loughnan, Norhardi Ismael, Julia Phillips, Dean Harder, Claudia Keitel and Ralf Ohlemüller, as well as Mark Tilson, Kate Neill, Louise Kregting, Katja Schweikert, and Rochelle Dewdney. Moreover, I would not have been able to get all the interesting endophytes without all those people who did the diving for me, so thank you so much, Stu, Chris, and Sheryl, Dr. Mike Stuart and Jim Fyfe!

I am also very grateful to Dr. Judy Broom, Biochemistry Department, for supporting my molecular work in her lab, and for discussions and very helpful comments on my thesis. The staff from the South Campus EM Unit was very helpful in dealing with the up and downs of electronmicroscopy: Thank you, Richard Lander, Mark Gould, Andrew McNaughton, Bronwyn Smail, Richard Easingwood, and Allan Mitchell.

Many thanks furthermore go to great people only indirectly involved with my work: Dr. Wendy Nelson, NIWA, Wellington, and Glenys Knight (then TePapa Museum of New Zealand,

Welligton) invited me to Wellington and to join them on that great expedition to the Chatham Islands. Susanne Wolff, Department of Botany, University of Konstanz, Germany, introduced me to the EM techniques for brown algae. Prof. Dr. Margaret Clayton, Dr. Monika Schoenwaelder, and Chris Ashburner from Monash University, Victoria, Australia, invited me to Melbourne and gave valuable comments on electron microscopy. Prof. Dr. Johannes Imhoff, Department of Marine Microbiology, Institute for Marine Science, Kiel, Germany, provided sequencing facilities. The University of Auckland, Botany Department, allowed me to examine the type specimen of *Mikrosyphar pachymeniae* LINDAUER 1960. Thomas Meyer, MariLim, Kiel, Germany, provided a slide scanner. Dr. Greg Collings commented on statistics, Drs. Eric Henry, Willem Prud'homme van Reine, Stefano Draisma, Peter Lockhart, Dieter Müller and Joe Zucharello made personal communications.

Thanks also go to the Departments of Geography and of Marine Science, Otago University, for providing equipment for field trips, and to Dr. Kim Currie from NIWA, Dunedin, as well as Dr. John Jillett from Department of Marine Science, Otago University, who kindly provided temperature data, as well as to the late Dr. Lücke, then Bayer AG, Brunsbüttel, Germany, who taught me a lot about the art of writing.

The German Academical Exchange Service DAAD and the University of Otago financially supported me with HSP III and Otago Postgraduate scholarships, respectively, which I gratefully acknowledge.

Moreover, I wish to thank all those nice people in New Zealand, who made me feel welcome and helped me not to feel overly homesick while being 20,000 km away from my family: the staff and students from Toroa International House, where I found a home far from home, all my friends and my "New Zealand family" from Gore, especially my "New Zealand grandmother", the late Jean Smith. My friends in Germany, on the other hand, kept in close contact despite the geographical distance. Many thanks to you - and also to whoever invented email!

Last but not least, I thank my family for their constant love, support and understanding, especially my mother. Thank you for never loosing trust and faith in me!

APPENDICES

A MEDIA AND SOLUTIONS

A 1 Cultivation of pigmented endophytes

A 1.1 PROVASOLI's enriched seawater (PES)

'PROVASOLI's enriched sea water' or PES consists of marine natural seawater enriched with nutrients, metals and vitamins after PROVASOLI (STARR & ZEIKUS 1993). For three litres enrichment, the following four solutions were prepared separately, each in 500 mL dd H_2O or millipore water:

Solution I (iron mixture)

0.3510 g	ferrous ammonium sulphate hexahydrate	$(NH_4)_2Fe(SO_4)_2 \times 6\ H_2O$
0.3000 g	Titriplex III (EDTA)	

Solution II (metal mixture)

0.5700 g	boric acid	H_3BO_3
0.0147 g	ferric chloride anhydrate	$FeCl_3$
0.1081 g	manganese sulphate tetrahydrate	$MnSO_4 \times 4\ H_2O$
0.0110 g	zinc sulphate heptahydrate	$ZnSO_4 \times 7\ H_2O$
0.0024 g	cobalt-II-sulphate heptahydrate	$CoSO_4 \times 7H_2O$
0.5000 g	Titriplex III (EDTA)	

This solution needed to be heated up to solve the boric acid.

Solution III (vitamin mixture)

0.0002 g	Vitamin B_{12}
0.0100 g	Thiamine
0.0001 g	Biotin
10 g	Tris(hydroxy methyl)methylamine

For the vitamin mixture, 1% solutions of Vitamin B_{12} and Biotin (10 mg in 10 mL) were made up. Together with Thiamine and Tris, 0.2 mL of the Vitamin B_{12} and 0.1 mL of the Biotin solution were added to 500 ml dd H_2O or Millipore water.

Solution IV	(nutrient mixture)	
7 g	sodium nitrate	$NaNO_3$
1 g	sodium-β-glycerophosphate	$C_3H_7Na_2O_6P$ x 5 H_2O

All four solutions were mixed together. The pH was brought to 7.8 by adding 1 M HCl. The volume was made up to three litres with dd H_2O or Millipore water. The enrichment was transferred to 20 mL glass scintillation vials (Whatman, England) with metal lids (source unknown) and sterilised in an autoclave (121°C, 1.5 bar, 15 minutes). Enrichment was stored in darkness, at RT. For the culture medium, 10 mL Provasoli-enrichment were added to one litre sterilised natural seawater ('half-strength PES').

A 1.2 Germanium dioxide solution

60 mg of GeO_2 were dissolved in 200 mL dd H_2O or Millipore water and sterilized by autoclaving (121°C, 1.5 bar, 25 mins). One drop of this solution was added to each millilitre PES to reach a final concentration of approximately 6 mg GeO_2 L^{-1} in the culture medium.

A 2 Electron microscopy

A 2.1 Scanning electron microscopy

Fixation after I. MAIER (S. WOLFF, Uni Konstanz, Germany, personal communication)

Stock solutions:

Stock solutions were stored at 4 °C.

buffer stock

substance	molecular weight [g m^{-1}]	amount [g]	concentration in stock solution
sodium cacodylate $C_2H_6AsO_2Na$ water free;	160	2.0	125mM
dd H_2O		made up to 100 mL	

salt stock solution

substance	molecular weight [g m^{-1}]	amount [g]	concentration in stock solution
NaCl	58.44	21.3	3.65 M
KCl	74.55	0.63	85 mM
$CaCl_2$ x 2 H_2O	147.02	6.25 g	425 mM
dd H_2O		made up to 100 mL	

solutions:

fixative: 4 % glutardialdehyde with 100 mM Na-cacodylate, containing 146 mM NaCl, 3.4 mM KCl and 17 mM $CaCl_2$

volume / substance	1 mL	6.25 mL	12.5 mL	25 mL	50 mL
buffer stock		5	10	20	40
salt stock		1	2	4	8
glutardialdehyde 25 %		0.25	0.5	1	2

To remove polymerised glutardialdehyde, the solution was transferred to a disposable syringe (20 mL volume) and pressed through a filter (Schleicher & Schüll, Einweg-Filter Rotrand, pore size 0,2 µm). The pH was adjusted from ca. pH 7.1 to pH 7.7 using concentrated NaOH.

wash buffer: 100mM Na-cacodylate, containing 313.9 mM NaCl, 7.3 mM KCl and 36.6 mM $CaCl_2$ (osmolality: ca 950 mmol kg^{-1})

volume / substance	1 mL	10 mL	20mL	40 mL	60 mL
buffer stock	0.8	8	16	32	48
salt stock	0.086	0.86	1.72	3.44	5.16
ddH_2O	0.114	1.14	2.28	4.56	6.84

The pH was adjusted from ca. pH 8.1 to pH 7.7 using concentrated HCl.

A 2.2 Transmission electron microscopy

A 2.2.1 Standard chemical fixation recipes

A 2.2.1.1 Fixative I

A 2.2.1.1.1 Fixative Ia

Fixation in 2.5% glutardialdehyde, in 0.05 M sodium cacodylate buffered seawater (70%), 0.2% caffeine (I. MAIER & S. WOLFF, University of Konstanz, Germany, personal communication).

Stock solutions (store at 4°C)

fixation buffer stock: 250 mM sodium cacodylate (214.02 g m^{-1}) + 1% caffeine in dd H_2O; pH 7.5

volume / substance	50 mL	100 mL
sodium cacodylate	2.65 g	5.3 g
caffeine ($C_8H_{10}N_4O_2$)	0.5 g	1.0 g
made up with dd H_2O to	50 mL	100 mL

wash buffer stock: 250 mM sodium cacodylate in dd H_2O (no caffeine); pH 7.5

volume / substance	50 mL	100 mL
sodium cacodylate	2.65 g	5.3 g
made up with dd H_2O to	50 mL	100 mL

post fixation buffer stock: 66 mM sodium cacodylate in seawater (diluted with OsO_4 solution => 50 mM)

volume / substance	40 mL	80 mL
sodium cacodylate	0.53 g	1.06 g
seawater (steril filtrered)	37.5 mL	75 mL
dd H_2O	2.5 mL	5 mL

'en bloc' staining: 2% uranyl acetate in dd H_2O (diluted 1:1 with wash buffer)

Solutions

fixation buffer (2.5% glutardialdehyde and 0.2% caffeine in 70% sterile filtered seawater buffered with 50 mM sodium cacodylate, pH 7.4 - 7.5; 2 mL solution were required per sample)

volume / substance	1mL	4 mL	10 mL
25% glutardialdehyde	0.1 mL	0.4 mL	1.0 mL
seawater	0.7 mL	2.8 mL	7.0 mL
fix buffer stock	0.2 mL	0.8 mL	2.0 mL

wash buffer (70% seawater buffered with 50 mM sodium cacodylate, pH 7.4 - 7.5; 5*2 mL = 10 mL solution were required per sample)

volume / substance	1mL	10 mL	20 mL	30 mL	40 mL	50 mL
dd H_2O	0.1 mL	1 mL	2 mL	3 mL	4 mL	5 mL
seawater	0.7 mL	7 mL	14 mL	21 mL	28 mL	35 mL
wash buffer stock	0.2 mL	2 mL	4 mL	6 mL	8 mL	10 mL

post fix buffer (1% osmiumtetroxide in 70% seawater buffered with 50 mM sodium cacodylate; 4 mL solution were required per sample)

volume / substance	1mL	4 mL	8 ml
4% osmiumtetroxide	0.25 mL	1 mL	2 mL
post fix buffer stock	0.75 mL	3 mL	6 mL

A 2.2.1.1.2 Fixative Ib

Fixation in 2.5% glutardialdehyde, in 0.05M sodium cacodylate buffered seawater (50%), 0.2% caffeine (modified after I. MAIER & S. WOLFF, with a reduced seawater concentration).

buffer stock solution (0.1 M sodium cacodylate buffered seawater)

For 100 mL stock solution, 2.14 g sodium cacodylate (water free; 160 g m^{-1}) were dissolved in 80 mL seawater. Seawater was added to make up 100 mL. The pH was adjust to pH 7.2 using 1 N HCl.

fixative

For 50 ml solution, 5 ml glutaraldehyde stock solution (25%) were mixed with 20 ml dd H_2O and 25 ml buffer stock solution. Additionally, 0.1 g caffeine was added and dissolved by stiring and heating up the solution (final concentration: 50% seawater, 0.05M Na-cacodylate).

wash buffer

The buffer stock solution was diluted 1:1 with dd H_2O. 1 g caffeine was added to 100 mL buffer and solved by stiring and heating up.

postfixation

The buffer stock solution was diluted 2:1:1 with dd H_2O and OsO_4 stock (4%).

A 2.2.1.2 Fixative II

Fixation in 4% paraformaldehyde and 4% glutaraldehyde in 0.05 M sodium cacodylate buffered seawater (50%; osmolality 20.6.1999: 971 mmol kg^{-1}) + 1% caffeine (modified after KARNOVSKY 1965).

buffer stock solution (0.1 M sodium cacodylate buffered seawater; osmolality 20.6.1999:1182 mmol kg^{-1}). For 100 mL solution, 2.14 g sodium cacodylate were dissolved in 80 mL seawater. The pH was adjust to pH 7.2 using 1 N HCl. Seawater was added to make up 100 mL, before checking the final pH.

fixative (50% SW in 0.05 M sodium cacodylate; osmolality 20.6.1999: 1243 mmol kg^{-1}). For 100 mL solution, 4 g paraformaldehyde were dissolved in 34 mL dd H_2O by heating the solution up to 60°C and addition of NaOH (ca. 10 drops). The solution was then filtered. 16 mL glutaraldehyde stock solution (25%) and 1 g caffeine were and dissolved by stiring and warming. Buffer stock solution was added to make up to 100 mL. The solution was then allowed to cool to RT, before checking the final pH.

wash buffer

The buffer stock solution was diluted 1:1 with dd H_2O. 1 g caffeine was added to 100 mL buffer and solved by stiring and warming.

postfixation

The buffer stock solution was diluted 2:1:1 with dd H_2O and OsO_4 stock (4%).

A 2.2.1.3 Fixative III

Fixation in 2% glutardialdehyde and 1% formaldehyde in 0.1M sodium cacodylate buffer (fixative IIIb: 0.05M sodium cacodylate) containing 2% NaCl and 0.1% $CaCl_2$ + 1% caffeine (after CLAYTON & ASHBURNER 1994; SCHOENWAELDER & CLAYTON 1998a, 1998b).

buffer stock (4% NaCl and 0.2% $CaCl_2$ in 0.2 M sodium cacodylate in dd H_2O). For 200 mL solution, 8.56 g of sodium cacodylate were dissolved in 160 mL ddH_2O. Eight gram NaCl and 0.4 g $CaCl_2$ were added and dissolved. The pH was adjusted to pH 7.2 by adding 1 N HCL. The volume was made up to 200 mL with dd H_2O, before checking the final pH. For a reduced buffer concentration (i. e. 4% NaCl and 0.2% $CaCl_2$ in 0.1M sodium cacodylate in ddH_2O; fixative IIIb), the amount of sodium cacodylate was reduced to 4.28 g dissolved in 160 mL ddH_2O.

fixative (2% glutardialdehyde, 1% formaldehyde, 1% caffeine). For 100 mL solution, 1 g of paraformaldehyde was dissolved in 34 mL ddH_2O by heating up to 60°C and adding some drops of 1 N NaOH. The solution was stirred and filtered, before adding 8 mL glutardialdehyde solution (25%). The volume was brought to 50 mL by adding ddH_2O. One gram caffeine was dissolved by stirring and warming. Then buffer stock was added to make up 100 mL solution. The solution was allowed to reach RT before checking the final pH. For 100 mL fixative containing 4% glutardialdehyde, 4% formaldehyde, 4 g of paraformaldehyde and 16 mL glutardialdehyde solution (25%) were added.

wash buffer (before postfixation). For 100 mL wash buffer, 50 mL buffer stock were diluted with 50 mL ddH_2O. One gram of caffeine was added and dissolved by stirring and warming the solution.

postfixation buffer. For 4 mL buffer, 2 mL buffer stock were diluted with 1 mL ddH_2O and 1 mL 4% OsO_4 (in water).

wash buffer (after postfixation). For 100 mL wash buffer, 50 mL buffer stock were diluted with 50 mL ddH_2O.

Suppliers of chemicals

Chemicals for electron microscopy were obtained from the following suppliers (for suppliers of chemicals used in molecular systematics see Section 2.2.3): Agar Scientific, UK (paraformaldehyde), Alltech, Auckland (sodium cacodylate), BDH Ltd. England ($CaCl_2$; KCl; NaCl; ethanol; aceton; propylene oxide), Electronmicroscopy Science (glutardialdehyde, 70%

EM grade; Quetol 651), Pro Sci Tech, Australia (Formvar; glutardialdehyde, 25% EM grade; LR White; ERL-4206; DER-732; NSA; DMAE; NMA; DMP-30).

A 2.2.2 Recipes for epoxy resins

component	function	chemical description	amount
SPURR's Low-Viscosity Resin, standard grade (SPURR 1969)			
ERL-4206	resin base	vinylcyclohexene dioxide	10 g
NSA	hardener	nonenylsuccinic anhydride	26 g
DER-732	flexibilizer	diglycidylether of polypropylene glycol	6 g
DMAE	accelerator	dimethylaminoethanol	0.4 g
Quetol 651 resin (KUSHIDA 1974)			
Quetol 651	resin base	ethyleneglycoldiglycidyl ether	10 g
NSA	hardener	nonenylsuccinic anhydride	10 g
NMA	flexibilizer	nadicmethyl anhydride	10 g
DMP-30	accelerator	2,4,8-tridimethylaminoethyl phenol	0.45 g

A 2.2.3 Stain for semi-thin sections

Methylen Blue-Azure-II stain

1.6 g Methylen Blue, 1 g Azure II, 17 g Na_2HPO_4 and 5.6 g KH_2PO_4 were dissolved in dd H_2O. Destilled water was added to make up 1000 mL solution.

B MORPHOLOGICAL DATA OF PIGMENTED ENDOPHYTES

B 1 Cell sizes of isolates

Table B 1.1: Cell and sporangia sizes of the New Zealand endophyte isolates. Data combined for species and varieties confirmed by DNA analyses. Lengths and widths are given in µm ± standard deviation. The numbers of cells or sporangia measured are set in brackets. *: taxon ined.; n. d.: not determined.

isolate	classification		cells			sporangia		
no.	species	variety	length [µm]	width [µm]	length/width ratio	length [µm]	width [µm]	no. of loculi
1-18	*Laminariocolax*	(all)	18.1 ± 7.5 (n = 180)	7 ± 2.3 (n = 180)	2.9 ± 1.5 (n = 180)	57.2 ± 30.5 (n = 126)	6.9 ± 1.2 (n = 126)	19 ± 9 (n = 115)
1-10	*macrocystis*	*radiatae**	17.8 ± 7.8 (n = 100)	7.4 ± 2.5 (n = 100)	2.7 ± 1.6 (n = 100)	67 ± 34.7 (n = 64)	7.2 ± 1 (n = 64)	20 ± 10 (n = 64)
11, 12		*macrocystis**	18.2 ± 8 (n = 20)	6.5 ± 1.3 (n = 20)	3 ± 1.6 (n = 20)	61 ± 17.1 (n = 20)	7.2 ± 0.8 (n = 20)	18 ± 5 (n = 20)
13-18		*novae-zelandiae**	18.6 ± 6.9 (n = 60)	6.4 ± 2 (n = 60)	3.1 ± 1.4 (n = 60)	40.5 ± 20.3 (n = 42)	6.5 ± 1.5 (n = 42)	15 ± 7 (n = 40)
19-27	*Microspongium*	(all)	11.6 ± 6.5 (n = 90)	4.6 ± 1.3 (n = 90)	2.6 ± 1.5 (n = 90)	35 ± 21.2 (n = 70)	4.4 ± 0.7 (n = 70)	11 ± 7 (n = 65)
19-26	*tenuissimum*	*tenuissimum*	11.6 ± 6.8 (n = 80)	4.7 ± 1.4 (n = 80)	2.5 ± 1.5 (n = 80)	36.7 ± 22.7 (n = 60)	4.4 ± 0.7 (n = 60)	11 ± 7 (n = 60)
(20-22)		(*tenuissimum* type 1)	6.2 ± 2.8 (n = 30)	3.9 ± 0.8 (n = 30)	1.6 ± 0.6 (n = 30)	18.6 ± 6 (n = 30)	4.3 ± 0.7 (n = 30)	6 ± 2 (n = 30)
(24-26)		(*tenuissimum* type 2)	16 ± 7.2 (n = 30)	5 ± 1.5 (n = 30)	3.5 ± 1.8 (n = 30)	54.8 ± 18.3 (n = 30)	4.4 ± 0.8 (n = 30)	17 ± 6 (n = 30)
27		*radians*	11.7 ± 4.2 (n = 10)	4.2 ± 0.9 (n = 10)	3 ± 1.3 (n = 10)	31.1 ± 6.4 (n = 10)	4.6 ± 0.7 (n = 10)	8 ± 2 (n = 5)
28-34	*Xiphophorocolax*	(all)	8.3 ± 2.9 (n = 70)	5.8 ± 1.3 (n = 70)	1.5 ± 0.7 (n = 70)	n. d.	n. d.	n. d.
28-33	*aotearoae**	*aotearoae**	8.6 ± 2.9 (n = 60)	5.8 ± 1.4 (n = 60)	1.6 ± 0.8 (n = 60)	27.6 ± 13.5 (n = 20)	5.5 ± 0.6 (n = 20)	10 ± 5 (n = 20)
34		*willanae**	6.8 ± 1.8 (n = 10)	5.6 ± 0.9 (n = 10)	1.3 ± 0.5 (n = 10)	n. d.	n. d.	n. d.

Table B 1.2: New Zealand endophyte isolates. Cell and sporangia size comparisons between species. Results of 1 way-ANOVAs (per species: $n_{cells} = 70$; $n_{sporangia} = 20$).

dependent	source	df	sum of squares	mean square	F-value	p-value
cell length	species	2	3555.268	1777.634	48.165	0.0001
	residual	207	7639.837	36.907		
cell width	species	2	245.003	122.501	36.908	0.0001
	residual	207	687.064	3.319		
cell length to cell width ratio	species	2	61.772	30.886	19.427	0.0001
	residual	207	329.091	1.590		
sporangia length	species	2	9297.835	4648.917	8.230	0.0007
	residual	57	32198.459	564.885		
sporangia width	species	2	51.749	25.874	27.470	0.0001
	residual	57	53.689	0.942		

Table B 1.3: *Laminariocolax macrocystis*: Cell and sporangia size comparisons between varieties. Results of 1 way-ANOVAs (per variety: n = 10).

dependent	source	df	sum of squares	mean square	F-value	p-value
cell length	variety	2	44.164	22.082	0.374	0.6913
	residual	27	1592.869	58.995		
cell width	variety	2	14.960	7.480	1.720	0.1982
	residual	27	117.442	4.350		
sporangia length	variety	2	2520.214	1260.257	3.300	0.0522
	residual	27	10312.608	381.948		
sporangia width	variety	2	3.702	1.851	1.595	0.2215
	residual	27	31.336	1.161		

Table B 1.4: *Microspongium tenuissimum*: Cell and sporangia size comparisons between varieties. Results of 1 way-ANOVAs (per variety: n = 10).

dependent	source	df	sum of squares	mean square	F-value	p-value
cell length	variety	1	99.681	99.681	4.843	0.0411
	residual	18	370.510	20.584		
cell width	variety	1	0.677	0.677	0.471	0.5015
	residual	18	25.899	1.439		
sporangia length	variety	1	194.002	194.002	0.928	0.3482
	residual	18	3763.658	209.092		
sporangia width	variety	1	1.250E-4	1.250E-4	2.568E-4	0.9874
	residual	18	8.762	0.487		

Table B 1.5: *Microspongium tenuissimum*: Cell and sporangia size comparisons between types of *Microspongium tenuissimum* var. *tenuissimum* (group M_1). Results of 1 way-ANOVAs (per type: n = 30).

dependent	source	df	sum of squares	mean square	F-value	p-value
cell length	type	1	1443.120	1443.120	48.035	0.0001
	residual	58	1742.493	30.043		
cell width	type	1	16.452	16.452	11.349	0.0013
	residual	58	84.078	1.450		
sporangia length	type	1	19640.379	19640.379	106.133	0.0001
	residual	58	10733.171	185.055		
sporangia width	type	1	0.362	0.362	0.643	0.4259
	residual	58	32.660	0.563		

Table B 1.6: *Xiphophorocolax aotearoae* gen. et sp. ined.: Cell size comparisons between varieties. Results of 1 way-ANOVAs (per variety: n = 10).

dependent	source	df	sum of squares	mean square	F-value	p-value
cell length	variety	1	23.526	23.526	5.408	0.3190
	residual	18	78.307	4.350		
cell width	variety	1	0.543	0.543	0.458	0.5073
	residual	18	21.362	1.187		

B 2 Distribution maps

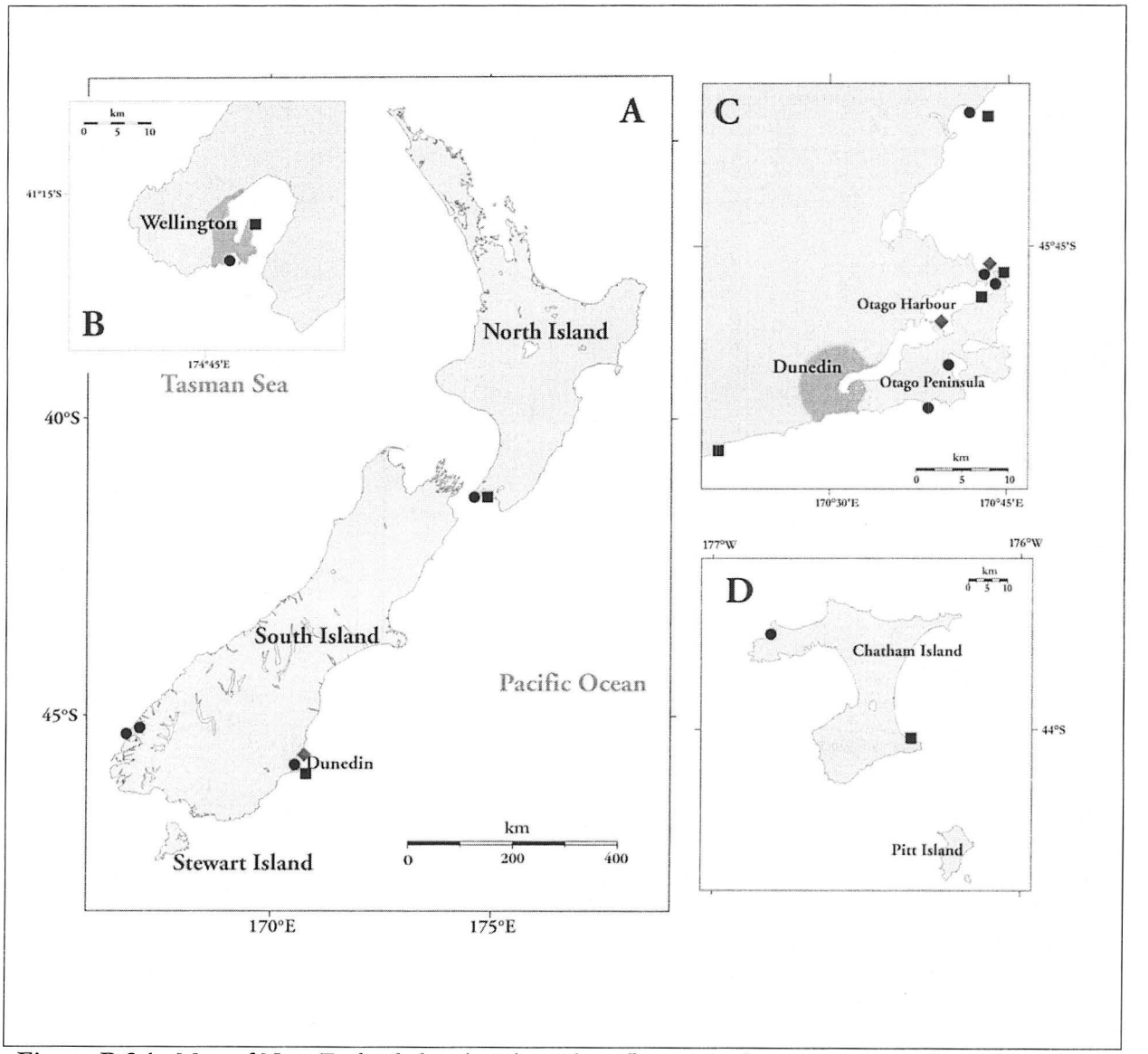


Figure B 2.1: Map of New Zealand showing sites where *Laminariocolax macrocystis* was found growing in macroalgae. ● *L. macrocystis* var. *radiatae** (group L_1); ◆ *L. macrocystis* var. *macrocystis* (group L_2); ■ *L. macrocystis* var. *novae-zelandiae** (group L_3). Taxa ined. are marked with *. A: New Zealand; B: Wellington area; C: Greater Dunedin area; D: Chatham Island.

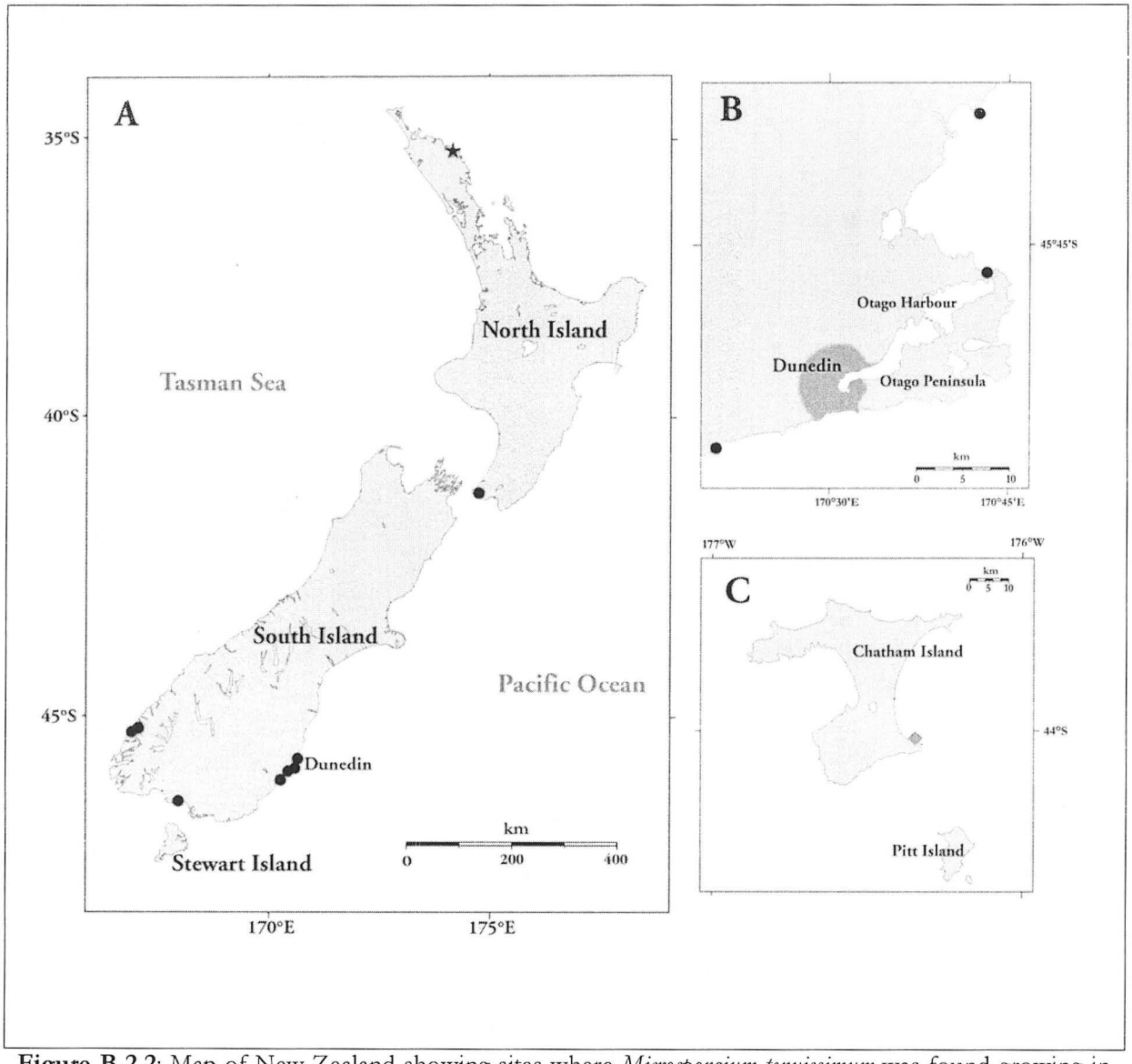


Figure B 2.2: Map of New Zealand showing sites where *Microspongium tenuissimum* was found growing in macroalgae. ● *M. tenuissimum* var. *tenuissimum* (group M_1); ◆ *M. tenuissimum* var. *radians* (group M_2). ★ type locality of *Mikrosyphar pachymeniae* LINDAUER (1960). A: New Zealand; B: Greater Dunedin area; C: Chatham Island.

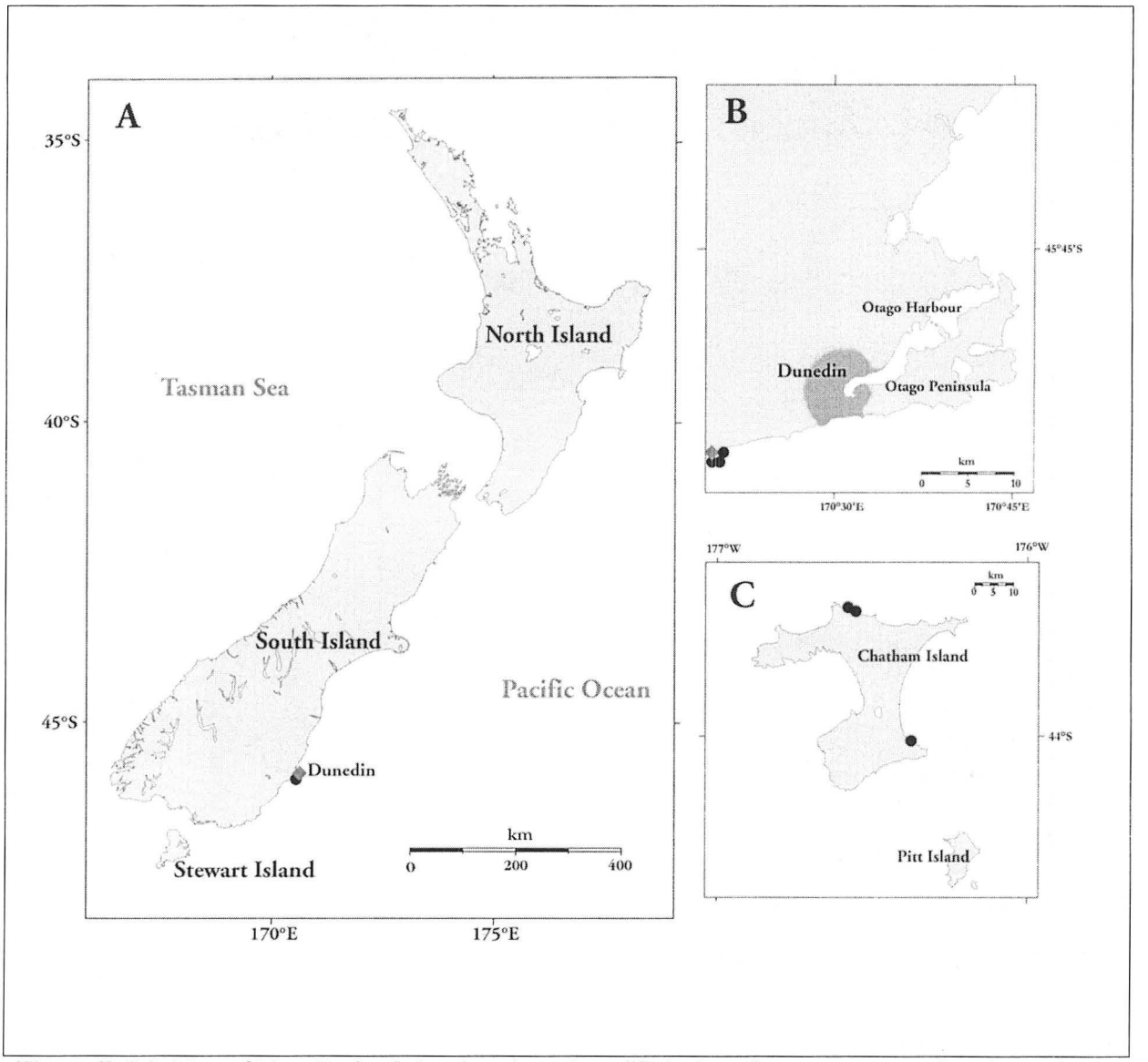


Figure B 2.3: Map of New Zealand showing sites where *Xiphophorocolax aotearoae** was found growing in macroalgae. ● *X. aotearoae* var. *aotearoae** (group X_1); ◆ *X. aotearoae* var. *willanae** (group X_2). Taxa ined. are marked with *. A: New Zealand; B: Greater Dunedin area; C: Chatham Island.

C FIELD DATA

C 1 Collection data

Table C 1.1.1: Data of *Macrocystis pyrifera* collected at Aramoana in 2000.

site	season	date	sub-sample no.	thallus	no. fronds	maximal length (m)	fresh weight (kg)	holdfast size (cm*cm*cm)	disease category	no. mature fronds	no. infected mature fronds	no. subcanopy fronds	no. infected subcanopy fronds	no. senescent fronds	no. infected senescent fronds	no. juvenile fronds	no. infected juvenile fronds
Aramoana	summer	19.01.00	1	1	3	2.4	1.8	4*10*2	2	2	1	0	0	0	0	1	0
surface temperature:				2	5	2.7	3.0	9*14*6	2	3	2	0	0	0	0	2	0
17.2°C				3	2	4	2.5	12*7*4	1	2	2	0	0	0	0	0	0
salinity:				mean	3.3	3.0	2.4		1.7	2.3	1.7	0		0		1	0
31.2 PSU				SD	1.5	0.9	0.6		0.6	0.6	0.6					1	
			2	4	10	4	6.6	9*10*16	3	6	4	3	0	1	1	0	0
				5	5	4.5	3.9	6*22*16	3	4	3	0	0	1	0	0	0
				6	8	4.8	13.9	19*5*13	2	4	3	4	1	0	0	0	0
				mean	7.7	4.4	8.1		2.7	4.7	3.3	2.3	0.3	0.7	0.3	0	
				SD	2.5	0.4	5.2		0.6	1.2	0.6	2.1	0.6	0.6	0.6		
			3	7	6	4.1	7.1	8*10*23	1	4	3	0	0	1	1	1	0
				8	13	6	8.9	20*8*5	3	3	3	3	0	4	4	3	0
				9	10	5	5.2	10*16*26	2	2	2	3	1	3	3	2	0
				mean	9.7	5.0	7.1		2	3	2.7	2	0.3	2.7	2.7	2	0
				SD	3.5	1.0	1.8		1	1	0.6	1.7	0.6	1.5	1.5	1	
			mean		6.9	4.2	5.9		2.1	3.3		1.4		1.1		1	
			SD		3.6	1.1	3.8		0.8	1.3		1.7		1.5		1.1	
		percentage infected							100		76.7		15.4		90		0
	autumn	12.04.00	1	1	6	4.6	3.0	20*4*5	2	3	2	0	0	0	0	3	0
	surface temperature:			2	4	4.5	1.6	3*9*6	2	1	1	0	0	1	1	2	0
	n.d.			3	8	4.4	3.4	11*13*18	2	3	3	1	0	4	4	0	0
				mean	6.0	4.5	2.7		2	2.3	2	0.3	0	1.7	1.7	1.7	0
				SD	2.0	0.1	0.9		0	1.2	1	0.6		2.1	2.1	1.5	
			2	4	4	6.5	2.5	8*9*3	2	2	1	1	0	0	0	1	0
				5	3	6	2.7	12*6*3	2	2	2	1	0	0	0	1	0
				6	6	5.4	3.9	7*9*11	2	4	3	0	0	0	0	2	0
				mean	4.3	6.0	3.1		2	2.7	2	0.7	0	0		1.3	0
				SD	1.5	0.6	0.7		0	1.2	1	0.6				0.6	
			3	7	4	2.1	1.6	4*5*9	2	2	2	1	0	1	1	0	0
				8	5	3.7	1.6	4*10*10	2	2	1	1	1	0	0	1	0
				9	4	6.1	2.5	4*7*8	2	2	2	2	1	0	0	0	0
				mean	4.3	4.0	1.9		2	2	1.7	1.3	0.7	0.3	0.3	0.3	0
				SD	0.6	2.0	0.5		0	0	0.6	0.6	0.6	0.6	0.6	0.6	
			mean		4.9	4.8	2.5		2	2.3		0.8		0.7		1.1	
			SD		1.5	1.4	0.8		0	0.9		0.7		1.3		1.1	
		percentage infected							100		81		28.6		100		0

Table C 1.1.1: Data of *Macrocystis pyrifera* collected at Aramoana in 2000 (continued).

site	season	date	sub-sample no.	thallus	no. fronds	maximal length (m)	fresh weight (kg)	holdfast size (cm*cm*cm)	disease category	no. mature fronds	no. infected mature fronds	no. subcanopy fronds	no. infected subcanopy fronds	no. senescent fronds	no. infected senescent fronds	no. juvenile fronds	no. infected juvenile fronds
Aramoana	winter	17.07.00	1	1	7	7.3	4.3	6*14*18	2	1	1	3	2	2	1	1	0
surface temperature:				2	9	7.7	3.4	7*19*18	2	2	2	1	0	3	1	3	0
10.5°C				3	5	9.5	3.2	2*10*12	2	2	2	0	0	1	1	2	0
				mean	7.0	8.2	3.7		2	1.7	1.7	1.3	0.7	2	1	2	0
				SD	2.0	1.2	0.6		0	0.6	0.6	1.5	1.2	1	0	1	
			2	4	5	6.3	2.7	5*13*16	2	1	1	2	2	0	0	2	0
				5	5	9.4	2.3	3*7*11	2	1	1	1	1	2	0	1	0
				6	7	8.8	3.4	12*14*6	2	1	1	2	2	2	2	2	0
				mean	5.7	8.2	2.8		2	1	1	1.7	1.7	1.3	0.7	1.7	0
				SD	1.2	1.6	0.6		0	0	0	0.6	0.6	1.2	1.2	0.6	
		26.07.00	3	7	7	7.1	3.9	14*10*5	2	2	2	2	0	1	1	2	0
				8	5	6.8	3.0	18*17*5	2	2	2	0	0	2	1	1	0
				9	8	4.1	3.0	10*12*3	2	1	1	5	2	0	0	2	0
				mean	6.7	6.0	3.3		2	1.7	1.7	2.3	0.7	1	0.7	1.7	0
				SD	1.5	1.7	0.5		0	0.6	0.6	2.5	1.2	1	1	0.6	
				mean	6.4	7.4	3.3		2	1.4		1.8		1.4		1.8	
				SD	1.5	1.7	0.6		0	0.5		1.6		1		0.7	
				percentage infected					100		100		56.3		53.8		0
	spring	06.11.00	1	1	5	6.1	3.9	12*10*6	2	2	2	1	0	2	2	0	0
	surface temperature:			2	8	5.2	5.7	12*6*16	1	3	1	1	0	3	3	1	0
	13.8°C			3	7	3.7	3.4	13*16*6	2	2	1	0	0	4	4	1	0
				mean	6.7	5.0	4.3		1.7	2.3	1.3	0.7	0	3	3	0.7	0
				SD	1.5	1.2	1.2		0.6	0.6	0.6	0.6		1	1	0.6	
			2	4	5	6.4	4.3	17*15*6	2	2	2	1	0	1	1	1	0
				5	3	7.3	6.2	18*9*7	3	1	1	2	2	0	0	0	0
				6	7	3.2	5.2	14*17*10	3	3	2	1	0	3	3	0	0
				mean	5.0	5.6	5.2		2.7	2.0	1.7	1.3	0.7	1.3	1.3	0.3	0
				SD	2.0	2.2	0.9		0.6	1.0	0.6	0.6	1.2	1.5	1.5	0.6	
			3	7	4	5.5	4.3	11*15*4	2	1	1	1	1	0	0	2	0
				8	5	5.2	4.8	9*13*4	2	3	3	2	0	0	0	0	0
				9	4	3.4	2.1	11*13*5	2	1	1	0	0	1	1	2	0
				mean	4.3	4.7	3.7		2	1.7	1.7	1.0	0.3	0.3	0.3	1.3	0
				SD	0.6	1.1	1.5		0	1.2	1.2	1.0	0.6	0.6	0.6	1.2	
				mean	5.3	5.1	4.4		2.1	2		1		1.6		0.8	
				SD	1.7	1.4	1.2		0.6	0.9		0.7		1.5		0.8	
				percentage infected					100		77.8		33.3		100		0

Table C 1.1.2: Data of *Macrocystis pyrifera* collected at Quarantine Point in 2000.

site	season	date	sub-sample no.	thallus	no. fronds	maximal length (m)	fresh weight (kg)	holdfast size (cm*cm*cm)	disease category	no. mature fronds	no. infected mature fronds	no. subcanopy fronds	no. infected subcanopy fronds	no. senescent fronds	no. infected senescent fronds	no. juvenile fronds	no. infected juvenile fronds
Quarantine Point	summer	19.01.00	1	1	4	3.5	3.0	4*28*19	2	1	0	0	0	0	0	3	0
	surface temperature:			2	3	0.9	1.2	5*21*11	1	1	1	2	1	0	0	0	0
	16.9°C			3	5	5	5.7	10*15*16	2	2	1	1	0	0	0	2	0
	salinity:			mean	4.0	3.1	3.3		1.7	1.3	0.7	1	0.3	0		1.7	0
	29.1 PSU			SD	1.0	2.1	2.3		0.6	0.6	0.6	1	0.6			1.5	
			2	4	5	1.2	3.0	15*17*6	1	2	1	1	1	0	0	2	0
				5	3	4.1	2.3	9*28*13	2	1	1	1	1	0	0	1	0
				6	5	6.5	4.3	18*24*8	2	1	1	0	0	1	1	3	0
				mean	4.3	3.9	3.2		1.7	1.3	1.0	0.7	0.7	0.3	0.3	2.0	0.0
				SD	1.2	2.7	1.0		0.6	0.6	0.0	0.6	1	0.6	0.6	1.0	0.0
			3	7	6	2.05	1.6	11*4*2	2	2	0	1	1	2	2	1	0
				8	3	2.5	1.6	18*5*2	1	1	1	1	0	0	0	1	0
				9	3	2.4	3.0	12*9*4	0	1	0	1	0	0	0	1	0
				mean	4.0	2.3	2.1		1	1.3	0.3	1.0	0.3	0.7	0.7	1.0	0.0
				SD	1.7	0.2	0.8		1	0.6	0.6	0.0	0.6	1.2	1.2	0.0	0.0
				mean	4.1	3.1	2.8		1.4	1.3		0.9		0.3		1.6	
				SD	1.2	1.8	1.4		0.7	0.5		0.6		0.7		1	
		percentage infected							89.9		50		50		100		0
	autumn	12.04.00	1	1	5	1.4	1.6	6*7*14	2	2	2	2	1	1	0	0	0
	surface temperature:			2	5	2.2	3.0	4*27*15	2	2	2	1	0	2	2	0	0
	n.d.			3	5	1.6	1.8	10*9*10	2	3	3	0	0	1	1	1	1
				mean	5.0	1.7	2.1		2	2.3	2.3	1	0.3	1.3	1	0.3	0.3
				SD	0.0	0.4	0.7		0	0.6	0.6	1	0.6	0.6	1	0.6	0.6
			2	4	5	2.5	1.6	5*9*4	2	2	2	0	0	2	1	1	1
				5	5	1.6	2.3	7*8*11	2	1	1	1	1	3	3	0	0
				6	3	1.9	2.5	17*22*7	2	1	1	1	1	1	1	0	0
				mean	4.3	2.0	2.1		2	1.3	1.3	0.7	0.7	2	1.7	0.3	0.3
				SD	1.2	0.5	0.5		0	0.6	0.6	0.6	0.6	1	1.2	0.6	0.6
			3	7	4	3.8	2.5	10*16*17	2	1	1	2	2	1	1	0	0
				8	6	2.5	2.1	8*6*3	2	2	2	0	0	1	1	3	0
				9	5	2.6	2.1	9*18*15	2	2	2	0	0	1	1	2	1
				mean	5.0	3.0	2.2		2	1.7	1.7	0.7	0.7	1	1	1.7	0.3
				SD	1.0	0.7	0.3		0	0.6	0.6	1.2	1.2	0	0	1.5	0.6
				mean	4.8	2.2	2.2		2	1.8		0.8		1.4		0.8	
				SD	0.8	0.7	0.5		0	0.7		0.8		0.7		1.1	
		percentage infected							100		100		71.4		84.6		42.9

Table C 1.1.2: Data of *Macrocystis pyrifera* collected at Quarantine Point in 2000 (continued).

site	season	date	sub-sample no.	thallus	no. fronds	maximal length (m)	fresh weight (kg)	holdfast size (cm*cm*cm)	disease category	no. mature fronds	no. infected mature fronds	no. subcanopy fronds	no. infected subcanopy fronds	no. senescent fronds	no. infected senescent fronds	no. juvenile fronds	no. infected juvenile fronds
Quarantine Point	winter	12.07.00	1	1	2	1.9	n.d.	13*9*4	2	2	1	0	0	0	0	0	0
	surface temperature:			2	5	2.8	n.d.	12*8*2	2	1	1	1	1	1	1	2	0
	10.0°C			3	6	2.3	n.d.	15*9*6	2	1	1	2	1	1	1	2	0
				mean	4.3	2.3			2	1.3	1	1	0.7	0.7	0.7	1.3	0
				SD	2.1	0.5			0	0.6	0	1	0.6	0.6	0.6	1.2	
			2	4	4	2.2	n.d.	20*20*8	3	2	2	1	1	0	0	1	0
				5	4	1.4	n.d.	7*16*7	2	2	2	1	1	0	0	1	0
		26.07.00		6	5	1.6	2.1	12*14*7	3	1	1	0	0	3	3	1	0
				mean	4.3	1.7			2.7	1.7	1.7	0.7	0.7	1	1	1	0
				SD	0.6	0.4			0.6	0.6	0.6	0.6	0.6	1.7	1.7	0	
			3	7	10	2.1	4.8	9*18*6	2	1	1	3	2	2	1	4	0
				8	2	1.8	1.6	9*10*4	2	1	1	0	0	0	0	1	1
				9	8	4.1	7.5	18*15*5	2	2	1	4	0	0	0	2	0
				mean	6.7	2.7	4.6		2	1.3	1	2.3	0.7	0.7	0.3	2.3	0.3
				SD	4.2	1.3	3.0		0	0.6	0	2.1	1.2	1.2	0.6	1.5	0.6
				mean	5.1	2.2	4.0		2.2	1.4		1.3		0.8		1.6	
				SD	2.6	0.8	2.7		0.4	0.5		1.4		1.1		1.1	
				percentage infected					100		84.6		50.0		85.7		7.1
	spring	01.11.00	1	1	3	2.1	3.9	11*21*20	2	2	2	0	0	1	1	0	0
	surface temperature:			2	3	1.7	2.5	13*7*3	2	2	1	0	0	0	0	1	0
	14.0°C			3	5	2	1.6	14*7*4	2	1	1	1	1	1	0	2	0
				mean	3.7	1.9	2.7		2	1.7	1.3	0.3	0.3	0.7	0.3	1	0
				SD	1.2	0.2	1.1		0	0.6	0.6	0.6	0.6	0.6	0.6	1	
			2	4	5	1.1	2.1	10*15*22	2	4	2	0	0	1	1	0	0
				5	6	1.3	3.4	9*17*8	1	3	0	2	0	1	1	0	0
				6	5	1.3	3.0	13*12*6	2	2	0	0	0	3	1	0	0
				mean	5.3	1.2	2.8		1.7	3	0.7	0.7	0	1.7	1	0	0
				SD	0.6	0.1	0.7		0.6	1	1.2	1.2	0	1.2	0	0	
			3	7	3	1.2	2.1	6*3*10	2	1	0	0		1	1	1	0
				8	4	2.5	3.9	19*22*5	2	1	0	1	0	0	0	1	0
				9	5	1.3	2.5	11*4*2	2	1	0	1	1	0	0	3	0
				mean	4.0	1.7	2.8		2	1	0	0.7	0.3	0.3	0.3	1.7	0
				SD	1.0	0.7	0.9		0	0		0.6	0.7	0.6	0.6	1.2	
				mean	4.3	1.6	2.8		1.9	1.9		0.6		0.9		0.9	
				SD	1.1	0.5	0.8		0.3	1.1		0.7		0.9		1.1	
				percentage infected					100		35.3		40		62.5		0

Table C 1.1.3: Data of *Macrocystis pyrifera* collected at Cornish Head in 2000.

site	season	date	sub-sample no.	thallus	no. fronds	maximal length (m)	fresh weight (kg)	holdfast size (cm*cm*cm)	disease category	no. mature fronds	no. infected mature fronds	no. subcanopy fronds	no. infected subcanopy fronds	no. senescent fronds	no. infected senescent fronds	no. juvenile fronds	no. infected juvenile fronds
Cornish Head	summer	14.01.00	1	1	2	8	4.3	20*21*23	0	1	0	1	0	0	0	0	0
	surface temperature:			2	5	10.3	13.9	16*23*17	2	3	1	0	0	1	1	1	0
	16.2°C			3	4	11.2	7.1	18*20*22	1	2	2	1	0	0	0	1	0
	salinity:			mean	3.7	9.8	8.4		1	2	1	0.7	0	0.3	0.3	0.7	0
	31.6 PSU			SD	1.5	1.7	4.9		1	1	1	0.6		0.6	0.6	0.6	
			2	4	10	8	3.7	13*25*21	0	5	0	2	0	1	0	2	0
				5	4	8.5	8.4	17*11*15	2	3	0	1	0	0	0	0	0
				6	4	5.3	4.3	11*25*20	1	4	1	0		0	0	0	0
				mean	6.0	7.3	5.5		1	4	0.3	1	0	0.3	0	0.7	0
				SD	3.5	1.7	2.6		1	1	0.6	1		0.6		1.2	
			3	7	7	8.7	11.1	18*15*25	1	3	0	1	0	1	1	2	0
				8	2	7.7	4.3	10*18*12	0	2	0	0	0	0	0	0	0
				9	4	10.5	11.6	14*28*26	0	3	0	0	0	1	0	0	0
				mean	4.3	9.0	9.0		0.3	2.7	0	0.3	0	0.7	0.3	0.7	0
				SD	2.5	1.4	4.1		0.6	0.6		0.6		0.6	0.6	1.2	
				mean	4.7	8.7	7.6		0.8	2.9		0.7		0.4		0.7	
				SD	2.5	1.8	3.8		0.8	1.2		0.7		0.5		0.9	
		percentage infected							55.6		15.4		0		50		0
	winter	25.7.00	1	1	8	13.6	5.7	16*25*20	0	2	0	1	0	2	0	3	0
	surface temperature:			2	4	9.8	3.4	9*15*20	0	2	0	0	0	1	0	1	0
	10.5°C			3	3	11.2	2.5	8*6*3	0	1	0	0	0	0	0	2	0
				mean	5	11.5	3.9		0	1.7	0	0.3	0	1	0	2	0
				SD	2.6	1.9	1.6			0.6		0.6		1		1	
			2	4	4	10.9	3.7	10*18*21	0	1	0	1	0	2	0	0	0
				5	2	12.8	3.4	10*15*35	0	1	0	1	0	0	0	0	0
				6	5	13.4	3.9	10*16*10	0	1	0	1	0	3	0	0	0
				mean	3.7	12.4	3.7		0	1	0	1	0	1.7	0	0	0
				SD	1.5	1.3	0.2			0		0		1.5			
			3	7	5	6.6	2.1	11*12*5	1	3	1	1	0	0	0	1	0
				8	6	11.1	3.9	10*15*35	0	1	0	2	0	1	0	2	0
				9	4	n.d.	n.d.	10*16*10	1	1	1	1	0	1	0	1	0
				mean	5	5.9	2		0.7	1.7	0.7	1.3	0	0.7	0	1.3	0
				SD	1	3.2	1.3		0.6	1.2	0.6	0.6		0.6		0.6	
				mean	4.6	11.2	3.6		0.2	1.4		0.9		1.1		1.1	
				SD	1.7	2.3	1.1		0.4	0.7		0.6		1.1		1.1	
		percentage infected							22.2		15.4		0		0		0

Table C 1.2.1: Data of *Macrocystis pyrifera* collected at sites along the Otago Coast between 1997 and 1999.

site	season	date	thallus	no. fronds	maximal length (m)	fresh weight (kg)	holdfast size (cm*cm*cm)	disease category	no. mature fronds	no. subcanopy fronds	no. senescent fronds	no. juvenile fronds
Aramoana	summer	03.12.97	1	9	4	3.0	18*13*7	2	4	n.d.	4	1
			2	5	5	1.3	15*14*5	1	3	n.d.	2	0
			3	3	2.1	0.3	5*3*3	3	1	n.d.	2	0
			4	2	2.6	1.0	12*11*4	1	2	n.d.	0	0
			5	8	4.8	3.8	20*12*7	1	5	n.d.	3	0
			6	5	3	0.3	7*6*4	2	2	n.d.	1	2
			7	7	5.4	2.5	14*17*4	1	5	n.d.	1	1
			8	11	4.6	2.8	19*18*5	1	8	n.d.	2	1
			9	5	3.4	1.8	20*15*5	1	4	n.d.	0	1
			10	8	1.5	0.8	21*15*8	2	5	n.d.	1	2
			11	5	1.2	0.3	12*6*15	2	3	n.d.	1	1
			12	8	4.5	3.0	27*20*5	2	7	n.d.	0	1
			13	6	1.9	1.3	14*15*8	2	5	n.d.	1	0
			14	6	1.5	0.5	11*9*6	2	3	n.d.	1	2
			15	3	1.1	0.2	8*6*4	2	2	n.d.	0	1
	autumn	5.03.98	1	11	3.1	2.3	26*9*27	0	5	n.d.	5	1
			2	4	3	1.0	14*5*7	2	3	n.d.	1	0
			3	4	3	1.0	16*4*11	2	4	n.d.	0	0
			4	6	2.9	1.0	18*7*14	2	5	n.d.	0	1
			5	6	3.4	2.5	12*23*12	2	2	n.d.	3	1
			6	16	3.3	3.0	15*18*12	2	4	n.d.	2	10
			7	9	2.9	1.5	16*18*11	1	7	n.d.	1	1
			8	4	3.8	2.3	10*4*14	2	2	n.d.	2	0
			9	4	2.6	0.5	20*5*8	2	2	n.d.	2	0
			10	3	4.7	0.5	13*4*11	3	0	n.d.	2	1
	spring	05.10.99	1	7	1.7	2.1	6*7*7	1	1	2	3	2
			2	11	3.8	5.5	7*16*8	2	3	1	3	4
			3	7	7.2	3.9	2.5*9*3	2	2	2	1	2
			4	10	3.2	2.5	6*16*11	2	1	3	1	4
			5	5	3.7	2.1	12*15*6	2	2	0	2	1
			6	7	4	4.8	13*8*4	2	1	2	2	2
			7	10	6.2	8.4	15*13*7	2	3	2	2	3
Quarantine Point	spring	11.10.99	1	12	7.4	3.0	16*10*7	2	2	1	8	1
			2	5	1.1	1.6	17*6*4	2	2	0	2	1
			3	9	3.4	6.2	16*8*8	3	3	1	2	3
			4	5	1	2.1	15*7*3	0	2	0	2	1
			5	7	4.7	5.2	28*15*17	2	1	0	3	3
Cornish Head	spring	07.10.99	1	9	14	5.2	13*14*6	0	2	3	3	1
			2	6	13	9.3	13*15*7	1	2	2	0	2
			3	9	15	10.7	15*8*3	0	2	3	1	3
			4	6	5.7	6.6	15*11*4	0	1	1	2	2
			5	9	15	15.7	14*12*4	0	4	2	1	2

Table C 1.3.1: Data of *Macrocystis pyrifera* collected at Harington Point on 27.04.1999.

site	season	date	thallus	no. fronds	maximal length (m)	fresh weight (kg)	holdfast size (cm*cm*cm)	disease category[1]	no. mature fronds	no. subcanopy fronds	no. senescent fronds	no. juvenile fronds	no. infected pieces			
													cauloid	pneumatocyst	phylloid	sporophyll
Harington Point	autumn	27.04.99	1	4	2.7	2.1	13*10*9	2	1	0	1	2	0	0	0	0
			2	3	3.4	1.8	4*3*4	2	2	0	0	1	1	1	0	0
			3	9	3.5	2.5	10*7*5	2	1	3	2	3	1	0	0	0
			4	4	4.8	2.1	50*8*4	2	2	0	0	2	0	0	0	0
			5	5	6.9	6.2	30*25*15	1	2	1	1	1	1	0	0	0
			6	8	5.9	5.2	20*13*13	0	4	0	3	1	0	0	0	0
			7	6	8.3	3.9	20*12*7	1	3	0	2	1	1	0	0	0
			8	10	8.1	7.1	16*20*15	1	5	0	2	3	1	0	0	0
			9	6	12	4.8	11*15*6	0	5	0	0	1	0	0	0	0

[1]: according to test piece

Table C 1.3.2: Data of *Macrocystis pyrifera* collected Aramoana on 01.02.2001.

site	season	date	thallus	disease category[1]	health status of frond	no. infected pieces			
						cauloid	pneumatocyst	phylloid	sporophyll
Aramoana	summer	01.02.01	1	2	infected	1	1	1	0
				1	healthy'	0	0	0	0
			2	2	infected	0	0	0	0
				0	healthy'	0	0	0	0
			3	2	infected	0	0	0	0
				0	healthy'	0	0	0	0
			4	2	infected	0	0	0	0
				0	healthy'	0	0	0	0
			5	2	infected	0	0	0	0
				0	healthy'	0	0	0	0
			6	2	infected	0	0	0	0
				0	healthy'	0	1	0	0
			7	2	infected	0	0	0	0
				0	healthy'	0	0	0	0
			8	2	infected	0	0	0	0
				0	healthy'	1	0	0	0
			9	2	infected	0	0	0	0
				0	healthy'	0	0	0	0
			10	2	infected	0	0	0	0
				0	healthy'	0	0	0	0

[1]: according to test piece

C 2 Statistical tables

Tables present sums of squares of nested ANOVAs and results of tests for variance homogeneity after COCHRAN (SACHS 1984). Significant factors are indicated by an asterix. Critical values for $G_{max\ 0.05\ [k,v]}$ are according to SACHS (1984, Table 152 on page 383).

C 2.1 Aramoana

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
season	3	55.170	18.390	6.259	0.0171*
sub-sample (season)	8	23.507	2.938	1.731	0.1422
residual	24	40.733	1.697		
Dependent variable: **thallus length**					

Test after COCHRAN: $G_{max} = 0.2281 < G_{max\ 0.05\ [12,2]} = 0.3924$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
season	3	23.556	7.852	0.779	0.5381
sub-sample (season)	8	80.667	10.083	2.975	0.0183*
residual	24	81.333	3.389		
Dependent variable: **number of fronds**					

Test after COCHRAN: $G_{max} = 0.3032 < G_{max\ 0.05\ [12,2]} = 0.3924$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
season	3	17.000	5.667	4.163	0.0474*
sub-sample (season)	8	10.889	1.361	1.885	0.1099
residual	24	17.333	0.722		
Dependent variable: **number of canopy fronds**					

Test after COCHRAN: $G_{max} = 0.1539 < G_{max\ 0.05\ [12,2]} = 0.3924$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
season	3	4.306	1.435	0.401	0.7566
sub-sample (season)	8	28.667	3.583	3.0	0.0176*
residual	24	28.667	1.194		
Dependent variable: **number of senescent fronds**					

Test after COCHRAN: $G_{max} = 0.3023 < G_{max\ 0.05\ [12,2]} = 0.3924$

C 2.2 Quarantine Point

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
season	3	10.505	3.502	3.281	0.0795
sub-sample (season)	8	8.538	1.067	0.864	0.5593
residual	24	29.658	1.236		
Dependent variable: **thallus length**					

Test after COCHRAN: $G_{max} = 0.1979 < G_{max\ 0.05\ [12,2]} = 0.3924$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
season	3	1.889	0.630	0.630	0.6161
sub-sample (season)	8	8.0	1.0	2.769	0.0254*
residual	24	8.667	0.361		
Dependent variable: **number of canopy fronds**					

Test after COCHRAN: $G_{max} = 0.2310 < G_{max\ 0.05\ [12,2]} = 0.3924$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
season	3	5.639	1.880	2.819	0.1072
sub-sample (season)	8	5.333	0.667	0.828	0.5869
residual	24	19.333	0.806		
Dependent variable: **number of senescent fronds**					

Test after COCHRAN: $G_{max} = 0.3103 < G_{max\ 0.05\ [12,2]} = 0.3924$

C 2.3 Cornish Head

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
season	1	1.389	1.389	3.125	0.1518
sub-sample (season)	4	1.778	0.444	1.0	0.4449
residual	12	5.333	0.444		
Dependent variable: **disease category**					

Test after COCHRAN: $G_{max} = 0.3751 < G_{max\ 0.05\ [2,6]} = 0.6161$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
season	1	0.058	0.058	1.8	0.2508
sub-sample (season)	4	0.128	0.032	1.25	0.3420
residual	12	0.308	0.026		
Dependent variable: **arcsine of squareroot of infection rate**					

Test after COCHRAN: $G_{max} = 0.25 < G_{max\ 0.05\ [2,6]} = 0.6161$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
season	1	20.480	20.480	2.398	0.1964
sub-sample (season)	4	34.164	8.541	2.785	0.0756
residual	12	36.8	3.067		
Dependent variable: **thallus length**					

Test after COCHRAN: $G_{max} = 0.2813 < G_{max\ 0.05\ [2,6]} = 0.6161$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
season	1	760467	76.467	13.391	0.0216*
sub-sample (season)	4	22.842	5.711	0.67	0.6252
residual	12	102.287	8.524		
Dependent variable: **fresh weight**					

Test after COCHRAN: $G_{max} = 0.4766 < G_{max\ 0.05\ [2,6]} = 0.6161$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
season	1	0.056	0.056	0.018	0.8993
sub-sample (season)	4	12.222	3.056	0.591	0.6754
residual	12	62.0	5.167		
Dependent variable: **number of fronds**					

Test after COCHRAN: $G_{max} = 0.3870 < G_{max\ 0.05\ [2,6]} = 0.6161$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
season	1	9.389	9.389	5.281	0.0831
sub-sample (season)	4	7.111	1.778	2.667	0.0842
residual	12	8.0	0.667		
Dependent variable: **number of canopy fronds**					

Test after COCHRAN: $G_{max} = 0.3335 < G_{max\ 0.05\ [2,6]} = 0.6161$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
season	1	2.0	2.0	4.5	0.1012
sub-sample (season)	4	1.778	0.444	0.571	0.6885
residual	12	9.333	0.778		
Dependent variable: **number of senescent fronds**					

Test after COCHRAN: $G_{max} = 0.5003 < G_{max\ 0.05\ [2,6]} = 0.6161$

C 2.4 Comparisons among sites

C 2.1.2 Quarantine Point vs. Aramoana (four seasons)

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
site	1	0.5	0.5	1.565	0.2289
season	3	1.056	0.352	1.101	0.3774
site*season	3	1.944	0.648	2.029	0.1504
sub-sample (site*season)	16	5.111	0.319	1.643	0.0934
residual	48	9.333	0.194		
Dependent variable: **disease category**					

Test after COCHRAN: $G_{max} = 0.2144 < G_{max\ 0.05\ [24,2]} = 0.2354$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
site	1	170.663	170.663	85.212	0.0001*
season	3	24.774	8.258	4.123	0.0241*
site*season	3	40.901	13.634	6.807	0.0036*
sub-sample (site*season)	16	32.045	2.003	1.366	0.1994
residual	48	70.392	1.466		
Dependent variable: **thallus length**					

Test after COCHRAN: $G_{max} = 0.2002 < G_{max\ 0.05\ [24,2]} = 0.2354$

C 2.1.1 Quarantine Point vs. Aramoana vs. Cornish Head (two seasons)

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
site	2	0.474	0.237	18.5	0.0002*
season	1	0.009	0.009	0.667	0.4301
site*season	2	0.056	0.028	2.167	0.1573
sub-sample (site*season)	12	0.154	0.013	1.2	0.3203
residual	36	0.385	0.011		
Dependent variable: **arcsine squareroot of infection rate**					

Test after COCHRAN: $G_{max} = 0.2 < G_{max\ 0.05\ [18,2]} = 0.29614$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
site	2	25.481	12.741	29.913	0.0001*
season	1	0.019	0.019	0.043	0.8383
site*season	2	4.148	2.074	4.870	0.0283*
sub-sample (site*season)	12	5.111	0.426	1.211	0.3135
residual	36	12.667	0.352		
Dependent variable: **disease category**					

Test after COCHRAN: $G_{max} = 0.1580 < G_{max\ 0.05\ [18,2]} = 0.29614$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
site	2	451.863	225.931	49.172	0.0001*
season	1	30.751	30.751	6.693	0.0238*
site*season	2	41.587	20.794	4.526	0.0343*
sub-sample (site*season)	12	55.136	4.595	2.050	0.0480*
residual	36	80.678	2.241		
Dependent variable: **thallus length**					

Test after COCHRAN: $G_{max} = 0.1746 < G_{max\ 0.05\ [18,2]} = 0.29614$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
site	2	65.534	32.767	4.155	0.0426*
season	1	66.667	66.667	8.453	0.0131*
site*season	2	41.041	20.521	2.602	0.1152
sub-sample (site*season)	12	94.644	7.887	1.446	0.1907
residual	36	196.307	5.453		
Dependent variable: **fresh weight**					

Test after COCHRAN: $G_{max} = 0.2726 < G_{max\ 0.05\ [18,2]} = 0.29614$

Source	df	Sum of Squares	Mean Square	*F*-value	p-value
site	2	50.704	25.352	3.414	0.0670
season	1	0.296	0.296	0.040	0.8450
site*season	2	5.148	2.574	0.347	0.7139
sub-sample (site*season)	12	89.111	7.426	1.536	0.1563
residual	36	174.000	4.833		
Dependent variable: **number of fronds**					

Test after COCHRAN: $G_{max} = 0.1992 < G_{max\ 0.05\ [18,2]} = 0.29614$

C 3 Environmental data

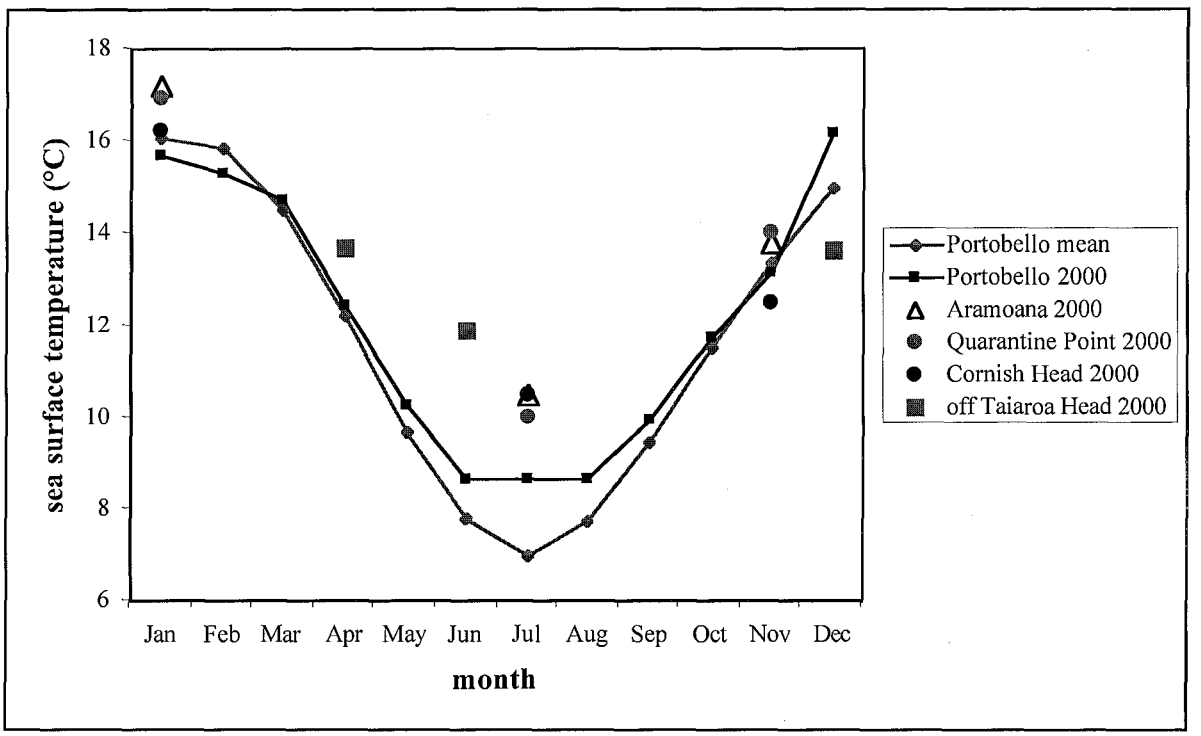


Figure C 3.1: Sea surface temperatures at the Otago coast in 2000. Mean monthly surface temperatures at Portobello Marine Laboratory (courtesy of Dr. J. JILLETT, Marine Science Department, Otago University), and surface temperatures measured on single dates 4.5 km outside the Harbour entrance, off Taiaroa Head (courtesy of Dr. K. CURRIE, NIWA, Dunedin) in comparison to surface temperatures measured at the three study sites on the respective sampling dates.

D Molecular data

D 1 Accession numbers for published sequences

Table D 1.1: GenBank accession numbers and references for sequences used in the present study. n/a: not applicable.

class/order	family	species	GenBank accession number			
			nrDNA			
			18S	ITS	26S	*rbc*L
Xanthophyceae						
Tribonematales	Tribonemataceae	*Tribonema aequale* PASCHER	M55286	n/a	Y07979	AF084611
Phaeophyceae						
Ascoseirales	Ascoseiraceae	*Ascoseira mirabilis* SKOTTSBERG	AJ229125	n/a	AJ229141	n/a
Cutleriales	Cutleriaceae	*Cutleria multifida* (J. E. SMITH) GREVILLE	AF073326	n/a	AF053119	n/a
Desmarestiales	Desmarestiaceae	*Desmarestia aculeata* (L.) LAMOUROUX	Z99451	n/a	AJ229143	AJ287847
		Himantothallus grandifolius (A. GEPP & E. GEPP) ZINOVA	AJ287432	n/a	AJ287433	AJ287850
		Phaeurus antarcticus C. SKOTTSBERG	AJ229111	n/a	AJ229146	n/a
Dictyotales	Dictyotaceae	*Dictyota dichotoma* (HUDSON) LAMOUROUX	AF130702	n/a	AF130715	AJ287852
		Dictyota cervicornis KÜTZING	n/a	n/a	n/a	AJ287851
		Taonia atomaria (WOODWARD) J. AGARDH	L17021	n/a	AF130714	n/a
Ectocarpales	Acinetosporaceae	*Pylaiella littoralis* (L.) KJELLMAN	n/a	n/a	AF071782	X55372
	Adenocystaceae	*Adenocystis utricularis* (BORY) SKOTTSBERG	n/a	n/a	AF071779	AJ295823
		Caepidium antarcticum J. AGARDH	n/a	n/a	AJ295827	AJ295826
	Chordariaceae	*Ascoseirophila violodora* PETERS	n/a	n/a	AJ439834	AJ439835
		Chordaria flagelliformis (MÜLLER) C. AGARDH	AJ229129	AJ229129	AJ229129	AF207798
		Dictyosiphon foeniculaceus (HUDSON) GREVILLE	Z99463	n/a	AJ229137	AF055397
		Laminariocolax tomentosoides (FARLOW) KYLIN	n/a	Z98566	(A. F. PETERS, pers. com.)	AF055404
		Laminariocolax tomentosoides ssp. *deformans* (DANGEARD) PETERS	n/a	AJ439852	n/a	n/a
		Laminariocolax aecidioides (ROSENVINGE) PETERS (Kiel)	n/a	AJ002353	n/a	n/a
		Laminariocolax aecidioides (ROSENVINGE) PETERS (Maine)	n/a	AJ439850	n/a	n/a
		Laminariocolax aecidioides (ROSENVINGE) PETERS (France)	n/a	AJ439851	n/a	n/a
		Laminariocolax macrocystis (PETERS) PETERS	n/a	AJ002359	n/a	n/a
		Laminariocolax eckloniae (PETERS) PETERS (South Africa)	n/a	AJ002357	n/a	n/a
		Laminariocolax eckloniae (PETERS) PETERS (Antarctica)	n/a	AJ439842	n/a	n/a
		Laminarionema elsbetiae KAWAI & TOKUYAMA	n/a	n/a	Z98567	AJ439858
		Microspongium alariae (PEDERSEN) PETERS	n/a	AJ439843	n/a	n/a
		Microspongium radians (HOWE) PETERS	n/a	Z98581	n/a	n/a

Table D 1.1: GenBank accession numbers and references for sequences used in the present study (continued). n/a: not applicable.

class/order	family	species	GenBank accession number			
			nrDNA			
			18S	ITS	26S	*rbc*L
Ectocarpales	Chordariaceae	*Microspongium tenuissimum* (HAUCK) PETERS	n/a	AJ439848	n/a	n/a
		Myrionema strangulans GREVILLE	n/a	n/a	AJ439857	AF055407
		Punctaria latifolia GREVILLE	AF115432	n/a	AF115429	n/a
		Punctaria plantaginea (ROTH) GREVILLE	n/a	n/a	n/a	AF055410
	Ectocarpaceae	*Ectocarpus siliculosus* (DILLWYN) LYNGBYE	L43062	n/a	U38776	X52503
	Scytosiphonaceae	*Scytosiphon lomentaria* (LYNGBYE) LINK	L43066	n/a	AF071159	AB022238
Fucales	Durvillaeaceae	*Durvillaea antarctica* (CHAMISSO) HARIOT	AF130706	n/a	AF130719	n/a
	Fucaceae	*Ascophyllum nodosum* (L.) LEJOLIS	AF091297	n/a	AF053106	AJ287853
		Fucus vesiculosus L.	AF091296	n/a	AF053105	n/a
		Xiphophora chondrophylla (R. BROWN *ex* TURNER) MONTAGNE *ex* HARVEY	AF091289	n/a	AF091271	n/a
	Notheiaceae	*Notheia anomala* HARVEY & BAILEY	(SAUNDERS & KRAFT 1995)	n/a	AF091282	n/a
	Sargassaceae	*Sargassum muticum* (YENDO) FENSHOLT	AF091295	n/a	AF053109	AJ287854
Laminariales	Alariaceae	*Alaria esculenta* (L.) GREVILLE	AF115427	n/a	AF071151	AF064745
		Undaria pinnatifida (HARVEY) SURINGAR	n/a	n/a	AF071152	n/a
	Laminariaceae	*Laminaria digitata* (L.) STACKHOUSE	AF091286	n/a	AF071153	n/a
	Lessoniaceae	*Macrocystis pyrifera* (L.) C. AGARDH	AF115430	n/a	AF053116	AJ287856
Ralfsiales	Ralfsiaceae	*Nemoderma tingitanum* SCHOUSBOE *in* BORNET	AF130709	n/a	AF130722	n/a
Scythotham-nales	Scytothamnaceae	*Scytothamnus australis* (J. AGARDH) HOOKER & HARVEY	AF073325	n/a	AF071780	AJ295833
	Splachnidiaceae	*Splachnidium rugosum* (L.) GREVILLE	AF073327	n/a	AJ229133	AJ295834
Sphacelariales	Cladostephaceae	*Cladostephus spongiosus* (HUDSON) C. AGARDH	AF091298	n/a	AF053115	AJ287836
	Sphacelariaceae	*Sphacelaria arctica* HARVEY	n/a	n/a	n/a	AJ287881
		Sphacelaria caespitula LYNGBYE	n/a	n/a	n/a	AJ287870
		Sphacelaria californica SAUVAGEAU ex SETCHELL & GARDNER	n/a	n/a	n/a	AJ287893
		Sphacelaria cirrosa (ROTH) C. AGARDH	AF115428	n/a	AF071150	AJ287865
		Sphacelaria divaricata MONTAGNE	n/a	n/a	n/a	AJ287889
		Sphacelaria nana NAEGELI ex KÜTZING	n/a	n/a	n/a	AJ287875
		Sphacelaria plumosa LYNGBYE	n/a	n/a	n/a	AJ287879
		Sphacelaria plumigera HOLMES	n/a	n/a	n/a	AJ287878
		Sphacelaria racemosa GREVILLE	n/a	n/a	n/a	AJ287880
		Sphacelaria radicans (DILLWYN) C. AGARDH	n/a	n/a	n/a	AJ287874
		Sphacelaria rigidula KÜTZING	n/a	n/a	n/a	AJ287883

Table D 1.1: GenBank accession numbers and references for sequences used in the present study (continued). n/a: not applicable.

class/order	family	species	GenBank accession number			
			nrDNA			
			18S	ITS	26S	*rbc*L
Sphacelariales	Sphacelariaceae	*Sphacelaria tribuloides* MENEGHINI	n/a	n/a	n/a	AJ287891
		Sphacelaria yamadae SEWAGA	n/a	n/a	n/a	AJ287890
		Sphacella subtillisima REINKE	n/a	n/a	n/a	AJ287931
	Stypocaulaceae	*Alethocladus corymbosus* (DICKIE) SAUVAGEAU	AJ287439	n/a	AJ287440	AJ287860
		Halopteris filicina (GRATELOUP) KÜTZING (1)	n/a	n/a	n/a	AJ287894
		Halopteris filicina (GRATELOUP) KÜTZING (2)	n/a	n/a	n/a	AJ287895
		Stypocaulon durum (RUPRECHT) OKAMURA	n/a	n/a	n/a	AJ287897
		Stypocaulon scoparium (L.) KÜTZING	AF091299	n/a	AF091285	AJ287866
Sporochnales	Sporochnaceae	*Sporochnus pedunculatus* (HUDSON) C. AGARDH	AF130711	n/a	AF130723	n/a
		Sporochnus scoparius HARVEY	n/a	n/a	n/a	AB037142
		Haplospora globosa KJELLM.	AF130712	n/a	AF130724	n/a
Syringoderma-tales	Syringodermata-ceae	*Syringoderma phinneyi* HENRY & MÜLLER	L17017	n/a	AJ243782	AJ287868
Tilopteridales	Tilopteridaceae	*Tilopteris mertensii* (TURNER in SMITH) KÜTZING	n/a	n/a	AF130726	AB045260
Incertae sedis	Choristocarpaceae	*Choristocarpus tenellus* (KÜTZING) ZANARDINI	AJ287441	n/a	AJ287442	AJ287861
Incertae sedis	Onslowiaceae	*Onslowia endophytica* SEARLES in SEARLES & LEISTER	AJ287443	n/a	AJ287444	AJ287864
		Verosphacela ebrachia HENRY	n/a	n/a	AJ287445	AJ287867
Incertae sedis		*Asteronema rhodochortonoides* (BOERGESEN) MÜLLER & PARODI	AJ229117	n/a	AJ229135	n/a
Incertae sedis		*Asterocladon lobatum* MÜLLER *et al.*	AJ229120	n/a	AJ229136	AJ295824

D 2 Sequence statistics

Table D 2.1: Statistics for ITS1 sequences of pigmented endophytic Phaeophyceae. *: partial sequence.

isolate			base frequencies [%]			
no.	abbreviation	length [bp]	A	C	G	T
1	EMa A 3/98	679	0.22907	0.25698	0.27313	0.24082
2	EMa HP 5/98	678	0.22941	0.25735	0.27353	0.23971
3	EMa H 4/99	678	0.22963	0.25630	0.27407	0.24000
4	EMa SP 5/98	679	0.22874	0.25806	0.27273	0.24047
5	EMa WW 5/99	679	0.22633	0.25888	0.27515	0.23964
6	EMa HI 10/99	678	0.22669	0.25723	0.26849	0.24759
7	EEck W 3/98	678	0.22614	0.26285	0.27313	0.23789
8	EEck DS 5/99	171*	0.18966	0.26437	0.24713	0.29885
9	EEck Waki 10/00	684	0.22482	0.25839	0.27445	0.24234
10	EEck OTS 11/99	677	0.22794	0.25735	0.27206	0.24265
11	EMa A 10/97	507	0.24951	0.26523	0.26326	0.22200
12	EMa QP 7/97	511	0.24902	0.26667	0.26275	0.22157
13	EMa PB 7 6/97	190*	0.20000	0.25405	0.25405	0.29189
14	EMa BB 10/97	479	0.24532	0.26611	0.25780	0.23077
15	EMa CH 5/98	481	0.24431	0.26708	0.25673	0.23188
16	EMa W 3/98	476	0.24274	0.25066	0.27704	0.22955
17	EMa OW 5/99	481	0.24587	0.26860	0.25413	0.23140
18	EMa WR 4/00	478	0.24792	0.26667	0.25417	0.23125
19	EPa A 10/97	288	0.24742	0.30241	0.25086	0.19931
20	EPa Riv 9/99	288	0.25086	0.30241	0.25086	0.19588
21	EPa BB 4/99	288	0.25086	0.30241	0.25086	0.19588
22	EPa BS 10/00	288	0.25086	0.30584	0.25086	0.19244
23	EGra BB 2/98	288	0.24742	0.30241	0.25086	0.19931
24	EKal CC 10/00	288	0.24742	0.30584	0.25086	0.19588
25	EUpi W 4/98	288	0.24742	0.30241	0.25086	0.19931
26	EEck Kari 5/99	288	0.24742	0.30584	0.25086	0.19588
27	EPa OW 5/99	258	0.26054	0.29502	0.24521	0.19923
28	ELes OW 5/99	582	0.21784	0.28988	0.27101	0.22127
29	ELes Wk 5/99	583	0.21672	0.29010	0.27133	0.22184
30	EXi BB 11/97	182*	0.17391	0.27174	0.25000	0.30435
31	EXi BB 8/99	581	0.21747	0.29110	0.27226	0.21918
32	EMu Wk 5/99	583	0.21843	0.29181	0.26962	0.22014
33	EDan BB 4/99	583	0.22014	0.29010	0.27304	0.21672
34	EDwil BB 8/00	553	0.22302	0.29676	0.27158	0.20863

Table D 2.2: Statistics for the partial nrDNA sequence of *Xiphophorocolax aotearoae* (isolate no. 29).

		base frequencies [%]			
sequence	length [bp]	A	C	G	T
18S	394	0.23333	0.22353	0.26607	0.27647
26S	502	0.22400	0.23200	0.31600	0.22800

D 3 Alignments

Alignments are numbered as follows:

D 3.1: ITS1 of the nrDNA gene (18S-5.8S), pigmented endophytes

D 3.2: 18S nrDNA gene

D 3.3: 26S nrDNA gene

D 3.4: *rbc*L gene.

Within the alignments, dots indicate the same base as in the first line, and dashes indicate missing bases. Unidentified bases are indicated by a 'N', unidentified positions by a question mark.

D 3.1: ITS1 of the nrDNA gene (18S-5.8S) of the pigmented endophytes.

D 3.1: ITS1 alignment

```
                                positions excluded
                                **********************************************************************************************************
                                **********************************************************************************************************
                                **********************************************************************************************************
                                **********************************************************************************************************
                                00000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000001
                                00000000011111111112222222222333333333344444444455555555556666666666777777777788888888899999999990
                                12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901
                                /      AFP2(F)     /
Chordaria flagelliformis        ???????????????????????????????????????????????????????????????????????????????????????????????????????
1 EMa A 398                     .....................................CTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
2 EMa HP 598                    ...................GGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
3 EMa H 499                     ..............................................TGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
4 EMa SP 598                    ..........................................GGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
5 EMa WW 599                    ....................................CTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
6 EMa HI 1099                   ..........................ATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
7 EEck W 398                    ......................................TAGTTGGNGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
8 EEck DS 599                   ..........................ATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
9 EEck Waki 1000                ..........................CATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
10 EEck OTS 1199                ..............................................AGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
L. eckloniae South Africa       ................................................................................................
L. eckloniae Antarctica         ................................................................................................
11 EMa A 1097                   ........................GCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
12 EMa QP 797                   ..........................................GGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
L. macrocystis Chile            ................................................................................................
13 EMa PB 697                   ..........................ATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
14 EMa BB 1097                  ..........................ATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
15 EMa CH 598                   AGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
16 EMa W 398                    ................................................................................................
17 EMa OW 599                   .....................................CTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
18 EMa WR 400                   AGCTCTTTCTTGATTCTATGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
L. tomentosoides Helgoland      ................................................................................................
L. tomentosoides deformans Bretagne ............................................................................................
L. aecidioides Kiel              ................................................................................................
L. aecidioides Maine            ................................................................................................
L. aecidioides Bretagne         ................................................................................................
19 EPa A 1097                   ..............................TGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
20 EPa Riv 999                  ....................GGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
21 EPa BB 499                    ........................GCATGGCCGTTCNTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
22 EPa BS 1000                  ....................GGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
23 EGra BB 298                  .....................................CTTAGTTGGNGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
24 EKal CC 1000                 ..........................ATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
25 EUpi W 498                   ..........................ATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
26 EEck Kari 599                .....................................TAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
Microspongium tenuissimum       ................................................................................................
27 EPa OW 599                   ..........................ATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
Microspongium radians           ................................................................................................
Microspongium alariae           ................................................................................................
28 ELes OW 599                  ..........................ATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
29 ELes Wk 599                  .....................................CTTAGTTGNTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
30 EXi BB 1197                   ..........................TGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
31 EXi BB 899                   ..................................CGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
32 EMu Wk 599                   ..........................................GGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
33 EDan BB 499                  ..........................TGGCCGTTCTTAGTTGGNGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
34 EDwil BB 800                 ..............................................GGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCT
```

D 3.1: ITS1 of the ntDNA gene (18S-5.8S) of the pigmented endophytes (continued).

D 3.1: ITS1 alignment (continued)

```
positions excluded
all endophytes:                  **********************************************************************************************************
Laminariocolax clade:            **********************************************************************************************************
Microspongium clade:             **********************************************************************************************************
Xiphophorocolax clade:           **********************************************************************************************************
                                 11111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111112
                                 00000000011111111112222222222333333333344444444455555555556666666666777777777788888888899999999990
                                 12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890

Chordaria flagelliformis         ??????????????????????????????????????????TTCTTAGAGGGACTTCTGGT-ACTAA-CAGAG-AAGTTGGGGGCAATAACAGGTCTGTGATGCC
1 EMa A 398                      AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
2 EMa HP 598                     AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
3 EMa H 499                      AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
4 EMa SP 598                     AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
5 EMa WW 599                     AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
6 EMa HI 1099                    AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
7 EEck W 398                     AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
8 EEck DS 599                    AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
9 EEck Waki 1000                 AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
10 EEck OTS 1199                 AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
L. eckloniae South Africa        .....................................??????????????????????????????????????????????????????????????????
L. eckloniae Antarctica          .....................................??????????????????????????????????????????????????????????????????
11 EMa A 1097                    AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
12 EMa QP 797                    AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
L. macrocystis Chile             .....................................??????????????????????????????????????????????????????????????????
13 EMa PB 697                    AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
14 EMa BB 1097                   AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
15 EMa CH 598                    AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
16 EMa W 398                     .....................................??????????????????????????????????????????????????????????????????
17 EMa OW 599                    AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
18 EMa WR 400                    AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
L. tomentosoides Helgoland       .....................................??????????????????????????????????????????????????????????????????
L. tomentosoides deformans Bretagne .....................................??????????????????????????????????????????????????????????????????
L. aecidioides Kiel              .....................................??????????????????????????????????????????????????????????????????
L. aecidioides Maine             .....................................??????????????????????????????????????????????????????????????????
L. aecidioides Bretagne          .....................................??????????????????????????????????????????????????????????????????
19 EPa A 1097                    AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
20 EPa Riv 999                   AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
21 EPa BB 499                    AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................NG.....C.....G.............................
22 EPa BS 1000                   AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
23 EGra BB 298                   AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
24 EKal CC 1000                  AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
25 EUpi W 498                    AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
26 EEck Kari 599                 AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
Microspongium tenuissimum        .....................................??????????????????????????????????????????????????????????????????
27 EPa OW 599                    AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
Microspongium radians            .....................................??????????????????????????????????????????????????????????????????
Microspongium alariae            .....................................??????????????????????????????????????????????????????????????????
28 ELes OW 599                   AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
29 ELes Wk 599                   AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
30 EXi BB 1197                   AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
31 EXi BB 899                    AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
32 EMu Wk 599                    AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
33 EDan BB 499                   AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................G.....C.....G.............................
34 EDwil BB 800                  AAATAGTGTGGCTTACGCTTTTGCGTAGGTGCTCGC.....................N.....G.....C.....G.............................
```

D 3.1: ITS1 of the nrDNA gene (18S-5.8S) of the pigmented endophytes (continued).

D 3.1: ITS1 alignment (continued)

```
                                positions excluded
                                **********************************************************************************************************
                                **********************************************************************************************************
                                **********************************************************************************************************
                                **********************************************************************************************************
                                22222222222222222222222222222222222222222222222222222222222222222222222222222222222222222222222222222223
                                00000000011111111112222222222333333333344444444455555555556666666666777777777788888888899999999990
                                12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901
Chordaria flagelliformis        CTTAGATGTCCTGGGCCGCACGCGCGCTACACTGATGCATGCAACGAGTTTTTTTTCCTGGGTCGAGAGGCCCGGGTAATCTGTTGAACGTGCATCGTGA
1 EMa A 398                     ........................................................................................................
2 EMa HP 598                    ........................................................................................................
3 EMa H 499                     ........................................................................................................
4 EMa SP 598                    ........................................................................................................
5 EMa WW 599                    ........................................................................................................
6 EMa HI 1099                   ........................................................................................................
7 EEck W 398                    ........................................................................................................
8 EEck DS 599                   ........................................................................................................
9 EEck Waki 1000                ........................................................................................................
10 EEck OTS 1199                ........................................................................................................
L. eckloniae South Africa        ????????????????????????????????????????????????????????????????????????????????????????????????????
L. eckloniae Antarctica         ????????????????????????????????????????????????????????????????????????????????????????????????????
11 EMa A 1097                   ........................................................................................................
12 EMa QP 797                   ........................................................................................................
L. macrocystis Chile            ????????????????????????????????????????????????????????????????????????????????????????????????????
13 EMa PB 697                   ........................................................................................................
14 EMa BB 1097                  ........................................................................................................
15 EMa CH 598                   ........................................................................................................
16 EMa W 398                    ????????????????????????????????????????????????????????????????????????????????????????????????????
17 EMa OW 599                   ........................................................................................................
18 EMa WR 400                   ........................................................................................................
L. tomentosoides Helgoland      ????????????????????????????????????????????????????????????????????????????????????????????????????
L. tomentosoides deformans Bretagne ????????????????????????????????????????????????????????????????????????????????????????????????????
L. aecidioides Kiel             ????????????????????????????????????????????????????????????????????????????????????????????????????
L. aecidioides Maine            ????????????????????????????????????????????????????????????????????????????????????????????????????
L. aecidioides Bretagne         ????????????????????????????????????????????????????????????????????????????????????????????????????
19 EPa A 1097                   ........................................................................................................
20 EPa Riv 999                  ........................................................................................................
21 EPa BB 499                   ........................................................................................................
22 EPa BS 1000                  ........................................................................................................
23 EGra BB 298                  ........................................................................................................
24 EKal CC 1000                 ........................................................................................................
25 EUpi W 498                   ........................................................................................................
26 EEck Kari 599                ........................................................................................................
Microspongium tenuissimum      ????????????????????????????????????????????????????????????????????????????????????????????????????
27 EPa OW 599                   ........................................................................................................
Microspongium radians           ????????????????????????????????????????????????????????????????????????????????????????????????????
Microspongium alariae           ????????????????????????????????????????????????????????????????????????????????????????????????????
28 ELes OW 599                  ........................................................................................................
29 ELes Wk 599                  ........................................................................................................
30 EXi BB 1197                  ........................................................................................................
31 EXi BB 899                   ........................................................................................................
32 EMu Wk 599                   ........................................................................................................
33 EDan BB 499                  ........................................................................................................
34 EDwil BB 800                 ........................................................................................................
```

D 3.1: ITS1 of the nrDNA gene (18S-5.8S) of the pigmented endophytes (continued).

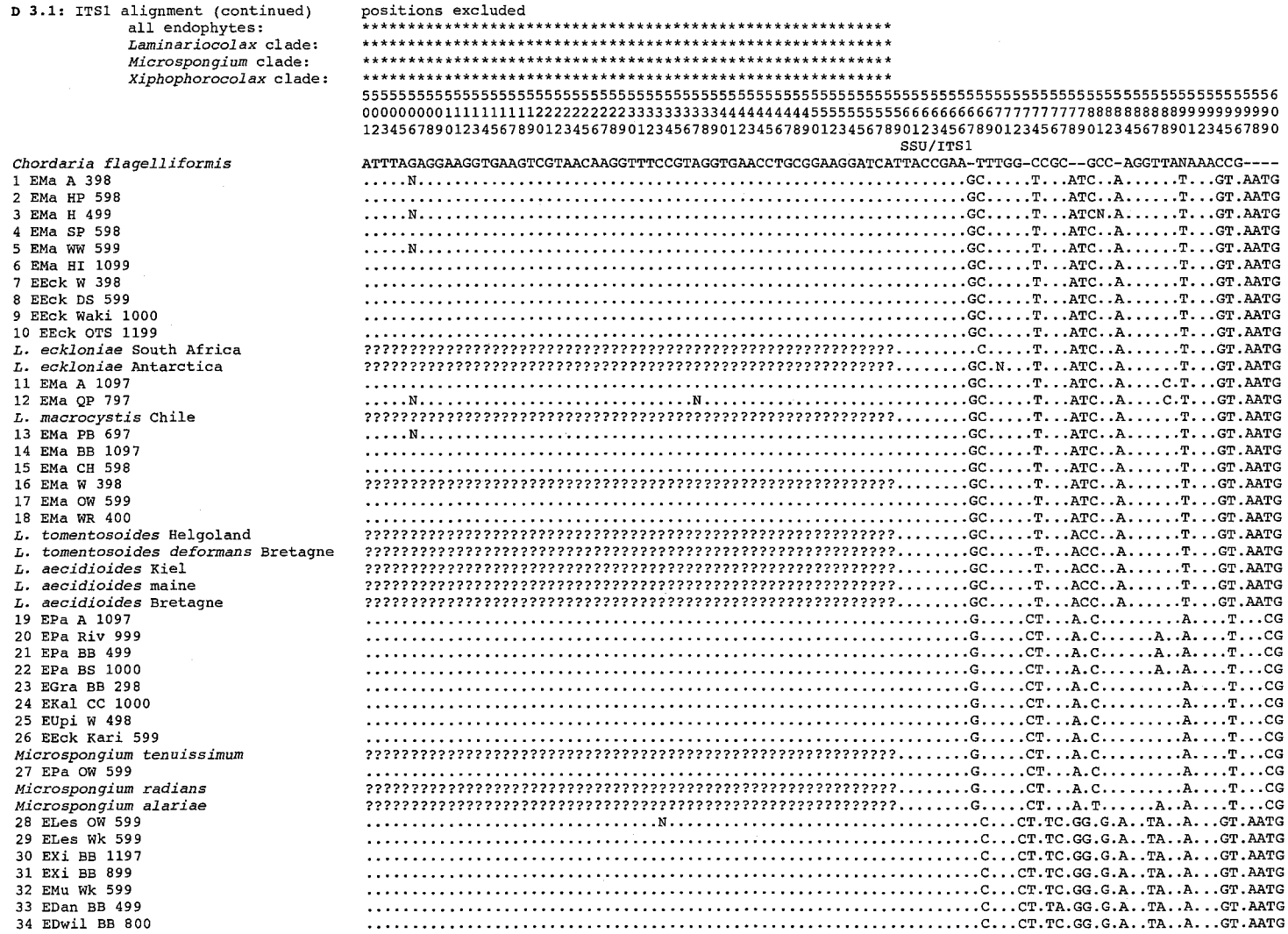
```
D 3.1: ITS1 alignment (continued)         positions excluded
         all endophytes:                  ****************************************************************************************************
         Laminariocolax clade:            ****************************************************************************************************
         Microspongium clade:             ****************************************************************************************************
         Xiphophorocolax clade:           ****************************************************************************************************
                                          3333333333333333333333333333333333333333333333333333333333333333333333333333333333333333333333333334
                                          0000000001111111111222222222233333333334444444444555555555566666666667777777777888888888899999999990
                                          1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890

Chordaria flagelliformis                  AGGGATAGATCATTGCAATTATTGATCTTGAACGAGGAATTCCTAGTAAACGCGAGTCATCAGCTCGCATTGATTACGTCCCTGCCCTTTGTACACACC
1 EMa A 398                               ....................................................................................................
2 EMa HP 598                              ....................................................................................................
3 EMa H 499                               ....................................................................................................
4 EMa SP 598                              ....................................................................................................
5 EMa WW 599                              ....................................................................................................
6 EMa HI 1099                             ....................................................................................................
7 EEck W 398                              ....................................................................................................
8 EEck DS 599                             ....................................................................................................
9 EEck Waki 1000                          ....................................................................................................
10 EEck OTS 1199                          ....................................................................................................
L. eckloniae South Africa                 ????????????????????????????????????????????????????????????????????????????????????????????????????
L. eckloniae Antarctica                   ????????????????????????????????????????????????????????????????????????????????????????????????????
11 EMa A 1097                             ....................................................................................................
12 EMa QP 797                             ....................................................................................................
L. macrocystis Chile                      ????????????????????????????????????????????????????????????????????????????????????????????????????
13 EMa PB 697                             ....................................................................................................
14 EMa BB 1097                            ....................................................................................................
15 EMa CH 598                             ....................................................................................................
16 EMa W 398                              ????????????????????????????????????????????????????????????????????????????????????????????????????
17 EMa OW 599                             ....................................................................................................
18 EMa WR 400                             ....................................................................................................
L. tomentosoides Helgoland                ????????????????????????????????????????????????????????????????????????????????????????????????????
L. tomentosoides deformans Bretagne       ????????????????????????????????????????????????????????????????????????????????????????????????????
L. aecidioides Kiel                       ????????????????????????????????????????????????????????????????????????????????????????????????????
L. aecidioides Maine                      ????????????????????????????????????????????????????????????????????????????????????????????????????
L. aecidioides Bretagne                   ????????????????????????????????????????????????????????????????????????????????????????????????????
19 EPa A 1097                             ....................................................................................................
20 EPa Riv 999                            ....................................................................................................
21 EPa BB 499                             ....................................................................................................
22 EPa BS 1000                            ....................................................................................................
23 EGra BB 298                            ....................................................................................................
24 EKal CC 1000                           ....................................................................................................
25 EUpi W 498                             ....................................................................................................
26 EEck Kari 599                          ....................................................................................................
Microspongium tenuissimum                 ????????????????????????????????????????????????????????????????????????????????????????????????????
27 EPa OW 599                             ....................................................................................................
Microspongium radians                     ????????????????????????????????????????????????????????????????????????????????????????????????????
Microspongium alariae                     ????????????????????????????????????????????????????????????????????????????????????????????????????
28 ELes OW 599                            ....................................................................................................
29 ELes Wk 599                            ....................................................................................................
30 EXi BB 1197                            ....................................................................................................
31 EXi BB 899                             ....................................................................................................
32 EMu Wk 599                             ....................................................................................................
33 EDan BB 499                            ....................................................................................................
34 EDwil BB 800                           ....................................................................................................
```

D 3.1: ITS1 of the nrDNA gene (18S-5.8S) of the pigmented endophytes (continued).

D 3.1: ITS1 alignment (continued)	positions excluded
all endophytes:	**
Laminariocolax clade:	**
Microspongium clade:	**
Xiphophorocolax clade:	**
	4444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444445
	0000000001111111111222222222233333333334444444444555555555566666666667777777777888888888899999999990
	1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
Chordaria flagelliformis	GCCCGTCGCACCTACCGATTGAATCATTCGGTGAGGATTCCGGATTCTGTAGCTTACGCTTTACGGCGTTTTTACGAAAGAAGTTATCCAAACCTCATG
1 EMa A 398	....C..TAT........C.........C.G.T........................
2 EMa HP 598	....C..TAT........C.........C.G.T........................
3 EMa H 499	....C..TAT........C.........C.G.T........................
4 EMa SP 598	......N..C..TAT........C.........C.G.T........................
5 EMa WW 599	........................N......................C..TAT........C.........C.G.T....................N...
6 EMa HI 1099	....C..TAT........C.........C.G.T........................
7 EEck W 398	....C..TAT........C.........C.G.T........................
8 EEck DS 599	....C..TAT........C.........C.G.T........................
9 EEck Waki 1000	....C..TAT........C.........C.G.T........................
10 EEck OTS 1199	....C..TAT........C.........C.G.T........................
L. eckloniae South Africa	??
L. eckloniae Antarctica	??
11 EMa A 1097	....C..TAT........C.........C.G.T........................
12 EMa QP 797	....C..TAT........C.........C.G.T........................
L. macrocystis Chile	??
13 EMa PB 697	.N...C..TAT........C.........C.G.T........................
14 EMa BB 1097	....C..TAT........C.........C.G.T........................
15 EMa CH 598	....C..TAT........C.........C.G.T........................
16 EMa W 398	??
17 EMa OW 599	....C..TAT........C.........C.G.T........................
18 EMa WR 400	....C..TAT........C.........C.G.T........................
L. tomentosoides Helgoland	??
L. tomentosoides deformans Bretagne	??
L. aecidioides Kiel	??
L. aecidioides Maine	??
L. aecidioides Bretagne	??
19 EPa A 1097	....TAT........C.........C.G..........................
20 EPa Riv 999	....TAT........C.........C.G..........................
21 EPa BB 499	....TAT........C.........C.G..........................
22 EPa BS 1000	....TAT........C.........C.G..........................
23 EGra BB 298	....TAT........C.........C.G..........................
24 EKal CC 1000	....TAT........C.........C.G..........................
25 EUpi W 498	....TAT........C.........C.G..........................
26 EEck Kari 599	....TAT........C.........C.G..........................
Microspongium tenuissimum	??
27 EPa OW 599	....TAT........C.........C.G..........................
Microspongium radians	??
Microspongium alariae	??
28 ELes OW 599	........................G......................C.............C.............T.......................C
29 ELes Wk 599	........................G......................C.............C.............T.......................C
30 EXi BB 1197	........................G......................C.............C.............T.......................C
31 EXi BB 899	........................G......................C.............C.............T.......................C
32 EMu Wk 599	........................G......................C.............C.............T.......................C
33 EDan BB 499	........................G......................C.............C.............T.......................C
34 EDwil BB 800	........................G......................C.............C.............T.......................C

D 3.1: ITS1 of the nrDNA gene (18S-5.8S) of the pigmented endophytes (continued).



```
D 3.1: ITS1 alignment (continued)      positions excluded
           all endophytes:                                                         ***********************************************
           Laminariocolax clade:
           Microspongium clade:                                                                                             *********
           Xiphophorocolax clade:
                                       666666666666666666666666666666666666666666666666666666666666666666666666666666666666666666667
                                       000000000111111111122222222223333333333444444444455555555556666666666777777777788888888889999999990
                                       123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
                                                                                                                            /L'colax2(F)
Chordaria flagelliformis               -----CGAACAACAA---A--GCGCCCCG--CC-----G-CC---------CCCCGTTTTCTT-GT-CT---------CTTTTT----ATAGAGACAGAC
1 EMa A 398                            TGTTAG...A.G.G.....CT..CAG.T....GTTT...T...GCTATT......C......C.C.........GGAG.......TCTGGC..AGGTAGT
2 EMa HP 598                           TGTTAG...A.G.G.....CT..CAG.T....GTTT...T...GCTATT..-...C......C.C.........GGAG.......NCTGGC..AGGTAGT
3 EMa H 499                            TGTTAG...A.G.G.....CT..CAG.T....GTTT...T...GCTATT..-...C......C.C.........GGAG.......NNTGGC..AGGTANN
4 EMa SP 598                           TGTTAG...A.G.G.....CT..CAG.T....GTTT...T...GCTATT......C......C.C.........GGAG.......TCTGGC..AGGTAGT
5 EMa WW 599                           TGTTAG...A.G.G.....CN..CAG.T....GTTT...T...GCTATT......C......C.C.........GGAN.......NCTGGC..NGGTAGT
6 EMa HI 1099                          TGTTAG...A.G.G.....CT..CAG.T....GTTT...TN.CGCTATT......C......C.C.........GGAG.......TCTGGC..AGGTAGT
7 EEck W 398                           TGTTAG...A.G.G.....CT..CAG.T....GTTT...T..CGCTATT......C......C.C.........GGAG.......TCTGGC..AGGTAGT
8 EEck DS 599                          TGTTAG...A.G.G.....CT..CAG.T....GTTT...T...GCTATTT.....C......C.C.........GTAG.......TCTGGC..AGGTAGT
9 EEck Waki 1000                       TGTTAG...A.G.G.....CT..CAG.T....GTTT...T...GCTATTTT....C......C.C.........GGAG.......TCTGGC..AGGTNGT
10 EEck OTS 1199                       TGTTAG...A.G.G.....CT..CAG.T....GTTT...T...GCTATTT.-...C......C.C.........GTAG.......TCTGGC..AGGTAGT
L. ecklonige South Africa              CGTTAG...A.-.G.....CT..CAG.T....GTTT...T...GCTATT......C......C.C.........GGAG..G....TCTGGC..AGGTAGT
L. ecklonige Antarctica                TGTTAG...A.G.G.....CT..CAG.T....GTTT...T...GCTATT......C......C.C.........GGAG.......TCTGGC..AGGTAGT
11 EMa A 1097                          TGTTAG...A.G.G.....CT..CAG.T....GTTT...T...GCTATT......C.....CC.C.........GGAG........CTGGC..AGGTAGT
12 EMa QP 797                          TGTTAN...A.G.G.....CT..CAG.T....GTTT...N...GCTATT......C....NCC.C.........GGAG......TTCTGGC..AGGTAGT
L. macrocystis Chile                   CGTTAG...A.-.G.....CT..CAG.T....GTTT...T...GCTATT......C.....CC.C.........GGAG.......TCTGGC..AGGTAGT
13 EMa PB 697                          TGTTAGA..A-G.G.....CT.NCNG.T....GTTT...T...GCTATTT.-...C......C.C.........GGAG.......TCTNGC..AGGNAGT
14 EMa BB 1097                         TGTTAG...A.G.G.....CT..CAG.T....GTTT...T...GCTATT......CC.....C.C.........GGAG.......NCTGGC..AGGTAGT
15 EMa CH 598                          TGTTAG...A.G.G.....CT..CAG.T....GTTT...T..CGCTATT......CC.....C.C.........GGAG.......TCTGGC..AGGTAGT
16 EMa W 398                           TGTTAG...A.G.G.....CT..CAG.T....GTTT...T...GCTATT......CC.....C.C.........GGAG.......TCTGGC..AGGTAGT
17 EMa OW 599                          TGTTAG...A.G.G.....CT..CAG.T....GTTT...T..CGCTATT......CC.....C.C.........GGAG.......TCTGGC..AGGTAGT
18 EMa WR 400                          TGTTAG...A.G.G.....CT..CAG.T....GTTT...T...GCTAT.......CC.....C.C.........GGAG.......NCTGGC..AGGTAGT
L. tomentosoides Helgoland             CGTTAG...A.G.......CT..CAG.T....GTTT..AT...GCTATT......C......C.C...C.....TATG........CTGG...A.GGAGT
L. tomentosoides deformans Bretagne    CGTTAG...A.G.......CT..CAG.T....GTTT..AT...GCTATT......C......C.C...C.....TATG........CTGG...A.GGAGT
L. aecidioides Kiel                    CGTTAG...A.G.......CT..CAG.T....GTTT...T...GCTATT.C....C......C.C.C..CCTCCTATG........CTGGGT.A.GGAGT
L. aecidioides Maine                   CGTTAG...A.G.......CT..CAG.T....GTTT...T...GCTATT.C....C......C.C.C..CCTCCTATG........CTGGGT.A.GGAGT
L. aecidioides Bretagne                CGTTAG...A.G.......CT..CAG.T....GTTT...T...GCTATT.C....C......C.C.C..CCTCCTATG........CTGGGT.A.GGAGT
19 EPa A 1097                          CG...AA.GA.T.--...TCT..C........GTTCA..G..TCCGTT..TAT.G.--GG.ACGT.TGGGGCGGA...-----GCGTTCG.---------
20 EPa Riv 999                         CG...AA.GA.T.--...TTT..C........GTTCA..G..TCCGTT..CAT.G.--GG.ACGT.TGGGGCGGA...-----GCGTTCG.---------
21 EPa BB 499                          CG...AA.GA.T.--...TTT..C........GTTCA..G..TCCGTT..CAT.G.--GG.ACGT.TGGGGCGGA...-----GCGTTCG.---------
22 EPa BS 1000                         CG...AA.GA.T.--...TTT..C........GTTCA..G..TCCGTT..CAT.G.--GG.ACGT.TGGGGCGGA...-----GCGTTCG.---------
23 EGra BB 298                         CG...AA.GA.T.--...TCT..C........GTTCA..G..TCCGTT..TAT.G.--GG.ACGT.TGGGGCGGA...-----GCGTTCG.---------
24 EKal CC 1000                        CG...AA.GA.T.--...TCT..C........GTTCA..G..TCCGTT..CAT.G.--GG.ACGT.TGGGGCGGA...-----GCGTTCG.---------
25 EUpi W 498                          CG...AA.GA.T.--...TCT..C........GTTCA..G..TCCGTT..TAT.G.--GG.ACGT.TGGGGCGGA...-----GCGTTCG.---------
26 EEck Kari 599                       CG...AA.GA.T.--...TCT..C........GTTCA..G..TCCGTT..CAT.G.--GG.ACGT.TGGGGCGGA...-----GCGTTCG.---------
Microspongium tenuissimum              CG...AA.GA.T.--...TCT..C........GTTCA..G..TCCGTT..CAT.G.--GG.ACGT.TGGGGCGGA...------CGTTCG.---------
27 EPa OW 599                          CG...AA.GA.T.--...TCT..C..--..-GTT...-.--.........------------.--.GGGTCGGA...-----GCGTTCG.---------
Microspongium radians                  CG...AA.GA.T.--...TCT..C..--..-GTT...-.--.........------------.--.-GGTCGGA...-----GCGTTCG.---------
Microspongium alariae                  CG...AA.GA.T.--...TCT..C........GTTCAT.C.ATGCCTTGCC.T.G.CGGCGGCCCCTGGCGGGAGGGGCGGAGTGTTTG.---------
28 ELes OW 599                         TTTTGT..TT.T.TCGGG.CT.AAG..T.G..GTTTTTTT.GAGTTTCTGGAGT.TGGAAGGAC..GTGATGTCTACTAC....CGTCGCTCC.C..CT.
29 ELes Wk 599                         TTTTGT..TT.T.TCGGG.CT.AAG..T.G..GTTTTTTT.GAGTTTCTGGAGT.TGGAAGGAC..GTGATGTCTACTAC....CGTCGCTCC.C..CT.
30 EXi BB 1197                         TTTTGT..TT.G.TCGGG.CT.AAG..T.G..GTTTTTTT.GAGTTTCTGGAGT.TGGAAGGAC..GTGATGTCTACTAC....CCTCGCTCC.C..CT.
31 EXi BB 899                          TTTTGT..TT.G.TCGGG.CT.AAG..T.G..GTTTTTTT.GAGTTTCTGGAGT.TGGAAGGAC..GTGATGTCTACTAC....CCTCGCTCC.C..CT.
32 EMu Wk 599                          TTTTGT..TT.T.TCGGG.CT.AAG..T.G..GTTTTTTT.GAGTTTCTGGAGT.TGGAAGGAC..GTGATGTCTACTAC....CCTCGCTCC.C..CT.
33 EDan BB 499                         TTTTGT..TT.T.TCGGG.CT.AAG..T.GG.GTTTTTTT.GAGTTTCTGGAGT.TGGAAGGAC..GTGATGTCTACTAC....CGTCGCTCC.C..CT.
34 EDwil BB 800                        TTTTGT..TT.G.TCGGG.CT.A------..--.....-.--.........-GT.TGGAAGGAC..GTGATGTCTACTAC....CCTCGCTCC.C..CT.
```

D 3.1: ITS1 of the nrDNA gene (18S-5.8S) of the pigmented endophytes (continued).

D 3.1: ITS1 alignment (continued)

```
                positions excluded
                ****************************************************************************************************
all endophytes:
Laminariocolax clade:
Microspongium clade:
Xiphophorocolax clade:

                777777777777777777777777777777777777777777777777777777777777777777777777777777777777777777777778
                000000000111111111122222222223333333334444444445555555556666666667777777778888888888999999999990
                123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890123456789
                   L'colax2(F)    /
Chordaria flagelliformis        GAACGAGAGGCAGCATGGTGCG----------------------------------------------------------------------------------
1 EMa A 398                     A.-..TCTTA......--...-..GCTGTGCGATCACC.GTCGGTCT..CGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CAAC.
2 EMa HP 598                    A.-..TCTTA......--...-..GCTGTGCGATCACC.GTCGGTCT..CGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CAAC.
3 EMa H 499                     N.-..TCTTA......--...-..GCTGTGCGATCACC.GTCGGTCT..CGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CAAC.
4 EMa SP 598                    A.-..TCTTA......--...-..GCTGTGCGATCACC.GTCGGTCT..CGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CAAC.
5 EMa WW 599                    A.-..TCTTA......--..N-..GCTGTGCGATCACC.GTCGGTCT..CGCGACCTGACCGTGTTCTCTTTGACGAGA.AAC............CAAC.
6 EMa HI 1099                   A.-..TCTTN......--...-..GCTGTGNGATCACC.GTCGGGCT..NGCGACCTGNCTGNGTTCTNTTNNANNANA.ANC............CNAC.
7 EEck W 398                    A.-..TCTTA......--...-..GCTGTGCGATCACC.GTCGGTCT..CGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CACC.
8 EEck DS 599                   A.-..TCTTA......--...-..GCTGTGCGATCACC.GTCGGTCT..CGCGACCTGACTG??????????????????????????????????????????
9 EEck Waki 1000                N.-..TCTTA......--...-..GCTGTGCGATCACC.GTCGGTCT..CGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CAAC.
10 EEck OTS 1199                A.-..TCTTA......--...-..GCTGTGCGATCACC.GTCGGTCT..CGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CAAC.
L. eckloniae South Africa       A.-..TCTTA......--...-..GCTGTGCGATCACC.GACGGTCT..CGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CAACG
L. eckloniae Antarctica         A.-..TCTTA......--...-..GCTGTGCGATCACC.GTCGGTCT..CGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CAACG
11 EMa A 1097                   A.-..TCTTA......--....C.GCTGTNCGATCACC.GTCGGGAT..CGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CAACG
12 EMa QP 797                   A.-..TCTTA......--....CCGCTGTGCGANCACCCGTCGGGAT..CGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CAACG
L. macrocystis Chile            A.-..TCTTA......--...C..GCTGTGCGATCACC.GTCGGGAT..CGCGACCTGACTGTGTTCTCTTCGACGAGA.AAC............CAACG
13 EMa PB 697                   NN-..TCNTA......--...N--..GCTGTGCGA.AACC.GGCGGTCT..TGCGACCTGGCTGTGTTC.CTTTG.C.AGAAAAC............CAAC?
14 EMa BB 1097                  A.-..TCTTA......--...-..GCTGTGCGATCACC.GTCGGTCTCTCGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CAACG
15 EMa CH 598                   A.N..TCTTA......--...-..GCTGTGCGATCACC.GTCGGTCTCTCGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CAACG
16 EMa W 398                    A.-..TCTTA......--...-..GCTGTGCGATCACC.GTCGGTCTCTCGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CAACG
17 EMa OW 599                   A.C..TCTTA......--...-..GCTGTGCGATCACC.GTCGGTCTCTCGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CAACG
18 EMa WR 400                   A.-..TCTTA......--...-..GCTGTGCGATCACC.GTCGGTCTCTCGCGACCTGACTGTGTTCTCTTTGACGAGA.AAC............CAACG
L. tomentosoides Helgoland      C.-..TCTTA......-C..-..GCTGTGCGATCACC.GTCGGTCT..CGCGACCTGACTGTGTTCCCTTTGACAAGA.AACTTGACTAGAATCCGATG
L. tomentosoides deformans Bretagne C.-..TCTTA.....--C..-..GCTGTGC.ATCACC.GTCGGTCT..CGCGACCTGACTGTGTTCCCTTTGACAAGA.AACTTGACTAGAATCCGATG
L. aecidioides Kiel             C.-..TCTTA....G.--...-..GCTGTGCGATCACC.GTCGGTCT..CGCGACCTGACTGTGTTCCCTTTGACAAGA.AACTTGACTAGAATCCGACG
L. aecidioides Maine            C.-..TCTTA....G.--...-..GCTGTGCGATCACC.GTCGGTCT..CGCGACCTGACTGTGTTCCCTTTGACAAGA.AACTTGACTAGAATCCGACG
L. aecidioides Bretagne         C.-..TCTTA....G.--...-..GCTGTGCGATCACC.GTCGGTCT..CGCGACCTGACTGTGTTCCCTTTGACAAGA.AACTTGACTAGAATCCGACG
19 EPa A 1097                   ----------------------.....................................................................................
20 EPa Riv 999                  ----------------------.....................................................................................
21 EPa BB 499                   ----------------------.....................................................................................
22 EPa BS 1000                  ----------------------.....................................................................................
23 EGra BB 298                  ----------------------.....................................................................................
24 EKal CC 1000                 ----------------------.....................................................................................
25 EUpi W 498                   ----------------------.....................................................................................
26 EEck Kari 599                ----------------------.....................................................................................
Microspongium tenuissimum      ----------------------.....................................................................................
27 EPa OW 599                   ----------------------.....................................................................................
Microspongium radians           ----------------------.....................................................................................
Microspongium alariae           ----------------------.....................................................................................
28 ELes OW 599                  C.T.CGC....C.TTCTCC.G.GGCCTCTAACCCTCCGACTTCTTTACNGAAGTCCCGGGGGCGAGCCCGGACCAAGAATCTGATCTCCATACGTTGCGA
29 ELes Wk 599                   C.T.CGC....C.TTCTCC.G.GGCCTCTAACCCTCCGACTTCTTTACCGAAGTCCCGGGGGCGAGCCCGGACCAAGAATCTGATCTCCATACGTTGCGA
30 EXi BB 1197                   C.T.CGC....C.TTCTCC.G.GGCCTCTAACC.TNCGACTTCTTTTACC????????????????????????????????????????????????????
31 EXi BB 899                   C.T.CGC....C.TTCTCC.G.GGCCTCTAACC.TCCGACTTCTTTACCGAAGTCCCGGGGGCGAGCCCGGACCAAGAATCTGATCTCCATACGTTGCGA
32 EMu Wk 599                   C.T.CGC....C.TTCTCC.G.GGCCTCTAACCCTCCGACTTCTTTACCGAAGTCCCGGGGGCGAGCCCGGACCAAGAATCTGATCTCCATACGTTGCGA
33 EDan BB 499                  C.T.CGC....C.TTCTCC.G.GGCCTCTAACCCTCCGACTTCTTTACCGAAGTCCCGGGGGCGAGCCCGGACCAAGAATCTGATCTCCATACGTTGCGA
34 EDwil BB 800                 C.T.CGC....C.TTCTCC.G.GGCCTCTAACC.TCCGACTTCTTTACCGAAGTCCCGGGGGCGAGCCCGGACCAAGAATCTGATCTCCATACGTTGCGA
```

D 3.1: ITS1 of the nrDNA gene (18S-5.8S) of the pigmented endophytes (continued).

D 3.1: ITS1 alignment (continued)

```
                                positions excluded
                                ********************************************************************************************************************
                                all endophytes:
                                Laminariocolax clade:
                                Microspongium clade:    ********************************************************************************************************************
                                Xiphophorocolax clade:
                                88888888888888888888888888888888888888888888888888888888888888888888888888888888888888888888888888888888888889
                                00000000011111111112222222222333333333344444444455555555556666666667777777777888888888999999999990
                                12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
Chordaria flagelliformis        ----------------------------------------------------------------------------------------------------------------------
1 EMa A 398                     ......................................................................................................................
2 EMa HP 598                    ......................................................................................................................
3 EMa H 499                     ......................................................................................................................
4 EMa SP 598                    ......................................................................................................................
5 EMa WW 599                    ......................................................................................................................
6 EMa HI 1099                   ......................................................................................................................
7 EEck W 398                    ......................................................................................................................
8 EEck DS 599                   ??????????????????????????????????????????????????????????????????????????????????????????????????????????????
9 EEck Waki 1000                ......................................................................................................................
10 EEck OTS 1199                ......................................................................................................................
L. eckloniae South Africa        CTAGCCGTGT.CATGTTCAACGGGCCCTTTCATTCTTTACGCAGGTACGGCGGTCCCGTCTTCGGTGGCGGGTGGCCAGAGCACCGATT...GGTGTACG
L. eckloniae Antarctica         CTAGCCGNNNNNNNNNNCAACGGGCCCTTTCATTCTTTACGCAGGTACAGCGGTCCCGTCTTGGTTGGCGGGTGGCCAGAGCACCGATTCGCGGTGTACG
11 EMa A 1097                    CTAGCCGTGT.CATGTTCAACGGGCCC.....................................................................................
12 EMa QP 797                   CTAGCCGTGT.CATGTTCAACGGGCCC.....................................................................................
L. macrocystis Chile            CTAGCCGTGT.CATGTTCAACGGGCCC.....................................................................................
13 EMa PB 697                   ??????????????????????????????????????????????????????????????????????????????????????????????????????????????
14 EMa BB 1097                  CTAGCCGTGT......................................................................................................
15 EMa CH 598                   CTAGCCGTGT......................................................................................................
16 EMa W 398                    CTAGCCGTGT......................................................................................................
17 EMa OW 599                   CTAGCCGTGT......................................................................................................
18 EMa WR 400                   CTAGCCGTGG......................................................................................................
L. tomentosoides Helgoland      CTAGCCGTGTTTATGTTCAACGGGCCCTTTCTTTCTTTACGCAGGTAC.....................................GTACG
L. tomentosoides deformans Bretagne CTAGCCGTGTTTATGTTCAACGGGCCCTTTCTTTCTTTACGCAGGTAC.....................................GTACG
L. aecidioides Kiel              CTAGCCGTG.......................................................................................................
L. aecidioides Maine            CTAGCCGTG.......................................................................................................
L. aecidioides Bretagne         CTAGCCGTG.......................................................................................................
19 EPa A 1097                   ......................................................................................................................
20 EPa Riv 999                  ......................................................................................................................
21 EPa BB 499                   ......................................................................................................................
22 EPa BS 1000                  ......................................................................................................................
23 EGra BB 298                  ......................................................................................................................
24 EKal CC 1000                 ......................................................................................................................
25 EUpi W 498                   ......................................................................................................................
26 EEck Kari 599                ......................................................................................................................
Microspongium tenuissimum      ......................................................................................................................
27 EPa OW 599                   ......................................................................................................................
Microspongium radians          ......................................................................................................................
Microspongium alariae          ......................................................................................................................
28 ELes OW 599                  CGCCGACTTCTTCACCNAAGGCGGCGGGGCGCGTTCGGAGCAGGCCTGAGACGGACCGAGTGGGCGTTTGCGACGGCCGAGTAGAGCTCTGCGGCAGGGG
29 ELes Wk 599                   CGCCGACTTCTTCACCGAAGGCGGCGGGGCGCGTTCGGAGCAGGCCTGAGACGGACCGAGTGGGCGTTTGCGACGGCCGAGTAGAGCTCTGCGGCAGGGG
30 EXi BB 1197                   ??????????????????????????????????????????????????????????????????????????????????????????????????????????????
31 EXi BB 899                   CGCCGACTTCTTCACCGAAGGCGGCGGGGCGCGTTCGGAGCAGGCCTGAGACGGACCGAGTGGGCGTTTGCGACGGCCGAGTAGAGCTCTGCGGCAGGGG
32 EMu Wk 599                   CGCCGACTTCTTCACCGAAGGCGGCGGGGCGCGTTCGGAGCAGGCCTGAGACGGACCGAGTGGGCGTTTGCGACGGCCGAGTAGAGCTCTGCGGCAGGGG
33 EDan BB 499                  CGCCGACTTCTTCACCGAAGGCGGCGGGGCGCGTTCGGAGCAGGCCTGAGACGGACCGAGTGGGCGTTTGCGACGGCCGAGTAGAGCTCTGCGGCAGGGG
34 EDwil BB 800                 CGCCGACTTCTTCACCGAAGGCGGCGGGGCGCGTTCGGAGCAGGCCTGAGACGGACCGAGTGGGCGTTTGCGACGGCCGAGTAGAGCTCTGCGGCAGGGG
```

D 3.1: ITS1 alignment (continued)

```
                                positions excluded
                                **************************************************************************************************
                 all endophytes:
                 Laminariocolax clade:
                 Microspongium clade:  ******************************************************************************************************
                 Xiphophorocolax clade:                                                                 ***************************************
                                9999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999990
                                0000000001111111111222222222233333333334444444445555555556666666667777777778888888889999999999990
                                1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
                                                                                                        / L'colax2 (F)     /
Chordaria flagelliformis        ----------------------------------------------------------------------------------------------------------
1 EMa A 398                     ................................GGCGTGCATCTGCTAGGCAGG..TACTAAGG...AAGTAACGTCTTACAGTATTGTACTGTGCGATC
2 EMa HP 598                    ................................GGCGTGCATCTGCTAGGCAGG..TACTAAGG...AAGTAACGTCTTACAGTATTGTACTGTGCGATC
3 EMa H 499                     ................................GGCGTGCATCTGCTAGGCAGG..TACTAAGG...AAGTAACGTCTTACAGTATTGTACTGTGCGATC
4 EMa SP 598                    ................................GGCGTGCATCTGCTAGGCAGG..TACTAAGG...AAGTAACGTCTTACAGTATTGTACTGTGCGATC
5 EMa WW 599                    ................................GGCGTGCGTCTGCTAGGCAGG..TACTAAGG...AAGTAACGTCTTACAGTATTGTACTGTGCGATC
6 EMa HI 1099                   ................................NGNGTGCATNTGNTAGGCAGG..TACTAAGG...AAGTAANGTCTTNCAGTATTGTCCNGNGCNNTC
7 EEck W 398                    ................................GGCGTGCATCTGCTAGGCAGG..TACTAAGG...AAGTAACGTCTTACAGTATTGTACTGTGCGATC
8 EEck DS 599                   ????????????????????????????????????????????????????????????????????????????????????????????????
9 EEck Waki 1000                ................................GGCGTGCATCTGCTAGGCAGG..TACTAAGGAGGAAGTAACGTCTTACAGTATTGTACTGTGCGATC
10 EEck OTS 1199                ................................GGCGTGCATCTGCTAGGCAGG..TACTAAGG...AAGTAACGTCTTACAGTATTGTACTGTGCGATC
L. eckloniae South Africa       ..TACCTGTTATATCAGCAGAAAGGGAACGGAGGGCGTGCATCTGCTAGGCAGG..TACTAAGG...AAGTAACGTCTTACAGTATTGTACTGTGCGATC
L. eckloniae Antarctica         ..TACNTGTTATATCAGCAGAAAGGGAACGGAGGGCGTGCATCTGCTAGGCAGG..TACTAAGG...AAGTAACGTCTTACAGTATTGTACTGTGCGATC
11 EMa A 1097                   ................................................................................................................
12 EMa QP 797                   ................................................................................................................
L. macrocystis Chile            ................................................................................................................
13 EMa PB 697                   ????????????????????????????????????????????????????????????????????????????????????????????????
14 EMa BB 1097                  ................................................................................................................
15 EMa CH 598                   ................................................................................................................
16 EMa W 398                    ................................................................................................................
17 EMa OW 599                   ................................................................................................................
18 EMa WR 400                   ................................................................................................................
L. tomentosoides Helgoland      CGTACCTG.TATATCAGAAGAAAGGGAACGGAGGGCGTACATCTGCTAGGCAGGTATACTAAGG...AAGTAACGTCTTACAGTATTGTGCTGTGCGATC
L. tomentosoides deformans Bretagne CGTACCTGCTATATCAGAAGAAAGGGAACGGAGGGCGTACATCTGCTAGGCAGGTATACTAAGG...AAGTAACGTCTTACAGTATTGTGCTGTGCGATC
L. aecidioides Kiel             ..................................CATCTGCTAGGCAGG..TACTGAGG...AAGTAACGTCTTACAGCATTGTGCTGTGCGATC
L. aecidioides Maine            ..................................CATCTGCTAGGCAGG..TACTGAGG...AAGTAACGTCTTACAGCATTGTGCTGTGCGATC
L. aecidioides Bretagne         ..................................CATCTGCTAGGCAGG..TACTGAGG...AAGTAACGTCTTACAGCATTGTGCTGTGCGATC
19 EPa A 1097                   ................................................................................................................
20 EPa Riv 999                  ................................................................................................................
21 EPa BB 499                   ................................................................................................................
22 EPa BS 1000                  ................................................................................................................
23 EGra BB 298                  ................................................................................................................
24 EKal CC 1000                 ................................................................................................................
25 EUpi W 498                   ................................................................................................................
26 EEck Kari 599                ................................................................................................................
Microspongium tenuissimum      ................................................................................................................
27 EPa OW 599                   ................................................................................................................
Microspongium radians          ................................................................................................................
Microspongium alariae          ................................................................................................................
28 ELes OW 599                  ATCTCCAGAAATATCATTCGTCCAGGACGGTTGAACTACCCCGTTGGGTTACCCGAGTGTTT................................................
29 ELes Wk 599                  ATCTCCAGAAATATCATTCGTCCAGGACGGTTGAACTACCCCGTTGGGTTACCCGAGTGTTT................................................
30 EXi BB 1197                  ????????????????????????????????????????????????????????????????????????????????????????????????
31 EXi BB 899                   ATCTCCAGAAATATCATTCGTCCAGGACGGTTGAACTACCCCGTTGGGTTACCCGAGTGTTT................................................
32 EMu Wk 599                   ATCTCCAGAAATATCATTCGTCCAGGACGGTTGAACTACCCCGTTGGGTTACCCGAGTGTTT................................................
33 EDan BB 499                  ATCTCCAGAAATATCATTCGTCCAGGACGGTTGAACTACCCCGTTGGGTTACCCGAGTGTTT................................................
34 EDwil BB 800                 ATCTCCAGAAATATCATTCGTCCAGGACGGTTGAACTACCCCGTTGGGTTACCCGAGTGTTT................................................
```

D 3.1: ITS1 of the ntDNA gene (18S-5.8S) of the pigmented endophytes (continued).

D 3.1: ITS1 of the nrDNA gene (18S-5.8S) of the pigmented endophytes (continued).

D 3.1: ITS1 alignment (continued)

```
                                positions excluded
                                **********************************************************************************************************
all endophytes:                 **********************************************************************************************************
Laminariocolax clade:           
Microspongium clade:            **********************************************************************************************************
Xiphophorocolax clade:          **********************************************************************************************************
                                11111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111
                                00000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000001
                                00000000011111111112222222222333333333344444444455555555566666666677777777778888888889999999990
                                12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890

Chordaria flagelliformis        ----------------------------------------------------------------------------------------------------------
1 EMa A 398                     GATCACGGCG.CGGAGTCTC..CTTC.....AGCGGTTGGTTGCGTGGGGGTCGTTGATCGTTAGGATCGACTGCCCCAGCGTCTCCCTCGGCTGATCGA
2 EMa HP 598                    GATCACGGCG.CGGAGTCTC..CTTC.....AGCGGTTGGTTGCGTGGGGGTCGTTGATCGTTAGGATCGACTGCCCCAGCGTCTCCCTCGGCTGATCGA
3 EMa H 499                     GATCACGGCG.CGGAGTCTC..CTTC.....AGCGGTTGGTTGCGTGGGGGTCGTTGATCGTTAGGATCGACTGCCCCAGCGTCTCCCTCGGCTGATCGA
4 EMa SP 598                    GATCACGGCG.CGGAGTCTC..CTTC.....AGCGGTTGGTTGCGTGGGGGTCGTTGATCGTTAGGATCGACTGCCCCAGCGTCTCCCTCGGCTGATCGA
5 EMa WW 599                    GATCACGGCG.CGGAGTCTCTCCTTC.....AGCGGTTGGTTGCGTGGGGGTCGTTGATCGTTAGGATCGACTGCCCCAGCGTCTCCCTCGGCTGATCGA
6 EMa HI 1099                   NATCNCGGCN.NGNAGTCTC..CTTC.....AGCGGTTGGTTGCGTGNNGNTCGNTGATCGNTAGGATTGCCTGCNNCAGCGTCTCCCTCGNGTGATCNA
7 EEck W 398                    GACCACGGCG.CGGAGTCTC..CTTC.....AGCGGTTGGTTGCGTGGGGGTCGTTGATCGTTAGGATCGACTGCCCCAGCGTCTCCCTCGGCTGATCGA
8 EEck DS 599                   ??????????????????????????????????????????????????????????????????????????????????????????????????????
9 EEck Waki 1000                GATCACGGCG.CGGAGTCTCTCCTTC.....AGCGGTTGGTTGCGTGGGGGTCGTTGATCGTTAGGATCGACTGCCCCAGCGTCTCCCTCGGCTGATCGA
10 EEck OTS 1199                GATCACGGCG.CGGAGTCTC..CTTC.....AGCGGTTGGTTGCGTGGGGGTCGTTGATCGTTAGGATCGACTGCCCCAGCGTCTCCCTCGGCTGATCGA
L. eckloniae South Africa       GATCACGGCG.CGGAGTCTC..CTTC.....AGCG.TTGGTTGCNTGGGNNTCGTCGATCGTTAGGATCGACTGCCCCAGCGTCTCCCTCGGCTGATCGA
L. eckloniae Antarctica         GATCACGGCG.CGGAGTCTC..CTTC.....AGCGGTTGGTTGCGTGGGGGTCGTTGATCGTTAGGATCGACTGCCCCAGCGTCTCCCTCGGCTGATCGA
11 EMa A 1097                   ........................................................................................................................
12 EMa QP 797                   ........................................................................................................................
L. macrocystis Chile            ........................................................................................................................
13 EMa PB 697                   ????????????????????????????????????????????????????????????????????????????????????????????????????????
14 EMa BB 1097                  ........................................................................................................................
15 EMa CH 598                   ........................................................................................................................
16 EMa W 398                    ........................................................................................................................
17 EMa OW 599                   ........................................................................................................................
18 EMa WR 400                   ........................................................................................................................
L. tomentosoides Helgoland      GATCACNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
L. tomentosoides deformans Bretagne GATCACGGCG.CGGAGTCT...CCTCCAGCTAGG......TAGCGTGGGGGTCGTTGATCGTTAGGATCGACTGCCCCAGCGTCTCCCTCGGCTGATTGA
L. aecidioides Kiel              GATCACGGCGACGGAGTCT...CTTCCATCCAGCGGT.CGTTGCGTGGGGGTCGTTGATCGTTAGGATCGACTGCCCCAGCGTCTCCCTCGGCTGATCGA
L. aecidioides Maine             GATCACGGCG.CGGAGTCT...CTTCCATCCAGCGGTCGGTTGCGTGGGGGTCGTTGATCGTTAGGATCGACTGCCCCAGCGTCTCCCTCGGCTGATCGA
L. aecidioides Bretagne          GATCACGGCG.CGGAGTCT...CTTCCATCCAGCGGTCGGTTGCGTGGGGGTCGTTGATCGTTAGGATCGACTGCCCCAGCGTCTCCCTCGGCTGATCGA
19 EPa A 1097                   ........................................................................................................................
20 EPa Riv 999                  ........................................................................................................................
21 EPa BB 499                   ........................................................................................................................
22 EPa BS 1000                  ........................................................................................................................
23 EGra BB 298                  ........................................................................................................................
24 EKal CC 1000                 ........................................................................................................................
25 EUpi W 498                   ........................................................................................................................
26 EEck Kari 599                ........................................................................................................................
Microspongium tenuissimum      ........................................................................................................................
27 EPa OW 599                   ........................................................................................................................
Microspongium radians          ........................................................................................................................
Microspongium alariae          ........................................................................................................................
28 ELes OW 599                  ........................................................................................................................
29 ELes Wk 599                  ........................................................................................................................
30 EXi BB 1197                  ????????????????????????????????????????????????????????????????????????????????????????????????????????
31 EXi BB 899                   ........................................................................................................................
32 EMu Wk 599                   ........................................................................................................................
33 EDan BB 499                  ........................................................................................................................
34 EDwil BB 800                 ........................................................................................................................
```

D 3.1: ITS1 of the nrDNA gene (18S-5.8S) of the pigmented endophytes (continued).

D 3.1: ITS1 alignment (continued)

```
                                positions excluded
                                **********************************************************************************************************
                all endophytes: **********************************************************************************************************
          Laminariocolax clade: 
          Microspongium clade: **********************************************************************************************************
          Xiphophorocolax clade: **********************************************************************************************************
                                11111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111
                                11111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111112
                                00000000011111111112222222222333333333344444444455555555556666666666777777777788888888899999999990
                                12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890

Chordaria flagelliformis        ------------------------------------------------------------------------------------------------------------
1 EMa A 398                     GAGACCGGGCTGTGCGCGTTCTCTTTGACGGGAAACCATCAATCTACCTGC...TA.TAGTGGCTCGGCTGGCCGCGCTCGGAAAGAAGAA.GCA.....
2 EMa HP 598                    GAGACCGGGCTGTGCGCGTTCTCTTTGACGGGAAACCATCAATCTACCTGC...TA.TAGTGGCTCGGCTGGCCGCGCTCGGAAAGAAGAA.GCA.....
3 EMa H 499                     GAGACCGGGCTGTGCGCGTTCTCTTTGACGGGAAACCATCAATCTACCTGC...TA.TAGTGGCTCGGCTGGCCGCGCTCGGAAAGAAGAA.GCA.....
4 EMa SP 598                    GAGACCGGGCTGTGCGCGTTCTCTTTGACGGGAAACCATCAATCTACCTGC...TA.TAGTGGCTCGGCTGGCCGCGCTCGGAAAGAAGAA.GCA.....
5 EMa WW 599                    GAGACCGGGCTGTGCGCGTTCTCTTTGACGGGAAACCATCAATCTACCTGC.....TAGTGGCTCGGCTGGCCGCGCTCGGAAAGAAGAA.GCA.....
6 EMa HI 1099                   NAGACCGGGCTGTGCGCGTTNTCTNTGNCNNGNANCCANCNNTCTACNNGC.....TAGTGGNTCGGCNGTCCGNGNTNNGAAACAANAA.NCA.....
7 EEck W 398                    GAGACCGGGCTGTGCGCGTTCTCTTTGACGGGAAACCATCAATCTACCTGC.....TAGTGGCTCGGCTGGCCGCGCTCGGAAAGAAGAA.GCA.....
8 EEck DS 599                   ????????????????????????????????????????????????????????????????????????????????????????????????????
9 EEck Waki 1000                GAGACCGGGCTGTGCGCGTTCTCTTTGACGGGAAACCATCAATCTACCTGC.....TAGTGGCTCGGCTGGCCGCGCTCGGAAAGAAGAA.GCA.....
10 EEck OTS 1199                GAGACCGGGCTGTGCGCGTTCTCTTTGACGGGAAACCATCAATCTACCTGC.....TAGTGGCTCGGCTGGCCGCGCTCGGAAAGAAGAA.GCA.....
L. eckloniae South Africa       GAGACCGGGCTGTGCGCGTTCTCTTTGACGGGAAACCATCAATCTACCTAC.....TAGTGGCTCGGCTGGCCG..CTCGGAAAGAAGAAGGCA.....
L. eckloniae Antarctica         GAGACCGGGCTGTGCGCGTTCACTTTGACGGGAAACCATCAATCTACCTAC.....TAGTGGCTCGGCTGGCCGCGCTCGGAAAGAAGAA.GCA.....
11 EMa A 1097                   .....TAGTGGCTCGGCTGGCCGCGCTCGGAAAGAAGAA.GCAGAAGA
12 EMa QP 797                   .....TAGTGGCTCGGCTGGCCGCGCTCGGAAAGAAGAA.GCAGAAGA
L. macrocystis Chile            .....TAGTGGCTCGGCTGGCCGCGCTCGGAAAGAAGAAGGCAGAAGA
13 EMa PB 697                   ????????????????????????????????????????????????????????????????????????????????????????????????????
14 EMa BB 1097                  .....CTCGGCTGGCCGCGCTCGGAAAGAAGAA.GCG.....
15 EMa CH 598                   .....CTCGGCTGGCCGCGCTCGGAAAGAAGAA.GCG.....
16 EMa W 398                    .....CTCGGCTGGCCGCGCTCGGAAAGAAGAA.GCA.....
17 EMa OW 599                   .....CTCGGCTGGCCGCGCTCGGAAAGAAGAA.GCA.....
18 EMa WR 400                   .....CTCGGCTTGCCGCGCTCGGAAAGAATAA.GCA.....
L. tomentosoides Helgoland      NNNNNNNNNNNNNNNGAGTTCTCTT.GACGG.AA.CCATCAATCTACCTAC..CTACTAGTGGCTCGGCTGGCCG..CTCGGAAGCTTG...GGAGAA..
L. tomentosoides deformans Bretagne GAGACCGGGCTGTGCGCGTTCTCTTTGACGGGAAACCATCAATCTACCTAC..CTACTAGTGGCTCGGCTGGCCG..CTCGGAAGCTTG...GCAGAA..
L. aecidioides Kiel             GAGACCGG..TGTG.GCGTTCTCTTTGACGGGAACCCAT...TGTACCTAC...TACTAGTGGCTCGGCTGGCCG..CTCGGAAGCTTG...GCAGAA..
L. aecidioides Maine            GAGACCGGGCTGTGCGGGTTCTCTTTGACGGGAACCCAT...TGTACCTACTACTACTAGTGGCTCGGCTGGCCG..CTCGGAAGCTTG...GCAGAA..
L. aecidioides Bretagne         GAGACCGGGCTGTGCGCGTTCTCTTTGACGGGAACCCAT...TGTACCTAC...TACTAGTGGCTCGGCTGGCCG..CTCGGAAGCTTG...GCAGAA..
19 EPa A 1097                   ................................................................................................................
20 EPa Riv 999                  ................................................................................................................
21 EPa BB 499                   ................................................................................................................
22 EPa BS 1000                  ................................................................................................................
23 EGra BB 298                  ................................................................................................................
24 EKal CC 1000                 ................................................................................................................
25 EUpi W 498                   ................................................................................................................
26 EEck Kari 599                ................................................................................................................
Microspongium tenuissimum      ................................................................................................................
27 EPa OW 599                   ................................................................................................................
Microspongium radians          ................................................................................................................
Microspongium alariae          ................................................................................................................
28 ELes OW 599                  ................................................................................................................
29 ELes Wk 599                  ................................................................................................................
30 EXi BB 1197                  ????????????????????????????????????????????????????????????????????????????????????????????????????
31 EXi BB 899                   ................................................................................................................
32 EMu Wk 599                  ................................................................................................................
33 EDan BB 499                  ................................................................................................................
34 EDwil BB 800                 ................................................................................................................
```

D 3.1: ITS1 of the nrDNA gene (18S-5.8S) of the pigmented endophytes (continued).

D 3.1: ITS1 alignment (continued)

```
                                positions excluded
all endophytes:                 **********************************************************
Laminariocolax clade:
Microspongium clade:            **********************************************************
Xiphophorocolax clade:          **********************************************************
                                11111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111
                                22222222222222222222222222222222222222222222222222222222222222222222222222222222222222222222222222223
                                00000000011111111112222222222333333333344444444455555555556666666666777777777788888888899999999990
                                12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890

Chordaria flagelliformis        ------------------------------------------------------------CGATGAACCCCGTGAAAGA-AT-TGCGTTATGCGATGTCGGGC
1 EMa A 398                     ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
2 EMa HP 598                    ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
3 EMa H 499                     ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
4 EMa SP 598                    ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
5 EMa WW 599                    ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
6 EMa HI 1099                   ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
7 EEck W 398                    ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
8 EEck DS 599                   ????????????????????????????????????????????????????????????????????????????????????????????????????
9 EEck Waki 1000                ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
10 EEck OTS 1199                ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
L. eckloniae South Africa       ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............-.
L. eckloniae Antarctica         ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
11 EMa A 1097                   GCGAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
12 EMa QP 797                   GCGAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
L. macrocystis Chile            GCGAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......-.............A.............-.
13 EMa PB 697                   ????????????????????????????????????????????????????????????????????????????????????????????????????
14 EMa BB 1097                  ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
15 EMa CH 598                   ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
16 EMa W 398                    ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
17 EMa OW 599                   ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
18 EMa WR 400                   ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTCGTAATATGGAA.GA.......C.............A.............
L. tomentosoides Helgoland      ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTTGTAATATGGAA.GA.......C.............A.............-.
L. tomentosoides deformans Bretagne ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTTGTAATATGGAA.GA.......C.............A.............
L. aecidioides Kiel             ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTTGTAATATGGAA.GA.......-.............A.............---
L. aecidioides Maine            ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTTGTAATATGGAA.GA.......C.............A.............
L. aecidioides Bretagne         ..GAAGAGAGAAGGCGGAGTACCGAGGACGAACCCGGAATGGCTATTTGTAACATGGAA.GA.......C.............A.......?????????
19 EPa A 1097                   ..........................................................ATGGA.......C.....................................
20 EPa Riv 999                  ..........................................................ATGGA.......C.....................................
21 EPa BB 499                   ..........................................................ATGGA....T..C.....................................
22 EPa BS 1000                  ..........................................................ATGGA.......C.....................................
23 EGra BB 298                  ..........................................................ATGGA.......C.....................................
24 EKal CC 1000                 ..........................................................ATGGA.......C.....................................
25 EUpi W 498                   ..........................................................ATGGA.......C.....................................
26 EEck Kari 599                ..........................................................ATGGA.......C.....................................
Microspongium tenuissimum       ..........................................................ATGGA.......C.....................................
27 EPa OW 599                   ..........................................................ATGGA.......C.....................................
Microspongium radians           ..........................................................ATGGA.......C.....................................
Microspongium alariae           ..........................................................ATGGA.......C.....................................
28 ELes OW 599                  ..........................................................AAGA........C......T..A.C.A........C.......
29 ELes Wk 599                  ..........................................................AAGA........C......T..A.C.A........C.......
30 EXi BB 1197                  ??????????????????????????????????????????????????????????????????????????????????????????????????
31 EXi BB 899                   ..........................................................AAGA........C......T..A.C.A........C.......
32 EMu Wk 599                   ..........................................................AAGA........C......T..A.C.A........C.......
33 EDan BB 499                  ..........................................................AAGA........C......T..A.C.A........C.......
34 EDwil BB 800                 ..........................................................AAGA........C......T..A.C.A........C.......
```

D 3.1: ITS1 alignment (continued)

```
                                        positions excluded
          all endophytes:                                                          ***********************************************
          Laminariocolax clade:                                                    ***********************************************
          Microspongium clade:
          Xiphophorocolax clade:

                                        1111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111
                                        3333333333333333333333333333333333333333333333333333333333333333333333333333333333333333333333333334
                                        0000000001111111111222222222233333333334444444444555555555566666666667777777777888888888899999999990
                                        1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890

Chordaria flagelliformis                GAGAGGCG-TCT-CGCCGAG-CTTTTCC---AAGCTCGAATCAAAGCGTGCCCCCACATTTCAACCCC-ACTAAACTCTGAATCTGAACTCAAAAGGAGG
1 EMa A 398                             .........................G.T.....................A..--...C...N........AC..................A.........
2 EMa HP 598                            .........................G.T.....................A..--...C............AC..................A.........
3 EMa H 499                             .........................G.T.....................A..--...C............AC..................A.........
4 EMa SP 598                            .........................G.T.....................A..--...C............AC..................A.........
5 EMa WW 599                            .........................G.T......T..............A..--...C...N........AC..................A.........
6 EMa HI 1099                           .........................G.T.....................A..--...C............AC..................A.........
7 EEck W 398                            .........................G.T.....................A..--...C............AC..................A.........
8 EEck DS 599                           ????????????????????????????????????????????????????????????????????????????????????????????????????
9 EEck Waki 1000                        .........................G.T.....................A..--...C............AC..................A.........
10 EEck OTS 1199                        .........................G.T.....................A..--...C............AC..................A.........
L. ecklonia South Africa                ....-....................G.T.....................A..--...C............AC..................A.........
L. ecklonia Antarctica                  ....-....................G.T...........N.........A..N--T.C..G..TG.....AC.......?????????????????????
11 EMa A 1097                          .........................G.T.....................A..--...C............AC..................A.........
12 EMa QP 797                          .........................G.T.....................A..--...C............AC..................A.........
L. macrocystis Chile                    ....-........N...........G.T..................A..A..--...C............AC..................A.........
13 EMa PB 697                           ????????????????????????????????????????????????????????????????????????????????????????????????????
14 EMa BB 1097                          .........................G.T.....................A..--...C............AC..................A.........
15 EMa CH 598                           .........................G.T.....................A..--...C............AC..................A.........
16 EMa W 398                            .............N----.......G.T.....................A...???????????????????????????????????????????????
17 EMa OW 599                           .........................G.T.....................A..--...C............AC..................A.........
18 EMa WR 400                           .........................G.T.....................A..--...C............AC..................A.........
L. tomentosoides Helgoland              ....-........-.-.........G.T..................-..A..--......????????????????????????????????????????
L. tomentosoides deformans Bretagne     .........................G.T............C........A..--................AC..................A.........
L. aecidioides Kiel                     ....-...G...-.-..........G.T..................-..A..--................AC..................A........-
L. aecidioides Maine                    ....-.GCG................G.T.....................A..--................AC..................A.........
L. aecidioides Bretagne                 ????????????????????????????????????????????????????????????????????????????????????????????????????
19 EPa A 1097                          ........................CG..AAG..................A...-....C.........A.AC............................
20 EPa Riv 999                          ........................CG..AAG..................A...-....C.........A.AC............................
21 EPa BB 499                           ........................CG..AAG..................A...-....C.........A.AC............................
22 EPa BS 1000                          ........................CG..AAG..................A...-....C.........A.AC............................
23 EGra BB 298                          ........................CG..AAG..................A...-....C.........A.AC............................
24 EKal CC 1000                         ........................CG..AAG..................A...-....C.........A.AC............................
25 EUpi W 498                           ........................CG..AAG..................A...-....C.........A.AC............................
26 EEck Kari 599                        ........................CG..AAG..................A...-....C.........A.AC............................
Microspongium tenuissimum               .............--.........CG..AAG..................A...-....C.........A.AC...........................?
27 EPa OW 599                           ........................CG..AAG..................A...-....C.........A.AC....................G.......
Microspongium radians                   .............---....G...CG..AAG..................A...-....C.........A.AC............................
Microspongium alariae                   ........................CG..AAG..................A...-....C.........A.AC...........................C
28 ELes OW 599                          .........................G..............A........CA..--...C.........A.AC..........G.................
29 ELes Wk 599                          .........................G..............A........CA..--...C.......T.A.AC..........G.................
30 EXi BB 1197                          ????????????????????????????????????????????????????????????????????????????????????????????????????
31 EXi BB 899                           .........................G..............A........CA..--...C.........A.AC..........G.................
32 EMu Wk 599                           .........................G..............A........CA..--...C.........A.AC..........G.................
33 EDan BB 499                          .........................G..............A........CA..--...C.C.......A.AC..........G.................
34 EDwil BB 800                         .........................G..............A........CA..--...C.........A.AC..........G.................
```

```
D 3.1: ITS1 alignment (continued)       positions excluded
          all endophytes:               ****************************************************************************************************
          Laminariocolax clade:         ****************************************************************************************************
          Microspongium clade:          ****************************************************************************************************
          Xiphophorocolax clade:                                                         ***************************************************
                                        1111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111
                                        4444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444445
                                        0000000001111111111222222222233333333334444444444555555555566666666667777777777888888888899999999990
                                        1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
                                                                                         ITS1/5.8S
Chordaria flagelliformis                CGCGTGCC--GTATGCCGCTSC-----GCCTTCTCAACCTTTAACGTTGTAAAACTTTCAGCGACGGATGTCTTGGCTCCCACAACGATGAAGAACGCAG
1 EMa A 398                             ....C.T...C.C...GA..G.TG.C..........................................................................
2 EMa HP 598                            ....C.T...C.C...GA..G.TG.C..........................................................................
3 EMa H 499                             ....C.T...C.C...GA..G.TG.C..........................................................................
4 EMa SP 598                            ....C.T...C.C...GA..G.TG.C..........................................................................
5 EMa WW 599                            ....C.T...C.C...GA..G.TG.C..........................................................................
6 EMa HI 1099                           ....C.T...C.C...GA..G.TG.C..........................................................................
7 EEck W 398                            ....C.T...C.C...GA..G.TG.C..........................................................................
8 EEck DS 599                           ????????????????????????????????????????????????????????????????????????????????????????????????????
9 EEck Waki 1000                        ....C.T...C.C...GA..G.TG.C..........................................................................
10 EEck OTS 1199                        ....C.T...C.C...GA..G.TG.C..........................................................................
L. ecklonia South Africa                ...--.T...C.C...GA.---....--.......?????????????????????????????????????????????????????????????????
L. ecklonia Antarctica                  ????????????????????????????????????????????????????????????????????????????????????????????????????
11 EMa A 1097                           ....C.T...C.C...GA..G.TG.C..........................................................................
12 EMa QP 797                           ....C.T...C.C...GA..G.TG.C..........................................................................
L. macrocystis Chile                    G...G.T...C.C...GA.--.TGGC.................................?????????????????????????????????????????
13 EMa PB 697                           ????????????????????????????????????????????????????????????????????????????????????????????????????
14 EMa BB 1097                          ....C.T...C.C...GA..G.TG.C..........................................................................
15 EMa CH 598                           ....C.T...C.C...GA..G.TG.C..........................................................................
16 EMa W 398                            ????????????????????????????????????????????????????????????????????????????????????????????????????
17 EMa OW 599                           ....C.T...C.C...GA..G.TG.C..........................................................................
18 EMa WR 400                           ....C.T...C.C...GA..G.TG.C..........................................................................
L. tomentosoides Helgoland              ????????????????????????????????????????????????????????????????????????????????????????????????????
L. tomentosoides deformans Bretagne     ....C.T...C.C...GA.CG.CG.C...............................................???????????????????????????
L. aecidioides Kiel                     -----.T...C.C.--GA.CG.CG..................??????????????????????????????????????????????????????????
L. aecidioides Maine                    ....C.T...C.C...GA.CG.CG.C...........................................???????????????????????????????
L. aecidioides Bretagne                 ????????????????????????????????????????????????????????????????????????????????????????????????????
19 EPa A 1097                           ....-CG...T..AC.GC--G.CG.C..........................................................................
20 EPa Riv 999                          ....-CG...C..AC.GC--G.CG.C......T...................................................................
21 EPa BB 499                           ....-CG...C..AC.GC--G.CG.C..........................................................................
22 EPa BS 1000                          ....-CG...C..AC.GC--G.CG.C..........................................................................
23 EGra BB 298                          ....-CG...T..AC.GC--G.CG.C..........................................................................
24 EKal CC 1000                         ....-CG...T..AC.GC--G.CG.C..........................................................................
25 EUpi W 498                           ....-CG...T..AC.GC--G.CG.C..........................................................................
26 EEck Kari 599                        ....-CG...T..AC.GC--G.CG.C..........................................................................
Microspongium tenuissimum               .?.?-CG.????????????????????????????????????????????????????????????????????????????????????????????
27 EPa OW 599                           ....-CG...T..AC.GC--G.CG.T..........................................................................
Microspongium radians                   ????????????????????????????????????????????????????????????????????????????????????????????????????
Microspongium alariae                   G...CCG.GCT..AC.GC.-G.GC............................................................................
28 ELes OW 599                          ....ACG...C.....GC---.CG.C...CC.....................................................................
29 ELes Wk 599                          ....ACG...C.....GC.--.CG.C...CC.....................................................................
30 EXi BB 1197                          ????????????????????????????????????????????????????????????????????????????????????????????????????
31 EXi BB 899                           ....ACG...C.....GC---.CG.C...CC.....................................................................
32 EMu Wk 599                           ....ACG...C.....GC---.CG.CA..CC...................................................................??
33 EDan BB 499                          ....ACG...C.....GC---.CG.C...CC.........A...........................................................
34 EDwil BB 800                         ....ACG...C.....GC---.CG.C...CC.....................................................................
```

D 3.1: ITS1 of the ntDNA gene (18S-5.8S) of the pigmented endophytes (continued).

D 3.1: ITS1 alignment (continued)

```
                                positions excluded
                 all endophytes: ****************************************
                 Laminariocolax clade: ****************************************
                 Microspongium clade: ****************************************
                 Xiphophorocolax clade: ****************************************
                                1111111111111111111111111111111111111111
                                5555555555555555555555555555555555555555
                                0000000001111111111222222222233333333344
                                1234567890123456789012345678901234567890

Chordaria flagelliformis        CGAAATGCGATACGTCTTGCGACTTGCAGAATCCAGTGAAT
1 EMa A 398                     ........................................
2 EMa HP 598                    ........................................
3 EMa H 499                     ........................................
4 EMa SP 598                    ............??????????????????????????????
5 EMa WW 599                    ........................................
6 EMa HI 1099                   ............??????????????????????????????
7 EEck W 398                    ............??????????????????????????????
8 EEck DS 599                   ????????????????????????????????????????
9 EEck Waki 1000                ............??????????????????????????????
10 EEck OTS 1199                ........................................
L. eckloniae South Africa       ????????????????????????????????????????
L. eckloniae Antarctica         ????????????????????????????????????????
11 EMa A 1097                   ........................................
12 EMa QP 797                   ........................................
L. macrocystis Chile            ????????????????????????????????????????
13 EMa PB 697                   ????????????????????????????????????????
14 EMa BB 1097                  ........................................
15 EMa CH 598                   ........................................
16 EMa W 398                    ????????????????????????????????????????
17 EMa OW 599                   .......................................?
18 EMa WR 400                   ........................................
L. tomentosoides Helgoland      ????????????????????????????????????????
L. tomentosoides deformans Bretagne ????????????????????????????????????
L. aecidioides Kiel             ????????????????????????????????????????
L. aecidioides Maine            ????????????????????????????????????????
L. aecidioides Bretagne         ????????????????????????????????????????
19 EPa A 1097                   ....????????????????????????????????????
20 EPa Riv 999                  ............??????????????????????????????
21 EPa BB 499                   ...........???????????????????????????????
22 EPa BS 1000                  ............A???????????????????????????
23 EGra BB 298                  ............AC..?????????????????????????
24 EKal CC 1000                 .......????????????????????????????????????
25 EUpi W 498                   ............A???????????????????????????
26 EEck Kari 599                ......??????????????????????????????????
Microspongium tenuissimum       ????????????????????????????????????????
27 EPa OW 599                   ............??????????????????????????????
Microspongium radians           ????????????????????????????????????????
Microspongium alariae           ........................???????????????????
28 ELes OW 599                  ............??????????????????????????????
29 ELes Wk 599                  ....????????????????????????????????????
30 EXi BB 1197                  ????????????????????????????????????????
31 EXi BB 899                   .......??????????????????????????????????
32 EMu Wk 599                   ????????????????????????????????????????
33 EDan BB 499                  ....????????????????????????????????????
34 EDwil BB 800                 ..????????????????????????????????????????
```

D 3.2: Alignment of the 18S nrDNA gene.

D 3.2: 18S alignment
positions excluded:

```
                                ********************************************************************************************************************
                                00000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000001
                                00000000011111111112222222222333333333344444444445555555555666666666677777777778888888889999999999000
                                12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890

Tribonema aequale               GTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGTTAACGAACGAGACCCCCGCCTGCTAAATAGCTCCCCGCACGCATTTGCGTG
Chordaria flagelliformis        ????????????????????????????????????????????????????????????????????????????????????????????????????????????????
Dictyosiphon foeniculaceus      ????????????????????????????????????????????????????????????????????????????????????????????????????????????????
Ectocarpus siliculosus          ..........................................................................TGTGG.TT....T.......-
Punctaria sp.                   ??????????????????????????????????.....................................A.....TGTGG.TT....T.......-
Scytosiphon lomentaria          ..........................................................................TGTGG.TT....T....-..-
Xiphophorocolax aotearoae       ????????????????????????????.............................................TGTGG.TT....T.......-
Asterocladon lobatum            ????????????????????????????????????????????????????????????????????????????????????????????????????T.......-
Asteronema rhodochortonoides    ????????????????????????????????????????????????????????????????????????????????.....TGTGG.TT....T.......-
Alaria esculenta                ????????????????????????????????????????????????????.....................................TGTGG.TT....T.C..T..-
Laminaria digitata              ????????????????????????????????????????????????????.....................................TGTGG.TT....T.C..T..-
Macrocystis pyrifera            ????????????????????????????????????????????????????.....................................TGTGG.TT....T.C..T..-
Sporochnus pedunculatus         ??????????????????????????????.....................N.....................................TGTGG.TT....T.......-
Haplospora globosa              ??????????????????????????????.............N.......NN.....................N......TGTGG.TT....T.......-
Desmarestia aculeata            ????????????????????????????????????????????????????????????????????????????????????????????????????????????????
Himantothallus grandifolius     ????????????????????????????????????????????????????.....................................TGTGG.TA....T.......-
Phaeurus antarcticus            ????????????????????????????????????????????????????.....................................TGTGG.TT....T.......-
Scytothamnus australis          ..........................................................................TGTGG.TT....T.......-
Splachnidium rugosum            ????????????????????????????????????????????????????.....................................TGTGGTTT....T.......-
Cutleria multifida              ????????????????????????????????????????????????????.....................................TGTGGTCT....T.......-
Ascophyllum nodosum             ??????????????????????????????????.......................................................TGGGG.TT...TT.C.A...-
Durvillaea antarctica           ????????????????????????????????????????.................................................TGGGG.TT....T.C.....-
Fucus vesiculosus               ????????????????????????????????????????????.............................................TGGGG.TT...TT.C.A...-
Sargassum muticum               ??????????????????????????????????.......................................................GTGG.TT.......C....-
Xiphophora chondrophylla        ????????????????????????????????.........................................................TGGGG.TT...TT.C.A...-
Notheia anomala                 ????????????????????????????????????????????????????.....................................GAGG.TT.......C....-
Ascoseira mirabilis             ????????????????????????????????????????????????????????????????????????????????????.....TGTGG.TT....T.......-
Nemoderma tingitanum            ??????????????????????????????.............NNNNN....N.....................................GTGG.TT.......CC....-
Alethocladus corymbosus         ????????????????????????????????????????.................................................TGTGGNTT..K.T.....N.-
Cladostephus spongiosus         ??????????????????????????????????.......................................................TGTGG.TT....T.......-
Herpodiscus durvillaeae         ..........................................................................TGTGG.TT....T.......-
Sphacelaria cirrosa             ????????????????????????????????????????.....N..N.....N..N.....................................TG.GG.NN....T.......-
Stypocaulon scoparium           ??????????????????????????????????.......................................................TGTGG.TT....T.......-
Syringoderma phinneyi           ..........................................................................TGTGG.TT....T.......-
Choristocarpus tenellus         ????????????????????????????????????????.................................................GGGG..T....T.GC....-
Onslowia endophytica            ????????????????????????????????????????.................................................TGTGG.TT....T.......-
Dictyota dichotoma              ??????????????????????????????????.......................................................GGGG.TT....GC.C....-
Taonia atomaria                 ..........................................................................TGGGG.TT....T....-..-
```

D 3.2: Alignment of the 18S nrDNA gene (continued).

D 3.2: 18S alignment (continued)

positions excluded:

```
**********************************************************************************************************
11111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111112
00000000011111111112222222222333333333344444444445555555556666666666777777777788888888899999999990
12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890

Tribonema aequale                ACGGGCGGCGCTTCTTAGAGGGACTTTCAGTGATTAACTGAAGGAAGTTGGGGGCAATAACAGGTCTGTGATGCCCTTAGATG-TCCTGGGCCGCACGCG
Chordaria flagelliformis         ??????????????????????????????????????????????????.....................................................
Dictyosiphon foeniculaceus       ????????????????????????????????????????????????????????????????????????????????.....................
Ectocarpus siliculosus           .G.TA.T-.................CTG....C....CAG.....................................................
Punctaria sp.                    .G.T..T-.................CTG....C....CAG.....................................................
Scytosiphon lomentaria           .G.T..T-.................CTG....C....CAG.....................................A...............
Xiphophorocolax aotearoae        .G.T..T-................NCTG....C....CAG.....................................................
Asterocladon lobatum             .G.C..N-.A..............G....C....-----------------------------------------.----------
Asteronema rhodochortonoides     .G.C..T-.A..............G....C....C...........................................................
Alaria esculenta                 .G.T..T-................G....C....C.........N................................................
Laminaria digitata               .G.T..T-................G....C....C...........................................................
Macrocystis pyrifera             .G.T..T-................G....C....C...........................................................
Sporochnus pedunculatus          .G.T..T-................G....C....C...........................................................
Haplospora globosa               .G.TA.T-................G....C....C...........................................................
Desmarestia aculeata             ????????????????????????????????????????????????????????????????????????????????????????????????
Himantothallus grandifolius      .G.TA.T-................G....C....CA..........................................................
Phaeurus antarcticus             .G.TA.T-................G....C....C...........................................................
Scytothamnus australis           .G.T..T-................G....C....C...........................................................
Splachnidium rugosum             .GAT..T-.A..............G....C....C...........................................................
Cutleria multifida               .G.TA.T-................G....C....C...........................................................
Ascophyllum nodosum              .G.CAGCT................G....C....C...........................................................
Durvillaea antarctica            .G.TATT-................G....C....C...........................................................
Fucus vesiculosus                .G.CAGCT................G....C....C...........................................................
Sargassum muticum                .G.T.G.-................G....C....C...........................................................
Xiphophora chondrophylla         .G.CATTT................G....C....C...........................................................
Notheia anomala                  .G.CA.T-................G....C....C...........................................................
Ascoseira mirabilis              .G.TA.T-................G....C....C...........................................................
Nemoderma tingitanum             .G.TA.T-................G....C....C...........................................................
Alethocladus corymbosus          .S.C..T-................G....C...............................................................
Cladostephus spongiosus          .G.T..T-................G....C....C...........................................................
Herpodiscus durvillaeae          .G.T..T-................G....C....C...........................................................
Sphacelaria cirrosa              .G.C.TN-.N..............G....C....C...........................................................
Stypocaulon scoparium            .G.T..T-................G....C....C...........................................................
Syringoderma phinneyi            .G.TA.T-................G....C....C...........................................................
Choristocarpus tenellus          .G.C..C-................G....C....C...........................................................
Onslowia endophytica             .G.C..T-................G....C....C...........................................................
Dictyota dichotoma               .G.T..C-A...A...........G....C....C...........................................................
Taonia atomaria                  .G.TA.T-................G....C....C...........................................................
```

D 3.2: Alignment of the 18S nrDNA gene (continued).

D 3.2: 18S alignment (continued)

```
positions excluded:  **********
                     22222222222222222222222222222222222222222222222222222222222222222222222222222222222222222222222223
                     00000000011111111112222222222333333333344444444445555555556666666666777777777788888888899999999990
                     12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678
Tribonema aequale    CGCTACACTGACACCTGCAACGAG--CGT--AGAACCTTGGCCGAGAGGCCCGGGTAATCTTTTG-AACGGGTGTCGTGATAGGGACAGATTATTGCAAT
Chordaria flagelliformis  ..........TG.A.........TTT--.TTTTT...G..T..................G........T.CA............T....C........
Dictyosiphon foeniculaceus  ..........TG.A.........TT---TTTTTT...G..T..................G........T.CA............T....C........
Ectocarpus siliculosus  ..........TG.A.........TTTT-ACTTTT...G..T..................G........T.CA............T....C........
Punctaria sp.        ..........TG.A.........TTT--.TTTTT...G..T..................G........T.CA............T....C........
Scytosiphon lomentaria  ..........TG.A.........TTTT-ATTTTT...A..T.........T...-.............T.CA............T....C........
Xiphophorocolax aotearoae  ..........TG.A.........TTT--.TTTTT...G..T..................G........T.CA............T....C........
Asterocladon lobatum  ---NNNNNN..TG.A.........TTTT-ACTTCT...G..T..................G........T.CA............T....C........
Asteronema rhodochortonoides  ..........TG.A.........TTT-CACTTTT...G..T..................G........T.CA............T....C........
Alaria esculenta     ..........TG.A.........TT.T.TTTTTT...G..T..................G........T.CA............T....C........
Laminaria digitata   ..........TG.A.........TT.T.TTTTTT...G..T..................G........T.CA............T....C........
Macrocystis pyrifera  ..........TG.A.........TT.T.TTTTTT...G..T..................G........T.CA............T....C........
Sporochnus pedunculatus  ...N.......TG.A.........TT.T.TCTTTT...G..T..................G........T.CA............T....C........
Haplospora globosa   ..........TG.A.........TT.T.TTTTTC...G..T..................G........T.CA............T....C........
Desmarestia aculeata  ????????????????????.....TT.T..TTTTT...G..T..................G........T.CA............T....C........
Himantothallus grandifolius  ..........TG.A.........TT.T-TTTTTT...G..T..................G........T.CA............T....C........
Phaeurus antarcticus  ..........TG.A.........TT.T..TTTT-...G..T.........T.........G........T.CA............T....C........
Scytothamnus australis  ..........TG.A.........TTTT-ACTTTT...G..T..................G........T.CA............T....C........
Splachnidium rugosum  ..........TG.A.........TTTT-ACTTTT...G..T..................G........T.CA............T....C........
Cutleria multifida   ..........TG.A.........TT.T.CTTTTC...G..T..................G........T.CA............T....C........
Ascophyllum nodosum  ..........TG.A.........TT.TCAATCTC...G.....GA...T.........C..........T.CA............T.....T...T..A
Durvillaea antarctica  ..........TG.A.........TT.T.CTTTTC...G..T.......T..........G........T.CA............T....C........
Fucus vesiculosus    ..........TG.A.........TT.TCAATCTC...G.....GA...T.........C..........T.CA............T.....T...T..A
Sargassum muticum    ..........TG.A.........TT.C.TTTTTC...G..T........T............G......T.CA............T....C.......C
Xiphophora chondrophylla  ..........TG.A.........TT.TCAATCTC...G.A....A.....T.............G....T.CA............T.....T...T..A
Notheia anomala      ..........TG.A.........TT.T.CTTTTC...G..T..................G........T.CA............T....C....G..C
Ascoseira mirabilis  ..........TG.A.........TTTT.TATTTC...G..T..................G........T.CA............T....C........
Nemoderma tingitanum  ..........TG.A.........TT.T.CTTTTC...G..T.............................T.CA............T....C........
Alethocladus corymbosus  ..........TG.A.........TTT-CATT.TT...G..T..................G........T.CA............T....C........
Cladostephus spongiosus  ..........TG.A.........TTT-CATT.TT...G..T..................G........T.CA............T....C........
Herpodiscus durvillaeae  ..........TG.A.........TTT-CATT.TT...G..T..................G........T.CA............T....C........
Sphacelaria cirrosa  ..........TG.A.........TNT-CATT.TT...G..T..................G........T.CA.G..........T....C........
Stypocaulon scoparium  ..........TG.A.........TTT-CATT.TT...G..T..................G........T.CA............T....C........
Syringoderma phinneyi  ..........TG.A.........TTT--ATT.TT...G.CT.......G..........G........T.CA............T....C........
Choristocarpus tenellus  ..........TG.A.........TTT-CGTTTG.......T..................G........T.CA............T....C........
Onslowia endophytica  ..........TG.A.........TCTTCAC-.TT...G..T..................G........T.CA............T....C........
Dictyota dichotoma   ..........TG.A.........TG.-CAAC.CT...G..T.................C..G........T.CA............T....CT.......
Taonia atomaria      ..........TG.A.........TTT-CGAC.CT...G..T.........-......C..........T.CA............T....CT...T...
```

D 3.2: 18S alignment (continued)
positions excluded:

```
                                3333333333333333333333333333333333333333333333333333333333333333333333333333333333333333333333333334
                                0000000001111111111222222222233333333334444444445555555556666666666777777777788888888899999999990
                                1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890

Tribonema aequale               TATTAATCTCGAACGAGGAATTCCTAGTAAACGCGAATCATCAGTTCGCATTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCACCTACCGATT
Chordaria flagelliformis        ....G....T.........................G.......C.....................................................
Dictyosiphon foeniculaceus      ....G....T.........................G.......C.....................................................
Ectocarpus siliculosus          ....G....T.........................G.......C.....................................................
Punctaria sp.                   ....G....T.........................G.......C.....................................................
Scytosiphon lomentaria          ....G....T.........................G.......C.....................................................
Xiphophorocolax aotearoae       ....G....T.........................G.......C.....................................................
Asterocladon lobatum            ....G....T.....................NN...G.......C.....................................................
Asteronema rhodochortonoides    ....G....T.........................G.......C.....................................................
Alaria esculenta                ....G....T.........................G.......C.....................................................
Laminaria digitata              ....G....T.........................G.......C.....................................................
Macrocystis pyrifera            ....G....T.........................G.......C.....................................................
Sporochnus pedunculatus         ....G....T.........................G.......C.....................................................
Haplospora globosa              ....G....T.........................G.......C.....................................................
Desmarestia aculeata            ....G....T.........................G.......C.....................................................
Himantothallus grandifolius     ....G....T.........................G.......C.....................................................
Phaeurus antarcticus            ....G....T.........................G.......C.....................................................
Scytothamnus australis          ....G....T.........................G.......C.....................................................
Splachnidium rugosum            ....G....T.........................G.......C.....................................................
Cutleria multifida              ....G....T.........................G.......C.....................................................
Ascophyllum nodosum             .........T.........................G.......C.....................................................
Durvillaea antarctica           ....G....T.........................G.......C.....................................................
Fucus vesiculosus               .........T.........................G.......C.....................................................
Sargassum muticum               ....G....T.........................G.......C.....................................................
Xiphophora chondrophylla        .........T.........................G.......C.....................................................
Notheia anomala                 ....G....T.........................G.......C.....................................................
Ascoseira mirabilis             ....G....T.........................G.......C.....................................................
Nemoderma tingitanum            ....G....T.........................G.......C.....................................................
Alethocladus corymbosus         ....G....T.........................G.......C.....................................A...............
Cladostephus spongiosus         ....G....T.........................G.......C.....................................................
Herpodiscus durvillaeae         ....G....T.........................G.......C.....................................................
Sphacelaria cirrosa             ....G....T.........................G.......C.....................................................
Stypocaulon scoparium           ....G....T.........................G.......C.....................................................
Syringoderma phinneyi           ....G....T.........................G.......C.....................................................
Choristocarpus tenellus         ....G....T.....................R.G.......C.....................................................
Onslowia endophytica            ....G....T.........................G.......C.....................................................
Dictyota dichotoma              ...CG....T.........................G.......C.....................................................
Taonia atomaria                 ...CG....T.........................G.......C.....................................................
```

D 3.2. Alignment of the 18S nrDNA gene (continued).

D 3.2: 18S alignment (continued)

```
      positions excluded:                                       *************************************************************
                                 4444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444445
                                 0000000001111111111222222222233333333334444444444555555555566666666667777777777888888888899999999990
                                 1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890

Tribonema aequale                GAATGATTCGGTGAAGATTCGGGACCGTGCC--AGCTCCGTTCACGCGGCGCT-GGC-GTGGGAACTTGTCTGAACCTCATCATTTAGAGGAAGGTGAAG
Chordaria flagelliformis         ....C.........G.....C...TTC..TA..GCT.A..C.TTACG.CGTT.TTTAC.AAA...G..A..CA.......G...................
Dictyosiphon foeniculaceus       ....C.........G.....C...TTCC.TA..GCT.A..C.TTGCG.CGTT.TTTAT.AAA...G..A..CA.......G...................
Ectocarpus siliculosus           ....C.........G...CTC...TTT..TT..-...TACG.TCACG.CGTT.TC.AC.A.A...G.CA..CA.......G...................
Punctaria sp.                    ....C.........G.....C...TTCC.TA..GCT.A..C.TCACG.CGTT.TTTAC.AAA...G..A..CA.......G......????????????
Scytosiphon lomentaria           ....C.........G...CTC...TTT..TT..TTT.A..C.TCACG.CGTT.CA.AC.A.A...G.CA..CA.......G...................
Xiphophorocolax aotearoae        ..............G.....C...TTCC.TA..GCT.A..C.TCACG.CGTT.TTTAT.AAA...G..A..CA...........................
Asterocladon lobatum             ....................C.......T...GT.AT..C.TCACG.CG.TCTT.AC......G..A...A.............................
Asteronema rhodochortonoides     ....AT..............C......A..G...TGATT.C.TT..G.CGA.GTT.TT-C.....G..A...A.......A...................
Alaria esculenta                 .....T..............C....T...G...TCGCGT.C.TCACG.CGCTCTT..C.......G..A...A.......A....???????????????
Laminaria digitata               .....T..............C....T...G...TCGCGT.C.TCACG.CGCTCCT..C.......G..A...A......?????????????????????
Macrocystis pyrifera             .....T..............C....T...G...TCGCGT.C.TCACG.CGCNCCT..C.......G..A...A.......A..????????????????
Sporochnus pedunculatus          .....T..............C....T...G...TCGCG..C.TCACG.CGCTCCT..C.......G..A...A.......A.....??????????????
Haplospora globosa               .....T..............C........G...TCGCG..C.TTACG.CGCTCTT..C.......G..A.??????????????????????????????
Desmarestia aculeata             .....T..............C........G...TTGCG..C.TCACG.CGCTCTT..C.......G..A...A.......A...................
Himantothallus grandifolius      .....T..............C........G...TTGCG..C.TCACG.CGCTCTT..C.......G..A...A.......A.??????????????????
Phaeurus antarcticus             .....T..............C........G...TTGCG..C.TCACG.CG.TCTT..C.......G..A...A.......A...................
Scytothamnus australis           .....T..............C....T...G...TCGC.G.C.TCACG.CGCTCTT..C.......G..A...A.......A...................
Splachnidium rugosum             .....T..............C....T...G...TCGGG..C.TCACG.CGCTCTT..C.......G..A...A.......A.....??????????????
Cutleria multifida               .....T..............C........G...TCGCG..C.TCACG.CGCTCTT..C.......G..A...A.......A.....??????????????
Ascophyllum nodosum              .....T..............C.....ACAA...TTGCG..C.TCACG.CGCTCTC..C.C.....G..A...A.......A.......????????????
Durvillaea antarctica            .....T..............C........T...GATCG..C.TCACG.CGCTGTC.TT.......G..A...A.......A......?????????????
Fucus vesiculosus                .....T..............C....T.GAA...TTGGG..C.TCACG.CGCT.G..GGAAA....G..A...A.......A...................
Sargassum muticum                .....T..............C......C.A.GCCAGAG..C.TCACG.CGC.CT..TC.......G......A.......A.......????????????
Xiphophora chondrophylla         .....T..............C....T...A....AGCG..C.TCACT.CGCTCTC.TC.......G..A...A.......A.....??????????????
Notheia anomala                  ....AT..............C........A...TTGCG..C.TCACG.CGCTCT..TC.......G..A...........AT..............???
Ascoseira mirabilis              .....T........G.....C....T...G...G.GAG..C.TCACG.CGCTCTT..C.......G..A..CT.......A...................
Nemoderma tingitanum             .....T..............C........G...TCGCG..C.TCACG.CGCTCTT..C.......G..N...A.......A..?????????????????
Alethocladus corymbosus          .....T..............C........G...TCG.G..C.TCACG.CGCTCTT..C.......G..A...A.......A.??????????????????
Cladostephus spongiosus          .....T..............C........G...TCG.G..C.TCACG.CGCGCTT..C.......G..A...A.......A.......????????????
Herpodiscus durvillaeae          .....T..............C........G...TCG.G..C.TCACG.CGCGCTC..C.......G..A...A.......A...................
Sphacelaria cirrosa              .....T..............C........G...TCG.G..C.TCACG.CGCGCTT..C.......???????????????????????????????????
Stypocaulon scoparium            .....T..............C........G...TCG.G..C.TCACG.CGCTCTT..C.......G..A...A.......A.....??????????????
Syringoderma phinneyi            .....T..............C........G...TCG.G..C.TCACG.CGT.CT-..C.......G..A...A.......A...................
Choristocarpus tenellus          .....T..............C........G...TTGGGG.C.TCGCG.C...CGC..C.......G..A...A.......A.??????????????????
Onslowia endophytica             .....T..............C........G...TCG.G..C.TCACG.CGCTCTT..C.......G......A.......A.??????????????????
Dictyota dichotoma               .....T..............C........T...-TGCG..C.TCACG.CGCGCT...C.......G..A...A.......A......?????????????
Taonia atomaria                  .....T..............C........G...TAGCG..C.TCACG.CGCTCT...C.......G..A...A.......A...................
```

D 3.2: Alignment of the 18S nrDNA gene (continued).

D 3.2: 18S alignment (continued)

```
positions excluded:     ****************************************
                        55555555555555555555555555555555555555555555
                        00000000011111111112222222222333333333334444
                        12345678901234567890123456789012345678901234

Tribonema aequale       TCGTAACAAGGTTTCCGTAGGTGAACCTGCAGAAGGATCAAGC
Chordaria flagelliformis ..........................G.........TTA
Dictyosiphon foeniculaceus ........................G.........???
Ectocarpus siliculosus ............??????????????????????????
Punctaria sp.           ????????????????????????????????????????
Scytosiphon lomentaria  ............??????????????????????????
Xiphophorocolax aotearoae .........................G.........TTA
Asterocladon lobatum    .........................G.........TTA
Asteronema rhodochortonoides ......................G.........TTA
Alaria esculenta        ????????????????????????????????????????
Laminaria digitata      ????????????????????????????????????????
Macrocystis pyrifera    ????????????????????????????????????????
Sporochnus pedunculatus ????????????????????????????????????????
Haplospora globosa      ????????????????????????????????????????
Desmarestia aculeata    .........................G.........T??
Himantothallus grandifolius ????????????????????????????????????
Phaeurus antarcticus    .........................G.........TTA
Scytothamnus australis  ..........??????????????????????????????
Splachnidium rugosum    ????????????????????????????????????????
Cutleria multifida      ????????????????????????????????????????
Ascophyllum nodosum     ????????????????????????????????????????
Durvillaea antarctica   ????????????????????????????????????????
Fucus vesiculosus       ............??????????????????????????
Sargassum muticum       ????????????????????????????????????????
Xiphophora chondrophylla ???????????????????????????????????????
Notheia anomala         ????????????????????????????????????????
Ascoseira mirabilis     .........................G.........TTA
Nemoderma tingitanum    ????????????????????????????????????????
Alethocladus corymbosus ????????????????????????????????????????
Cladostephus spongiosus ????????????????????????????????????????
Herpodiscus durvillaeae .........................G.........???
Sphacelaria cirrosa     ????????????????????????????????????????
Stypocaulon scoparium   ????????????????????????????????????????
Syringoderma phinneyi   ............?????????????????????????????
Choristocarpus tenellus ????????????????????????????????????????
Onslowia endophytica    ????????????????????????????????????????
Dictyota dichotoma      ????????????????????????????????????????
Taonia atomaria         ............?????????????????????????????
```

D 3.3: Alignment of the 26S nrDNA gene.

D 3.3: 26S alignment

positions excluded: **********

```
                                  0000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000001
                                  0000000001111111112222222222333333333344444444455555555556666666666777777777788888888889999999990
                                  1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890

Tribonema aequale                 CTAACCAGGATTCCCCTAGTAACGGCGAGTGAAGCGGGAAGAGCCCACGATGGTAATCGATGCGCCCCTGCGG--CCACCGAATTGTAGTCTGTAGAGGG
Adenocystis utricularis           ................C.........................T....T..TA....TGAC-----T.-.....--T.............G.....C
Ascoseirophila violodora          ................C.........................T....T..TA....TGAC----TT.-...G.--T.............G.....C
Caepidium antarcticum             ................C.........................T....T..TA....TGAC-----T.-.....--T.............G.....C
Chordaria flagelliformis          ................C.........................T....T..TA....TGAC----TT.-...G.--T.............G.....C
Dictyosiphon foeniculaceus        ................C.........................TNNNNT..TA....TGAC----TT.-...G.--T.............G.....C
Ectocarpus siliculosus            ................C.................-.......T....T..TA....TGAC-----T.-.....--T.............G.....C
Laminariocolax tomentosoides      ................C.........................T....T..TA....TGAC----TT.-...G.--T.............G.....C
Laminarionema elsbetiae           ................C.........................T....T..TA....TGAC----T..-...G.--T.............G.....C
Myrionema strangulans             ................C.........................T....T..TA....TGAC----TT.-...G.--T.............G.....C
Punctaria sp.                     ................C.........................T....T..TA....TGAC----TT.-...G.--T.............G.....C
Pylaiella littoralis              ................C.........................T....T..TA....TGAC------.-.C.G.--T.............G.....C
Scytosiphon lomentaria            ................C.........................T....T..TA....TGAC-----T.-.....--T.............G.....C
Xiphophorocolax aotearoae         ................C.........................T....T..TA....TGAC----TT.-...G.--T.............G.....C
Asterocladon lobatum              ................C.........................T...AC..TA....TGGC-----T.-...C.---.............G.....C
Asteronema rhodochortonoides      ................C.........................T....T..TA....TGGC-----T.-...C.---.............G.....C
Alaria esculenta                  ................C.........................T....T..TA....TGCC-----T.-...G.---.............G.....C
Laminaria digitata                ................C.........................T....T..TA....TGCC-----T.-...G.---.............G.....C
Macrocystis pyrifera              ................C.....................G....T....T..TA....TGCC-----T.-...G.---.............G.....C
Undaria pinnatifida               ................C.........................T....T..TA....TGCC-----T.-...G.---.............G.....C
Sporochnus pedunculatus           ................C.........................T....T..TA....TGGC-----.T.-...G.--C.............G.....C
Haplospora globosa                ................C.........................T....T..TA....TGGC-----.T.-...C.---.............G.....C
Tilopteris mertensii              ................C.........................T....T..TA....TGGC----TT.-...G.--C.............G.....C
Desmarestia aculeata              ................C.........................T....T..TA....TGGC-----T.-...C.---.............G.....C
Himantothallus grandifolius       ................C.........................T....T..TA....TGGC-----.T.-...G.--C.............G.....C
Phaeurus antarcticus              ??????????????????????????????.....T..T.T..TA....TGGC-----T.-NN.GG--C.............G.....C
Scytothamnus australis            ................C.........................T....T..TA....TGAC------.-.ACG.--T.............G.....C
Splachnidium rugosum              ................C.........................T....T..TA....TGAC-----T.-.....--T.............G.....C
Cutleria multifida                .....A..........C.........................T....T..TA....TGGC----TT.-...G.--C.............G.....C
Ascophyllum nodosum               ................C.........................T...AT.TAA....TGGC----A..-..TG.---.............AA.....T
Durvillaea antarctica             ................C.........................T...AT..TA....TGCC-----T.-..TG.--G.............C
Fucus vesiculosus                 ................C.........................T...AT.TAA....TGGC----A..-..TG.---.............AA.....T
Sargassum muticum                 ................C.........................T..T....TA..C.CGGC----...-...G.--T.G.G-.........G.....C
Xiphophora chondrophylla          ................C.........................T...AT..TA...TTG.C--A.AA.-G.TG.--..A.............A.....C
Notheia anomala                   ................C.........................T...AC..TA....C.C.-----.T....-..---TG...........C.A.....C
Ascoseira mirabilis               ................C.........................T....T..TA....TGGC-----T.-...G.--T.............G.....C
Nemoderma tingitanum              ................C.........................T....T..TA....CGCC--.T.T.-..AGG--.TG...........G.....C
Alethocladus corymbosus           .....A..........C.........................T....T..TA....TGGC----...-...G.--C.............C
Cladostephus spongiosus           ................C.........................T....T..TA....TGGC----...-...G.--C.............C
Herpodiscus durvillaeae           ................C.........................T....T..AA....TGGC----...-...G.--C.............C
Sphacelaria cirrosa               ................C.........................T....T..TA....TGGC----.T.-...G.--T.............C
Stypocaulon scoparium             .....A..........C.........................T....T..TA....TGGC----.T.-...G.--C.............C
Syringoderma phinneyi             ................C.........................T....T..TA....TGGC----TT.-...G.--C.............G.....C
Choristocarpus tenellus           ??????????????????????????????????????????...T....T..TG....CG.C-C.....-...GGGGT.............G.....C
Onslowia endophytica              ................C.........................T...CT..TA....TGGC----TT.-...G.--C.............G.....C
Verosphacela ebrachia             .....A..........................................T...AT..AA....TGGC----...-A..C.---.............G.....C
Dictyota dichotoma                .....A..........C.........................T....T..TG....TGGC----...-...G.---.........CA...G.....C
Taonia atomaria                   .....A..........C.........................T.......TG......GC----...-A..G.--C.............G.....C
```

D 3.3: Alignment of the 26S nrDNA gene (continued).

D 3.3: 26S alignment (continued)
positions excluded:

```
                        1111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111112
                        0000000001111111111222222222233333333334444444444555555555566666666667777777777888888888999999999990
                        1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890

Tribonema aequale       TCG-TCC-ACTGCGCACCGCCGA-CTCAAGTCC-C-TTGGAAGAGGGCGCCAGGGAGGGTGACAGCCCCGTCCATGGTTGGCA----GCGTGT-GCG-GA
Adenocystis utricularis GT..A....GC-T.G.GTCGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T.CCCCC..G....-.TCCA.-..C.T
Ascoseirophila violodora GT..A....GC-T.GGGACGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCAC....T.CCCA.-..C.T
Caepidium antarcticum   GT..A....GC-T.G.G.CGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCGT....C.TCCA.-..C.T
Chordaria flagelliformis GT..A....GC-T.G.GACGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCAC....T.TCCA.-..C.T
Dictyosiphon foeniculaceus GA..A....GC-T.GGGACGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCAC....T.CCCA.-..C.T
Ectocarpus siliculosus  GT..A....GC-T.G.GACGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCGC....TTTCCA.-T.C.T
Laminariocolax tomentosoides GT..A....GC-T.GGGACGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCAC....T.CCCA.-..C.T
Laminarionema elsbetiae GT..A....GC-T.G.GGCGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCAC....C.TCCA.-..C.T
Myrionema strangulans   GT..A....GC-T.G.GACGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCAC....T.TCCA.-..C.T
Punctaria sp.           GT..A....GC-T.GGGACGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCTAG....T.CCCA.-..C.T
Pylaiella littoralis    GT..A....GC-T.G.G.CGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCG.....T.TCCA.-..C.T
Scytosiphon lomentaria  GT..A....GC-T.G.GTCGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CC.CCGC......TCCA.-..C.T
Xiphophorocolax aotearoae GT..A....GC-T.GGGACGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCAC....T.CCCA.-..C.T
Asterocladon lobatum    GT..A....GC-..G.GGCGGAG.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCGC....TAACCG.-..C.T
Asteronema rhodochortonoides GT..A....GC-..G.GGTGG.G.G.....-T........A..A..AT..A...........AT.....T.T..CCCCCGC....T..CCG.-..C.T
Alaria esculenta        G...G....GC-...TG.C.G.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCAGG.......GCG.-T.C.T
Laminaria digitata      G...A....GC-..TGG.CGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCAC......CGCG.-..C.T
Macrocystis pyrifera    GA..G....GC-...GG.CGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCAC......CGTG.-..C.T
Undaria pinnatifida     G...G....GC-...TG.C.G.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCA.G......AGCG.-..C.T
Sporochnus pedunculatus GA.CG....GC-..TCGGCGG.G.GCG.....-T........A..A..AT..A...........AT.....T.C..CCCCCTCC...C...CG.-..C.T
Haplospora globosa      GA..G....GC-..TCGGCGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCTGATT.T..GCG.-..C.T
Tilopteris mertensii    GA..G....GC-..TCGGCGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCTGATT.T..GCG.-..C.T
Desmarestia aculeata    GA..G....GC-..GCAGCGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCAG.TT.CT.CCG.-..C.T
Himantothallus grandifolius GA..G....GC-..GCAGCGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCAGCTT.CT.CCG.-..C.T
Phaeurus antarcticus    GA..G....GC-..GCAGCGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCAGC...CT.CCG.-..C.T
Scytothamnus australis  GA..G....GC-..GCGGAGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCATG...TT..CG.-..C.T
Splachnidium rugosum    GA..A....GC-..GCGGCGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCATC...T...CG.-..C.T
Cutleria multifida      GA..A....GC-..GCGGCGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCTGATT.T...CG.-..C.T
Ascophyllum nodosum     AG..G..C.GC-A.ACGA.GT..TTCG......T..........A..AT..A...........AT......G.ACACC.TA.....TT..C..-T.C.T
Durvillaea antarctica   GT..A....GC-..TCGGCGA.G.GCG.....-T........A..A..AT..A...........AT.....GAT..CCCCCGC....TT.ACG.-..C.T
Fucus vesiculosus       AG..A..C.GC-A.ACGA.GT.CTTCG.....-T........A..A..AT..A...........AT.....TG.ACACC.CA.....T...C..-T.C.T
Sargassum muticum       GTT.G....GC-..GCGGCGG.G.TCG.....-T........A..A..AT..A...........AT.....T.CG.TCCCCGC....T...TG.-T.C.T
Xiphophora chondrophylla GA..A....GC-G.ATGA.AT.-.GCT.....-T........A..A..AT.............AT.......GGCT..T.T.C....ACCAA.-G.C.T
Notheia anomala         GT..G....GC-..TCGGCGGAG.AA.......-C........A..A..AT..T.........T.AT.....AT...TCCCCGC.......ACG.-T.C.T
Ascoseira mirabilis     GT..G....GC-..TTGGCGG.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCG.C...T.T.CG.-..C.T
Nemoderma tingitanum    GA..G....GC-..GCG.TG..G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCCCA........CCG.-..C.T
Alethocladus corymbosus GT..G....GC-..GCGAC.GAG.GCG.....-T........A..A..AT.GA..........AT.....T.T..CCCC.GTT...T..CCG.-..C.T
Cladostephus spongiosus GT..G....GC-..GCGAC.GAG.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCC.GCC...C..CCG.-..C.T
Herpodiscus durvillaeae GA..G....GC-..GTGAC.GAG.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCC.GCC...C..CCG.-..C.C
Sphacelaria cirrosa     GT..G....GC-..GCG.C.GAG.GCG.....-T........A..A..AT.GA..........AT.....T.T..CCCC.GCA......CCG.-..C.T
Stypocaulon scoparium   GT..G....GC-..GCGAT.GAG.G.G.....-T........A..A..AT..A.........T.AT.....T.T..CCCC.GT......AGTG.-..C.T
Syringoderma phinneyi   GA..G....GC-..TTGTC.G.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCC.GTATTGTATCAGC.T.C.T
Choristocarpus tenellus GAT.G....GC-...CGGC.G.G.GGT.....-T..C......G.A..AT.GT.........G.AT.....T.G.TCCCC.GC.....TCGCGC...C.T
Onslowia endophytica    GT..G....GC-...CG.C.G.G.GCG.....-T........A..A..AT..T...........AT.....T.T..CC.C.GT......CG.CG-..C.G
Verosphacela ebrachia   G...G...TGC-.A.CGGC.G.G.GCG.....-T........A..A..AT..A...........AT.....T.T..CCCC.GC.....GTC.GT...C.T
Dictyota dichotoma      GT..G....GC-...TGTC.GCG.GC.......-TGA.....AT.A..AT..A...........AT..........CCGC.GTACCGATCA.CG-..C.T
Taonia atomaria         GA..G...TGC-...TGTC.GTG.GC.......-TGA.....AT.A..AT..T.........T.AT.....T.G..CCGC.GTAT.C.ATCAGC...CAG
```

D 3.3: Alignment of the 26S nrDNA gene (continued).

D 3.3: 26S alignment (continued)
positions excluded:

```
                                2222222222222222222222222222222222222222222222222222222222222222222222222222222222222222222222222223
                                0000000001111111111222222222233333333334444444444555555555566666666667777777778888888889999999990
                                1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
Tribonema aequale               TCGGTCGGGCCTCGACGAGTCGAGTTGCTTGGGATTGCAGCTCAAAGCGGGTGGTAAATTCCATCCAAGGCTAAATATGGGTGGGAGACCGATAGCGAAC
Adenocystis utricularis         A.....C.TTT..T.....................................................T..A......C.C.....................
Ascoseirophila violodora        A.....C.TTT..T.....................................................T..A......C.C.....................
Caepidium antarcticum           A.....C.TTT..T.....................................................T..A......C.C.....................
Chordaria flagelliformis        A.....C.TTT..T.....................................................T..A......C.C.....................
Dictyosiphon foeniculaceus      A.....C.TTT..T.....................................................T..A......C.C.....................
Ectocarpus siliculosus          A.....C.TTT..T.....................................................T..A......C.C.....................
Laminariocolax tomentosoides    A.....C.TTT..T.....................................................T..A......C.C.....................
Laminarionema elsbetiae         A.....C.TTT..T.....??????????????????????????????????????????????????????????????????????????????
Myrionema strangulans           A.....C.TTT..T.....................................................T..A......C.C.....................
Punctaria sp.                   A.....C.TTT..T.....................................................T..A......C.C.....................
Pylaiella littoralis            A.....C.TTT..T.....................................................T..A......C.C.....................
Scytosiphon lomentaria          A.....C.TTT..T.....................................................T..A......C.C.....................
Xiphophorocolax aotearoae       A.....C.TTT..T.....................................................T..A......C.C.....................
Asterocladon lobatum            A.....C.TTT..T.....................................................T..A......C.C.....A...............
Asteronema rhodochortonoides    A.....C.TTT..T.....................................................T..A......C.C.....................
Alaria esculenta                A.....C.TTT..T.A...................................................T..A......C.C.....................
Laminaria digitata              A.....C.TTT..T.A...................................................T..A......C.C.....................
Macrocystis pyrifera            A.....C.TTT..T.A...................................................T..A......C.C.....................
Undaria pinnatifida             A.....C.TTT..T.A...................................................T..A......C.C.....................
Sporochnus pedunculatus         A...C.C.TTT..T.....................................................T..A......C.C.....................
Haplospora globosa              A.....C.TTT..T.....................................................T..A......C.C.....................
Tilopteris mertensii            A.....C.TTT..T.....................................................T..A......C.C.....................
Desmarestia aculeata            A...C.C.TTT..T.....................................................T..A......C.C.....................
Himantothallus grandifolius     A...C.C.TTT..T.....................................................T..A......C.C.....................
Phaeurus antarcticus            A...C.C.TTT..T.....................................................T..A......C.NNNNNNNNNNNNNNN.....
Scytothamnus australis          A...C.C.TTT..T.....................................................T..A......C.C.....................
Splachnidium rugosum            A.....C.TTT..T.....................................................T..A......C.C.....................
Cutleria multifida              A.....C.TTT..T.A...................................................T..A......C.C.....................
Ascophyllum nodosum             G.....C.CTT..T.T.....................T.............T..A........C.C.....................
Durvillaea antarctica           AT....C.TTT..A.....................T.............T..A......C.C.C.....................
Fucus vesiculosus               G.....C.CTT..T.T.....................T.............T..A........C.C.....................
Sargassum muticum               A....GC.CTT..T.....................................................T..A........CC.....................
Xiphophora chondrophylla        A.....C.CTT..T.....................T.............T..A......C.C.C.G.....................
Notheia anomala                 A...C.C.CTT........................T.............T..A......C.C.A.....................
Ascoseira mirabilis             A...C.C.TTT..T.....................................................T..A......C.C.....................
Nemoderma tingitanum            A...C.C.TTT..T.A...................................................T..A......C.C.....................
Alethocladus corymbosus         A...C.C.TTT..T.A...................................................T..A......C.C...G.................
Cladostephus spongiosus         A.....C.TTT..T.A...................................................T..A......C.C...G.................
Herpodiscus durvillaeae         A...C.C.TTT..T.A...................................................T..A......C.C...G.................
Sphacelaria cirrosa             A...C.C.TTT..T.A...................................................T..A......C.C...G.................
Stypocaulon scoparium           A.....C.TTT..T.A...................................................T..A......C.C...G.................
Syringoderma phinneyi           A...C.C.TTT..T.A.....................................A.....T..A.........T.....................
Choristocarpus tenellus         A.....C.CTT..T.....................G.............T..A......C.G.T.C.....................
Onslowia endophytica            A...C.C.TTT....A.....................T.............T..A......C.C...G.....................
Verosphacela ebrachia           A...C.C.TTT....A...................................................T..A......C.C...G.....................
Dictyota dichotoma              C...C.C.CTT.....................................A.........C.........A......C.C...G.....................
Taonia atomaria                 AA..C.C.TTT.....................................TG.........C.........A......C.C...G.....................
```

D 3.3: 26S alignment (continued)
positions excluded:

```
                                  3333333333333333333333333333333333333333333333333333333333333333333333333333333333333333333333333334
                                  0000000001111111111222222222233333333334444444444555555555566666666667777777777888888888899999999990
                                  1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890

Tribonema aequale                 AAGTACCGTGAGGGAAAGATGAAAAGAACTTTGGAAAGAGAGTTAAA-AGTGCTTGAAATTGCTGAAAGGGAACCGAAGGG-AGGCAGTG-CTGGTTCAG
Adenocystis utricularis           .....................C...........A.............G.....C...................G......A..AC.....T....CGGTT
Ascoseirophila violodora          .....................C...........A.............G.....C...................G......A..AC.....T...A.AGTT
Caepidium antarcticum             .....................C...........A.............G.....C...................G......A..AC.....T....CGGTT
Chordaria flagelliformis          .....................C...........A.............G.....C...................G......A..AC.....T...ACAGTT
Dictyosiphon foeniculaceus        .....................C...........A.............G.....C...................G......A..AC.....T...ACAGTT
Ectocarpus siliculosus            .............-.......C..-.......-A.............G.....C.......-...........G......A..AC.....T...-CGGTT
Laminariocolax tomentosoides      .....................C...........A.............G.....C...................G......A..AC.....T...ACAGTT
Laminarionema elsbetiae           ????????????????????????????????????????????????????????????????????????????????????????????????????
Myrionema strangulans             .....................C...........A.............G.....C...................G......A..AC.....T....CG.TT
Punctaria sp.                     .....................C...........A.............G.....C...................G......A..AC.....T...ACAGTT
Pylaiella littoralis              .....................C...........A.............G.....C...................G......A..AC.....T...ACAGTC
Scytosiphon lomentaria            .....................C...........A.............G.....C...................G......A..AC.....T....CGGTC
Xiphophorocolax aotearoae         .....................C...........A.............G.....C...................G......A..AC.....T...ACAGTT
Asterocladon lobatum              .....................C...........A.............G.....C...................G......A..AC.....T...ACAGTT
Asteronema rhodochortonoides      .....................C...........A.............G.....C...................G......A..AC.....T...ACGTTT
Alaria esculenta                  .....................C...........A.............G.....C...................G......A..AC.....TA...CGTC.
Laminaria digitata                .....................C...........A.............G.....C...................G......A..AC.....T....CGTC.
Macrocystis pyrifera              .....................C...........A.............G.....C...................G......A..AC.....T....CGTC.
Undaria pinnatifida               .....................C...........A.............G.....C...................G......A..AC.....TA...CGTC.
Sporochnus pedunculatus           .....................C...........A.............G.....C...................G......A..AC.....T....CGGT.
Haplospora globosa                .....................C...........A.............G.....C...................G......A..AC.....T...TCAG.T
Tilopteris mertensii              .....................C...........A.............G.....C...................G......A..AC.....T...TCAG.T
Desmarestia aculeata              .....................C...........A.............G.....C...................G......A..AC.....T...AC...T
Himantothallus grandifolius       .....................C...........A.............G.....C...................G......A..AC.....T...ACATCT
Phaeurus antarcticus              ....NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN.NAC.....T...ACNTCA
Scytothamnus australis            .....................C...........A.............G.....C...................G......A..AC.....T....CGGCT
Splachnidium rugosum              .....................C...........A.............G.....G...................G......A..AC.....T....CGTTT
Cutleria multifida                .....................C...........A.............G.....C...................G......A..AC.....T...TCA.GT
Ascophyllum nodosum               .....................C...........A.........G...A.....C...................G......AT.AC.....T....CGGTT
Durvillaea antarctica             .....................C...........A.............G.....C...................G......A..AT.....T..TCGGTT
Fucus vesiculosus                 .....................C...........A.........G...G.....C...................G......ATTAT.....T....CGGTT
Sargassum muticum                 .....................C...........A.............G.....C...................G......A...C.....T....CGGTT
Xiphophora chondrophylla          .............A.......C...........A.............G.....C......G............G......AA.AT.....T....CGTTT
Notheia anomala                   .....................C...........A.............G.....C...................G......A..AT.....T.....GTTT
Ascoseira mirabilis               .....................C...........A.............G.....C...................G......A..AC.....T...ACAGTT
Nemoderma tingitanum              .....................C...........A.............G.....C...................G......A..AC.....T...TCGGTT
Alethocladus corymbosus           .....................C...........A.............G.....C...................G......A..A......T....CGGCT
Cladostephus spongiosus           .....................C...........A.........G...G.....C...................G......A..A......T.....GGGT
Herpodiscus durvillaeae           .....................C...........A.............G.....C...................G......A..A......T....CGG.T
Sphacelaria cirrosa               .....................C...........A.............G.....C...................G......A..A......T....CGG.T
Stypocaulon scoparium             .....................C...........A.............G.....C...................G......A..A......T....CGGTT
Syringoderma phinneyi             .....................C...........A.............G.....C...................G......A..A......T.....AGTC
Choristocarpus tenellus           .....................C...........A.............G.....C...........G.......G.........A.......-....GGC.
Onslowia endophytica              .....................C...........A.............G.....C...................G......A..A......T...ACGGTC
Verosphacela ebrachia             .....................C...........A.............G.....C...................G......A..A......T...ACGGTC
Dictyota dichotoma                .............-.......C...........A.............G.....C.T.........G.......G......A..A.AG...TGC....GCC
Taonia atomaria                   .............-.......C...........A.............G.....C...........G.......G......A..A.AG...TGC...CGCC
```

D 3.3: Alignment of the 26S nrDNA gene (continued).

D 3.3: 26S alignment (continued)
positions excluded:

```
                                4444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444445
                                0000000001111111111222222222233333333334444444444555555555566666666677777777778888888888999999999990
                                1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890

Tribonema aequale               TGCGTC--TTGCCCCTGGG-CC-TCGGCCCCGGGTGCTGCTCG-CTGAGCCTTG-TCAGGGC--GGGTTCTCTGTCCGCGGGAT-CTGTCGTCGTCGGAG
Adenocystis utricularis         CCGC--....CT..T...-...CCTC.GGG.CTT-.G.TTG..TT.CC...G..GC......C.....AG-TGT.--.......GC...CGC.TC-G.A..
Ascoseirophila violodora        .CGT--....CT..TCC.-...C.TC.GGG..TT-.GCTTG..TTCCTAT.G..GC......T.T...GG-.GT.--.......GC...CGCCT.-G.A..
Caepidium antarcticum           AAGC--....TTT.TC..-...CCTC.GGG.CTT-.G.TTG..TT.CC...G..GC......C.....AG-TGT.--.......GC...CGCCTC-G.A..
Chordaria flagelliformis        CCGC--....CG....T.-...C.TC.GG.GTT.-.G.TTG..TTCCT.T.G..GC......C.....GG-.GT.--...N..GC...CGCCGT-G....
Dictyosiphon foeniculaceus      .CGT--....C...ACC.-.T.C.TC.GGGTT.T-.GCTTG..TTCCT.T.G..GC......C.....AG-.GT.--...T..GC...CGCCGT-G....
Ectocarpus siliculosus          CCGC--....CT..T.T.-.T.CCTC.GG-.ATT--G.TT-..TT.CC...G..GC......C.....AG-TGT.--....-.-T...CGCCTC-G.A..
Laminariocolax tomentosoides    .CGC--....CA..T...-...C.TC.GGG.TTT-.G.TTG..T.CCTAT.G..GC......T.T...AG-.GT.--......GT...CGCCTT-G.A..
Laminarionema elsbetiae         ????????????????????????????????????????????????????????????????????????????????????????????????????
Myrionema strangulans           ACGT--....CT..TCC.-...CCTC.GGG.TCT-.G.TTG..TT..T.T.G..GC......T.....AG-.GT.--......GC...CGCCTT-G.A..
Punctaria sp.                   .CGC--....CT....C.-...C.TC.GGG.TTT-.G.TTG..TTCCT.T.G..GC......C.....GG-.GT.--......GC...CGCCTC-G....
Pylaiella littoralis            .TGC--....CT..TCC.-...CCTC.GGG..TT-.G.TTG..TT.CT.T.G..GC......C.....AG-.GT.--......GT...CGC.TC-G.A..
Scytosiphon lomentaria          .CGC--....CT..T...-...CCTC.GGG.CTT-.G.TTG..TT.CC...G..GC......C.....AG-.GT.--......GG...CGC.TC-G.A..
Xiphophorocolax aotearoae       .CG---....T....CTT....CCTC.GGG.CT.-.GCTTG..TTCCT.T.G..GC......C.....AG-.GT.--......GC...CGCCTT-G.A..
Asterocladon lobatum            ATGC--....CT..T.T.-...CCTC.GGG..CT-.G.TTG..T..CT.T.G..GC......C.....GG-TGC.--...A...C...CGCCTC-G.A..
Asteronema rhodochortonoides    AAGC--....CT..T.T.-...CCTC.GG-..CT-.G.CTG.TTT.CT.T.G.AGC......C.....GT-TGCC--......GC...CGCCTC-G.A..
Alaria esculenta                AAGC--....T...TCC.-...CCTC.GGG.TCT-.GGT.G..T..TC......GC.....TC.....AT-GGT.--......GT...CGTCG.-G.A..
Laminaria digitata              AAGC--....T...TCC.-...C.TC.GGG.TCT-.GGT.G..T..TC...G..GC.....TC.....AT-GGT.--......GC...CGTCGT-G.A..
Macrocystis pyrifera            AAGC--....T...ACC.-...CCTC.GGG.T.C-.GGT.G..T..TC...G..GC......C.....A.-GGT.--......GC...CGCCGC-G....
Undaria pinnatifida             AAGC--....T...TCC.-...CCTC.GGG.TCT-.GGT.G..T..TC......GC.....TC.....A.-GGT.--......GC...CGCCG.-G.A..
Sporochnus pedunculatus         AAGC--....CT..TCC.-...CCTC.GGG..TT-.G..TG..T..CC...G..GC......C....CAG-GGT.--.T....GT...CGCC.C-G....
Haplospora globosa              CCGC--....C...TCC.-...C.TC.GGG..TT-.GCTTG..TT.TT.T.G..GC......C.....AG-TGT.--......GT...CGCCTC-G.A..
Tilopteris mertensii            CCGC--....CT..TCC.-...C.TC.GGG..TT-.GCTTG..TT.TT.T.G..GC......C.....AG-.GT.--......GT...CGCCTC-G.A..
Desmarestia aculeata            CCGC--....CT..T.C.-...CCTC.----NTT-.G.TTG..TT....T.G..GC......C.....CG-AGT.--...N..GC...CACCTC-G.AR.
Himantothallus grandifolius     CCGC--....CT..TCT.-...C.TC.GGG..TT-.G.TTG..TT.TT.T.G..GC......C.....GG-AGT.--......GC...CACCTC-G.A..
Phaeurus antarcticus            ACRC--....CT..TCT.-...CCTC.GSG-.TT-.G.TTG..TT.TT.T.G..GC......C.....GG-AGT.--......GT...CACCTC-G.AR.
Scytothamnus australis          CCGC--....C.T.A...-...CCTC.GGG.CTT-.AAT.G..TTGCC.T.G..GC......T.....AG-AGTC--......GC...CGCCTC-G.A..
Splachnidium rugosum            .CGC--....A..TT.T.-...CCTC.GGG.ATT-.GATTG..TT.AT.T.G..GC......C.....AG-AGT.--......GC...CGCCTC-G.A..
Cutleria multifida              CCGC--....CT..TCT.-...CCTC.GGG.TCT-.GATTG..TT.CT.G.G..GC....A.C.....AG-TGT.--......GT...CGCCTC-G.A..
Ascophyllum nodosum             CCGC--....CT..TGC.-.TTCCTC.GAA..CT-.GAT.G..TT.CC.T.G..G....T..C....C.AG-.GTC--...A..GC...CGT.AC-G....
Durvillaea antarctica           .TGC--....CT..G..T.T.TT.T..GA-..TTC.GATTG..TT.CC.T.G..GC..TA..C.....GT-..T.--......GC...CGTCG.-G....
Fucus vesiculosus               CCGC--....CT..TGC.-.TTCCTC.GAA..CT-.GAT.G..TT.CC.T.G..G....T..C...C.AG-..T.--......GC...CGTCAC-G....
Sargassum muticum               CTGC--....CT..GAC.-.GTC..C.GA...TC-.GGTTG..CT.CC..TG.TGC......C.....CG-GGCC--......AT.G.CGTC.C.G....
Xiphophora chondrophylla        CCGC--....CT..T.C.-.TTCCTC.GAA..TT-.GAT.G..TT.CC.T.G..GC......T.....AT-TGTC--......GC...CGTAAT-G....
Notheia anomala                 .TGC--....CT..GAC.-.GTC.TC.GAT..CC-.GGC.G..TT..C...G..G.......C.....A..T..AT-.......C..T.GTCTT-G....
Ascoseira mirabilis             CCGC--....CG..T.C.-...CC.C.GGG..TT-.G.TTG..TT.CT.T.G..GC......C.....AG-GGT.--......GT...CGCCTC-G.A..
Nemoderma tingitanum            CCGC--....CT..TCC.-...C..C.GGG..TT-.G.TTG..TT.CC.T.G..GC......C.....AG-..T.--......GT.G.CGCCTC-G....
Alethocladus corymbosus         .CGC--....CT..GG..C.G.C.TC.GGT.CTC-.G.C.G..CT.CC...G..GC...C..C.....CT-T.TC--.......G..TCGCCTC-G....
Cladostephus spongiosus         .CGC--....AT..GGA.-.A.C.TC.GGT.CCC-.GCT.G..CTCCC...G..GC...C..C........-GGT.--.......G..TCGCCTC-G....
Herpodiscus durvillaeae         CCGC--..C.CT..GG..-TA.C.TC.GGT.CCC-.GCTTG..CT.CC...G..GC...C..C.....CG-GGTC--......CG..TCGTCTC-G....
Sphacelaria cirrosa             .CGC--....AT..GA.A-.G.CCTC.GG.TCCC-.GCT.G..CT.CC...G.AGC...C..C.....C.-GGCC--.......G..TCGCCTC-G....
Stypocaulon scoparium           .CGC--....CT..GG.A-TA.C.TC.GGTTCTC-.G.CTG..TT.CC...G..GC...C..C.....CT-T.TC--.......AG..TCGCCTC-G....
Syringoderma phinneyi           .CGC--....CT..TCTA-...C.TC.GGGT.TT-.G.CTG..TT.TTA..G..GC......C.....AT-GGT.--...CC.AG.GAAT..TCGG.A..
Choristocarpus tenellus         .C....GTC.....AA.C.......C.GG.GCT.G.GC..G..T.G.C...G..GC..............-.C.GG-......CG.G..G.GGCGG.T..
Onslowia endophytica            .CGC--....CT..G.T.-...CCTC.GGG...CG.GCTTG..T..CC.T.G..GC....AGC.....GA-GGTC--.......G..TCG.GTC-G.A..
Verosphacela ebrachia           .CGC--..C.C...G.C.-..TT.TC.AAG...CG.GCTTG..T..CC.T.G..GC....A.C.....GG-GGC.--......CG...CGCG.CGG.T..
Dictyota dichotoma              C.G.CGCCAAC...TACC-.-TG.TC..AGGT..G.G.AGG.CG.CCG...GC.G......GTA.C.CCG-GGC..-.G....AGAGCCGTCG.GGATC.
Taonia atomaria                 G.A.CGCCAACT...ATT....C...TGGTGT..G.G.AGG.CG..CC...GCTG....C.GTT.C.CCG-GGC..-......GG.CCCGCG.CGGATC.
```

D 3.3: Alignment of the 26S nrDNA gene (continued).

D 3.3: 26S alignment (continued)
positions excluded:

```
                                5555555555555555555555555555555555555555555555555555555555555555555555555555555555555555555555555556
                                0000000001111111111222222222233333333334444444445555555556666666666777777777788888888899999999990
                                1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890

Tribonema aequale               GTAGG---GTGC--------------------TT-------GCATCTGAA--ACGTCGTCG-TCTT-GT-CGCGGGCTGGACCGAGGCAGGGGAGTGGTAT
Adenocystis utricularis         ....CAT.C.C-.....................CG......A..G.....G..CGT.G....G.C...CGCGAC.....----------------------
Ascoseirophila violodora        ....CAT.C.C-.....................CG......AG.G.....G..C.G.G....G.C...CGCGAT.....----------------------
Caepidium antarcticum           ....CAT.C.C-.....................CG......AG.G.....G..CGT.G....T.....CGCGAC.....----------------------
Chordaria flagelliformis        ....CAT.C.C-.....................CG......AG.G.....G..C...G....G.C...CGCGAT..C..----------------------
Dictyosiphon foeniculaceus      ....CAT.C.C-.....................CG......AG.G.....G..C...G....G.CC..CGCGAC.....----------------------
Ectocarpus siliculosus          ....CAT.C.C-.....................CG......AG.G.....G..CGT.G....G.C...CGCGAC.....----------------------
Laminariocolax tomentosoides    ....CAT.C.C-.....................CG......AG.G.....T..C.G.G....G.C...CGCGAT.....----------------------
Laminarionema elsbetiae         ??????????????????????????????????????????????????????????????????????????????????????????????????
Myrionema strangulans           ....CAT.A.C-.....................CG......AT.G.....T..C...G....G.C...CGCGAC.....----------------------
Punctaria sp.                   ....CAT.C.C-.....................CG......AG.G.....G..C.G.G....G.C...CGCGAC.G...----------------------
Pylaiella littoralis            ....CGT.C.C-.................TC..TT......AGCG.....G..CGT.G....G.C...CGCGAT.....----------------------
Scytosiphon lomentaria          ....CGT.C.C-.............GA...C..GTC.....AGCG.....G..CGT.G....T.C...CGCGAT.....----------------------
Xiphophorocolax aotearoae       ....CAC.A.C-.....................CG......AG.G.....G..C.NNG....G.C...CNNGAC.....C--------------------
Asterocladon lobatum            ....CTT.C.C-.....................GCG.....AGAG.....T..CGT.G....G.C...CGCG.C.GC..----------------------
Asteronema rhodochortonoides    ....CTT.A.C-.....................CG......ATAG.....T..CGT.G....GAC...CGCG.C.GC..----------------------
Alaria esculenta                ....CCT.C.C-.....................CG......AGGG.....G..CC..G....G.C...CGCGACTG...----------------------
Laminaria digitata              ....CCT.C.C-...................C.CG......AGGG.....T..CG..G....G.C...CGCGACTG...----------------------
Macrocystis pyrifera            ....CCT.-.C-.................CC.CGG.....A.GG.....TG.CG..G....G.C...CGCGACTG...----------------------
Undaria pinnatifida             ....CCT.C.C-.....................CG......AGGG.....G..CC..G....G.C...CGCGACTG...----------------------
Sporochnus pedunculatus         ....CCT.C.C-...................C.CG......AGGG.....T..CG..GGCG.G.C..CCGCGA.C....----------------------
Haplospora globosa              ..G.CCT.A.C-...................C.CG......ATGG.....T..CGT.G....T.C...CGCGAC.....----------------------
Tilopteris mertensii            ....CCT.A.C-...................C.CG......ATGG.....T..CGT.GT...T.C...CGCGAC.....----------------------
Desmarestia aculeata            ....CCT.C.C-.....................CG......AGGG.....T..CGT.GT...G.C...NGCGATT....----------------------
Himantothallus grandifolius     ....CCT.C.C-...................C.CG......AGGG.....T..CGT.GT...G.C...CGCGATT....----------------------
Phaeurus antarcticus            ....CNT.C.C-...................N.CG.....NAGGG.....G..CGT.GT...G.C...CGCGATT....----------------------
Scytothamnus australis          .....CA...C-...................C.CG......ACG......T..CGT.G....G.C...CGCGA.T....----------------------
Splachnidium rugosum            ....CTC...C-...................C.CG......ACGG.....T..CGT.GT...G.C...CGCGATT....----------------------
Cutleria multifida              ....CTC.C.C-...................C.CG......AGGG.....T..CGT.G....TAC...CGCGAC.....----------------------
Ascophyllum nodosum             ....-CGT.C.C-...................C.CG......AGATG....TG.CGT.G....G.C...CGCGA.....T--------------------
Durvillaea antarctica           ...C-GT.C.C-.....................CG......AGAAG....TG.CCTTG....G.C...CGCGA..A??????????????????????????
Fucus vesiculosus               ...C.T..C.C-.....................C.CG......AGATG....TG.CGT.G....G.C...CGCGA.....----------------------
Sargassum muticum               ...-CGC.C.C-.....................CG......AGGTG....TG.CGG.GT.C...CC..CGCG.CCT...G--------------------
Xiphophora chondrophylla        ...C-GT.C.C-.....................CG......AGAGG....TG.CAT.A....T.....CGCGAT.T...----------------------
Notheia anomala                 ...C.T..C.C-.....................CG......AGAGG....TG.C.A.AT...GAC...CGCGTTTG...----------------------
Ascoseira mirabilis             ....CCT.C.C-.....................CG......AGGG.....T..CGT.G....G.C...CGCGACT....----------------------
Nemoderma tingitanum            ...C.T..C.C-.....................CG......AGAGG....T..CGT.G..T.T..T..CGCGA.....----------------------
Alethocladus corymbosus         ....CGT.C.C-.............ACTC..TTG.T...AGCG.....T..CGT.GGCG.T.....CGCGATTGG..----------------------
Cladostephus spongiosus         ....CGC.C.C-.....................A..C.G.T...AGCG.....G..CGT.G....T.....CGCGACCGG..----------------------
Herpodiscus durvillaeae         ....CGC.C.C-.............ACC.CGG.T...AGCG.....T..CGT.G....T.....CGCGACCGG..----------------------
Sphacelaria cirrosa             ....CGN.C.C-.................GTC.CGGC....AGCG.....T..CGT.G....T.....CG.GCC.GG.C--------------------
Stypocaulon scoparium           ....CGT.T.C-.............ATTCT..TTGGT...A.CG.....T..CGT.G....T.....CGCGA.AGG..----------------------
Syringoderma phinneyi           ....-...-.C-.....................TT.....TA.GA.....T..CCG.A....TCA...CGCGACTG...----------------------
Choristocarpus tenellus         .....GT.C...GGCCGGCGCCCCGAGC.G.-.CGGTT..CAGC.C....T-GCCGCC.C........T...ATT.GCCG...CCCGG...T--------
Onslowia endophytica            ........C.C-.....................G.CCGC....AG..-....T..CGTCC....T.C...CGCGATCTC..----------------------
Verosphacela ebrachia           .....CT.C-C-.....................C.CGG.....AG.-.....TG.CGGCG....T.C..CCGCG..C.C..----------------------
Dictyota dichotoma              .....C..----.............GAGCCGC..CGGTG...TGCGCTGTC..G.C.CCG...G--...TGTT.CC..-..G.----------------------
Taonia atomaria                 ..-..CC.C.C-.................CCC-.CGG.G...AGGG-TGTC..G.C..G....---...G..GC.C.GC..GACGACTC..----------
```

D 3.3: Alignment of the 26S nrDNA gene (continued).

D 3.3: 26S alignment (continued)
positions excluded:

```
                                  6666666666666666666666666666666666666666666666666666666666
                                  0000000001111111111222222222233333333334444444444455555
                                  1234567890123456789012345678901234567890123456789012345

Tribonema aequale                 TTATTTTGCTGCTTCCTCCG-GCC-----CTGTCGAAAT--GCTTCCCTTTAC
Adenocystis utricularis           ----------------.T...TT........A.........G..TT......
Ascoseirophila violodora          ----------------.A...TT........A.........G..TT......
Caepidium antarcticum             ----------------.T...TT........A.........G..TT......
Chordaria flagelliformis          ----------------.....TT........A.........G..TT......
Dictyosiphon foeniculaceus        ----------------.....TT........A.........G..TT......
Ectocarpus siliculosus            ----------------.T...TT........A.........G..TT......
Laminariocolax tomentosoides      ----------------.A...TT........A.........G..TT......
Laminarionema elsbetiae           ??????????????????????????????????????????????????????
Myrionema strangulans             ----------------.....TT........A.........G..TT......
Punctaria sp.                     ----------------.....TT........A.........G..TT......
Pylaiella littoralis              ----------------.....TACC......A.........G..TT......
Scytosiphon lomentaria            ----------------.....TT........A.........G..TT......
Xiphophorocolax aotearoae         ----------------.....TT........A.........G..TT......
Asterocladon lobatum              ----------------....T.........A.........G..TT......
Asteronema rhodochortonoides      ----------------.....T.........A.........G..TT......
Alaria esculenta                  ----------------......T........A.........G..TT......
Laminaria digitata                ----------------......T........A.........G..TT......
Macrocystis pyrifera              ----------------......T........A.........G..TT......
Undaria pinnatifida               ----------------......T........A.........G..TT......
Sporochnus pedunculatus           ----------------.....T.......NNA.........G..TT......
Haplospora globosa                ----------------.....TT........A.........G..TT......
Tilopteris mertensii              ----------------.....TT........A.........G..TT......
Desmarestia aculeata              ----------------.....TT........A.........G..TT......
Himantothallus grandifolius       ----------------.....TT........A.........G..TT......
Phaeurus antarcticus              ----------------....NN-........A.........G..TT......
Scytothamnus australis            ----------------.....TT........A.....N...G..TT...N..
Splachnidium rugosum              ----------------.....TT........A.........G..TT...C..
Cutleria multifida                ----------------...............A.........G..TT......
Ascophyllum nodosum               ----------------.....G.........A.........G..TT...C..
Durvillaea antarctica             ??????????????????????????????????????????????????????
Fucus vesiculosus                 ----------------.....T.........A.........G.ATT...C..
Sargassum muticum                 ----------------.T...T.........A.A.......GC.TT...C..
Xiphophora chondrophylla          ----------------.T...TT........A.........G..TT...C..
Notheia anomala                   ----------------...............A.........G..TT......
Ascoseira mirabilis               ----------------.....TT........A.........G..TT......
Nemoderma tingitanum              ----------------.T...T.........A.........G..TT...C..
Alethocladus corymbosus           ----------------...C..G........A.........T..TT......
Cladostephus spongiosus           ----------------...C..G........A.........???????????
Herpodiscus durvillaeae           ----------------...C..G........A............TT......
Sphacelaria cirrosa               ----------------.GC...G........A.........N..TT......
Stypocaulon scoparium             ----------------...C..G......NNA......??????????????
Syringoderma phinneyi             ----------------.....TT........A............TT......
Choristocarpus tenellus           ----------------..C...G........A............TT......
Onslowia endophytica              ----------------.....T.........A............TT...C..
Verosphacela ebrachia             ----------------...............A.........T..TT......
Dictyota dichotoma                ----------------.......CCCGT...G..GTCGCG....TT...C..
Taonia atomaria                   ----------------..C...T........G....CGCC....TT...C..
```

D 3.4: Alignment of the *rbc*L gene.

D 3.4: *rbc*L alignment

```
                                  000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000001
                                  000000000011111111112222222222333333333344444444455555555556666666667777777777888888888899999999990
                                  123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890123456789
Tribonema aequale                 AGTAGTATGGACGGATTTATTAACAGCTTGTGATATCTACCGTGCGAAAGCATACCGTGTAGATCCTGTACCTGGTACAACTGATCAATTCTTCGCTTAC
Adenocystis utricularis           G.....T.....A..........T........C..T..TA.A..A........TA.A........G.....G..A....G...........T......
Ascoseirophila violodora          ......T.....A..........T..........T..TA.A..A.....G..TA.A........A...........G...........T.....T
Caepidium antarcticum             T.....T.....A..........T........C.....TA.A..........TA.A........A........A....G...........T..C..T
Chordaria flagelliformis          ......T.....A..........T........C.....TA.A..........TA.A........A........A....G...............T
Dictyosiphon foeniculaceus        ......T.....A..........T............TA.A.......T..TA.A........A.....G...C........T.....T
Ectocarpus siliculosus            ...G..T.....A..........T............TA.A..A........TA.A........A.....A..C....G...........T.....T
Laminariocolax tomentosoides      ......T.....A..........T............TA.A..........TA.G........A........A....G...........T.....T
Laminarionema elsbetiae           ......T.....A..........T............TA.A..........TA.A........A.....G....G...........T.....T
Myrionema strangulans             G.....T.....A..........T............TA.A..........TA.A........A.....C..A....G...........T.....T
Punctaria sp.                     ......T.....A..........T..........T..TA.A..........TA.A........C........A....G...........T.....T
Pylaiella littoralis              T.....T.....A..........T.............A.A........T...A.A........A...........G...........T.....T
Scytosiphon lomentaria            G.....T.....A..C.......T..........T..TA.A..A........TA.A........A.....C..A....G..C.........T......
Xiphophorocolax aotearoae         ??????????????????????????????????????????????????????????????????????????????????????????????????
Asterocladon lobatum              T.....T.....A..C........T........C..T...A.A..A........TA.A..................A....GC.............G..T
Alaria esculenta                  ......T.....A..C........T.....C........TA.A..A........TA.A........A........A....GC..C....A...T..A...
Macrocystis pyrifera              G.....T.....A..C........T.....C........TA.A..A........TA.A........A........A....GC..C.........T..A..T
Sporochnus pedunculatus           ......T............C....T........C.....TA.A..A.....C..TA.A..G.....A........A....G......G.....T..A...
Tilopteris mertensii              ......T.....A......C.....................A.A..A.....T.....A..T.....A........A....AC.....G.AT.........
Desmarestia aculeata              G.....T.................T.....C........TA.A.......T..TA.A..G.............C....G...........T......
Himantothallus grandifolius       G.....T.................T..G..C........TA.A........T..TA.A..G.............C....G......G.....T.....T
Scytothamnus australis            ......T.....A.........................TA.A.......T..TA.A..T..C..A..T.....G....GC..C.....T.....A...
Splachnidium rugosum              ......T.....A.....................C.....TA.A..A.....C..TA.A..T.....A..T.....A....G......G........A..T
Ascophyllum nodosum               ............A..........T........C........A...........T.....G........C..A..A....A........AT..T..A...
Sargassum muticum                 ............A......C.G..G.................A...........T.....C........T..A..A....AC.......AT...T.A..T
Alethocladus corymbosus           ......T.....A..........T..C............A.A..A.....G.....A........A.............G...........T......
Cladostephus spongiosus           ......T.....A..........T..C............A.A..A.....G.....A........A.............G...........T......
Herpodiscus durvillaeae           ......T.....A..............A.C.........TA.A..A.....G.....A........C.............A.........T..T......
Sphacelaria arctica               ......T.....A..........T..C............A.A..A.....G.....A........A.............G...........T......
Sphacelaria caespitula            ......T.....A..........T..C............A.A..A.....G.....A........A.............G...........T......
Sphacelaria californica           ......T.....A..........T...........T..TA.A..A.....T..T..A........A..T..........GC.......A...T.....T
Sphacelaria cirrosa               ......T.....A..........T...........T..TA.A..A.....G..TA.A........A..T..........G.........A...T.....T
Sphacelaria divaricata            ..C...T.....A..........T...........T..TA.A..A.....T..T..A........A..T..........G........A...T.....T
Sphacelaria nana                  ......T.....A..........T..C.....C......A.A..A.....G.....A........A.............G............T..C..T
Sphacelaria plumosa               ......T.....A..........T..C............A.A..A.....G.....A........A.............G...........T......
Sphacelaria plumigera             ......T.....A..........T..C............A.A..A.....G.....A........A.............G...........T......
Sphacelaria racemosa              ......T.....A..........T..C............A.A..A.....G.....A........A.............G...........T......
Sphacelaria radicans              ......T.....A..........T.NN.........A...A.A..A.....A.....A........A.............G.........T..T......
Sphacelaria rigidula              ......T.....A..........T...........T..TA.A..A.....T..T..G........A..T..........G.........A...T.....T
Sphacelaria tribuloides           ......T.....A..........T...........T..TA.A..A.....T..T..A.....C..A..T..........G.........A...T.....T
Sphacelaria yamadae               ......T.....A..........T...........T..TA.A..A.....T..T..A.....C..A..T..........G.........A...T.....T
Sphacella subtilissima            ..C...T.....A..........T..C........T...A.A..A.....G.....A........A.............C.G...........T.....T
Halopteris filicina 1             ??????????????.........T..C............A.A..A.....G.....A........A.............G.........T..T.....T
Halopteris filicina 2             ......T.....A......C....T..C............A.A..A.....G.....A........A.............G.........T..T......
Stypocaulon durum                 ......T.....A..........T..C............A.A..A.....G.....A........A.............GC........T..T......
Stypocaulon scoparium             ......T.....A..........T..C............A.A..A.....G.....A........A.............G......G..T..T.....T
Syringoderma phinneyi             ......T................T..............A.A..A.....T..TA.A..............C..C....G............T..A...
Choristocarpus tenellus           ...T..T.....A.........................T..TA.A..A.....T..TA.A..............AA....G.AA.......T..T.....T
Onslowia endophytica              ...T..T.....A..........................A.A..A.....T..TA.A..............A..C....GC...........T..CC.T
Verosphacela ebrachia             ...T..T.....C..........................TA.A..A.....T.....A..............A....G.............T.....
Dictyota cervicornis              T..T..T.....T..............C..C..C..T..TA.A..A.....T..T..A..........T.....G.............T..A...
Dictyota dichotoma                T..T........T..............A..C..C..T..TA.A..A.....T..TA.A........C.........N...A..N.........T..A..T
```

D 3.4: Alignment of the *rbc*L gene (continued).

D 3.4: *rbc*L alignment (continued)

```
                                 1111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111112
                                 0000000001111111111222222222233333333334444444444555555555566666666667777777777888888888899999999990
                                 1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
Tribonema aequale                ATTGCATACCAATGTGAATTATTTGAAGAAGGTTCAATCGCAAACTTAACTGCATCTATTATTGGTAACGTTTTCGGCTTCAAAGCTGTTAAAGCGTTAC
Adenocystis utricularis          ..A..T..TG.....T.............GC.T..T.........G..A..C..C.............T.............A....
Ascoseirophila violodora         ..A..T..TG.....T.............TC.T..T.....A.....................T..T.................
Caepidium antarcticum            ..A..T..TG.....T...........A..TC.T..T.....A.........C..........T..T.....C.....AC...
Chordaria flagelliformis         ..A..G..TG.....C.............TC.T..T.....A.............T.....T..T.....C.....C....
Dictyosiphon foeniculaceus       ..A..T...G.....T.....G.....TC.T..T.....A.....................T..T.............A....
Ectocarpus siliculosus           ..A..T...G.....T.............TC.T..T..T....A.....................A.................
Laminariocolax tomentosoides     ..A..T...G.....T.....G.....TC.T..T.....A.....................T..T.................
Laminarionema elsbetiae          ..A..T...G.....T.....G.....TC.T..T.....G.....................T..T..G..........C...
Myrionema strangulans            ..A..T...G.....T.........G....TC.T..T.....A.....................T..T.................
Punctaria sp.                    ..A..T...G.....T.............TC.T..T.....A.....................T..T.............A....
Pylaiella littoralis             ..A....TG.....T.....G.....TC.T..T.....A.....................T.............C....
Scytosiphon lomentaria           ..A..T...G.....T.............TC.T..T.....A.....................T.............C....
Xiphophorocolax aotearoae        ??????????????????????????????????????????????????????????????????????????????????????????????????????
Asterocladon lobatum             ..C..T..TG.....T.............TC.T..T.....A.....................T..T.................
Alaria esculenta                 G.C..T..TG.....T.....G.....TC.T..G.....A..C.....................T..T.............T....
Macrocystis pyrifera             .....T..TG.....T.............TC.T..G..TC....A..T.....................T..T.............T....
Sporochnus pedunculatus          ..A..G..TG.....T.........G....TC.T.....A.....................T..T.....C.....A....
Tilopteris mertensii             ..A.....TG.....TC.G..........TC.T.....A.....C.....T.....T..T.............A...A
Desmarestia aculeata             ..A..T...G.....T.............TCAG..T.....A.....C.....T.....T..T.....G.....T....
Himantothallus grandifolius      ..A..T...G.....T.............TC.T..T..T....A.....C.....T.....T..T.....G.....T....
Scytothamnus australis           ..A..T..TG.....T..G..........TC.T.....A.....C.....T..T.....................G.
Splachnidium rugosum             ..A..C..TG.....T.............TC.T..C..T....A..C.....T.....T..T.....A.....A....
Ascophyllum nodosum              .....T..TG.....T.............TC.T..T.....C.....T..A..T..T..T.....A....
Sargassum muticum                .....TG.....T.....G.....TC.T.....C..C.....A..T..T..T.....A....
Alethocladus corymbosus          G.A..T...G.....T.....C.....G..A..TC.T..G..T.....G..C.....T..A.....T..T.....A.....T....
Cladostephus spongiosus          G.A..T..TG.....T.....C....G..A..TC.A..G..T....A.....C.....T..A.....T..T.....T....
Herpodiscus durvillaeae          G.A..T..TGG.....C.....G....A..TC.T.TG..T.....A..C.....T..G..T..T..T.....A....G..T....
Sphacelaria arctica              G.A..T..TG.....T.....C.....G..A..TC.T..G..T....A.....C.....T..A.....T..T.....T....
Sphacelaria caespitula           ..C..T..TG.....T.....C.....G..A...C.T..G..T....A.....C.....T..A.....T..T.....T....
Sphacelaria californica          G.A..T..TG.....T.....G..A..TC.T....T....A.....T..G..T..T.....G..T....
Sphacelaria cirrosa              G.A..T..TG.....T.....G.....TC.T....T....A.....T..A..T..T.....A..A.....T....
Sphacelaria divaricata           G.A..T..TG.....T.....G..A..TC.T....T....A.....T..G..T..T.....T....
Sphacelaria nana                 G.A..T..TG.....T.....C....G..A..TC.T..G..T....A.....C.....T..A.....T.....G..T....
Sphacelaria plumosa              G.A..T..TG.....T.....C.....G..A..TC.T..G..T....A.....C.....T..A.....T..T.....T....
Sphacelaria plumigera            G.A..T..TG.....T.....C.....G..A..TC.T..G..T....A.....C..C....T..A.....T..T.....T....
Sphacelaria racemosa             ..A..T..TG.....T.....C.....G..A..TC.T..G..T....A.....C.....T..A.....T..T.....T....
Sphacelaria radicans             G....T..TG.....T.....C.....G..A..TC.T..G..T....A....A..C..C..G..T..A.....T..T.....A....T....
Sphacelaria rigidula             .....C..TG.....T.....G..A..TC.T..G..T....A..C.....T..G..T..T.....T....
Sphacelaria tribuloides          ..A..T..TG.....T.....G.....TC.T....T....A.....T..A..T..T.....T....
Sphacelaria yamadae              ..A..T..TG.....T.....G.....TC.T....T....A.....T..G..T..T.....T....
Sphacella subtilissima           G.A..T..TG.T..C..T.....C.....A..TC.T..G..T....A..C.....T..A..T..T..T.....T....
Halopteris filicina 1            G....T...G.....T.....G..A..TC.T....T....A....A..C.....T..A.....T.....T....
Halopteris filicina 2            G....T...G.....T.....C.....G..A..TC.T....T....A.....G..C.....T..A.....T..T.....A....T....
Stypocaulon durum                G.C..T..TG.....T.....C.....G..A..TC.T..G..T....A.....G..C.....T..A.....T..T.....A....T....
Stypocaulon scoparium            G.A..T...G.....T.....C.....G..A..TC.T..G..T....A.....G..C.....T..A.....T..T.....A....T....
Syringoderma phinneyi            G.A..T..TG.....T..G.....G..C..TC.T..C.....A.....C.....T..A.....G..T.....A..A....T....
Choristocarpus tenellus          G....T..TG.....T.............TC.T....T....A..G..A.....T..A..T..T.....A...GT..A....
Onslowia endophytica             ..A..T..TG....C..T.....C.....C..TC.T..G..T....A..T.....T..A..T.....A.....TC...
Verosphacela ebrachia            .....T..TG.....T.............TC.T..G..T....A..T.....C.....T.....T.....T....
Dictyota cervicornis             ...K.T..TG....C..T.....C.....T.A.....A..T..A.....G..T..T..T.....T....
Dictyota dichotoma               .....TG.....T.....C.....T.A.....T..GN.A.....A.....T.....T..T..T.....A....
```

D 3.4: Alignment of the *rbc*L gene (continued).

D 3.4: *rbc*L alignment (continued)

```
                                 2222222222222222222222222222222222222222222222222222222222222222222222222222222222222222222222222223
                                 0000000001111111111222222222233333333334444444444555555555566666666677777777778888888889999999990
                                 1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
Tribonema aequale                GTTTAGAAGATATGCGAATTCCTTTTGCATACTTAAAAACTTTCCAAGGTCCTGCTACAGGTCTAGTTGTGGAAAGAGAAAGAATGGACAAATTTGGTCG
Adenocystis utricularis          .........C...A.........A...T.....................A..C.........G.......A.............T.A..T.........A..
Ascoseirophila violodora         ..............A.........A...T.....................A..A......G.T.................GT.A..T...........
Caepidium antarcticum            .........C.............A...T.....................A..A..G......G....C..A..G.........GT.A..T.........A..
Chordaria flagelliformis         .............A.........A...T.....................A..A......G.T.....A............GT.A..T.........C..
Dictyosiphon foeniculaceus       .............A.........AC..T..T.....................A..A......G.T.................GT.A..T.........C..
Ectocarpus siliculosus           .............A....C....A...T.....................C..A..A......G.G.................GT.A..T...........
Laminariocolax tomentosoides     .............A.........AC..T.....................A..A......G.T.................GT.A..T.........C..
Laminarionema elsbetiae          .............A.........A...T...C.....................C..A......G.T.........C.......GT.A..T.........C..
Myrionema strangulans            .............A.......C.AC..T.....................A..A......G.T.................GT.A..T.........C..
Punctaria sp.                    .............A.........A...T.....................A..A......G.T.................GT.A..T.........C..
Pylaiella littoralis             .............A.........A...T...........A.........A..A......G.......A............T.A..T.....C.....
Scytosiphon lomentaria           .........C...A....C....AC..T.....................C..A..A......G.G.................T.A..T...........
Xiphophorocolax aotearoae        ????????????????????????????????????????????????????????????????????????????????????????????????????
Asterocladon lobatum             .............A.........A...T.....................A..A..A.....GG.TA.................T.A..T.........G..
Alaria esculenta                 ..C.......C...A.............T.....................A..A..T...G.T.................T.A..............
Macrocystis pyrifera             ..C.......C...A.............T.....................A.....T...G..A.................T.A..............
Sporochnus pedunculatus          .............A.........A...T..TC.................C.....A......G..A.................T.A..............
Tilopteris mertensii             .A............A.........AC..T...C.....................A.A....G.TA.................T.A..............
Desmarestia aculeata             .......G......A.........AC..T.....................C.....A......G..A.................T.A..............
Himantothallus grandifolius      ....G..G..C...A.........AC..T.....................A.....A......G..A.................T.A...M..........
Scytothamnus australis           ..C...........A....C....A.....................A.....A......G.TA........C.........T.A..T...........
Splachnidium rugosum             ..................C....A...T.....................A.....A......G..A.................T....T...........
Ascophyllum nodosum              .............A.........AC..T..TC.....................G.......A...C.........T.A..T.....C..A..
Sargassum muticum                .............A.........AC..T...C.T.....G...........C.........G.C..C.................T.A..T.........A..
Alethocladus corymbosus          .............A....C.....C..T..T.....................A.....A......T.......T.............T.A..T...........
Cladostephus spongiosus          .C............A.........C.....................A.....A........GA....A.............T.A..............
Herpodiscus durvillaeae          ..C...........A.....A..C.....TC.......C........G..C..A.....GT..A....A.............T.A..T.........A..
Sphacelaria arctica              .C............A....C.....C.....T.................G.....A..G......A....A.............T.A..............
Sphacelaria caespitula           .............A....C.....C.....T.................A.....G.........A....A.............T.A..............
Sphacelaria californica          .............A.....A..C.....T.................A.....A.....G...A....A.............T.A.............C..
Sphacelaria cirrosa              .............A.........C.....T.................A.....A...........C..A.............T.A..............
Sphacelaria divaricata           .............A.........C.....T.................A..G..A...........W...A............GT.A.....G........
Sphacelaria nana                 .C............A....C.....C.....................A.....A.........A....A..G.........T.A..............
Sphacelaria plumosa              .C............A....C....AC.....T.................G.....A.........A.................T.A..............
Sphacelaria plumigera            .C............A....C.....C.....T.................G.....A..G......A....A.............T.A..............
Sphacelaria racemosa             .C............A....C.....C.....T.................G.....A..G......A.............G...T.A..............
Sphacelaria radicans             .............A.........C.....T.................A.....A.......T.......A.............T.A...........C..
Sphacelaria rigidula             .............A.........C.....T.................A.....A.............A.............T.A.....G........
Sphacelaria tribuloides          ..C...........A.........C.....T.................A.....A.........A....A.............T.A.....G.....C..
Sphacelaria yamadae              .............A.........C.....T.................A.....A.......T.......A.............T.A...........C..
Sphacella subtilissima           .C............A....C...........T.................A.....G......G..A.C..............T.A..T..G........
Halopteris filicina 1            .............A....A.....C......NNNNNN...........A.....A......T..A...NT............T.A..T...........
Halopteris filicina 2            .............A....A.....C..T..T.....................A.....A......T..A.............T.A..T...........
Stypocaulon durum                .............A....C.....C..T..T.....................A......T.......A.............T.A..T...........
Stypocaulon scoparium            .............A....C...........T.................A.....A......T..A.................T.A..T...........
Syringoderma phinneyi            ..C...........A.........AC..T..T.....................C.....A.....AG..A.................T.A..............
Choristocarpus tenellus          .............A.............T..T.....................G......T..A.C..A.............T.A..T........A..
Onslowia endophytica             .......G.....................................................A......G..A.C..A.............T.A..............
Verosphacela ebrachia            ..C...........A.........A.....................C.....A.....G.GA.C..A..G.........T.A....C...........
Dictyota cervicornis             .............A.........C.....T.....................A.....T..GG..A....A...C.....C..T.A..T..G.........
Dictyota dichotoma               .............A...YY..A.A......TC.G.....A.....................GG.GA....A.............T.A..T.C......A..
```

D 3.4: Alignment of the *rbc*L gene (continued).

D 3.4: *rbc*L alignment (continued)

```
                                  3333333333333333333333333333333333333333333333333333333333333333333333333333333333333333333333333334
                                  0000000001111111112222222223333333333444444444455555555556666666666777777777788888888899999999990
                                  1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
Tribonema aequale                 TCCATTATTAGGTGCAACTGTAAAACCAAAATTAGGTCTTTCAGGTAAAAACTACGGTCGTGTAGTATATGAAGGTCTTCGTGGTGGTCTTGACTTCTTA
Adenocystis utricularis           C..G...C....A..G..........T..........G..C..........T..A.....T..T.........T.AAC.............T...C.G
Ascoseirophila violodora          ...T........G..T..........T.............T.............A.....T..T.........T.AAC.............T...C.T
Caepidium antarcticum             ...C........A..T.....G.....T........C.....T........T.....G.....T..T.........T.AAC.............C.T
Chordaria flagelliformis          ...T........G..T..C........T.............T.............A.....T..T.........T.AAC.............T...C.T
Dictyosiphon foeniculaceus        ...T........G..T..........T.............T.............A.....T..T.........T.AAC......C.....T...C.T
Ectocarpus siliculosus            ...T..T.....A..T..........T.............T..........T.......T..T.........T.AAC.............C.T
Laminariocolax tomentosoides      ...T........G..T..........T...G..........T..........C...A.....T..T.........T.AAC.............T...C.T
Laminarionema elsbetiae           ...T........G..T..C........T.............T.............A.....T..T.........CT.AAC.............T..TC.T
Myrionema strangulans             ...T........G..T..........T........C.....T.............A.....T..T.........T.AAC.............T...C.T
Punctaria sp.                     ...T........G..T..........T.............T.............A.....T..T.........T.AAC.............T...C.T
Pylaiella littoralis              ...T........G..T..........T.............T..........T........T.............T.AGC.................C.T
Scytosiphon lomentaria            ...T........G..T..........T.............T.............A.....T..T.........T.AAC.................C.T
Xiphophorocolax aotearoae         ??????????????????????????????????????????????????????????????????????????????????????????????????????
Asterocladon lobatum              ...T..C........T..A..G..G..T.....................G.....T..........T........AT.AACA..............C.T
Alaria esculenta                  ...T..C........T..........T.............................A........T........T.AT..............T...C.T
Macrocystis pyrifera              ...T..C........T..........T.............................A........T........T.AAC.............T...C.T
Sporochnus pedunculatus           ...T........A..T.....................................T..A.....T.....C......T.AT.............T...C.T
Tilopteris mertensii              ...TC.......A..............T.........................T..A.....T..T.........T.AGCA.....C.....T...C..
Desmarestia aculeata              ...T........A..T..........T.........................T..A.....T..T.........T.A..............T...C.T
Himantothallus grandifolius       ...T........A..T..........T.........................T..A.....T..T.........T.A..............T...C.T
Scytothamnus australis            C...C.T..............T.....T........A.....T..............A.....G..T...........AT.....................C.G
Splachnidium rugosum              C..T...........C..........T.............................T..A.....G..T...........AT.......A.....T...C.T
Ascophyllum nodosum               ...TC.....N.A..T..........T.........................T..G.....T..T.........T.AACA..........T..TC.T
Sargassum muticum                 ...T........A..T.....T.....T.....................T..T........T..T..C......T.AACA..........T...C.T
Alethocladus corymbosus           ...T..C.....A..T..........T.................A........T..A.....G..T...........G..A..C.........T..TC.T
Cladostephus spongiosus           ...T..TC....A..T..........T.................A........T..A.....T..T...........G..A..C.........T..TC.T
Herpodiscus durvillaeae           ...T........A..G..........................A.....T..T..G.....T..T.....G...T.A..C...........T..TC.T
Sphacelaria arctica               ...T..C.....A..T..........T.................A........T..A.....T..T...........G..A..C.........T..TC.T
Sphacelaria caespitula            ...T..C........T..........T.................A........T..A.....T..T...........G..A..C.........T..TC.T
Sphacelaria californica           ...T..C.....G..T.....G.....T.................A........T........T..T.........A..G..C...........T...C.T
Sphacelaria cirrosa               ...T..C.....A..T..........T.................A.....T..T..C.....T..T...........A..A.............T...C.T
Sphacelaria divaricata            ...T..C.....A..T..........T.................A........T........T..T.........A..A.............T...C.T
Sphacelaria nana                  ...T..CC....A..T........G..T.................A........T..G.....T..T...........G..A..C.........T..TC.T
Sphacelaria plumosa               ...T..TC....A..T..........T.................A........T..A.....T..T...........G.AA..C.........T..TC.T
Sphacelaria plumigera             ...T..CC....A..T..........T.................A........T..A.....T..T...........G.AA..C.........T..TC.T
Sphacelaria racemosa              ...T..CC....A..T..A.......T.................A........T..A.....T..T...........G..A..C.........T..TC.T
Sphacelaria radicans              ...T..C.....G..T..........................A........T..C.....T..T...........A.NA..C.........T..TC.G
Sphacelaria rigidula              ...T..C.....A..T..........................A........T........T..T.........A..A..C...........T...C.T
Sphacelaria tribuloides           ...T..C.....G..T..........T.................A........T..C.....T..T.........A..A.............T...C.T
Sphacelaria yamadae               ...T..C.....A..T..........T.................A........T........T..T.........A..A.............T...C.T
Sphacella subtilissima            ...T..C.....A..T..........T.................A........T..A.....T..T...........AA.A...........T..TC.T
Halopteris filicina 1             ...T..CC....A..T..........T.................A........T..A.....G..T...........G..A..C.........T..TC.T
Halopteris filicina 2             ...T..C.....A..T..........T.................A........T..A.....G..T...........G..A..C.........T..TC.T
Stypocaulon durum                 ...T..C.....A..T..........T.................A........T..A.........T...........G..A..C.........T..TC.T
Stypocaulon scoparium             ...T..C.....A..T..........T.................A........T..A.........T..C........G..A............T..TC.T
Syringoderma phinneyi             ...T........A..T.....T.....T.....................A........T..A.........T...........A..............T......
Choristocarpus tenellus           ..............T.....T.....T.....................T........T..T.........T.A.........T.A.........
Onslowia endophytica              ............A..T..........T........A........A........T..A.....T..T.....G.....A.NA..G.........T..NC.T
Verosphacela ebrachia             ...T........A..T..........T........A........A........T..A..A..G............GT.AA.A..G.........T......
Dictyota cervicornis              C.........N....T.....T..G..T..G..........T..........T..........T.........T.AT.C.....A.....T......
Dictyota dichotoma                ...T.............A..T.....T.............T..........T.........T..T.........AT.A.........AT.A.....T...
```

D 3.4: Alignment of the *rbc*L gene (continued).

D 3.4: *rbc*L alignment (continued)

```
                                  4444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444444445
                                  0000000001111111111222222222233333333334444444444555555555566666666667777777778888888888999999999990
                                  1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
Tribonema aequale                 AAAGATGATGAAAACATTAACTCTCAACCATTCATGCGTTGGAGAGAACGTTTCCTTTACTGTGTTGAAGGTATTAACCGTTCATCTGCATCTACAGGTG
Adenocystis utricularis           ..G.........T........A.....C.............A...G......T.A..T...A.G......G.......AG.TG....TG.A.GT....
Ascoseirophila violodora          ..G.........T........A.....................A...........A......A.G......G........G.TG.A..TG.A..T....
Caepidium antarcticum             ..G.........T.....T..A.....................A..........T.A......A.G......G.....A.AG.TG....CG.A..T....
Chordaria flagelliformis          ..G.........T........A.....................A..........A..T...A.G......G.........G.TG....TG.A..C....
Dictyosiphon foeniculaceus        ..G.........T........A.....................A..........T.A......A.G......G........G.TG.A..CG.A..C....
Ectocarpus siliculosus            ............T.....T..A........T..........A..........T.A......A.G......G........G.TG.A..TG.A.......
Laminariocolax tomentosoides      ..G.........T.....T..A........T..........A...........A......A.G......G.......AG.TG.A..TG.........
Laminarionema elsbetiae           ..G.........T........A..............C....A.........T..A......A.G......G.......G.TG....TG.A..C..C.
Myrionema strangulans             ..G..C......T........A..G...............AG.....C...T.A......A.G......G........G.TG.A..TG.A..T....
Punctaria sp.                     ..G.........T........A.....................A..........T..A......A.G......G........G.TG.A..TG.A..C....
Pylaiella littoralis              ..G.........T........A.....................A...........A......A.G......G.......AG.CG....TG.A..T....
Scytosiphon lomentaria            ..G.........T.....T..A.....G..T..........A..........T.A......A.G......G.......AG.TG....TG.A.......
Xiphophorocolax aotearoae         ????????????????????????????????????????????????????????????????????????????????????????????????????
Asterocladon lobatum              ..G.....................A.....................A.....................A.G.....GG........G.TG....TG.A..T....
Alaria esculenta                  ..G........G...........A.....................A..........T.A......A.G......G........G.CG....TG.A.......
Macrocystis pyrifera              ..G........G...........A.....................A...G......T.A......A.G......G........G.TG....TG.A.......
Sporochnus pedunculatus           ..G.........T........A.....................A...G....ATT.A......A.G......G..........TG....TG.A.......
Tilopteris mertensii              ....................T..A.....................A...........A......A.G......G........G.TG....TG.........
Desmarestia aculeata              .....................A.....................A...........A......A.G......G........G.TG....TG.A..T....
Himantothallus grandifolius       .....................A.....................A...........A......A.G......G........G.TG....TG.A.......
Scytothamnus australis            ............T........A.....................A...G......T.A......A.G......G........G.TG....TG.A..T..G.
Splachnidium rugosum              ..........G..T.....T..A........T..........A...G......T.A......A.G......G........G.TG....TG.A..G....
Ascophyllum nodosum               ............T.....T..A.....T..T..........AG.........T.A..T...A.G.....CG........G.TG....TG.A..T....
Sargassum muticum                 ....................T..G........T...A.A....A...G......T.G..T...A.G......G..........G.A...G.........
Alethocladus corymbosus           ............T.....T..A.....T.............A..........T.A......A.G......G.A......G.TG....TG.G.......
Cladostephus spongiosus           ............T.....T..A.....T........A....A...G......T.A......A.G......G.A......G.GG....TG.A..T....
Herpodiscus durvillaeae           ............T.....T...........T..........A.........TT.A......A.G......G.A..T...G.TG....CG.C..T....
Sphacelaria arctica               ............T.....T..A.....T..T..........A.........TT.A..T...A.G......G.A......G.TG....TG.G..T....
Sphacelaria caespitula            ............T.....T..A.....T.............A..........T.A..T...A.G......G.A......G.TG....TG.A.......
Sphacelaria californica           ............T..............T..T..........A...G.....T..A......A.G.....CG.A......G.TG....TG....T....
Sphacelaria cirrosa               ..G.....................A.....T.............A..........T.A......A.G......G.A......G.CG.A..T.....T....
Sphacelaria divaricata            ............T.....T........T..T..........A.........T..A......A.G......G.A......G.CG....TG.G..T....
Sphacelaria nana                  ............T.....T..A.....T.............A..........T.A......A.G..G...G.A......G.TG....TG.A..T..C.
Sphacelaria plumosa               ............T.....T..G.....T.............A..........T.A......A.G......G.A......G.TG....TG.G..T....
Sphacelaria plumigera             ............T.....T..A.....T..T..........A.............A......A.G......G.A......G.TG....TG.A..T....
Sphacelaria racemosa              ............T.....T..A.....T..T..........A..........T.A......A.G......G.A......G.TG....TG.G..T....
Sphacelaria radicans              ............T.....T..A.....T.............A..........T.A..T...A.G......G.A..NNNNNNNNNNNNNNNNNNNNN
Sphacelaria rigidula              ............T.....T........T..T..........A.........T..A......A.G......G.A......G.CG....TG.G..T....
Sphacelaria tribuloides           ............T.....T........T.......A.....A...G.....T..A......A.G......G.A......G.C.....TG....T....
Sphacelaria yamadae               ..........................T.....................A...G.....T..A......A.G......G.A......G.CG....TG.A..T....
Sphacella subtilissima            .....C..............T..A.....................AG.........T.A.....CA.G......G........G.TG.A..TG.A..T....
Halopteris filicina 1             ....................T..A.....T..T..........A..........T.A......A.G......G.A......G.TG....TG.A.......
Halopteris filicina 2             ............T.....T..A..G..T.............A..........T.A......A.G......G.A......G.TG....TG....G....
Stypocaulon durum                 ............T.....T..A.....T.............A..........T.A......A.G......G.A......G.CG....TG....G....
Stypocaulon scoparium             ............T.....T..A.....T.............A..........T.A......A.G......G.A......G.CG....TG....T....
Syringoderma phinneyi             ............T.....T..A........T..........A.........T.....T...A.G......G.A......G.TG....TG.A.......
Choristocarpus tenellus           ............T.....T...........T..........A....A.A..TT.A..T...A.G......G........G.TG.....GG........
Onslowia endophytica              ............T........A........T..........A.........ATT.A......A.G......G.A..T..C..GG....TG.A..T....
Verosphacela ebrachia             ........C..G........T.....................A.........A...A..T...A.G..G...G.A......G.TG....TG.A.......
Dictyota cervicornis              ............T.....T..A.....T..T..........A..........T.A......A.G.....NG.A...A.AG.TG....TG.........
Dictyota dichotoma                ....................T..A..G............C....A.........T..A......A.G......G.A......G.TG.A..TG.........
```

D 3.4: Alignment of the *rbcL* gene (continued).

D 3.4: *rbcL* alignment (continued)

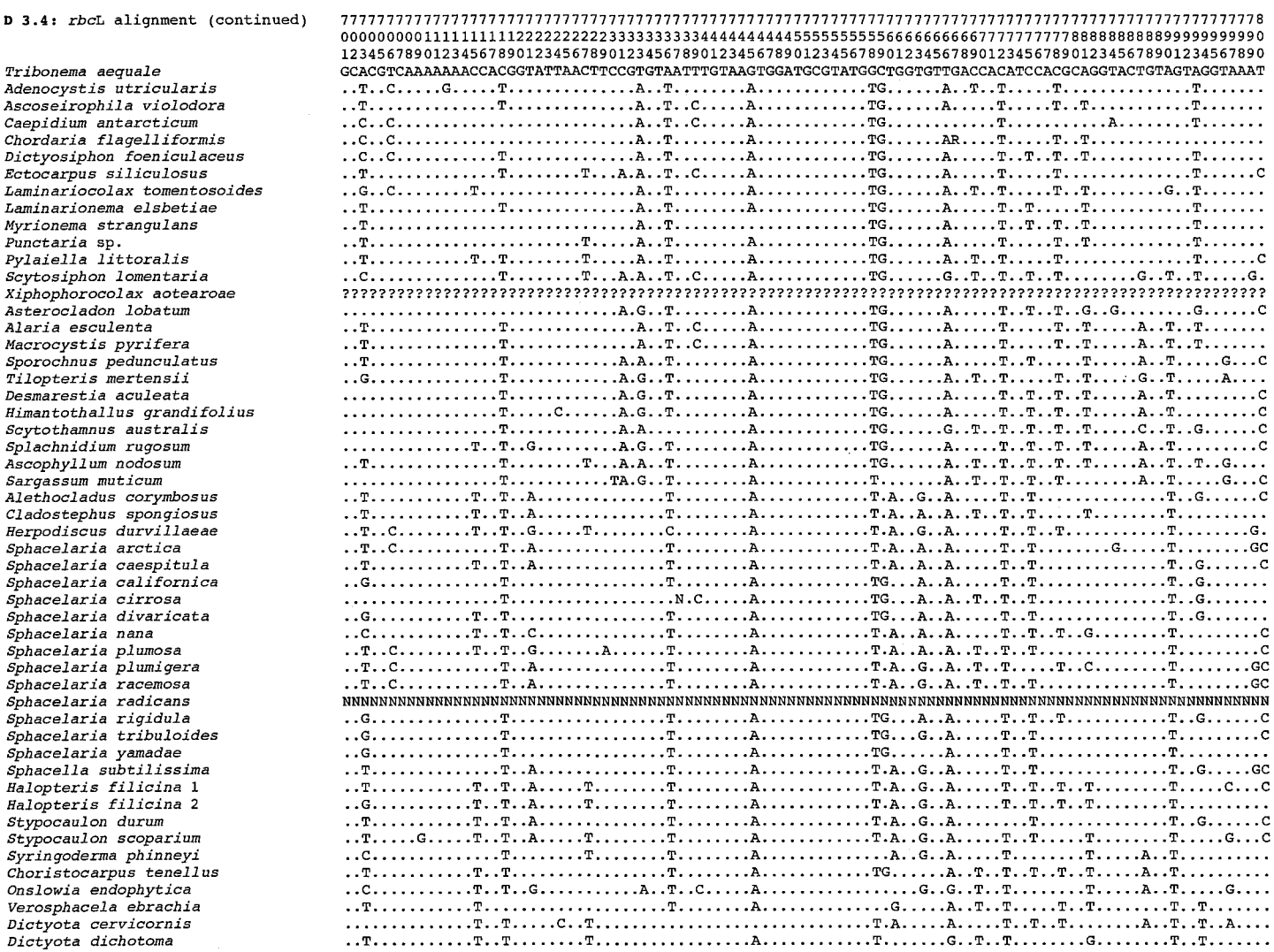
```
                              5555555555555555555555555555555555555555555555555555555555555555555555555555555555555555555555555556
                              0000000001111111111222222222233333333334444444444555555555566666666667777777777888888888899999999990
                              1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
Tribonema aequale             AAGTTAAAGGTTCTTACTTAAACGTAACAGCTGGTACAATGGAAGACATGTACGAACGTGCTGAATACGCAAAAACTTTAGGCTCAATCATTATTATGAT
Adenocystis utricularis       ................C...TA.T....A.CA..T.......A.....C.......A..G..T..TC.CT.AA.C..T...G.T...G.......
Ascoseirophila violodora      ................C....A.T..T...CA..T.......A....T.......A....T...C.TT.AA.C..T...G.T...G.G.....
Caepidium antarcticum         ................C....A.T..T...CA..T.......A....TC......A....T..TC.TT.AA.T..T..CG.T...G.......
Chordaria flagelliformis      ..............TC...TA.T..T...CA..T.....C.A....T.......G....T..GC.TT.AA.T..T...G.T...G.G.....
Dictyosiphon foeniculaceus    ................C.G..TA.T..T...CA..T.....C.A....T.......A....T...C.TT.AA.T..T...G.T...G.G.....
Ectocarpus siliculosus        ..............TC.T..A.T....C.CA..TG.A..GC.A....T.......A..G..T..TC.TG.AA.T..T...G.T...G.......
Laminariocolax tomentosoides  ................C...TA.T..T...CA..T.......G....T.......A....T...C.TT.AA.C..T...G.T...G.G.....
Laminarionema elsbetiae       ................C...TA.T..T...C...T.....C.A....T....C..A........C.TT.AA.T..T...G.T...G.G.....
Myrionema strangulans         ................C...TA.T..T...CA..TG.A....A....T.......A..G..T...C.TT.AA.T..T...G.T...G.A.....
Punctaria sp.                 .........G..........TA.T..T...CA..T.....C.A....T.......A....T...C.TT..A.T..T...G.T...G.A.....
Pylaiella littoralis          ....................TA.T......CA..T....GC.A....T.......A....T..TC.TT.AA.T..T...G.G...G.......
Scytosiphon lomentaria        ..............TC.T..A.T..C....C...T.....C.A....T.......A.......GC.TT.AA.T..T...G.T...G.......
Xiphophorocolax aotearoae     ????????????????????????????????????????????????????????????????????????????????????????????????????
Asterocladon lobatum          ..............T....TA.T..T...CA..G..T...C.A....T.......A....T...T.TT..A.C..T...G.T...G.G.....
Alaria esculenta              ................C.......T..T...CA........C.A..A..T.......A..G....TC.TT.AA.T..TA.TG.T...G.A...G.
Macrocystis pyrifera          ................C.T..A.T..T...CA....A...C.A....T.......A.......TC.TT.AA.T..TA.TG.T...G.A.....
Sporochnus pedunculatus       ................C.T..T..T..T...CA....C...C.A....T.......A....T..T..TGACC.T..TAGTG.T...G.A...G.
Tilopteris mertensii          ................C.T....T..T...CA........A..............A....TT.TC..G.AA.C..TAGTG.T...TG......
Desmarestia aculeata          ................C.T.......TT...CA........C.A............A....T..TC.TT.AA.T..TAGT..T...G.A...G.
Himantothallus grandifolius   ................C.T....T..T...CA........C.A............A....T..TC.T..AA.T..TAGTG.T...G.A...G.
Scytothamnus australis        ......G.....A..TC.T..TA.T..T...CA..C..T..GC.A............A....T..TC.TG.AA.T..TAGTG.T...G.A...G.
Splachnidium rugosum          ...A........G...C.T..TA.T..T..A.CA..T..T..GC.A....T..........G....TC.TG.GA.T..TAGTG.T...G.A.....
Ascophyllum nodosum           ..............TC.T..T..T..C..A.CA........A.T....T..............T.TC.TG.GA.T..TAGTG.....TG......
Sargassum muticum             ......G........TC.T..T..T..T..A.CA........A.T....T.......A......T.TC.TG..A.T..TAGTG.T...TG......
Alethocladus corymbosus       ................C.T.......T...C.........A.T..C..........A...TT..TG.CT.AG.T..TA..G.T...G.A...G.
Cladostephus spongiosus       ................C.T.......T...CA....C...C.T....Y.......A...TT...G..T.AG.T..TA..G.T...G.......
Herpodiscus durvillaeae       ............C...C.T..T....T..G.CA....A..GC.A....T..G....G...TT...G..T.GA.T..TA..G.T...TG....G.
Sphacelaria arctica           ................C.T..T....T..A.CA........C.A..A..T.......A...TT..TG..T.AG....TA..G.T...G.......
Sphacelaria caespitula        ............A...C.T.......T...CA..T.....C.A..C..T.......A....T..TG..T.AG.T..TA..G.T...G.A.....
Sphacelaria californica       ................C.T..TA.T..T...C...T..A...A.T....T..G....A...TT..TG..T.AG.T..TA..G.T...TG......
Sphacelaria cirrosa           ...A............C.T...A.T..T...C...T..C...C.G....T..N....A...TT..TG..T.AG.T..TA..G.T...TG......
Sphacelaria divaricata        ................C.T..TA.T..T...C...T..A...C.A....T..G....A...TT..TG..T.AG.T..TA..G.T...TG......
Sphacelaria nana              ................C.T..T....T...CA........C.A..C..T..G....G...TT...G..T.CG.T..TA.GG.T...G.....G.
Sphacelaria plumosa           ................C.T.......T...CA........C.A..A..T..G....A...TT..TG..T.AG.T..TA..G.....G.....G.
Sphacelaria plumigera         ................C.T.......T...CA........C.A..A..T..G.........TT..TG..T.AG.T..TA..G.T...G.......
Sphacelaria racemosa          .............NC.C.T..T....T..A.CA........C.A....T..G..........T..TG..T.AG.G..TA..G.T...G.......
Sphacelaria radicans          NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
Sphacelaria rigidula          ................C.T..TA.T..T...C...T..A...A.T....T..G....AN...TT..TG..T.AN.T..TA..G.T...TG......
Sphacelaria tribuloides       ................C.T...A.T..T...C...T..A...A.T....T..G....A...TT..TG..T.AG.T..TA..G.T...TG......
Sphacelaria yamadae           ................C.T..TA.T..T...C...T..A...A.T....T..G....A...TT..TG..GAAG.T..TA..G.T...TG......
Sphacella subtilissima        ..............TC.T.......T...CA..G..T...C.A....T..G....A...TT..TG..T.AG.T..TA..G.T...TG....G.
Halopteris filicina 1         ................C.T.......T.........C...A.T.............A...TT..TG..T.GG.T..TA..G.T...GCA...G.
Halopteris filicina 2         ................C.T.......T....C..G..T...A.T....T.......A...TT..TG..T.AG.T..TA..G.T...GCA...G.
Stypocaulon durum             ................C.T.......T...C...G.....A.T.CT..T.......A...TT..TG.TT.AA.T..TA..G.T...G.A...G.
Stypocaulon scoparium         .........G......C.T..T....T...C.....T...C.A.............AN...TT..TG.TT.AA.T..TA.TG.T...G.G.....
Syringoderma phinneyi         ..A.........A...C.T.......T..G..A.AC.....A......T..G....A....T..TG.TT.AA.C..TA.TG.T...G.A.....
Choristocarpus tenellus       ......G.............T.......A..A.........A.T................T..T.....CAAA.T..TA.TG.T.....C.....
Onslowia endophytica          .G..........A...C.T...A.T.....TCA..T..C..........T.......A....T..TG.TT..C.T..TA.TG.A...G.A.....
Verosphacela ebrachia         ..........A.A...C.T.......TT...C.........A.T....T..G....A.......T..TG.AG.T..TA.TG.T...G.A.....
Dictyota cervicornis          ............A..TC...T.........C.........A.......N.G.......T........CT.A....TA.TG.T...G.......
Dictyota dichotoma            .........G..G...C.T..T....G...CA........A.T..............A....T...G.GT..A.T..TA..G.T...G.......
```

D 3.4: *rbc*L **alignment (continued)**

```
                                6666666666666666666666666666666666666666666666666666666666666666666666666666666666666666666666666667
                                0000000001111111111222222222233333333334444444444555555555566666666667777777777888888888899999999990
                                1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
Tribonema aequale               CGACCTTGTAATTGGTTACAGTGCTATCCAATCAATGGGTATTTGGAGTCGTAAAAATGACATGATCCTTCACTTACACCGTGCTGGTAACTCAACTTAC
Adenocystis utricularis         ...TT.A..T..C.....T.C...A..T...AGT....CA......GCA..A...GC...A.....TT.A..T.....T..............G.....T
Ascoseirophila violodora        ...TT.A..T..C.......CA..A......AGT....CA..A...GCA......T....A.....TT.A..T.....T.....A..A.....T......
Caepidium antarcticum           ...TT.A..T..........CG..A..T...AGT....CA......GCA..A...GC...A.........A..T.....T...................T
Chordaria flagelliformis        ...TT.A..T..........CA..A......AGT....CA..A...GCA......T....A.....TT.A..T.....T.....A..A..T..T......
Dictyosiphon foeniculaceus      T..TT.A..T..........CA..A......AGT....CA..C...GCA......T....A.....TT.A..T.....T.....A..A.....T......
Ectocarpus siliculosus          ...TT.A..T........T.CA..A..T...AGT....CA..C...GCA..A...GC...AG....TT.A..T.....T..............T.....T
Laminariocolax tomentosoides    ...TT.A..T........T.CA..A......AGT....CA..A...GCA......T....A.....TT.A..T.....T.....A..A.....T......
Laminarionema elsbetiae         ...TT.A..T..C.....T.CA..A..A...AGT....CA..A...GCA......T....A.....TT.A..T.....T.....A..A.....T......
Myrionema strangulans           T..TT.A..T........T.CA..A......AGT....CA..A...GCA......T....A.....TT.A..T.....T........A.....T......
Punctaria sp.                   ...TT.A..T..C.......CA..A......AGT....CA..A...GCA......T....A.....TT.A..T.....T.....A..G.....T......
Pylaiella littoralis            ...TT.A..T........T.CA..A......AGT....CA..A...GCAA.A..GT.C..A.....TT.A..T.....T........A.........A..T
Scytosiphon lomentaria          ...TT.A..T..C.....T.CA..A......AGT....CA..A...GCAA.A...GC...A.....TT.A..T.....T.....A.....T..T.....T
Xiphophorocolax aotearoae       ????????????????????????????????????????????????????????????????????????????????????????????????????
Asterocladon lobatum            ...TT.A..T..C.....T.CA..A..T..GA......CA......GC...A...GC...AG.A...T.A..T....................T.....T
Alaria esculenta                ...TT.A..T..C.....T.C......T...A.T....C...C...GCA..A...GC...G.....TT.A..T.....T.....A.....T..T.....T
Macrocystis pyrifera            ...TT.A..T..C.....T.C......T...A.T....C...C...GCA..A...GC...G.....TT.A..T.....T.....A..G.....T.....T
Sporochnus pedunculatus         T...T.A..T........T.CA.....T...A.T....C...A...GCAA.A...GC.C.A.....TT.A..T...........A.....T..T......
Tilopteris mertensii            T..TT.A..TG.......T.CA.....T...A.T....C.......GCA..A...GC...A.....TT.A..T.....T.....A..............T
Desmarestia aculeata            ...TT.A..T..C.......CA.....T...A......C.......GC.......GC...A.....TT.A..T.....T.....A........T......
Himantothallus grandifolius     ...TT.A..T..C.....T.CA.....T...A......C.......GC...A...GC...A.....TT.A..T.....T.....A........T......
Scytothamnus australis          ...TT.A..T........T.CA.....T...A......C...C...GCG..A...GC...A.....TT.A..T.....T.....G..G.....T.....T
Splachnidium rugosum            ...TT.A..T........T.CA..G..T...A.T....C...A...GCA..A...GC...A.....TT.A..T.....T.....C.....T..T......
Ascophyllum nodosum             ...TT.A..TG.......T.CA.....T...AGT....C.......GCA..A...GC...A.....TT.A..T.....T.....A..A..T..C..A..T
Sargassum muticum               T..TT.A..GG....A..T.CA..A......A.T....CA......GC...A...GC...G.....TT.A..T.....T.....G........T.....T
Alethocladus corymbosus         ...TT.A..T..C.......C......T..........CA......GC...A...GC.C.A.....TT.A..T.....T.....A.....T..T.....T
Cladostephus spongiosus         ...TT.A..T........T.C......T...A......C.......GC.A.A...GC.C.A.....TT.A..T.....T.....A.....T..T.....T
Herpodiscus durvillaeae         ...TT.A..T........T.C......T...A......C.......GC.A.A...GC.C.A.....TT.A..T.....T.....A.....T..T.....T
Sphacelaria arctica             T..TT.A..T..C.....T.C......T...A......C.......GC.A.A...GC.C.A.....TT.A..T.....T.....A.....T..T.....T
Sphacelaria caespitula          ...TT.A..T........T.C......T...A......C.......GC...A...GC.C.A.....TT.A..T.....T.....A.....T..T.....T
Sphacelaria californica         T..TT.A..T........T.C......T...A......C.......GC.A.A...GC.C.AT.A..TT.A..T.....T.....A.....T..T......
Sphacelaria cirrosa             T..TT.A..T........T.C..........A......C.......GC...A...GC.C.AT.A..TT.A..T....................T.....T
Sphacelaria divaricata          ...TT.A..T........T.C..........A......C.......GC.A.A...GC.C.AT.A..TT.A..T.....T.....A.....T..T......
Sphacelaria nana                ...TT.A..T........T.C......T...A......CG......GC.A.A...GC.C.A.....TT.A..T.....T.....A.....T..T.....T
Sphacelaria plumosa             T..TT.A..T........T.C......T...A......C.......GC.A.A...GC.C.A.....TT.A..T.....T.....A.....T..T..A..T
Sphacelaria plumigera           ...TT.A..G..C.....T........T...A......C.......GC.A.A...GC.C.A.....T..A..T.....T....GG........T.....T
Sphacelaria racemosa            ...TT.A..T........T.C......T...A......C.......GC.A.A...GC.C.A.....TT.A..T.....T.....A.....T..T......
Sphacelaria radicans            NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
Sphacelaria rigidula            A..TT.A..T........T.C..........A......C.......GC.A.A...GC.C.AT.A..TT.A..T.....T.....A.....T..T......
Sphacelaria tribuloides         ...TT.A..T........T.C..........A......C...C...GC.A.A...GC.C.AT.A..TT.A..T.................T..T.....T
Sphacelaria yamadae             ...TT.A..T........T.C..........A......C.......GC...A...GC.C.AC.A..TT.A..T...........A.....T..T.....T
Sphacella subtilissima          ...TT.A..T........T.C......T...A......C.......GC.A.A...GC.C.A.....TT.A..T..G..T.....A.....T..T.....T
Halopteris filicina 1           T..TT.A..T.....G..T.C......T...A......CG......GC.......GC.C.A.....TT.A..T.....T.....A.....T..T.....T
Halopteris filicina 2           T..TT.A..T..........C......T..GA.T....CG......GC......GGCGC.A.....TT.A..T.....T.....A.....T..T.....T
Stypocaulon durum               T..TT.A..T.....C....C......T...A......CG......GC.......GC.C.A.....TT.A..T.....T.....A.....T..T.....T
Stypocaulon scoparium           ...TT.AT.T..........CA.....T...A......C.......GC.......GCAC.A.....TT.A..T.................T..T..G..T
Syringoderma phinneyi           T..TT.A..T........TTCA.....T..G.......CA......GC...A...GC.C.A.....TT.A..T.....T.....A.....T..T......
Choristocarpus tenellus         T.................T........T..G..T....C.G...ATGC.......GC.C.AT.A.....A..T...........A........T..A..T
Onslowia endophytica            T...T.A..T..C.....T.CA.....T...A......CA......GC...A...GCAC.A.....T.....T..G.....................T
Verosphacela ebrachia           T..TT.A................A.......A.T....C.......GCAA.A...GC.C.A.....TT.A..T...........A.....T..T..A..T
Dictyota cervicornis            .N.TT.A..T........T.C......T..........C.......GCAA.A...GCAC.A.....TT.A..T.....T.....A..A..T.....M..T
Dictyota dichotoma              ...TT.A..T........T.CA..A.....GA......CA......GCAA.A...GC.C.A.....T..A..T.....T.....A.....T.....A..T
```

D 3.4: Alignment of the *rbc*L gene (continued).

D 3.4: *rbc*L alignment (continued)



D 3.4: Alignment of the *rbc*L gene (continued).

D 3.4: *rbc*L alignment (continued)

```
                                 888888888888888888888888888888888888888888888888888888888888888888888888888888888888888888888888888888888889
                                 000000000111111111122222222223333333334444444445555555556666666667777777778888888889999999999
                                 123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890123456789
Tribonema aequale                TAGAAGGTGACCCTTTAATGGTTAAAGGTTTCTACCACACTTTATTAGACGTTACAACAGACGTTAACCTTCCTCAAGGTTTATTCTTCGCAATGGACTG
Adenocystis utricularis          ....G..A..T...C.......A...........A...G.......TTAAC.CATTT.A.AA.......AG.AG.............T.AT.....T..
Ascoseirophila violodora         .......A..T.........................A...G....C..TTAAC.CATTT.A.AA.......AT..G.....C..........AT.....T..
Caepidium antarcticum            .......G..T...C.......A...........TA.T.G....C..TTAAC.CATTT.A.AA.......AG.AG.............T.AT.....T..
Chordaria flagelliformis         .......A..T..G.....................TA...GC...C..TTAAC.CATTT.A.AA.....T.AG..G.............T.AT.....T..
Dictyosiphon foeniculaceus       .......A..T.........................A.T.G....C..TTAAC.CATTT.A.AA....T..AG..G..............AC.....T..
Ectocarpus siliculosus           .......A..T..C.............A......A...G.......TTAAC.CATTT.A.AA....TT.AG..G....C........T.AT.....T..
Laminariocolax tomentosoides     .......A..T.........................TA...G....C..TTAAC.CATTT.A.AA.......AG..G.....C..........AT.....T..
Laminarionema elsbetiae          .......A..T..................G.....A.T.G.......TTAAC.CATTT.A.AA.......AG..G..............T.AT.....T..
Myrionema strangulans            .......A.............................A...G....C..TTAAC.CATTT.A.AA.......AG..G..............AT.....T..
Punctaria sp.                    .......A..T.........................A...G.......TTAAC.CATTT.A.AA.......AG..G..............T.AT.....T..
Pylaiella littoralis             .G.....A..T.................A......A...G.......TTAAC.CATTT.A.AA.....T.AG..G..............AC.....T..
Scytosiphon lomentaria           .......G..T..A.............A......A.T.G.......TTAAC.CATCTGA.AA....TT.AG.CG.....C........T.AT.....T..
Xiphophorocolax aotearoae        ????????????????????????????????????????????????????????????????????????????????????????????????????
Asterocladon lobatum             ............A.........CG...A.....TA.....C.....TTAAC.CATTT.A.A.....TT.AG.AG.............T.AT.........
Alaria esculenta                 .......A..T.................A......A...G.......TTAAC.CA.CTTA.AA....TT.A...G..............AC.....T..
Macrocystis pyrifera             .......A..T.............G...A......A...G.......TTAAC.CA.CTTA.AA....T..AG..G..............AC.....T..
Sporochnus pedunculatus          .......A..............C.G...A.....TA..TG.......TTAAC.CAGTT.A.AA.....T.AG.AG.....C........T.AT.....T..
Tilopteris mertensii             ....G..A..T...........C.G...A.....TA....G......TTAAC.GAGTT.A.AA....TT.AG..G..............AT.....T..
Desmarestia aculeata             .......A..T.............G..........A...G.......TTAAC.CA.CT.A.A.....TT.AG.AG..............AC.....T..
Himantothallus grandifolius      .......A..T.............G..........A...G.......TTAAC.CA.CT.A.A.....TT.AG.AG..............AC.....T..
Scytothamnus australis           .......A..T..C........C.....G.....TA.T.GC......TTAAC.CATTT.A.AA........AG.GG..............AT.....T..
Splachnidium rugosum             ....G..A..T.............G...A..T...A.T.G.......TTAAC.CACTT.A.AA....TT.AG.AG....C........T.AT.....T..
Ascophyllum nodosum              .......G..T.........................T...A....AC.....TTAACAGAGCT.A.AA....TT.AG.AG.......G.......AT.....T..
Sargassum muticum                .......A..T.....G.......G...A.....TA.T..GC.....TTAAC.GAGTT.A.AA....TT.AG..G....GC....T....AT.....T..
Alethocladus corymbosus          .........T............CG...A.....TA...........TTAAC.GA.TT.A.A.....TT.AG..G.............AT.AT.....T..
Cladostephus spongiosus          .......A..T...........CG...A..T...A....A...C..CTAAC.GA.CT.A.A.....TT.AG.GG.....C........T.AT.....T..
Herpodiscus durvillaeae          .......A..T...........CG...A.....TA....A......TTAAC.GA.TTGT.T..??????????????????????????????????????
Sphacelaria arctica              .......A..T...........CG...A.....TA....AC.....TTAAC.GA.TT.A.A.....TT.AG..G.............T.AT.....T..
Sphacelaria caespitula           .........T...........CG...A.....TA...G.......TTAAC.GA.TT.A.A.....TT.AG..G.............T.AT.....T..
Sphacelaria californica          .......A..T...........CG...A.....TA....A......TTAAC.GA.TT.A.A.....TT.AG..G.............T.AT.....T..
Sphacelaria cirrosa              .......A..T...........CN...A.....TA....A......TTAACAGA.TT.A.A......T.AG..G.............T.AT.....T..
Sphacelaria divaricata           .......A..T...........CG...A......A....A......TTAAC.GA.CT.A.A.....TT.AG..G.....C........T.AT.....T..
Sphacelaria nana                 .........T...........CG...A.....TA....AC..C..TTAAC.GA.TT.A.A..A..TT.AG..G..............T.AC.....T..
Sphacelaria plumosa              .......A..T...........CG...A......A.T..A......TTAAC.GA.TT.A.A.....TT.AG..G.....C........T.AT.....T..
Sphacelaria plumigera            .......A.GT...........CG...A......A.T..A......TTAAC.GA.TT.A.A.....TT.AG..G.............T.AT.....T..
Sphacelaria racemosa             .......A..T...........CG...A.....TA....A......TTAAC.GA.TT.A.A.....TT.AG..G.............T.AT.....T..
Sphacelaria radicans             NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
Sphacelaria rigidula             .......A..T...........NG...A.CN..TA....A......TTAAN.GA.TT.A.A.....TT.AGN.G.....N....N..T.AT.....T..
Sphacelaria tribuloides          .......A..T...........CG...A.....TA....A......TTAAC.GA.TT.A.A.....TT.AG..G.....C........T.AT.....T..
Sphacelaria yamadae              .......A..T...........CG...A.....TA....A......TTAAC.GA.TT.A.A...G.TT.AG..G.....C........T.AT.....T..
Sphacella subtilissima           .......A..T...........CG...A.....TA....A......TTAAC.GA.TT.A.A.....TT.AG.GG.............T.AT.....T..
Halopteris filicina 1            .........T...........CG...A.....TA........C..TTAAC.GA.TT.A.A....GTT.AG..G.....A........T.AT.....T..
Halopteris filicina 2            .........T...........CG...A.....TA........C..TTAAC.GA.TT.A.A.....T..AG..G..............T.AT.....T..
Stypocaulon durum                .........T...........CG...A.....TA...G.......TTAAC.GA.TT.A.A.....TT.AG..G.............T.AT.....T..
Stypocaulon scoparium            .T........T...........CGT..A.....TA..........TTAAC.GA.TT.A.T.....TT.AG..G.....C........T.AT.....T..
Syringoderma phinneyi            .......A..T..G.........CG.........TA.T......C..TTAAC.GA.TT.A.GA....TT.AG.AG..............AT.....T..
Choristocarpus tenellus          .......A..T.........................TA.T..G......TTAACA.GTTT...AA..G.TT.AG.........A.T.....T.AT.....T..
Onslowia endophytica             ..............C.......ACGC..A.....TA.T.........TTATCAGA.TT.A.A......T.AG..G.............T.AT.....T..
Verosphacela ebrachia            ............A.....................TA.T...C.....TTATC.GAGTT.A.A......T.AG.CG.....C.T.....T.AT.....T..
Dictyota cervicornis             .......A..T...........CG...A.....TA.T...C..C..TTAAC.GA.TT.A.A.....TT.AG.AG....GA........T.AT.....T..
Dictyota dichotoma               .........T...........A.G...A.....TA.T..A...C.TTTAAC.GA.TT.A.A..A..TT.AG..G.............T.AT.....T..
```

D 3.4: Alignment of the *rbc*L gene (continued).

D 3.4: *rbc*L alignment (continued)

```
                                 0000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000001
                                 9999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999999990
                                 0000000001111111111222222222233333333334444444444555555555566666666667777777777888888888899999999990
                                 1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
Tribonema aequale                GGCTCTTTTACGTAAAACTGTACCAGTAGCTTCTGGTGGTATCCACTGTGGTCAAATGCACCAATTACTTTACTACTTAGGTGATGACGTAGTTCTTCAA
Adenocystis utricularis          ...AGC.C.TA.A...TG...T..T..........A..A.....T.................C.T.....T.............T..G.....A...
Ascoseirophila violodora         ...AGC.C.TA.A...TG...T..T.........C..........T.....C...........C.T.....T........C.....T..G.....A...
Caepidium antarcticum            ...AGC.C.TA.A...TG...T..T..........A..A.....T.................C.T.....T........A.....T........A...
Chordaria flagelliformis         ...AGC.C..A.A...TG...C..T.....................T.................C.T.....T.............T..G.....A...
Dictyosiphon foeniculaceus       ...AGC.C.TA.A...TG...T..T.....................T.....C...........C.T.....................T..T.....A...
Ectocarpus siliculosus           ...ATC.C.TA.A...TG...T..G..........G..A.....T.................C.T.....T..T...........T........A...
Laminariocolax tomentosoides     ...GGC.C.TA.A...TG......T.................T..T.....C...........C.T.....T........C.....T..G.....A...
Laminarionema elsbetiae          ...AGC.C.TA.A...TG...T..T.....................T.................C.T.....T..T...........T..G.....A...
Myrionema strangulans            ...AGC.C.TA.A...TG...T..T.....................T.....C...........C.T.....T.............T..G.....A...
Punctaria sp.                    ...AGC.C.TA.A...TG...T..T.....................T.....C...........C.T.....T..T.....G.....T..G.....A...
Pylaiella littoralis             ...AGC.C.TA.A...TG...T..C..........A..A.....T.....C...........C.T.....T.............T........A...
Scytosiphon lomentaria           ...GGC.C.TA.A...TG...T..T..........G..A.....T.................C.T.....T..T...........T........A...
Xiphophorocolax aotearoae        ??????????????????????????????????????????????????????????????????????????????????????????????????????
Asterocladon lobatum             ...ATCGC.TA.A...TG...G........C.........A..T.....................C.T.....................T...........G
Alaria esculenta                 ...ATC.C.TA.A...TG...T..T.............A.....T.................C.T....T...T.........T..G.....A...
Macrocystis pyrifera             ...ATC.C.TA.A...TG...T.................A.....T.................C.T........T........C..T..G.....A...
Sporochnus pedunculatus          ...ATC.C.TA.A...TG...T..T........A........T.....................C.T.....T.............T..G.....A...
Tilopteris mertensii             ...ATCAC.TA.A...TG...T..T........A.....A.....T.................C.T.....T.............T..G.....A...
Desmarestia aculeata             ...GACAC.TA.A...TG...T..T..............A.....T.................C.T.....T.............T..G.....A...
Himantothallus grandifolius      ...GTCAC.TA.A...TG...T..T..........G..A..T.....................C.T.....T.............T..T.....A...
Scytothamnus australis           ...ATC.C.T..A...TG...T.....T.....A..C.....T..T.....C........T...C.T........T.....................T.A...
Splachnidium rugosum             ...ATC.C.TA.A...TG...T..T........A.....A.....T.....A........T...C.T........T........C.........T.A...
Ascophyllum nodosum              ...ATC.C.TA.A...TG...T..T.................TT.T.............T...C.T........T.....................A...
Sargassum muticum                ...ATCGC.T......TG...T..T.....G..A........T..T.............T...C.T.....T..T.....C.....T..T.....A...
Alethocladus corymbosus          ....TCA...A.A...TG...T.................A..T..T.............T...C.T.....T.............T.....A..A...
Cladostephus spongiosus          ....TCA...A.A...TG...T.................A..T..T.............T...C.T.C...T.............T..G.....A...
Herpodiscus durvillaeae          ??????????????????????????????????????????????????????????????????????????????????????????????????????
Sphacelaria arctica              ....TCAC..A.A...TG...T.................A..T..T.............T...C.T.....T.............T........A...
Sphacelaria caespitula           ...ATCA...A.A...TG...T........A..A.....A.....T.................C.T.....T.............T........A...
Sphacelaria californica          ....TCA...A.A...TG...T........A........A.....T.............T...C.T.....T.............T........A...
Sphacelaria cirrosa              ....TCAC..A.A...TG...T........A........A.....T.................C.T.........T.........T........A...
Sphacelaria divaricata           ....TCA...A.A...TG...T........A........A..T..N..............T...C.T.....T.............T........A...
Sphacelaria nana                 ....TCA...A.A...TG...T..........A.....A.CT..T.............T...C.T.....T.............T........A...
Sphacelaria plumosa              ....TCAC..A.A...TG...T.....T...........A..T..T.............T...C.T.....T.............T........A...
Sphacelaria plumigera            ....TCAC..A.A...TG...T.................A..T..T.............T...C.T.....T.............T........A.G.
Sphacelaria racemosa             ...CTCA...A.A...TG...T.................A..T..T.............T...C.T.....T.............T........A...
Sphacelaria radicans             NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
Sphacelaria rigidula             ..N.TCA...A.A...TG...T........A........A..T..T.................C.T.....T.............T........A...
Sphacelaria tribuloides          ....TCA...A.A...TG...T........A........A..T..T.............T...C.T.....T.............T......T.A...
Sphacelaria yamadae              ....TCA...A.A...TG...T........A........A..T..T.............T...C.T.....T.............T........A...
Sphacella subtilissima           ....TCCC..A.A...TG...T.................A.....T.............T...C.T.....T.............T........A...
Halopteris filicina 1            ....TCAC..A.A...TG...T.................A..T.................T...C.T.....T.............T..G..A..A...
Halopteris filicina 2            ....TCAC..A.A...TG...T.................A..T.................T...C.T..A..T.....................G..A...
Stypocaulon durum                ....TCA...A.A...TG...T.................A..T..T.............T...C.T.....................T.....A..A...
Stypocaulon scoparium            ....TCA...A.A...TG...T.................A..T.....................C.T.....T.............T.....AT.A...
Syringoderma phinneyi            ...GTCAC.TA.A...TG...T........A........A..T..T.....C...........C.T.....T..T.........C..T..G..A..A...
Choristocarpus tenellus          ....TCA.........TG...T..T........A........T.................T...C.T.....T..T.............T......T.A...
Onslowia endophytica             ...ATCAC.TA.A...TG......T..T..A.....................A.............C.T.........................AT.A...
Verosphacela ebrachia            ...ATCAC.TA.A...TG......T........C..G..C..T..T.....C...........C.T........T.....G.....T.....A..A...
Dictyota cervicornis             ....TC.C.TA.A...TG...T..T.....................G..A..T..T.....................C.TT.A..T..T.....T..T...T.A...
Dictyota dichotoma               ....TC....A.A...TG...T..T.....A.....A.....T.....................C.TT.A..T..T.....A.....T..T..AT.A...
```

D 3.4: *rbcL* alignment (continued)

```
                                1111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111
                                0000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000001
                                0000000001111111111222222222233333333334444444444555555555566666666667777777777888888888899999999990
                                1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
Tribonema aequale               TTTGGTGGTGGTACTATTGGTCACCCAGATGGTATCCAAGCAGGTGCAACAGCAAACCGTGTAGCTTTAGAATCAATGGTATTAGCTCGTAACGAAGGTC
Adenocystis utricularis         .......................T...............T.G.................C..T...........T.....T...........T.....A.
Ascoseirophila violodora        ....................G..T...........T...T...................G..T...........T....GTC..........T.....A.
Caepidium antarcticum           ..C.................A..T...............T.G...........C..T.....T...........T.....T................AA.
Chordaria flagelliformis        .................C.....T...........T...T...................A..T...........T.....TC..........T.....A.
Dictyosiphon foeniculaceus      .......................T...............T.......C..............T...........T.....TC................A.
Ectocarpus siliculosus          .......................T...........T...T.......G.....T........T...........T.....TC..........T.......
Laminariocolax tomentosoides    .......................T...............T......................T...........T.....CC................A.
Laminarionema elsbetiae         .......................T..G........T...T.......C...........A..T...C.......T.....TC..........T.....A.
Myrionema strangulans           ..C....................T.....C.....T...T.C....................T...........T.....T...........T.....A.
Punctaria sp.                   ....................C..T...........T...T......................G...........T.....TC................A.
Pylaiella littoralis            ...................................T...T.............T........T..CC....G..T.....T...........T.....A.
Scytosiphon lomentaria          ..C....................T...........T..GT......................T...C.......T.....T...........T.....A.
Xiphophorocolax aotearoae       ????????????????????????????????????????????????????????????????????????????????????????????????????
Asterocladon lobatum            ..............A..C...........C.....T...T.G.....C..............T...........T.....TC..........T..G..A.
Alaria esculenta                .................???????????????????????????????????????????????????????????????????????????????????
Macrocystis pyrifera            ..........................T........T...T.C.....G..............T...C.......T.....TC..........T.......
Sporochnus pedunculatus         ...........K..A...........T........T.....T.....T..............T.........G.T.....T...........T.....C.
Tilopteris mertensii            ..C...........A...........T........T...T.T.....T..............T.........G.T.....T.....A.....T.....C.
Desmarestia aculeata            ..............A........T..T........T...T.......T........T.....T.........G.......T...........T.....A.
Himantothallus grandifolius     ..............A....................T...T.......T.....G..T.....T.........G.......T...........T.....A.
Scytothamnus australis          ..C...........A........T..G........T...T.G.....T........T.....T..C......G.T.....TC.G..G.............
Splachnidium rugosum            ..............A.....C..T..T........T...T.......T........T...............G.T.....T.....C.....T.......
Ascophyllum nodosum             ..............A...........T.....G..A...........T.....G..T.....T..G......G.T..C..T...........T..G....
Sargassum muticum               .....A........A...........T........A.....C.....G........T...............G.......TC.....A.A..T.......
Alethocladus corymbosus         ..............A....................T...T.......T.....T........T.........G.T.....T.....A.....T....AA.
Cladostephus spongiosus         ..............A........T...........T...T.......T.....T..T.....T..A......G.T.....T...........T....AA.
Herpodiscus durvillaeae         ????????????????????????????????????????????????????????????????????????????????????????????????????
Sphacelaria arctica             ..C...........A........T...........T...T.......T.....T..T.....T..A......G.T.....C...........T....AA.
Sphacelaria caespitula          ..............A....................T...T.......T.....T........T.........G.T.....T...........T....AA.
Sphacelaria californica         ..............A.....A..T..T........T...T.T.....T.....T..T.....T..A......G.T.....T...........T....AG.
Sphacelaria cirrosa             ..............A.....A..T..T........T...........T.....T........T.........G.T.....T...........T....AG.
Sphacelaria divaricata          ..............A.....A..T..T........T...T.T.....T.....T..T.....T..A......G.T.....T...........T....AG.
Sphacelaria nana                ..C...........A........T...........T...T.......T.....T..T.....T..A......G.T.....T...........T....AA.
Sphacelaria plumosa             ..............A.....C..T...........T...T.......T.....T........T..A......G.T.....T...........T....AA.
Sphacelaria plumigera           ..C...........A........T...........T..GT.......T.....T........T..A......G.T.....T...........T....AA.
Sphacelaria racemosa            ..C...........A........T...........T...T.......T.....T..T.....T..A......G.T.....T.....C.....T....AA.
Sphacelaria radicans            NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN......G.T.....T...........T....AA.
Sphacelaria rigidula            ..............A.....A..T..T........T...T.T.....T.....T..T.....T..A......G.T.....T.....G.....T....A..
Sphacelaria tribuloides         ..............A.....A..T..T........T...TTT.....T.....T..T.....T..A......G.T.....T...........T....AG.
Sphacelaria yamadae             ..............A.....G..T..T........T...T.T.....T.....T..T.....T..A......G.T.................T....AG.
Sphacella subtilissima          ..............A.....G..T...........T...T.......C.....T..T..A..T..A......G.T.....T...........T....AGG
Halopteris filicina 1           ...........A..A..............C.....T...T.G........T...........T.........G.T.....T.....A.....T.......
Halopteris filicina 2           ...........A..A....................T...T.C........T..G..T.....T.........G.T.....T.....A.....T.......
Stypocaulon durum               ..............A........T.....C.....T...T.......T.....T........T...C.....G.T.....T.....A.....T....AA.
Stypocaulon scoparium           ..............A....................T...T.G...........T........T........GG.T.....T.....A.....T....AA.
Syringoderma phinneyi           ..............A....................T...T.T.....T.....T........T.........G.T.....T...........T.....A.
Choristocarpus tenellus         ..C...........A.....A..T...........T.....T.....T..............T.........AGT.................T......G
Onslowia endophytica            .....A........A...........T........T...C.T..A..T.....T..T........A......G.T.....T...........T.....A.
Verosphacela ebrachia           ..............A.....A.....T........T...C.......T.....T..................G.T.....T...........T.......
Dictyota cervicornis            ..........................T.....A..A...T.T.....T.....G..T..A............G.T.....TC.T..GA.A..T.....C.
Dictyota dichotoma              ..............A........T..T........T...T......GT...........A............G.T.....GNNN..A..A..T.......
```

D 3.4: Alignment of the *rbcL* gene (continued).

D 3.4: *rbc*L alignment (continued)

```
                                  1111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111
                                  1111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111111112
                                  0000000001111111111222222222233333333334444444444555555555566666666667777777777888888888999999999900
                                  1234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
Tribonema aequale                 GTGACTACTTAAACGAAGGACCTCAAATCTTACGTGATGCTGCTAAAATGTGTGGACCACTAAAAACAGCATTAGATTTATGGAAAGATATTACTTTTGA
Adenocystis utricularis           .C.....TG.TGG......T..AG....T......A.A.....ATCT.GC.....T..TT.....G....C.....C.....................
Ascoseirophila violodora          ....T...G..GGT.....T...G....T......AGA..A..A.CT.CT.....T..TT.....G....T.....................C..
Caepidium antarcticum             .......TG.TGG......T...G.G..T......AGA.....A.CT.CT.....C..CT.....G....T.....CC.................
Chordaria flagelliformis          ....T..TG..GGT.....T...G....T......AGA..C..A.CW.CT.....T..TT.....G....T.....................C..
Dictyosiphon foeniculaceus        ....T..TG..GGT.....T...G....T......AGA..C..AGCT.CT.....T..TT.....G....C.....................C..
Ectocarpus siliculosus            ....T..TG..GG......T...G.T..T......CGA..A..ATCT.CT.....C..TT.....G.G..T.....................C..
Laminariocolax tomentosoides      ....T..TG..GGT.....T..CG....T......AGA..C..A.CT.CT.....T..TT.....G....T.....................C..
Laminarionema elsbetiae           ....T..TG..GGT.....T...G....T......AGA.....A.CT.CT.....T..TT.....G....T.....................C.....
Myrionema strangulans             ....T...G..GGT.....T...G....T......AGA.....A.CT.CT.....T..TT.....G....T.....................C..
Punctaria sp.                     ....T..TG..GGT.....T...G....T......AGA.....A.CT.CT.....T..TT.....G....T.....................C.....
Pylaiella littoralis              .A.....TG.TGGT..G..C...G..........AGA.....AGCT.CT.....T..TT.....G.G..T.....................A.....
Scytosiphon lomentaria            ........G.TGGT.....T..AG.....C.....A.A.....A.CT.CT.....G..TT.....G.T..C.....................C..
Xiphophorocolax aotearoae         ??????????????????????????????????????????????????????????????????????????????????????????????????
Asterocladon lobatum              .C..T...G.TGGT.....T...G....T......C.C.....CGCT.CT.....T...T.....G....TC....C.....................
Alaria esculenta                  ??????????????????????????????????????????????????????????????????????????????????????????????????
Macrocystis pyrifera              ....T..TG.TGG......T...G..........A.C..A..GGCT.CT.....T..TT.....G....G.....................C..
Sporochnus pedunculatus           ....T..TG.TGGT.....T...G....T.....CA.......A.CT.CT.....T..TT.....G....G.....................
Tilopteris mertensii              .......TG.TGGT.....T...G....T......A.......A.GT.CT.....T..TT.....G....T.............G.............
Desmarestia aculeata              ....T...TG..GG......C...G..........A.C.....A.GT.CT.....T..??????????????????????????????????????
Himantothallus grandifolius       ....T...G..GGT.....T...G....T......A.......A.GT.CT.....T..TT.....G....T.....................C..
Scytothamnus australis            ....T..TG.TGGA.....T........TC.....A....A..A..CTCT.....T...T.....G.T..C.................C.....A.....
Splachnidium rugosum              ....T..TG.TGGA.....T........T......A.C..G..A..T.GT.....T...T.....G....T.....................C.....
Ascophyllum nodosum               ....T..TG.TGGT.....T...G.G..T......ACA....G..GT.CT.........T.....G....T.....................
Sargassum muticum                 ....T..TG.TGGT........GG....T......ACC..G..A.GT.CT........GT.....G....T......C.....................
Alethocladus corymbosus           ....T..TG.TGCT.....T...G....T......AC...A..A....CT.....T...T.....G.....................
Cladostephus spongiosus           ....T...G.T.GT.....T...G..........T.......GCG.CT.....T...T.....G....T.....................
Herpodiscus durvillaeae           ??????????????????????????????????????????????????????????????????????????????????????????????????
Sphacelaria arctica               .......TG.T.GT.....T...G..........AT.......GCG.CT.....T...T.....G....T.....................
Sphacelaria caespitula            ....T..TG..GCT.....T...G....T......AC......A.G..CT.........T.....G.....................
Sphacelaria californica           ....T..TG.T.GT.....G.G.G....T.......C...C...TC..CT.....T..TT.....G....T.....................
Sphacelaria cirrosa               ....T..TG.TGGT.....G.G.G....T.......T....T.A.C..CA.....T..TT.....G.T..T.....................
Sphacelaria divaricata            ....T...G.T.GT.....G.G.G..........C...C...TC..CT.....T..TT.....G....T.....................C..
Sphacelaria nana                  .......TG...GT.....T...G....T.......T.......GCG.CT.....T..GT.....G....C.....................
Sphacelaria plumosa               .......TG.T.GT.....T...G..........AT.......GCG.CT.....T...T.....G....T.....................
Sphacelaria plumigera             .......TG.T.GT.....T...G....T.......TA......GC..CT.....T..C......G....T.....................
Sphacelaria racemosa              .C..T..TG.T.GT.....T...G....T.......T.......GCC.CT.....T..GT.....G....T.............G.............
Sphacelaria radicans              .......TG..GCT.....T..GG....T.......T...CT.A....CT.....T...T.....G....T....R.............A.....C..
Sphacelaria rigidula              ....T...G.G.GT.....G.G.G....T.......C......CTC..CT.....T..TT.....G....T.....................
Sphacelaria tribuloides           ....T...G.T.GT.....T.G.G....T........C..C...TC..CT.....T...T.....G....T.....................
Sphacelaria yamadae               ....T...G.T.GT.....T.G.G....T......AC...C...TC..CT.....T..TT.....G....T.....................
Sphacella subtilissima            T...T..TG.TGCTA.T..T...G..........T.......NC..CT.....T...TNN...G....T.......NN.............C.....
Halopteris filicina 1             .......TG..GCT.....T..AG....T......AC......G....CT.....T...T.....G.T.....................
Halopteris filicina 2             .......TG..GCT..G..T..AG....T.....C.GC.....A....CT.....T...T.....G....T.............C.....
Stypocaulon durum                 .......TG..GCA.....T...G....T......AT......A....CA.....T...T.....G....C.....................C..
Stypocaulon scoparium             .......TG..GCT.....T...G.G..T......AC...A..A....CT.....T..TT.....G..........C.....................
Syringoderma phinneyi             ....T..TG...CT.....T...G....T......A....A..A.CT.CT.....T...T.....G.....................
Choristocarpus tenellus           TA..T..TG.TG.AC.G..T...G....TC.....A.......A.G.G.A.....T..TT.....G.T..T.....................
Onslowia endophytica              .......TG.TGCT.....T...GG...T......AC......A....CT.....T..TT.........G.....................A.....
Verosphacela ebrachia             ....T..TG.TGCA.....T..AG....T......ACA..A........AC........TT.............C.................A..C..
Dictyota cervicornis              ....T..TG..GCA.....AG....T......ACA.....GCT.CA.....T...T.....................C.............G....A.
Dictyota dichotoma                ....T..TG.TGGT........CG....T......ACA.....GCT.CA.....T...T....G.....T.....................A.....
```

D 3.4: Alignment of the *rbc*L gene (continued).

D 3.4: *rbc*L alignment (continued)

```
                                  1111111111111111111111111111111111111111111111111111111111111
                                  2222222222222222222222222222222222222222222222222222222222222
                                  0000000001111111111222222222233333333334444444444555555
                                  1234567890123456789012345678901234567890123456789012345
Tribonema aequale                 TTACACTTCTACAGATACTCCAGATTTCGTAGAAACTGCA???????????????
Adenocystis utricularis           ...T.....G..T.....A..T..C.............GACAGAAAGTAGATAA
Ascoseirophila violodora          .........A..T.....A..T........T...GT....ACAGAAAGTAGATAA
Caepidium antarcticum             .........A........A..T..C.....T.........ACAGAAAGTAGATAA
Chordaria flagelliformis          ...T.....A..T.....A..T........C...GT....ACAGAAAGTAGATAA
Dictyosiphon foeniculaceus        ...T.....A..T.....A..T........T...GT....ACAGAAAGTAGATAA
Ectocarpus siliculosus            ...T.....A..G.........T............GT....ACAGGAAGTAGATAA
Laminariocolax tomentosoides      ...T.....A..T.....A..T........T...GT....ACAGAAAGTAGATAA
Laminarionema elsbetiae           ...T.....A..T.....A..T........T...GT....ACAGAAAGTAGATAA
Myrionema strangulans             .........A..T.....A..T........T...GT....ACAGATATGAGATAA
Punctaria sp.                     .........A........A..T........T...GT....ACAGAAAGTAGATAA
Pylaiella littoralis              .........A..T.....A..T............GT....ACAGAAAGTAGATAA
Scytosiphon lomentaria            ...T.....G........A..T........T...GT....ACAGGAAGTCGATAA
Xiphophorocolax aotearoae         ??????????????????????????????????????????????????????????
Asterocladon lobatum              .........A........A??????????????????????????????????????
Alaria esculenta                  ??????????????????????????????????????????????????????????
Macrocystis pyrifera              .........A........A..T..C.....T...GT....ACTGAAAGCAACTAG
Sporochnus pedunculatus           ......A..A........A..T........T...GT....ACTGAAAGTAAATAA
Tilopteris mertensii              .........A........A..T........T...GT....ACTGAAAGTAACTAA
Desmarestia aculeata              ??????????????????????????????????????????.............
Himantothallus grandifolius       ......A..A........A..T........T...GT....ACTGAAAGCAATTAA
Scytothamnus australis            ...T.....A...........T........T...GTGT..ACTGAATCAAAATAG
Splachnidium rugosum              ...T.....A...........T........T......T..ACAGAATCTAGATAA
Ascophyllum nodosum               A..T.....A........A..T......ACT...GTG...ACTGAAAGTAACTAA
Sargassum muticum                 A..T.....A.....C..A..T.....T..T...GTA...ACTGAAAATSSTTAG
Alethocladus corymbosus           A........G........A...........T...GT....ACTGAAAGTAATTAA
Cladostephus spongiosus           A..T..A...........A..T........T...GT...GACTGAAAGTAATTAA
Herpodiscus durvillaeae           ??????????????????????????????????????????????????????????
Sphacelaria arctica               A..T..A...........A..T........T...GT....ACTGAAAGTAATTAA
Sphacelaria caespitula            A..T..A...........A..T........T...GT....ACTGAAAGTAACTAA
Sphacelaria californica           A..T..............A..T..C..T..T...GT....ACTGGAAGTAACTAA
Sphacelaria cirrosa               A..T..................T.....T............ACTCAAAGTAACTAA
Sphacelaria divaricata            A.................A..T..C..T..T...GT....ACTGGAAGTAACTAA
Sphacelaria nana                  A..T..C...........A..T........T...GT...GACTGAGAGTAATTAA
Sphacelaria plumosa               A..T..A.....G.....A..T........T...GT....ACTGAAAGTAATTAA
Sphacelaria plumigera             A..T..A...........A..T........T...GT....ACTGGAAGTAATTAA
Sphacelaria racemosa              A..T..A.....T.....G..T..C..T..T...GT....ACAGAGAGTAAATAG
Sphacelaria radicans              A..T..A...........A...........T...GY....ACTGAAAGTAACTAA
Sphacelaria rigidula              A.................A..T..C..T..T...GTC..GACCGGAAGCAAATAA
Sphacelaria tribuloides           A..T..............A..T..C..T..T...GT....ACTCAAAGTAAATAA
Sphacelaria yamadae               A..T..............A..T..C.....T...GT....ACTGAAGCTAAATAA
Sphacella subtilissima            A..T..A...........A..T........T...GT....ACTGAAAGCAAATAA
Halopteris filicina 1             A.................G..T.....T..G.....A...ACTGAAAGCAAATAA
Halopteris filicina 2             A.................A..T..C..T..G...GT..G.ACTGAAAGTAACTAA
Stypocaulon durum                 A..............T...A..T........T...GT....CCTGAAAGTAATTAA
Stypocaulon scoparium             G.....A...........A..T............GT...TACTGGAAGTAACTAA
Syringoderma phinneyi             A..T..A..A........A..T........T...GT....ACAGGAAGTAATTAA
Choristocarpus tenellus           A..T..............A.....C..TA.C.........ACAGAAGCAAAATAA
Onslowia endophytica              A..T.....A...........T........T...GT....ACAGAAAGTAAATAA
Verosphacela ebrachia             A..T.....A..T.....A..T........T...GT....ACAGAAAGTAAATAA
Dictyota cervicornis              ...T..A..A..T.....A........T.........C..ACAGGTAGTAAGTAA
Dictyota dichotoma                A..T.....A...........T.....T..G.....A..TACAACTAATCCATAA
```

D 4 Distance matrices

The following tables show pairwise distance comparisons of sequences (upper set: Kimura-2-parameter distances; lower set: absolute distances).

D 4.1 Pigmented endophytes

In the distance matrices presented in Tables D 4.1.1 - D 4.1.4, sequences included in the respective analyses are set in bold. Distance matrices are numbered as follows:

Table D 4.1.1: ITS1 with adjacent 18S-5.8S: All endophytes (partial ITS1)

Table D 4.1.2: ITS1 with adjacent 18S-5.8S: *Laminariocolax* clade (complete ITS1)

Table D 4.1.3: ITS1 with adjacent 18S-5.8S: *Microspongium* clade (complete ITS1)

Table D 4.1.4: ITS1 with adjacent 18S-5.8S: *Xiphophorocolax* clade (complete ITS1)

Table D 4.1.5: Partial *rbc*L (including *Xiphophorocolax aotearoae*)

Table D 4.1.6: Partial 26S nrDNA (including *Xiphophorocolax aotearoae*).

D 4.2 *Herpodiscus durvillaeae*

Distance matrices are numbered as follows:

Table D 4.2.1: Partial 18S nrDNA

Table D 4.2.2: Partial 26S nrDNA

Table D 4.2.3: Partial *rbc*L (Phaeophyceae)

Table D 4.2.4: Partial *rbc*L (Sphacelariales).

Table D 4.1.1

		1	2	3	4	5	6	7	8	9	10	11	12
		Chordaria flagelliformis	isolate no. 1 EMa A 3/98	isolate no. 2 EMa HP 5/98	isolate no. 3 EMa HP 4/99	isolate no. 4 EMa SP 5/98	isolate no. 5 EMa WW 5/99	isolate no. 6 EMa HI 10/99	isolate no. 7 EEck W 3/98	isolate no. 9 EEck Waki 10/00	isolate no. 10 EEck OTS 11/99	*Laminariocolax eckloniae* South Africa	*Laminariocolax eckloniae* Antarctica
1	*Chordaria flagelliformis*		0.182	0.182	0.184	0.182	0.193	0.184	0.182	0.182	0.182	0.176	0.188
2	isolate no. 1 EMa A 3/98	23		0	0	0	0.006	0	0	0	0	0.006	0
3	isolate no. 2 EMa HP 5/98	23	0		0	0	0.006	0	0	0	0	0.006	0
4	isolate no. 3 EMa HP 4/99	23	0	0		0	0.006	0	0	0	0	0.006	0
5	isolate no. 4 EMa SP 5/98	23	0	0	0		0.006	0	0	0	0	0.006	0
6	isolate no. 5 EMa WW 5/99	24	1	1	1	1		0.006	0.006	0.006	0.006	0.012	0.006
7	isolate no. 6 EMa HI 10/99	23	0	0	0	0	1		0	0	0	0.006	0
8	isolate no. 7 EEck W 3/98	23	0	0	0	0	1	0		0	0	0.006	0
9	isolate no. 9 EEck Waki 10/00	23	0	0	0	0	1	0	0		0	0.006	0
10	isolate no. 10 EEck OTS 11/99	23	0	0	0	0	1	0	0	0		0.006	0
11	*Laminariocolax eckloniae* South Africa	22	1	1	1	1	2	1	1	1	1		0.006
12	*Laminariocolax eckloniae* Antarctica	23	0	0	0	0	1	0	0	0	0	1	
13	isolate no. 11 EMa A 10/97	24	1	1	1	1	2	1	1	1	1	2	1
14	isolate no. 12 EMa QP 7/97	23	1	1	1	1	2	1	1	1	1	2	1
15	*Laminariocolax macrocystis* Chile	22	2	2	2	2	3	2	2	2	2	1	2
16	isolate no. 14 EMa BB 10/97	23	0	0	0	0	1	0	0	0	0	1	0
17	isolate no. 15 EMa CH 5/98	23	0	0	0	0	1	0	0	0	0	1	0
18	isolate no. 16 EMa W 3/98	23	0	0	0	0	1	0	0	0	0	1	0
19	isolate no. 17 EMa OW 5/99	23	0	0	0	0	1	0	0	0	0	1	0
20	isolate no. 18 EMa WR 4/00	23	0	0	0	0	1	0	0	0	0	1	0
21	*Laminariocolax tomentosoides* Helgoland	23	4	4	4	4	5	4	4	4	4	3	4
22	*Laminariocolax tomentosoides* ssp. *deformans* Bretagne	24	5	5	5	5	6	5	5	5	5	4	5
23	*Laminariocolax aecidioides* Kiel	21	3	3	3	3	4	3	3	3	3	2	3
24	*Laminariocolax aecidioides* Maine	24	5	5	5	5	6	5	5	5	5	4	5
25	*Laminariocolax aecidioides* Bretagne	19	3	3	3	3	3	3	3	3	3	2	3
26	isolate no. 19 EPa A 10/97	20	23	23	23	23	24	24	24	23	23	21	23
27	isolate no. 20 EPa Riv 9/99	21	25	25	25	25	26	26	26	25	25	23	25
28	isolate no. 21 EPa BB 4/99	22	26	26	26	26	27	27	27	26	26	24	26
29	isolate no. 22 EPa BS 10/00	21	25	25	25	25	26	26	26	25	25	23	25
30	isolate no. 23 EGra BB 2/98	20	23	23	23	23	24	24	24	23	23	21	23
31	isolate no. 24 EKal CC 10/00	20	23	23	23	23	24	24	24	23	23	21	23
32	isolate no. 25 EUpi W 4/98	20	23	23	23	23	24	24	24	23	23	21	23
33	isolate no. 26 EEck Kari 5/99	20	23	23	23	23	24	24	24	23	23	21	23
34	*Microspongium tenuissimum*	20	23	23	23	23	24	24	24	23	23	21	23
35	isolate no. 27 EPa OW 5/99	20	17	17	17	17	18	17	17	17	17	15	17
36	*Microspongium radians*	20	17	17	17	17	18	17	17	17	17	15	17
37	*Microspongium alariae*	22	25	25	25	25	26	26	26	25	25	23	25
38	isolate no. 28 ELes OW 5/99	34	36	36	35	36	37	37	37	36	36	36	36
39	isolate no. 29 ELes Wk 5/99	34	36	36	35	36	37	37	37	36	36	36	36
40	isolate no. 31 EXi BB 8/99	34	35	35	34	35	36	36	36	35	35	36	35
41	isolate no. 32 EMu Wk 5/99	34	36	36	35	36	37	37	37	36	36	36	36
42	isolate no. 33 EDan BB 4/99	34	36	36	35	36	37	37	37	36	36	36	36
43	isolate no. 34 EDwil BB 8/00	28	27	27	26	27	28	27	27	27	27	28	27

Table D 4.1.1 (cont.)

		13	14	15	16	17	18	19	20	21	22	23	24
		isolate no. 11 EMa A 10/97	isolate no. 12 EMa QP 7/97	*Laminariocolax macrocystis* Chile	isolate no. 14 EMa BB 10/97	isolate no. 15 EMa CH 5/98	isolate no. 16 EMa W 3/98	isolate no. 17 EMa OW 5/99	isolate no. 18 EMa WR 4/00	*Laminariocolax tomentosoides* Helgoland	*Laminariocolax tomentosoides* ssp. *deformans* Bretagne	*Laminariocolax aecidioides* Kiel	*Laminariocolax aecidioides* Maine
1	*Chordaria flagelliformis*	0.191	0.185	0.179	0.182	0.182	0.191	0.182	0.182	0.191	0.191	0.178	0.191
2	isolate no. 1 EMa A 3/98	0.006	0.006	0.012	0	0	0	0	0	0.025	0.031	0.018	0.030
3	isolate no. 2 EMa HP 5/98	0.006	0.006	0.012	0	0	0	0	0	0.025	0.031	0.018	0.030
4	isolate no. 3 EMa HP 4/99	0.006	0.006	0.012	0	0	0	0	0	0.025	0.031	0.019	0.031
5	isolate no. 4 EMa SP 5/98	0.006	0.006	0.012	0	0	0	0	0	0.025	0.031	0.018	0.030
6	isolate no. 5 EMa WW 5/99	0.012	0.012	0.018	0.006	0.006	0.006	0.006	0.006	0.031	0.037	0.025	0.037
7	isolate no. 6 EMa HI 10/99	0.006	0.006	0.013	0	0	0	0	0	0.025	0.031	0.019	0.031
8	isolate no. 7 EEck W 3/98	0.006	0.006	0.012	0	0	0	0	0	0.025	0.030	0.018	0.030
9	isolate no. 9 EEck Waki 10/00	0.006	0.006	0.012	0	0	0	0	0	0.025	0.031	0.018	0.030
10	isolate no. 10 EEck OTS 11/99	0.006	0.006	0.012	0	0	0	0	0	0.025	0.031	0.018	0.030
11	*Laminariocolax eckloniae* South Africa	0.012	0.012	0.006	0.006	0.006	0.006	0.006	0.006	0.018	0.024	0.012	0.025
12	*Laminariocolax eckloniae* Antarctica	0.006	0.006	0.013	0	0	0	0	0	0.025	0.031	0.019	0.031
13	isolate no. 11 EMa A 10/97		0	0.019	0.006	0.006	0.006	0.006	0.006	0.032	0.037	0.025	0.037
14	isolate no. 12 EMa QP 7/97	0		0.019	0.006	0.006	0.007	0.006	0.006	0.032	0.037	0.026	0.037
15	*Laminariocolax macrocystis* Chile	3	3		0.012	0.012	0.013	0.012	0.012	0.019	0.031	0.012	0.032
16	isolate no. 14 EMa BB 10/97	1	1	2		0	0	0	0	0.025	0.031	0.018	0.030
17	isolate no. 15 EMa CH 5/98	1	1	2	0		0	0	0	0.025	0.030	0.018	0.030
18	isolate no. 16 EMa W 3/98	1	1	2	0	0		0	0	0.025	0.032	0.019	0.032
19	isolate no. 17 EMa OW 5/99	1	1	2	0	0	0		0	0.025	0.030	0.018	0.030
20	isolate no. 18 EMa WR 4/00	1	1	2	0	0	0	0		0.025	0.031	0.018	0.030
21	*Laminariocolax tomentosoides* Helgoland	5	5	3	4	4	4	4	4		0.006	0.006	0.019
22	*Laminariocolax tomentosoides* ssp. *deformans* Bretagne	6	6	5	5	5	5	5	5	1		0.013	0.024
23	*Laminariocolax aecidioides* Kiel	4	4	2	3	3	3	3	3	1	2		0.013
24	*Laminariocolax aecidioides* Maine	6	6	5	5	5	5	5	5	3	4	2	
25	*Laminariocolax aecidioides* Bretagne	4	4	2	3	3	3	3	3	1	1	0	0
26	isolate no. 19 EPa A 10/97	24	22	22	23	24	23	24	23	23	24	22	24
27	isolate no. 20 EPa Riv 9/99	26	24	24	25	26	25	26	25	25	26	24	26
28	isolate no. 21 EPa BB 4/99	27	25	25	26	27	26	27	26	26	27	25	27
29	isolate no. 22 EPa BS 10/00	26	24	24	25	26	25	26	25	25	26	24	26
30	isolate no. 23 EGra BB 2/98	24	22	22	23	24	23	24	23	23	24	22	24
31	isolate no. 24 EKal CC 10/00	24	22	22	23	24	23	24	23	23	24	22	24
32	isolate no. 25 EUpi W 4/98	24	22	22	23	24	23	24	23	23	24	22	24
33	isolate no. 26 EEck Kari 5/99	24	22	22	23	24	23	24	23	23	24	22	24
34	*Microspongium tenuissimum*	24	22	22	23	24	23	24	23	23	24	22	24
35	isolate no. 27 EPa OW 5/99	18	17	16	17	17	17	17	17	16	17	16	18
36	*Microspongium radians*	18	17	16	17	17	17	17	17	16	17	16	18
37	*Microspongium alariae*	26	24	24	25	26	25	26	25	25	26	24	26
38	isolate no. 28 ELes OW 5/99	37	36	37	36	37	36	37	36	37	37	37	39
39	isolate no. 29 ELes Wk 5/99	37	36	37	36	37	36	37	36	37	37	37	39
40	isolate no. 31 EXi BB 8/99	36	35	37	35	36	35	36	35	36	36	36	38
41	isolate no. 32 EMu Wk 5/99	37	36	37	36	37	36	37	36	37	37	37	39
42	isolate no. 33 EDan BB 4/99	37	36	37	36	37	36	37	36	37	37	37	39
43	isolate no. 34 EDwil BB 8/00	28	27	29	27	27	27	27	27	28	28	28	30

Table D 4.1.1 (cont.)

		25	26	27	28	29	30	31	32	33	34	35	36
		Laminariocolax aecidioides Bretagne	isolate no. 19 EPa A 10/97	isolate no. 20 EPa Riv 9/99	isolate no. 21 EPa BB 4/99	isolate no. 22 EPa BS 10/00	isolate no. 23 EGra BB 2/98	isolate no. 24 EKal CC 10/00	isolate no. 25 EUpi W 4/98	isolate no. 26 EEck Kari 5/99	*Microspongium tenuissimum*	isolate no. 27 EPa OW 5/99	*Microspongium radians*
1	*Chordaria flagelliformis*	0.262	0.157	0.166	0.175	0.166	0.157	0.157	0.157	0.157	0.159	0.164	0.168
2	isolate no. 1 EMa A 3/98	0.027	0.163	0.179	0.186	0.179	0.163	0.163	0.163	0.163	0.164	0.128	0.129
3	isolate no. 2 EMa HP 5/98	0.027	0.163	0.179	0.186	0.179	0.163	0.163	0.163	0.163	0.164	0.128	0.129
4	isolate no. 3 EMa HP 4/99	0.027	0.164	0.180	0.188	0.180	0.164	0.164	0.164	0.164	0.166	0.129	0.131
5	isolate no. 4 EMa SP 5/98	0.027	0.163	0.179	0.186	0.179	0.163	0.163	0.163	0.163	0.164	0.128	0.129
6	isolate no. 5 EMa WW 5/99	0.028	0.172	0.189	0.197	0.189	0.172	0.172	0.172	0.172	0.173	0.138	0.140
7	isolate no. 6 EMa HI 10/99	0.027	0.171	0.186	0.194	0.186	0.171	0.171	0.171	0.171	0.172	0.128	0.129
8	isolate no. 7 EEck W 3/98	0.027	0.169	0.185	0.193	0.185	0.169	0.169	0.169	0.169	0.170	0.128	0.129
9	isolate no. 9 EEck Waki 10/00	0.027	0.163	0.179	0.186	0.179	0.163	0.163	0.163	0.163	0.164	0.128	0.129
10	isolate no. 10 EEck OTS 11/99	0.027	0.163	0.179	0.186	0.179	0.163	0.163	0.163	0.163	0.164	0.128	0.129
11	*Laminariocolax eckloniae* South Africa	0.018	0.151	0.167	0.175	0.167	0.151	0.151	0.151	0.151	0.153	0.115	0.116
12	*Laminariocolax eckloniae* Antarctica	0.027	0.167	0.184	0.192	0.184	0.167	0.167	0.167	0.167	0.169	0.132	0.134
13	isolate no. 11 EMa A 10/97	0.037	0.171	0.187	0.195	0.187	0.171	0.171	0.171	0.171	0.172	0.136	0.138
14	isolate no. 12 EMa QP 7/97	0.038	0.156	0.172	0.180	0.172	0.156	0.156	0.156	0.156	0.157	0.128	0.130
15	*Laminariocolax macrocystis* Chile	0.019	0.161	0.177	0.185	0.177	0.161	0.161	0.161	0.161	0.162	0.124	0.125
16	isolate no. 14 EMa BB 10/97	0.027	0.163	0.179	0.186	0.179	0.163	0.163	0.163	0.163	0.164	0.128	0.129
17	isolate no. 15 EMa CH 5/98	0.027	0.169	0.185	0.193	0.185	0.169	0.169	0.169	0.169	0.170	0.128	0.129
18	isolate no. 16 EMa W 3/98	0.027	0.169	0.186	0.194	0.186	0.169	0.169	0.169	0.169	0.169	0.133	0.133
19	isolate no. 17 EMa OW 5/99	0.027	0.169	0.185	0.193	0.185	0.169	0.169	0.169	0.169	0.170	0.128	0.129
20	isolate no. 18 EMa WR 4/00	0.027	0.163	0.179	0.186	0.179	0.163	0.163	0.163	0.163	0.164	0.128	0.129
21	*Laminariocolax tomentosoides* Helgoland	0.008	0.168	0.185	0.193	0.185	0.168	0.168	0.168	0.168	0.169	0.124	0.124
22	*Laminariocolax tomentosoides* ssp. *deformans* Bretagne	0.008	0.170	0.186	0.194	0.186	0.170	0.170	0.170	0.170	0.171	0.127	0.129
23	*Laminariocolax aecidioides* Kiel	0	0.164	0.180	0.188	0.180	0.164	0.164	0.164	0.164	0.165	0.127	0.128
24	*Laminariocolax aecidioides* Maine	0	0.169	0.185	0.193	0.185	0.169	0.169	0.169	0.169	0.171	0.135	0.137
25	*Laminariocolax aecidioides* Bretagne		0.224	0.250	0.263	0.250	0.224	0.224	0.224	0.224	0.224	0.170	0.170
26	isolate no. 19 EPa A 10/97	20		0.012	0.018	0.012	0	0	0	0	0	0	0
27	isolate no. 20 EPa Riv 9/99	22	2		0.006	0	0.012	0.012	0.012	0.012	0.012	0.013	0.013
28	isolate no. 21 EPa BB 4/99	23	3	1		0.006	0.018	0.018	0.018	0.018	0.018	0.020	0.020
29	isolate no. 22 EPa BS 10/00	22	2	0	1		0.012	0.012	0.012	0.012	0.012	0.013	0.013
30	isolate no. 23 EGra BB 2/98	20	0	2	3	2		0	0	0	0	0	0
31	isolate no. 24 EKal CC 10/00	20	0	2	3	2	0		0	0	0	0	0
32	isolate no. 25 EUpi W 4/98	20	0	2	3	2	0	0		0	0	0	0
33	isolate no. 26 EEck Kari 5/99	20	0	2	3	2	0	0	0		0	0	0
34	*Microspongium tenuissimum*	20	0	2	3	2	0	0	0	0		0	0
35	isolate no. 27 EPa OW 5/99	14	0	2	3	2	0	0	0	0	0		0
36	*Microspongium radians*	14	0	2	3	2	0	0	0	0	0	0	
37	*Microspongium alariae*	22	6	6	7	6	6	6	6	6	6	2	2
38	isolate no. 28 ELes OW 5/99	33	43	43	44	43	43	43	43	43	43	32	32
39	isolate no. 29 ELes Wk 5/99	33	43	43	44	43	43	43	43	43	43	32	32
40	isolate no. 31 EXi BB 8/99	32	44	44	45	44	44	44	44	44	44	33	33
41	isolate no. 32 EMu Wk 5/99	33	43	43	44	43	43	43	43	43	43	32	32
42	isolate no. 33 EDan BB 4/99	33	43	43	44	43	43	43	43	43	43	32	32
43	isolate no. 34 EDwil BB 8/00	24	31	31	32	31	31	31	31	31	31	31	31

Table D 4.1.1 (cont.)

		37	38	39	40	41	42	43
		Microspongium alariae	isolate no. 28 ELes OW 5/99	isolate no. 29 ELes Wk 5/99	isolate no. 31 EXi BB 8/99	isolate no. 32 EMu Wk 5/99	isolate no. 33 EDan BB 4/99	isolate no. 34 EDwil BB 8/00
1	*Chordaria flagelliformis*	0.174	0.299	0.299	0.297	0.299	0.299	0.250
2	isolate no. 1 EMa A 3/98	0.180	0.253	0.253	0.245	0.253	0.254	0.211
3	isolate no. 2 EMa HP 5/98	0.180	0.253	0.253	0.245	0.253	0.254	0.211
4	isolate no. 3 EMa HP 4/99	0.182	0.247	0.247	0.238	0.247	0.247	0.203
5	isolate no. 4 EMa SP 5/98	0.180	0.253	0.253	0.245	0.253	0.254	0.211
6	isolate no. 5 EMa WW 5/99	0.190	0.264	0.264	0.255	0.264	0.264	0.223
7	isolate no. 6 EMa HI 10/99	0.188	0.262	0.262	0.253	0.262	0.262	0.211
8	isolate no. 7 EEck W 3/98	0.187	0.260	0.260	0.252	0.260	0.261	0.211
9	isolate no. 9 EEck Waki 10/00	0.180	0.253	0.253	0.245	0.253	0.254	0.211
10	isolate no. 10 EEck OTS 11/99	0.180	0.253	0.253	0.245	0.253	0.254	0.211
11	*Laminariocolax eckloniae* South Africa	0.167	0.260	0.260	0.259	0.260	0.260	0.227
12	*Laminariocolax eckloniae* Antarctica	0.185	0.261	0.261	0.252	0.261	0.261	0.218
13	isolate no. 11 EMa A 10/97	0.188	0.262	0.262	0.253	0.262	0.263	0.221
14	isolate no. 12 EMa QP 7/97	0.174	0.258	0.258	0.249	0.258	0.259	0.213
15	*Laminariocolax macrocystis* Chile	0.178	0.272	0.272	0.272	0.272	0.272	0.239
16	isolate no. 14 EMa BB 10/97	0.180	0.253	0.253	0.245	0.253	0.254	0.211
17	isolate no. 15 EMa CH 5/98	0.187	0.260	0.260	0.252	0.260	0.261	0.211
18	isolate no. 16 EMa W 3/98	0.187	0.263	0.263	0.254	0.263	0.263	0.221
19	isolate no. 17 EMa OW 5/99	0.187	0.260	0.260	0.252	0.260	0.261	0.211
20	isolate no. 18 EMa WR 4/00	0.180	0.253	0.253	0.245	0.253	0.254	0.211
21	*Laminariocolax tomentosoides* Helgoland	0.186	0.272	0.272	0.263	0.272	0.272	0.230
22	*Laminariocolax tomentosoides* ssp. *deformans* Bretagne	0.188	0.262	0.262	0.253	0.262	0.262	0.220
23	*Laminariocolax aecidioides* Kiel	0.181	0.279	0.279	0.270	0.279	0.279	0.237
24	*Laminariocolax aecidioides* Maine	0.186	0.281	0.281	0.272	0.281	0.282	0.241
25	*Laminariocolax aecidioides* Bretagne	0.251	0.377	0.377	0.362	0.377	0.376	0.325
26	isolate no. 19 EPa A 10/97	0.037	0.330	0.330	0.339	0.330	0.330	0.268
27	isolate no. 20 EPa Riv 9/99	0.037	0.329	0.329	0.339	0.329	0.330	0.268
28	isolate no. 21 EPa BB 4/99	0.043	0.338	0.338	0.348	0.338	0.339	0.279
29	isolate no. 22 EPa BS 10/00	0.037	0.329	0.329	0.339	0.329	0.330	0.268
30	isolate no. 23 EGra BB 2/98	0.037	0.330	0.330	0.339	0.330	0.330	0.268
31	isolate no. 24 EKal CC 10/00	0.037	0.330	0.330	0.339	0.330	0.330	0.268
32	isolate no. 25 EUpi W 4/98	0.037	0.330	0.330	0.339	0.330	0.330	0.268
33	isolate no. 26 EEck Kari 5/99	0.037	0.330	0.330	0.339	0.330	0.330	0.268
34	*Microspongium tenuissimum*	0.037	0.334	0.334	0.344	0.334	0.334	0.272
35	isolate no. 27 EPa OW 5/99	0.013	0.263	0.263	0.274	0.263	0.264	0.266
36	*Microspongium radians*	0.013	0.269	0.269	0.279	0.269	0.269	0.272
37	*Microspongium alariae*		0.309	0.309	0.318	0.309	0.310	0.258
38	isolate no. 28 ELes OW 5/99	41		0	0.006	0	0.006	0.008
39	isolate no. 29 ELes Wk 5/99	41	0		0.006	0	0.006	0.008
40	isolate no. 31 EXi BB 8/99	42	1	1		0.006	0.011	0
41	isolate no. 32 EMu Wk 5/99	41	0	0	1		0.006	0.008
42	isolate no. 33 EDan BB 4/99	41	1	1	2	1		0.013
43	isolate no. 34 EDwil BB 8/00	30	1	1	0	1	2	

Table D 4.1.2

		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
		isolate no. 1 EMa A 3/98	isolate no. 2 EMa HP 5/98	isolate no. 3 EMa HP 4/99	isolate no. 4 EMa SP 5/98	isolate no. 5 EMa WW 5/99	isolate no. 6 EMa HI 10/99	isolate no. 7 EEck W 3/98	isolate no. 9 EEck Waki 10/00	isolate no. 10 EEck OTS 11/99	*Laminariocolax eckloniae* South Africa	*Laminariocolax eckloniae* Antarctica	isolate no. 11 EMa A 10/97	isolate no. 12 EMa QP 7/97	*Laminariocolax macrocystis* Chile	isolate no. 14 EMa BB 10/97	isolate no. 15 EMa CH 5/98	isolate no. 16 EMa W 3/98	isolate no. 17 EMa OW 5/99	isolate no. 18 EMa WR 4/00	*Laminariocolax tomentosoides* Helgoland	*Laminariocolax tomentosoides* ssp. *deformans*	*Laminariocolax aecidioides* Kiel	*Laminariocolax aecidioides* Maine	*Laminariocolax aecidioides* Bretagne
2	isolate no. 1 EMa A 3/98		0	0	0	0.005	0.01	0	0	0	0.009	0	0.011	0.011	0.017	0.01	0.01	0	0	0.01	0.060	0.055	0.050	0.055	0.057
3	isolate no. 2 EMa HP 5/98	0		0	0	0.005	0.01	0	0	0	0.009	0	0.011	0.011	0.017	0.01	0.01	0	0	0.01	0.060	0.055	0.050	0.055	0.057
4	isolate no. 3 EMa HP 4/99	0	0		0	0.005	0.01	0	0	0	0.009	0	0.011	0.011	0.017	0.01	0.01	0	0	0.01	0.058	0.053	0.048	0.053	0.055
5	isolate no. 4 EMa SP 5/98	0	0	0		0.005	0.01	0	0	0	0.009	0	0.011	0.011	0.017	0.01	0.01	0	0	0.01	0.060	0.055	0.050	0.055	0.057
6	isolate no. 5 EMa WW 5/99	3	3	3	3		0.020	0.009	0.005	0.007	0.014	0.009	0.017	0.017	0.023	0.011	0.011	0.009	0.009	0.014	0.068	0.061	0.056	0.061	0.062
7	isolate no. 6 EMa HI 10/99	7	7	7	7	10		0.02	0.01	0.02	0.024	0.02	0.015	0.015	0.018	0.01	0.01	0.01	0.01	0.02	0.064	0.065	0.058	0.063	0.066
8	isolate no. 7 EEck W 3/98	2	2	2	2	5	9		0	0.01	0.012	0.01	0.014	0.014	0.019	0.01	0.01	0.01	0.01	0.01	0.065	0.059	0.054	0.059	0.061
9	isolate no. 9 EEck Waki 10/00	0	0	0	0	3	7	2		0	0.009	0	0.011	0.011	0.017	0.01	0.01	0	0	0.01	0.058	0.053	0.050	0.055	0.057
10	isolate no. 10 EEck OTS 11/99	1	1	1	1	4	8	3	1		0.010	0.01	0.014	0.014	0.020	0.01	0.01	0.01	0.01	0.01	0.060	0.055	0.050	0.055	0.057
11	*Laminariocolax eckloniae* South Africa	5	5	5	5	8	12	7	5	6		0.012	0.018	0.018	0.018	0.013	0.013	0.011	0.011	0.019	0.056	0.056	0.051	0.056	0.058
12	*Laminariocolax eckloniae* Antarctica	2	2	2	2	5	9	4	2	3	8		0.011	0.011	0.016	0.01	0.01	0	0	0.01	0.055	0.053	0.049	0.054	0.056
13	isolate no. 11 EMa A 10/97	4	4	4	4	6	5	5	4	5	7	4		0	0.012	0.016	0.016	0.013	0.013	0.021	0.075	0.074	0.067	0.071	0.080
14	isolate no. 12 EMa QP 7/97	4	4	4	4	6	5	5	4	5	7	4	0		0.012	0.016	0.016	0.014	0.013	0.022	0.076	0.074	0.067	0.072	0.081
15	*Laminariocolax macrocystis* Chile	6	6	6	6	8	6	7	6	7	7	6	5	5		0.021	0.021	0.019	0.019	0.027	0.072	0.074	0.064	0.072	0.077
16	isolate no. 14 EMa BB 10/97	2	2	2	2	4	5	3	2	3	5	2	6	6	8		0	0	0	0.01	0.071	0.069	0.062	0.067	0.075
17	isolate no. 15 EMa CH 5/98	2	2	2	2	4	5	3	2	3	5	2	6	6	8	0		0	0	0.01	0.071	0.069	0.062	0.067	0.075
18	isolate no. 16 EMa W 3/98	1	1	1	1	3	4	2	1	2	4	1	5	5	7	1	1		0	0.01	0.068	0.067	0.060	0.065	0.072
19	isolate no. 17 EMa OW 5/99	1	1	1	1	3	4	2	1	2	4	1	5	5	7	1	1	0		0.01	0.067	0.066	0.059	0.064	0.072
20	isolate no. 18 EMa WR 4/00	3	3	3	3	5	5	4	3	4	7	3	8	8	10	4	4	3	3		0.076	0.075	0.065	0.070	0.079
21	*Laminariocolax tomentosoides* Helgoland	27	27	26	27	30	26	29	26	27	30	29	28	28	27	25	25	24	24	27		0.005	0.026	0.030	0.031
22	*Laminariocolax tomentosoides* ssp. *deformans*	30	30	29	30	33	32	32	29	30	35	33	28	28	28	25	25	24	24	27	3		0.032	0.036	0.034
23	*Laminariocolax aecidioides* Kiel	27	27	26	27	30	28	29	27	27	28	27	24	24	23	22	22	21	21	23	12	18		0.007	0
24	*Laminariocolax aecidioides* Maine	30	30	29	30	33	31	32	30	30	31	30	26	26	26	24	24	23	23	25	14	21	4		0
25	*Laminariocolax aecidioides* Bretagne	28	28	27	28	30	29	30	28	28	29	28	25	25	24	23	23	22	22	24	13	18	2	2	

Table D 4.1.3

		26	27	28	29	30	31	32	33	34	35	36	37
		isolate no. 19 EPa A 10/97	isolate no. 20 EPa Riv 9/99	isolate no. 21 EPa BB 4/99	isolate no. 22 EPa BS 10/00	isolate no. 23 EGra BB 2/98	isolate no. 24 EKal CC 10/00	isolate no. 25 EUpi W 4/98	isolate no. 26 EEck Kari 5/99	*Microspongium tenuissimum*	isolate no. 27 EPa OW 5/99	*Microspongium radians*	*Microspongium alariae*
26	isolate no. 19 EPa A 10/97		0.013	0.017	0.013	0	0	0	0	0	0.01	0	0.083
27	isolate no. 20 EPa Riv 9/99	3		0.004	0	0.013	0.008	0.013	0.008	0.008	0.019	0.014	0.079
28	isolate no. 21 EPa BB 4/99	4	1		0.004	0.017	0.013	0.017	0.013	0.013	0.024	0.019	0.083
29	isolate no. 22 EPa BS 10/00	3	0	1		0.013	0.008	0.013	0.008	0.008	0.019	0.014	0.079
30	isolate no. 23 EGra BB 2/98	0	3	4	3		0	0	0	0	0.01	0	0.083
31	isolate no. 24 EKal CC 10/00	1	2	3	2	1		0	0	0	0.01	0	0.078
32	isolate no. 25 EUpi W 4/98	0	3	4	3	0	1		0	0	0.01	0	0.083
33	isolate no. 26 EEck Kari 5/99	1	2	3	2	1	0	1		0	0.01	0	0.078
34	*Microspongium tenuissimum*	1	2	3	2	1	0	1	0		0.01	0	0.076
35	isolate no. 27 EPa OW 5/99	2	4	5	4	2	2	2	2	2		0	0.038
36	*Microspongium radians*	1	3	4	3	1	1	1	1	1	1		0.034
37	*Microspongium alariae*	19	18	19	18	19	18	19	18	17	8	7	

Table D 4.1.4

		38	39	40	41	42	43
		isolate no. 28 ELes OW 5/99	isolate no. 29 ELes Wk 5/99	isolate no. 31 EXi BB 8/99	isolate no. 32 EMu Wk 5/99	isolate no. 33 EDan BB 4/99	isolate no. 34 EDwil BB 8/00
38	isolate no. 28 ELes OW 5/99		0	0.002	0	0.003	0.002
39	isolate no. 29 ELes Wk 5/99	1		0.003	0	0.004	0.003
40	isolate no. 31 EXi BB 8/99	2	3		0.001	0.004	0
41	isolate no. 32 EMu Wk 5/99	1	2	1		0.004	0.001
42	isolate no. 33 EDan BB 4/99	3	4	5	4		0.005
43	isolate no. 34 EDwil BB 8/00	2	3	0	1	5	

Table D 4.1.5

		1	2	3	4	5	6	7	8	9	10	11	13	14	15	16	17
		Tribonema aequale	*Adenocystis utricularis*	*Ascoseirophila violodora*	*Caepidium antarcticum*	*Chordaria flagelliformis*	*Dictyosiphon foeniculaceus*	*Ectocarpus siliculosus*	*Laminariocolax tomentosoides*	*Myrionema strangulans*	*Pylaiella littoralis*	*Scytosiphon lomentaria*	*Asterocladon lobatum*	*Alaria esculenta*	*Macrocystis pyrifera*	*Sporochnus pedunculatus*	*Desmarestia aculeata*
1	*Tribonema aequale*		0.223	0.202	0.218	0.209	0.210	0.222	0.207	0.208	0.207	0.228	0.215	0.213	0.213	0.221	0.199
2	*Adenocystis utricularis*	239		0.088	0.080	0.094	0.096	0.099	0.096	0.096	0.098	0.097	0.128	0.110	0.114	0.129	0.118
3	*Ascoseirophila violodora*	220	104		0.087	0.041	0.038	0.072	0.035	0.039	0.069	0.078	0.111	0.083	0.087	0.105	0.095
4	*Caepidium antarcticum*	235	94	102		0.088	0.088	0.099	0.091	0.091	0.101	0.100	0.126	0.120	0.122	0.134	0.129
5	*Chordaria flagelliformis*	226	110	50	103		0.042	0.081	0.044	0.052	0.077	0.086	0.111	0.099	0.104	0.104	0.101
6	*Dictyosiphon foeniculaceus*	227	112	46	103	51		0.077	0.036	0.042	0.074	0.080	0.119	0.087	0.089	0.108	0.094
7	*Ectocarpus siliculosus*	238	116	86	115	96	91		0.083	0.079	0.080	0.071	0.114	0.093	0.094	0.117	0.107
8	*Laminariocolax tomentosoides*	224	113	43	107	53	44	98		0.045	0.078	0.083	0.124	0.103	0.106	0.116	0.108
9	*Myrionema strangulans*	225	112	47	107	63	51	94	55		0.079	0.086	0.116	0.099	0.098	0.108	0.101
10	*Pylaiella littoralis*	224	114	83	117	91	88	95	92	93		0.090	0.123	0.103	0.109	0.121	0.114
11	*Scytosiphon lomentaria*	244	113	93	116	101	95	85	98	101	105		0.124	0.088	0.096	0.119	0.106
13	*Asterocladon lobatum*	227	143	126	140	125	134	128	139	130	138	139		0.125	0.115	0.129	0.124
14	*Alaria esculenta*	188	104	80	112	94	83	89	97	94	98	84	116		0.030	0.100	0.084
15	*Macrocystis pyrifera*	230	132	103	140	121	105	110	123	115	127	112	129	30		0.099	0.087
16	*Sporochnus pedunculatus*	237	149	123	153	122	126	136	134	126	140	137	144	95	116		0.087
17	*Desmarestia aculeata*	202	126	103	136	109	102	115	116	109	122	114	132	81	95	95	
18	*Himantothallus grandifolius*	221	145	114	149	123	114	122	124	120	134	124	130	82	101	94	30
19	*Scytothamnus australe*	254	177	157	173	161	149	149	163	161	172	159	162	114	145	146	133
20	*Splachnidium rugosum*	250	153	143	161	139	139	127	151	149	157	146	155	100	129	117	111
21	*Ascophyllum nodosum*	256	167	163	171	155	154	155	160	163	163	156	172	120	155	143	133
22	*Sargassum muticum*	259	202	186	199	185	176	177	174	190	196	188	180	144	175	163	157
23	*Alethocladus corymbosus*	226	173	155	178	161	159	154	164	168	166	159	162	117	150	142	117
24	*Cladostephus spongiosus*	234	174	156	184	162	163	162	165	167	165	160	168	118	149	141	117
25	*Herpodiscus durvillaeae*	185	140	136	147	136	133	132	137	145	135	137	146	128	130	127	119
26	*Sphacelaria cirrosa*	226	170	154	165	159	158	148	166	164	162	161	155	113	151	147	130
27	*Stypocaulon scoparium*	236	192	166	188	169	171	162	175	177	167	177	166	130	163	142	129
28	*Syringoderma phinneyi*	239	179	149	180	151	149	153	151	159	172	159	163	120	147	128	107
29	*Onslowia endophytica*	241	192	177	175	184	181	176	185	184	177	188	182	142	168	149	152
30	*Verosphacela ebrachia*	233	189	165	187	168	173	171	169	182	180	172	187	129	162	154	151
31	*Dictyota dichotoma*	246	198	188	199	185	188	187	187	200	187	192	181	158	198	186	167

Table D 4.1.5 (continued)

		18	19	20	21	22	23	24	25	26	27	28	29	30	31
		Himantothallus grandifolius	*Scytothamnus australe*	*Splachnidium rugosum*	*Ascophyllum nodosum*	*Sargassum muticum*	*Alethocladus corymbosus*	*Cladostephus spongiosus*	*Herpodiscus durvillaeae*	*Sphacelaria cirrosa*	*Stypocaulon scoparium*	*Syringoderma phinneyi*	*Onslowia endophytica*	*Verosphacela ebrachia*	*Dictyota dichotoma*
1	*Tribonema aequale*	0.204	0.240	0.235	0.242	0.246	0.209	0.218	0.256	0.210	0.220	0.223	0.226	0.216	0.232
2	*Adenocystis utricularis*	0.126	0.157	0.133	0.147	0.182	0.153	0.154	0.184	0.150	0.172	0.159	0.172	0.168	0.178
3	*Ascoseirophila violodora*	0.097	0.137	0.124	0.143	0.165	0.135	0.136	0.178	0.134	0.146	0.129	0.157	0.145	0.168
4	*Caepidium antarcticum*	0.130	0.153	0.142	0.151	0.179	0.158	0.164	0.195	0.145	0.168	0.160	0.155	0.167	0.179
5	*Chordaria flagelliformis*	0.106	0.141	0.120	0.135	0.164	0.141	0.142	0.178	0.139	0.149	0.132	0.164	0.148	0.165
6	*Dictyosiphon foeniculaceus*	0.097	0.130	0.120	0.135	0.155	0.139	0.143	0.173	0.138	0.151	0.130	0.161	0.153	0.168
7	*Ectocarpus siliculosus*	0.105	0.130	0.109	0.135	0.156	0.134	0.142	0.171	0.129	0.142	0.133	0.156	0.150	0.167
8	*Laminariocolax tomentosoides*	0.106	0.143	0.132	0.140	0.153	0.144	0.145	0.179	0.146	0.155	0.131	0.165	0.149	0.167
10	*Myrionema strangulans*	0.103	0.141	0.130	0.143	0.169	0.148	0.147	0.192	0.144	0.157	0.139	0.164	0.161	0.180
12	*Pylaiella littoralis*	0.116	0.152	0.137	0.143	0.175	0.146	0.145	0.176	0.142	0.147	0.152	0.157	0.159	0.167
13	*Scytosiphon lomentaria*	0.107	0.139	0.127	0.136	0.168	0.139	0.140	0.180	0.141	0.157	0.139	0.168	0.152	0.172
15	*Asterocladon lobatum*	0.116	0.147	0.140	0.157	0.165	0.147	0.153	0.194	0.140	0.151	0.148	0.167	0.172	0.167
16	*Alaria esculenta*	0.086	0.122	0.106	0.129	0.157	0.125	0.126	0.166	0.121	0.141	0.129	0.155	0.140	0.175
17	*Macrocystis pyrifera*	0.085	0.126	0.111	0.136	0.155	0.131	0.130	0.170	0.132	0.143	0.128	0.148	0.142	0.178
18	*Sporochnus pedunculatus*	0.079	0.127	0.100	0.124	0.143	0.123	0.122	0.164	0.128	0.123	0.110	0.130	0.134	0.166
20	*Desmarestia aculeata*	0.026	0.125	0.103	0.125	0.150	0.109	0.109	0.153	0.122	0.121	0.099	0.145	0.144	0.162
21	*Himantothallus grandifolius*		0.129	0.107	0.122	0.148	0.102	0.105	0.155	0.124	0.114	0.097	0.138	0.143	0.167
22	*Scytothamnus australe*	148		0.104	0.155	0.168	0.163	0.166	0.192	0.160	0.172	0.157	0.165	0.169	0.192
23	*Splachnidium rugosum*	124	121		0.132	0.154	0.141	0.145	0.170	0.144	0.149	0.136	0.148	0.153	0.170
24	*Ascophyllum nodosum*	141	175	152		0.111	0.144	0.140	0.161	0.151	0.161	0.146	0.170	0.156	0.175
25	*Sargassum muticum*	168	189	174	129		0.174	0.180	0.188	0.169	0.189	0.169	0.188	0.173	0.180
26	*Alethocladus corymbosus*	120	183	161	164	195		0.062	0.110	0.096	0.053	0.094	0.132	0.132	0.158
27	*Cladostephus spongiosus*	123	186	165	160	200	74		0.105	0.092	0.081	0.107	0.139	0.144	0.154
28	*Herpodiscus durvillaeae*	120	145	131	125	143	88	84		0.131	0.117	0.144	0.176	0.175	0.156
29	*Sphacelaria cirrosa*	143	180	164	171	189	113	108	103		0.109	0.128	0.130	0.150	0.161
30	*Stypocaulon scoparium*	132	192	169	181	209	64	96	93	127		0.110	0.133	0.153	0.167
31	*Syringoderma phinneyi*	114	177	156	166	190	111	125	113	147	128		0.126	0.129	0.161
32	*Onslowia endophytica*	158	185	168	190	208	151	158	135	149	152	145		0.132	0.174
33	*Verosphacela ebrachia*	163	190	173	176	194	152	164	134	170	173	149	151		0.162
34	*Dictyota dichotoma*	187	211	190	195	199	178	174	121	180	187	181	193	182	

Table D 4.1.6

		1	2	3	4	5	6	7	8	9	10	11	**12**	13	14	15
		Tribonema aequale	*Adenocystis utricularis*	*Ascoseirophila violodora*	*Caepidium antarcticum*	*Chordaria flagelliformis*	*Dictyosiphon foeniculaceus*	*Ectocarpus siliculosus*	*Laminariocolax tomentosoides*	*Myrionema strangulans*	*Pilayella littoralis*	*Scytosiphon lomentaria*	***Xiphophorocolax aotearoae***	*Asterocladon lobatum*	*Alaria esculenta*	*Macrocystis pyrifera*
1	*Tribonema aequale*		0.311	0.329	0.322	0.315	0.329	0.334	0.326	0.315	0.318	0.307	**0.321**	0.334	0.305	0.302
2	*Adenocystis utricularis*	132		0.062	0.026	0.058	0.069	0.025	0.058	0.048	0.050	0.032	**0.056**	0.080	0.099	0.099
3	*Ascoseirophila violodora*	139	32		0.064	0.044	0.036	0.057	0.022	0.043	0.054	0.066	**0.034**	0.091	0.118	0.116
4	*Caepidium antarcticum*	136	14	33		0.066	0.070	0.030	0.064	0.050	0.050	0.038	**0.054**	0.080	0.090	0.093
5	*Chordaria flagelliformis*	134	30	23	34		0.034	0.049	0.040	0.047	0.060	0.064	**0.036**	0.082	0.118	0.110
6	*Dictyosiphon foeniculaceus*	138	35	19	36	18		0.059	0.042	0.044	0.064	0.074	**0.030**	0.096	0.108	0.092
7	*Ectocarpus siliculosus*	137	13	29	16	25	30		0.050	0.046	0.047	0.038	**0.051**	0.070	0.096	0.106
8	*Laminariocolax tomentosoides*	138	30	12	33	21	22	26		0.044	0.056	0.060	**0.038**	0.086	0.114	0.110
9	*Myrionema strangulans*	134	25	23	26	25	23	24	23		0.056	0.058	**0.040**	0.082	0.097	0.091
10	*Pilayella littoralis*	134	26	28	26	31	33	24	29	29		0.032	**0.057**	0.076	0.105	0.112
11	*Scytosiphon lomentaria*	131	17	34	20	33	38	20	31	30	17		**0.062**	0.086	0.103	0.107
12	***Xiphophorocolax aotearoae***	**135**	**29**	**18**	**28**	**19**	**16**	**26**	**20**	**21**	**29**	**32**		**0.090**	**0.108**	**0.098**
13	*Asterocladon lobatum*	140	41	46	41	42	48	35	44	42	39	44	**45**		0.120	0.118
14	*Alaria esculenta*	130	50	59	46	59	54	48	57	49	53	52	**54**	60		0.052
15	*Macrocystis pyrifera*	129	50	58	47	55	46	52	55	46	56	54	**49**	59	27	
16	*Sporochnus pedunculatus*	135	45	54	42	54	52	43	51	47	45	45	**54**	51	46	41
17	*Desmarestia aculeata*	132	34	41	38	39	42	33	40	36	38	38	**42**	41	51	49
18	*Himantothallus grandifolius*	142	39	39	41	40	44	37	41	41	38	43	**42**	42	50	46
19	*Scytothamnus australe*	138	42	52	44	52	51	43	48	46	51	47	**46**	59	58	49
20	*Splachnidium rugosum*	139	37	43	39	43	43	36	40	36	40	39	**35**	47	53	46
21	*Ascophyllum nodosum*	145	77	88	85	86	83	74	89	82	79	82	**88**	86	91	88
22	*Sargassum muticum*	149	76	83	76	81	83	67	83	83	77	78	**84**	78	84	80
23	*Alethocladus corymbosus*	138	60	72	58	68	73	58	69	71	60	55	**72**	66	68	66
24	*Cladostephus spongiosus*	131	56	62	53	62	62	53	66	65	61	54	**58**	61	60	59
25	*Herpodiscus durvillaeae*	140	58	71	57	67	67	56	70	67	67	58	**66**	64	64	61
26	*Sphacelaria cirrosa*	138	60	74	57	71	71	59	73	70	69	59	**66**	63	66	61
27	*Stypocaulon scoparium*	135	58	74	58	69	72	59	71	70	65	56	**70**	67	71	64
28	*Syringoderma phinneyi*	142	63	68	66	73	71	62	69	68	61	60	**69**	72	66	72
29	*Onslowia endophytica*	137	64	69	65	69	72	63	68	72	62	58	**66**	64	69	67
30	*Verosphacela ebrachia*	135	72	76	75	74	74	70	75	79	72	69	**76**	65	76	69
31	*Dictyota dichotoma*	156	122	123	124	121	120	120	123	123	125	125	**121**	125	113	121

Table D 4.1.6 (continued)

		16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
		Sporochnus pedunculatus	*Desmarestia aculeata*	*Himantothallus grandifolius*	*Scytothamnus australe*	*Splachnidium rugosum*	*Ascophyllum nodosum*	*Sargassum muticum*	*Alethocladus corymbosus*	*Cladostephus spongiosus*	*Herpodiscus durvillaeae*	*Sphacelaria cirrosa*	*Stypocaulon scoparium*	*Syringoderma phinneyi*	*Onslowia endophytica*	*Verosphacela ebrachia*	*Dictyota dichotoma*
1	*Tribonema aequale*	0.321	0.318	0.340	0.331	0.331	0.348	0.361	0.327	0.315	0.333	0.328	0.330	0.338	0.325	0.317	0.382
2	*Adenocystis utricularis*	0.089	0.067	0.076	0.083	0.072	0.159	0.158	0.120	0.116	0.116	0.121	0.120	0.128	0.130	0.148	0.279
3	*Ascoseirophila violodora*	0.108	0.081	0.076	0.104	0.084	0.185	0.174	0.146	0.128	0.144	0.151	0.156	0.138	0.140	0.156	0.279
4	*Caepidium antarcticum*	0.083	0.075	0.080	0.087	0.076	0.178	0.158	0.116	0.109	0.114	0.114	0.119	0.134	0.132	0.155	0.284
5	*Chordaria flagelliformis*	0.108	0.077	0.078	0.104	0.084	0.180	0.169	0.138	0.128	0.135	0.144	0.145	0.150	0.140	0.152	0.274
6	*Dictyosiphon foeniculaceus*	0.104	0.084	0.087	0.103	0.085	0.174	0.175	0.150	0.129	0.137	0.145	0.153	0.146	0.149	0.153	0.273
7	*Ectocarpus siliculosus*	0.087	0.066	0.073	0.086	0.071	0.155	0.140	0.119	0.111	0.114	0.121	0.125	0.128	0.130	0.147	0.278
8	*Laminariocolax tomentosoides*	0.101	0.079	0.080	0.095	0.078	0.187	0.174	0.140	0.137	0.142	0.149	0.150	0.140	0.138	0.154	0.280
10	*Myrionema strangulans*	0.093	0.071	0.080	0.091	0.070	0.170	0.173	0.144	0.135	0.135	0.142	0.147	0.138	0.147	0.163	0.280
12	*Pilayella littoralis*	0.089	0.075	0.074	0.102	0.078	0.164	0.161	0.120	0.127	0.135	0.140	0.135	0.124	0.125	0.148	0.283
13	*Scytosiphon lomentaria*	0.089	0.075	0.084	0.093	0.076	0.170	0.162	0.109	0.111	0.115	0.118	0.115	0.121	0.116	0.141	0.284
14	***Xiphophorocolax aotearoae***	**0.109**	**0.084**	**0.083**	**0.092**	**0.068**	**0.187**	**0.178**	**0.148**	**0.120**	**0.134**	**0.134**	**0.149**	**0.142**	**0.135**	**0.158**	**0.276**
15	*Asterocladon lobatum*	0.102	0.081	0.083	0.119	0.093	0.180	0.162	0.133	0.126	0.129	0.127	0.140	0.148	0.129	0.132	0.285
16	*Alaria esculenta*	0.091	0.102	0.099	0.116	0.105	0.191	0.175	0.138	0.124	0.129	0.133	0.150	0.135	0.141	0.156	0.252
17	*Macrocystis pyrifera*	0.081	0.099	0.091	0.097	0.091	0.185	0.167	0.133	0.121	0.122	0.122	0.133	0.149	0.136	0.140	0.272
18	*Sporochnus pedunculatus*		0.074	0.070	0.095	0.087	0.169	0.141	0.126	0.128	0.118	0.138	0.140	0.144	0.134	0.127	0.257
20	*Desmarestia aculeata*	37		0.021	0.069	0.057	0.163	0.154	0.118	0.127	0.114	0.127	0.135	0.129	0.132	0.136	0.269
21	*Himantothallus grandifolius*	36	11		0.072	0.056	0.171	0.161	0.119	0.126	0.113	0.137	0.140	0.122	0.129	0.138	0.282
22	*Scytothamnus australe*	48	35	37		0.058	0.158	0.169	0.131	0.142	0.129	0.138	0.145	0.156	0.137	0.153	0.281
23	*Splachnidium rugosum*	44	29	29	30		0.169	0.158	0.137	0.130	0.133	0.140	0.143	0.141	0.134	0.157	0.300
24	*Ascophyllum nodosum*	81	78	82	76	81		0.185	0.203	0.194	0.184	0.206	0.216	0.237	0.209	0.211	0.346
25	*Sargassum muticum*	69	73	77	80	76	88		0.173	0.161	0.154	0.161	0.182	0.204	0.189	0.182	0.301
26	*Alethocladus corymbosus*	63	58	60	65	68	96	83		0.058	0.060	0.069	0.052	0.141	0.127	0.147	0.274
27	*Cladostephus spongiosus*	62	60	61	68	63	90	76	29		0.043	0.061	0.074	0.153	0.122	0.142	0.270
28	*Herpodiscus durvillaeae*	59	56	57	64	66	88	75	32	22		0.063	0.077	0.148	0.118	0.125	0.261
29	*Sphacelaria cirrosa*	68	62	68	68	69	97	78	36	31	33		0.086	0.161	0.126	0.145	0.281
30	*Stypocaulon scoparium*	67	64	67	69	68	98	84	27	37	39	43		0.132	0.131	0.157	0.302
31	*Syringoderma phinneyi*	70	63	61	75	69	109	95	69	72	72	78	63		0.148	0.180	0.284
32	*Onslowia endophytica*	66	64	64	67	66	98	90	63	59	59	63	63	72		0.101	0.251
33	*Verosphacela ebrachia*	63	66	68	74	76	99	87	72	68	62	71	74	85	51		0.244
34	*Dictyota dichotoma*	115	118	125	123	130	146	131	123	118	118	125	129	126	113	111	

Table D 4.2.1

		1	2	3	4	5	6	8	9	10	11	12	13	14	15	16	17	18	19	20
		Tribonema aequale	*Chordaria flagelliformis*	*Dictyosiphon foeniculaceus*	*Ectocarpus siliculosus*	*Punctaria* sp.	*Scytosiphon lomentaria*	*Asterocladon lobatum*	*Asteronema rhodochortonoides*	*Alaria esculenta*	*Laminaria digitata*	*Macrocystis pyrifera*	*Sporochnus pedunculatus*	*Haplospora globobosa*	*Desmarestia aculeata*	*Himantothallus grandifolius*	*Phaeurus antarctica*	*Scytothamnus australe*	*Splachnidium rugosum*	*Cutleria multifida*
1	*Tribonema aequale*		0.226	0.228	0.225	0.232	0.232	0.176	0.192	0.190	0.190	0.185	0.184	0.179	0.179	0.180	0.174	0.190	0.189	0.178
2	*Chordaria flagelliformis*	45		0.013	0.066	0.008	0.062	0.085	0.124	0.094	0.099	0.099	0.098	0.094	0.099	0.099	0.104	0.093	0.089	0.099
3	*Dictyosiphon foeniculaceus*	45	3		0.085	0.013	0.081	0.104	0.123	0.104	0.109	0.110	0.109	0.104	0.110	0.109	0.115	0.113	0.108	0.114
4	*Ectocarpus siliculosus*	45	15	19		0.066	0.052	0.085	0.129	0.113	0.118	0.119	0.113	0.128	0.113	0.118	0.119	0.098	0.098	0.123
5	*Punctaria* sp.	46	2	3	15		0.062	0.085	0.129	0.094	0.099	0.099	0.099	0.104	0.099	0.099	0.104	0.093	0.089	0.099
6	*Scytosiphon lomentaria*	46	14	18	12	14		0.104	0.149	0.118	0.113	0.114	0.113	0.129	0.109	0.113	0.105	0.113	0.108	0.123
8	*Asterocladon lobatum*	36	19	23	19	19	23		0.080	0.066	0.071	0.071	0.061	0.066	0.048	0.052	0.048	0.052	0.048	0.061
9	*A. rhodochortonoides*	39	27	27	28	28	32	18		0.098	0.103	0.099	0.103	0.098	0.089	0.089	0.095	0.084	0.085	0.103
10	*Alaria esculenta*	39	21	23	25	21	26	15	22		0.004	0.004	0.013	0.017	0.013	0.013	0.021	0.021	0.021	0.017
11	*Laminaria digitata*	39	22	24	26	22	25	16	23	1		0	0.008	0.021	0.017	0.017	0.026	0.026	0.026	0.021
12	*Macrocystis pyrifera*	38	22	24	26	22	25	16	22	1	0		0.008	0.021	0.017	0.017	0.026	0.026	0.026	0.021
13	*Sporochnus pedunculatus*	38	22	24	25	22	25	14	23	3	2	2		0.021	0.017	0.017	0.026	0.021	0.017	0.021
14	*Haplospora globobosa*	37	21	23	28	23	28	15	22	4	5	5	5		0.013	0.013	0.017	0.034	0.030	0.008
15	*Desmarestia aculeata*	37	22	24	25	22	24	11	20	3	4	4	4	3		0	0.008	0.026	0.021	0.009
16	*H. grandifolius*	37	22	24	26	22	25	12	20	3	4	4	4	3	0		0.008	0.030	0.025	0.013
17	*Phaeurus antarctica*	36	23	25	26	23	23	11	21	5	6	6	6	4	2	2		0.034	0.030	0.013
18	*Scytothamnus australe*	39	21	25	22	21	25	12	19	5	6	6	5	8	6	7	8		0.013	0.030
19	*Splachnidium rugosum*	39	20	24	22	20	24	11	19	5	6	6	4	7	5	6	7	3		0.025
20	*Cutleria multifida*	37	22	25	27	22	27	14	23	4	5	5	5	2	2	3	3	7	6	
21	*Ascophyllum nodosum*	45	39	40	39	37	38	29	34	25	26	26	25	23	21	21	22	25	24	22
22	*Durvillaea antarctica*	39	23	24	27	23	28	16	23	13	14	14	14	11	10	11	11	16	15	9
23	*Fucus vesiculosus*	46	38	39	41	37	38	34	37	30	30	30	29	30	28	28	29	30	27	29
24	*Sargassum muticum*	39	30	30	34	28	33	21	25	16	17	16	17	14	14	14	15	20	18	14
25	*X. chondrophylla*	41	36	39	36	36	35	27	33	22	23	23	22	22	21	21	20	22	21	21
26	*Notheia anomala*	40	26	29	29	26	28	17	23	11	12	12	12	9	7	8	8	14	13	7
27	*Ascoseira mirabilis*	39	20	23	23	20	26	13	23	8	9	9	8	8	8	8	9	9	7	8
28	*Nemoderma tingitanum*	36	23	26	28	23	26	15	24	5	6	6	6	3	3	4	4	8	7	1
29	*Alethocladus corymbosus*	37	21	25	25	21	25	13	21	8	9	9	9	8	6	6	8	7	5	7
30	*Cladostephus spongiosus*	36	21	25	25	21	25	13	20	8	9	8	9	8	6	6	8	7	5	7
31	***H. durvillaeae***	**36**	**22**	**26**	**24**	**22**	**25**	**14**	**21**	**9**	**10**	**9**	**10**	**9**	**7**	**7**	**9**	**8**	**6**	**8**
32	*Sphacelaria cirrosa*	37	22	26	26	22	26	14	21	9	10	9	10	9	7	7	9	8	6	8
33	*Stypocaulon scoparium*	36	20	24	24	20	24	12	20	7	8	8	8	7	5	5	7	6	4	6
34	*Syringoderma phinneyi*	35	22	26	25	22	25	15	21	10	11	10	11	10	8	9	9	10	8	9
35	*Choristocarpus tenellus*	34	30	32	31	30	30	18	24	16	16	15	17	16	13	13	12	15	14	15
36	*Onslowia endophytica*	36	22	26	24	22	26	12	20	9	10	10	8	9	7	7	9	6	4	8
37	*Dictyota dichotoma*	37	30	33	32	30	32	16	26	16	17	16	16	16	12	12	14	16	15	15
38	*Taonia atomaria*	39	31	35	33	31	31	19	29	16	17	17	16	16	13	13	14	15	14	15

Table D 4.2.1 (cont.)

		21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
		Ascophyllum nodosum	*Durvillaea antarctica*	*Fucus vesiculosus*	*Sargassum muticum*	*Xiphophora chondrophylla*	*Notheia anomala*	*Ascoseira mirabilis*	*Nemoderma tingitanum*	*Alethocladus corymbosus*	*Cladostephus spongiosus*	***Herpodiscus durvillaeae***	*Sphacelaria cirrosa*	*Stypocaulon scoparium*	*Syringoderma phinneyi*	*Choristocarpus tenellus*	*Onslowia endophytica*	*Dictyota dichotoma*	*Taonia atomaria*
1	*Tribonema aequale*	0.222	0.190	0.227	0.189	0.200	0.195	0.189	0.173	0.179	0.173	**0.172**	0.178	0.173	0.169	0.163	0.173	0.178	0.191
2	*Chordaria flagelliformis*	0.188	0.104	0.181	0.139	0.171	0.119	0.088	0.104	0.094	0.094	**0.099**	0.099	0.089	0.099	0.139	0.099	0.139	0.144
3	*Dictyosiphon foeniculaceus*	0.194	0.109	0.186	0.139	0.187	0.134	0.103	0.119	0.113	0.113	**0.118**	0.119	0.109	0.119	0.148	0.119	0.155	0.165
4	*Ectocarpus siliculosus*	0.186	0.123	0.196	0.159	0.169	0.132	0.103	0.128	0.114	0.114	**0.109**	0.119	0.109	0.114	0.144	0.109	0.150	0.155
5	*Punctaria* sp.	0.177	0.104	0.175	0.128	0.171	0.119	0.089	0.104	0.094	0.094	**0.099**	0.099	0.089	0.099	0.139	0.099	0.139	0.144
6	*Scytosiphon lomentaria*	0.183	0.128	0.182	0.155	0.165	0.128	0.118	0.118	0.114	0.114	**0.114**	0.120	0.109	0.114	0.139	0.120	0.152	0.147
8	*Asterocladon lobatum*	0.134	0.071	0.159	0.094	0.123	0.075	0.057	0.066	0.057	0.057	**0.062**	0.062	0.053	0.067	0.080	0.053	0.072	0.086
9	*A. rhodochortonoides*	0.159	0.103	0.175	0.113	0.153	0.103	0.103	0.108	0.093	0.089	**0.093**	0.094	0.089	0.094	0.108	0.089	0.118	0.133
10	*Alaria esculenta*	0.114	0.056	0.137	0.070	0.098	0.048	0.034	0.021	0.034	0.034	**0.039**	0.039	0.030	0.044	0.070	0.039	0.071	0.071
11	*Laminaria digitata*	0.119	0.061	0.137	0.075	0.103	0.052	0.038	0.025	0.039	0.039	**0.043**	0.044	0.034	0.048	0.070	0.043	0.076	0.075
12	*Macrocystis pyrifera*	0.120	0.061	0.138	0.071	0.103	0.052	0.039	0.026	0.039	0.035	**0.039**	0.039	0.035	0.044	0.066	0.044	0.072	0.076
13	*Sporochnus pedunculatus*	0.114	0.061	0.132	0.075	0.098	0.052	0.034	0.025	0.039	0.039	**0.043**	0.043	0.034	0.048	0.075	0.034	0.071	0.071
14	*Haplospora globobosa*	0.104	0.047	0.137	0.061	0.098	0.039	0.034	0.013	0.034	0.034	**0.039**	0.039	0.030	0.044	0.070	0.039	0.071	0.071
15	*Desmarestia aculeata*	0.095	0.043	0.128	0.061	0.094	0.030	0.034	0.013	0.026	0.026	**0.030**	0.030	0.021	0.035	0.057	0.030	0.053	0.057
16	*H. grandifolius*	0.094	0.048	0.127	0.061	0.093	0.034	0.034	0.017	0.026	0.026	**0.030**	0.030	0.021	0.039	0.057	0.030	0.053	0.057
17	*Phaeurus antarctica*	0.100	0.048	0.133	0.066	0.089	0.034	0.039	0.017	0.035	0.034	**0.039**	0.039	0.030	0.039	0.052	0.039	0.062	0.062
18	*Scytothamnus australe*	0.114	0.070	0.138	0.089	0.098	0.061	0.038	0.034	0.030	0.030	**0.034**	0.035	0.026	0.043	0.066	0.026	0.071	0.066
19	*Splachnidium rugosum*	0.110	0.066	0.123	0.080	0.093	0.057	0.030	0.030	0.021	0.021	**0.026**	0.026	0.017	0.035	0.061	0.017	0.067	0.062
20	*Cutleria multifida*	0.099	0.038	0.132	0.061	0.093	0.030	0.034	0.004	0.030	0.030	**0.034**	0.034	0.026	0.039	0.065	0.034	0.066	0.066
21	*Ascophyllum nodosum*		0.112	0.052	0.118	0.061	0.098	0.118	0.094	0.109	0.109	**0.104**	0.114	0.104	0.115	0.138	0.109	0.100	0.095
22	*Durvillaea antarctica*	25		0.136	0.070	0.098	0.047	0.061	0.043	0.070	0.070	**0.066**	0.075	0.066	0.071	0.093	0.075	0.084	0.094
23	*Fucus vesiculosus*	12	30		0.143	0.083	0.122	0.137	0.127	0.138	0.138	**0.138**	0.143	0.133	0.144	0.158	0.138	0.129	0.124
24	*Sargassum muticum*	26	16	31		0.103	0.052	0.070	0.056	0.080	0.075	**0.075**	0.080	0.075	0.075	0.098	0.089	0.093	0.095
25	*X. chondrophylla*	14	22	19	23		0.088	0.098	0.088	0.103	0.103	**0.098**	0.108	0.098	0.109	0.137	0.103	0.104	0.090
26	*Notheia anomala*	22	11	27	12	20		0.061	0.034	0.061	0.061	**0.061**	0.066	0.057	0.066	0.084	0.066	0.076	0.080
27	*Ascoseira mirabilis*	26	14	30	16	22	14		0.038	0.047	0.047	**0.052**	0.052	0.043	0.057	0.079	0.047	0.080	0.075
28	*Nemoderma tingitanum*	21	10	28	13	20	8	9		0.034	0.034	**0.039**	0.039	0.030	0.043	0.070	0.039	0.071	0.062
29	*Alethocladus corymbosus*	24	16	30	18	23	14	11	8		0.008	**0.013**	0.013	0.004	0.021	0.065	0.013	0.062	0.057
30	*Cladostephus spongiosus*	24	16	30	17	23	14	11	8	2		**0.004**	0.004	0.004	0.017	0.061	0.013	0.053	0.056
31	***H. durvillaeae***	**23**	**15**	**30**	**17**	**22**	**14**	**12**	**9**	**3**	**1**		**0.008**	**0.008**	**0.017**	**0.056**	**0.017**	**0.052**	**0.057**
32	*Sphacelaria cirrosa*	25	17	31	18	24	15	12	9	3	1	**2**		0.008	0.021	0.065	0.013	0.053	0.061
33	*Stypocaulon scoparium*	23	15	29	17	22	13	10	7	1	1	**2**	2		0.017	0.061	0.009	0.057	0.052
34	*Syringoderma phinneyi*	25	16	31	17	24	15	13	10	5	4	**4**	5	4		0.061	0.026	0.067	0.066
35	*Choristocarpus tenellus*	30	21	34	22	30	19	18	16	15	14	**13**	15	14	14		0.070	0.094	0.089
36	*Onslowia endophytica*	24	17	30	20	23	15	11	9	3	3	**4**	3	2	6	16		0.053	0.053
37	*Dictyota dichotoma*	22	19	28	21	23	17	18	16	14	12	**12**	12	13	15	21	12		0.035
38	*Taonia atomaria*	21	21	27	21	20	18	17	14	13	13	**13**	14	12	15	20	12	8	

Table D 4.2.2

		1	5	6	7	11	13	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
		Tribonema aequale	*Chordaria flagelliformis*	*Dictyosiphon foeniculaceus*	*Ectocarpus siliculosus*	*Punctaria* sp.	*Scytosiphon lomentaria*	*Asterocladon lobatum*	*Asteronema rhodochorton.*	*Alaria esculenta*	*Laminaria digitata*	*Macrocystis pyrifera*	*Undaria pinnatifida*	*Sporochnus pedunculatus*	*Haplospora globobosa*	*Tilopteris mertensii*	*Desmarestia aculeata*	*Himantothallus grandifolius*	*Phaeurus antarctica*	*Scytothamnus australe*	*Splachnidium rugosum*	*Cutleria multifida*
1	*Tribonema aequale*		0.345	0.362	0.366	0.351	0.336	0.367	0.359	0.333	0.343	0.328	0.327	0.351	0.357	0.363	0.350	0.374	0.448	0.363	0.363	0.369
5	*Chordaria flagelliformis*	133		0.037	0.053	0.033	0.069	0.089	0.092	0.129	0.108	0.117	0.122	0.117	0.096	0.091	0.084	0.085	0.105	0.113	0.091	0.098
6	*Dictyosiphon foeniculaceus*	137	18		0.064	0.035	0.081	0.104	0.111	0.118	0.092	0.098	0.111	0.114	0.085	0.083	0.092	0.095	0.113	0.112	0.092	0.094
7	*Ectocarpus siliculosus*	136	25	30		0.053	0.042	0.076	0.077	0.105	0.102	0.112	0.109	0.094	0.075	0.077	0.071	0.080	0.092	0.094	0.077	0.074
11	*Punctaria* sp.	135	16	17	25		0.065	0.085	0.087	0.115	0.098	0.106	0.107	0.110	0.082	0.075	0.077	0.078	0.097	0.106	0.084	0.087
13	*Scytosiphon lomentaria*	130	33	38	20	31		0.094	0.092	0.112	0.103	0.114	0.109	0.096	0.087	0.084	0.081	0.092	0.103	0.101	0.082	0.089
15	*Asterocladon lobatum*	139	42	48	35	40	44		0.052	0.131	0.115	0.127	0.123	0.111	0.094	0.096	0.088	0.090	0.103	0.130	0.101	0.082
16	*Asteronema rhodochortonoides*	136	43	51	36	41	43	25		0.124	0.110	0.120	0.124	0.107	0.085	0.087	0.088	0.088	0.104	0.116	0.088	0.080
17	*Alaria esculenta*	129	59	54	48	53	52	60	57		0.035	0.054	0.012	0.099	0.100	0.100	0.111	0.108	0.117	0.127	0.114	0.103
18	*Laminaria digitata*	132	50	43	47	46	48	53	51	17		0.033	0.035	0.085	0.087	0.087	0.102	0.089	0.114	0.110	0.094	0.091
19	*Macrocystis pyrifera*	127	54	45	51	49	53	58	55	26	16		0.045	0.085	0.091	0.092	0.105	0.096	0.118	0.103	0.096	0.101
20	*Undaria pinnatifida*	127	56	51	50	50	51	57	57	6	17	22		0.099	0.100	0.101	0.107	0.103	0.117	0.122	0.110	0.103
21	*Sporochnus pedunculatus*	134	54	52	43	51	45	51	49	46	40	40	46		0.079	0.074	0.080	0.076	0.085	0.104	0.095	0.087
22	*Haplospora globobosa*	136	45	40	35	39	41	44	40	47	41	43	47	37		0.012	0.058	0.053	0.077	0.085	0.069	0.051
23	*Tilopteris mertensii*	138	43	39	36	36	40	45	41	47	41	43	47	35	6		0.054	0.047	0.067	0.088	0.069	0.047
24	*Desmarestia aculeata*	131	39	42	33	36	38	41	41	51	47	48	49	37	28	26		0.022	0.032	0.075	0.061	0.072
25	*Himantothallus grandifolius*	141	40	44	37	37	43	42	41	50	42	45	48	36	26	23	11		0.014	0.079	0.061	0.068
26	*Phaeurus antarctica*	132	40	42	34	37	39	38	39	44	43	44	44	33	30	26	13	6		0.105	0.076	0.082
27	*Scytothamnus australe*	137	52	51	43	49	47	59	53	58	51	48	56	48	40	41	35	37	39		0.063	0.097
28	*Splachnidium rugosum*	138	43	43	36	40	39	47	41	53	44	45	51	44	33	33	29	29	29	30		0.069
29	*Cutleria multifida*	140	46	44	35	41	42	39	38	48	43	47	48	41	25	23	34	33	31	45	33	
30	*Ascophyllum nodosum*	144	86	83	74	82	82	86	86	91	87	87	93	81	82	79	78	82	78	76	81	79
31	*Durvillaea antarctica*	136	58	62	57	57	60	58	60	70	64	68	72	65	61	59	61	62	63	65	63	64
32	*Fucus vesiculosus*	145	82	79	71	79	80	84	82	90	83	85	92	78	79	76	78	81	78	74	74	73
33	*Sargassum muticum*	148	81	83	67	78	78	78	75	84	82	79	89	69	74	72	73	77	74	80	76	76
34	*Xiphophora chondrophylla*	148	83	81	70	80	75	89	84	88	84	86	89	90	85	85	80	86	81	84	83	87
35	*Notheia anomala*	136	93	92	85	90	90	81	80	85	80	81	87	84	89	88	82	85	84	92	87	88
36	*Ascoseira mirabilis*	136	34	38	26	31	33	36	37	44	42	45	44	32	24	24	24	26	27	37	32	32
37	*Nemoderma tingitanum*	134	54	54	44	49	47	57	53	61	56	57	61	47	41	40	43	43	43	49	49	43
38	*Alethocladus corymbosus*	137	67	72	57	62	54	65	62	67	66	64	68	62	64	63	57	59	57	64	67	63
39	*Cladostephus spongiosus*	130	62	62	53	56	54	61	63	60	60	58	59	62	61	60	60	61	56	68	63	56
40	***Herpodiscus durvillaeae***	**138**	**67**	**67**	**56**	**63**	**58**	**64**	**64**	**64**	**61**	**60**	**66**	**59**	**58**	**57**	**56**	**57**	**57**	**64**	**66**	**55**
41	*Sphacelaria cirrosa*	137	71	71	59	67	59	63	61	66	64	60	65	68	64	64	62	68	64	68	69	61
42	*Stypocaulon scoparium*	132	68	71	58	63	55	66	58	70	68	62	69	66	62	62	63	66	63	68	67	64
43	*Syringoderma phinneyi*	141	73	71	62	66	60	72	70	66	68	71	69	70	60	57	63	61	58	75	69	64
44	*Choristocarpus tenellus*	137	104	105	104	107	101	114	114	98	97	94	97	101	103	104	101	104	96	97	106	111
45	*Onslowia endophytica*	136	69	72	63	65	58	64	65	69	68	66	69	66	66	63	64	64	60	67	66	62
46	*Verosphacela ebrachia*	134	72	72	68	70	67	63	70	74	70	66	73	61	64	67	64	66	64	72	74	69
47	*Dictyota dichotoma*	154	120	119	119	121	124	124	127	112	116	119	113	114	125	125	117	124	117	122	129	127
48	*Taonia atomaria*	161	120	126	120	124	118	123	126	116	119	117	115	112	122	122	116	118	113	121	127	121

Table D 4.2.2 (cont.)

		30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
		Ascophyllum nodosum	*Durvillaea antarctica*	*Fucus vesiculosus*	*Sargassum muticum*	*Xiphophora chondrophylla*	*Notheia anomala*	*Ascoseira mirabilis*	*Nemoderma tingitanum*	*Alethocladus corymbosus*	*Cladostephus spongiosus*	***H. durvillaeae***	*Sphacelaria cirrosa*	*Stypocaulon scoparium*	*Syringoderma phinneyi*	*Choristocarpus tenellus*	*Onslowia endophytica*	*Verosphacela ebrachia*	*Dictyota dichotoma*	*Taonia atomaria*
1	*Tribonema aequale*	0.383	0.392	0.387	0.397	0.395	0.353	0.357	0.347	0.358	0.346	**0.363**	0.360	0.358	0.372	0.337	0.356	0.348	0.417	0.431
5	*Chordaria flagelliformis*	0.197	0.136	0.186	0.185	0.188	0.217	0.071	0.117	0.148	0.140	**0.148**	0.158	0.156	0.164	0.253	0.153	0.161	0.300	0.296
6	*Dictyosiphon foeniculaceus*	0.191	0.147	0.180	0.191	0.185	0.216	0.081	0.117	0.161	0.141	**0.149**	0.159	0.165	0.160	0.259	0.162	0.162	0.299	0.317
7	*Ectocarpus siliculosus*	0.170	0.136	0.161	0.153	0.158	0.200	0.055	0.096	0.127	0.122	**0.124**	0.132	0.134	0.139	0.261	0.142	0.156	0.304	0.304
11	*Punctaria* sp.	0.186	0.133	0.178	0.177	0.180	0.209	0.065	0.105	0.135	0.125	**0.138**	0.147	0.143	0.146	0.261	0.143	0.156	0.302	0.307
13	*Scytosiphon lomentaria*	0.186	0.141	0.181	0.177	0.168	0.208	0.069	0.101	0.116	0.121	**0.125**	0.128	0.123	0.132	0.243	0.126	0.149	0.310	0.289
15	*Asterocladon lobatum*	0.197	0.136	0.191	0.178	0.205	0.185	0.076	0.124	0.143	0.138	**0.141**	0.138	0.150	0.162	0.282	0.141	0.139	0.311	0.306
16	*Asteronema rhodochortonoides*	0.197	0.141	0.186	0.170	0.192	0.183	0.078	0.115	0.136	0.143	**0.141**	0.134	0.130	0.156	0.284	0.144	0.156	0.322	0.316
17	*Alaria esculenta*	0.209	0.168	0.206	0.192	0.202	0.195	0.094	0.134	0.148	0.136	**0.141**	0.146	0.162	0.147	0.236	0.154	0.165	0.275	0.284
18	*Laminaria digitata*	0.199	0.151	0.188	0.187	0.192	0.182	0.089	0.122	0.145	0.135	**0.133**	0.141	0.156	0.152	0.233	0.151	0.156	0.287	0.294
19	*Macrocystis pyrifera*	0.199	0.162	0.194	0.180	0.198	0.185	0.096	0.125	0.140	0.130	**0.131**	0.131	0.140	0.160	0.226	0.146	0.146	0.293	0.286
20	*Undaria pinnatifida*	0.214	0.173	0.211	0.205	0.205	0.200	0.094	0.134	0.150	0.133	**0.145**	0.143	0.158	0.155	0.233	0.154	0.163	0.277	0.281
21	*Sporochnus pedunculatus*	0.185	0.154	0.177	0.154	0.208	0.193	0.067	0.101	0.135	0.140	**0.128**	0.150	0.150	0.157	0.244	0.146	0.134	0.280	0.271
22	*Haplospora globobosa*	0.186	0.143	0.178	0.167	0.194	0.206	0.050	0.087	0.140	0.137	**0.126**	0.140	0.140	0.130	0.250	0.146	0.141	0.311	0.302
23	*Tilopteris mertensii*	0.178	0.138	0.170	0.161	0.194	0.203	0.049	0.085	0.137	0.135	**0.123**	0.140	0.140	0.123	0.252	0.138	0.148	0.311	0.301
24	*Desmarestia aculeata*	0.179	0.144	0.179	0.168	0.184	0.191	0.050	0.093	0.126	0.139	**0.125**	0.139	0.145	0.141	0.251	0.144	0.144	0.294	0.290
25	*Himantothallus grandifolius*	0.187	0.146	0.184	0.176	0.197	0.196	0.054	0.092	0.128	0.137	**0.123**	0.150	0.150	0.133	0.253	0.141	0.146	0.309	0.288
26	*Phaeurus antarctica*	0.231	0.191	0.230	0.208	0.240	0.249	0.068	0.115	0.152	0.157	**0.154**	0.178	0.183	0.164	0.287	0.162	0.177	0.376	0.355
27	*Scytothamnus australe*	0.172	0.154	0.167	0.185	0.192	0.217	0.078	0.106	0.141	0.155	**0.141**	0.150	0.157	0.170	0.235	0.149	0.162	0.307	0.301
28	*Splachnidium rugosum*	0.185	0.149	0.166	0.173	0.189	0.202	0.067	0.105	0.148	0.143	**0.145**	0.153	0.153	0.154	0.260	0.146	0.167	0.328	0.319
29	*Cutleria multifida*	0.178	0.151	0.162	0.172	0.199	0.203	0.067	0.092	0.137	0.125	**0.118**	0.133	0.145	0.140	0.272	0.136	0.153	0.317	0.297
30	*Ascophyllum nodosum*		0.168	0.037	0.203	0.152	0.224	0.174	0.174	0.220	0.213	**0.201**	0.225	0.235	0.261	0.332	0.229	0.226	0.380	0.415
31	*Durvillaea antarctica*	70		0.152	0.185	0.173	0.170	0.135	0.131	0.170	0.173	**0.174**	0.179	0.167	0.213	0.272	0.179	0.170	0.336	0.347
32	*Fucus vesiculosus*	18	64		0.198	0.150	0.202	0.166	0.151	0.217	0.205	**0.201**	0.230	0.229	0.260	0.341	0.223	0.221	0.379	0.404
33	*Sargassum muticum*	88	76	86		0.222	0.186	0.147	0.156	0.186	0.177	**0.169**	0.176	0.198	0.224	0.287	0.207	0.194	0.329	0.342
34	*Xiphophora chondrophylla*	69	72	68	95		0.208	0.181	0.170	0.214	0.220	**0.208**	0.228	0.219	0.243	0.309	0.201	0.232	0.364	0.375
35	*Notheia anomala*	96	70	88	81	90		0.193	0.176	0.182	0.193	**0.203**	0.196	0.202	0.239	0.282	0.214	0.222	0.339	0.352
36	*Ascoseira mirabilis*	77	58	74	66	80	84		0.082	0.130	0.133	**0.123**	0.138	0.141	0.122	0.250	0.127	0.134	0.284	0.281
37	*Nemoderma tingitanum*	77	56	68	70	76	78	39		0.140	0.143	**0.136**	0.147	0.138	0.163	0.256	0.148	0.159	0.321	0.300
38	*Alethocladus corymbosus*	95	71	94	82	93	80	60	64		0.061	**0.063**	0.073	0.057	0.151	0.224	0.136	0.158	0.302	0.284
39	*Cladostephus spongiosus*	90	72	87	76	93	82	59	63	28		**0.046**	0.066	0.078	0.168	0.223	0.133	0.150	0.296	0.277
40	***Herpodiscus durvillaeae***	**88**	**73**	**88**	**75**	**91**	**88**	**57**	**62**	**31**	**22**		**0.069**	**0.082**	**0.161**	**0.227**	**0.128**	**0.131**	**0.285**	**0.259**
41	*Sphacelaria cirrosa*	97	74	99	78	98	86	63	67	35	31	**33**		0.091	0.176	0.208	0.137	0.153	0.307	0.270
42	*Stypocaulon scoparium*	97	70	95	83	92	85	62	61	27	36	**38**	42		0.142	0.249	0.141	0.169	0.334	0.295
43	*Syringoderma phinneyi*	109	86	109	95	103	101	56	73	68	72	**72**	78	62		0.259	0.161	0.192	0.311	0.287
44	*Choristocarpus tenellus*	130	103	133	116	123	113	103	106	95	92	**96**	89	101	106		0.236	0.229	0.301	0.303
45	*Onslowia endophytica*	98	74	96	90	88	92	58	67	62	59	**59**	63	62	72	99		0.106	0.273	0.265
46	*Verosphacela ebrachia*	97	71	95	85	99	95	61	71	71	66	**60**	69	73	83	97	49		0.265	0.258
47	*Dictyota dichotoma*	145	122	145	130	140	133	115	127	123	117	**117**	124	129	125	124	112	110		0.168
48	*Taonia atomaria*	156	127	153	135	144	137	115	121	118	112	**109**	112	117	117	127	110	108	77	

Table D 4.2.3

		1	5	6	7	11	13	15	16	17	18	19	20	21	22	23	24
		Tribonema aequale	*Chordaria flagelliformis*	*Dictyosiphon foeniculaceus*	*Ectocarpus siliculosus*	*Punctaria* sp	*Scytosiphon lomentaria*	*Asterocladon lobatum*	*Alaria esculenta*	*Macrocystis pyrifera*	*Sporochnus pedunculatus*	*Tilopteris mertensii*	*Desmarestia aculeata*	*Himantothallus grandifolius*	*Scytothamnus australe*	*Splachnidium rugosum*	*Ascophyllum nodosum*
1	*Tribonema aequale*		0.209	0.210	0.222	0.198	0.228	0.215	0.213	0.213	0.221	0.215	0.199	0.204	0.240	0.235	0.242
5	*Chordaria flagelliformis*	226		0.042	0.081	0.041	0.086	0.111	0.099	0.104	0.104	0.119	0.101	0.106	0.141	0.120	0.135
6	*Dictyosiphon foeniculaceus*	227	51		0.077	0.037	0.080	0.119	0.087	0.089	0.108	0.118	0.094	0.097	0.130	0.120	0.135
7	*Ectocarpus siliculosus*	238	96	91		0.074	0.071	0.114	0.093	0.094	0.117	0.126	0.107	0.105	0.130	0.109	0.135
11	*Punctaria* sp	216	50	45	88		0.079	0.108	0.088	0.090	0.102	0.119	0.094	0.098	0.133	0.117	0.135
13	*Scytosiphon lomentaria*	244	101	95	85	94		0.124	0.088	0.096	0.119	0.127	0.106	0.107	0.139	0.127	0.136
15	*Asterocladon lobatum*	227	125	134	128	122	139		0.125	0.115	0.129	0.138	0.124	0.116	0.147	0.140	0.157
16	*Alaria esculenta*	188	94	83	89	84	84	116		0.030	0.100	0.112	0.084	0.086	0.122	0.106	0.129
17	*Macrocystis pyrifera*	230	121	105	110	106	112	129	30		0.099	0.109	0.087	0.085	0.126	0.111	0.136
18	*Sporochnus pedunculatus*	237	122	126	136	119	137	144	95	116		0.088	0.087	0.079	0.127	0.100	0.124
19	*Tilopteris mertensii*	232	138	137	145	138	146	153	105	127	104		0.092	0.087	0.130	0.117	0.108
20	*Desmarestia aculeata*	202	109	102	115	102	114	132	81	95	95	100		0.026	0.125	0.103	0.125
21	*Himantothallus grandifolius*	221	123	114	122	115	124	130	82	101	94	103	30		0.129	0.107	0.122
22	*Scytothamnus australe*	254	161	149	149	153	159	162	114	145	146	149	133	148		0.104	0.155
23	*Splachnidium rugosum*	250	139	139	127	136	146	155	100	129	117	135	111	124	121		0.132
24	*Ascophyllum nodosum*	256	155	154	155	155	156	172	120	155	143	126	133	141	175	152	
25	*Sargassum muticum*	259	185	176	177	181	188	180	144	175	163	137	157	168	189	174	129
26	*Alethocladus corymbosus*	226	161	159	154	158	159	162	117	150	142	140	117	120	183	161	164
27	*Cladostephus spongiosus*	234	162	163	162	166	160	168	118	149	141	142	117	123	186	165	160
28	***Herpodiscus durvillaeae***	**185**	**136**	**133**	**132**	**136**	**137**	**146**	**128**	**130**	**127**	**126**	**119**	**120**	**145**	**131**	**125**
32	*Sphacelaria cirrosa*	226	159	158	148	158	161	155	113	151	147	147	130	143	180	164	171
46	*Stypocaulon scoparium*	236	169	171	162	167	177	166	130	163	142	150	129	132	192	169	181
47	*Syringoderma phinneyi*	239	151	149	153	151	159	163	120	147	128	134	107	114	177	156	166
48	*Choristocarpus tenellus*	228	203	205	205	200	212	204	160	207	188	188	179	193	206	197	200
49	*Onslowia endophytica*	241	184	181	176	176	188	182	142	168	149	166	152	158	185	168	190
50	*Verosphacela ebrachia*	233	168	173	171	168	172	187	129	162	154	161	151	163	190	173	176
52	*Dictyota dichotoma*	246	185	188	187	177	192	181	158	198	186	181	167	187	211	190	195

Table D 4.2.3 (continued)

		25	26	27	**28**	32	46	47	48	49	50	52
		Sargassum muticum	*Alethocladus corymbosus*	*Cladostephus spongiosus*	***Herpodiscus durvillaeae***	*Sphacelaria cirrosa*	*Stypocaulon scoparium*	*Syringoderma phinneyi*	*Choristocarpus tenellus*	*Onslowia endophytica*	*Verosphacela ebrachia*	*Dictyota dichotoma*
1	*Tribonema aequale*	0.246	0.209	0.218	**0.256**	0.210	0.220	0.223	0.212	0.226	0.216	0.232
5	*Chordaria flagelliformis*	0.164	0.141	0.142	**0.178**	0.139	0.149	0.132	0.183	0.164	0.148	0.165
6	*Dictyosiphon foeniculaceus*	0.155	0.139	0.143	**0.173**	0.138	0.151	0.130	0.185	0.161	0.153	0.168
7	*Ectocarpus siliculosus*	0.156	0.134	0.142	**0.171**	0.129	0.142	0.133	0.185	0.156	0.150	0.167
11	*Punctaria* sp	0.160	0.138	0.146	**0.177**	0.138	0.147	0.131	0.180	0.156	0.148	0.157
13	*Scytosiphon lomentaria*	0.168	0.139	0.140	**0.180**	0.141	0.157	0.139	0.192	0.168	0.152	0.172
15	*Asterocladon lobatum*	0.165	0.147	0.153	**0.194**	0.140	0.151	0.148	0.189	0.167	0.172	0.167
16	*Alaria esculenta*	0.157	0.125	0.126	**0.166**	0.121	0.141	0.129	0.177	0.155	0.140	0.175
17	*Macrocystis pyrifera*	0.155	0.131	0.130	**0.170**	0.132	0.143	0.128	0.187	0.148	0.142	0.178
18	*Sporochnus pedunculatus*	0.143	0.123	0.122	**0.164**	0.128	0.123	0.110	0.168	0.130	0.134	0.166
19	*Tilopteris mertensii*	0.118	0.121	0.123	**0.163**	0.128	0.130	0.115	0.168	0.146	0.141	0.161
20	*Desmarestia aculeata*	0.150	0.109	0.109	**0.153**	0.122	0.121	0.099	0.174	0.145	0.144	0.162
21	*Himantothallus grandifolius*	0.148	0.102	0.105	**0.155**	0.124	0.114	0.097	0.173	0.138	0.143	0.167
22	*Scytothamnus australe*	0.168	0.163	0.166	**0.192**	0.160	0.172	0.157	0.186	0.165	0.169	0.192
23	*Splachnidium rugosum*	0.154	0.141	0.145	**0.170**	0.144	0.149	0.136	0.176	0.148	0.153	0.170
24	*Ascophyllum nodosum*	0.111	0.144	0.140	**0.161**	0.151	0.161	0.146	0.180	0.170	0.156	0.175
25	*Sargassum muticum*		0.174	0.180	**0.188**	0.169	0.189	0.169	0.193	0.188	0.173	0.180
26	*Alethocladus corymbosus*	195		0.062	**0.110**	0.096	0.053	0.094	0.172	0.132	0.132	0.158
27	*Cladostephus spongiosus*	200	74		**0.105**	0.092	0.081	0.107	0.180	0.139	0.144	0.154
28	***Herpodiscus durvillaeae***	**143**	**88**	**84**		**0.131**	**0.117**	**0.144**	**0.177**	**0.176**	**0.175**	**0.156**
32	*Sphacelaria cirrosa*	189	113	108	**103**		0.109	0.128	0.158	0.130	0.150	0.161
46	*Stypocaulon scoparium*	209	64	96	**93**	127		0.110	0.181	0.133	0.153	0.167
47	*Syringoderma phinneyi*	190	111	125	**113**	147	128		0.172	0.126	0.129	0.161
48	*Choristocarpus tenellus*	213	192	200	**136**	178	201	192		0.192	0.176	0.173
49	*Onslowia endophytica*	208	151	158	**135**	149	152	145	212		0.132	0.174
50	*Verosphacela ebrachia*	194	152	164	**134**	170	173	149	197	151		0.162
52	*Dictyota dichotoma*	199	178	174	**121**	180	187	181	193	193	182	

Table D 4.2.4

		26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
		Alethocladus corymbosus	*Cladostephus spongiosus*	***Herpodiscus durvillaeae***	*Sphacelaria arctica*	*Sphacelaria caespitula*	*Sphacelaria californica*	*Sphacelaria cirrosa*	*Sphacelaria divaricata*	*Sphacelaria nana*	*Sphacelaria plumigera*	*Sphacelaria plumosa*	*Sphacelaria racemosa*	*Sphacelaria radicans*	*Sphacelaria rigidula*	*Sphacelaria tribuloides*	*Sphacelaria yamadae*
26	*Alethocladus corymbosus*		0.062	**0.110**	0.055	0.040	0.090	0.096	0.090	0.068	0.057	0.067	0.067	0.057	0.094	0.093	0.090
27	*Cladostephus spongiosus*	74		**0.105**	0.040	0.054	0.078	0.092	0.073	0.046	0.036	0.049	0.050	0.070	0.086	0.078	0.079
28	***Herpodiscus durvillaeae***	**88**	**84**		**0.093**	**0.109**	**0.108**	**0.131**	**0.108**	**0.102**	**0.098**	**0.106**	**0.095**	**0.108**	**0.111**	**0.118**	**0.115**
29	*Sphacelaria arctica*	66	48	**75**		0.046	0.073	0.091	0.075	0.045	0.028	0.033	0.032	0.064	0.083	0.081	0.080
30	*Sphacelaria caespitula*	49	65	**87**	56		0.080	0.085	0.084	0.057	0.051	0.060	0.056	0.050	0.087	0.086	0.085
31	*Sphacelaria californica*	106	93	**86**	87	95		0.058	0.025	0.087	0.081	0.086	0.079	0.103	0.033	0.034	0.033
32	*Sphacelaria cirrosa*	113	108	**103**	107	101	70		0.061	0.104	0.092	0.098	0.099	0.087	0.069	0.061	0.063
33	*Sphacelaria divaricata*	106	87	**86**	89	99	31	73		0.088	0.078	0.087	0.081	0.102	0.027	0.034	0.033
34	*Sphacelaria nana*	81	56	**82**	54	69	102	121	103		0.049	0.054	0.053	0.077	0.092	0.092	0.092
35	*Sphacelaria plumigera*	69	44	**79**	34	62	96	108	92	59		0.036	0.042	0.069	0.090	0.084	0.086
36	*Sphacelaria plumosa*	80	59	**85**	40	72	101	115	102	65	44		0.041	0.065	0.094	0.089	0.094
37	*Sphacelaria racemosa*	80	60	**77**	39	68	94	116	96	64	51	50		0.087	0.084	0.084	0.087
38	*Sphacelaria radicans*	36	44	**47**	40	32	63	54	62	48	43	41	54		0.110	0.098	0.101
39	*Sphacelaria rigidula*	110	100	**88**	97	102	40	81	33	107	105	109	98	67		0.041	0.041
40	*Sphacelaria tribuloides*	110	93	**94**	96	102	41	73	42	108	99	105	99	60	49		0.028
41	*Sphacelaria yamadae*	106	94	**92**	95	101	40	75	40	108	101	110	103	62	49	34	
42	*Sphacella subtilissima*	91	80	**85**	73	81	102	107	99	92	76	85	82	61	106	102	104
43	*Halopteris filicina* 1	66	93	**86**	96	83	123	124	125	97	96	105	105	49	121	127	124
44	*Halopteris filicina* 2	68	98	**93**	97	86	121	131	126	104	99	108	106	42	128	125	129
45	*Stypocaulon durum*	42	85	**92**	75	60	114	114	116	87	77	90	92	35	117	119	117
46	*Stypocaulon scoparium*	64	96	**93**	93	77	126	127	121	102	94	103	103	43	127	125	124
47	*Syringoderma phinneyi*	111	125	**113**	117	107	133	147	140	137	121	127	127	64	142	139	139
48	*Choristocarpus tenellus*	192	200	**136**	192	188	179	178	186	206	198	200	197	100	184	182	170
49	*Onslowia endophytica*	151	158	**135**	155	140	160	149	167	167	156	159	165	74	167	164	159
50	*Verosphacela ebrachia*	152	164	**134**	167	144	173	170	172	174	158	165	173	86	169	168	161
51	*Dictyota cervicornis*	174	179	**124**	176	167	175	191	180	182	181	187	178	104	176	184	185
52	*Dictyota dichotoma*	178	174	**121**	182	172	174	180	181	184	181	188	182	116	177	177	174

Table D 4.2.4 (continued)

		42	43	44	45	46	47	48	49	50	51	52
		Sphacella subtilissima	*Halopteris filicina* 1	*Halopteris filicina* 2	*Stypocaulon durum*	*Stypocaulon scoparium*	*Syringoderma phinneyi*	*Choristocarpus tenellus*	*Onslowia endophytica*	*Verosphacela ebrachia*	*Dictyota cervicornis*	*Dictyota dichotoma*
26	*Alethocladus corymbosus*	0.077	0.056	0.056	0.034	0.053	0.094	0.172	0.132	0.132	0.154	0.158
27	*Cladostephus spongiosus*	0.067	0.080	0.083	0.071	0.081	0.107	0.180	0.139	0.144	0.159	0.154
28	***Herpodiscus durvillaeae***	**0.107**	**0.110**	**0.117**	**0.116**	**0.117**	**0.144**	**0.177**	**0.176**	**0.175**	**0.159**	**0.156**
29	*Sphacelaria arctica*	0.061	0.082	0.082	0.062	0.078	0.100	0.172	0.135	0.147	0.156	0.162
30	*Sphacelaria caespitula*	0.068	0.071	0.072	0.049	0.064	0.091	0.167	0.121	0.125	0.147	0.152
31	*Sphacelaria californica*	0.087	0.107	0.103	0.097	0.108	0.114	0.159	0.140	0.153	0.155	0.154
32	*Sphacelaria cirrosa*	0.091	0.108	0.113	0.097	0.109	0.128	0.158	0.130	0.150	0.171	0.161
33	*Sphacelaria divaricata*	0.084	0.109	0.108	0.099	0.104	0.121	0.166	0.147	0.152	0.160	0.161
34	*Sphacelaria nana*	0.078	0.083	0.088	0.073	0.086	0.118	0.186	0.147	0.154	0.162	0.165
35	*Sphacelaria plumigera*	0.064	0.082	0.084	0.064	0.079	0.103	0.178	0.136	0.138	0.161	0.161
36	*Sphacelaria plumosa*	0.072	0.091	0.092	0.076	0.087	0.109	0.180	0.139	0.145	0.167	0.168
37	*Sphacelaria racemosa*	0.069	0.091	0.090	0.077	0.087	0.109	0.177	0.145	0.153	0.158	0.163
38	*Sphacelaria radicans*	0.100	0.081	0.067	0.055	0.069	0.104	0.168	0.122	0.143	0.177	0.201
39	*Sphacelaria rigidula*	0.091	0.107	0.111	0.101	0.110	0.124	0.165	0.149	0.150	0.157	0.159
40	*Sphacelaria tribuloides*	0.087	0.111	0.107	0.102	0.107	0.120	0.162	0.144	0.148	0.164	0.157
41	*Sphacelaria yamadae*	0.089	0.108	0.111	0.100	0.106	0.120	0.150	0.139	0.141	0.165	0.154
42	*Sphacella subtilissima*		0.096	0.096	0.086	0.095	0.126	0.180	0.144	0.152	0.164	0.168
43	*Halopteris filicina* 1	111		0.048	0.064	0.070	0.114	0.166	0.147	0.147	0.159	0.155
44	*Halopteris filicina* 2	112	57		0.058	0.070	0.113	0.174	0.149	0.155	0.167	0.170
45	*Stypocaulon durum*	101	75	70		0.060	0.106	0.174	0.136	0.141	0.167	0.168
46	*Stypocaulon scoparium*	111	82	84	72		0.110	0.181	0.133	0.153	0.162	0.167
47	*Syringoderma phinneyi*	145	130	131	124	128		0.172	0.126	0.129	0.148	0.161
48	*Choristocarpus tenellus*	199	183	194	194	201	192		0.192	0.176	0.186	0.173
49	*Onslowia endophytica*	163	164	169	156	152	145	212		0.132	0.183	0.174
50	*Verosphacela ebrachia*	172	164	175	161	173	149	197	151		0.161	0.162
51	*Dictyota cervicornis*	184	176	187	187	182	168	206	203	181		0.127
52	*Dictyota dichotoma*	187	172	190	188	187	181	193	193	182	145	

E TEM PROTOCOLS

Table E 1: TEM protocols for chemical fixation.

	protocol no.		1	2	3	4	5	6
		date	21.10.97	17.11.97	8.3.98	7.4.98	21.6.99	4.8.99
		species	*D. antarctica*	*D. antarctica*	*D. antarctica*	*D. antarctica*	*D. antarctica*	*D. antarctica*
		site	Brighton Beach	Brighton Beach	Brighton Beach	Brighton Breach	St. Kilda	St. Kilda
step	**substance**							
fixation	glutardialdehyde		2.5%	2.5%	2.5%	2.5%	4%	4%
	paraformaldehyde						4%	4%
	sodium cacodylate		0.05 M	0.05 M	0.05 M	0.05 M	0.05 M	0.05 M
	seawater		70%	70%	70%	70%	50%	100%
	NaCl							
	$CaCl_2$							0.02%
	KCl							
	caffeine		0.2%	0.2%	0.2%	0.2%	1%	1%
temperature			4°C	RT	RT/4°C	RT/4°C	RT/4°C	RT
time			19 h	overnight	overnight	overnight	overnight	overnight
osmolarity							1267, 1219	
pH			7.4	7.4	7.4	7.4	7.2	7.2
microwave?				one batch MW		one batch MW		
1. wash	sodium cacodylate		0.05 M	0.05 M	0.05 M	0.05 M	0.1 M	0.05 M
	seawater		70%	70%	70%	70%	50%	100%
	NaCl							
	$CaCl_2$							0.02%
	KCl							
	caffeine							1%
no. and duration of steps			3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min
osmolarity								1171, 1192
pH			7.4	7.4	7.4	7.4	7.2	7.2
break	**in buffer**							
	temperature							
postfixation	osmium tetroxide		1%	1%	1%	1%	1%	1%
	sodium cacodylate		0.05 M	0.05 M	0.05 M	0.05 M	0.05 M	0.05 M
	seawater		70%	70%	70%	70%	50%	100%
	NaCl							
	$CaCl_2$							0.02%
	KCl							
time			2 h	2 h	2 h	2 h	2 h	2 h
2. Wash	sodium cacodylate		0.05 M	0.05M	0.05M	0.05 M	0.05 M	0.05 M
(buffer, 1:1,	seawater		70%	70%	70%	70%	100%	100%
dd H2O)	NaCl							
	$CaCl_2$							0.02%
	KCl							
no. and duration of steps			3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 *30 min	3 *30 min
addit. Wash	maleate buffer					0.05M		
no. and duration of steps						3* 15 min		
en bloc stain.	uranyl acetate		1%	1%	1%	1% in buffer	1%	1%
time			1 h	1 h	1 h	1 h	1 h	1 h
3. Wash			ddH_2O	ddH_2O	ddH_2O	0.05M buffer	ddH_2O	ddH_2O
no. and duration of steps			3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min
dehydration	solvent		ethanol	ethanol	ethanol	ethanol	ethanol	acetone
no. and duration of steps		1%						
		3%						
		6%						
		10%						
		15%						
		20%						
		25%	1 * 15 min	1 * 15 min	1 * 15 min	1 * 15 min	1 * 20 min	1 * 20 min
		30%						
		40%						
		50%	1 * 15 min	1 * 15 min	1 * 15 min	1 * 15 min	1 * 20 min	1 * 20 min
		60%						
		70%	1 * 15 min	overnight (4°C)	overnight (4°C)	overnight (4°C)	overnight (4°C)	overnight (4°C)
		75%						
		80%					1 * 20 min	1 * 20 min
		85%	1 * 15 min	1 * 15 min	1 * 15 min	1 * 15 min		
		90%					1 * 20 min	1 * 20 min
		95%	1 * 15 min	1 * 15 min	1 * 15 min	1 * 15 min	1 * 20 min	1 * 20 min
		100%	3 * 10 min	3 * 10 min	3 * 10 min	3 * 10 min	3 * 20 min	3 * 20 min
	propylene oxide (PO)		2 * 20 min	2 * 20 min	2 * 20 min	2 * 20 min	2 * 20 min	
tissue processor								

Table E 1: TEM protocols for chemical fixation (continued).

	protocol no.		1	2	3	4	5	6
		date	21.10.97	17.11.97	8.3.98	7.4.98	21.6.99	4.8.99
		species	*D. antarctica*	*D. antarctica*	*D. antarctica*	*D. antarctica*	*D. antarctica*	*D. antarctica*
		site	Brighton Beach	Brighton Beach	Brighton Beach	Brighton Breach	St. Kilda	St. Kilda
step	**substance**							
embedding	resin		Spurr's	Spurr's	Spurr's	Quetol	Spurr's	Spurr's
	solvent		PO	PO	PO	PO	PO	PO
	steps/changes	1%						
		3%						
		6%						
		10%						
		15%						
		20%						
		25%	overnight	4 h	over night	over night	over night	overnight
		30%						
		40%						
		50%	overnight or 1 h	overnight	3 days	over night	over night	
		60%						
		70%						
		75%	4 h or 1 h	overnight	overnight	over night	over night	2 days
		80%						
		85%						
		90%						
		95%						
		100%	overnight	4 *1 day	7 *1 day	8 *1 day	8 *1 day	7 or 14 *1 day
	days							
gradient								
tissue processor								
microwaving				one batch MW				1 x per day

Table E 1: TEM protocols for chemical fixation (continued).

	protocol no.		7	8	9			10
		date	25.8.99	14.9.99	22.11.99			24.2.2000
		species	*D. antarctica*	*D. antarctica*	*D. antarctica*			*D. antarctica*
		site	Brighton Beach	Brighton Beach	Brighton Beach			Brighton Beach
step	**substance**				**1**	**2**	**3**	**1**
fixation	glutardialdehyde		2.5%	4%	2.50%	4%	4%	4%
	paraformaldehyde			4%		4%	4%	4%
	sodium cacodylate		0.05M	0.05M	0.08 M	0.05M	0.05M	0,05 M
	seawater		70%	70%	70%	70%	50%	50%
	NaCl							
	$CaCl_2$							0.1%
	KCl							
	caffeine		0.2%	1%	0.2%	1%	1%	1%
temperature			RT/4°C	RT/4°C	RT/4°C	RT/4°C	RT/4°C	RT
time			overnight	overnight	overnight (18 for MW, 20:30 h for no MW)			overnight
osmolarity					1058, 1042	1658, 1621	1275, 1268	
pH			7.2	7.2	7.2	7.2	7.2	7.2
microwave?			X		MW, noMW	MW, noMW	MW, noMW	MW, noMW
1. wash	sodium cacodylate		0.05 M	0.05 M	0.08 M	0.05M	0.05M	0.05 M
	seawater		70%	70%	70%	70%	50%	50%
	NaCl							
	$CaCl_2$							0.1%
	KCl							
	caffeine				0.2%	1%	1%	1%
no. and duration of steps			3 * 15 min	3 * 15 min	3 * 20 min	3 * 20 min	3 * 20 min	3 * 12 min
osmolarity					811, 807	1153, 1166	575, 564	
pH			7.2	7,1 (stock)	7.2	7.2	7.2	7.2
break	**in buffer**			6 days				
	temperature		fridge	fridge				
postfixation	osmium tetroxide		1%	1%	1%	1%	1%	1%
	sodium cacodylate		0.05 M	0.05 M	0.08 M	0.05M	0.05M	0.05M
	seawater		70%	70%	70%	70%	50%	50%
	NaCl							
	$CaCl_2$							0.1%
	KCl							
time			2:15 h	2 h	2:15 h	2:15 h	2:15 h	2 h
2. Wash	sodium cacodylate		0.05 M	0.05 M	0.08 M	0.05M	0.05M	0.05 M
(buffer, 1:1,	seawater							50%
dd H2O)	NaCl							
	$CaCl_2$							0.10%
	KCl							
no. and duration of steps			3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min
addit. Wash	maleate buffer							
no. and duration of steps								
en bloc stain.	uranyl acetate		1%	1%	1%	1%	1%	
time			1h	1 h	1 h	1 h	1 h	
3. Wash			ddH_2O	ddH_2O	ddH_2O	ddH_2O	ddH_2O	
no. and duration of steps			3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	
dehydration	solvent		ethanol	ethanol	ethanol	ethanol	ethanol	ethanol
no. and duration of steps		1%						12 min
		3%						12 min
		6%						12 min
		10%						12 min
		15%						12 min
		20%						12 min
		25%	1 * 15 min	1 * 15 min	1 * 15 min	15 min	15 min	12 min
		30%						12 min
		40%						12 min
		50%	1 * 15 min	1 * 15 min	1 * 15 min	15 min	15 min	12 min
		60%						12 min
		70%	overnight (4°C)	overnight (4°C)	1 * 15 min	15 min	15 min	12 min
		75%						12 min
		80%						12 min
		85%	1 * 15 min	1 * 15 min	1 * 15 min	15 min	15 min	12 min
		90%						12 min
		95%	1 * 15 min	1 * 15 min	1 * 15 min	15 min	15 min	12 min
		100%	3 * 10 min	3 * 10 min	3 * 10 min	3 * 10 min	3 * 10 min	3 * 10 min
propylene oxide (PO)			2 * 20 min (for Q.	2 * 20 min (for Q.)				
tissue processor								

Table E 1: TEM protocols for chemical fixation (continued).

	protocol no.		7	8	9			10
		date	25.8.99	14.9.99	22.11.99			24.2.2000
		species	*D. antarctica*	*D. antarctica*	*D. antarctica*			*D. antarctica*
		site	Brighton Beach	Brighton Beach	Brighton Beach			Brighton Beach
step	**substance**				1	2	3	1
embedding	resin		Quetol/LRWhite	Quetol/LRWhite	LR White	LR White	LR White	LR White
	solvent		PO/Ethanol	PO/Ethanol	Ethanol	Ethanol	Ethanol	Ethanol
	steps/changes	1%						1 day
		3%						1 day
		6%						1 day
		10%						1 day
		15%						1 day
		20%						1 day
		25%	4 h	4 h	overnight, fridge			1 day
		30%						1 day
		40%						1 day
		50%	4 h	4 h		7 h, RT		1 day
		60%						1 day
		70%						1 day
		75%			overnight, fridge			1 day
		80%						1 day
		85%						1 day
		90%						1 day
		95%						1 day
		100%	Quetol: 17 *1 day			14-17 *1 day		7 * 1 day
	days		LRWhite: 21*1 day					
gradient								
tissue processor								
microwaving								

Table E 1: TEM protocols for chemical fixation (continued).

	protocol no.		10			11		
		date	24.2.2000			8.3.2000		
		species	*D. antarctica*			*D. antarctica*		
		site	Brighton Beach			Brighton Beach		
step	**substance**		**2**	**3**	**4**	**1**	**2**	**3**
fixation	glutardialdehyde		4%	4%	4%	4%	4%	4%
	paraformaldehyde		4%	4%	4%	4%	4%	4%
	sodium cacodylate		0,05 M	0,05 M	0,05 M	0,05 M	0,05 M	0,05 M
	seawater		50%	50%	50%	50%	50%	50%
	NaCl							
	$CaCl_2$		0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
	KCl							
	caffeine		1%	1%	1%	1%	1%	1%
temperature			RT	RT	RT	RT	RT	RT
time			overnight	4 h	4 h	overnight	overnight	4 h
osmolarity								
pH			7.2	7.2	7.2	7.2	7.2	7.2
microwave?			MW, noMW	MW, noMW	MW, noMW	all four batches: half of samples 5x MW, half no		
1. wash	sodium cacodylate		0,05 M	0,05 M	0,05 M	0,05 M	0,05 M	0,05 M
	seawater		50%	50%	50%	50%	50%	50%
	NaCl							
	$CaCl_2$		0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
	KCl							
	caffeine		1%	1%	1%	1%	1%	1%
no. and duration of steps			3 * 12 min	3 * 12 min	3 * 12 min	3 * 12 min	3 * 12 min	3 * 12 min
osmolarity								
pH			7.2	7.2	7.2	7.2	7.2	7.2
break	**in buffer**							
	temperature							
postfixation	osmium tetroxide		1%	1%	1%	1%	1%	1%
	sodium cacodylate		0.05M	0.05M	0.05M	0.05M	0.05M	0.05M
	seawater		50%	50%	50%	50%	50%	50%
	NaCl							
	$CaCl_2$		0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
	KCl							
time			2 h	2 h	2 h	2 h	2 h	2 h
2. Wash	sodium cacodylate		0.05 M	0.05 M	0.05 M	0.05 M	0.05 M	0.05 M
(buffer, 1:1,	seawater		50%	50%	50%	50%	50%	50%
dd H2O)	NaCl							
	$CaCl_2$		0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
	KCl							
no. and duration of steps			3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min
addit. Wash	maleate buffer							
no. and duration of steps								
en bloc stain.	uranyl acetate							
time								
3. Wash								
no. and duration of steps								
dehydration	solvent		ethanol	ethanol	ethanol	ethanol	ethanol	ethanol
no. and duration of steps		1%		12 min		12 min		12 min
		3%		12 min		12 min		12 min
		6%		12 min		12 min		12 min
		10%		12 min		12 min		12 min
		15%		12 min		12 min		12 min
		20%		12 min		12 min		12 min
		25%	15 min	12 min	15 min	12 min	15 min	12 min
		30%		12 min		12 min		12 min
		40%		12 min		12 min		12 min
		50%	15 min	12 min	15 min	12 min	15 min	12 min
		60%		12 min		12 min		12 min
		70%	15 min	overnight (4°C)	15 min	12 min	15 min	overnight (4°C)
		75%		12 min		12 min		12 min
		80%		12 min		12 min		12 min
		85%	15 min	12 min	15 min	12 min	15 min	12 min
		90%		12 min		12 min		12 min
		95%	15 min	12 min	15 min	12 min	15 min	12 min
		100%	3 * 10 min	3 * 10 min	3 * 10 min	3 * 10 min	3 * 10 min	3 * 10 min
	propylene oxide (PO)					2 * 20 min	2 * 20 min	2 * 20 min
tissue processor								

Table E 1: TEM protocols for chemical fixation (continued).

	protocol no.		10			11		
		date	24.2.2000			8.3.2000		
		species	*D. antarctica*			*D. antarctica*		
		site	Brighton Beach			Brighton Beach		
step	**substance**		**2**	**3**	**4**	**1**	**2**	**3**
embedding	resin		LR White	LR White	LR White	Quetol	Quetol	Quetol
	solvent		Ethanol	Ethanol	Ethanol	PO	PO	PO
	steps/changes	1%	1 day	1 day	1 day	1 day	1 day	1 day
		3%	1 day	1 day	1 day	1 day	1 day	1 day
		6%	1 day	1 day	1 day	1 day	1 day	1 day
		10%	1 day	1 day	1 day	1 day	1 day	1 day
		15%	1 day	1 day	1 day	1 day	1 day	1 day
		20%	1 day	1 day	1 day	1 day	1 day	1 day
		25%	1 day	1 day	1 day	1 day	1 day	1 day
		30%	1 day	1 day	1 day	1 day	1 day	1 day
		40%	1 day	1 day	1 day	1 day	1 day	1 day
		50%	1 day	1 day	1 day	1 day	1 day	1 day
		60%	1 day	1 day	1 day	1 day	1 day	1 day
		70%	1 day	1 day	1 day	1 day	1 day	1 day
		75%	1 day	1 day	1 day	1 day	1 day	1 day
		80%	1 day	1 day	1 day	1 day	1 day	1 day
		85%	1 day	1 day	1 day	1 day	1 day	1 day
		90%	1 day	1 day	1 day	1 day	1 day	1 day
		95%	1 day	1 day	1 day	1 day	1 day	1 day
		100%	7 * 1 day	7 * 1 day	7 * 1 day	5 * 1 day	5 * 1 day	5 * 1 day
	days							
gradient								
tissue processor								
microwaving								

Table E 1: TEM protocols for chemical fixation (continued).

	protocol no.		11	12			13	
		date	8.3.2000	21.6.2000			4.7.2000	
		species	*D. antarctica*	*D. antarctica*			*D. antarctica*	
		site	Brighton Beach	Brighton Beach			Brighton Beach	
step	**substance**		**4**	**1**	**2**	**3**	**1**	**2**
fixation	glutardialdehyde		4%	4%	4%	2%	4%	4%
	paraformaldehyde		4%	4%	4%	1%	4%	4%
	sodium cacodylate		0,05 M	0.05 M	0.1 M	0.1 M	0.05 M	0.1 M
	seawater		50%	50%			50%	
	NaCl				2%	2%		2%
	$CaCl_2$		0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
	KCl							
	caffeine		1%	1%	1%	1%	1%	1%
temperature			RT	RT/4°C	RT/4°C	RT/4°C	4°C	4°C
time			4 h	overnight	overnight	overnight	overnight	overnight
osmolarity							1234, 1228	
pH			7.2	7.2	7.2	7.2	7.2	7.2
microwave?								
1. wash	sodium cacodylate		0,05 M	0.05 M	0.1 M	0.1 M	0.05 M	0.1 M
	seawater		50%	50%			50%	
	NaCl				2%	2%		2%
	$CaCl_2$		0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
	KCl							
	caffeine		1%	1%	1%	1%	1%	1%
no. and duration of steps			3 * 12 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min
osmolarity								
pH			7.2	7.2	7.2	7.2	7.2	7.2
break	**in buffer**							
	temperature							
postfixation	osmium tetroxide		1%	1%	1%	1%	1%	1%
	sodium cacodylate		0.05M	0.05 M	0.1 M	0.1 M	0.05 M	0.1 M
	seawater		50%	50%			50%	
	NaCl				2%	2%		2%
	$CaCl_2$		0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
	KCl							
time			2 h	2 h	2 h	2 h	2 h	2 h
2. Wash	sodium cacodylate		0.05 M				0.05 M	0.1 M
(buffer, 1:1,	seawater		50%				50%	
dd H2O)	NaCl							2%
	$CaCl_2$		0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
	KCl							
no. and duration of steps			3 * 15 min	3 * 20 min	3 * 20 min	3 * 20 min	3 * 15 min	3 * 15 min
addit. Wash	maleate buffer							
no. and duration of steps								
en bloc stain.	uranyl acetate							
time								
3. Wash								
no. and duration of steps								
dehydration	solvent		ethanol	ethanol	ethanol	ethanol	ethanol	ethanol
no. and duration of steps		1%		2:30 h	2:30 h	2:30 h	1 h	1 h
		3%		2:30 h	2:30 h	2:30 h	1 h	1 h
		6%		2:30 h	2:30 h	2:30 h	1 h	1 h
		10%		2:30 h	2:30 h	2:30 h	1 h	1 h
		15%		2:30 h	2:30 h	2:30 h	1 h	1 h
		20%		2:30 h	2:30 h	2:30 h	1 h	1 h
		25%	15 min	2:30 h	2:30 h	2:30 h	1 h	1 h
		30%		2:30 h	2:30 h	2:30 h	1 h	1 h
		40%		2:30 h	2:30 h	2:30 h	1 h	1 h
		50%	15 min	2:30 h	2:30 h	2:30 h	1 h	1 h
		60%		2:30 h	2:30 h	2:30 h	1 h	1 h
		70%	15 min	2:30 h	2:30 h	2:30 h	1 h	1 h
		75%		2:30 h	2:30 h	2:30 h	1 h	1 h
		80%		2:30 h	2:30 h	2:30 h	1 h	1 h
		85%	15 min	2:30 h	2:30 h	2:30 h	1 h	1 h
		90%		2:30 h	2:30 h	2:30 h	1 h	1 h
		95%	15 min	2:30 h	2:30 h	2:30 h	1 h	1 h
		100%	3 * 10 min	3 * 2:30 h	3 * 2:30 h	3 * 2:30 h	1 h	1 h
	propylene oxide (PO)		2 * 20 min	2 * 20 min	2 * 20 min	2 * 20 min		
tissue processor				X	X	X	X	X

Table E 1: TEM protocols for chemical fixation (continued).

	protocol no.		11	12			13	
		date	8.3.2000	21.6.2000			4.7.2000	
		species	*D. antarctica*	*D. antarctica*			*D. antarctica*	
		site	Brighton Beach	Brighton Beach			Brighton Beach	
step	**substance**		**4**	**1**	**2**	**3**	**1**	**2**
embedding	resin		Quetol	Quetol	Quetol	Quetol	Quetol	Quetol
	solvent		PO	PO	PO	PO	ethanol	ethanol
	steps/changes	1%	1 day	12 h	12 h	12 h	12 h	12 h
		3%	1 day	12 h	12 h	12 h	12 h	12 h
		6%	1 day	12 h	12 h	12 h	12 h	12 h
		10%	1 day	12 h	12 h	12 h	12 h	12 h
		15%	1 day	12 h	12 h	12 h	12 h	12 h
		20%	1 day	12 h	12 h	12 h	12 h	12 h
		25%	1 day	12 h	12 h	12 h	12 h	12 h
		30%	1 day	12 h	12 h	12 h	12 h	12 h
		40%	1 day	12 h	12 h	12 h	12 h	12 h
		50%	1 day	12 h	12 h	12 h	12 h	12 h
		60%	1 day	12 h	12 h	12 h	12 h	12 h
		70%	1 day	12 h	12 h	12 h	12 h	12 h
		75%	1 day	12 h	12 h	12 h	12 h	12 h
		80%	1 day	12 h	12 h	12 h	12 h	12 h
		85%	1 day	12 h	12 h	12 h	12 h	12 h
		90%	1 day	12 h	12 h	12 h	12 h	12 h
		95%	1 day	12 h	12 h	12 h	12 h	12 h
		100%	5 * 1 day	12 h	12 h	12 h	12 h	12 h
	days							
gradient								
tissue processor				X	X	X	X	X
microwaving								

Table E 1: TEM protocols for chemical fixation (continued).

	protocol no.		11	12			13	
		date	8.3.2000	21.6.2000			4.7.2000	
		species	*D. antarctica*	*D. antarctica*			*D. antarctica*	
		site	Brighton Beach	Brighton Beach			Brighton Beach	
step	**substance**		**4**	**1**	**2**	**3**	**1**	**2**
fixation	glutardialdehyde		4%	4%	4%	2%	4%	4%
	paraformaldehyde		4%	4%	4%	1%	4%	4%
	sodium cacodylate		0,05 M	0.05 M	0.1 M	0.1 M	0.05 M	0.1 M
	seawater		50%	50%			50%	
	NaCl				2%	2%		2%
	$CaCl_2$		0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
	KCl							
	caffeine		1%	1%	1%	1%	1%	1%
temperature			RT	RT/4°C	RT/4°C	RT/4°C	4°C	4°C
time			4 h	overnight	overnight	overnight	overnight	overnight
osmolarity							1234, 1228	
pH			7.2	7.2	7.2	7.2	7.2	7.2
microwave?								
1. wash	sodium cacodylate		0,05 M	0.05 M	0.1 M	0.1 M	0.05 M	0.1 M
	seawater		50%	50%			50%	
	NaCl				2%	2%		2%
	$CaCl_2$		0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
	KCl							
	caffeine		1%	1%	1%	1%	1%	1%
no. and duration of steps			3 * 12 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min
osmolarity								
pH			7.2	7.2	7.2	7.2	7.2	7.2
break	**in buffer**							
	temperature							
postfixation	osmium tetroxide		1%	1%	1%	1%	1%	1%
	sodium cacodylate		0.05M	0.05 M	0.1 M	0.1 M	0.05 M	0.1 M
	seawater		50%	50%			50%	
	NaCl				2%	2%		2%
	$CaCl_2$		0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
	KCl							
time			2 h	2 h	2 h	2 h	2 h	2 h
2. Wash	sodium cacodylate		0.05 M				0.05 M	0.1 M
(buffer, 1:1,	seawater		50%				50%	
dd H2O)	NaCl							2%
	$CaCl_2$		0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
	KCl							
no. and duration of steps			3 * 15 min	3 * 20 min	3 * 20 min	3 * 20 min	3 * 15 min	3 * 15 min
addit. Wash	maleate buffer							
no. and duration of steps								
en bloc stain.	uranyl acetate							
time								
3. Wash								
no. and duration of steps								
dehydration	solvent		ethanol	ethanol	ethanol	ethanol	ethanol	ethanol
no. and duration of steps		1%		2:30 h	2:30 h	2:30 h	1 h	1 h
		3%		2:30 h	2:30 h	2:30 h	1 h	1 h
		6%		2:30 h	2:30 h	2:30 h	1 h	1 h
		10%		2:30 h	2:30 h	2:30 h	1 h	1 h
		15%		2:30 h	2:30 h	2:30 h	1 h	1 h
		20%		2:30 h	2:30 h	2:30 h	1 h	1 h
		25%	15 min	2:30 h	2:30 h	2:30 h	1 h	1 h
		30%		2:30 h	2:30 h	2:30 h	1 h	1 h
		40%		2:30 h	2:30 h	2:30 h	1 h	1 h
		50%	15 min	2:30 h	2:30 h	2:30 h	1 h	1 h
		60%		2:30 h	2:30 h	2:30 h	1 h	1 h
		70%	15 min	2:30 h	2:30 h	2:30 h	1 h	1 h
		75%		2:30 h	2:30 h	2:30 h	1 h	1 h
		80%		2:30 h	2:30 h	2:30 h	1 h	1 h
		85%	15 min	2:30 h	2:30 h	2:30 h	1 h	1 h
		90%		2:30 h	2:30 h	2:30 h	1 h	1 h
		95%	15 min	2:30 h	2:30 h	2:30 h	1 h	1 h
		100%	3 * 10 min	3 * 2:30 h	3 * 2:30 h	3 * 2:30 h	1 h	1 h
	propylene oxide (PO)		2 * 20 min	2 * 20 min	2 * 20 min	2 * 20 min		
tissue processor				X	X	X	X	X

Table E 1: TEM protocols for chemical fixation (continued).

	protocol no.		11	12			13	
		date	8.3.2000	21.6.2000			4.7.2000	
		species	*D. antarctica*	*D. antarctica*			*D. antarctica*	
		site	Brighton Beach	Brighton Beach			Brighton Beach	
step	**substance**		**4**	**1**	**2**	**3**	**1**	**2**
embedding	resin		Quetol	Quetol	Quetol	Quetol	Quetol	Quetol
	solvent		PO	PO	PO	PO	ethanol	ethanol
	steps/changes	1%	1 day	12 h	12 h	12 h	12 h	12 h
		3%	1 day	12 h	12 h	12 h	12 h	12 h
		6%	1 day	12 h	12 h	12 h	12 h	12 h
		10%	1 day	12 h	12 h	12 h	12 h	12 h
		15%	1 day	12 h	12 h	12 h	12 h	12 h
		20%	1 day	12 h	12 h	12 h	12 h	12 h
		25%	1 day	12 h	12 h	12 h	12 h	12 h
		30%	1 day	12 h	12 h	12 h	12 h	12 h
		40%	1 day	12 h	12 h	12 h	12 h	12 h
		50%	1 day	12 h	12 h	12 h	12 h	12 h
		60%	1 day	12 h	12 h	12 h	12 h	12 h
		70%	1 day	12 h	12 h	12 h	12 h	12 h
		75%	1 day	12 h	12 h	12 h	12 h	12 h
		80%	1 day	12 h	12 h	12 h	12 h	12 h
		85%	1 day	12 h	12 h	12 h	12 h	12 h
		90%	1 day	12 h	12 h	12 h	12 h	12 h
		95%	1 day	12 h	12 h	12 h	12 h	12 h
		100%	5 * 1 day	12 h	12 h	12 h	12 h	12 h
	days							
gradient								
tissue processor				X	X	X	X	X
microwaving								

Table E 1: TEM protocols for chemical fixation (continued).

	protocol no.		13	14				15	
	date		4.7.2000	29.8.2000				13.12.2000	
	species		*D. antarctica*	*D. antarctica*				*D. antarctica*	
	site		Brighton Beach	Brighton Beach				Brighton Beach	
step	**substance**		**3**	**1**	**2**	**3**	**4**	**1**	**2**
fixation	glutardialdehyde		2%	2.5%	2%	4%	2%	2%	2%
	paraformaldehyde		1%		1%		1%	1%	1%
	sodium cacodylate		0.1 M	0.05 M	0.05 M	0.05 M	0.05 M	0.1 M	0.05 M
	seawater			50%					
	NaCl		2%		2%	146 mM	146 mM	2%	2%
	$CaCl_2$		0.1%		0.1%	17 mM	17 mM	0.1%	0.1%
	KCl					3.4 mM	3.4 mM		
	caffeine		1%	0.2%	1%	1%	1%	1%	1%
temperature			4°C	4°C	4°C	4°C	4°C	4°C	4°C
time			overnight	18 h	18 h	18 h	18 h	2, 4, 8 or 24 h	2, 4, 8 or 24 h
osmolarity			1068, 1102	850, 834	1083, 1079	1172, 1176	1071, 1070	1184, 1181	1049, 1048
pH			7.2	7.2	7.2	7.2	7.2	7.2	7.2
microwave?									
1. wash	sodium cacodylate		0.1 M	0.05 M	0.05M	0.05 M	0.05 M	0.1 M	0.1 M
	seawater			50%					
	NaCl		2%		2%	313.9 mM	313.9 mM	2%	2%
	$CaCl_2$		0.1%		0.1%	36.6 mM	36.6 mM	0.10%	0.10%
	KCl					7.3 mM	7.3 mM		
	caffeine		1%					1%	1%
no. and duration of steps			3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min
osmolarity				569, 584	712, 700	724, 732	724, 732	871, 892	786, 785
pH			7.2	7.2	7.2	7.2	7.2	7.2	7.2
break	**in buffer**								
	temperature								
postfixation	osmium tetroxide		1%	1%	1%	1%	1%	1%	1%
	sodium cacodylate		0.1 M	0.05M	0.05M	0.05M	0.05M	0.1 M	0.1 M
	seawater								
	NaCl		2%	2%	2%	2%	2%	2%	2%
	$CaCl_2$		0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
	KCl								
time			2 h	2 h	2 h	2 h	2 h	2 h	2 h
2. Wash	sodium cacodylate		0.1 M	0.05 M	0.05M	0.05 M	0.05 M	0.1 M	0.1 M
(buffer, 1:1,	seawater			50%					
dd H2O)	NaCl		2%		2%	313.9 mM	313.9 mM	2%	2%
	$CaCl_2$		0.1%		0.1%	36.6 mM	36.6 mM	0.10%	0.10%
	KCl					7.3 mM	7.3 mM		
no. and duration of steps			3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min	3 * 15 min
addit. Wash	maleate buffer								
no. and duration of steps									
en bloc stain.	uranyl acetate								
time									
3. Wash									
no. and duration of steps									
dehydration	solvent		ethanol	ethanol	ethanol	ethanol	ethanol	ethanol	ethanol
no. and duration of steps		1%	1 h	1 h	1 h	1 h	1 h	30 min	30 min
		3%	1 h	1 h	1 h	1 h	1 h	30 min	30 min
		6%	1 h	1 h	1 h	1 h	1 h	30 min	30 min
		10%	1 h	1 h	1 h	1 h	1 h	30 min	30 min
		15%	1 h	1 h	1 h	1 h	1 h	30 min	30 min
		20%	1 h	1 h	1 h	1 h	1 h	30 min	30 min
		25%	1 h	1 h	1 h	1 h	1 h	30 min	30 min
		30%	1 h	1 h	1 h	1 h	1 h	30 min	30 min
		40%	1 h	1 h	1 h	1 h	1 h	30 min	30 min
		50%	1 h	1 h	1 h	1 h	1 h	30 min	30 min
		60%	1 h	1 h	1 h	1 h	1 h	30 min	30 min
		70%	1 h	1 h	1 h	1 h	1 h	13 h	13 h
		75%	1 h	1 h	1 h	1 h	1 h	20 min	20 min
		80%	1 h	1 h	1 h	1 h	1 h	20 min	20 min
		85%	1 h	1 h	1 h	1 h	1 h	20 min	20 min
		90%	1 h	1 h	1 h	1 h	1 h	20 min	20 min
		95%	1 h	1 h	1 h	1 h	1 h	20 min	20 min
		100%	1 h	3 * 1 h	3 * 1 h	3 * 1 h	3 * 1 h	3 * 20 min	3 * 20 min
	propylene oxide (PO)								
tissue processor			X	X	X	X	X	X	X

Table E 1: TEM protocols for chemical fixation (continued).

	protocol no.		13	14				15	
		date	4.7.2000	29.8.2000				13.12.2000	
		species	*D. antarctica*	*D. antarctica*				*D. antarctica*	
		site	Brighton Beach	Brighton Beach				Brighton Beach	
step	**substance**		**3**	**1**	**2**	**3**	**4**	**1**	**2**
embedding	resin		Quetol	Quetol	Quetol	Quetol	Quetol	Quetol	Quetol
	solvent		ethanol	ethanol	ethanol	ethanol	ethanol	ethanol	ethanol
	steps/changes	1%	12 h	12 h	12 h	12 h	12 h	0.5 h	0.5 h
		3%	12 h	12 h	12 h	12 h	12 h	1 h	1 h
		6%	12 h	12 h	12 h	12 h	12 h	1.5 h	1.5 h
		10%	12 h	12 h	12 h	12 h	12 h	2 h	2 h
		15%	12 h	12 h	12 h	12 h	12 h	2.5 h	2.5 h
		20%	12 h	12 h	12 h	12 h	12 h	2.5 h	2.5 h
		25%	12 h	12 h	12 h	12 h	12 h		
		30%	12 h	12 h	12 h	12 h	12 h	5 h	5 h
		40%	12 h	12 h	12 h	12 h	12 h	5 h	5 h
		50%	12 h	12 h	12 h	12 h	12 h	5 h	5 h
		60%	12 h	12 h	12 h	12 h	12 h	5 h	5 h
		70%	12 h	12 h	12 h	12 h	12 h	5 h	5 h
		75%	12 h	12 h	12 h	12 h	12 h	5 h	5 h
		80%	12 h	12 h	12 h	12 h	12 h	5 h	5 h
		85%	12 h	12 h	12 h	12 h	12 h	5 h	5 h
		90%	12 h	12 h	12 h	12 h	12 h	5 h	5 h
		95%	12 h	12 h	12 h	12 h	12 h	2.5 h	2.5 h
		100%	12 h	12 h	12 h	12 h	12 h	3 x 5 h	3 x 5 h
	days								
gradient								2% per hour	2% per hour
tissue processor			X	X	X	X	X	X	X
microwaving									

Table E 2: Protocol for cryosubstitution.

Embedding of cryo samples		**protocol no.**	**1**	**2**	**3**
		date	20.4.2000	21.6.2000	4.7.2000
		species	*D. antarctica*	*D. antarctica*	*D. antarctica*
		site	Brighton Beach	Brighton Beach	Brighton Beach
step	**substance**				
embedding	resin		Quetol	Quetol	Quetol
	solvent		methanol	methanol	methanol
	steps/changes	1%	1 day	12 h	12 h
		3%	1 day	12 h	12 h
		6%	1 day	12 h	12 h
		10%	1 day	12 h	12 h
		15%	1 day	12 h	12 h
		20%	1 day	12 h	12 h
		25%	1 day	12 h	12 h
		30%	1 day	12 h	12 h
		40%	1 day	12 h	12 h
		50%	1 day	12 h	12 h
		60%	1 day	12 h	12 h
		70%	1 day	12 h	12 h
		75%	1 day	12 h	12 h
		80%	1 day	12 h	12 h
		85%	1 day	12 h	12 h
		90%	1 day	12 h	12 h
		95%	1 day	12 h	12 h
		100%	5 * 1 day	12 h	12 h
tissue processor			X	X	X