Molecular evolution, phylogenetics and biogeography in southern hemispheric bryophytes with special focus on Chilean taxa.

Dissertation

zur

Erlangung des Doktorgrades (Dr. rer. nat.)

der Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

Rolf Blöcher

aus

Biedenkopf/Lahn

Bonn 2004

Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn

1. Referent: Prof. Dr. Jan-Peter Frahm
2. Referent: Prof. Dr. Wilhelm Barthlott

Tag der Promotion: 20. Dezember 2004

meinen Eltern,

Doris und Horst Blöcher

1 Introduction

Biologists have long been fascinated by the existence of disjunct distributions of certain plant and animal taxa. Especially the southern temperate disjunctions between southern South America and New Zealand have attracted their attention. The taxa characterized by these distribution patterns are assumed to share a common history. Generally two different hypotheses are used to explain their disjunct distribution. The first can be described by the term 'vicariance' which refers to disjunct distribution patterns as a result of the splitting of populations by e.g. the fragmentation of landmasses (e.g. Croizat et al., 1974). The second hypothesis explains the existing distribution patterns based on long distance dispersal events.

For the first explanation based on vicariance events an understanding of the past fragmentation processes of the continental landmasses is necessary. The former connection of the recent southern continents in a large landmass, the Gondwana continent, is nowadays widely accepted. Over a period of *c*. 180 Myr mainly continental drift led to the recent formation of the continents (e.g. McLoughlin, 2001). During the Permian to Jurassic period the supercontinent Pangea consisted of a northern land mass, Laurasia and a southern land mass Gondwana, that were partly separated by an ocean, the Tethys. During that time Pangea extended from high northern to high southern latitudes covering substantial climatic gradients (McLoughlin, 2001). The early Cretaceous floras of Gondwana were conifer and pteridosperm dominated and differed little from that of the Jurassic. By the mid-Cretaceous angiosperms were already important elements of the cool temperate flora of the southern Gondwana continent. These forest types appear to be quite similar to that found in the temperate regions of the southern hemisphere today, possibly offering good conditions for the ancestors of recent temperate rainforest taxa. The breakup of the Gondwana continent started in the late Jurassic (*c*. 152 Myr BP) with sea-floor spreading between Africa and Madagascar (2004; Scotese & McKerrow, 1990). The separation of Africa from a landmass comprising e.g. recent South America, Antarctica, Australia and New Zealand was completed about 105 Myr BP. New Zealand as part of the continental block 'Tasmantia' separated about 80 Myr BP from Australia which was at the time still connected via Antarctica to South America.

Lastly, the separation of the continents South America, Antarctica, and Australia was completed about 30 Myr BP (McLoughlin, 2001).

Southern temperate disjunct taxa presumably once had a continuous distribution range on the Gondwana continent, their recent distribution caused by separation of populations concomitant with the breakup of Gondwana (e.g. Darlington, 1965; Du Rietz, 1960; Godley, 1960; Skottsberg, 1960). The term 'vicariance' Recent taxa are the result of evolutionary processes which since then have taken place since in the disjunct populations. A southern hemispheric disjunction caused by vicariance is also assumed for many bryophyte taxa (e.g. Schuster, 1969). In a later review of the phytogeography of bryophytes, Schuster (1983) gives many examples of mosses and liverworts with Gondwana distribution patterns (e.g. *Dendroligotrichum*, Lepidoleanaceae, and *Polytrichadelphus magellanicus*). Matteri (1986), Seki (1973), and most recently Villagrán (2003) give detailed information of phytogeographical relationships of bryophytes from specific areas of southern South America. They classify the bryophyte taxa according to their overall distribution pattern. A detailed study about the evolution of the Gondwana relict moss family Hypopterygiaceae is provided by Kruijer (2002).

The other hypothesis explaning the disjunct distribution of southern hemispheric taxa is long distance dispersal defined by van Zanten & Pócs (1981) as dispersal over more than 2,000 km distance. Van Zanten (1976) designed germination experiments in which bryophyte spores received a treatment comparable to the conditions of long distance disperal by wind (jet stream) in the southern hemisphere. Van Zanten (1978) proved experimentally that especially widespread species had spores which were still able to germinate after the experimental treatment and were therefore assumed able to survive long distance dispersal. Species confined to a small distribution range, e.g. *Catharomnion ciliatum* restricted to New Zealand/Australia, did not germinate after two months of treatment.

Most recently Muñoz et al. (2004) tested the correlation between near-surface wind direction and speed and floral similarity of certain areas in mosses, liverworts, lichens and pteridophytes. They found a stronger correlation between floristic similarity and maximum wind connectivity in mosses, liverworts and lichens than with geographic proximity. They concluded that wind is the main force determining current plant distribution.

After introducing the two principal explanation models of southern hemispheric disjunct distribution patterns, methods of phylogenetic reconstruction are presented. Traditionally, morphological similarities are used as indicators of close relationship. Decisions on which characters are regarded as conserved or derived are supported by the analysis of fossils. Also, determination of the timing of evolutionary processes is based on the fossil record. Fossil pollen has helped to reconstruct historical distribution ranges, especially in trees. In the last 20 years the use of molecular methods have gained more and more importance. Today, molecular data in combination with the fossil record are used to estimate relative clade divergence or calibrate data for age estimates of certain clades. In bryophytes molecular sequence data have proven indispensable for phylogenetic analyses on different taxonomic levels (for review see: Quandt & Stech, 2003). However, especially when fossils are rare one relies on indicative methods for studying the time scale of evolution. For instance, the breakup sequence of the Gondwana continent can be used as a time sequence (McLoughlin, 2001) to fit the cladograms of phylogenetic analyses (e.g. Frey et al., 1999; Schaumann et al., 2003). Additional geological events possibly relevant for understanding the history of disjunct southern temperate rainforest taxa are e.g. temporary flooding of parts of South America, the formation of the Andes, the Isthmus of Panama and the Atacama desert.

The classical example of a disjunct distribution in the southern hemisphere is that of the southern beech *Nothofagus* (e.g. van Steenis, 1971). There are contrasting opinions on whether vicariance or dispersal events are responsible for the distribution of *Nothofagus*. Manos (1997) analyses molecular sequence data and fossil records and concludes that *Nothofagus* was widely distributed in the southern hemisphere before the breakup of Gondwana. The disjunction of *Nothofagus* is interpreted by him as vicariance, for the Australasian taxa in combination with multiple extinction events. In contrast, Swenson et al. (2001) explained Australasian disjunctions by colonization, i.e. long distance dispersal, and extinction events. The colonization hypothesis is supported by findings of Pole (1994; 2001) who questions the persistence of continous temperate forest in New Zealand during the Tertiary on the basis of periodic ‘gaps’ in pollen records especially of plant taxa commonly

associated with temperate rainforest vegetation. He therefore suggests that the New Zealand flora is mainly a result of long distance dispersal.
Another example of a taxon with a mainly southern hemispheric disjunct distribution range is the angiosperm genus *Gunnera*. This taxon has an even wider distribution than *Nothofagus*, including Africa and extending into North America. Wanntorp & Wanntorp (2003) based their reconstruction of *Gunnera* evolution on genetic as well as on morphological analyses supported by fossil and pollen data. Most of the phylogenetic results were in accordance with the chronology of the Gondwana breakup. Only few phenomena were interpreted as dispersal events in the late Tertiary.

In bryophytes only few of the recent taxa can be related to fossils in order to predict their evolutionary age (e.g. Pallaviciniaceae, Frey, 1990; Schuster, 1982). Well preserved fossils are very rare. The earliest moss fossils were reported from the carboniferous (e.g. Goffinet & Hedderson, 2000; Krassilov & Schuster, 1984). *Muscites guescelini* from the Triassic (South Africa) is sometimes regarded as the earliest known representative of the pleurocarpous lineage in bryophytes (Krassilov & Schuster, 1984). Most of the younger fossils originate from tertiary Baltic and Saxon amber (e.g. Frahm, 2004). Only few examples were reported from the Early Pleistocene (e.g. Weymouthia mollis, Jordan & Dalton, 1995) and from the Late Pleistocene/Holocene (e.g. Hylocomiaceae, Willerslev et al., 2003).
The only example of DNA sequences of fossil mosses was reported only recently. Willerslev et al. (2003; 2004) used samples from ice cores from Sibiria as template in PCRs for animal and plant taxa. They successfully presented partial rbcL sequence data of 300,000 to 400,000 year old bryophyte taxa related to the Hylocomiaceae and Bryales, respectively. However, this is a rare case where very old plant material is sufficiently well preserved for use in molecular phylogeny. Also, the fossils are difficult to relate to living taxa and most of them do not provide a time record for interpreting bryophyte evolution.

For disjunct southern hemispheric bryopyhte taxa few molecular based studies addressing their distribution exist. An example is the liverwort genus *Monoclea* which occurs in southern temperate rainforests of New Zealand and Chile and in tropical rainforests in northern South to Central America. Analysis of cpDNA sequence data

(Meißner et al., 1998) suggests that this genus is of Gondwana origin and its current disjunct distribution is best explained as a result of vicariance. It is assumed that the common ancestor was widely distributed in Gondwana and that the split of the Gondwana continent resulted in the evolution of two geographically distinct species, one occurring in South America and the other in New Zealand. According to Meißner (1998) the South American populations extended their distribution range into the tropical region resulting in two geographically and genetically distinct subspecies.
The genus *Lopidium* occurs in three regions which were formerly part of Gondwana: South America, Africa and Australia/New Zealand. Based on corresponding sequence data of cpDNA in *Lopidium concinnum* from South American and New Zealand populations and restricted long distance spore dipersal ability, Frey et al. (1999) regarded this species as an old Gondwanan relict of stenoevolutionary character. A low genetic differention between New Zealand and Chilean taxa is also reported by Pfeiffer (2000a) for *Hypopterygium didictyon*.

However, not all taxa show the pattern of low genetic differentiation between the geographically distinct regions of Chile and New Zealand/Australia. The geographical separation of the ancient taxon *Polytrichadelphus magellanicus* populations from New Zealand and Chile for example was followed by divergent evolution. This resulted according to Stech et al. (2002) in two morphologically and genetically distinct subspecies of *Polytrichadelphus magellanicus*.
Based on cpDNA and nrDNA sequence data together with paleobotanical evidence Schaumann et al. (2003) suggest that the dendroid liverworts of the genus *Symphyogyna* had their origin on Gondwana well before the separation of Africa.
Schaumann et al. (2004) found low sequence variation (cpDNA, nrDNA) in the genus *Jensenia*. They observed a regional pattern in which taxa from South America were more closely related to each other than to the Australasian taxa. They proposed a possible Gondwanan origin for the genus *Jensenia*.
McDaniel & Shaw (2003) found no morphological differentiation between populations from different geographical origins (southern South America, northern South America, Australia/New Zealand) but a high genetic differentiation (‘cryptic speciation’) correlated with geographical patterns in the moss *Pyrrhobryum mnioides*. Based on genetic separation of southern South American and northern South American populations they used geological evidence (establishment of the Atacama

dessert, 14 Myr BP) to calibrate a molecular clock, and concluded that the South American and Australasian populations of *Pyrrhobryum mnioides* were fragmented by the Gondwana breakup 80 Myr BP.

All the above mentioned authors used the breakup sequence of Gondwana and further geological evidence together with the pattern of genetically based data to explain the evolution of certain bryophyte taxa. There is yet no genetic evidence for long-distance dispersal in bryophytes. Van Zanten & Pócs (1981) put forward the example of subantarctic Marion Island situated in the southern Indian Ocean 2,300 km from Capetown whose moss flora was probably established by long-distance dispersal as the island was nearly entirely covered by ice during the Riss-glaciation (276,000 – 100,000 yr BP). Although the authors consider the possibility that some species may have survived these extreme conditions on nunataks they suppose that the majority of the species arrived on the island afterwards by long-distance dispersal. Van Zanten (1978) also found a strong correlation between germination rates of moss spores after they had been experimentally exposed to desiccation and freezing and geographical distribution range: the greater the resistance to conditions similar to those experienced in long-distance dispersal the larger the distribution range. These results also indicate that long-distance dispersal may play a more important part than commonly believed.

Study objectives. This study adresses phylogenetic relationships within four southern hemispheric bryophyte taxa (two families, two genera) using molecular genetic methods. The data are related to the timing of historical/geological processes in order to test the hypothesis whether the recent distribution patterns of the taxa can be attributed to a Gondwanan origin. Alternative explanation models, especially long distance dispersal by wind are also discussed. In a first step similarities between the moss flora of southern temperate rainforests of Chile and New Zealand were identified in order to select appropriate taxa for closer study (chapter 2). For this purpose existing taxa lists from Chile (He, 1998) and New Zealand (Fife, 1995) were compared and analysed (Blöcher & Frahm, 2002). The Ptychomniaceae and Lepyrondontaceae as well as the genera *Acrocladium* and *Catagonium* were chosen. The family Lepyrodontaceae consists of two genera, the monotypic genus *Dichelodontium* endemic to New Zealand and the genus *Lepyrodon* which consists of

seven species, five of which are restricted to South America and two occurring only in New Zealand/Australia. The genus *Lepyrodon* was studied because of its typical southern temperate distribution range with outliers in Central America and southern Mexico. The widespread South American species *Lepyrodon tomentosus* is reported as a characteristic epiphyte of upper montane rainforests of tropical South America (Gradstein et al., 2001) and is also widely distributed in temperate rainforests. During my field studies in Chile *Lepyrodon tomentosus* also proved to be one of the characteristic epiphytes in subandean *Nothofagus* forests. The genus *Lepyrodon* was also an important element of the epiphytic bryophyte communities studied in New Zealand by Lindlar & Frahm (2002).

The family Ptychomniaceae occurs in southern South America and is widely distributed in the Australasian region. Its evolution is probably connected with the genus *Dichelodontium* (Lepyrodontaceae). One aim of this study was to determine if the genus *Dichelodontium* placed in the family Lepyrodontaceae by Allen (1999) might be more closely related to the Ptychomniaceae, as indicated by Fleischer (1908).

The genus *Acrocladium* was chosen because there are only two species described in the genus, each geographically restricted to either southern South America or New Zealand/Australia. By studying the genetic relationships of several specimens of *Acrocladium* the author aimed at clarifying the doubtful status within the genus (e.g. Karczmarz, 1966). The main question was if two genetically distinct species exist and if the genetic distances between them as well as in relation to their closest relatives indicate a Gondwanan origin.

The genus *Catagonium* was selected for this study because it occurs on three major continents of Gondwanan origin, i.e. in South America, Australia/New Zealand and, in contrast to the other taxa studied, also in Africa.

Most of the specimens used for this study were collected by the author on a field trip to Chile (BryoAustral project) in temperate rainforests or originate from former field work of colleagues within the BryoAustral and BryoTrop projects.

After the taxa were chosen it was then necessary to circumscribe their closest relatives in order to find a reference for the results of molecular genetic analysis as well as evolution. In chapters 3 and 4 the closest relatives of the taxa are identified by phylogenetic analysis. Chapter 3 deals with the Ptychomniaceae focussing on the status of *Dichelodontium* as well as on *Ptychomnion ptychocarpon*. In chapter 4 the

systematic position of the genera *Lepyrodon*, *Acrocladium*, and *Catagonium* within the Hypnales is analysed and presented with special emphasis on their relation to the Plagiotheciaceae. Chapters 5 to 7 concentrate on the phylogenetic relationships within the single genera (chapter 5: *Lepyrodon*, chapter 6: *Acrocladium*, chapter 7: *Catagonium*). Within each taxon the genetic distances between disjunct taxa were determined and the phylogeny was constructed based on molecular sequence data obtained by using different molecular markers.

In chapter 8 the data of all taxa are brought together in order to find possible common patterns as well as differences in their molecular evolution. The data are placed in a wider biogeographical context.

2 A comparison of the moss floras of Chile and New Zealand.

(Published in *Tropical Bryology* 2002, vol. 21, p. 81-92)

Summary: Chile and New Zealand share a common stock of 181 species of mosses in 94 genera and 34 families. This number counts for 23.3 % of the Chilean and 34.6 % of the New Zealand moss flora. If only species with austral distribution are taken into account, the number is reduced to 113 species in common, which is 14.5 % of the Chilean and 21.6 % of the New Zealand moss flora. This correlation is interpreted in terms of long distance dispersal resp. the common phytogeographical background of both countries as parts of the palaeoaustral floristic region and compared with disjunct moss floras of other continents as well as the presently available molecular data.

2.1 Introduction

Herzog (1926) called disjunctions the “most interesting problems in phytogeography and their explanation the greatest importance for genetic aspects”. One of these interesting disjunctions is that between the southern part of Chile, New Zealand (and also southeastern Australia, Tasmania and southern Africa). Herzog (1926) wrote: “The strange fact that the southern part of South America south of 40° S lat. is an extraneous element as compared with the rest of South America and is more related to the remote flora of the southeastern corner of Australia, Tasmania and New Zealand, allows to include these regions into an floristic realm of its own”. Herzog called it the austral-antarctic floristic realm.

Herzog (1926) made no attempts to explain the floristic similarity of these regions, although Wegener (1915) had published his continental drift theory already 11 years before the publication of Herzog´s textbook. This theory was, however, not accepted by scientists and therefore not even discussed by Herzog but simply ignored. It took 50 more years until Wegener´s theory was confirmed by the results of the studies on

sea floor spreading and successfully used for the explanation of disjunctions of bryophytes.

Southern Chile and New Zealand share the same geological history: both were parts of the *Nothofagus* province of the palaeoaustral region until about 82 mio years ago, at a time, when Africa had already separated from the former Gondwana continent (Hill, 1994; White, 1990). In contrast to other parts of this continent such as India, Antarctica or Australia, Chile and New Zealand remained since in a humid-temperate climate belt. Whereas in Australia the continental drift to the tropic of Capricorn revealed in an explosive speciation of dry adapted species, Chile and New Zealand preserved parts of the late cretaceous flora in their humid temperate forests. This concerns *Nothofagus* forest as well as ancient conifer forest, which consist of genera such as *Agathis, Podocarpus, Libocedrus, Dacrydium, Dacrycarpus, Fitzroya, Pilgerodendron* among others. The floristic similarity between these former parts of the Gondwana continent, does, however, not only concern flowering plants but also bryophytes, which show much more affinities between Chile and New Zealand than flowering plants. The disjunctions in flowering plants are on a genus level, which demonstrates that even these ancient genera such as *Nothofagus* (Hill & Dettmann, 1996) have evolved new species in these separate parts of the world. In contrast, bryophytes have a common stock of identical species. This raises the question whether the species identical in both parts are remnants of late cretaceous forests and have survived morphologically unchanged, or are identical because they have genetic exchange through the west-wind drift, which could disperse spores from New Zealand westwards over a distance of 10,000 km to Chile.

2.2 Comparison

A first estimation of the genera of bryophytes common in New Zealand and Chile was presented by van Balgooy (1960), who indicated 128 genera (=75 %) as common to both regions. Seki (1973) in an evaluation of his collections in Patagonia indicated 14.7 % of the mosses as circumsubantarctic (including S. Africa, Tasmania, Australia, New Guinea highlands, northern Andes and Central America). Van Zanten & Pócs (1981) calculated the relationship on the species level and indicated 122

species (=27 %) in common. Matteri (1986) calculated the percentage of circumsubantarctic species from collections along a transect through Patagonia with 15.4 %. An exact determination of the degree of conformity of the moss floras of New Zealand and Chile was so far really impossible due to the lack of checklists. However, in the past checklists of mosses were published by Fife (1995) for New Zealand and He (1998) for Chile, which provided the base for the present more exact comparison.

The moss flora of Chile (He, 1998) comprises 778 species and 88 subspecific taxa in 203 genera and 63 families. For New Zealand, Fife (1995) recorded 523 species and 23 varieties in 208 genera and 61 families. Both checklists were compared to identify the

taxa identical in the floras of both regions.

2.3 Results

The comparison revealed that 181 species (+ 3 varieties) in 94 genera are identical in Chile and New Zealand (see tab. 1). The species common in Chile and New Zealand are listed in tab. 2. These are 23.3 % of the species and 63.1 % of the genera of the Chilean moss flora. It is, however, better to base the comparison on the moss flora of New Zealand, because Chile has also part of the neotropical flora. New Zealand shares 34.6 % of its species and of 61.5 % genera with Chile. If the species are excluded from this comparison, which are not confined to the austral region but are cosmopolitan or also occur e.g. in the tropical mountains or the holarctic (marked with asterix in tab. 1), the number of species disjunct between Chile and New Zealand is reduced to 113, that are 21.6 % of the New Zealand moss flora and 14.5 % of the Chilean moss flora. If the mosses of Chile would be reduced to austral region and the neotropical species would not be taken into account, the percentage would probably as high as in New Zealand. On the genus level, Chile and New Zealand have 127 genera in common, which are 63 % of the flora of Chile and 61 % of the flora of New Zealand. Thirty-three of the 127 genera have no species in common.

The conformity is accordingly higher on the family level and concerns 76 % of the genera of Chile and 78 % of the genera of New Zealand.

The species in common belong to 34 families (tab. 3). Most of the species belong to the Bryaceae, followed by Dicranaceae , Pottiaceae, Orthotrichaceae and Amblystegiaceae.

2.4 Discussion

Bryophytes can absolutely not be compared with higher plants in terms of their phytogeography. In a most recent comparison of the flora of New Zealand and the southern Andes, Wardle et al. (2001) indicate the percentage of realm endemics of both parts with 90 % of the species (465 species of the southern Andes and 522 of New Zealand) and 30 % of the genera, however, only forty species or closely related pairs of species are shared. Half of the number of species is not identical but closely related, half of the rest belongs to the coastal vegetation, most of the remaining species are ferns and others (*Deschampsia cespitosa*, *Trisetum spicatum*) may ultimately be introduced from the northern hemisphere. It can therefore be generalized that higher plants of the austral realm are disjunct on a genus level, bryophytes on a species level.

The percentage of conformity of disjunct floras may be the result of long distance dispersal or relicts of a former closed range. A detailed discussion of this topic is given by van Zanten & Pócs (1981). It is still difficult to decide which factor is crucial. A molecular analysis can only state whether base sequences of certain genes of populations of the same species in disjunct populations are identical or not. Identical base sequences can, however, be the result of gene exchange but also of relict population, which have not undergone genetic changes since the separation of the populations (stenoevolution sensu Frey et al., 1999). Additional arguments are required to decide whether the species are able for long distance dispersal or not tolerance to frost or UV-radiation, see van Zanten (1976; 1978; 1983; 1984), sterility or rarety of sporulation, morphological arguments (spore size, cleistocarpy), habitats (epiphytes in the understory of forests as opposed to species from open habitats), life strategies (colonists vs. perennial stayers).

Nevertheless calculations of the degree of conformity of disjunct floras give an almost perfect correlation with the duration of separation (tab. 4) and not with the distance. If

long distance dispersal would be the essential factor for explaining these disjunctions, tropical South America and tropical Africa would have more species in common than Chile and New Zealand, because both continents are closer than Chile and New Zealand. It could also be argued that tropical species are not as able for long distance dispersal as cool temperate species.

A further tool for differentiating relicts from species with gene exchange could be the interpretation of life strategies and habitats preferences. It could be argued that agressive colonists colonizing roadside banks (*Campylopus clavatus, C. introflexus*) are more likely dispersed by long distance dispersal than epiphytes in forests. About 30 species of the 187 common in Chile and New Zealand are epiphytic and therefore candidates for species with relict status.

Attempts have been made to solve the question experimentally (van Zanten, 1976; 1978; 1983; 1984) and very recently by molecular studies (Frey et al., 1999; Meißner et al., 1998; Pfeiffer, 2000b; Pfeiffer et al., 2000; Quandt et al., 2001; Quandt et al., 2000; Stech et al., 1999; Stech et al., 2002).

Van Zanten (1976; 1978) tested 139 disjunct bryophyte species for their ability for long distance dispersal (germination experiments with wet- and dry-freezing). Amongst these species there were 38 species occurring in Chile and New Zealand. Sixty-six species did not germinate, with a considerable high percentage (67 %) of diocious species. This might give an estimation of the percentage of species disjunct in Chile and New Zealand but with no genetic exchange. In contrast, only 23 % of the 48 tested species occurring “closer” in New Zealand and Australia did not germinate. Of the 29 the species occurring in Chile and New Zealand und used in the germination tests (van Zanten, 1978), most species were able to germinate after 1-3 years of desiccation. Only three species tolerated less than one year of desiccation: *Weymouthia mollis* and *Fissidens rigidulus* half a year and *Lopidium concinnum* only one month. Weymouthia and Lopidium are epiphytes, Fissidens is a hygrophyt.

It has, however, to be kept in mind that these spore germination experiments were necessarily based on species which are producing sporophytes and a certain percentage of species is only known in sterile condition. Therefore the percentage of species with presumably no genetic exchange is in fact much higher than the results of the germination experiments suggest.

The molecular studies were all made with the BryoAustral project using the *trn*L intron of cp DNA, which has proved to be most suitable for this purpose, with the following results:

1. ***Hypopterygium*** (Pfeiffer, 2000b; Stech et al., 1999)

Hypopterygium "rotulatum" (Hedw.) Brid. from primary rain forests in New Zealand shows 100 % sequence identity with *H. didictyon* from Chile. This disjunction is interpreted as palaeoaustral origin. Long distance dispersal is regarded as less likely because the species is dioiceous and has no vegetative reproduction. Even if the comparably small spores (10-17µm) are dispersed, a population cannot be established if not spores of both sexes land on the same spot. The existing stands are all bisexual. In addition it is difficult that this species growing on the floor of rain forests releases spores into higher air currents.

2. ***Polytrichadelphus*** (Stech et al., 2002)

Base sequences of *Polytrichadelphus magellanicus* from Chile and *P. innovans* from New Zealand show only small differences. Both taxa are therefore regarded as subspecies of *P. magellanicus.* The andine *P. longisetus* and *P. umbrosus* show a higher sequence variation and maybe derived from the latter. Genetic exchange can be excluded because the spores cannot tolerate dry or wet freezing (van Zanten 1978).

3. ***Lopidium*** (Frey et al., 1999)

A comparison of populations of the epiphytic Hypopterygiaceae *Lopidium concinnum* from Chile and New Zealand showed no genetic differences. The relict status is supported by van Zanten´s experiments (van Zanten 1978) which showed a desiccation tolerance of the spores of less than one month.

4. ***Weymouthia*** (Quandt et al., 2001)

The sequences of *Weymouthia cochleariifolia* described from New Zealand and *W. billardieri* described from Chile show no differences. The closely related species *W. mollis* had a desiccation tolerance of spores of less than half a year (van Zanten 1978).

5. ***Monoclea*** (Meißner et al., 1998)

Monoclea gottschei from South America and *M. forsteri* from New Zealand, two species morphologically very similar, have differences in base sequences on a species level (Meißner et al. 1998). This shows that both have originated from the same anchestor but have undergone a separate evolution after the separation of the populations. The evolution went on in South America, where *M. gottschei* ssp. *elongata* developed from ssp. *gottschei* by migration into the northern parts of the Andes.

In conclusion, the molecular data of species disjunct between Chile and New Zealand show three cases (see also tab. 5):

1. There are species with apparently no genetic interchange and no apparent evolution within the last 80 mio years (*Lopidium concinnum, Weymouthia cochleariifolia, Hypopterygium didictyon*). Interestingly, the two first species concern epiphytes in rain forests.
2. There are subspecies derived from the same anchestor originated in Chile and New Zealand during 80 mio years with low molecular and morphological differences (*Polytrichadelphus magellanicus* ssp. *magellanicus* and ssp*. innovans*).
3. There are two species originated from the same anchestor (*Monoclea forsteri/gottschei*). Case two and three concerns epigaeic bryophytes.

Acknowledgements. This study is part of the project BRYO AUSTRAL supported by the German Research Foundation with grants to J.-P. Frahm and W. Frey.

Tab. 1 Comparison of the moss flora between Chile and New Zealand.

	taxa		shared taxa	percentage of conformity [%]	
	Chile	New Zealand		Chile	New Zealand
total species	778	523	181	23.3	34.6
austral species	778	523	113	14.5	21.6
genera	203	208	127	63.1	61.5
families	63	61	48	76.2	78.7

Tab. 2 Moss species common in Chile and New Zealand according to He (1998) and Fife (1995). The nomenclature has been homologized to He (1998). The list includes 181 species and three varieties. Questionable records of *Brachymenium exile, Bruchia hampeana, Bryum coronatum, Cyclodictyon sublimbatum* and *Ptychomnion aciculare* are included. Species marked with * are not confined to the austral region but have wider ranges.

Achrophyllum dentatum	Hookeriaceae
Acrocladium auriculatum	Amblystegiaceae
Amblystegium serpens *	Amblystegiaceae
Amblystegium varium *	Amblystegiaceae
Amphidium tortuosum	Orthotrichaceae
Andreaea acutifolia	Andreaeaceae
Andreaea mutabilis	Andreaeaceae
Andreaea nitida	Andreaeaceae
Andreaea subulata	Andreaeaceae
Aulacomnium palustre *	Aulacomniaceae
Barbula calycina	Pottiaceae
*Barbula unguiculata**	Pottiaceae
*Bartramia halleriana**	Bartramiaceae
Blindia contecta	Seligeriaceae
Blindia magellanica	Seligeriaceae
Blindia robusta	Seligeriaceae
*Brachythecium albicans**	Brachytheciaceae
Brachythecium paradoxum	Brachytheciaceae
*Brachythecium plumosum**	Brachytheciaceae
Brachythecium rutabulum *	Brachytheciaceae
Brachythecium subpilosum	Brachytheciaceae
Breutelia elongata	Bartramiaceae
Breutelia pendula	Bartramiaceae
Breutelia robusta	Bartramiaceae
Bryoerythrophyllum jamesonii	Pottiaceae
*Bryum algovicum**	Bryaceae
*Bryum amblyodon**	Bryaceae
*Bryum argenteum**	Bryaceae
Bryum australe	Bryaceae
Bryum biliardieri	Bryaceae
*Bryum caespiticium**	Bryaceae
Bryum campylothecium	Bryaceae
*Bryum capillare**	Bryaceae
Bryum clavatum	Bryaceae
Bryum dichotomum	Bryaceae
Bryum laevigatum	Bryaceae
Bryum mucronatum	Bryaceae
*Bryum muehlenbeckii**	Bryaceae
Bryum pachytheca	Bryaceae
Bryum pallescens *	Bryaceae
Bryum perlimbatum	Bryaceae
*Bryum pseudotriquetrum**	Bryaceae
*Bryum rubens**	Bryaceae
Calliergidium austro-stramineum	Amblystegiaceae
*Calliergon stramineum**	Amblystegiaceae
*Calliergonella cuspidata**	Amblystegiaceae
Calyptopogon mnioides	Pottiaceae
Calyptrochaeta apiculata	Hookeriaceae
Calyptrochaeta flexicollis	Hookeriaceae
Camptochaete gracilis	Lembophyllaceae
*Campyliadelphus polygamum**	Amblystegiaceae

Campylopodium medium	Dicranaceae
Campylopus acuminatus	Dicranaceae
Campylopus clavatus	Dicranaceae
Campylopus incrassatus	Dicranaceae
Campylopus introflexus	Dicranaceae
Campylopus purpureocaulis	Dicranaceae
Campylopus pyriformis	Dicranaceae
Campylopus vesticaulis	Dicranaceae
Catagonium nitens ssp. *nitens*	Phyllogoniaceae
*Ceratodon purpureus**	Ditrichaceae
Ceratodon purpureus ssp. *convolutus*	Ditrichaceae
Chorisodontium aciphyllum	Dicranaceae
Conostomum tetragonum	Bartramiaceae
*Cratoneuron filicinum**	Amblystegiaceae
Cratoneuropsis relaxa	Amblystegiaceae
Dendrocryphaea lechleri	Cryphaeaceae
Dendroligotrichum dendroides	Polytrichaceae
Dicranella cardotii	Dicranaceae
Dicranella jamesonii	Dicranaceae
Dicranoloma billardieri	Dicranaceae
Dicranoloma menziesii	Dicranaceae
Dicranoloma robustum	Dicranaceae
Dicranoweisia antarctica	Dicranaceae
Didymodon australasiae	Pottiaceae
Distichium capillaceum	Distichaceae
Distichophyllum krausei	Hookeriaceae
Distichophyllum rotundifolium	Hookeriaceae
Ditrichum austro-georgicum	Ditrichaceae
Ditrichum brotherusii	Ditrichaceae
Ditrichum cylindricarpum	Ditrichaceae
Ditrichum difficile	Ditrichaceae
Ditrichum strictum	Ditrichaceae
*Drepanocladus aduncus**	Amblystegiaceae
*Drepnocladus exannulatus**	Amblystegiaceae
*Drepanocladus fluitans**	Amblystegiaceae
*Drepanocladus uncinatus**	Amblystegiaceae
*Encalypta rhaptocarpa**	Encalyptaceae
*Encalypta vulgaris **	Encalyptaceae
Entosthodon laxus	Funariaceae
*Fissidens adianthoides**	Fissidentaceae
*Fissidens asplenioides **	Fissidentaceae
Fissidens curvatus	Fissidentaceae
Fissidens oblongifolius	Fissidentaceae
Fissidens rigidulus	Fissidentaceae
Fissidens serratus	Fissidentaceae
*Fissidens taxifolius**	Fissidentaceae
*Funaria hygrometrica**	Funariaceae
Glyphothecium sciuroides	Ptychomniaceae
Goniobryum subbasilare	Rhizogoniazeae
Grimmia grisea	Grimmiaceae
*Grimmia levigata**	Grimmiaceae
*Grimmia pulvinata**	Grimmiaceae
*Grimmia trichophylla**	Grimmiaceae
*Gymnostomum calcareum**	Pottiaceae
*Hedwigidium integrifolium**	Hedwigiaceae
Hennediella arenae	Pottiaceae
*Hennediella heimii**	Pottiaceae

Hennediella serrulata	Pottiaceae
*Hymenostylium recurvirostrum**	Pottiaceae
Hypnum chrysogaster	Hypnaceae
*Hypnum cupressiforme Hedw. var. cupressiforme**	Hypnaceae
*Hypnum cupressiforme var. filiforme**	Hypnaceae
Hypnum cupressiforme var. mossmanianum	Hypnaceae
*Hypnum revolutum**	Hypnaceae
Hypopterygium didctyon	Hypopterygiaceae
*Isopterygium pulchellum**	Plagiotheciaceae
Kiaeria pumila	Dicranaceae
*Kindbergia praelonga **	Brachytheciaceae
*Leptobryum piriforme**	Bryaceae
*Leptodictyum riparium**	Amblystegiaceae
*Leptodon smithii**	Neckeraceae
Leptotheca gaudichaudii	Aulacomniaceae
Lepyrodon lagurus	Lepyrodontaceae
Lopidium concinnum	Hypopterygiaceae
Macromitrium longirostre	Orthotrichaceae
Macromitrium microstomum	Orthotrichaceae
Muelleriella angustifolia	Orthotrichaceae
Muelleriella crassifolia	Orthotrichaceae
Oligotrichum canaliculatum	Polytrichaceae
Orthodontium lineare	Byaceae
Orthotrichum assimile	Orthotrichaceae
*Orthotrichum cupulatum**	Orthotrichaceae
Orthotrichum hortense	Orthotrichaceae
*Orthotrichum rupestre**	Orthotrichaceae
Papillaria flexicaulis	Meteoriaceae
Philonotis scabrifolia	Bartramiaceae
*Plagiothecium denticulatum**	Plagiotheciaceae
Plagiothecium lucidum	Plagiotheciaceae
*Pohlia cruda**	Bryaceae
*Pohlia nutans**	Bryaceae
*Pohlia wahlenbergii**	Bryaceae
Polytrichadelphus magellanicus	Polytrichaceae
*Polytrichastrum alpinum**	Polytrichaceae
*Polytrichastrum longisetum**	Polytrichaceae
*Polytrichum juniperinum**	Polytrichaceae
Pseudocrossidium crinitum	Pottiaceae
Ptychomnion densifolium	Ptychomniaceae
Pyrrhobryum mnioides	Rhizogoniaceae
Racomitrium crispipilum	Grimmiaceae
Racomitrium crispulum	Grimmiaceae
*Racomitrium lanuginosum**	Grimmiaceae
Racomitrium pruinosum	Grimmiaceae
Racomitrium ptychophyllum	Grimmiaceae
*Rhacocarpus purpurascens**	Hedwigiaceae
Rhaphidorrhynchium amoenum	Sematophyllaceae
Rhizogonium novae-hollandiae	Rhizogoniaceae
Rhynchostegium tenuifolium	Brachytheciaceae
*Sarmentypnum sarmentosum**	Amblystegiaceae
Sauloma tenella	Hookeriaceae
*Schistidium apocarpum **	Grimmiaceae
*Schistidium rivulare **	Grimmiaceae
Sematophyllum uncinatum	Sematophyllaceae
Sphagnum falcatulum	Sphagnaceae
*Sphagnum subnitens **	Sphagnaceae

Syntrichia andersonii	Pottiaceae
Syntrichia papillosa *	Pottiaceae
Syntrichia princeps *	Pottiaceae
Syntrichia robusta	Pottiaceae
*Tetrodontium brownianum**	Tetraphidaceae
Thuidium furfurosum	Thuidiaceae
Thuidium sparsum	Thuidiaceae
Tortula atrovirens *	Pottiaceae
*Tortula muralis**	Pottiaceae
*Trichostomum brachydontium**	Pottiaceae
Ulota rufula	Orthotrichaceae
*Weissia controversa**	Pottiaceae
Weymouthia cochlearifolia	Meteoriaceae
Weymouthia mollis	Meteoriaceae
Zygodon gracillimus	Orthotrichaceae
Zygodon hookeri	Orthotrichaceae
Zygodon intermedius	Orthotrichaceae
Zygodon menziesii	Orthotrichaceae
Zygodon obtusifolius	Orthotrichaceae

Tab. 3: Number of species per families occurring disjunct in Chile and New Zealand.

Amblystegiaceae (14)
Andreaeaceae (4)
Aulacomniaceae (2)
Bartramiaceae (5)
Brachytheciaceae (7)
Byaceae (23)
Cryphaeaceae (1)
Dicranaceae (20)
Ditrichaceae (4)
Encalyptaceae (2)
Fissidentaceae (7)
Funariaceae (2)
Grimmiaceae (11)
Hedwigiaceae (2)
Hookeriaceae (6)
Hypnaceae (6)
Hypopterygiaceae (2)
Lembophyllaceae (1)
Lepyrodontaceae (1)
Meteoriaceae (3)
Neckeraceae (1)
Orthotrichaceae (15)
Phyllogoniaceae (1)
Plagiotheciaceae (2)
Polytrichaceae (6)
Pottiaceae (20)
Ptychomniaceae (2)
Rhizogoniaceae (3)
Seligeriaceae (3)
Sematophyllaceae (2)
Sphagnaceae (2)
Tetraphidaceae (1)
Thuidiaceae (2)

Tab. 4 Degree of conformity of the mosses of various disjunct floras. The percentage is correlated with the time span of separation.

Disjunction	Percentage of species in common	Author	Age mio years	Distance (approx.) km
Europe – North America	70 % of the species of North America	Frahm & Vitt (1991)	50	6,000
Africa – South America	8 % of the neotropical flora[2]	Delgadillo (1993)	180	6,000
Chile – New Zealand	33 % of the species of New Zealand[1]	this paper	80	10,000[2]

[1] The percentage is calculated on the flora of New Zealand because Chile is also part of the neotropical flora.
[2] The distance across the South Pacific Ocean is given, because it correlates with the prevailing wind systems.

Tab. 5 Genetic distances between disjunct populations or taxa in the austral temperate region using the *trn*L-Intron of cp DNA.

	differences in trnL-Intron [%]	Disjunction	Separation [Myr BP]	Reference
Monoclea forsteri/gottschei	5.5	Chile – New Zealand	80	Meißner et al, 1998
M gottschei ssp. *gottschei/* ssp. *elongata*	1.0	S – N South America	? (<80)	Meißner et al, 1998
Hypopterygium didictyon	0.0	Chile – New Zealand	80	Pfeiffer 2000
H. didictyon/debile	3.4			Pfeiffer et al, 2000
H. didictyon/muelleri	4.1			Pfeiffer et al, 2000
Lopidium concinnum	0.0	New Zealand – S Brazil- Chile		Frey et al. 1999
L. concinnum/struthiopteris	3.0	New Zealand/Chile – Zaire		Frey et al, 1999
Polytrichadelphus magellanicus ssp. *m,/*ssp. *innovans*	1.1	Chile – New Zealand	80	Stech et al, 2002
Polytrichadelphus magellanicus/ longisetus	2.3	Patagonia – N, Andes		Stech et al, 2002
P. magellanicus/umbrosus	4.5	Patagonia N, Andes		Stech et al, 2002
Weymouthia cochleariifolia	0.0	Chile – New Zealand	80	Quandt et al, 2001

3 A preliminary study on the phylogeny and molecular evolution of the Ptychomniaceae M. Fleisch. (Bryopsida) with special emphasis on *Ptychomnion ptychocarpon* and *Dichelodontium*.

3.1 Introduction

Systematics of the family Ptychomniaceae. During our field studies on bryophytes in the temperate rainforest in Chile, one of the most remarkable species we encountered was *Ptychomnion ptychocarpon* (Schwaegr.) Mitt., a member of the Ptychomniaceae Fleisch. Because of its pendent life form, unique within the genus, and its endemic status (southern temperate rainforests of Chile and Argentina) the evolution of this species was of special interest to us.

Fleischer (1906-1908) established the family Ptychomniaceae based e.g. on the character of the ribbed capsules. He separated the family into two tribes (‘tribus’), based on the orientation of the capsule. The group with erect capsules, ‘tribus’ Cladomnieae, comprises *Hampeella* C. Müll., *Glyphothecium* Hamp., *Dichelodontium* Hook.f. & Wils., *Cladomniopsis* Fleisch., and *Cladomnion* Hook.f. & Wils. The second ‘tribus’, Ptychomnieae, with inclined to curved capsules consists of the single genus *Ptychomnion*.

Brotherus (1909b) describes fourteen species in seven genera for the Ptychomniaceae, based on the same characters used by Fleischer (1908). In contrast to Fleischer Brotherus (1909b) divides the family Ptychomniaceae into two subfamilies (Cladomnioideae and Ptychomnioideae) and subsequently includes in his later treatment of the family (Brotherus, 1925c) the newly established genus *Tetraphidopsis* Broth. & Dix. in the Ptychomniaceae (Cladomnioideae).

According to Hattaway (1984) the family Ptychomniaceae consists of 16 species in seven genera. He uses the two subdivisions already described in Fleischer (1908)

but adopts the rank ‘subfamily’ for the subdivisons made by Brotherus (1909b) to divide the family Ptychomniaceae. He recognizes that several synapomorphic characters unite the genera *Ptychomnion* and *Cladomniopsis* and transfers the latter genus to the subfamily Ptychomnioidae which since then consists of the genera *Ptychomnion* and *Cladomniopsis*. The synapomorphic characters uniting these genera are among others that they are inclined to having suberect capsules and a well developed basal membrane of the endostome.

The second subfamily Cladomnioidae consists of the genera *Cladomnion, Glyphothecium, Hampeella* and *Tetraphidopsis*. According to Hattaway (1984) the genus *Dichelodontium* does not belong to the Ptychomniaceae, but as he could not define its most closely related taxa he provisionally included *Dichelodontium* in the Ptychomniaceae (subfamily Cladomnioidae). The shared characters of the five genera are e.g. the erect, straight and symmetric capsules and the special exostome ornamentation.

Brotherus (1909b; 1925c) already recognizes the special status of *Ptychomnion ptychocarpon* and separates it from the remaining species of *Ptychomnion* Hook.f. & Wils., by placing the latter in the ‘section’ Eu-Ptychomnion and *P. ptychocarpon* in the ‘section’ Ptychomniella of the genus *Ptychomnion*.

Kühnemann & Carralves (1975) present a monography of the Ptychomniaceae of the temperate rainforests of Chile and Argentina and list nine species of the family occurring in this area. They share the view of Brotherus (1909b) and separate *P. ptychocarpon* from the remaining species of the genus.

Morphological characterisation and systematics of Dichelodontium. As already mentioned above, Hattaway (1984) only provisionally places the monospecific genus *Dichelodontium* within the Ptychomniaceae but also states that further studies should be made to determine its familial placement. Based on morphological studies using characters of the gametophyte and the sporophyte Magill (1987) retains *Dichelodontium* in the Ptychomniaceae whereas Allen (1999) transfers the New Zealand endemic *Dichelodontium nitidum* (Hook. & Wilson) Broth. to the Lepyrodontaceae. The shared characters which justify this placement are according to Allen (1999) the single peristome of endostomial nature and rhizoids which arise from initials around branch buds (or leaf axels). Both taxa have similar leaf cells,

weakly developed costae, sheathing perichaetial leaves, and a cucullate calyptra. Also the branching pattern of *Dichelodontium nitidum* resembles that of some species in *Lepyrodon*. As the status of *Dichelodontium nitidum* remains questionable we also included it in our study.

Recent research studying Ptychomniaceae has mainly focussed on single taxa (e.g. Ochyra, 2002; Tangney & Fife, 1997) or has included members of the family in molecular phylogenetic studies in order to reveal relationships in pleurocarpous mosses as a whole (e.g. Buck et al., 2000b). In 2003, Shaw et al. (2003) used the term 'Ptychomniales' for a new order in pleurocarpous mosses but did not specify which taxa belong to that order. A specification of this category can be expected from Buck et al. (in press). Shaw and Renzaglia (2004) already mention some results of this paper (Buck et al., in press) which suggest that based on molecular data the Garovagliaceae are now included in the Ptychomniaceae which is the only family in the order Ptychomniales of the suborder Ptychomnianae. For this reason, representatives of the Garovagliaceae are included in the present study. The second suborder Hypnanae according to Shaw and Renzaglia (2004) comprises the orders Hookeriales and Hypnales.

Morphological characterisation of the Ptychomniaceae. The pleurocarpous Ptychomniaceae are described by Beever (1992) as having leaves which are 'often of papery texture'. The leaves are erect spreading and usually plicate and/or rugose. The leaf shape is ovate-lanceolate to broadly ovate with a slender leaf apex. The costae are short and double. The most prominent sporophytic character is the capsule carrying eight ribs when ripe. The peristome is usually double and the endostome has a basal membrane.

Morphological characterisation and systematics of Ptychomnion. Characters of the gametophyte and sporophyte which separate the genus from the remaining six genera are e.g. a well developed primary stem, the lack of gemmae and a well developed exostome with primary and secondary banding.
Brotherus (1909b; 1925c) distinguishes two 'sections' within the genus. Hattaway (1984) gave these sections the rank of a subgenus. According to Hattaway (1984) the subgenus *Ptychomnion* consists of the taxa *P. aciculare* (Brid.) Mitt., *P.*

cygnisetum (C. Müll.) Kindb.*, P. densifolium* (Brid.) Jaeg., and *P. falcatulum* Broth. The second subgenus '*Ptychomniella*' consists of only one species *Ptychomnion ptychocarpon* (Schwaegr.) Mitt.

The most important characters Brotherus (1909b; 1925c) describes to separate *P. ptychocarpon* from the remaining species of the genus, are the moderate size, the slender habitus and the long creeping stem which can be very long and pendent, the curved secondary and irregularly branched stems as well as the more or less spreading leaves. Hattaway (1984) adds three sporophytic characters to these characters to justify the separation. He considered the genus *Ptychomnion* as the ancestral genus within the family. The genus is restricted in its distribution to the southern hemisphere.

Morphological characterisation and systematics of Cladomniopsis. The genus is monotypic and its representative species *Cladomniopsis crenato-obtusa* Fleisch. is endemic to southern South America. One of the outstanding characters of this monotypic genus is the presence of a rudimentary central strand as reported in Hattaway (1984).

Morphological characterisation and systematics of Cladomnion. This monotypic genus is according to Hattaway (1984) closely related to the genus *Ptychomnion* based on the plicate leaves, and incrassate porose laminal cells. *Cladomnion ericoides* is endemic to New Zealand.

Morphological characterisation and systematics of Glyphothecium. There are three species described within this genus (Hattaway, 1981) which occur in southern South America and Australasia. The characters used to circumscribe the genus *Glyphothecium* are the basal membrane in the peristome and the rhizoids which can be both smooth and papillose within one plant.

The genus is one of two genera in the family Ptychomniaceae of which some species occur north of the equator. Species belonging to this genus occur in Australasia from New Zealand and southeast Australian temperate rainforests, to further north on the Phillipines and Sri Lanka. In South America it is restricted to the southern temperate forests of Chile and Patagonia.

Morphological characterisation and systematics of Hampeella. This genus, comprising four species, has an Australasian distribution and follows mainly the same distribution pattern as observed in *Glyphothecium* with the difference that it does not occur in southeast Australia and Sri Lanka.
The genus is characterized by a double peristome (without cilia) and the absence of paraphyllia. Unique within the family is its polymorphism in leave characters.

Morphological characterisation and systematics of Tetraphidopsis. The monotypic genus occurs from New Zealand and Australia to Tasmania and Victoria.
The peristome in *Tetraphidopsis* lacks a basal membrane. Further characters to distinguish this genus from the other genera of the family are the short single costa and the presence of pseudoparaphyllia instead of paraphyllia.

***Morphological characterisation and systematics of* Garovagliaceae**. Recent phylogenetic analyses resolved representatives of the Garovagliaceae Buck & Vitt to be closely allied with the Ptychomniaceae (e.g. Buck et al., 2000b). Therefore two species representing the Garovagliaceae are included in this analysis.

This study is aimed at clarifying the familial relationship of *Dichelodontium* (Lepyrodonataceae) and the relationship of *P. ptychocarpon* to other representatives of the genus *Ptychomnion*.

3.2 Material & Methods

***Plant material*.** Plant material of *Lepyrodon tomentosus* and *Ptychomnion ptychocarpon* was collected by the author during a field trip of the BryoAustral project to Chile in 2001. Material of *Ptychomnion cygnisetum* from Chile was kindly provided by Dr. Friederike Schaumann (Freie Universität, Berlin). The remaining specimens used in this study originate from herbarium specimens. Specimens of *Acrocladium chlamydophyllum* and *Lepyrodon pseudolagurus* were collected during the BryoAustral project expedition to New Zealand in 1998. Duplicates are preserved in the herbaria in Christchurch (CHR), Bonn (BONN) and Berlin (B). We also used

sequences available in GenBank. All specimens used in our analyses are listed in Appendix I including further voucher information.

Using cpDNA sequences of the *trn*L-Intron and the *rps*4-gene the phylogenetic relationships of *Dichelodontium* (Lepyrodontaceae) and *Ptychomnion ptychocarpon* and its relation to the species within the Ptychomniaceae sensu Hattaway (1984) were analysed.

At least one species of each genus of the Ptychomniaceae as well as *Dichelodontium nitidum* were included in this analysis. The following species of the Ptychomniaceae were included in this study: *Hampeella alaris, Ptychomnion cygnisetum, Ptychomnion ptychocarpon, Cladomnion ericoides, Tetraphidopsis pusilla, Cladomniopsis crenato-obtusa*. Additionally, sequences from GenBank of all available taxa of the Ptychomniaceae (*Hampeella alaris, Hampeella pallens, Ptychomnion cygnisetum,* two specimens of *Ptychomnion aciculare, Ptychomnion ptychocarpon, Cladomnion ericoides,* and *Tetraphidopsis pusilla*) and of *Dichelodontium nitidum* were used to test the variability within a species.

The following taxa were chosen as outgroup taxa for this study: representative species of the Lepyrodontaceae (*Lepyrodon tomentosus*, *L. pseudolagurus*), two species of the genus *Acrocladium* (*A. auriculatum, A. chlamydophyllum*) belonging to the Plagiotheciaceae according to Pedersen & Hedenäs (2002); two representatives of the Hypopterygiacae (*Hypopterygium didictyon, Lopidium concinnum*), four representatives of the core of the Hookeriales (*Hookeria lucens, Schimperobryum splendidissimum, Distichophyllum pulchellum* and *Daltonia gracilis*) and two species representing the Garovagliaceae (*Garovaglia elegans, Euptychium robustum*) as well as *Hypnum cupressiforme* and *Neckera crispa*.

The Ptychomniaceae were represented in this study by at least one species of each genus described by Hattaway (1984). The genus *Hampeella* is represented by two species (*H. alaris, H. pallens*) out of the four described as belonging to the genus (Hattaway, 1984). The genus *Glyphothecium*, comprising a total of three species, is represented by *G. sciuroides*, and the genus *Ptychomnion*, comprising six species, is represented by *P. aciculare, P. cygnisetum* and *P. ptychocarpon*. Also, one specimen each of the monotypic genera *Cladomnion*, *Cladomniopsis* and *Tetraphidopsis* were included.

Additional sequences of the genera of Ptychomniaceae (*trn*L region and *rps*4 gene) were extracted from GenBank and used in the phylogenetic analysis as well as for

the calculation of genetic distances (p-distance) in order to analyse intrapecific differences in the Ptychomniaceae.

DNA isolation, PCR and sequencing. Prior to DNA extraction the plant material was thoroughly cleaned with distilled water and additionally treated by ultrasonic waves for 2-4 minutes. Success of cleaning was checked by examining the plants under a binocular microscope. Remaining contaminations e.g. with algae and fungi were removed mechanically. Isolation of DNA was carried out following the CTAB technique described in Doyle & Doyle (1990).

PCR amplifications (Biometra TriBlock thermocycler, PTC-100 MJ Research) were performed in 50 µl–reactions containing 1.5 U *Taq* DNA polymerase (PeqLab), 1 mM dNTPs-Mix, nucleotide concentration 0.25 mM each (PeqLab), 1x buffer (PeqLab), 1.5 mM $MgCl_2$ (PeqLab) and 12.5 pmol of each amplification primer. PCR products were purified using the QIAquick purification kit (Qiagen). Cycle sequencing reactions (half reactions) were performed using a PTC-100 Thermocycler (MJ Research) in combination with the ABI Prism™ Big Dye Terminator Cycle Sequencing Ready Reaction Kit with Amplitaq-DNA polymerase FS (Perkin Elmer), applying a standard protocol for all reactions. Extension products were precipitated with 40 µl 75 % (v/v) isopropanol for 15 min at room temperature, centrifuged with 15.000 rpm at 25°C, and washed with 250 µl of 75 % (v/v) isopropanol. These purified products were loaded on an ABI 310 automated sequencer (Perkin Elmer) and electrophoresed. For cycle sequencing 10 µl–reactions were used containing 3 µl of Big Dye Terminator Cycle Sequencing premix. Sequencing reactions were performed on two independent PCR products generated from each sample in order to verify the results.

Table 6: Primer sequences used for amplification and sequencing of the *trn*L region and *rps*4 gene. Underlined nucleotides represent changes (Quandt et al., 2000) with respect to the original primers of Taberlet (1991).

Primer	Sequence	Data source
*trn*S	TAC CGA GGG TTC GAA TC	Nadot et al. 1994
*rps*5	ATG TCC CGT TAT CGA GGA CCT	Nadot et al. 1994
*trn*L-C_mosses	CGR AAT TGG TAG ACG CTA CG	Quandt et al. 2000
*trn*L-F	ATT TGA ACT GGT GAC ACG AG	Taberlet et al. 1991

All PCR products were sequenced using two primers. For amplifying and sequencing the non-coding regions of the chloroplast DNA a modification of primer C (Quandt et

al., 2000) as well as primer F, originally designed by Taberlet et al. (1991) were employed.

Primers used to amplify the *rps*4 gene were those described in Nadot et al. (1994), 'trnS' and 'rps5' (table 6).

For the *trn*L-F region and the *rps*4 gene the PCR program was performed with the following settings: 2 min. 94ºC, 35 cycles (1 min. 94ºC, 1 min. 55ºC, 1 min. 72ºC) and a 5 min. 72ºC extension time, cycle sequencing settings: 29 cycles (5 sec. 96ºC, 4 min. 50ºC).

All sequences will be deposited in EMBL, accession numbers are listed in Appendix 1, the alignments are available on request from the author.

***Phylogenetic analyses*.** Heuristic searches under the parsimony criterion were carried out under the following options: all characters unweighted and unordered, multistate characters interpreted as uncertainties, gaps coded as missing data, performing a tree bisection reconnection (TBR) branch swapping, collapse zero branch length branches, MulTrees option in effect, random addition sequence with 1000 replicates.

Furthermore the data sets were analysed using *win*PAUP 4.0b10 (Swofford, 2002) executing the command files generated by 'PRAP' (Parsimony Ratchet Analyses using PAUP Müller, 2004), employing the implemented parsimony ratchet algorithm (Nixon, 1999). For the parsimony ratchet the following settings were employed: 10 random addition cycles of 200 iterations each with a 40 % upweighting of the characters in the PRAP iterations. Heuristic bootstrap searches (BS Felsenstein, 1985) under parsimony criterion were performed with 1000 replicates, 10 random addition cycles per bootstrap replicate and the same options in effect as the heuristic search for the most parsimonious tree (MPT). The consistency index (CI, Kluge & Farris, 1969), retention index (RI), and rescaled consistency index (RC, Farris, 1989) were calculated to assess homoplasy.

Maximum Likelihood analyses were executed assuming a general time reversible model (GTR+G+I), and a rate variation among sites following a gamma distribution (four categories represented by the mean), with the shape being estimated and the molecular clock not enforced. According to Akaike Information Criterion (AIC, Akaike, 1974) GTR+G+I was chosen as the model that best fits the data by Modeltest v3.06 (Posada & Crandall, 1998), employing the windows front-end (Patti, 2002). The

proposed settings by Modeltest v3.06 (tab. 7) were executed in *win*PAUP 4.0b10. In addition to our MP analyses we performed Bayesian Inferences with MrBayes3.0 (Huelsenbeck & Ronquist, 2001). Modeltest 3.5 (Posada, 2004) was used to select DNA substitution models for our data set (gamma shape distribution,

Table 7: Substitution models selected for the combined *trn*L and *rps*4 data set.

	***trn*L and *rps*4 data set**
Model selected	GTR+G -lnL = 4596.3706
Base frequencies	freqA = 0.4154 freqC = 0.1167 freqG = 0.1351 freqT = 0.3329
Substitution model	R(a) [A-C] = 1.6344 R(b) [A-G] = 4.5384 R(c) [A-T] = 0.2791 R(d) [C-G] = 0.3519 R(e) [C-T] = 4.5384 R(f) [G-T] = 1.0000
Among-site rate variation	
Proportion of invariable sites (I)	0
Variable sites (G, Gamma distribution shape parameter)	0.2676

six substitution types). The Markov Chain Monte Carlo (MCMC) analyses were run for 1,000,000 generations with four simultaneous MCMCs and one tree per 100 generations was saved. The 'burn-in' values were determined empirically from the likelihood values. The analyses were repeated three times to assure sufficient mixing by confirming that the program converged to the same posterior probability (PP).

The program Treegraph (Müller & Müller, 2004) was used to edit trees directly from PAUP-treefiles.

MEGA2.1 (Kumar et al., 2001) was used to calculate GC-content, sequence length and distance measure ('p-distance'). In the following the term 'genetic distance' is used beside the term 'p-distance'.

3.3 Results

3.3.1 Sequence Variation

In this study eight species representing all genera of the Ptychomniaceae and *Dichelodontium nitidum* were successfully sequenced for the *trn*L region and the *rps*4 gene. The GC-content and sequence length are listed for all taxa used in phylogenetic analysis (tab. 8). The variability of the data sets are shown in table 9.
The length of the total sequence of the *rps*4 gene ranged between 547 basepairs (bp) in *Tetraphidopsis pusilla* (specimen 126) and 592 bp in e.g. *Ptychomnion cygnisetum* (sp. 130) and *Cladomniopsis crenato-obtusa* (sp. 127). The length of the *rps*4 gene was on average 581 base pairs (standard deviation 13.2) for all taxa of the Ptychomniaceae - Garovagliaceae included - and *Dichelodontium*. In the following these 21 taxa are referred to as Ptychomniaceae s.l.
The average GC-content in the data set comprising the Ptychomniaceae s.l. was 27.4 % (standard deviation 0.8).
The average length in the *trn*L intron of the Ptychomniaceae s.l. (21 taxa) was 309 bp (standard deviation 11.5). The shortest *trn*L intron sequence was found in *Cladomniopsis crenato-obtusa* (sp. 127) with 267 bp, the longest sequence (314 bp) was obtained for *Tetraphidopsis pusilla* (sp. 126), and three taxa where sequences were extracted from GenBank.
The average GC-content in the *trn*L intron of the Ptychomniaceae s.l. was 31.1 % (standard deviation 1.2).
The length of the *trn*L-*trn*F spacer varied considerably, as can be seen from a high standard deviation (tab. 8). The average GC-content in the *trn*L-*trn*F spacer of the Ptychomniaceae s.l. was 32.3 % (standard deviation 4.7).

Variability of the data set. Table 9 presents the information for the different regions in the alignment. The data presented here were derived from all 34 taxa included in the phylogenetic analysis.
The highest proportion of variable sites was found in the *rps*4 gene where 31.8 % of the 595 aligned positions were variable within the data set including the outgroup. The proportion of parsimony informative positions in this data set was 20.8 %.

The aligned *trn*L intron revealed 21.9 % variable sites for 424 aligned positions (13.9 % parsimony informative positions). The lowest values of variable positions (16.7) and parsimony informative position (9.6 %) were found within the 156 positions of the *trn*L-*trn*F spacer.

Table 8: Sequence lengths [base pairs, bp] of selected gene regions and GC-content [%] of the *trn*L intron, *trn*L-*trn*F spacer and *rps4* gene studied for 34 bryophyte taxa. Average sequence lengths and standard deviations are also given. For origin of the data refer appendix 1. Abbreviations: n. d. = no data available.

Taxon	*trn*L intron sequence length [bp]	*trn*L intron G/C content [%]	*trn*L-*trn*F spacer Length [bp]	*trn*L-*trn*F spacer G/C content	*rps*4 Length [bp]	*rps*4 G/C content spacer
Hypnum cupressiforme	315	30.8	62	22.6	592	27.4
Neckera crispa	258	29	n. d.	n. d.	592	28.2
Leucodon sciuroides	316	30.1	70	21.4	592	27.2
Acrocladium auriculatum (sp.78)	314	30.2	64	20.3	558	26.3
Acrocladium chlamydophyllum (sp.12)	315	30.8	64	25	570	26.7
Lepyrodon tomentosus (sp.64)	314	32.5	63	22.2	540	28.5
Lepyrodon pseudolagurus (sp.67)	315	31.7	64	23.4	576	27.9
Lopidium concinnum	304	21.4	n. d.	n. d.	595	27.5
Hypopterygium didictyon	296	23	n. d.	n. d.	592	27.6
Hookeria lucens	303	24.1	n. d.	n. d.	592	27.4
Schimperobryum splendidissimum	366	26.2	63	20.6	573	26.7
Daltonia gracilis	347	27.4	46	19.6	587	24.9
Distichophyllum pulchellum	378	25.9	62	19.4	585	25.3
Hampeella pallens	308	31.2	42	19	587	26
Hampeella alaris (sp.128)	307	31.6	n. d.	n. d.	553	25.7
Hampeella alaris (sp.2)	306	31.7	52	15.3	592	26.4
Ptychomnion ptychocarpon (sp.132)	319	30.4	n. d.	n. d.	559	27
Ptychomnion ptychocarpon (sp.2)	320	30	53	28.3	587	27.2
Cladomniopsis creanato-obscura (sp.127)	267	27.4	n. d.	n. d.	592	26.7
Glyphothecium sciuroides (sp.158)	312	32	n. d.	n. d.	571	28
Ptychomnion cygnisetum (sp.130)	313	31.3	n. d.	n. d.	592	27.9
Ptychomnion cygnisetum (sp.2)	314	31.2	47	21.2	586	27.3
Ptychomnion aciculare (sp.1)	314	30.8	47	23.4	586	27.6
Ptychomnion aciculare (sp.2)	314	30.8	52	23.1	586	27.6
Cladomnion ericiodes (sp.125)	313	32.2	47	25.5	586	27.3
Cladomnion ericiodes (sp.2)	313	32.2	47	25.5	586	27.3
Glyphothecium sciuroides (sp.123)	288	29.1	n. d.	n. d.	570	28.2
Glyphothecium sciuroides (sp.2)	311	31.8	47	23.4	586	28.1
Euptychium robustum	313	31.3	53	22.6	592	27.7
Garovaglia elegans	313	32.9	n. d.	n. d.	587	28.5
Dichelodontium nitens (sp.81)	308	32.5	47	25.5	586	28.5
Dichelodontium nitidum (sp.2)	313	31.9	47	23.4	586	28.5
Average	***312.2***	***29.7***	***54.2***	***22.4***	***581.7***	***27.3***
SD	***21.6***	***3.0***	***8.4***	***2.9***	***13.4***	***0.9***

Table 9: Number of taxa, total number of aligned characters; variable characters and number of parsimony informative sites and %-value of variable sites for the partial data sets of 34 taxa.
(* includes part of the *trn*F and *rps*4-*trn*S spacer).

	Com-bined*	Varia-bility [%]	*trn*L intron	Varia-bility [%]	*trn*L-F spacer	Varia-bility [%]	*rps*4	Varia-bility [%]
Number of sites	1.175		424		156		595	
Variable sites	308	26.2	93	21.9	26	16.7	189	31.8
Parsimony Informative	199	17.0	59	13.9	15	9.6	124	20.8

3.3.2 Genetic distances

Distance between *Dichelodontium* and *Lepyrodon.* The distance of *Dichelodontium nitidum* (sp. 81) to the representative species of the Lepyrodontaceae (*Lepyrodon pseudolagurus* and *L. tomentosus*) is 6.7 % in the *trn*L-intron (Appendix 2) and between 9.5 and 9.6 % in the *rps4* gene (Appendix 3). The distance of the sequence of *Dichelodontium nitidum* (#2) obtained from GenBank to both *Lepyrodon* species is 8.2 % for the *trn*L intron and between 9.5 and 9.6 % for the *rps4* gene.

Interspecific distances in the genus Ptychomnion. Sequence variation of *P. ptychocarpon* (sp. 132, 2) and *P. cygnisetum* (sp. 130, 2) ranges from 4.2-4.5 % in the intron (Appendix 2) and 6.3-6.8 % in the *rps*4 gene (Appendix 3). The p-distance of *P. ptychocarpon* (sp. 132, 2) to *P. aciculare* (sp. 1, 2) is between 3.8 and 4.2 % in the intron and between 6.8 and 7.3 % in the *rps*4 gene.

Intraspecific distances. No difference was detected between the two sequences of *Hampeella alaris*, sp. 128 and 2, neither in the *trn*L nor in the *rps*4 sequences. This same pattern was found for *Ptychomnion cygnisetum*, *Ptychomnion aciculare* and *Cladomnion ericoides*.

The variation between the sequences of *Ptychomnion ptychocarpon* (sp. 132, 2) was 0.2 % in the *rps4* gene (Appendix 3) and 0.3 % in the *trn*L intron. The same differences were found between the two sequences of *Glyphothecium sciuroides* (sp. 123, 2). In *Tetraphidopsis pusilla* (sp. 126, 2) the two sequences differed in 0.3 % of

the positions within the *trn*L intron (Appendix 2). There was no difference detected between specimens 126 and 2 in the *rps*4 gene.
The sequences of *Dichelodontium nitidum* (sp. 81, 2) differed in 1.6 % of the positions within the *trn*L intron (Appendix 2). No difference was found between the two obtained *rps*4 sequences (Appendix 3) of the different specimens of this species.

3.3.3 Phylogenetic analysis

In figure 1 the result of the Bayesian Inference (BI) analysis is presented as cladogram with posterior probabilities (PP) indicated as percent values above branches and bootstrap (BS) values below branches. The Maximum Parsimony (MP) analysis with the parsimony ratchet revealed the same topology in the strict consensus tree as the BI analysis with an exception discussed below.
Hypnum cupressiforme and *Neckera crispa* were used as outgroup taxa. A clade consisting of *Leucodon sciuroides* (Leucodontaceae) and the specimens of *Acrocladium* (Plagiotheciaceae) and *Leucodon* (Leucodontaceae) is sister to a clade consisting of the remaining 27 taxa. In this study the genus *Acrocladium* (*A. auriculatum, A. chlamydophyllum*) was identified as the closest relative to the Lepyrodontaceae (represented by *Lepyrodon pseudolagurus* and *L. tomentosus*) based on a posterior probability of 93 % (BS 65 %).

The monophyly of the clade with the remaining 27 taxa (clades labeled ‘Hypopterygiaceae’, ‘Hookeriales’, and ‘P’) had a posterior probability of 100 % (87 % BS). The basal clade within this group consists of the members of the Hypopterygiaceae. The monophyly of the remaining two clades, the Hookeriales and ‘P’ clade is supported with 98 % PP (72 % BS). The monophyly of the four species representing the Hookeriales has a PP of 96 % (58 % BS). The terminal taxa is the monophyletic ‘P’ clade with a posterior probability of 100 % (100 % BS).
The ‘P’ clade consists of *Dichelodontium nitidum* and the species representing the Ptychomniaceae and Garovagliaceae. The most basal taxon within this clade is the genus *Hampeella*. The genus is represented by two specimens of *H. alarix* and one specimen of *H. pallens*. The next most basal clade within the ‘P’group consists of two specimens of *Ptychomnion ptychocarpon* basal to the remaining taxa. The probability of this clade including *Ptychomnion ptychocarpon* is 100 % (94 % BS).

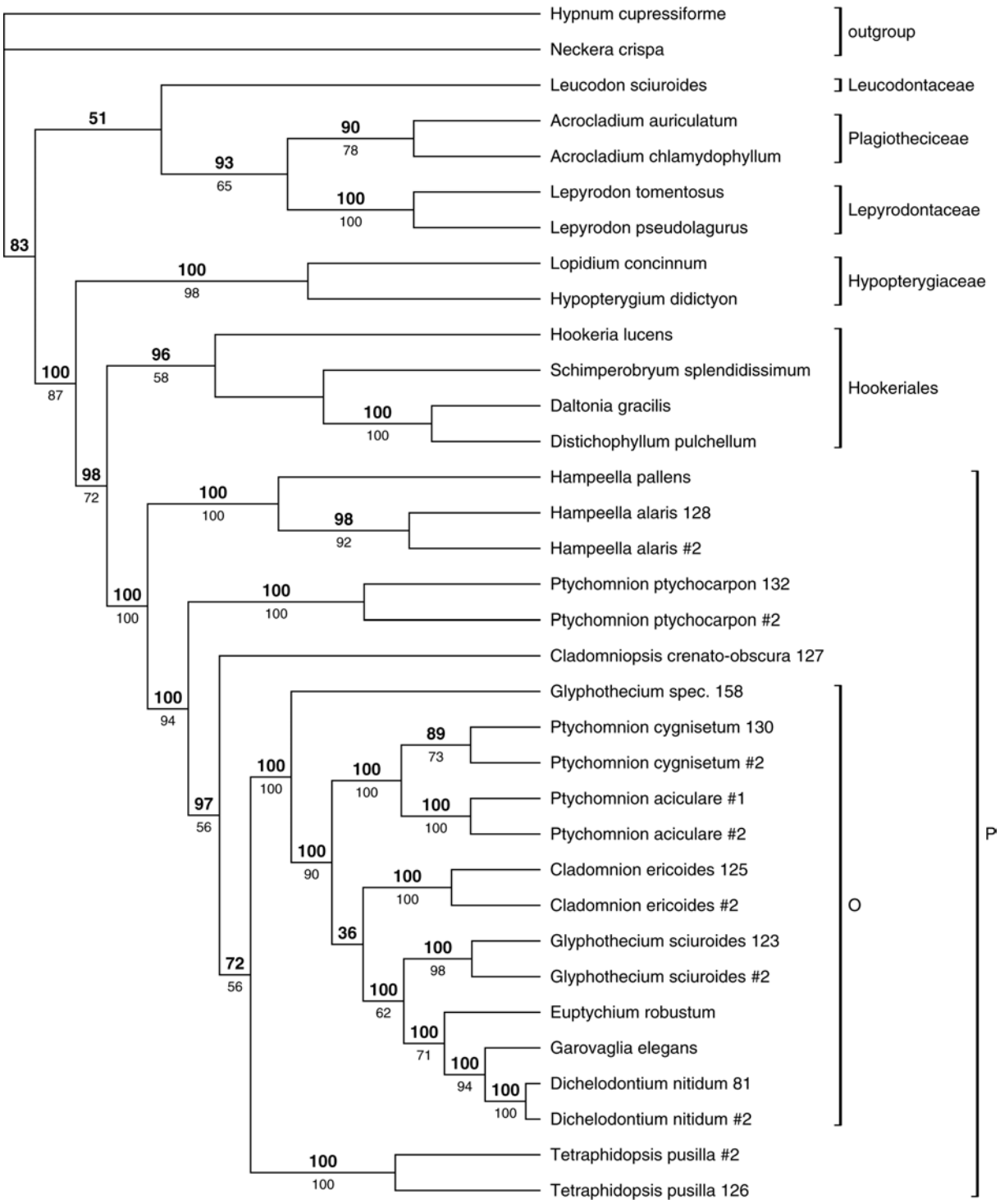

Figure 1: Cladogram resulting from a Bayesian Inference analysis of the complete data set (*rps*4 and *trn*L sequence data). Numbers above branches indicate the posterior probabilities as a percentage value.
A strict consensus cladogram of six trees found during the parsimony ratchet of the same data set revealed the same topology (Length= 554; CI: 0.671, RI: 0.829; RC: 0.557) and is not shown separately (see discussion in the text). Bootstrap values below branches are the result of a Maximum Parsimony analysis. For explanation of the clades referred to as 'outgroup', O, and P see text.

The following clade with the remaining fourteen specimens is supported by a 97 % PP (56 % BS). The next taxon branching off is *Cladomniopsis crenato-obscura*. The basal position of *Tetraphidopsis pusilla* to clade 'O' has a posterior probability of 72 % (BS 72 %).

Clade 'O' consists of thirteen taxa (100 % PP, BS 100 %). The basal position in this clade is occupied by a specimen of *Glyphothecium* (specimen 158). The remaining group consists of three clades. The first clade is that of *Ptychomnion* (100 % PP, 100 % BS) with the species *Ptychomnion aciculare* and *P. cygnisetum*, each represented by two specimens. The relationship of *Ptychomnion* to the second clade *Cladomnion* and third clade (consisting of six taxa) is ambiguous as the PP is only 36 % (BS < 50 %). The monophyly of the third clade within 'O' is well supported (99 % PP, 62 % BS). *Glyphothecium sciuroides* retains a basal position in this clade, followed by the two representatives of the Garovagliaceae, *Euptychium robustum* (100 % PP, 71 % BS) and *Garovaglia elegans*, which is sister to *Dichelodontium nitidum* (100 % PP, 94 % BS).

The phylogram of the Maximum Likelihood (ML) analysis (fig. 2) supports the main clades of the topology as detected with the Bayesian Inference (BI) and Maximum Parsimony (MP) analysis.

Based on the phylogenetic analyses conducted in this study the following relationships remain ambiguous. Due to the low BS values neither the relationship of *Leucodon* to the Plagiotheciaceae and Lepyrodontaceae nor the relationship between *Leucodon* and clade 'P' were resolved.

Also the relationship between the clade compromising *Ptychomnion aciculare/P. cygnisetum* to *Cladomnion*, as well as *Glyphothecium, Dichelodontium* and the species representing the Garovagliaceae remains unresolved. This same result was obtained in a strict consensus of six trees found in a MP analysis. Due to these identical results this latter analysis is not presented in this study.

Furthermore, the analyses of this study show that the monotypic genus *Dichelodontium* does not belong to the Lepyrodontaceae; the closest relative of *Lepyrodon* is *Acrocladium* (Plagiotheciaceae) whereas *Dichelodontium* appears within a clade of the representatives of the Ptychomniaceae and Garovagliaceae.

All analyses in this study indicate that the family Ptychomniaceae is polyphyletic as the genus *Glyphothecium* is sister to a clade at the terminal end of phylogram, which

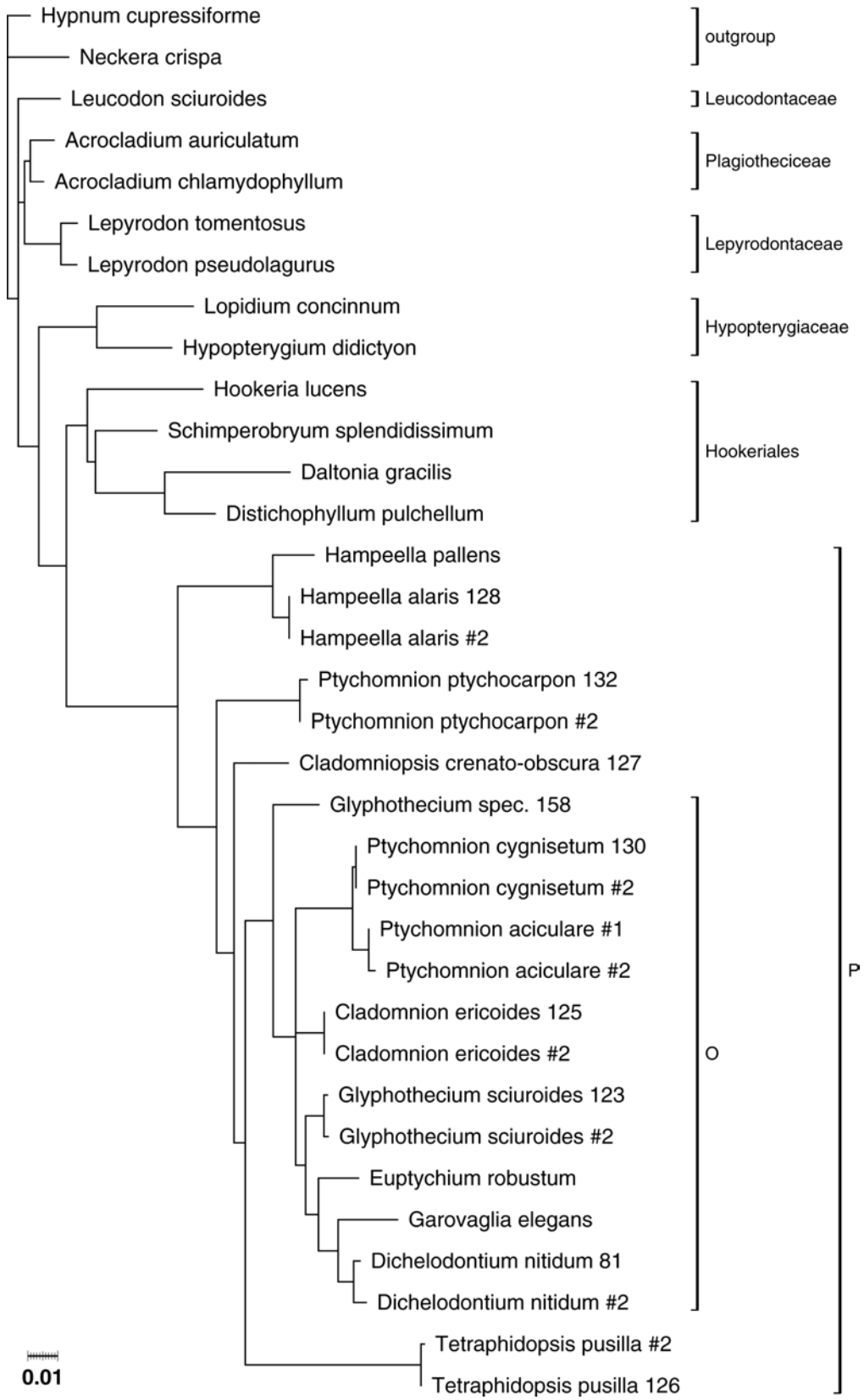

Figure 2: Maximum Likelihood (ML) phylogram of the combined data set of *rps*4 and *trn*L sequence data (L score = - 4596.3706). Branch lengths are proportional to genetic distance between taxa. Scale bar equals 1% distance under the assumed substitution model (GTR+G). For explanation of the clades referred to as 'outgroup', H, and A see text.

consists of representatives of the Garovagliaceae and *Dichelodontium* recently transferred in the Lepyrodontaceae.

Ptychomnion ptychocarpon is more closely related to *Hampeella* and *Cladomniopsis* than to the representative species of the genus *Ptychomnion* (*P. aciculare, P. cygnisetum*).

In all analyses *Hampeella* was identified as the most basal taxon within Ptychomniaceae s.l.

Furthermore, the genus *Glyphothecium* is polyphyletic, with respect to the position of the unspecified specimen 158.

3.4 Discussion

The results of this study are based on a data set comprising 34 taxa, which represent species of the orders Hypnales, Leucodontales and Hookeriales. The conducted analyses with these 34 taxa revealed a higher variability in the *rps*4 (31.8 %) than in the *trn*L intron (21.9 %). This stands in contrast to the pattern obtained by Buck et al. (2000b) for a data set of 90 taxa, comprising representatives of Bryales, Hookeriales, Leucodontales and Hypnales. Buck et al. (2000b) found a 51.86 % variability in the *trn*L intron, 7.3 % variability in the *trn*L-*trn*F spacer, and 46.5 % in the *rps4* gene. The higher variabilities found by Buck et al. (2000b) can be explained by the higher number of species involved as well as the broader taxonomic representation of the taxa, i.e. that also representatives of the Bryales are included.

On the other hand this study here is the first to use species that represent all genera of the Ptychomniaceae as well as *Dichelodontium nitidum* (Lepyrodontaceae) in an analysis that addresses the systematic position and intrafamilial relationship of the Ptychomniaceae as circumscribed by Hattaway (1984).

Since a common pattern reported for phylogenetic analyses is that the posterior probability values resulting from Bayesian Inference analyses (Huelsenbeck & Ronquist, 2001) are generally higher than values obtained from non-parametric bootstrapping (e.g. Erixon et al., 2003; Felsenstein, 1985; Suzuki et al., 2002), we chose to apply both methods. The lower bootstrap values compared with the higher posterior probability support do not question the main results of this study.

The monophyly of the Hypopterygiaceae, Hookeriales and the Ptychomniaceae s.l. ('P' clade) in this study comprising species representative of Ptychomniaceae and Garovagliaceae, is highly supported by the results of both methods. The monophyletic group of the Hookeriales has a posterior probability of 96 %, with only 58 % bootstrap support.

The sister relationship of the Hookeriales and the Ptychomniaceae s.l. is supported by different values from the Bayesin Inference analysis (98 % PP) and non-parametric bootstrapping (72 % BS). Based on characters derived from the sporophyte already Robinson (1975) regarded the Ptychomniaceae as closely related to the hookeroide taxa (Robinson, 1971). The sister relationship between species representing the Ptychomniaceae and the Hookeriales is also obtained by Kruijer & Blöcher (2004) who use a combined data set of cpDNA, nrDNA and morphological data, by Buck et al. (2000b) using cpDNA, and by Buck et al. (in press; cit in Shaw & Renzaglia, 2004) based on nrDNA, cpDNA and mtDNA sequence data. In contrast, Kruijer (2002) suggests a closer relationship between Hypopterygiaceae and Ptychomniaceae. However, the results of the study at hand resolve the clade of Ptychomniaceae and Garovagliaceae as a monophyletic group with high support values. This result confirms the placement of both families in a separate suborder Ptychomniieae (order Hookeriales) as described by Buck & Goffinet (2000) which they based on earlier molecular phylogenetic studies (Buck et al., 2000a; Buck et al., 2000b).

Both specimens of each species investigated in this study were always resolved as a monophyletic group in the phylogenetic analysis. The intraspecific variability was quite low (0.0 to 0.3 %) and there were only slight differences (+/- 0.1 %) between the *trn*L and *rps*4 region. This low variability within one species was expected as the specimens of the same species used for the analyses were derived from similar geographical regions and reported intraspecific sequence variation of the regions used is commonly very low (Quandt & Stech, 2004). Only the two specimens of the New Zealand endemic *Dichelodontium nitidum* had a high intraspecific variation for the *rps*4 gene (1.6 %). There was no intraspecific differentiation between the two specimens based on the *trn*L intron (0.0 %). A common pattern observed is, that substitution rates between regions are quite variable (e.g. Clegg et al., 1994; Palmer, 1990; Quandt, 2002), although it is rather unusual that coding regions have a higher variation than a group I intron as observed here.

However, the differences should be in proportion and if the *rps*4 shows a high variability within one species compared to others one would expect that also the *trnL* intron would show some genetic difference between the *Dichelodontium nitidum* specimens. Therefore the large genetic distance between the *rps*4 gene of the two *Dichelodontium nitidum* specimens might well by an artefact and needs justification by additional sequences.

Earlier research on the Garovagliaceae (e.g. Buck & Vitt, 1986; During, 1977) and Ptychomniaceae (e.g. Hattaway, 1984) does not mention any relationship between the two taxa. The Garovagliaceae was considered to be closely related to the Pterobryaceae (Buck & Vitt, 1986) or was placed within the Pterobryaceae (e.g. During, 1977). More recent studies (e.g. Buck et al., 2000b), however, indicate a closer relationship between the Garovagliaceae and the Ptychomniaceae. Results of the study at hand confirm an affinity between the two groups. Due to the position of *Dichelodontium* as sister to the representatives of the Garovagliaceae (*Garovaglia elegans* and *Euptychium robustum*) in this analysis the family Ptychomniaceae is paraphyletic.

Since based on the results of this study taxa of the Garovagliaceae appear within the Ptychomniaceae and the genus *Ptychomnion*, subfamily Ptychomnioideae, shows a closer relationship to taxa belonging to the subfamily Cladomnioideae (e.g. *Cladomnion* and *Garovaglia*) than to its formerly allied *Cladomniopsis* (Ptychomnioideae) (Hattaway, 1984), the subfamilial concept needs revision.

The most basal lineage of the Ptychomniaceae s.l. in this study is the genus *Hampeella*. This is in contrast to Hattaway (1984) who suggests that the genus *Ptychomnion*, based on morphological data and the wide geographical distribution of *P. aciculare*, might be the most basal genus within the Ptychomniaceae. Furthermore, the study at hand has indicated a close relationship between the genus *Hampeella* and the species *P. ptychocarpon*. As *Hampeella*, identified as the most basal taxon of the Ptychomniaceae s.l., is restricted to the Australasian region and *P. ptychocarpon* is endemic to southern South America this may indicate an origin of the Ptychomniaceae on the Gondwana continent as also suggested by e.g. Kruijer (2002) for the Hypopterygiaceae. In addition, the epiphytic life form found in *Hampeella* as well as in *P. ptychocarpon* (Hattaway, 1984) could be regarded as an basal characteristic of the family.

The position of *Ptychomnion aciculare* and its sister species *P. cygnisetum* as closest relatives to *Cladomnion ericoides* and to a clade consisting among others of *Glyphothecium sciuroides, Garovaglia elegans* and *Dichelodontium nitidum* is strongly supported by this analysis. In contrast, the southern South American endemic *P. ptychocarpon* is one of the basal taxa of the Ptychomniaceae s.l. clade, and thus separated from the other species of the same genus. The morphological differences between *P. ptychocarpon* and the remaining species of *Ptychomnion* have already been recognized by different authors (Brotherus, 1909b; Hattaway, 1984; Kühnemann, 1975). However, these morphological characters were not regarded as valid in order to justify a generic separation of *P. ptychocarpon*.

As the type specimen of the genus *Ptychomnion, P*. *aciculare* (Brid.) Mitten (as *Hypnum aciculare* S.E. Bridel), is sister to *Ptychomnion cygnisetum*, and P. *ptychocarpon* does not indicate any affinity to another taxon included in this analysis, it is suggested from the result of this study that *P. ptychocarpon* should be transferred to a separate genus.

Based on this study *Dichelodontium nitidum* should be removed from the Lepyrodontaceae, where it is placed by Allen (1999). According to our results this species retains a terminal position within the Ptychomniaceae s.l. clade. The observed strongly supported position of *Dichelodontium* as sister to *Garovaglia elegans* should be examined with more taxa from the Garovagliaceae. The distinction between the representatives of the Lepyrodonataceae and their sister taxon *Acrocladium* is obvious from the phylogenetic results as well as the genetic distances, separating *Dichelodontium* from the Lepyrodontaceae. The result of this study stands in contrast to the view that *Dichelodontium* should be placed within the former ‘Leucodontales’ as stated by Hattaway (1984) and the view on the relationships within the Lepyrodontaceae as described by Allen (1999). Instead, our results are in good concordance with Magill (1987) and the original description of the Lepyrodontaceae by Fleischer (1908).

The results of this study put a question mark by the monophyletic status of the genus *Glyphothecium*. Specimen 158 ‘*Glyphothecium* spec.’ could not be identified further (as either *G. gracile* (Hampe) Broth. or *G. sciuroides*) as the voucher lacked sporophytes (Hattaway, 1981; 1984). But to whatever species of *Glyphothecium* the specimen no. 158 might belong, the genus *Glyphothecium* is polyphyletic according to our study, as *G. sciuroides* is sister to a clade comprising the Garovagliaceae and

Dichelodontium with high branch support, whereas *Glyphothecium* specimen 158 is at a basal position to a highly supported monophyletic group consisting of e.g. *Ptychomnium aciculare*, *Cladomnion ericoides*, and the formerly mentioned *Glyphothecium-Garovagliaceae-Dichelodontium* clade. Moreover, the genetic distances support the outstanding position of *Glyphothecium* sp. 158. So far, no one has questioned a close relationship between species within the genus *Glyphothecium* since Hattaway revised it (Hattaway, 1981; 1984). We recommend further studies including specimens of *G. gracile*, and *G. pendulum* Zant. to shet light on the taxonomic status of *Glyphothecium*. Further molecular studies are also needed to avoid the influence of e.g. lineage sorting and hybridization on the phylogenetic results (e.g. Doyle, 1992; Hidalgo et al., 2004; Vanderpoorten et al., 2004).

4 The systematic affinities of selected Gondwanan bryophyte taxa based on molecular sequence data

4.1 Introduction

For studies on the Lepyrodontaceae as well as on the genera *Catagonium* and *Acrocladium* it is indispensable to know the closest relatives of each of these taxa in order to reduce the possibility of homoplasy in the phylogenetic constructions within the family or genus.

A monophyletic group formed by *Lepyrodon* and representatives of the Stereophyllaceae (Buck et al., 2000a; Buck et al., 2000b) as well as recent phylogenetic studies indicating a closer relationship of the genus *Lepyrodon* with the genus *Acrocladium* (Quandt et al., 2004b) suggest an ambiguous position of the Lepyrodontaceae within the superorder Hypnanae.

Combined analyses of morphological and genetic sequence data show a basal position of *Acrocladium* within the Plagiotheciaceae (Pedersen & Hedenäs, 2002).

The dataset used in this study consisted of species of the genera *Lepyrodon, Acrocladium*, and *Catagonium*. Furthermore, the data comprised representative species of the Plagiotheciaceae as described in Pedersen & Hedenäs (2002). *Rhizofabronia* and *Myurella* were excluded, but *Isopterygiopsis* and *Platydictya* which are considered their closest relatives, respectively, were included. Thus, the species of the Plagiotheciaceae used in the analyses were: *Platydictya jungermannioides, Orthothecium chryseum, O. intricatum, Struckia zerovii, Plagiothecium denticulatum, P. undulatum, Isopterygiopsis pulchella, I. muelleriana, Herzogia seligeri, and Pseudotaxiphyllum elegans*. One representative of the Pterobryaceae (*Pterobryon densum*) was chosen as well as three species of the Stereophyllaceae (*Entodontopsis leucostega, Pilosium chlorophyllum*, and *Stereophyllum radiculosum*). Additional ingroup taxa covered a selection of the main clades within the Hypnanae (e.g. Amblystegiaceae, Brachytheciaceae, Neckeraceae, Leucodontaceae, Hypnaceae, Meteoriaceae, Lembophyllaceae).

In addition to a broader taxon sampling with respect to possible relatives of *Lepyrodon*, *Catagonium* and *Acrocladium* than in previous analyses (Buck et al., 2000a; Pedersen & Hedenäs, 2002; Quandt et al., 2004b), in this study I also used a wider range of molecular markers. A combined sequences of cpDNA (*trn*L-F, *rps*4, *psb*T-H) and nrDNA (ITS region) was used.

A brief historical outline of the systematic placement of the taxa used in our analyses

Lepyrodontaceae. The genus *Lepyrodon* was established by Hampe in 1865 (cit in Allen, 1999) and comprises diplolepideous moss species with a single peristome of endostomial origin and a cucullate calyptra. He placed the genus within the Leskeaceae. In 1908 Brotherus introduced the monotypic family Lepyrodontaceae and placed it near the Neckeraceae. Since then the genus *Lepyrodon* is considered to belong to either to the Ptychomniaceae (e.g. Brotherus, 1925b; Buck & Vitt, 1986; Fleischer, 1915-1922) or the Pterobryaceae (Buck, 1998; Crum, 1994). Allen (1999) revised the family Lepyrodontaceae and transferred the monotypic genus *Dichelodontium* into the Lepyrodontaceae which since then consists of the genera *Lepyrodon* and the monotypic genus *Dichelodontium*, with the New Zealand endemic *D. nitidum*. Allen (1999) considered the Lepyrodontaceae as possibly related to the Pterobryaceae based on morphological characters e.g. the reduced diplolepideous peristom and the absence of an exostome. In his monographic study of *Lepyrodon*, Allen (1999) considered seven species within this genus. These species are mostly distributed in the southern hemisphere. The exception is *L. tomentosus* which occurs from Mexico via Central America, to northern and even southern South America. *L. hexastichus, L. parvulus*, *L*. *lagurus* and *L. patagonicus* occur only in southern South America (Argentina, Chile, and the Juan Fernandez Islands) whereas the species *L. pseudolagurus* and *L. australis* are restricted to Australia, Tasmania, New Zealand and Campbell Island.

Catagoniaceae. As *Catagonium nitens* ssp. *nitens* is one of the prominent species of the Chilean temperate rainforest, in this study a special interest was taken in the evolution of this species and the relationship to its sister taxa.

Earlier the genus *Catagonium* was placed either in or near the Plagiotheciaceae (Brotherus, 1925c; Lin, 1984) or Phyllogoniaceae (Fleischer, 1915-1922; Vitt, 1984). Then Buck (1985) revised the Plagiotheciaceae and transferred the genus *Catagonium* in the monotypic family Catagoniaceae. Recently, based on cpDNA sequences and morphological data, Pedersen & Hedenäs (2002) transferred the genus back to the Plagiotheciaceae.

The genus *Catagonium*, comprising only four species, presents a distribution pattern which implies a very old Gondwanan origin of the genus. The subspecies *Catagonium nitens* ssp. *maritimum* occurs in South Africa, *Catagonium nitens* ssp. *nitens* in eastern Africa, New Zealand/Australia, and southern South America as well as on a few subantarctic islands. *Catagonium nitidum* is reported from southern South America, the Falkland Islands and Tristan Da Cunha Island. *Catagonium brevicaudatum* is known from Brazil, Bolivia, Columbia, Costa Rica, Ecuador, Guatemala, Jamaica, Mexico, Peru and Venezuela, and *Catagonium emarginatum* from Brazil and Bolivia (Lin, 1984).

Acrocladium. Brotherus (1925c) described two species in the genus *Acrocladium*: *A. auriculatum* (Mont.) Mitt. from southern South America and *A. chlamydophyllum* (Hook.f. & Wils.) Broth. from New Zealand, eastern Australia, Tasmania and some adjacent islands. Since that time there has been disagreement among bryologists whether the genus includes one or two species, and wether the populations in Chile and Argentina are identical with those in New Zealand, Australia, and Tasmania. Accordingly, collections of *Acrocladium* from Chile were either named *A. auriculatum* (e.g. Brotherus, 1925c; Deguchi, 1991; Mitten, 1869) or *A. chlamydophyllum* (Cardot, 1908). Brotherus (1925c) distinguished two species, and Andrews (1949), Karczmarz (1966) and Fife (1995) supported the view that the two taxa are different species. In contrast, Dixon (1928), Sainsbury (1955) and He (1998) considered both taxa as variations of the same species, using the name '*A. auriculatum*' as the older epitheton.

In fact, the variability of the *Acrocladium* specimens from southern South America and Australia/New Zealand is quite high. Brotherus (1925c) differentiated both species by leaf auricles and characters of the leaf costa. Karczmarz (1966) omitted the character of the costa and distinguished both species by leaf shape and by presence versus absence of auricles.

Despite the early recognition of the genus *Acrocladium* (Mitten, 1869), its familial position has been very much a subject of discussion. The genus was shifted from the Lembophyllaceae (Brotherus, 1925a; Fleischer, 1915-1922) to the Amblystegiaceae (Ochyra & Matteri, 2001; Vitt, 1984) and most recently to the Plagiotheciaceae (Pedersen & Hedenäs, 2002).

Due to the problematic distinction of the two species based on anatomical and morphological characters described above, an attempt has been made to evaluate the differences based on molecular data.

Stereophyllaceae. The family Stereophyllaceae was formerly regarded as a subfamily of the Plagiotheciaceae (Stereophylloidae, Fleischer, 1915-1922) with the Plagiotheciaceae serving as 'catch-all' family for pleurocarpous mosses with complanate habit and a hypnoid peristome (Buck & Ireland, 1985).

Buck & Ireland (1985) raised the Stereophyllaceae to family status. Therefore three representatives of the family (*Stereophyllum radiculosum*, *Entodontopsis leucostega* and *Pilosium chlorophyllum*) were included in this study. Furthermore, the data set used included taxa of the genus *Catagonium* that was also considered closely related to *Plagiothecium* (Fleischer, 1915-1922) before Buck & Ireland (1985) gave it the rank of a family.

4.2 Material and Methods

***Plant material*.** Plant material was either collected by the author during a field trip of the BryoAustral project to Chile in 2001, or originatesfrom herbarium specimens. Specimens of *Acrocladium chlamydophyllum*, *Lepyrodon pseudolagurus*, and *Catagonium nitens* were collected during the BryoAustral project expedition to New Zealand in 1998. Duplicates are preserved in the herbaria in Christchurch (CHR), Bonn (BONN) and Berlin (B). We also used sequences available in GenBank. All specimens used in our analyses are listed in (Appendix 4) including further voucher information.

DNA isolation, PCR and sequencing. Prior to DNA extraction the plant material was thoroughly cleaned with distilled water and additionally treated by ultrasonic waves for 2-4 minutes. Success of cleaning was checked by examining the plants under a binocular microscope. Remaining contaminations e.g. with algae and fungi were removed mechanically. Isolation of DNA was carried out following the CTAB technique described in Doyle & Doyle (1990).

PCR amplifications (Biometra TriBlock thermocycler, PTC-100 MJ Research) were performed in 50 µl–reactions containing 1.5 U *Taq* DNA polymerase (PeqLab), 1 mM dNTPs-Mix, nucleotide concentration 0.25 mM each (PeqLab), 1x buffer (PeqLab), 1.5 mM $MgCl_2$ (PeqLab) and 12.5 pmol of each amplification primer. PCR products were purified using the QIAquick purification kit (Qiagen). Cycle sequencing reactions (half reactions) were performed using a PTC-100 Thermocycler (MJ Research) in combination with the ABI Prism™ Big Dye Terminator Cycle Sequencing Ready Reaction Kit with Amplitaq-DNA polymerase FS (Perkin Elmer), applying a standard protocol for all reactions. Extension products were precipitated with 40 µl 75 % (v/v) isopropanol for 15 min at room temperature, centrifuged with 15,000 rpm at 25°C, and washed with 250 µl of 75 % (v/v) isopropanol. These purified products were loaded on an ABI 310 automated sequencer (Perkin Elmer) and electrophoresed. For cycle sequencing 10 µl–reactions were used containing 3 µl of Big Dye Terminator Cycle Sequencing premix. Sequencing reactions were performed on two independent PCR products generated from each sample in order to verify the results. All PCR products were sequenced using two primers. For amplifying and sequencing the non-coding regions of the chloroplast DNA (table 10) a modification of primer C (Quandt et al., 2000) as well as primer F, originally designed by Taberlet et al. (1991) were employed. Primers used to amplify the *rps*4 gene (table 11) were those described in Nadot et al. (1994), ‘trnS’ and ‘rps5’.

Table 10: Primer sequences used for amplification and sequencing of the *trn*L region and *rps*4 gene. Underlined nucleotides represent changes Quandt et al. 2000 with respect to the original primers of Taberlet et al 1999.

Primer	Sequence	Data source
*trn*S	TAC CGA GGG TTC GAA TC	Nadot et al. 1994
*rps*5	ATG TCC CGT TAT CGA GGA CCT	Nadot et al. 1994
*trn*L-C_mosses	CGR AAT TGG TAG ACG CTA CG	Quandt et al. 2000
*trn*L-F	ATT TGA ACT GGT GAC ACG AG	Taberlet et al. 1991

Table 11: Primer sequences used for amplification and sequencing of the ITS region. Underlined nucleotides represent changes with respect to the original primers of Blattner 1999.

Primer	Sequence	Data source
ITS-C bryo	GCA ATT CAC ACT ACG TAT CGC	Blattner 1999
ITS-D bryo	CTC TCA GCA ACG GAT ATC TTG	Blattner 1999
ITS4-bryo	TCC TCC GCT TAG TGA TAT GC	Stech 1999
ITS5-bryo	GGA AGG AGA AGT CGT AAC AAG G	Stech 1999

Table 12: Primer sequences used for amplification and sequencing of the *adk* gene.

Primer	Sequence	Data source
F	GAA GAA GCC AGA AAA CTG GGC	Vanderpoorten et al. 2004
R	GTC ACC CCA TCT TCA GCA AC	Vanderpoorten et al. 2004
1F	AAG CTT TTC CCG TAA GT	Vanderpoorten et al. 2004
2R	ACT TAC GGG AAA AGC TT	Vanderpoorten et al. 2004
3R	GGT CCC CTG GGT AAT AAC	Vanderpoorten et al. 2004
4F	TTT CAT CCC ATC GGT GG	Vanderpoorten et al. 2004

Primers for amplifying and sequencing the ITS region (ITS4-bryo and ITS5-bryo, table 12) based upon the primers “ITS4” and “ITS5” respectively, designed and named by White et al.(1990), were slightly modified with respect to bryophytes (Stech, 1999).The primers ITS-C and ITS-D (Blattner, 1999) were modified for our study (ITS-D_bryo and ITS-C_bryo) and additionally used for sequencing reactions.

For amplifying and sequencing the chloroplast and nuclear region different protocols have been applied. For the *trn*L-F region and the *rps*4 gene the PCR program was performed with the following settings: 2 min. 94ºC, 35 cycles (1 min. 94ºC, 1 min. 55ºC, 1 min. 72ºC) and a 5 min. 72ºC extension time, cycle sequencing settings: 29 cycles (5 sec. 96ºC, 4 min. 50ºC).

The ITS region was amplified using a protocol consisting of: 5 min. 94ºC, 35 cycles (1 min. 94ºC, 1 min. 48ºC, 1 min. 72ºC) and a 5 min. 72ºC extension time, cycle sequencing settings: 25 cycles (30 sec. 96ºC, 15 sec. 50ºC, 4 min. 60ºC).

All sequences are deposited in EMBL, accession numbers are listed in Appendix 3, the alignments are available on request from the author.

***Phylogenetic analyses*.** Heuristic searches under the parsimony criterion were carried out under the following options: all characters unweighted and unordered, multistate characters interpreted as uncertainties, gaps coded as missing data, performing a tree bisection reconnection (TBR) branch swapping, collapse zero

branch length branches, MulTrees option in effect, random addition sequence with 1000 replicates.

Furthermore, the data sets were analysed using *win*PAUP 4.0b10 (Swofford, 2002) executing the command files generated by 'PRAP' (Parsimony Ratchet Analyses using PAUP Müller, 2004), employing the implemented parsimony ratchet algorithm (Nixon, 1999). For the parsimony ratchet the following settings were employed: 10 random addition cycles of 200 iterations each with a 40 % upweighting of the characters in the PRAP iterations. Heuristic bootstrap (BS Felsenstein, 1985) searches under parsimony criterion were performed with 1000 replicates, 10 random addition cycles per bootstrap replicate and the same options in effect as the heuristic search for the most parsimonious tree (MPT). A further measurement of support, the Bremer support (DC, Bremer, 1994), for the individual clades was obtained using PRAP (Müller, 2004), employing the implemented parsimony ratchet algorithm (Nixon, 1999).

The consistency index (CI, Kluge & Farris, 1969), retention index (RI), and rescaled consistency index (RC, Farris, 1989) were calculated to assess homoplasy.

In addition to our MP analyses we performed Bayesian Inferences with MrBayes3.0 (Huelsenbeck & Ronquist, 2001). Modeltest 3.5 (Posada, 2004) was used to select a DNA substitution model for our data set (gamma shape distribution, six substitution types). The Markov Chain Monte Carlo (MCMC) analyses were run for 1,000,000 generations with four simultaneous MCMC and one tree per 100 generations was saved. The 'burn-in' values were determined empirically from the likelihood values. The analyses were repeated four times to assure sufficient mixing by confirming that the program converged to the same posterior probability (PP). The program Treegraph (Müller & Müller, 2004) was used to edit trees directly from PAUP-treefiles. MEGA2.1 (Kumar et al., 2001) was used to calculate GC-content, sequence length and distance measure ('p-distance'). In the following the term 'genetic distance' is used instead of 'p-distance'.

4.3 Results

4.3.1 Sequence variation

Results of the data set analysis are shown in Appendix 5. The alignment of a combined data set of *trn*L, *psb*T-H, ITS and *rps*4 sequence data comprises 3,221 characters.

The GC-content is higher in the nrDNA regions than in the cpDNA regions. The coding region in the nrDNA, the 5.8S, has a lower GC-content compared to the spacers, ITS1 and ITS2. The average GC-content in the 32 sequences of the *psb*T-H region is 28.2 %; that of the *trn*L-F region (N=53 species) is 29.0 % in average (Appendix 5).

The average GC-content in the *rps*4-sequences (N=38 species) is 27.5 %. There are striking differences in the GC-content when looking at the codon positions. For the first codon position a GC-content of 8.5 % was calculated, for the second codon position the GC-content is 41 % in average and for the third codon position 33 % in average. The average GC-content in the intergenic spacer (IGS) of the *trn*S-*rps*4 is 20 % (N=27 taxa). In the nrDNA of the ITS1 region (N=17 species) the average GC-content is 63 %. The 5.8S nrDNA (N=37 taxa) contains an average of 54 % of GC nucleotides. In the nrDNA the ITS2 region (N=39 species) the average GC-content is 65 %.

4.3.2 Phylogenetic analysis.

Results of the parsimony analysis.

In the Maximum Parsimony (MP) analysis 1,223 most parsimonious trees (MPT) were found. Each tree with a length of 1,686 steps (CI 0.643, RI 0.613, RC 0.394). Figure 3 presents the strict consensus of all trees, with bootstrap support value (BS) and Bremer support value (BO).

Using *Pyrrhobryum latifolium*, *Orthotrichum anomalum* and *Orthotrichum stramineum* as outgroup taxa, the ingroup taxa form a well supported clade as shown in figure 3 (100 % BS, DC value 19). *Trachyloma planifolium* is the most basal taxon of the ingroup, followed by a clade comprising *Ctenidium molluscum* and *Myurium*

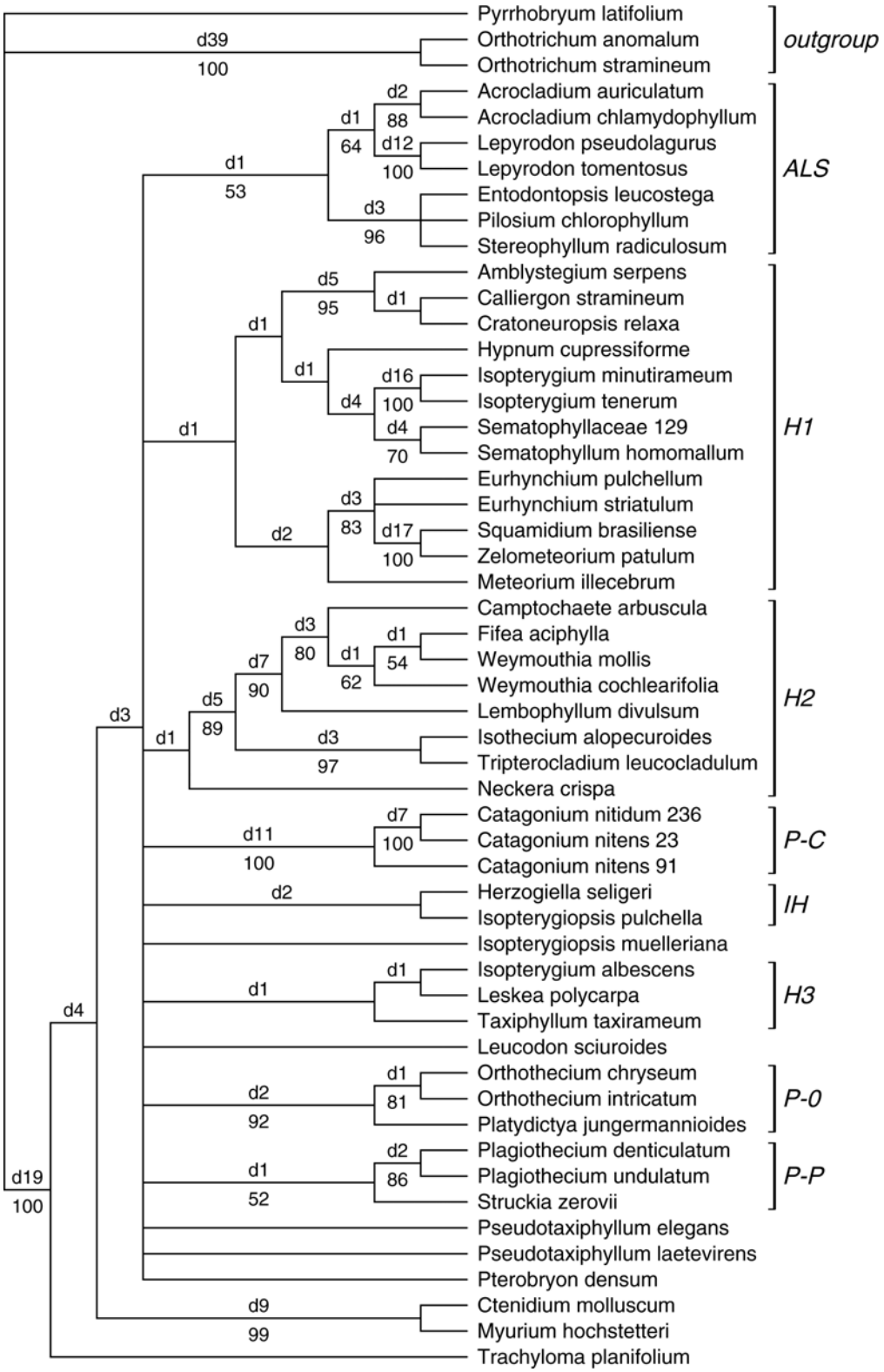

Figure 3: Strict consensus of 1223 most parsimonious trees (Length: 1,686, CI: 0.643, RI: 0.613, RC: 0.394) found during the parsimony ratchet of the combined data set. Values above branches ('d-value') are Bremer support values (DC). Values below branches are bootstrap (BS) support values (1000 repeats). For explanation of the clades referred to as 'outgroup', ALS, H1, H2, P-C, IH, P-O, and P-P see text.

hochstetteri (fig. 3: BS: 99 %, DC: 9), then by *Pseudotaxiphyllum laetevirens*.

The next clade branching off consists of *Acrocladium* (two species), *Lepyrodon* (two species) and three representative species of the Stereophyllaceae. This clade will be referred to as 'ALS' clade in the following. The clade is weakly supported by a BS value of 56 % and a DC value of one (fig. 3). This clade is sister to polytomous group which consists of four clades and *Pseudotaxiphyllum elegans*. One clade comprises nine representative species of the Plagiotheciaceae, here named clade P, a second clade (P-O) is formed by two species of *Orthothecium* and *Platydiction jungermannioides*. A third clade comprises *Leucodon sciuroides* and *Pterobryum densum* and finally a clade here named H, which consists of further representatives of the Hypnanae.

In clade P, the genus *Catagonium* retains a basal position among the core representatives of the Plagiotheciceae. The *Catagonium* clade is followed by a clade formed by *Herzogiella seligeri* and *Isopterygiopsis pulchella* (DC 2) and is sister to a clade which consists of *Isopterygiopsis muelleriana*, *Struckia zerovii* and the two included *Plagiothecium* species.

Orthothecium chryseum, *O. intricatum* and *Platydictya jungermannioides* form a well-supported monophyletic group (BS: 92 %, DC: 2).

Lepyrodon sciuroides and *Pterobryon densum* form a clade which lacks branch support (DC 1, BS 0 %) or bootstrap support. Clade H consists of two sister clades, one with the representatives of the Amblystegiaceae, Hypnaceae, Sematophyllaceae, Meteoriaceae and Brachytheciaceae, and a second comprising, among others, representatives of the Lembophyllaceae, Neckeraceae, Leskeaceae and Hypnaceae.

Results of the Bayesian approach.

A 50 % majority rule consensus tree of 9,501 trees generated with the settings as described above resulted in the cladogram depicted in figure 4. Using *Pyrrhobryum latifolium*, *Orthotrichum anomalum* and *Orthotrichum stramineum* as outgroup taxa, the ingroup taxa form a well supported clade (100 %). *Trachyloma planifolium* is the most basal taxon, next to it is the *Ctenidium molluscum* - *Myurium hochstetteri* clade (100 %).

As with the Maximum Parsimony analysis also in the Bayesian analysis clades H and ALS were obtained. The monophyly of clade P was not detected in the Bayesian

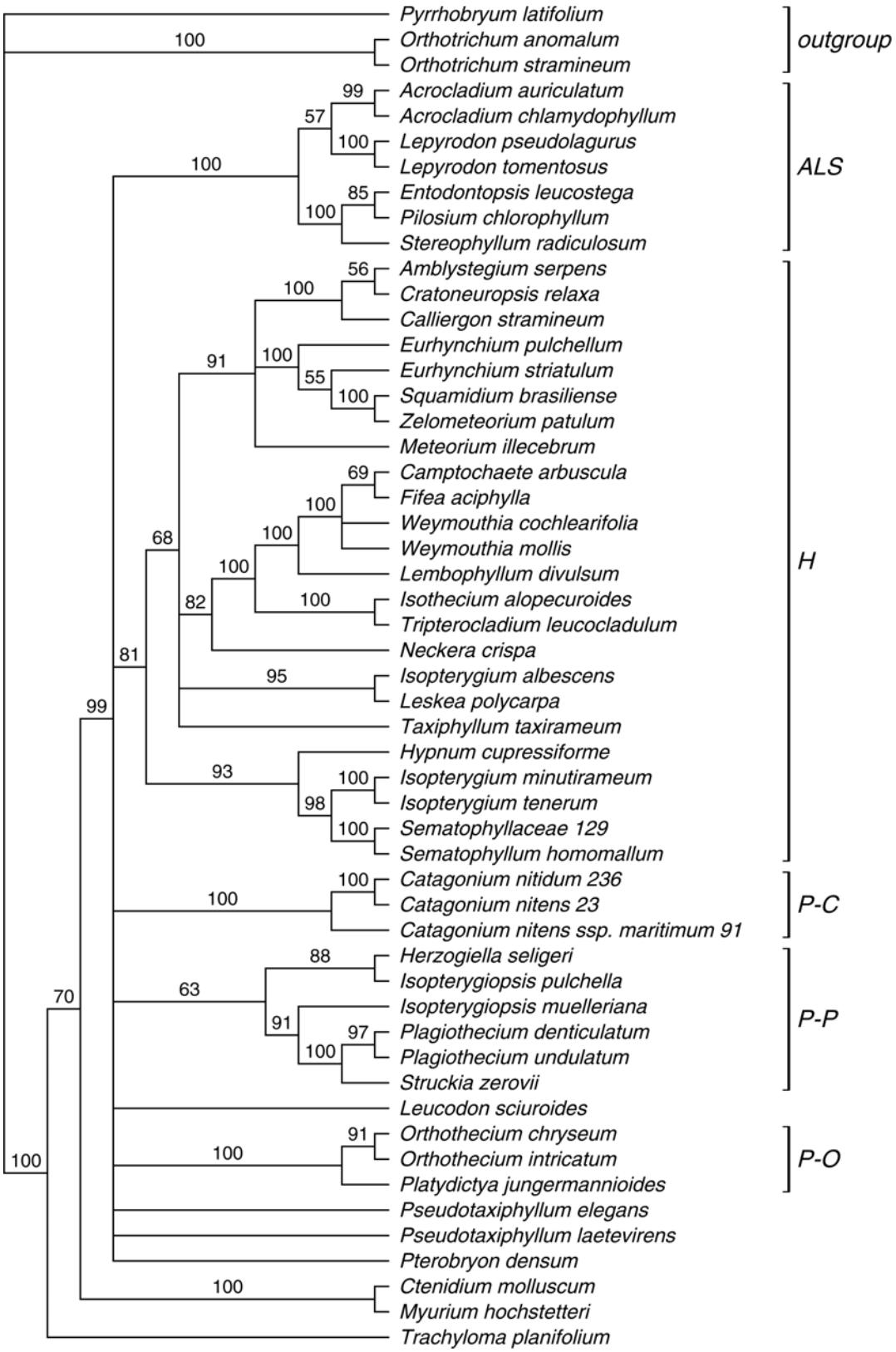

Figure 4: 50%-majority rule consensus cladogram resulting from a Bayesian Inference analysis. Numbers above branches indicate the posterior probabilities support as a percentage value. For explanation of the clades referred to as ‘outgroup’, ALS, H1, H2, P-C, IH, P-O, and P-P see text.

analysis. Instead this clade split into two, here referred to as clade, P-C, and P-P. Furthermore the relationships of these clades to any other clade were not resolved. This resulted in a polytomous branching pattern of five clearly discernible clades and an additional four single taxa: *Leucodon sciuroides, Pterobryon densum, Pseudotaxiphyllum elegans, Pseudotaxiphyllum laetevirens*. The clade formed by *Orthothecium chryseum*, *Orthothecium intricatum* and *Platydictya jungermannioides* (clade P-O) was found in 100 % of the 9,501 trees. Another group was obtained consisting of *Herzogiella seligeri, Isopterygiopsis pulchella, Isopterygiopsis muelleriana, Struckia zerovii, Plagiothecium denticulatum* and *Plagiothecium undulataum* (clade P-P).

The monophyly of the representatives of the genus *Catagonium* (clade P-C) was resolved in all cladograms. Also, the monophyly of *Acrocladium, Lepyrodon* and Stereophyllaceae (clade ALS) was detected in all of the 9,501 cladograms. The rest of the major clades of hypnalean mosses (clade H) was found as a monophyletic group in 81 % of the trees.

4.3.3 Synthesis.

According to the results of a combined data analysis of four different genetic markers (*trn*L-F-, *psb*T-H-, *rps*4- and ITS- region) using Maximum Parsimony (MP) and Bayesian Inference, the genera *Acrocladium* and *Lepyrodon* are more closely related to the representatives of the Stereophyllaceae than either to the Plagiotheciaceae or the Pterobryaceae. In both analyses the Plagiotheciaceae as circumscribed by Pedersen & Hedenäs (2002) is polyphyletic. In the MP analysis the genera *Acrocladium* (A. *auriculatum*, *A. chlamydophyllum*) and *Pseudotaxiphyllum* (*P. elegans*, *P. laetevirens*) as well as a clade of the genera *Orthothecium*/*Platydictya* (P-O) do not belong to the main Plagiotheciaceae clade (clade P). The Bayesian analysis shows a polytomous branching pattern between a clade P-P, *Catagonium* (P-C), *Orthothecium*/*Platydictya* (P-O) and two representatives of the genus *Pseudotaxiphyllum*.

The genus *Catagonium* as indicated in the MP analysis retains a basal position in the Plagiotheciaceae, but without support for this position in neither the parsimony trees nor in the Bayesian analysis. The position of the remaining species of the Plagiotheciaceae subject to Baysian analysis is left ambiguous due to the polytomous character of the single clades. A main clade of the Plagiotheciaceae

comprising six species was detected, with *Herzogiella seligeri* and *Isopterygiopsis pulchella* forming a basal clade to *Isopterygiopsis muelleriana, Struckia zerovii, Plagiothecium denticulatum* and *Plagiothecium undulataum*. A clade of *Orthothecium*/*Platydictya* (P-O) is well resolved. However, its relation to any other clade especially of the representatives of Plagiotheciaceae (P-P, P-C) is ambiguous. The systematic position of *Pseudotaxiphyllum elegans* and *Pseudotaxiphyllum laetevirens*, also remains unresolved in relation to the remaining Plagiotheciaceae.

4.4 Discussion

Based on the results of the sequence data analysis performed in this study the family Plagiotheciaceae as circumscribed in Pedersen & Hedenäs (2002) appear to be polyphyletic in the MP and Bayesian analyses. The results described above suggest that the genus *Acrocladium* is sister to the monotypic the family of the Lepyrodontaceae and both clades together are sister to the Stereophyllaceae.

Parts of the results were already suggested by the analyses by Buck et al. (2000a; Buck et al., 2000b) and Quandt et al. (2004b). In the analysis by Buck et al. (2000a; Buck et al., 2000b) based on *rps*4 and *trn*L sequence data the genus *Lepyrodon* was closely related to representatives of the Stereophyllaceae whereas the representatives of the Plagiotheciaceae were closely related to a clade comprising representatives of the Brachytheciace. However, *Acrocladium* was not included in their analysis. Quandt et al. (2004b) analysed the systematic position of the Lembophyllaceae. They included *Acrocladium* and additionally representatives of the Lepyrodontaceae and Plagiotheciaceae. Based on sequence data of three different regions, the *trn*L-F, *psb*T-H and ITS2-regions the investigated species of Plagiotheciaceae, Lepyrodontaceae and *Acrocladium* formed a moderately supported monophyletic group.

Our evidence for a close relationship between *Acrocladium* and *Lepyrodon* and the Stereophyllaceae is based on a synthesis of a broad taxon as well as data sampling. The taxon sampling was aimed at detecting the closest relatives of *Catagonium*, *Acrocladium* and the Lepyrodontaceae rather than to re-evaluate the Plagiotheciaceae as defined by Pedersen & Hedenäs (2002).

In the phylogenetic analysis of sequence data based on *rps*4- and *trn*L-sequences Pedersen & Hedenäs (Pedersen & Hedenäs, 2002) resolved two sister clades with a polyphyletic position of several genera of Plagiotheciaceae. The combined data set analysis, including morphological, anatomical and genetic sequence data revealed the genus *Acrocladium* as sister clade to the genera *Catagonium, Herzogiella, Isopterygiopsis, Rhizofabronia, Orthothecium, Myurella, Bardunovia, Platydictya, Pseudotaxiphyllum, Plagiothecium*, and *Struckia*. This clade was supported with a bootstrap value of 64 %. Nevertheless, Pedersen & Hedenäs (2002) also included the genus *Acrocladium* in the Plagiotheciaceae based on the fact that *Acrocladium* and the remaining eleven genera of the Plagiotheciacea were resolved as a monophyletic group in the strict consensus tree. Regarding morphological characters the synapomorphies for the Plagiotheciaceae including the genus *Acrocladium* are the absence of pseudoparaphyllia and of rhizoids on the stem; shared characters are the lowermost abaxial costa, the purplish and granular-papillose axillary rhizoids and an exostome with a whitish yellow basal part. However, some of these characters are not shared by all taxa investigated in the study (Pedersen & Hedenäs, 2002).

The morphological characters of *Acrocladium* not shared with any representative within the Plagiothecicaeae are the firmly attached as opposed to easily detached leaves, and the exostome margin which is dentate above but more or less entire in the remaining genera of Plagiotheciaceae (Pedersen & Hedenäs, 2001).

In fact it occurs to me as rather difficult to find synapomorphies supporting the clade consisting of Stereophyllaceae, Lepyrodontaceae and *Acrocladium* based on morphological/anatomical characters. Ireland & Buck (1994) state that there seem to be no morphological relationships between the Stereophyllaceae and Plagiotheciaceae. In their opinion the family Brachytheciaceae is probably a close relative of Stereophyllaceae based on shared morphological characters such as the single costa, elongate median leaf cells and the hypnoid peristom. Buck (1998) points out that the most possible relatives of Lepyrodontaceae, might well be found within the Pterobryaceae based on the similar growth pattern shared between these families and the presence of filamentous pseudoparaphyllia around branch primordial in some species of *Lepyrodon*. Allen (1999) as well as Buck (1998) point towards the Pterobryaceae as putative relatives of the Lepyrodontaceae but suggest a critical revision of the Pterobryaceae before comparing the taxa. Although I included *Pterobryon densum* as representative of the family Pterobryaceae in the analyses, no

closer relationship of this taxon to the taxa investigated within the ALS clade was found. However, a better resolution of the relationships between members of the ALS clade and the Pterobryaceae can be expected if additional members of this large familiy (24 genera) are studied.

The interpretation of both morphological characters and genetic sequence data can lead to erroneous phylogenetic trees. Huttunen et al. (2004) demonstrate that morphological adaptations to special habitat conditions in epiphytes, e.g. the specialized or reduced exostomes (Hedenäs, 2001), which were formerly regarded as synapopmorphic characters, have lead to a misplacement of certain taxa of epiphytic mosses e.g. within the Meteoriaceae.

Even the use of sequence based data, especially in the order Hypnales, might cause problems in resolving interfamilial relationships as this clade shows rapid radiation at the beginning of its differentiation resulting in short or even unresolved branches at deeper phylogenetic levels e.g. families (Shaw et al., 2003).

The morphological characters of the species found to form the ALS-clade in the study at hand differ considerably according to Pedersen and Hedenäs (2002), not suggesting a close relationship of these taxa. The Plagiotheciaceae, in contrast, form a morphologically well-defined group. Only the genetic data cause polytomies within the Plagiotheciaceae (Pedersen & Hedenäs, 2002). Unfortunately *Lepyrodon* was not used in the morphological data analyses to trace possible synapomorphic characters with the Plagiotheciaceae (Pedersen & Hedenäs, 2002). At least this study was informative in so far as *Acrocladium* and the representative of the Stereophyllaceae (*Stereophyllum radiculosum*) were in none of the analyses resolved as sister clades.

In contrast to the combined analysis and the cpDNA analyses the Plagiotheciaceae described by Pedersen & Hedenäs (2002) are paraphyletic rather than polyphyletic. If this result could be confirmed with at least one additional marker it would be interesting to investigate morphological and anatomical characters in terms of corresponding results. Only furthers studies can clarify whether the phylogenetic position of *Lepyrodon* and *Acrocladium* as closest relatives of the Plagiotheciceae can be justified.

In this study tried to minimize the problem that morphological and genetically based data can give different evolutionary scenarios (e.g. Doyle, 1992) by using four different regions (*trn*L, *rps*4, *psb*T-H and ITS) from two different genome types

(nrDNA and cpDNA). Due to problems obtaining sequences for all four regions of every species included in the analysis (compare appendix 4) the systematic position of e.g. three Stereophyllaceae taxa and *Acrocladium/Lepyrodon* is based on only two genes (*trn*L, *rps*4). That means there is in this respect no difference between data sets used by Buck et al. (2000a; Buck et al., 2000b), Pedersen & Hedenäs (2002) and the data set used in the study at hand. Although the analyses where cp and nrDNA were analysed separately should be considered a preliminary result due to a lack of a complete ITS data set for some of the taxa especially for taxa respresenting the Stereophyllaceae or e.g. *Herzogiella*, the results of the cpDNA analysis gave an ambiguous phylogenetic position which might be due to introgression for example (Doyle, 1992) or an extremely low sequence divergence in the investigated chloroplast markers as pointed out by (Shaw et al., 2003). It also might be useful to investigate new markers which are more variable than the *trn*L, *rps*4 or *psb*T-H regions like the *nad*5 region from the mtDNA, for example.

5 Molecular evolution, phylogenetics and biogeography of the genus *Lepyrodon* (Lepyrodontaceae, Bryopsida)

5.1 Introduction

5.1.1 The genus *Lepyrodon*

The genus *Lepyrodon* was established by Hampe in 1865 (cit. Allen, 1999). It comprises diplolepideous moss species with a single peristome of endostomial origin and a cucullate calyptra. He placed the genus within the Leskeaceae. In 1908 Brotherus introduced the monotypic family Lepyrodontaceae and placed it near the Neckeraceae. Since then the genus *Lepyrodon* has been considered to belong to either the Ptychomniaceae (e.g. Brotherus, 1925b; Buck & Vitt, 1986; Fleischer, 1923a) or the Pterobryaceae (Buck, 1998; Crum, 1994). Allen (1999) revised the family Lepyrodontaceae and transferred the monotypic genus *Dichelodontium* into the Lepyrodontaceae which consists since then of the genera *Lepyrodon* and *Dichelodontium* (*D. nitidum* endemic to New Zealand). He considered the Lepyrodontaceae as possibly related to the Pterobryaceae based on morphological characters e.g. the reduced diplolepideous peristome and the absence of an exostome. In his monograph of *Lepyrodon* Allen (1999) placed seven species within this genus. These species are mostly distributed in the southern hemisphere. The exception is *L. tomentosus* that occurs from Mexico via Central America, to northern and even southern South America. *L. hexastichus*, *L. parvulus*, *L. lagurus* and *L. patagonicus* occur only in southern South America (Argentina, Chile, and Juan Fernandez Islands) whereas the species *L. pseudolagurus* and *L. australis* are restricted to Australia, Tasmania, New Zealand and Campbell Island.

5.1.2 Morphological relationships within the genus

Some of the species are morphologically difficult to separate from possibly related species. In this study an attempt was therefore made to test the species concept

proposed by Allen (1999) based on molecular data. According to Allen (1999) *Lepyrodon lagurus* from Chile is a polymorphic species throughout its range. Plants of higher elevations are generally smaller, having erect leaves and less tomentum than those from lower elevations e.g. 'Tierra del Fuego'. The smaller plants from high elevations resemble *L. parvulus* in size but are distinguishable by their longer leaves that are stiffly erect rather than imbricate as in *L. parvulus*.

Allen (1999) distinguishes three morphological expressions in *Lepyrodon tomentosus*, which are more or less geographically restricted, with intermediate expressions where their areas of distribution overlap.

The type expression occurs in the Andean range of western South America as well as in Mexico and northwest Argentina. The northern expression occurs in southern Mexico, Panama, the Dominican Republic and southeast Brazil. This expression has '*lagurus*'-type branches which often cover more than half of the plant According to Allen (1999) this is especially the case in the type specimen of *L. duellii* which consists almost entirely of *lagurus*-type branches. The third expression, the '*L. gunckeli*'-type expression, occurs in Chile and southwest Argentina.

5.1.3 The systematic position of Lepyrodontaceae

The systematic position of the Lepyrodontaceae and its genera *Lepyrodon* and *Dichelodontium* were investigated in a previous study (compare chapters 3 and 4).

Results from chapters 3 and 4 based on cpDNA analysis identified the genus *Lepyrodon* as a monophyletic group with high branch support (> 95 %) in all analyses performed. Its closest relative is the genus *Acrocladium.* Furthermore, I identified *Dichelodontium nitidum* as belonging to the Ptychomniaceae. According to analyses described in previous chapters (3 & 4) the Ptychomniaceae are nested within a group of taxa closely related to the Hookeriales, whereas the Lepyrodontaceae belong to the Hypnales together with the genus *Acrocladium*. Based on these results the family Lepyrodontaceae was treated as a monotypic family with the single genus *Lepyrodon*.

This study aims at

a) verifying the species concept within the genus *Lepyrodon*

b) bringing to light the evolution and the historical biogeography of *Lepyrodon*

5.2 Material & Methods

***Plant material*.** Plant material was either collected by the author during a field trip of the BryoAustral project to Chile in 2001, or originates from herbarium specimens (Appendix 6). Specimens of *Acrocladium chlamydophyllum*, *Lepyrodon pseudolagurus* were collected during the BryoAustral project expedition to New Zealand in 1998. Duplicates are preserved in the herbaria in Christchurch (CHR), Bonn (BONN) and Berlin (B). Sequences available in GenBank were also used. All specimens used in the analyses are listed in (Appendix 6) including further voucher information.

The study includes 26 specimens from all of the seven *Lepyrodon* species recently described as belonging to the genus (Allen, 1999). Each of the seven species was represented by at least two specimens. Within each species, specimens were selected to span a wide range of geographically distinct populations (e.g. including specimens from the Juan Fernández Islands) and different morphological expressions of the widespread species *L. tomentosus* (Allen, 1999). Unfortunately, I was not able to gather enough DNA from all specimens (table 13) for successful PCR and successive sequencing.

At least one specimen of every species described in the genus *Lepyrodon* (Allen, 1999) was investigated in this study. I analysed one specimen each of *L. parvulus* and *L. patagonicus*, two specimens each of *L. lagurus*, *L. pseudolagurus* and *L. australis*, and three specimens each of *L. tomentosus* and *L. hexastichus.* The geographical origin of the specimens of *Lepyrodon* successfully sequenced is shown in figure 5 on a global scale and in figure 6 (New Zealand) and figure 7 (South America) on a regional scale.

In a previous study (chapter 4), the genus *Acrocladium* was identified as as sister taxon to *Lepyrodon*. Therefore two species of *Acrocladium*, as closest relatives, were selected as outgroup for the analysis within the genus *Lepyrodon*.

Table 13: List of investigated specimens of *Lepyrodon* with EMBL accession numbers for the regions sequenced. Voucher numbers and the herbaria where the specimens are kept and country of origin are listed. ITS2 sequences of *L. pseudolagurus* and *L. tomentosus* were kindly provided by Dr. Dietmar Quandt (Dresden). For detailed voucher information see Appendix 6.

No.	taxon	rps4	ITS complete	adk	country of origin	voucher label	herbarium
33	*Lepyrodon lagurus* (Hook.) Mitt.	AJ862336	AJ862513	submitted to EMBL	Chile	BryoAustral Rolf Blöcher no. 90 det. Bruce Allen 01/2003	J.-P. Frahm, Bonn
64	*Lepyrodon tomentosus* (Hook.) Mitt.	AJ862337	AJ862688 (ITS1) AF509839 (ITS2)	AJ862663	Chile	BryoAustral Rolf Blöcher no. 74 det. Bruce Allen 01/2003	J.-P. Frahm, Bonn
66	*Lepyrodon lagurus* (Hook.) Mitt.		AJ862514	AJ862669	Chile	BryoAustral Rolf Blöcher no. 82 det. Bruce Allen 01/2003	J.-P. Frahm, Bonn
67	*Lepyrodon pseudolagurus* (Hook.) Mitt. [originally labelled *Lepyrodon lagurus* (Hook.) Mitt.]	AJ862335	AJ862687 (ITS1) AF188044 (ITS2)	AJ862664	New Zealand	BryoAustral J.-P. Frahm No. 10-12	J.-P. Frahm, Bonn
83	*Lepyrodon australis* Hpe ex Broth.		AJ862509	submitted to EMBL	New Zealand	Musci Australasiae Exsiccati H. Streimann 51277 det. J.Beever, 07/1993	J.-P. Frahm, Bonn
84	*Lepyrodon patagonicus* (Card. & Broth.) Allen [orig. labelled *Lepyrodon implexus* (Kze.) Paris]		AJ862516	AJ862668	Chile	Plantae Chilenensis H. Roivainen 2934 det. Bruce Allen 1995	Berlin
85	*Lepyrodon parvulus* Mitt.		AJ862515	AJ862667	Chile	Plantae Chilenensis H. Roivainen 3129 det. Bruce Allen 1995	Berlin
106	*Lepyrodon hexastichus* (Mont.) Wijk &Marg.		AJ862510	AJ862662	Chile	BryoAustral Rolf Blöcher no. 77 det. Bruce Allen 01/2003	J.-P. Frahm, Bonn
107	*Lepyrodon hexastichus*		AJ862511	AJ862666	Chile	BryoAustral Rolf Blöcher no. 87 det. Bruce Allen 01/2003	J.-P. Frahm, Bonn
112	*Lepyrodon pseudolagurus* (Hook.) Mitt. [originally labelled *Lepyrodon lagurus* (Hook.) Mitt.]		AJ862517	submitted to EMBL	New Zealand	Musci Australasiae Exsiccati H. Streimann 51045 det. H. Streimann	J.-P. Frahm, Bonn
113	*Lepyrodon tomentosus* (Hook.) Mitt. [originally labelled *Lepyrodon lagurus* (Hook.) Mitt.]		AJ862519	no data	Mexico	Düll 2/248	J.-P. Frahm, Bonn
207	*Lepyrodon australis* Hpe ex Broth.		AJ862508	AJ862670	New Zealand	H. Streimann 58133	Bot. Mus. Helsinki, Finland
208	*Lepyrodon hexastichus* (Mont.) Wijk &Marg.		AJ862512	AJ862661	Chile	Marshall R. Crosby 11,631 det. B. H. Allen 1985	Leiden, Nat. Herb. Netherlands
214	*Lepyrodon tomen-tosus* (Hook.) Mitt.		AJ862520	AJ862665	Costa Rica	J. Eggers CR 6,17	J.-P. Frahm, Bonn

Distribution map. Regional maps of the origin of *Lepyrodon* specimens were constructed using the web-page www.planiglobe.com (Körsgen et al., 2004). Dots

were generated by adding geographical coordinates of collection localities as indicated on the voucher labels of the specimens.

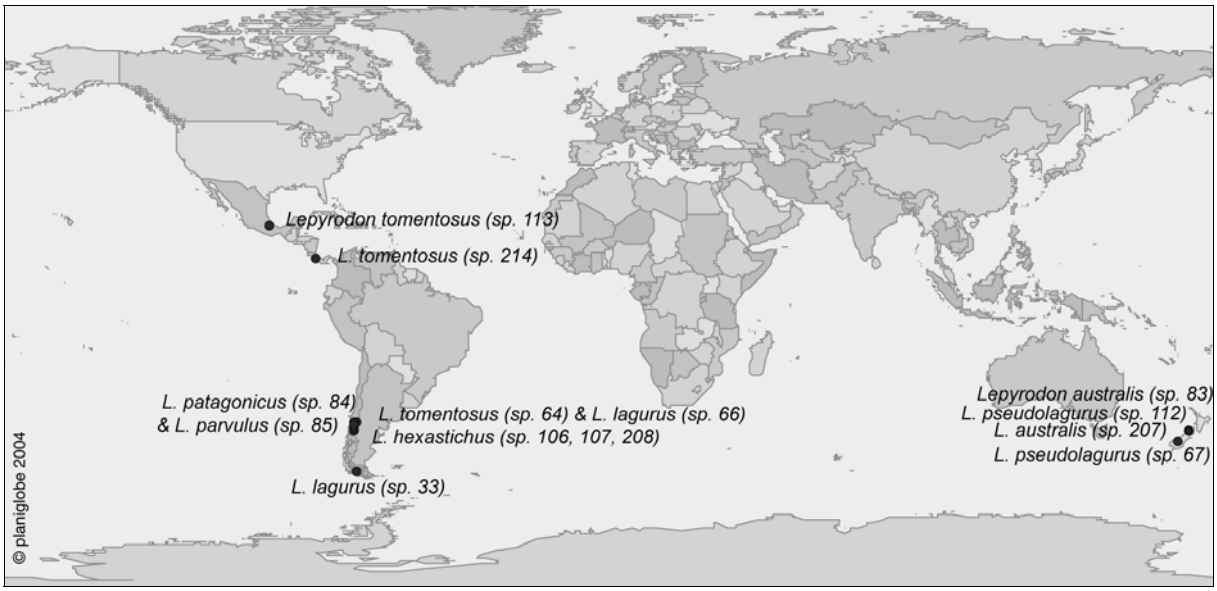

Figure 5: Geographical origin of all *Lepyrodon* specimens used for this study. Numbers in brackets are specimen numbers. For detailed information of the collection localities see figures 6 & 7.

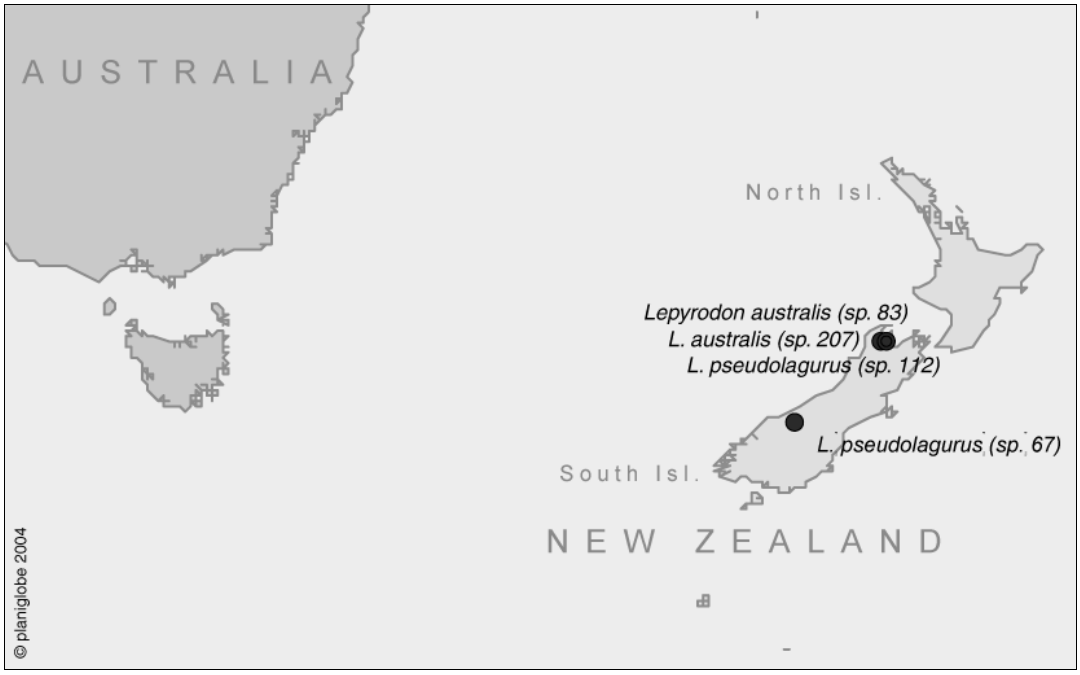

Figure 6: Geographical origin of the *Lepyrodon* specimens from New Zealand used for this study. Numbers in brackets are specimen numbers.

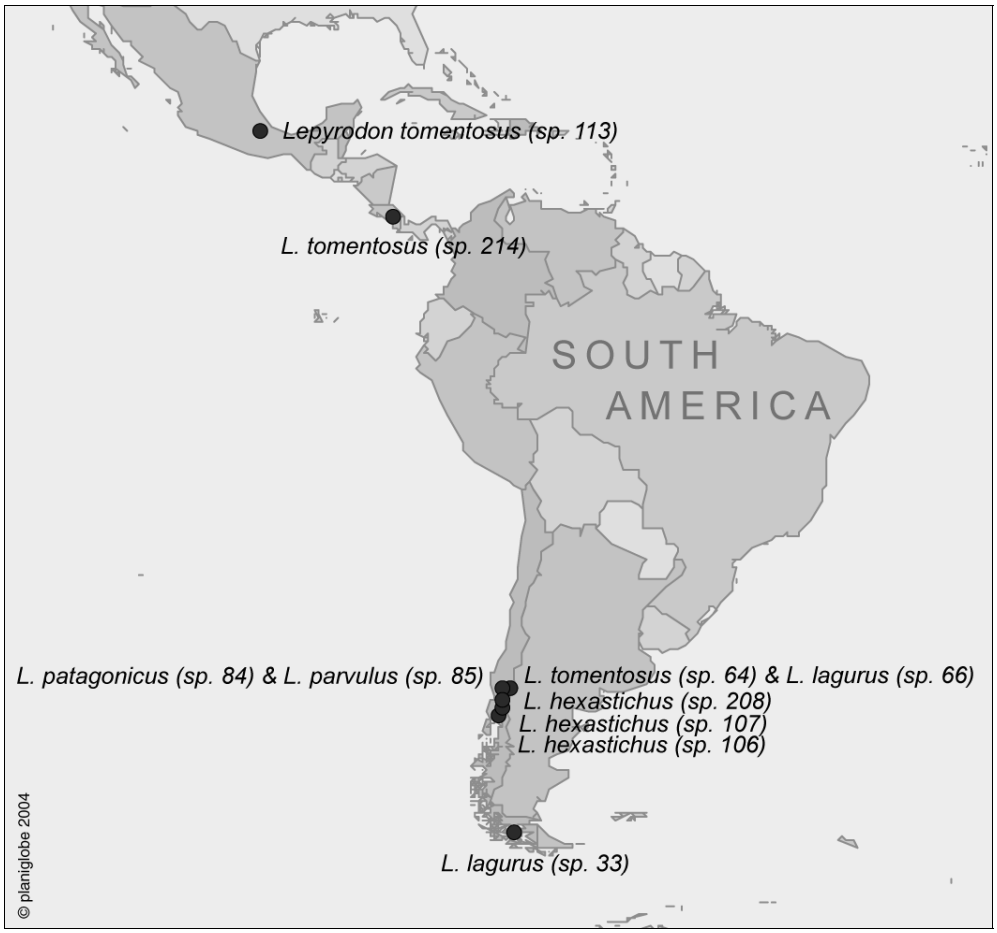

Figure 7: Geographical origin of the *Lepyrodon* specimens from South and Central America used for this study. Numbers in brackets are specimen numbers.

DNA isolation, PCR and sequencing. Prior to DNA extraction the plant material was thoroughly cleaned with distilled water and additionally treated by ultrasonic waves for 2-4 minutes. Success of cleaning was checked by examining the plants under a binocular microscope. Remaining contaminations e.g. with algae and fungi were removed mechanically. Isolation of DNA was carried out following the CTAB technique described in Doyle & Doyle (1990).

PCR amplifications (Biometra TriBlock thermocycler, PTC-100 MJ Research) were performed in 50 µl–reactions containing 1.5 U *Taq* DNA polymerase (PeqLab), 1 mM dNTPs-Mix, nucleotide concentration 0.25 mM each (PeqLab), 1x buffer (PeqLab), 1.5 mM $MgCl_2$ (PeqLab) and 12.5 pmol of each amplification primer. PCR products were purified using the QIAquick purification kit (Qiagen). Cycle sequencing reactions (half reactions) were performed using a PTC-100 Thermocycler (MJ Research) in

combination with the ABI Prism™ Big Dye Terminator Cycle Sequencing Ready Reaction Kit with Amplitaq-DNA polymerase FS (Perkin Elmer), applying a standard protocol for all reactions. Extension products were precipitated with 40 µl 75 % (v/v) isopropanol for 15 min at room temperature, centrifuged with 15,000 rpm at 25°C, and washed with 250 µl of 75 % (v/v) isopropanol. These purified products were loaded on an ABI 310 automated sequencer (Perkin Elmer) and electrophoresed. For cycle sequencing 10 µl–reactions were used containing 3 µl of Big Dye Terminator Cycle Sequencing premix. Sequencing reactions were performed on two independent PCR products generated from each sample in order to verify the results. Primers for amplifying and sequencing the ITS region (ITS4-bryo and ITS5-bryo) based upon the primers “ITS4” and “ITS5” respectively, designed and named by White et al.(1990), were slightly modified with respect to bryophytes (Stech, 1999).The primers ITS-C and ITS-D (Blattner, 1999) were modified for this study (ITS-D_bryo and ITS-C_bryo) and additionally used for sequencing reactions (table 14).

Table 14: Primer sequences used for amplification and sequencing of the ITS region. Underlined nucleotides represent changes with respect to the original primers of Blattner 1999.

Primer	Sequence	Data source
ITS-C bryo	GCA ATT CAC ACT ACG TAT CGC	Blattner 1999
ITS-D bryo	CTC TCA GCA ACG GAT ATC TTG	Blattner 1999
ITS4-bryo	TCC TCC GCT TAG TGA TAT GC	Stech 1999
ITS5-bryo	GGA AGG AGA AGT CGT AAC AAG G	Stech 1999

Table 15: Primer sequences used for amplification and sequencing of the *adk* gene.

Primer	Sequence	Data source
F	GAA GAA GCC AGA AAA CTG GGC	Vanderpoorten et al. 2004
R	GTC ACC CCA TCT TCA GCA AC	Vanderpoorten et al. 2004
1F	AAG CTT TTC CCG TAA GT	Vanderpoorten et al. 2004
2R	ACT TAC GGG AAA AGC TT	Vanderpoorten et al. 2004
3R	GGT CCC CTG GGT AAT AAC	Vanderpoorten et al. 2004
4F	TTT CAT CCC ATC GGT GG	Vanderpoorten et al. 2004

The amplified *adk* region started about 196 bp downstream of the 155th codon and ended at the 257th codon of the *adk* gene isolated from the moss species *Physcomitrella patens* (Y15430, Schwartzenberg et al., 1998). Coding and non-coding regions were identified by comparison with moss sequences available from

GenBank (e.g. Vanderpoorten et al., 2004). Primers used for amplification of the *adk* gene (table 15) were those described in Vanderpoorten (2004).

For amplifying and sequencing the nuclear region different protocols have been applied.

The ITS region was amplified using a protocol consisting of: 5 min. 94ºC, 35 cycles (1 min. 94ºC, 1 min. 48ºC, 1 min. 72ºC) and a 5 min. 72ºC extension time, cycle sequencing settings: 25 cycles (30 sec. 96ºC, 15 sec. 50ºC, 4 min. 60ºC). According to Vanderpoorten et al. (2004) the following PCR protocol was used to amplify parts of the *adk* gene : 2 min. 97ºC, 30 cycles (1 min. 97ºC, 1 min. 50ºC, 3 min. 72ºC) and a 7 min. 72ºC extension time. For more detailed information compare Vanderpoorten et al.(2004).

All sequences will be deposited in EMBL, accession numbers are listed in Appendix 6, the alignments are available on request from the author.

Phylogenetic analyses. Heuristic searches under the parsimony criterion were carried out under the following options: all characters unweighted and unordered, multistate characters interpreted as uncertainties, gaps coded as missing data, performing a tree bisection reconnection (TBR) branch swapping, collapse zero branch length branches, MulTrees option in effect, random addition sequence with 1000 replicates.

Furthermore, the data sets were analysed using *win*PAUP 4.0b10 (Swofford, 2002) executing the command files generated by 'PRAP' (Parsimony Ratchet Analyses using PAUP Müller, 2004), employing the implemented parsimony ratchet algorithm (Nixon, 1999). For the parsimony ratchet the following settings were employed: 10 random addition cycles of 200 iterations each with a 40 % upweighting of the characters in the PRAP iterations. Heuristic bootstrap (BS Felsenstein, 1985) searches under parsimony criterion were performed with 1000 replicates, 10 random addition cycles per bootstrap replicate and the same options in effect as the heuristic search for the most parsimonious tree (MPT). The consistency index (CI, Kluge & Farris, 1969), retention index (RI), and rescaled consistency index (RC, Farris, 1989) were calculated to assess homoplasy.

Maximum Likelihood analyses were executed assuming a general time reversible model (GTR+G+I), and a rate variation among sites following a gamma distribution (four categories represented by the mean), with the shape being estimated and the

molecular clock not enforced. According to Akaike Information Criterion (AIC, Akaike, 1974) GTR+G+I was chosen as the model that best fits the data by Modeltest v3.06 (Posada & Crandall, 1998), employing the windows front-end (Patti, 2002). The proposed settings by Modeltest v3.06 (table 16) were executed in *win*PAUP 4.0b10. In addition to the MP analyses Bayesian Inferences with MrBayes3.0 (Huelsenbeck & Ronquist, 2001) were performed. Modeltest 3.5 (Posada, 2004) was used to select DNA substitution models for the data set (gamma shape distribution, six substitution types). The Markov Chain Monte Carlo (MCMC) analyses were run for 2,000,000 generations with four simultaneous MCMCs and one tree per 100 generations was saved. The 'burn-in' values were determined empirically from the likelihood values. The analyses were repeated three times to assure sufficient mixing by confirming that the program converged to the same posterior probability (PP).

Table 16: Substitution models selected for the different data sets in Maximum Likelihood analyses in the *Lepyrodon* data sets.

	combined	**non-coding region in *adk* gene**
Model selected	GTR+G+I -lnL = 3103.1511	GTR+I -lnL = 1260.0568
Base frequencies	freqA = 0.2066 freqC = 0.2588 freqG = 0.2527 freqT = 0.2818	freqA = 0.2112 freqC = 0.2167 freqG = 0.1933 freqT = 0.3788
Substitution model	R(a) [A-C] = 1.0000 R(b) [A-G] = 1.8159 R(c) [A-T] = 0.6009 R(d) [C-G] = 0.6009 R(e) [C-T] = 1.8159 R(f) [G-T] = 1.0000	R(a) [A-C] = 1.0000 R(b) [A-G] = 1.0023 R(c) [A-T] = 0.4932 R(d) [C-G] = 0.5324 R(e) [C-T] = 1.0023 R(f) [G-T] = 1.0000
Among-site rate variation		
Proportion of invariable sites (I)	0	0.5324
Variable sites (G, Gamma distribution shape parameter)	0.1410	equal rates for all sites

The program Treegraph (Müller & Müller, 2004) was used to edit trees directly from PAUP-treefiles. MEGA2.1 (Kumar et al., 2001) was used to calculate GC-content, sequence length and distance measure ('p-distance'). In the following the term 'genetic distance' is used instead 'p-distance'.

5.3 Results

5.3.1 Sequence variation

Sequence length and GC-content of the ITS region. For this study fourteen specimens of *Lepyrodon* and two specimens of *Acrocladium* were successfully sequenced. The statistical data on the obtained sequences are depicted in table 17 for ITS1, ITS2 and the total *adk* sequence. The data for the coding and non-coding regions in *adk* are presented in appendix 7.

The observed size of the total sequence of ITS1 ranged between 246 bp for *Lepyrodon tomentosus* (sp. 64) and *L. hexastichus* (sp. 106 & 107) and 255 bp found in the two outgroup species *Acrocladium auriculatum* and *A. chlamydophyllum*.

The obtained length for the ITS1 region was on average 248 base pairs (bp) with a standard deviation of 3.2 bp. For two specimens only a partial sequence of the ITS1 was obtained. In *Lepyrodon lagurus* (sp. 33) only the first 134 bp and in the specimen of *Lepyrodon tomentosus* from Mexico only 206 bp could be read. The average GC-content in the data set was 64.1 % (standard deviation 1.2).

The entire ITS2 region was obtained for all 16 specimens. The average length was 260 bp (standard deviation 9.8). The shortest ITS2 sequence was found in both outgroup specimens *Acrocladium chlamydophyllum* (233 bp) and *A. auriculatum* (236 bp). This difference in length, apart from several short indels, ranged from one to four nucleotides, mainly due to an indel of 20 bp in length which was found in all specimens of *Lepyrodon* but not in *Acrocladium*. The length of the ITS2 region within *Lepyrodon* was between 260 and 266 bp. The average GC-content in the ITS2 region was 65.5 % (standard deviation 0.5).

Sequence length and GC-content of the adk gene. In the *adk* data set four of the fifteen investigated specimens could only be partially sequenced (both species of *Acrocladium* as well as two specimens of *Lepyrodon hexastichus*; specimens no. 106 & 208). These species were excluded from the total length presentation in the coding as well as the non-coding region of the *adk* gene (appendix 7). For the remaining thirteen species 312 bp were obtained in the coding region spanning the entire exons 1 to 3 and parts of exon 4. The GC-content was 48.9 % on average (standard

deviation 1.7). The GC-content of the different codon positions differed

Table 17: Sequence lengths [base pairs, bp] and GC-content [%] of selected gene regions (ITS1, ITS2, and *adk* gene) of fourteen *Lepyrodon* specimens and two outgroup taxa. Average sequence lengths and standard deviations are also given. For origin of the data refer tab. xz. Abbreviations: n. d. = no data available. (* partial sequences were excluded when determining the average sequence length).

	ITS1 sequence length [bp}	**ITS1 GC-content [%]**	**ITS2 sequence length [bp]**	**ITS2 GC-content [%]**	***adk* gene sequence length [bp}**	***adk* gene GC-content [%]**
A. auriculatum (sp. 78)	255	64,3	236	64,9	689*	45,6
A. hlamydophyllum (sp. 12)	255	62,7	233	63,9	544*	41,5
L. australis (sp. 83)	249	63,8	266	65,5	866	42,4
L. australis (sp. 207)	249	63,8	266	65,5	835	42,7
L. hexastichus (sp. 107)	246	63,8	264	65,9	846	43,5
L. hexastichus (sp. 106)	246	63,8	260	65,8	588*	41,5
L. hexastichus (sp. 208)	247	64	265	65,3	511*	44,2
L. lagurus (sp. 66)	247	63,2	262	65,3	891	43,1
L. lagurus (sp. 33)	134*	68	265	65,7	874	43,0
L. parvulus (sp. 85)	247	64	265	65,7	866	43,1
L. patagonicus (sp. 84)	247	64	264	65,5	867	43,2
L. pseudolagurus (sp. 67)	249	64,6	264	65,9	871	42,6
L. pseudolagurus (sp. 112)	249	65	266	65,8	867	42,4
L. tomentosus (sp. 113)	206*	66	265	65,7	n. d.	n. d.
L. tomentosus (sp. 64)	246	63,4	266	65,4	890	42,8
L. tomentosus (sp. 214)	247	64	264	65,9	868	42,9
Average	248.1	64,1	260,8	65,5	867,4	43,0
S.D.	3.2	1.2	9.8	0,5	16,2	1,0

considerably. The lowest GC-content was found in the second codon position with 38.1 % (standard deviation 2.1) followed by the first codon position with 50.7 % (standard deviation 1.7), and the highest GC-content in the third codon position with 57.9 % (standard deviation 2.4). The differences in sequence length resulted from the exclusion of sites (character state "?" in the alignment) where different nucleotide states were in conflict with each other.

In *Lepyrodon australis*, for example, the amplified region started at 196 bp downstream of the 155th codon and ended at the 257th codon of the *adk* cDNA compared to *Physcomitrella patens* (Y15430, Schwartzenberg et al., 1998).

Table 18: Number of taxa, total number of aligned characters; variable characters and number of parsimony informative sites and %-value of variable sites for the partial data sets of *Lepyrodon* data set (* Including the outgroup taxa).

Data set	Number of taxa included	Total number of aligned characters [bp]	Variable characters [bp]	parsimony informative [bp]	Variable sites [%]
adk	15*	897	90	30	10.1
adk	13	897	34	19	3.8
adk coding	15*	312	16	5	5.1
adk coding	13	312	2	2	0.6
adk non-coding	15*	585	74	25	12.6
adk non-coding	13	585	32	17	5.5
ITS	16*	694	40	27	5.8
ITS	14*	694	18	12	2.6
ITS1	16*	260	24	16	9.2
ITS1	14	260	13	7	5.0
5.8S	16*	160	0	0	0
5.8S	14	160	0	0	0
ITS2	16*	274	16	11	5.8
ITS2	14	274	5	5	1.8

Table 18 presents the information for the different regions in the alignment. The highest proportion of variable sites was found in the *adk* non-coding region where 12.6 % of the 585 aligned positions were variable with the data set including the outgroup (5.5 % variability within the specimens of *Lepyrodon*). The coding region of the *adk* data set revealed only 5.1 % variable sites (0.6 % without outgroup) in the alignment with 312 positions. Within the ITS region the ITS1 was the most variable with 9.2 % of the characters in 260 positions. The variability of the ITS1 data set without the two outgroup taxa was 5.0 %.The ITS2 region was less variable than ITS1, i.e. 5.8 % when the outgroup was included, and only 1.8 % of its 274 characters when the outgroup was excluded.

Indel matrix. In the ITS1 region three indels of one bp length were detected within the fourteen accessions of *Lepyrodon* (Table 19):

- both specimens of *L. lagurus* (sp. 33 and 66) share a C with *L. australis* (in 83 and 207) and claim another C of their own;

In the ITS2 region four one nucleotide indels were identified:

- the New Zealand/Australian distributed species *L. australis* and *L. pseudolagurus* share a synapomorphic indel of a single C;
- a single T indel occurred in *L. tomentosus* from Costa Rica (sp. 214);

- one indel , a single T, in the ITS2 region was observed in *L. tomentosus* from Chile (sp. 64).

Table 19: Indelmatrix of 15 specimens of *Lepyrodon* of the ITS- and adk-region. Indel number 1-3 in the ITS1 region, no. 4-7 in the ITS2 region, and no. 8-11 is in the *adk* gene.

Indel no.	1	2	3	4	5	6	7	8	9	10	11
L. australis sp. 83	C		A		C	T			CCTT		
L. australis sp. 207	C		A		C	T			CCTT		
L. hexastichus sp. 106						T		TACT	CCTT	G	T
L. hexastichus sp. 107						T		TACT			
L. hexastichus sp. 208						T		TACT	CCTT		
L. lagurus sp. 33	C	C				T		TACT	CCTT		
L. lagurus sp. 66	C	C		N		T		TACT	CCTT		
L. parvulus sp. 85						T		TACT	CCTT		
L. patagonicus sp. 84						T		TACT	CCTT		
L. pseudolagurus sp. 67					C	N		TACT	CCTT		
L. pseudolagurus sp. 112					C	T		TACT	CCTT		
L. tomentosus sp. 64						T	T	TACT	CCTT		
L. tomentosus sp. 113						T		TACT	CCTT		
L. tomentosus sp. 214								TACT	CCTT		

Indels in the *adk*-region occurred in non-coding regions only. Two indels of four nucleotides and two of one nucleotide were identified within the sequenced part of the region. The TACT indel occurred in all investigated specimens except both specimens of *L. australis*. The second 4-base indel, CCTT, was only missing in *L. hexastichus* (sp. 107) whereas the two single nucleotide indels G and T were only found in one specimen of *L. hexastichus* (sp. 106).

5.3.2 Phylogenetic analysis

Maximum Parsimony and Maximum Likelihood analyses. The result of the Maximum Likelihood (ML) as well as Maximum Parsimony (MP) analysis of the combined (*adk*, ITS), data set with *Acrocladium auriculatum* and *A. chlamydophyllum* as outgroup taxa is depicted in figure 8. The result of the Maximum Parsimony (MP) analysis is not depicted separately as the resolution in the cladograms was quite low. The clades with which the MP and ML analysis correspond are marked (#) in the ML cladograms (fig. 8). The values above branches (fig. 8) are the result of a heuristic bootstrap analysis (1000 repeats) of the combined data set with PAUP. The phylogram of the ML analysis is depicted in figure 9.

One result of the statistical analyses of the combined data set was the striking difference in variability between the single regions (tab. 19). Due to these large

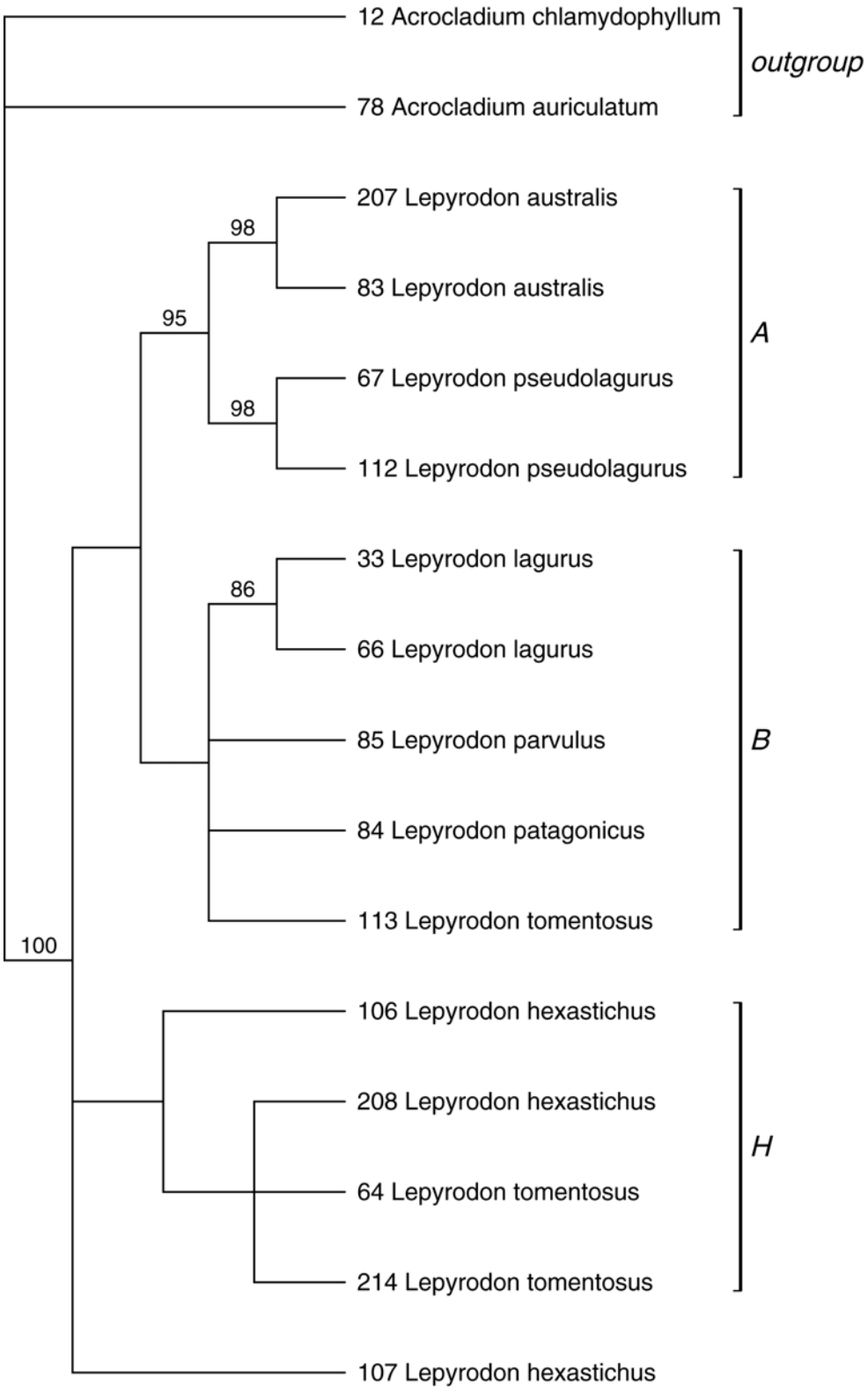

Figure 8: Cladogram resulting from a Maximum Likelihood analysis of 14 species of *Lepyrodon* and the outgroup species based on a combined data analysis (*adk* gene and ITS data). Bootstrap values above branches are the result of a Maximum Parsimony analysis of the data set. For explanation of the clades referred to as 'outgroup', H, and A see text.

differences in variability I analysed the *adk* non-coding region separately. The result of this analysis of the *adk* is depicted in figure 10 as a cladogram resulting from the ML analyses with bootstrap values taken from the MP analyses. The resulting topologies of the ML and MP analysis were identical, therefore only the ML cladograms of the analysis (fig. 10) are presented. The values above branches (fig. 10) are the result of a heuristic bootstrap analysis (1000 repeats) of the combined data set with PAUP.

The fourteen ingroup taxa investigated in this study are a monophyletic group with 100 % bootstrap support in the analysis. The specimens investigated in this study are separated in a polytomy consisting of three clades (fig. 8) named H, B, and A and a single taxon (*L. hexastichus*, specimen 107). Clade H consists of two samples of *L. hexastichus* (sp. 106 & 208) and two samples of *L. tomentosus* (sp. 64 & 214). Clade A consists of two samples each of *L. pseudolagurus* (sp. 67 & 112) and *L. australis* (sp. 83 & 207). This clade is sister to clade B which contains five specimens: *L. patagonicus* (sp. 84), *L. parvulus* (sp. 85), two samples of *L. lagurus* and one sample of *L. tomentosus* from Mexico. The relationships of the species in clade H do not resolve the specimens of *L. tomentosus* or those of *L. hexastichus* as monophyletic. *L. hexastichus* (sp. 106, Puerto Montt) is at the basal position of the clade whereas the other sample of *L. hexastichus* (sp. 208, Valdivia) is sister to the specimens of *L. tomentosus* from Costa Rica (sp. 214) and Chile (sp. 64).

Within clade B merely the close relationship between *L. lagurus* from Conquillio National Park near Temuco (sp.66) and sample 33 from southern Chile near Punta Arenas becomes obvious whereas the relationship of two further species from Chile, *L. patagonicus* (sp. 84) and *L. parvulus* (sp. 85) and the Mexican specimen of *Lepyrodon tomentosus* (sp. 113) remains unresolved among each other as well as in relation to *Lepyrodon lagurus*. Clade A consists of the only two species which occur in New Zealand and Australia, *L. australis* (sp. 83 & 207) and *L. pseudolagurus*. The relationship within clade A, the sister clade to B, shows the two specimens of *L. australis* (sp. 83 & 207) and of *L. pseudolagurus* (sp. 67 & 112) as a monophyletic group, respectively. The monophyly of each species is supported with a 98 % bootstrap value. Furthermore, the monophyly of this clade has a strong bootstrap support of 95 %. The branch lengths in the phylogram of the ML analysis (fig. 9) are very short at the base of clade H, A and B indicate a lower differentiation (supporting autapomorphic characters).

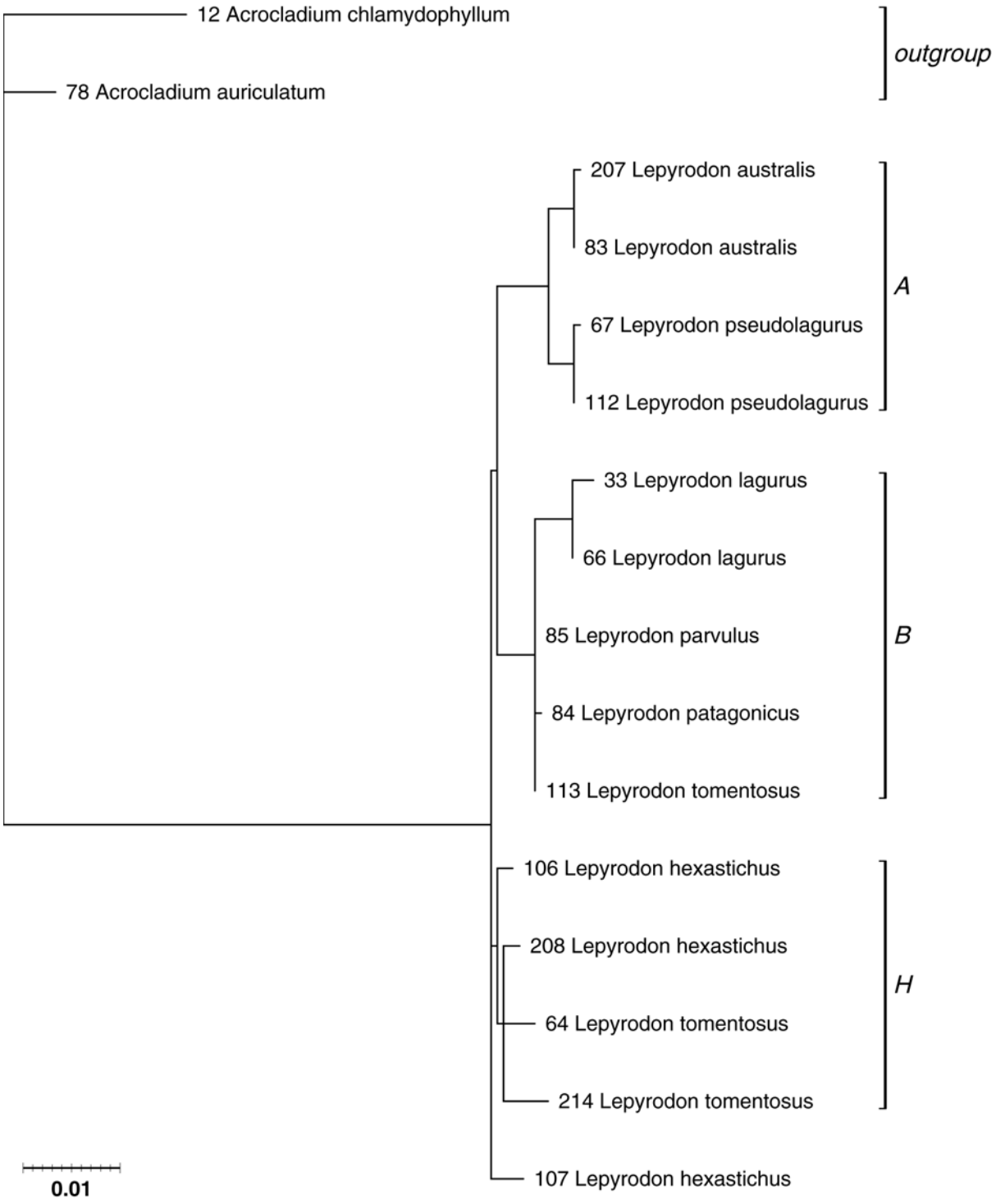

Figure 9: Maximum Likelihood (ML) phylogram of the combined data set of *adk* gene and ITS data (L score = -3103.1511). Branch lengths are proportional to genetic distance between taxa. Scale bar equals 1% distance under the assumed substitution model (GTR+G+I). For explanation of the clades referred to as 'outgroup', H, and A see text.

The results of the ML and MP analyses based on the *adk*-intron are shown in figure 10. *L. tomentosus* was removed from the data set as sequence data were lacking for this specimen. The analyses revealed three well-supported clades also strongly supported in a succeeding bootstrap analysis. The main clades A and H are the same clades as in the combined analysis. Clade B from the combined analysis (fig. 8, 9, 11) lacked *L. tomentosus* from Mexico for the reason described above. There are differences in bootstrap support compared to the former analysis. The clade consisting of *L. hexastichus* (sp. 106 & 208) and *L. tomentosus* (sp. 64 & 214) now has a bootstrap support of 53 %. Within this clade the monophyly of the two *L. tomentosus* specimens and *L. hexastichus* (sp. 208) is also supported with 53 %. A bootstrap support for a clade consisting of *L. lagurus* (sp. 66), *L. parvulus* and *L. patagonicus* was detected. The support for the species *L. australis* dropped to 82 % and that of *L. pseudolagurus* to 52 %. The position of *L. hexastichus* (sp. 107) remains ambiguous with respect to the three clades mentioned above.

Bayesian Inference analysis. Figure 11 presents the result of a Bayesian Inference of molecular phylogenetic data. The data set included the combined ITS and *adk* data of fourteen specimens of *Lepyrodon* and two outgroup taxa used in the ML analysis depicted in figures 8 and 9. The values above branches are the posterior probabilities supporting the corresponding clade.

The ‘east austral’ clade (clade A) consisting of the two species from New Zealand has a probability of 100 %. Within this clade, the monophyly of the investigated specimens of *L. australis* (sp. 83 & 207) and *L. pseudolagurus* (sp. 67 & 112) is supported with 100 % probability.

A clade consisting of three species from Chile, *Lepyrodon lagurus* (sp. 33 & 66), *L. parvulus* (sp. 85) and *L. patagonicus* (sp. 84) is supported with 90 %. The monophyly of *L. lagurus* is supported with 100 % probability. Two specimens of *L. hexastichus* (sp. 106 & 208) and *L. tomentosus* (sp. 64 & 214) form a clade H with 58 % probability, within which the specimens 208, 64 and 214 are monophyletic with a probability of 68 %, thus both clades lack significant support. The taxonomic status of *L. tomentosus* from Mexico (sp. 113) and one specimen of *L. hexastichus* (sp. 107) remains unresolved with respect to the former clades.

The investigated specimens of the seven species of the genus *Lepyrodon* indicate polyphyletic cryptic relationships with respect to distribution and taxonomy. The

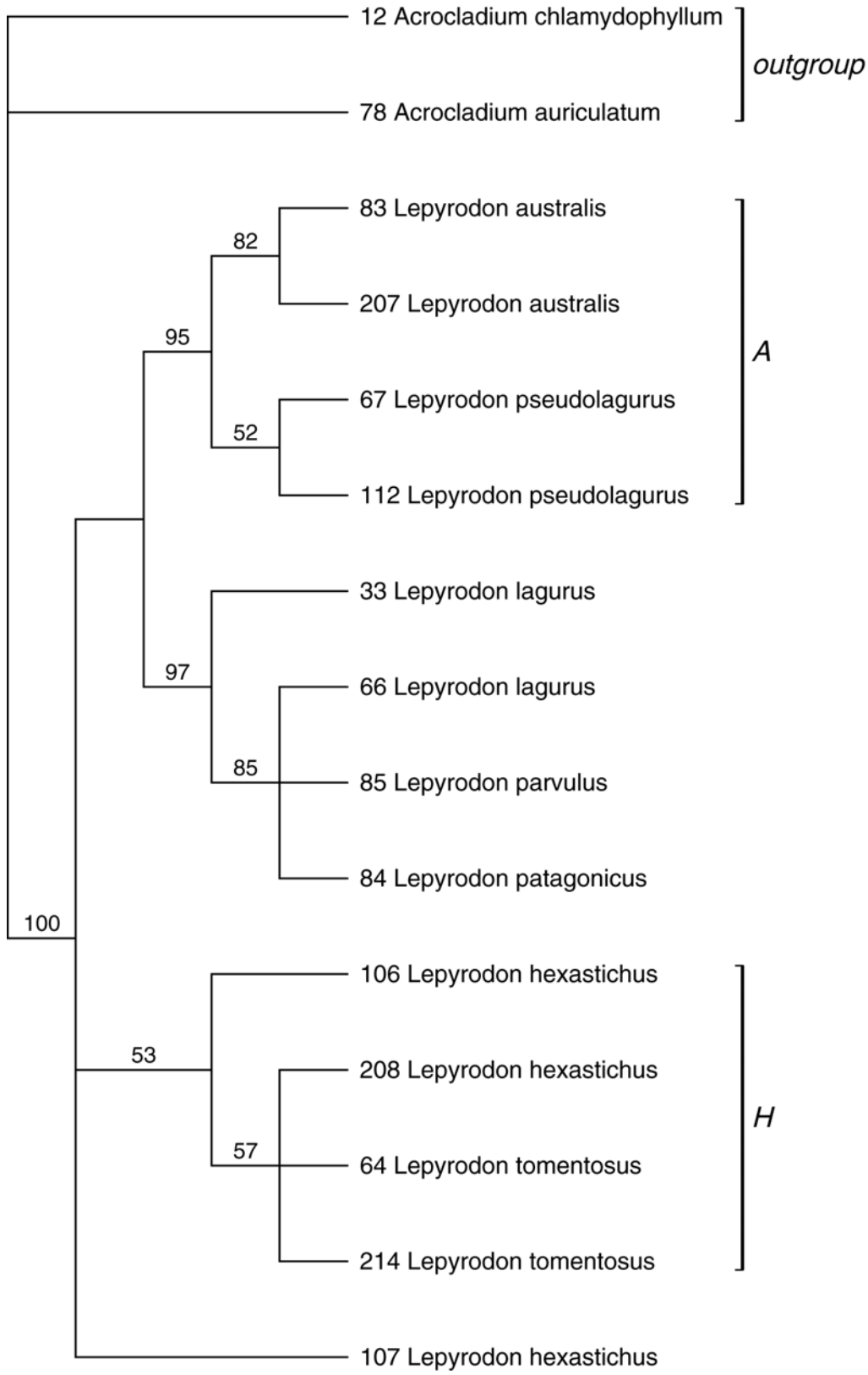

Figure 10: Maximum Likelihood (ML) cladogram of the *adk* non-coding regions of thirteen species of *Lepyrodon* and the outgroup species (Lscore: -1260.0568). Bootstrap values above branches are the result of a Maximum Parsimony analysis. For explanation of the clades referred to as 'outgroup', A, and H see text.

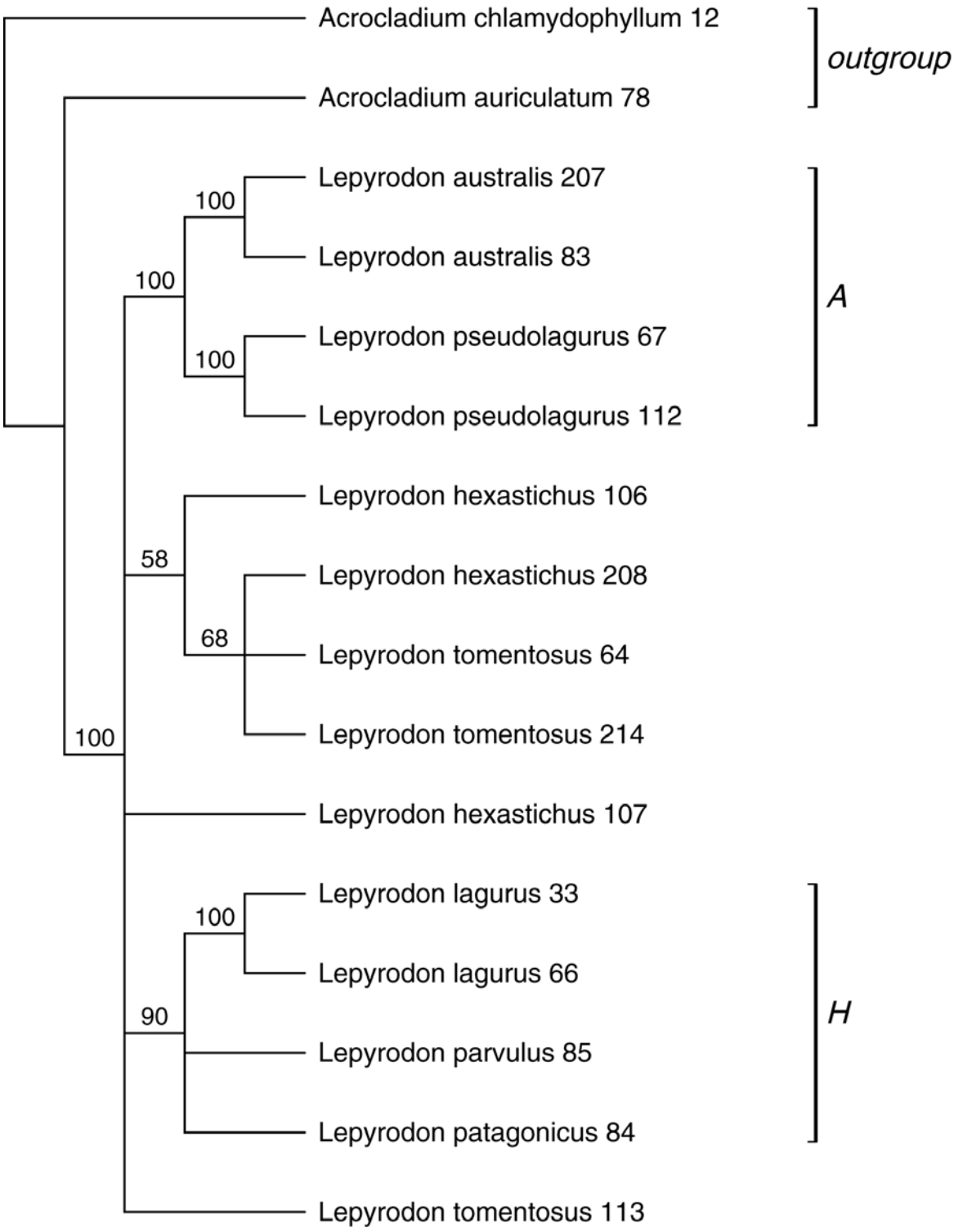

Figure 11: 50%-majority rule consensus cladogram resulting from a Bayesian Inference analysis of the complete data set (*adk* gene and ITS sequence data). Numbers above branches indicate the posterior probabilities as a percentage value. For explanation of the clades referred to as 'outgroup', H, and A see text.

exception is the monophyly of the Austral distributed taxa of *L. australis* and *L. pseudolagurus*.

The three specimens of *L. hexastichus* (sp. 106 Puerto Montt, sp. 107 Osorno, sp. 208 Valdivia) do not appear as a monophyletic group as one would expect. Two of the specimens (sp. 106 & 208) show close relationships to *L. tomentosus* from Costa Rica (sp. 214) and Chile (sp. 64). The third specimen (sp. 107) is in an ambiguous position to all taxa investigated in this study. The specimen of *L. tomentosus* from Mexico (sp. 113) does not appear in the group of the other two specimens of *L. tomentosus* (sp. 64 & 214), but belongs to a clade consisting of *L. lagurus* (sp. 84 & 85), *L. parvulus* (sp. 85) and *L. patagonicus* (sp. 84), of which all specimens originate from Chile (fig. 8 & 9).

Determining genetic distances. As mentioned above one result of the statistical analyses of the combined data set (tab. 18) performed in this study were the striking differences in variability between the single regions. Therefore I tested the variability of the combined data set to the *adk* non-coding region as the most variable data set.

The genetic distance within the genus *Lepyrodon* and in relation to its outgroup are depicted in appendix 8 and appendix 9. Results are listed as p-distances with standard errors. In appendix 8 the distance was computed from the combined ITS1, 5.8S nrDNA, ITS2 and *adk* data sets. Appendix 9, in contrast, shows the p-distances of the *adk* intron for the successfully sequenced specimens.

Combined data set. The genetic distances (p-distances) between the *Lepyrodon* specimens as well as between the genus and the outgroup species as derived from the phylogenetic analysis of the combined data set are described in the following paragraph (also compare appendix 8 and appendix 9).

The genetic distance separating *Acrocladium auriculatum* (N=1) from Chile and *Acrocladium chlamydophyllum* (N=1) is 1.40 %.

The genetic distance within *L. australis* from New Zealand (South Island, N=2) is 0.15 %.

The three specimens of *L. hexastichus* show a genetic distance of 0.15 % between specimens 107 and 106 as well as between specimens 106 and 208; the distance between specimens 107 and 208 is 0.30 %.

No a genetic distance (0.00 %) was found between the two samples of *L. lagurus* from southern Chile.

The difference within *L. pseudolagurus* from the South Island of New Zealand is 0.15 %.

The genetic distance within *L. tomentosus* is between 0.00 and 0.30 %. The genetic distance between the specimens from Costa Rica and Mexico is 0.15 % (sp.113 vs. sp. 214) and between the specimens from Costa Rica and Chile it is 0.30 %, whereas the two specimens which are geographically most widely separated (Chile and Mexico) were identical.

adk data set. The results of genetic distance of the separately analysed data set of *adk* non-coding regions (appendix 9) differ from those of the combined (*adk* & ITS) data set (appendix 8). The greatest genetic distances of all the pairs computed were those between *Acrocladium chlamydophyllum* and the specimens of *Lepyrodon*, ranging from 14.8 to 19.7 % (standard errors between 4.5 and 5.1 %). The genetic distance separating the two outgroup taxa, the Chilean species *Acrocladium auriculatum* and the New Zealand species *A. chlamydophyllum*, is 6.6 %. The relatively high standard error (3.2 %) for this distance is possibly caused by the low number of successfully sequenced nucleotides.

The genetic distances within the thirteen specimens of *Lepyrodon* ranged between 0.0 and 8.2 %. There was no infra-genomic variation within *L. australis* (0.0 %), *L. pseudolagurus* (0.0 %), and *L. lagurus* (0.0 %). A low infra-genomic distance was detected between the specimens of *L. tomentosus* (1.6 %) from Chile and Costa Rica, whereas the variation between the three specimens of *L. hexastichus* from Chile ranged between 1.6 and 3.3 %. No genetic distance was observed between the specimens of *L. parvulus* and *L. patagonicus*.

With 8.2 %, the distance between either *L. australis* or *L. pseudolagurus*, the taxa from New Zealand, to *L. tomentosus* from Costa Rica was the highest distance observed in the data set. In general, the samples from New Zealand were genetically quite distinct from the specimens from South America, pairs tested reaching mainly between 4.9 and 6.6 % distance. Within the group formed by the species *L. lagurus*, *L. patagonicus* and *L. parvulus* there was no difference detected between the four specimens under study. *Lepyrodon hexastichus* from Lago Riñihue (Prov. Valdivia,

specimen 208) was identical to *L. tomentosus* from Chile, and only a low variation to the specimen from Costa Rica was observed.

Conclusion. The results of this study, based on *adk* and ITS data and subsequent Maximum Likelihood (ML) analysis, show that the Australian/New Zealand species, *L. australis* and *L. pseudolagurus*, are monophyletic and sister to a second clade consisting of *L. lagurus*, *L. patagonicus*, *L. parvulus* from Chile and a specimen of *Lepyrodon tomentosus* from Mexico. The relationships within this clade remained unresolved. The third clade consists of two specimens of *L. hexastichus* from Chile, one specimen of *L. tomentosus* from Costa Rica, and another specimen of this species from southern Chile.

5.4 Discussion

5.4.1 Genetic results

When comparing the variability of the *Lepyrodon* data set in this study with the only published investigation of the *adk* gene in bryophyte taxonomy so far (Vanderpoorten et al., 2004), there are striking differences between the two studies.

In this study the same primers described in Vanderpoorten et al. (2004) were used to amplify parts of the *adk* gene. Therefore, results concerning length variation and variability should be comparable.

The data set of Vanderpoorten et al. (2004) comprised four outgroup species (7 accessions) and five ingroup species (25 accessions), whereas in the analysis described here two outgroup species and seven ingroup species (13 accessions) were used.

For the exons the *Lepyrodon* alignment revealed 312 nucleotides in length compared to 291 in *Hygroamblystegium* as sequenced by Vanderpoorten et al. (2004). The aligned intron sequences were 585 nucleotides in length in the *Lepyrodon* alignment whereas Vanderpoorten et al. (2004) aligned 618 nucleotides. This difference in intron length might be the result of several indels within the extremely variable data set in *Hygroamblystegium*.

There are big differences in variability between the data sets of *Lepyrodon* and *Hygroamblystegium*. Vanderpoorten et al. (2004) found 47.5 % variability in the *adk* gene, and, as expected, a higher variability in the introns (56.1 %) than in the exons (29.2 %). Even without the outgroup taxa there was a high variability within the *adk* data sets: 38.1 % for the *adk* and 22.0 and 45.6 % in the exon and intron alignment, respectively. In contrast, the results obtained from the data set of *Lepyrodon*, subject of this study, shows only 10.1 % variability in the *adk* region. Considering introns and exons separately, 12.6 % of the positions in the intron are variable and 5.1 % of those in the exon if a complete data set comprising all ingroup and outgroup taxa is used. Within the genus *Lepyrodon* and its 13 accessions the variability in the intron is 5.5 %.

Vanderpoorten et al. (2004) identified multiple copies of the *adk* gene within all individuals of *Hygroamblystegium* analysed. This is in contrast to the sequences of the *adk* gene in other bryophytes e.g. *Physcomitrella* (Schwartzenberg et al., 1998). Vanderpoorten et al. (2004) suggest that the high polyploid state of *Hygroamblystegium* enables the DNA to evolve independently and therefore may account for the presence of multiple copies of the *adk* gene within the individuals of *Hygroamblystegium*.

Unfortunately, there is no information available on the polyploidy status of *Lepyrodon*. An independent evolution of gene copies in *Hygroamblystegium* may well account for the high variability in the data set when compared to *Lepyrodon*. In the original sequences of the taxa used in this study only very few ambiguous positions appeared. They were therefore not identified further but rated as 'N' in the following analysis.

The ITS1 and ITS2 regions of *Hygroamblystegium* are also more variable including outgroup taxa (11.2 and 15.2 %) as well as analysed separately (9.7 % and 10.1 %) than in the data set of *Lepyrodon* with 9.2 % in ITS1 (ingroup alone 5.0 %) and 5.8 % (ingroup alone 1.8 %) in the ITS2. In contrast to the results of the ITS1 and ITS2 sequence variation in *Hygroamblystegium* (Vanderpoorten et al., 2004) in the *Lepyrodon* data set analysed here the ITS1 region revealed a higher degree of variation than the ITS2.

The length of the ITS1 region as reported by Vanderpoorten et al. (2001) for a data set of 39 species of pleurocarpous mosses, mainly representatives of the Amblystegiaceae, ranged from 280-340 bp in length and was therefore larger than in

the *Lepyrodon* data set. Also the variability in this region was higher in the data set of Vanderpoorten et al. (2001) than in this study.

A comparison of the GC-content with other nuclear regions is no possible as sequence data of other nuclear, especially non-coding regions, is lacking so far. However, compared to non-coding cpDNA the ITS displays a GC-content twice as high, similar to structural DNA such as tRNAs (compare Quandt & Stech, 2004), that might be attributed to the functional constrains of the ITS region (see Hershkovitz & Zimmer, 1996; Musters et al., 1990; van der Sande et al., 1992)

The length variation and GC-content in ITS2 sequences of *Lepyrodon* (compare tab. 17) as revealed by this study lies in the range reported by Quandt et al. (2004a) for a data set consisting of 63 species representing major lineages of pleurocarpous mosses. The authors describe length variations between 251 and 360 bp (mean 282.83) and a GC-content between 58.72 and 70.71 % (mean 65.53). The variability of the ITS2 in the genus *Lepyrodon* (1.8 %) seems quite low compared to that found e.g. in *Papillaria* (2.95 %) and *Meteorium* (4.27 %) by Quandt et al. (2004a). Taking into account that the genus *Lepyrodon* actually represents the family Lepyrodontaceae, the variability of the ITS2 appears even lower when compared to the ITS2 alignments of other families (Quandt et al., 2004a). The taxa of Brachytheciaceae investigated in their study revealed a variability of 9.83 %, the Lembophyllaceae 5.16 %, and the Meteoriaceae 8.64 %. The Lepyrodontaceae, however, are a very small family, comprising only seven species, compared to more than 500 species in the Brachytheciaceae, approx. 100 species in the Lembophyllaceae, and 100-150 species in the Meteoriaceae

In order to get an impression of the magnitude of the GC-content of the *adk* gene in *Lepyrodon*, this content is compared to that of another protein coding gene, the *rps*4 gene (cpDNA) in the pleurocarpous moss family Hypopterygiaceae (Blöcher, 2000). The GC-content of the coding regions of the *adk* in *Lepyrodon* is quite different from that of the *rps*4 sequence data observed in the Hypopterygiaceae. The mean GC-content in the *rps*4 gene of the Hypopterygiaceae comprising 612 bp was 28.3 %, whereas the mean GC-content of the *adk* in *Lepyrodon* is considerably higher reaching a value of 48.9 %. Also, the pattern in the GC-content is different in the two genes compared. In the *rps*4 gene the GC-content of the first codon position was highest with 42.0 %, that taking in the second position was 33.9 %, and the lowest content was found in the third position with 8.9 % (Blöcher, 2000). In contrast to

these results, the parts of the codons sequenced from the *adk* gene in the *Lepyrodon* data set show their highest GC-content in third codon position. Both studies used a comparable number of taxa.

5.4.2 Phylogenetic and taxonomic results

Lepyrodon australis. Hooker (cit in Allen, 1999; 1867), Brotherus (1909a), Dixon (cit in Allen, 1999; 1927), and Sainsbury (1955) considered *L. australis* as morphologically closely related to *L. hexastichus. L. hexastichus* was formerly described as *L. implexus* by Mitten (in Hooker, 1867). Allen (1999), in contrast, found these two species *L. australis* and *L. hexastichus* distinguishable e.g. by characters of the leaf apices as well as the occurrence of flagellate branches in *L. australis*. Instead, Allen (1999) drew attention to the similarities between *L. australis* and the widespread South American species *L. tomentosus*. He found that *L. australis* united characters of the three expressions of *L. tomentosus* he described (Allen, 1999). Allen (1999) justifies the separation of *L. australis* as a distinct species rather than as a variety of *L. tomentosus* by endostome characters and a geographic isolation of the taxa. Our genetic data, based on a combined data analysis of the ITS1 and 2 and the *adk* gene as well as a separate analysis of the respective genes, revealed *L. australis* as the closest relative of *L. pseudolagurus* with high bootstrap support for the Australian/New Zealand clade.

Lepyrodon hexastichus. *L. hexastichus* was seen as a minor expression of *L. tomentosus* by Mitten (1869). In Allen's (1999) view *L. hexastichus* has more morphological characters in common with *L. patagonicus* e.g. its short pointed leaves. Especially some plants from the Juan Fernández Islands appeared unusually large and therefore closely resembled some expressions of *L. patagonicus* and *L. tomentosus*. However, according to Allen (1999) *L. hexastichus* is distinguished from *L. patagonicus* by its smooth, narrow upper leaf cells and its plane to incurved leaf margins. It is delimited from *L. tomentosus* by the lack of hair-points and by having very strong leaf margin serrations. The three accessions of *L. hexastichus* from the region Los Lagos (Chile) used in the study at hand showed genetically close affinities to two accessions of *L. tomentosus*.

Lepyrodon patagonicus. The newly described species *L. patagonicus* (Allen, 1999) from Chile belongs to a group of species with plicate leaves. It was formerly regarded as a variety of *Lepyrodon tomentosus* (*L. tomentosus* var. *patagonicus* Card. & Broth) and shares some characters, e.g. leaf form, with the type expression of *L. tomentosus*. *L. patagonicus* is distinguished from the other species, especially from *L. tomentosus*, by the galeate leaf apex which has short, broad prorate leaf cells. The robust colonies it forms in the area near its northern limit of distribution and on the Juan Fernández Islands closely resemble those of *L. tomentosus*. In the phylogenetic analysis at hand *Lepyrodon patagonicus* belongs to a clade consisting of two representatives of the 'smooth leaved' species *L. lagurus* and *L. parvulus*. The Maximum Likelihood analysis revealed no further relationship within this clade.

Lepyrodon tomentosus. Allen (1999) states that *L. tomentosus* is a remarkably variable species. He distinguishes three morphological expressions of *L. tomentosus* which are more or less separated geographically but with intermediate expressions where their areas of distribution overlap.

The type expression of *L. tomentosus* occurs in the Andes of western South America and is described as a robust plant with large, strongly plicate leaves but also with 'smooth' branch leaves like those found in *L. lagurus* (Allen, 1999). The accession no. 214 from Costa Rica with strongly plicate leaves represents the type expression in the study at hand.

The northern expression, *L. tomentosus* var. *latifolius*, occupies an area from southern Mexico through Panama to southeast Brazil. The size of the plant is moderate, and the "*lagurus*-type" branch leaves can occupy more than half of the branch. An extreme expression of *L. tomentosus* var. *latifolius* (Allen, 1999) is the expression identical to the type specimen of *L. duellii* as described by Crum (1984) which is almost entirely covered with *lagurus*-type branches. This type is represented in this study (sp. 113) by the isotype of *L. duellii*.

The distribution range of the southern type expression in *L. tomentosus* covers southern Chile and southwestern Argentina. The plants are usually smaller than in the other two expressions. The specimen no. 64 in the study at hand resembles this southern type.

Two specimens of *L. tomentosus*, one from Chile, the other from Costa Rica, representing the type expression and the southern expression as described in Allen (1999), are closely related on the base of the sequence data used in the analysis. However, they form a clade together with two specimens of *L. hexastichus* that is not well resolved concerning the monophyletic status of either one of the species.
The northern expression, *L. tomentosus* from Mexico, the type locality of *L. duellii*, is within the clade of *Lepyrodon lagurus*, *L. parvulus* and *L. patagonicus*. That means this specimen, which has entirely "*lagurus* type" branches as described by Allen (1999), is closer related to *L. lagurus* than to *L. tomentosus* in this study

Lepyrodon lagurus, L. pseudolagurus. The group of smooth leaved *Lepyrodon* species consists of three species, i.e. *L. lagurus, L. pseudolagurus, and L. parvulus* (Allen, 1999).
L. lagurus plants from South America have formerly been considered conspecific with specimens from New Zealand as plants from the two areas are difficult to distinguish based on morphological characters. Justified by differences in peristomal characters the material from New Zealand is treated as *L. pseudolagurus* by Allen (1999). *L. lagurus* is polymorphic throughout its range, e.g. plants from higher elevations are in general smaller and have less tomentum than those from lower elevations, e.g. Tierra del Fuego. The separation of *L. pseudolagurus* with Australian/New Zealand distribution from material of *L. lagurus* from Chile based on morphological and anatomical data by Allen (1999) is supported by genetic data in this study.

Lepyrodon parvulus. The smaller high elevation plants of *Lepyrodon lagurus* approach *L. parvulus* in size, but differ e.g. in leaf form. *L. parvulus* is mostly stenotypic throughout its range and differs from the other smooth leaved species e.g. by its smaller size, a more pronounced creeping habitus and by the existence of full sized male plants. The smaller leaves almost always separate it from *L. lagurus*. *L. lagurus* from high elevations in the northern part of its Chilean range occasionally has similarly small leaves. These collections of *L. lagurus*, however, differ from *L. parvulus* in having ovate leaves with inflexed upper leaf margins that are weakly serrate. As in other species the specimens of *L. parvulus* found on the Juan Fernández Islands were morphologically different from the mainland taxa (Allen, 1999).

In this study, *Lepyrodon parvulus* appears within a monophyletic group of four species which include two accessions of *L. lagurus* from Chile as well as one accession each of *L. patagonicus* from Chile and *L. tomentosus* from Mexico. The relationship within this group is not resolved, except for the monophyly of the *L. lagurus* specimens. The geographical distance between the two samples of *L. lagurus* investigated was quite high. Specimen no. 33 is from Punta Arenas at 53º 24' S and specimen no. 66 from Parque Nacional Conquillio at 38º 39' S, but they appear still more closely related to each other than either of them to *L. parvulus*, *L. patagonicus* or the Mexican specimen of *L. tomentosus*. Thus, the results of the genetic analysis support the species status of *L. patagonicus* (Allen, 1999) and *L. parvulus*. This is possibly also true for *L. tomentosus*, the holotype of *L. duellii*, but this has to be confirmed by further investigations of at least one more genetic marker and additional material of *L. tomentosus* from Mexico.

On "preliminary and superficial examination" (Buck, 1998) the Lepyrodontaceae split into two clearly distinguishable groups, one represented by *L. lagurus* and the other by *L. tomentosus*. According to Buck (1998) these groups might even deserve consideration on a higher taxonomic level. These suggestions are not further discussed by Allen (1999). However, when closely analysing Allen's descriptions of the *Lepyrodon* species and the affinities between them it is notable that morphological similarities only occur within two distinct groups. Within the 'plicate leaved' group, an overlapping of characters occurs between *L. australis* and *L. tomentosus*, between *L. tomentosus* and *L. hexastichus*, between *L. tomentosus* and *L. patagonicus*, and between *L. hexastichus* and *L. patagonicus*. Within the 'smooth leaved' group Allen (1999) detected similarities between *L. parvulus* and *L. lagurus* as well as between *L. lagurus* and *L. pseudolagurus*.
However, results of Hedenäs (2001), who investigated the relationship between morphological characters and habitat, indicated that the character 'plicate stem leaves' was highly significant for taxonomic grouping rather than related to environmental factors. Similarly, this was one of the characters Buck (1998) suggested as being useful for distinguishing taxonomic groups within *Lepyrodon*. Allen (1999) described the occurrence of smooth leaves in the type expression of *L. tomentosus*, a species with plicate leaves. This might reflect the morphological transparency within *Lepyrodon*.

Another character, 'dwarf males' as suggested in Buck (1998) valuable for grouping within the genus *Lepyrodon*, turned out to be not significantly related to taxonomic grouping nor to environmental factors in Hedenäs' analysis (2001).
On the other hand the double peristome in *L. pseudolagurus*, proved to be valuable to separate this taxon from *L. lagurus* (Allen, 1999). All other species in the genus lack a double peristom, and have only the endostome left. The reduction of the peristom is regarded as an adaption to epiphytism (Hedenäs, 2001). All species including *L. pseudolagurus* grow epiphytically, also *L. pseudolagurus* is known to grow as epiphyte as well as on soil and rock. *L. lagurus* and *L. tomentosus* are also known to grow on rock and soil.

The genetic data are in contradiction with the species concept proposed for *Lepyrodon* in Allen (1999) but this analysis also failed to resolve an unambiguous phylogeny within *Lepyrodon*.
Genetic relationships were identified between rather than within the former mentioned plicate and smooth leaved group. A monophyletic group consists of the plicate *L. australis* and the smooth leaved *L. pseudolagurus*. Also the smooth leaved species *L. lagurus*, *L. parvulus* and plicate leaved *L. patagonicus* form a well-supported monophyletic group and perhaps include the isotype of the former recognized species *L. duellii* Crum (Crum, 1984).
A correspondence between genetic and morphological data can be found between *L. hexastichus* and *L. tomentosus*. Also on the basis of genetic data, so far the species status of *L. hexastichus* could no be confirmed.

5.4.3 Biogeographical implications

The most obvious result of this study is the monophyly of the Australian/New Zealand species *L. pseudolagurus* and *L. australis*. They form two well separated sister species in an 'east austral' clade supported by high bootstrap values and low genetic distances. The distribution of *L. pseudolagurus*, a species which is commonly found with sporophytes (Allen, 1999), comprises a greater area (Tasmania, Victoria, New Zealand, Campbell Island) than that of *L. australis* (Tasmania/ New Zealand) suggesting that the distribution pattern of the former might be related to its ability of spore dispersal. Germination data for *L. australis* from van Zanten (1978) suggests

that this species is unable to tolerate any treatment correlated with long distance dispersal (e.g. desiccation and freezing) for longer than seven months. This restricts the species in extending its distribution range to South America. There were no data available for any other species in the genus *Lepyrodon*, but possibly the fact that there are different species in South America and Australia/New Zealand may be explained by the restricted ability this genus hasin long distance dispersal. The same pattern was found in the southern temperate *Hypopterygium rotulatum* s.l. (Pfeiffer, 2000b). Based on the inability of spore survival after long distance dispersal Frey et al. (1999) concluded that *Lopidium concinnum* which occurs in southern South America as well as in Australia/New Zealand was separated between these regions since *c*. 80 Myr BP.

In contrast to the former vicariance based explanation for disjunct patterns in the southern temperate hemisphere, Muñoz et al. (2004) tested with statistical methods if the floristic affinities among southern hemispheric landmasses outside the tropics could be better explained by near-surface wind transport (direction dependent) or geographic proximity (direction independent). They used four different data sets: mosses with 601 species, liverworts (461 species), lichens (597 species) and the pteridophytes represented by 192 species. They found a stronger correlation between floristic similarity and maximum wind connectivity, in mosses, liverworts and lichens than with geographic proximity. From their analyses they concluded that wind is the main force driving current plant distributions in these groups.

A recent analysis of the distribution of southern hemispheric plant taxa indicated that most plant distribution patterns are not congruent with the geological sequence of breakup history Gondwana (Africa(NZ(sSAM, AUS))) as most plant distribution patterns (sSAM(AUS,NZ)) exhibit a closer relationship between Australia and New Zealand (Sanmartín & Ronquist, 2004). This suggests dispersal events between Australia and New Zealand as already discussed (Pole, 1994; Pole, 2001) but not necessarily between southern South America and Australia/New Zealand.

The sister clade to the east austral clade comprises four species restricted to southern Chile, and maybe also the isotype of *L. duellii* from southern Mexico. If the specimen of *L. duelli* is included in this clade the clade would show a southern temperate – northern tropical disjunct distribution pattern as also reported in e.g. *Pyrrhobryum* (McDaniel & Shaw, 2003).

Perhaps the forming of an 'arid diagonal' (Villagrán et al., 1998 and discussion therein) separating southern and central Chile from tropical South America caused the separation of the specimens of the *L. lagurus*-clade from *L. duelli*, resulting in a distinct taxon *L. duelli* in the north.

One could conclude, that the clade consisting of the Australia/New Zealand *Lepyrodon* species and its sister clade consisting of *L. lagurus, L. patagonicus, parvulus*, (and perhaps to *L. duellii*) was separated by the breakup of Gondwana and the separation of the fragments of the continent starting ca. 80 Myr BP (McLoughlin, 2001). Thus the distribution pattern can be seen as a result of vicariance.

As another specimen of *L. duelli* was reported from Honduras (specimen 109) a survey of this specimen as well as a variety of *L. tomentosus* specimens is needed to clarify its taxonomic position. Although dispersal events can account for the similarities between e.g. the Central American and South American moss floras as suggested by Delgadillo (2000).

An inclusion of *L. duellii* in the clade of *L. tomentosus* and *L. hexastichus* despite its taxonomic status (low probabilities for this with Bayesian statistic), would be in concordance with the existing distribution pattern of *L. tomentosus* occurring from southern South America continuously along the Andes, central America to Mexico with an outlier in southeast Brazil. The morphological differentiation within *L. tomentosus* resulting in the description of morphological distinct expressions ('northern', 'southern' and 'type' expressions, Allen, 1999) may well show a species which is in the process of speciation. Intermediate forms in the area where the morphological expressions overlap may account for speciation in progress. *L. tomentosus* shows a similar distribution pattern as *Monoclea gottschei* in South America (Meißner et al., 1998). A temperate ancestor may have spread north along the Andean range and to southeast Brazil. The habitats in northern South America are well above the lowland rainforest, in the upper montane forest and the páramo/puna region (Gradstein et al., 2001). Thus the spread of *L. tomentosus* must be related to the uplift of the Andes *c*. 10 Myr ago (Hartley, 2003) which provided a suitable habitat for its spread to the north and *L. tomentosus* is the most recent taxon within *Lepyrodon*. However the phylogenetic results show either a polyphyletic relationship of the South American clades (*L. tomentosus* and *L. lagurus*) in the Bayesian analysis or a starlike cladogram with five separate clades.

6 Molecular circumscription and biogeography of the genus *Acrocladium* (Bryopsida)

6.1 The genus Acrocladium

6.1.1 Status of *Acrocladium*

Despite the early recognition of the genus *Acrocladium* (Mitten, 1869), its familial position has been discussed controversially since. It has been shifted from the Lembophyllaceae (Brotherus, 1925a; Fleischer, 1923a) to the Amblystegiaceae (Ochyra & Matteri, 2001; Vitt, 1984) and most recently to the Plagiotheciaceae (Pedersen & Hedenäs, 2002).

Brotherus (1925a) described two species in the genus *Acrocladium*: *A. auriculatum* (Mont.) Mitt. from southern South America and *A. chlamydophyllum* (Hook.f. & Wils.) Broth. from New Zealand, eastern Australia, Tasmania and adjacent islands. Since then there has been disagreement among bryologists whether the genus includes one or two species and whether the populations in Chile and Argentina are identical with those in New Zealand, Australia, and Tasmania. Accordingly, collected specimens of *Acrocladium* from Chile were either named *A. auriculatum* (e.g. Brotherus, 1925a; Deguchi, 1991; Mitten, 1869) or *A. chlamydophyllum* (e.g. Cardot, 1908). Brotherus (1925a) distinguishes two species and Andrews (1949), Karczmarz (1966) and Fife (1995) supported the view that the two taxa are different species. In contrast, Dixon (1928), Sainsbury (1955) and He (1998) considered both taxa as variations of the same species, using the name '*A. auriculatum*' as the older epitheton.

In fact, the variability of the specimens of *Acrocladium* from southern South America and Australia/New Zealand is quite high. Brotherus (1925a) differentiated between two species based on leaf auricles and characteristics of the leaf costa. Karczmarz (1966) did not take into account the characteristics of the costa and distinguished two species based on leaf shape and presence versus absence of auricles.

Due to the problematic distinction of the two species based on anatomical and morphological characters described above, an attempt has been made in this study to evaluate the differences based on molecular data.

6.1.2 Distribution of *Acrocladium*

When studying phylogenetic relationships, biogeography and historical dispersal events also play an important part in understanding current conditions.
Acrocladium auriculatum occurs in Chile from the Cautín in the north to Magallanes in the south as well as on the Juan Fernández Islands (Robinson, 1975). In Argentina the species occurs from Neuquén toTierra del Fuego (Ochyra & Matteri, 2001). Van Zanten (1971) and Gremmen (1981) additionally report a disjunct population of the species from subantarctic Marion Island.

6.1.3 Ecology of *Acrocladium*

Acrocladium chlamydophyllum occurs epiphytically (on branches), epilithically (on rocks) as well as on rotten logs and soil on the forest floor (e.g. Beever et al., 1992; Sainsbury, 1955).
Pfeiffer (2001) describes an *Acrocladium chlamydophyllum*-dominated bryophyte community on the South Island of New Zealand. She states that the species dominates the forest floor at montane and subalpine altitudes "[…] on moderately shaded sites on west-orientated slopes […]".
On the subantarctic Macquarie Island the species occurs at altitudes between 10-200 m (Seppelt, 2004). Voucher information from the selected specimens in Seppelt (2004) e.g. "wet grassland", "boggy herbfield", suggests rather moist habitat conditions.
Gremmen (1981) provides the following voucher information for the specimen of *Acrocladium* (Gremmen 02.03; 19-12-1974) collected on Marion Island: "forming a mat under herb layer of *Acaena*, sheltered".
The locations where specimens of *Acrocladium auriculatum* were found and collected by the author indicate that this species can take on epiphytic and epilithic growth forms, and might as well grow on rotten logs and bare soil of the forest floor (own observations, Karczmarz, 1966; Ochyra & Matteri, 2001; Robinson, 1975).

6.2 Material & Methods

***Plant material*.** Plant material was either collected by the author during a field trip of the BryoAustral project to Chile in 2001, or originates from herbarium specimens. Specimens of *Acrocladium chlamydophyllum* as well as *A. auriculatum*, especially the specimen from Marion Island were kindly provided by Dr. B. O. van Zanten (Herbarium and University of Groningen). Specimens of *Acrocladium chlamydophyllum* and *Lepyrodon pseudolagurus* were collected during the BryoAustral project expedition to New Zealand in 1998. Duplicates are preserved in the herbaria in Christchurch (CHR), Bonn (BONN) and Berlin (B). Sequences available in GenBank were also used. All specimens used in the analyses are listed in (Appendix 10) including further voucher information.

Twenty-four specimens of *Acrocladium* were selected. The selection consisted of nine accessions from Chile, two from Argentina, five from Australia (two from New South Wales, three from Tasmania) and six specimens that represent the North and South Island of New Zealand. Furthermore, a specimen from Macquarie Island and a specimen from Marion Island (1.800 km southeast of Africa) were included. Thus, the taxon sampling took into account the geographical provenance of the genus with respect to the description of two disjunctly distributed species, one from southern South America and the second one from Australia and New Zealand (Andrews, 1949; Brotherus, 1925a; Fife, 1995; Karczmarz, 1966).

The following six species were selected as outgroup to *Acrocladium* and were included in the analyses: *Herzogiella seligeri, Plagiothecium undulatum, Plagiothecium denticulatum, Taxiphyllum taxirameum* and two taxa of *Lepyrodon*, in previous analyses identified as sister genus to *Acrocladium* (e.g. Quandt et al., 2004b, own data compare chapter 4).

The sequences of the *rps*4 and *trn*L used in this analysis were extracted from GenBank for the following taxa: *Herzogiella seligeri, Plagiothecium undulatum, Plagiothecium denticulatum, Taxiphyllum taxirameum*. Furthermore, for the taxa *Acrocladium chlamydophyllum, A. auriculatum* and *Lepyrodon* sequences of the *trn*L and ITS2 were kindly provided by Dr. Dietmar Quandt, Dresden (table 20). The

geographical origin of the specimens of *Acrocladiumn* successfully sequenced is shown in figure 12 on a global scale and in figure 13 (South America) and figure 14 (New Zealand) on a regional scale.

Table 20: List of investigated specimens of *Acrocladium* with EMBL accession numbers for the regions sequenced. Voucher numbers and the herbaria where the specimens are kept and country of origin are listed. ITS2 sequences of *A. auriculatum* and *A. chlamydophyllum* were kindly provided by Dr. Dietmar Quandt (Dresden). For detailed voucher information see Appendix 10.

No.	*taxon*	*trn*L-*trn*F	Rps4	ITS	adk	country of origin	Voucher label	herbarium
12	*Acrocladium chlamydophyllum* (Hook.f. & Wilson) Muell. Hal. & Broth.		AJ862339	AJ862495 (ITS1) AF509863 (ITS2)	AJ863571	New Zealand	BRYO AUSTRAL W. Frey 98-T154 B	W. Frey, Berlin
78	*Acrocladium auriculatum* (Mont.) Mitt.		AJ862338	AJ862491 (ITS1) AF543550 (ITS2)	AJ854491	Chile	Rolf Blöcher No. 49	J.-P. Frahm, Bonn
162	*Acrocladium chlamydophyllum* (Hook.f. & Wilson) Muell. Hal. & Broth.	AJ862672				Australia	R. D. Seppelt 15801	J.-P. Frahm, Bonn
165	*Acrocladium auriculatum* (Mont.) Mitt.	AJ862671				Argentina	J. Eggers ARG 1/3	J.-P. Frahm, Bonn
171	*Acrocladium cf. chlamydophyllum* (Hook.f. & Wilson) Muell. Hal. & Broth.	AJ862676		AJ862690		New Zealand	Ben O. van Zanten 00 11 376	B. O. v. Zanten, Groningen, Netherlands
178	*Acrocladium cf. chlamydophyllum* (Hook.f. & Wilson) Muell. Hal. & Broth.			Submitted to EMBL		Australia	Ben O. van Zanten 82.02.812A	B. O. v. Zanten, Groningen, Netherlands
185	*Acrocladium auriculatum* (Mont.) Mitt.	AJ862674		AJ862692		Chile	BRYO AUSTRAL Rolf Blöcher no. 261	J.-P. Frahm, Bonn
186	*Acrocladium auriculatum* (Mont.) Mitt.	AJ862675		AJ862693		Chile	BRYO AUSTRAL Rolf Blöcher no. 50	J.-P. Frahm, Bonn
189	*Acrocladium auriculatum* (Mont.) Mitt.	AJ862673				Chile	BRYO AUSTRAL J.-P. Frahm no. 2-7	J.-P. Frahm, Bonn

Distribution maps. Regional maps of the origin of *Acrocladium* specimens were constructed using the web-page www.planiglobe.com (Körsgen et al., 2004). Dots were generated by adding geographical coordinates of collection localities as indicated on the voucher labels of the specimens. The map showing the world wide distribution of *Acrocladium* was constructed using 'online map creation' OMC (www.aquarius.geomar.de) provided by M. Weinelt, (2004) which uses 'The Generic Mapping Tools' (GMT, Wessel & Smith, 1995).

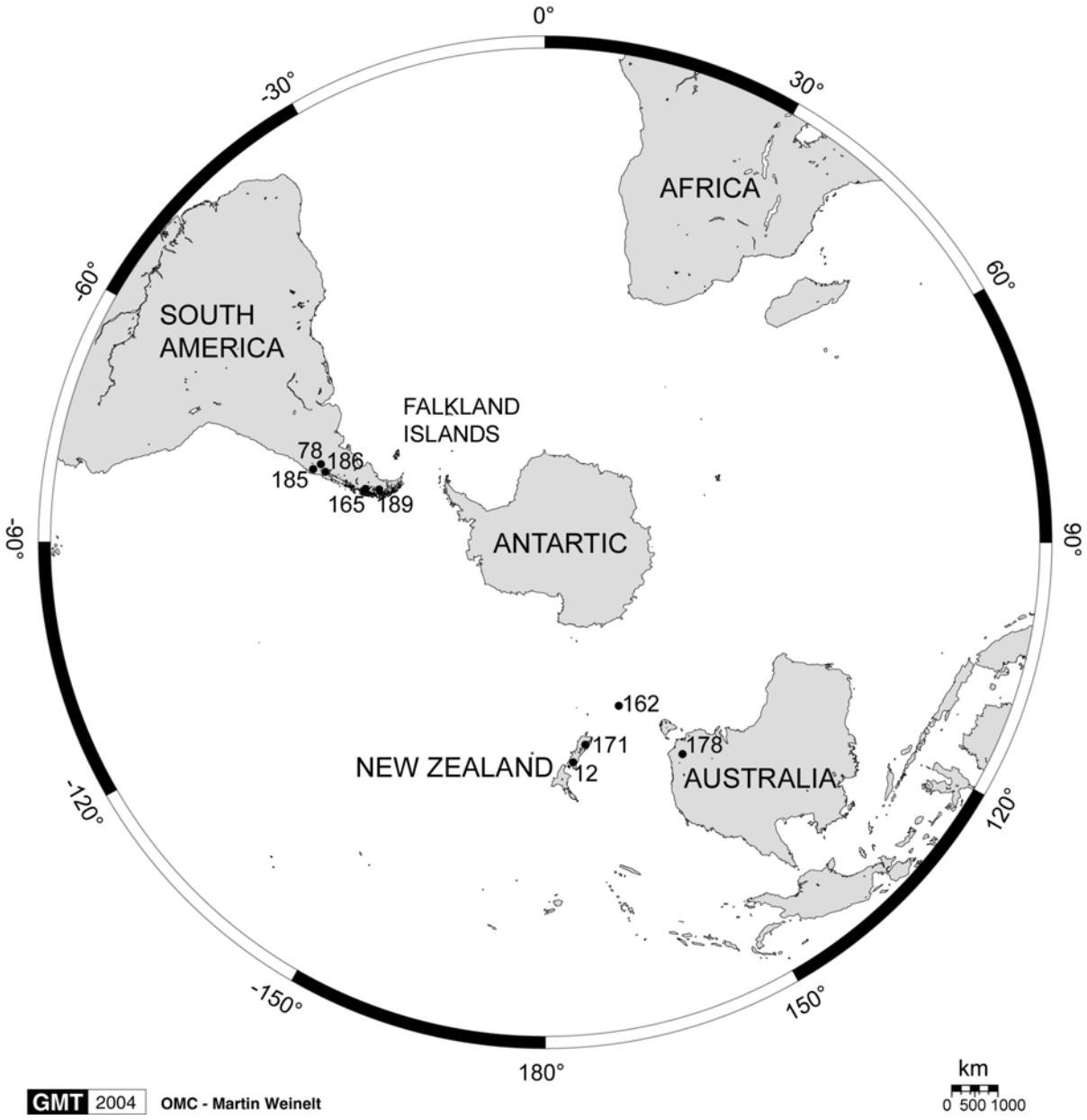

Figure 12: Geographical origin of all *Acrocladium* specimens used for this study. Specimens from South America are *Acrocladium auriculatum*, specimens from Australia, New Zealand and Macquarie Island are *A. chlamydophyllum*. Numbers are specimen numbers.

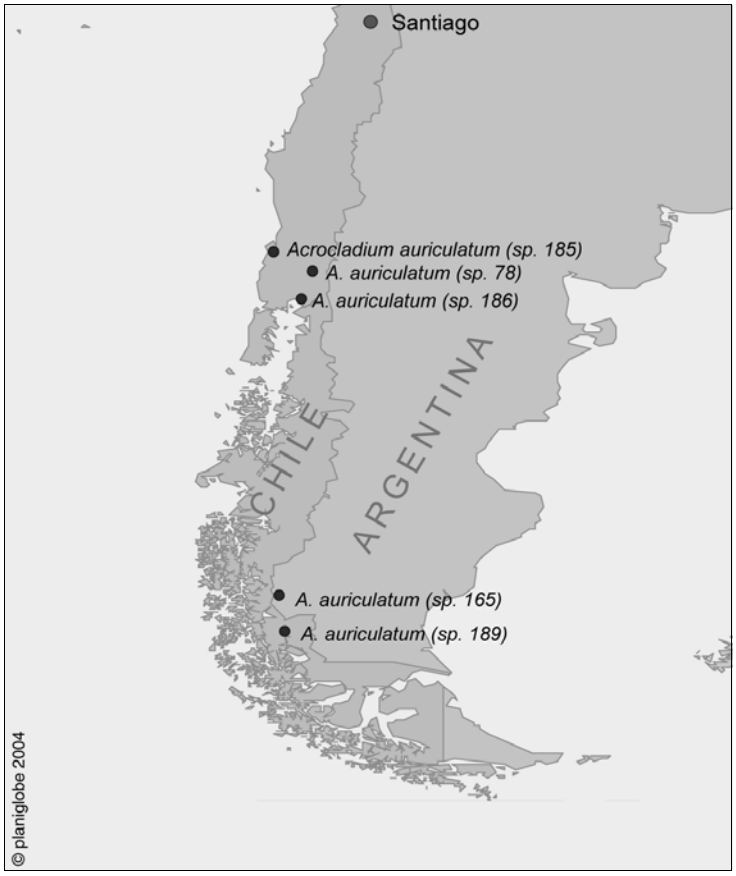

Figure 13: Geographical origin of the *Acrocladium* specimens from South America used for this study. Numbers in brackets are specimen numbers.

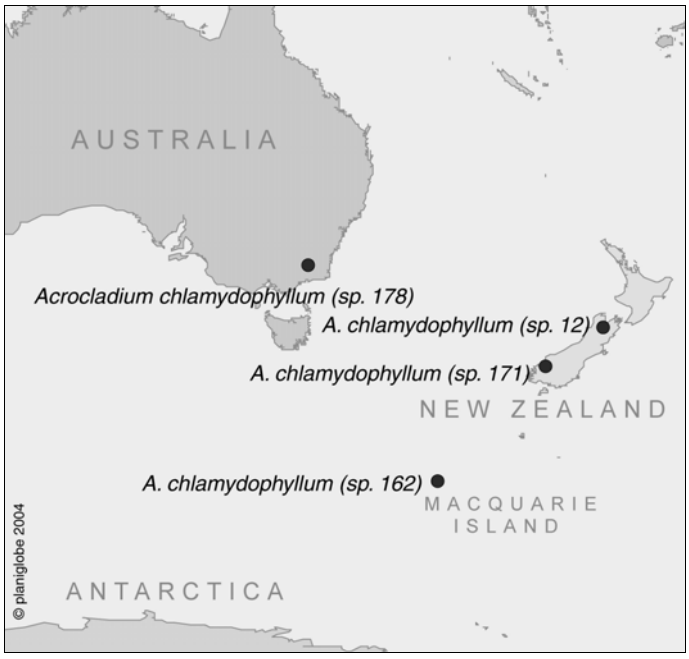

Figure 14: Geographical origin of the *Acrocladium* specimens from Australia, New Zealand and Macquarie Island used for this study. Numbers in brackets are specimen numbers.

DNA isolation, PCR and sequencing. Prior to DNA extraction the plant material was thoroughly cleaned with distilled water and additionally treated by ultrasonic waves for 2-4 minutes. Success of cleaning was checked by examining the plants under a binocular microscope. Remaining contaminations e.g. with algae and fungi were removed mechanically. Isolation of DNA was carried out following the CTAB technique described in Doyle & Doyle (1990).

PCR amplifications (Biometra TriBlock thermocycler, PTC-100 MJ Research) were performed in 50 µl–reactions containing 1.5 U *Taq* DNA polymerase (PeqLab), 1 mM dNTPs-Mix, nucleotide concentration 0.25 mM each (PeqLab), 1x buffer (PeqLab), 1.5 mM $MgCl_2$ (PeqLab) and 12.5 pmol of each amplification primer. PCR products were purified using the QIAquick purification kit (Qiagen). Cycle sequencing reactions (half reactions) were performed using a PTC-100 Thermocycler (MJ Research) in combination with the ABI Prism^{TM} Big Dye Terminator Cycle Sequencing Ready Reaction Kit with Amplitaq-DNA polymerase FS (Perkin Elmer), applying a standard protocol for all reactions. Extension products were precipitated with 40 µl 75 % (v/v) isopropanol for 15 min at room temperature, centrifuged with 15,000 rpm at 25°C, and washed with 250 µl of 75 % (v/v) isopropanol. These purified products were loaded on an ABI 310 automated sequencer (Perkin Elmer) and electrophoresed. For cycle sequencing 10 µl–reactions were used containing 3 µl of Big Dye Terminator Cycle Sequencing premix. Sequencing reactions were performed on two independent PCR products generated from each sample in order to verify the results.

All PCR products were sequenced using two primers. For amplifying and sequencing the non-coding regions of the chloroplast DNA a modification of primer C (Quandt et al., 2000) as well as primer F, originally designed by Taberlet et al. (1991) were employed. Primers used to amplify the *rps*4 gene were those described in Nadot et al. (1994), ‘trnS’ and ‘rps5’ (table 21). Primers for amplifying and sequencing the ITS region (ITS4-bryo and ITS5-bryo) based upon the primers “ITS4” and “ITS5” respectively, designed and named by White et al.(1990), were slightly modified with respect to bryophytes (Stech, 1999). The primers ITS-C and ITS-D (Blattner, 1999) were modified for this study (ITS-D_bryo and ITS-C_bryo) and additionally used for sequencing reactions (table 22).

The amplified *adk* region started about 196 base pairs (bp) downstream of the 155th codon and ended at the 257th codon of the *adk* gene isolated from the moss species

Physcomitrella patens (Y15430, Schwartzenberg et al., 1998). Coding and non-coding regions were identified by comparison with moss sequences available from GenBank (e.g. Vanderpoorten et al., 2004). Primers used for amplification of the *adk* gene (table 23) were those described in Vanderpoorten (2004).

Table 21: Primer sequences used for amplification and sequencing of the *trn*L region and *rps*4 gene. Underlined nucleotides represent changes (Quandt et al., 2000) with respect to the original primers of Taberlet (1991).

Primer	Sequence	Data source
*trn*S	TAC CGA GGG TTC GAA TC	Nadot et al. 1994
*rps*5	ATG TCC CGT TAT CGA GGA CCT	Nadot et al. 1994
*trn*L-C_mosses	CGR AAT TGG TAG ACG CTA CG	Quandt et al. 2000
*trn*L-F	ATT TGA ACT GGT GAC ACG AG	Taberlet et al. 1991

Table 22: Primer sequences used for amplification and sequencing of the ITS region. Underlined nucleotides represent changes with respect to the original primers of Blattner (1999).

Primer	Sequence	Data source
ITS-C bryo	GCA ATT CAC ACT ACG TAT CGC	Blattner 1999
ITS-D bryo	CTC TCA GCA ACG GAT ATC TTG	Blattner 1999
ITS4-bryo	TCC TCC GCT TAG TGA TAT GC	Stech 1999
ITS5-bryo	GGA AGG AGA AGT CGT AAC AAG G	Stech 1999

Table 23: Primer sequences used for amplification and sequencing of the *adk* gene.

Primer	Sequence	Data source
F	GAA GAA GCC AGA AAA CTG GGC	Vanderpoorten et al. 2004
R	GTC ACC CCA TCT TCA GCA AC	Vanderpoorten et al. 2004
1F	AAG CTT TTC CCG TAA GT	Vanderpoorten et al. 2004
2R	ACT TAC GGG AAA AGC TT	Vanderpoorten et al. 2004
3R	GGT CCC CTG GGT AAT AAC	Vanderpoorten et al. 2004
4F	TTT CAT CCC ATC GGT GG	Vanderpoorten et al. 2004

For amplifying and sequencing the chloroplast and nuclear region different protocols have been applied. For the *trn*L-F region and the *rps*4 gene the PCR program was performed with the following settings: 2 min. 94ºC, 35 cycles (1 min. 94ºC, 1 min. 55ºC, 1 min. 72ºC) and a 5 min. 72ºC extension time, cycle sequencing settings: 29 cycles (5 sec. 96ºC, 4 min. 50ºC).

The ITS region was amplified using a protocol consisting of: 5 min. 94ºC, 35 cycles (1 min. 94ºC, 1 min. 48ºC, 1 min. 72ºC) and a 5 min. 72ºC extension time, cycle sequencing settings: 25 cycles (30 sec. 96ºC, 15 sec. 50ºC, 4 min. 60ºC). According to Vanderpoorten et al. (2004) the following PCR protocol was used to amplify parts

of the *adk* gene : 2 min. 97ºC, 30 cycles (1 min. 97ºC, 1 min. 50ºC, 3 min. 72ºC) and a 7 min. 72ºC extension time. For more detailed information compare Vanderpoorten et al. (2004).

All sequences will be deposited in EMBL, accession numbers are listed in table 20, the alignments are available on request from the author.

***Phylogenetic analyses*.** Heuristic searches under the parsimony criterion were carried out under the following options: all characters unweighted and unordered, multistate characters interpreted as uncertainties, gaps coded as missing data, performing a tree bisection reconnection (TBR) branch swapping, collapse zero branch length branches, MulTrees option in effect, random addition sequence with 1000 replicates.

Furthermore the data sets were analysed using *win*PAUP 4.0b10 (Swofford, 2002) executing the command files generated by 'PRAP' (Parsimony Ratchet Analyses using PAUP Müller, 2004), employing the implemented parsimony ratchet algorithm (Nixon, 1999). For the parsimony ratchet the following settings were employed: 10 random addition cycles of 200 iterations each with a 40 % upweighting of the characters in the PRAP iterations. Heuristic bootstrap searches (BS Felsenstein, 1985) under parsimony criterion were performed with 1000 replicates, 10 random addition cycles per bootstrap replicate and the same options in effect as the heuristic search for the most parsimonious tree (MPT). The consistency index (CI, Kluge & Farris, 1969), retention index (RI), and rescaled consistency index (RC, Farris, 1989) were calculated to assess homoplasy.

In addition to MP analyses Bayesian Inferences with MrBayes3.0 (Huelsenbeck & Ronquist, 2001) were performed. Modeltest 3.5 (Posada, 2004) was used to select DNA substitution models for the data set (gamma shape distribution, six substitution types). The Markov Chain Monte Carlo (MCMC) analyses were run for 1,000,000 generations with four simultaneous MCMCs and one tree per 100 generations was saved. The 'burn-in' values were determined empirically from the likelihood values. The analyses were repeated three times to assure sufficient mixing by confirming that the program converged to the same posterior probability (PP).

The program Treegraph (Müller & Müller, 2004) was used to edit trees directly from PAUP-treefiles.

MEGA2.1 (Kumar et al., 2001) was used to calculate GC-content, sequence length and distance measure ('p-distance'). In the following the term 'genetic distance' is used beside the term 'p-distance'.

6.3 Results

6.3.1 Sequence variation

Sequencing success. Results on sequence length and GC-content for ITS1, ITS2, *trn*L intron, and *rps*4 are listed in table 24. Only partial sequences of *Acrocladium auriculatum* (specimen 78) and *A. chlamydophyllum* (specimen 12) for the *adk* intron as well as exon were obtained and are therefore not listed. We obtained the complete sequence of the *trn*L intron for six of the 24 specimens of *Acrocladium*. As the *trn*L-*trn*F spacer was sequenced only partially these results are not discussed in detail (table 24).

Table 24: Sequence lengths [base pairs, bp] and GC-content [%] in the ITS1, ITS2, trnL intron and rps4 gene of eight *Acrocladium* specimens and six outgroup taxa. Average sequence lengths and standard deviations are also given. For origin of the data refer tab. xz. Abbreviations: n.d. = no data available, *A.=Acrocladium.*

Taxon	ITS1 sequence length [bp}	ITS1 GC-content [%]	ITS2 sequence length [bp]	ITS2 GC-content [%]	*trn*L intron sequence length [bp]	*trn*L intron GC-content [%]	*rps*4 sequence length [bp]	*rps*4 GC-content [%]
Herzogiella seligeri (sp.120)	244	62.30	259	62.5	312	31.1	570	29.3
Plagiothecium undulatum	240	62.90	183	63.4	265	28.7	570	28
Plagiothecium denticulatum	248	62.50	255	64.7	315	31.4	570	28.2
Taxiphyllum taxirameum (sp.117)	286	65.40	250	67.2	318	31.2	571	26.9
Lepyrodon tomentosus (sp.64)	246	63.40	266	65.4	314	32.5	540	28.5
Lepyrodon pseudolagurus (sp.67)	249	64.60	264	65.9	315	31.7	571	27.9
A. chlamydophyllum (sp.12)	255	62.70	233	63.9	315	30.8	570	26.7
A. chlamydophyllum (sp.171)	255	62.70	234	64.1	315	30.8	n.d.	n.d.
A. chlamydophyllum (sp.162)	n.d.	n.d.	n.d.	n.d.	315	30.8	n.d.	n.d.
A. auriculatum (sp.165)	n.d.	n.d.	n.d.	n.d.	315	30.5	n.d.	n.d.
A. auriculatum (sp.78)	255	64.30	236	64.9	314	30.2	558	26.3
A. chlamydophyllum (sp.185)	230	65.60	236	64.9	315	30.2	n.d.	n.d.
A. auriculatum (sp.186)	255	64.30	236	64.9	315	30.2	n.d.	n.d.
A. auriculatum (sp.189)	n.d.	n.d.	n.d.	n.d.	315	30.2	n.d.	n.d.
Average	*251*	*63.70*	*241*	*64.7*	*311*	*30.7*	*565*	*27.7*
SD	*13.9*	*1.2*	*23.0*	*1.3*	*12.9*	*0.9*	*11.0*	*1.0*

Sequence lengths and GC-content. The sequence length of the complete *trn*L intron in the genus *Acrocladium* ranged from 314 base pairs (bp; *A. auriculatum*, sp. 78) to 416 bp (*A. chlamydophyllum*, specimen 12). The GC-content ranged from 30.2 (all specimens from Chile) to 30.8 % (all specimens from New Zealand and Macquarie Island).

We successfully sequenced the ITS1 region for five specimens of *Acrocladium*. The sequence length of the ITS1 in the genus *Acrocladium* was 255 bp. At the 5'-end of ITS1 of specimen 185 the signal from the sequencer was very low resulting in a readable length of 230 bp only. The GC-content was 62.7 % for the specimens from New Zealand and 64.3 % for two specimens from Chile. For specimen 185 from Chile the GC-content was 65.6 %. The average GC-content within the genus *Acrocladium* was 63.7 % (standard deviation 1.2). For five species of *Acrocladium* from Chile and New Zealand the complete sequence of the ITS2 region was obtained. The sequence length ranged between 233 bp (specimen 12) and 236 bp (all specimens from Chile). The GC-content in the ITS2 region was 63.9 % in specimen 12 and 64.9 % (all specimens from Chile).

The length difference between the two successfully sequenced *rps*4 genes from *Acrocladium auriculatum* (specimen 78) and *A. chlamydophyllum* (specimen 12) is due to a low signal in the sequence analysis of these specimens, which prevented 12 bp from being read at the 3'-end of the *rps*4 gene of the former specimen.

Only the first *adk* exon (99 bp) and *adk* intron (124 bp) of the two *Acrocladium* species were successfully sequenced. The length of both the exons and introns differed considerably between the two species. For A. *auriculatum* from Chile (sp. 78) more unambiguous positions in the sequences than for the specimen from New Zealand (sp. 12) were obtained. In the sequences of *A. auriculatum* 26 bp at the 5'-end of the second exon, 115 bp at the 3'-end of the second intron as well as 52 bp at the 5'-end of the third exon were unambiguous. In both specimens 84 positions at the 3'end of the third intron as well as 43 bp of the fourth exon revealed signals of one nucleotide.

Variability of the regions in the combined data set. Table 25 presents the information on the different regions in the alignment. The highest proportion of variable sites was found in the ITS2 region where 12.6 % of the 326 aligned positions

were variable within the data set including the outgroup (1.5 % variability between the specimens of *Acrocladium*). In the ITS1 region the variability in the data set including the outgroup taxa was 6.7 % for the 315 aligned positions. The variability of the ITS1 data set without the two outgroup taxa was 5.1 %. In the *trn*L region the variability of the data set comprising 421 positions was 1.9 % (9.3 % including the outgroup), whereas in the *rps*4 region (571 characters) it was only 0.7 % (8.1 % including the outgroup). The *adk* gene had a variability of 2.5 % in the intron and 0.8 % in the exon, in 476 and 241 aligned nucleotides respectively. The coding region of the *adk* data set revealed only 5.1 % variable sites (0.6 % without outgroup) in 312 aligned positions.

Table 25: Number of taxa, total number of aligned characters; variable characters and number of parsimony informative sites and %-value of variable sites for the partial data sets of *Acrocladiu*m. Numbers in brackets refers to the data set including the outgroup taxa.

	Com-bined	*trn*L	Varia-bility [%]	*rps*4	Varia-bility [%]	*adk*-intron	Varia-bility [%]	*adk*-exon	Varia-bility [%]	ITS1	Varia-bility [%]	ITS2	Varia-bility [%]
Number of sites	2698	421		571		476		241		315		326	
Variable sites	35 (244)	8 (39)	1.9 (9.3)	4 (46)	0.7 (8.1)	12	2.5	2	0.8	16 (21)	5.1 (6.7)	5 (39)	1.5 (12.0)
Parsimony Informative	13 (97)	4 (15)	1.0 (3.7)	0 (20)	(3.5)					12 (48)	3.8 (15.2)	5 (28)	1.5 (8.6)

Indel and substitution matrix.Within eight variable positions of the *trn*L intron five substitutions (table 26) clearly support the genetic separation between the South American (specimens 78, 165, 185, 186, 189) and New Zealand and Macquarie Island (specimens 12, 171, 162) samples. Two substitutions different from the remaining specimens group the specimen from Argentina (specimen 165) clearly with those from New Zealand and Macquarie Island. One substitution event occurs only in the specimen from Argentina.

The four substitutions found for the ITS1 as well as ITS2 region support the genetic distinction between the two specimens from New Zealand (sp. 12, 171) and those from Chile (specimens 78, 185, 186).

The most promising region concerning the variability is the *adk* gene. Within the 884 aligned base pairs thirteen positions and an additional ambiguous one, separate the New Zealand specimen 12 from the Chilean specimen 78. Within the *rps*4 gene four substitutions were identified which separate Chile (specimen 78) from New Zealand

(specimen 78). Overall 34 substitutions support the genetic differentiation of the two geographical regions.

Additionally, three indels support the separation between these regions - two indels from the ITS1 region, each consisting of one nucleotide and one indel in the ITS2 region consisting of two nucleotides (table 27).

Table 26: Substitution matrix in the combined data set (trnL, ITS1, ITS2, adk, and rps4) within the genus *Acrocladium*. 35 sites were found to be variable. Substitutions in *trn*L: no. 1-8; in ITS1: no. 9-12; in ITS2: no. 13-17; in *adk*: 18-31; in *rps*4: 32-35. Abbreviations: A.a.: *Acrcocladium auriculatum*, A.c.: *A. chlamydophyllum*.

Substituion no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
A.c. 12	G	C	G	C	A	C	C	C	T	T	G	A	T	T	C	T	G	A	A	C
A.c. 171	G	C	G	C	A	C	C	?	T	T	G	A	T	T	C	T	G	?	?	?
A.c. 162	G	C	G	C	A	C	C	C	?	?	?	?	?	?	?	?	?	?	?	?
A.a. 165	G	C	A	A	G	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
A.a. 78	A	T	A	C	G	A	T	A	C	G	C	G	C	C	T	C	A	C	G	T
A.a. 185	A	T	A	C	G	?	?	?	C	G	C	G	C	C	T	C	A	?	?	?
A.a. 186	A	T	A	C	G	?	?	?	C	G	C	G	C	C	T	C	A	?	?	?
A.a. 189	A	T	A	C	G	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?

Substituion no.	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**
A.c. 12	C	A	G	T	C	G	C	A	t	G	A	A	G	C	A
A.c. 171	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
A.c. 162	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
A.a. 165	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
A.a. 78	A	T	A	G	G	C	A	T	C	A	G	C	A	A	G
A.a. 185	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
A.a. 186	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
A.a. 189	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?

Table 27: Indelmatrix of the combined data set of Acrocladium (Indel no. I and II from ITS1 region, indel no. III from ITS2 region).

Position in the alignment [%]	491 (ITS1)	631 (ITS1)	900/1 (ITS2)
Indel no.	I	II	III
New Zealand 12		T	
New Zealand 171		T	
Chile 78	C		CC
Chile 185	C		CC
Chile 186	C		CC

6.3.2 Genetic distances

Within the *trn*L data set (appendix 11) including outgroup the average genetic distance (p-distance) was 2.3 % (standard error 0.4). Within the four specimens from Chilean localities (specimens 189, 186, 185, 78) investigated in this study no genetic variation in the *trn*L intron was detectable. Similarly the specimens from New Zealand

(sp. 12, 171) and Macquarie Island (sp. 162) were all identical. An equal distance of 1.0 % (standard error 0.5) separates the southern Argentinean specimen (specimen 165) from both the Chilean and New Zealand specimens .

The genetic distances in the *trnL* intron separating the Chilean specimens from those from New Zealand was 1.3 % (standard error 0.6).

For the ITS1 (appendix 12) data set including outgroup an average genetic distance of 6.0 % (standard error 0.9) was observed. No genetic variation was detected in the ITS1 region within the three specimens from Chilean localities (specimens 186, 185, 78) nor within the two from New Zealand (specimens 12, 171).

In the ITS2 (appendix 13) region the sequence variation separating the Chilean specimens from those collected in New Zealand range from 1.6 to 1.7 % (standard error 0.9). The ITS2 data set including outgroup had an average genetic distance of 5.4 % (standard error 0.9).

The three specimens from Chilean localities (specimens 186, 185, 78) as well as both specimens from New Zealand (specimens 12, 171) had identical ITS2 regions. The genetic distances in the ITS2 region separating the Chilean specimens from those in New Zealand was 2.1 % (standard error 0.9).

Within the *rps*4 data set (appendix 14) including outgroup the average genetic distance was 2.7 % (standard error 0.4). The genetic distances in the *rps*4 region separating the Chilean specimen from those in New Zealand was 0.7 % (standard error 0.3).

Within the two partial sequences of the *adk* gene of *Acrocladium* sequence variation was 3.3 % (standard error 0.9) in the intron and 1.2 % in the exon (standard error 0.8).

The complete data set including four sequenced regions reveals different values for the p-distance between the geographical regions investigated. The reason is that this data set includes the *trn*L-*trn*F spacer region (63 characters). Computing the p-distance for three specimens (two specimens from New Zealand and one from Chile) of which the complete *trn*L-*trn*F spacer region (60 bp) was successfully sequenced a genetic distance of 4.9 % between the specimen 78 from Chile and specimen 12 from New Zealand was found. No difference was found between the specimens from New Zealand and from Macquarie Island (specimen 162).

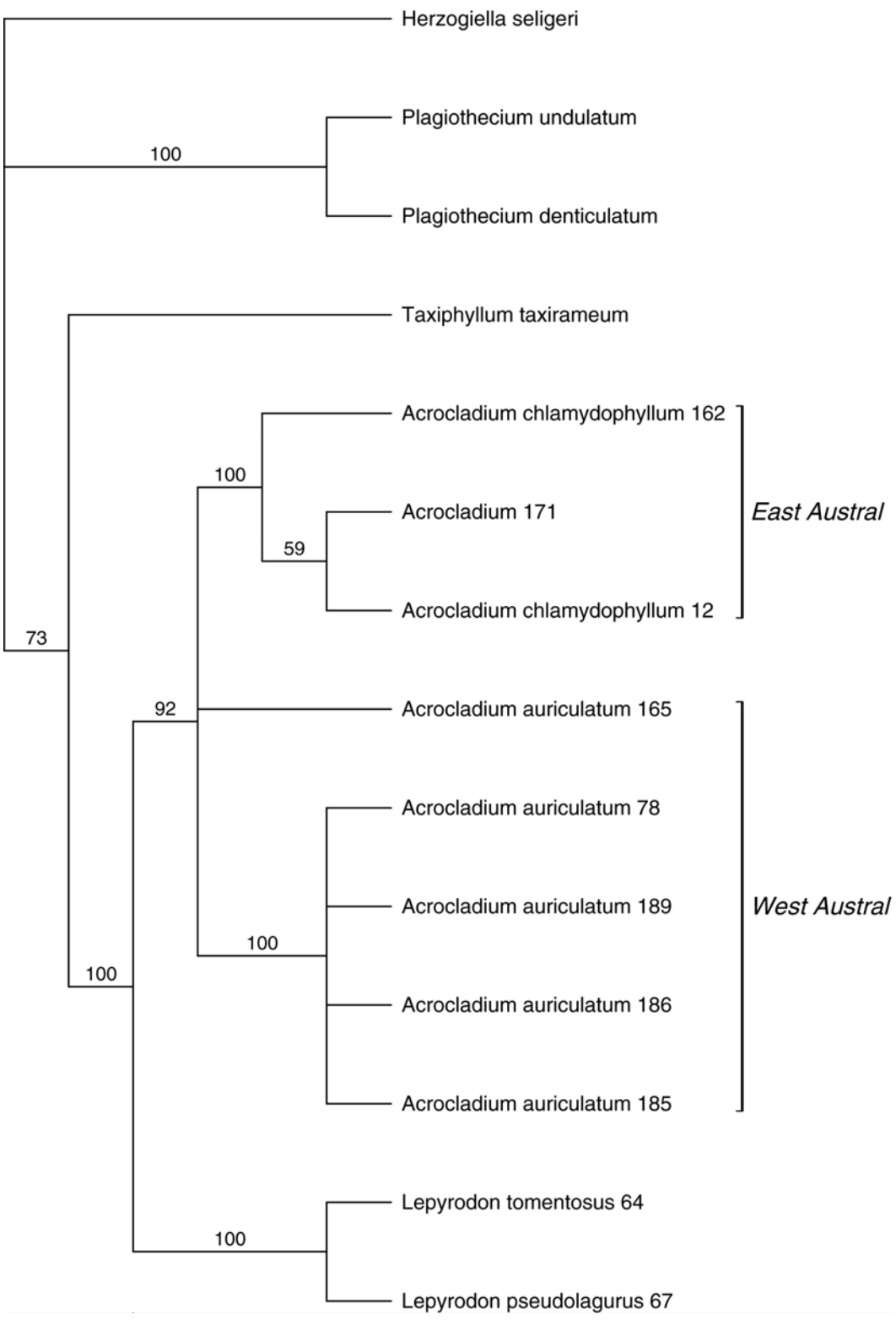

Figure 15: Cladogram resulting from a Bayesian Inference analysis of *trn*L intron, ITS1, ITS2, *adk*, and *rps*4 sequence data of *Acrocladium* specimens from different geographical locations. Numbers above branches indicate the posterior probabilities support as a percentage value. Clade 'East Austral'consists of specimens from New Zealand and Macquarie Island, clade'West Austral' consists of specimens from Chile and Argentina.

6.3.3 Phylogenetic analysis

Figure 15 depicts the cladogram resulting from a Bayesian Inference analysis using MrBayes (Huelsenbeck & Ronquist, 2001) resulting from 9,900 trees. The data set includes six outgroup taxa and eight specimens of *Acrocladium* representing the two geographical provenances, one covering southern South America (west austral) and the other New Zealand, Australia and the ancient (subantarctic) islands (east austral). The outgroup taxa comprise two species of the genus *Lepyrodon*, proposed as sister taxon to the genus *Acrocladium* (chapter 4, Quandt et al., 2004b), three representatives of the Plagiotheciaceae to which the genus *Acrocladium* belongs according to Pedersen and Hedenäs (2002) and *Taxiphyllum taxirameum*.

Herzogiella seligeri is the most basal taxon in the cladograms. The clade comprising two representatives of the genus *Plagiothecium* is supported with a posterior probability of 100 %). The clade which has *Taxiphyllum taxirameum* as its most basal taxon and also includes the representatives of *Lepyrodon* and *Acrocladium* has a posterior probability of 73 %. The sistergroup relationship between the genera *Acrocladium* and *Lepyrodon* is supported with a posterior probability of 100 %. The monophyly of both genera *Acrocladium* and *Lepyrodon* is supported with a posterior probability of 92 % and 100 % respectively. The specimens 171 and 12 derived from New Zealand and the specimen from subantarctic Macquarie Island no. 162, here referred to as ‘east austral’ clade are monophyletic with a 100 % probability. The specimens from Chile (sp. 78, 189, 186, and 185) are also monophyletic (PP 100 %). However, the relationship of the specimens from southern South America, here referred to as ‘west austral’ clade, including the four taxa from Chile as well as one taxon from east of the Andes in Argentina are polyphyletic.

The figure 16 depicts the 50 %-majority rule tree of 39 MPTs (length 282, CI 0.929, RI 0.877, RC 0.815) as a phylogram. The phylogram was obtained with the branch and bound search option based on the combined data set of the genus *Acrocladium* including the outgroup taxa. Values above branches refer to bootstrap support (1,000 iterations), whereas numbers below branches indicate the number of characters supporting each clade. A high bootstrap support (100 %) was found for the genus *Plagiothecium*. Its monophyly is also supported by 20 autapomorphic characters. A clade consisting of *Herzogiella seligeri*, a putative member of the Plagiotheciaceae

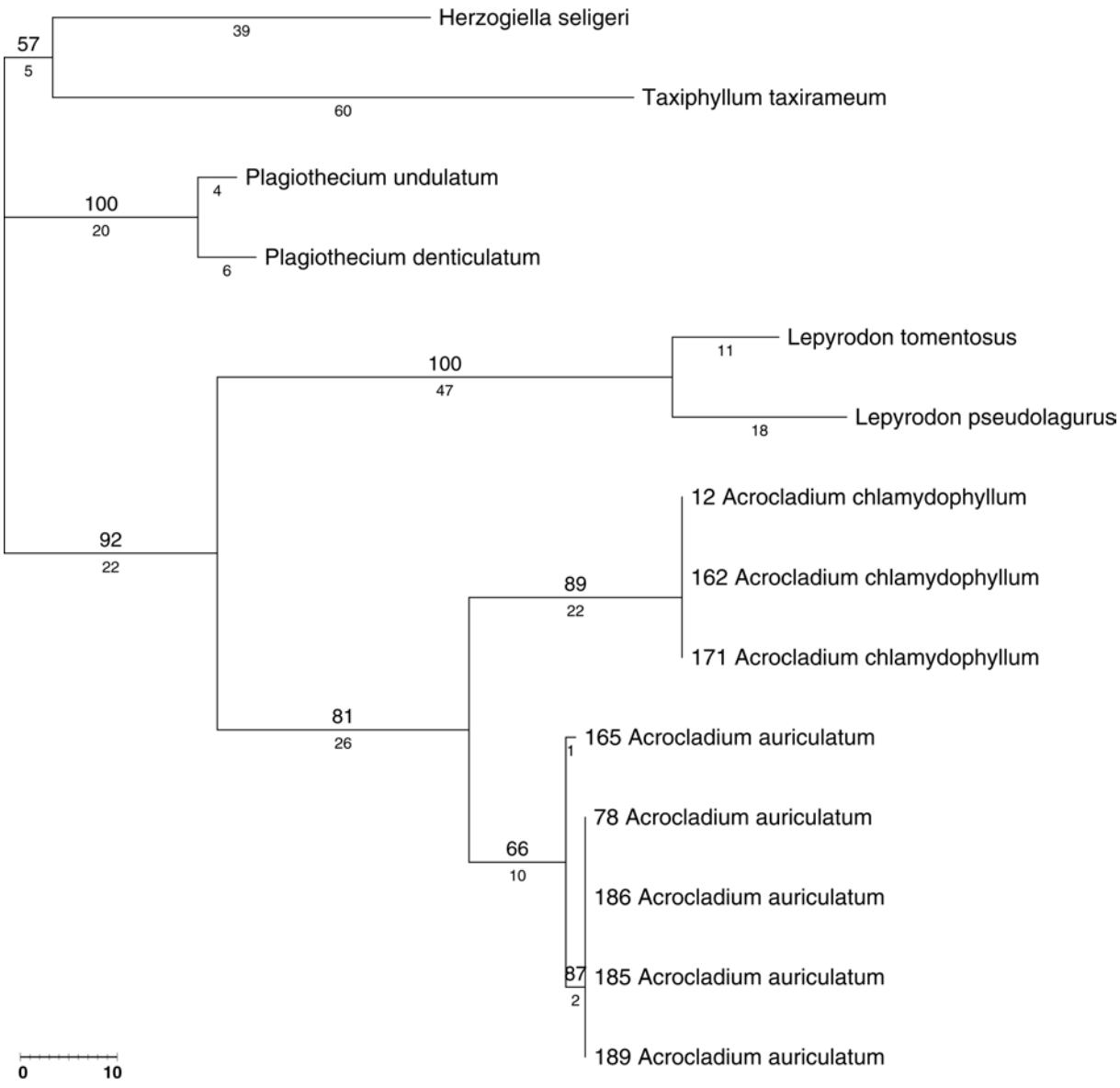

Figure 16: Phylogram of 39 MPTs (Length 282, CI 0.929, RI 0.877, RC 0.815) found during the parsimony ratchet of the combined sequence data (ITS, *trn*L, *adk* and *rps*4) of specimens the genus *Acrocladium* and outgroup taxa. Numbers above branches are bootstrap values (1000 iterations) numbers below branches is the number of characters supporting each clade. Length of the scale bar in the lower left corner of the phylogram equals 10 characters.

(Pedersen & Hedenäs, 2002) and *Taxiphyllum taxirameum* (Buck & Goffinet, 2000) is indicated by five autapomorphic characters though weakly supported (BS 57 %). Both species are characterised by a high amount of apomorphic characters, *Taxiphyllum taxirameum* having 60 and *Herzogiella seligeri* 39 characters.

The clade of *Lepyrodon* and *Acrocladium* is supported by 22 characters and a bootstrap value of 92 %. The two species of *Lepyrodon* are characterised by 47 characters and a 100 % bootstrap value supporting their monophyly.

The monophyletic position of the genus *Acrocladium* is supported by a bootstrap value of 81 % and 26 autapomorphic characters. The taxonomic sovereignty of the east austral clade is supported by 89 % BS and 22 autapomorphic characters. There are 10 autapomorphic characters supporting the monophyly of the west austral clade (66 % BS). In this clade the specimen from Argentina, no. 165 is the most basal one, and also the only specimen of *Acrocladium* with a unique apomorphic character. The four specimens from Chile are separated by two apomorphic characters and an 87 % bootstrap support from the east Andean taxon.

6.4 Discussion

6.4.1 The status of *A. auriculatum* and *A. chlamydophyllum*

As stated in the results there were problems involved in obtaining sequence data for large parts of the exons and introns. A possible explanation is offered by Vanderpoorten et al. (2004) who report high infra-genomic polymorphism in the *adk* gene of *Hygroamblystegium*. Within-organism polymorphism is usually associated with a divergent evolution of gene arrays, hybridization or formation of pseudogenes (for a detailed discussion see e.g. Campbell et al., 1997; Doyle, 1992; Hugall et al., 1999). In *Hygroamblystegium* as well as in related genera e.g. *Amblystegium* polyploids are quite common (e.g. Fritsch, 1991). Vanderpoorten et al. (2004) therefore suggest that “repeated events of gene duplication and losses may account for the observed polymorphism of *adk* in *Hygroamblystegium*”. There are two chromosome counts reported for *Acrocladium chlamydophyllum* (Ramsay, 1974, cit. in Fritsch, 1991; Przywara et al., 1992). Ramsay (1974, cit. in Fritsch, 1991) report n=11 (10+m) for material from Australia. According to Ramsay (1983) the loss or addition of such m-chromosomes occurs together with aneuploidy which may lead to polyploid taxa (Ramsay, 1983). On the other hand, the analysis of material from New Zealand (Przywara et al., 1992) resulted in n=11, revealing no additional m-chromosome.

Taking the above mentioned problems into account, the difficulties in obtaining sequences in large parts of some introns and exons in the *adk* gene in this study may be due to the existence of different copies of the *adk* gene with mutation events in these regions which resulted in ambiguous sequencing signals. A possible solution for this problem may be the cloning of the PCR products prior sequencing. The obtained results would give insight into possible hybridization events or the occurrence of pseudogenes.

There has been a lot of discussion on the status of the taxa described in the genus *Acrocladium* based on morphological characters. The holotype of *Acrocladium auriculatum* (Mont.) Mitt. was described by Montagne in 1843 as *Hypnum auriculatum* Mont. based on material collected in southern South America (Karczmarz, 1966). The holotype of *Acrocladium chlamydophyllum* (Hook.f. et Wils.) Muell. Hal. & Brotherus was described as *Hypnum chlamydophyllum* Hook.f. et Wils. based on material which originated from Campbell Island and Tasmania (Karczmarz, 1966). The genus *Acrocladium* first was established by Mitten (1869), and included besides *A. auriculatum* (Mont.) Mitt. a second species *Acrocladium politum* (Hook.f. & Wils.) Mitt., now known as *Catagonium nitens* (Brid.) Cardot. In 1879 Lindberg (cit. in Andrews, 1949) united the northern *Acrocladium cuspidatum* (L.) Lindb. with the southern hemisphere species of *Acrocladium*. Kindberg in 1897, included *A. cuspidatum* in the genus *Calliergon* (Sull.). The east southern hemispheric *A. chlamydophyllum* was established in 1900 by C. Müller and Brotherus (Karczmarz, 1966).

Brotherus (1909b) distinguishes three species in the genus *Acrocladium*, which he classifies into two different systematic categories. In ‘section I’, ‘Eu-*Acrocladium*’ he includes the southern hemispheric species *A. auriculatum* (Mont.) Mitt. from southern South America and *A. chlamydophyllum* (Hook.f. & Wils.) Broth. from New Zealand, eastern Australia, Tasmania and adjacent islands. ‘Section II’ contains the northern hemispheric *A. cuspidatum* (L.) Lindb. Brotherus (1909b) distinguishes the two sections among others based on form and shape of the perichaetal leaves and differences in stem anatomy. The separation of *A. auriculatum* and *A. chlamydophyllum* was based on the presence or absence of leaf auricles and the extension of the leaf costa. In a later treatment of the genus *Acrocladium* Brotherus (1925a) adopts the view that only the southern hemispheric species belong to the genus *Acrocladium*.

Andrews (1949), Karczmarz (1966) and Fife (1995) support the view of Brotherus (1909b; 1925c) that *A. auriculatum* (Mont.) Mitt. and *A. chlamydophyllum* (Hook.f. & Wils.) Broth. are two morphologically well distinct taxa, where *A. chlamydophyllum* deserves the rank of a species. Karczmarz (1966) omits the character of the costa and distinguishes both species by leaf shape and by presence versus absence of auricles. Furthermore, he states that each species is restricted in its distribution. *A. auriculatum* occurs in the western part of the distribution range of the genus whereas *A. chlamydophyllum* is restricted to the eastern part.

In contrast, Mitten (cit. in Karczmarz, 1966; 1869), Dixon (1928), Sainsbury (1955) and He (1998) consider both taxa as geographical variations of the same species, using the name '*A. auriculatum*' as the older epitheton.

Both the phylogenetic results as well as the genetic distances obtained in this study clearly distinguish between the specimens labeled *Acrocladium auriculatum*, originating from Chile and Argentina and the specimens representing *A. chlamydophyllum* from New Zealand and Macquarie Island.

The specimens of *A. auriculatum* on the one hand and those of *A. chlamydophyllum* on the other hand form two well supported monophyletic clades. The obtained genetic distances between *A. auriculatum* and *A. chlamydophyllum* (e.g. 1.3 % in the *trn*L intron) are comparable with the genetic distances used to distinguish between the Gondwanan taxa *Polytrichadelphus magellanicus* and *P. innovans* (Stech et al., 2002). Additionally, there were three indels found which separated between the populations from New Zealand and Macquarie Island (*A. chlamydophyllum*) and Chile/Argentina (*A. auriculatum*).

6.4.2 Possible explanations for the disjunct distribution of *Acrocladium*

There are two possible explanations for the disjunct distribution of the two *Acrocladium* species which are discussed in the following. On the one hand the genus may have originally only occurred in one of the two disjunct areas: southern South America or New Zealand/Australia. After a long distance dispersal event the two species developped by divergent evolution. Regarding the high genetic differentiation found in this study this putative event must have happened a very long time ago. On the other hand a common ancestor of both species may originate from the former Gondwana continent. After the continent broke apart two isolated populations evolved independently resulting in two species.

Muñoz et al. (2004) test with statistical methods whether the floristic affinities among southern hemispheric landmasses outside the tropics could be explained better by a near-surface wind transport (direction dependent) or geographic proximity (direction independent). They used four different data sets: a set with 601 species of mosses, 461 species of liverworts, 597 species of lichens, and 192 species of pteridophytes. They found a stronger correlation between floristic similarity and maximum wind connectivity than between floristic similarity and geographic proximity in mosses, liverworts and lichens. From their analyses they concluded that wind is the main force driving current plant distributions in these groups.

Van Zanten (1976; 1978) designed experiments to test for the ability of bryophyte spores to germinate after being exposed to the same conditions as in a long distance transport by jet streams. *Acrocladium auriculatum* was one of the taxa of which the spores tolerated the experimental conditions of long distance dispersal for only one year. Based on this result van Zanten (1978) ruled out long distance dispersal as an option for this species and concluded that *Acrocladium auriculatum* may consist of more than one taxon each occurring in different isolated areas.

Taking into account van Zanten's results (1976; 1978) a long distance dispersal via jet streams is rather unlikely, however a dispersal event via near-surface winds might be possible according to the correlation found by Muñoz et al. (2004). However, a comparison of the observed genetic variation with published values (e.g. Quandt et al., 2001; Quandt & Stech, 2004; Stech et al., 2002) argues for the establishment of two clearly separated species, as shown in the phylogenetic analyses. Hence the large genetic differentiation between the species *Acrocladium auriculatum* and *A. chlamydophyllum* found in the study at hand, indicates an early separation of the two species, with a common ancestor of the two species on the Gondwana continent.

A possible example for long distance dispersal either in jet streams as tested in van Zanten (1978) or by near-surface winds (Muñoz et al., 2004) is the occurrence of *Acrocladium* along with other bryophytes on Marion Island (Gremmen, 1981; van Zanten, 1971). As Marion Island was never part of the former Gondwanan landmass, its recent flora must have different origins. Gremmen (1981) assumed long distance dispersal by wind to be the most important factor for the establishment of the cryptogamic flora on this island. The island is only *c.* 500,000 years old and probably suffered several glaciation events during the Pleistocene probably destroying most of the flora at the time (Gremmen, 1981). However, he stated that some of the

angiosperms were brought in accidentally by seal hunters during the last 300 years. Therefore, it can not be ruled out that some bryophytes on Marion Island are of anthropogenic origin, and given the habitat preferences of *Acrocladium* this scenario represents a likely option. Unfortunately, no sequence data were obtained from samples from Marion Island. Thus, the interesting question concerning the origin of the genus *Acrocladium* on Marion Island remains unresolved.

7 Molecular evolution, phylogenetics and biogeography of the genus *Catagonium* (Plagiotheciaceae, Bryopsida)

7.1 Introduction

The genus *Catagonium* consists of four species described by Lin (1984). The plants have a shiny appearance and the stems have complanate leaf orientation. The plants form mats mainly on soil in tropical montane forest and temperate rain forests of the southern hemisphere. On subantarctic islands they also occur in open, subantarctic vegetation types. The distribution pattern implies an old Gondwanan origin of the genus.

Within *Catagonium nitens* (Brid.) Card. two subspecies were described (Lin, 1984). The subspecies *Catagonium nitens* (Brid.) Card. ssp. *maritimum* (Hook.) Lin is restricted to South Africa, *Catagonium nitens* (Brid.) Card. ssp. *nitens* occurs in eastern Africa, New Zealand, Australia, and southern South America as well as on some subantarctic islands. There are two varieties of the subspecies *nitens* described by Lin (1989), *C. nitens* (Brid.) Card. ssp. *nitens* var. *myurum* (Card. & Thér.) S.-H. Lin occurring in Chile and *C. nitens* (Brid.) Card. ssp. *nitens* var. *nitens*. If not stated otherwise in the text “*C. nitens* ssp. *nitens*” refers to the variety *nitens*.

Catagonium nitidum (Hook.f. & Wils.) Broth. is reported from southern South America, the Falkland Islands and Tristan Da Cunha Island.

Catagonium brevicaudatum C. Müll. ex Broth. is known from Brazil, Bolivia, Columbia, Costa Rica, Ecuador, Guatemala, Jamaica, Mexico, Peru and Venezuela, and *Catagonium emarginatum* S.-H. Lin. from Brazil, Bolivia (Lin, 1984) and Peru (Lin, 1989).

As *Catagonium nitens* ssp. *nitens* is one of the prominent species of the Chilean temperate rainforest I took special interest in the evolution of this species and the relationship to its sister taxa. First the molecular conditions within the *Catagonium nitens*-group using ITS sequences were investigated in order to obtain the genetic divergence between *Catagonium nitens* ssp. *nitens* from Chile and New Zealand as well as the genetic divergence of these taxa to *Catagonium nitens* ssp. *maritimum*

from South Africa. It was also tried to confirm the taxonomic status of the variety *Catagonium nitens* ssp. *nitens* var. *myurum* in relation to *Catagonium nitens* ssp. *nitens* var. *nitens* based on molecular data.

Secondly I aimed at understanding the biogeographical evolution of the genus by investigating the genetic relationship between the four species *Catagonium nitens, Catagonium brevicaudatum, Catagonium emarginatum,* and *Catagonium nitidum* and possible related taxa using ITS data sets.

7.1.1 Morphological characterisation

The genus *Catagonium* is characterised by its short creeping primary stem and a secondary irregularly branched stem. Stems and branches are complanately to teretely foliate. The plants are yellow-green to brown-green and form dense mats over rocks and on the forest floor or grow epiphytically on bark. They are small to medium sized, with branches between 1 and 5 cm in length. The leaves are appressed on their dorsiventral faces and either erect spreading laterally or erect on all sides. The costa is short, double or absent. The plants are dioicous.

Catagonium nitens (Brid.) Card. Lin (1984). described *Catagonium nitens* (Brid.) Card. as a highly polymorphic species with respect to e.g. plant size, leaf shape, and foliation. He recognized two subspecies within *C. nitens*, but stated that he also found plants with intermediate characters. However, Lin (1984) found that the morphological characters highly correlated with the geographical distribution of the two subspecies.

The plants in the subspecies *maritimum* are between 5.5-10 cm long and generally teretely foliate. The leaves are between 1.3-2.5 mm wide and concave. The apices of the leaves are distinctly mucronate. The subspecies is restricted to South Africa.

The subspecies *maritimum* can be distinguished from the ssp. *nitens* by the concave, mucronate leaves and the terete foliation.

The subspecies *nitens* is very variable in its appearance and has a wider distribution range than the ssp. *maritimum*. It occurs in southern South America, some subantarctic islands, southeastern Africa, Réunion, New Zealand, Australia and New Guinea.

The plants are between 4-12 cm long and generally complanately foliate. The leaves are between 2-3 mm wide, strongly conduplicate, cuspidate to acuminate and have a

narrow, long, acute apex. Lin (1984) observed a correlation between plant size, size and shape of the leaves and latitude in *C. nitens* ssp. *nitens*. In subantarctic areas julaceous or minute plants with small leaves occur, whereas well developed plants occur in southern South America, southeastern Africa, Réunion, Australia, New Guinea, and New Zealand. Lin (1984) was also able to correlate these morphological differences with altitude, i.e. the higher the elevation the smaller the plant.

According to Lin (1984) the type specimen of *C. myurum* Card. & Thér. (from Punta Arenas) is characterized by minute, julaceous stems and branches with erect-spreading, oblong lanceolate and gradually acuminate leaves. In 1989 Lin (1989) pointed out that ‘intermediates between *Catagonium nitens* ssp. *nitens* and *C. myurum* can occasionally be found on the same plant'. Because of the similarities of the two he recognized *C. myurum* Card. & Thér. as a variety of the subspecies *nitens*, *C. nitens* (Brid.) Card. ssp. *nitens* var. *myurum* (Card. & Thér.) S.-H. Lin. It is separated from *Catagonium nitens* (Brid.) Card. ssp. *nitens* var. *nitens* by terete branches, concave leaves, the attenuate leaf apex and shorter leaf cells. These characters of *C. nitens* ssp. *nitens* var. *myurum* in Lin's view (Lin, 1989) might express adaptations to the environment. *Catagonium nitens* (Brid.) Card. ssp. *nitens* var. *nitens*, in contrast, is characterized e.g. by the complanate branches and conduplicate leaves with recurved apices.

Lin (1984) described a close relationship of *Catagonium nitens* with *C. brevicaudatum* based on the abruptly narrowed leave apices appearing in *C. nitens* ssp. *maritimum* as well as in plants of the ssp. *nitens* from New Guinea and are also a characteristic feature of *C. brevicaudatum*. The concave leaves found in *C. nitens* and the absence of leaf auricles distinguish this species from *C. brevicaudatum* (Lin, 1984). Lin also found some plants belonging to the ssp. *nitens* which resembled *C. nitidum* in their long and slender leaf apices. In contrast to *C. nitidum*, however, the leaves in *C. nitens* ssp. *nitens* are complanate and conduplicate.

***Catagonium nitidum* (Hook.f. & Wilson) Broth.** According to Lin (1984), the plants of this species are up to 12 cm long, with 2.5-5 cm long branches, growing in dense mats. Furthermore, they are characterized by julaceous foliation with few slender branches. The leaves are strongly concave with erect and long-cuspidate apices. In his investigation Lin (1984) found in some of the specimens dwarf vegetative plants with long rhizoids on the adaxial surface of the leaves. He states that *C. nitidum* is

very close to the dwarf forms of *C. nitens* ssp. *nitens* from subantarctic islands. Lin (1984) distinguished the species by the oblong leaves with abruptly long-cuspidate apices found in *C. nitidum*.

C. nitidum is found in Argentina, Chile, the Falkland Island, and Tristan da Cunha Island. It occurs mainly on soil, rarely on bark.

***Catagonium brevicaudatum* C. Müll. ex Broth.** The diagnostic characters of *C. brevicaudatum* are the sparse and complanate foliation. The species has ovate-oblong, distinctly and minutely auriculate, cucullate-concave leaves that are more or less undulate, rounded to broadly obtuse. The apices of the leaves end in a short and soft recurved hair (Lin, 1984).

According to Lin (1984), *C. brevicaudatum* occurs mainly on wet or shaded rocks or soil in cloud forests at altitudes between 1,700 and 3,930 m. The species was reported from Brazil, Bolivia, Columbia, Peru, Ecuador, Costa Rica, Guatemala, Jamaica, and Mexico.

***Catagonium emarginatum* Lin** is distinguishable from its closest relative *C. brevicaudatum* by its emarginated leaf apices with recurved soft short hairs at the terminal end of the leaves. The species was so far only reported from Brazil, Peru and Bolivia.

Catagonium emarginatum occurs on soil at altitudes between 2,200 m (Brazil) and 3,900 m (Bolivia).

The systematic position of *Catagonium*. The genus *Catagonium* had been placed either in or near the Plagiotheciaceae (Brotherus, 1925c; Fleischer, 1923b; Lin, 1984) or Phyllogoniaceae (Vitt, 1984), before Buck & Ireland (1985) revised the Plagiotheciaceae and transferred the genus *Catagonium* in the monotypic family Catagoniaceae. Recently, based on cpDNA sequences and morphological data, Pedersen & Hedenäs (2002) transferred the genus back to the Plagiotheciaceae.

7.2 Material & Methods

Plant material. Plant material was either collected by the author during a field trip of the BryoAustral project to Chile in 2001, or originates from herbarium specimens. The specimen of *Catagonium nitens* ssp. *nitens* var. *myurum* was kindly provided by Dr. Friederike Schaumann (Freie Universität Berlin) and a specimen of *C. nitidum* was kindly provided by Dr. Frank Müller (Technische Universität Dresden). Specimens of *Acrocladium chlamydophyllum*, *Lepyrodon pseudolagurus*, and *Catagonium nitens* were collected during the BryoAustral project expedition to New Zealand in 1998. Duplicates are preserved in the herbaria in Christchurch (CHR), Bonn (BONN) and Berlin (B). Sequences available in GenBank were also used. All specimens used in the analyses are listed in Appendix 15 including further voucher information.

The study included 20 specimens of all four *Catagonium* species described as belonging to the genus including representatives of the two subspecies of *C. nitens* (Lin, 1984). Each of the taxa was represented by at least one specimen. The selection comprises four specimens of *Catagonium brevicaudatum* C. Müll. ex Broth. from Venezuela and Columbia and three specimens of *Catagonium emarginatum* Lin originating from Brazil, Bolivia, and Peru. Taking into account the wide geographical range and morphological variation of *Catagonium nitens* (Brid.) Card. several specimens of this species were selected. The subspecies *Catagonium nitens* (Brid.) Card. ssp. *maritimum* (Hook.) Lin was represented by three specimens from South Africa. The specimens of *Catagonium nitens* (Brid.) Card. ssp. *nitens* came from Australia, Tanzania (2x), New Zealand and from Chile (four specimens) including the variety *Catagonium nitens* (Brid.) Card. ssp. *nitens* var. *myurum* (Card. & Thér.) Lin. The specimens of the fourth species, *Catagonium nitidum* (Hook.f. & Wilson) Broth., originated from Tierra de Fuego, the Falkland Islands and from southern Chile. The geographical origin of the specimens of *Catagonium* successfully sequenced is shown in figure 17 on a global scale and in figure 18 (South America), figuren 19 (Africa) and figure 20 (New Zealand) on a regional scale.

We selected the two species *Lepyrodon pseudolagurus* and *L. tomentosus* as outgroup for the analysis and also included six species representing the family Plagiotheciaceae as the closest relatives of *Catagonium* described in Pedersen & Hedenäs (2002). The specimen selection within the genus *Catagonium* was based

on the principle of different morphological expressions of a species as well as wide spanning geographical derivation. Unfortunately, I was not able to gather enough DNA from all of the specimens for successful PCR and successive sequencing.

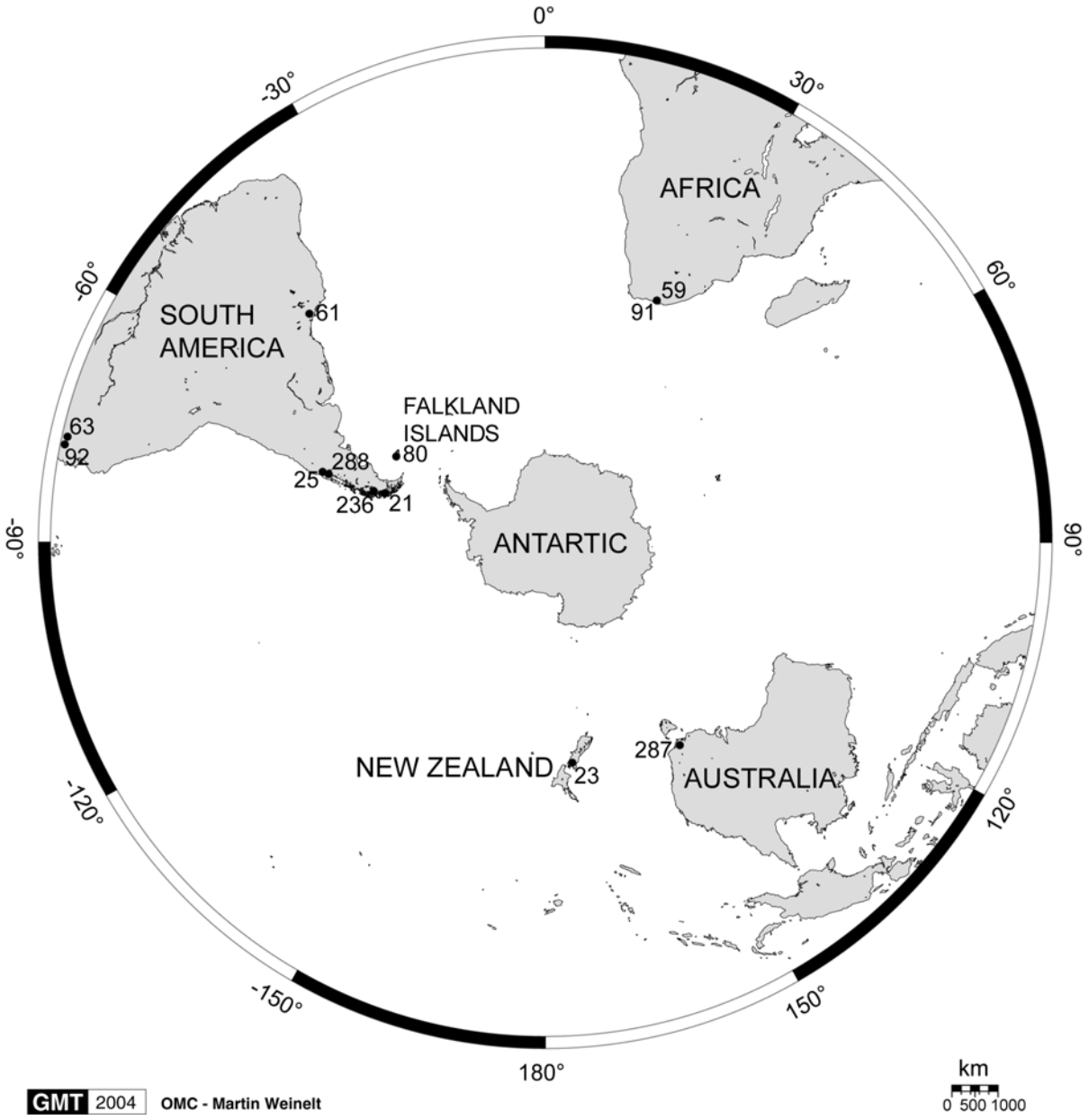

Figure 17: Geographical origin of all *Catagonium* specimens used for this study. Numbers are specimen numbers.

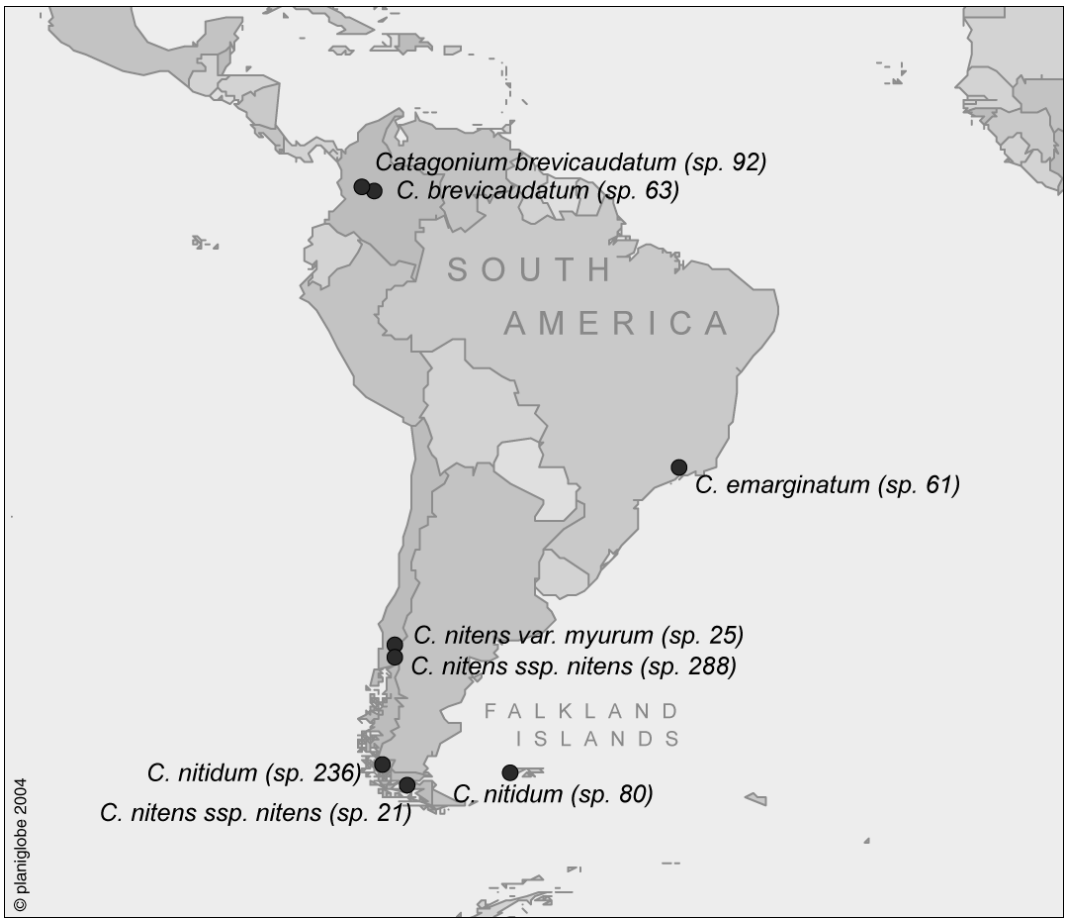

Figure 18: Geographical origin of the *Catagonium* specimens from South America used for this study. Numbers in brackets are specimen numbers.

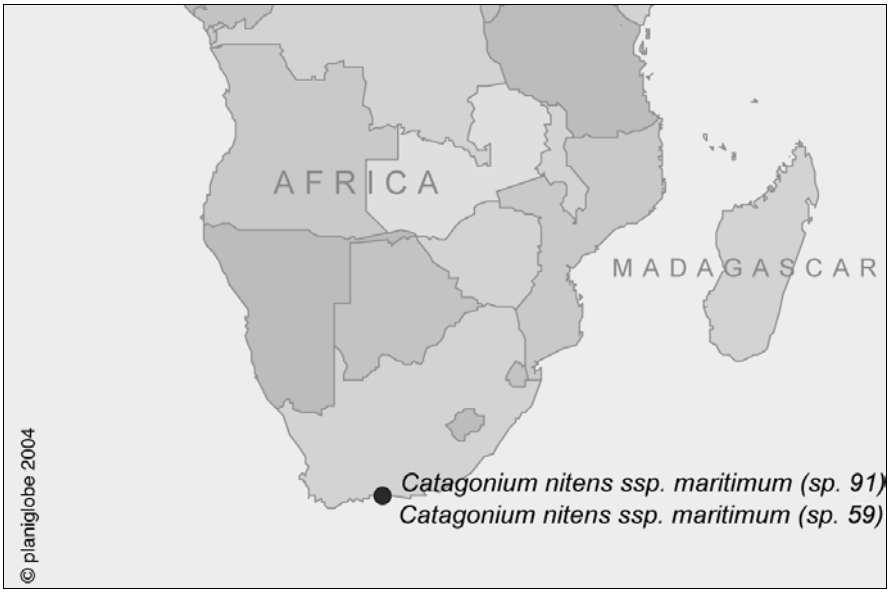

Figure 19: Geographical origin of the *Catagonium* specimens from South Africa used for this study. Numbers in brackets are specimen numbers.

Distribution Maps. Regional maps of the origin of *Catagonium* specimens were constructed using the web-page www.planiglobe.com (Körsgen et al., 2004). Dots were generated by adding geographical coordinates of collection localities as indicated on the voucher labels of the specimens. The map showing the world wide distribution of *Catagonium* were constructed using 'online map creation' OMC (www.aquarius.geomar.de) provided by M. Weinelt, (2004) which uses 'The Generic Mapping Tools' (GMT, Wessel & Smith, 1995).

Figure 20: Geographical origin of the *Catagonium* specimens from Australia/New Zealand used for this study. Numbers in brackets are specimen numbers.

DNA isolation, PCR and sequencing. Prior to DNA extraction the plant material was thoroughly cleaned with distilled water and additionally treated by ultrasonic waves for 2-4 minutes. Success of cleaning was checked by examining the plants under a binocular microscope. Remaining contaminations e.g. with algae and fungi were removed mechanically. Isolation of DNA was carried out following the CTAB technique described in Doyle & Doyle (1990).

PCR amplifications (Biometra TriBlock thermocycler, PTC-100 MJ Research) were performed in 50 µl–reactions containing 1.5 U *Taq* DNA polymerase (PeqLab), 1 mM dNTPs-Mix, nucleotide concentration 0.25 mM each (PeqLab), 1x buffer (PeqLab), 1.5 mM $MgCl_2$ (PeqLab) and 12.5 pmol of each amplification primer. PCR products were purified using the QIAquick purification kit (Qiagen). Cycle sequencing reactions (half reactions) were performed using a PTC-100 Thermocycler (MJ Research) in combination with the ABI Prism™ Big Dye Terminator Cycle Sequencing Ready Reaction Kit with Amplitaq-DNA polymerase FS (Perkin Elmer), applying a standard protocol for all reactions. Extension products were precipitated with 40 µl 75 % (v/v) isopropanol for 15 min at room temperature, centrifuged with 15,000 rpm at 25°C, and washed with 250 µl of 75 % (v/v) isopropanol. These purified products were loaded on an ABI 310 automated sequencer (Perkin Elmer) and electrophoresed. For cycle sequencing 10 µl–reactions were used containing 3 µl of Big Dye Terminator Cycle Sequencing premix. Sequencing reactions were performed on two independent PCR products generated from each sample in order to verify the results. All PCR products were sequenced using two primers.

Primers for amplifying and sequencing the ITS region (ITS4-bryo and ITS5-bryo) based upon the primers “ITS4” and “ITS5” respectively, designed and named by White et al.(1990), were slightly modified with respect to bryophytes (Stech, 1999). The primers ITS-C and ITS-D (Blattner, 1999) were modified for this study (ITS-D_bryo and ITS-C_bryo) and additionally used for sequencing reactions (table 28).

Table 28: Primer sequences used for amplification and sequencing of the ITS region. Underlined nucleotides represent changes with respect to the original primers of Blattner (1999).

Primer	Sequence	Data source
ITS-C bryo	GCA ATT CAC ACT ACG TAT CGC	Blattner 1999
ITS-D bryo	CTC TC<u>A</u> GCA ACG GAT ATC T<u>T</u>G	Blattner 1999
ITS4-bryo	TCC TCC GCT TAG TGA TAT GC	Stech 1999
ITS5-bryo	GGA AGG AGA AGT CGT AAC AAG G	Stech 1999

The ITS region was amplified using a protocol consisting of: 5 min. 94ºC, 35 cycles (1 min. 94ºC, 1 min. 48ºC, 1 min. 72ºC) and a 5 min. 72ºC extension time, cycle sequencing settings: 25 cycles (30 sec. 96ºC, 15 sec. 50ºC, 4 min. 60ºC). All sequences will be deposited in EMBL, accession numbers are listed in table 29, the alignments are available from the author on request.

Table 29: List of investigated specimens of *Catagonium* with EMBL accession numbers for the regions sequenced. Voucher numbers and the herbaria where the specimens are kept and country of origin are listed.

No.	*taxon*	ITS	Country/island of origin	Voucher label	herbarium
21	*Catagonium nitens* (Brid.) Card. ssp. *nitens*	AJ862497	Chile	Rolf Blöcher No. 1/14.2.01	J.-P. Frahm, Bonn
23	*Catagonium nitens* (Brid.) Cardot cf. ssp. *nitens*	AJ862505	NZ	BRYO AUSTRAL J.-P. Frahm no. 27-8	J.-P. Frahm, Bonn
25	*Catagonium nitens* (Brid.) Card. var. *myurum (*Card. & Thér.) Lin	AJ862504	Chile	BRYO AUSTRAL W. Frey & F. Schaumann no. 01-223	W. Frey, Berlin
59	*Catagonium nitens* (Brid.) Card. ssp. *maritimum* (Hook.) Lin	AJ862501	South Africa	S. M. Perold 936	Helsinki, Finland
61	*Catagonium emarginatum* Lin	AJ862496	Brazil	leg. A. Schäfer-Verwimp det. A. Schäfer-Verwimp & B. H. Allen 11193	Helsinki, Finland
63	*Catagonium brevicaudatum* C. Müll. ex Broth.	AJ862494	Columbia	Flora de Colombia Edgar Linares C. & Steven Churchill 3821	Helsinki
80	*Catagonium nitidum* (Hook. f. & Wilson) Broth.	AJ862496	Argentina	John J. Engel no. 3368 det. S. H. Lin 1981	Bot. Mus. Berlin
91	*Catagonium nitens* (Brid.) Card. ssp. *maritimum* (Hook.) Lin	AJ862503	South Africa	S. M. Perold 902 det. R. E. Magill 1988	Helsinki, Finland
92	*Catagonium brevicaudatum* C. Müll. ex Broth.	AJ862495	Columbia	Steven P. Churchill, Alba Luz Arbeláez, Wilson Rengifo no. 16297	Helsinki, Finland
236	*Catagonium nitidum* (Hook. f. & Wilson) Broth.	AJ862506	Chile	Frank Müller C 1501	F. Müller, Dresden
287	*Catagonium nitens* (Brid.) Card. ssp. *nitens*	AJ862498	Australia	H. Streimann 50457MUSCI	J.-P. Frahm, Bonn
288	*Catagonium nitens* (Brid.) Cardot cf. ssp.*nitens*	AJ862500	Chile	Holz & Franzaring CH 00-152 det. W. R. Buck	J.-P. Frahm, Bonn
289	*Catagonium nitens* (Brid.) Card. ssp. *nitens*	AJ862499	Chile	BRYO AUSTRAL Rolf Blöcher no. 46	J.-P. Frahm, Bonn

Phylogenetic analyses. Heuristic searches under the parsimony criterion were carried out under the following options: all characters unweighted and unordered, multistate characters interpreted as uncertainties, gaps coded as missing data, performing a tree bisection reconnection (TBR) branch swapping, collapse zero branch length branches, MulTrees option in effect, random addition sequence with 1000 replicates.

Furthermore the data sets were analysed using *win*PAUP 4.0b10 (Swofford, 2002) executing the command files generated by 'PRAP' (Parsimony Ratchet Analyses using PAUP Müller, 2004), employing the implemented parsimony ratchet algorithm (Nixon, 1999). For the parsimony ratchet the following settings were employed: 10

random addition cycles of 200 iterations each with a 40 % upweighting of the characters in the PRAP iterations. Heuristic bootstrap (BS Felsenstein, 1985) searches under parsimony criterion were performed with 1000 replicates, 10 random addition cycles per bootstrap replicate and the same options in effect as the heuristic search for the most parsimonious tree (MPT). The consistency index (CI, Kluge & Farris, 1969), retention index (RI), and rescaled consistency index (RC, Farris, 1989) were calculated to assess homoplasy.

Maximum Likelihood analyses were executed assuming a general time reversible model (GTR+G+I), and a rate variation among sites following a gamma distribution (four categories represented by the mean), with the shape being estimated and the molecular clock not enforced. According to Akaike Information Criterion (AIC, Akaike, 1974) GTR+G+I was chosen as the model that best fits the data by Modeltest v3.06 (Posada & Crandall, 1998), employing the windows front-end (Patti, 2002). The proposed settings by Modeltest v3.06 (table 30) were executed in *win*PAUP 4.0b10.

Table 30: Substitution models selected for the ITS data set *Catagonium* data set and 8 outgroup taxa.

	ITS data set
Model selected	GTR+I -lnL = 1921.4596
Substitution model	R(a) [A-C] = 1.0000 R(b) [A-G] = 2.3445 R(c) [A-T] = 0.4343 R(d) [C-G] = 0.8075 R(e) [C-T] = 2.3445 R(f) [G-T] = 1.0000
Among-site rate variation	
Proportion of invariable sites (I)	0.8075
Variable sites (G, Gamma distribution shape parameter)	equal rates for all sites

In addition to the MP analyses Bayesian Inferences with MrBayes3.0 (Huelsenbeck & Ronquist, 2001) were performed. Modeltest 3.5 (Posada, 2004) was used to select DNA substitution models for the data set (gamma shape distribution, six substitution types). The Markov Chain Monte Carlo (MCMC) analyses were run for 1,000,000 generations with four simultaneous MCMCs and one tree per 100 generations was saved. The ‘burn-in’ values were determined empirically from the likelihood values. The analyses were repeated three times to assure sufficient mixing by confirming that the program converged to the same posterior probability (PP).

The program Treegraph (Müller & Müller, 2004) was used to edit trees directly from PAUP-treefiles.
MEGA2.1 (Kumar et al., 2001) was used to calculate sequence length and distance measure ('p-distance'). In the following the term 'genetic distance' is used besides the term 'p-distance'.

7.3 Results

7.3.1 Phylogenetic results.

The results of the Maximum Likelihood (ML) analysis are presented in figure 21 as a phylogram where branch lengths are proportional to the number of substitutions per site. The data set consists of 21 taxa. Thirteen taxa of *Catagonium* were successfully sequenced and used in the analysis. Eight taxa were used as outgroup taxa, six of them belong to the same family as *Catagonium*, the Plagiotheciaceae (Pedersen & Hedenäs, 2002). Additionally, two species of the genus *Lepyrodon* (Lepyrodontaceae) were chosen as phylogenetically more distant outgroup taxa.

The eight outgroup taxa are well separated from the monophyletic clade of *Catagonium* (fig. 21). The most basal clade within the genus *Catagonium* consists of two taxa, *C. emarginatum* and *C. brevicaudatum*, which occur in northern South America and Brazil, here referred to as the 'Northern South America' clade. This clade is sister to a clade consisting of the representatives of *Catagonium nitidum* and two subspecies and one variety of *C. nitens*.
Within this clade the specimens of *C. nitens* ssp. *maritimum* from South Africa (sp. 51, 91) are the first to branch off. The specimens of this subspecies form the 'South African' clade. The long branch leading to these two specimens indicates a higher substitution rate compared to the following species (fig. 22).

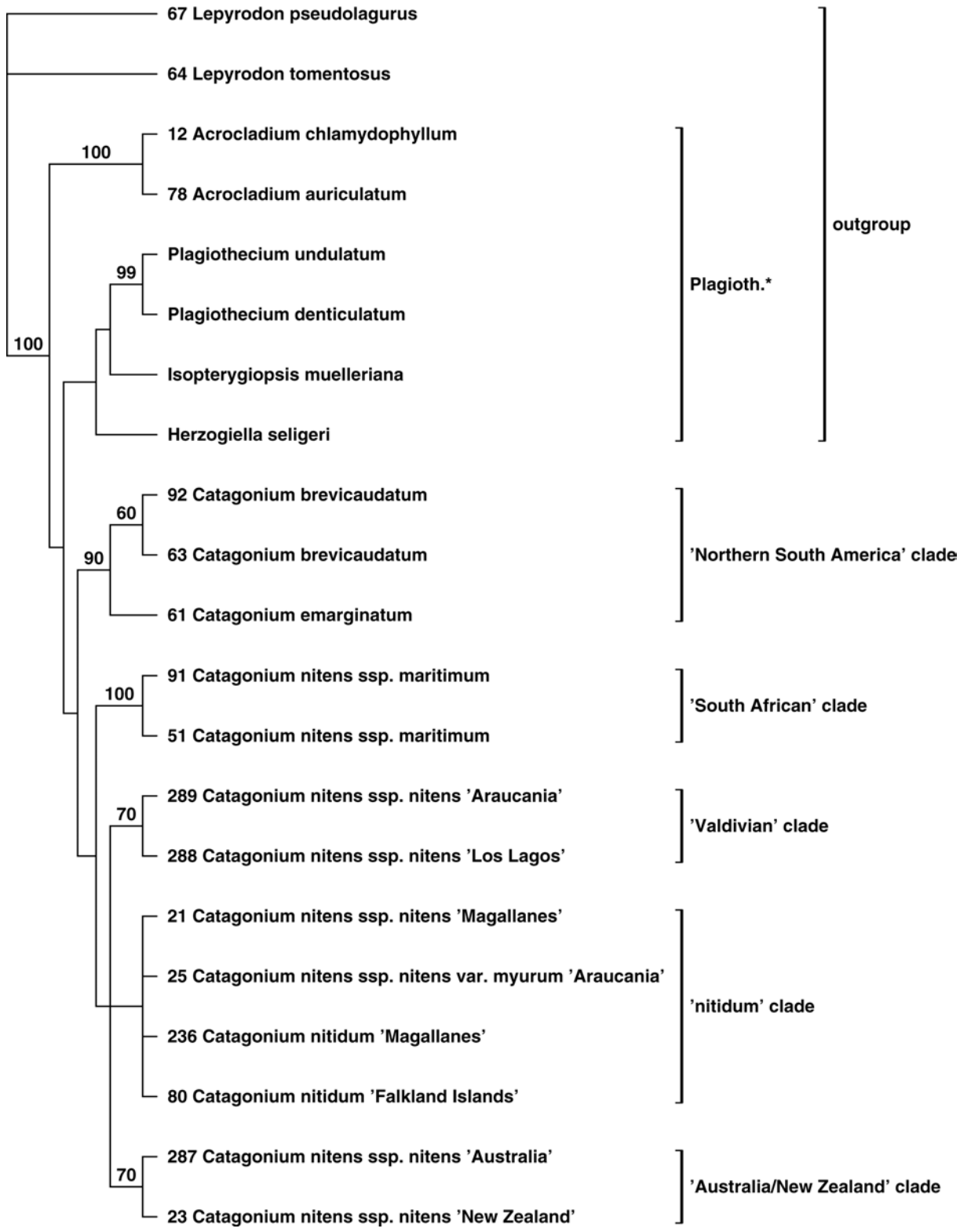

Figure 21: Maximum Likelihood (ML) cladogram of the ITS sequence data (L score = -1921.4596) of thirteen specimens of the genus *Catagonium* and two outgroup taxa. Bootstrap support values shown above branches result from a Maximum Parsimony analysis. For explanation of the clades referred to as 'outgroup', H, and A see text. Plagioth.*: Plagiotheciaceae *sensu* Pedersen & Hedenäs 2002.

The following monophyletic group consists of three clades, the 'Valdivian' clade, the 'Australia/New Zealand' clade and the '*myurum*' clade. The ‘Valdivian’ clade consists of two specimens of *C. nitens* ssp. *nitens* from Chile, one specimen from the Araucanian region (sp. 288), the second specimen from the Los Lagos region (sp. 289). The ‘Australia/New Zealand' clade consists of *C. nitens* ssp. *nitens* from Australia (sp. 287) and a second specimen from New Zealand (sp. 23). The '*myurum*' clade consists of four specimens with an ambiguous relationship. It comprises two specimens of *C. nitens* ssp. *nitens* (sp. 21, 25), including the variety ‘*myurum*‘ collected in the Araucanian region (sp. 25), and two specimens of *C. nitidum* (sp. 80, 236).

The Bayesian analysis (fig. 23) supports the monophyletic status of the genus *Catagonium* with 94 % posterior probability (PP). The ‘Northern South America’ clade is supported with 100 % PP, as well as the clade of the specimens of *Catagonium nitens* ssp. *maritimum* (‘South African’ clade).

In contrast to the ML analysis, the specimen of *C. nitidum* (sp. 80) from the Falkland Islands is the next taxon branching off. The following clade, supported with 91 % PP, consists of two specimens of *C. nitens* ssp. *nitens* from Chile, one specimen from the Araucanian region (sp. 288), the other from the Los Lagos region (sp. 289). The monophyly of the ‘Australian/New Zealand’ clade of *C. nitens* ssp. *nitens* is supported with 97 % PP. In contrast to the ML analyses the Bayesian Inference analyses resolved a clade consisting of *C. nitens* ssp. *nitens* from the Magallanes region (sp. 21), *C. nitens* ssp. *nitens* var. *myurum* (sp. 25) from the Araucanian and *C. nitidum* (sp. 236) from the Magallanes region. The specimen *C. nitidum* (sp. 80) from the Falkland Islands is clearly separated from this monophyletic group. In contrast to the other specimen of *C. nitidum*, specimen 80 from the Falkland Islands has a solitary basal position within the entire *C. nitens* clade.

Maximum Parsimony analyses resulted in a polytomy of five clades within the genus *Catagonium* (figure not shown). These clades were also resolved in a subsequent bootstrap analysis (fig. 21). The first clade consists of the ‘Northern South America’ clade (compare fig. 21-23) with 90 % bootstrap support (BS). In this clade the monophyly of the two specimens of *C. brevicaudatum* was weakly supported with

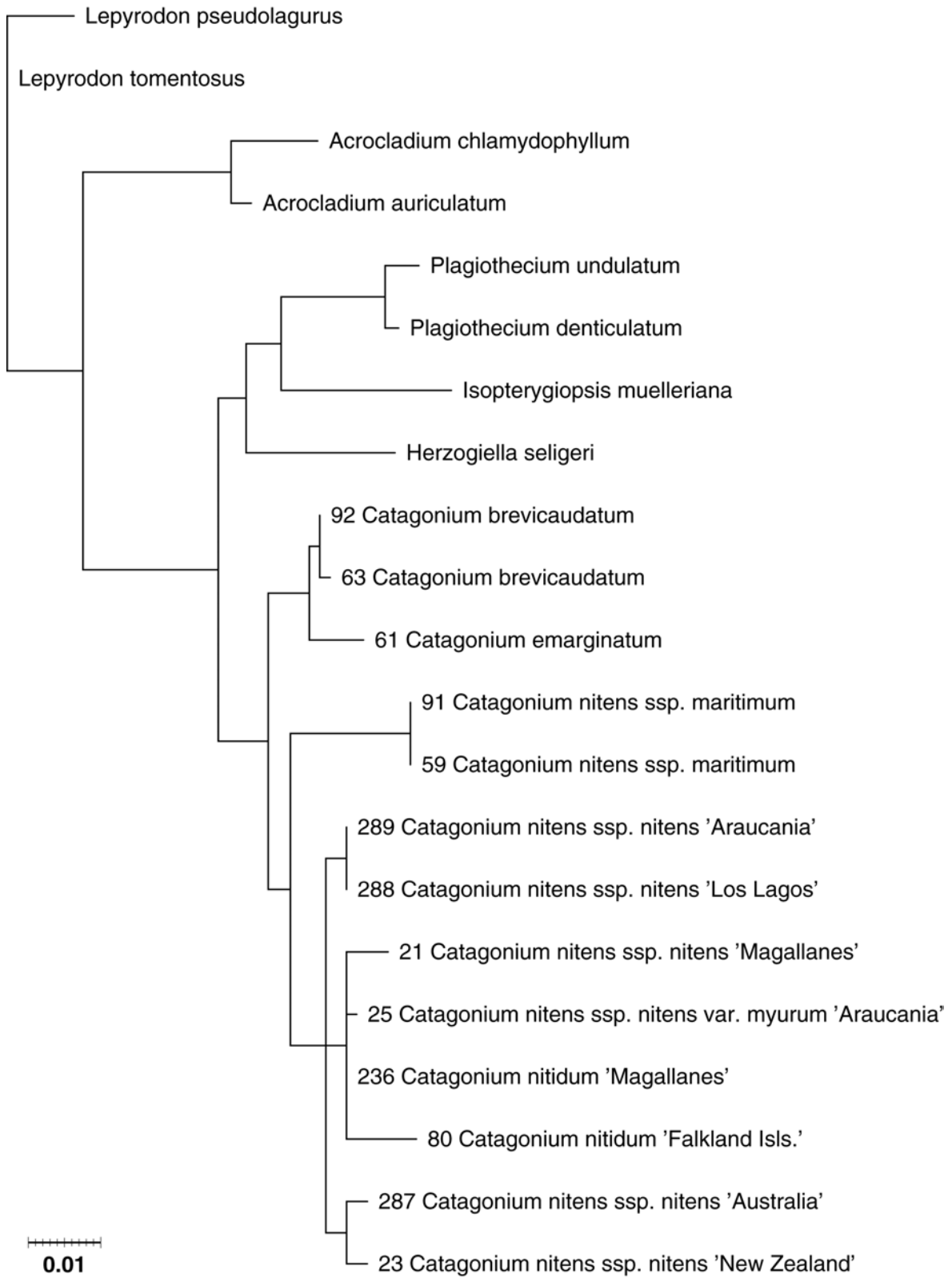

Figure 22: Maximum Likelihood (ML) phylogram of the ITS sequence data (L score = -1921.4596) of thirteen specimens of the genus *Catagonium* and two outgroup taxa. Branch lengths are proportional to genetic distance between taxa. Scale bar equals 1% distance under the assumed substitution model (GTR+I).

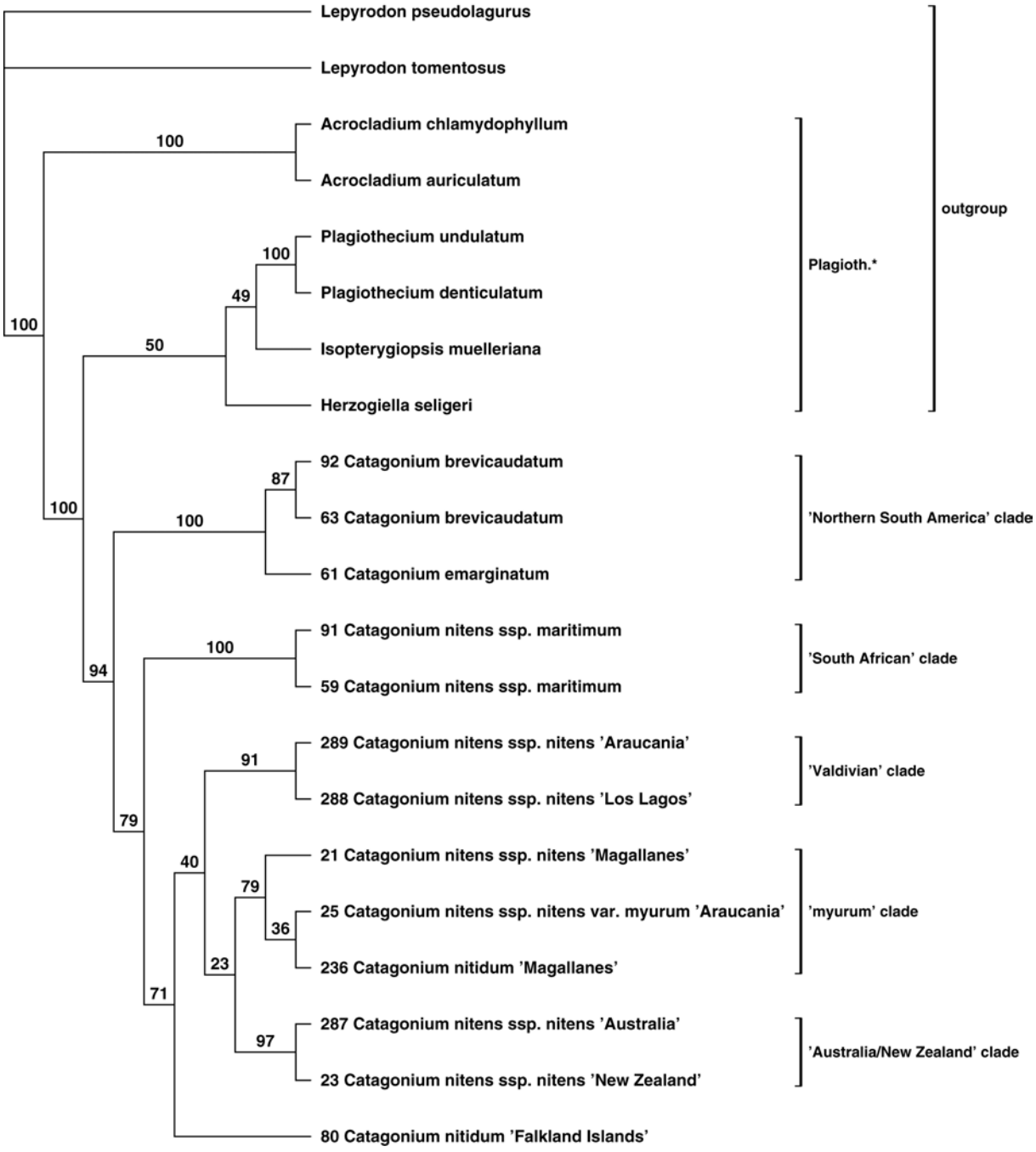

Figure 23: Cladogram resulting from a Bayesian Inference analysis of the ITS sequence data of thirteen specimens of the genus *Catagonium* and two outgroup taxa. Numbers above branches indicate the posterior probabilities as a percentage value. For explanation of the clades referred to as 'outgroup', 'Northern South America', 'South African', 'Valdivian', 'nitidum' and 'Australia/New Zealand see text. Plagioth.*: Plagiotheciaceae sensu Pedersen & Hedenäs 2002.

60 %. The second clade consists of the two specimens of *C. nitens* ssp. *maritimum* (100 % BS). The 'Valdivian' clade, and the 'New Zealand/Australia' clade are supported with 70 % BS each. A monophyletic group of *C. nitens* sp. 21, *C. nitidum* sp. 236 and *C. nitens* var. *myurum* is weakly supported with 58 % BS. The relationship of *C. nitidum* from the Falkland Islands to all these previously described clades remains ambiguous.

Both analyses resulted in the following clades with high branch support:
The basal position of the 'Northern South America' clade was found with ML analyses and with high support from Bayesian Inference (100 %) as well as bootstrap support (90 %).
The position of the clade of *C. nitens* ssp. *maritimum* from South Africa, following the 'Northern South America' clade in the cladograms and the phylogram (fig. 21-23), and as a sister group to a clade consisting of *C. nitens* and *C. nitidum*, has a posterior probability of 71 %.
The monophyly of the specimens of *C. nitens* ssp. *nitens* from Australia/New Zealand (sp. 23, 287) is supported with 97 % PP and the 'Valdivian' clade (sp. 288, 289) with 91 %. Each of the two clades is further supported with a bootstrap value of 70 %.

In this study the ITS region of 13 specimens of *Catagonium* was successfully sequenced. For specimen 80 (*Catagonium nitidum*) only the ITS 1 and part of the 5.8S rRNA were obtained. For the other specimen of *C. nitidum* (sp. 236), however, the full data set is available. ITS sequences of the specimens of *Acrocladium, Lepyrodon* and *Herzogiella seligeri* were taken from the results described in chapters 4-6. The ITS sequence data for *Plagiothecium undulatum*, *P. denticulatum* and *Isopterygiopsis muelleriana* were extracted from GenBank (table 29). The statistical data on the obtained sequences are depicted in table 31 for ITS1, 5.8S rRNA, and ITS2 sequences.
The observed sequence length of ITS1 within the genus *Catagonium* ranged between 248 basepairs (bp) for *Catagonium nitens* ssp. *nitens* (specimen 21) and 252 bp in *Catagonium nitens* var. *myurum* (sp. 25), *Catagonium nitidum* (sp. 236), and *Catagonium brevicaudatum* (sp. 63). The length of the ITS1 region was on average 250.3 bp with a standard deviation of 1.4 for the thirteen specimens of *Catagonium*. For the complete data set consisting of 21 taxa the average length of

Table 31: Sequence lengths [base pairs, bp] and GC-content [%] for the ITS region of thirteen *Catagonium* specimens and eight outgroup taxa. Average sequence lengths and standard deviations are given for the data set with 21 species. Average sequence lengths and standard deviations are also given for the thirteen species separately ('Average Cat.'). For origin of the data refer tab. xz. Abbreviations: A.: *Acrocladium*; C.: *Catagonium*; n. d. = no data available. (* partial sequences were excluded when determining the average sequence length)

	Total sequence length[%] ITS1	G/C content ITS1	Total sequence length[%] 5.8S	G/C content 5.8S	Total sequence length[%] ITS2	G/C content ITS2
Lepyrodon pseudolagurus (sp.67)	249	64.6	160.0	64.6	264.0	65.9
Lepyrodon tomentosus (sp.64)	246	63.4	159.0	63.4	266.0	65.4
A. chlamydophyllum (sp.12)	255	62.7	160.0	62.7	236.0	63.6
A. auriculatum (sp.78)	255	64.3	160.0	64.3	239.0	64.5
Plagiothecium undulatum	240	62.9	n.d.	n.d.	183.0	63.4
Plagiothecium denticulatum	248	62.5	94.0	62.5	258.0	64.4
Isopterygiopsis muelleriana	248	64.9	160.0	64.9	262.0	64.9
Herzogiella seligeri	244	62.3	160.0	62.3	262.0	62.2
C. brevicaudatum (sp. 92)	252	65.1	160.0	65.1	292.0	65.4
C. brevicaudatum (sp. 63)	252	65.1	160.0	65.1	292.0	65.4
C. emarginatum (sp. 61)	249	64.2	160.0	64.2	292.0	65.7
C. nitens (sp. 91)	249	64.6	160.0	64.6	303.0	65.3
C. nitens (sp. 59)	249	64.6	160.0	64.6	303.0	65.3
C. nitens (sp. 289)	250	64.0	160.0	64.0	299.0	65.9
C. nitens (sp. 21)	248	62.9	160.0	62.9	300.0	66.0
C. nitens (sp. 288)	250	64.0	160.0	64.0	300.0	66.0
C. nitens (sp. 287)	251	63.4	160.0	63.4	299.0	67.5
C. nitens (sp. 23)	249	63.4	160.0	63.4	299.0	67.2
C. nitens (sp. 25)	252	62.7	160.0	62.7	301.0	66.2
C. nitidum (sp. 236)	252	63.1	160.0	63.1	302.0	66.3
C. nitidum (sp. 80)	251	62.6	79.0	62.6	n.d.	n.d.
Average	*249.5*	*63.7*	*159.9*	*49.7*	*277.6*	*65.3*
SD	*3.4*	*0.9*	*0.2*	*1.9*	*31.4*	*1.3*
Average Cat.	*250.3*	*63.8*	*160.0*	*50.0*	*298.5*	*66.0*
SD Cat.	*1.4*	*0.9*	*0.0*	*0.0*	*4.2*	*0.7*

the ITS1 region was 249.5 bp with a standard deviation of 3.4. For *Plagiothecium undulatum* from GenBank only part of the ITS1 sequence was available.

The GC-content of the thirteen specimens of *Catagonium* ranged between 62.6 % in *Catagonium nitidum* (sp. 80) and 65.1 % observed in both specimens of *Catagonium brevicaudatum* (sp. 63, 92). The average GC-content in the data set was 63.8 % (standard deviation 1.2). For the complete data set (21 taxa) the average GC-content in the ITS1 was 63.7 % (standard deviation 0.9).

The observed size of the sequence length of ITS2 within the genus *Catagonium* ranged between 292 basepairs (bp) for *Catagonium brevicaudatum* (sp. 63, 92) and *Catagonium emarginatum* (sp. 61) and 303 bp found in *Catagonium nitens* ssp. *maritimum* (sp. 59, 91). The obtained length for the ITS2 region was on average 298.5 bp with a standard deviation of 4.2 for the thirteen specimens of *Catagonium*. For the data set consisting of 20 taxa the average length of the ITS2 was 277.6 bp with a standard deviation of 31.4. For *Plagiothecium undulatum* from GenBank only part of the ITS2 sequence was available.

The GC-content of the thirteen specimens of *Catagonium* was between 65.4 % in *Catagonium brevicaudatum* (sp. 63, 92) and 67.5 % observed in the specimens of *Catagonium nitens* ssp. *nitens* from Australia (sp. 288). The average GC-content in the data set was 66.0 % (standard deviation 0.7). For the complete data set (20 taxa) the average GC-content in the ITS2 was 65.3 % (standard deviation 1.3).

Table 32 presents the information for the different regions in the alignment. The complete data set of the entire ITS region of 21 taxa revealed a variability of 11.2 % in 805 aligned positions (basepairs). Within the thirteen specimens of *Catagonium*, the intrageneric variability was 4.8 %.

Table 32: Number of taxa, total number of aligned characters; variable characters and number of parsimony informative sites and %-value of variable sites for the partial data sets of *Catagonium*. (* Including the outgroup taxa).

Data set	Number of taxa included	Total number of aligned characters [bp]	Variable characters [bp]	parsimony informative [bp]	Variable sites [%]
ITS	21*	805	90	61	11.2
ITS	13	805	39	25	4.8
ITS1	21*	273	40	23	14.7
ITS1	13	273	15	8	5.5
5.8S	21*	160	1	1	0.6
5.8S	13	160	1	1	0.6
ITS2	21*	371	49	38	13.2
ITS2	13	371	23	17	6.2

The highest proportion of variable sites was found in the ITS1 region where 14.7 % of the 273 aligned positions (basepairs) were variable in the data set including the outgroup (intrageneric variability of *Catagonium* 5.5 %). The ITS2 region is less variable than ITS1, bearing only 13.2 % variable positions within 371 aligned

basepairs, but offers a higher degree of intrageneric variability of 6.2 % within the genus *Catagonium*.

7.3.2 Indel matrix

Table 33 lists a summary of the specific indels supporting single clades in the genus *Catagonium*. 21 indels were recognized in the ITS region. Six were found in the ITS1 and fifteen in the ITS2 region. The length of these indels ranged from one to four nucleotides. Fifteen indels were uniquely found in certain clades and can therefore be interpreted as synapomorphies of these clades (figure 21 & 23).

Table 33: Indelmatrix for the ITS1 and ITS2 data set of thirteen specimens of *Catagonium*. Indels I to VI were found in the ITS1 region, Indels VII were found in the ITS2 region. Abbreviations: *C.=Catagonium*, *brev.=brevicaudatum*, *emargin.=emarginatum.*

Indel no./ Species	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
C. brevicaud .(sp. 92)	CC	TCG					CTTT									
C. brevicaud. (sp. 63)	CC	TCG					CTTT									
C. emargin. (sp. 61)	CC	TCG					CTTT									
C. nitens (sp. 91)			CA	GT	A	AGT	CTTT	GC	CGTT	GC						
C. nitens (sp. 59)			CA	GT	A	AGT	CTTT	GC	CGTT	GC						
C. nitens (sp. 289)								GC	CGTT	GC	G	GC	C	T	T	
C. nitens (sp. 21)								GC	CGTT	GC	G	GC	C	T	T	
C. nitens (sp. 288)								GC	CGTT	GC	G	GC	C	T	T	
C. nitens (sp. 287)								GC	CGTT	GC	G	GC	C	T	T	
C. nitens (sp. 23)								GC	CGTT	GC	G	GC	C	T	T	
C. nitens (sp. 25)								GC	CGTT	GC	G	GC	C	T	T	AAT
C. nitidum (sp. 236)								GC	CGTT	GC	G	GC	C	T	T	AAT
C. nitidum (sp. 80)								NN	NNNN	NN	G	NN	N	N	N	AAT

Two indels, with two and three nucleotides in length, respectively (I, II, table 33) are found as synapomorphies of the three specimens from Brazil/northern South America, *C. brevicaudatum* and *C. emarginatum* (sp. 61, 63, 92) investigated in this study. Four indels (III-VI) are synapomorphic in the specimens of *Catagonium nitens* ssp. *maritimum* from South Africa (sp. 91, 51). One indel (VII) with four nucleotides in length is shared between the species from Brazil/northern South America (sp. 61, 63, 92) and South Africa (sp. 91, 51). Three indels (VIII-X, table 33) are synapomorphic to the specimens of *Catagonium nitens* ssp. *maritimum* (sp. 91, 51) and those of *Catagonium nitens* (sp. 21, 23, 25, 287, 288, 289) from southern South America, Australia and New Zealand as well as *Catagonium nitidum* (sp. 80, 236) from the Falkland Islands and from Chile. The lengths of these three indels are two and four

nucleotides, respectively. Five indels (XI-XV), 1-2 nucleotides in length, are synapomorphies for eight specimens comprising *Catagonium nitens* from southern South America, Australia and New Zealand (sp. 21, 23, 25, 287, 288, 289) and *Catagonium nitidum* from the Falkland Islands and Chile (sp. 80, 236). Another indel (XVI) comprising 3 bp is syapomorphic to the clade '*myurum*' in figure 23 comprising *Catagonium nitens* (sp. 21) from southern Chile, *C. nitens* ssp. *nitens* var. *myurum* (sp. 25) and a specimen of *C. nitidum* (sp. 236) from southern Chile.

7.3.3 Genetic distances

Genetic distance revealed by ITS1 sequence data. Results of the pairwise distance comparison (model: 'p-distance') with MEGA (Kumar et al., 2001) are depicted in appendix 16 for the ITS1 region and in appendix 17 for ITS2.

The average genetic distance in the data of the ITS1 region for 21 specimens is 3.4 % (standard error 0.6). The average genetic distance of the thirteen specimens of the genus *Catagonium* is 1.6 % (standard error 0.5).

The highest genetic distances in the ITS1 were obtained separating *Herzogiella seligeri* from *Lepyrodon tomentosus* (7.4 %) and representative species of the Plagiotheciaceae (e.g. 7.4 % to *Acrocladium auriculatum*, 6.6 % to *Isopterygiopsis muelleri*, 5.4 % to *Plagiothecium denticulatum*). Low values in the outgroup taxa comprising the genus *Lepyrodon* and representatives of the Plagiotheciaceae were obtained when comparing intrageneric distances. The genetic distance separating the two species of *Acrocladium* is 1.6 %, *Lepyrodon pseudolagurus* and *L. tomentosus* are separated by 1.6 % difference in substitutions, and between the two species of *Plagiothecium* the difference is 0.8 %.

Genetic distance of Catagonium to the outgroup taxa. The genetic distance of *Catagonium* to *Acrocladium* is between 2.4 % in *Catagonium nitens* ssp. *nitens* (sp. 21) and 6.1 % in *Catagonium nitens* ssp. *maritimum* (sp. 59, 91). The distance to *Acrocladium* is between 4.1 % in *Catagonium nitens* ssp. *nitens* (sp. 21) and 6.9 % in *Catagonium nitens* ssp. *maritimum* (sp. 59, 91). *Catagonium nitens* (sp. 288, 289) and *Catagonium nitidum* (sp. 236) show the lowest genetic distance to the genus *Plagiothecium* with 2.5 % each, and *C. brevicaudatum* (sp. 63, 92) the highest with 4.3 % each.

Genetic distance to *Isopterygiopsis muelleriana* is lowest (2.4 %) in *Catagonium nitens* (sp. 288, 289) and *Catagonium nitidum* (sp. 236). The greatest distance was observed in relation to *Catagonium nitens* ssp. *maritimum* (sp. 59, 91) with 4.0 %. The genetic distance of the species *Herzogiella seligeri* to *Catagonium* ranged between 4.1 % (*Catagonium nitens*, sp. 288, 289 and *Catagonium nitidum*, sp. 236) and 5.3 % (*Catagonium emarginatum*, sp. 61).

Genetic distances within the genus Catagonium

***Catagonium brevicaudatum* and *C. emarginatum*.** The genetic distance between *C. brevicaudatum* (sp. 63, 92) from Columbia and *C. emarginatum* from Brazil (sp. 61) is 0.4 %. There is no genetic difference between the two specimens of *C. brevicaudatum*, i.e. between specimens 63 and 92.

The genetic distance of the 'Northern South America' species to *C. nitens* ssp. *nitens* is between 1.2 (*C. emarginatum*) and 1.6 % (*C. brevicaudatum*) for the specimens of *C. nitens* ssp. *nitens* from Chile and Australia (sp. 21, 288, 287, 289). The specimen of *C. nitens* ssp. *nitens* from New Zealand (sp. 23) and the variety '*myurum*' from Chile (sp. 25) have a distance of 1.6 % (to *C. emarginatum*) and 2.0 % (to *C. brevicaudatum*) to the 'Northern South America' species.

The two specimens of *C. nitidum* show different distances to the 'Northern South America' species. *C. nitidum* from the Torres del Paine National Park shows the same distance to *C. emarginatum* (1.2 %) and to *C. brevicaudatum* (1.6 %) as most of the specimens of *C. nitens* ssp. *nitens* whereas *C. nitidum* from the Falkland Islands (sp. 80) shows a higher distance with 2.0 and 2.4 % to *C. emarginatum* and *C. brevicaudatum*, respectively.

The genetic distances between the 'Northern South America' specimens (*C. brevicaudatum*, sp. 63, 92 and *C. emarginatum*, sp. 61) and the South African specimens of *Catagonium nitens* ssp. *maritimum* (sp. 59, 91) is between 2.8 % (*C. brevicaudatum*) and 3.2 % (*C. emarginatum*).

***Catagonium nitens* ssp. *maritimum*.** Both specimens of *C. nitens* ssp. *maritimum* were identical, whereas the genetic distance of the South African specimens of *Catagonium nitens* ssp. *maritimum* to *C. nitens* ssp. *nitens* ranges from 2.0 % to 3.2 %.

The distance of *Catagonium nitens* ssp. *maritimum* is lowest to the specimens 288 and 289 of *C. nitens* ssp. *nitens* from the Chilean Los Lagos and Araucanian region, intermediate to the variety '*myurum*' of *C. nitens* from Chile (sp. 25) with 2.4 %, to the Australian specimen (sp. 287) and to the southern Chilean specimen (sp. 21). The genetic distance is highest to the specimen from New Zealand (sp. 23) with 3.2 %.
The two specimens of *C. nitidum* show different distances to the South African specimens. *C. nitidum* from the Torres del Paine National Park shows the same distance to *Catagonium nitens* ssp. *maritimum* (2.0 %) as the specimens 288 and 299 of *C. nitens* ssp. *nitens*, whereas *C. nitidum* from the Falkland Islands (sp. 80) shows a higher distance of 2.9 % to *Catagonium nitens* ssp. *maritimum* (sp. 59, 91).

Distances between the specimens of *Catagonium nitens* ssp. *nitens*. No mutations were detected between the specimens of *C. nitens* ssp. *nitens* from the Chilean Los Lagos and the Araucanian region, sp. 288 and 289, respectively. These specimens showed a genetic distance of 0.8 % to specimen 21 from Punta Arenas (Chile). The genetic distance of the *C. nitens* ssp. *nitens* specimens from Chile (sp. 21, 288, 289) showed a distance of 0.8 % to the specimen from Australia (sp. 287), and a distance of 1.2 % to the specimen from New Zealand (sp. 23). The genetic distance of *C. nitens* ssp. *nitens* var. *myurum* from the Araucanian region to the specimens of *C. nitens* ssp. *nitens* var. *nitens* from Los Lagos and the Araucanian region was 0.4 %. The distance of this variety is 1.2 % to the subspecies *nitens* from Australia and that of Punta Arenas.

Catagonium nitidum. *Catagonium nitidum* (sp. 236) from the Torres del Paine National Park and *Catagonium nitidum* (sp. 80) from the Falkland Islands show a distance of 0.8 %.
There was no genetic distance (0.000 %) detected between specimen 236 of *C. nitidum* and the specimens of *C. nitens* ssp. *nitens* from the Chilean Los Lagos and Araucanian region. It is separated by a distance of 1.2 % from *C. nitens* ssp. *nitens* from New Zealand (sp. 23).
The specimen from the Falkland Islands (sp. 80) shows highest distances to the specimens of *C. nitens* from New Zealand (2.0 %) and Australia (1.6). The distance of sp. 80 to the specimen of *C. nitens* from Punta Arenas (sp. 21) is 1.6 %. The

distance to *C. nitens* ssp. *nitens* from the Chilean Los Lagos and Araucanian region is 0.8 %.

Genetic distance as determined from ITS2 sequence data

The average genetic distance in the data of the ITS2 region for 20 specimens is 5.0 % (standard error 0.8). The average genetic distance between the thirteen specimens of the genus *Catagonium* is 2.6 % (standard error 0.6). Note that no genetic sequences of the ITS2 region were obtained for the specimen of *C. nitidum* from the Falkland Islands (sp. 80).

The highest genetic distances in the ITS2 were obtained separating *Plagiothecium denticulatum* from *Acrocladium chlamydophyllum* (8.2 %) and *A. auriculatum* (7.7 %). Low values in the outgroup taxa comprising *Lepyrodon* and representatives of the Plagiotheciaceae were obtained when comparing intrageneric distances. The genetic distance separating the two species of *Acrocladium* is 2.1 %, *Lepyrodon pseudolagurus* and *L. tomentosus* are separated by 0.8 % differences in substitutions, and between the two species of *Plagiothecium* the difference is 0.5 %.
The genetic distance of *Catagonium* to *Acrocladium* ranges from 6.1 % in *C. brevicaudatum* (sp. 92) and *C. emarginatum* (sp. 61) to the *Acrocladium* species to 8.9 % in *C. nitens* ssp. *nitens* from southern Chile (sp. 21).
In relation to the genus *Plagiothecium* the species *Catagonium brevicaudatum* (sp. 92), *Catagonium nitens* ssp. *nitens* (sp. 23, 25, 287, 288, 289) and *C. nitidum* (sp. 236) show the lowest genetic distance with 3.4 %. *Catagonium emarginatum* (sp. 61) and *C. nitens* ssp. *maritimum* (sp. 59, 91) show the highest distance to *Plagiothecium* with 4.3 %.
Genetic distance to *Isopterygiopsis muelleriana* is lowest (5.3 %) in *C. brevicaudatum* (sp. 92). The greatest difference was observed to *C. nitens* ssp. *nitens* from southern Chile (sp. 21) with 8.2 %.
The genetic distance of the species *Herzogiella seligeri* to *Catagonium* ranged between 3.7 % in *C. brevicaudatum* (sp. 92) and 6.1 % (*Catagonium nitidum,* sp. 236 and *Catagonium nitens* (sp. 25).

Genetic distance within the genus *Catagonium*

The genetic distance between the Andean specimens of *C. brevicaudatum* (sp. 63, 92) from Columbia and *C. emarginatum* from southeastern Brazil (sp. 61) is between 1.7 % (sp. 63) and 2.1 % (sp. 92). Genetic distance between the two specimens of *C. brevicaudatum* is 0.3 %.

The genetic distance of the Andean specimens of *C. brevicaudatum* (sp. 63, 92) to *C. nitens* ssp. *nitens* is between 2.5 % (sp. 23 from New Zealand) and 3.9 % (sp. 21 from southern Chile). The distance of *C. emarginatum* (sp. 61) to *C. nitens* is lowest to *C. nitens* ssp. *maritimum* (sp. 59, 91) and *C. nitens* ssp. *nitens* from New Zealand with 4.2 % whereas it is 4.6 % to all the other specimens.

The specimen of *C. nitidum* from the Torres del Paine National Park (sp. 236) shows the same distance to *C. emarginatum* (2.8-3.2 %) and to *C. brevicaudatum* (4.6 %) as the specimen of *C. nitens* ssp. *nitens* var. *myurum*.

The genetic distance between the specimens of the 'Northern South America' clade (*C. brevicaudatum*, sp. 63, 92, and *C. emarginatum*, sp. 61) to the South African specimens of *Catagonium nitens* ssp. *maritimum* (sp. 59, 91) is between 2.8 % (*C. brevicaudatum*, sp. 92) and 4.2 % (*C. emarginatum*). The genetic distance between the two specimens of *C. nitens* ssp. *maritimum* is 0.000 %.

The genetic distance of the South African specimens of *Catagonium nitens* ssp. *maritimum* to *C. nitens* ssp. *nitens* ranges from 3.1 % to 4.4 %.

The distance of the subspecies *maritimum* is lowest to the specimens 288 and 289 of ssp. *nitens* from the Chilean Los Lagos and Araucanian region (3.1 %), intermediate to *C. nitens* ssp. *nitens* from New Zealand (sp. 23) with 3.4 %, to the Australian specimen (sp. 287) and to the variety '*myurum*' of *C. nitens* ssp. *nitens* from Chile (sp. 25) with 3.7 %. The genetic distance is highest to the specimen from southern Chile (sp. 21) with 4.4 %.

The specimen of *C. nitidum* (sp. 236) shows a difference of 3.7 % to *Catagonium nitens* ssp. *maritimum*.

There was no genetic distance (0.000 %) detected between the specimens of *C. nitens* ssp. *nitens* from the Chilean Los Lagos and Araucanian region, specimens 288 and 289, respectively. These specimens showed 2.0 % genetic distance to the specimen from Punta Arenas (sp. 21).

The genetic distance of the *C. nitens* ssp. *nitens* specimens from the Chilean Los Lagos (sp. 288) and Araucanian region (sp. 289) to the specimen from Australia sp.

287, is 1.3 and 1.4 %, respectively. The distance of sp. 21 from Punta Arenas to the Australian specimen is 2.0 %.

The genetic distance of the *C. nitens* ssp. *nitens* specimens from the Chilean Los Lagos (sp. 288) and Araucanian (sp. 289) region to the specimen from New Zealand (sp. 23) is 1.0 %. The distance of sp. 21 from Punta Arenas to the New Zealand specimen is 1.7 %.

The genetic distance between *Catagonium nitens* ssp. *nitens* from New Zealand (sp. 23) and Australia (sp. 287) is 0.3 %.

The genetic distance of *C. nitens* ssp. *nitens* var. *myurum* (sp. 25) from the Araucanian region to the specimens of *C. nitens* ssp. *nitens* from Los Lagos (sp. 288), the Araucanian region (sp. 289) and Australia (sp. 287) is 1.3 %. The distance of this variety to *C. nitens* ssp. *nitens* from New Zealand (sp. 23) is 1.0 %, the distance to ssp. *nitens* from Punta Arenas (sp. 21) is 0.7 %.

There was no genetic difference detected between *C. nitens* ssp. *nitens* var. *myurum* from the Araucanian region and *C. nitidum* (sp. 236) from the Magallanes region. Furthermore, the genetic difference of *C. nitidum* (sp. 236) to the specimens of *C. nitens* ssp. *nitens* from Los Lagos (sp. 288), the Araucanian region (sp. 289), Australia (sp. 287), New Zealand (sp. 23), and Punta Arenas (sp. 21) is the same as described for *C. nitens* ssp. *nitens* var. *myurum*.

7.4 Discussion

Phylogenetic results.

7.4.1 The 'Northern South American' species

Lin (1984) described a new species *Catagonium emarginatum* Lin from the Andes and stated that this species is closely related but morphological quite distinct from *C. brevicaudatum*. In the genetically based analysis presented here *C. brevicaudatum* is represented by two specimens originating from Columbia (sp. 63, 92) and *C. emarginatum* from southeastern Brazil (sp. 61). The two species are sister taxa in a clade at the most basal position of the specimens of the genus *Catagonium* investigated in this study. Although *C. brevicaudatum* and *C. emarginatum* are

closely related as indicated in the phylogenetic analysis, they are genetically distinct taxa. One could argue that the genetic differentiation between *C. brevicaudatum* and *C. emarginatum* is caused by geographical variation of one species, as both specimens of *C. brevicaudatum* originate from Columbia and that of *C. emarginatum* from southeastern Brazil. An additional analysis of the two species using material from the same area e.g. southeastern Brazil, might give further information about the taxonomic status of the 'Northern South America' clade obtained in this study.
The closest relative to the 'Northern South America' taxa is *C. nitens* ssp. *maritimum* in the next following clade.

7.4.2 The systematic position of *C. nitens* ssp. *maritimum*

Lin (1984) described a close relationship of *Catagonium nitens* with *C. brevicaudatum* based on the abruptly narrowed leaf apices appearing in the ssp. *maritimum* as well as in plants of ssp. *nitens* from New Guinea and are also characteristic for *C. brevicaudatum*. Unfortunately, no fresh material for DNA extraction from New Guinea could be obtained for this study. The two specimens of *C. nitens* ssp. *maritimum* from South Africa (sp. 51, 91) included in this study were genetically distinct from the other specimens of *C. nitens* as well as from *C. nitidum*, *C. emarginatum*, and *C. brevicaudatum*. However, according to Lin (1984) *C. nitens* ssp. *maritium* is morphologically well separated from *C. nitens* ssp. *nitens* and also from *C. brevicaudatum*. The characters separating *C. nitens* ssp. *maritium* from subspecies *nitens* is e.g. the terete foliation and the mucronate leaf apex of the subspecies from South Africa compared to the complanate foliation and the narrow, acute leaf apex in *C. nitens* ssp. *nitens*. The concave leaves found in *C. nitens* and the absence of leaf auricles distinguish this species from *C. brevicaudatum* (Lin, 1984).
Based on morphological as well as on the genetic evidence summarized above the status of *C. nitens* ssp. *maritium* as a subspecies of *C. nitens* should be revised. The data presented here and also the morphological data by Lin (1984) suggest that a species status might be justified.

7.4.3 The relationship within *Catagonium nitens*

In this study *Catagonium nitens* sensu Lin (1984) is paraphyletic with respect to the position of *C. nitidum* (sp. 80).

Both analyses resolve a clade comprising all representatives of *C. nitens* ssp. *nitens* and of *C. nitidum*. Within this clade two geographically distinct clades are well supported, one clade consisting of the specimens from Chile ('Valdivian' clade), the other of the specimens from Australia and New Zealand ('Australian/New Zealand' clade). These two clades are genetically separated from the representatives of *C. nitidum* as well as from the variety *myurum*.

The genetic data in this case give information not obtained by morphological analysis. Lin (1984; 1989) did not detect any further separation of the variety *nitens*, e.g. geographically.

The two clades were found in the ML analysis in an ambiguous position to each other as well as to a third clade consisting of the two specimens of *C. nitidum*, *C. nitens* ssp. *nitens* var. *myurum* and one more specimen of the variety *nitens*. The Bayesian Inference (BI), in contrast, indicated a sister relationship between one specimen of *C. nitidum*, and one specimen each of the varieties *nitens* and *myurum*. In fact even Lin (1989), who was the first to describe the variety *myurum* of *C. nitens* ssp. *nitens*, pointed out that the separation between the two varieties is not always clear and that intermediate forms exist. In the species *C. nitidum* Lin (1984) observed dwarf plants attached with rhizoids on the leaf surface of full sized plants. According to Lin (1984) *Catagonium nitidum* is morphologically very close to the dwarf forms of *C. nitens* ssp. *nitens* from subantarctic islands (which resembles *C. myurum* Card. & Thér.). *C. nitidum* is separated by its oblong leaves with an abruptly long-cuspidate apices from the dwarf forms of ssp. *nitens* which Lin (1989) described as the variety *myurum*.

The specimen 236 investigated in this study was a dwarf expression of either *C. nitidum* as labelled or *C. nitens* ssp. *nitens* with which it shares characters of the leaf apex and would in the later case represent another specimen of the variety *myurum* (like sp. 25). Both specimens appear as sister taxa in the Bayesian analysis (although with low probability), and show low genetic variability (0-0.4 %) between the two specimens.

The ability to develop dwarf plants may reflect adaptations to the environment (Hedenäs, 2001; Lin, 1989) and needs further investigation.

The specimen of *C. nitidum* from the Falkland Islands (sp. 80) is a normal sized plant which was identified by Lin in 1981. In the BI analysis it retains a basal position to *C. nitens* ssp. *nitens* implying that this taxon is genetically distinct from *C. nitens*.

However, this position is based on ITS1 data only and more specimens are needed for a final statement on the *C. nitidum* and *C. nitens* ssp. *nitens* clade.

8 The 'Gondwana connection' and their genetic patterns in bryophytes

The expression ‚Gondwana connection’ as used for example on the title of vol. 49, issue 3 of the Austral Journal of Botany in 2001 refers to the different areas formerly connected in the ‘supercontinent’ Gondwana, which are now disjunct, i.e. South America, Africa, Antarctica, and parts of Australasia.

The results of the phylogenetic analysis and the genetic distances are used to circumscribe a scenario of evolution of the genus *Catagonium*. Furthermore, common patterns between the evolution of the southern hemispheric disjunct distributed taxa *Acrocladium*, *Catagonium* and *Lepyrodon* are pointed out.

For the genus *Catagonium* the phylogenetic results of this study resolved the northern South American (*C. brevicaudatum* und *C. emarginatum*) and South African taxa (*C. nitens* ssp. *maritimum*) as basal within the genus. The remaining clade comprises taxa with specimens of the taxa *C. nitens* ssp. *nitens* var. *nitens* and *C. nitidum*. The analysis showed ambiguous results concerning the taxonomic identity of one *C. nitidum* specimen (sp. 236). The position of *C. nitidum* from the Falkland Islands basal to *C. nitens* ssp.*nitens* is uncertain probably because of the missing sequence data from the ITS2 region. The obtained phylogenetic results are in the following used to explain the evolution within the genus *Catagonium*.

Many species occur disjunctly in northern South America and in Africa and there are discussions whether the disjunct distribution patterns result from a vicariance event such as the break-up of the Gondwana continent or whether they are the result of dispersal events e.g. *Calymperes venezuelanum, Squamidium brasiliense* (Delgadillo M., 1993; Orbán, 2000). In this analysis, the basal position of the South African clade and South American clade is consistent with the break-up history of Gondwana during which the first continental blocks to separate were those of Africa and South America in the Early and late Cretaceous *c*. 105 Myr BP (e.g. McLoughlin, 2001; Sanmartín & Ronquist, 2004). From this study it is concluded that the common

ancestor of *C. brevicaudatum/C. emarginatum* and *C. nitens* ssp. *maritimum* originated from the former Gondwana continent, and the split of the African and South American landmasses as a vicariance event resulted in a divergent evolution of the taxon in the geographically separated areas. The strong genetic separation, as shown by the genetic distances, separating the northern South American taxa from the South African taxa on the one hand as well as separating these two groups of species from the remaining species *C. nitidum* and *C. nitens* ssp. *nitens* supports the hypothesis that populations of a common ancestor of *C. brevicaudatum/C. emarginatum* and *C. nitens* ssp. *maritimum* were separated by Gondwana vicariance *c*. 105 Myr BP.

Evidence of vicariance events related to the early split of the landmasses of Africa and South America as found here in *Catagonium* has also been found based on molecular data of certain angiosperm taxa, e.g. in *Gunnera* (for a review also see Sanmartín & Ronquist, 2004; data by Wanntorp & Wanntorp, 2003) as well as in bryophytes, most recently e.g. in *Campylopus pilifer* (Dohrmann, 2003) and the liverwort genus *Symphyogyna* (Schaumann et al., 2003). In *Catagonium* the northern South American taxa were found to be evolutionary older than both the southern South American species and the other specimens of the genus, the dispersal in South America therefore supposed to have taken place from north to south. In contrast there is the example of the liverwort genus *Monoclea* where the dispersal of a taxon has started from the southern, temperate zone into the northern, tropical zone of South America (Meißner et al., 1998).

Furthermore, the phylogenetic results of this study make a distinction between the South African specimens on the one hand and the South American and New Zealand/Australian specimens on the other hand. This pattern is well-known (e.g. Frey et al., 1999; McDaniel & Shaw, 2003; Meißner et al., 1998; Schaumann et al., 2004) and has been explained with a second Gondwanan break-up, during which first South America and New Zealand were separated from the rest of Gondwana *c*. 80 Myr BP followed by the separation of Australia from South America *c*. 30 Myr BP.

Apart from the northern South American and South African taxa the remaining taxa consist of the species *C. nitens* ssp. *nitens* that is widespread throughout the southern hemisphere, and a second species, *C. nitidum*, which seems to be

restricted to the southernmost islands of Chile and Argentina (Lin, 1984). The performed analysis distinguishes two clades: one with the New Zealand/Australian specimens, the other with the Chilean specimens of *C. nitens* ssp. *nitens*. The genetic distances between the Chilean and New Zealand/Australian populations of *C. nitens* ssp. *nitens* suggest a somewhat later split of these populations, and no recent genetic exchange via long distance dispersal. Interestingly there is evidence for a genetic separation between the populations from New Zealand and Australia. Furthermore, the genetic distance between the Chilean and Australian populations is lower than between the Chilean and New Zealand populations of *C. nitens* ssp. *nitens*. In contrast to the close relationship of the New Zealand and Australian *Catagonium* taxa found in the phylogenetic analysis, which contradicts the vicariance hypothesis, the results of the genetic distances can be considered consistent with the documented time sequence of the Gondwanan break-up. The strong genetic differentiation of the New Zealand taxa from the Australian and Chilean taxa fits with the early splitting off of the New Zealand landmass, *c*. 80 Myr BP, leading to a long period of isolation. The smaller genetic distances between the *Catagonium* taxa from Chile and Australia than between those from Chile and New Zealand could be explained by the longer connection of South America to Australia via Antarctica. The separation of these continents only took place *c*. 30 Myr BP.

The break-up sequence of Gondwana, with the early split of New Zealand and the later separation of Australia and New Zealand is not consistently reflected in phylogentic analyses in plants (Sanmartín & Ronquist, 2004). Instead, closer relationships between the areas of New Zealand and Australia are recognized. This frequently documented result should not be seen as a contradiction between geological records and evolutionary history, but can be interpreted in terms of evidence for dispersal events between New Zealand and Australia (e.g. Sanmartín & Ronquist, 2004; Swenson et al., 2001). More data are needed to trace the possible dispersal events within the evolutionary history of *Catagonium*.

Although the genetic distance data of this study are in concordance with the geological history of Gondwana, using genetic distances to interpret sequences in time remains methodologically problematic.

This phylogenetic analysis gives an ambiguous relationship within *C. nitens* ssp. *nitens* as well as to *C. nitidum* from the Falkland Islands. With the inclusion of more

specimens especially of *C. nitens* ssp. *nitens* from east Africa and from subantarctic Marion Island, as well as of the variety *myurum* and of *C. nitidum*, more clearly resolved relationships can be expected that allow to assess more accurately the role of vicariance and dispersal events in the evolution of the genus. For example, the occurrence of *C. nitens* ssp. *nitens* on the remote subantarctic Marion Island, situated in the southern Indian Ocean halfway between South America and New Zealand/Australia is best explained by long distance dispersal (Gremmen, 1981) as this island is supposed to be only 500,000 years old, and its vegetation may have repeatedly been influenced by glaciation events (Gremmen, 1981; van Zanten, 1971). This can be seen as evidence for the ability of *C. nitens* ssp. *nitens* to disperse over long distances with the wind as vector.

Summarizing, the disjunct distribution of *Catagonium* in northern South America and South Africa is best explained as a result of a vicariance event in the form of the break-up of Gondwana, i.e. the separation of Africa from South America, *c*. 105 Myr BP. Furthermore, from the results of the analysis presented here the wide distribution of *C. nitens* ssp. *nitens* can be interpreted as a result of the further fragmentation of the Gondwana continent as well as long distance dispersal by wind to subantarctic islands e.g. the Kerguelen Islands and Marion Island.

The genus *Acrocladium* consists of only two taxa. It is evident from this analysis that these are two genetically and geographically distinct species. One species, *A. auriculatum* is confined to southern South America. The second species, *A. chlamydophylum* occurs in Australia and New Zealand. Like in *Catagonium nitens* ssp. *nitens*, one of the species occurs on remote subantarctic Marion Island, which can be regarded as evidence for the ability of this species to disperse over long distances.

The genus *Acrocladium* is genetically clearly separated from its sister genus *Lepyrodon*, which may suggest an ancient age for *Acrocladium* and *Lepyrodon*. On the one hand one cannot rule out that the disjunct distribution of the *Acrocladium* species is caused by long distance dispersal. Regarding the strong genetic differentiation between the two *Acrocladium* taxa it could be concluded that the separation must have occurred a long time ago, perhaps during times when Gondwana already was about to rift apart. Considering the results at hand vicariance

is here seen as the most parsimonious solution (e.g. Ronquist, 1997; Wanntorp & Wanntorp, 2003) for explaining the disjunct distribution pattern of *Acrocladium*.

The disjunct distribution of the genus *Lepyrodon* is restricted to New Zealand/Australia and South America. The phylogenetic analysis revealed a sister relationship between taxa from New Zealand/Australia and Chile. However, these taxa are genetically clearly separated which could be interpreted as the result of an extremely long separation time related to a vicariance event when the Gondwana continent split apart *c.* 80 Myr BP. The specimens of taxa with an Australian/New Zealand distribution analysed in this study all originate from New Zealand and therefore the genetic relationship between the New Zealand and Australian region cannot be discussed.

So far only a few bryophytes with a disjunct distribution in the temperate region of the southern hemisphere have been investigated in molecular studies. For example, *Lopidium concinnum* (Frey et al., 1999) and *Hypopterygium didictyon* (Pfeiffer, 2000b), are regarded as ancient Gondwana relict species within which no genetic differentiation occurred. For most of the taxa with a disjunct distribution, however, genetic differentiation is reported (e.g. Meißner et al., 1998; Schaumann et al., 2004; Stech et al., 2002).

In the phylogenetic analysis presented here, there is one clade which comprises *L. hexastichus* as well as the wide-spread taxon *L. tomentosus* which occurs throughout South America up to Mexico. The relationships within this clade are not well-resolved. The short branches found in the Maximum Likelihood analysis together with the genetic distances suggest a low genetic differentiation of these taxa. The southern South American populations of *L. tomentosus* are separated from the northern South American populations by two arid areas. The Atacama desert separates the temperate southern South America from northern South America and the Gran Chaco east of the Andes forms a barrier to the populations in southeast Brazil. The separation between temperate southern South America and southeast Brazil may have already started in the Lower Miocene (24.7 – 15.3 Myr BP) when a sea transgression of a former “atlantic” ocean flooded east Patagonia and roughly separated the western and the eastern part of South America (Hinojosa & Villagran, 1997). The habitat of the species in northern South America where it is characteristically an epiphyte in the subalpine rain forests (Gradstein et al., 2001)

suggests a more recent spread into this region i.e. during the Tertiary along with the proto Andean mountain ridge *c.* 10 Myr BP (Hartley, 2003) where there may have been temperate conditions before the establishment of the hyperarid Atacama 5 Myr BP (Hartley, 2003). The spread of populations of *L. tomentosus* into Mexico and the establishment in Central America started later when the Isthmus of Panama had formed 4.6 to 3.6 Myr BP (Haug & Tiedemann, 1998). Allen (1999) describes morphologically and geographically distinct forms in this widespread species with intermediate forms in overlapping areas. This may indicate that the separation between the populations of the so called ‘expression’ (Allen, 1999) of *L. tomentosus* took place in the Upper Miocene.

Common genetic patterns in the Gondwana connection

The disjunct distribution of the taxa under study is reflected in molecular phylogenetic analyses as well as in genetic distances. The genetically based data mostly separate between a southern South American temperate region on one side and an Australian/New Zealand region on the other side resulting in a reciprocal monophyly between these two areas in each of the taxa. Based on the high degree of genetic distinction between the taxa the disjunct distribution patterns are interpreted as vicariance events from the break-up of the former Gondwana continent.

However, ambiguous relationships between taxa and therefore area relationships in phylogenetic analysis in *C. nitens* ssp. *nitens* suggest that a broader taxon sampling considering underrepresented areas and taxa is needed as well as additional molecular markers to get a better resolution of the clades in order to identify dispersal events which probably occurred after the Gondwanan break-up. Dispersal might especially explain the occurrence of *C. nitens* ssp. *nitens* and *Acrocladium auriculatum* on remote subantarctic Marion Island.

9 Summary

Researchers have long been fascinated by disjunct distribution patterns of plant and animal species. Especially the disjunctly distributed species occurring in the temperate Chilean and New Zealand rainforests of the southern hemisphere are considered interesting due to the common history these locations share. These areas were originally part of the former Gondwana landmass. There are also moss species from temperate forest habitats revealing such a disjunct distribution.

The native moss flora of Chile comprises about 780 species. According to a study on the Chilean and New Zealand mosses 113 of these 780 species reveal a disjunct austral distribution pattern and also occur in New Zealand. The majority of the species common to both countries are inhabitants of temperate rainforests.

This study investigates phylogenetic relationships within four southern hemispheric bryophyte taxa characteristic for the Chilean and New Zealand temperate rainforests. These taxa consisted of the families Lepyrodontaceae and Ptychomniaceae as well as the genera *Acrocladium* and *Catagonium*. The results are discussed within the context of historical and geological processes in order to test the hypothesis whether the distribution patterns can be attributed to a common Gondwanan origin or to long distance dispersal as an alternative explanation.

Molecular phylogenetic analyses using molecular markers from nrDNA (ITS region, *adk* gene) and cpDNA (*trn*L-*trn*F region, *rps*4 gene) were conducted for a large number of specimens representing the taxa under study. Most of these specimens originated from the BryoAustral and the BryoTrop projects. The resulting molecular data set was used to reconstruct phylogenies. Additionally, genetic distances were determined to compliment the phylogenetic results.

Firstly, phylogenetic relationships within the Ptychomniaceae and within a taxa group consisting of the Plagiotheciaceae, Lepyrodontaceae and related taxa were investigated. For this purpose phylogenetic analyses based on DNA sequence data were conducted for several data sets. Concerning the family Ptychomniaceae the

results showed that the species *Ptychomnion ptychocarpon*, endemic to the Valdivian rainforest, does not belong to the genus *Ptychomnion.* In contrast to the other representatives of this genus *Ptychomnion ptychocarpon* occupies a basal position within the family showing no close relationship to any of the other genera within the family. Further results of this study placed the genus *Dichelodontium* in the family Ptychomniaceae. This genus was formerly considered a member of the Lepyrodontaceae.

Further analyses were performed using specimens of the southern hemispheric genus *Lepyrodon*. This genus comprises seven species, two of which only occur in New Zealand and Australia and another four which are only found in southern Chile and southern Argentina. In contrast, *Lepyrodon tomentosus* has a distribution area which covers the southernmost tip of the American continents and expands northwards over Central America up to Mexico. The genetic analyses showed that the two New Zealand-Australian species form a common clade and that the most closely related species originate from Chile. Furthermore, based on the results of both phylogenetic analyses and genetic distances it is concluded that populations of *Lepyrodon tomentosus* occurring in southern and northern South America, respectively, probably already became separated during the tertiary.

Analyses aimed at clarifying the phylogenetic relationships of the genus *Acrocladium* revealed a close relationship between this genus and the genus *Lepyrodon*. There has been much discussion on whether the genus *Acrocladium* comprises a single species or whether a distinction can be made between two species. In this study clear evidence was found for the existence of two genetically distinct species, a Chilean-Argentinian species (*A. auriculatum*) and a New Zealand-Australian species (*A. chlamydophyllum*).

The genus *Catagonium* occupies a very basal position within the family Plagiotheciaceae. The study of this genus revealed a high genetic similarity between two species only occurring in northern South America on the one hand and a taxon only found in South Africa on the other hand. Based on this phylogenetic result the conclusion is made that the recent taxa had a common ancestor which occurred on

the former Gondwana continent. When this landmass split apart the *Catagonium* populations found on today's African and South American continents were separated.

Not all phylogenetic relationships resulting from analyses of molecular markers found in this study could be explained by vicariance events. Therefore, long distance dispersal is discussed as an explanation for the disjunct distribution of specific taxa.

10 Acknowledgements

I would like to express a special thanks to Prof. Dr. Jan-Peter Frahm for all the supervision he gave me and for always being open for a helpful discussion. He offered me a position within the BryoAustral project of the German Research Foundation which included financing of the laboratory analyses which were crucial for my research.
I am also very grateful to Prof. Wilhelm Barthlott who kindly agreed to take the Korreferat and offered numerous critical comments especially during the last phase of the study.

A very special thank-you goes to Dr. Friederike Schaumann (Berlin), who sadly recently passed away. Thanks a lot Friederike for the shared experiences we had during the excursion to Chile and for the many motivating discussions and talks we had during the past four years.

In particular, I would like to thank Dr. Dietmar Quandt (Dresden) for the valuable comments he made and all the time he was willing to spend when critically reading final versions of this manuscript. Thank-you.

A very warm thank-you is expressed to Dr. Hans Kruijer (Leiden) for his very helpful comments and the interesting discussions we had on the evolution of bryophytes.

A sincere thank-you goes to the colleagues of the AG Bryologie, especially to Dr. Dietmar Quandt and Dr. Andreas Solga for all the interesting discussions and comments of the last four years. Thanks Dietmar and Andreas also for the fun shared in life outside the institute.
A special thank-you goes to Dr. Thomas Borsch, Kim Govers, Conny Löhne, Kai Müller and Andreas Worberg of the ‘Molecular Systematics Working Group’ at the Nees-Institute for the very good cooperation we had in the molecular lab.

Furthermore, I am very grateful to the colleagues at the Nees-Institute for providing a nice atmosphere and for always being helpful when I came along with various problems.

Prof. Dr. Ingrid Essigmann-Capesius (Heidelberg) I would like to thank for all the time and patience she had while introducing me to the molecular labwork.

It is with great pleasure that I remember the time I spent at the Botanical Museum in Helsinki. I thank Dr. Sanna Huttunen (Helsinki) very much for her hospitality during my stay there.
I also warmly thank Prof. Dr. Ben van Zanten for his hospitality during a visit to Groningen. He kindly provided access to his personal as well as to the institutional bryophyte collection.
Another very motivating trip for my research were the two weeks I spent at the Institute of Biology in Berlin, enabled by Prof. Dr. Wolfgang Frey. I am very grateful to him for this opportunity.

Additionally, I must express my thanks to Dr. Bruce Allen (Missouri Botanical Garden) who identified my specimens of *Lepyrodon* from Chile.

Plant material was kindly provided by Volker Buchbender (Bonn), Prof. Dr. Frey (Berlin), Dr. Frank Müller (Dresden), Dr. Friederike Schaumann (Berlin), Dr. Andreas Solga (Bonn) and Prof. Dr. Ben van Zanten (Groningen).

A sincere appreciation is due to Dr. Dietmar Quandt (Dresden) and Dr. Sanna Huttunen (Helsinki) for sharing some of the sequences with me.

I am indebted to the curators of the herbaria at the Botanical Museum Berlin Dr. H. Nowak-Krawietz, from the Botanical Museum Helsinki, J. Heino, Dr. Viivi Virtanen and Dr. Sanna Huttunen and from the National Herbarium Leiden Dr. Hans Kruijer, for the loan of specimens and the permission for use in the molecular systematic studies.

I wish to express my warmest thanks to Celia Nitardy (Marburg) and Petra Daniels (Groningen) for being around during the last weeks and for all the support they provided. Especially for carefully reading the manuscript and for all valuable comments. Celia Nitardy and Nicole Scheifhacken (Konstanz) are also acknowledged for the drawing of distribution maps.

Finally, I would like to express a very important thank-you to my parents who have given me invaluable support and who have shown a patience that I greatly appreciate.

This study was embedded in the BryoAustral project financed by the German Research Foundation (DFG) which granted the project money to Prof. Dr. J.-P-Frahm (481/9-2, 481/9-49) and to Prof. Dr. W. Frey (DFG 404/3-1), for which I am grateful.

11 References

Akaike, H. 1974. A new look at the statistical model identification. *IEEE Trans. Autom. Contr.*, 19:716-723.

Allen, B. 1999. A revision of the moss genus *Lepyrodon* (Leucodontales, Lepyrodontaceae). *Bryobrothera*, 5:23-48.

Andrews, A. L. 1949. Taxonomic notes. VIII. The genus *Acrocladium*. *The Bryologist*, 52:72-77.

Beever, J., K. W. Allison, and J. Child. 1992. *The mosses of New Zealand.* second edition ed. University of Otago Press.

Blattner, F. R. 1999. Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. *BioTechniques*, 27:1180-1186.

Blöcher, R. 2000. Molekular-systematische Untersuchungen zur Stellung der Hypopterygiaceae Mitt. s.l. (Musci) im System der Bryidae. Diplom thesis, Philipps-Universität Marburg.

Blöcher, R., and I. Capesius. 2002. The systematic position of the Hpopterygiaceae (Bryopsida) inferred from *rps*4 gene sequences. *Cryptogamie Bryologie*, 23:191-207.

Blöcher, R., and J.-P. Frahm. 2002. A comparison of the moss floras of Chile and New Zealand. Studies in austral temperate rain forest bryophytes 17. *Tropical Bryology*, 21:81-92.

Bremer, K. 1994. Branch support and tree stability. *Cladistics*, 10:295-304.

Brotherus, V. F. 1909a. Lepyrodontaceae. In A. Engler and K. Prantl (eds.), *Die natürlichen Pflanzenfamilien*, Vol. I. Teil, Abteilung 3, II. Hälfte (=I.3b), pp. 771-773. W. Engelmann, Leipzig.

—. 1909b. Musci (Laubmoose). In A. Engler and K. Prantl (eds.), *Die natürlichen Pflanzenfamilien*, Vol. 1. Teil, 3. Abteilung, 2. Hälfte, pp. 701-1246. W. Engelmann, Leipzig.

—. 1925a. Lembophyllaceae. In A. Engler and K. Prantl (eds.), *Die natürlichen Pflanzenfamilien*, Vol. 11. Band, 2. Hälfte, pp. 207-208. W. Engelmann, Leipzig.

—. 1925b. Lepyrodontaceae. In A. Engler and K. Prantl (eds.), *Die natürlichen Pflanzenfamilien*, Vol. 11. Band, 2. Hälfte, pp. 109-111. W. Engelmann, Leipzig.

—. 1925c. Musci (Laubmoose). In A. Engler and K. Prantl (eds.), *Die natürlichen Pflanzenfamilien*, Vol. 11. Band, 2. Hälfte, pp. 542 p. W. Engelmann, Leipzig.

Buck, W. R. 1998. Pleurocarpous mosses of the West Indies. *Memoirs of the New York Botanical Garden*, 82:1-.

Buck, W. R., C. J. Cox, A. J. Shaw, and B. Goffinet. in press. Ordinal relationships of pleurocarpous mosses, with special emphasis on the Hookeriales. *Systematics and Biodiversity*.

Buck, W. R., and B. Goffinet. 2000. Morphology and classification of mosses. In A. J. Shaw and B. Goffinet (eds.), *Bryophyte Biology*, pp. 71-123. Cambridge University Press, Cambridge.

Buck, W. R., B. Goffinet, and A. J. Shaw. 2000a. Novel relationships in pleurocarpous mosses as revealed by cpDNA sequences. *The Bryologist*, 103:774-789.

—. 2000b. Testing morphological concepts of orders of pleurocarpous mosses (Bryophyta) using phylogenetic reconstructions based on trnL-trnF and rps4 sequences. *Molecular Phylogenetics and Evolution*, 16:180-198.

Buck, W. R., and R. R. Ireland. 1985. A reclassification of the Plagiotheciaceae. *Nova Hedwigia*, 41:89-125.

Buck, W. R., and D. H. Vitt. 1986. Suggestions for a new familial classification of pleurocarpous mosses. *Taxon*, 35:21-60.

Campbell, C. S., M. F. Wojciechowski, B. G. Baldwin, L. A. Alice, and M. J. Donoghue. 1997. Persistent nuclear ribosomal DNA sequence polymorphism in the Amelanchier agamic complex (Rosaceae). *Molecular Biology and Evolution*, 14:81-90.

Cardot, J. 1908. La flore bryolologique des Terres Magellaniques, de la Géorgie du Sud et de l'Antarctide., *Wissenschaftliche Ergebnisse der Schwedischen Südpolar-Expedition 1901-1903. Unter Leitung von Dr. O. Nordenskjöld*, Vol.

4, Lieferung 8, pp. 1-298. Lithographisches Institut des Generalstabs, Stockholm.

Clegg, M. T., B. S. Gaut, G. H. j. Learn, and B. R. Morton. 1994. Rates and patterns of chloroplast DNA evolution. *91*.

Croizat, L., G. J. Nelson, and D. E. Rosen. 1974. Centers of origin and related concepts. *Systematic Zoology*, 23:265-287.

Crum, H. 1984. Notes on tropical American mosses. *The Bryologist*, 87:203-216.

—. 1994. Lepyrodontaceae. In A. J. Sharp and H. Crum (eds.), *The moss flora of Mexico*, Vol. 2, pp. 703-704, Mem. New York Bot. Garden 69.

Darlington, P. J. J. 1965. *Biogeography of the southern end of the world*. Harvard University Press, Cambridge, Massachusetts.

Deguchi, H. 1991. A list of moss collection made during the expeditions to Chile in 1981 and 1987 (1). *Bull. Natn. Sci. Mus., Tokyo, Ser. B*, 17:15-34.

Delgadillo M., C. 1993. The Neotropical-African moss disjunction. *The Bryologist*, 96:604-615.

—. 2000. Mosses and the Caribbean connection between North and South America. *The Bryologist*, 103:82-86.

Dixon, H. N. 1927. Studies in the bryology of New Zealand with special reference to the herbarium of Robert Brown. *New Zealand Inst. Bull.*, 3:239-298.

—. 1928. Studies in the bryology of New Zealand part IV. *Bull. New Zealand Inst.*, 3:299-372.

Dohrmann, J. 2003. Molekulare Systematik und Biogeographie von *Campylopus* pilifer Brid. (Dicranaceae, Bryopsida). Diplomarbeit thesis, Freie Universität Berlin.

Doyle, J. J. 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. *Systematic Botany*, 17:144-163.

Doyle, J. J., and J. L. Doyle. 1990. Isolation of plant DNA from fresh tissues. *Focus*, 12:13-15.

Du Rietz, G. E. 1960. Remarks on the botany of the southern cold temperate zone. *Proceedings of the Royal Society London Series B*, 152:500-507.

During, H. J. 1977. A taxonomic revision of the Garovaglioideae (Pterobryaceae, Musci). *Bryophytorum Bibliotheca*, 12:244.

Erixon, P., B. Svennblad, T. Britton, and B. Oxelmann. 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic Biology*, 52:665-673.

Farris, J. S. 1989. The retention index and the rescaled consistency index. *Cladistics*, 5:417-419.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap.

Fife, A. J. 1995. Checklist of the mosses of New Zealand. *The Bryologist*, 98:313-337.

Fleischer, M. 1906-1908. *Bryales Metacranaceales i. p. Isobryinae i. p., Hookerinae*. E.J. Brill, Leiden.

—. 1908. *Bryales Metacranaceales i. p. Isobryinae i. p., Hookerinae*. E.J. Brill, Leiden.

—. 1915-1922. *Bryales: Hypnobryales, Buybaumiales, Diphysciales, Polytrichales*. E.J. Brill, Leiden.

—. 1923a. *Bryales: Hypnobryales, Buybaumiales, Diphysciales, Polytrichales*. E.J. Brill, Leiden.

—. 1923b. *Plagiotheciaceae (Bryales: Hypnobryales, Buybaumiales, Diphysciales, Polytrichales)*. E.J. Brill, Leiden.

Frahm, J.-P. 2004. A new contribution to the moss flora of baltic and saxon amber. *Review of Palaebotany and Palynology*, 129:81-101.

Frey, W. 1990. Genoelemente prä-angiospermen Ursprungs bei bryophyten. *Botanische Jahrbücher für Systematik*, 111:433-456.

Frey, W., M. Stech, and K. Meissner. 1999. Chloroplast DNA-relationship in palaeoaustral *Lopidium concinnum* (Hypopterygiaceae, Musci). An example of stenoevolution in mosses. Studies in austral temperate rain forest bryophytes 2. *Plant Systematics and Evolution*, 218:67-75.

Fritsch, R. 1991. Index to bryophyte chromosome counts. In J.-P. Frahm and S. R. Gradstein (eds.), *Bryophytorum Bibliotheca*, Vol. 40, pp. 352. J. Cramer, Berlin.

Godley, E. J. 1960. The botany of southern Chile in relation to New Zealand and the Subantarctic. *Proceedings of the Royal Society London Series B*, 152:457-475.

Goffinet, B., and T. A. J. Hedderson. 2000. Evolutionary Biology of the Bryopsida (Mosses): A synthesis-introductory comments. *Bryologist*, 103: 185-186.

Gradstein, S. R., S. P. Churchill, and N. Salazar-Allen. 2001. Guide to the bryophytes of tropical America. *Memoirs of the New York Botanical Garden*, 86:1-577.

Gremmen, N. J. M. 1981. *The vegetation of the subantarctic islands Marion and Prince Edward*. W. Junk, The Hague.

Hartley, A. J. 2003. Andean uplift and climate change. *Journal of the Geological Society, London*, 160:7-10.

Hattaway, R. A. 1981. Commentary on the genus Glyphothecium (Ptychomniaceae). *The Bryologist*, 84:344-347.

—. 1984. A monograph of the Ptychomniaceae (Bryopsida). Pennsylvania State University.

Haug, G. H., and R. Tiedemann. 1998. Effect of the formation of the Isthmus of Panama on Atlantic Ocean thermohaline circulation. *Nature*, 393:673-676.

He, S. 1998. A checklist of the mosses of Chile. *Journal of the Hattori Botanical Laboratories*, 85:103-189.

Hedenäs, L. 2001. Environmental factors potentially affecting character states in pleurocarpous mosses. *The Bryologist*, 104:72-91.

Hershkovitz, M. A., and E. A. Zimmer. 1996. Conservation patterns in angiosperm rDNA-ITS2 sequences. *Nucleic Acids Research*, 24:2857-2867.

Herzog, T. 1926. *Geographie der Moose*. Gustav Fischer Verlag, Jena.

Hidalgo, O., T. Garnatje, A. Susanna, and J. Mathez. 2004. Phylogeny of Valerianaceae based on *mat*K and ITS markers; with reference to *mat*K individual polymorphism. *Annals of Botany*, 93:283-293.

Hill, R. S. 1994. The history of selected Australian taxa. In R. S. Hill (ed.), *History of the Australian Vegetation: cretaceous to recent.*, pp. 390-419. Cambridge University Press, Cambridge.

Hill, R. S., and M. E. Dettmann. 1996. Origin and diversification of the genus *Nothofagus*. In T. T. Veblen, R.S. Hill & J. Read (ed.), *The ecology and biogeography of Nothofagus forests.*, pp. 11-24. Yale University Press, New Haven.

Hinojosa, L. F., and C. Villagran. 1997. Historia de los bosques del sur de Sudamerica, I.: antecedentes paleobotanicos, geologicos y climaticos del

Terciario del cono sur de America. *Revista Chilena de Historia Natural*, 70:225-239.

Hooker, J. D. 1867. *Handbook of the New Zealand flora*. Reeve & Co., London.

Huelsenbeck, J. P., and F. Ronquist. 2001. MrBayes: A program for the Bayesian inference of phylogeny. *Bioinformatics*, 17:754-755.

Hugall, A., j. Stanton, and C. Moritz. 1999. Reticulate evolution and the origins of ribosomal transcribed spacer diversity in apomictic Melaidogyne. *Molecular Biology and Evolution*, 16:157-164.

Huttunen, S., and M. S. Ignatov. 2004. Phylogenetic analyses of Brachytheciaceae (Bryophyta) based on morphology and sequence level data. *Cladistics*, 20:151-183.

Huttunen, S., M. S. Ignatov, K. Müller, and D. Quandt. 2004. Phylogeny and evolution of epiphytism in the three moss families Meteoriaceae, Brachytheciaceae and Lembophyllaceae. *Monographs in Systematic Botany*, in press.

Ireland, R. R. W. R. B. 1994. Stereophyllaceae. In O. f. F. Neotropica (ed.), *Flora Neotropica*, Vol. 65, pp. 49. The New York Botanical Garden, New York.

Jordan, G. J., and P. J. Dalton. 1995. Mosses from Early Pleistocene sediments in western Tasmania. *Alcheringa*, 19:291-296.

Karczmarz, K. 1966. Taxonomic studies on the genus *Acrocladium* Mitt. *Nova Hedwigia*, 11:499-505.

Kluge, A. G., and J. S. Farris. 1969. Quantitative phyletics and the evolution of anurans. *Systematic Zoology*, 18:1-32.

Körsgen, S., R. Kantz, and M. Weinelt. 2004. Planiglobe - online map creation. http://www.planiglobe.com.

Krassilov, and R. M. Schuster. 1984. Paleozoic and Mesozoic fossils. In R. M. Schuster (ed.), *New manual of bryology*, Vol. 2, pp. 1172-1193. Nichinan, Japan.

Kruijer, H. 2002. Hypopterygiaceae of the World. *Blumea*, Supplement 13:1-388.

Kruijer, H., and R. Blöcher. 2004. Re-evaluation of the phylogeny of the Hypopterygiaceae (Bryophyta) based on morphological and molecular data. In A. C. Newton, R. S. Tangney and E. DeLuna (eds.), *Symposium 'Evolution of the Pleurocarpous Mosses' , September 6-8*, Cardiff.

Kühnemann, O. M. F. G. C. 1975. Monografia preliminar para la flora criptogamica fueguina: Los musgos de la familia Ptychomniaceae (Bryophyta). *Darwiniana*, 19:583-617.

Kumar, S., K. Tamura, I. B. Jakobsen, and M. Nei. 2001. MEGA2.1: Molecular Evolutionary Genetics Analysis software. Arizona State University, . Tempe, Arizona, USA.

Lin, S.-H. 1984. A taxonomic revision of Phyllogoniaceae (Bryopsida). Part II. *Journal of the Taiwan Museum*, 37:1-54.

—. 1989. *Catagonium* from Chile and Peru collected by Dr. H. Deguchi. *Yushania*, 6:19-22.

Lindlar, A., and J.-P. Frahm. 2002. Epiphytic bryophyte communities in New Zealand temperate rainforests along selected altitudinal transects. *Phytocoenologia*, 32:251-316.

Magill, R. E. 1987. On the endostomial nature of the Dichelodontium (Ptychomniaceae) peristome. *Memoirs of the New York Botanical Garden*, 45:87-94.

Manos, P. S. 1997. Systematics of Nothofagus (Nothofagaceae) based on rDNA spacer sequences (ITS): taxonomic congruence with morphology and plastid sequences. *American Journal of Botany*, 84:1137-1155.

Matteri, C. M. 1986. Overview on the phytogeography of the moss flora from southern Patagonia, at 51º-52º south latitude. *Journal of the Hattori Botanical Laboratories*, 60:171-174.

McDaniel, S. F., and A. J. Shaw. 2003. Phylogeographic structure and cryptic speciation in the trans-antarctic moss *Pyrrhobryum mnioides*. *Evolution*, 57:205-215.

McLoughlin, S. 2001. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany*, 49:271-300.

Meißner, K., J.-P. Frahm, M. Stech, and W. Frey. 1998. Molecular divergence patterns and infrageneric relationship of Monoclea (Monocleales, Hepaticae). Studies in austral temperate rainforest bryophytes 1. *Nova Hedwigia*, 67:289-302.

Mitten, G. 1869. Musci Austro-Americani. Enumeratio Muscorum omnium Austro-Americanorum auctori huiusque cognitorum. *The Journal of the Linn. Soc. Bot.*, 12:270-550.

Müller, J., and K. Müller. 2004. Treegraph: automated drawing of complex tree figures using an extensible tree description format. *Molecular Ecology Notes*, 0:doi: 10.1111/j.1471-8286.2004.00813.x.

Müller, K. 2004. PRAP - calculation of Bremer support for large data sets. *Molecular Phylogenetics and Evolution*, 31:780-782. http://www.botanik.uni-bonn.de/system/downloads/.

Muñoz, J., A. M. Felicísimo, F. Cabezas, A. R. Burgaz, and I. Martínez. 2004. Wind as along-distance dispersal vehicle in the southern hemisphere. *Science*, 304:1144-1147.

Musters, W., K. Boon, C. A. F. M. van der Sande, H. van Heerikhuizen, and R. J. Planta. 1990. Functional analysis of transcribed spacers of yeast ribosomal DNA. *EMBO*, 9:3989-3996.

Nadot, S., R. Bajon, and B. Lejeune. 1994. The chloroplast gene *rps*4 as a tool for the study of Poaceae phylogeny. *Plant Systematics and Evolution*, 191:27-38.

Nixon, K. C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics*, 15:407-414.

Ochyra, R. 2002. *Ptychomnion ringianum* Broth. & Kaal, synonymus with *P. densifolium* (Brid.) A.Jaeger (Ptychomniaceae). *Journal of Bryology*, 24:87-88.

Ochyra, R., and C. M. Matteri. 2001. Bryophyta, Musci, Amblystegiaceae. *Flora Criptogámica de Tierra del Fuego*, XIV:1-96.

Orbán, S. 2000. *Calymperes venezuelanum*, a newly discovered American-African disjunct element in the flora of Madagascar. *The Bryologist*, 103:145-146.

Palmer, J. D. 1990. Contrasting modes and tempos of genome evolution in land plant organelles. *Trends in genetics*, 6:115-120.

Patti, F. P. 2002. WinModeltest front-end v4b. Program distributed by the author, Harvard.

Pedersen, N., and L. Hedenäs. 2001. Phylogenetic relationships within the Plagiotheciaceae. *Lindbergia*, 26:62-76.

—. 2002. Phylogeny of the Plagiotheciaceae based on molecular and morphological evidence. *The Bryologist*, 105:310-324.

Pfeiffer, T. 2000a. Molecular relationship of *Hymenophyton* species (Metzgeriidae, Hepaticophytina) in New Zealand and tasmania. Studies in austral temperate rain forest bryophytes 5. *New Zealand Journal of Botany*, 38:415-423.

—. 2000b. Relationships and divergence patterns in *Hypopterygium* '*rotulatum*' s.l. (Hypopterygiaceae, Bryopsida) inferred from trnL intron sequences. Studies in austral temperate rainforest bryophytes 7. *Edinburgh Journal of Botany*, 57:291-307.

—. 2001. Terricolous bryophyte vegetation of New Zealand temperate rain forests-Communities, adaptive strategies and divergence patterns, Freie Universität Berlin.

Pfeiffer, T., J. D. Kruijer, W. Frey, and M. Stech. 2000. Systematics of the Hypopterygium tamarisci complex (Hypopterygiaceae, Bryopsida): implications of molecular and morphological data. Studies in austral temperate rain forest bryophytes 9. *Journal of the Hattori Botanical Laboratory*, 89:55-70.

Pole, M. 1994. The New Zealand flora - entirely long-distance dispersal. *Journal of Biogeography*, 21:625-635.

Pole, M. S. 2001. Can long-distance dispersal be inferred from the New Zealand plant fossil record. *Australian Journal of Botany*, 49:357-366.

Posada, D. 2004. Modeltest 3.5, *Free software. Available at http://darwin.uvigo.es/software/modeltest.html*. dposada@uvigo.es.

Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics*, 14:817-818.

Przywara, L., E. Kuta, and R. Ochyra. 1992. Chromosome numbers in some New Zealand bryopyhtes: I. *Fragmenta Floristica et Geobotanica*, 37:395-405.

Quandt, D. 2002. Molecular evolution and phylogenetic utility of non-coding DNA: addressing relationships among pleurocarpous mosses, RheinischeFriedrich-Wilhelms-Universität.

Quandt, D., J.-P. Frahm, and W. Frey. 2001. Patterns of molecular divergence within the palaeoaustral genus Weymouthia Broth. (Lembophyllaceae, Bryopsida). *Journal of Bryology*, 23:305-311.

Quandt, D., S. Huttunen, H. Streimann, J.-P. Frahm, and W. Frey. 2004a. Molecular phylogenetics of the Meteoriaceae s. str.: focusing on the genera *Meteorium* and *Papillaria*. *Molecular Phylogenetics and Evolution*, 32:435-461.

Quandt, D., S. Huttunen, R. S. Tangney, and M. Stech. 2004b. A generic revision of Lembophyllaceae based on molecular data. *Systematic Botany*, in press.

Quandt, D., and M. Stech. 2003. Molecular systematics of bryophytes in context of land plant evolution. In A. K. Sharma and A. Sharma (eds.), *Plant Genome:*

Biodiversity and Evolution, Vol. 1, pp. 267-295. Science Publishers, Enfield, New Hampshire.

—. 2004. Molecular evolution of the trnT(UGU)-trnF(GAA) region in bryophytes. *Plant Biology*, 6:545-554.

Quandt, D., R. S. Tangney, J.-P. Frahm, and W. Frey. 2000. A molecular contribution for understanding the Lembophyllaceae (Bryopsida) based on noncoding Chloroplast regions (cpDNA) and ITS2 (nrDNA) sequence data. *Journal of the Hattori Botanical Laboratory*, 89:71-92.

Ramsay, H. P. 1974. Cytological studies of Australian mosses. *Australian Journal of Botany*, 22:293-348.

—. 1983. Cytology in mosses. In R. M. Schuster (ed.), *New manual of bryology*, Vol. 1, pp. 149-221. The Hattori Botanical Laboratory, Nichinan, Japan.

Robinson, H. 1971. A Revised classification for the orders and families of mosses. *Phytologia*, 21: 289-293.

—. 1975. The mosses of Juan Fernandez Islands. *Smithsonian Contributions to Botany*, 27:1-88.

Ronquist, F. 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology*, 46:195-203.

Sainsbury, G. O. K. 1955. A handbook of the New Zealand mosses. *Royal Society of New Zealand Bulletin*, 5:1-490.

Sanmartín, I., and F. Ronquist. 2004. Southern hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Systematic Biology*, 53:216-243.

Schaumann, F., W. Frey, G. G. Hässel de Menéndez, and T. Pfeiffer. 2003. Geomolecular divergence in the Gondwanan dendroid *Symphyogyna* complex (Pallaviciniaceae, Hepaticophytina, Bryophyta). *Flora*, 198:404-412.

Schaumann, F., T. Pfeiffer, and W. Frey. 2004. Molecular divergence patterns within the gondwanan liverwort genus *Jensenia* (Pallaviciniaceae, Hepaticophytina, Bryophyta). Studies in austral temperate rainforest bryophytes 25. *Journal of the Hattori Botanical Laboratory*, 96:231-244.

Schuster, R. M. 1969. Problems of antipodal distribution in lower land plants. *Taxon*, 18:46-91.

—. 1982. Generic and familial endemism in the hepatic flora of gondwanaland: origin and causes. *Journ. Hattori Bot. Lab.*, 52:3-35.

—. 1983. Phytogeography of the bryophyta. In R. M. Schuster (ed.), *New manual of bryology*, Vol. 1, pp. 463-626. Nichinan, Japan.

Schwartzenberg, K. v., S. Kruse, R. Reski, B. Moffatt, and M. Laloue. 1998. Cloning and characterization of an adenosine kinase from *Physcomitrella* involved in metabolism. *The Plant Journal*, 13:249-257.

Scotese, C. R. 2004. PALEOMAP Project. homepage: www.scotese.com.

Scotese, C. R., and W. S. McKerrow. 1990. Revised world maps and introduction. In W. S. McKerrow and C. R. Scotese (eds.), *Palaeozoic palaeogeography and biogeography.*, Vol. 12, pp. 1-21.

Seki, T. 1973. Distributional patterns of the Patagonian mosses. *Proceedings of the Japan Society of plant taxonomists.*, 3:13-15.

Seppelt, R. D. 2004. The moss flora of Macquarie Island. In D. Bergstrom (ed.), *Antarctic Terrestrial Biodiversity Series*, pp. 328. Australian Antarctic Division, Kingston, Tasmania, Australia.

Shaw, A. J., C. J. Cox, B. Goffinet, W. R. Buck, and S. B. Boles. 2003. Phylogenetic evidencen of a rapid radiation of pleurocarpous mosses (Bryophyta). *Evolution*, 57:2226-2241.

Shaw, J., and K. Renzaglia. 2004. Phylogeny and diversification of bryophytes. *American Journal of Botany*, 9:1557-1581.

Skottsberg, C. 1960. Remarks on the plant geography of the southern cold temperate zone. *Proceedings of the Royal Society London Series B*, 152:447-457.

Stech, M. 1999. Molekulare Systematik haplolepider Laubmoose (Dicrananae, Bryopsida). PhD thesis, Freie Universität Berlin.

Stech, M., T. Pfeiffer, and W. Frey. 1999. Molecular systematic relationship of temperate austral Hypopterygiaceae (Bryopsida): implications for taxonomy and biogeography. Studies in austral temperate rain forest bryophytes 3. *Haussknechtia*, 9:359-367.

—. 2002. Chloroplast DNA relationship in palaeoaustral *Polytrichadelphus magellanicus* (Hedw.) Mitt. (Polytrichaceae, Bryopsida). *Botanisches Jahrbuch für Systematik*, 124:217-226.

Suzuki, Y., G. V. Glazko, and M. Nei. 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. *Proceedings of the American Academy of Arts and Sciences*, 99:16138-16143.

Swenson, U., A. Backlund, S. McLoughlin, and R. S. Hill. 2001. *Nothofagus* biogeography revisited with special emphasis on the enigmatic distribution of subgenus *Brassospora* in New Caledonia. *Cladistics*, 17:28-47.

Swofford, D. L. 2002. PAUP*4b10. Phylogenetic Analysis Using Parsimony (*and other Methods). Sinauer Associates, Sunderland.

Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of the chloroplast DNA. *Plant Molecular Biology*, 17:1105-1109.

Tangney, R. S., and A. J. Fife. 1997. The occurence of *Ptychomnion densifolium* in new Zealand. *Journal of Bryology*, 19:819-825.

van Balgooy, M. M. J. 1960. Preliminary plant geographical analysis of the Pacific. *Blumea*, 10:385-430.

van der Sande, C. A. F. M., M. Kwa, R. W. van Nues, H. van Heerikhuizen, H. A. Rauke, and R. J. Planta. 1992. Functional analysis of transcribed spacer 2 Saccharomyces cerevisiae ribosomal DNA. *Journal of Molecular Biology*, 223:899-910.

van Steenis, C. G. 1971. Nothofagus, key genus of plant geography, in time and space, living and fossil, ecology and phylogeny. *Blumea*, 19:65-98.

van Zanten, B. O. 1971. Musci. In E. M. van Zinderen Bakker, J. M. Winterbottom and R. A. Dyer (eds.), *Marion and Prince Edward Islands*, pp. 173-227. Balkema, Cape Town.

—. 1976. Preliminary report on germination experiments designed to estimate the survival chances of moss spores during aerial trans-oceanic long-range dispersal in the southern hemisphere, with particular reference to New Zealand. *Journal of the Hattori Botanical Laboratory*, 41:133-140.

—. 1978. Experimental studies on trans-oceanic long-range dispersal of moss spores in the southern hemisphere. *Journal of the Hattori Botanical Laboratory*, 44:455-482.

—. 1983. Possibilities of long-range dispersal in bryophytes with special reference to the Southern Hemisphere. *Sonderbände des Naturwiss. Ver. Hamburg*, 7:49-64.

—. 1984. Some considerations on the feasibility of long-distance transport in bryophytes. *Acta Botanica Neerlandica*, 33:231-232.

van Zanten, B. O., and T. Pócs. 1981. Distribution and dispersal of bryophytes, *Advances in Bryology*, Vol. 1, pp. 479-561. J. Cramer.

Vanderpoorten, A., A. J. Shaw, and C. J. Cox. 2004. Evolution of multiple paralogous adenosine kinase genes in the moss genus *Hygroamblystegium*: phylogenetic implications. *Molecular Phylogenetics and Evolution*, 31:505-516.

Vanderpoorten, A., A. J. Shaw, and B. Goffinet. 2001. Testing controversial alignments in Amblystegium and related genera (Amblystegiaceae: Bryopsida). Evidence from rDNA ITS sequences. *Systematic Botany*, 26:470-479.

Villagrán, C., E. Barrera, J. Cuvertino, and N. García 2003. Musgos de la Isla Grande de Chiloé, X. región, Chile: lista de especies y rasgos fitogeográficos. *Boletín del Museo Nacional de Historia Natural, Chile*, 52:17-44.

Villagrán, C., C. Le-Quesne, J. C. Aravena, H. Jiménez, and F. Hinojosa. 1998. El rol de los cambios de clima del cuanternario en la distribucíon actual de la vegetacíon de Chile central-sur. In K. Garleff and H. Stingl (eds.), *Landschaftsentwicklung, Paläoökologie und Klimageschichte der ariden Diagonale Südamerikas im Jungquartär.*, Vol. 15, pp. 227-242. Selbstverlag Fach Geographie, Universität Bamberg, Bamberg.

Vitt, D. H. 1984. Classification of Bryopsida. In R. M. Schuster (ed.), *New manual of bryology*, Vol. 2, pp. 696-759. Nichinan, Japan.

Wanntorp, L., and H.-E. Wanntorp. 2003. The biogeography of *Gunnera* L.: vicariance and dispersal. *Journal of Biogeography*, 30:979-987.

Wardle, P., C. Ezcurra, C. Ramírez, and S. Wagstaff. 2001. Comparison of the flora and vegetation of the southern Andes and New Zealand. *New Zealand Journal of Botany*, 39:69-108.

Wegener, A. 1915. *Die Entstehung der Kontinente und Ozeane*, Braunschweig.

Weinelt, M. 2004. OMC - Online map creation. http://www.aquarius.geomar.de.

Wessel, P., and W. H. F. Smith. 1995. New Version of the Generic Mapping Tools Released, *http://www.agu.org/eos_elec/95154e.html*. Copyright 1995 by the American Geophysical Union.

White, M. E. 1990. *The Flowering of Gondwana*. Princeton University Press, Princeton.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. Innis, D.

Gelfand, J. Sninsky and T. J. White (eds.), *PCR protocols: a guide to methods and applications*, pp. 315-322. Academic Press, San Diego.

Willerslev, E., A. J. Hansen, J. Binladen, T. B. Brand, M. T. P. Gilbert, B. Shapiro, M. Bunce, C. Wiuf, D. A. Gilichinsky, and A. Cooper. 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science*, 300:791-795.

Willerslev, E., A. J. Hansen, and H. N. Poinar. 2004. Isolation of nucleic acids and cultures from fossil ice and permafrost. *Trends in Ecology & Evolution*, 19:141-147.

Index to tables

Index to figures

Appendix 1: List of investigated specimens, with EMBL accession numbers for the regions sequenced. Voucher numbers and the herbaria where the specimens are kept are listed only for those specimens where sequence was not downloaded from EMBL/GenBank. Accession numbers marked [rb] were especially sequenced for this analysis. The remaining sequences were obtained from GenBank.

Taxon	family	rps4	*trn*L-F intron/spacer	origin	Voucher no.	herbarium
Acrocladium auriculatum (Mont.) Mitt. sp. 78	Plagiotheciaceae*	AJ862338[rb]	AF543546	Chile	BryoAustral W. Frey 98-T154 B	W. Frey, Berlin
Acrocladium chlamydophyllum (Hook. f. & Wilson) Müll. Hal. & Broth. sp. 12	Plagiotheciaceae*	AJ862339[rb]	AF509543	New Zealand	BryoAustral Rolf Blöcher No. 49	J.-P. Frahm, Bonn
Hypnum cupressiforme Hedw.	Hypnaceae	AJ269690	AF397812	Europe	EMBL/GenBank	
Lepyrodon pseudolagurus (Hook.) Mitt. sp. 67	Lepyrodontaceae	AJ862337[rb]	AF187239/ AF187255	New Zealand	BryoAustral J.-P. Frahm no. 10-12	J.-P. Frahm, Bonn
Lepyrodon tomentosus (Hook.) Mitt. sp. 64	Lepyrodontaceae	AJ862337[rb]	AF509541	Chile	BryoAustral Rolf Blöcher no. 74	J.-P. Frahm, Bonn
Leucodon sciuroides (Hedw.) Schwägr.	Leucodontaceae	AJ269688	AF397786	Europe	EMBL/GenBank	
Neckera crispa Hedw.	Neckeraceae	AJ269692	AY050280/ AY050287	Europe	EMBL/GenBank	
Dichelodontium nitidum (Hook.f. & Wils.) Broth.#2	Lepyrodontaceae	AY306917	AY306751	New Zealand	EMBL/GenBank	
Hampeella alaris *(Dix. & Sainsb.) Sainsb. #2*	Ptychomniaceae	AY306920	AY306754	New Zealand	EMBL/GenBank	

Appendix 1: continued

Taxon	family	rps4	*trn*L-F intron/spacer	origin	Voucher no.	herbarium
Ptychomnion cygnisetum (C. Müll..) Kindb. #2	Ptychomniaceae	AY306984	AY306818	Chile	EMBL/GenBank	
Ptychomnion ptychocarpon (Schwaegr.) Mitt. #2	Ptychomniaceae	AY 306985	AY306819	Chile	EMBL/GenBank	
Cladomnion ericoides (Hook.) Wils. in Hook.f. #2	Ptychomniaceae	AY 306884	AY306718	New Zealand	EMBL/GenBank	
Tetraphidopsis pusilla (Hook.f. & Wils.) Dix. #2	Ptychomniaceae	AY307001	AY306835	New Zealand	EMBL/GenBank	
Cladomniopsis crenato-obtusa Fleisch.	Ptychomniaceae	AY 306883	AY306717	Chile	EMBL/GenBank	
Glyphothecium sciuroides (Hook.) Hamp. #2	Ptychomniaceae	AY306919	AY306753	Australia	EMBL/GenBank	
Ptychomnion aciculare (Brid.) Mitt. #1	Ptychomniaceae	AY306983	AY306817	Australia	EMBL/GenBank	
Hampeella pallens (Lac.) Fleisch.	Ptychomniaceae	AY306921	AY306755	Australia	EMBL/GenBank	
Dichelodontium nitidum (Hook.f. & Wils.) Broth. Sp. 81	Lepyrodontaceae	n.d.	AJ862683[rb]	New Zealand	Bryo 267448 (Sainsbury 5. Jan. 1942)	Berlin
Hampeella alaris *(Dix. & Sainsb.) Sainsb. sp. 128*	Ptychomniaceae	AJ862334	AJ862684[rb]	New Zealand	Zanten 93.10.1528	B. van Zanten, Groningen
Ptychomnion cygnisetum (C. Müll..) Kindb. sp. 131	Ptychomniaceae	AJ862331	AJ862681	Chile	BryoAustral, Rolf Blöcher 247	J.-P. Frahm, Bonn
Ptychomnion ptychocarpon (Schwaegr.) Mitt. sp. 130	Ptychomniaceae	AJ862330	AJ862682[rb]	Chile	BryoAustral, Rolf Blöcher 249	J.-P. Frahm, Bonn
Cladomnion ericoides (Hook.) Wils. sp. 125	Ptychomniaceae	n.d.	AJ862680[rb]	New Zealand	H. Streimann 51478	Helsinki
Tetraphidopsis pusilla (Hook.f. & Wils.) Dix. sp. 126	Ptychomniaceae	AJ862329 [rb]	AJ862679[rb]	New Zealand	Zanten 00.11.712	B. van Zanten, Groningen

Appendix 1: continued

Taxon	family	rps4	*trn*L-F intron/spacer	origin	Voucher no.	herbarium
Cladomniopsis crenato-obtusa Fleisch. sp. 127	Ptychomniaceae	submitted to EMBL	submitted to EMBL	Chile	Matteri CM 2696	J.-P. Frahm, Bonn
Glyphothecium sciuroides (Hook.) Hamp. sp. 123	Ptychomniaceae	AJ862333[rb]	AJ862677[rb]	Chile	Zanten 00.11.378	B. van Zanten, Groningen
Glyphothecium sciuroides (Hook.) Hamp. sp. 158	Ptychomniaceae	AJ862332[rb]	AJ862677[rb]	Chile	BryoAustral, Frahm 16-0	J.-P. Frahm, Bonn
Ptychomnion aciculare (Brid.) Mitt. #2	Ptychomniaceae	AF143015	AF161108	New Zealand		
Schimperobryum splendidissimum Margad.	Hookeriaceae	AJ315873	AJ507770	Chile		
Daltonia gracilis Mitt.	Daltoniaceae	AY306894	AY306728	Ecuador		
Distichophyllum pulchellum (Hampe) Mitt.	Daltoniaceae	AY306902	AY306736	New Zealand		
Hookeria lucens (Hedw.) Sm.	Hookeriaceae	AJ269689	AF152380	Europe		
Lopidium concinnum (Hook.) Wilson	Hypopterygiaceae	AJ252289	AF033233	New Zealand		
Hypopterygium didictyon Müll.Hal.	Hypopterygiaceae	AJ252292	AF170592	Chile		
Euptychium robustum Hampe	Garovagliaceae	AY306907	AY306741	Australia		
Garovaglia elegans (Dozy & Molk) Bosch & Lac.	Garovagliaceae	AY306915	AY306749	Papua New Guinea		

Appendix 2: P-distances of the *trn*L intron of the successfully sequenced specimens of Ptychomniaceae including the outgroup, and standard errors. P-distances are shown in the lower left triangle, standard errors in the upper right triangle. The mean p-distance for the full dataset including the outgroup is 0.05 (SE 0.007). The mean p-distance for dataset comprising only the taxa of Ptychomniaceae s.l. (see text) is 0.05 (SE 0.007). Abbreviations: *C. cr.-obscura=Cladomniopsis creanato-obscura*, *Clad.=Cladomnion*, *Dich.=Dichrlodontium*, *Gly.=Glyphothecium*, *Hamp.=Hampeella*, *Lep.=Lepyrodon*, *P.=Ptychomnion*, *Tet.=Tetraphidopsis*.

	Specimens	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	Hookeria lucens		0.018	0.018	0.017	0.017	0.017	0.018	0.017	0.016	0.020	0.019	0.019	0.018	0.018	0.018	0.018	0.019	0.019	0.018	0.019	0.018	0.019	0.019	0.019
2	L. tomentosus (sp. 64)	0.086		0.005	0.015	0.015	0.015	0.015	0.015	0.016	0.016	0.016	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.016	0.017	0.014	0.016
3	L. pseudolagurus (sp. 67)	0.090	0.010		0.015	0.015	0.015	0.015	0.015	0.016	0.016	0.016	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.017	0.014	0.016
4	Hamp. pallens	0.080	0.070	0.069		0.006	0.007	0.012	0.012	0.012	0.014	0.014	0.012	0.012	0.012	0.011	0.011	0.011	0.011	0.012	0.012	0.012	0.013	0.012	0.013
5	Hamp. alaris (sp. 128)	0.076	0.076	0.076	0.013		0.000	0.013	0.012	0.013	0.015	0.015	0.013	0.013	0.013	0.013	0.013	0.012	0.012	0.012	0.012	0.012	0.014	0.012	0.013
6	Hamp. alaris (sp. 2)	0.076	0.077	0.076	0.013	0.000		0.013	0.012	0.014	0.015	0.014	0.013	0.013	0.013	0.013	0.013	0.012	0.012	0.013	0.012	0.012	0.014	0.012	0.013
7	P. ptychocarpon (sp.132)	0.086	0.076	0.076	0.049	0.052	0.053		0.003	0.012	0.012	0.012	0.011	0.012	0.012	0.011	0.011	0.010	0.010	0.010	0.011	0.011	0.013	0.010	0.011
8	P. ptychocarpon (sp. 2)	0.082	0.073	0.072	0.046	0.049	0.049	0.003		0.011	0.012	0.011	0.011	0.011	0.011	0.011	0.011	0.010	0.010	0.010	0.010	0.011	0.012	0.010	0.011
9	C. cr.-obscura (sp.127)	0.074	0.066	0.066	0.040	0.048	0.048	0.036	0.032		0.014	0.014	0.012	0.013	0.013	0.013	0.013	0.011	0.011	0.010	0.011	0.012	0.015	0.011	0.013
10	Tet. pusilla (sp. 126)	0.110	0.090	0.089	0.066	0.072	0.069	0.048	0.045	0.056		0.003	0.014	0.013	0.013	0.013	0.013	0.012	0.012	0.014	0.013	0.014	0.015	0.012	0.014
11	Tet. pusilla (sp. 2)	0.106	0.090	0.089	0.066	0.069	0.066	0.045	0.042	0.056	0.003		0.014	0.013	0.013	0.013	0.013	0.013	0.013	0.014	0.013	0.014	0.015	0.012	0.014
12	Gly. sciuroides (sp.158)	0.102	0.073	0.073	0.049	0.052	0.053	0.042	0.038	0.040	0.061	0.061		0.010	0.010	0.009	0.009	0.007	0.007	0.006	0.006	0.008	0.010	0.006	0.009
13	P. cygnisetum (sp. 130)	0.094	0.076	0.076	0.046	0.056	0.056	0.045	0.042	0.047	0.058	0.058	0.032		0.000	0.003	0.003	0.009	0.009	0.009	0.009	0.010	0.012	0.010	0.011
14	P. cygnisetum (sp. 2)	0.094	0.076	0.076	0.046	0.056	0.056	0.045	0.042	0.047	0.058	0.058	0.032	0.000		0.003	0.003	0.009	0.009	0.009	0.009	0.010	0.012	0.010	0.011
15	P. aciculare (sp. 1)	0.094	0.073	0.072	0.042	0.052	0.052	0.042	0.038	0.043	0.055	0.055	0.029	0.003	0.003		0.000	0.009	0.009	0.008	0.008	0.010	0.012	0.009	0.011
16	P. aciculare (sp. 2)	0.094	0.073	0.072	0.042	0.052	0.052	0.042	0.038	0.043	0.055	0.055	0.029	0.003	0.003	0.000		0.009	0.009	0.008	0.008	0.010	0.012	0.009	0.011
17	Clad. ericioides (sp. 125)	0.098	0.069	0.069	0.042	0.046	0.046	0.035	0.032	0.032	0.048	0.051	0.016	0.029	0.029	0.026	0.026		0.000	0.005	0.006	0.007	0.010	0.000	0.010
18	Clad. ericioides (sp. 2)	0.098	0.069	0.069	0.042	0.046	0.046	0.035	0.032	0.032	0.048	0.051	0.016	0.029	0.029	0.026	0.026	0.000		0.005	0.006	0.007	0.010	0.000	0.010

Appendix 2: continued

	Specimens	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
19	Gly. sciuroides (sp. 123)	0.094	0.072	0.072	0.043	0.046	0.046	0.031	0.028	0.028	0.056	0.056	0.010	0.024	0.024	0.021	0.021	0.007	0.007		0.003	0.006	0.010	0.005	0.009
20	Gly. sciuroides (sp. 2)	0.098	0.070	0.070	0.043	0.046	0.046	0.035	0.032	0.032	0.055	0.055	0.013	0.026	0.026	0.023	0.023	0.010	0.010	0.003		0.006	0.010	0.006	0.009
21	Euptychium robustum	0.094	0.079	0.079	0.046	0.049	0.050	0.042	0.039	0.040	0.061	0.061	0.019	0.032	0.032	0.029	0.029	0.016	0.016	0.010	0.010		0.010	0.007	0.010
22	Garovaglia elegans	0.105	0.092	0.092	0.059	0.066	0.066	0.051	0.048	0.060	0.071	0.071	0.029	0.048	0.048	0.045	0.045	0.032	0.032	0.028	0.029	0.035		0.008	0.007
23	Dich. nitens (sp. 81)	0.096	0.067	0.067	0.043	0.047	0.047	0.033	0.029	0.032	0.046	0.049	0.013	0.029	0.029	0.026	0.026	0.000	0.000	0.007	0.010	0.016	0.020		0.007
24	Dich. nitidum (sp. 2)	0.098	0.082	0.082	0.052	0.059	0.059	0.042	0.039	0.047	0.065	0.061	0.026	0.042	0.042	0.039	0.039	0.029	0.029	0.024	0.026	0.032	0.016	0.016	

Appendix 3: P-distances of the *rps*4 gene of the successfully sequenced specimens of Ptychomniaceae including the outgroup, and standard errors. P-distances are shown in the lower left triangle, standard errors in the upper right triangle. The mean p-distance for the full dataset including the outgroup is 0.065 (SE 0.005). The mean p-distance for dataset comprising only the taxa of Ptychomniaceae s.l. (see text) is 0.048 (SE 0.005). *C. cr.-obscura=Cladomniopsis creanato-obscura*, *Clad.=Cladomnion*, *Dich.=Dichrlodontium*, *Gly.=Glyphothecium*, *Hamp.=Hampeella*, *Lep.=Lepyrodon*, *P.=Ptychomnion*, *Tet.=Tetraphidopsis*.

nr.	Specimens	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	Hookeria lucens		0.010	0.009	0.011	0.011	0.010	0.011	0.011	0.011	0.012	0.011	0.011	0.011	0.011	0.012	0.012	0.011	0.011	0.011	0.011	0.011	0.012	0.012	0.012
2	L. tomentosus (sp. 64)	0.054		0.004	0.012	0.011	0.011	0.012	0.012	0.011	0.012	0.012	0.011	0.012	0.012	0.012	0.012	0.011	0.011	0.012	0.011	0.012	0.013	0.012	0.012
3	L. pseudolagurus (sp. 67)	0.057	0.009		0.011	0.011	0.010	0.011	0.011	0.010	0.012	0.012	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011	0.011
4	Hamp. pallens	0.085	0.093	0.089		0.006	0.005	0.011	0.010	0.010	0.012	0.011	0.011	0.011	0.011	0.011	0.011	0.010	0.010	0.011	0.011	0.011	0.011	0.011	0.011
5	Hamp. alaris (sp. 128)	0.072	0.080	0.078	0.020		0.000	0.010	0.010	0.009	0.011	0.011	0.011	0.010	0.010	0.010	0.010	0.009	0.009	0.010	0.010	0.010	0.011	0.011	0.011
6	Hamp. alaris (sp. 2)	0.073	0.080	0.076	0.019	0.000		0.010	0.010	0.009	0.011	0.011	0.011	0.010	0.010	0.010	0.010	0.009	0.009	0.010	0.010	0.010	0.011	0.011	0.011
7	P. ptychocarpon (sp.132)	0.088	0.095	0.089	0.081	0.067	0.068		0.002	0.009	0.011	0.011	0.010	0.011	0.011	0.011	0.011	0.010	0.010	0.010	0.010	0.010	0.011	0.011	0.011
8	P. ptychocarpon (sp. 2)	0.087	0.091	0.085	0.080	0.065	0.068	0.002		0.009	0.011	0.010	0.010	0.010	0.010	0.010	0.010	0.009	0.009	0.010	0.010	0.010	0.011	0.010	0.010
9	C. cr.-obscura (sp.127)	0.074	0.080	0.076	0.066	0.054	0.056	0.054	0.051		0.010	0.009	0.008	0.009	0.008	0.009	0.009	0.007	0.007	0.009	0.008	0.009	0.009	0.009	0.009
10	Tet. pusilla (sp. 126)	0.084	0.097	0.095	0.095	0.083	0.086	0.079	0.079	0.060		0.000	0.010	0.011	0.011	0.011	0.011	0.010	0.010	0.011	0.011	0.011	0.011	0.011	0.011
11	Tet. pusilla (sp. 2)	0.082	0.095	0.092	0.092	0.083	0.083	0.079	0.077	0.057	0.000		0.010	0.010	0.010	0.011	0.011	0.010	0.010	0.011	0.010	0.010	0.010	0.010	0.010

Appendix 3: continued

nr.	Specimens	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
12	Gly. sciuroides (sp.158)	0.089	0.091	0.091	0.081	0.067	0.068	0.063	0.060	0.044	0.068	0.066		0.008	0.008	0.008	0.008	0.007	0.007	0.007	0.007	0.008	0.009	0.008	0.008
13	P. cygnisetum (sp. 130)	0.090	0.091	0.089	0.078	0.063	0.068	0.068	0.063	0.049	0.069	0.068	0.037		0.000	0.003	0.003	0.006	0.006	0.007	0.006	0.007	0.007	0.007	0.007
14	P. cygnisetum (sp. 2)	0.089	0.091	0.089	0.078	0.063	0.067	0.068	0.063	0.048	0.069	0.068	0.037	0.000		0.003	0.003	0.006	0.006	0.007	0.006	0.007	0.007	0.007	0.007
15	P. aciculare (sp. 1)	0.094	0.094	0.092	0.085	0.071	0.073	0.073	0.068	0.055	0.077	0.075	0.044	0.007	0.007		0.000	0.007	0.007	0.008	0.007	0.008	0.008	0.007	0.007
16	P. aciculare (sp. 2)	0.094	0.094	0.092	0.085	0.071	0.073	0.073	0.068	0.055	0.077	0.075	0.044	0.007	0.007	0.000		0.007	0.007	0.008	0.007	0.008	0.008	0.007	0.007
17	Clad. ericioides (sp. 125)	0.085	0.087	0.087	0.073	0.056	0.061	0.063	0.060	0.038	0.066	0.066	0.039	0.026	0.026	0.032	0.032		0.000	0.006	0.006	0.007	0.007	0.007	0.007
18	Clad. ericioides (sp. 2)	0.085	0.087	0.087	0.073	0.056	0.061	0.063	0.060	0.038	0.066	0.066	0.039	0.026	0.026	0.032	0.032	0.000		0.006	0.006	0.007	0.007	0.007	0.007
19	Gly. sciuroides (sp. 123)	0.091	0.091	0.091	0.081	0.067	0.068	0.068	0.065	0.047	0.073	0.072	0.037	0.028	0.028	0.035	0.035	0.025	0.025		0.002	0.006	0.008	0.007	0.007
20	Gly. sciuroides (sp. 2)	0.090	0.089	0.089	0.080	0.065	0.068	0.066	0.061	0.046	0.071	0.070	0.035	0.026	0.026	0.032	0.032	0.024	0.024	0.002		0.006	0.007	0.006	0.006
21	Euptychium robustum	0.095	0.094	0.095	0.083	0.069	0.071	0.066	0.061	0.052	0.077	0.075	0.049	0.039	0.038	0.041	0.041	0.032	0.032	0.028	0.026		0.008	0.006	0.006
22	Garovaglia elegans	0.101	0.107	0.102	0.089	0.074	0.077	0.082	0.077	0.061	0.073	0.071	0.056	0.036	0.036	0.043	0.043	0.038	0.038	0.040	0.038	0.036		0.007	0.007
23	Dich. nitens (sp. 81)	0.097	0.096	0.095	0.082	0.069	0.072	0.073	0.068	0.053	0.073	0.071	0.049	0.032	0.032	0.039	0.039	0.027	0.027	0.026	0.024	0.022	0.027		0.000
24	Dich. nitidum (sp. 2)	0.097	0.096	0.095	0.082	0.069	0.072	0.073	0.068	0.053	0.073	0.071	0.049	0.032	0.032	0.039	0.039	0.027	0.027	0.026	0.024	0.022	0.027	0.000	

Appendix 4: List of investigated specimens, with EMBL accession numbers for the regions sequenced. Voucher numbers and the herbaria where the specimens are kept are listed only for those specimens where sequence was not downloaded from EMBL/GenBank. Accession numbers marked [rb] were especially sequenced for this analysis. The remaining sequences were obtained from GenBank. Abbreviations: [+] Huttunen & Ignatov 2004; [++]/ [$$]Quandt et al. 2004; [*] Shaw et al. 2003; [**] Blöcher & Capeisus 2002; [$] Stech et al 2003; [§§] Pedersen, & Hedenäs 2002.

Taxon	family	*trn*L intron	*trn*L-trnF region	*psb*T-H	ITS complete	ITS1/ITS2	rps4
Pyrrhobryum latifolium (Bosch. & Lac.) Mitt.	Rhizogoniaceae	AY044077[++]/		AF417406[++]		AF395643[++]	
Orthotrichum anomalum Hedw.	Orthotrichaceae	AF130314[++]/	AF129580[++]	AF508318[++]		AF144129[++]	
Orthotrichum stramineum Hornsch. ex Brid.	Orthotrichaceae	AF127183[++]/	AF129579[++]	AF508317[++]		AF144130[++]	
Acrocladium auriculatum (Mont.) Mitt.	Plagiotheciaceae*		AF543546[++]	AF543556[++]		AJ862695[rb]/ AF543550	AJ862338[rb]
Acrocladium chlamydophyllum (Hook. f. & Wilson) Müll. Hal. & Broth.	Plagiotheciaceae*		AF509543[++]	AF543555[++]		AJ862491[rb]/ AF509863	AJ862339[rb]
Amblystegium serpens (Hedw.) Schimp.	Amblystegiaceae		AF397836[+]	AF417420[+]		AF403633[+]	
Calliergon stramineum (Dicks. ex Brid.) Kindb.	Amblystegiaceae		AY429495[$$]	AY429485[$$]		AY429501[$$]	
Camptochaete arbuscula (Sm.) Reichdt.	Lembophyllaceae	AF187250[++]/	AF187266[++]	AF543559[++]		AF188056[++]	
Catagonium nitidum (Hook. f. & Wilson) Broth. CH236b	Plagiotheciaceae*				AJ862506[rb]		AJ862341[rb]
Catagonium nitens (Brid.) Cardot NZ23	Plagiotheciaceae*		AF472449[§§]		AJ862505[rb]	rb	AF469810[§§]
Catagonium nitens (Brid.) Cardot MA91	Plagiotheciaceae*		AF472450[§§]		AJ862503[rb]	rb	AF469811[§§]
Cratoneuropsis relaxa (Hook. & Wilson) M.Fleisch.	Amblystegiaceae		AY429494[$$]	AY429484[$$]		AF152391[++]	
Ctenidium molluscum (Hedw.) Mitt.	Hypnaceae		-[+]	AF417414[+]		AF403632[+]	
Entodontopsis leucostega (Brid.) W.R. Buck & Ireland	Stereophyllaceae	AF161153[*]/	#[*]				AF143060[*]

Appendix 4: continued

Taxon	family	*trn*L intron	*trn*L-trnF region	*psb*T-H	ITS complete	ITS1/ITS2	rps4
Eurhynchium pulchellum (Hedw.) Jenn.	Brachytheciaceae		AY044069[+]	AF417384[+]		AF395635[+]	
Eurhynchium striatum (Hedw.) Schimp.	Brachytheciaceae		AY184788[+]	AY184769[+]		AF503538[+]	
Fifea aciphylla (Dix. & Sainsb.) H.A.Crum	Lembophyllaceae	AF295041[++]/	AF295042[++]	-[++]		AF295043[++]	
Herzogiella seligeri (Brid.) Z. Iwats.	Plagiotheciaceae*	AF472453[§§]/			AJ862507[rb]		AF469814[§§]
Hypnum cupressiforme Hedw.	Hypnaceae		AF397812[+]	AF417361[+]		AF403607[+]	AJ269690[**]
Isopterygiopsis muelleriana (Schimp.) Z. Iwats.	Plagiotheciaceae	AF472455[§§]/					AF469816[§§]
Isopterygiopsis pulchella (Hedw.) Z. Iwats.	Plagiotheciaceae	AF472456[§§]/					AF469817[§§]
Isopterygium albescens (Hook.) A. Jaeger	Hypnaceae	AF472457[§§]/					AF469818[§§]
Isopterygium minutirameum (Müll. Hal.) A. Jaeger	Hypnaceae	AF472458[§§]/					AF469819[§§]
Isopterygium tenerum (Sw.) Mitt.	Hypnaceae	AF161130[*]/	#[*]				AF143037[*]
Isothecium alopecuroides (Dubois) Isov.	Lembophyllaceae		AY044065[+]	AF417353[+]		AF395636[+]	
Lembophyllum divulsum (Hook.f. & Wilson) Lindb.	Lembophyllaceae	AF187249[++]/	AF187265[++]	AF397887[++]		AF188055[++]	AY306936[*]
Lepyrodon pseudolagurus (Hook.) Mitt.	Lepyrodontaceae	AF187239[++]/	AF187255[++]	-[++]		AJ862687[rb]/ AF188044[++]	AJ862335[rb]
Lepyrodon tomentosus (Hook.) Mitt.	Lepyrodontaceae	AF509541[++]/	#[++]	AF509938[++]		AJ862688[rb]/ AF509839[++]	AJ862337[rb]
Leskea polycarpa Hedw.	Leskeaceae		AF397810[+]	AF417367[+]		AF403604[+]	
Leucodon sciuroides (Hedw.) Schwägr.	Leucodontaceae		AF397786[+]	AF417398[+]		AF403634[+]	AJ269688[**]
Meteorium illecebrum (Hedw.) Broth.	Meteoriaceae	AF187241[++]/	AF187257[++]	AF508319[++]		AF188046[++]	AY306952[*]
Myurium hochstetteri (Schimp.) Kindb.	Myuriaceae	AF161111[*]/	#[*]				AF143018[*]
Neckera crispa Hedw.	Neckeraceae	AY050280[§]/	AY050287[§] (spacer)	AY122283[§]		AY050296[§]	AJ269692[**]
Orthothecium chryseum (Schwägr.) Schimp.	Hypnaceae	AF472462[§§]/					AF469823[§§]

Appendix 4: continued

Taxon	family	*trn*L intron	*trn*L-trnF region	*psb*T-H	ITS complete	ITS1/ITS2	rps4
Orthothecium intricatum (Hartm.) Schimp.	Hypnaceae	AF472463[§§]/					AF469824[§§]
Pilosium chlorophyllum (Hornsch.) Müll. Hal.	Hookeriaceae	AF161152[*]					AF143059[*]
Plagiothecium denticulatum (Hedw.) Schimp.	Plagiotheciaceae		AF397845[+]	AF417419[+]		AF403635[+]	AF469828[§§]
Plagiothecium undulatum (Hedw.) Schimp.	Plagiotheciaceae	AF215905[§§]/					AJ251315[§§]
Platydictya jungermannioides (Brid.) H.A. Crum	Amblystegiaceae	AF472472[§§]/					AF469833[§§]
Pseudotaxiphyllum elegans (Brid.) Z. Iwats.	Plagiotheciaceae	AF472473[§§]/					AF469834[§§]
Pseudotaxiphyllum laetevirens (Dixon & Luisier ex F. Koppe & Düll) Hedenas	Plagiotheciaceae	AF472474[§§]/					AF469835[§§]
Pterobryon densum Hornsch.	*Pterobryaceae*	AY050283[§]/	AY050291[§] (spacer)	AF417432[§]		AY050294[§]	AF143013[*]
Sematophyllaceae CH129	Sematophyllaceae		AJ862343[rb]		AJ862342[rb]		
Sematophyllum homomallum (Hampe) Broth.	*Sematophyllaceae*		AF509540[++]	AF509937[++]		AF509838[++]	
Squamidium brasiliense (Hornsch.) Broth.	Brachytheciaceae		AY044063[+]	AF417393[+]		AF395637[+]	AY306991*
Stereophyllum radiculosum (Hook.) Mitt.	Stereophyllaceae		AF472484[§§]				AF469846[§§]
Struckia zerovii (Lazarenko) Hedenas	Sematophyllaceae	AF472478[§§]/					AF469839[§§]
Taxiphyllum taxirameum (Mitt.) M. Fleisch.	Hypnaceae	AF472480[§§]/			AJ862522[rb]		AF469841[§§]
Trachyloma planifolium (Hedw.) Brid.	Trachylomataceae	AF187238[++]/	AF187254[++]	AF543553[++]		AF188042[++]	

Appendix 4: continued

Taxon	family	*trn*L intron	*trn*L-trnF region	*psb*T-H	ITS complete	ITS1/ITS2	rps4
Tripterocladium leucocladulum (Müll. Hal.) A. Jaeger	Lembophyllaceae			AY429492[++]			
Weymouthia cochlearifolia (Hedw.) Broth.	Lembophyllaceae	AF187248[++]/	AF187264[++]	AF397883[++]		AJ862693[rb]/ AF188054[++]	AY307012*
Weymouthia mollis (Hedw.) Broth.	Meteoriaceae	AF187246/		AF417422		AJ862694[rb]/ AF188051	AY307013*
Zelometeorium patulum (Hedw.) Manuel	Brachytheciaceae		AF397787[+]	AF417362[+]		AF509862[+]	AY307016*

Appendix 5: Sequence lengths [base pairs, bp] of selected gene regions (*trn*L, *rps*4, *rps*4-*trn*S spacer, ITS region) and GC-content [%] of the regions studied for 53 bryophyte taxa. Average sequence lengths and standard deviations are also given. For origin of the data refer tab. xz. (n. d. = no data available)

gene / gene region	***trn*L**		***rps*4**		***rps*4-*trn*S spacer**		**ITS1**		**5.8S**		**ITS2**	
Taxon	**sequence length [bp]**	**GC-content [%]**	**sequence length [bp]**	**GC-content [%]**	**sequence length [bp]**	**GC-content [%]**	**sequence length [bp]**	**GC-content [%]**	**sequence length [bp]**	**GC-content [%]**	**sequence length [bp]**	**GC-content [%]**
Pyrrhobryum latifolium	465	28.6	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	83	55.4	368	66.3
Orthotrichum anomalum	441	27.4	587	28.1	60	26.6	n. d.	n. d.	80	57.5	258	66.7
Orthotrichum stramineum	422	28.9	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	80	56.3	250	64.4
Acrocladium auriculatum (sp. 78)	403	29.8	558	26.3	n. d.	n. d.	259	63.3	156	51.2	245	64.5
Acrocladium chlamydophyllum (sp. 12)	416	31.2	570	26.7	n. d.	n. d.	259	61.8	156	51.2	245	63.6
Amblystegium serpens	421	31.8	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	83	56.6	272	69.5
Calliergon stramineum	421	31.6	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	83	56.6	273	70.3
Camptochaete arbuscula	416	32.0	587	25.0	58	25.8	n. d.	n. d.	83	55.4	278	64.1
Catagonium nitidum (sp. 236)	303	30.1	557	27.6	20	10.0	256	62.1	156	51.2	308	66.2
Catagonium nitens (sp. 23)	418	30.6	589	28.1	n. d.	n. d.	253	62.4	156	51.2	307	67.1
Catagonium nitens (sp. 91)	430	30.0	592	27.7	51	25.4	253	63.6	156	51.2	311	65.3
Cratoneuropsis relaxa	422	31.9	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	83	56.6	275	69.4
Ctenidium molluscum	275	27.3	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	83	55.4	332	62.9
Entodontopsis leucostega	415	31.8	463	25.0	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.

Appendix 5: continued

gene / gene region	*trn*L		*rps*4		*rps*4-*trn*S spacer		ITS1		5.8S		ITS2	
Eurhynchium pulchellum	407	32.2	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	83	56.6	296	64.2
Eurhynchium striatulum	409	31.8	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	83	56.6	291	66.4
Fifea aciphylla	416	32.2	n. d.	n. d.	n. d.	n. d.	247	63.5	156	51.2	280	64.3
Herzogiella seligeri	412	31.6	584	29.5	n. d.	n. d.	248	61.2	156	51.2	270	62.2
Hypnum cupressiforme	414	31.9	592	27.9	78	26.9	n. d.	n. d.	83	55.4	275	68.4
Isopterygiopsis muelleriana	416	32.0	591	28.6	60	23.4	252	63.9	156	51.3	270	64.8
Isopterygiopsis pulchella	415	31.3	591	27.9	60	21.6	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Isopterygium albescens	418	31.3	592	26.4	61	21.3	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Isopterygium minutirameum	414	29.7	584	28.3	57	17.5	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Isopterygium tenerum	415	30.6	576	27.4	43	9.4	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Isothecium alopecuroides	416	31.7	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	83	55.4	269	65.5
Lembophyllum divulsum	416	32.0	573	26.7	55	10.9	n. d.	n. d.	83	55.4	279	63.8
Lepyrodon tomentosus (sp. 64)	384	31.0	540	28.5	n. d.	n. d.	250	62.4	155	51.6	277	65.0
Leskea polycarpa	416	32.9	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	83	55.4	284	64.7
Leucodon sciuroides	423	30.1	592	27.2	59	27.1	n. d.	n. d.	83	56.6	297	66.4
Meteorium illecebrum	416	30.8	574	25.8	52	15.4	n. d.	n. d.	83	55.4	278	60.1
Myurium hochstetteri	423	30.2	587	27.9	60	26.6	n. d.	n. d.	n. d.	n. d.	288	64.6
Neckera crispa	409	33.0	592	28.2	78	32.0	n. d.	n. d.	83	55.4	267	64.8
Orthothecium chryseum	415	31.0	587	27.2	60	18.3	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Orthothecium intricatum	422	30.3	569	27.1	62	22.6	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Pilosium chlorophyllum	418	31.6	576	26.5	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Plagiothecium denticulatum	416	32.7	591	28.7	62	21.0	252	61.5	90	54.4	266	64.3
Plagiothecium undulatum	265	28.7	591	28.6	35	8.6	240	62.9	n. d.	n. d.	183	63.4
Platydictya jungermannioides	414	30.9	587	27.1	51	23.5	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Pseudotaxiphyllum elegans	415	31.0	592	27.7	59	22.1	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Pseudotaxiphyllum laetevirens	412	31.3	588	28.4	62	20.9	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Pterobryon densum	395	32.2	n. d.	n. d.	n. d.	n. d.	292	62.3	74	45.9	181	59.1

Appendix 5: continued

gene / gene region	***trn*L**		***rps*4**		***rps*4-*trn*S spacer**		**ITS1**		**5.8S**		**ITS2**	
Sematophyllaceae 129	396	27.5	559	27.8	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Sematophyllum homomallum	423	28.9	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	83	56.6	304	72.4
Squamidium brasiliense	416	31.5	567	28.2	31	13.0	n. d.	n. d.	83	56.6	323	70.3
Stereophyllum radiculosum	415	32.3	592	26.9	62	21.0	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Struckia zerovii	406	33.2	592	28.2	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Taxiphyllum taxirameum	421	32.1	591	27.4	n. d.	n. d.	290	64.5	156	51.2	261	66.7
Trachyloma planifolium	458	29.0	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	83	56.6	290	71.4
Tripterocladium leucocladulum	417	31.6	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	83	55.4	279	64.2
Weymouthia cochlearifolia	416	32.2	587	27.4	37	10.8	247	62.3	156	51.2	278	62.6
Weymouthia mollis	416	32.2	580	27.0	54	14.9	249	63.8	156	51.2	282	64.2
Zelometeorium patulum	416	31.5	589	28.5	29	13.8	293	65.5	156	51.9	288	70.5
Average	409.3	31.0	578.6	27.5	53.93	19.6	258.4	63.0	36.1	53.9	278.1	65.6
Standard deviation	34.2	1.4	23.0	1.0	13.61	6.5	16.5	1.1	110.4	2.7	32.7	2.9

Appendix 6: Lepyrodon species considered in this study. Species names, voucher information and the herbarium where the voucher is deposited are listed. Fourteen specimens were successfully sequenced. Accession numbers of the successfully sequenced specimens are listed in Appendix 1 in alphabetical order.

No.	taxon	country of origin	collection locality	habitat	altitude	grid	decimal	voucher label	herbarium
33	Lepyrodon lagurus *(Hook.) Mitt.*	Chile	XII. Región; Prov. Magallanes, R.N. Lago Parrillar, 50 km S of Punta Arenas	epiphytic	270 m	71° 15' 44'' W, 53° 24' 25'' S	-71.262, -53.407	BryoAustral Rolf Blöcher no. 90 det. Bruce Allen 01/2003	J.-P. Frahm, Bonn
64	Lepyrodon tomentosus *(Hook.) Mitt.*	Chile	IX. Región; P.N. Conquillio; path from Laguna Conquillio to Sierra Nevada	epiphytic	1565 m	71° 37' 9.5'' W, 38° 39' 2.3'' S	-71.619, -38.651	BryoAustral Rolf Blöcher no. 74 det. Bruce Allen 01/2003	J.-P. Frahm, Bonn
66	Lepyrodon lagurus *(Hook.) Mitt.*	Chile	IX. Región; P.N. Conquillio; path from Laguna Conquillio to Sierra Nevada	epiphytic	1420 m	71° 37' 9.5'' W, 38° 39' 2.3'' S	-71.619, -38.651	BryoAustral Rolf Blöcher no. 82 det. Bruce Allen 01/2003	J.-P. Frahm, Bonn
67	Lepyrodon pseudolagurus *(Hook.) Mitt. [originally labelled* Lepyrodon lagurus *(Hook.) Mitt.]*	NZ	South Island: Haast Pass	epiphytic	775 m	169° 21' E 44° 07' S	169.35, -44.117	BryoAustral J.-P. Frahm No. 10-12	J.-P. Frahm, Bonn
83	Lepyrodon australis *Hpe ex Broth.*	NZ	South Island: Track between Peel Ridge and Cobb Valley, North West Nelson Forest Reserve, 32 km W of Motueka	epiphytic	1090 m	172° 37' E, 41° 08' S	172.617, -41.133	Musci Australasiae Exsiccati H. Streimann 51277 det. J.Beever, 07/1993	J.-P. Frahm, Bonn
84	Lepyrodon patagonicus *(Card. & Broth.) Allen [orig. labelled* Lepyrodon implexus *(Kze.) Paris]*	Chile	Prov. de Cautin, Temuco, Cerro Ñielol	epiphytic	250 m	72° 35' W, 38° 43'S	-72.583, -38.717	Plantae Chilenensis H. Roivainen 2934 det. Bruce Allen 1995	Berlin
85	Lepyrodon parvulus *Mitt.*	Chile	Prov. de Cautin, Temuco, Cerro Ñielol	epiphytic	220 m	72° 35' W, 38° 43'S	-72.583, -38.717	Plantae Chilenensis H. Roivainen 3129 det. Bruce Allen 1995	Berlin
106	Lepyrodon hexastichus *(Mont.) Wijk &Marg.*	Chile	X. Región, R.N. de Llanquihue, 50 km WSW Puert Montt, Sector Rio Blanco, path to Calbuco volcano	epiphytic	Relevé no. 138	72° 38' 7.4'' W, 41° 20' 41.3'' S	-72.635, -41.345	BryoAustral Rolf Blöcher no. 77 det. Bruce Allen 01/2003	J.-P. Frahm, Bonn
107	Lepyrodon hexastichus	Chile	X. Región, P.N. Puyehue, 50 km E of Osorno, Sector Antillanca, Sendero El Pionero	epiphytic	610 m	72º 18' 53.3'' W, 40° 44' 15.9'' S	-72.315, -40.738	BryoAustral Rolf Blöcher no. 87 det. Bruce Allen 01/2003	J.-P. Frahm, Bonn

Appendix 6: continued

No.	taxon	country of origin	collection locality	habitat	altitude	grid	decimal	voucher label	herbarium
112	Lepyrodon pseudolagurus *(Hook.) Mitt. [originally labelled* Lepyrodon lagurus *(Hook.) Mitt.]*	NZ	South Island: Flora Saddle-Mt Arthur Hut track, North West Nelson Forest Reserve, 25 km SSW of Motueka	epiphytic	950 m	172° 44' E, 41° 11' S	172.733, -41.183	Musci Australasiae Exsiccati H. Streimann 51045 det. H. Streimann	J.-P. Frahm, Bonn
113	*Lepyrodon tomentosus* (Hook.) Mitt. [originally labelled *Lepyrodon lagurus* (Hook.) Mitt.]	Mexico	Prov. Veracruz, near the pass "Porto de Aire", 10 km from Acultzingo	epiphytic	2300 m	97° 19' W, 18° 43' N (Acultzingo)	-97.317, 18.717	Düll 2/248	J.-P. Frahm, Bonn
207	*Lepyrodon australis* Hpe ex Broth.	NZ	South Island: Flora Saddle-Mt Arthur Track, North West Nelson Forest Reserve, 25 km SSW of Motueka	epiphytic	950 m	172° 44' E, 41° 11' S	172.733, -41.183	H. Streimann 58133	Bot. Mus. Helsinki, Finland
208	*Lepyrodon hexastichus* (Mont.) Wijk &Marg.	Chile	Prov. Valdivia, near south shore of Lago Riñihue, 8.2 km by road east of Riñihue	epiphytic	150 m	72° 22' W, 39° 49' S	-72.367, -39.817	Marshall R. Crosby 11,631 det. B. H. Allen 1985	Leiden, Nat. Herb. Netherlands
214	Lepyrodon tomentosus *(Hook.) Mitt.*	Costa Rica	Prov. San José, Cordillera de Talamanca, not far from the Panamercian Highway, near pass Asunción	epiphytic	3300 m	83° 44' W, 09° 34' N (Cerro La Asunción)	-83.733, 09.567	J. Eggers CR 6,17	J.-P. Frahm, Bonn
65	*Lepyrodon tomentosus* (Hook.) Mitt.	Chile	X. Región, P.N. Puyehue, 50 km E of Osorno, Sector Antillanca, near Centro de Ski	epiphytic, *Nothofagus* forest	ca. 1100 m	72° 18' 53.3'' W , 40° 44' 15.9'' S		BryoAustral leg. Rolf Blöcher det. Bruce Allen 01/03 No. 75	J.-P. Frahm, Bonn
79	*Lepyrodon lagurus* (Hook.) Mitt.	Chile	XII. Región, Prov. Magallanes, R.N. Lago Parrillar, 50 km S of Punta Arenas	epiphytic, *Nothofagus* forest	270 m	71° 15' 44'' W, 53° 24' 25'' S		BryoAustral leg. Rolf Blöcher det. Bruce Allen 01/03 No. 89	J.-P. Frahm, Bonn
108	*Lepyrodon tomentosus* (Hook.) Mitt.	Peru	Dep. Ancash, Cordillera Blanca, P.N. Huascaran, Laguna Llanganuco	meadows and rock	3850 m			J.-P. Frahm 29.9.1982 (31) 823984	J.-P. Frahm, Bonn
109	*Lepyrodon tomentosus* (Hook.) Mitt.	Honduras	Lempira Department, Montana de Celaque, Filo Seco, 13 km SW of Gracias	epiphytic	2700-2730 m	88° 41' W, 14° 32' N		Mosses of Honduras Bruce Allen 12086	J.-P. Frahm, Bonn
159	Lepyrodon hexastichus *(Mont.) Wijk &Marg.*	Chile	Juan Fernández Islands, Cordon E of Yunque	forest floor	500 m			Flora von Juan Fernández (Chile) leg. G. Kunkel det. Bruce Allen No. 312/6	Berlin

Appendix 6: continued

No.	taxon	country of origin	collection locality	habitat	altitude	grid	decimal	voucher label	herbarium
160	*Lepyrodon parvulus* Mitt.	Chile	Juan Fernández Islands, Quebrada E of Plazoleta	epiphytic	300 m			Flora von Juan Fernández (Chile) leg. G. Kunkel det. Bruce Allen No. 322/15	Berlin
161	*Lepyrodon patagonicus* (Card. & Broth.) Allen	Chile	Juan Fernández Islands, path to Camote	-----	500 m			Flora von Juan Fernández (Chile) leg. G. Kunkel det. Bruce Allen No. 330/8	Berlin
209	*Lepyrodon pseudolagurus* B.H. Allen	NZ	South Island, Canterbury: Craigieburn Range	roots and rocks	1200 m			det. I. Froehlich (*L. lagurus*) revised Bruce Allen 1995	Leiden, Nat. Herb. Netherlands
210	*Lepyrodon patagonicus* (Card. & Broth.) Allen	Chile	Juan Fernández Islands, path to Camote	-----	500 m			Flora von Juan Fernández (Chile) leg. G. Kunkel det. Bruce Allen, 1995 No. 330/19	Berlin
211	*Lepyrodon parvulus* Mitt.	Chile	Juan Fernández Islands, Quebrada E of Plazoleta	epiphytic	300 m			Flora von Juan Fernández (Chile) leg. G. Kunkel det. Bruce Allen, 1995 No. 322/15/1	Berlin
212	*Lepyrodon parvulus* Mitt.	Chile	Juan Fernández Islands, path to Camote	epiphytic	350-450 m			Flora von Juan Fernández (Chile) leg. G. Kunkel det. Bruce Allen, 1995 No. 327/5	Berlin
213	*Lepyrodon tomentosus* (Hook.) Mitt.	Brazil	Rio de Janeiro, P.N. Itatiaia, Agulhas Negras	rock fissures	2500 m			Bryophyta Brasiliensis J.-P. Frahm no. 1508	J.-P. Frahm, Bonn

R.N. = Reserva Nacional, Nature Reserve

P.N. = Parque Nacional, National Park

Appendix 7: Sequence lengths [base pairs, bp] and GC-content [%] in the coding (exon) and non-coding (intron) region of the *adk* gene of fourteen *Lepyrodon* specimens and two outgroup taxa. Average sequence lengths and standard deviations are also given. For origin of the data refer tab. xz. Abbreviations: n. d. = no data available. (* partial sequences were excluded when determining the average sequence length).

	adk-intron sequence length [bp]	**GC-content [%]**	**adk-exon sequence length [bp]**	**GC-content [%]**	**1st codon position sequence length [bp]**	**1st codon position GC-content [%]**	**2nd codon position sequence length [bp]**	**2nd codon position GC-content [%]**	**3rd codon position sequence length [bp]**	**3rd codon position GC-content [%]**
A. auriculatum (sp. 78)	461*	63,5	231	52,8	78*	53,8	77*	42,9	76*	61,8
A. hlamydophyllum (sp. 12)	376*	59,8	171	48,5	58*	50	57*	40,3	56*	55,3
L. australis (sp. 83)	553	60,0	312	49	104	51	104	38,5	104	57,7
L. australis (sp. 207)	523	60,4	311	48,9	104	51	103	37,8	104	57,7
L. hexastichus (sp. 107)	537	60,9	309	49,9	103	51,4	103	38,8	103	59,2
L. hexastichus (sp. 106)	384*	60,7	204	44,1	68*	45,6	68*	33,8	68*	52,9
L. hexastichus (sp. 208)	298*	63,4	212	49	71*	49,3	70*	34,3	71*	63,4
L. lagurus (sp. 66)	578	60,4	312	49	104	51	104	38,5	104	57,7
L. lagurus (sp. 33)	562	60,5	311	48,9	104	51	103	37,8	104	57,7
L. parvulus(sp. 85)	554	60,6	311	48,9	104	51	103	37,8	104	57,7
L. patagonicus (sp. 84)	554	60,6	312	49	104	51	104	38,5	104	57,7
L. pseudolagurus (sp. 67)	558	60,2	312	49	104	51	104	38,5	104	57,7
L. pseudolagurus (sp. 112)	556	60,3	310	48,7	104	51	103	37,8	103	57,2
L. tomentosus (sp. 64)	577	60,4	312	49	104	51	104	38,5	104	57,7
L. tomentosus (sp. 214)	556	61,2	311	48,9	104	51	103	37,8	104	57,7
Avg,	555	60,8	311	48,9	104	50,7	103	38,1	104	57,9
S.D.	15.6	1,1	1.0	1,7	0.3	1,7	0.5	2,1	0.4	2,4

Appendix 8: P-distances of the complete data set of ITS1, ITS2 and *adk* gene of the successfully sequenced specimens of *Lepyrodon* including the outgroup, and standard errors. P-distances are shown in the lower left triangle, standard errors in the upper right triangle. Mean p-distances are 0.002 (SE 0.002) for the full dataset including the outgroup and 0.009 (SE 0.001) for the ingroup only. Abbreviations: SE=standard error.

Specimens	sp. 12	sp. 78	sp. 83	sp. 207	sp. 106	sp. 107	sp. 208	sp. 33	sp. 66	sp. 85	sp. 84	sp. 67	sp. 112	sp. 64	sp. 113	sp. 214
Acrocladium chlamydophyllum (sp. 12)		0.004	0.006	0.006	0.007	0.006	0.007	0.007	0.006	0.006	0.006	0.006	0.006	0.006	0.008	0.006
Acrocladium auriculatum (sp. 78)	0.020		0.006	0.006	0.007	0.006	0.008	0.007	0.006	0.006	0.006	0.006	0.006	0.006	0.007	0.006
Lepyrodon australis (sp. 83)	0.045	0.055		0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.002	0.002	0.003	0.003	0.003
Lepyrodon australis (sp. 207)	0.046	0.056	0.001		0.003	0.003	0.003	0.004	0.003	0.003	0.003	0.002	0.002	0.003	0.003	0.003
Lepyrodon hexastichus (sp. 106)	0.048	0.055	0.010	0.011		0.002	0.002	0.003	0.003	0.002	0.003	0.003	0.003	0.002	0.002	0.003
Lepyrodon hexastichus (sp. 107)	0.045	0.051	0.011	0.011	0.005		0.002	0.003	0.003	0.002	0.002	0.003	0.003	0.002	0.000	0.002
Lepyrodon hexastichus (sp. 208)	0.046	0.061	0.012	0.013	0.004	0.004		0.004	0.003	0.003	0.002	0.003	0.003	0.002	0.002	0.002
Lepyrodon lagurus (sp. 33)	0.051	0.059	0.016	0.017	0.010	0.012	0.013		0.001	0.002	0.002	0.003	0.003	0.003	0.004	0.003
Lepyrodon lagurus (sp. 66)	0.045	0.053	0.016	0.017	0.012	0.012	0.013	0.002		0.002	0.002	0.003	0.003	0.003	0.004	0.003
Lepyrodon parvulus (sp. 85)	0.041	0.049	0.012	0.013	0.007	0.008	0.008	0.006	0.004		0.001	0.003	0.003	0.002	0.000	0.003
Lepyrodon patagonicus (sp. 84)	0.041	0.049	0.012	0.013	0.008	0.009	0.007	0.007	0.005	0.001		0.003	0.003	0.002	0.002	0.003
Lepyrodon pseudolagurus (sp. 67)	0.046	0.055	0.006	0.007	0.011	0.013	0.013	0.015	0.016	0.012	0.012		0.001	0.003	0.004	0.003
Lepyrodon pseudolagurus (sp. 112)	0.045	0.054	0.005	0.006	0.010	0.012	0.012	0.015	0.016	0.012	0.012	0.001		0.003	0.003	0.003
Lepyrodon tomentosus (sp. 64)	0.044	0.050	0.012	0.012	0.003	0.007	0.005	0.012	0.012	0.008	0.009	0.012	0.012		0.000	0.002
Lepyrodon tomentosus (sp. 113)	0.037	0.029	0.005	0.006	0.002	0.000	0.002	0.009	0.008	0.000	0.002	0.008	0.006	0.000		0.002
Lepyrodon tomentosus (sp. 214)	0.047	0.053	0.014	0.015	0.008	0.009	0.006	0.015	0.014	0.010	0.011	0.015	0.014	0.008	0.002	

Appendix 9: P-distances of the *adk* intron of the successfully sequenced specimens of *Lepyrodon* including the outgroup, and standard errors. P-distances are shown in the lower left triangle, standard errors in the upper right triangle. Mean p-distances are 0.034 (SE 0.004) for the full dataset including the outgroup. and 0.019 (SE 0.004) for the ingroup only.

specimens	sp. 12	sp. 78	sp. 83	sp. 207	sp. 106	sp. 107	sp. 208	sp. 33	sp. 66	sp. 85	sp. 84	sp. 67	sp. 112	sp. 64	sp. 214
Acrocladium chlamydophyllum (sp. 12)		0.009	0.013	0.013	0.016	0.013	0.025	0.012	0.012	0.012	0.012	0.013	0.013	0.013	0.013
Acrocladium auriculatum (sp. 78)	0.033		0.013	0.014	0.016	0.013	0.024	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.014
Lepyrodon australis (sp. 83)	0.065	0.088		0.000	0.007	0.006	0.010	0.006	0.006	0.006	0.006	0.003	0.003	0.006	0.007
Lepyrodon australis (sp. 207)	0.067	0.092	0.000		0.007	0.006	0.010	0.007	0.007	0.007	0.007	0.003	0.003	0.006	0.008
Lepyrodon hexastichus (sp. 106)	0.078	0.106	0.021	0.021		0.005	0.008	0.006	0.007	0.007	0.007	0.007	0.007	0.004	0.007
Lepyrodon hexastichus (sp. 107)	0.063	0.087	0.021	0.019	0.011		0.007	0.006	0.006	0.006	0.006	0.006	0.006	0.005	0.006
Lepyrodon hexastichus (sp. 208)	0.093	0.152	0.030	0.030	0.012	0.013		0.009	0.009	0.009	0.009	0.009	0.009	0.006	0.007
Lepyrodon lagurus (sp. 33)	0.059	0.083	0.022	0.023	0.016	0.019	0.023		0.003	0.003	0.003	0.006	0.006	0.006	0.007
Lepyrodon lagurus (sp. 66)	0.054	0.083	0.024	0.025	0.021	0.020	0.027	0.005		0.000	0.000	0.006	0.006	0.006	0.007
Lepyrodon parvulus (sp. 85)	0.054	0.084	0.024	0.025	0.021	0.021	0.027	0.005	0.000		0.000	0.006	0.006	0.006	0.007
Lepyrodon patagonicus (sp. 84)	0.054	0.084	0.024	0.025	0.021	0.021	0.027	0.005	0.000	0.000		0.006	0.006	0.006	0.007
Lepyrodon pseudolagurus (sp. 67)	0.064	0.088	0.005	0.006	0.018	0.020	0.027	0.020	0.022	0.022	0.022		0.000	0.006	0.007
Lepyrodon pseudolagurus (sp. 112)	0.064	0.088	0.005	0.006	0.018	0.020	0.027	0.020	0.022	0.022	0.022	0.000		0.006	0.007
Lepyrodon tomentosus (sp. 64)	0.065	0.086	0.020	0.019	0.005	0.015	0.010	0.020	0.021	0.020	0.020	0.018	0.018		0.005
Lepyrodon tomentosus (sp. 214)	0.070	0.092	0.029	0.031	0.021	0.022	0.017	0.025	0.027	0.027	0.027	0.027	0.027	0.016	

Appendix 10: Acrocladium species considered in this study. Species names, voucher information and the herbarium where the voucher is deposited are listed. Nine specimens were successfully sequenced. Accession numbers of the successfully sequenced specimens are listed in Appendix 1 in alphabetical order.

No.	taxon	country of origin	collection locality	habitat	altitude	grid	decimal	Voucher label	herbarium
12	Acrocladium chlamydophyllum *(Hook.f. & Wilson) Muell. Hal. & Broth.*	NZ	South Island, Nelson Lakes National Park, St. Arnaud, St. Arnaud Range track		800 m	41° 49' S, 172° 52' E	172.867, -41.817	BRYO AUSTRAL W. Frey 98-T154 B	W. Frey, Berlin
78	Acrocladium auriculatum *(Mont.) Mitt.*	Chile	X. Región, P.N. Puyehue, 50 km E of Osorno, Sector Antillanca, above Lago El Toro	epiphytic	750 m	40° 44' 15.9'' S, 72° 18' 53.3'' W	-72.315, -40.738	Rolf Blöcher No. 49	J.-P. Frahm, Bonn
162	Acrocladium chlamydophyllum *(Hook.f. & Wilson) Muell. Hal. & Broth.*	Australia	Macquarie Island, NW side of Green Gorge, 150 m W of lake	wet grassland		54° 30' S, 158° 57' E	158.95, -54.5	R. D. Seppelt 15801	J.-P. Frahm, Bonn
165	Acrocladium auriculatum *(Mont.) Mitt.*	Argentina	Prov. Santa Cruz, 80 km WNW Calafate, P.N. Los Glaciares, Lago Argentino near Onelli-Gletscher	*Nothofagus* forest	220 m		-73.30, -50.03,	J. Eggers ARG 1/3	J.-P. Frahm, Bonn
171	Acrocladium *cf.* chlamydophyllum *(Hook.f. & Wilson) Muell. Hal. & Broth.*	NZ	South Island, Milford Track, Glade House	forest floor, on soil and rotten wood	200 m		167.91 -44.91	Ben O. van Zanten 00 11 376	B. O. v. Zanten, Groningen, Netherlands
178	Acrocladium *cf.* chlamydophyllum *(Hook.f. & Wilson) Muell. Hal. & Broth.*	Australia	New South Wales, Kosciusko National Park, Wilson's Valley	on stones along creek; shade, rather dry, gully in sclerophyll forest	approx. 1200 m	36° 30' S, 148° 16' E (central coordinates of Kosciusko National Park)	148.27, -36.50	Ben O. van Zanten 82.02.812A	B. O. v. Zanten, Groningen, Netherlands
185	Acrocladium auriculatum *(Mont.) Mitt.*	Chile	X. Región, Cordillera Pelada, S Valdivia, road from La Union to Puiculla	forest floor	approx. 800 m	40° 10' 13.4'' S, 73° 27' 17.2'' W	-73.455, -40.17	BRYO AUSTRAL Rolf Blöcher no. 261	J.-P. Frahm, Bonn

Appendix 10: continued

No.	taxon	country of origin	collection locality	habitat	altitude	grid	decimal	Voucher label	herbarium
186	Acrocladium auriculatum *(Mont.) Mitt.*	Chile	X. Región, P.N. Alerce Andino, approx. 45 km WSW Puerto Montt, path to Laguna Sargazo	evergreen broad-leaf forest	350-400 m	41° 30' 51'' S, 72° 38' 38'' W	-72.644, -41.514	BRYO AUSTRAL Rolf Blöcher no. 50	J.-P. Frahm, Bonn
189	Acrocladium auriculatum *(Mont.) Mitt.*	Chile	XII. Región, P.N. Torres del Paine, 2 km NW Refugio Pingo at Rio Pingo	epiphytic	200 m	51° 06' 28'' S, 73° 06' 28'' W	-73.108, -51.108	BRYO AUSTRAL J.-P. Frahm no. 2-7	J.-P. Frahm, Bonn
163	*Acrocladium chlamydophyllum* (Hook.f. & Wilson) Muell. Hal. & Broth.	Australia	Victoria, Binns Road, Aire River, Otway State Forest, 10 km NW of Apollo Bay	epiphytic	480 m	38° 41' S, 143° 35' E		MUSCI AUSTRALASIAE EXSICCATI H. Streimann 58715	J.-P. Frahm, Bonn
164	*Acrocladium auriculatum* (Mont.) Mitt.	Australia	Tasmania, South of Devonport, King Soloman Cave	on soil and rock		41° 33' S, 146° 15' E		Dale H. Vitt 29371	J.-P. Frahm, Bonn
172	*Acrocladium* cf. *chlamydophyllum* (Hook.f. & Wilson) Muell. Hal. & Broth.	NZ	North Island, Bay of Plenty, Kaingaroa Plantation, forest SE of Rotorva	on soil	600 m			B. O. van Zanten No. 1261	B. O. v. Zanten, Groningen, Netherlands
173	*Acrocladium* cf. *chlamydophyllum* (Hook.f. & Wilson) Muell. Hal. & Broth.	Australia	Tasmania, King Soloman Cave	limestone				H. Ramsay 9-12-1981/2	B. O. v. Zanten, Groningen, Netherlands
174	*Acrocladium* cf. *chlamydophyllum* (Hook.f. & Wilson) Muell. Hal. & Broth.	Australia	N.S.W., Kosciusko N.P., Wilson's Valley	rock	ca. 1200 m			B. O. van Zanten No. 82.02.819	B. O. v. Zanten, Groningen, Netherlands
175	*Acrocladium* cf. *chlamydophyllum* (Hook.f. & Wilson) Muell. Hal. & Broth.	Australia	Tasmania	*Nothofagus* forest				H. Ramsay No. 40	B. O. v. Zanten, Groningen, Netherlands
176	*Acrocladium* cf. *chlamydophyllum* (Hook.f. & Wilson) Muell. Hal. & Broth.	NZ	North Island, Urevera N.P., near Ngaputaki	on bark				B. O. van Zanten No. 82.02.244	B. O. v. Zanten, Groningen, Netherlands
177	*Acrocladium* cf. *chlamydophyllum* (Hook.f. & Wilson) Muell. Hal. & Broth.	NZ	North Island, Taranaki, Mt. Egmont N.P. above Dawson Falls, Tourist Lodge	on branchlets on forest floor				B. O. van Zanten No. 82.02.170	B. O. v. Zanten, Groningen, Netherlands
179	*Acrocladium* cf. *chlamydophyllum* (Hook.f. & Wilson) Muell. Hal. & Broth.	NZ	South Island, Jack's Blowhole, ca. 60 km E of Invercargill along coast near Owaka	on rotten wood	ca. 100 m			B. O. van Zanten No. 00.11.155	B. O. v. Zanten, Groningen, Netherlands

Appendix 10: continued

No.	taxon	country of origin	collection locality	habitat	altitude	grid	decimal	Voucher label	herbarium
180	*Acrocladium* cf. *auriculatum* (Mont.) Mitt.	Chile	Isla Navarino, near Puerto Williams, Camina a la Cascada	on stones and litter on forest floor				B. O. van Zanten No. 86.01.147	B. O. v. Zanten, Groningen, Netherlands
181	*Acrocladium* cf. *auriculatum* (Mont.) Mitt.	Argentina	Tierra del Fuego, above Ushuaia	*Nothofagus* forest	ca. 200 m			R. Krisai 5-1-1990/5	B. O. v. Zanten, Groningen, Netherlands
182	*Acrocladium* cf. *auriculatum* (Mont.) Mitt.		Marion Island, Black Haglett River near Kildalkey campsite	on soil	70 m			N. J. M. Gremmen 02.03	B. O. v. Zanten, Groningen, Netherlands
183	*Acrocladium* cf. *auriculatum* (Mont.) Mitt.	Chile	Patagonia, Laguna Parrillar, ca. 50 km S of Punta Arenas	*Nothofagus* forest	300 m			B. O. van Zanten No. 86.01.674	B. O. v. Zanten, Groningen, Netherlands
184	*Acrocladium* cf. *auriculatum* (Mont.) Mitt.	Chile	Puerto Montt area, Lago Todos los Santos, forest Cayutué	rotten wood on forest floor	200-250 m			B. O. van Zanten No. 79.01.489	B. O. v. Zanten, Groningen, Netherlands
187	*Acrocladium auriculatum* (Mont.) Mitt.	Chile	XII. Región, Prov. Magallanes, Punta Arenas, Reserva Forestal Magallanes	*Nothofagus* forest		53° 09' 10'' S, 71° 01' 34.9'' W		J.-P. Frahm No. 1-12	J.-P. Frahm, Bonn
188	*Acrocladium auriculatum* (Mont.) Mitt.	Chile	XII. Región, Prov. Magallanes, Punta Arenas, Reserva Forestal Magallanes	*Nothofagus* forest		53° 09' 10'' S, 71° 01' 34.9'' W		J.-P. Frahm No. 1-11	J.-P. Frahm, Bonn

R.N. = Reserva Nacional, Nature Reserve

P.N. = Parque Nacional, National Park

N.P. = National Park

Appendix 11: P-distances of the *trn*L intron of the successfully sequenced specimens of *Acrocladium* including the outgroup, and standard errors. P-distances are shown in the lower left triangle, standard errors in the upper right triangle. The mean p-distance for the full dataset including the outgroup is 0.023 (SE 0.004). The mean p-distance for dataset comprising only the eight taxa of *Acrocladium* is 0.008 (SE 0.004). Abbreviations: *A.=Acrocladium*, *H.=Herzogiella*, *L.=Lepyrodon*, *P.=Plagiothecium*, *T.=Taxiphyllum*, *A. chlamyd.=Acrocladium chlamydophyllum*,

Specimens	sp.120	*P.und.*	*P.den.*	sp.117	sp.64	sp.67	sp.12	sp.171	sp.162	sp.165	sp.78	sp.185	sp.186	sp.189
H. seligeri (sp.120)		0.012	0.009	0.010	0.010	0.010	0.008	0.008	0.008	0.009	0.010	0.009	0.009	0.009
P. undulatum	0.038		0.009	0.013	0.012	0.012	0.011	0.011	0.011	0.012	0.012	0.012	0.012	0.012
P. denticulatum	0.029	0.023		0.011	0.010	0.010	0.009	0.009	0.009	0.010	0.010	0.010	0.010	0.010
T. taxirameum (sp.117)	0.032	0.049	0.038		0.011	0.011	0.010	0.010	0.010	0.009	0.010	0.010	0.010	0.010
L. tomentosus (sp.64)	0.035	0.042	0.035	0.041		0.005	0.008	0.008	0.008	0.008	0.009	0.009	0.009	0.009
L. pseudolagurus (sp.67)	0.032	0.038	0.032	0.041	0.010		0.007	0.007	0.007	0.008	0.008	0.008	0.008	0.008
A. chlamyd. (sp.12)	0.019	0.034	0.029	0.032	0.019	0.016		0.000	0.000	0.005	0.006	0.006	0.006	0.006
A. chlamyd. (sp.171)	0.019	0.034	0.029	0.032	0.019	0.016	0.000		0.000	0.005	0.006	0.006	0.006	0.006
A. chlamyd. (sp.162)	0.019	0.034	0.029	0.032	0.019	0.016	0.000	0.000		0.005	0.006	0.006	0.006	0.006
A. auriculatum (sp.165)	0.026	0.038	0.032	0.029	0.022	0.019	0.010	0.010	0.010		0.005	0.005	0.005	0.005
A. auriculatum (sp.78)	0.029	0.042	0.035	0.032	0.026	0.022	0.013	0.013	0.013	0.010		0.000	0.000	0.000
A. auriculatum (sp.185)	0.029	0.042	0.035	0.032	0.025	0.022	0.013	0.013	0.013	0.010	0.000		0.000	0.000
A. auriculatum (sp.186)	0.029	0.042	0.035	0.032	0.025	0.022	0.013	0.013	0.013	0.010	0.000	0.000		0.000
A. auriculatum (sp.189)	0.029	0.042	0.035	0.032	0.025	0.022	0.013	0.013	0.013	0.010	0.000	0.000	0.000	

Appendix 12: P-distances of the ITS1 region of the successfully sequenced specimens of *Acrocladium* including the outgroup, and standard errors. P-distances are shown in the lower left triangle, standard errors in the upper right triangle. The mean p-distance for the full dataset including the outgroup is 0.060 (SE 0.009). The mean p-distance for dataset comprising only the five taxa of Acrocladium is 0.01 (SE 0.005). Abbreviations: *A.*=*Acrocladium*

Specimens	sp. 120	*P.und.*	*P.den.*	sp. 117	sp. 64	sp. 67	sp. 12	sp. 171	sp. 78	sp. 185	sp. 186
Herzogiella seligeri (sp. 120)		0.015	0.015	0.023	0.016	0.017	0.017	0.017	0.017	0.018	0.017
Plagiothecium undulatum	0.056		0.006	0.022	0.013	0.015	0.016	0.016	0.015	0.016	0.015
Plagiothecium denticulatum	0.054	0.008		0.021	0.013	0.014	0.016	0.016	0.015	0.016	0.015
Taxiphyllum taxirameum (sp. 117)	0.137	0.129	0.120		0.021	0.022	0.022	0.022	0.021	0.023	0.021
Lepyrodon tomentosus (sp. 64)	0.062	0.043	0.041	0.112		0.008	0.013	0.013	0.012	0.013	0.012
Lepyrodon pseudolagurus (sp. 67)	0.074	0.055	0.049	0.124	0.016		0.015	0.015	0.014	0.015	0.014
A. chlamydophyllum (sp. 12)	0.074	0.068	0.066	0.129	0.045	0.061		0.000	0.008	0.009	0.008
A. chlamydophyllum (sp. 171)	0.074	0.068	0.066	0.129	0.045	0.061	0.000		0.008	0.009	0.008
A. auriculatum (sp. 78)	0.074	0.059	0.057	0.121	0.037	0.053	0.016	0.016		0.000	0.000
A. auriculatum (sp. 185)	0.078	0.059	0.059	0.130	0.041	0.054	0.017	0.017	0.000		0.000
A. auriculatum (sp. 186)	0.074	0.059	0.057	0.121	0.037	0.053	0.016	0.016	0.000	0.000	

Appendix 13: P-distances of the ITS2 region of the successfully sequenced specimens of *Acrocladium* including the outgroup, and standard errors. P-distances are shown in the lower left triangle, standard errors in the upper right triangle. The mean p-distance for the full dataset including the outgroup is 0.054 (SE 0.009). The mean p-distance for dataset comprising only the five taxa of Acrocladium is 0.013 (SE 0.005). Abbreviations: *A.=Acrocladium*.

Specimens	sp. 120	*P.und.*	*P.den.*	sp. 117	sp. 64	sp. 67	sp. 12	sp. 171	sp. 78	sp. 185	sp. 186
Herzogiella seligeri (sp. 120)		0.013	0.011	0.016	0.015	0.015	0.017	0.017	0.015	0.015	0.015
Plagiothecium undulatum	0.033		0.005	0.021	0.018	0.018	0.017	0.017	0.017	0.017	0.017
Plagiothecium denticulatum	0.036	0.005		0.019	0.017	0.017	0.019	0.019	0.018	0.018	0.018
Taxiphyllum taxirameum (sp. 117)	0.073	0.090	0.096		0.020	0.020	0.022	0.022	0.021	0.021	0.021
Lepyrodon tomentosus (sp. 64)	0.064	0.057	0.076	0.090		0.005	0.014	0.014	0.012	0.012	0.012
Lepyrodon pseudolagurus (sp. 67)	0.068	0.057	0.081	0.089	0.008		0.014	0.014	0.012	0.012	0.012
A. chlamydophyllum (sp. 12)	0.069	0.052	0.083	0.108	0.053	0.052		0.000	0.009	0.009	0.009
A. chlamydophyllum (sp. 171)	0.069	0.052	0.083	0.108	0.052	0.052	0.000		0.009	0.009	0.009
A. auriculatum (sp. 78)	0.064	0.052	0.078	0.099	0.035	0.035	0.021	0.021		0.000	0.000
A. auriculatum (sp. 185)	0.064	0.052	0.078	0.099	0.035	0.035	0.021	0.021	0.000		0.000
A. auriculatum (sp. 186)	0.064	0.052	0.078	0.099	0.035	0.035	0.021	0.021	0.000	0.000	

Appendix 14: P-distances of the *rps*4 gene of the successfully sequenced specimens of *Acrocladium* including the outgroup, and standard errors. P-distances are shown in the lower left triangle, standard errors in the upper right triangle. The mean p-distance for the full dataset including the outgroup is 0.027 (SE 0.004).

Specimens	sp. 120	*P.und.*	*P.den.*	sp. 117	sp. 64	sp. 67	sp. 12	sp. 78
Herzogiella seligeri (sp. 120)	0.007	0.007	0.008	0.008	0.008	0.008	0.008	
Plagiothecium undulatum	0.035		0.002	0.007	0.007	0.007	0.006	0.006
Plagiothecium denticulatum	0.032	0.002		0.007	0.007	0.007	0.006	0.007
Taxiphyllum taxirameum (sp. 117)	0.042	0.028	0.028		0.008	0.007	0.006	0.007
Lepyrodon tomentosus (sp. 64)	0.044	0.028	0.03	0.033		0.004	0.006	0.006
Lepyrodon pseudolagurus (sp. 67)	0.044	0.032	0.033	0.033	0.009		0.006	0.006
Acrocladium chlamydophyllum (sp. 12)	0.039	0.021	0.021	0.025	0.02	0.025		0.003
Acrocladium auriculatum (sp. 78)	0.041	0.022	0.023	0.027	0.02	0.025	0.007	

Appendix 15: *Catagonium* species considered in this study. Species names, voucher information and the herbarium where the voucher is deposited are listed. thirteen specimens were successfully sequenced. Accession numbers of the successfully sequenced specimens are listed in Appendix 1 in alphabetical order.

No.	taxon	Country/island of origin	collection locality	habitat	altitude	grid	Voucher label	herbarium
21	*Catagonium nitens* (Brid.) Card. ssp. *nitens*	Chile	Reg. Magallanes , NW Punta Arenas, Reserva Forestal Magallanes	*Nothofagus pumilio*- forest	350-430 m.	53° 09´ 10´´ S, 71° 01´ 34.9´´ W	Rolf Blöcher No. 1/14.2.01	J.-P. Frahm, Bonn
23	Catagonium nitens *(Brid.) Cardot cf. ssp.* nitens	New Zealand	South Island, Nelson Lakes National Park, St. Arnaud, St. Arnaud Range track	*Nothofagus fusca* forest, in cave	800 m	41° 49' S, 172° 52' E	BRYO AUSTRAL J.-P. Frahm no. 27-8	J.-P. Frahm, Bonn
25	Catagonium nitens *(Brid.) Card. var.* myurum *(Card. & Thér.) Lin*	Chile	X. Región, P.N. Villarica, volcano Villarica, S Pucón, road to skiing area	on soil	1420 m	39° 23' 50.3'' S, 71° 58' 3.9'' W	BRYO AUSTRAL W. Frey & F. Schaumann no. 01-223	W. Frey, Berlin
59	Catagonium nitens *(Brid.) Card. ssp.* maritimum *(Hook.) Lin*	South Africa	Cape Prov., near Fairy Knowe Railway Station	on rock wall		34° 03' S, 23° 03' E (Knysna)	S. M. Perold 936	Helsinki, Finland
61	Catagonium emarginatum *Lin*	Brazil	Minas Gerais, Mt. Itatiaia N.P., rain forest at Brejo da Lapa	on soil	2130 m	22° 22' S, 44° 41' W	leg. A. Schäfer-Verwimp det. A. Schäfer-Verwimp & B. H. Allen 11193	Helsinki, Finland
63	*Catagonium brevicaudatum* C. Müll. ex Broth.	Columbia	Departamento de Cundinamarca, Municipio de El Charquito, Salto del Tequendama, Portero al lado del Río Bogotá	rock	2420 m	ca. 04° 34' N, 74° 17' W	Flora de Colombia Edgar Linares C. & Steven Churchill 3821	Helsinki
80	Catagonium nitidum *(Hook. f. & Wilson) Broth.*	Argentina	Falkland Islands, Weddell Island, rock dome on summit of peak NE of Mt. Weddell	on vegetation hanging over rock	approx. 350 m	UTM Grid 21F TC 2941	John J. Engel no. 3368 det. S. H. Lin 1981	Bot. Mus. Berlin
91	Catagonium nitens *(Brid.) Card. ssp.* maritimum *(Hook.) Lin*	South Africa	Cape Prov., Gouna Forest Reserve, N of Knysna	on earthwall next to road		33° 58' S, 23° 02' E (Gouna Forest Station)	S. M. Perold 902 det. R. E. Magill 1988	Helsinki, Finland

Appendix 15: continued

No.	taxon	Country/island of origin	collection locality	habitat	altitude	grid	Voucher label	herbarium
92	Catagonium brevicaudatum *C. Müll. ex Broth.*	Columbia	Department of Caldas, municipality Villamaria, road from Manizales to Bogotá		3920 m	04° 55' N, 75° 21' W	Steven P. Churchill, Alba Luz Arbeláez, Wilson Rengifo no. 16297	Helsinki, Finland
236	Catagonium nitidum *(Hook. f. & Wilson) Broth.*	Chile	P.N. Torres del Paine, eastern border of 'Glaciar Grey' at Campamento Paso	acidic rock	approx. 600 m	50° 57' S, 73° 15' W	Frank Müller C 1501	Frank Müller, Dresden
287	*Catagonium nitens* (Brid.) Card. ssp. *nitens*	Australia	Victoria, Tarra National Park, 27 km S of Traralgon	*Nothofagus* roots ans track cutting	450 m	38° 27' S, 146° 32' E	MUSCI AUSTRALASIAE EXSICCATI H. Streimann 50457	J.-P. Frahm, Bonn
288	*Catagonium nitens* (Brid.) Cardot cf. ssp.*nitens*	Chile	X. Región de los Lagos, Osorno, between Lagos, Parque Nacional Puyehue Salto del Indio, Salto de la Princesa, RN 215	Trail in primary forest, waterfalls, rocks, small cave		40° 40' 7.3'' S, 72° 10' 20.1'' W	Holz & Franzaring CH 00-152 det. W. R. Buck	J.-P. Frahm, Bonn
289	*Catagonium nitens* (Brid.) Card. ssp. *nitens*	Chile	IX. Región, P.N. Conquillio, path from Laguna Conquillio to Sierra Nevada	on soil	1200-1400 m	38° 39' 2.3'' S, 71° 37' 9.5'' W	BRYO AUSTRAL Rolf Blöcher no. 46	J.-P. Frahm, Bonn
18	*Catagonium brevicaudatum* C. Müll. ex Broth.	Venezuela	Mérida, Teleférico, Loma Redonda	rock fissures	4100 m		J.-P. Frahm febuary 1997	J.-P. Frahm, Bonn
19	*Catagonium emarginatum* Lin	Bolivia	Departmento La Paz, Prov. Inquisivi, Quime-Molinos road, 3 km W of Quime, waterfalls 'Cascadas de Naranjani'	humus on dirt bank	3490-3570 m	16° 39' S, 67° 14' W	Marko Lewis 87635	J.-P. Frahm, Bonn
20	*Catagonium nitidum* (Hook.fil. & Wils.) Broth.	Argentina	Tierra del Fuego, Bahía buen Suceso, slope south of Monte Béccar	*Nothofagus* forest	200-300 m	54° 47' S, 65° 15' W	leg. Matteri-Schiavone det. Matteri/86 CM no. 3622	J.-P. Frahm, Bonn
22	*Catagonium nitens* (Brid.) Card. ssp. *nitens*	Tanzania	S-Uluguru Mts. Kilangala, top of the main ridge SE of Bunduki	epiphytic, on tree fern stem	1750-1950 m		Flora of Tanzania leg. T. Pócs & P. Mwanjabe det. T. Pócs 6464/BI	J.-P. Frahm, Bonn
24	*Catagonium nitens* (Brid.) Card. ssp. *maritimum* (Hook.) Lin	South Africa	Cape: Diep River picnic area, N of Buffels Neck Forest Station, on hills above road, just N of Kruis Valley	dry forest		grid ref. 3323 CC	South Africa R.E. Magill 5979	J.-P. Frahm, Bonn

Appendix 15 continued

No.	taxon	Country/island of origin	collection locality	habitat	altitude	grid	Voucher label	herbarium
92	*Catagonium brevicaudatum* C. Müll. ex Broth.	Columbia	Departamento de Caldas, Municipio de Villamaria, road Manizales-Bogotá, near the road leading to Nevado del Ruiz (km 213), wasteland	on the embankment	3920 m	ca. 4° 55' N, 75° 21' W	Flora de Colombia Steven P. Churchill, Alba Luz Arbeláez, Wilson Rengifo no. 16297	Helsinki
93	*Catagonium emarginatum* Lin	Peru	between Marcapata and Achubamba, Prov. Quispicanchis, Dept. Cuzco	on moist rocks	ca. 2700 m		Bryophyta Selecta Exsiccata leg. H. Inoue det. H. Deguchi (*C. nitidum*) revised Shan-Hsiung Lin 1989 no. 931	Helsinki
94	*Catagonium nitens* (Brid.) Card.	Tanzania	University Forest Reserve of Mazumbai, West Usambara Mts.	on moist soil	1620 m		Bryophyta Selecta Exsiccata leg. T. Pócs, E. W. Jones & Mrs. Tanner det. T. Pócs 629	Berlin

R.N. = Reserva Nacional (Nature Reserve); P.N. = Parque Nacional (National Park) ; N.P. = National Park

Appendix 16: P-distances of the ITS1 region of the successfully sequenced specimens of *Catagonium* including the outgroup, and standard errors. P-distances are shown in the lower left triangle, standard errors in the upper right triangle. The mean p-distance for the full dataset including the outgroup is 0.034 (SE 0.006). The mean p-distance for dataset comprising only the taxa of *Catagonium* is 0.016 (SE 0.005).Abbreviations: *Acro.=Acrocladium*, *Cat.=Catagonium*, *Lep.=Lepyrodon*

Specimens	sp. 67	sp. 64	sp. 12	sp. 78	*P.und.*	*P.den.*	*I.mue.*	H.sel.	sp. 92	sp. 63	sp. 61	sp. 91	sp. 59	sp. 289	sp. 21	sp. 288	sp. 287	sp. 23	sp. 25	sp. 236	sp. 80
Lep. pseudolagurus (sp. 67)		0.008	0.015	0.014	0.015	0.014	0.015	0.017	0.014	0.014	0.014	0.015	0.015	0.013	0.012	0.013	0.013	0.014	0.014	0.013	0.014
Lep. tomentosus (sp. 64)	0.016		0.013	0.012	0.013	0.013	0.013	0.016	0.012	0.012	0.011	0.013	0.013	0.011	0.010	0.011	0.011	0.011	0.011	0.011	0.012
Acro. chlamydophyllum (sp. 12)	0.061	0.045		0.008	0.016	0.016	0.016	0.017	0.015	0.015	0.015	0.016	0.016	0.014	0.014	0.014	0.014	0.015	0.015	0.014	0.015
Acro. auriculatum (sp. 78)	0.053	0.037	0.016		0.015	0.015	0.015	0.017	0.014	0.014	0.014	0.015	0.015	0.013	0.013	0.013	0.013	0.014	0.014	0.013	0.014
Plagiothecium undulatum	0.055	0.043	0.068	0.059		0.006	0.013	0.015	0.013	0.013	0.013	0.013	0.013	0.010	0.011	0.010	0.012	0.013	0.011	0.010	0.012
Plagiothecium denticulatum	0.049	0.041	0.066	0.057	0.008		0.012	0.015	0.013	0.013	0.012	0.013	0.013	0.010	0.011	0.010	0.011	0.012	0.011	0.010	0.012
Isopterygiopsis muelleriana	0.061	0.045	0.069	0.061	0.038	0.037		0.016	0.011	0.011	0.011	0.013	0.013	0.010	0.011	0.010	0.011	0.011	0.011	0.010	0.011
Herzogiella seligeri	0.074	0.062	0.074	0.074	0.056	0.054	0.066		0.014	0.014	0.014	0.013	0.013	0.013	0.014	0.013	0.014	0.014	0.013	0.013	0.014
Cat. brevicaudatum (sp. 92)	0.052	0.037	0.057	0.049	0.043	0.041	0.033	0.049		0.000	0.004	0.011	0.011	0.008	0.008	0.008	0.008	0.009	0.009	0.008	0.010
Cat. brevicaudatum (sp. 63)	0.052	0.037	0.057	0.049	0.043	0.041	0.033	0.049	0.000		0.004	0.011	0.011	0.008	0.008	0.008	0.008	0.009	0.009	0.008	0.010
Cat. emarginatum (sp. 61)	0.049	0.033	0.057	0.049	0.038	0.037	0.029	0.053	0.004	0.004		0.011	0.011	0.007	0.007	0.007	0.007	0.008	0.008	0.007	0.009
Cat. nitens (sp. 91)	0.061	0.045	0.069	0.061	0.042	0.041	0.040	0.045	0.028	0.028	0.032		0.000	0.009	0.011	0.009	0.011	0.011	0.010	0.009	0.011
Cat. nitens (sp. 59)	0.061	0.045	0.069	0.061	0.042	0.041	0.040	0.045	0.028	0.028	0.032	0.000		0.009	0.011	0.009	0.011	0.011	0.010	0.009	0.011
Cat. nitens (sp. 289)	0.044	0.028	0.053	0.045	0.026	0.025	0.024	0.041	0.016	0.016	0.012	0.020	0.020		0.006	0.000	0.006	0.007	0.004	0.000	0.006
Cat. nitens (sp. 21)	0.040	0.024	0.049	0.041	0.030	0.029	0.029	0.049	0.016	0.016	0.012	0.028	0.028	0.008		0.006	0.006	0.007	0.007	0.006	0.008
Cat. nitens (sp. 288)	0.044	0.028	0.053	0.045	0.026	0.025	0.024	0.041	0.016	0.016	0.012	0.020	0.020	0.000	0.008		0.006	0.007	0.004	0.000	0.006
Cat. nitens (sp. 287)	0.044	0.028	0.053	0.045	0.034	0.033	0.033	0.049	0.016	0.016	0.012	0.028	0.028	0.008	0.008	0.008		0.007	0.007	0.006	0.008
Cat. nitens (sp. 23)	0.048	0.033	0.057	0.049	0.038	0.037	0.029	0.053	0.020	0.020	0.016	0.032	0.032	0.012	0.012	0.012	0.012		0.008	0.007	0.009
Cat. nitens (sp. 25)	0.048	0.033	0.057	0.049	0.030	0.029	0.029	0.045	0.020	0.020	0.016	0.024	0.024	0.004	0.012	0.004	0.012	0.016		0.004	0.007
Cat. nitidum (sp. 236)	0.044	0.028	0.053	0.045	0.026	0.025	0.024	0.041	0.016	0.016	0.012	0.020	0.020	0.000	0.008	0.000	0.008	0.012	0.004		0.006
Cat. nitidum (sp. 80)	0.053	0.037	0.062	0.053	0.034	0.033	0.033	0.050	0.024	0.024	0.020	0.029	0.029	0.008	0.016	0.008	0.016	0.020	0.012	0.008	

Appendix 17: P-distances of the ITS2 region of the successfully sequenced specimens of *Catagonium* including the outgroup, and standard errors. P-distances are shown in the lower left triangle, standard errors in the upper right triangle. The mean p-distance for the full dataset including the outgroup is 0.050 (SE 0.008). The mean p-distance for dataset comprising only the taxa of *Catagonium* is 0.026 (SE 0.006). Abbreviations: *Acro.=Acrocladium*, *Cat.=Catagonium*

Specimens	sp. 67	sp. 64	sp. 12	sp. 78	*P.und.*	*P.den.*	*I.mue.*	H.sel.	sp. 92	sp. 63	sp. 61	sp. 91	sp. 59	sp. 289	sp. 21	sp. 288	sp. 287	sp. 23	sp. 25	sp. 236
Lepyrodon pseudolagurus (sp. 67)		0.005	0.014	0.012	0.017	0.018	0.015	0.016	0.015	0.016	0.015	0.018	0.018	0.018	0.017	0.018	0.018	0.018	0.017	0.017
Lepyrodon tomentosus (sp. 64)	0.008		0.015	0.012	0.017	0.018	0.015	0.016	0.015	0.016	0.016	0.018	0.018	0.018	0.017	0.018	0.018	0.017	0.017	0.017
Acro. chlamydophyllum (sp. 12)	0.052	0.053		0.009	0.016	0.018	0.017	0.017	0.016	0.017	0.017	0.018	0.018	0.018	0.018	0.018	0.019	0.019	0.018	0.018
Acro. auriculatum (sp. 78)	0.035	0.035	0.021		0.017	0.018	0.017	0.016	0.016	0.016	0.016	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018
Plagiothecium undulatum	0.057	0.057	0.052	0.052		0.005	0.016	0.013	0.013	0.014	0.015	0.016	0.016	0.013	0.014	0.013	0.013	0.013	0.013	0.013
Plagiothecium denticulatum	0.081	0.076	0.082	0.077	0.005		0.014	0.012	0.013	0.014	0.015	0.015	0.015	0.013	0.014	0.013	0.013	0.013	0.013	0.013
Isopterygiopsis muelleriana	0.064	0.064	0.064	0.068	0.044	0.052		0.011	0.014	0.014	0.015	0.016	0.016	0.016	0.017	0.016	0.016	0.016	0.016	0.016
Herzogiella seligeri	0.068	0.064	0.068	0.063	0.033	0.036	0.035		0.012	0.012	0.014	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
Cat. brevicaudatum (sp. 92)	0.061	0.061	0.066	0.062	0.034	0.042	0.053	0.037		0.003	0.007	0.009	0.009	0.009	0.010	0.009	0.010	0.009	0.009	0.009
Cat. brevicaudatum (sp. 63)	0.065	0.065	0.071	0.066	0.04	0.046	0.057	0.041	0.003		0.008	0.010	0.010	0.010	0.011	0.010	0.010	0.010	0.010	0.010
Cat. emarginatum (sp. 61)	0.061	0.069	0.075	0.062	0.04	0.058	0.07	0.057	0.017	0.021		0.011	0.011	0.012	0.012	0.012	0.012	0.012	0.012	0.012
Cat. nitens (sp. 91)	0.085	0.085	0.088	0.084	0.052	0.058	0.066	0.057	0.028	0.031	0.042		0.000	0.009	0.011	0.009	0.011	0.010	0.010	0.010
Cat. nitens (sp. 59)	0.085	0.085	0.088	0.084	0.052	0.058	0.066	0.057	0.028	0.031	0.042	0.000		0.009	0.011	0.009	0.011	0.010	0.010	0.010
Cat. nitens (sp. 289)	0.081	0.081	0.084	0.079	0.034	0.042	0.07	0.057	0.028	0.032	0.046	0.031	0.031		0.008	0.000	0.006	0.006	0.006	0.006
Cat. nitens (sp. 21)	0.082	0.089	0.093	0.088	0.04	0.054	0.082	0.07	0.035	0.039	0.046	0.044	0.044	0.020		0.008	0.008	0.007	0.004	0.004
Cat. nitens (sp. 288)	0.081	0.081	0.084	0.079	0.034	0.042	0.07	0.057	0.028	0.032	0.046	0.031	0.031	0.000	0.020		0.006	0.006	0.006	0.006
Cat. nitens (sp. 287)	0.085	0.085	0.088	0.084	0.034	0.042	0.07	0.057	0.028	0.032	0.046	0.037	0.037	0.014	0.02	0.013		0.003	0.006	0.006
Cat. nitens (sp. 23)	0.081	0.081	0.088	0.084	0.034	0.042	0.07	0.057	0.025	0.028	0.042	0.034	0.034	0.01	0.017	0.010	0.003		0.006	0.006
Cat. nitens (sp. 25)	0.081	0.081	0.088	0.084	0.034	0.046	0.074	0.061	0.028	0.032	0.046	0.037	0.037	0.013	0.007	0.013	0.013	0.010		0.000
Cat. nitidum (sp. 236)	0.081	0.081	0.088	0.084	0.034	0.046	0.074	0.061	0.028	0.032	0.046	0.037	0.037	0.013	0.007	0.013	0.013	0.010	0.000	