MULTIPLICITY OF VIRAL INFECTION IN BROWN ALGAE

by

KIM STEVENS

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Kim Stevens

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Abstract

Brown algae are important primary producers and habitat formers in coastal environments and are believed to have evolved multicellularity independently of the other eukaryotes. The phaeoviruses that infect them form a stable lysogenic relationship with their host via genome integration, but have only been extensively studied in two genera: *Ectocarpus* and *Feldmannia*. In this study I aim to improve our understanding of the genetic diversity, host range and distribution of phaeoviruses.

Sequencing and phylogenetic analysis of amplified fragments of three core phaeoviral genes (encoding major capsid protein (MCP), DNA polymerase and superfamily III helicase) of phaeovirus infected algae confirmed the suspected phaeoviral identity of viruses infecting *E. fasciculatus*, *F. simplex*, *Pilayella littoralis*, *Myriotrichia clavaeformis* and *Hincksia hincksiae*. Furthermore, this approach revealed multiple virus sequence variants within individual strains, and moreover that the variants formed two distinct subgroups. Subgroup A was highly conserved and observed in multiple algal genera, whereas subgroup B was much more diverse, but only found in *Feldmannia* species. Transcriptome sequencing of an actively infected *F. irregularis* strain revealed polymorphisms within key viral genes, suggesting that multiple variants were indeed active within this strain.

High resolution melt curve (HRM) technology was used to develop a high throughput screening method for detecting phaeoviral MCP as a proxy for detection of phaeoviruses. This technique was also able to assign 88% of those detected to one of the subgroups, based on their differing melting temperature distributions. This was then applied to 1034 *Ectocarpus* isolates collected from around Europe and South America, and in accordance with previous studies of phaeoviral infection, 43-79% of strains contain virus sequence (depending on species). 17% of the isolates tested even contained sequence from both subgroups.

82 Laminariales strains, close relatives of the Ectocarpales, were also screened because they comprise commercially important kelp species but are not known to be infected by viruses. 10-17% of these tested positive for phaeoviral MCP, which when sequenced formed a separate group within the phaeoviruses. This finding could have a major impact on the kelp farming industry if the viruses are found to affect reproduction as happens in the Ectocarpales.

The discovery of two subgroups is contrary to current beliefs that the phaeoviruses are a single monophyletic group, and that each species of alga has its own phaeovirus, casting doubt on the usefulness of the current convention of naming each phaeovirus after its host. It appears that the subgroup B viruses have begun to evolve away from the stable, *K*-selected subgroup A viruses towards a more *r*- type strategy with higher mutation and diversification. This study has identified potential mechanisms that may influence this shift, including mutations in a region of the DNA polymerase known to negatively affect DNA replication fidelity, combined with an active integrase and lack of a proofreading exonuclease, along with the observed infection of individuals with both phaeovirusal subgroups. The resulting mutations and recombinations could lead to the diversity observed here, and may provide a suitable model for the study of other emergent virus infections.

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Signed .....

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List of symbols and abbreviations

% Percent

°C Degrees Celsius

µg Microgram

µl Microlitre

µM Micromolar

3'

454 Next generation sequencer manufacturer

5'

A Adenosine

ABI Applied Biosystems (a next generation sequencer manufacturer)

ATPase adenosine triphosphatase

B cells Lymphocyte (white blood cell) with antigen binding receptor

BLAST Basic local alignment search tool

BLASTn Blast search of nucleotide sequence against nucleotide database

BLASTp Blast search of protein sequence against protein database

bp Base (nucleotide) pairs

BRCA1 Human gene involved in breast cancer

BSA Bovine serum albumen

C Cytosine

CCAP Culture collections of algae and protozoa (marine) reference number

$CD14^+$ monocyte Blood monocyte displaying the cluster of differentiation 14 protein

CD4 Helper T cell surface glycoprotein

cDNA Complementary DNA synthesised from mRNA template

CDS Coding sequence

cI Bacteriophage lambda lysogenic maintenance protein

cro Bacteriophage lambda early lytic protein

DNA Deoxyribonucleic acid

DnaJ A chaperone protein also known as HPS40 (heat shock protein of 40kiloDalton weight)

DNA pol DNA polymerase

dsDNA Double-stranded DNA

DWV Deformed wing virus

EBV Epstein-Barr virus

EDTA Ethylenediaminetetraacetic acid

Efas *Ectocarpus fasciculatus*

EfasV-1 *Ectocarpus fasciculatus* virus 1

EhV-86 *Emiliania huxleyi* virus 86

Esil *Ectocarpus siliculosus*

EsV-1 *Ectocarpus siliculosus* virus 1

EsV-1-XXX Refers to the gene number given to EsV-1 genes in the Delaroque 2001 paper[1]

Firr *Feldmannia irregularis*

FirrV-1 *Feldmannia irregularis* virus 1

Flex Feldmannia simplex

Flex8 Subgroup A virus found in *Feldmannia simplex*

FsV-158 *Feldmannia* sp. Virus 158

g Acceleration due to gravity

G Guanine

GC content Proportion of guanine-cytosine to adenine-thymine nucleotides in a sequence

GIY-YIG Protein domain consisting of glycine, isoleucine, tyrosine then a further tyrosine, isoleucine, glycine

gp Viral glycoprotein

H_5N_1 Influenza virus A subtype commonly known as bird flu

HAS *Ectocarpus siliculosus* strain isolated from Hastings

HGT Horizontal gene transfer

Hinc *Hincksia hincksiae*

HincV-1 Hincksia *hincksiae* virus 1

HIV-1 Human immunodeficiency virus 1

HCMV Human cytomegalovirus

HNH Protein motif of two β-sheets surrounded by an α-helix, with a metal binding site in the middle

HRM High resolution melt

HsV-1 Herpes simplex virus

IGV Integrative genomics viewer

K Carrying capacity of the environment in evolutionary model

kb kilobases (1000 base pairs)

kDa Kilodalton

LB Liquid broth

LUCA Last universal common ancestor

m Metre

M13 Plasmid derived from bacteriophage M13 used for cloning

Ma Million years before present time

Mcla *Myriotrichia clavaeformis*

MclaV-1 *Myriotrichia clavaeformis* virus 1

MCP Major capsid protein

mg Milligram

Mg Magnesium

$MgCl_2$ Magnesium chloride

MGE Mobile genetic element

ml Millilitre

mRNA Messenger ribonucleic acid

N Population size in evolutionary model

NCBI National Centre for Biotechnology Information

NCLDV Nuclear-cytoplasmic large DNA virus

ng Nanogram

p Probability

P1 Phage

p450 Respiratory pathway

PBCV-1 *Paramecium bursaria* chlorella virus 1

PCNA Proliferating cell nuclear antigen

PCR Polymerase chain reaction (A DNA amplification technique)

pers. comms. Personal communication

Plit *Pylaiella littoralis*

PlitV-1 *Pylaiella littoralis* virus 1

pmol Picomolar

Pfam Protein family homology search

psbA Gene encoding chlorophyll binding protein

r Growth rate in evolutionary model

R_0 Average number of secondary infections arising from one infected host in an otherwise uninfected population

R696W DNA polymerase mutation in yeast; arginine to tryptophan in position 696

Rad50 DNA repair protein

RAT *Ectocarpus crouaniorum* isolated from Rattray Head

Rec-A *Escherichia coli* recombinatorial repair subunit A

Rec-BCD *Escherichia coli* recombinatorial repair comprising subunits B, C and D

RFC Replication factor

RLT Buffer used in Quaigen RNeasy RNA extraction kit

RNA Ribonucleic acid

RNase Ribonuclease

rt-PCR Real time polymerase chain reaction

s Second

SNP Single nucleotide polymorphism

sp. Species

SV40 Simian virus 40

t Time in evolutionary model

T Thymine

T cell Lymphocyte originating from the thymus gland

TonB Escherichia coli protein involved in outer-membrane receptor protein function

UDP Uridine diphospho-

unEV Unknown *Ectocarpus* virus

unFV Unknown *Feldmannia* virus

unPV Unknown phaeovirus

VAC-D5 Vaccina virus helicase gene

Vp Virus protein

VPU HIV-1 encoded protein

X-gal 5-bromo-4-chloro-3-indolyl-beta-D-galacto-pyranoside

CHAPTER 1 INTRODUCTION

1.1 What is evolution?

The vast diversity of life-forms, from the smallest microbes to the largest mammals, have all appeared as a product of evolution. A collection of gradual changes build up over time in response to environmental pressures, resulting in the huge species richness we see today, as was first proposed by Charles Darwin and Alfred Wallace from observations on Galapagos mockingbirds and the animals of Malaysia and Indonesia, respectively[2]. Evolution centres on the fact that biological replication is not 100% accurate, and mutations lead to slight changes in the offspring of all organisms. Environmental selection pressures may act preferentially on such mutations, either increasing or decreasing that individual's chance of reproductive success. In this manner, beneficial mutations tend to be more likely to be passed to subsequent generations, and deleterious mutations are more likely to be removed from the gene pool by a process commonly referred to as "natural selection", until eventually the mutation becomes fixed and the population is said to have evolved.

1.1.1 Evolutionary mechanisms

Mutations, the driving force of evolution, occur due to a variety of mechanisms such as unrepaired DNA damage[3], errors in replication[4], or the insertion[5] or deletion[6] of DNA by various mobile genetic elements (MGE).

Transposons, or transposable elements, are one example of a mobile genetic element which are capable of insertion into the genome of their hosts. Class 1 transposons, or retrotransposons, have an intermediate RNA stage, usually have long terminal repeats and typically encode reverse transcriptase[7]. Class 2 elements on the other hand transpose via a DNA intermediate. These have inverted terminal repeats and typically encode a transposase that allows them to excise themselves and reinsert elsewhere[7]. The insertion of transposons has been found to not only alter genome and gene structure[8], but also gene regulation and expression[9].

Inteins are fragments of protein which include their own homing endonuclease; they insert into specific sequences in essential host proteins, and are transcribed and translated along with the host protein, excising themselves at the protein level[10]. They are selfish mobile genetic elements which are not beneficial to their host, but neither are they very detrimental since they catalyse their own removal, however, their position in essential host genes makes them very difficult for the host to remove by the usual DNA repair mechanisms[11]. They encode various other genes and they may be a mechanism for genetic exchange between their various hosts.

The age of insertion of any MGE can be estimated by comparing its GC content with its surrounding gene; recent acquisitions tend to be accompanied by different GC content since there would not have been time for mutations to arise to homogenise the DNA composition[11].

The fidelity of DNA polymerase is a crucial factor in ensuring accurate DNA replication, and depends on a combination of polymerase base selectivity, 3' – 5' exonucleolytic proofreading, mismatch correction and DNA damage repair[12]. The loss of either polymerase proofreading capability or mismatch repair causes strong mutator phenotypes which may be caused by as little as one point mutation.

As in evolution, the formation of cancerous tumours depends on mutation and selection. Mutated DNA polymerase with decreased nucleotide specificity and proofreading capability has been proven in mice to increase the mutation rate in studies of tumourigenesis and causes fatal genome instability in yeast[4]. The crucial mutated amino acid in that study (R696W, arginine to tryphtophan in position 696) was not in the base-pair binding pocket which has the main role in fidelity, but in the fingers domain which was postulated to affect the partition between polymerase and proofreading domains.

1.1.2 Evolutionary strategies

One of the early models proposed to explain various evolutionary strategies employed by organisms is that of *r*- and *K*- selection which was developed in the 1960s[13], based on the formula dN/dt=rN(1-N/K) where r is the growth rate, t is the time, N is the population size and K represents the carrying capacity. Essentially, *r*- selection is experienced by organisms in changing conditions

where a high mortality rate would be balanced by rapid reproduction, favouring an ability to rapidly acquire resources and convert them into offspring. At the opposite end of the continuum, in stable environments close to carrying capacity, *K*- selection favours the efficient utilisation of resources; those organisms that display slow growth, low mortality and production of a lower number of offspring with a high survival rate will tend to dominate. This theory has since been displaced by demographic models focussing on mortality patterns which indicate that the selective effect of a variable environment is more likely to be significant when reproduction has a high cost on adult survival, or in the case of extreme life histories such as very low or high rates of reproduction[14]. Nonetheless *r*- and *K*- selection theory does provide a basic means of interpreting many evolutionary scenarios, providing it is always considered that this is an over-simplification of extremes, and that most organisms fall between the two.

It is important to understand in studies of evolution that it is not the individuals that evolve, but the genetic makeup of the population as a whole. The quasi-species concept refers to an infinitely large population that has reached steady state due to equilibrium of mutation and selection[15]. The fitness of the quasi-species depends on reproduction rate, adaptability to changes in the environment and genetic robustness to withstand deleterious mutations. Genetic robustness is favoured in constant environments and is characterised by high neutrality; that is the ability to withstand mutations by mechanisms

such as gene redundancy and over-expression of chaperones. High neutrality favours a high growth rate, but this can be detrimental to populations which suffer frequent bottlenecks due to changing environments. In this case adaptability is more beneficial and results in a reduction in time taken to regenerate the equilibrium population from a smaller number of individuals. It is difficult to measure the amount of beneficial and deleterious mutations in populations, since lethal and highly deleterious mutations are generally eliminated rapidly from the population, and conversely a beneficial mutation may be fixed rapidly leading to the loss of others that were also spreading in the same population[15].

The evolutionary history of parasitism and mutualism are particularly interesting, having resulted in a huge number of highly specialised relationships. It may seem like the deleterious effects of parasites and parasitoids (parasites that kill their hosts) would be an evolutionary dead end since the infected individual would be less fit and less likely to reproduce, and certainly on an individual level this appears true. However, the vast diversity of parasitic wasps (order Hymenoptera) is just one example of the power of parasitism in driving evolution. The phylogenies of these wasps, combined with life-history trait analysis, suggest that the evolution of parasitism in Hymenoptera has a single origin in the common ancestor between the families Orussoidea and Apocrita, resulting first in ectoparasitoids which develop outside the body of the host, and then the endoparasitoids which develop inside their host[16]. Other

descendents of this putative ancestor have formed similarly close relationships with their host plants, for example fig-pollinating wasps have developed a mutualistic relationship with their host, and show clear signs of co-evolution[16]. The host-parasite relationship is just one scenario selecting for increased mutation rates, since the adaptation in one species is often detrimental to the other[17], as characterised in the Red Queen hypothesis[18] which proposes that organisms must constantly evolve in order to remain competitive with those around them.

1.2 Viruses

Viruses could be considered to be the ultimate parasites; they are incredibly abundant[19], yet they depend on their host's cellular machinery to facilitate replication of their own genome and packaging molecules to continue the cycle of transmission to a new host cell[20]. They were first reported in 1892 by Ivanovsky when he found that the causative agent of tobacco mosaic disease was too small to be retained by a filter[21]. Since then our understanding of viruses has changed a great deal, shifting from considering them as disease causing agents, into understanding their value in global processes such as the marine food web and global carbon cycle[22].

The question of whether viruses are a life form is hotly debated from both sides: they are obligate cellular parasites and therefore incapable of reproducing

outside their specific host; however, they can survive independently of their host cell in much the same way as a dormant plant seed. Recently it has been proposed that the living "virus" is not the protein packaged genome, but the infected cell which has been transformed to produce virions[23]. Nonetheless, the sheer abundance of viruses, particularly in aquatic environments[19] shows that they are clearly very important biological entities; whether they live or not depends entirely on how one chooses to define life and thus becomes more of a philosophical debate. This debate has prompted the suggestion of a "virus-friendly" definition of life as being "a collection of integrated organs (molecular machines or structures) producing individuals evolving through natural selection[23]".

1.2.1 Viral life cycles

There are two main strategies of viral reproduction: lytic, whereby the virus reproduces rapidly and causes the cell to burst thus releasing new viral particles, and latent or lysogenic whereby the virus integrates its genome with the host cell and remains there, dormant until some factor triggers production of viral particles. There are grey areas between these two main cycles, including pseudolysogeny which has been observed in *Chlorella* viruses[24], but is most commonly observed in bacteriophage infecting starved bacteria[25]. In pseudolysogeny, the phage remain inactive within the cell, with the phage

genome either not replicating, or doing so in synchronisation with the host genome, until the cell is fed and has sufficient energy to support reproduction of the phage, which then enter their normal lytic cycle[25]. In addition, some viruses cause chronic latent infections which do not kill the host cell; rather, viral particles are produced by budding or extrusion over some time[26].

Lysis is perhaps the best characterised life cycle mechanism, since its effects on the susceptible host (death or disease) are often overt and thus quantifiable. The first stage in the cycle is the recognition of the host cell by the virus, which usually involves molecules on the outside of the cell and virus particle. The virus nucleic acid then enters the cell, where it is reproduced by the cell's DNA replication machinery. Transcription of the virus coat proteins also occurs, followed by assembly of the progeny virions. The lytic cycle ends when the virions cause the cell to burst (lysis), killing the cell and releasing the progeny virus particles. This is a common approach used by viruses of unicellular organisms, such as *Emiliania huxleyi* virus-86 (EhV-86)[27] or *Paramecium bursaria* chlorella virus-1 (PBCV-1)[24].

Latent viruses have similar mechanisms of cell entry to lytic viruses, but once inside the cell they enter an inactive state; either their genome integrates with that of the host to become a provirus[28], or they are maintained in an episomal form within the host cell[29]. Host mechanisms then replicate the viral genome every time the host cell divides. If the integration occurs in the germline of the host, or in the spores (for example in the brown algal virus EsV-

1), the provirus is passed on from parent to offspring with little sign of the infection. A trigger, such as stress to the host, can cause the provirus to become active once again and the infection becomes more aggressive, causing the production of virus particles which often exit the cell by bursting it in the same way as the lytic viruses.

As stated above, the Red Queen Hypothesis posits that the pathogen does not eradicate the host population. This is due to the complex host immune responses to eliminate or destroy infecting viruses. Often the virus is able to survive the host defences just long enough to replicate and be transmitted to the next host. However, latency is one of the myriad of mechanisms exploited by viruses to evade the host immune defences. The virus remains in an inactive state, which may involve genome integration, sometimes in immune-privileged sites such as the brain, until being reactivated by some trigger[30].

Virus addiction can also occur in latent infections, whereby the fitness of the infected organism is greater than those without the virus[31]. This can be seen when a population that has been exposed to a virus and therefore has developed immunity encounters a separate population that has no immunity. The non-immune population can be wiped out in a dramatic fashion as can be seen with many laboratory maintained organisms, for example laboratory strain mice exposed to mouse hepatitis virus from wild mice with immunity to the disease[31]. Some phage also possess an addiction module; for example, the phage P1 which infects *Escherichia coli* becomes integrated into the bacterium

as a plasmid and remains dormant while expressing two genes, one long lived toxin and one short lived antidote. If the bacterium eliminates the phage, it dies since the antidote degrades before the toxin. A similar addiction mechanism can promote group identity, by killing individuals lacking the addiction module by lysis, leading to the hypothesis that viruses may be involved in the recognition of self in some organisms[32].

1.2.2 Viruses in evolution

Viruses can drive evolution. Ancestral retroviral genomes make up around 5 – 8% of the human genome[20] and have been proposed to play a major role in the evolution of eukaryotes[33], by transferring their genes into the genomes of their hosts. Indeed there are proposals that viruses play a much more fundamental role even than that; some scientists believe that viruses are the source of major eukaryotic genes such as DNA replication machinery[33] and potassium channels[34], that the cell wall has evolved in response to selection pressure to prevent virion entry[35] or even that the eukaryotic nucleus evolved from endosymbiosis of a complex DNA virus[36].

Initial virus classification was split into bacteriophage and viruses according to the domain of life which they were observed to infect; Eukarya and Prokarya, respectively. This in turn led to assumptions that phage originated from bacterial plasmids, and viruses from eukaryotic genomes, such as retroviruses

from retro-elements. Most virally encoded proteins actually have no specific relationship with those of their hosts, and many viruses share similar proteins which have no homologues in cellular life[37]. The identification of a third domain, the Archaea[38], with associated unique viruses began to suggest that the last universal common ancestor of cells was a victim of viral attack, and that viruses originated before the last universal common ancestor (LUCA) when some cells had RNA genomes instead of the DNA genomes we see today, possibly even triggering the emergence of the three cellular domains by inducing the transmission of cells with RNA genomes into cells with DNA genomes[23].

Moreover, Boyer[39] goes as far as to suggest that nuclear-cytoplasmic large dsDNA viruses (NCLDVs) which have double-stranded DNA genomes and infect a diverse range of eukaryotes, form a fourth kingdom of life based on the observation that the phylogeny of their DNA replication machinery shows them forming a distinct group from the Bacteria, Eukarya and Archaea. All of this points towards a much more central role of viruses in all kingdoms of life than was previously believed. Indeed it does seem that the most abundant microbial hosts in the marine environment are not those that grow the fastest (the *r*-strategists), but those that are resistant to viruses and show slow growth and adaptation, and therefore a *K*- type evolutionary strategy. In contrast to this, the most abundant marine viruses are those that are highly virulent and short lived, with rapid replication, small genomes and high burst sizes[40].

Viral metagenomics has shown that marine viruses contain many genes which may augment their host's metabolism, immunity, distribution and evolution. Marine viral cyanophage commonly carry genes for photosynthesis which ensure continued cell survival during virion production even after the normal host processes have ceased to function. It has been suggested by comparative genomics that phage *psbA* genes have been exchanged between hosts[41], and 60% of *psbA* genes in the marine environment that could be assigned to an origin came from phage, making it possible that 10% of the global photosynthesis could be the result of a gene originating from phage[42].

Metagenomic comparisons of microbial and viral fractions showed that the viral fractions contained less respiration genes but more nucleic acid metabolism genes[42], along with genes associated with vitamin and cofactor synthesis and stress response genes such as chaperones, with a roughly equal split of carbohydrate and protein metabolism genes. It has also become clear that viral functional diversity and its potential for host adaptation has been seriously underestimated[42]. Thus viruses may act as a store of genes involved in microbial adaptation to the various niches found in marine environments since a high rate of gene transfer has been found between some viruses and their hosts.

Some genes which are important to the host's survival have been shown to have originated from viruses, for example mitochondrial RNA polymerase[23], DNA polymerase[23] and DNA helicase[23] originated from a virus that was

integrated into the genome of bacteria at the origin of the endosymbiotic event resulting in their integration into cells[23]. The cholera toxin gene is another example of a viral gene that augments its host's survival, since *Vibrio cholerae* is usually harmless unless infected by phage with this gene[42]. In addition, a temperate phage in *Clostridium difficile* appears to contribute to host pathogenicity either by encoding novel toxins or by differentially regulating the expression of bacterial toxins[43]. In fact it appears possible that the presence of two separate phage may confer even more evolutionary advantage, since simultaneous infection by two types of phage has been observed[43].

Horizontal gene transfer (HGT) is less common in large eukaryotic viruses than in phage[44]. However, more examples of NCLDVs [45] and other giant viruses [46,47] indicate that these viruses have an important role to play in their host's evolution. Moreover, latent viruses maintain a stable relationship with their hosts by integration of novel genes into the host genome which could facilitate HGT, as such, proviruses can be considered to be a specialised form of transposon.

The above theories of the influence of viral infection in the genetic makeup of their hosts all deal with the transfer of specific genes between virus and host. However, perhaps the most basic mechanism of gene transfer is the release of dissolved DNA during lytic events, the uptake of which is not subject to any requirement of host specificity and is possibly the source of some of the more far-reaching gene transfer events[48].

1.2.3 Virus evolution

As host populations grow and adapt, they put selection pressure on the viruses that infect them. Equally, the converse is also true; viruses can be a selective force in the evolution of host populations[18]. The host-parasite interaction theory developed in 1983[49] states that selection acts to maximise R_0 – the average number of secondary infections arising from one infected host in an otherwise uninfected population. An R_0 of less than one means an epidemic cannot occur and it may even be possible to eliminate the disease, typified by an infection with low exposure time, low yield of infectious virus or a short duration of replication. On the other hand, a large R_0 almost certainly results in an epidemic and typically has "superspreaders", single hosts that can infect a large number of individuals.

Viruses are constantly evolving to adapt to changing selective pressures in their environments by processes such as mutation, recombination and re-assortment. Virus evolution is considered in terms of the population, or quasi-species, not the individual, since it is the diversity that is essential in the survival of the population and this is maintained by high mutation rates. Broadly speaking there are two evolutionary strategies employed by viruses: acute and persistent[50]. The acute strategy is common among lytic viruses and is typically characterised by short reproductive cycles producing many progeny that are effective when resources are scarce (similar to an *r*- replication strategy). In spite of the *r/K* evolutionary theory originating in the 1960s, it only

appears to have been applied to viruses in the last decade or so, being initially described in the RNA vesicular stomatitis virus[51] and bacteriophage[52]. This theory has only been applied to marine viruses by Suttle in his review in 2007[40], based on observations in marine bacteriophage[53,54,55,56], and to our knowledge has not been used in the description of eukaryotic marine viruses until now.

Some lytic, but mostly latent viruses follow a persistent life strategy (akin to *K*-replication strategy), with a lower reproductive output but better competition for resources; they tend to demonstrate low pathogenesis and produce fewer progeny with higher survival rate. These viruses generally infect complex multicellular organisms such as seaweed[57], plants[58] and animals[59] and tend to survive as long as their host survives. That said, temperate/lysogenic phage are also persistent life strategists: persistent when integrated stably in their host cell and acute during virulent replication and lysis[40] and are therefore able to take advantage of both ends of the *r/K* spectrum.

The replication of viruses with RNA genomes is much more error prone than those with DNA genomes, and large DNA genomes are usually reproduced more faithfully than smaller DNA genomes, therefore viruses with large DNA genomes are much more likely to follow the persistent strategy than the acute strategy[60].

There are currently three main theories about the origin of viruses: regressive theory states that they came from intracellular parasites that have lost all but the most essential genes, the cellular origin theory states they came from cellular components that developed autonomous replication within the cell, and the independent entity theory is that viruses have co-evolved with cells from the origin of life[60]. A cellular origin for viruses seems unlikely since several of the common viral genes, such as the major capsid protein, have no homologs in the cellular world[61].

The independent entity theory is currently the most popular and seems to be the most likely since some core viral genes are not present in any other known organisms, and evolutionary links have been discovered between distantly related viruses infecting hosts from different kingdoms. For example structural analysis of the major capsid protein shows that dsDNA herpesviruses share a characteristic unique fold with the tailed DNA bacteriophage of the family Caudovirales[62]. This may represent an infection predating the incorporation of the prokaryotically derived nucleus into the eukaryotic cell and suggests that extant viruses have arisen from a small number of primordial progenitors[62]. This possibility was investigated by Koonin[63] who used comparative genomics to determine the origin of various common viral genes and found no evidence of the monophyletic origin of all viruses, although he did demonstrate monophyly in several large virus classes. He defines the ancient virus world as consisting of “selfish genetic elements, including viruses, viroids, and mobile

genetic elements, which initially predated cellular life but ultimately have come to depend on certain cellular processes for replication[63].”

1.2.3.1 NCLDV evolution

Virus classification is generally based on such characteristics as host range, genome composition (double-stranded or single-stranded, DNA or RNA), or the morphological characteristics of the virion particle. There is no evidence for the monophyly of all viruses since no one gene is common to all; however, the NCLDVs are one of the most diverse virus divisions which appear to be monophyletic [64]. They include the families Phycodnaviridae, Mimiviridae, Poxviridae, Iridoviridae, Ascoviridae and Asfarviridae[65], as well as a potential new family comprising Marseillevirus[66] and Lausannevirus[67]. The NCLDVs multiply in the cytoplasm and sometimes partly in the nucleus of the cells which they infect. They share a common set of genes and include some of the largest known viruses, the family Mimiviridae, which encode over a thousand genes and are a similar size to bacteria[68].

Around 40 genes have been mapped to their common ancestor[69], although there is some dispute as to the exact number[61,64]. Some of these ancestral genes are shared with other dsDNA viruses such as DNA polymerase, DNA primase and superfamily II helicase [64], whilst other characteristic NCLDV genes are unique to the NCLDVs, such as the superfamily III helicase, packaging

ATPase and disulphide oxidoreductase [64]. The large size and diversity of NCLDV genomes is probably a result of HGT acquisitions from cellular organisms, combined with duplication and lineage specific expansion of gene families[61].

A more comprehensive study into the origins of a subset of common NCLDV genes has shown that many derive from cellular sources, although the majority of those considered to be viral hallmark genes are indeed of monophyletic viral origin and seem likely to have been vertically transmitted from a common ancestor[37,70]. The genes from a cellular origin follow a general trend; viruses infecting protists appear to have acquired the majority of their horizontally transferred genes from the bacterial prey of their hosts, whereas viruses infecting metazoa have a higher proportion of host derived genes[70]. Thus it does indeed seem that the most likely evolutionary scenario for the origin of NCLDVs is one of a common viral ancestor with a small number of core genes which has been augmented by the acquisition of cellular genes from a variety of sources.

1.2.3.2 Phycodnavirus evolution

The members of family Phycodnaviridae are viruses that infect algae. This is a somewhat arbitrary grouping since the term algae covers an evolutionary diverse group of photosynthetic eukaryotes from both freshwater and marine

environments[44]; however, the viruses in this group do share a similar icosahedral capsid morphology and have been proven by genetic means to be related[37]. They share many similarities both in terms of structure and genome content, which is surprising since the algae that they infect are polyphyletic. This suggests that they are a very ancient group of viruses which arose before the division of the algae and infected their common ancestor[71], thus they have a roughly equivalent diversity to that between viruses of plants and animals[72]. When one considers that the putative first eukaryotic cell was a unicellular green alga[73], it seems plausible that if viruses appeared and evolved with their host, then phycodnaviruses could date back more than 1.2 million years. Indeed this scenario could provide an explanation for the presence of both bacterial and eukaryotic genes in NCLDV genomes in spite of their surprisingly constant GC content, indicating that these genes are not the result of relatively recent horizontal acquisitions[74]. DNA polymerase[75,76,77] and MCP[75,78,79,80,81] are the most commonly used genes in the study of phycodnaviral phylogeny, and these studies agree on their monophyletic origin within the NCLDVs[82].

The chlorella viruses (*Chlorovirus)* are the most extensively studied of the phycodnaviruses[82,83,84], and clear similarities in gene order and nucleotide conservation have been demonstrated between viral strains infecting the same host species but less so between viruses infecting different species. Moreover,

comparisons of host and virus phylogeny indicate that it is likely that these viruses have changed host during their evolutionary history[85].

There is a section at the left end of the PBCV-1 (*Chlorovirus*) genome which seems to act as a recombinatorial hotspot, being prone to deletions and rearrangements[74]. In fact it is this section which encodes most of the genes which have an apparently cellular origin in these viruses[70]. Mobile genetic elements (MGEs) are generally associated with HGT, and the *Chlorovirus* and *Mimivirus* genomes have insertion sequences which are typically found in bacteria and archaea, and have probably been acquired as a result of MGE activity[61]. Their genomes also have multiple mobile endonucleases, sometimes with self-splicing introns, and also some inteins[61]. However, HGT appears to be less common in large eukaryotic viruses than it is in phage based on nucleotide composition studies[86].

Studies on phyacodnaviral evolution to date have relied on phylogenetic analysis and comparison of gene composition. For example, the fact that EsV-1 and FirrV-1 have different but overlapping subsets of histidine kinase genes[87], and have concluded that they have all evolved by gene loss from a common ancestor with a larger genome[87,88]. Delaroque *et al*.[87] even suggested that the phaeoviruses may have evolved from a primitive single-celled symbiont in an algal host which through subsequent gene loss adopted a viral lifestyle, although this seems unlikely due to the presence of unique viral genes such as the major capsid protein.

1.2.3.3 Phaeoviruses

Phaeoviruses are a group of dsDNA viruses within the family Phycodnavirideae which infect members of the class Phaeophyceae (brown algae). The phaeophytes form part of the division Heterokontophyta from the kingdom Chromalveolata and consist of mostly marine macroalgae which evolved multicellularity separately to the other major multicellular eukaryotic groups (Metazoa, Plantae and Fungi)[89,90,91]. They vary in complexity from chains of single cells (filaments) in the order Ectocarpales to the orders Fucales and Laminariales which have evolved thalli of differentiated tissues[92]. The class Phaeophyceae comprises 14 orders as shown in Figure 1.1. Among the most commonly studied of these classes are the Ectocarpales, due to the adoption of *Ectocarpus siliculosus* as a model brown alga [90,93], which are infected by the well studied phaeoviruses[94], and their sister class Laminariales, some members of which are very valuable, both ecologically as primary producers and habitat formers, and commercially for food and chemical production[95,96].

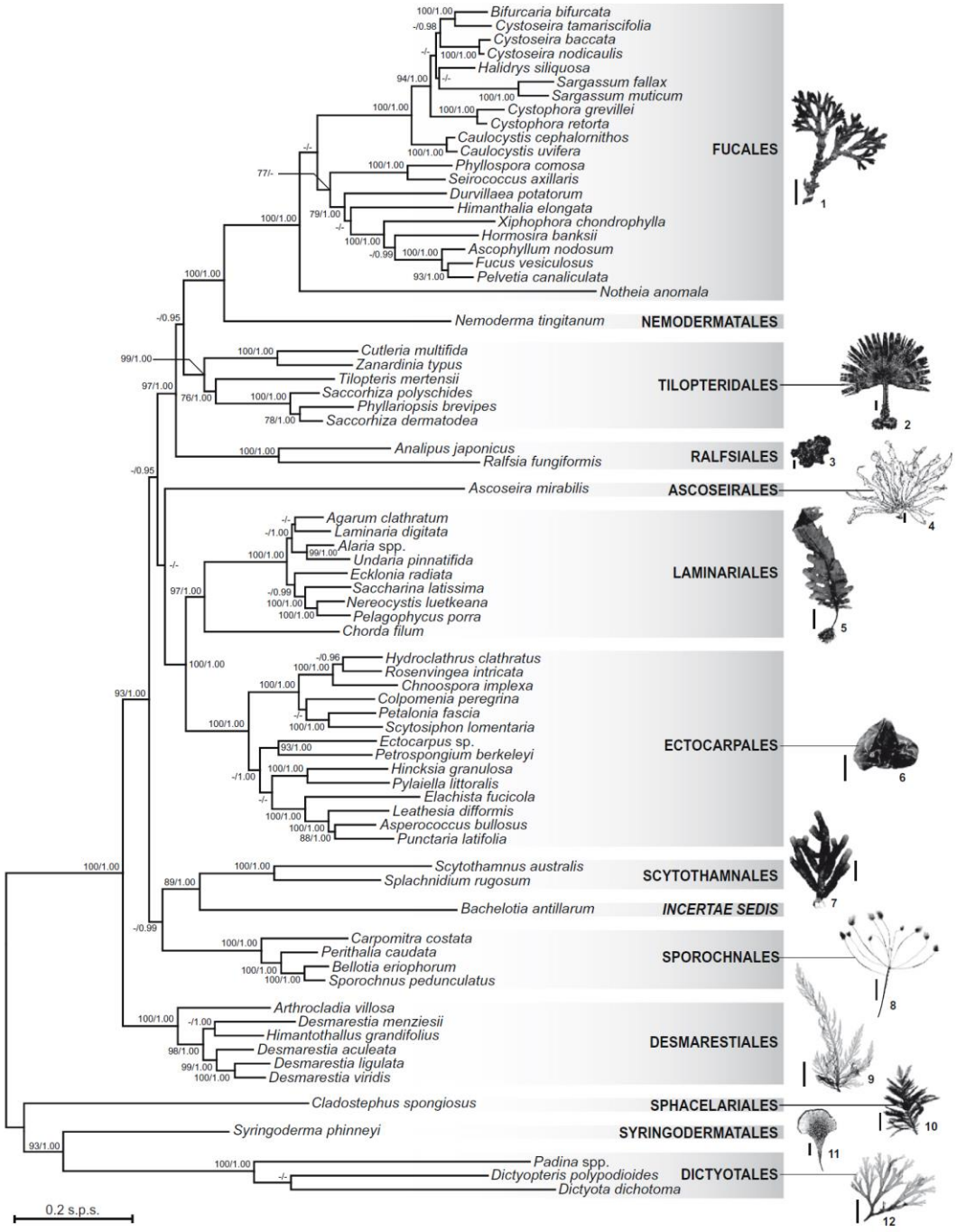

Figure 1.1: Maximum likelihood phylogeny of the class Phaeophyceae based on a concatenation of 10 genes. First node label indicates ML bootstrap percentage (only those above 75 are shown), second value indicates Bayesian posterior probability (only those above 0.95 are shown). Permission to reproduce this figure was granted by http://www.elsevier.com (from [92])

However, in spite of their inherent value, relatively little research has been carried out on members of the Phaeophyceae[93]. This, combined with their evolutionary distance from the more intensely studied eukaryotes such as land plants and animals, means that little is known about their physiology and responses to stress. Therefore there is a real potential to discover novel cellular processes through studying these organisms and their viruses. The recently sequenced ectocarpoid, *Ectocarpus siliculosus*[91], is currently a widely accepted model for the study of brown algae and is known to be infected by a latent dsDNA phaeovirus, EsV-1[97].

Phaeoviruses, in spite of their name, have so far only been studied in detail in the order Ectocarpales (filamentous brown algae commonly referred to as maidens hair) and not throughout the whole brown algal class[44]. However, their presence in other members of the brown algae cannot be ruled out simply because they have not been seen, as symptom suppression is known to be common[98]. Indeed, viruses have been observed in *Chorda tomentosa* Lyngbye[99] and *Leptonematella fasciculata* (Reinke) Silva[98], which suggests that virus infections may be a general feature in the entire Phaeophyceae class[98]. Viruses in the order Laminariales (the sister order of the Ectocarpales[100]) for example would be of great interest due to the ecological and commercial importance of some species of kelp, and so discovering anything that may potentially influence their reproductive capacity would be very valuable.

To date, three phaeoviral genomes have been sequenced: *Ectocarpus siliculosus* virus-1 (EsV-1)[1], *Feldmannia irregularis* virus-1 (FirrV-1)[87] and *Feldmannia* sp. virus 158 (FsV-158)[88], as well as an inactive provirus in the *Ectocarpus* genome[91]. Five additional phaeoviruses, identified solely on morphology and life cycle, infecting *Ectocarpus fasciculatus* (Harvey)[101], *Feldmannia simplex* (Crouan & Crouan) Hamel[102], *Hincksia hincksiae* (Harvey) Silva[103], *Pylaiella littoralis* (Linnaeus) Kjellman[104] and *Myriotrichia clavaeformis* (Harvey)[103], infecting three different Phaeophyceaen families; Ectocarpaceae, Acinetosporaceae and Chordariaceae have also been described in the literature.

1.2.3.4 Ectocarpus siliculosus virus-1

EsV-1 (Figure 1.2) is the most intensely studied phaeovirus [80,87,97,105,106,107] and infects *Ectocarpus siliculosus* [97]. It is pandemic, and estimates of infection rates range from 10% in the closely related alga *Ectocarpus fasciculatus*[79], to 40-100% of natural *Ectocarpus* populations[105,108], depending on the detection technique used. EsV-1 infection inhibits the reproduction of *E. siliculosus* and is believed to be a major factor in controlling the extent of wild populations of the alga[109].

Figure 1.2 has been removed due to Copyright restrictions.

1.2.3.4.1 Infection process

EsV-1, as with other phaeoviruses, can only infect the free-swimming wall-less gametes and spores of the filamentous brown alga *Ectocarpus siliculosus*[109]. The virus particles attach to the plasma membrane which fuses with the viral internal capsid membrane and the viral core enters the cytoplasm[111]. Müller[109] suggests that the cell wall either acts as a physical barrier against virus entry or lacks the molecules involved in host recognition by the virus. However, in a previous study it was observed that protoplasts could not be infected[112] so it is likely to be a different factor preventing the infection of vegetative cells, probably a cell surface recognition molecule. The infection strategy of a phycodnavirus from the chlorellavirideae, *Paramecium bursaria chlorella* virus-1 (PBCV-1), is very different; it enters the host cell by digesting a portion of the wall and injecting its DNA and is even capable of binding to purified cell wall fragments therefore it seems the cell wall does play an important part in host recognition for this virus[113].

Once inside the cell, EsV-1 integrates its genome with that of the host which was shown by Delaroque *et al.* [57] by a study of agarose gel extracted DNA from a phaeovirus-infected host alga, followed by virus-specific PCR. When the Ectocarpus genome was sequenced in full [91], it was discovered that a virus very similar to EsV-1 was integrated into the algal genome, in almost one complete fragment on contig 0052, however the viral integrase gene was found in a different host contig and was replaced in the large viral fragment by a homologous sequence, suggesting that homologous transposition had occurred at some point [91] and may possibly explain the inactivity of this provirus. After genome integration, the provirus enters an inactive, latent state during which it is transmitted to all cells of the alga by mitosis during vegetative growth and displays Mendelian segregation during meiosis, suggesting that it is linked to a host autosome[78].

Although a few populations of infected *E. siliculosus* showed retarded growth, the majority showed no apparent symptoms during vegetative growth[105]. However, *Feldmannia simplex* and *F. irregularis* did show a growth decrease in infected strains due to reduced photosynthetic performance[114]. The previously mentioned EsV-1 provirus in the sequenced strain of *E. siliculosus* has been shown not to be transcriptionally active (except for one gene of unknown function) [91], which explains why no viral symptoms have been observed in this strain (Akira Peters, pers. comm.). This is surprising however, when one considers that the viral genome makes up around 1% of the genes and 0.1% of

the sequence length [91], and raises the question of why the alga is using its resources to maintain such a large but apparently redundant section of DNA.

1.2.3.4.2 Latent/lytic switch

At the end of the latent period, an unknown trigger causes EsV-1 to become active during the production of the host's reproductive structures, the zoidangia[97], and the cell switches to viral replication and virion production as shown in Figure 1.3. This trigger is likely to be a factor involved in the development of reproductive cells[1], because this is the only type of cell which allows viral replication. The reactivation of latent viruses has been intensely studied in the bacteriophage λ and in human herpesviruses such as cytomegalovirus (HCMV), Epstein-Barr virus (EBV) and herpes simplex virus (HsV-1). These are all capable of maintaining long periods of latency, and reactivation relies on transcriptional regulators, such as the *cI* and *cro* genes which have opposing functions and together control the transcription of viral genes for either the lytic cycle or lysogeny in the bacteriophage λ[115].

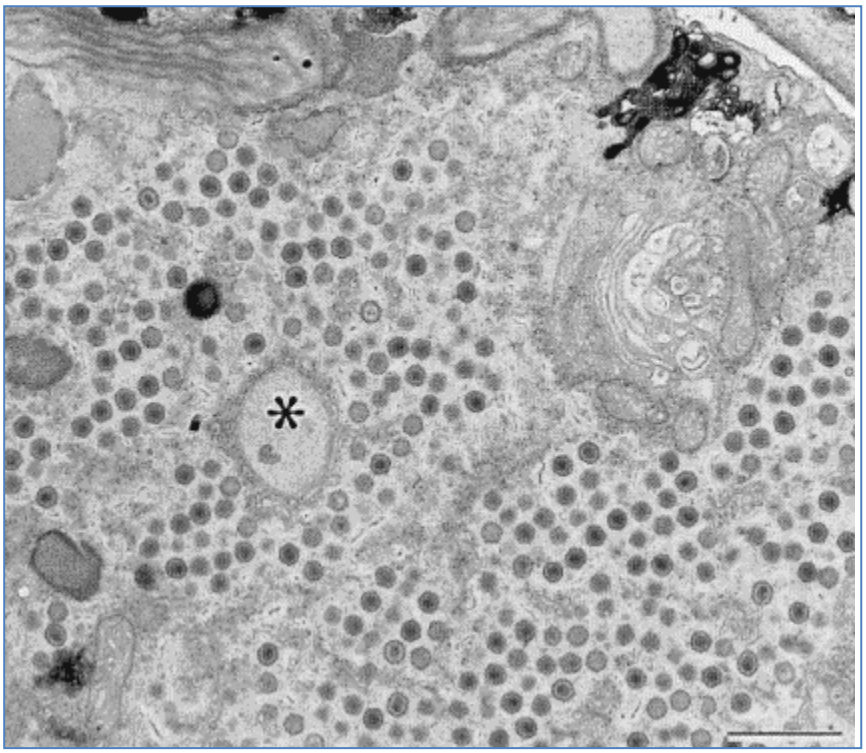

Figure 1.3: Infected *Ectocarpus siliculosus* cell containing arrays of EsV-1 particles. Scale bar represents 1µm, asterisk shows degenerating nucleus. Permission to reproduce this figure was granted by John Wiley and Sons (from [97]).

The reactivation of human latent viruses generally occurs during a profound depression in the host's immune status[116]. Such changes usually occur as a result of some form of stress, which has also been shown in symbiotic zooxanthellae of coral exposed to ultra-violet light to induce the release of virus-like particles which may be related to bleaching events[117]. Although the human immune system is very different to that of plants and algae, analysis of the *E. siliculosus* genome has raised the suggestion that it may be capable of adaptive immunity in the form of exon shuffling, which is hypothesised to allow the rapidly changing structure of pathogenicity receptors such as leucine-rich

repeats[118]. This form of immunity is not likely to occur in specific cell types, such as are involved in animals' immune responses; therefore, it seems unlikely that any potential algal immunity can be depressed in the same way.

Another possible trigger for the reactivation of EsV-1 is the differentiation of cells during the formation of zoidangia, since these are the only locations where infection symptoms are observed. Some factor in the mitotic/meiotic switch may be responsible for activating this, or the differentiation itself may be the trigger, since differentiation of the host cell is also important in the early stages of the reactivation of other dsDNA viruses such as the herpesviruses[119,120]. Human cytomegalovirus (HCMV) infects the cells of the immune system directly, entering a latent state in $CD14^{+}$ monocytes, which are the progenitors of phagocytes[121]. Reactivation of the virus depends on the differentiation of its host cell into macrophages which allow virus replication[119]. Epstein-Barr virus (EBV) reactivation *in vitro* also depends on cell differentiation and it is postulated that *in vivo* reactivation occurs due to differentiation of memory B cells as a response to antigen stimulation[120].

1.2.3.4.3 Virion production

As with other phaeoviruses[122,123], EsV-1 virion production starts with a failure in the cell division resulting in incomplete wall formation and multinucleate cells, followed by nuclear hypertrophy as the virus genome is

replicated[97]. The zoidangia cells swell, becoming unstructured as can be seen in Figure 1.4 and producing virus particles[109]. In the replication of the related *Hincksia hincksiae* virus (HincV-1), empty capsid particles bud from cisternae which are thought to be derived from the endoplasmic reticulum. Packaging of the nucleoprotein genome subsequently occurs, and then the virus particles are released by cell lysis[122]. From observations of the infection process of EsV-1[97] and other phaeoviruses[104,124,125,126] it is likely that the process of EsV-1 particle assembly is similar to that of HincV-1[122].

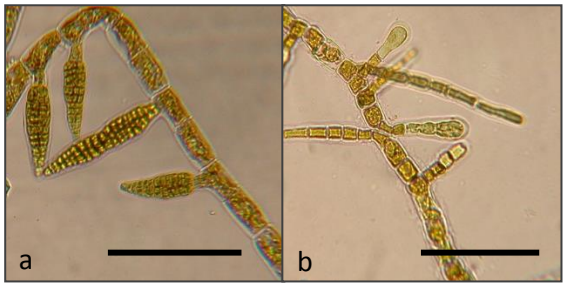

Figure 1.4: Asymptomatic (a) and symptomatic (b) strains of *E. siliculosus* showing normal zoidangia containing developing zoids, and deformed zoidangia containing virions respectively. (Bars represent 100µm)

1.2.3.4.4 Virion release

In *Ectocarpus*, the release of virus particles can be induced by fresh medium and an increase in temperature from 12°C to 18°C; the same conditions that cause spore release[127]. This allows both virus and spores to be released at the same time, thus maximising the chance of infection by the virions which only

retain their infectious capacity for two to three days[109]. Spore release from the unilocular sporangia of *Pilayella littoralis* has been suggested to be due to a combination of digestion of the inner cell wall and increased turgour pressure due to production of extracellular polysaccharides and their absorption of water[128]. The release of virus particles from infected *E. siliculosus* zoidangia appears to occur in a similar way, with the infected zoidangia cells bursting and releasing a spherical mass of virus particles which disperses by Brownian motion[105,127]. This lytic mechanism of viral release is common among non-enveloped viruses, although little is known about the mechanisms involved[20]. However, some non-enveloped viruses, for example some picornaviruses, have developed mechanisms to exit the host cell without lysis, possibly by the action of vesicles which fuse with the cell membrane[20]. This may be related to the release mechanism of enveloped viruses which either assemble at the plasma membrane and bud from it, or are formed from intracellular membranes and exit through the cell membrane[20].

1.2.3.4.5 Symptom suppression

EsV-1 infection symptoms (deformed zoidangia) are more pronounced at lower temperatures. Their suppression in a strain from Naples, resulting in the production of normal zoidangia, was much greater at 18°C compared to 15°C[109]. Partial viral suppression may also occur which results in a mosaic

plant producing both spores and virions as a result of developing both normal and abnormal zoidangia or sometimes even normal and abnormal sections within the same zoidangium producing a mosaic pattern of cells. Plantlets growing from spores produced by an infected plant show infection symptoms at sexual maturity and therefore still contain the viral genome[105]. The only mechanism by which the host can actually get rid of the viral genome is meiotic elimination, whereby the viral genome is transmitted to the offspring in a Mendelian fashion, resulting in some offspring being virus-free[78].

1.2.3.5 Phaeovirus evolution

Phaeoviruses are the only members of the family Phycodnaviridae known to follow a persistent life strategy. They favour stable integration into their host's genome and only reproduce in large numbers at certain stages during the host's life cycle. In contrast, the lytic viruses infecting *Emiliania huxleyi* follow an acute strategy with huge diversity among strains infecting the same host that may help them overcome strong evolutionary pressures faced by their boom and bust life cycle[129].

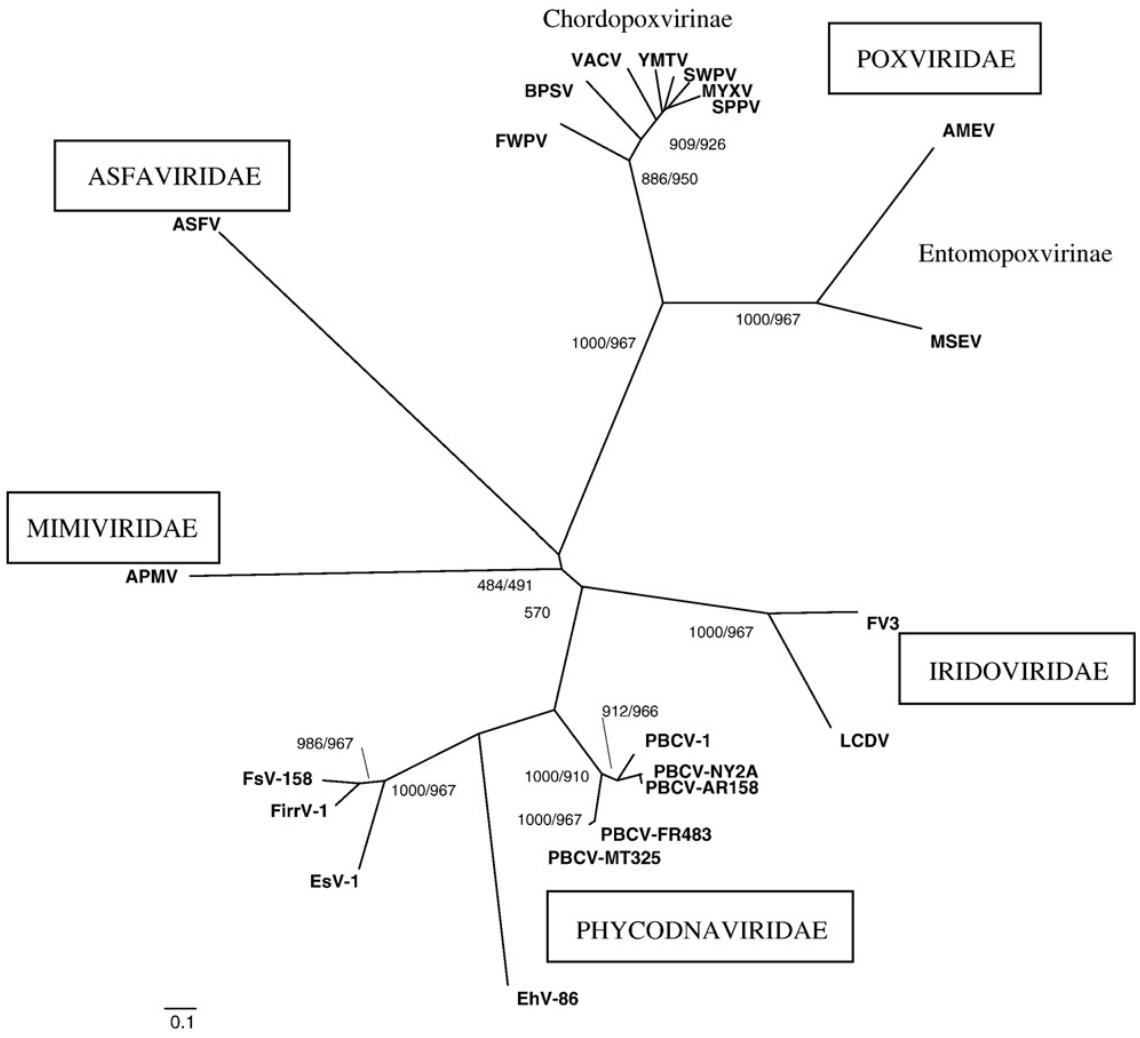

Figure 1.5: Phylogenetic inference tree derived from concatenations of conserved domains of NCLDV group I core genes. Node numbers represent bootstrap values from 1000 replicates for neighbour-joining and where possible parsimony analyses. Virus abbreviations not described already mentioned are: ASFV, African swine fever virus; FWPV, Fowlpox virus; BPSV, Bovine papular stomatitis virus; VACV, Vaccinia virus; YMTV, Yaba monkey tumour virus; SWPV, Swinepox virus; MYXV, Myxoma virus; SPPV, Sheeppox virus; AMEV, *Amsacta moorei* virus; MSEV, *Melanoplus sanguinipes* entomopoxvirus; FV3, Frog virus 3; LCDV, Lymphocystis disease virus. The bar depicts 1 base substitution per 10 amino acids. (This figure was published in Virology 384, Schroeder DC, Park Y, Yoon H-M, Lee YW, Kang SW et al. Genomic analysis of the smallest giant virus – Feldmannia sp.virus 158 p223-232, Copyright Elsevier 2009 [88]).

Phylogenetic relationships between conserved genes amongst the NCLDVs suggest that phaeoviruses have a more recent evolutionary history than most [88]. Green algal viruses (for example the chloroviruses) split from heterokont

algal viruses (haptophytes/brown algae) which further separate into coccolithoviruses and phaeoviruses [88] as shown in Figure 1.5. Even within the phaeoviruses, host specific patterns emerge; the two *Feldmannia* viruses (FsV-158 and FirrV-1) are more genetically similar to each other than to EsV-1, with gene order generally being maintained, albeit with some evidence of genome recombination and inversion[88]. Moreover, the *Feldmannia* viruses even seem to have evolved further than EsV-1 by reducing their genome size probably as a result of gene losses via recombination[88] suggesting there is a selection pressure for smaller genomes in these viruses.

1.2.3.6 Phaeovirus genetics

The EsV-1 genome is 335 kb double-stranded DNA, separated by some single stranded regions. It has inverted repeats at the ends that allow it to form a cruciform structure that effectively produces a circular molecule[130]. The genome sequence revealed 231 putative CDSs in gene dense regions separated by repeats and noncoding regions[1]. 149 of the 231 putative genes (65%) have no sequence homology to other identified genes, or only have similarities to various identified domains but cannot be assigned a function; therefore, studies of these genes have great potential to reveal novel algal or viral functions.

In spite of the general lack of transcriptome studies and, therefore, knowledge of the genetic activity of these viruses, some conclusions can be formed

regarding the molecular mechanisms involved in the viral life cycle by comparing EsV-1 genes with common genes found in the various algal virus genomes which have been sequenced to date: the latent phaeoviruses FirrV-1[87], FsV-158[88], as well as the lytic Coccolithovirus EhV-86[45] and Chlorovirus PBCV-1[24]. Perhaps the most informative genes in NCLDVs, and phaeoviruses in particular, are those that are common to all members. As previously mentioned, only 14 genes are common to all phycodnaviruses sequenced to date, and when considering the entire NCLDV group this number drops to nine[65] which are mainly involved in DNA replication, transcription and virion packaging.

1.2.3.6.1 DNA metabolism

Most of the NCLDV genes involved in DNA processing are considered to encode core proteins essential to viral life cycles, and therefore many of these are present in all five sequenced phycodnaviruses (EsV-1, FirrV-1, FsV-158, PBCV-1 and EhV-86). However, none of the viruses encode all of the necessary proteins to replicate their own genome, each rely on different host proteins to complete the process.

DNA replication in eukaryotes relies on the co-ordinated activity of a suite of enzymes. Initially helicases separate the two strands, and a primase creates short primers to allow the DNA polymerase to begin replication. A replication

factor (RFC) assembles a clamp-like structure between proliferating cell nuclear antigen (PCNA) and the polymerase to enable the latter to remain on the DNA strand, increasing its efficiency. The primers are removed by a further helicase and cut out by a nuclease, with the resulting fragments being joined by a DNA ligase. Telomerase delays cell aging due to the loss of the ends of the chromosomes by ligating repeats to the end[131].

The EsV-1 genome encodes many of these proteins, including a superfamily III helicase (EsV-1-109) which may have a primase function[1]. DNA polymerase (EsV-1-93) is present and highly conserved in all five viral genomes, and for this reason has been used in PCR-based studies to detect the presence of integrated viral DNA [132,133]. PCNA is also encoded by the five viruses considered here, suggesting that both DNA polymerase and PCNA are essential to viral replication. Indeed EhV-86 has two copies of PCNA. Each virus has varying numbers of the RFC subunits; EsV-1 encodes all four small subunits (EsV-1-87, 182, 187, 224) as well as the large subunit (EsV-1-138), whereas FsV-158 and FirrV-1 both lack the 4 small subunits. The PBCV-1 large subunit has no sequence homology to the phaeovirus versions[87], so although EhV-86 appears to lack the large subunit[45], it is also possible that the sequence is so different that it has not yet been identified. Both EhV-86 and PBCV-1 have DNA ligase, as well as DNA topoisomerase II [24,45] which separates DNA during mitosis; the phaeoviruses lack both of these genes. Although viral genomes do not contain telomeres, all three phaeoviruses encode a protelomerase (EsV-1-175) which is

potentially involved in the circularisation or linearisation of the viral genome[87].

Helicases are a group of enzymes which are involved in the separation of DNA strands for various purposes such as replication and repair. EsV-1 encodes a rec-BCD like helicase (EsV-1-29) which is responsible for splitting and digesting DNA ends after a double stranded break, thus initiating repair mechanisms[134]. This gene is not present in FsV-158 or FirrV-1. EsV-1 and FirrV-1 both encode further viral DNA repair mechanisms in the form of two exonucleases (one of which is missing in FsV-158); one is involved in recombination (EsV-1-64)[65] and the other potentially codes for a proofreading exonuclease (EsV-1-126)[1]. The Rec-A family profile 2 protein (EsV-1-95) may be involved in recombinatorial DNA repair, since these proteins control the recombination of DNA and its exchange from one strand to another[135].

DNA methylation is an important method of controlling gene expression by preventing the transcription machinery from binding to the DNA. Chlorellavirus genomes typically contain extensive adenine and cytosine base methylation, therefore unsurprisingly PBCV-1 codes for 3 cytosine DNA methylases and 2 adenine DNA methylases [136]. The EsV-1 genome contains few methylated bases, and correspondingly only codes for the one adenine DNA methylase (EsV-1-129)[1].

1.2.3.6.2 DNA integration and transposition

The phaeoviruses encode proteins involved in integration and transposition, which are essential for their lysogenic lifestyle. EsV-1 encodes two HNH endonucleases, which are rare cutting enzymes that make site specific cuts in DNA[137], although FirrV-1 lacks these. EsV-1 also encodes a protein similar to a site-specific integrase (EsV-1-213), and there are two copies of this in FsV-158. EsV-1 also encodes a potential viral transposon consisting of two transposases (EsV-1-155, 170) flanking a thaumatin-like protein (EsV-1-169)[1], although this is not present in the other phaeoviruses: FirrV-1 lacks transposases, and FsV-158 only has one that is different from those in EsV-1.

In spite of their lytic lifestyle, EhV-86 and PBCV-1 code for one and six HNH endonucleases, respectively, and PBCV-1 also encodes two transposases, in addition to 7 further GIY-YIG endonucleases which are all transcribed at various points in the lifecycle[136]. This suggests that they are still capable of integrating parts of their genomes with the host's, perhaps with a transposon-like system.

1.2.3.6.3 Transcription

Transcription, the production of messenger RNA from the DNA template, relies on a variety of transcription factors to facilitate binding of RNA polymerase to the DNA. EsV-1 is probably able to regulate the transcription of its own genes,

as well as potentially those of the host, since it encodes two transcription regulators (EsV-1-40, 28) and a variety of transcription factors (EsV-1-96, 193, 196), only one of which is also encoded by FirrV-1 and FsV-158. EsV-1, FirrV-1 and FsV-158 also encode an oligoribonuclease (EsV-1-139) which may play a role in mRNA degradation. The lytic PBCV-1 encodes three putative transcription factors (one of which is also present in the EhV-86 genome [45]) as well as RNase III [136] which probably controls transcription by determining the termination site.

1.2.3.6.4 Nucleotide metabolism

Genes encoding proteins with nucleotide metabolism functions are essential to produce free nucleotides for the replication of the viral genome. EsV-1 encodes both the small (EsV-1-128) and large (EsV-1-180) subunits of ribonucleotide reductase, as do FsV-158 and FirrV-1 and PBCV-1[136]. These four viruses also encode a viral ATPase (EsV-1-26) which is important in nucleotide metabolism. EhV-86 however does not encode any of these proteins [45] and therefore must rely on the host's own mechanisms to provide sufficient nucleotides for viral replication. PBCV-1 encodes more than 12 nucleotide metabolism proteins in total, perhaps due to its lytic lifecycle which requires large quantities of nucleotides for its reproduction[24].

1.2.3.6.5 Structural proteins

Three viral structural proteins have been identified in EsV-1: the major capsid protein (EsV-1-116) which is common to all phaeoviruses studied, Vp55 (EsV-1-58) and Vp74 (EsV-1-144)[1] which are all likely to be transcribed late in the viral replication cycle, during virion assembly. In addition, Vp27 (EsV-1-143) may also be a structural protein [138].

1.2.3.6.6 Signalling

Signalling genes are potentially important for sensing environmental stimuli and determining the time of the switch from lysogenic to lytic stages of the viral life cycle. Potential signal transduction proteins in EsV-1, FirrV-1 and FsV-158 consist of putative serine/threonine kinases (EsV-1-11, 82, 104, 111, 156), along with putative hybrid histidine kinases (EsV-1-14, 38, 65, 88, 112, 181, 186), which are unusual for viruses in having the sensing histidine kinase region linked directly to the response region, rather than having two separate enzymes for sensing and response as is more common in *Escherichia coli*[1]. These may potentially be involved in regulation of the latent state since they are also present in FsV-158 and FirrV-1, but not the lytic PBCV-1[1] which may be a symptom of their earlier evolutionary separation from the phaeoviruses. Further proteins which may be involved in signalling and sensing are a viral phosphoshuttle (EsV-1-113) and a potassium ion channel (EsV-1-223).

Additional EsV-1 proteins which are potentially involved in cell signalling or membrane transport include the von Willebrand factor domain (EsV-1-176)[139], the TonB dependent receptor protein (EsV-1-152)[140], lipid peroxidise (EsV-1-147)[141] and a calcium binding protein (EsV-1-56). The four fibronectin type III domains (EsV-1-25, 39, 50, 159) may play an important role in sensing zoidangia development, or some other host-related cue, since fibronectin binds to integrins which have many signalling roles within the cell, and are important for cell adhesion and differentiation[142].

1.2.3.6.7 Miscellaneous

EsV-1 encodes two proteins with similarities to both a viral repressor (EsV-1-197) and antirepressor (EsV-1-117) of the lysogenic cycle. These may function in a similar manner to the cI and cro proteins in bacteriophage λ which have already been mentioned. To date neither of these proteins has been identified in the other phaeoviruses studied, lytic or lysogenic.

EsV-1 contains genes encoding two proteins with homology to HIV proteins and therefore may be important in its lysogenic infection strategy: EsV-1-85 resembles the HIV VPU protein which is important in enhancing the release of virions and delaying cell death[143], while EsV-1-92 is similar to the HIV gp120 envelope surface glycoprotein which binds to CD4[144], a helper T cell surface

glycoprotein, therefore it is possible that EsV-1 utilises some of the same mechanisms as HIV-1.

EsV-1 also contains genes involved in a variety of genetic control mechanisms, such as encoding the bacterial regulatory protein (EsV-1-97) which is involved in gene activation[145]. Two proteins with DnaJ domains (EsV-1-80, 173) potentially have a chaperone function[1], since similar proteins have been shown to be important in viral DNA replication in SV40[146]. A further gene that has a potentially interesting function encodes a regulator of chromosome condensation (EsV-1-24) which may be a viral method for inhibiting cell division, since chromosome condensation occurs in metaphase, before cell division.

EsV-1 encoded sugar metabolism proteins include the coat glycoprotein gp1 (EsV-1-226) which has also been used as a marker to detect the presence of viral DNA by PCR in various *Ectocarpus* strains[80,147,148] since it is highly conserved within the phaeoviruses (PlitV-1)[148]. The gp1 is similar to bacterial mannuronan C-5-epimerases[1], and together with the sugar lyase (EsV-1-164), glycosyltransferase (EsV-1-84), UDP-glucose dehydrogenase (EsV-1-83) and one protein with similarities to a fungal cellulose binding domain (EsV-1-166) may be involved in modifying or degrading the algal alginate, possibly during virion release[1].

1.2.4 Phaeovirus ecology

In spite of the current practice of naming phaeoviruses according to the host they infect, a number of studies have shown that some cross-species infection can occur within this group. Some cause symptom-like deformities in the host in spite of being unable to produce infectious virions (EsV-1 infecting *F. simplex[149]*, or EfasV infection of *E. siliculosus[101]* and *M. clavaeformis[147]*). Indeed it has even been shown that a complete infection cycle of EsV-1 can occur in *Kuckuckia kylinii* (Cardinal) which produces virions that are infectious to the original host[150], so perhaps the host ranges of these viruses are not as clearly defined as is generally believed.

This host species plasticity has also been observed in cyanophage from the Gulf of Mexico[151] which was able to infect several different *Synechococcus* species, but not all of them. However, in the majority of cases it appears that each type of virus has its own individual host; for example, a virus observed to infect a *Vibrio* strain was not even able to infect other very closely related *Vibrio* species[22], the viruses of braconid wasps also have very specific host species[16] as do chloroviruses[82]. Moreover some viruses, such as the coccolithoviruses which infect *Emiliania huxleyi*, are even limited to infecting certain strains within the host species[129]. Indeed, there are some reports of catastrophic consequences for new host survival where emerging viruses cross species boundaries (for example, Human Immunodeficiency virus (HIV-1)[152], Ebola[153], bird flu (H_5N_1)[154] and honey-bee deformed wing virus

(DWV)[155]), and therefore the ability of phaeoviruses to infect a range of species without such consequences is unusual.

1.2.4.1 Phaeovirus prevalence

Studies of the prevalence of phaeovirus infection in natural host populations produce very different results depending on the technique used to detect the virus. As previously mentioned, PCR based techniques detecting integrated viral DNA showed that viruses were present in between 40-100% of natural *Ectocarpus* populations in the North Atlantic, Gran Canaria and Chile[105,108], whereas observations of physical symptoms are much lower, being up to 20% in a population of *E. siliculosus* on the coast of Brittany[98]. In the closely related alga *Ectocarpus fasciculatus*, symptoms were only observed in less than 10% of filaments examined in the field[79]. Each of these techniques have their own limitations; the high level of symptom suppression in these algae means that checking for overt symptoms will fail to detect all infected individuals, while the PCR based tests for one or more viral genes only guarantees that specific fragments of viral DNA are present and are not necessarily indicative of a fully functional virus. Nonetheless, these various techniques have revealed that phaeoviruses are very common, occurring in all sites where filamentous brown algae are found[80]. However, for a more accurate observation of their prevalence it would be necessary to combine PCR screening with laboratory

observations, with the addition of sequencing data to further our understanding of their population genetics.

1.2.4.2 Phaeovirus diversity

Analyses of the diversity and distribution of marine viruses generally do not include studies of phaeoviruses due to the lack of similarity between their sequences and other phycodnaviruses[75,76]; however it may be possible to draw some conclusions about phaeoviruses from studies into other viruses. DNA polymerase is often used as a single-gene screen for phycodnaviruses, and in spite of the limitations of using just one gene in such studies, it has been shown that DNA polymerase phylogenies do accurately reflect whole genome phylogenies, at least in *Micromonas pusilla* viruses[156]. Further screening and phylogenetic studies have used the major capsid protein (MCP) [75,80,81].

Short *et al*.[77] found the unexpected result that the DNA polymerase sequences obtained from North American freshwater lakes were more closely related to the marine phycodnaviruses of the genus *Prasinovirus* than to the freshwater genus *Chlorovirus*, although all sequences were still more closely related to phycodnaviruses than to the asfarviruses and mimiviruses. However, they did observe that the genetic distances between all the viruses in freshwater group II were more closely related to each other than EsV-1 is to FsV-158, suggesting that phaeoviral diversity is even greater than that of the

viruses in this study. Genomic comparison studies have shown that 24 genomes from four phycodnavirus genera contained over 1000 unique genes, with only fourteen being common to all genera[85].

In spite of recent trends towards sequencing marine viral metagenomes, these studies have so far failed to find any NCLDV genes within the virally enriched fractions, although NCLDV genes, including some core genes, were found in the fractions that were intended to have been enriched for bacteria[157]. It may be that new isolation techniques are necessary in order to study phycodnavirus, and thus phaeovirus, diversity on a large scale.

1.2.4.3 Multiplicity of phaeoviral infection

On a much smaller scale, the naming convention of phaeoviruses from their host species or strain is based on the assumption that each individual is only infected with one virus. This may not be the case since Ivey *et al*.[158] demonstrated that two (and potentially four) different size variants (158bp and 178bp) of a phycodnavirus were present in cultures of a *Feldmannia* species which originated from a single cell. The size class produced is dependent on temperature: 178kb at 5-10°C, both sizes at 15°C and 158kb at 20°C. They also demonstrated by Southern hybridization analysis that the different viruses have highly conserved sequences and that the differences between the sizes are likely due to duplications and/or deletions. Delaroque *et al.*[87] suggest that

this multiple infection may be the reason why they were unable to join up all the contigs when sequencing the virus from *Feldmannia irregularis*, FirrV-1.

1.3 Aims

Although there have been numerous investigations into the biology of phaeoviruses and their distribution in wild populations, many unknowns still remain. For example, screening methods to date rely on the detection of a section of a viral gene through PCR, with no detail on the type of virus being detected [75,80,81]; results are based on the assumption that the detected virus is the one that is being screened for. In addition, there are several viruses that have been observed and are assumed to be phaeoviruses, but no genetic evidence of this has been obtained [102,103,104,159]. The relationships of these viruses to the sequenced phaeoviruses could yield interesting results about their evolution. Furthermore, there is currently very little evidence of the existence of viruses in wider members of the family Phaeophyceae as stated in section 1.2.3.3, such as the more commercially important order Laminariales. The prevalence of viruses in the sea, and their ability to infect the majority of living organisms, suggests that other algae are likely to have their own viruses, which simply have not been yet identified.

Another gap in our knowledge of these phaeoviruses is what they actually do at a genetic level as described in section 1.2.3.6. We have several genome

sequences which allow us to draw some conclusions about important common genes; however, the majority of these genes are of unknown function.

The overarching objective of this study is to gain a more detailed understanding of phaeovirus biology - their genetic diversity, distribution, infection frequency and some of the genetic mechanisms involved in the infection process.

My specific objectives are to:

1) Determine the genetic diversity and phylogenetic relationships of phaeoviruses obtained from infected algal strains in culture, including both the well-studied phaeoviruses from EsV-1 and FirrV-1, as well as the additional viruses which are hypothesised to be phaeoviruses from their structure and life history. This should reveal much about the evolution of these closely related viruses.
2) Obtain a general overview of the active viral genes in an infected strain of *Feldmannia irregularis*, using next generation sequencing technology. This will provide a great deal of information both about the activity of viral genes and the nature of the infection.
3) Develop a cheap, high throughput diagnostic test using a PCR-based method in order to investigate both the presence and genetic variation in viruses present in environmental samples of brown algae. This test will then be used to rapidly screen algal samples for viruses, enabling an

understanding of the prevalence and distribution of these viruses in field populations.

CHAPTER 2 THE DIVERSITY AND EVOLUTION OF PHAEOVIRUSES INFECTING ECTOCARPOID ALGAE

2.1 Abstract

In this chapter, I use a combination of PCR, cloning and sequencing of three core phaeoviral genes (major capsid protein, DNA polymerase and superfamily III helicase) to demonstrate that individual laboratory maintained strains of *Ectocarpus siliculosus, E. fasciculatus, Feldmannia* sp. and *Feldmannia irregularis* contain multiple phaeoviral sequence variants, and moreover that these variants should be split into two subgroups based on sequence as well as genome size and host range, where previously phaeoviruses were assumed to be a single monophyletic group within the phycodnaviruses. One subgroup is conserved and was observed in three of the genera studied here, whereas the other subgroup is much more diverse but was limited to infecting the *Feldmannia* genus only. The difference in diversity between these two groups suggests a shift in evolutionary strategy of subgroup B from the currently accepted *r*-type strategy of the phaeoviruses towards a more *K*-type strategy due to increased mutation rates caused by a lack of proofreading exonuclease and DNA polymerase mutations in this subgroup. In addition, I confirm the identity of five additional putative phaeoviruses (EfasV-1, FlexV-1, PlitV-1, MclaV-1, and HincV-1), using single and multi-gene phylogenies of the three NCLDV core single copy genes mentioned above.

2.2 Introduction

Viruses display various life-history strategies, from the highly virulent, short-lived *r*-selected or acute viruses to the more stable *K*-selected or persistent viruses[33]. Broadly speaking, the *r*-selected viruses are characterised by high mutation rates and rapid reproductive strategies, infecting hosts which also adopt an *r*- like strategy, as typified by bacteria and their phage[40]. At the other end of the spectrum are the *K*-selected viruses, which tend to cause persistent infections in longer-lived, multicellular organisms, often integrating their genomes with that of the host as in the case of herpesviruses and their mammalian hosts, generally forming a stable relationship and only causing significant host mortality when outside their usual host range[40].

The majority of known algal viruses (family Phycodnaviridae) follow the acute lytic *r*-selected strategy and as such include many important algal bloom terminators such as the coccolithoviruses [45,160,161]. The only exception are the phaeoviruses[1,88,91] that integrate their genomes with that of the host to form a latent provirus and have very little impact on the host other than the transmission of its genome to all host cells during normal cellular division[97]. As such they are the only known example of *K*-selected or persistent viruses in the family Phycodnaviridae[40], probably because their multicellular hosts are much longer lived and provide a more stable environment than the unicellular bloom forming algae which are infected by the other phycodnaviruses.

To date, phaeovirus identity has been confirmed only by genome sequencing for viruses infecting three species of filamentous brown algae: *Ectocarpus siliculosus* (Dillwyn) Lyngbye, *Feldmannia* sp. and *Feldmannia irregularis* (Kützing) Hamel infected by EsV-1[130], FsV-158[125] and FirrV-1[103], respectively. In addition, the genome of an *Ectocarpus* strain was found to contain a transcriptionally inactive copy of an EsV-1-like phaeovirus which is potentially a relic from an ancient viral infection [91]. These genomes vary in size from 180 – 336 kb [1,87,88] (see Table 2.1), and have been found to contain a limited number of common single copy core genes[88], as well as many unique genes. It is generally accepted that these viruses have a narrow host range, with each virus having its own host and each host having only one virus; EsV-1 is the only virus known to infect *E. siliculosus*, and Delaroque *et al*.[87] reported the FirrV-1 genome with no evidence of multiple viruses within *F. irregularis*.

A number of studies have shown that some cross-species infection of virus-free host gametes/spores by isolated phaeovirus particles can occur, some causing symptom-like deformities in the host in spite of being unable to produce infectious virions (EsV-1 infecting *F. simplex* [149], or EfasV-1 infecting *E. siliculosus* [101] and *M. clavaeformis* [147]). Indeed it has even been shown that a complete infection cycle of EsV-1 can occur in *Kuckuckia kylinii* (Cardinal) which produces virions that are infectious to the original host [150]. So perhaps

the host ranges of these viruses are not as clearly defined as is generally believed.

Other potential phaeoviruses have been identified, based solely on their microscopically observed morphology, life cycle and symptoms produced. These infect *Ectocarpus fasciculatus* (Harvey)[101], *Feldmannia simplex* (Crouan & Crouan) Hamel[102], *Hincksia hincksiae* (Harvey) Silva[103], *Pylaiella littoralis* (Linnaeus) Kjellman[104] and *Myriotrichia clavaeformis* (Harvey)[103]; the naming of these viruses (EfasV-1, FlexV-1, HincV-1, PlitV-1, MclaV-1) reflects the host species in which they were originally found. However, in contrast to the currently accepted belief that each host species has its own unique phaeovirus, Ivey *et al*.[158] reported two (and potentially four) different size variants (158kb and 178kb) of phaeoviruses present in cultures of *Feldmannia* sp.

This chapter aims to confirm the identity of the five potential phaeoviruses mentioned above from the strains in which they were originally identified, by sequencing and phylogenetic analysis of three core phaeoviral genes encoding major capsid protein (MCP), DNA polymerase and superfamily III helicase. In addition, I aim to determine the phylogenetic relationships between these and the remaining sequenced phaeoviruses. (EsV-1, FirrV-1, FsV-158 and the Ectocarpus provirus).

This approach was successful in confirming that the additional five viruses did indeed belong to the phaeoviruses. Moreover, it revealed a huge diversity of

phaeovirus sequences, both within individual algal strains as well as within the phaeovirus group as a whole, revealing that the phaeoviruses could be subdivided into two subgroups based on their phylogenetic relationships, as has previously been suggested based on their genome size and membrane composition [74].

Table 2.1: Ectocarpoid strains used for phaeovirus screening.

Strain code	Species	Family	Location	Date collected	Reported virus genome size (kb)	Ref	Number of sequence variants of virus genes found in this study*			Possible concatenation permutations (DNApol +MCP) for Figure 2.5
							DNApol	MCP	Helicase	
Esil CCAP1310/48	*Ectocarpus siliculosus*	Ectocarpaceae	Kaikoura, New Zealand	Aug-88	336	[162]	1 (1)	1 (1)	1 (1)	1
Efas CCAP1310/20	*Ectocarpus fasciculatus*	Ectocarpaceae	Plouescat, Brittany	Mar-93	320	[163]	2 (2)	1 (1)	2 (2)	2
Plit	*Pylaiella littoralis*	Acinetosporaceae	Savoonga, St. Lawrence Island, Alaska	May-96	280	[148]	1 (1)	1 (1)	1 (1)	1
Hinc	*Hincksia hincksiae*	Acinetosporaceae	Plouescat, Brittany	Mar-93	240	[163]	1 (1)	-	2 (1)	-
Mcla CCAP1325/1	*Myriotrichia clavaeformis*	Chordariaceae	Las Grutas, Argentina	Jan-95	320	[159]	1 (1)	2 (2)	-	2
Firr	*Feldmannia irregularis*	Acinetosporaceae	Isla Hierro, Canary Islands	Jun-95	180	[94]	2 (2)	3 (2)	2 (2)	4
Flex	*Feldmannia simplex*.	Acinetosporaceae	Killleany Bay, Aran Islands, Ireland	Sep-90	220	[102]	9 (8)	6 (4)	8 (3)	22

*: variant in DNA sequence with amino acid variation indicated in parentheses. A negative PCR result is indicated by a minus symbol**.**

**CCAP, Culture Collections of Algae and Protozoa (marine) reference number, Dunstaffnage Marine Laboratory, Oban, Scotland.

2.3 Materials and Methods

2.3.1 Strains

See Table 2.1 for a list of the phaeovirus-infected cultures used in this study. These are the same infected strains which were used to generate the genome sequences of EsV-1 and FirrV-1, as well as those in which EfasV-1, PlitV-1, MclaV-1, HincV-1 and FlexV-1 were originally described.

2.3.2 Culture conditions

Each strain was cultured in a 40ml petri dish at 15°C, 16:8 light-dark cycle, approximately 100 µmol photons m^{-2} s^{-1}. Culture medium was filtered (30kDa) natural sea water from the L4 sampling station close to the Eddystone Lighthouse near Plymouth, enriched with Provasoli's enrichment [164]. Sub-culturing into a new dish with fresh media was carried out every 14 days, when the cultures were pulled apart using forceps to separate out filaments in order to encourage production of zoidangia and virions.

2.3.3 DNA extraction method

50 – 200 mg wet weight fresh algal material was transferred to an Eppendorf tube, frozen in liquid nitrogen and ground using Eppendorf grinders with 10µl saturated ≤106 microns acid washed glass bead solution before proceeding with

the Qiagen DNeasy protocol for Genomic DNA purification from cultured animal cells, starting with the proteinase K treatment. 40µl proteinase K and 200µl Buffer AL were added to the sample and incubated at 56°C for 30 minutes, before centrifuging for 2 minutes at maximum speed to separate out the beads. 200µl ethanol was added to the resulting supernatant, vortexed and pipetted onto the spin column, to proceed with the first centrifugation step. For the final step, DNA was eluted using 100µl water, instead of 200µl in order to obtain a more concentrated sample.

Table 2.2: Primers used for PCRs

Primer name	Sequence	Gene	Product size (bp)
PAVS For1.1	GRGGNCAGCAGATYAAGTG	DNA polymerase	643
PAVS Rev1.1	GARTCCGTRTCSCCRTA	DNA polymerase	643
vMCP_F4	CVGCGTACTGGGTGAACGC	Major Capsid Protein	268
vMCP_R3	AGTACTTGTTGAACCAGAACGG	Major Capsid Protein	268
vhelic_F	GTGGCAGGTSATYCCYTTC	Helicase	303
vhelic_R	GTTKCCGGCCATGATYCC	Helicase	303

2.3.4 Primers used

Degenerate primers were designed for three active viral genes encoding DNA polymerase, helicase and major capsid protein (MCP) (See Table 2.2). All three sets of primers were designed against a consensus of published sequences from

EsV-1, FirrV-1, FsV-158 and the provirus from the sequenced *Ectocarpus* genome.

2.3.5 PCR conditions and product purification

Degenerate PCR was carried out using Promega GoTaq® Flexi DNA polymerase kit according to the manufacturer's instructions, with an addition of 0.8mg/ml bovine serum antigen (BSA). Cycling conditions were 95°C for 5 minutes, followed by 35 cycles of 95°C for 1 minute, a 30 second annealing step, an extension step at 72°C, and a final elongation step at 72°C for 10 minutes (see

Table 2.3 for oligonucleotide and magnesium concentrations, annealing temperatures and extension times). Post-PCR samples were run on a 2% agarose gel at 80V to achieve maximum separation between the bands. Samples with more than one product were purified by gel extraction; the band of the correct size was cut out of the gel and purified using the Qiaex II® Gel Extraction Kit according to the manufacturer's instructions. Samples with clean bands were purified using GenElute™ PCR Clean-Up Kit from Sigma according to the manufacturer's instructions.

Table 2.3: PCR conditions

	[Mg^{2+}] (mM)	[Oligonucleotide] (pmol/µl)	Annealing temperature(°C)	Extension time (s)
DNApol	1.25	4	50	10
MCP	1.5	8	55	30
Helicase	1.5	8	55	10

2.3.6 Cloning and sequencing

Purified PCR product was cloned into pCR®2.1 vector according to the manufacturer's instructions, incubated overnight at 15°C before storing at -20°C until used. 4µl ligation mixture was added to 0.2ml competent cells and mixed. The cells were then incubated on ice for 40 minutes, heat shocked at 42°C for 2 minutes and returned to the ice for 5 minutes. 0.7ml pre-warmed LB medium was added to the cells which were then incubated at 37°C for one hour. The cells were concentrated by spinning at 8000g for 5 minutes, removing 0.5ml supernatant, and re-suspended gently with a pipette before being plated out onto LB agar plates containing 5µg/ml ampicillin, with 40µl of 20 X-gal spread on each plate. Plates were incubated overnight at 37°C.

Single cloned colonies were picked from agar plates into a 0.2ml tube containing 5µl molecular grade water and heated to 95°C for 5 minutes to denature the cells before adding 10µl 5x buffer, 5µl 25mM $MgCl_2$, 5µl 2.5mM dNTPs, 2µl

each of 10 pmol/µl M13 forward and reverse primers, 0.2 µl Taq polymerase, 20.8 µl molecular grade H_2O. Cycling conditions consisted of 30 cycles of 95°C for 45 seconds, 56°C for 45 seconds and 72°C for 45 seconds, followed by a final extension step of 72°C for 5 minutes.

PCR products were purified using the Qiaex II® Gel Extraction Kit and then sequenced using the BigDye® Terminator v3.1. The mix consisted of 3.5µl 5x BigDye buffer, 1µl Ready Reaction Mix, 2µl template (6 – 14 ng μl^{-1} concentration), 1µl primers (either M13 forward or reverse) at a concentration of 3.2pmol μl^{-1} and 12.5 µl dH_2O. Cycling conditions were 95°C for 2 minutes, followed by 30 cycles of 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 30 seconds, then a final elongation at 72°C for 5 minutes. Sequenced reactions were precipitated by adding 5µl 125mM EDTA and 65 µl cold 100% ethanol and incubated in the dark at room temperature for 15 minutes. They were then spun for 30 minutes at 2200g, the supernatant removed and the pellet washed with 60 µl cold 70% ethanol, and spun for a further 15 minutes at 2200g. The supernatant was removed again and the pellet air dried. Sequencing was carried out by Source Bioscience in Cambridge. Sequences were submitted to the European Nucleotide Archive with accession numbers (HG003317 – HG003355).

2.3.7 Single gene tree production

Initial alignments were carried out in BioEdit using the ClustalW alignment method, followed by a manual check of any unresolved areas, then forward and reverse sequences were combined into one read for each clone. Any clones that were uniquely different by four or fewer single nucleotides were ignored as being due to experimental variation. Translation to amino acid sequence was carried out using the ExPASY translate tool http://web.expasy.org/translate/, removing any containing stop codons, followed by further removal of any identical amino acid sequences post translation. Alignment was carried out using the MergeAlign website http://mergealign.appspot.com/[165,166], and then manual deletion of any unresolved areas around gaps. Bayesian analysis of phylogenetic trees was carried out using MrBayes v3.2.1, running the analysis until the standard deviation of split frequencies reached <0.01 and the number of generations was > 100 000. Maximum Likelihood analysis was carried out using MEGA5.05 with 500 bootstrap replications, using the Jones-Taylor-Thornton model and Nearest-Neighbour-Interchange heuristic method. Where the topology from these two methods agreed, combined posterior probability/bootstrap values are indicated. Where topologies differed, posterior probability was used on the main tree based on Bayesian analysis, and the inset tree shows the Maximum Likelihood topology.

2.3.8 Multiple gene phylogeny

Since the presence of multiple sequence variants of each gene means it is impossible to accurately predict which variants of each gene belong together, gene concatenations were carried out allowing for all possible combinations of variants. Only the major capsid protein and DNA polymerase gene were used for this, since the polyphyletic nature of the helicase gene[37] in phaeoviruses led us to discount it as being useful for this study. These concatenations were then aligned to produce large multi-gene phylogenies from both the Bayesian and Maximum Likelihood analyses. Where multiple Flex variants grouped closely together, these branches were collapsed and the number of variants indicated.

2.3.9 Distance analysis

Nucleotide sequences were obtained for the various groups of phycodnaviruses that have been sequenced to date by carrying out a BLAST search of known genome sequences from each group. The phaeovirus sequences obtained in this study were split into two subgroups according to their phylogenies as shown in sections 2.4.2 and 2.4.3. Chloroviruses, being the only green algal viruses, were considered as a separate group, and the remaining viral groups (coccolithoviruses, prymnesioviruses, prasinoviruses, raphidoviruses) were considered together since they are all lytic viruses of stramenopiles. Pairwise distances were computed using Mega 5.05.

2.4 Results

2.4.1 DNA extraction and PCR conditions

Total genomic DNA was extracted from each of the phaeovirus infected strains, as shown in Table 2.1. Various PCR conditions were tested in order to determine the optimal concentrations of oligonucleotide and magnesium, as well as the annealing temperature and extension time for the most reliable PCR amplification of the different genes (

Table 2.3).

It was not possible to obtain sequences for all genes from all strains screened (Table 2.1). This is most likely to be because the degeneracy of the primers was insufficient to amplify the more distantly related viruses, since the primers were designed against the known sequenced *Ectocarpus* and *Feldmannia* virus genomes, all of which did amplify.

2.4.2 Cloning and sequencing

Direct sequencing of many PCR amplicons produced unresolved sequence at many positions; therefore, the PCR products were cloned before sequencing in both directions using M13 primers. Sequences for all three genes tested revealed the presence of multiple sequence variants within most, but not all, strains (Table 2.1).

The sequence alignment for DNApol (Figure 2.1a) shows viral protein sequences from EsiI matched perfectly with reference gene sequences for EsV-1. The FirrV-1 DNApol sequence was also confirmed within the *Feldmannia irregularis* isolate; however, at least one other additional variant could also be identified (Table 2.1). Most of the other ectocarpoid strains contained two or more viral sequence variants, with the *Feldmannia simplex* (Flex) isolate containing at least eight variants which differ from each other in at least five nucleotide positions (Table 2.1). These trends (one EsiI variant, two Firr variants, and multiple

variants in some of the other strains) were also repeated in the MCP (Figure 2.1b) and helicase sequences (Figure 2.1c), albeit with fewer Flex variants.

The DNApol alignment suggests that the phaeovirus variants sequenced here can be subdivided into two groups (hereupon referred to as subgroup A and subgroup B) according to their shared sequence variations. Furthermore, it appears that the Flex 8 variant shares features with both of the subgroups, whilst, unsurprisingly, being more closely related to subgroup B (Figure 2.2) since this subgroup only appears to infect members of the *Feldmannia* genus. A closer look at the DNApol sequence (Figure 2.1a) shows not only the high degree of amino acid conservation (32% are identical) across all the phaeoviruses, but also how certain amino acids in the Flex 8 variant can be assigned to belong to either subgroup A (5 - triangles) or subgroup B (10 - inverted triangles). The MCP and helicase sequences are more conserved than the DNApol sequence (66% and 57% identical amino acids, respectively), but the division between the subgroups can still be seen, and where the two subgroups differ, the “Flex 8” variant tends to share the amino acid with subgroup A for both of these genes; 5 out of 5 for MCP and 10 out of 11 for helicase (Figure 2.1).

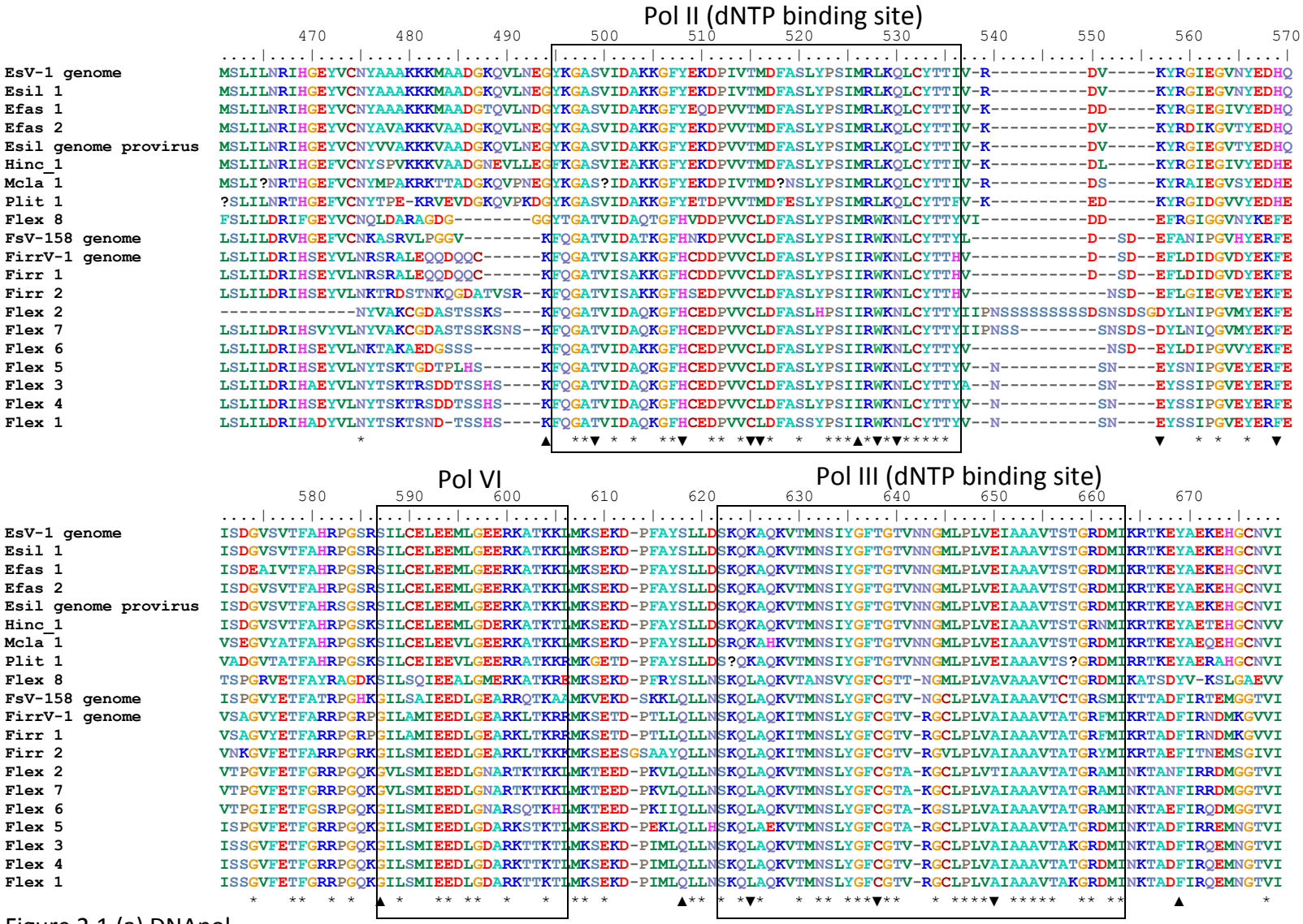

Figure 2.1 (a) DNApol

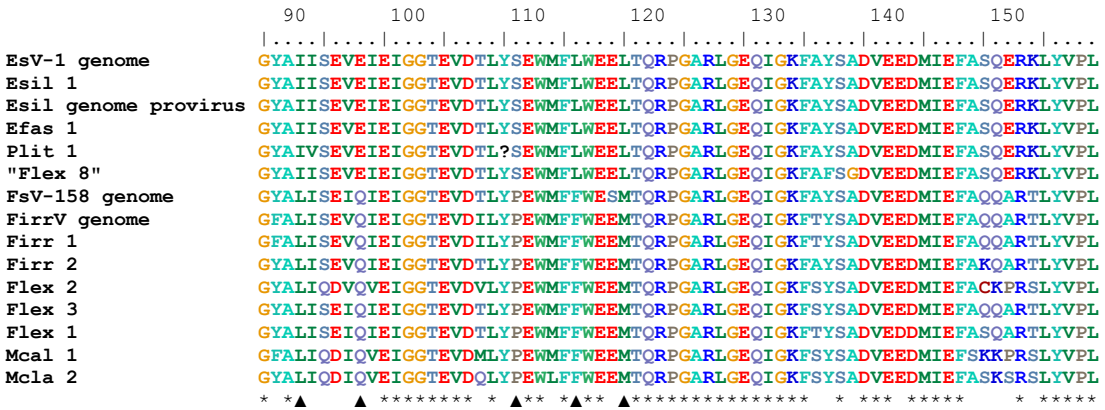

Figure 2.1 (b) MCP

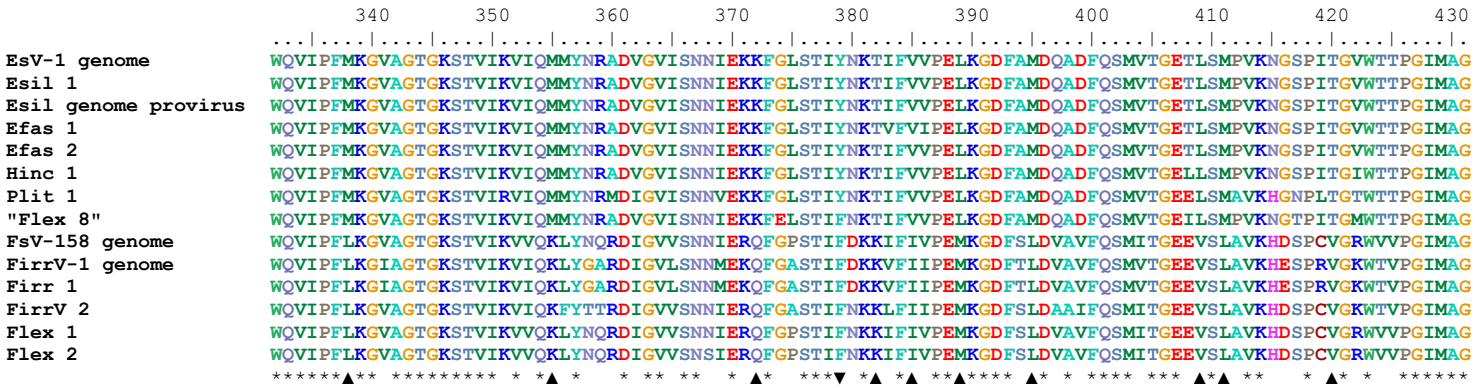

Figure 2.1 (c) helicase

Figure 2.1: Partial predicted amino acid alignment of cloned fragments of the viral (a) DNA polymerase, (b) Major capsid protein and (c) helicase genes. Numbers refer to amino acid position in the complete EsV-1 DNApol, MCP or helicase genes respectively taken from Delaroque *et al*. 2001[1] (GenBank accession numbers NC_002687.1, NP_077601.1 and NP_077594.1). Boxed regions in the DNApol gene indicate conserved polymerase domains[12]. * indicates conserved positions between all sequences, ▲ shows where the Flex 8 variant shares an amino acid with the larger viruses of subgroup A, ▼ shows where the Flex 8 variant shares an amino acid with the smaller genomed viruses of subgroup B.

2.4.3 Phylogenetic analysis

For the most part, the Bayesian and Maximum Likelihood inference trees confirm the observations from the alignments (Figure 2.2, Figure 2.3, Figure 2.4 and Figure 2.5); the phaeovirus sequence variants group into two distinct subgroups, forming a virus subgroup A that infects multiple species across three Ectocarpales families. A second subgroup B contains members infecting the genus *Feldmannia*, demonstrating much greater sequence variation than in the first subgroup. In addition, there is also a corresponding grouping which can be created based on genome sizes (Table 2.1); the larger viral genomes from Esil, Efas, Plit, Mcla and Hinc (240-336kb) fall within subgroup A and the smaller viruses from Firr, Flex and FsV-158 (158-220kb) within subgroup B.

However, there are two notable differences between the DNApol and other phylogenies. Firstly, the MCP alignment Figure 2.1 (b) shows the two Mcla sequences belonging to subgroup B whereas the DNApol alignment shows it belongs to subgroup A. It was not possible to successfully amplify and clone the Mcla helicase fragment which would have assisted in the placement of the Mcla viral sequences. Similarly amplification of the Hinc helicase fragment was unsuccessful. In addition, the intermediate position of the “Flex 8” variant from the amino acid sequence alignments is confirmed in all phylogenies. In the DNApol phylogeny it is clearly positioned between the two subgroups, as is also the case with the concatenated phylogeny. However, both the MCP and

helicase phylogenies show “Flex 8” positioned within subgroup A which agrees with the amino acid conservation shown in Figure 2.1 b & c.

The position of the *E. siliculosus* genome provirus also varies slightly according to the gene being analysed; DNApol (and also the concatenated tree) indicates a greater degree of homology with one of the Efas variants (Efas2) than with the EsV-1 genome sequence. MCP and helicase sequences are much more conserved and therefore such a grouping is not obvious in their phylogenies.

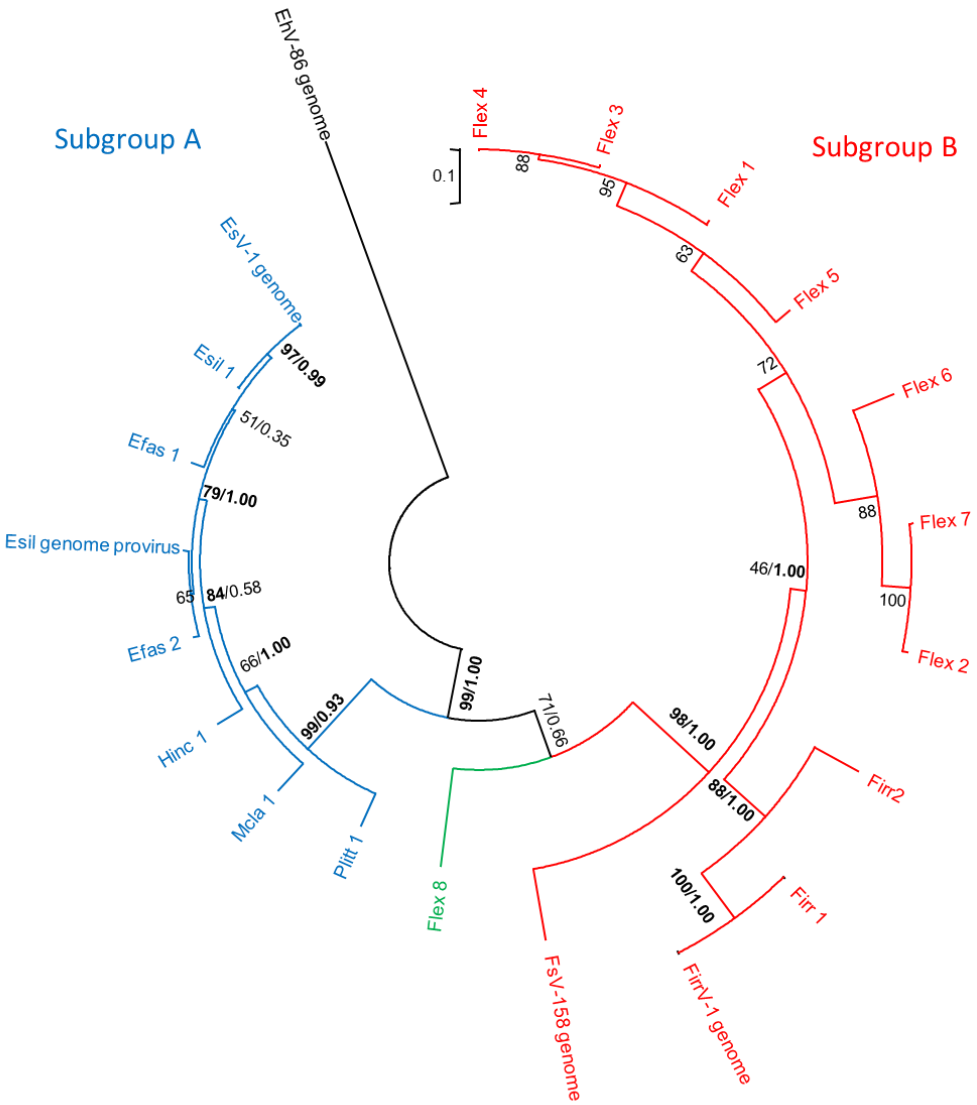

Figure 2.2: Maximum Likelihood analysis of the phylogenetic relationship between variants of the predicted phaeoviral amino acid sequences of DNA polymerase, with EhV-86 being used as an outgroup. Single value node labels represent ML bootstrap values. Where nodes are labelled with two values, this indicates that both ML and Bayesian topologies agree (whole numbers represent ML bootstrap values, decimals indicate Bayesian posterior probability). Subgroup A viruses are labelled in blue, subgroup B viruses are red and the intermediate Flex virus variant is green. Bold values are those greater than 75% bootstrap or probability.

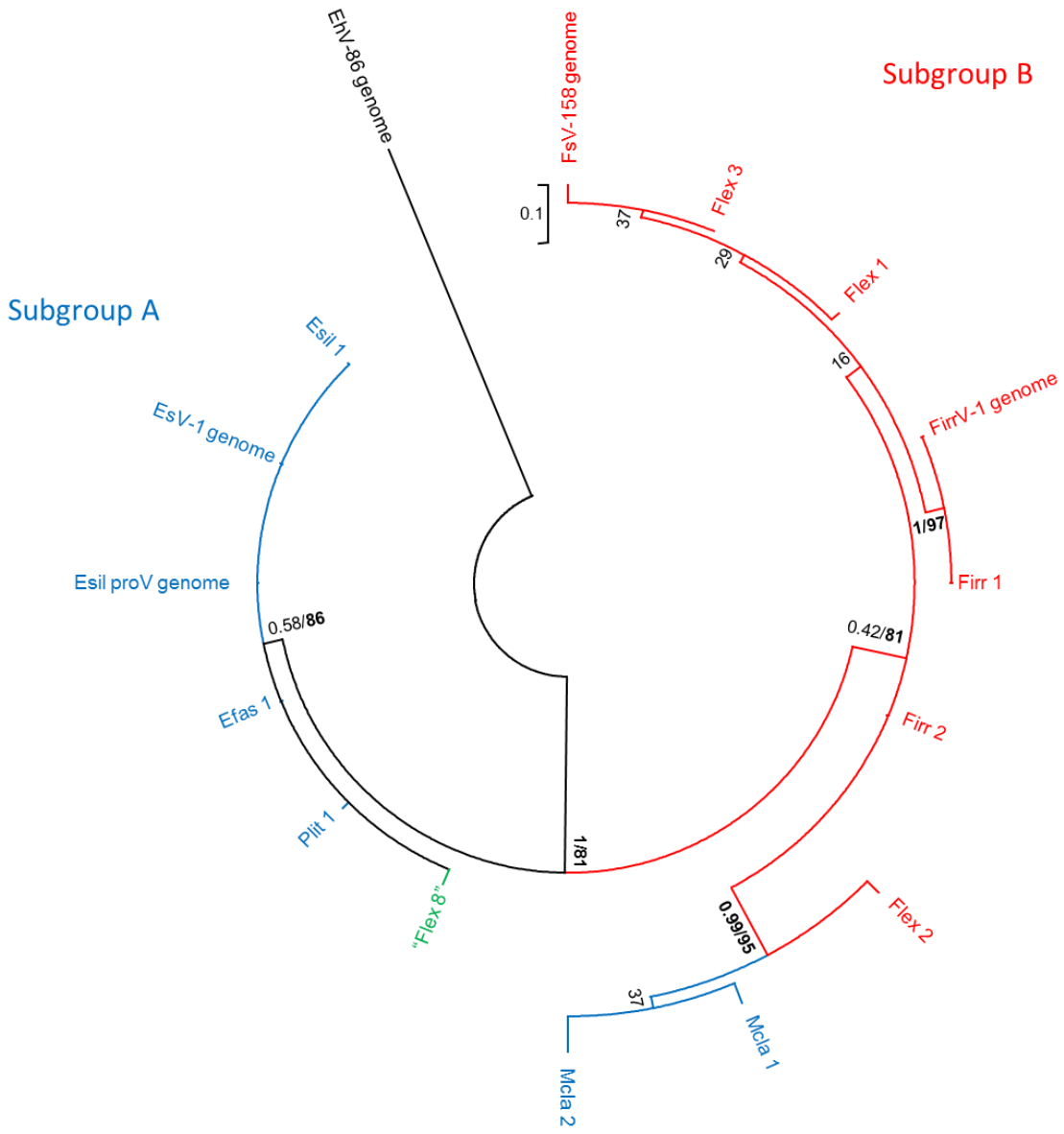

Figure 2.3: Maximum Likelihood analysis of the phylogenetic relationship between variants of the predicted phaeoviral amino acid sequences of major capsid protein, with EhV-86 being used as an outgroup. Single value node labels represent ML bootstrap values. Where nodes are labelled with two values, this indicates that both ML and Bayesian topologies agree (whole numbers represent ML bootstrap values, decimals indicate Bayesian posterior probability). Subgroup A viruses are labelled in blue, subgroup B viruses are red and the intermediate Flex virus variant is green. Bold values are those greater than 75% bootstrap or probability.

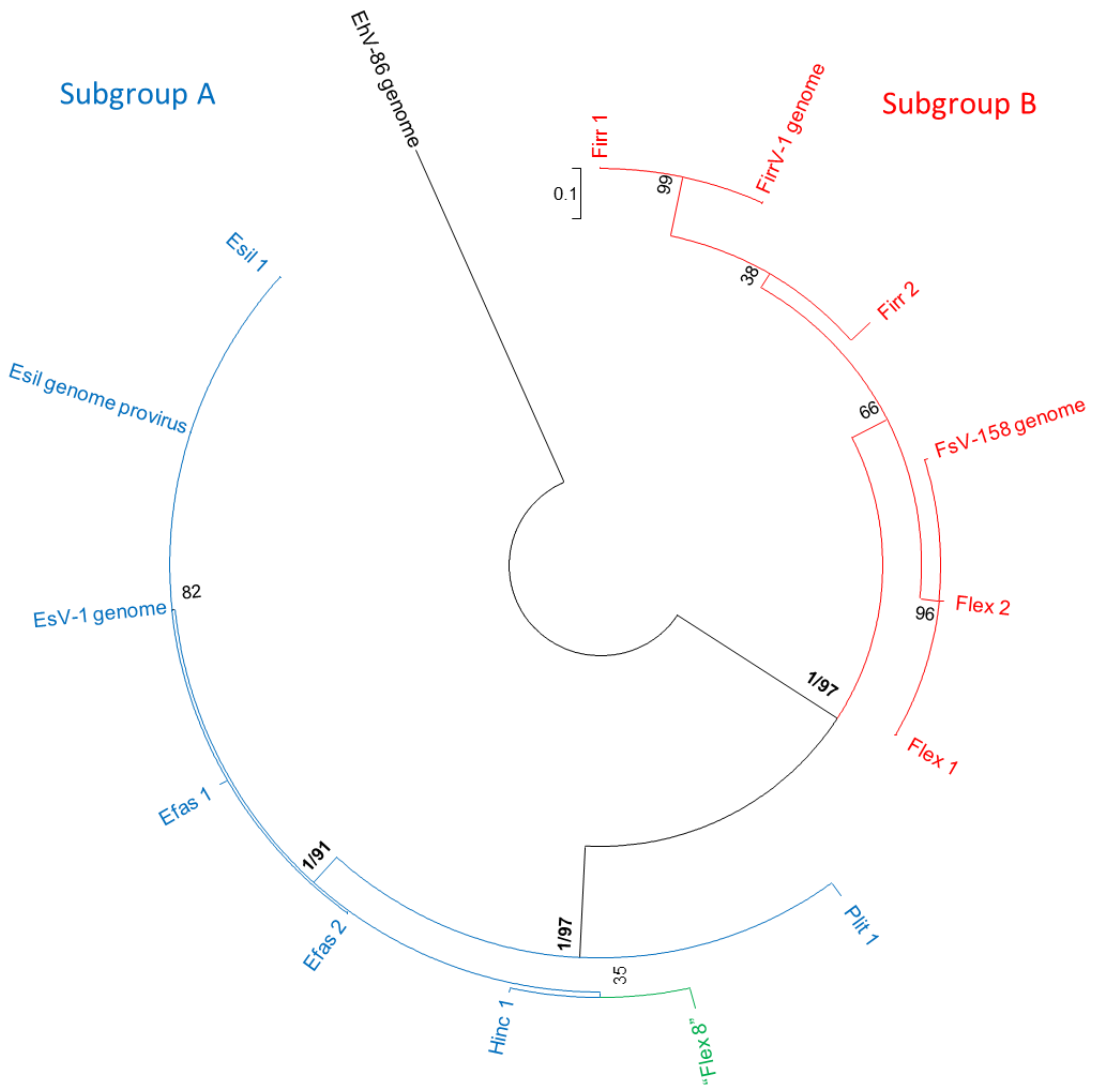

Figure 2.4: Maximum Likelihood analysis of the phylogenetic relationship between variants of the predicted phaeoviral amino acid sequences of superfamily III helicase, with EhV-86 being used as an outgroup. Single value node labels represent ML bootstrap values. Where nodes are labelled with two values, this indicates that both ML and Bayesian topologies agree (whole numbers represent ML bootstrap values, decimals indicate Bayesian posterior probability). Subgroup A viruses are labelled in blue, subgroup B viruses are red and the intermediate Flex virus variant is green. Bold values are those greater than 75% bootstrap or probability.

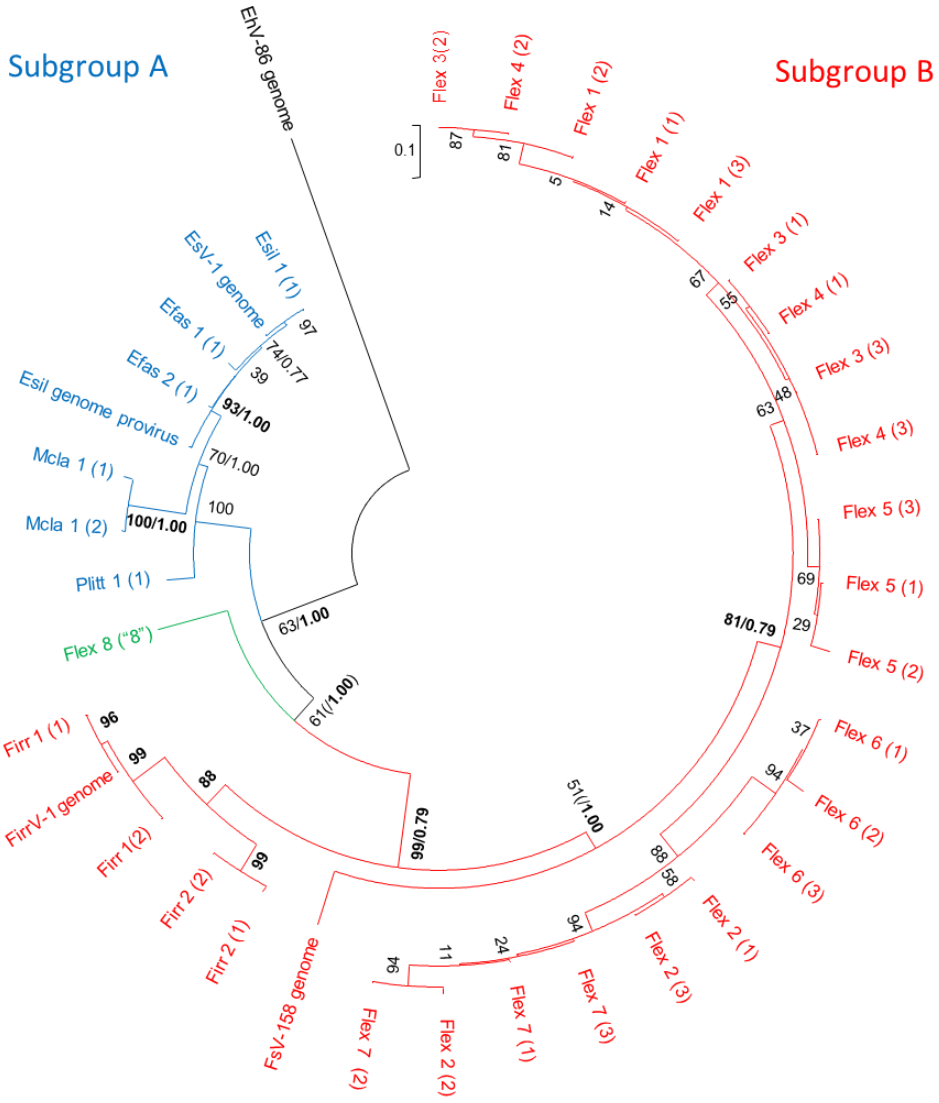

Figure 2.5: Maximum Likelihood analysis of the phylogenetic relationship between variants of the predicted phaeoviral amino acid sequences of concatenations of DNA polymerase and Major Capsid Protein, with EhV-86 being used as an outgroup. Variants are labelled according to DNApol identifier initially, followed by the MCP variant number in brackets. In order to slightly reduce the number of combinations of sequences, where individual gene phylogenies show a clear separation of individual variants, these are concatenated together and excluded from the other combinations. Single value node labels represent ML bootstrap values. Where nodes are labelled with two values, this indicates that both ML and Bayesian topologies agree (whole numbers represent ML bootstrap values, decimals indicate Bayesian posterior probability). Subgroup A viruses are labelled in blue, subgroup B viruses are red and the intermediate Flex virus variant is green. Bold values are those greater than 75% bootstrap or probability.

2.4.4 Distance analysis

Due to the dependence on sequences in the public databases on previous studies, the DNApol alignment for chloroviruses and stramenopile viruses were relatively easy to assemble with large numbers of isolates; however, MCP and helicase are much less studied and therefore were represented by far fewer sequences. MCP was also much more difficult to align correctly due to its high divergence among the stramenopiles, consisting of short conserved regions separated by highly variable regions.

The analysis of evolutionary divergence for the DNApol gene within the various phycodnavirus groups (Figure 2.6) revealed that the *r*-selected lytic viruses in the chlorovirus and general stramenopile groups have a much higher divergence than the phaeovirus subgroup A, whilst subgroup B is clearly more divergent than A, and falls within the values of the lytic viruses of chlorella and *r*-selected stramenopile viruses. For the purposes of the figure, the Mcla MCP sequence was included with subgroup B based on the phylogenetic grouping in Figure 2.3. When it was included in subgroup A, the divergence increased, becoming much closer to the other viral groups.

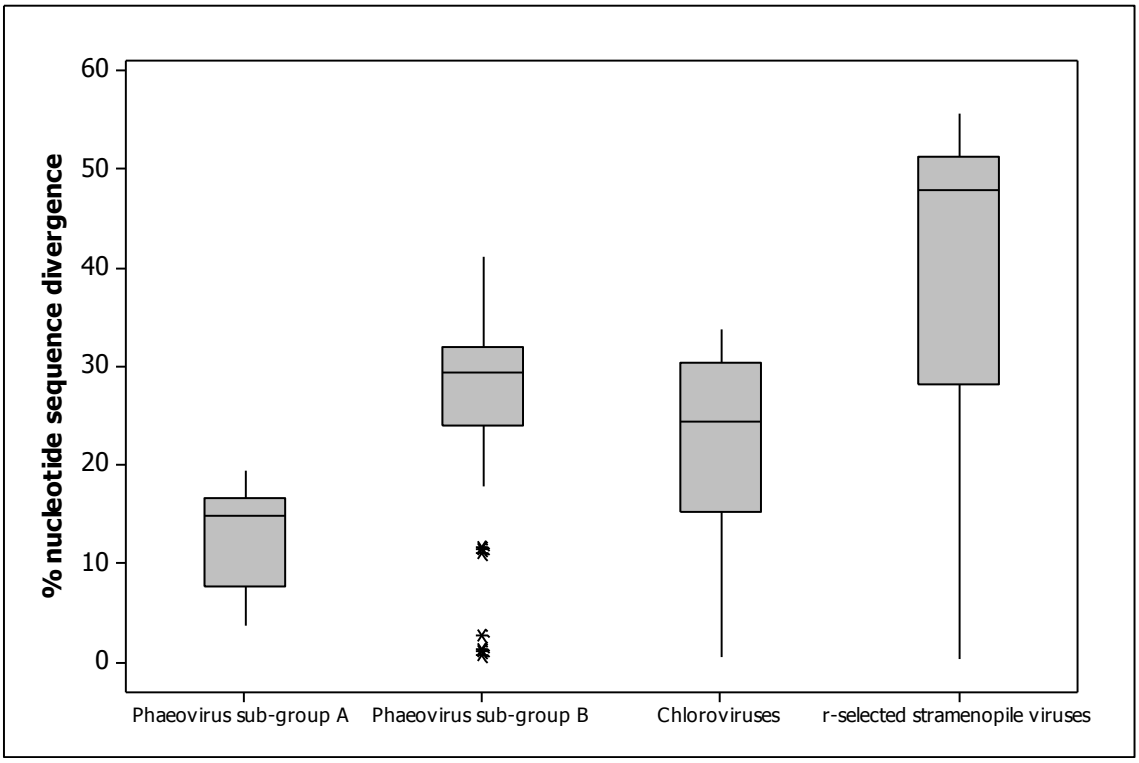

Figure 2.6: Box and whiskers plot of evolutionary divergence between nucleotide sequences of the DNApol fragment used in this study for the various virus groups infecting algae sequenced to date, showing the percentage base pair difference. Identical sequences were not included more than once. The box represents the interquartile range which shows the middle 50% of the data, the bottom line being the first quartile, the middle line being the median and the upper line being the third quartile. The whiskers represent the maximum (or minimum) data point up to 1.5 times the box height above (or below) the top (or bottom) of the box. Outliers beyond the whiskers are shown as a *. Phaeovirus sub-groups are as shown in Figure 2.2, Figure 2.3, Figure 2.4 and Figure 2.5, with Flex 8 being included in sub-group B. Chloroviruses consist of thirteen viral isolates from *Paramecium bursaria* Chlorella (AF344202, AF344203, AF344211, AF344212, AF344215, AF344226, AF344230, AF344231, AF344235, AF344238, AF344239, M86837, U32985) and one from *Acanthocystis turfacea* Chlorella (AY971002). The r-selected stramenopile group consists of three viral isolates from *Emiliania huxleyi* (AF453961, AF453867, AF472534), three from *Micromonas pusilla* (U32975, U32982, U32976), five from *Ostreococcus tauri* (FJ67503, FJ884758, FJ884763, FJ884773, FJ884776), two from *Ostreococcus lucimarinus* (GQ412090, GQ412099), six from *Phaoecystis globosa* (A345136-AY345140, DQ401030), one from *Chrysochromulina brevifilum* (U32983), one from *Chrysochromulina ericina* (EU006632) and one from *Heterosigma akashiwo* (AB194136).

2.5 Discussion

The results reported in this chapter strongly suggest that the phaeoviruses should be further divided into two additional subgroups as shown in Figure 2.2. The combined evidence of the amino acid sequence alignments and phylogenetic trees, as well as the nucleotide divergence analyses suggest that the two subgroups may have developed different evolutionary strategies: subgroup A being highly conserved across three genera (*Ectocarpus*, *Pilayella* and *Hincksia*) from two families (Ectocarpaceae and Acinetosporaceae) and subgroup B being much more divergent whilst being limited to infecting one genus, *Feldmannia*.

The gene encoding DNApol is the generally accepted barcoding gene equivalent for NCLDVs, so it is reassuring that the viral DNApol gene sequences from Esil matched perfectly with the reference gene sequence for EsV-1 (Figure 2.1a) and that no additional sequence variations could be found in the Esil strain for any of the genes sequenced in this experiment. This increases our confidence that the sequence variants found in the other algal strains are true sequence variants and not the result of the PCR and cloning procedures. The observation of at least one additional variant in the *Feldmannia irregularis* strain (Firr) where these have not previously been reported is the likely explanation for the inability of Delaroque *et al*. [87] to assemble the FirrV-1 genome, since polymorphic regions would impede assembly.

Daee *et al*.[4] found that a single amino acid mutation (R696W) in the pol III region of DNApol resulted in extreme rates of spontaneous mutation in yeast due to vastly reduced DNA synthesis fidelity. The three amino acid polymorphisms observed here in this region (Figure 2.1a, positions 618, 621, 625) could be a contributing factor to the large number of variants observed amongst the *Feldmannia* viruses due to a decrease in DNA replication fidelity, since this region affects the partition between polymerase and proofreading domains[4].

Another key observation is that virus variant Flex 8 is likely to be the progenitor virus to the *Feldmannia* subgroup B viruses since it seems to be an intermediate between the two subgroups, which therefore gives us a unique insight into the emergence of a new phaeovirus subgroup probably as a result of the genome reduction of an ancestral member from subgroup A from genome sizes between 280-336 kb down to between 180-220 kb in subgroup B. Both FirrV-1[87] and FsV-158[88] reported the loss of the DNA proofreading exonuclease gene (EsV-126) known to be present in EsV-1. This, in conjunction with the modifications in DNA polymerase Pol III region, may have resulted in the key life strategy shift, thereby utilizing the high mutation rates more associated with *r*- selection. If the DNApol mutations are the driver for further mutations across the viral genomes, it follows that the other genes would be more conserved, and this appears to be the case for the MCP and helicase genes studied here with amino

acid conservation being 66% and 57% respectively, compared to 32% for DNApol.

It is already known that phaeoviruses have the potential for cross-species infection as mentioned previously (Section 1.2.4). Here it can be seen that, for DNApol at least, the provirus from the *Ectocarpus* genome [91] appears to be more closely related to an *E. fasciculatus* variant than the EsV-1 (Figure 2.2). This suggests an *E. fasciculatus* virus infected an *Ectocarpus* species more closely related to *E. siliculosus[167]*.

M. clavaeformis is the only representative of the family Chordariaceae observed to be infected by a virus to date and, therefore, it is not surprising that its virus seems to have a different evolutionary history. A cross-species infection of the ancestral strain by different viruses from both subgroups may explain its phylogeny being different depending on gene (subgroup A for DNApol, B for MCP). Nonetheless, this study confirms the life-history and morphometric data that all of the viruses infecting Efas, Mcla, Plit and Hinc do indeed belong in the phaeovirus group.

In screening the genomes of a further five ectocarpoid species known to be infected by phaeoviruses that are believed to belong to this group, due to their structure and life-history, a remarkable diversity of viral gene sequence variants has been revealed, especially amongst the smaller phaeoviral genomes.

To our knowledge, this is the first study to show a multiplicity of virus infections by different members of a monophyletic group of viruses within a single host genome - synonymous with, but nonetheless atypical of, an *r*-selection strategy. Moreover, both multigene and individual gene phylogenies infer that the sequenced variants can be separated into two subgroups: the first represents the phaeoviruses with larger genomes found within all families of filamentous brown algae tested and a second subgroup of viruses with smaller genomes that, whilst showing the greatest intrinsic genetic diversity, is restricted to one genus of filamentous brown algae. The only exceptions are the Mcla MCP sequence which more closely resembles that of subgroup B than A, and the variant Flex 8 which shares features from both these subgroups. It seems unlikely that the intermediate position of Flex 8 is a result of convergent evolution since the three different genes studied here produce very similar topologies and it would be unusual for three individual traits to converge in this manner; it is more likely to be a result of divergence acting on the entire viral genome in this case.

Given that the filamentous ectocarpoids separated from the Laminariales lineage around 100 Ma[92], to our knowledge Flex 8 provides the earliest example on record for an emergent virus exploiting the reduction of its giant genome and accompanying apparent loss of DNA proofreading capability to shift towards a more *r*- like evolutionary strategy in the subgroup B phaeoviruses, whilst still essentially remaining a stable latent infection within

the *Feldmannia* hosts. A pairwise analysis of the evolutionary divergence in nucleotide sequences within the various groups of phycodnaviruses (Figure 2.6) illustrates this shift by subgroup B towards a more *r*-like evolutionary strategy. Subgroup B has a higher nucleotide divergence than subgroup A in the DNApol gene fragment and is comparable to that of the other *r*- selected lytic phycodnavirus groups (chloroviruses and the lytic viruses of stramenopiles). Subgroup A has maintained the classic *K* – selection life strategy with a much lower divergence.

Taken together, this study has provided some remarkable observations about the diversity, life histories and host specificities of phaeoviruses. Moreover, unlike previous reports on emerging viruses which cross species boundaries (e.g. HIV[152], Ebola[153], H_5N_1[154] and DWV[155]) with catastrophic consequences for new host survival, this study suggests a very different scenario. It is one in which the integration and diversification of the viruses has happened over a long period of time, allowing the relationship to have developed into a stable state of co-existence, both between different viral strains within the host, and between the host and its viruses.

CHAPTER 3 POLYMORPHISM ANALYSIS OF VIRAL GENES IN THE TRANSCRIPTOME OF AN INFECTED STRAIN OF *FELDMANNIA IRREGULARIS*

3.1 Abstract

This chapter aims to build on the observations of diverse sequence variants in phaeoviruses infecting *Feldmannia* which were detected in Chapter 2. The transcriptome sequence of an actively infected strain of *Feldmannia irregularis* revealed that 97% of the genome was actively transcribed, indicating little, if any, redundant genes in this virus. Moreover, polymorphic regions, including two base differences in the helicase gene which were identified during the sequencing described in Chapter 2, confirmed that multiple phaeoviral variants are not only present in this strain, but also simultaneously active.

A comparison with a previous microarray study of EsV-1 transcription, as well as the previously sequenced FirrV-1 and EsV-1 genomes, revealed differences between EsV-1 and FirrV-1 genetic activity which may account for the different evolutionary strategies of these viruses. Firstly, a proofreading exonuclease is active in EsV-1 and absent from FirrV-1, and an integrase is active in FirrV-1 which is present but not transcribed in EsV-1. These differences, combined with the potential decrease in replication fidelity due to the DNA polymerase mutations identified in Chapter 2 will result in a higher mutation rate in FirrV-1 and more potential for recombination than in EsV-1, potentially resulting in the greater diversity observed among the subgroup B phaeoviruses.

3.2 Introduction

A great deal is already known about the life cycle of phaeoviruses due to extensive studies involving light microscopy and infection experiments[97,105], however, little is known about the molecular mechanisms involved in the infection cycle of these viruses. A previous MRes project using microarrays to examine the difference in mRNA populations between EsV-1 infected and healthy strains of *E. siliculosus* (Dillwyn) Lyngbye provided inconclusive results[168] which was likely due to non-specific hybridization and insufficient wash stringency. A further tiling array analysis of the *Ectocarpus* genome showed that the EsV-1-like provirus was not being expressed and is likely to be an inactive remnant of an ancient infection [91]. Microarray analysis, which by its very nature requires prior knowledge of gene sequences[169], is less suitable than sequencing based analyses for identifying polymorphisms since the results usually only show presence or absence of sequence complementary to the probe[170] and tend not to highlight the different sequences present. Thus, the multiple phaeoviral sequence variants detected in Chapter 2 would be difficult to interpret by microarray analysis, and another approach is necessary to identify which variants are active in these virus infected strains.

Next generation sequencing, or massively parallel sequencing as it is also known, has revolutionised the world of genomics[171], allowing the rapid production of vast amounts of sequence data which can be used in a wide range of applications. This type of sequencing is the latest development in a long line

of improvements to the accuracy and throughput of the original Sanger sequencing method derived in 1977[172], which has evolved from radioactively labelled nucleotides, through the use of fluorescence and automated capillary sequencing. Next generation sequencing technologies use a synthetic adaptor which is ligated to each sequence in a library of fragments[173]. This adaptor binds each fragment to a solid surface, either a bead [174,175] or a flat glass microfluidics channel [176], where the sequencing reaction takes place. The reaction occurs in a series of steps, from the addition of a fluorescently labelled nucleotide, to a detection step which determines which nucleotide was added and finally a wash step to remove the fluorescent label or blocking agent to allow the addition of subsequent nucleotides[173]. The three most commonly used approaches (Illumina[176], 454[174] and ABI[175]) rely on the production of vast quantities of sequences due to the short reads produced, and therefore require a scaffold of known sequence in the form of a reference genome in order to allow assembly of the new sequence data.

One of the range of uses of next generation sequencing technologies is transcriptome sequencing[177], which is rapidly increasing in popularity because it not only has the potential to identify new transcripts which may not have been annotated[178], but also highlights polymorphisms which would be impossible on a microarray[179]. Furthermore, next generation sequencing allows the analysis of de novo genomes (genomic[180] or transcriptome[181]

analysis), with subsequent gene identification being carried out by similarity searches to sequences in the published databases[181].

This study is a perfect candidate for next generation transcriptome sequencing, since the FirrV-1 genome has been sequenced[1], and can be used as a scaffold for alignment of the short reads produced by this technology. The Illumina technology[182] is the best method for this study due to the vast number of sequencing reads that can be produced, compared with the 454 technology[183]. Moreover, since the discovery of two viral variants in *Feldmannia irregularis* (Kützing) Hamel[184], the origin strain of FirrV-1, its transcriptome has the potential to reveal the activity of these multiple variants.

This chapter aims to determine whether one or more viral sequence variants are active in the infected *Feldmannia irregularis* strain used in Chapter 2, as well as providing some information about the important viral genes in the infection process, by sequencing the entire transcriptome of a highly symptomatic culture and checking for polymorphisms by mapping the reads against the published FirrV-1 genome. In addition, the results will be compared to a previous microarray study of EsV-1 transcriptome activity described by Stevens et al. 2009[168] in order to elucidate any potential genetic mechanisms that may influence the different evolutionary strategies of these two viruses.

The FirrV-1 transcriptome revealed polymorphisms in several key genes and suggests that the multiple phaeoviral variants found in Chapter 2 are indeed

actively transcribed. The comparison with the EsV-1 transcription study showed two key differences between the two viruses that may account for the higher mutation rate in subgroup B viruses compared to subgroup A: a proofreading exonuclease which is highly transcribed in EsV-1 but not present in the FirrV-1 genome, and an integrase which is transcribed in FirrV-1 but not in EsV-1 although it is present in the genome.

3.3 Methods

3.3.1 Strain used

The infected *Feldmannia irregularis* strain from Chapter 2 was used because it was the strain that most reliably produced abundant symptoms of viral infection, indicating that the virus was actively reproducing at the time of RNA extraction. Culture conditions were as described in section 2.3.2 and RNA extractions were carried out at one time point, from filaments checked microscopically to contain abundant infected zoidangia, i.e. actively reproducing virions, in order to ensure high levels of viral transcription.

3.3.2 RNA extraction

5 – 15 mg wet weight fresh algal material was transferred to an Eppendorf tube, frozen in liquid nitrogen and ground using Eppendorf grinders with 10µl saturated ≤106 microns acid washed glass bead solution. The sample was then subjected to the Qiagen RNeasy Mini Protocol for Isolation of Total RNA from Yeast, beginning with the addition of 350µl Buffer RLT according to the manufacturer's instructions, with the exception that beads and other insoluble material were removed by centrifuging at maximum speed for 2 minutes after vortexing in RLT and only using the resulting supernatant in further steps.

3.3.3 cDNA preparation

cDNA was prepared for sequencing from 6ng RNA, using the Clontech™ SMARTer PCR cDNA Synthesis Kit. The quality of the resulting cDNA was tested by running on an agarose gel, PCR using the helicase and MCP primers used in Chapter 2 and concentration analysis on a NanoDrop 1000. The cDNA was then sent to the University of Exeter, where it was fragmented into small fragments, attached to oligonucleotide adaptors and subjected to paired end sequencing on the Illumina Genome Analyzer HighSeq-2000.

3.3.4 Sequence analysis

Initial quality control was carried out on the data by the University of Exeter who trimmed and filtered the reads, removing adaptor sequences and reads containing low quality bases (http://biosciences.exeter.ac.uk/facilities/ sequencing/postprocessing/). The 16 contigs comprising the published FirrV-1 genome[87] were concatenated into a single sequence using the 'cat' command on the Biolinux shell in order to facilitate alignment of the reads. All subsequent data analysis steps are represented in the flowchart in Figure 3.1. The quality of the read data was summarised using FastQC and some initial quality filtering was also carried out at this stage, then the remaining reads were assembled to the reference FirrV-1 genome using TopHat, with a mean inner distance between mate pairs of 100bp +/- 100 standard deviation, the junction search disabled since viruses do not usually have introns, and using the Illumina library

type. SAMtools was used to check for correctly paired reads, correct alignment to the reference genome, and finally to detect and remove false SNPs caused by PCR errors. After this, Cufflinks was used to detect expressed genes in the dataset. Finally the unmapped reads from the TopHat output were assembled using Velvet (in order to detect potential host genes which would not have aligned to the FirrV-1 reference genome) and genes identified via a BLAST search for sequence homology and a Pfam search to detect protein families.

3.3.5 Homology search for gene expression

The expression levels for the viral genes were checked using a BLAST search of the reads against the FirrV-1 database. After the FastQC step shown in Figure 3.1, a BLASTn homology search was carried out in the BioLinux platform against the FirrV-1 genome which was downloaded from the NCBI database. The top hit for each read (with an e-value cut-off of 10e-05) was then exported to a spread sheet containing read position and match quality information. The number of matching reads for each gene was then divided by the length of the gene and the log calculated. Genes in the lower and upper quartile were classified as having low and high expression respectively, with the interquartile range having medium expression. Any genes with a positive log value were classified as very highly expressed.

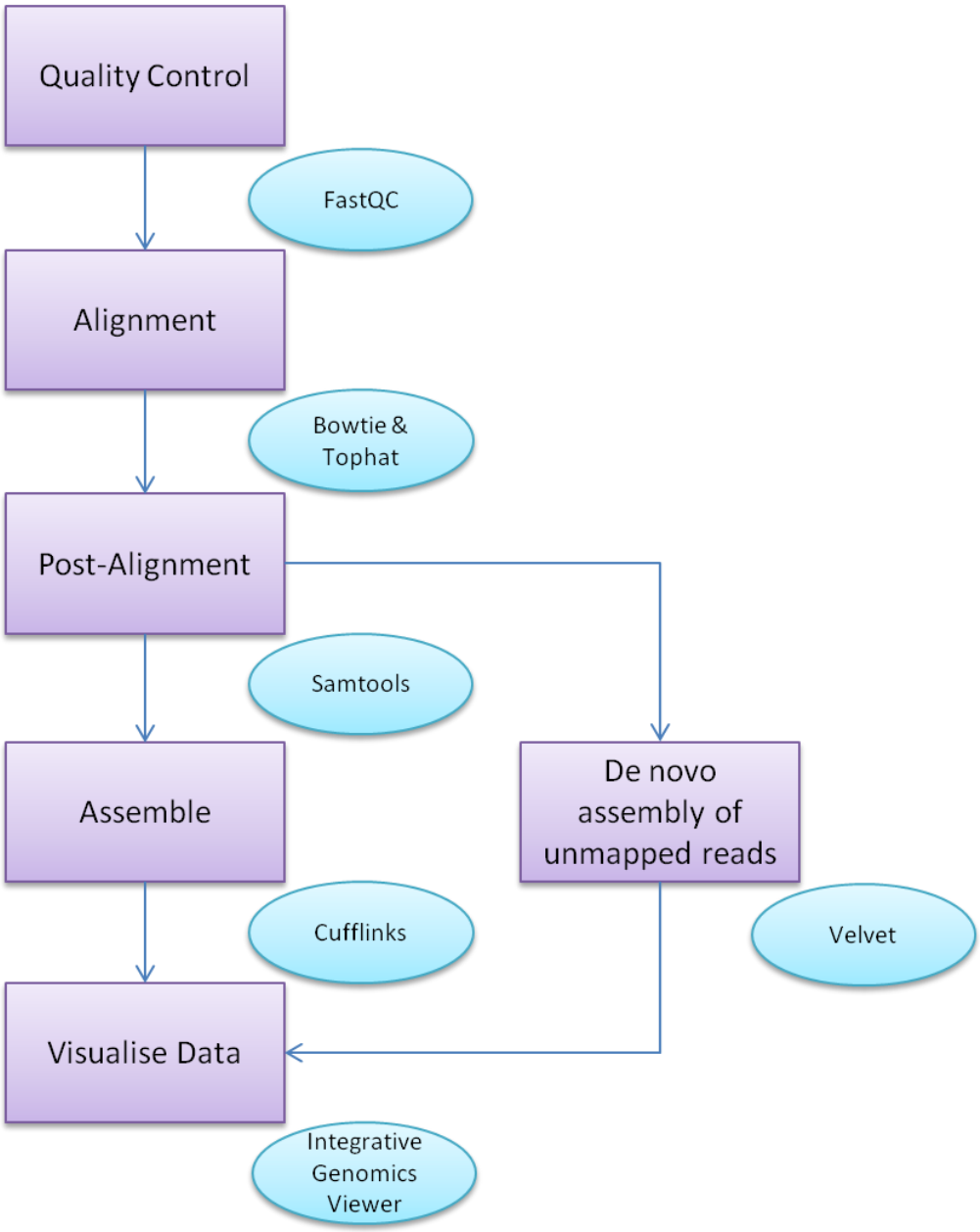

Figure 3.1: Flowchart demonstrating the steps involved in analysing the transcriptome data. Blue ovals represent the programs used and purple boxes indicate the steps.

3.3.6 Polymorphism detection

Expressed genes were also examined for polymorphisms in IGV; regions of at least 10 reads depth with at least 3 reads containing the same polymorphism were considered to be polymorphic.

3.4 Results

3.4.1 cDNA quality

Running the cDNA on a gel resulted in a smear of varying sized fragments between 300 and 1300bp (Figure 3.2) which corresponds with the gene sizes identified in the FirrV-1 genome[87]. PCR reactions for the helicase and MCP fragments used in Chapter 2 were also positive (data not shown), and the concentration was determined to be 56.73ng/µl on a NanoDrop 1000 (Figure 3.3).

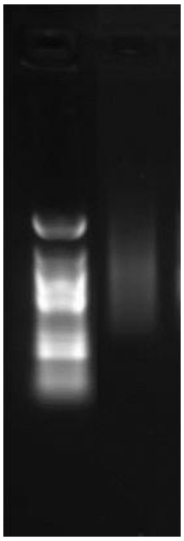

Figure 3.2: Agarose gel image of amplified cDNA used to generate the transcriptome.

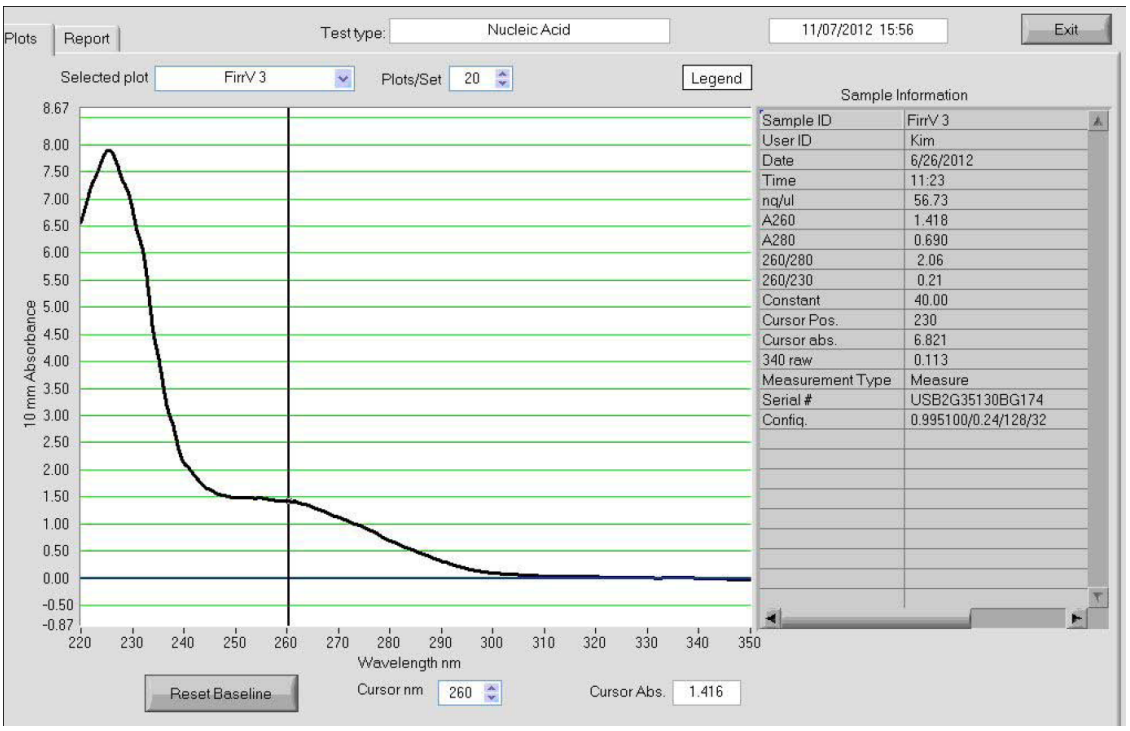

Figure 3.3: NanoDrop 1000 analysis of amplified cDNA used to generate the transcriptome, showing the concentration to be 56.73 ng/µl.

3.4.2 Transcriptome QC data

The various processes involved in the analysis of the transcriptome data all involved filtering out low quality or non-matching reads. The numbers of reads lost via this entire pipeline are indicated in Table 3.1.

Table 3.1: Numbers of reads passing through the various filtering and alignment processes.

Process	Number of reads
Illumina HiSeq 2000	15 203 864
After filtering by FastQC	6 681 194
Reads used by TopHat	6 560 298
Reads discarded by TopHat	120 896
Reads mapped to FirrV by TopHat	22 134
Mapped reads after duplicates removed	10 127
BLASTn matches to FirrV-1 genome	71 882

3.4.3 Transcriptome analysis – homology search for expression levels

The full results of this analysis would be impractical to include in this thesis and so a sample of the best 20 hits is shown in Table 3.2. The complete set of results was used to generate the individual FirrV-1 gene expression levels shown in full in Table 3.3.

Table 3.2: An example of the output of the BLASTn match, showing the top 20 hits generated by this process

Query name	Query length	Accession number	Matched contig length	Matched contig description	E value	Bit score	Frame	Query start	Query end	Hit start	Hit end	Positive matches	Identical nucleotides
D3P26HQ1:173:D14GLACXX:4:1101:14101:83380	101	AY225135	24235	Feldmannia irregularis virus a strain FirrV-1 contig C, partial sequence	6.00E-54	200	0	1	101	6234	6334	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1101:7263:55996	101	AY225135	24235	Feldmannia irregularis virus a strain FirrV-1 contig C, partial sequence	6.00E-54	200	0	1	101	10834	10934	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1101:7567:72828	101	AY225133	55981	Feldmannia irregularis virus a strain FirrV-1 contig A, partial sequence	6.00E-54	200	0	1	101	52054	52154	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1101:8001:51395	101	AY225135	24235	Feldmannia irregularis virus a strain FirrV-1 contig C, partial sequence	6.00E-54	200	0	1	101	6105	6205	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1101:9718:81685	101	AY225135	24235	Feldmannia irregularis virus a strain FirrV-1 contig C, partial sequence	6.00E-54	200	0	1	101	6339	6439	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1102:14363:26864	101	AY225133	55981	Feldmannia irregularis virus a strain FirrV-1 contig A, partial sequence	6.00E-54	200	0	1	101	52371	52471	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1102:15169:61402	101	AY225133	55981	Feldmannia irregularis virus a strain FirrV-1 contig A, partial sequence	6.00E-54	200	0	1	101	52309	52409	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1102:17167:19627	101	AY225135	24235	Feldmannia irregularis virus a strain FirrV-1 contig C, partial sequence	6.00E-54	200	0	1	101	5884	5984	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1102:19336:57689	101	AY225135	24235	Feldmannia irregularis virus a strain FirrV-1 contig C, partial sequence	6.00E-54	200	0	1	101	6024	6124	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1102:7252:40909	101	AY225135	24235	Feldmannia irregularis virus a strain FirrV-1 contig C, partial sequence	6.00E-54	200	0	1	101	5767	5867	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1102:7887:20045	101	AY225134	48352	Feldmannia irregularis virus a strain FirrV-1 contig B, partial sequence	6.00E-54	200	0	1	101	20209	20309	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1102:8385:61276	101	AY225133	55981	Feldmannia irregularis virus a strain FirrV-1 contig A, partial sequence	6.00E-54	200	0	1	101	52614	52714	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1106:4445:41717	101	AY225135	24235	Feldmannia irregularis virus a strain FirrV-1 contig C, partial sequence	6.00E-54	200	0	1	101	6121	6221	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1108:12711:57338	101	AY225135	24235	Feldmannia irregularis virus a strain FirrV-1 contig C, partial sequence	6.00E-54	200	0	1	101	5816	5916	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1112:11171:64647	101	AY225135	24235	Feldmannia irregularis virus a strain FirrV-1 contig C, partial sequence	6.00E-54	200	0	1	101	21773	21873	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1116:17745:10733	101	AY225133	55981	Feldmannia irregularis virus a strain FirrV-1 contig A, partial sequence	6.00E-54	200	0	1	101	37382	37482	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1116:5531:53210	101	AY225135	24235	Feldmannia irregularis virus a strain FirrV-1 contig C, partial sequence	6.00E-54	200	0	1	101	4952	5052	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1202:14082:15027	101	AY225135	24235	Feldmannia irregularis virus a strain FirrV-1 contig C, partial sequence	6.00E-54	200	0	1	101	6488	6588	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1203:13608:80963	101	AY225133	55981	Feldmannia irregularis virus a strain FirrV-1 contig A, partial sequence	6.00E-54	200	0	1	101	52614	52714	100.00%	100.00%
D3P26HQ1:173:D14GLACXX:4:1203:15302:47011	101	AY225133	55981	Feldmannia irregularis virus a strain FirrV-1 contig A, partial sequence	6.00E-54	200	0	1	101	52433	52533	100.00%	100.00%

3.4.4 Transcriptome analysis – FirrV-1 gene expression

The full table of expression results from the transcriptome sequencing, together with a comparison of EsV-1 orthologue expression from the previous microarray study carried out by Stevens[168], is shown in Table 3.3. Also included are FsV-158 orthologues, where known [88]. The overall level of transcription of FirrV-1 genes is much higher than was observed in the EsV-1 microarray study; 151 of the 156 FirrV-1 genes (97%) were expressed to some extent, whereas of the EsV-1 orthologues considered in this study only 37 of the 86 (43%) were considered to be transcribed. Of the five FirrV-1 genes that were not transcribed in this study, three were also not expressed in EsV-1. Only one of the two EsV-1 orthologues of the unexpressed FirrV-1-P1 was expressed. The only real discrepancy between the expression profiles was that FirrV-1-C7, a gene of unknown function, was not transcribed whereas EsV-1-207 was, albeit only at a low level.

3.4.4.1 DNA metabolism

Of the fourteen FirrV-1 genes in this category, only one (FirrV-1-H4, putative adenine-specific methyltransferase) was not expressed, whereas two (EsV-1-101 methylase and EsV-1-132 PCNA) out of eleven EsV-1 genes were not expressed, although both showed medium expression in FirrV-1 (FirrV-1-B20 and FirrV-1-A6, respectively). The two very highly expressed genes in this group were the superfamily III helicase (FirrV-1-B27) and DNA adenine methyltransferase

(FirrV1-B29), that were also expressed in EsV-1 (EsV-1-109 and EsV-1-129, respectively). Both orthologues of DNA primase were expressed; FirrV-1-A46 showed medium expression, and EsV-1-45 was also expressed. The same levels of expression were also found for DNA polymerase (FirrV-1-A18 and EsV-1-93), and an additional putative DNA polymerase III (FirrV-1-B47) was also expressed at a low level. The large subunit of replication factor C was also expressed at the same levels as the DNA primase in both viruses (FirrV-1-A26 and EsV-1-138). A putative protelomerase, FirrV-1-B51 and EsV-1-175, showed medium and low expression respectively. The putative ATP-dependent nuclease FirrV-1-A45 was highly expressed, although no orthologue for this gene exists in EsV-1. The putative exonuclease was expressed at a low level (FirrV-1-B43 and EsV-1-64) and the remaining two nucleases (FirrV-1-B2 and FirrV-1-P2) had medium and low expression; their EsV-1-168 orthologue was also expressed at a low level.

A further DNA metabolism gene worth mentioning here is the proofreading exonuclease gene (EsV-1-127) mentioned in Chapter 2 that was present in the EsV-1 genome but absent from the Feldmannia viruses. This gene was highly expressed in the EsV-1 microarray experiment[168].

3.4.4.2 DNA integration and transposition

In spite of EsV-1 containing several genes for DNA integration and transposition (as discussed in section 1.2.3.6.2), FirrV-1 appears to only have one, the

integrase FirrV-1-B4/EsV-1-213. This gene had medium expression in FirrV-1, but was not found to be expressed in the EsV-1 transcriptome experiment.

3.4.4.3 Nucleotide metabolism

All six FirrV-1 nucleotide metabolism genes showed at least medium expression, whereas only two of the three EsV-1 orthologues were expressed. The large (FirrV-1-A20) and small (FirrV-1-A19) subunits of ribonucleotide reductase were highly expressed in FirrV-1, but showed no and low expression, respectively in EsV-1. The Rad50 ATPase (FirrV-1-C2) was very highly expressed whereas the cytidine deaminase and ATPase beta-2-subunit showed medium expression. Both orthologues of the VVA32-like ATPase (FirrV-1-A12 and EsV-1-26) had a medium expression level.

3.4.4.4 Structural proteins

All four structural genes were present in both FirrV-1 and EsV-1, and were all found to be expressed to some extent. The major capsid protein (FirrV-1-B50 and EsV-1-116) showed a medium level of expression in both viruses, but an additional FirrV-1 gene similar to a phage MCP was highly expressed (FirrV-1-B28). The mannuronan-C5-epimerase was highly expressed in FirrV-1 (FirrV-1-A15) but only found at a low rate in EsV-1 (EsV-1-226).

3.4.4.5 Signalling

All of the twelve FirrV-1 genes associated with cell signalling or membrane transport were expressed, whereas only four of the seven EsV-1 genes were. Two (FirrV-1-F1 and FirrV-1-H1) of the three hybrid histidine kinases had low expression and the other (FirrV-1-B9) had medium expression, although the equivalent EsV-1-181 was not expressed. Of the two FirrV-1 serine/threonine protein kinases, one (FirrV-1-B25) had medium and one (FirrV-1-B44) had low expression and EsV-1-111 was expressed. The serine/threonine protein phosphatase FirrV-1-B33 was highly expressed, and EsV-1-68 was also expressed. The calcium binding protein was also highly expressed in FirrV-1 (FirrV-1-B38) and one of its orthologues (EsV-1-56) was expressed, but the other (EsV-1-71) was not.

3.4.4.6 Transcription

All six FirrV-1 genes with a transcriptional function were expressed, although the same was only true for one of the three EsV-1 orthologues. Both FirrV-1 orthologues of the putative VLTF2 transcription factor (FirrV-1-B14 and FirrV-1-I5) were expressed (medium and low expression, respectively). The remaining four FirrV-1 genes (FirrV-1-B21 putative alanine tRNA ligase, FirrV-1-A3 putative oligoribonuclease, FirrV-1-D3 putative RNA-binding protein, FirrV-1-A41 putative transcription regulator) all showed a medium level of expression, whereas the putative transcription regulator (EsV-1-40) was the only EsV-1 gene

in this group to be expressed, the putative oligoribonuclease (EsV-1-139) was not.

3.4.4.7 Protein metabolism

All seven FirrV-1 genes with a potential role in protein metabolism were expressed to some extent, as were three of the five EsV-1 protein metabolism genes. Ubiquitin ligase was highly expressed in FirrV-1 (FirrV-1-A31) but only at a low level in EsV-1 (EsV-1-142), and an additional putative ubiquitin ligase was expressed at a low level in FirrV-1 (FirrV-1-D5) but not in EsV-1 (EsV-1-172).

3.4.4.8 Miscellaneous

There are a further 41 FirrV-1 genes which have similarities to other genes, but cannot be easily assigned to any of the above categories. 39 of these were expressed in FirrV-1 whereas only 12 of the 20 EsV-1 orthologues were expressed. Five of the very highly expressed FirrV-1 genes belong to this category, and none of them have EsV-1 orthologues; these are: putative adrenoxin reductase (FirrV-1-B5), putative bestrophin-1 (FirrV-1-C9), putative glycosyl transferase (FirrV-1-A14), putative integral membrane protein (FirrV-1-C3) and putative methyltransferase (FirrV-1-B7).

3.4.4.9 Unknown functions

FirrV-1-B6 was very highly expressed, but no significant BLAST matches were found. FirrV-1-A37 was also very highly expressed and its phaeoviral orthologues EsV-1-184 and FirrV-1-A36 were expressed and highly expressed, respectively. There are a total of 64 FirrV-1 genes of unknown function, 62 of which were expressed at some level, whereas 22 of the 43 orthologous EsV-1 genes were not expressed.

Table 3.3: Transcription levels of FirrV-1 genes showing their putative functions and functional groups, along with EsV-1, FsV-158 and FirrV-1 homologues (those with a BLAST search e-value of less than 1). EsV-1 orthologue transcription levels were obtained from Stevens 2009[168].

Gene	Expression	EsV-1 orthologues	FirrV-1 orthologues	FsV-158 orthologues	Functional group	Function
A1	++		FirrV-1-A2			Unknown
A2	+++		FirrV-1-A1			Unknown
A3	++	EsV-1-139 -		FsV-158-077	Transcription	Putative oligoribonuclease
A4	+	EsV-1-140 ++		FsV-158-078		Unknown
A5	++	EsV-1-141 -		FsV-158-079		Unknown
A6	++	EsV-1-132 -		FsV-158-080	DNA metabolism	PCNA
A7	++					Unknown
A8	+++	EsV-1-130 -		FsV-158-082		Unknown
A9	+++	EsV-1-125 -		FsV-158-083		Unknown
A10	++				Miscellaneous	Putative ricin-type beta-trefoil lectin domain protein
A11	++			FsV-158-084	Nucleotide metabolism	Putative ATPase beta-2 subunit
A12	++	EsV-1-26 ++		FsV-158-087	Nucleotide metabolism	VVA32-like ATPase
A13	+++			FsV-158-088		Unknown
A14	++++				Miscellaneous	Putative glycosyl transferase
A15	+++	EsV-1-226 +			Structural	Mannuronan-C5- epimerase
A16	++			FsV-158-090		Unknown
A17	++			FsV-158-91/92	Protein metabolism	Putative collagenase/protease
A18	++	EsV-1-93 ++		FsV-158-093	DNA metabolism	DNA-dependent DNA polymerase
A19	+++	EsV-1-128 +		FsV-158-094	Nucleotide metabolism	Putative small subunit ribonucleotide reductase
A20	+++	EsV-1-180 -		FsV-158-096	Nucleotide metabolism	Putative large subunit ribonucleotide reductase
A21	+	EsV-1-135 +		FsV-158-099		Unknown
A22	++	EsV-1-136 ++		FsV-1-100		Unknown

Table 3.3 (continued)

Gene	Expression	EsV-1 orthologues	FirrV-1 orthologues	FsV-158 orthologues	Functional group	Function
A23	+++	EsV-1-137 -	FirrV-1-O1	FsV-158-101		Unknown
A24	+++			FsV-158-102		Unknown
A25	+			FsV-158-103		Unknown
A26	++	EsV-1-138 ++		FsV-158-105	DNA metabolism	Replication factor C large subunit
A27	+++	EsV-1-50 +	FirrV-1-B56/N1	FsV-158-065/086/106/119/125	Miscellaneous	Putative cell wall adhesion protein
A28	++					Unknown
A29	++			FsV-158-107	Nucleotide metabolism	Cytidine deaminase
A30	++	EsV-1-91 ++		FsV-158-108	Structural	Putative cell surface glycoprotein
A31	+++	EsV-1-142 +		FsV-158-001/109	Protein metabolism	Ubiquitin ligase
A32	++	EsV-1-11 -	FirrV-1-G3			Unknown
A33	++	EsV-1-210/211 -/++	FirrV-1-A34/B3/G1/K1/L1/P1	FsV-158-004/5/69/70/71/72/75/76/92/134/135	Miscellaneous	Putative 5-azacytidine-induced protein
A34	++	EsV-1-210/211 -/++	FirrV-1-A33/B3/G1/K1/L1/P1	FsV-158-004/5/69/70/71/72/75/76/90/91/92/134/135		Unknown
A35	++			FsV-158-110		Unknown
A36	+++	EsV-1-184 ++	FirrV-1-A37	FsV-158-111/112		Unknown
A37	++++	EsV-1-184 ++	FirrV-1-A36	FsV-158-111/112		Unknown
A38	++	EsV-1-52 ++		FsV-158-113	Miscellaneous	Putative viral nucleoprotein
A39	+	EsV-1-51/52 ++/++		FsV-158-114	Miscellaneous	Arginine methyltransferase
A40	+++				Miscellaneous	Putative serine-rich repeat protein
A41	++	EsV-1-40 ++		FsV-158-115	Transcription	Putative transcription regulator
A42	++	EsV-1-41/99 -/+	FirrV-1-B18	FsV-158-116		Unknown
A43	+	EsV-1-42 -		FsV-158-117		Unknown
A44	++	EsV-1-43 -		FsV-158-118		Unknown
A45	+++			FsV-158-120	DNA metabolism	Putative ATP-dependent nuclease
A46	++	EsV-1-45 ++		FsV-158-121	DNA metabolism	DNA primase

Table 3.3 (continued)

Gene	Expression	EsV-1 orthologues	FirrV-1 orthologues	FsV-158 orthologues	Functional group	Function
A47	+			FsV-158-123	Miscellaneous	Putative ribonuclease Y
A48	++	EsV-1-75 -		FsV-158-126	Protein metabolism	Putative cysteine protease
A49	++			FsV-158-127	Miscellaneous	Putative membrane anchored cell surface protein
A50	+			FsV-158-009		Unknown
A51	+++	EsV-1-39/159/160 -/-/+	FirrV-1-C1/E1	FsV-158-012/55/74/124	Signalling	Fibronectin type III domain/LamG-like jellyroll
B1	++	EsV-1-217 ++	FirrV-1-F4			Unknown
B2	++	EsV-1-168 +	FirrV-1-P2	FsV-158-148	DNA metabolism	Putative nuclease
B3	++	EsV-1-210/211 -/++	FirrV-1-A33/A34/G1/K1	FsV-158-004/5/69/70/72/75/76/134/135		Unknown
B4	++	EsV-1-213 -		FsV-158-013	DNA integration and transposition	Putative integrase (FsV)
B5	++++				Miscellaneous	Adrenoxin reductase
B6	++++					No blast matches
B7	++++				Miscellaneous	Putative methyltransferase
B8	+				Miscellaneous	Putative tetratricopeptide
B9	++			FsV-158-017	Signalling	Putative sensor hybrid histidine kinase
B10	+++	EsV-1-76 ++	FirrV-1-I1	FsV-158-018		Unknown
B11	+++	EsV-1-77 –	FirrV-1-I2	FsV-158-019		Unknown
B12	+++	EsV-1-79 -	FirrV-1-I3	FsV-158-020		Unknown
B13	+++	EsV-1-95 -	FirrV-1-I4	FsV-158-021		Unknown
B14	++	EsV-1-96 -	FirrV-1-I5	FsV-158-022	Transcription	Putative VLTF2 transcription factor
B15	+			FsV-158-023	Miscellaneous	Putative dnaG/RNA recognition motif
B16	++	EsV-1-146 -		FsV-158-024	Miscellaneous	Putative peroxide operon regulator
B17	++	EsV-1-98 ++		FsV-158-025		Unknown
B18	++	EsV-1-99 +	FirrV-1-A42	FsV-158-026		Unknown
B19	+	EsV-1-100 -		FsV-158-027		Unknown

Table 3.3 (continued)

Gene	Expression	EsV-1 orthologues	FirrV-1 orthologues	FsV-158 orthologues	Functional group	Function
B20	++	EsV-1-101 -		FsV-158-028	DNA metabolism	Methylase
B21	++			FsV-158-029	Transcription	Putative alanine tRNA ligase
B22	++	EsV-1-74/103 ++/++		FsV-158-030		Unknown
B23	++	EsV-1-105 -		FsV-158-031	Miscellaneous	6-carboxy-5,6,7,8-tetrahydropterin synthase
B24	++			FsV-158-032		Unknown
B25	++				Signalling	Putative serine/threonine protein kinase
B26	++	EsV-1-108 -		FsV-158-033		Unknown
B27	++++	EsV-1-109 ++		FsV-158-034	DNA metabolism	Superfamily III helicase
B28	+++	EsV-1-110 +		FsV-158-035	Structural	Phage MCP
B29	++++	EsV-1-129 ++		FsV-158-037	DNA metabolism	DNA adenine methyltransferase
B30	+	EsV-1-164 -		FsV-158-038	Miscellaneous	Putative nosD copper-binding domain
B31	+++			FsV-158-036		Unknown
B32	+++	EsV-1-67 ++		FsV-158-039		Unknown
B33	+++	EsV-1-68 ++		FsV-158-040	Signalling	Putative serine/threonine protein phosphatase
B34	++	EsV-1-70 -		FsV-158-042		Unknown
B35	++			FsV-158-043		Unknown
B36	++			FsV-158-044		Unknown
B37	+	EsV-1-57 -		FsV-158-045		Unknown
B38	+++	EsV-1-56/71 ++/-	FirrV-B45	FsV-158-046	Signalling	Calcium binding protein
B39	++	EsV-1-55 ++		FsV-158-047	Miscellaneous	Putative lipase
B40	++	EsV-1-61 ++		FsV-158-049		Unknown
B41	++	EsV-1-62 +		FsV-158-050		Unknown
B42	+	EsV-1-63 -		FsV-158-051		Unknown
B43	++	EsV-1-64 ++		FsV-158-052	DNA metabolism	Putative exonuclease

Table 3.3 (continued)

Gene	Expression	EsV-1 orthologues	FirrV-1 orthologues	FsV-158 orthologues	Functional group	Function
B44	+	EsV-1-111 ++		FsV-158-053	Signalling	Putative serine-threonine protein kinase
B45	++	EsV-1-56/71 ++/-	FirrV-1-B38	FsV-158-046	Miscellaneous	Putative outer membrane autotransporter
B46	+				Miscellaneous	Putative collagen-like protein
B47	+			FsV-158-056	DNA metabolism	Putative DNA polymerase III
B48	++	EsV-1-161 -		FsV-158-057	Miscellaneous	Putative thiol oxidoreductase
B49	-					Unknown
B50	++	EsV-1-116 ++		FsV-158-059	Structural	MCP
B51	++	EsV-1-175 +		FsV-158-060	DNA metabolism	Putative protelomerase
B52	++			FsV-158-061	Signalling	Putative signal transduction histidine kinase
B53	++	EsV-1-72 ++	FirrV-1-J2	FsV-158-062		Unknown
B54	++	EsV-1-28 +	FirrV-1-J1	FsV-158-063	Miscellaneous	Deoxyribonuclease II family protein
B55	+	EsV-1-78 -		FsV-158-064		Unknown
B56	++	EsV-1-50/159 -/-	FirrV-1-A27/N1	FsV-158-065/086/106/119/125	Miscellaneous	Putative lamG-like jellyroll protein
B57	++	EsV-1-47 -		FsV-158-067		Unknown
B58	+			FsV-158-068	Miscellaneous	Putative oligosaccharyl transferase
C1	++	EsV-1-39 -	FirrV-1-E1	FsV-158-012/55/74/124	Miscellaneous	Putative lamG-like jellyroll protein
C2	++++				Nucleotide metabolism	Rad50 ATPase
C3	++++		FirrV-1-H3/C4	FsV-158-011	Miscellaneous	Putative integral membrane protein
C4	+		FirrV-1-C3/H3	FsV-158-011	Signalling	Fibronectin type III domain protein
C5	++			FsV-158-143	Miscellaneous	Contains ankyrin repeats
C6	+++					Unknown
C7	-	EsV-1-207 +		FsV-158-139		Unknown
C8	++					Unknown
C9	++++				Miscellaneous	Putative bestrophin-1

Table 3.3 (continued)

Gene	Expression	EsV-1 orthologues	FirrV-1 orthologues	FsV-158 orthologues	Functional group	Function
D1	+			FsV-158-128	Miscellaneous	Glutamate/valine rich protein
D2	++			FsV-158-129	Miscellaneous	Phage tail tape measure protein
D3	++				Transcription	Putative RNA-binding protein
D4	+++	EsV-1-183 +		FsV-158-131		Unknown
D5	+	EsV-1-172 -		FsV-158-132	Protein metabolism	Putative ubiquitin-protein ligase
D6	+					Unknown
D7	+				Miscellaneous	Sperm nuclear basic protein PL-I
E1	+	EsV-1-39/159/160 -/-/+	FirrV-1-A51/C1	FsV-158-012/55/74/124	Miscellaneous	LamG-like jellyroll
E2	+			FsV-158-133		Unknown
E3	+		FirrV-1-E5	FsV-158-140/144/145	Protein metabolism	Putative ubiquitin-like cysteine protease
E4	+++					Unknown
E5	++	EsV-1-153 ++	FirrV-1-E3		Miscellaneous	Putative histone
F1	+			FsV-158-141	Signalling	Hybrid sensor histidine kinase
F2	++	EsV-1-185 +			Miscellaneous	Putative lipase
F3	++				Miscellaneous	Contains ankyrin repeats
F4	+	EsV-1-217 ++	FirrV-1-B1			Unknown
G1	++	EsV-1-210 -	FirrV-1-A33/A34	FsV-158-004/5/69/70/71/72/75/76/90/91/92/134/135	Signalling	Cell surface protein
G2	++	EsV-1-158 ++		FsV-158-136	Protein metabolism	Putative lysine methyltransferase
G3	++		FirrV-1-A32		Miscellaneous	ATPase
H1	+	EsV-1-181 -		FsV-158-149	Signalling	Putative hybrid sensor histidine kinase
H2	-				Miscellaneous	Putative beta galactosidase small chain
H3	+		FirrV-1-C3/C4	FsV-158-011	Miscellaneous	Putative autotransporter-associated beta strand repeat
H4	-				DNA metabolism	Putative adenine-specific methyltransferase
I1	+	EsV-1-76 ++	FirrV-1-B10	FsV-158-018		Unknown

Table 3.3 (continued)

Gene	Expression	EsV-1 orthologues	FirrV-1 orthologues	FsV-158 orthologues	Functional group	Function
I2	+	EsV-1-77 -	FirrV-1-B11	FsV-158-019		Unknown
I3	+	EsV-1-79 -	FirrV-1-B12	FsV-158-020		Unknown
I4	+	EsV-1-95 -	FirrV-1-B13	FsV-158-021		Unknown
I5	+	EsV-1-96 -	FirrV-1-B14	FsV-158-022	Transcription	Putative VLTF2 transcription factor
J1	++	EsV-1-28 +	FirrV-1-B54	FsV-158-063	Protein metabolism	Putative histone-lysine N-methyltransferase
J2	+++		FirrV-1-B53	FsV-158-062	Miscellaneous	Putative PhoH family protein
K1	++	EsV-1-210/211 -/++	FirrV-1-A33/A34/B3/G1/L1/P1	FsV-158-004/5/69/70/71/72/75/76/134/135	Miscellaneous	Putative viral A-type inclusion protein
L1	++	EsV-1-210/211 -/++	FirrV-1-A33/A34/B3/G1/K1/P1	FsV-158-004/5/69/70/71/72/75/76/134/135	Signalling	Putative signal recognition particle receptor
M1	++	EsV-1-178/222 ++/++			Miscellaneous	Putative phosphoserine phosphatase
N1	++	EsV-1-50 -	FirrV-1-A27/B56	FsV-158-065/106/119/125	Miscellaneous	Putative chitinase
N2	+	EsV-1-13 -		FsV-158-066		Unknown
O1	+	EsV-1-137 -	FirrV-1-A23	FsV-158-101		Unknown
P1	-	EsV-1-210/211 -/++	FirrV-1-A33/K1/L1	FsV-158-004/72/134/135	Miscellaneous	Putative amino acid transporter
P2	+	EsV-1-168 +	FirrV-1-B2	FsV-158-148	DNA metabolism	Putative nuclease

Where EsV-1 orthologues are known, the expression of these is indicated (-, + or ++ after the name) from the transcriptome study reported by Stevens et al.[168]: - not above background, + above background but lower than 2x the expression of the host tubulin housekeeping gene, ++ expressed. Level of FirrV-1 expression from the transcriptome is indicated as follows: - not expressed (i.e. no reads on the assembly to the FirrV-1 reference genome), + slightly expressed (one to twenty reads), ++ expressed (greater than twenty reads but not full deep coverage), +++ highly expressed (full deep coverage across the whole length of the gene), ++++ very highly expressed (log of standardized read counts is greater than 0).

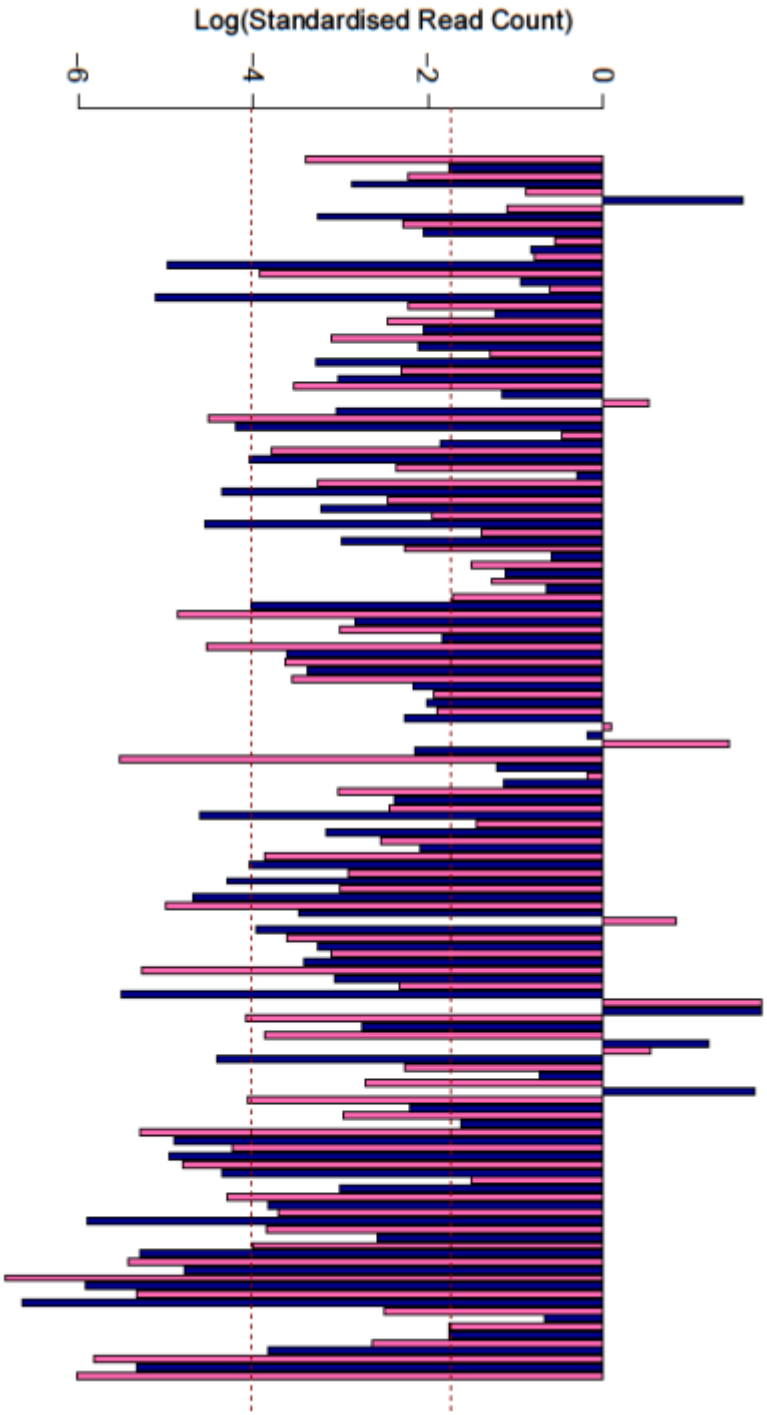

Figure 3.4: Barchart showing the transcription levels of FirrV-1 genes represented by the log of the number of BLAST matched reads from the transcriptome sequencing divided by the length of the gene. Genes are in the same order as in table 3.3, but those with no matching reads are not included.

3.4.5 Transcriptome analysis - polymorphisms

Polymorphisms were found in the mapped transcriptome reads, examples of which are shown in Figure 3.5. The results of the polymorphism search carried out for the mapped FirrV-1 genes in IGV are shown in Table 3.4. 28 genes were found to contain polymorphisms (a single nucleotide polymorphism present in at least three reads out of ten), at levels ranging from 77 to 1 polymorphism per kilobase. Ten of these polymorphic reads are of unknown function; two are involved in core viral DNA replication functions (superfamily III helicase and RFC large subunit), one structural gene (mannuronan-C5-epimerase) is polymorphic, as are four genes with a potential role in cell signalling (two putative serine/threonine kinases, a calcium binding protein and a putative signal recognition particle receptor). The remaining polymorphic genes have a range of functions including adrenoxin reductase, putative glycosyl transferase, putative bestrophin-1, Rad50 ATPase, putative integral membrane protein and a phage tape measure protein orthologue.

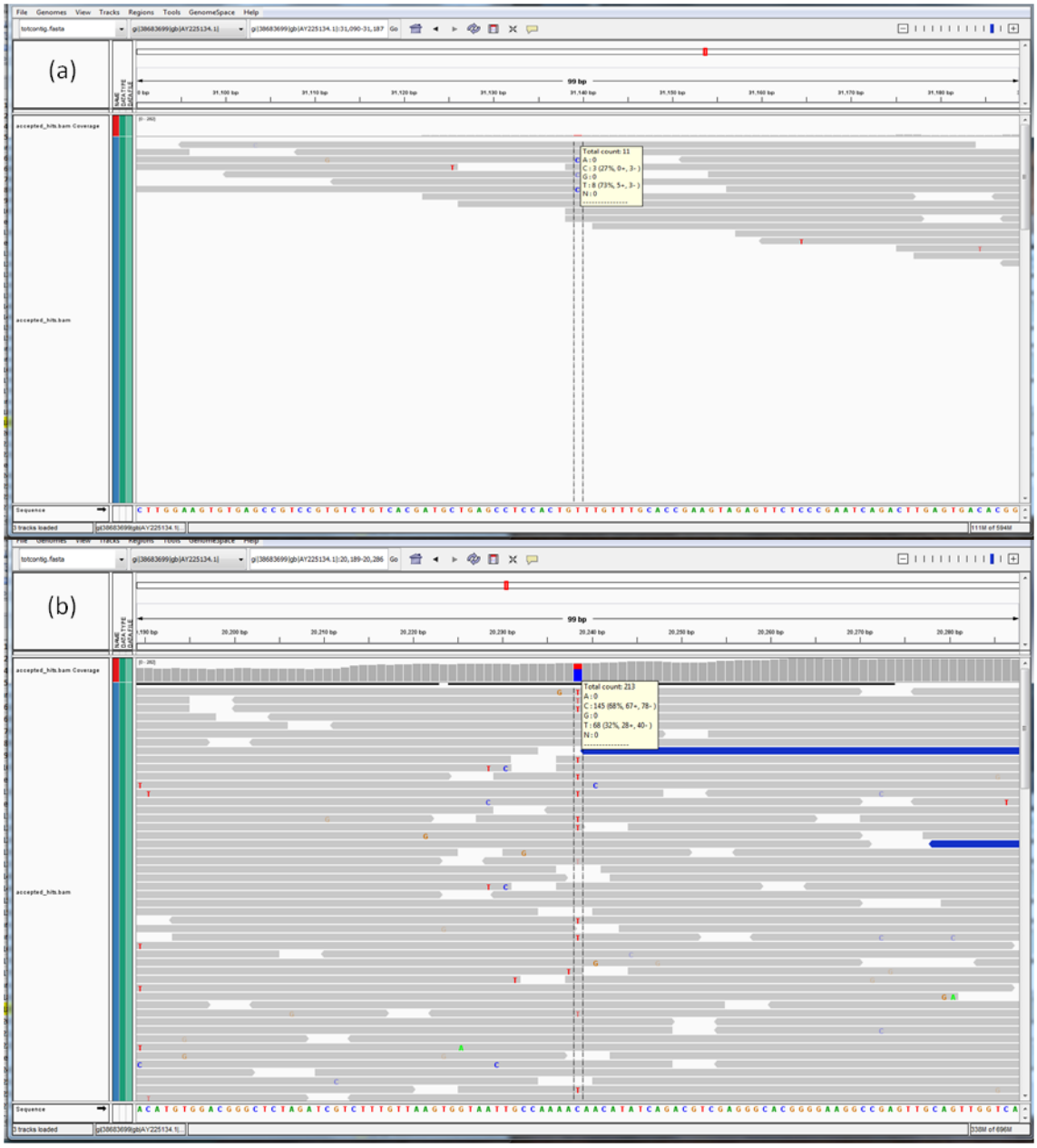

Figure 3.5: Example IGV view of typical polymorphisms found in mapped transcriptome genes. Scale along the top of the window refers to position of the screenshot within contig B in these examples, and the reference FirrV-1 genome sequence is along the bottom. Grey bars represent individual transcriptome reads which mapped to the reference genome. Letters within the bars indicate sequence differences between mapped reads and the reference. (a) shows a 27% T→C polymorphism in the gene FirrV-1-B38 (putative membrane anchored cell surface membrane protein), an area of low transcriptome coverage (11 reads), (b) shows a 32% C→T polymorphism in the gene FirrV-1-B27 (superfamily III helicase), an area of high coverage (213 reads).

Of the four genes studied in Chapter 2, superfamily III helicase was the only one observed to have polymorphisms. Unfortunately the PCR fragment sequenced for Figure 2.1 covered an area from 332 – 431 bp (positions 19235-19334 in Figure 3.6) that had low transcriptome coverage, and therefore it was not possible to validate the polymorphisms observed in the transcriptome with the sequencing data already obtained. However, when the sequenced fragment was compared with the IGV output, two of the polymorphisms were confirmed: the T→G/C at position 516 (Figure 3.7 (a)) and the C→T at position 672 (Figure 3.7 (b)).

Figure 3.6: IGV output of the superfamily III helicase gene showing the transcriptome read coverage, as well as the area covered by the PCR fragment sequenced in Figure 2.1.

Table 3.4: Results of the IGV analysis to detect polymorphisms, showing number of polymorphisms per kilobase of open reading frame. The genes in the table are ordered from most to least polymorphic.

Gene	Polymorphisms per kb	Expression level	FirrV-1 orthologues	Functional group	Function
A40	76.92	+++		Miscellaneous	Putative serine-rich repeat protein
B5	40.82	++++		Miscellaneous	Adrenoxin reductase
C9	34.70	++++		Miscellaneous	Putative bestrophin-1
A37	31.63	++++	FirrV-1-A36		Unknown
B32	20.35	+++			Unknown
B27	17.95	++++		DNA metabolism	Superfamily III helicase
C2	11.66	++++		Nucleotide metabolism	Rad50 ATPase
A15	11.37	+++		Structural	Mannuronan-C5-epimerase
C6	10.11	+++			Unknown
B25	8.81	++		Signalling	Putative serine/threonine protein kinase
A14	8.71	++++		Miscellaneous	Putative glycosyl transferase
A13	6.11	+++			Unknown
B33	5.68	+++		Signalling	Putative serine/threonine protein phosphatase
B38	4.93	+++	FirrV-B45	Signalling	Calcium binding protein
B31	4.57	+++			Unknown
D4	4.15	+++			Unknown
B18	4.08	++	FirrV-1-A42		Unknown
C3	3.75	++++	FirrV-1-H3/C4	Miscellaneous	Putative integral membrane protein
A26	2.97	++		DNA metabolism	Replication factor C large subunit
L1	2.63	++	FirrV-1-A33/A34/B3/G1/K1/P1	Signalling	Putative signal recognition particle receptor
A42	2.53	++	FirrV-1-B18		Unknown
B22	1.97	++			Unknown
A5	1.91	++			Unknown
B37	1.66	+			Unknown
D2	1.41	++		Miscellaneous	Phage tail tape measure protein
B24	1.16	++			Unknown
B11	1.13	+++	FirrV-1-I2		Unknown

3.4.6 Transcriptome analysis – unmapped genes

The results of the BLASTp and Pfam searches of the unmapped contigs which did not match the FirrV-1 reference genome revealed one phaeoviral BLASTp match, to the gene EsV-1-7 of unknown function (which represents a potential match to FirrV-1).

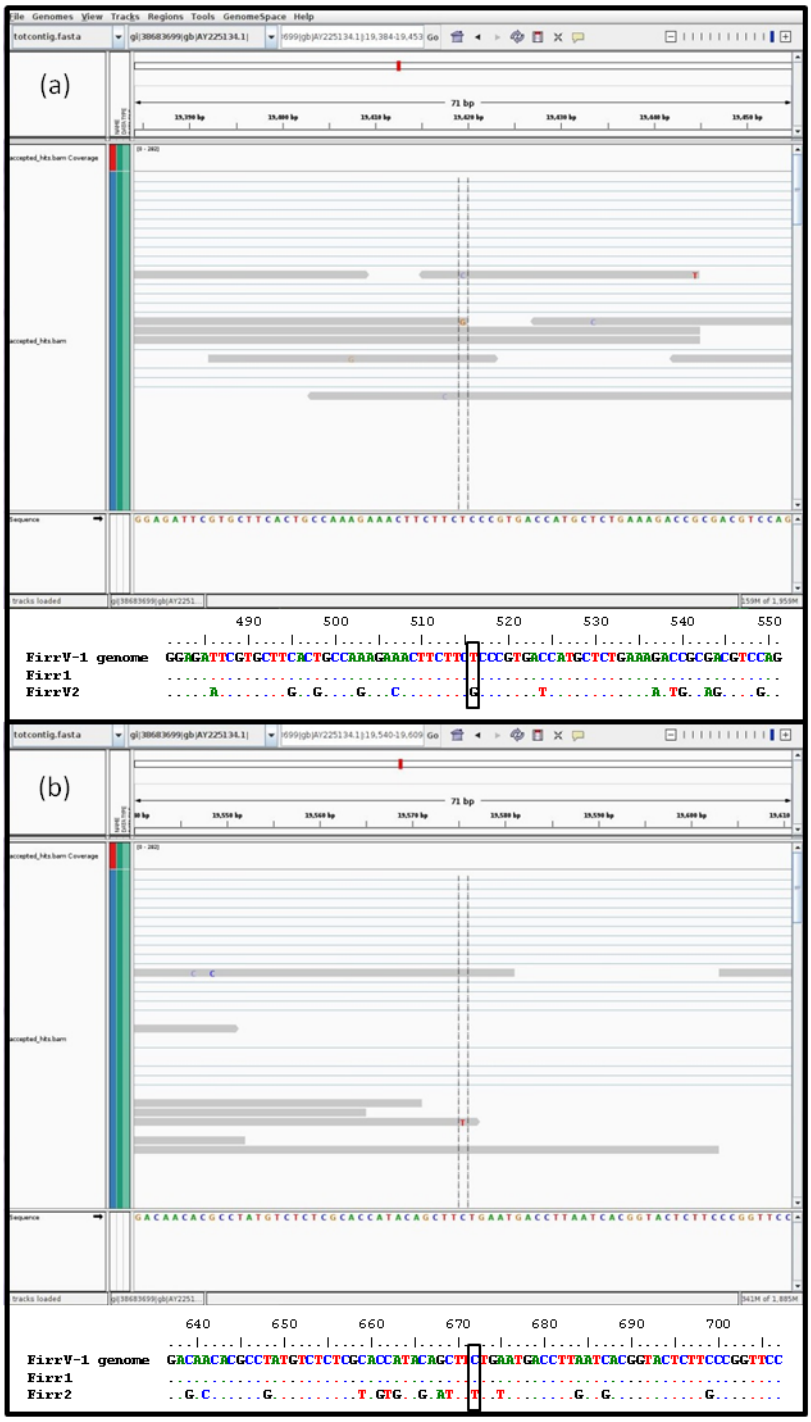

Figure 3.7: Confirmation of two polymorphisms in the superfamily III helicase gene from Figure 2.1 in the transcriptome sequence as visualized in IGV with the nucleotide sequence generated from clones in Chapter 2.

3.5 Discussion

The aim of this chapter was to build on the results of Chapter 2 which discovered multiple sequence variants within individual strains of brown algae infected with phaeoviruses. Indeed, the transcriptome analysis of this infected *F. irregularis* strain which was shown to have two main variants[184], has confirmed that multiple viral sequence variants are active. The observations of two previously known polymorphisms in the superfamily III helicase gene (

Figure 3.6), in addition to the well represented polymorphism in the same gene as shown in Figure 3.5 (b), support this conclusion. Furthermore, several other genes crucial to viral reproduction are polymorphic, including the structural mannuronan-C5-epimerase and large subunit of replication factor C which is involved in DNA replication, as well as several signalling genes. This suggests that the different viruses are not only actively replicating, as observed by the extent of physical symptoms mentioned in section 3.3.1, but are also potentially communicating with other infected host cells, perhaps to coordinate viral release, and potentially also zoid release to ensure the presence of susceptible cells for further infection.

These results build on the observation of multiple virus variants observed in Chapter 2 by confirming that these variants are not only present, but also transcriptionally active, providing a convenient pool of similar sequences during virus multiplication which would facilitate recombination and the packaging of

different variants into the offspring virions. Indeed it seems likely that the diversity of transcribed virus genes is much higher than detected here due to limitations in our detection methods, both in the mapping process and in our definition of a polymorphic region. Further investigations, using reverse-transcription PCR combined with sequencing were not possible due to time constraints, but would confirm more accurately how many variants were active in this strain. Since the polymorphisms that have already been observed in the sequencing in Chapter 2 were not all observed in the transcriptome, and the fact that the cloned fragment corresponds to the under-represented section of the helicase gene, it suggests that this area is too polymorphic for most of the reads to map correctly.

Firstly, many reads would have been rejected from the mapping procedure since only paired reads are used, meaning that any reads whose partner has failed for some reason would not be considered in this analysis. Further polymorphic reads would also fail due to the stringency of the mapping process, therefore only those most similar to the reference genome would be mapped and examined in this analysis. In addition to the mapping methods, the stringency with which we have defined a region as polymorphic would not normally have picked up the two helicase polymorphisms identified in

Figure 3.6 since both are only represented by one read; they were only observed in this case because we already had the sequence available.

The BLAST search was used to detect levels of expression because this is a less stringent search method which would detect more polymorphic matches than the IGV analysis. In addition, this analysis starts with a much larger data set of 6 681 194 reads, of which 71 882 matches were found, instead of the 10 127 (out of 22 134 reads) which were eventually mapped to IGV (Table 3.1). This method found that 97% of the FirrV-1 genome was actively transcribed, which is comparable to previous studies on phycodnavirus transcriptomes which discovered that 86% of EhV-86 genes were expressed during the lytic phase[45] and 99% of PBCV-1 genes were expressed at some point in its life cycle. The previous microarray analysis of EsV-1 transcription revealed a much lower level of active genes (43% in those genes orthologous to FirrV-1) and in general those that were not expressed in FirrV-1 were also not expressed in EsV-1 with the exception of one gene of unknown function.

The fact that the majority of the FirrV-1 genes were found to be transcribed in this study confirms that the viruses were actively reproducing. It also indicates that there are very few gene redundancies in the viral genome, which is an observation common to all phaeoviruses which tend to keep their genome as small as possible in order to remain energetically efficient in spite of containing some repetitive sequences that may have a role in host genome integration[1,87,88].

Those genes of a known function that were very highly expressed include three DNA metabolism genes (superfamily III helicase and two methyltransferases),

the Rad50 ATPase which has a role in DNA double-strand break repair, one sugar biosynthesis gene, as well as the adrenoxin reductase which has a role in the p450 respiration pathway, and two genes involved in cell transport and signalling (a putative integral membrane protein and a putative calcium activated anion channel (putative bestrophin-1)). This supports the previous conclusion that these viruses are actively reproducing, and even replacing some of the host cells' functions in order to ensure their survival until the virion replication has finished. Of special note is the FirrV-1-B6 gene which is very highly transcribed, but a BLAST search revealed no known orthologues. This is obviously an important gene for FirrV-1, but its lack of similarity to any other known genes, even within other phaeoviruses, is highly unusual and may be a function of the strange evolutionary history of these viruses. The DNA proofreading exonuclease gene mentioned in Chapter 2 (EsV-1-126) is highly transcribed in EsV-1 and therefore must play an important role in its DNA replication fidelity, since it is missing from the much more diverse group of viruses infecting *F. irregularis* and *F. simplex*. A further difference between EsV-1 and FirrV-1 is the integrase (EsV-1-213/FirrV-1-B4) activity; this is expressed in FirrV-1 and therefore must play a role in genomic recombination, further adding to its genomic diversity. This gene is not active in the invariant EsV-1.

Of the five genes that were not transcribed, two were of unknown function. One of the remaining three was involved in DNA metabolism (the putative adenine-specific methyltransferase FirrV-1-H4) and may have a role in

controlling gene expression by methylating the DNA; however, since other methyltransferases were active, including one that was highly transcribed, the inactivity of this gene is probably unimportant. The other two inactive genes, putative beta galactosidase small chain FirrV-1-H2 and putative amino acid transporter FirrV-1-P1 are involved in carbohydrate and protein metabolism, respectively which may have many potential uses within the cell and therefore reveal little about the virus infection process.

Not only has this chapter provided useful data about the FirrV-1 genome activity and polymorphisms, this transcriptome could also prove to be an invaluable resource for the study of the *Feldmannia irregularis* genome. Although the host genome has not yet been sequenced, de novo assemblies with Pfam and BLAST searches to detect potential gene functions and orthologues, combined with comparisons of unmapped reads to the recently sequenced *Ectocarpus* genome could reveal much about the *F. irregularis* genome and its activity.

CHAPTER 4 THE PREVALENCE OF VIRUSES IN WILD POPULATIONS OF BROWN ALGAE

4.1 Abstract

The aim of this chapter was to establish a high throughput, low cost method to screen brown algal isolates for phaeoviral infection, in such a way as to provide some information about the type of virus present in the light of the observation of the sequence diversity found in Chapter 2. We found that the High Resolution Melt (HRM) Curve technology using degenerate primers designed against the phaeoviral MCP sequence was able to detect phaeoviral infection, which was common in *Ectocarpus* species: 43% - 79% of isolates studied were infected, depending on species. Additionally it was able to detect viral sequences in kelp species, albeit at a much lower rate (10% - 17% of tested isolates were infected). Sequence and phylogenetic analysis from the kelps revealed that the amplified MCP fragment formed a separate group within the phaeoviruses, more similar to subgroup B sequences than subgroup A. HRM was even sensitive enough to separate 88% of the detected viruses in various species of *Ectocarpus* into the two subgroups identified in Chapter 2, since the two groups had different melting temperature distributions. Futhermore, 17% of isolates studied were infected by at least one virus from both subgroup A and B simultaneously.

In addition, our results show that the degree of wave exposure and the host species have an effect on both the frequency and type of viral infection present.

Isolates from shores exposed to greater wave action were more likely to be infected than those from more sheltered locations. *E. crouaniorum* had a higher rate of phaeoviral infection than both *E. fasciculatus* and *E. siliculosus*, and also had a higher rate of infection by subgroup A viruses, whereas *E. fasciculatus* was more likely to be infected by subgroup B viruses than the other two species.

4.2 Introduction

The phylogeny of the *Ectocarpus* genus has been subject to several changes over the years. Species within the genus were separated from other small brown algae by their ribbon-shaped plastids and lack of phaeophycean hairs[167]. Since then, the classification of species within the genus has changed several times as more accurate taxonomical tools have become available. The most recent phylogenetic relationships, using molecular taxonomy confirmed with crossing experiments to determine hybrid viability, have been proposed by Peters *et al*.[167,185] to contain *E. siliculosus* (Dillwyn) Lyngbye*, E. fasciculatus* Harvey and *E. crouaniorum* Thuret. They also suggest a further two potential genotypes in a study focussing mainly on strains from Western Europe; one closely related to *E. fasciculatus* and isolated in France and one previously isolated from New Zealand (CCAP1301/47) which sits between *E. siliculosus* and *E. crouaniorum* (both marked with * in Figure 4.1). An additional paper published in the same year[185], identified a total of six potential new genotypes from Chile and Peru, one of which contains the genome sequenced strain, but for the purposes of this chapter we will be concentrating on the three main species: *E. siliculosus*, *E. crouaniorum* and *E. fasciculatus*.

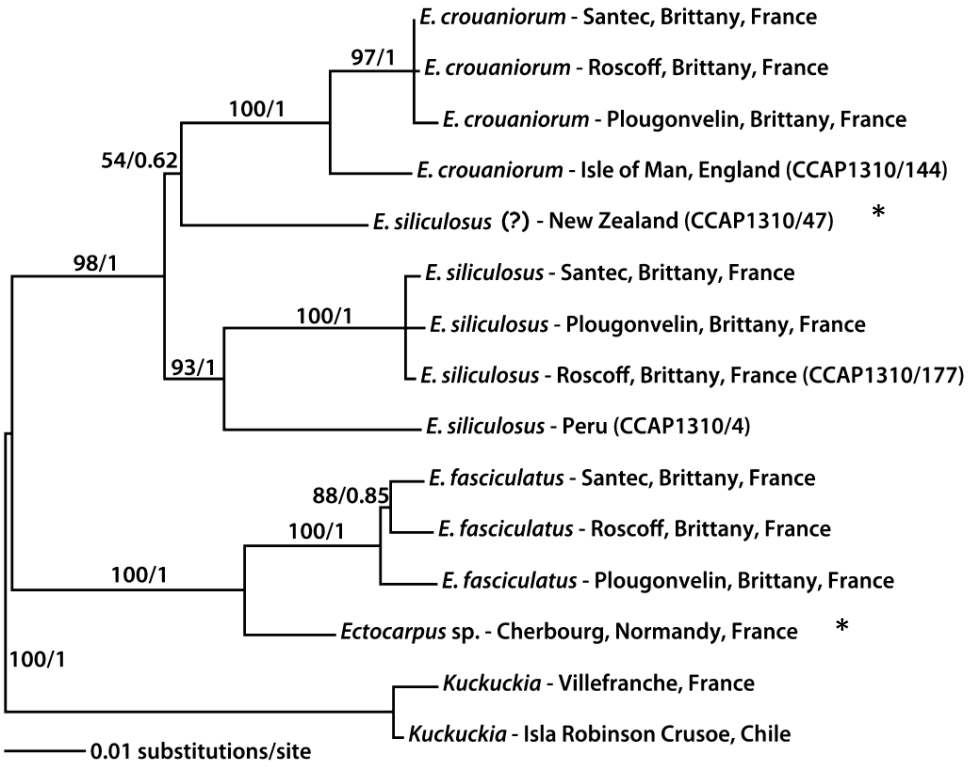

Figure 4.1: Current proposal for taxonomy of *Ectocarpus* and *Kuckuckia* isolates showing the three species, *E. siliculosus*, *E. crouaniorum* and *E. fasciculatus*, along with two potential additional species (*). (This figure was reproduced with permission from [167] © 2010 Japanese Society of Phycology)

The *Ectocarpus* genus is widely distributed in temperate marine environments, growing on rocky shores from high intertidal pools to the sublittoral zone[90]. The three main *Ectocarpus* species, collected for this study, occupy slightly different niches[167] on the rocky shores from which they were collected and this may affect their susceptibility to viral infection. *Ectocarpus siliculosus* is found growing epiphytically on *Sargassum*, *Ulva*, *Porphyra*, *Gracilaria*, *Saccharina*, *Himanthalia* and *Zostera marina* collected from mid-intertidal pools to subtidal. *Ectocarpus fasciculatus* is found on *Laminaria*, *Saccharina*, *Saccorhiza*, *Himanthalia* and *Zostera marina* from the lower intertidal to subtidal and *Ectocarpus crouaniorum* sporophytes were found on rocks and the

gametophytes, only in spring, found epiphytic on *Scytosyphon,* both in high-intertidal pools[167], as shown in Figure 4.2. Other brown algae of note which also inhabit these zones include the two *Feldmannia* species, discussed in Chapter 2, and members of the order Laminariales which are frequently basiphytes (anchors for epiphytes) for members of the order Ectocarpales[167] as mentioned above.

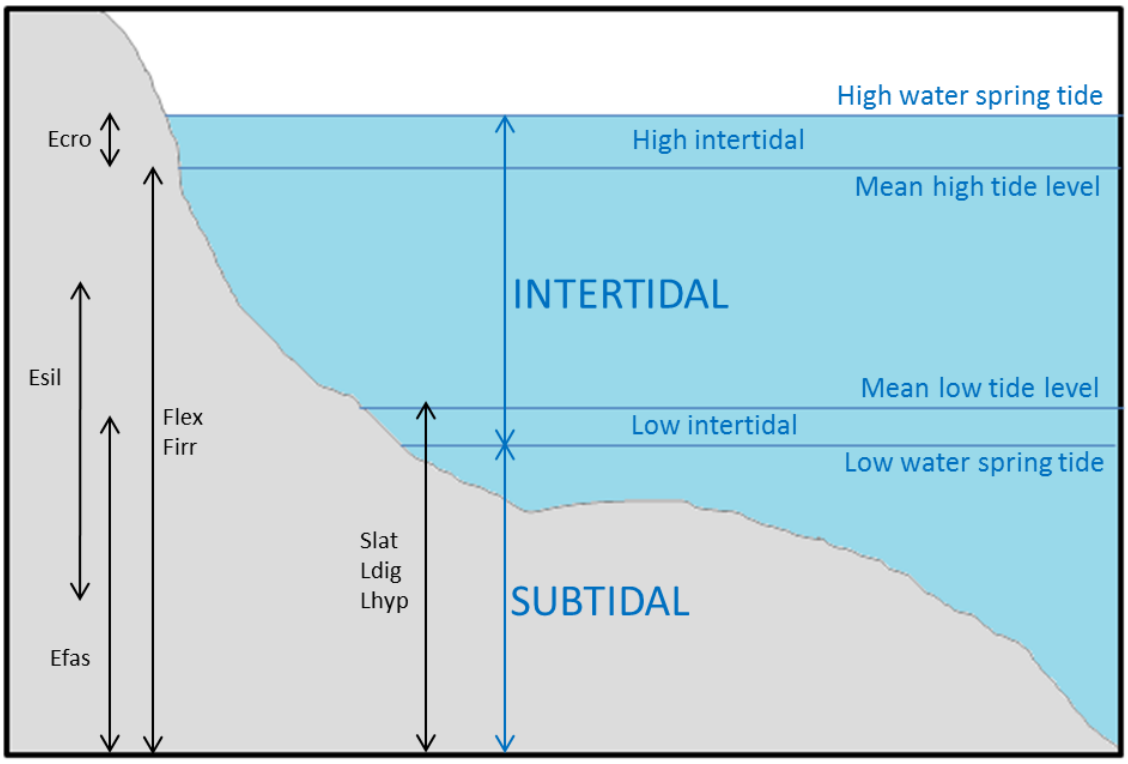

Figure 4.2: Habitat zonation on the rocky shore, and the preferred habitats for *E. siliculosus* (Esil), *E. fasciculatus* (Efas) and *E. crouaniorum* (Ecro)[167], *Feldmannia irregularis*[186,187] (Firr) and *F. simplex* [186,187] (Flex) and three kelp species *Saccharina latissima* (Slat), *Laminaria digitata* (Ldig) and *L. hyperborea* (Lhyp) in the "Laminaria zone"[167].

Investigations in the 1990s into the prevalence of phaeoviruses in wild populations of their hosts concluded that they are highly ubiquitous, occurring

in all populations of filamentous brown algae studied to date[80,98,188], as discussed in detail in sections 1.2.4.1 and 1.2.4.2. However, these studies using PCR-based techniques were limited in both the host range (the genus *Ectocarpus*) and in only detecting the presence or absence of phaeovirus infection in wild populations [79,108,188]. Moreover, numerous studies aiming to investigate the diversity of algal viruses more generally, i.e. screening for phycodnaviruses, have not included the phaeoviruses due to the lack of similarity between their sequences and other phycodnaviruses[75,76]. In addition, the huge diversity of phaeovirus sequence within individual strains discovered in Chapter 2 would not have been revealed by this type of screen. Therefore, a different approach which distinguishes different viral variants is necessary in order to examine the true diversity within environmental isolates.

Sengco *et al.*[80] focused specifically on those phaeoviruses infecting *Ectocarpus* species by extracting total DNA from collected algal strains, rather than using seawater samples. However, even this study did not deal with potential phaeoviruses infecting the remaining brown algal genera, even though viruses are already well characterized in *Feldmannia* [87,88]. Indeed, the "Flex8" variant discovered in Chapter 2 appears to be an evolutionary intermediate originating in an *Ectocarpus* strain, but having evolved to infect *Feldmannia* strains[184] while maintaining some sequence similarities to subgroupA. Although this has only been discovered in just one laboratory strain to date, it seems likely that it will also occur in field samples.

Moreover, large scale viral screening studies on other members of the class Phaeophyceae have not been carried out to date due to a lack of evidence of infection in the larger brown algal species, although viruses have been observed in *Chorda tomentosa* Lyngbye and *Leptonematella fasciculata* Reinke (Silva)[98]. This observation, combined with the fact that symptom suppression is common[101], suggests that virus infections may be a general feature in the entire Phaeophyceae class, and that the virus and host have evolved together into a stable, persistent relationship which would facilitate the transmission of viral DNA to uninfected hosts, potentially across the globe. If this is indeed the case, it would be crucial to understand whether other members of the class are affected, particularly in the ecologically and commercially important species within the order Laminariales (kelps), the sister order of the Ectocarpales, as shown in **Error! Reference source not found.**.

Various methods have been employed to determine the extent of viral infection in the order Ectocarpales, including the identification of viral symptoms by light and electron microscopy[98]. However, the PCR based techniques searching for specific fragments of viral DNA appear to be the most useful since they eliminate the need to rely on observations of symptoms which are commonly suppressed in infected cultures[105]. This allows the use of more targeted PCR-based approaches using primers specific to known core viral genes, such as DNA polymerase or major capsid protein[76,77,79,80,81,189], combined with DNA sequencing in order to allow identification (as demonstrated in Chapter 2).

High resolution melting curve (HRM) analysis allows researchers to both detect amplified product and distinguish between different sequences[190]. This technology uses an intercalating fluorescent dye which binds to double- but not single-stranded DNA[191]. When the PCR product is subjected to incremental temperature increases, the subsequent fluorescence profiles obtained will vary depending on the GC content and other physical properties of the sequence, thus allowing the identification of sequence variations down to a single base pair difference[192]. In combination with a real time PCR cycler, it is possible to quantify, amplify and identify differences in one closed-tube reaction, reducing the potential for errors in processing and eliminating the need for sequencing, making this a highly desirable technique for large-scale and rapid screening[193].

HRM already has a variety of medical diagnostic uses, such as analyzing genetic variants of *Salmonella* in order to determine the most appropriate treatment[194], or patient genotyping to detect mutations in the BRCA1 gene in breast cancer testing[195], as well as screening for new variants of influenza virus[196]. In the aquatic environment this technique has also been used to differentiate harmful algal bloom species[197] and to identify viruses in the freshwater prawn *Macrobrachium rosenbergii*[198], although to our knowledge HRM analysis has not yet been employed in the study of algal viruses.

This study aims to use a combination of reverse-transcription PCR to detect phaeoviral MCP and HRM to build on previous PCR-based studies by

distinguishing between the two groups of phaeoviruses discovered in Chapter 2. This will provide a cheap, high-throughput method which does not rely on sequencing to identify the virus type. The method will be tested on 1034 phaeophyte specimens collected by Akira Peters (Station Biologique Roscoff) for an unrelated study on *Ectocarpus* phylogeny from various locations around Europe, Chile and Peru (see Figure 4.3). In addition, 48 *Laminaria digitata* (Hudson) J.V.Lamouroux, 10 *Laminaria hyperborea* (Gunnerus) Foslie and 24 *Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders isolates were also sampled from Perharidy (location 35 in Figure 4.3) and will be tested in order to determine whether this screening method could detect potential viruses in the commercially important order Laminariales and therefore possibly in other members of the Phaeophyceae.

HRM detected phaeovirus MCP in 63% of the *Ectocarpus* isolates tested, as well as in 15% of the Laminariales isolates. Moreover, it was able to successfully distinguish between the two phaeoviral subgroups since they have differing melting temperature distributions, assigning 88% of those detected to one of the two subgroups.

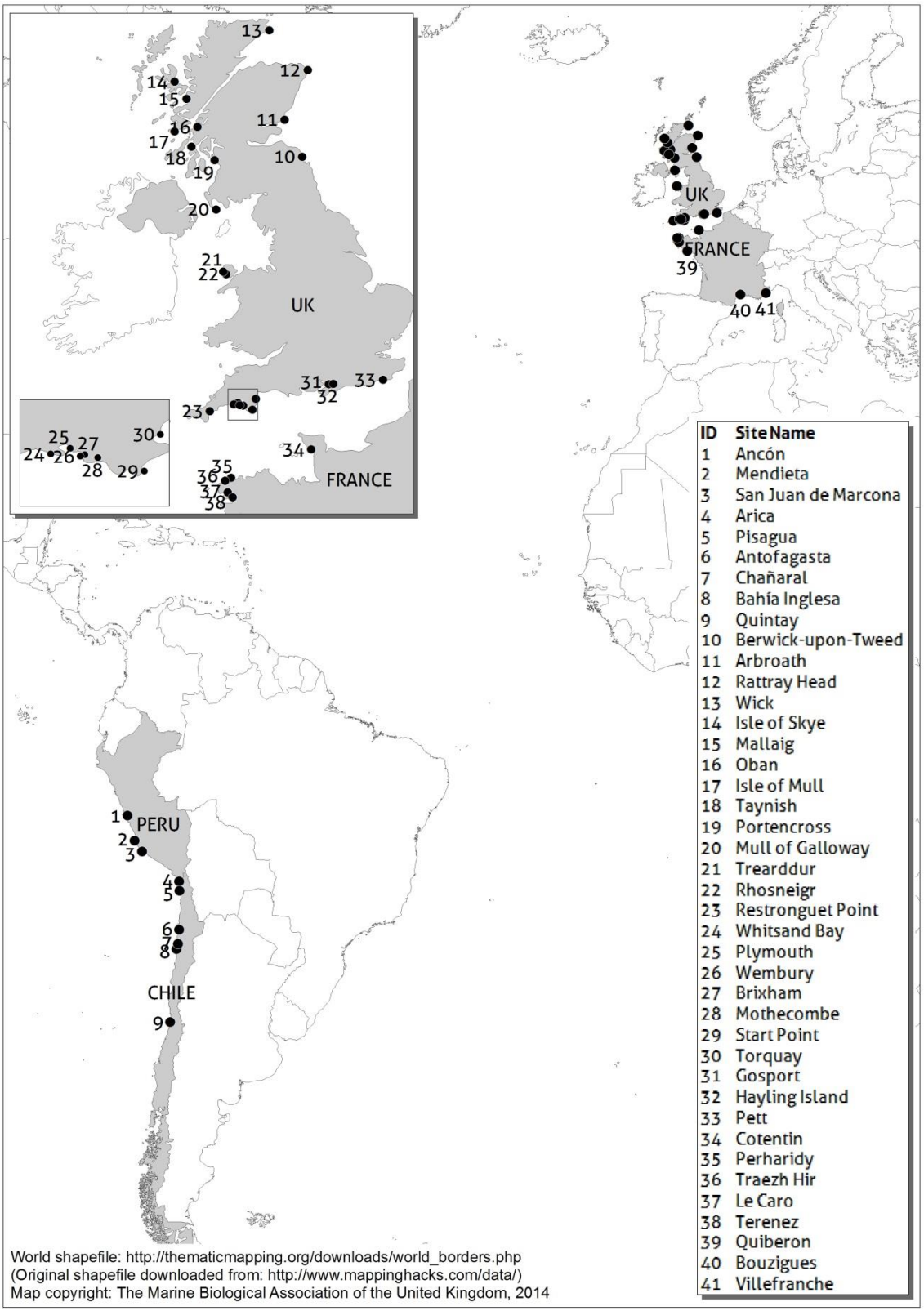

Figure 4.3: Sample collection sites

4.3 Methods

4.3.1 Isolates and DNA extractions

The isolates used in this study, and the locations from which they were collected, are shown in Appendix A.1. The samples consisted of 1034 *Ectocarpus* isolates, focussing on *E. crouaniorum*, *E. fasciculatus* and *E. siliculosus*, collected from a variety of locations throughout the Europe and South America, as well as some kelp species (10 *Laminaria hyperborea,* 48 *L. digitata,* 24 *Saccharina latissima*) from Perharidy in Northern France. Collection and isolation of the cultures and DNA extractions from most isolates were carried out by Akira Peters, the kelp DNA extractions were carried out from uniparental gametophytes by Frank Ehrlich according to the method described in section 2.3.3 (Figure 4.4 step 1). Since these strains were collected for studies on host ecology and phylogenetic relationships, no observations were carried out regarding the presence of viral infection symptoms.

4.3.2 Genotypes used in study

Genotypes Esil1, Firr1 and Flex1 generated in Chapter 2, as well as clones of three environmental screens (HAS08-17B, HAS08-20A and RAT08-5C) (Table 4.1) were used to generate training data (both HRM and DNA sequence) to allow corrections of between run differences (Figure 4.4 steps 6-8) and allow subgroup assignment (steps 6, 7 & 9-11 of Figure 4.4) which will be further explained in section 4.3.5.

Table 4.1: Clones subjected to sequencing, phylogenetic analysis and HRM used to generate training data to assess the effectiveness of the HRM to distinguish between the two groups of viruses. Esil1, Firr1 and Flex1 are the sequenced clones generated in Chapter 2. Numbers in brackets show the location from which each isolate was collected, as shown in Figure 4.3.

Genotype	Source DNA	Phylogenetic subgroup	Reference
Esil1	*E. siliculosus*	A	[184]
Firr1	*F. irregularis*	B	[184]
Flex1	*F. simplex*	B	[184]
HAS08-17B.2	Hastings *E. siliculosus* (33)	A	This study
HAS08-20A.1	Hastings *E. siliculosus* (33)	A	This study
HAS08-17B.3	Hastings *E. siliculosus* (33)	B	This study
HAS08-17B.4	Hastings *E. siliculosus* (33)	B	This study
HAS08-20A.2	Hastings *E. siliculosus* (33)	B	This study
RAT08-5C.1	Rattray Head *E. crouaniorum* (12)	Unassigned	This study
RAT08-5C.2	Rattray Head *E. crouaniorum* (12)	Unassigned	This study
LdigPH10-18.1	Perharidy *L. digitata* (35)	Kelp	This study
LdigPH10-18.2	Perharidy *L. digitata* (35)	Kelp	This study
LhypPH10-3	Perharidy *L. hyperborea* (35)	Kelp	This study
SlatPH10-7.1	Perharidy *S. latissima* (35)	Kelp	This study
SlatPH10-7.2	Perharidy *S. latissima* (35)	Kelp	This study

4.3.3 Real–time PCR and High Resolution Melt Curve analysis

The MCP primers used in this study were as used in Chapter 2, Table 2.2: Primers used for PCRs.

Real-Time PCR (rt-PCR) and High Resolution Melt (HRM) curve analysis (Figure 4.4 steps 2, 3 & 7) were carried out using the SensiMix™ HRM kit on both environmental screening samples and purified colony PCR products (see

sections 2.3.5 and 2.3.6 for PCR cleanup and cloning methods) of the strains mentioned in Table 4.1.

The protocol was used according to the manufacturer's instructions, but decreasing the volumes accordingly to a final mix volume of 10 µl for each reaction, using 0.4 µl 10 µM primers and 0.7 µl 50mM $MgCl_2$ per reaction. To ensure the highest reproducibility between reactions, they were set up with a CAS1200™ automated liquid handling robot from Corbett Life Science. PCR and melt conditions consisted of a 10 minutes initial denaturation and enzyme activation step at 95°C, followed by 40 cycles of 15 seconds at 95°C, 10 seconds at 55°C and 10 seconds at 72°C. To obtain the melting curve, the temperature was ramped from 75°C to 90°C, increasing by 0.1°C per step, with a 90 second wait for pre-melt on step one and 5 seconds for each subsequent step. Reactions were removed from the cycling conditions in the exponential phase of amplification to allow more reliable calculations of the melt temperature.

Each run was initially analysed individually – threshold values for peak calling were set by eye to cut off any peaks lower than the negative controls whilst attempting to minimize false negatives. Any peak above the threshold value indicates the presence of viral DNA in the sample. This process provided the raw melting temperatures which were subsequently used in Figure 4.4 steps 4 & 8.

4.3.4 Cloning, sequencing and phylogeny

Sequencing of cloned HRM products was carried out for two reasons; firstly to determine whether multiple melting temperature peaks corresponded to different sequence variants or were simply due to experimental artefact, and secondly to allow the creation of a training data set to provide a correction factor to calibrate for differences between HRM runs.

Cloning, sequencing and phylogenetic analysis were carried out for the strains in Table 4.1 according to the methods described in Chapter 2, sections 2.3.6 and 2.3.7. Clones were sequenced in triplicate across two different runs to allow an estimation of the technical variability between samples of the same and different runs.

These extra analyses created a training data set which was used for two purposes. Firstly, since the melting temperatures of the HAS08-17B and HAS08-20A isolates were obtained for both individual clones (step 7) and the genomic DNA (step 3), and both should be the same because they originate from the same sequences; any differences were considered to be part of the between run variation and applied to the main screening data set in order to calibrate the results.

The second use of the training data was to determine the phylogenetic relationships between the viral sequences in order to assign a range of melting temperatures to the two subgroups (Figure 4.4 steps 10-11) using clones of Esil, Firr, Flex, HAS08-17B and HAS08-20A (subgroup A and B strains in Table 4.1). The phylogenetic analysis was used to assign a distribution of melting

temperatures to the following viral types: EsV (*Ectocarpus* virus), FirrV (*Feldmannia irregularis* virus), FlexV (*F. simplex* virus), unEV (unknown *Ectocarpus*-like virus belonging to subgroup A), unFV (unknown *Feldmannia*-like virus belonging to subgroup B) or unPV (unknown phaeovirus belonging to neither subgroup A or B).

A normal mixture model with two groups was fitted to the corrected melting points for the environmental isolates. This model assumes that there are two different virus groups with different melting point distributions underlying the data set. The distribution of melting points is different for each virus subgroup, since subgroup B was observed to be much more diverse than subgroup A, and these can be approximated by normal distributions with different mean and variance parameters. These parameters were estimated using iterative maximum likelihood techniques in order to fit the model to the test data. Initial parameters were set to reflect the distributions of melting points observed in the sequenced subgroup A and subgroup B viruses in the training data set. Using these parameter estimates, together with an estimate for the proportion of subgroup A and B virus infections overall, the posterior probability that the virus belonged to subgroup A or subgroup B was computed as follows:

$$\mathrm{p}(\mathrm{subgroup} = \mathrm{A}) = \frac{\lambda \mathrm{f}(\mathrm{x}|\,\mathrm{subgroupA})}{\left[\lambda \mathrm{f}\left(\mathrm{x}|\,\mathrm{subgroupA}\right) + (1-\lambda)\mathrm{f}(\mathrm{x}|\,\mathrm{subgroupA})\right]}$$

λ represents the estimate of the proportion of subgroup A and B infections, and f is the normal likelihood function with mean parameters μ_A and μ_B, and parameters σ_A^2 and σ_B^2 estimated as part of the model fitting process. The

groups had different variances to allow for the differences in variability between the sequences in the two subgroups (i.e. that subgroup B is more diverse than subgroup A). These posterior model probabilities were computed for each melting point and used to assign a viral subgroup, providing it could be assigned with at least 90% probability, otherwise the virus remained unclassified.

4.3.5 Screening pipeline

Figure 4.4 shows the process of assigning a viral subgroup to each of the algal isolates (steps 1-5).First DNA was extracted from the isolates, then rt-PCR and HRM analyses were carried out on each. The raw melting temperatures obtained were first calibrated (step 4) by applying the correction factor obtained from the comparison of the HAS08-17B and HAS08-20A clones and genomic DNA (step 8) and then each corrected melting temperature was assigned to a viral subgroup (step 5) using the posterior group probabilities determined from comparing the phylogenetic grouping with the melting temperatures (step 11).

4.3.6 Association analyses

After assignment of melting temperatures to virus subgroups, various association analyses were carried out to determine whether any relationships existed between viral infection rate, species and degree of wave exposure (as defined by Akira Peters (pers. comms.) of the collection sites.

4.3.6.1 Chi-squared

A Chi-squared (χ^2) test is used to determine whether a set of observed data fits to what would be expected from the hypothesis being tested. It is calculated using the formula:

$$\chi^2 = \sum \frac{(obs - exp)^2}{exp}$$

If this calculated value is greater than the critical value for the appropriate number of degrees of freedom (related to the sample size). In this case a two way classification was used to determine whether the number of infections was the same between exposed and sheltered environments.

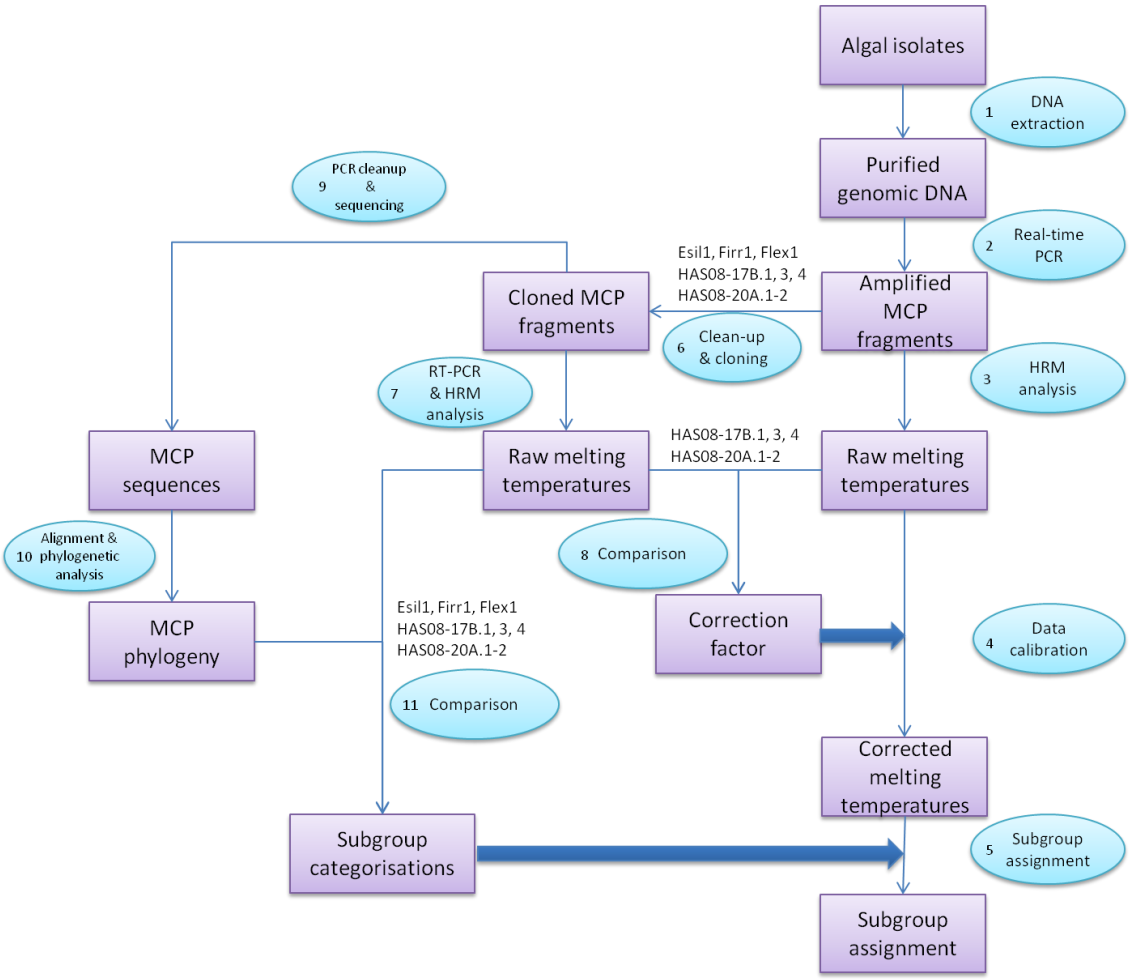

Figure 4.4: Pipeline for assigning viral subgroup to the environmental algal isolates (steps 1-5), showing how the training data was produced and used to calculate the correction factor to allow comparisons between the training data and the environmental samples (steps 6-8), and to assign a viral subgroup to each isolate (steps 6 & 9-11). Purple boxes represent biological samples or data, blue ovals represent processes and unboxed text indicates the clones used for training data in steps 6-11.

4.3.6.2 Logistic regression and odds ratios

A logistic regression model can be fitted when the outcome is dichotomous (i.e. failure and success are coded as a binary variable taking values 0 and 1 respectively) and there is a set of dependent variables which aim to explain differences in the probability of success or failure. In this case to investigate the variables affecting infection rate, the response variable was given the value 1 if

there was a viral infection, and 0 otherwise. In the analysis to compare the rates of group A and B infection in the infected isolates, the response variable was 1 if a group virus was present, and 0 otherwise.

A logistic regression model assumes that there is an underlying bernoulli distribution explaining the dependent variable, i.e. that it takes the value 1 with probability (p) and 0 with the probability (1-p). In a logistic regression the probability (p) is not explained as a function of the dependent variables directly, but instead seeks to explain the log of the odds, calculated as

$$log_e(Odds\ of\ infection) = log_e\left(\frac{P(\text{Infection})}{1 - P(\text{Infection})}\right)$$

as a linear function of the dependent variables. Since the probability (p) takes a value between 0 and 1, the log of the odds takes values between –infinity and infinity. This allows this quantity to be modelled as a linear function of the dependent variables. As a result, probabilities (p) for different variables are not compared, rather the odds via computation of the odds ratio:

$$\text{Odds ratio Group1 : Group2} = \frac{\text{Odds in group 1}}{\text{Odds in group 2}}$$

The odds of infection in group one are said to be equal to the odds ratio times the odds of group two, all other things being equal (e.g. all other factors being controlled for). The hypothesis test to determine whether the odds of infection are different between two groups tested whether the odds ratio is significantly different from 1.

4.3.6.3 Cochran-Mantel-Haenszel test

The Cochran-Mantel-Haenszel test is used to determine whether there are differences in overall viral infection rate in the exposed and sheltered shore environments. Since there may be differences in infection rates according to species, it was necessary to test whether there is a common effect for exposed environments relative to sheltered environments within each host species. Hence a common odds ratio for exposed and sheltered shore environments was fitted within each host species group and permutations were used to determine whether this odds ratio is significantly different from 1. In a permutation test the labels of exposed and sheltered environments were swapped within host species and the common odds ratio recomputed. The p-value of the test is estimated to be the proportion of permutated odds ratio statistics which exceeded the observed odd ratio.

4.3.6.4 Mantel-Haenszel (exact) test

This test was carried out on the 2x2 tables of exposure versus presence/absence of virus infection in order to estimate the common odds ratio of the probability of virus infection occurring in exposed or sheltered environments, and whether this ratio was independent of host species.

4.4 Results

4.4.1 Virus detection by real time-PCR

Real-time PCR screening found that the MCP DNA fragment is highly prevalent in *Ectocarpus* species, infecting 63% of isolates across the three main species as shown in Table 4.2 (79% *E. crouaniorum* (out of 242), 64% *E. siliculosus* (out of 479) and 43% *E. fasciculatus* (out of 215)). Figure 4.5 shows a typical screening run; the fluorescence increases in the majority of the samples, demonstrating the number of positive results in each run.

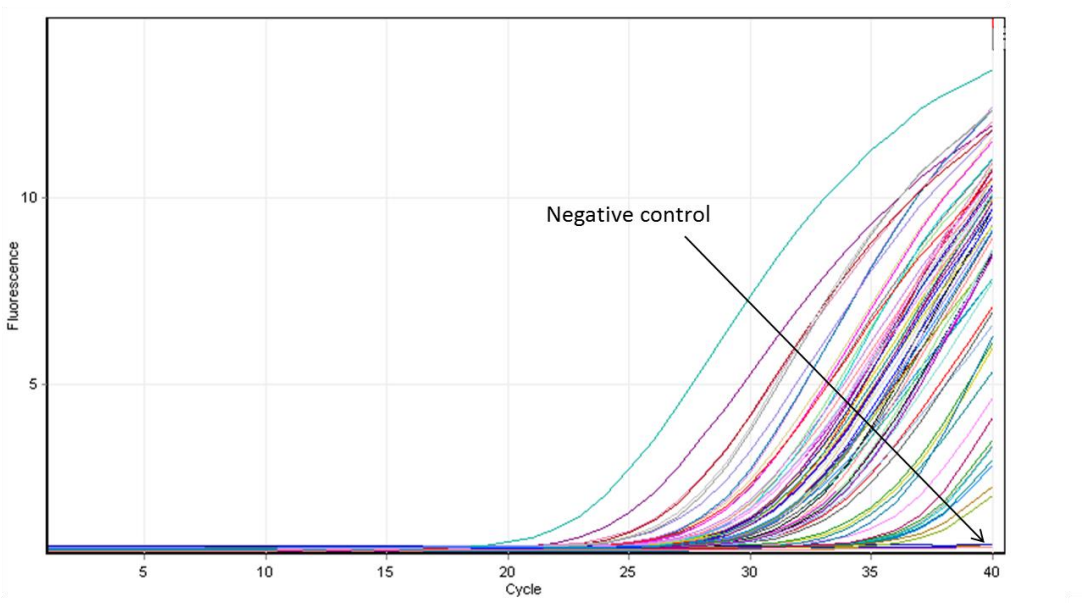

Figure 4.5: A typical real-time PCR screening run demonstrating the increase in fluorescence (y-axis) as the cycle number increases (x-axis) as each PCR product is amplified. The negative control is a DNA-free reaction mix and any product that amplifies above this level is considered to be a positive result.

Table 4.2: Percentage of positive MCP real-time PCR test for *Ectocarpus* species

Species	Total	MCP +	%
Ectocarpus crouaniorum	242	191	78.926
Ectocarpus fasciculatus	215	93	43.256
Ectocarpus siliculosus	479	305	63.674
Totals	936	589	62.927

4.4.2 Training data set

The HRM analysis of the clones created in Chapter 2 (Table 4.1) indicates that it may be possible to separate the two viral subgroups (A and B) if their melting temperature is known. However, further information was needed since the melting points for Flex1 (subgroup B) are close to those for Esil1 (subgroup A). The combination of sequence data and melting point information from the other *E. siliculosus* clones (HAS08-17B and HAS08-20A) isolated from Hastings (Table 3.1) provided greater confidence for separating the subgroups (based on the maximum likelihood analysis in Figure 4.9.

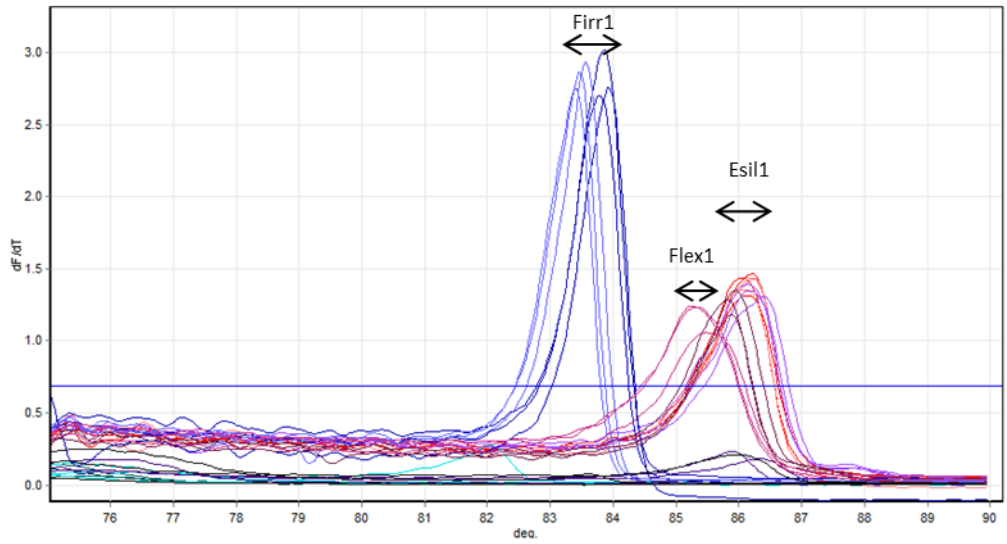

Figure 4.6: HRM analysis of technical replicates for the Chapter 2 clones (Table 4.1. The threshold bar was set at 0.7 to allow calling of the main peaks but no false peaks. The melting temperature in °C is along the X axis, and the Y axis represents a measure of the change in fluorescence.

HRM analysis of the cloned MCP fragments from the infected *Ectocarpus* and *Feldmannia* strains used in Chapter 2, as well as the other clones mentioned in Table 4.1, demonstrated that the melting temperatures could indeed be used to distinguish between the two viral subgroups groups (Figure 4.77). These boxplots clearly indicate that although subgroup B has a wide distribution, the median is skewed to the lower temperatures.

An unexpected observation from the HRM analysis of the HAS08-17B and HAS08-20A genomic DNA and their cloned MCP fragments described in Table 4.1 was that multiple melt temperatures, corresponding to different sequences, were observed in both isolates as shown in Figure 4.88 (a) for the genomic DNA and for both genomic and cloned MCP (Appendix A.2). The maximum likelihood analysis (Figure 4.99) produced from the clone sequences (Table 4.1) shows that

not only do the HAS08-17B and HAS08-20A melting temperature peaks correspond to multiple sequences, but each isolate contains variants from both subgroups A and B. Their melting points are represented in Figure 4.77 (b) and Appendix A.3 as unEV (HAS17B.2 and HAS08-20A.1: unknown *Ectocarpus* virus from subgroup A) and unFV (HAS0817B.4 and HAS08-20A.2: unknown *Feldmannia* virus from subgroup B). The melt points of the unEV and unFV cover almost the entire range of melt points in the training data as shown in Figure 4.77, and the medians of their melt points differ from their equivalent in the environmental data by 0.77 and 0.80 respectively (Appendix A.2). Consequently, the entire set of environmental data was shifted by a mean of 0.79 in order to account for this shift. The calibrated environmental data then aligned with the distribution of the training data as shown Figure 4.88 (b), and therefore gives confidence in our ability to assign groups.

After the data correction was applied, Subgroup A and B medians and variances shown in Appendix A.3 (86.42 & 0.063 and 84.08 & 0.501, respectively) were used as the starting parameters for calculating the posterior probabilities used for subgroup assignment. 88% of the melting temperatures were successfully assigned to one or other of the subgroups using this method (see Appendix A.1 for the full table of results of the screening experiment with subgroup predictions).

It can also be seen that the RAT08-5C sample did contain two sequences with distinct peaks which were detected after cloning, although in the original HRM

analysis (Figure 4.8 (a)) the second peak was below the threshold and therefore not detected. These two RAT08-5C (*E. crouaniorum*) sequences were not obviously assignable to either subgroup A or B, and were instead assigned as unPV and unPV2 (unknown phaeovirus) in Figure 4.7 (b). Both Figure 4.7 and Figure 4.9 demonstrate the diversity of subgroup B viruses compared to subgroup A viruses which have a much more tightly packed distribution of both sequences and melting temperatures.

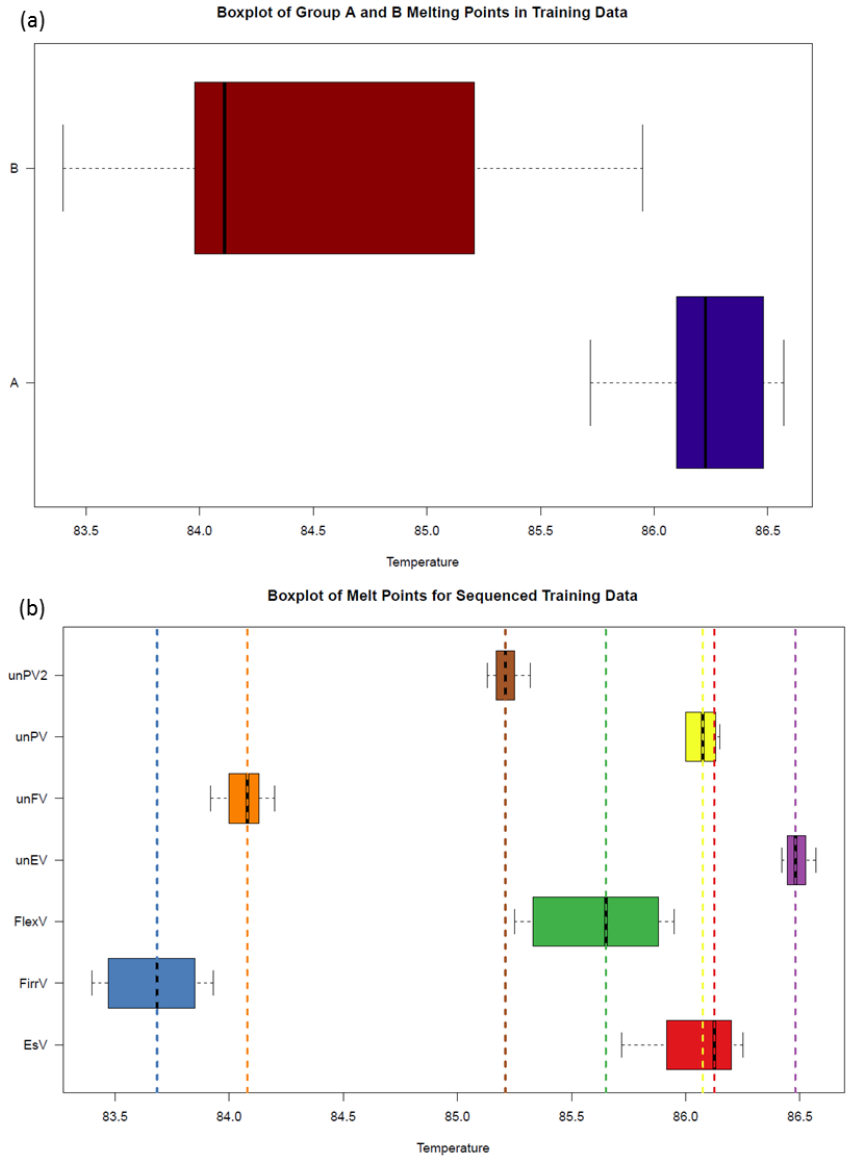

Figure 4.7: Box and whiskers plot of melting temperatures obtained from cloned MCP fragments. The box represents the interquartile range which shows the middle 50% of the data, the left line being the first quartile, the middle line being the median and the right line being the third quartile. The whiskers represent the maximum (or minimum) data point up to 1.5 times the box width to either side of the box.

(a) the variation between replicates and different sequences of the two subgroups A and B.

(b) the variation between replicates of the various virus sequences as defined in Figure 4.9 (EsV and unEV (unknown *Ectocarpus* virus) belong to subgroup A, FirrV, FlexV and unFV (unknown *Feldmannia* virus) belong to subgroup B. unPV and unPV2 (unknown phaeovirus) were not assigned to either group phylogenetically).

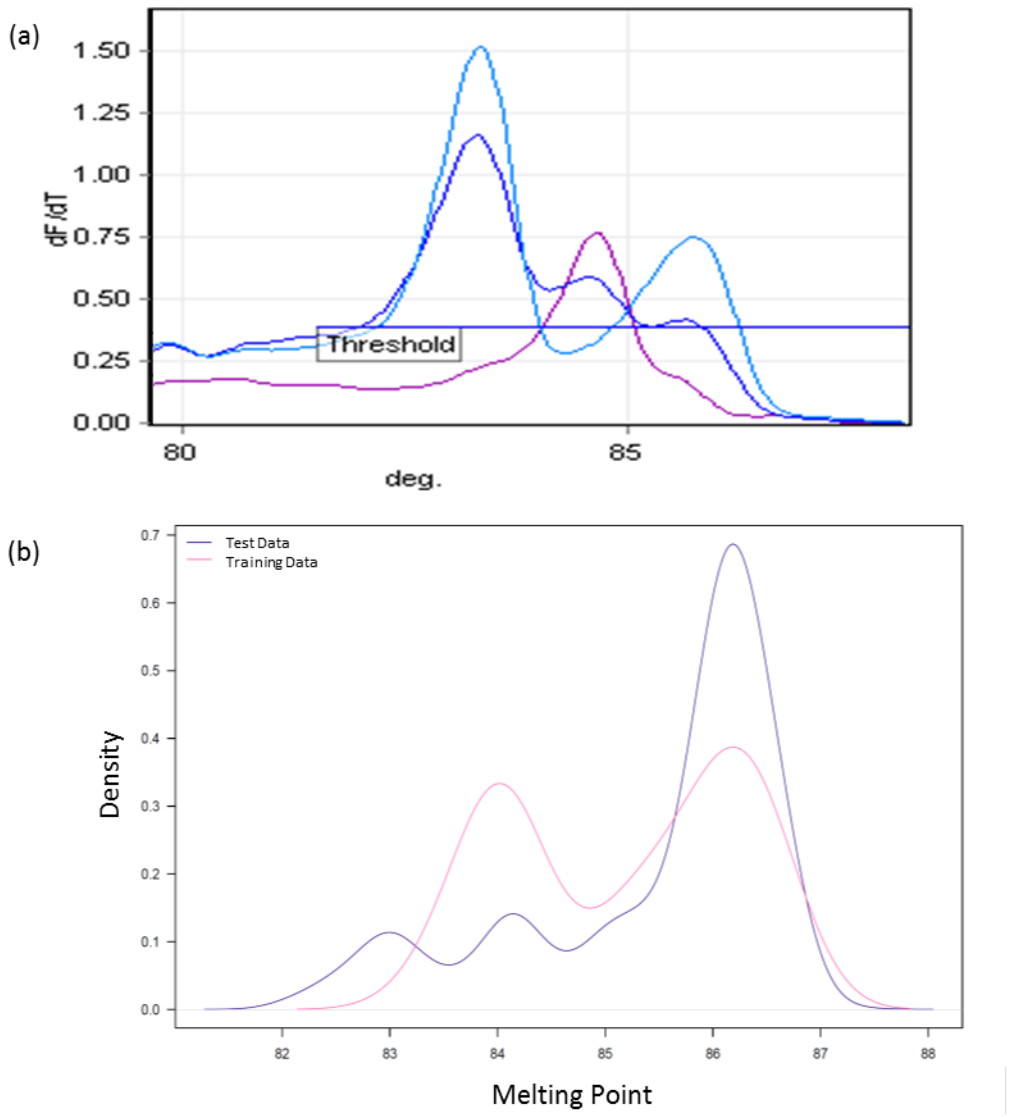

Figure 4.8 (a): MCP HRM analysis of two *E. siliculosus* (HAS08-17B and HAS08-20A) and one *E. crouaniorum* (RAT08-5C) isolate showing that multiple peaks do indeed occur in these wild populations. Key: Dark blue is HAS08-17B, lighter blue is HAS08-20A and purple is RAT08-5C. The threshold bar was set at 0.38 to allow calling of the main peaks but no false peaks. The melting temperature in °C is along the X axis, and the Y axis represents a measure of the change in fluorescence.

(b) Distributions of melting points for the training data (pink) and all environmental screening results after calibration (blue). The peaks represent the most common melting temperatures for each dataset.

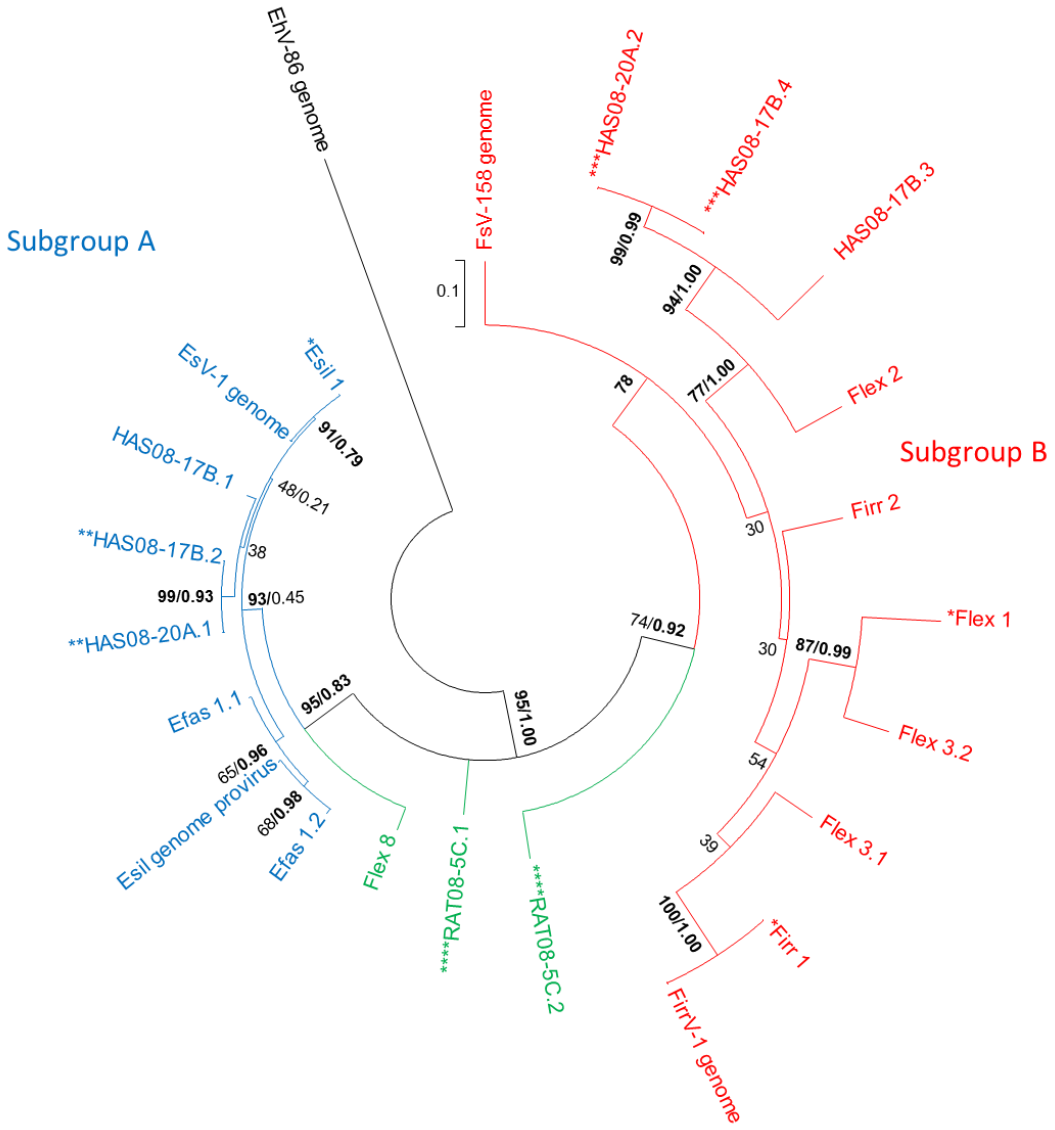

Figure 4.9: Maximum likelihood analysis of cloned phaeoviral MCP fragment as in Figure 2.1, including all sequences used for training data, with EhV-86 being used as an outgroup. Single value node labels represent ML bootstrap values. Where nodes are labelled with two values, this indicates that both ML and Bayesian topologies agree (whole numbers represent ML bootstrap values, decimals indicate Bayesian posterior probability). Subgroup A viruses are labelled in blue, subgroup B viruses are red and the intermediate Flex and Ecro virus variants are green. Bold values are those greater than 75% bootstrap or probability. Sequences marked with asterisks are those used in the training data. *indicates sequences from laboratory maintained strains, ** indicates unknown *Ectocarpus* virus (unEV) sequences, *** indicates unknown *Feldmannia* virus (unFV) sequences, **** indicates unknown phaeoviruses (unPV and unPV2).

4.4.3 Infection rates vs. host species and exposure to waves

The fact that the data sets studied in this chapter were of unequal sizes means that a simple comparison of the number of infections in each species would be misleading, and therefore it was decided to use a chi-squared test to account for the differences in sample sizes. This was carried out based on the null hypothesis that the number of infections (characterized as the number of melting temperature peaks) is the same between sheltered and exposed environments based on the data in Table 4.3, which revealed that the sheltered environments have lower than expected infection rates compared to the exposed environments ($p = 2.5e^{-07}$). For example, if the distribution was homogeneous between the two environments, the number of isolates with a single viral infection would be expected to be 105.7 in exposed environments and 298.3 in sheltered environments, but the observed numbers were 143 and 261, respectively. An additional odds ratio estimated by the fitting of a logistic regression model indicated that the odds of a sample being infected by one or more viruses is 2.03 (95% confidence interval 1.47-2.81) times greater than for a sample from a sheltered environment.

Table 4.3: Number of infections in all isolates from exposed and sheltered environments. Numbers in brackets represent the expected values if infection rates were homogeneous between the two types of environments.

Number of infections (HRM peaks)	**Exposed**	**Sheltered**	**Total number of isolates**
0	63 (91.3)	286 (257.7)	**349**
1	143 (105.7)	261 (298.3)	**404**
2	34 (37.7)	110 (106.3)	**144**
3	6 (11.3)	37 (31.7)	**43**
Total number of isolates	**246**	**694**	**940**

Concentrating on the most abundant species, i.e. *E. siliculosus*, *E. fasciculatus* and *E. crouaniorum*, a further Chi-squared test for homogeneity of virus type with species also rejected the null hypothesis ($p = 3.96e^{-19}$). Table 4.4 shows that *E. fasciculatus* had fewer subgroup A infections than expected (30 observed, 69.3 expected) but more from subgroup B (19 observed, 8.6 expected), *E. crouaniorum* showed the reverse pattern (118 observed and 80.8 expected in subgroup A; 7 observed and 10 expected in subgroup B), and the number of subgroup A infections in *E. siliculosus* were roughly as expected (158 observed, 156 expected) but the subgroup B infection rate was lower (12 observed, 19.4 expected).

Table 4.4: Incidence of infection in *E. siliculosus*, *E. fasciculatus* and *E. crouaniorum* by subgroup A and B viruses. Numbers in brackets represent the expected values if infection rates were homogeneous between the three species.

Viral subgroup present	***E. siliculosus***	***E. crouaniorum***	***E. fasciculatus***	**Total number of isolates**
A	158 (156)	118 (80.8)	30 (69.3)	**306**
B	12 (19.4)	7 (10.0)	19 (8.6)	**38**
Both	77 (71.8)	43 (37.2)	21 (31.9)	**141**
None	174 (173.8)	50 (90)	117 (77.2)	**341**
Total number of isolates	**421**	**218**	**187**	**826**

However, a further chi-squared test revealed that the null hypothesis of equal distribution of species between sheltered and exposed sites can be rejected ($p = 1.2e^{-45}$). From Table 4.5 it can be seen that the distribution of species is not uniform between exposed and sheltered sites: *E. crouaniorum* and *E. fasciculatus* are more abundant than expected in exposed areas and less common in sheltered areas (104 observed, 63.9 expected and 109 observed, 54.6 expected, respectively), whereas the converse is true for *E. siliculosus* (31 observed, 127.5 expected).

Table 4.5: The distribution of *E. siliculosus*, *E. fasciculatus* and *E. crouaniorum* in exposed and sheltered environments. Numbers in brackets represent the expected values if the three species were distributed evenly between exposed and sheltered environments.

Species	**Sheltered**	**Exposed**	**Total number of isolates**
E. siliculosus	448 (359.8)	31 (127.5)	**479**
E. crouaniorum	136 (180.2)	104 (63.9)	**240**
E. fasciculatus	96 (154.0)	109 (54.6)	**205**
Total number of isolates	**680**	**244**	**924**

Because both algal host species and exposure have an effect on the infection rates of these viruses, a further test was carried out to take both of these factors into consideration. Table 4.6 shows the incidence of infection of the two subgroups of viruses by exposure, subdivided into species which demonstrates the variability in viral subgroup between both the different species and the exposure levels.

The observed frequency of the different viral subgroups within *E. siliculosus* are roughly as would be expected if the distribution were homogeneous between the different subgroups and exposure levels; for example, the expected amounts of subgroup B in sheltered and exposed locations were 11.2 and 0.8 respectively, and the observed amounts were 12 and 0 (Table 4.6 (a)).

However, *E. crouaniorum* had many more uninfected isolates in sheltered locations than expected (observed 43, expected 27.3) and fewer uninfected isolates in exposed areas than expected (observed 7, expected 22.7).

Consequently the number of subgroup A infections were much lower than expected in sheltered areas (observed 46, expected 63.9) and higher in exposed areas (observed 71, expected 53.1). The number of subgroup B and infections by both subgroups were roughly as expected in this species (Table 4.6 (b)).

Conversely, in spite of *E. fasciculatus* also being enriched for uninfected isolates in sheltered shores (observed 69, expected 58.8) compared to exposed (observed 46, expected 56.2), it shows the opposite subgroup infection pattern to *E. crouaniorum*, having more subgroup B than expected in exposed shores (observed 18 expected9.3) and fewer in sheltered areas (observed 1, expected 9.7). However, it does also have approximately the expected numbers of the other subgroup (A) and both subgroups in both types of locations, as was seen for *E. crouaniorum* (Table 4.6 (c)).

A Cochran-Mantel-Haenszel test for homogeneity across sheltered and exposed sites with respect to species based on Table 4.6 led to rejection of the null hypothesis ($p = 8.583e^{-11}$), i.e. both species and exposure have an effect on the type of virus infection.

The data from Table 4.6 was reduced to a binary form considering only isolates which tested positive for virus infection, with those only infected by subgroup A viruses represented by 0 and those infected by subgroup B, or both A and B, as 1. Fitting a logistic regression model to this binary data shows that the odds of

subgroup B infection is significantly more likely (p = 0.00302) in infected isolates of *E. fasciculatus* than in either *E. crouaniorum* or *E. siliculosus*.

A Mantel-Haenszel (exact) test, considering the presence or absence of virus infection for each species individually, rejected the hypothesis of infection rates for each species being independent of exposure (p = 8.8e-11) with an estimated common odds ratio of 3.74 (95% confidence interval 2.42-5.89). When each species was considered individually, they each had different odds ratios as shown in Table 4.7. Although the 95% confidence intervals for each species (1.19 – 8.35 for *E. siliculosus*, 2.84-15.45 for *E. crouaniorum*, 1.62-5.20 for *E. fasciculatus*) contain the common odds ratio (3.74), this test still suggests that the infection rate of *E. crouaniorum* is higher than that in *E. siliculosus* and *E. fasciculatus*, and that exposure increases the chance of phaeoviral infection in all three species.

Table 4.6: A three-way contingency table showing the viral subgroups in sheltered and exposed samples divided by species: (a) *E. siliculosus* (b) *E. crouaniorum* and (c) *E. fasciculatus.* Numbers in brackets represent the expected values if the viral types were distributed evenly between exposed and sheltered environments for each algal host species.

(a) *E. siliculosus*

Infection type	**Sheltered**	**Exposed**	**Total number of isolates**
None	169 (162.0)	5 (12)	**174**
Subgroup A Only	139 (147.1)	19 (10.9)	**158**
Subgroup B Only	12 (11.2)	0 (0.8)	**12**
Both	72 (71.7)	5 (5.3)	**77**
Total number of isolates	**392**	**29**	**421**

(b) *E. crouaniorum*

Infection type	**Sheltered**	**Exposed**	**Total number of isolates**
None	43 (27.3)	7 (22.7)	**50**
Subgroup A Only	46 (63.9)	71 (53.1)	**117**
Subgroup B Only	4 (3.8)	3 (3.2)	**7**
Both	25 (23.0)	17 (19.0)	**42**
Total number of isolates	**118**	**98**	**216**

(c) *E. fasciculatus*

Infection type	**Sheltered**	**Exposed**	**Total number of isolates**
None	69 (58.8)	46 (56.2)	**115**
Subgroup A Only	13 (14.3)	15 (13.7)	**28**
Subgroup B Only	1 (9.7)	18 (9.3)	**19**
Both	9 (9.2)	9 (8.8)	**18**
Total number of isolates	**92**	**88**	**180**

Table 4.7: Odds ratios of infection rates for *E. siliculosus*, *E. fasciculatus* and *E. crouaniorum* from exposed and sheltered environments, including the 95% confidence intervals and p-value.

Species	Odds Ratio	Odds Ratio (95% Conf. Int.)	p-value
E. siliculosus	3.15	[1.19, 8.35]	1.9e-02
E. crouaniorum	6.63	[2.84, 15.45]	9.4e-07
E. fasciculatus	2.91	[1.62, 5.20]	3.6e-04

4.4.4 Laminariales screening

Some of the kelp isolates also tested positive for the phaeoviral MCP fragment, albeit at a lower rate than in the *Ectocarpus* isolates, as shown in Table 4.8: 10% *Laminaria hyperborea* (out of 10), 17% *L. digitata* (out of 48) and 13% *Saccharina latissima* (out of 24) were infected. Table 4.9 provides the full results, including melting temperatures. Since no signs of virus infection have ever been observed in this order, it was decided to sequence one MCP positive isolate from each species (Table 4.1), in order to determine whether these could be due to contamination from ectocarpoid endophytes. Phylogenetic analysis showed that the Laminariales viruses group together (Figure 4.10) and are more closely related to subgroup B than to subgroup A, but still form a separate group, with a bootstrap value of 60, posterior probability of 0.70. The group prediction based on these melting temperatures fits within the group B viruses with high significance, except for two peaks which do not significantly belong to either subgroup A or B (see Table 4.9.)

Table 4.8: Percentage of positive MCP real-time PCR tests for members of the Laminariales.

Species	Total	MCP +	%
Laminaria digitata	48	8	16.67
Laminaria hyperborea	10	1	10.00
Saccharina latissima	24	3	12.50
Totals	82	12	14.63

Table 4.9: Positive real-time PCR results from the kelp isolates collected from Perharidy, France (location 35 of Figure 4.3 in 2010. Isolates marked * were sequenced for the phylogeny in Figure 4.100. Total number of isolates for *L. digitata* = 48, *L. hyperborea* = 10, *S. latissima* = 24. Posterior probabilities indicate the chance that the peak belongs to subgroup A instead of subgroup B.

Genotype	Species	Peak 1	Peak 2	Predicted Group	Posterior probability
LdigPH10-10	*Laminaria digitata*	83.38		B	0.0012
LdigPH10-11	*Laminaria digitata*	83.25		B	2.00E-04
LdigPH10-18*	*Laminaria digitata*	83.15		B	0
LdigPH10-22	*Laminaria digitata*	83.27		B	2.00E-04
LdigPH10-24	*Laminaria digitata*	83.25		B	2.00E-04
LdigPH10-31	*Laminaria digitata*	82.97		B	0
LdigPH10-33	*Laminaria digitata*	83.3		B	4.00E-04
LdigPH10-44	*Laminaria digitata*	83.27		B	2.00E-04
LhypPH10-3*	*Laminaria hyperborea*	82.4	83.45	B/B	0/0.0033
SlatPH10-10*	*Saccharina latissima*	83.22	83.98	B/NA	0.0001/0.5443
SlatPH10-14	*Saccharina latissima*	84		NA	0.5849
SlatPH10-20	*Saccharina latissima*	83.53		B	0.0096

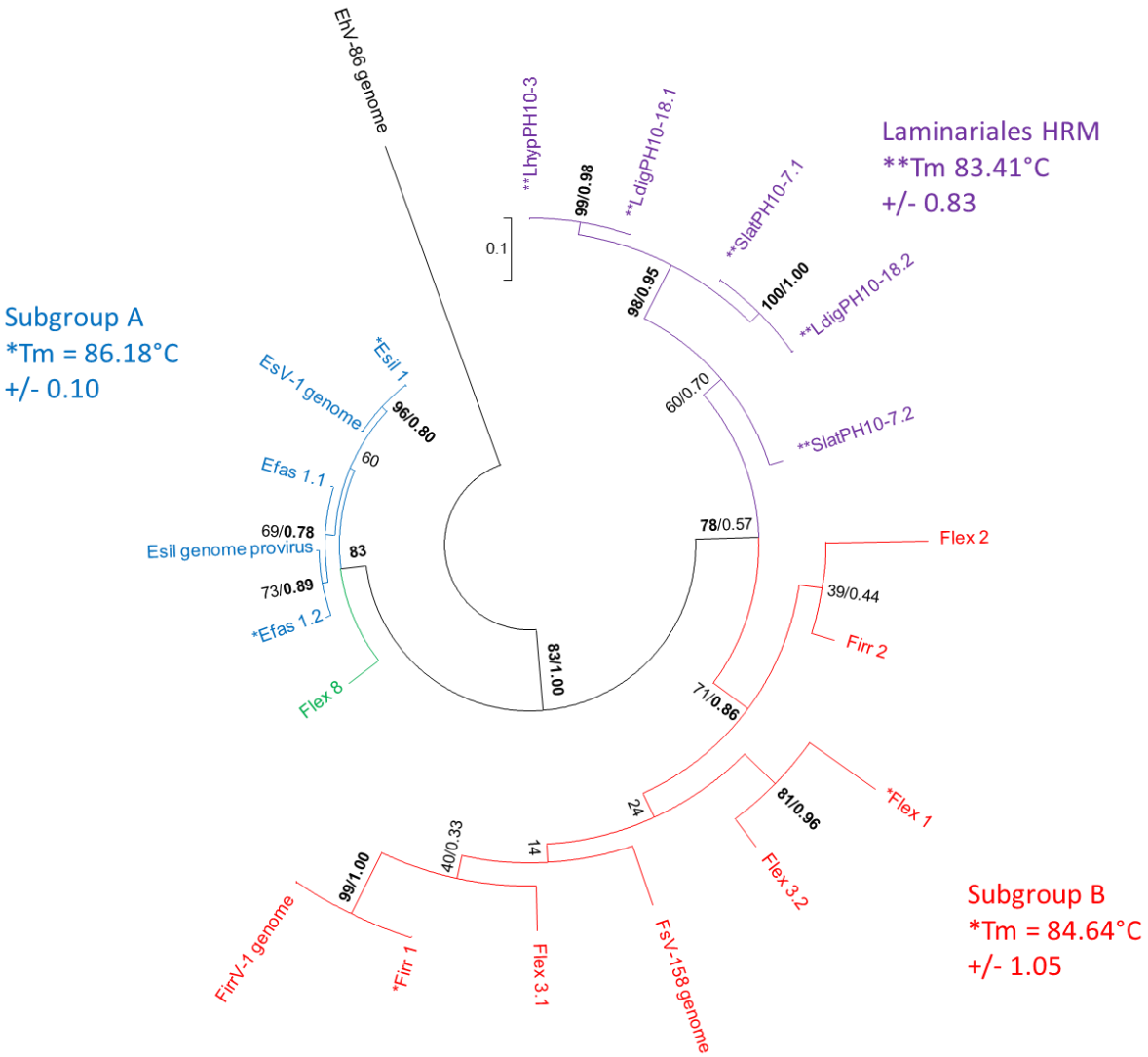

Figure 4.10: Maximum likelihood analysis of cloned phaeoviral MCP fragment as in Figure 2.1, including the positive Laminariales results from the screening study, with EhV-86 being used as an outgroup. Single value node labels represent ML bootstrap values. Where nodes are labelled with two values, this indicates that both ML and Bayesian topologies agree (whole numbers represent ML bootstrap values, decimals indicate Bayesian posterior probability). Subgroup A viruses are labelled in blue, subgroup B viruses are red, the intermediate Flex virus variant is green and the Laminariales sequences are in purple. Bold values are those greater than 75% bootstrap or probability.

4.5 Discussion

This study has successfully expanded on previous phaeoviral screening studies by using HRM analysis combined with a newly designed pair of MCP primers to not only detect the presence of viruses, but also to assign 88% of the detected viruses to one of the two viral subgroups (*Ectocarpus* virus-like or *Feldmannia* virus-like[184]). In addition, we have successfully detected phaeoviruses in the kelps which, to our knowledge, have not been previously reported to be infect by these viruses.

This study showed that 43%, 64% and 79% isolates of *E. fasciculatus*, *E. siliculosus* and *E. crouaniorum*, respectively are infected with at least one phaeovirus, i.e. HRM amplification of phaeoviral PCR revealed at least one melting temperature peak. This falls well within the amounts found by previous PCR-based studies which detected viral DNA in between 40 – 100% of natural *Ectocarpus* populations[105,108]. In addition, the previous studies of physical symptoms in field populations confirm the infection rate being proportionately lower in *E. fasciculatus* (less than 10%[79]) than in *E. siliculosus* (up to 20%[98]).

The training data revealed that the two viral subgroups do indeed have different melting temperatures and that these can be used to assign viral types to the melting temperatures obtained from environmental isolates of various *Ectocarpus* species. Although we are confident of our ability to assign viral subgroups within the *Ectocarpus* species, further work would be necessary before we could confidently apply this technique to more widespread screening

of other members of the brown algae such as kelp or *Feldmannia*, since our training data did not consider these other algae.

The discovery of multiple peaks in the environmental isolates was not wholly unexpected given the results of the sequencing studies of Chapter 2, which revealed multiple virus sequences within laboratory maintained strains of ectocarpoid algae[184]. However, the extent to which both subgroup A and B viruses are present in the same environmental isolate, as revealed by melting temperature analysis, as well as cloning and sequencing, was completely unexpected based on previous assumptions that each host strain is infected by only one phaeovirus. Nonetheless, we have already discovered an intermediate virus sequence ("Flex8") present in the same Flex isolate as subgroup B virus variants which may be a sign of the emergent evolution of new phaeoviruses; infections by viruses from both subgroups could be considered as a further necessary step in the evolution of phaeoviruses, allowing for exchange of genomic material.

The fact that *E. crouaniorum* and *E. fasciculatus* are enriched on exposed shores may be because their preferred high and low shore locations[167] (**Error! Reference source not found.**) are somewhat more stable than the mid-intertidal range preferred by *E. siliculosus* which would be particularly vulnerable to wave action for a higher proportion of the time. Although the result was not statistically significant, our analyses still suggest that *E. crouaniorum* is more likely to be infected by one or more phaeoviruses than the

other two species studied here. In spite of the enrichment of *E. crouaniorum* on exposed shores, it still has a higher rate of overall phaeoviral infection than *E. fasciculatus* which is also enriched on exposed shores. This is perhaps counter-intuitive since its high shore location would be less exposed to water-borne viruses; however this could be a sign of a higher rate of an early persistent infection of this species after the split from the other *Ectocarpus* species.

The virus type (i.e. the subgroup present) is not homogeneous between the 3 most prevalent *Ectocarpus* species when considering both sheltered and exposed locations together. Although the differences in type of viral infection within infected isolates is only statistically significant in showing that *E. fasciculatus* has a higher proportion of subgroup B than the other two species, our analysis still suggests that *E. fasciculatus* and *E. siliculosus* are enriched for subgroup B, whereas *E. crouaniorum* has more A and less B than expected. *Feldmannia irregularis* and *F. simplex* (subgroup B viral hosts) are found in the mid- and low-intertidal and subtidal zones[186,187] (**Error! Reference source not found.**2), closer to the populations of *E. fasciculatus* and *E. siliculosus* which may explain the enrichment of subgroup B viruses in these species since the closer contact will facilitate cross species infections. We cannot draw any conclusions about whether the converse has occurred, i.e. whether subgroup A viruses have infected *Feldmannia* species, since the sampling effort was targeting *Ectocarpus* hosts, although it seems likely that this has occurred, given the "Flex8" virus grouping with subgroup A sequences shown in the previous

chapter. A similar study targeting *Feldmannia* species would reveal whether subgroup A viruses have infected this genus to the same extent.

When considering the effect of wave exposure on subgroup type, *E. crouaniorum* and *E. fasciculatus* showed opposite trends, i.e. that the former had fewer A than expected in sheltered, whereas the latter had more B than expected in exposed areas. This suggests that wave exposure is an important factor for the spread of both phaeoviral subgroups, since *E. fasciculatus* will be exposed for longer periods since it occupies the lower shore level and thus is more likely to come into contact with new phaeoviral infections. Conversely, *E. croaniorum* on the high shore will be exposed to waves for less time in each tidal cycle and therefore has a lower infection level.

Regardless of viral subgroup, this HRM assay has shown that the frequency of viral infection is affected by both the host species and the exposure to wave energy of the shore from which the isolate was collected. However, since exposure also affects species distribution, it is very difficult to determine which of these is the most important factor for determining rate of phaeovirus infection.

The common odds ratio showing that exposed isolates are 3.74 times more likely to be infected by a phaeovirus than those from sheltered sites is a fairly large effect. Other studies of factors affecting viral infection have found odds values of similar orders of magnitude, from 1.2-2.69 (the odds of injecting drug

users being infected with Hepatitis C virus living in Glasgow instead of London[199]) to 4.4 (the odds of West Nile virus infection in humans in US counties with less forest cover (a proxy for urbanisation) than heavily forested counties[200]), up to greater than 10 (the odds of Epstein-Barr virus infection in humans with systemic lupus erythematosus[201]).

It was decided that due to the confounding factors of differences in infection between different species and also exposure, and the fact that not all species were represented in all locations and that the proportions of exposed and sheltered habitats vary with location, studies of virus prevalence and type with location would prove inconclusive. More sampling effort with an experimental design to specifically target this question is necessary.

Finally, we have successfully detected what appears to be a new group of viruses infecting the kelps. In spite of the melting points of the kelp MCP fragments identifying them as belonging to subgroup B, they still formed a phylogenetically distinct division within this subgroup. It seems likely, therefore, that these are indeed unique to the kelps and not due to potential contamination from endophytic members of the order Ectocarpales. This potential virus group is very interesting and worth further investigation, since the kelps are a group of economically important algae, and therefore anything potentially affecting their growth and fertility would be of great scientific and commercial interest. Indeed it may prove possible to use this screening test to

extend the range of phaeoviruses detected even further to include more members of the brown algae, such as the order Fucales.

CHAPTER 5 FINAL DISCUSSION

The importance of viruses in the marine environment is apparent when one considers their abundance and the fact that they are probably the most diverse group of organisms on the planet[202], playing such crucial roles as controlling host abundance[40], biogeochemical processes[40], horizontal gene transfer and thus host evolution[18]. In spite of the wealth of information about the physical infection process of EsV-1 in *Ectocarpus siliculosus* from investigations by Dieter Müller[97,101], little is understood about the true diversity, and thus evolution, of phaeoviruses, since most phycodnaviral studies only grant them a cursory investigation[75,76].

5.1 Phaeoviruses are extremely diverse

In attempting to further our understanding of the genetic diversity, distribution, infection frequency and infection mechanisms within the phaeoviruses specifically, this study has revealed a great deal more than originally expected by revealing the diversity of phaeoviruses in the strains studied here. The true extent of phaeoviral diversity has only been hinted at previously from suggestions that viral size variants in a *Feldmannia* species are due to differences in repeat regions[158]. However, the results from this study show that this is only the tip of the iceberg. The phaeoviruses were once considered a single virus group based on a shared phylogeny [64] and classified according to the host in which they are found [88]. Based on our observations they need to

be redefined into two subgroups; one conserved group that infects at least three genera within the Ectocarpales, and one much more diverse group which has so far only been observed within the *Feldmannia* genus. While these two groups have already been suggested, based on previous observations of the genome size as well as observed differences in membrane composition[74], this study has provided a great deal more information to confirm this theory.

By carrying out a routine PCR and sequencing effort to enable us to carry out maximum likelihood analysis to confirm the classification of the putative phaeoviruses EfasV-1, FlexV-1, PlitV-1, HincV-1 and MclaV-1, considerably more has been revealed; specifically, up to eight phaeoviral sequence variants were present in a single algal strain and the presence of polymorphisms in key phaeoviral genes in the transcriptome implies that multiple variants are active within an individual strain.

It is likely that phaeoviral diversity is even greater than discovered here, and this would be confirmed by a further study considering more genes, or even whole viral genomes after virion extraction. Chromosome walking off the known ends of the virus sequence, followed by cloning and sequencing, could reveal the virus insertion position within the host genome, but was not carried out due to time constraints. Complete genome sequencing of host strains would also demonstrate whether multiple complete viruses are indeed inserted into the genome or just multiple viral fragments, as well as allowing identification of the site(s) of viral integration.

However, the diversity of variants found here definitely suggests the existence of two phaeoviral subgroups; subgroup A is a conserved group of viruses that infect at least 3 genera (*Ectocarpus*, *Pilayella* and *Hincksia*) across 2 families (Ectocarpaceae and Acinetosporaceae), whereas the viruses of the much more divergent subgroup B are limited to hosts from just one genus (*Feldmannia*) in the laboratory strains tested here. The observation of both subgroups of viruses in Ectocarpus from the HRM screen suggests that the host boundaries of these subgroups are not so clearly defined, and a similar screening study focussing on Feldmannia isolates would reveal whether these species are also susceptible to infection from both virus subgroups.

To confirm the existence of these two subgroups, further sequence information is necessary; the full host genome sequence mentioned previously would show whether the different gene sequence variants discovered by this study are part of different complete proviruses, or simply inactive fragments of viral genes. If the subgroups are as predicted here, sequencing would also reveal differences in virus genome size as well as gene content which could be used to split the viruses into their respective subgroups.

5.2 Potential phaeovirus evolutionary mechanisms revealed

Thus far, phaeoviruses have been considered to have a *K*-type, persistent evolutionary strategy, favouring stable integration into their host's genome [57]

rather than having the high mutation and reproduction rates associated with *r*-strategists. Our observations show that the diverse subgroup B viruses have adopted a strategy between these two extremes, and appear to be evolving away from their original hosts (from the families Ectocarpaceae and Acinetosporaceae) to infect members of the genus *Feldmannia*. This is demonstrated in the *F. simplex* strain, which contained the largest number of subgroup B variants, and was also infected by a subgroup A virus which appears to be an evolutionary intermediate since it shares some sequence similarities with subgroup B viruses.

Previous studies have suggested that phaeoviruses have evolved by gene loss from a common ancestor[87], since they both have different subsets of various DNA replication genes, but our results suggest a slightly different scenario whereby subgroup B phaeoviruses have evolved from subgroup A. Our analyses have even suggested various mechanisms by which the subgroup B diversity may have developed relative to the subgroup A viruses. It seems likely that the first stage was the development of polymorphisms in a region of DNA polymerase which is known to be important for controlling mutation rates in yeast[4], possibly as a result of the host switch from *Ectocarpus* to *Feldmannia*. This increased the mutation rate and could have led to the loss of a proofreading exonuclease in subgroup B viruses that is highly expressed in EsV-1 (subgroup A) and the re-activation of an integrase that is expressed in FirrV-1 (subgroup B) but is inactive in EsV-1. This combination of differences not only

allows spontaneous variations to occur by decreased fidelity of DNA replication by the mutated DNA polymerase and absence of the proofreading exonuclease, but the active integration allows increased recombination between the different variants.

Moreover, our observation from the HRM analysis, confirmed by the sequencing data and maximum likelihood analysis, shows that this infection with phaeoviruses from both subgroups is a reasonably common occurrence, even in environmental isolates (17%), and therefore is not simply an artefact of the prolonged laboratory culturing of the original four strains. Furthermore, the transcriptome results showed that these multiple variants are not merely inactive artefacts of mutation over time, but are actively being transcribed in *F. irregularis*. This provides a wealth of genetic variation within each virus-infected zoidangium, facilitating further mutation via the active integrase gene, and perhaps contributing to virally-mediated horizontal transfer of sections of the host genome as the viruses move from infecting their original hosts to invading their new ones.

The environmental screening results support previous studies [80,108] showing that up to 90% of individuals in natural populations of *Ectocarpus* can contain phaeoviral DNA without necessarily showing any symptoms. Although there is no obvious explanation as to the prevalence of phaeoviruses in these algae, since the only obvious phenotypic change from infection is a decrease or cessation of host reproduction, it is difficult to imagine why the host alga would

maintain such a large fragment of DNA if it were completely useless. One observation during the course of this study was that actively symptomatic cultures appeared "cleaner" under the microscope than those without symptoms, i.e. they had less obvious epiphytic growth associated with the filaments. Although this is a purely anecdotal observation, it may be a defensive benefit provided by the virus to the host in exchange for the provision of the stable intracellular habitat which the host provides.

Furthermore, we have discovered that infection rates, and even subgroups of viruses, differ depending on host species and whether the isolate comes from an exposed or sheltered shore. Of particular interest is that the two species (*E. fasciculatus* and *E. siliculosus*) which were enriched for subgroup B viruses favour the lower shore locations that are also inhabited by *Feldmannia* species. This suggests a scenario whereby the phaeoviruses originated in the *Ectocarpus* genus (subgroup A) and mutated to be able to infect both *Ectocarpus* and *Feldmannia* (subgroup B), based on the observation of both subgroups in *Ectocarpus* and only subgroup B in *Feldmannia*. However, the strains used in this screening study were selected for an unrelated investigation into *Ectocarpus* ecology and therefore research into the viruses infecting *Feldmannia* was limited to the original strains in which FsV-1 and FlexV-1 were found, which is probably not an accurate representation of the subgroup distribution in this genus. In order to further investigate this assumption, a much broader study considering the whole range of brown algae is necessary. If indeed the scenario

suggested above is true, there should be no (or very little) infection of *Feldmannia* species by subgroup A. In addition, this further study would determine whether this test, or a modification of it, could be used to screen more widely for phaeovirus diversity within the brown algae.

5.3 Explanation for fragmented FirrV-1 genome and *Feldmannia* virus diversity

The diverse nature of the *Feldmannia* viruses provides an explanation for the differences between two of the phaeoviral sequencing projects of EsV-1[1] and FirrV-1[87] in spite of them being carried out by the same authors. The EsV-1 genome is fully assembled and of the expected size, whereas the FirrV-1 genome could not be assembled in one contiguous sequence and was instead published as a series of smaller contigs, including some which contained orthologous genes to those on larger contigs, the total of which exceeded the expected size of the complete genome. Based on our findings of a single EsV-1 variant, but multiple FirrV-1 variants, within the strains used to generate these genomes, it becomes clear that this may well be the cause of the assembly problems found with FirrV-1.

Multiple lysogeny has been described for many years in bacteriophage studies, e.g. [203,204,205] however to our knowledge this has not been found in eukaryotic DNA viruses such as the phaeoviruses, with one exception: an infected strain of *Feldmannia* sp. was found to contain two different size

variants[158] with the active variant depending on the culture temperature, although these multiple viruses were not confirmed by sequence data and therefore the true diversity was not revealed until now. It is possible that this higher diversity is due to the lower position of *Feldmannia* species on the shore than *Ectocarpus* which potentially could increase the exposure of these hosts to the virus, however it seems more likely that it is due to the mutations in DNA polymerase and loss of proofreading genes in the smaller viral genomes as described above.

5.4 Transcriptome dataset is an excellent resource for future work

Phaeoviral transcriptome studies have so far been limited to the transcriptionally inactive EsV-1-like provirus in the *Ectocarpus* genome[91], and a microarray study of EsV-1[168] which demonstrated a pattern of gene expression. Our study of the entire FirrV-1 transcriptome sequence has provided many useful observations of FirrV-1 activity and genetic polymorphisms; however, by limiting our study to the viral genes as a result of mapping the reads to the FirrV-1 genome, we have automatically eliminated all the information about the host gene expression. Whilst the *F. irregularis* genome is not available to use as a reference, the *Ectocarpus* genome is likely to be sufficiently similar to allow mapping of some of the host genes, which will provide some information about the host gene content, sequences and activity. The analysis of this pre-existing dataset is for a future study which would reveal

a great deal of information about the active genes within this *F. irregularis* host, and potentially its evolutionary relationship to the *Ectocarpus* genus, allowing the identification of host transcripts without much further financial or time investment.

5.5 The discovery of kelp viruses

The discovery of a new subgroup of viruses within the kelps raises the interesting question of whether other orders within the brown algae are also infected with viruses that have not yet been detected, and if so, how many? We are confident that the phaeoviral sequences detected in the kelps are not due to potential contamination by endophytic *Ectocarpus* species since the maximum likelihood analysis showed that the kelp MCP sequences grouped separately from the other phaeoviruses considered here. However, there is still a great deal of potential for further study within this new group of phaeoviruses.

Due to the commercial importance of many kelp species, any factor that could potentially affect their reproduction, such as viruses, would be of great interest, therefore this is an important first step in the identification of viruses within these species. Although we have only shown the presence of phaeoviral MCP sequence in this study, genome and transcriptome sequencing would determine whether these species contain complete, active viruses or simply an inactive relic proviral infection. The design of kelp-specific viral primers would also give

a more accurate representation of the true extent of phaeoviral infection in these species, since this study used primers which were designed against ectocarpoid algal viruses and therefore it is impossible to say whether the lower infection rate in the kelps is a true result or simply because the primers used here were too specific to amplify the less- related kelp viruses. Close physical examination by microscopy may also reveal viral reproduction, either as arrays of virions in the cell, or physical abnormalities of the host. This could be followed by virion extraction and further sequencing to greatly expand knowledge of viruses in kelps and their potential effects.

5.6 Wider impact

Overall, the results presented in this thesis show that the relationship between brown algae and their phaeoviruses is far more complex than previously suspected. Until now the phaeoviruses have been believed to be a single monophyletic group of viruses, and the generally accepted practice of naming the virus for the host in which it was found relied on host specificity which we have shown is not necessarily as strict as that practice would suggest. Now we have shown that the phaeoviruses comprise at least two subgroups, and possibly three if the kelp viruses are taken into consideration, and that the host range of one of these subgroups is much less specific than previously thought, since they are able to infect multiple genera within the Ectocarpales. Nonetheless, the data presented here suggest an ancient relationship between

the phaeoviruses and their hosts in which integration and diversification has occurred over a long time period, resulting in a stable co-existence between not only the hosts and their phaeoviruses, but also between different phaeoviruses within the same host.

Currently phaeoviruses are believed to only infect members of the Ectocarpales, the small filamentous brown algae. The discovery of potential viruses in kelp could be of great interest to the commercial production of these species, since if the symptoms are similar to known phaeoviruses, they could potentially affect their host's reproduction, so it will be crucial to understand the effects of viral infection in these species, as well as potential triggers for the reactivation of virus reproduction in order to minimise their impact on the industries build up around them.

The findings of this study are not only of interest to kelp farmers and algal virologists, but also reveal much about the field of general viral evolution. Until now, studies of phaeoviral evolution have been limited to phylogenetic analysis and genetic composition. We have not only uncovered potential mechanisms for increased mutation rates caused by the DNA polymerase mutation and lack of a proofreading exonuclease, but also for increased chances of genetic recombination and horizontal gene transfer due to the simultaneous infection by multiple viral sequence variants and an active integrase gene.

The shift from the persistent *K*-type life strategy of the subgroup A viruses towards the greater diversity, but more limited host range, of the viruses within subgroup B, takes advantage of the higher mutation rates of *r*-strategists whilst maintaining stable relationships with their hosts. This a perfect example of evolution in action, and could provide a convenient model by which to study the mechanisms of more virulent emergent diseases such as HIV[152], H5N1[154] and DWV[155]. Due to the potential scale and covert nature of persistent infections, many similar scenarios could still be undetected, and the presence of these as yet unknown infections could potentially have a massive impact on global biogeochemical processes. Similarly, this infection strategy is likely to be more prevalent than currently suspected in related animal viruses, with equally huge impacts on our ability to control further emergent diseases.

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Appendices

A.1 Isolates screened for phaeoviral DNA and results of subgroup analysis

Key

“Tm”	Melting temperature peak
"Unclassified"	Infected with virus that cannot be classified at 90% threshold
"None"	No virus infections
"0"	Group B virus infection (with >90% certainty)
"1"	Group A virus infection (with >90% certainty)
"0*"	Group B virus infection and an unclassified virus
"1*"	Group A virus infection and an unclassified virus
"Both"	Group A and Group B infection (both classified with >90% certainty)

Table A.1: Isolates screened for phaeoviral DNA and results of subgroup analysis

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
ARB7C	3	83.45	84.3	85.55	AF Peters	*Ectocarpus crouaniorum*	UK	11	09/06/2004	Exposed	Both
TRE08-15C	3	82.75	83.07	85.57	AF Peters	*Ectocarpus crouaniorum*	UK	21	21/06/2008	Exposed	Both
TRE08-16C	3	81.75	82.68	85.42	AF Peters	*Ectocarpus crouaniorum*	UK	21	21/06/2008	Exposed	Both
WEM08-10C	3	83.78	84.4	85.35	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	Both
BUT08-11A	2	82.1	85.62	NA	AF Peters	*Ectocarpus crouaniorum*	UK	10	02/06/2008	Exposed	Both
ARB4C	2	84.5	85.45	NA	AF Peters	*Ectocarpus crouaniorum*	UK	11	09/06/2004	Exposed	Both
WIC08-25C	2	83.4	85.32	NA	AF Peters	*Ectocarpus crouaniorum*	UK	13	12/06/2008	Exposed	Both
SKY08-9	2	85.07	85.77	NA	AF Peters	*Ectocarpus crouaniorum*	UK	14	14/06/2008	Exposed	1*
TRE08-17A	2	82.63	85.45	NA	AF Peters	*Ectocarpus crouaniorum*	UK	21	21/06/2008	Exposed	Both
TRE08-4B	2	83.63	85.27	NA	AF Peters	*Ectocarpus crouaniorum*	UK	21	21/06/2008	Exposed	Both
TRE08-9B	2	84.25	85.5	NA	AF Peters	*Ectocarpus crouaniorum*	UK	21	21/06/2008	Exposed	Both
RHOS08-1C	2	82.37	85.28	NA	AF Peters	*Ectocarpus crouaniorum*	UK	22	20/06/2008	Exposed	Both
WEM08-15A	2	83.98	85.7	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	Both
WEM08-16A	2	83.17	86.02	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	Both
WEM08-19A	2	82.27	85.77	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	Both
WEM08-20B	2	84.9	85.85	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	1*
WEM08-21C	2	82.22	85.32	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	Both
WEM08-7A	2	84.83	85.75	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	1*
TOR4A	2	82.05	85.4	NA	AF Peters	*Ectocarpus crouaniorum*	UK	30	29/06/2008	Exposed	Both
GOS4c	2	84.15	85.52	NA	AF Peters	*Ectocarpus crouaniorum*	UK	31	28/05/2008	Exposed	Both
BUT08-13C	1	85.65	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	10	02/06/2008	Exposed	1
BUT08-1A	1	85.42	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	10	01/06/2008	Exposed	1
BUT08-4A	1	85.97	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	10	01/06/2008	Exposed	1
BUT08-5C	1	85.95	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	10	01/06/2008	Exposed	1
BUT28A	1	85.5	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	10	02/06/2008	Exposed	1
ARB1C	1	85.52	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	11	09/06/2004	Exposed	1
ARB8A3	1	85.53	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	11	09/06/2004	Exposed	1
RAT08-5C	1	84.65	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	12	10/06/2008	Exposed	Unclassified
RAT9B	1	85.67	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	12	11/06/2008	Exposed	1
WIC08-10C	1	85.4	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	13	12/06/2008	Exposed	1
WIC08-16C	1	84.98	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	13	12/06/2008	Exposed	Unclassified
WIC08-17A	1	85.7	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	13	12/06/2008	Exposed	1
WIC08-18A	1	85.4	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	13	12/06/2008	Exposed	1
WIC08-19C	1	85.58	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	13	12/06/2008	Exposed	1

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
WIC08-21B	1	85.45	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	13	12/06/2008	Exposed	1
WIC08-22C	1	85.8	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	13	12/06/2008	Exposed	1
WIC08-23A	1	85.38	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	13	12/06/2008	Exposed	1
WIC08-24B	1	85.45	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	13	12/06/2008	Exposed	1
SKY08-10C	1	85.33	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	14	14/06/2008	Exposed	1
SKY08-11C	1	85.42	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	14	14/06/2008	Exposed	1
SKY08-1C	1	85.5	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	14	14/06/2008	Exposed	1
SKY08-4C	1	85.4	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	14	14/06/2008	Exposed	1
SKY08-5C	1	85.38	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	14	14/06/2008	Exposed	1
SKY08-8C	1	85.45	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	14	14/06/2008	Exposed	1
SKY12B	1	85.9	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	14	14/06/2008	Exposed	1
LIA08-3B	1	85.85	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	15	15/06/2008	Exposed	1
LIA2A	1	85.18	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	15	15/06/2008	Exposed	1
GAL08-13A	1	85.4	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-14C	1	85.3	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-16B	1	85.32	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-17B	1	85.52	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-1A	1	85.22	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-21C	1	85.13	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-23C	1	85.37	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-24B	1	85.35	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-26C	1	85.38	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-27C	1	85.33	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-29C	1	85.27	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-2C	1	85.12	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-3C	1	85.28	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-5C	1	85.32	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-6C	1	85.45	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-7C	1	85.18	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-8C	1	85.18	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL08-9C	1	85.2	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL15A	1	85.07	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	Unclassified
GAL22	1	85.48	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1
GAL28A	1	85.48	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	20	17/06/2008	Exposed	1

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
TRE08-10B	1	85.52	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	21	21/06/2008	Exposed	1
TRE08-18C	1	85.45	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	21	21/06/2008	Exposed	1
TRE08-3C	1	85.2	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	21	21/06/2008	Exposed	1
TRE08-5A	1	85.3	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	21	21/06/2008	Exposed	1
TRE08-6C	1	85.47	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	21	21/06/2008	Exposed	1
TRE08-7A	1	85.47	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	21	21/06/2008	Exposed	1
TRE1B	1	85.47	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	21	21/06/2008	Exposed	1
TRE2C3	1	85.33	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	21	21/06/2008	Exposed	1
TRE8B4	1	85.5	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	21	21/06/2008	Exposed	1
RHOS08-2C	1	85.25	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	22	20/06/2008	Exposed	1
RHOS08-3C	1	85.33	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	22	20/06/2008	Exposed	1
RHOS08-4B	1	85.25	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	22	20/06/2008	Exposed	1
WEM08-11C	1	85.52	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	1
WEM08-12C	1	85.85	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	1
WEM08-13A	1	85.5	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	1
WEM08-14B	1	85.92	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	1
WEM08-17B	1	85.72	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	1
WEM08-18A	1	85.67	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	1
WEM08-22A	1	85.58	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	1
WEM08-23C	1	85.95	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	1
WEM08-24B	1	85.98	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	1
WEM08-25C	1	85.55	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	1
WEM08-26A	1	85.47	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	1
WEM08-27C	1	84.35	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	28/06/2008	Exposed	0
WEM08-6C	1	85.67	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	26	21/05/2008	Exposed	1
Tor 3c3	1	84.5	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	30	29/06/2008	Exposed	0
GOS1c	1	85.67	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	31	28/05/2008	Exposed	1
WIC08-11B	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	13	12/06/2008	Exposed	None
WIC08-12C	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	13	12/06/2008	Exposed	None
WIC08-13C	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	13	12/06/2008	Exposed	None
WIC08-15C	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	13	12/06/2008	Exposed	None
TRE08-19C	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	21	21/06/2008	Exposed	None
RHOS08-5C	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	22	20/06/2008	Exposed	None
RHOS08-6A	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	22	20/06/2008	Exposed	None

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
Mul(06-4)1	1	85.65	NA	NA	Claire Gachon	*Ectocarpus crouaniorum*	UK	17	23/05/2006	NA	1
TOR6B2	3	83.15	83.48	85.4	AF Peters	*Ectocarpus crouaniorum*	UK	27	29/06/2008	Sheltered	Both
EcPH11-14	3	83.65	84.6	86.02	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	0*
EcPH11-20	3	83.18	84.17	85.4	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	Both
EcPH11-25	3	83.05	83.5	85.08	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	0*
EcPH11-43	3	83.23	85.1	85.37	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	0*
EcPH11-5	3	81.8	84.2	84.62	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	0*
EcPH11-s#2A-31	3	81.75	82.22	85.45	AF Peters	*Ectocarpus crouaniorum*	France	35	26/01/2011	Sheltered	Both
EcPH11-s#2A-42	3	81.85	82.12	85.1	AF Peters	*Ectocarpus crouaniorum*	France	35	26/01/2011	Sheltered	0*
EcPH11-s#2A-44	3	82.33	82.55	85.22	AF Peters	*Ectocarpus crouaniorum*	France	35	26/01/2011	Sheltered	Both
VF08-3C	3	82.27	83.45	85.73	AF Peters	*Ectocarpus crouaniorum*	France	41	19/04/2008	Sheltered	Both
VF08-4A	3	82.42	83.05	85.95	AF Peters	*Ectocarpus crouaniorum*	France	41	19/04/2008	Sheltered	Both
TOR08-5C	2	82.53	85.25	NA	AF Peters	*Ectocarpus crouaniorum*	UK	27	29/06/2008	Sheltered	Both
TOR08-7B	2	82.5	85.4	NA	AF Peters	*Ectocarpus crouaniorum*	UK	27	29/06/2008	Sheltered	Both
EcPH10-35	2	82.55	85.42	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	Both
EcPH11-113	2	85.48	85.82	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/06/2011	Sheltered	1
EcPH11-16	2	82.1	85.2	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	Both
EcPH11-18	2	84.4	85.38	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	Both
EcPH11-2	2	81.72	84.35	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	0
EcPH11-26	2	82.13	85.25	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	Both
EcPH11-27	2	84.32	85.42	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	Both
EcPH11-3	2	83.98	85.25	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	Both
EcPH11-31	2	82.03	84.6	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	0*
EcPH11-46	2	83.35	85.52	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	Both
EcPH11-6	2	84.45	85.58	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	28/04/2011	Sheltered	Both
EcPH11-s#2A-19	2	84.35	85.45	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	26/01/2011	Sheltered	Both
EcPH11-s#2B-20	2	84.75	85.68	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1*
EcPH11-s#2B-22	2	82.25	85.15	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	Both
EcPH11-s#2B-30	2	81.92	85.17	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	Both
EcPH11-s#2B-47	2	83.25	85.12	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	Both
EcPH11-s#2B-7	2	81.95	85.27	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	Both
EcPH11-s#5-38	2	83.42	85.6	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	21/01/2011	Sheltered	Both
EcPH11-s#5-7	2	84.97	85.68	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	21/01/2011	Sheltered	1*
Ec244	2	84.3	85.12	NA	AF Peters	*Ectocarpus crouaniorum*	France	38	13/07/2011	Sheltered	Both

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
VF08-5A	2	82.17	85.57	NA	AF Peters	*Ectocarpus crouaniorum*	France	41	19/04/2008	Sheltered	Both
TOR08-8B	1	85.3	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	27	29/06/2008	Sheltered	1
LH6b	1	85.5	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	32	28/05/2008	Sheltered	1
LH8A	1	84.97	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	32	28/05/2008	Sheltered	Unclassified
HAS08-10B	1	85.38	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	33	30/05/2008	Sheltered	1
HAS08-1C	1	85.42	NA	NA	AF Peters	*Ectocarpus crouaniorum*	UK	33	30/05/2008	Sheltered	1
Ec195	1	85.4	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	13/01/2010	Sheltered	1
EcPH10-14	1	85.27	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	14/05/2010	Sheltered	1
EcPH10-24	1	84.75	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	Unclassified
EcPH10-28	1	85	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	Unclassified
EcPH10-29	1	85.53	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	1
EcPH10-31	1	85.23	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	1
EcPH10-33	1	85.25	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	1
EcPH10-37	1	85.5	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	1
EcPH10-60	1	84.58	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	Unclassified
EcPH10-63	1	85.38	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	1
EcPH11-1	1	85.52	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	1
EcPH11-106	1	85.4	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	06/06/2011	Sheltered	1
EcPH11-11	1	85.43	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	1
EcPH11-112	1	85.52	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/06/2011	Sheltered	1
EcPH11-114	1	85.4	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/06/2011	Sheltered	1
EcPH11-13	1	85.1	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	Unclassified
EcPH11-17	1	85.45	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	1
EcPH11-21	1	85.75	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	1
EcPH11-28	1	85.3	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	1
EcPH11-37	1	84.93	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	Unclassified
EcPH11-38	1	84.33	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	0
EcPH11-39	1	85.52	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	1
EcPH11-42	1	85.27	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	1
EcPH11-47	1	85.47	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	1
EcPH11-7	1	85.2	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	1
EcPH11-9	1	85.5	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	1
EcPH11-s#2A-1	1	85.3	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	26/01/2011	Sheltered	1
EcPH11-s#2A-14	1	85.05	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	26/01/2011	Sheltered	Unclassified

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
EcPH11-s#2A-29	1	85.48	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	26/01/2011	Sheltered	1
EcPH11-s#2A-46	1	85.23	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	26/01/2011	Sheltered	1
EcPH11-s#2B-1	1	85.2	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-10	1	85.73	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-17	1	85.1	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	Unclassified
EcPH11-s#2B-2	1	85.5	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-21	1	85.58	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-23	1	85.25	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-25	1	84.28	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	0
EcPH11-s#2B-28	1	85.33	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-3	1	85.48	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-35	1	85.35	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-37	1	85.2	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-4	1	85.4	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-42	1	85.37	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-52	1	85.2	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-53	1	85.35	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-54	1	85.3	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-9	1	84.57	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	Unclassified
Ec326	1	84.1	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	36	27/05/2006	Sheltered	0
Ec329	1	85.25	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	36	27/05/2006	Sheltered	1
Ec243	1	85.22	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	38	13/07/2011	Sheltered	1
VF08-2C	1	86.08	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	41	19/04/2008	Sheltered	1
Ec334hSP	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	14/04/2007	Sheltered	None
EcPH10-13	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	14/05/2010	Sheltered	None
EcPH10-22	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-23	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-25	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-32	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-34	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-36	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-4	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	01/03/2010	Sheltered	None
EcPH10-45	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-46	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
EcPH10-48	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-49	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-50	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-51	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-54	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-55	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-57	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-58	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-64	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-65	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-70	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	27/05/2010	Sheltered	None
EcPH10-8	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	28/04/2010	Sheltered	None
EcPH11-10	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	None
EcPH11-12	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	None
EcPH11-33	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	None
EcPH11-35	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	None
EcPH11-36	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	None
EcPH11-40	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	20/01/2011	Sheltered	None
EcPH11-s#2A-18	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	26/01/2011	Sheltered	None
EcPH11-s#2A-21	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	26/01/2011	Sheltered	None
EcPH11-s#2A-24	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	26/01/2011	Sheltered	None
EcPH11-s#2A-36	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	26/01/2011	Sheltered	None
EcPH11-s#2B-16	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	None
EcPH11-s#2B-2	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-26	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	None
EcPH11-s#2B-32	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	None
EcPH11-s#2B-34	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	None
EcPH11-s#2B-36	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	None
EcPH11-s#2B-39	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	None
EcPH11-s#2B-49	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	25/01/2011	Sheltered	None
EcPH11-s#5-44	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-47	0	NA	NA	NA	AF Peters	*Ectocarpus crouaniorum*	France	35	21/01/2011	Sheltered	None
Ec308	3	83.33	84.05	85.08	AF Peters	*Ectocarpus fasciculatus*	Peru	3	06/03/2006	Exposed	0*
RHOS08-15A	3	81.5	82.42	85.17	AF Peters	*Ectocarpus fasciculatus*	UK	22	20/06/2008	Exposed	Both

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
Ec310	2	84.02	85.05	NA	AF Peters	*Ectocarpus fasciculatus*	Chile	4	01/03/2009	Exposed	0*
BUT08-14C	2	81.7	82.97	NA	AF Peters	*Ectocarpus fasciculatus*	UK	10	02/06/2008	Exposed	0
BUT08-16A	2	81.73	85.35	NA	AF Peters	*Ectocarpus fasciculatus*	UK	10	02/06/2008	Exposed	Both
RHOS08-16A	2	82.18	85.1	NA	AF Peters	*Ectocarpus fasciculatus*	UK	22	20/06/2008	Exposed	0*
WEM08-2A	2	83.2	85.15	NA	AF Peters	*Ectocarpus fasciculatus*	UK	26	21/05/2008	Exposed	Both
GOS3b	2	82.27	84.3	NA	AF Peters	*Ectocarpus fasciculatus*	UK	31	28/05/2008	Exposed	0
(Ec)PHH9	2	84.45	85.42	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	13/08/2006	Exposed	Both
EcPH10-255	2	83.35	85.07	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	0*
EcPH10-273	2	82.5	85.22	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	Both
EcPH10-274	2	82.85	85.33	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	Both
EcPH10-279	2	82.33	85.25	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	Both
EcPH10-285	2	82.18	85.23	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	Both
QY16	1	84.8	NA	NA	Aaron Mann	*Ectocarpus fasciculatus*	Chile	9	05/10/2006	Exposed	Unclassified
QY19	1	85.43	NA	NA	Aaron Mann	*Ectocarpus fasciculatus*	Chile	9	05/10/2006	Exposed	1
QY21	1	84.73	NA	NA	Aaron Mann	*Ectocarpus fasciculatus*	Chile	9	05/10/2006	Exposed	Unclassified
QY22	1	84.77	NA	NA	Aaron Mann	*Ectocarpus fasciculatus*	Chile	9	05/10/2006	Exposed	Unclassified
QY23	1	85.45	NA	NA	Aaron Mann	*Ectocarpus fasciculatus*	Chile	9	05/10/2006	Exposed	1
BUT08-15B	1	81.68	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	10	02/06/2008	Exposed	0
BUT08-17B	1	81.85	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	10	02/06/2008	Exposed	0
BUT08-2B	1	85.23	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	10	01/06/2008	Exposed	1
BUT08-3C	1	85.13	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	10	01/06/2008	Exposed	1
BUT20A	1	83.1	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	10	02/06/2008	Exposed	0
WIC08-26C	1	85.17	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	13	12/06/2008	Exposed	1
WIC08-28A	1	81.2	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	13	12/06/2008	Exposed	0
WIC08-29C	1	81.65	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	13	12/06/2008	Exposed	0
WIC08-30C	1	85.2	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	13	12/06/2008	Exposed	1
GAL08-10C	1	85.02	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	20	17/06/2008	Exposed	Unclassified
GAL08-12A	1	81.47	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	20	17/06/2008	Exposed	0
GAL08-18A	1	81.35	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	20	17/06/2008	Exposed	0
GAL08-19C	1	81.32	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	20	17/06/2008	Exposed	0
GAL08-4C	1	81.5	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	20	17/06/2008	Exposed	0
TRE08-14B	1	81.45	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	21	21/06/2008	Exposed	0
RHO14C	1	84.68	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	22	20/06/2008	Exposed	Unclassified
W004	1	85.12	NA	NA	D Schroeder	*Ectocarpus fasciculatus*	UK	26	05/07/2004	Exposed	1

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
WEM08-1A	1	85.2	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	26	21/05/2008	Exposed	1
WEM4B	1	85.32	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	26	21/05/2008	Exposed	1
(Ec)PHL10	1	86.22	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	13/08/2006	Exposed	Unclassified
(Ec)PHL12	1	85.02	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	08/09/2006	Exposed	Unclassified
(Ec)PHL30	1	84.92	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	08/09/2006	Exposed	Unclassified
(Ec)PHL6	1	83.45	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	13/08/2006	Exposed	0
Ec680	1	84.55	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	13/08/2006	Exposed	0
Ec684	1	84.77	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	08/09/2006	Exposed	Unclassified
EcPH10-245	1	84.85	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	Unclassified
EcPH10-264	1	85.18	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	1
EcPH10-267	1	85.1	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	Unclassified
EcPH10-270	1	85.13	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	1
EcPH10-271	1	85.1	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	Unclassified
EcPH10-275	1	84.58	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	Unclassified
EcPH10-277	1	84.4	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	0
EcPH10-278	1	84.55	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	0
EcPH10-281	1	84.33	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	0
EcPH10-283	1	85.77	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	1
EcPH10-287	1	85.07	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	Unclassified
EcPH10-288	1	85.37	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	1
QY20	0	NA	NA	NA	Aaron Mann	*Ectocarpus fasciculatus*	Chile	9	05/10/2006	Exposed	None
BUT7B	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	10	01/06/2008	Exposed	None
RAT1C4	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	12	10/06/2008	Exposed	None
WICK27A	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	13	12/06/2008	Exposed	None
LIA08-1C	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	15	15/06/2008	Exposed	None
GAL08-11C	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	20	17/06/2008	Exposed	None
GAL08-20C	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	20	17/06/2008	Exposed	None
GAL08-25A	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	20	17/06/2008	Exposed	None
TRE08-12C	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	21	21/06/2008	Exposed	None
WEM08-3A	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	26	21/05/2008	Exposed	None
WEM08-5B	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	26	21/05/2008	Exposed	None
TOR08-1C	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	30	29/06/2008	Exposed	None
TOR08-2A	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	30	29/06/2008	Exposed	None
(Ec)PHH2	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	13/08/2006	Exposed	None

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
(Ec)PHH7	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	13/08/2006	Exposed	None
(Ec)PHL1	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2006	Exposed	None
(Ec)PHL2	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	13/08/2006	Exposed	None
(Ec)PHL26	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	08/09/2006	Exposed	None
Ec396	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	30/09/2003	Exposed	None
Ec578	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	29/09/2005	Exposed	None
Ec674	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	13/08/2006	Exposed	None
Ec683	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	08/09/2006	Exposed	None
EcPH10-227	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2007	Exposed	None
EcPH10-227	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2007	Exposed	None
EcPH10-228	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2007	Exposed	None
EcPH10-228	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2007	Exposed	None
EcPH10-234	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	None
EcPH10-234	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	None
EcPH10-240	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	None
EcPH10-240	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	None
EcPH10-245	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	Unclassified
EcPH10-248	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	None
EcPH10-248	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	None
EcPH10-255	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	0*
EcPH10-260	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	None
EcPH10-260	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	None
EcPH10-262	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	None
EcPH10-262	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	None
EcPH10-264	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	1
EcPH10-267	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	Unclassified
EcPH10-270	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	11/08/2010	Exposed	1
EcPH10-272	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	None
EcPH10-276	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	None
EcPH10-282	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	None
EcPH10-284	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	None
EcPH10-286	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	None
EcPH10-289	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	None
EcPH10-290	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	12/08/2010	Exposed	None

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
EcPH10-5	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	01/03/2010	Exposed	None
CH(05-)11	3	82.65	84.17	85.13	DG Müller	*Ectocarpus fasciculatus*	Chile	8	10/01/2004	NA	Both
M001	2	83.9	85.38	NA	D Schroeder	*Ectocarpus fasciculatus*	UK	28	23/11/2003	NA	Both
S001	2	82.97	84.75	NA	D Schroeder	*Ectocarpus fasciculatus*	UK	29	04/06/2004	NA	0*
S002	2	82.7	85.6	NA	D Schroeder	*Ectocarpus fasciculatus*	UK	29	04/06/2004	NA	Both
Caro(05-)12	1	85.25	NA	NA	DG Müller	*Ectocarpus fasciculatus*	Chile	8	10/01/2004	NA	1
P002	1	85.15	NA	NA	D Schroeder	*Ectocarpus fasciculatus*	UK	25	04/06/2004	NA	1
P005	1	84.87	NA	NA	D Schroeder	*Ectocarpus fasciculatus*	UK	25	24/02/2004	NA	Unclassified
P006	1	84.97	NA	NA	D Schroeder	*Ectocarpus fasciculatus*	UK	25	23/07/2004	NA	Unclassified
Y003	0	NA	NA	NA	D Schroeder	*Ectocarpus fasciculatus*	UK	24	30/07/2004	NA	None
P001	0	NA	NA	NA	D Schroeder	*Ectocarpus fasciculatus*	UK	25	04/06/2004	NA	None
SAL08-2C	3	81.5	83.6	85.03	AF Peters	*Ectocarpus fasciculatus*	UK	29	29/06/2008	Sheltered	0*
EcPH10-178	3	81.95	82.27	85.08	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	0*
EcQB10-2	3	83.67	83.98	85.48	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	Both
SAM08-8C	2	81.28	85.65	NA	AF Peters	*Ectocarpus fasciculatus*	UK	16	07/06/2008	Sheltered	Both
QAB08-4B	2	82.38	85.62	NA	AF Peters	*Ectocarpus fasciculatus*	UK	25	24/05/2008	Sheltered	Both
SAL08-1C	2	81.85	85.2	NA	AF Peters	*Ectocarpus fasciculatus*	UK	29	29/06/2008	Sheltered	Both
HAS08-2B	2	83.1	85.5	NA	AF Peters	*Ectocarpus fasciculatus*	UK	33	30/05/2008	Sheltered	Both
(Ec)PHZ5	2	82.25	85.35	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	24/08/2006	Sheltered	Both
EcQB10-11	2	83.93	85.57	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	Both
EcQB10-15	2	84.4	85.87	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	Both
EcQB10-18	2	84.2	85.3	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	Both
Ec165	1	85.2	NA	NA	AF Peters	*Ectocarpus fasciculatus*	Chile	4	01/03/2006	Sheltered	1
Ob07-2	1	84.93	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	16	07/11/2007	Sheltered	Unclassified
TOR08-15B	1	84.93	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	27	29/06/2008	Sheltered	Unclassified
HAS08-4C	1	85.48	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	33	30/05/2008	Sheltered	1
(Ec)PHZ17	1	85.48	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	24/08/2006	Sheltered	1
(Ec)PHZ35	1	85.38	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	30/08/2007	Sheltered	1
(Ec)PHZ6	1	85.48	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	24/08/2006	Sheltered	1
(Ec)PHZ8	1	85.47	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	24/08/2006	Sheltered	1
EcPH10-186	1	84.45	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	0
EcQB10-10	1	85.52	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	1
EcQB10-12	1	85.73	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	1
EcQB10-13	1	85.42	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	1

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
EcQB10-5	1	85.47	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	1
EcQB10-6	1	85.87	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	1
EcQB10-9	1	85.53	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	1
Ob07-12C	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	16	07/11/2007	Sheltered	None
Ob07-13C	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	16	07/11/2007	Sheltered	None
Ob07-7	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	16	07/11/2007	Sheltered	None
SAM08-1B	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	16	07/06/2008	Sheltered	None
SAM08-6C	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	16	07/06/2008	Sheltered	None
SAM2	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	16	07/06/2008	Sheltered	None
SAM7A	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	16	07/06/2008	Sheltered	None
SAM08-13C	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	18	08/06/2008	Sheltered	None
POR08-3C	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	19	17/06/2008	Sheltered	None
POR08-4C	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	19	17/06/2008	Sheltered	None
RHOS08-10C	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	22	20/06/2008	Sheltered	None
RHOS08-11B	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	22	20/06/2008	Sheltered	None
RHOS08-13B	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	22	20/06/2008	Sheltered	None
RHOS08-7C	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	22	20/06/2008	Sheltered	None
RHOS08-8B	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	22	20/06/2008	Sheltered	None
RHOS08-9B	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	22	20/06/2008	Sheltered	None
REP10-2	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	23	14/06/2010	Sheltered	None
REP10-3	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	23	14/06/2010	Sheltered	None
REP10-4	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	23	14/06/2010	Sheltered	None
REP10-5	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	23	14/06/2010	Sheltered	None
TOR08-17C	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	UK	27	29/06/2008	Sheltered	None
(Ec)PHZ1	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	24/08/2006	Sheltered	None
(Ec)PHZ29	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	24/08/2006	Sheltered	None
(Ec)PHZ36A	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	26/09/2007	Sheltered	None
(Ec)PHZ7	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	24/08/2006	Sheltered	None
Ec736	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	21/08/2009	Sheltered	None
EcPH10-175	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-176	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-179	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-18	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	14/05/2010	Sheltered	None
EcPH10-181	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
EcPH10-183	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-184	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-185	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-187	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-196	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-197	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-198	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-209	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-210	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-211	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-213	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-214	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-216	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-217	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH10-219	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	15/07/2010	Sheltered	None
EcPH11-120	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	02/08/2011	Sheltered	None
EcPH11-s#2A-10	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	26/01/2011	Sheltered	None
EcPH11-s#2A-15	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	26/01/2011	Sheltered	None
EcPH11-s#2A-3	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	26/01/2011	Sheltered	None
EcPH11-s#2A-30	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	26/01/2011	Sheltered	None
EcPH11-s#2A-51	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	26/01/2011	Sheltered	None
EcPH11-s#5-2	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-23	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-29	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-45	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	35	21/01/2011	Sheltered	None
Ec328	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	36	27/05/2006	Sheltered	None
EcQB10-14	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	None
EcQB10-17	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	None
EcQB10-27	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	None
EcQB10-29	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	None
EcQB10-3	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	None
EcQB10-31	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	None
EcQB10-33	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	None
EcQB10-34	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	None

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
EcQB10-4	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	None
EcQB10-7	0	NA	NA	NA	AF Peters	*Ectocarpus fasciculatus*	France	39	31/07/2010	Sheltered	None
Ec156	2	82.45	85.4	NA	AF Peters	*Ectocarpus siliculosus*	Chile	5	01/03/2006	Exposed	Both
REP10-59	2	83.2	85.37	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	Both
REP10-60	2	83.13	85.15	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	Both
REP10-61	2	83.55	85.35	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	Both
Ec286	1	85.77	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Exposed	1
Ec287	1	84.95	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Exposed	Unclassified
Ec288	1	85.28	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Exposed	1
Ec161	1	85.2	NA	NA	AF Peters	*Ectocarpus siliculosus*	Chile	4	01/03/2006	Exposed	1
Ec157	1	85.1	NA	NA	AF Peters	*Ectocarpus siliculosus*	Chile	5	01/03/2006	Exposed	Unclassified
Ec159	1	85.4	NA	NA	AF Peters	*Ectocarpus siliculosus*	Chile	5	02/03/2006	Exposed	1
QY24	1	85.45	NA	NA	Aaron Mann	*Ectocarpus siliculosus*	Chile	9	05/10/2006	Exposed	1
LIA08-4A	1	85.62	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	15	15/06/2008	Exposed	1
REP10-56	1	85.82	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	1
REP10-58	1	85.88	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	1
REP10-59	1	85.43	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	Both
REP10-62	1	85.38	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	1
REP10-64	1	85.35	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	1
REP10-65	1	85.35	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	1
REP10-67	1	85.3	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	1
REP10-68	1	85.32	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	1
REP10-69	1	85.32	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	1
REP10-70	1	85.85	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	1
REP10-75	1	85.5	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	1
REP10-77	1	85.5	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	1
REP10-78	1	85.37	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	1
REP10-79	1	85.3	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	1
Ec721	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Chile	4	01/03/2006	Exposed	None
WIC08-14C	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	13	12/06/2008	Exposed	None
REP10-80	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Exposed	None
W009	0	NA	NA	NA	D Schroeder	*Ectocarpus siliculosus*	UK	26	04/12/2004	Exposed	None
GOS2b	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	31	28/05/2008	Exposed	None
EcQAB10-1	3	84.35	85.55	85.82	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	Both

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
EcQAB10-3	3	83.03	84.15	85.38	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	Both
TOR08-10A	3	82.7	83.73	85.8	AF Peters	*Ectocarpus siliculosus*	UK	27	29/06/2008	Sheltered	Both
TOR08-14C	3	82.75	83.87	85.78	AF Peters	*Ectocarpus siliculosus*	UK	27	29/06/2008	Sheltered	Both
GOS7b	3	82.25	84.17	85.45	AF Peters	*Ectocarpus siliculosus*	UK	31	28/05/2008	Sheltered	Both
LH08-1B	3	83.33	83.93	85.8	AF Peters	*Ectocarpus siliculosus*	UK	32	28/05/2008	Sheltered	Both
LH10b	3	82.72	84.18	85.72	AF Peters	*Ectocarpus siliculosus*	UK	32	28/05/2008	Sheltered	Both
HAS08-17B	3	83.3	84.55	85.62	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	Both
HAS08-21A	3	83.78	85.03	86.1	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	0*
HAS08-22A	3	83.7	84.95	85.98	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	0*
HAS08-5A	3	83.42	84.68	85.77	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	0*
Ec669	3	82.25	82.55	85.07	AF Peters	*Ectocarpus siliculosus*	France	35	12/08/2006	Sheltered	0*
EcPH10-171	3	82.07	83.35	85.35	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	Both
EcPH10-9	3	82.87	83.1	84.98	AF Peters	*Ectocarpus siliculosus*	France	35	28/04/2010	Sheltered	0*
EcPH11-107	3	81.72	82.12	85.65	AF Peters	*Ectocarpus siliculosus*	France	35	06/06/2011	Sheltered	Both
EcPH11-s#2A-16	3	82.23	83.42	85.4	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	Both
EcPH11-s#2A-37	3	81.9	83.12	85.13	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	Both
EcPH11-s#2A-41	3	82.25	83.3	85.4	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	Both
EcPH11-s#2B-8	3	82.85	83.55	85	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	0*
EcTH10-18	3	83.6	84.33	85.07	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	0*
EcTH10-205	3	83.05	84.22	85.58	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	Both
EcTH10-8	3	83.57	84.23	85.1	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	0*
Ec278	2	84.57	86.05	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	1*
BUT08-12B	2	82.1	84.62	NA	AF Peters	*Ectocarpus siliculosus*	UK	10	02/06/2008	Sheltered	0*
SKY08-13C	2	84.15	85.87	NA	AF Peters	*Ectocarpus siliculosus*	UK	14	14/06/2008	Sheltered	Both
SAM08-9C	2	84.35	85.7	NA	AF Peters	*Ectocarpus siliculosus*	UK	16	07/06/2008	Sheltered	Both
SAM4	2	84.25	85.9	NA	AF Peters	*Ectocarpus siliculosus*	UK	16	07/06/2008	Sheltered	Both
REP10-13	2	83.63	85.5	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	Both
EcQAB10-15	2	83.48	85.37	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	Both
EcQAB10-16	2	83.2	85.18	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	Both
EcQAB10-19	2	85.57	85.8	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-27	2	84.35	85.45	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	Both
EcQAB10-40	2	83.45	85.33	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	Both
EcQAB10-46	2	83.45	85.37	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	Both
TOR08-9C	2	82.18	85.32	NA	AF Peters	*Ectocarpus siliculosus*	UK	27	29/06/2008	Sheltered	Both

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
GOS08-14B	2	83.7	86.07	NA	AF Peters	*Ectocarpus siliculosus*	UK	31	28/05/2008	Sheltered	Both
GOS08-8C	2	83.8	85.77	NA	AF Peters	*Ectocarpus siliculosus*	UK	31	28/05/2008	Sheltered	Both
GOS08-9A	2	83.77	86.17	NA	AF Peters	*Ectocarpus siliculosus*	UK	31	28/05/2008	Sheltered	0*
LH12c	2	83.53	84.73	NA	AF Peters	*Ectocarpus siliculosus*	UK	32	28/05/2008	Sheltered	0*
LH2b	2	84.17	85.92	NA	AF Peters	*Ectocarpus siliculosus*	UK	32	28/05/2008	Sheltered	Both
LH9c	2	83.47	84.65	NA	AF Peters	*Ectocarpus siliculosus*	UK	32	28/05/2008	Sheltered	0*
HAS08-11B	2	83.33	85.73	NA	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	Both
HAS08-12B	2	83.3	85.7	NA	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	Both
HAS08-13C	2	83.33	85.7	NA	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	Both
HAS08-14B	2	83.3	85.7	NA	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	Both
HAS08-15A	2	83.28	85.65	NA	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	Both
HAS08-16A	2	83.38	85.8	NA	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	Both
HAS08-18B	2	83.35	85.62	NA	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	Both
HAS08-19B	2	83.4	85.82	NA	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	Both
HAS08-20A	2	83.33	85.73	NA	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	Both
HAS08-6A	2	83.37	85.77	NA	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	Both
HAS08-7A	2	83.35	85.75	NA	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	Both
HAS08-8B	2	83.33	85.75	NA	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	Both
HAS08-9B	2	83.28	85.6	NA	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	Both
HAS12B	2	83.38	85.78	NA	AF Peters	*Ectocarpus siliculosus*	UK	33	30/05/2008	Sheltered	Both
(Ec)PHS2	2	81.95	85.55	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/11/2007	Sheltered	Both
Ec487	2	82	85.2	NA	AF Peters	*Ectocarpus siliculosus*	France	35	16/08/2004	Sheltered	Both
Ec673	2	82.38	85.07	NA	AF Peters	*Ectocarpus siliculosus*	France	35	27/09/2007	Sheltered	0*
Ec730	2	83.25	85.32	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/08/2009	Sheltered	Both
Ec731	2	85.48	85.9	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/08/2009	Sheltered	1
Ec732	2	83.27	85.37	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/08/2009	Sheltered	Both
EcPH10-127	2	82.02	85.7	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	Both
EcPH10-128	2	82.35	84.4	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	0
EcPH10-129	2	83.2	85.3	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	Both
EcPH10-132	2	82.03	85.57	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	Both
EcPH10-134	2	82.22	85.65	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	Both
EcPH10-139	2	81.93	85.37	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	Both
EcPH10-173	2	83.37	85.68	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	Both
EcPH10-292	2	82.15	85.57	NA	AF Peters	*Ectocarpus siliculosus*	France	35	12/08/2010	Sheltered	Both

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
EcPH11-100	2	82.3	85.42	NA	AF Peters	*Ectocarpus siliculosus*	France	35	06/06/2011	Sheltered	Both
EcPH11-108	2	82.22	85.65	NA	AF Peters	*Ectocarpus siliculosus*	France	35	06/06/2011	Sheltered	Both
EcPH11-115	2	82.47	84.98	NA	AF Peters	*Ectocarpus siliculosus*	France	35	03/07/2011	Sheltered	0*
EcPH11-116	2	83.37	85.18	NA	AF Peters	*Ectocarpus siliculosus*	France	35	03/07/2011	Sheltered	Both
EcPH11-117	2	82.3	85.1	NA	AF Peters	*Ectocarpus siliculosus*	France	35	03/07/2011	Sheltered	0*
EcPH11-118	2	82.37	85.05	NA	AF Peters	*Ectocarpus siliculosus*	France	35	03/07/2011	Sheltered	0*
EcPH11-s#2A-4	2	82.25	85.7	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	Both
EcPH11-s#2A-40	2	82.27	85.77	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	Both
EcPH11-s#2A-47	2	81.93	85.43	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	Both
EcPH11-s#2A-48	2	82	85.4	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	Both
EcPH11-s#2A-50	2	82.45	85.68	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	Both
EcPH11-s#2A-52	2	82.37	85	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	0*
EcPH11-s#2B-15	2	82.13	84.98	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	0*
EcPH11-s#2B-19	2	83.55	85.4	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	Both
EcPH11-s#2B-41	2	82.07	85.03	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	0*
EcPH11-s#5-17	2	83.4	85.28	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	Both
EcPH11-s#5-25	2	82.12	85.7	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	Both
EcPH11-s#5-30	2	84.57	85.33	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	1*
EcPH11-s#5-33	2	84.68	85.78	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	1*
Ec539	2	84.15	85.53	NA	AF Peters	*Ectocarpus siliculosus*	France	36	24/05/2005	Sheltered	Both
EcTH10-10	2	84.28	85.1	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	0*
EcTH10-17	2	84.17	85.05	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	0*
EcTH10-176	2	83.98	85.57	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	Both
EcQB10-21	2	84.07	85.5	NA	AF Peters	*Ectocarpus siliculosus*	France	39	31/07/2010	Sheltered	Both
EcQB10-22	2	84.07	85.85	NA	AF Peters	*Ectocarpus siliculosus*	France	39	31/07/2010	Sheltered	Both
EcQB10-23	2	84.37	85.9	NA	AF Peters	*Ectocarpus siliculosus*	France	39	31/07/2010	Sheltered	Both
(Ec)BIOCEAN	2	82.22	85.6	NA	AF Peters	*Ectocarpus siliculosus*	France	NA	28/03/2008	Sheltered	Both
Ec201	1	85.05	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	Unclassified
Ec202	1	85	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	Unclassified
Ec246	1	85.18	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	1
Ec266	1	85	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	Unclassified
Ec267	1	85.82	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	1
Ec269	1	85.15	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	1
Ec270	1	85.1	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	Unclassified

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
Ec274	1	85.05	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	Unclassified
Ec283	1	85.95	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	1
Ec284	1	86.03	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	1
Ec285	1	85.5	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	1
Ec289	1	84.97	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	Unclassified
Ec290	1	85.97	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	1
Ec291	1	85.88	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	1
Ec294	1	85.22	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	3	06/03/2006	Sheltered	1
Ec295	1	85.4	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	3	06/03/2006	Sheltered	1
Ec296	1	85.23	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	3	06/03/2006	Sheltered	1
Ec297	1	85.22	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	3	06/03/2006	Sheltered	1
Ec298	1	85.17	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	3	06/03/2006	Sheltered	1
EC298	1	85.4	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	3	06/03/2006	Sheltered	1
Ec299	1	85.2	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	3	06/03/2006	Sheltered	1
Ec300	1	85.22	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	3	06/03/2006	Sheltered	1
Ec302	1	85.17	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	3	06/03/2006	Sheltered	1
Ec304	1	85.27	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	3	06/03/2006	Sheltered	1
Ec305	1	85.27	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	3	06/03/2006	Sheltered	1
Ec306	1	85.22	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	3	06/03/2006	Sheltered	1
Ec307	1	85.35	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	3	06/03/2006	Sheltered	1
Ec147	1	85.25	NA	NA	AF Peters	*Ectocarpus siliculosus*	Chile	6	28/02/2006	Sheltered	1
Ec150	1	83.33	NA	NA	AF Peters	*Ectocarpus siliculosus*	Chile	6	28/02/2006	Sheltered	0
Ec151	1	83.45	NA	NA	AF Peters	*Ectocarpus siliculosus*	Chile	6	28/02/2006	Sheltered	0
Ec153	1	83.47	NA	NA	AF Peters	*Ectocarpus siliculosus*	Chile	6	28/02/2006	Sheltered	0
Ec154	1	83.45	NA	NA	AF Peters	*Ectocarpus siliculosus*	Chile	6	28/02/2006	Sheltered	0
Ec155	1	83.38	NA	NA	AF Peters	*Ectocarpus siliculosus*	Chile	6	28/02/2006	Sheltered	0
Ob07-10A	1	85.78	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	16	07/11/2007	Sheltered	1
Ob07-15A	1	85.72	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	16	07/11/2007	Sheltered	1
Ob07-4	1	85.75	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	16	07/11/2007	Sheltered	1
SAM08-5A	1	85.58	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	16	07/06/2008	Sheltered	1
RHOS08-12C	1	85.7	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	22	20/06/2008	Sheltered	1
REP10-18	1	85.42	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1
REP10-22	1	85.8	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1
REP10-23	1	85.55	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
REP10-26	1	84.4	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	0
REP10-28	1	85.85	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1
REP10-30	1	85.82	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1
REP10-32	1	85.85	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1
REP10-35	1	84.82	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	Unclassified
REP10-36	1	85.67	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1
REP10-38	1	85.83	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1
REP10-42	1	85.62	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1
REP10-45	1	85.77	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1
REP10-47	1	85.87	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1
REP10-48	1	85.77	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1
REP10-50	1	85.9	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1
REP10-51	1	85.4	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1
REP10-52	1	85.52	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1
REP10-54	1	85.67	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	1
EcQAB10-10	1	85.45	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-11	1	85.6	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-13	1	85.8	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-14	1	85.53	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-17	1	85.08	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	Unclassified
EcQAB10-18	1	85.5	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-2	1	85.4	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-20	1	85.35	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-24	1	85.6	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-25	1	85.4	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-28	1	85.45	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-29	1	85.48	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-30	1	85.53	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-32	1	85.47	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-33	1	85.35	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-34	1	85.2	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-35	1	85.38	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-38	1	85.47	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-39	1	85.48	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
EcQAB10-43	1	85.35	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-48	1	85.4	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-5	1	85.47	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-6	1	85.5	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-7	1	85.32	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-8	1	85.45	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
EcQAB10-9	1	85.5	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	1
QAB08-1C	1	85.7	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	24/05/2008	Sheltered	1
QAB08-2A	1	85.9	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	24/05/2008	Sheltered	1
QAB08-3A	1	85.78	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	24/05/2008	Sheltered	1
TOR08-13B	1	85.65	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	27	29/06/2008	Sheltered	1
GOS08-11B	1	85.98	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	31	28/05/2008	Sheltered	1
GOS08-12B	1	86.05	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	31	28/05/2008	Sheltered	1
GOS08-13B	1	86.1	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	31	28/05/2008	Sheltered	1
GOS08-17B	1	86.12	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	31	28/05/2008	Sheltered	1
GOS5c	1	85.8	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	31	28/05/2008	Sheltered	1
GOS6c	1	85.78	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	31	28/05/2008	Sheltered	1
LH11c	1	84.72	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	32	28/05/2008	Sheltered	Unclassified
LH5c	1	85.83	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	32	28/05/2008	Sheltered	1
(Ec)PHL21	1	85.2	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	08/09/2006	Sheltered	1
(Ec)PHS1	1	85.82	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	12/08/2006	Sheltered	1
(Ec)PHU(07)2	1	85.53	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	27/09/2007	Sheltered	1
Ec670	1	85.15	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	12/08/2006	Sheltered	1
Ec671	1	85.07	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/09/2007	Sheltered	Unclassified
EcPH10-11	1	84.32	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	14/05/2010	Sheltered	0
EcPH10-130	1	85.45	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	1
EcPH10-131	1	85.13	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	1
EcPH10-142	1	85.42	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	1
EcPH10-15	1	85.18	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	14/05/2010	Sheltered	1
EcPH10-151	1	84.72	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	Unclassified
EcPH10-152	1	84.65	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	Unclassified
EcPH10-157	1	85.35	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	1
EcPH10-160	1	85.55	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	1
EcPH10-162	1	85.03	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	Unclassified

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
EcPH10-165	1	85.32	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	1
EcPH10-166	1	84.97	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	Unclassified
EcPH10-167	1	84.95	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	Unclassified
EcPH10-168	1	85.67	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	1
EcPH10-170	1	85.57	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	1
EcPH10-294	1	85.03	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	12/08/2010	Sheltered	Unclassified
EcPH11-109	1	84.9	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	06/06/2011	Sheltered	Unclassified
EcPH11-124	1	85.47	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	02/08/2011	Sheltered	1
EcPH11-8	1	83.2	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	20/01/2011	Sheltered	0
EcPH11-97	1	85.8	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	06/06/2011	Sheltered	1
EcPH11-98	1	85.77	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	06/06/2011	Sheltered	1
EcPH11-99	1	85.42	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	06/06/2011	Sheltered	1
EcPH11-s#2A-17	1	84.72	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	Unclassified
EcPH11-s#2A-32	1	85.45	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	1
EcPH11-s#2A-35	1	85.33	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	1
EcPH11-s#2A-39	1	84.93	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	Unclassified
EcPH11-s#2A-49	1	85.3	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	1
EcPH11-s#2A-6	1	85.75	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	1
EcPH11-s#2A-9	1	85.75	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	1
EcPH11-s#2B-12	1	85.62	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-38	1	85.6	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-40	1	84.82	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	Unclassified
EcPH11-s#2B-43	1	85.15	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-45	1	85.47	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-46	1	85.25	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-48	1	85.58	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-5	1	85.35	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	1
EcPH11-s#2B-6	1	85.15	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	1
EcPH11-s#5-1	1	85.05	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	Unclassified
EcPH11-s#5-13	1	85.1	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	Unclassified
EcPH11-s#5-15	1	85.37	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	1
EcPH11-s#5-18	1	85.25	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	1
EcPH11-s#5-19	1	85.25	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	1
EcPH11-s#5-20	1	85.5	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	1

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
EcPH11-s#5-28	1	85.02	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	Unclassified
EcPH11-s#5-31	1	86.05	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	1
EcPH11-s#5-32	1	85.05	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	Unclassified
EcPH11-s#5-34	1	85.05	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	Unclassified
EcPH11-s#5-35	1	85.5	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	1
EcPH11-s#5-37	1	84.47	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	0
EcPH11-s#5-42	1	85.03	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	Unclassified
EcPH11-s#5-43	1	85.1	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	Unclassified
EcPH11-s#5-49	1	84.33	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	0
EcPH11-s#5-5	1	85.38	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	1
EcPH11-s#5-6	1	85.73	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	1
EcPH11-s#5-8	1	84.53	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	0
Ec531	1	85.45	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	24/05/2005	Sheltered	1
Ec533	1	84.75	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	24/05/2005	Sheltered	Unclassified
Ec540	1	85.52	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	24/05/2005	Sheltered	1
Ec543	1	85.9	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	22/08/2005	Sheltered	1
EcTH10-172	1	84.75	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	Unclassified
EcTH10-175	1	85.53	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	1
EcTH10-197	1	85.52	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	1
EcTH10-198	1	85.62	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	1
EcTH10-199	1	85.9	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	1
EcTH10-201	1	85.9	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	1
EcTH10-202	1	85.87	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	1
EcTH10-9	1	85.17	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	1
EcQB10-1	1	85.53	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	39	31/07/2010	Sheltered	1
EcQB10-16	1	85.92	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	39	31/07/2010	Sheltered	1
EcQB10-26	1	84.85	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	39	31/07/2010	Sheltered	Unclassified
EcQB10-8	1	85.9	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	39	31/07/2010	Sheltered	1
ET08-2A	1	85.93	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	40	15/04/2008	Sheltered	1
ET08-3A	1	85.9	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	40	15/04/2008	Sheltered	1
ET08-4B	1	85.87	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	40	15/04/2008	Sheltered	1
ET08-5B	1	85.78	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	40	15/04/2008	Sheltered	1
Ec311	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	1	07/03/2006	Sheltered	None
Ec312	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	1	07/03/2006	Sheltered	None

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
Ec314	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	1	07/03/2006	Sheltered	None
Ec247	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	None
Ec275	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	None
Ec276	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	None
Ec277	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	None
Ec279	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	None
Ec280	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	None
Ec281	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	None
Ec282	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	None
Ec292	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	None
Ec293	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	08/03/2006	Sheltered	None
Ec315	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	07/03/2006	Sheltered	None
Ec316	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	2	07/03/2006	Sheltered	None
Ec301	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	3	06/03/2006	Sheltered	None
Ec303	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	Peru	3	06/03/2006	Sheltered	None
REP10-1	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-10	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-11	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-12	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-14	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-16	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-17	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-19	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-21	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-25	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-27	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-29	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-33	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-34	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-39	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-40	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-43	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-44	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-46	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
REP10-53	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-6	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
REP10-7	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	23	14/06/2010	Sheltered	None
EcQAB10-21	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	None
EcQAB10-22	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	None
EcQAB10-23	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	None
EcQAB10-36	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	None
EcQAB10-37	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	None
EcQAB10-41	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	None
EcQAB10-42	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	None
EcQAB10-44	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	None
EcQAB10-45	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	None
EcQAB10-47	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	25	13/06/2010	Sheltered	None
GOS08-16B	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	31	28/05/2008	Sheltered	None
LH3C	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	32	28/05/2008	Sheltered	None
LH4A	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	UK	32	28/05/2008	Sheltered	None
Ec331	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	05/06/2007	Sheltered	None
Ec393	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	01/07/2003	Sheltered	None
Ec488	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	16/08/2004	Sheltered	None
Ec489	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	29/08/2004	Sheltered	None
Ec697	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	16/04/2007	Sheltered	None
EcPH10-12	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	14/05/2010	Sheltered	None
EcPH10-133	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-135	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-137	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-140	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-141	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-143	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-144	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-145	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-146	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-147	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-148	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-149	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
EcPH10-153	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-154	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-156	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-158	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-159	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-16	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	14/05/2006	Sheltered	None
EcPH10-161	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-164	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-17	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	14/05/2010	Sheltered	None
EcPH10-172	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-174	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	04/07/2010	Sheltered	None
EcPH10-20	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	14/05/2010	Sheltered	None
EcPH10-21	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	14/05/2010	Sheltered	None
EcPH10-291	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	12/08/2010	Sheltered	None
EcPH10-293	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	12/08/2010	Sheltered	None
EcPH10-296	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	12/08/2010	Sheltered	None
EcPH10-38	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	27/05/2010	Sheltered	None
EcPH10-40	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	27/05/2010	Sheltered	None
EcPH10-41	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	27/05/2010	Sheltered	None
EcPH10-43	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	27/05/2010	Sheltered	None
EcPH10-44	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	27/05/2010	Sheltered	None
EcPH11-102	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	06/06/2011	Sheltered	None
EcPH11-103	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	06/06/2011	Sheltered	None
EcPH11-104	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	06/06/2011	Sheltered	None
EcPH11-105	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	06/06/2011	Sheltered	None
EcPH11-110	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	06/06/2011	Sheltered	None
EcPH11-121	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	02/08/2011	Sheltered	None
EcPH11-s#2A-12	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	None
EcPH11-s#2A-20	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	None
EcPH11-s#2A-22	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	None
EcPH11-s#2A-28	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	None
EcPH11-s#2A-38	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	None
EcPH11-s#2A-43	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	None
EcPH11-s#2A-45	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	26/01/2011	Sheltered	None

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
EcPH11-s#2B-13	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	None
EcPH11-s#2B-14	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	None
EcPH11-s#2B-18	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	None
EcPH11-s#2B-51	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	25/01/2011	Sheltered	None
EcPH11-s#5-10	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-11	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-12	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-14	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-21	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-22	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-36	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-39	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-4	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-40	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-48	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-50	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-51	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	None
EcPH11-s#5-9	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	35	21/01/2011	Sheltered	None
Ec327	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	27/05/2006	Sheltered	None
Ec530	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	24/05/2005	Sheltered	None
Ec534	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	24/05/2005	Sheltered	None
Ec535	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	24/05/2005	Sheltered	None
Ec536	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	24/05/2005	Sheltered	None
Ec538	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	24/05/2005	Sheltered	None
Ec542	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	24/05/2005	Sheltered	None
Ec544	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	22/08/2005	Sheltered	None
Ec546	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	22/08/2005	Sheltered	None
EcTH10-1	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	None
EcTH10-11	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	None
EcTH10-12	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	None
EcTH10-13	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	None
EcTH10-14	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	None
EcTH10-15	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	None
EcTH10-16	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	None

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
EcTH10-170	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	None
EcTH10-171	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	None
EcTH10-173	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	None
EcTH10-174	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	None
EcTH10-177	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	None
EcTH10-178	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	None
EcTH10-2	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	None
EcTH10-200	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	None
EcTH10-203	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	None
EcTH10-204	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	None
EcTH10-206	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	13/08/2010	Sheltered	None
EcTH10-3	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	None
EcTH10-4	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	None
EcTH10-5	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	None
EcTH10-6	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	None
EcTH10-7	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	36	28/05/2010	Sheltered	None
Ec547	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	37	26/04/2005	Sheltered	None
Ec548	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	37	26/04/2005	Sheltered	None
Ec549	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	37	26/04/2005	Sheltered	None
Ec550	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	37	26/04/2005	Sheltered	None
Ec551	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	37	26/04/2005	Sheltered	None
Ec552	0	NA	NA	NA	DG Muller	*Ectocarpus siliculosus*	France	37	10/04/2005	Sheltered	None
EcQB10-19	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	39	31/07/2010	Sheltered	None
EcQB10-20	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	39	31/07/2010	Sheltered	None
EcQB10-24	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	39	31/07/2010	Sheltered	None
EcQB10-25	0	NA	NA	NA	AF Peters	*Ectocarpus siliculosus*	France	39	31/07/2010	Sheltered	None
BUT08-8C	2	84.85	85.78	NA	AF Peters	*Ectocarpus* sp.	UK	10	01/06/2008	Exposed	1*
RAT08-6A	1	85.78	NA	NA	AF Peters	*Ectocarpus* sp.	UK	12	11/06/2008	Exposed	1
QAB08-5D	2	82.27	84.97	NA	AF Peters	*Ectocarpus sp.*	UK	25	24/05/2008	Sheltered	0*
LH7c	2	82.8	85.8	NA	AF Peters	*Ectocarpus* sp.	UK	32	28/05/2008	Sheltered	Both
SAM08-11C	1	84.53	NA	NA	AF Peters	*Ectocarpus* sp.	UK	16	07/06/2008	Sheltered	0
SAM08-3B	1	84.12	NA	NA	AF Peters	*Ectocarpus* sp.	UK	16	07/06/2008	Sheltered	0
SAM11C4	1	84.5	NA	NA	AF Peters	*Ectocarpus* sp.	UK	16	07/06/2008	Sheltered	0
EcQAB10-26	1	84.47	NA	NA	AF Peters	*Ectocarpus* sp.	UK	25	13/06/2010	Sheltered	0

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
LH14a	1	85.97	NA	NA	AF Peters	*Ectocarpus* sp.	UK	32	28/05/2008	Sheltered	1
SAM08-10C	0	NA	NA	NA	AF Peters	*Ectocarpus* sp.	UK	16	07/06/2008	Sheltered	None
POR08-1B	0	NA	NA	NA	AF Peters	*Ectocarpus* sp.	UK	19	17/06/2008	Sheltered	None
POR08-2B	0	NA	NA	NA	AF Peters	*Ectocarpus* sp.	UK	19	17/06/2008	Sheltered	None
EcQAB10-31	0	NA	NA	NA	AF Peters	*Ectocarpus* sp.	UK	25	13/06/2010	Sheltered	None
Ec160	1	84.98	NA	NA	AF Peters	*Kuckuckia*	Chile	4	01/03/2006	Sheltered	Unclassified
BUT08-10C	3	81.62	83.62	85.3	NA	Unknown	NA	NA	NA	NA	Both
TOR08-11A	3	81.95	83.25	85.02	NA	Unknown	NA	NA	NA	NA	0*
W010	3	83.45	84.05	85.6	NA	Unknown	NA	NA	NA	NA	Both
L(els)PH(06)-4	2	84.07	86.55	NA	AF Peters	Unknown	France	35	07/11/2006	NA	0*
BRI08-3A	2	82.12	84.6	NA	NA	Unknown	NA	NA	NA	NA	0*
BRI1	2	84.7	85.55	NA	NA	Unknown	NA	NA	NA	NA	1*
Ec351	2	83.55	85.15	NA	NA	Unknown	NA	NA	NA	NA	Both
EcPH11-119	2	83.33	85.35	NA	NA	Unknown	NA	NA	NA	NA	Both
EcPH11-29	2	83.35	85.42	NA	NA	Unknown	NA	NA	NA	NA	Both
EcPH11-s#5-16	2	82.25	85.35	NA	NA	Unknown	NA	NA	NA	NA	Both
ET08-1B	2	82.53	85.82	NA	NA	Unknown	NA	NA	NA	NA	Both
FW Ecto	2	82.48	84.18	NA	NA	Unknown	NA	NA	NA	NA	0
HAS08-3C	2	84.08	85.55	NA	NA	Unknown	NA	NA	NA	NA	Both
J002	2	82.92	85.05	NA	NA	Unknown	NA	NA	NA	NA	0*
P003	2	81.65	84.7	NA	NA	Unknown	NA	NA	NA	NA	0*
RSA001	2	83.27	85.07	NA	NA	Unknown	NA	NA	NA	NA	0*
RSA002	2	83.7	85.15	NA	NA	Unknown	NA	NA	NA	NA	Both
SKY08-7C	2	84.45	85.53	NA	NA	Unknown	NA	NA	NA	NA	Both
W011	2	84.87	85.4	NA	NA	Unknown	NA	NA	NA	NA	1*
WEM08-9B	2	83.65	85.65	NA	NA	Unknown	NA	NA	NA	NA	Both
L(els)BR(02)D	1	84.3	NA	NA	AF Peters	Unknown	France	35	13/06/2002	NA	0
(Ec)PHG1	1	85.27	NA	NA	NA	Unknown	NA	NA	NA	NA	1
(Ec)PHG2	1	85.33	NA	NA	NA	Unknown	NA	NA	NA	NA	1
(Ec)PHH11	1	85.22	NA	NA	NA	Unknown	NA	NA	NA	NA	1
(Ec)PHL18	1	85.25	NA	NA	NA	Unknown	NA	NA	NA	NA	1
BER4A	1	85.4	NA	NA	NA	Unknown	NA	NA	NA	NA	1
BUT08-6B	1	85.17	NA	NA	NA	Unknown	NA	NA	NA	NA	1
CH(05-)2	1	84.72	NA	NA	NA	Unknown	NA	NA	NA	NA	Unclassified

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
Ec less	1	85.45	NA	NA	NA	Unknown	NA	NA	NA	NA	1
Ec Oban 06-29-7	1	85.3	NA	NA	NA	Unknown	NA	NA	NA	NA	1
Ec238hSP	1	83.33	NA	NA	NA	Unknown	NA	NA	NA	NA	0
Ec32m	1	85.17	NA	NA	NA	Unknown	NA	NA	NA	NA	1
Ec454f	1	85.7	NA	NA	NA	Unknown	NA	NA	NA	NA	1
EcJAP07-19	1	85.18	NA	NA	NA	Unknown	NA	NA	NA	NA	1
EcPH11-24	1	85.4	NA	NA	NA	Unknown	NA	NA	NA	NA	1
EcPH11-s#2B-24	1	85.35	NA	NA	NA	Unknown	NA	NA	NA	NA	1
EcPH11-s#2B-29	1	85.5	NA	NA	NA	Unknown	NA	NA	NA	NA	1
EcPH11-s#5-46	1	85.48	NA	NA	NA	Unknown	NA	NA	NA	NA	1
J001	1	84.65	NA	NA	NA	Unknown	NA	NA	NA	NA	Unclassified
KckCRO11-1	1	84.93	NA	NA	NA	Unknown	NA	NA	NA	NA	Unclassified
Na108f	1	83.18	NA	NA	NA	Unknown	NA	NA	NA	NA	0
REP10-20	1	85.7	NA	NA	NA	Unknown	NA	NA	NA	NA	1
REP10-66	1	85.35	NA	NA	NA	Unknown	NA	NA	NA	NA	1
S003	1	85.28	NA	NA	NA	Unknown	NA	NA	NA	NA	1
SKY3B	1	85.55	NA	NA	NA	Unknown	NA	NA	NA	NA	1
TOR08-12C	1	84.97	NA	NA	NA	Unknown	NA	NA	NA	NA	Unclassified
TOR08-16A	1	85.17	NA	NA	NA	Unknown	NA	NA	NA	NA	1
W012	1	85.05	NA	NA	NA	Unknown	NA	NA	NA	NA	Unclassified
wem 8c4	1	85.58	NA	NA	NA	Unknown	NA	NA	NA	NA	1
Y004	1	85.27	NA	NA	NA	Unknown	NA	NA	NA	NA	1
L(els)BR(02)C	0	NA	NA	NA	AF Peters	Unknown	France	35	13/06/2002	NA	None
L(els)BUT(08)	0	NA	NA	NA	AF Peters	Unknown	France	35	02/06/2008	NA	None
L(els)PH(06)-1	0	NA	NA	NA	AF Peters	Unknown	France	35	07/11/2006	NA	None
L(els)PH(06)-2	0	NA	NA	NA	AF Peters	Unknown	France	35	07/11/2006	NA	None
L(els)PH(06)-3	0	NA	NA	NA	AF Peters	Unknown	France	35	07/11/2006	NA	None
L(els)PH(06)-5	0	NA	NA	NA	AF Peters	Unknown	France	35	07/11/2006	NA	None
L(els)Seil(07)-1	0	NA	NA	NA	AF Peters	Unknown	France	35	04/11/2007	NA	None
L(els)Seil(07)-2	0	NA	NA	NA	AF Peters	Unknown	France	35	04/11/2007	NA	None
(Ec)PHH1	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
(Ec)PHL20	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
BUT08-9A	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
CH(05-)3	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None

Table A.1 (continued)

Name	No. of infections	Tm 1 / °C	Tm 2 / °C	Tm 3/ °C	Collector	Species	Country	Collection site	Date	Exposure	Virus Group
Dga	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
Ec08m	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
EcPH10-10	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
EcPH10-136	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
EcPH10-6	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
EcPH10-7	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
EcPH11-34	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
EcPH11-41	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
EcPH11-s#2A-2	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
EcPH11-s#2A-23	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
EcPH11-s#2B-50	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
L(els)HSoW15	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
Na166m	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
PHZ30B	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
PHZ37	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
RB1m	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
REP10-15	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
REP10-55	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
REP10-9	0	NA	NA	NA	NA	Unknown	NA	NA	NA	NA	None
EcPH11-4	1	84.97	NA	NA	AF Peters	Unknown	France	35	20/01/2011	Sheltered	Unclassified

A.2 Comparison of melting temperatures for cloned and genomic DNA from environmental isolates used as training data

Comparison of the melting temperatures for the HAS08-17B and HAS08-20A clones used as training data for the calculation of the correction factor used to calibrate the screening data in Figure 4.4 step 4.

Repeat	unFV		unEV	
	Cloned	Genomic	Cloned	Genomic
1	84.05	83.30	86.47	85.62
2	84.00	83.33	86.42	85.73
3	84.08		86.42	
4	84.08		86.55	
5	83.98		86.48	
6	84.05		86.50	
7	84.07		86.55	
8	83.92		86.57	
9	83.98		86.50	
10	84.18		86.47	
11	84.12		86.48	
12	84.20		86.42	
13	84.18			
14	84.10			
15	84.13			
16	84.17			
17	83.98			
18	84.10			
Median	84.08	83.32	86.48	85.68
Difference	0.77		0.80	

A.3 Melting temperatures of cloned DNA used for training data

The melting temperatures for the training data generated by HRM analysis of clones of known sequence (Figure 4.5 step 7). Abbreviations are as follows: EsV (*Ectocarpus* virus), FirrV (*Feldmannia irregularis* virus), FlexV (*F. simplex* virus), unEV (unknown *Ectocarpus*-like virus belonging to subgroup A), unFV (unknown *Feldmannia*-like virus belonging to subgroup B) or unPV (unknown phaeovirus belonging to neither subgroup A nor B). Medians and variances of the two subgroups and two unknown phaeovirus temperatures are also shown.

Repeat	Subgroup A		Subgroup B			Unassigned subgroup	
	EsV	unEV	FirrV	FlexV	unFV	unPV	unPV2
1	85.78	86.47	83.93	85.95	84.05	86.03	85.13
2	85.72	86.42	83.8	85.82	84	86	85.2
3	86.15	86.42	83.85	85.88	84.08	86	85.17
4	86.2	86.55	83.57	85.48	84.08	86.15	85.25
5	86.05	86.48	83.47	85.25	83.98	86.13	85.32
6	86.25	86.5	83.4	85.33	84.05	86.12	85.22
7	86.2	86.55			84.07		
8	86.1	86.57			83.92		
9		86.5			83.98		
10		86.47			84.18		
11		86.48			84.12		
12		86.42			84.2		
13					84.18		
14					84.1		
15					84.13		
16					84.17		
17					83.98		
18					84.1		
Median	86.42		84.08			86.075	85.21
Variance	0.062846316		0.500664368			0.00477667	0.00435

Publications

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A Novel Evolutionary Strategy Revealed in the Phaeoviruses

Kim Stevens[1,2], Karen Weynberg[1,2], Christopher Bellas[1,2], Sonja Brown[1,2], Colin Brownlee[1,2], Murray T. Brown[2], Declan C. Schroeder[1]*

1 Cell and Molecular Department, Marine Biological Association, Plymouth, Devon, United Kingdom, **2** School of Marine Science and Engineering, University of Plymouth, Plymouth, Devon, United Kingdom

Abstract

Phaeoviruses infect the brown algae, which are major contributors to primary production of coastal waters and estuaries. They exploit a Persistent evolutionary strategy akin to a *K*- selected life strategy via genome integration and are the only known representatives to do so within the giant algal viruses that are typified by *r*- selected Acute lytic viruses. In screening the genomes of five species within the filamentous brown algal lineage, here we show an unprecedented diversity of viral gene sequence variants especially amongst the smaller phaeoviral genomes. Moreover, one variant shares features from both the two major sub-groups within the phaeoviruses. These phaeoviruses have exploited the reduction of their giant dsDNA genomes and accompanying loss of DNA proofreading capability, typical of an Acute life strategist, but uniquely retain a Persistent life strategy.

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* E-mail: dsch@mba.ac.uk

Introduction

All viruses broadly follow one of two life strategies, Acute or Persistent [1,2,3]. Moreover, the switch from Persistent to Acute in animal systems underpins emerging new viral epidemiology, notable examples being influenza, measles and HIV [2]. This transformation is often triggered by viruses jumping from one species to another. Viruses that follow an Acute life strategy have characteristic features that associate them with a disease phenotype; high reproduction and mutation rates, and greater dependency on host population densities for transmission. Many animal viral infections that are responsible for emerging epidemic diseases follow this Acute infection dynamic that originated from a Persistent viral life strategist [2]. Despite their likely prevalence, Persistent viral life strategies are not well described. Persistence is defined as a stable coexistence in an individual host, seldom causing disease, and transmission is often from parent to offspring [1]. Phaeoviruses infect the Ectocarpales brown algae, which are major contributors to primary production of coastal waters and estuaries [4], and separated from the kelps around 100 Ma [5]. Viral infections in protists contribute significantly to the sheer abundance of viruses in our oceans [6], and have been shown to play important roles in some of the major oceanic processes, such as plankton mortality [7,8], nutrient cycling and carbon storage [9,10]. Their ubiquitous nature means that viruses affect every aspect of life in the marine environment, and their importance in such fundamental areas as evolution [3,11], the global food web and even climate change should not be underestimated [3,9,12].

Protist viruses belonging to the family *Phycodnaviridae* [13] are members of the wider grouping of nuclear cytoplasmic large dsDNA viruses (NCLDVs). The coccolitho-[11,14] and phaeoviruses [4,15] are two examples of NCLDVs having opposing life strategies Acute vs Persistent, respectively. The former are lytic algal bloom terminators [14], while the latter covertly infect and integrate their genomes via the gamete and/or spore life stages of the host, forming a latent provirus which is transmitted to all cells during adult development [4]. As with most persistent viruses, phaeoviruses have no noticeable negative impact on the life-cycle of the host; however, overt symptoms of phaeovirus infection can be seen when the reproductive organs become deformed and produce virions, instead of gametes or spores (Figure 1).

To date, phaeovirus identity has only been confirmed for viruses infecting three species of filamentous brown algae: *Ectocarpus siliculosus* (Dillwyn) Lyngbye (Esil), *Feldmannia* sp. and *Feldmannia irregularis* (Kützing) Hamel (Firr); infected by EsV-1, FsV and FirrV-1, respectively [16]. They vary in genome size from 180–336 kb (Table 1). In addition, the genome of an *Ectocarpus* strain was found to contain a transcriptionally inactive copy of an EsV-1-like provirus [4]. Complete genome sequences show that EsV-1, FirrV-1 and FsV-158 contain a limited number of common single copy core genes, as well as many unique genes [15]. Five phaeoviruses, identified by morphology and life cycle, infecting *Ectocarpus fasciculatus* (Harvey) (Efas), *Feldmannia simplex* (Crouan & Crouan) Hamel (Flex), *Hincksia hincksiae* (Harvey) Silva (Hinc), *Pylaiella littoralis* (Linnaeus) Kjellman (Plit) and *Myriotrichia clavaeformis* (Harvey) (Mcla) have also been described in the literature (Table 1) [16]. Here we report on the phylogenetic placement of these phaeoviruses, using single and multi-gene phylogenies for three NCLDV core single copy genes, namely the major capsid protein (MCP), DNA polymerase (DNApol) genes,

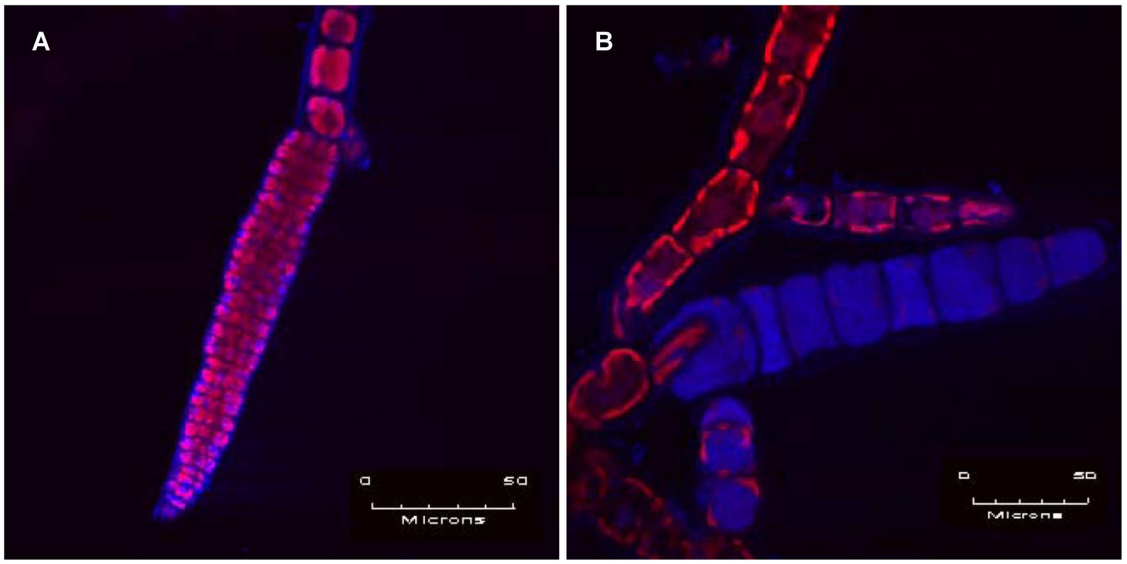

Figure 1. Epifluorescence microscope images of *E. siliculosus*. The pink stained individual spores (combination of DAPI stained blue DNA and red auto-fluorescence from nuclei and chloroplasts, respectively) are clearly visible within the normal zoidangium (A), whereas in (B) the zoidangium is misshapen and heavily stained showing that the space is filled with densely packed blue viral particles.
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and a hitherto untested viral superfamily III helicase (VACV D5-like) gene.

Results and Discussion

EsV-1 is the only virus known to infect *E. siliculosus*, while Ivey *et al.*[17] reported the presence of two (and potentially four) different size variants (158 kb and 178 kb) of phaeoviruses in cultures of *Feldmannia* sp. Delaroque *et al.* [18] reported an incomplete FirrV-1 genome, with no evidence of multiple variants, within *F. irregularis*. Our viral sequences from Esil matched perfectly with reference gene sequences for EsV-1 (Table 1 & Figure 2). Notably, no additional sequence variation for EsV-1 could be found. The other available DNApol gene sequences for the reference genome, FirrV-1, were identified within the *Feldmannia irregularis* (Firr 1) isolate (Figure 2); however, at least one additional variant could also be identified (Table 1). This result is the likely explanation for the inability of Delaroque *et al.* to assemble the FirrV-1 genome [18]. All the other ectocarpoid strains contained two or more viral sequence variants, with the *Feldmannia simplex* (Flex) isolate containing at least eight different variants (Table 1). Our Bayesian and Maximum Likelihood inference trees (DNApol or multigene) were largely in agreement that the phaeovirus sequence variants group should be split into two distinct sub-groups: a virus sub-group A that infect multiple species across three families of the Ectocarpales (Figures 3 & 4) and a second sub-group B containing members that infect the genus *Feldmannia*.

Furthermore, there are two unexpected observations from these phylogenies. Firstly, the Flex 8 variant shares features with both of the sub-groups, whilst, unsurprisingly, being more closely connected to sub-group B (Figure 3 & Figure 4). A closer look at the DNApol sequence (Figure 2) shows not only the overall conservation of amino acids (32%) across all the phaeoviruses and the wider eukaryote kingdom as a whole, but also how certain amino acids can be assigned to either sub-group A (triangles, Figure 2) or sub-group B (inverted triangles, Figure 2). Moreover, one important conserved region, Pol III dNTP binding site, is

Table 1. Ectocarpoid strains used for phaeovirus screening (adapted from Schroeder [16]).

Strain	Species	Family	Location	Genome	Number of sequence variants *			Concatenations**
				kb	DNApol	MCP	Helicase	
Esil	*Ectocarpus siliculosus*	Ectocarpaceae	New Zealand	336	1 (1)	1 (1)	1 (1)	1
Efas	*Ectocarpus fasciculatus*	Ectocarpaceae	France	320	2 (2)	1 (1)	2 (2)	2
Plit	*Pylaiella littoralis*	Acinetosporaceae	Alaska	280	1 (1)	1 (1)	1 (1)	1
Hinc	*Hincksia hincksiae*	Acinetosporaceae	France	240	1 (1)	-	2 (1)	-
Mcla	*Myriotrichia clavaeformis*	Chordariaceae	Argentina	320	1 (1)	2 (2)	-	2
Firr	*Feldmannia irregularis*	Acinetosporaceae	Canary Islands	180	2 (2)	3 (2)	2 (2)	4
Flex	*Feldmannia simplex.*	Acinetosporaceae	Ireland	220	9 (8)	6 (4)	8 (3)	22

*: variant in DNA sequence (HG003317 - HG003355) with amino acid variation indicated in parentheses. A negative PCR result is indicated by a minus symbol.
**: possible permutations for DNApol and MCP as seen in Figure 4.
doi:10.1371/journal.pone.0086040.t001

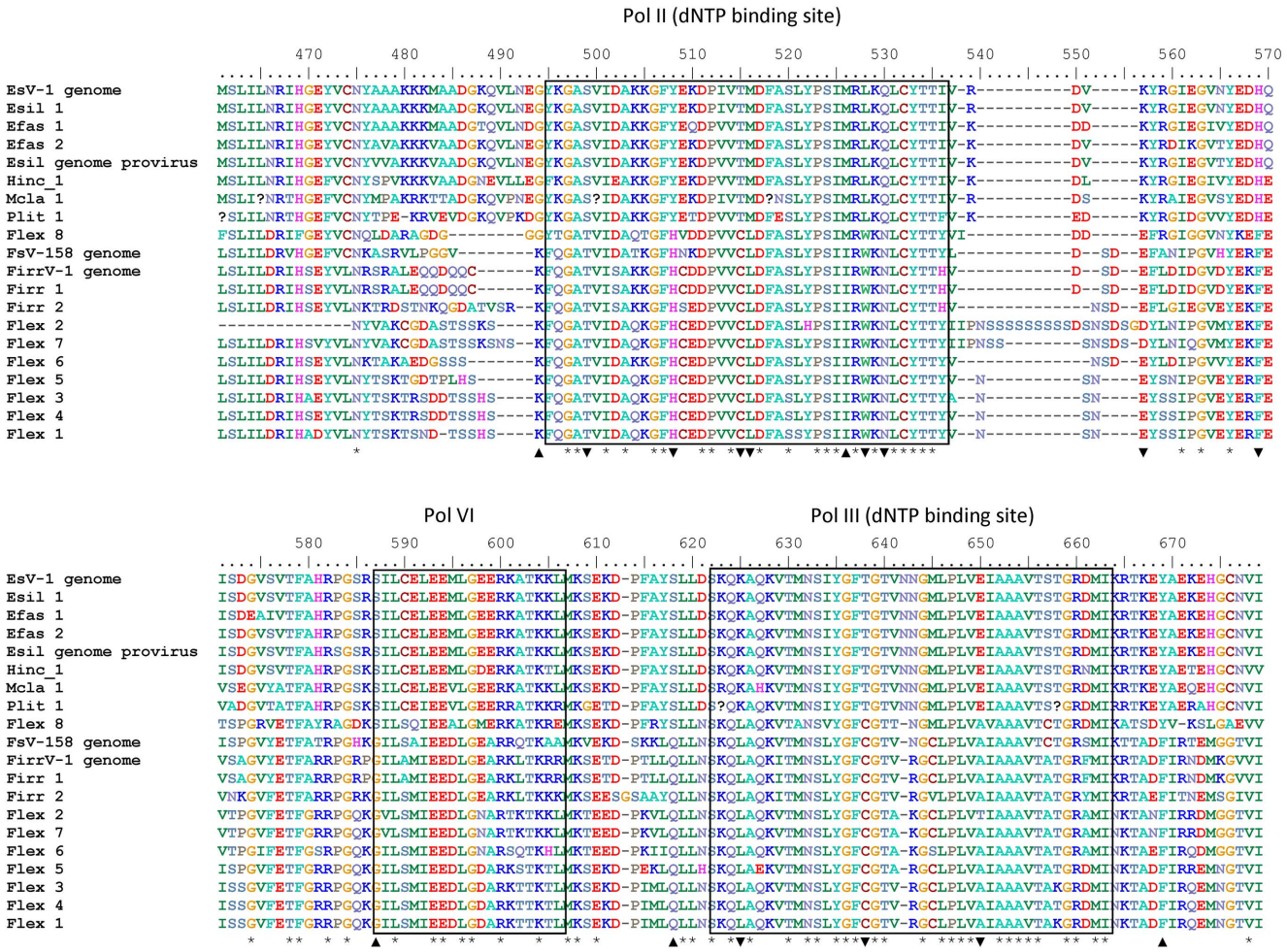

Figure 2. Partial nucleotide alignment of cloned fragment of the viral DNA polymerase gene. Numbers refer to amino acid position in the complete EsV-1 DNA polymerase gene taken from Delaroque *et al*. 2001 [20] (GenBank accession number NC_002687.1). Boxed regions indicate conserved polymerase domains [30]. * indicates conserved positions between all sequences, ▲ shows where the Flex 8 variant shares an amino acid with the larger viruses of sub-group A, ▼ shows where the Flex 8 variant shares an amino acid with the smaller genomed viruses of sub-group B.
doi:10.1371/journal.pone.0086040.g002

known to be important for genome stability [19]. Daee *et al*. found that a single amino acid mutation was associated with extreme rates of spontaneous mutation in yeast. Three amino acid polymorphisms in this region (Figure 2, positions 618, 621, 625) could be a contributing factor to the large number of variants observed amongst the *Feldmannia* viruses. The second key observation is that Flex 8, at the base of the sub-group B clades in all the phylogenetic trees, is probably the progenitor virus to the *Feldmannia* sub-group B viruses. This, therefore, gives us a unique insight into the emergence of a new phaeovirus sub-group, which is likely to be a result of the genome reduction of an ancestral member from sub-group A. Both FirrV-1 and FsV-158 [15] show the loss of the DNA proofreading exonuclease gene (EsV-126) known to be present in EsV-1 [20]. We therefore hypothesize that these genomic modifications could have resulted in the key life strategy shift, thereby utilizing the high mutation rates more associated with acute infections. When and how this happened is unclear; however, the expansion of localised genomic regions in poxviruses, causing gene duplications and mutations have been proposed to be a response to overcoming changing immune responses after a host switch [21]. Whilst gene duplications have not yet been discovered in the phaeoviruses sequenced thus far, these expansions are usually followed by a rapid gene reduction in order to minimise the burden of replicating and enlarged genome, therefore a similar mechanism may also be involved here.

A pairwise analysis of the evolutionary divergence in nucleotide sequences within the various groups of phycodnaviruses (Figure 5) illustrates the shift by sub-group B to a genome characteristic of an *r*-like evolutionary strategy. Sub-group B has a median nucleotide divergence of 29.3% in the DNApol gene fragment, comparable to that of the other *r*- selected lytic phycodnavirus groups (24.3–47.9%). Sub-group A has maintained the classic K – selection life strategy with a much lower divergence of 14.9%.

There have been several studies that reported on the host specificity of phaeoviruses. EsV-1 can successfully infect *Kuckuckia kylinii* (Cardinal) producing virions infectious to the original host [22]. Other cross-species infections do not produce infectious virions although the virus does induce symptom-like deformities in the host, for example EsV-1 in *F. simplex* [22], or EfasV in *E. siliculosus* [23] and *M. clavaeformis* [24]. This demonstrates not only the potential of phaeoviruses to jump between species but also that not all jumps result in successful infections. Another example of this unsuccessful jump can be seen by the presence of an inactive provirus in the *Ectocarpus* genome [4]. Here we show that the

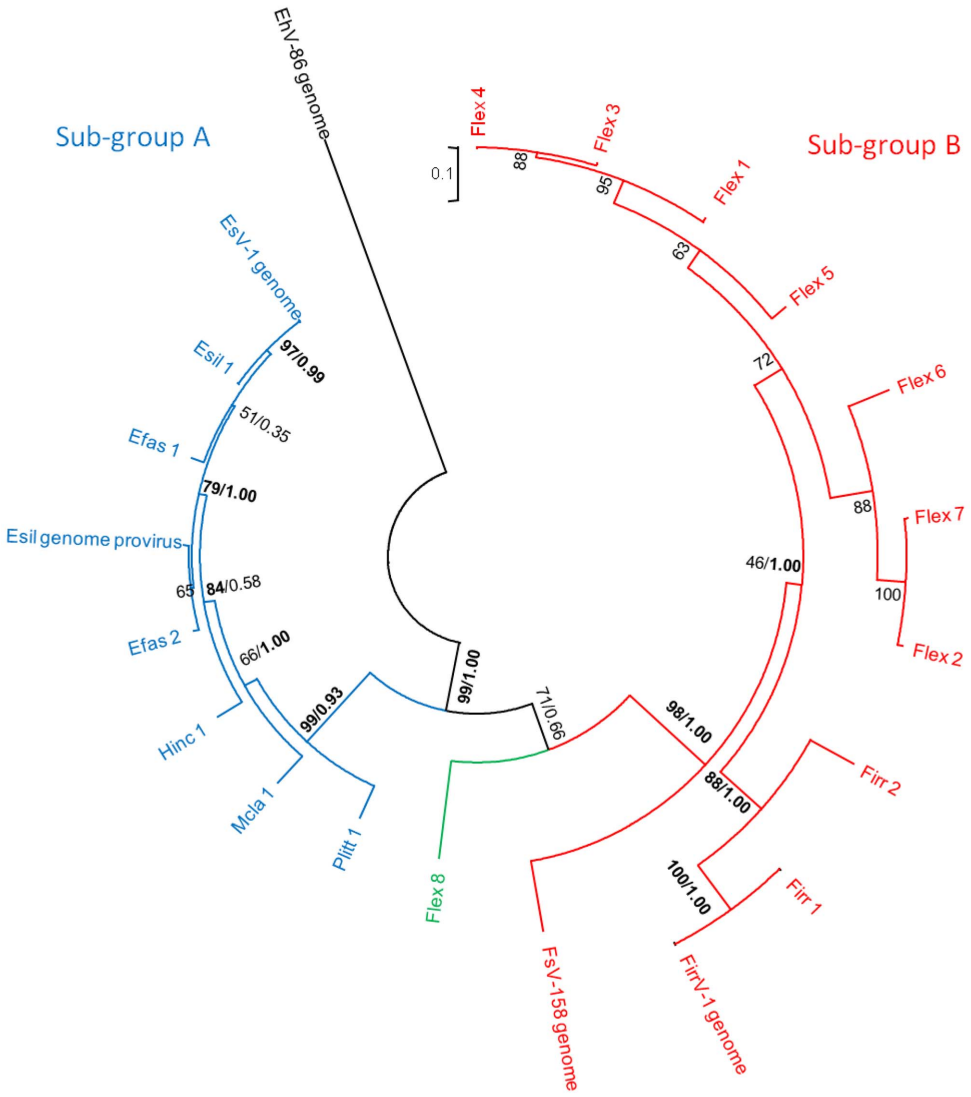

Figure 3. Maximum Likelihood analysis between variants of the phaeoviral sequences of DNA polymerase. Single value node labels represent ML bootstrap values. Where nodes are labelled with two values, this indicates that both ML and Bayesian topologies agree (whole numbers represent ML bootstrap values, decimals indicate Bayesian posterior probability). Sub-group A viruses are labelled in blue, sub-group B viruses are red and the intermediate Flex virus variant is green. Bold values are those greater than 75% bootstrap or probability.
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provirus appears to be more closely related to an *E. fasciculatus* variant than to EsV-1 (e.g. Figures 3 & 4). This suggests that an *E. fasciculatus* virus infected an *Ectocarpus* species more closely related to *E. siliculosus* [25]. This study also confirmed the life-history and morphometric data that the viruses infecting Efas, Mcla, Plit and Hinc do indeed belong in the phaeovirus group. Moreover, there is also a corresponding grouping which can be created based on genome sizes (see Table 1); the larger viral genomes from Esil, Efas, Plit, Mcla and Hinc (240–336 kb) fall within sub-group A and the smaller viruses from Firr, Flex and *Feldmannia* sp [15] (158–220 kb) into sub-group B.

This study provides the first example of an emergent virus system retaining a Persistent life strategy, but exploiting an Acute strategist's high genomic mutation rate. Moreover, unlike current reports on how emerging acute diseases develop where cryptic persistent viruses cross species boundaries (e.g. HIV [26], H5N1 [27] and DWV [28]), which can have catastrophic consequences for new host survival, this study suggests a very different scenario of one in which the integration and diversification of Persistent viruses has been stably maintained over a long period of time. Similarly, due to their evolutionary link to animal viruses this infection strategy is likely to also occur in these systems, and further studies in this field may help our understanding of the spread of new emergent diseases.

Materials and Methods

Isolates & culture conditions

See Table 1 for a list of the phaeovirus-infected cultures used in this study. Each strain was cultured in a 40 ml petri dish at 15°C, 16:8 light-dark cycle, approximately 100 µmol photons m^{-2} s^{-1}. The Western Channel Observatory (www.westernchannelobservatory.org.uk) is an oceanographic time-series and marine biodiversity reference site in the Western English Channel. In situ measurements are undertaken weekly at coastal station L4 (source of water for our study) and fortnightly at open

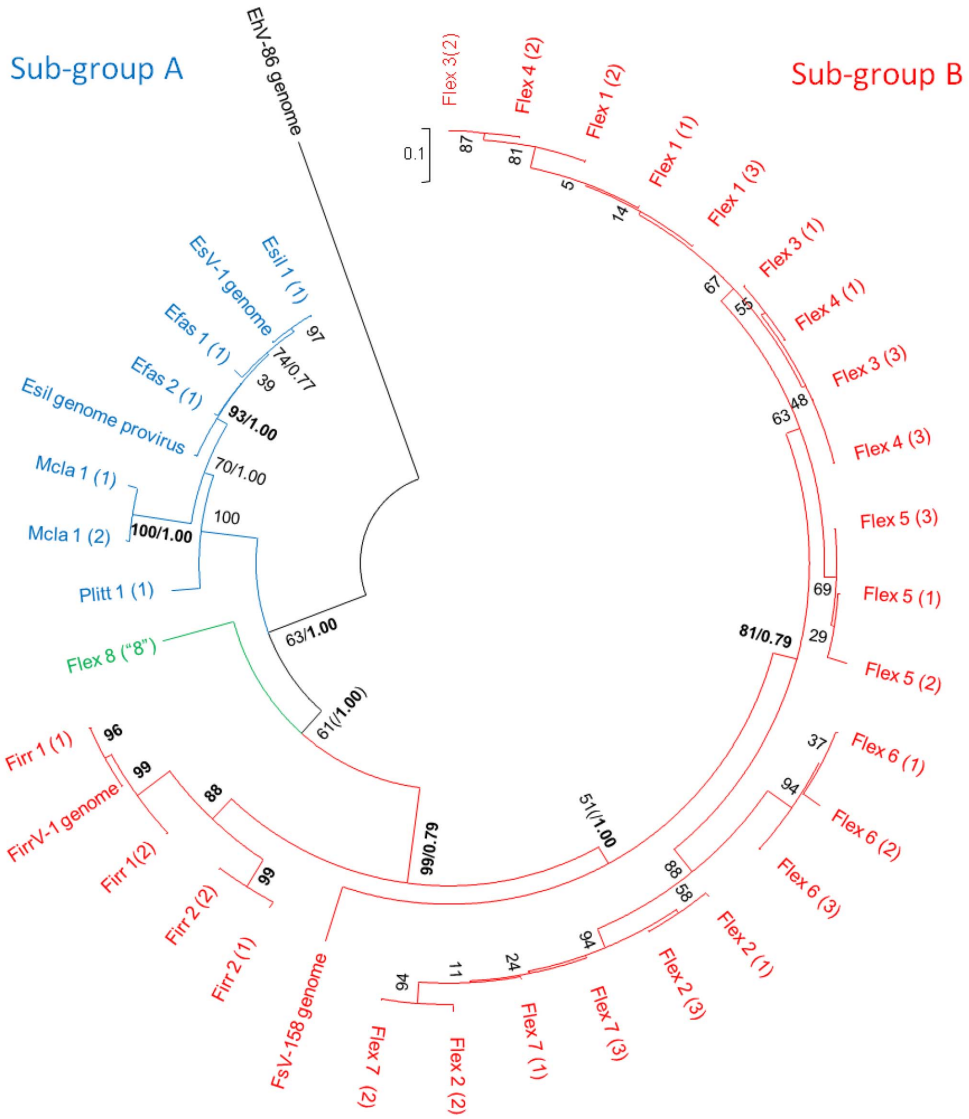

Figure 4. Maximum Likelihood analysis between variants of the phaeoviral sequences of concatenations of DNApol and MCP. Variants are labelled according to DNApol identifier initially, followed by the MCP variant number in brackets. In order to slightly reduce the number of combinations of sequences, where individual gene phylogenies show a clear separation of individual variants, these are concatenated together and excluded from the other combinations. Single value node labels represent ML bootstrap values. Where nodes are labelled with two values, this indicates that both ML and Bayesian topologies agree (whole numbers represent ML bootstrap values, decimals indicate Bayesian posterior probability). Sub-group A viruses are labelled in blue, sub-group B viruses are red and the intermediate Flex virus variant is green. Bold values are those greater than 75% bootstrap or probability.
doi:10.1371/journal.pone.0086040.g004

shelf station E1 using the research vessels of the Plymouth Marine Laboratory and the Marine Biological Association. THE DATA POLICY of the NERC National Capability funded Western Channel Observatory is to make the data freely available at the point of delivery. Culture medium was filtered (30 kDa) natural sea water from the L4 sampling station close to the Eddystone Lighthouse near Plymouth, enriched with Provasoli's enrichment [29]. Sub-culturing into a new dish with fresh media was carried out every 14 days, when the cultures were pulled apart using forceps to separate out filaments in order to encourage production of zoidangia and virions.

DNA extraction method

50–200 mg wet weight fresh algal material was transferred to an Eppendorf tube, frozen in liquid nitrogen and ground using Eppendorf grinders with 10 µl saturated ≤106 microns acid washed glass bead solution before proceeding with the Qiagen DNeasy protocol for Genomic DNA purification from cultured animal cells, starting with the proteinase K treatment. 40 µl proteinase K and 200 µl Buffer AL were added to the sample and incubated at 56°C for 30 minutes, before centrifuging for 2 minutes at maximum speed to separate out the beads. 200 µl ethanol was added to the resulting supernatant, vortexed and pipetted onto the spin column, to proceed with the first centrifugation step. For the final step, DNA was eluted using 100 µl water, instead of 200 µl in order to obtain a more concentrated sample.

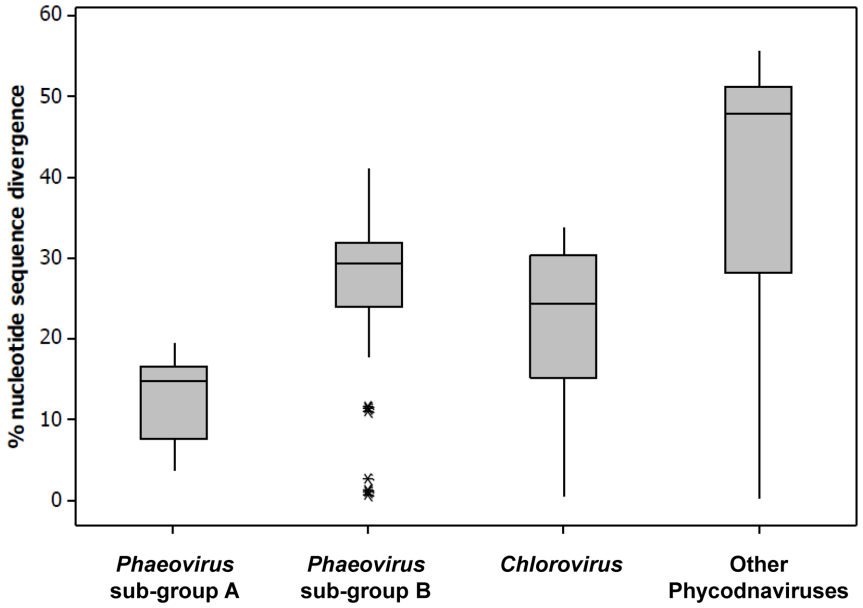

Figure 5. Box and whiskers plot of evolutionary divergence between nucleotide sequences of the DNApol. Identical sequences were not included more than once. The box represents the interquartile range which shows the middle 50% of the data, the bottom line being the first quartile, the middle line being the median and the upper line being the third quartile. The whiskers represent the maximum (or minimum) data point up to 1.5 times the box height above (or below) the top (or bottom) of the box. Outliers beyond the whiskers are shown as a *. Phaeovirus sub-groups are as shown in Fig. 3 & 4, with Flex 8 being included in sub-group B. Chloroviruses consist of thirteen viral isolates from *Paramecium bursaria Chlorella* (AF344202, AF344203, AF344211, AF344212, AF344215, AF344226, AF344230, AF344231, AF344235, AF344238, AF344239, M86837, U32985) and one from *Acanthocystis turfacea Chlorella* (AY971002). The other phycodnaviruses group consists of three viral isolates from *Emiliania huxleyi* (AF453961, AF453867, AF472534), three from *Micromonas pusilla* (U32975, U32982, U32976), five from *Ostreococcus tauri* (FJ67503, FJ884758, FJ884763, FJ884773, FJ884776), two from *Ostreococcus lucimarinus* (GQ412090, GQ412099), six from *Phaoecystis globosa* (A345136-AY345140, DQ401030), one from *Chrysochromulina brevifilum* (U32983), one from *Chrysochromulina ericina* (EU006632) and one from *Heterosigma akashiwo* (AB194136).
doi:10.1371/journal.pone.0086040.g005

PCR, cloning & Sequencing

Degenerate primers were designed for three active viral genes (DNA polymerase (GRGGNCAGCAGATYAAGTG forward, GARTCCGTRTCSCCRTA reverse), helicase (GTGGCAGGT-SATYCCYTTC forward, GTTKCCGGCCATGATYCC reverse) and major capsid protein (MCP) (CVGCGTACTGGGT-GAACGC forward, AGTACTTGTTGAACCAGAACGG reverse)) against a consensus of published sequences from EsV-1, FirrV-1, FsV-158 and the provirus from the sequenced *Ectocarpus* genome. Degenerate PCR was carried out using Promega GoTaq® Flexi DNA polymerase kit, with an addition of 0.8 mg/ml bovine serum antigen (BSA). Cycling conditions were 95°C for 5 minutes, followed by 35 cycles of 95°C for 1 minute, a 30 second annealing step, an extension step at 72°C, and a final elongation step at 72°C for 10 minutes. Oligonucleotide and magnesium concentrations, annealing temperatures and extension times varied for each gene: DNApol required 1.25 mM $MgCl^2$, 4 pmol/µl oligonucleotides, 50°C annealing temperature and 10 second extension time, MCP required 1.5 mM $MgCl^2$, 8 pmol/µl oligonucleotides, 55°C annealing temperature and 30 second extension time and helicase required 1.5 mM $MgCl^2$, 8 pmol/µl oligonucleotides, 55°C annealing temperature and 10 second extension time. Post-PCR samples were run on a 2% agarose gel at 80 V to achieve maximum separation between the bands. Samples with more than one product were purified by gel extraction; the band of the correct size was cut out of the gel and purified using the Qiaex II® Gel Extraction Kit. Samples with clean bands were purified using GenElute™ PCR Clean-Up Kit from Sigma. Purified PCR product was cloned into pCR®2.1, incubated overnight at 15°C before storing at −20°C until used. 4 µl ligation mixture was added to 0.2 ml competent cells and mixed. The cells were then incubated on ice for 40 minutes, heat shocked at 42°C for 2 minutes and returned to the ice for 5 minutes. 0.7 ml pre-warmed LB medium was added to the cells which were then incubated at 37°C for one hour. The cells were concentrated by spinning at 8000 g for 5 minutes, removing 0.5 ml supernatant, and re-suspended gently with a pipette before being plated out onto LB agar plates containing 5 µg/ml ampicillin, with 40 µl of 20 X-gal spread on each plate. Plates were incubated overnight at 37°C.

Single cloned colonies were picked from agar plates into individual 0.2 ml tubes containing 5 µl molecular grade water and heated to 95°C for 5 minutes to denature the cells before adding 10 µl 5× buffer, 5 µl 25 mM $MgCl_2$, 5 µl 2.5 mM dNTPs, 2 µl each of 10 pmol/µl M13 forward and reverse primers, 0.2 µl Taq polymerase, 20.8 µl molecular grade H_2O. Cycling conditions consisted of 30 cycles of 95°C for 45 seconds, 56°C for 45 seconds and 72°C for 45 seconds, followed by a final extension step of 72°C for 5 minutes.

PCR products were purified using the Qiaex II® Gel Extraction Kit and then sequenced using the BigDye® Terminator v3.1. The mix consisted of 3.5 µl 5× BigDye buffer, 1 µl Ready Reaction Mix, 2 µl template (6–14 ng μl^{-1} concentration), 1 µl primers (either M13 forward or reverse) at a concentration of 3.2 pmol μl^{-1} and 12.5 µl dH_2O. Cycling conditions were 95°C for 2 minutes, followed by 30 cycles of 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 30 seconds, then a final elongation at 72°C for 5 minutes. Sequenced reactions were precipitated by adding 5 µl

125 mM EDTA and 65 µl cold 100% ethanol and incubated in the dark at room temperature for 15 minutes. They were then spun for 30 minutes at 2200 g, the supernatant removed and the pellet washed with 60 µl cold 70% ethanol, and spun for a further 15 minutes at 2200 g. The supernatant was removed again and the pellet air dried. Sanger sequencing was carried out by Source Bioscience in Cambridge. Sequences were submitted to the European Nucleotide Archive with accession numbers (HG003317–HG003355).

Phylogeny

Bayesian analysis of phylogenetic trees was carried out using MrBayes v3.2.1, running the analysis until the standard deviation of split frequencies reached <0.01 and the number of generations was $>100\ 000$. Maximum Likelihood analysis was carried out using MEGA5.1 WAG model with 500 bootstrap replications and the Nearest-Neighbour-Interchange heuristic method. DNApol and MCP sequences were combined in all possible combinations (Table 1) in order to create concatenations which were used to create Figure 4.

Distance analysis

Nucleotide sequences were obtained for the various groups of phycodnaviruses that have been sequenced to data by carrying out a BLAST search of known genome sequences from each group. The phaeovirus sequences obtained in this study were split into two subgroups according to their phylogenies as shown in Figures 3 & 4. Chloroviruses were considered together with the prasinoviruses, and the remaining viral groups (coccolithoviruses, prymnesioviruses, raphidoviruses) were considered together since they are all lytic viruses of stramenopiles or coccolithophores. Pairwise distances were computed using Mega 5.05.

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Author Contributions

Conceived and designed the experiments: KS DCS. Performed the experiments: KS KW CBellas SB DCS. Analyzed the data: KS KW CBellas MTB DCS. Contributed reagents/materials/analysis tools: CBrownlee DCS. Wrote the paper: KS MTB DCS.

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