Investigating diversity, evolution, development and physiology of red algal parasites from New Zealand

BY

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Abstract

Red algal parasites have evolved independently over a 100 times and grow only on other red algal hosts. Most parasites are closely related to their host based on the similarity of their reproductive structures. Secondary pit connections between red algal parasites and their hosts are used to transfer parasite organelles and nuclei into host cells. Morphological and physiological changes in infected host cells have been observed in some species. Parasite mitochondrial genomes are similar in size and gene content to free-living red algae whereas parasite plastids are highly reduced. Overall, red algal parasites are poorly studied and thus the aim of this study was to increase the general knowledge of parasitic taxa with respect to their diversity, evolutionary origin, development, physiology, and organelle evolution. Investigation of the primary literature showed that most species descriptions of red algal parasites were poor and did not meet the criteria for defining a parasitic relationship. This literature study also revealed a lack of knowledge of many key parasitic processes including early parasite development, host cell "control", and parasite origin. Many of these poorly studied research areas were addressed in this thesis. Phylogenetic analyses, using a range of markers from all three genomes (cpDNA: *rbc*L, nDNA: actin, LSU rRNA; mtDNA: *cox*1), showed different patterns of phylogenetic relationships for the four new red algal parasites and their hosts. The parasites *Phycodrys novae-zelandiophila* sp. nov. and *Vertebrata aterrimophila* sp. nov. closest relative is its host species. *Cladhymenia oblongifoliophila* sp. nov. closest relative is its host species based on nuclear and mitochondrial markers whereas the plastid markers group the parasite with *Cladhymenia lyallii,* suggesting that the parasite plastid was acquired when previously parasitizing *C. lyallii*. *Judithia parasitica* sp. nov. grows on two *Blastophyllis* species but the parasites' closest relative is the non-host species *Judithia delicatissima*. Developmental studies of the parasite *Vertebrata aterrimophila*, showed a unique developmental structure ("trunk-like" cell) not known in other parasites, plus localised infection

and few changes in infected host cells. High-throughput-sequencing revealed mitochondrial genomes of similar size, gene content and order in the parasite *Pterocladiophila hemisphaerica* to its host *Pterocladia lucida*, and a reduced non-photosynthetic plastid in the parasite. Mitochondrial (mt) and plastid (cp) genome phylogenies placed *Pterocladiophila hemisphaerica* on long branches, either as sister to Ceramiales (mt) or Gracilariales (cp). Further analyses, filtering non-elevated plastid genes grouped the parasite neither with the Gracilariales (mt) or Gelidiales (cp) on shorter branches but without support. Nuclear phylogeny grouped *P. hemisphaerica* as sister to the Gelidiales and other red algal orders and was the only phylogenetic relationship with support. Investigations of photosystem II capacity using PAM fluorometry, and quantifying chlorophyll *a* content in three pigmented parasites, showed different host nutrient dependencies. *Rhodophyllis parasitica* and *Vertebrata aterrimophila* are not able to photosynthesize and are fully dependent on host nutrients. *Pterocladiophila hemisphaerica* is able to photosynthesize independently, even though it has a reduced non-photosynthetic plastid genome, and therefore is only partially dependent on its host. This study advances our current understanding of red algal parasites and highlights many possibilities for future research including genome evolution and understanding parasite diversity.

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Thesis declaration

I hereby declare that this thesis is my own work and that all sources quoted, paraphrased or otherwise referred to, have been acknowledged in the references at the end of this document. To the best of my knowledge, this thesis neither contains material previously published or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institutes of higher learning, except where due to the acknowledgement is has been made clear in the text.

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Chapter One

General Introduction

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1.1 Research interests and chapter outline

Parasitism is the most successful lifestyle on earth but our understanding of many key processes are still poorly understood or based on only a few selected taxa. The aim of this PhD thesis is to increase our general knowledge of parasitic taxa in regards to their diversity, evolutionary origin, development, physiology, and organelle evolution.

This general introduction chapter is divided into two parts: biodiversity and parasitism, and red algal parasites. The first part is an introduction to biodiversity and parasitism by defining diversity and symbiotic relationships and highlighting evolutionary trends. The second part provides a brief summary about parasitic red algae. This section is rather short as the second chapter is a literature review on all described red algal parasites and covers this topic more extensively.

1.2 Biodiversity and parasitism

Biodiversity describes the entirety of diversity at all biotic levels, from genetic variation to ecosystem function (Purvis & Hector 2000), and species are central to most measures of diversity. The complexity of species definitions challenges our ability to determine how many species are present (Agapow *et al.* 2004). There are many different species concepts; species may be defined by their sexual incompatibility (biological species concept), difference in niches (ecological species concept), and/or levels of genetic distinctness (phylogenetic species concept) (Hausdorf 2011). In algae, the morphological species concept is either the most dominant, or is used in combination with the phylogenetic species concept (Guiry 2012). It is important to understand biodiversity to fully appreciate important biological questions such as speciation, ecosystem function, interaction of species (competition, symbiosis, predator-prey), ecological importance (productivity, food webs) and economic importance for humans.

Symbiosis can lead to diversification of species and was first described as an intimate living together of dissimilar organisms (de Bary 1879) and can be further classified into: 1) commensalism: one organism benefits from the interaction and the other organism neither benefits nor gets harmed, 2) mutualism: both organisms benefit, and 3) parasitism: one organism benefits while harming the other organism (de Bary 1879). Symbiotic relationships can change over time (Neuhauser & Fargione 2004) or cannot be strictly determined as one of these three symbiotic relationships (Roossinck 2011). Nevertheless, parasitism is the most common lifestyle of organisms (Dobson *et al.* 2008) and has evolved independently over 223 times in animals (Weinstein & Kuris 2006). Parasitic species can be found in all eukaryotes, such as apicomplexans (Leander 2005), fungi (Quandt *et al.* 2015), oomycetes (Li *et al.* 2010), plants (Westwood *et al.* 2010) and red algae (Goff 1982). Parasites can influence the ecology, behaviour and evolution of free-living organisms (Poulin 1995; Hudson *et al.* 1998) and biodiversity (Karvonen & Seehausen 2012).

Parasites can share similar evolutionary trends with other parasites. Many parasites share phenotypic changes, i.e. reduced morphology (Keeling & Fast 2002) and complex sensory structures (Poulin 2011). Another common trend concerns changes in functions, for example reduction or loss of metabolic pathways (Revill *et al.* 2005; Müller *et al.* 2012) and genomic changes (reduction and compaction of genomes, Keeling 2004; Slamovits *et al.* 2004; Keeling *et al.* 2010). The interpretation of these evolutionary trends should be treated with caution because there are always exceptions and many parasitic taxa have yet to be studied.

1.3 Red algal parasites

Red algal parasites only grow on other red algae and have evolved over 100 times (Goff 1982; Salomaki & Lane 2014). Currently used characters to describe these parasites are: 1) reduction in size; 2) reduction in pigmentation; 3) presence of both gametophytes and sporophytes on the host, and 4) connection between parasite and host cell (via formation of secondary pit connections) (Wynne & Scott 1989). Most of those characters have been used to clarify the nature of structures such as galls and exclude misidentifications (i.e., epiphytes). The formation of secondary pit connections between parasite and host is the most important character for distinguishing these organisms as parasites (Goff & Coleman 1985).

Red algal parasites were initially grouped into "adelphoparasites" or "alloparasites" (Feldmann & Feldmann 1951). Eighty percent of red algal parasites were defined to be "adelphoparasites" or taxonomically closely related to their hosts, and parasites with a distant relationship to their hosts were described as "alloparasites" (Goff 1982). These two terms are still commonly used (Vérges *et al.* 2005; Kim & Cho 2010; Salomaki *et al.* 2015) but they are quite controversial because there seems to be a continuum between closely to distantly related parasite-host combinations (Zuccarello *et al.* 2004; Preuss & Zuccarello 2014).

Phylogenetic analyses are an essential tool to address questions about parasite origins, host switching and phylogenetic relationships. The patterns of host-parasite relationships can be quite complex. Some red algal parasites appear to have evolved from and continue to infect the same host species (e.g., *Gardneriella tuberifera* Kylin). Other parasites evolved from and infect one host but have also switched to a secondary host (e.g., *Faucheocolax attenuata* Setch.). While yet other parasites evolved on one host species, switched to a secondary closely related host and are not found on the original host (e.g., *Plocamiocolax pulvinata* Setch.) (Goff *et al.* 1996). The evolutionary relationships of red algal parasites to their hosts can range from closely related to distantly related but phylogenetic data are still quite limited on red algal parasites and further intensive studies are needed to address questions on their evolution pattern and complex organelle history.

Red algal parasites have unusual organelle relationships to their hosts. The mitochondria and nuclei of host and parasite can be either quite similar (Preuss & Zuccarello 2014) or quite different (Goff & Coleman 1995). Similar gene sequences would lead to the assumption that the parasite evolved from its host recently, whereas different gene sequences would indicate that the parasite retained its own mitochondria and nuclei and either evolved long ago or evolved from distantly related species and then switched to its secondary and current host. The plastid, on the other hand, is usually identical in most host and parasite combinations and this pattern suggests that the parasite has acquired and retained the plastid of its host (Goff & Coleman 1995). There are exceptions where a parasite has its own plastid genome (Salomaki *et al.* 2015). Phylogenetic studies using genes from all three genomes (*cox*1, ITS *rbc*L) compared parasites, *Gracilaria babae* (H.Yamam.) P.K.Ng, P.E.Lim *et* Phang, growing on host species in different genera (*Gracilaria* and *Hydropuntia*). All parasites genes were almost identical to the host *Gracilaria salicornia* (C.Agardh) E.Y.Dawson, even when growing on *Hydropuntia* (Ng *et al.* 2014). This close relationship between the parasite and *G. salicornia*

would indicate that the parasite evolved from a recent common ancestor of *G. salicornia* and did not capture the host plastid when growing on a *Hydropuntia* species.

Red algal parasites have a unique development. Usually germinating parasite spores produce an infection peg (Goff & Coleman 1984) which fuses either by connecting directly with an epidermal host cell after penetrating the host cuticle or first growing between host cells separately and then connecting to subepidermal host cells (Goff & Zuccarello 1994; Zuccarello & West 1994a). The fusion of parasite and host cells establishes a structural linkage, called a secondary pit connection (Goff & Coleman 1985) and this is an essential connection for early parasite development (Zuccarello *et al.* 2004) and organelle transfer, i.e. nuclei, to the host cell (Goff & Coleman 1995).

This organelle transfer is another unique process in red algal parasites and can give the parasite control over the host cell (host cell 'transformation') (Goff & Coleman 1987). Transformed host cells undergo unusual developmental processes (Goff & Coleman 1995). Only a few developmental studies have been conducted on red algal parasites (e.g., Nonomura 1979; Goff & Coleman 1987; Zuccarello & West 1994a) and further research is needed to understand different infection mechanisms and investigate differences in parasite infection and host cell transformation.

The understanding of parasite genome evolution improved with the use of Next-Generation Sequencing. Assembled whole genome data can be used to study genome size: if there is reduction (Jackson 2015) and compaction (Corradi *et al.* 2007); genetic changes, for example gene loss (Keeling & Slamovits 2005) and gene order (Corradi *et al.* 2007) and functional changes, such as loss of photosynthesis genes (Wicke *et al.* 2013). Red algal parasites are good model organisms to study parasite evolution as they have a close relationship to their hosts and have evolved independently multiple times. The genomic comparison of parasites with their closest relatives could unravel the changes an organism has to undergo to become parasitic (Hancock *et al.* 2010). Investigations of more examples of parasitic taxa are needed to explore these questions.

Genomic data of red algal parasites are limited to two mitochondrial genomes (Hancock *et al.* 2010) and one plastid genome (Salomaki *et al.* 2015). The mitochondrial genome of the red algal parasites *Gracilariophila oryzoides* Setch. *et* H.L.Wilson is reduced (mainly due to one deletion of a large intergenic spacer) but is not smaller in comparison with other red algal species (Hancock *et al.* 2010). The plastid genome of the parasite *Choreocolax polysiphoniae* Reinsch has lost photosynthesis genes (Salomaki *et al.* 2015).

Pigmentation in plants is associated with photosynthesis and whether or not red algal parasites can independently photosynthesize is a result of complex interactions between organelles and their genes/protein products. Both nuclear- and plastid-encoded genes are used for photosynthesis (Race 1999), and to function, signalling pathways between organelles and photosynthetic gene expression have to operate correctly (Queval & Foyer 2012). Loss of photosynthesis is often associated with loss of photosynthetic genes in plastid genomes (Suzuki *et al.* 2018). This loss of functionality can be explained by conflict between different genomes (for example, between nuclear and mitochondrial genomes) (Werren 2011) and is also known as intra-individual conflict. Further studies are needed to reveal if intra-individual/cell genome conflict occurs in red algal parasites.

The level of host dependency of red algal parasites is mostly unknown. The range of pigmentation in red algal parasites varies from unpigmented (Zuccarello & West 1994a; Salomaki *et al.* 2015) to pigmented (Maggs & Hommersand 1993; Wynne 2013; Preuss & Zuccarello 2014). Some parasites show variation in pigmentation depending on their host species (Goff *et al.* 1997) and other parasites undergo a juvenile non-pigmented stage followed by a mature pigmented stage (Nonomura & West 1981). In at least a few examples, parasites have been shown to receive photosynthetic products from their host (Evan *et al.* 1973; Goff 1979; Kremer 1983) but without further studies of pigmented parasites, questions about their level of host dependency remain.

1.4 Thesis aims

The aims of my PhD research were to increase our general knowledge of red algal parasites in regard to their diversity, evolutionary origin, development, physiology, and organelle evolution. Specific research questions were separately addressed in each research chapter.

i. Red algal parasites: a synopsis of described species, their hosts, distinguishing characters and areas for continued research (Chapter 2). The following questions were addressed:

- What is the current understanding of the diversity of red algal parasites?
- Do existing descriptions meet the criteria for defining these organisms as parasites?

ii. Three new red algal parasites from New Zealand: *Cladhymenia oblongifoliophila* sp. nov. (Rhodomelaceae), *Phycodrys novae-zelandiophila* sp. nov. (Delesseriaceae) and *Judithia parasitica* sp. nov. (Kallymeniaceae) (Chapter 3)

- What are morphological feature of the parasite?
- What is the phylogenetic relationship of the parasite to its host?
- In which genus should the parasite be placed?

iii. Development of the red algal parasite *Vertebrata aterrimophila* sp. nov. (Rhodomelaceae, Ceramiales) from New Zealand (Chapter 4)

- How does the development in the parasite *Vertebrata aterrimophila* compare with other known red algal parasites?
- What impacts does the parasite have on infected host cells?
- What is the phylogenetic relationship of the parasite *Vertebrata aterrimophila* to its host?

iv.   High mutation rates in a non-photosynthetic plastid hides phylogenetic relationships in the red algal parasite *Pterocladiophila hemisphaerica* (Gelidiales) (Chapter 5)

- Does *Pterocladiophila hemisphaerica* have a highly reduced plastid genome?
- What is the phylogenetic relationship of *Pterocladiophila hemisphaerica* and its hosts *Pterocladia lucida*?
- Is the current taxonomic position of *Pterocladiophila hemisphaerica* in the order Gracilariales correct?

v.   Comparative studies of photosynthetic capacity in three pigmented red algal parasites using PAM fluorometry and chlorophyll *a* concentration (Chapter 6)

- Can pigmented parasites photosynthesize independently?

1.5 Thesis structure

This PhD thesis was written in five individual research chapters (2-6), some of which are published or submitted to peer review journals. All published chapters are similar to the publications, and there is some repetition between chapters. All references and all supplementary materials were combined in a single reference list and appendices at the end of this thesis.

Chapter 2 has been published: **Preuss, M.**, Nelson, W. A. & Zuccarello G.C. 2017. Red algal parasites: a synopsis of described species, their hosts, distinguishing characters and areas for continued research. *Botanica Marina*. 60:13-25.

Author contributions: I conceived the idea, collect the literature, analyzed the data, wrote the manuscript and submitted it for publication. W. A. Nelson provided some literature and commented on the manuscript. G.C. Zuccarello helped with revisions on the manuscript and strengthening of the main arguments.

Chapter 3 has been published: **Preuss, M.** & Zuccarello G.C. 2018. Three new red algal parasites from New Zealand: *Cladhymenia oblongifoliaphila* sp. nov. (Rhodomelaceae), *Phycodrys novae-zelandiaephila* sp. nov. (Delesseriaceae) and *Judithia parasitica* sp. nov. (Kallymeniaceae). *Phycologia*. 57:9-19.

Author contributions: I collected the samples, analyzed the data and wrote the manuscript. G.C. Zuccarello helped collect some of the samples and helped to improve the manuscript significantly with his comments.

Chapter 4 has been accepted to the European Journal of Phycology: **Preuss, M.** & Zuccarello G.C. Development of the red algal parasites *Vertebrata aterrimophila* sp. nov (Rhodomelaceae, Ceramiales) from New Zealand.

Author contributions: I collected the samples, collected and analyzed the data and wrote the manuscript. G.C. Zuccarello helped to define the research ideas and improved the manuscript with critical comments.

Chapter 5 is in preparation to be submitted: **Preuss, M.**, Verbruggen, H. & Zuccarello G.C. High mutation rates in a non-photosynthetic plastid hides phylogenetic relationships in the red algal parasite *Pterocladiophila hemisphaerica*.

Author contributions: I collected the samples and analyzed the majority of the data and wrote the manuscript. H. Verbruggen analyzed the plastid data sets and improved the manuscript with helpful comments. G.C. Zuccarello helped significantly to improve the manuscript and clarify the research ideas.

Chapter 6 has been accepted to Phycological Research: **Preuss, M.** & Zuccarello G.C. Comparative studies of photosynthetic capacity in three pigmented red algal parasites using chlorophyll *a* concentrations and PAM fluorometry.

Author contributions: I collected the samples, designed the experiments, analyzed the data and wrote the manuscript. G.C. Zuccarello helped to define the research ideas and made many useful suggestions to improve the manuscript.

Chapter Two

**Red algal parasites: a synopsis of described species, their hosts,
distinguishing characters and areas for continued research**

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2.1 Abstract

Red algal parasites are diverse organisms that are unusual due to the fact that many are closely related to their hosts. Parasitism has developed many times within different red algal groups, but the full extent of parasite biodiversity is unknown, as parasites are easily overlooked due to their small size and often low abundance. Additionally, the literature on red algal parasites is dispersed and has not been compiled in over 30 years. Although criteria have been proposed to define what constitutes a red algal parasite, many parasites are poorly described, and the cellular interactions with their host are poorly known. A few studies have demonstrated that parasites transfer organelles to host cells, which can alter the physiology of the host to the benefit of the parasite. Here, we apply a set of defining criteria for parasites to a compiled list of all described red algal parasites. Our results highlight the lack of knowledge of many key parasitic processes including early parasite development, host cell "control", and parasite origin. Until the biology of more parasites is studied, generalisations on the processes of parasitism in red algae may be premature. We hope this synopsis will stimulate research into this fascinating group.

2.2 Introduction

Parasitism is defined as a relationship that is beneficial for the parasite but harms the host and is a common lifestyle in organisms. Approximately 40% of all known species across all phyla are parasitic and the actual number of parasites is thought to be higher than the number of free-living organisms (Dobson *et al.* 2008). The parasitic lifestyle occurs in a wide range of organisms such as fish (Le Roux & Avenant-Oldewage 2010), flatworms (Cribb *et al.* 2002), fungi (Quandt *et al.* 2015), plants (Westwood *et al.* 2010), 'protozoa' (Keeling & Rayner 2015) and algae (Blouin & Lane 2012; 2016). The importance parasites have for the ecology, behaviour and evolution of free-living organisms (e.g., Poulin 1995; Hudson *et al.* 1998) and biodiversity (e.g., Karvonen & Seehausen 2012) are well documented.

Red algal parasites are common on other red algae (Goff 1982) and are known from eight orders: Ceramiales, Corallinales, Gigartinales, Gracilariales, Halymeniales, Palmariales, Plocamiales, Rhodymeniales (Salomaki & Lane 2014; Blouin & Lane 2016). The majority of red algal parasites are taxonomically closely related to their hosts (designated as 'adelphoparasites'), while a lesser number are more distantly related to their hosts (designated 'alloparasites'; Goff 1982). While this dichotomy has been used in the past, there appears to be a continuum from closely related to more distantly related parasite-host combinations (Zuccarello *et al.* 2004, Blouin & Lane 2012). However, only a few red algal parasites have been investigated phylogenetically.

Red algal parasites are unique in that they transfer organelles (e.g., nuclei, mitochondria, plastids) into host cells, via host-parasite cell fusion by secondary pit connection formation (Goff & Coleman 1985; Salomaki *et al.* 2015) and thereby 'control' host cells for their benefit. A recent study showed that in one parasite these heterokaryotic cells not only contain the host plastid but also retain their own plastid ("ghost plastid") (Salomaki *et al.* 2015). The process of parasite-host cell fusion is unique to red algal parasite-host interactions, and has led to speculation as to their origin and how complete the 'control' is (Blouin & Lane 2012). The outcome of parasite organelles being transferred to host cells and the details of parasite development have been studied in very few parasites. However, the establishment of secondary pit connections appears to be essential for parasite development, and host resistance can occur as a response to incompatibility in parasite-host cell fusion (Zuccarello & West 1994a; b; c).

The descriptions of red algal parasites have been problematic for decades, in part because of the size of the parasite thallus and the infrequency with which they have been collected. Some described parasites have later been shown to be misidentifications of small red algal epiphytes, or bacterial infections, or even parts of the host thallus (Table 2.1). Given these problems, Setchell (1918) attempted to develop a set of characters to be used to characterise red algal parasites and to distinguish them from epiphytes and host outgrowths. This set of characters was: 1) penetration beyond the superficial layer of the host; 2) reduction in size of the thallus, and 3) loss of colour. These characters were later reviewed and modified by Wynne & Scott (1989) to: 1) reduction in size; 2) reduction in pigmentation; 3) formation of secondary pit connections between parasite and host cells, and 4) presence of both gametophytes and sporophytes on the same host stage. Most of these criteria if taken alone would not be sufficient to confirm that a red algal species was a parasite. For example, some parasites are pigmented during certain stages of their life cycle (Nonomura 1979; Goff & Coleman 1995).

Much of the current knowledge of red algal parasite species diversity is based on old lists and general statements. Setchell (1918) created the first list of described red algal parasites, and further lists followed (Evans *et al.* 1978; Goff 1982), while subsequent reviews have focused on general knowledge of red algal parasite biology (Blouin & Lane 2012; 2016; Salomaki & Lane 2014). The percentage of parasitic red algal genera has been estimated at 15% of all red algal genera by Goff (1982) or 8% of all florideophyte genera by Blouin & Lane (2012). The estimated number of red algal parasite species has more than doubled since Setchell's (1918) initial list of about 50 species to over a 100 species (Goff 1982; Salomaki & Lane 2014), 116 species (Blouin & Lane 2012) or 121 species (Blouin & Lane 2016).

The aim of this study was to create a comprehensive list of red algal parasite species, with associated primary literature, as such a list has not been produced for over 30 years. This paper 1) summarises the current understanding of the diversity of red algal parasites, and, 2) provides an analysis of whether existing descriptions meet the criteria for defining parasites. This synopsis highlights the lack of documentation available for many parasite species, as well as the scarcity of data about many key parasite characters and processes (i.e. host cell 'control', parasite origin), which may alter our notions of parasite biology, and suggest areas for future targeted research.

2.3 Materials and Methods

This list of red algal parasite species was created by examining recent literature and classifications, reviewing red algal classification for parasitic genera and consulting AlgaeBase (Schneider & Wynne 2007; Wynne & Schneider 2010; Schneider & Wynne 2013; Guiry & Guiry 2016). Over 200 papers in ten different languages were located, and the species were categorised, and tabulating criteria (i.e. level of pigmentation, reduced thallus, presence of secondary pit connections, penetration of host tissue, and descriptions of all life cycle stages) were used in defining red algal parasites. The number of these criteria that were met was then used to rank (e.g., all criteria met; only one criterion met) whether there are sufficient data on the described organism to meet the definition of a red algal parasite.

2.4 Results

Our list contains 120 species and two invalidly described species (Appendix 2.1, Fig. 2.1). Appendix 2.1 combines all available information on red algal parasite species and is organised in systematic order based on the presumed taxonomy of the parasite species. The entries include general information such as host species, year of description, type locality (based on primary literature), and distribution, and whether the criteria used to determine parasitic status (Goff 1982; Wynne & Scott 1989) were described. Recognized parasites are listed alphabetically in Table 2.2.

Many red algal parasites were described by Setchell (1914; 1923) and Pocock (1953; 1956). Based on the dates listed in Table 2.2, approximately 15% of all red algal parasites were described in the 19th century, 80% in the 20th century and 5% in the 21st century.

Red algal parasites are found in a number of families within the Florideophyceae. Parasitic genera are often small containing 1-4 species. The Pterocladiophilaceae is the only family containing solely parasitic genera (*Gelidiocolax*, *Holmsella* and *Pterocladiophila*).

Table 2.1. Alphabetical list of original name of species misidentified as red algal parasites, with changed name (if applicable), and current understanding of the described structure.

Original name of species	Changed name	Current understanding	Reference
Actinococcus aggregatus F.Schmitz	*Gymnogongrus griffithsiae* (Turner) Mart.	Nemathecium	Gregory 1930
Actinococcus chiton M.Howe	*Fredericqia chiton* (M.Howe) Maggs, LeGall, Mineur, Provan *et* G.W.Saunders	Nemathecium	McCandless & Vollmer 1984
Actinococcus latior F.Schmitz	*Gymnogongrus dilatatus* (Turner) J.Agardh	Tetrasporangial outgrowth	Silva *et al.* 1996
Actinococcus peltaeformis F.Schmitz	*Gymnogongrus crenulatus* (Turner) J.Agardh	Nemathecium	McCandless & Vollmer 1984
Actinococcus subcutaneus (Lyngb.) Rosenv.	*Coccotylus trunacates* (Pall.) M.J.Wynne *et* J.N.Heine	Carpotetrasporangial outgrowth	Dixon & Irvine 1995
Callilithophytum parcum (Setch. *et* Foslie) P.W.Gabrielson, W.H.Adey, G.P.Johnson *et* Hernández-Kantún	-	Epiphyte	Adey *et al.* 2015
Catenellocolax leeuwenii Weber Bosse	-	Fungal infection	Zuccarello 2008
Choreocolax cystoclonii Kylin	-	Bacterial infection	Dixon & Irvine 1995
Choreocolax delesseriae Reinsch	*Neuroglossum delesseriae* (Reinsch) M.J.Wynne	Early stages in lateral branch formation	Wynne 2013
Colacolepsis decipiens F.Schmitz	*Phyllophora herediae* (Clem.-Munoz) J.Agardh	Nemathecium	Goff 1982
Colacolepis incrustans F.Schmitz	*Phyllophora crispa* (W.E.Hudson) P.S.Dixon	Cystocarpic outgrowth	Dixon & Irvine 1995
Entocolax rhodymeniae Reinsch	-	Fungal infection	Edelstein 1972
Erythrocystis saccata (J.Agardh) P.C.Silva	-	Epiphyte	Melchionna & De Masi 1977
Fosliella paschalis (Me.Lemoine) Setch. *et* N.L.Gardner	-	Epiphyte	Setchell & Gardner 1930
Lobocolax deformans M.Howe	-	Bacterial infection	McBride *et al.* 1974; Ashen & Goff 1998

Loranthophycus californicus (E.Y.Dawson) E.Y.Dawson[1]	*Loranthophycus californicus* E.Y.Dawson	Tetrasporophytic outgrowth	Dawson 1945; Goff 1982; Wynne 2013
Neopolyporolithon reclinatum (Foslie) W.H.Adey *et* G.P.Johansen	-	Epiphyte	Adey *et al.* 2015
Phaeocolax kajimurae Hollenb.	-	Epiphyte	Apt 1984a
Pleurostichidium falkenbergii Heydr.	-	Epiphyte	Phillips 2000
Rhodymeniocolax austrina	*Halopeltis austrina* (Womersley) G.W.Saunders	Epiphyte	Saunders & McDonald 2010
Sterrocolax decipiens F.Schmitz	*Ahnfeltia plicata* (W.E.Hudson) Fr.	Gametangial outgrowth	Dixon & Irvine 1995

[1]described as an outgrowth on *Holmesia californica* (Dawson) Dawson

Approximately 60% of red algal parasites are known from only one host species, 30% have been reported on two or three host species, and only 10% on more than three host species (Fig 2.1). Four genera, *Gracilaria* (Gracilariaceae), *Gelidium* (Gelidiaceae), *Laurencia* and *Polysiphonia* (Rhodomelaceae), are the most common hosts of red algal parasites (Appendix 2.1). Surprisingly, approximately 54% of the parasites found on two or more hosts have host species from different genera (Appendix 2.1). For example, *Choreocolax polysiphoniae* Reinsch has been reported from *Cystoclonium purpureum* (W.E.Hudson) Batters, *Neosiphonia confusa* (Hollenb.) J.N.Norris, and *Vertebrata lanosa* (L.)T.R.Chr.

Table 2.2. Alphabetical list of red algal parasites, year of publication, and family to which they belong. For species authorities, refer to Appendix 2.1.

Parasite	Year	Family	Reference
Aiolocolax pulchella	1956	Rhodomelaceae	[1]
Antarctocolax lambii	1953	Rhodomelaceae	[2]
Apoglossocolax pusilla	1993	Delesseriaceae	[3]
Asterocolax denticulatus	1934	Delesseriaceae	[4,5]
Asterocolax erythroglossi	1951	Delesseriaceae	[5]
Asterocolax gardneri	1923	Delesseriaceae	[5,6]
Asterocolax hypophyllophilus	1970	Delesseriaceae	[7]
Benzaitenia yenoshimensis	1913	Rhodomelaceae	[8]
Bostrychiocolax australis	1994	Rhodomelaceae	[9]
Callocolax acicularis	1992	Kallymeniaceae	[10]
Callocolax fungiformis	1925	Kallymeniaceae	[11]
Callocolax japonica	-	Kallymeniaceae	[12]
Callocolax neglectus	1895	Kallymeniaceae	[13]
Centrocerocolax ubatubensis	1965	Ceramiaceae	[14]
Chamaethamnion pocockiae	1988	Rhodomelaceae	[15]
Chamaethamnion schizandra	1897	Rhodomelaceae	[16]
Champiocolax lobatus	1996	Champiaceae	[17]
Champiocolax sarae	1985	Champiaceae	[18]
Choreocolax americanus	1875	Rhodomelaceae	[19]
Choreocolax destructor	1875	Rhodomelaceae	[19]
Choreocolax polysiphoniae	1875	Rhodomelaceae	[19]
Choreocolax rabenhorstii	1875	Rhodomelaceae	[19]
Choreocolax rhodymeniae	1888	Rhodomelaceae	[20]
Choreocolax tumidus	1875	Rhodomelaceae	[19]
Choreonema thuretii	1889	Hapalidiaceaeae	[21]
Coccotylus hartzii	1898	Phyllophoraceae	[22,23]

Colacodasya australica	1998	Dasyaceae	[17]
Colacodasya californica	1970	Dasyaceae	[24]
Colacodasya inconspicua	1888	Dasyaceae	[8,20]
Colacopsis lophurellae	1919	Rhodomelaceae	[25]
Colacopsis pulvinata	1897	Rhodomelaceae	[8]
Colacopsis smitheniae	1988	Rhodomelaceae	[15]
Colacopsis velutina	1953	Rhodomelaceae	[15,26]
Dawsoniocolax bostrychiae	1967	Rhodomelaceae	[27]
Dipterocolax fernandezianus	1977	Rhodomelaceae	[28]
Episporium centroceratis	1885	Ceramiaceae	[21]
Epulo multipedes	2004	Hapalidiaceaeae	[29]
Ezo epiyessoense	1974	Corallinaceae	[30]
Faucheocolax attenuata	1923	Faucheaceae	[6]
Gardneriella tuberifera	1941	Solieriaceae	[8]
Gelidiocolax christianae	1963	Pterocladiophilaceae	[31]
Gelidiocolax deformans	1982	Pterocladiophilaceae	[32]
Gelidiocolax desikacharyi	1970	Pterocladiophilaceae	[33]
Gelidiocolax lyndae	1988	Pterocladiophilaceae	[15]
Gelidiocolax mammillatus	1959	Pterocladiophilaceae	[34]
Gelidiocolax margaritoides	1953	Pterocladiophilaceae	[34,35]
Gelidiocolax microsphaericus	1927	Pterocladiophilaceae	[8]
Gelidiocolax pustulatus	1984	Pterocladiophilaceae	[36]
Gelidiocolax suhriae	1953	Pterocladiophilaceae	[34,35]
Gelidiocolax verruculatus	-	Pterocladiophilaceae	[37]
Gloiocolax novae-zelandiae	1957	Faucheaceae	[38]
Gonimocolax australis	1919	Delesseriaceae	[8,25]
Gonimocolax corymbosus	1941	Delesseriaceae	[39]
Gonimocolax roscoffensis	1961	Delesseriaceae	[40]
Gonimophyllum africanum	1953	Delesseriaceae	[35]
Gonimophyllum buffhamii	1892	Delesseriaceae	[41]
Gonimophyllum insulare	1954	Delesseriaceae	[42]
Gonimophyllum skottsbergii	1923	Delesseriaceae	[6]
Gracilaria babae	1986	Gracilariaceae	[43,44]
Gracilariocolax deformans	1928	*Incertae sedis*	[45,46]
Gracilariocolax henriettae	1928	*Incertae sedis*	[45]
Gracilariocolax infidelis	1928	*Incertae sedis*	[45,46]
Gracilariocolax setchellii	1928	*Incertae sedis*	[45,46]
Gracilariocolax setchellii var. aggregata	1928	*Incertae sedis*	[45,46]
Gracilariocolax sibogae	1928	*Incertae sedis*	[45,46]
Gracilariophila oryzoides	1910	Gracilariaceae	[47]

Gracilariophila gardneri	1923	Gracilariaceae	[6]
Grateloupiocolax colombiana	1983	Halymeniaceae	[48]
Harveyella mirabilis	1875	Rhodomelaceae	[8,19]
Holmsella pachyderma	1875	Pterocladiophilaceae	[49]
Holmsella australis	1983	Pterocladiophilaceae	[50]
Hypneocolax stellaris	1920	Cystocloniaceae	[51]
Hypneocolax stellaris f. orientalis	1928	Cystocloniaceae	[52]
Janczewskia gardneri	1914	Rhodomelaceae	[53]
Janczewskia hawaiiana	1987	Rhodomelaceae	[54]
Janczewskia lappacea	1914	Rhodomelaceae	[53]
Janczewskia meridionalis	1953	Rhodomelaceae	[35]
Janczewskia moriformis	1914	Rhodomelaceae	[53]
Janczewskia morimotoi	1947	Rhodomelaceae	[55]
Janczewskia ramiformis	1978	Rhodomelaceae	[56]
Janczewskia solmsii	1914	Rhodomelaceae	[53]
Janczewskia tasmanica	1897	Rhodomelaceae	[57]
Janczewskia teysmannii	1923	Rhodomelaceae	[58]
Janczewskia verruciformis	1877	Rhodomelaceae	[53]
Jantinella sinicola	1924	Rhodomelaceae	[59,60]
Jantinella verruciformis	1911	Rhodomelaceae	[61,62]
Kintokiocolax aggregato-ceranthus	1960	Halymeniaceae	[63]
Kvaleya epilaeve	1971	Hapalidiaceaeae	[64]
Laurenciocolax polysporus	1964	Rhodomelaceae	[65]
Leachiella pacifica	1982	Rhodomelaceae	[66]
Levringiella gardneri	1923	Rhodomelaceae	[6,8]
Levringiella microscopica	1941	Rhodomelaceae	[8,67]
Masakiella bossiellae	2007	Corallinaceae	[68]
Meridiocolax bracteata	1983	Rhodomelaceae	[50]
Meridiocolax narcissus	1976	Rhodomelaceae	[69]
Meridiocolax polysiphoniae	1973	Rhodomelaceae	[50,70]
Microcolax africanus	1953	Rhodomelaceae	[35]
Microcolax botryocarpa	1845	Rhodomelaceae	[16,71]
Neohalosacciocolax aleutica	1978	Palmariaceae	[72]
Neotenophycus ichthyosteus	2002	Rhodomelaceae	[73]
Onychocolax polysiphoniae	1956	Rhodomelaceae	[1]
Phitycolax inconspicua	1989	Delesseriaceae	[74]
Plocamiocolax pulvinata	1923	Plocamiaceae	[6]
Plocamiocolax papenfussianus	1953	Plocamiaceae	[35]
Polycoryne compacta	1963	Delesseriaceae	[75]
Polycoryne radiata	1919	Delesseriaceae	[25]
Pterocladiophila hemisphaerica	1959	Pterocladiophilaceae	[34]

Rhodophyllis parasitica	2014	Cystocloniaceae	[76]
Rhodophysema kjellmanii	1959	Palmariaceae	[77,78]
Rhodymeniocolax botryoideus	1923	Rhodymeniaceae	[6]
Rhodymeniocolax mediterraneus	2005	Rhodymeniaceae	[79]
Scagelonema parasiticum	1969	*Incertae sedis*	[80,81]
Sorellocolax stellaris	1996	Delesseriaceae	[82]
Sporoglossum lophurellae	1919	Rhodomelaceae	[25]
Spyridiocolax capixabus	1966	Ceramiaceae	[83]
Stromatocarpus parasiticus	1897	Rhodomelaceae	[16]
Symphyocolax koreana	2010	Rhodomelaceae	[84]
Syringocolax macroblepharis	1875	Ceramiaceae	[19]
Tikvahiella candida	1983	Solieriaceae	[85]
Trichidium pedicellatum	1983	Rhodomelaceae	[50]
Tylocolax microcarpus	1897	Rhodomelaceae	[16]
Ululania stellata	1998	Rhodomelaceae	[86]

References: [1] Pocock 1956; [2] Skottsberg 1953; [3] Maggs & Hommersand 1993; [4] Tokida 1934; [5] Wynne 2013; [6] Setchell 1923; [7] Wynne 1970; [8] Kylin 1956; [9] Zuccarello & West 1994a; [10] Wynne & Heine 1992; [11] Abbott & Hollenberg 1992; [12] Goff 1982; [13] Batters 1895; [14] Joly 1966; [15] Norris 1988; [16] Schmitz & Falkenberg 1897; [17] Womersley 1998; [18] Bula-Meyer 1985; [19] Reinsch 1875; [20] Reinsch 1890; [21] Womersley 1996; [22] Rosenvinge 1931; [23] Le Gall & Saunders 2010; [24] Hollenberg 1970; [25] Kylin & Skottsberg 1919; [26] Pocock 1953; [27] Joly & Yamaguishi-Tomita 1969; [28] Morrill 1977; [29] Townsend & Huisman 2004; [30] Adey *et al.* 1974; [31] Feldmann & Feldmann 1963; [32] Seoane-Camba 1982; [33] Ganesan 1970; [34] Fan & Papenfuss 1959; [35] Martin & Pocock 1953; [36] Yoneshigue & de Oliveira 1984; [37] Ouahi 1993; [38] Sparling 1957; [39] Baardseth 1941; [40] Feldmann & Feldmann 1961; [41] Batters 1892; [42] Wagner 1954; [43] Yamamoto 1986; [44] Ng *et al.* 2014; [45] Weber-van Bosse 1928; [46] Gerung & Yamamoto 2002; [47] Wilson 1990; [48] Schnetter *et al.* 1983; [49] Fredericq & Hommersand 1990; [50] Noble & Kraft 1983; [51] Børgesen 1920; [52] Womersley 1994; [53] Setchell 1914; [54] Apt 1987; [55] Tokida 1947; [56] Chang & Xia 1978; [57] Womersley 2003; [58] Weber-van Bosse 1923; [59] Setchell & Gardner 1924; [60] Kylin 1941; [61] McFadden 1911; [62] Morrill 1976b; [63] Tanaka & Nozawa 1960; [64] Adey & Sperapani 1971; [65] Zinova 1967; [66] Kugrens 1982; [67] Levring 1941; [68] Guiry & Selivanova 2007; [69] Morrill 1976c; [70] De Oliveira & Ugadim 1973; [71] Harvey & Hooker 1845; [72] Lee & Kurogi 1978; [73] Kraft & Abbott 2002; [74] Wynne & Scott 1989; [75] Zinova 1963; [76] Preuss & Zuccarello 2014; [77] Edelstein 1972; [78] Saunders & Clayden 2010; [79] Vergés *et al.* 2005; [80] Norris & Wynne 1969 '1968'; [81] Wynne & Schneider 2010; [82] Yoshida & Mikami 1996; [83] Joly & Oliveira 1966; [84] Kim & Cho 2010; [85] Kraft & Gabrielson 1983; [86] Apt & Schlech 1998.

Classifying red algal parasites according to their pigments (Appendix 2.1) reveals that 38% are pigmented, 25% are unpigmented, and 14% are described as having both unpigmented and pigmented stages, whereas no information is available on pigmentation for the remaining 23%. In most cases it is not possible to determine from the literature if this pigment variation is due to the parasites being on different host species or is a consequence of a developmental stage (i.e. early development, reproductive stage).

Parasite characters by species
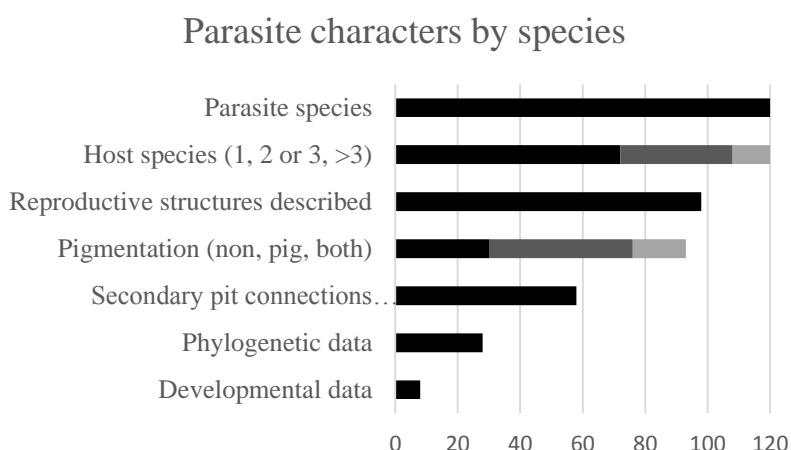
Fig. 2.1. Current knowledge of all 120 red algal parasite species. Host species: number of parasite species which infect one (black bar), two or three (grey bar), or more than three (light grey bar) host species. Reproductive structures described: number of species for which male/female gametophyte and tetrasporophyte described. Pigmentation: none (black), pigmented (grey), both unpigmented and pigmented stages (light grey). Secondary pit connections: number of species for which connections between parasite and host described. Phylogenetic and Developmental data: number of species with any phylogenetic or developmental data.

Based on an estimate of 861 genera of Florideophyceae (Schneider & Wynne 2007; 2013; Wynne & Schneider 2010; 2016), slightly over 7% of genera include parasitic species. Fewer than half of the species (approximately 45%) fulfil all the criteria used to define red algal parasites (Goff 1982; Wynne & Scott 1989). Approximately 45% of all descriptions of parasitic species do not mention secondary pit connections between parasite and host, which is a crucial criterion for establishing parasite status (Goff & Coleman 1985; Blouin & Lane 2012). Much of the missing data on secondary pit connections can be explained by the fact that many of these parasitic species were described before 1982, when this unusual developmental process was first highlighted (Goff 1982). A smaller percentage of species descriptions (approximately 10%) did not supply information on pigmentation or secondary pit connections, and lacked description of all reproductive structures (Appendix 2.1).

Table 2.3. List of red algal parasites (and host species) on which phylogenetic analyses have been conducted. * = parasites in which sequences are available from mitochondrial, nuclear and plastid genomes.

Parasite	Host	Reference
Aiolocolax pulchella	*Polysiphonia caespitosa*	Diaz-Tapia & Bárbara 2013
Asterocolax erythroglossi	*Erythroglossum laciniatum*	Goff *et al.* 1997
Asterocolax gardneri	*Anisocladella pacifica*	Goff *et al.* 1997
	Phycodrys isabelliae	Goff *et al.* 1997
	Phycodrys setchelli	Goff *et al.* 1997
	Nienburgia andersoniana	Goff *et al.* 1997
	Polyneura latissima	Goff et al. 1997
Benzaitenia yenoshimensis	*Chondria crassicaulis*	Kurihara *et al.* 2010
Bostrychiocolax australis	*Bostrychia radicans*	Zuccarello *et al.* 2004; Zuccarello & West 2006
Choreocolax polysiphoniae	*Vertebrata lanosa*	Zuccarello *et al.* 2004; Salomaki *et al.* 2015
Choreonema thuretii	*Jania micrarthrodia*	Harvey *et al.* 2003
Coccotylus hartzii	-	Le Gall & Saunders 2010
Dawsoniocolax bostrychiae	*Bostrychia radicans*	Zuccarello *et al.* 2004
Faucheocolax attenuate	*Gloiocladia laciniata*	Goff *et al.* 1996
	Gloiocladia fryeana	Goff *et al.* 1996
Gardneriella tuberifera	*Sarcodiotheca gaudichaudii*	Goff *et al.* 1996
Gonimophyllum skottsbergii	*Cryptopleura crispa*	Zuccarello *et al.* 2004
Gracilaria babae*	*Gracilaria salicornia*	Ng *et al.* 2013; Ng *et al.* 2014; Ng *et al.* 2015
	Hydropuntia sp.	Ng *et al.* 2014
Gracilariophila oryzoides	*Gracilariopsis lemaneiformis*	Goff & Zuccarello 1994; Goff *et al.* 1996; Hancock *et al.* 2010
Harveyella mirabilis	*Gonimophyllum skottsbergii*	Zuccarello *et al.* 2004
	Odonthalia floccosa	Zuccarello *et al.* 2004
	Odonthalia washingtoniensis	Zuccarello *et al.* 2004
	Rhodomela confervoides	Zuccarello *et al.* 2004
Holmsella pachyderma	*Gracilaria gracilis*	Zuccarello *et al.* 2004
	Gracilariopsis longissima	Zuccarello *et al.* 2004
Holmsella australis	*Gracilaria cliftonii*	Zuccarello *et al.* 2004
Hypneocolax stellaris f. orientalis	-	Sherwood *et al.* 2010
Janczewskia hawaiiana	*Laurencia mcdermidiae*	Kurihara *et al.* 2010
Janczewskia morimotoi*	*Laurencia nipponica*	Kurihara *et al.* 2010
Kintokiocolax aggregato-ceranthus	*Grateloupia angusta*	Yang & Kim 2015
Leachiella pacifica	*Neosiphonia paniculata*	Zuccarello *et al.* 2004
Plocamiocolax pulvinata	*Plocamium cartilagineum*	Goff *et al.* 1996
Rhodophyllis parasitica*	*Rhodophyllis membranacea*	Preuss & Zuccarello 2014

Rhodophysema kjellmanii	-	Clayden & Saunders 2010
Rhodymeniocolax botryoideus	*Rhodymenia pacifica*	Goff *et al.* 1996
Tikvahiella candida	*Solieria robusta*	Saunders *et al.* 2004
Ululania stellata	*Acanthophora pacifica*	Kurihara *et al.* 2010
	Acanthophora spicifera	Kurihara *et al.* 2010

The majority of type localities for red algal parasites are in the USA (26), South Africa (13) and Australia (11), and many type localities are on islands. The distribution data available are highly variable, ranging from records of single individuals and their host species to infrequent collections, and thus it is difficult to draw any conclusions about the distribution of most species.

Table 2.4. List of species of red algal parasites in which the parasite's development, parasite-host nuclear transfer and the fate of these parasite nuclei in the host heterokaryotic cell have been documented. Host transformation: + = changes observed in infected host cells, which can include: increased storage products in infected host cells, loss of host plastid fluorescence, host nuclear enlargement, infected host cell division. Parasite nuclear division: + = parasite nuclei known to divide in heterokaryotic host cell; - = parasite nuclei do not divide in heterokaryotic host cell; ? = data not available.

	Host transformation	Parasite nuclear division	Reference
Bostrychiocolax australis	+	-	Zuccarello & West 1994a
Leachiella pacifica	+	-	Goff & Coleman 1985;
(as *Choreocolax polysiphoniae*)			Zuccarello *et al.* 2004
Dawsoniocolax bostrychiae	+	-	Zuccarello & West 1994a
Gardneriella tuberifera	+	+	Goff & Zuccarello 1994
Gracilariophila oryzoides	+	+	Goff & Zuccarello 1994
Harveyella mirabilis	+	?	Goff 1976
Janczewskia gardneri	+	+	Goff & Coleman 1987
Janczewskia morimotoi	+	?	Nonomura 1979

There is limited knowledge of the phylogenetic relationships of red algal parasites. Phylogenetic sequences are available for only 27% of all red algal parasites (Table 2.3) and, in many cases, all their hosts have not been sequenced. Data from all three genomes (mitochondria, nuclear and plastid) are only available for a small percentage of parasites (2.5%; Table 2.3).

Only eight red algal parasites have been investigated with reference to host cell transformation, and in only three species are the nuclei known to divide after transfer into the host cell (Table 2.4).

2.5 Discussion

Although it has been stated that red algal parasites have evolved independently over a hundred times (Blouin & Lane 2012), this is based on the current morphological taxonomy rather than on phylogenetic analyses. The origin (i.e. taxonomy) of parasites is complicated by their reduced thalli and consequent lack of diagnostic morphological characters, leading to diversity being underreported (Zuccarello & West 1994a). The ability of many parasites to switch hosts and infect multiple hosts, and the propensity of phycologists to name parasites based on hosts, further complicate the interpretation of their phylogenetic origin (Goff *et al.* 1996; 1997), and have led to multiple names for some taxa that are found on multiple hosts (Zuccarello & West 1994a). An example is *Asterocolax gardneri* (Setch.) Feldmann *et* Feldm.-Maz., where phylogenetic results indicate that the species has three independent origins from *Phycodrys setchellii* Skottsb., *Phycodrys isabelliae* R.E.Norris *et* M.J.Wynne and *Polyneura latissima* (Harv.) Kylin (i.e. a polyphyletic *A. gardneri* (Goff *et al.* 1997)). Without further information on their phylogeny or more detailed morphological investigations, the origins of parasites and their true diversity remain to be uncovered. The few phylogenetic studies have revealed parasites that are nested within their host genera, requiring taxonomic changes which may involve parasites losing their distinct generic status to maintain monophyly of the host genus (Ng *et al.* 2014; Preuss & Zuccarello 2014).

Characters used to define the parasitic mode in red algae differ in their utility. Both size and pigmentation are not definitive because small epiphytes do exist, and reproductive structures in some red algae can have lighter pigmentation. The criteria that we feel are most useful are the cell-cell secondary pit connections between parasites and host cells, and finding all life history stages of parasites on the same host plant, which reduces the chance of mistaking host outgrowths as parasites.

Our summary highlights that our understanding of the parasitic process in these unique organisms is based on only a small handful of species that have been studied intensively (e.g., *Leachiella pacifica* Kugrens). Red algal parasites have been intriguing for scientists since the first reports of nuclear, and organelle, transfer between parasites and hosts (Goff 1982; Goff & Coleman 1985). This a unique phenomenon in eukaryotic parasitism, although in some non-parasitic florideophyte lineages there is nuclear transfer during carposporophyte development (Kugrens & Delivopoulos 1985; Delivopoulos & Diannelidis 1990). Summaries of these processes (early parasite development, host cell 'control') have been presented (Salomaki & Lane 2014) but generalizations about these processes are based on very few examples, and more data may show that different, and novel, infection mechanisms exist.

The classification of red algal parasites as parasites is rarely discussed but there is evidence that parasites alter hosts and many have detrimental effects on their hosts. This evidence includes: degradative changes in infected host cells include plasmolysis and hypertrophy (Goff 1982), host cell death (Goff 1976), breakdown of host nuclei and plastids (Goff 1982), and reduction in host growth (Apt 1984b). Another negative effect for the host is the loss of cell cycle regulation, shown by rapid division of plastids, nuclei and host cells (Goff 1976; Goff & Coleman 1985), and the infection spreading to surrounding host cells (Goff & Coleman 1995). Few studies have analyzed the effects of parasite infection on host fitness, and the results vary from negative effects on the host being either highly localized and minimal (Goff 1982) or appreciable (Martin & Pocock 1953). In contrast to its effects on the host, it is clear that the parasite depends on the host for nutrients (Evans *et al.* 1973; Goff 1982), for a habitat due to their host specificity (Goff 1982), and for cell-cell interactions during early development (Zuccarello & West 1994b; c). The degree of parasitism (i.e. damage to the host) may therefore vary among host species but further investigations are needed for a better understanding of parasite-host relationships.

We have produced a comprehensive list of described parasites, and characterized the available knowledge about these parasites. It is clear that much information is still lacking. We hope that this list will focus research on poorly studied parasites, and thereby add information about their taxonomy, origins, early development, distribution and effects on host fitness, and will contribute to species discovery. Guiry (2012) estimated that only half of all red algae are described to date. To illustrate this point, many red algal genera and species in New Zealand are continuing to be described (Nelson *et al.* 2014; Boo *et al.* 2015; D'Archino *et al.* 2015; Nelson *et al.* 2015; D'Archino *et al.* 2016). Currently, there are ten red algal parasites known from New Zealand. In addition, several undescribed parasitic species have been included in compilations of the flora (Dalen & Nelson 2013). We hope that molecular studies, especially studies using molecular markers from all three genomes, will be stimulated by this study, and that further work will also investigate host switching and cell-cell relationships between parasites and hosts. The diversity of parasite development has been barely explored, and current hypotheses about developmental processes need to be tested. We hope that this synopsis will aid and inspire further work on these organisms.

Chapter Three

Three new red algal parasites from New Zealand: *Cladhymenia oblongifoliophila* sp. nov. (Rhodomelaceae), *Phycodrys novae-zelandiophila* sp. nov. (Delesseriacease) and *Judithia parasitica* sp. nov. (Kallymeniaceae)

3.1 Abstract

There are over 120 species of red algal parasites (Florideophyceae), but they are often overlooked due to their small size and patchy distribution. Red algal parasites have mostly been described as independent genera, but recent phylogenetic studies have shown that parasites are related to free-living relatives, often their hosts, and have been named in these genera to maintain monophyly. We investigated the morphology, distribution and phylogeny, using diverse molecular markers (mitochondrial, nuclear, plastid), of three new red algal parasites in New Zealand. We describe the parasites using morphological and anatomical observations, and estimated their distribution by surveying herbarium vouchers. Analyses of reproductive structures and molecular phylogenies indicate that the closest relative of the parasite *Phycodrys novae-zelandiophila* sp. nov. is its host, *P. novae-zelandiae*. Based on nuclear and mitochondrial markers, the closest relative of the parasite *Cladhymenia oblongifoliophila* sp. nov. is its host *C. oblongifolia*, but plastid markers group it with *C. lyallii*, suggesting that this species was a past host and the source of parasite plastids. The parasite *Judithia parasitica* sp. nov. groups with *Judithia delicatissima* but infects *Blastophyllis* spp., suggesting that this parasite evolved as a free-living or parasitic *Judithia* species and host switching may have occurred. This study adds to our knowledge of New Zealand red algal parasites and highlights contrasting patterns of host-parasite relationships.

3.2 Introduction

Red algal parasites, a poorly studied polyphyletic category with many unique features, are found exclusively on red algal species in eight orders within the Florideophyceae (Blouin & Lane 2016; Chapter 2). Approximately 120 species have been described world-wide but their diversity is probably severely underestimated due to their small size and patchy distribution (Chapter 2). Four key characters are used to identify red algae as parasitic: 1) reduced size, 2) lack of or reduced pigmentation, 3) formation of secondary pit connection between parasite and host cells, and 4) both gametophytic and sporophytic parasite life stages on the same host stage (Wynne & Scott 1989). In the past, similarities in reproductive structures were used to indicate a close taxonomic relationship ('adelphoparasites') or more distant relationship ('alloparasites') between parasite and host combinations (Goff 1982), whereas more recent phylogenetic data indicates a continuum of relatedness between hosts and parasites (e.g., Zuccarello *et al.* 2004; Blouin & Lane 2012).

The close relationship between most red algal parasites and their hosts led to a hypothesis that these parasites evolved directly from their hosts (Setchell 1918), consistent with the entomological concept known as "Emery's rule" (Emery 1909). Later molecular evidence supported Emery's rule (Goff *et al.* 1997) but also revealed varied phylogenetic relationships. Several studies showed that some parasites are more closely related to their hosts than the host is to other species in the same genus (Goff *et al.* 1997; Zuccarello *et al.* 2004; Preuss & Zuccarello 2014), while parasites with multiple hosts in different genera (e.g., *Harveyella mirabilis* (Reinsch) F.Schmitz *et* Reinke) have undergone host switching (Zuccarello *et al.* 2004; Kurihara *et al.* 2010). Previous studies indicated that the plastid was mobile between hosts and parasites with the parasite 'capturing' the host plastid (Goff & Coleman 1995; Goff *et al.* 1996), a phenomenon that was not seem with the mitochondria (Goff & Coleman 1995). This lead to varied relationships between hosts and parasite using plastid sequence data and could be used to indicate parasite origins and host switching. For example, parasites can have similar, occasionally nearly identical, plastid gene sequences to the host (e.g., *Rhodophyllis parasitica* M.Preuss *et* Zuccarello; Preuss & Zuccarello 2014), matching the relationships of the nuclear and mitochondrial markers, indicating a recent evolution from the host species. Parasites can have plastids more closely related to another species of host from the host they are found on (e.g., *Gracilaria babae* (H.Yamam.) P.K.Ng, P.E.Lim *et* Phang; Ng *et al.* 2014), indicating that the parasite acquired its plastids from a previous host. Recent studies have also

shown that parasites can have a highly reduced plastid genome relative to that of the host (e.g., *Choreocolax polysiphoniae* Reinsch; Salomaki e*t al.* 2015), possibly indicating a long history of parasitism.

Previously, newly described parasites were grouped into independent parasitic genera (e.g., Kraft & Abbott 2002; Townsend & Huisman 2004; Vérges *et al.* 2005; Kim & Cho 2010). Several phylogenetic studies have now shown that parasites and hosts are often closely related to each other, using nuclear and mitochondrial markers, and parasites have origins within the host genus, but still distinct parasite generic names were retained (e.g., Goff *et al.* 1996; Kurihara *et al.* 2010). Newer studies support a strictly monophyletic scheme reflecting the integration of the parasites into the host genus based on phylogenetic support (Ng *et al.* 2014; Preuss & Zuccarello 2014).

Of the 120 recognised red algal parasite species, 10 are currently known from New Zealand (Chapter 2). Five species were described from New Zealand and the others were recorded for New Zealand but described from other parts of the world. The five described parasites from New Zealand are: *Colacopsis lophurellae* Kylin*, Gloiocolax novae-zelandiae* Sparling, *Gonimophyllum insulare* F.S.Wagner, *Pterocladiophila hemisphaerica* K.C.Fan *et* Papenf., and *Rhodophyllis parasitica*. The five remaining species are: *Callocolax neglectus* F.Schmitz *et* Batters, *Choreonema thuretii* (Bornet) F.Schmitz, *Colacodasya inconspicua* (Reinsch) F.Schmitz, *Microcolax botryocarpa* (Hook.f. *et* Harv.) F.Schmitz, and *Sporoglossum lophurellae* Kylin. Molecular data are available only for *Rhodophyllis parasitica* (Preuss & Zuccarello 2014).

In this study, we describe three new red algal parasite species from New Zealand: one found on *Cladhymenia oblongifolia* Hook.f. & Harv., one on *Phycodrys novae-zelandiae* Showe M.Lin *et* W.A.Nelson; and one species found on both *Blastophyllis calliblepharoides* (J.Agardh) D'Archino *et* W.A.Nelson and *B. hombroniana* (Mont.) D'Archino *et* W.A.Nelson.

3.3 Materials and Methods

Samples were collected mostly as drift around New Zealand (Appendix 3.1). All specimens were pressed as herbarium vouchers, dried in silica gel or fixed in 2% glutaraldehyde in phosphate buffer (0.1 M, pH 6.8) in 50% seawater.

For anatomical observations, sections were either embedded in resin following Preuss & Zuccarello (2014) or hand sectioning with a razor blade. Sections were stained with 1% acidified aniline blue in either water or 50% KARO syrup (Englewood Cliffs, New Jersey, USA). Samples were examined using Olympus AX-70 and Olympus BX53 microscopes (Tokyo, Japan) with integrated cameras (Olympus DP-70, Olympus SC100) and images were captured using Olympus cellSens software.

DNA was extracted either using 5% Chelex following Zuccarello *et al.* (1999) or following a modified CTAB protocol (Zuccarello & Lokhorst 2005). Mitochondrial (*cox*1), nuclear (actin, LSU rDNA, SSU rDNA) and plastid (*rbc*L) markers were used for analysis (Appendix 3.2). PCR conditions for actin amplification were as follows: initial denaturation at 94°C for 5 min, followed by 9 cycle of 94°C / 55°C / 72°C for 1 min each, followed by 29 cycles of 94°C for 30 sec, 45°C and 72°C for 1 min and a final step at 72°C for 10 min. PCR conditions for all other genes were carried out with an initial denaturation at 94°C for 5 min, followed by 36 cycles of 94°C / 45°C /72°C for 1 min each and a final step at 72 °C for 5 min. Successful amplifications were purified using ExoSAP-IT following manufactures instructions (USB product; Affymetrix, Santa Clara, CA, USA) and commercially sequenced (Macrogen Inc., Seoul, Korea).

New sequences were assembled and edited in Geneious 8.0.5 (http://www.geneious.com, Kearse *et al.* 2012). GenBank sequences were added to the alignments following D'Archino *et al.* (2017) or using the closest BLAST search hits (Appendix 3.3). MAFFT alignments implemented in Geneious were used and modified by eye. Bayesian inference was performed with MrBayes v.3.2.5 (Ronquist & Huelsenbeck 2003). Analyses consisted of two independent simultaneous runs of one cold and three incrementally heated chains, and 3×10^6 generations with sampling every 1000 generations. A "burn-in" of 5×10^5 generations was used and 25000 trees were saved to make the consensus tree. RAxML 7.2.8 (Stamatakis 2006) was used to construct maximum-likelihood trees (ML) to show the most likely tree from the data set.

RAxML was performed using the GTR+gamma model and 500 non-parametric bootstrap replicates (Felsenstein 1985). RAxML and Bayesian inference was performed with all three codons partitioned for *cox*1 and *rbc*L. Phylogenies of *cox*1, LSU and *rbc*L sequences of the parasite growing on *Blastophyllis* spp., and of *cox*1 and LSU sequences of the parasite growing on *Cladhymenia* sp. were congruent (Appendices 3.4-3.8) and the data sets were concatenated (with partition for LSU and partitioned codons for *cox*1 and *rbc*L) for a more robust phylogeny.

All alignments of the *Phycodrys* parasite and its host were analyzed for genetic diversity using TCS statistical parsimony networks (Clement *et al.* 2000) in PopArt 1.7 (http://popart.otago.ac.nz). *Phycodrys adamsiae* Showe M.Lin *et* W.A.Nelson was used as comparison of interspecific variation within *Phycodrys*. Unique sequences were deposited in GenBank (MF319122-MF319182).

Herbarium specimens of *Blastophyllis calliblepharoides*, *B. hombroniana*, *Cladhymenia oblongifolia* and *Phycodrys novae-zelandiae* at the Museum of New Zealand Te Papa Tongarewa (WELT vouchers) in Wellington were searched for parasites and observed parasites listed.

3.4 Results

Three unrecorded parasites were found throughout New Zealand with the exception of the host species *Blastophyllis hombroniana* (as *Callophyllis hombroniana*) from which previously a parasite was recorded (Cotton 1907).

3.4.1 Parasite on *Phycodrys novae-zealandiae*

All genetic markers showed the same pattern, and indicated a very close relationship between *Phycodrys novae-zelandiae* and its parasite.

Partial *cox*1 sequences (623 bp) were obtained for six samples of *Phycodrys novae-zelandiae* and three of its parasite. Genetic distances within *P. novae-zelandiae* ranged between 0.16-0.64% (1-4 bp), and between the parasite and host 0.0-1.12% (0-9 bp) and up to 0.8% (5 bp) between parasite specimens. Four haplotypes were found: C1-C4. Five hosts and two

parasites had Haplotype C3 while C1 and C2 were represented in one host specimen each. C4 was represented in one parasite specimen (Fig. 3.1A).
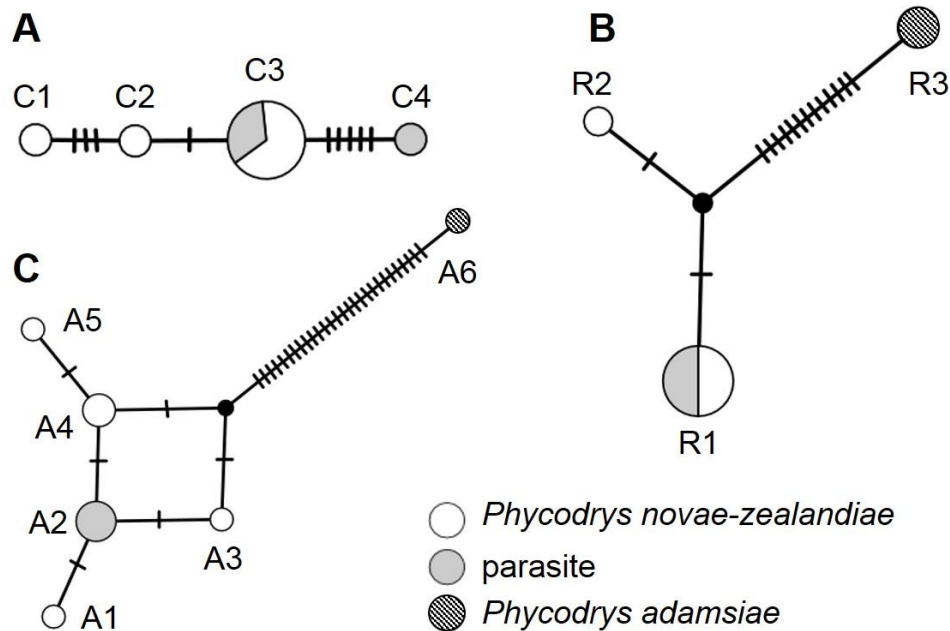
Figs 3.1A-C. DNA sequence networks of *Phycodrys novae-zelandiae*, its parasite *P. novae-zealandiophila* and *P. adamsiae*. **Fig. 3.1A.** *Cox*1 haplotype network with four different haplotypes (C1-C4). **Fig. 3.1B.** *Rbc*L haplotype network with three different haplotypes (R1-R3). **Fig. 3.1C.** Actin haplotype network with six haplotypes (A1-A6). Small dark circle represents missing intermediates, lines = one mutational step. Parasite, host and *P. adamsiae* haplotypes highlighted in white, gray and hatched, respectively.

Partial *rbc*L sequences of 530 bp were obtained from *Phycodrys novae-zelandiae* (n = 4), its parasite (n = 3) and *Phycodrys adamsiae* (n = 2). Three haplotypes were found: R1-R3. Three hosts and three parasites had haplotype R1 while R2 was represented in one host specimen and R3 in two specimens of *Phycodrys adamsiae* (Fig. 3.1B).

Actin sequences of 638 bp were obtained from *P. novae-zelandiae* (n = 4), its parasite (n = 3) and *P. adamsiae* (n = 2). Six haplotypes were found: A1-A6. All three parasites had haplotype A2, while A1, A3 A4, and A5 was represented in one host sample each, and A6 in two specimens of *Phycodrys adamsiae* (Fig. 3.1C).

The partial SSU alignment (827 bp) for *P. novae-zelandiae* (n = 3) and its parasite (n = 3) showed that all sequences of host and parasite were identical (data not shown).

The molecular data of the parasite and its host, *P. novae-zealandiae* showed the same pattern of low or no variation for all four genes from different genomes and demonstrate that the parasite is closely related to its host. This new parasite belongs in the genus *Phycodrys*.

Phycodrys novae-zelandiophila M.Preuss *et* Zuccarello *sp. nov.*
Figs 3.2A-I

DIAGNOSIS: Thalli lightly pigmented (pale red), size 1-2 mm across, with multiple simple branches. Dioecious gametophytes. Carposporophyte 430-530 µm in diameter, surrounded by a pericarp, with rows of carposporangia. Spermatangia unknown. Tetrasporangia 40 µm long x 32 µm wide, tetrahedrally divided, scattered on surface of stichidial branches. Parasitic on *Phycodrys novae-zelandiae* Showe M.Lin *et* W.A.Nelson.

HOLOTYPE: WELT A033494, collected 27 November 2015, deposited in the Museum of New Zealand Te Papa Tongarewa.

GENBANK ACCESSION NUMBERS: *cox*1: MF319155, MF319157; *rbc*L: MF319166; actin: MF319160; SSU: MF319164.

ISOTYPE: WELT A033494, collected 27 November 2015, deposited in the Museum of New Zealand Te Papa Tongarewa.

TYPE LOCALITY: 41°43.667'S, 174°12.917'E; drift, Marfells Beach, South Island, New Zealand.

ETYMOLOGY: *novae-zelandiophila* refers to the parasite's affinity to its host *Phycodrys novae-zelandiae*.
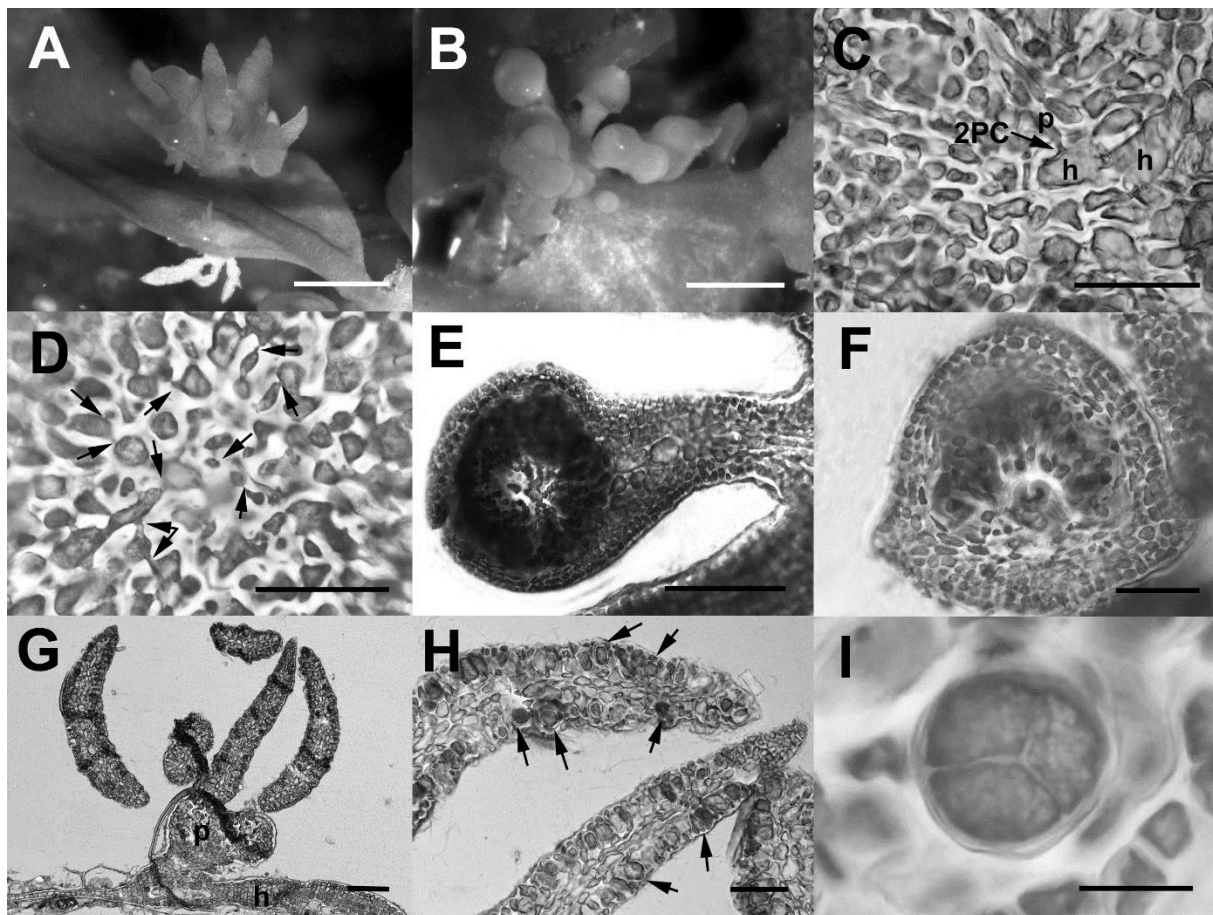
Figs 3.2A-I. Vegetative and reproductive structures of *Phycodrys novae-zealandiophila* sp. nov. on its host *Phycodrys novae-zealandiae*. **Fig. 3.2A.** Habit of a tetrasporophytic parasite growing on the central vein of its host. Scale bar = 1 mm. **Fig. 3.2B.** Habit of cystocarpic gametophyte growing on host thallus. Scale bar = 1 mm. **Fig. 3.2C.** Contact area between parasite and host. Parasite cell (p) forms secondary pit connection (2PC; arrow) with host cell (h). Scale bar = 100 µm. **Fig. 3.2D.** Parasite cells are highly connected with each other. Arrows indicate pit connections. Scale bar = 100 µm. **Fig. 3.2E.** Branch with mature cystocarp of parasite. Central fusion cell visible. Scale bar = 250 µm. **Fig. 3.2F.** Close-up of cystocarp of parasite, showing pericarp of approximately five cell layers. Scale bar = 100 µm. **Fig. 3.2G.** Cross section of parasite (p) tetrasporangial stichidia on its hosts (h). Scale bar = 200 µm. **Fig. 3.2H.** Tetraspores scattered on the surface of the tetrasporangial stichidia, tetrasporangia indicated by arrows. Scale bar = 100 µm. **Fig. 3.2I.** Mature tetrahedrally divided tetrasporangium. Scale bar = 20 µm.

DISTRIBUTION: The collection at Te Papa contained 52 specimens of *P. novae-zelandiae* of which red algal parasites were observed on nine. The parasite was found from Mataikona (40°47'S) on the North Island to Stewart Island (46°55'S), south of the South Island (Appendix 3.9).

Habitat and vegetative morphology

Phycodrys novae-zelandiophila grew on blades of *Phycodry novae-zelandiae* which had over 20 parasites on one blade, usually growing on the veins of the host (Fig. 3.2A). *P. novae-zelandiophila* was found in spring (September, November), summer (January, February) and autumn (March, April) (Appendices 3.1, 3.9).

The thallus was light red, 1-2 mm in size (Figs 3.2A-B). It had a single base that penetrated and disrupted the cell layers of the host. Host cells were embedded between parasite tissue in the contact area. Secondary pit connections were found between large host cells and smaller parasitic cells in the contact area (Fig. 3.2C). The cells within the main body of the parasite thallus were highly connected, by either primary or secondary pit connections (Fig. 3.2D).

Reproductive morphology

Female gametophytes and tetrasporophytes were observed. Thalli bear branches with either fusiform stichidia bearing tetraspores (Fig. 3.2A) or apical, rounded cystocarps (Fig. 3.2B). All observed parasites were reproductive, but males were not found, suggesting dioecious gametophytes.

The female gametophyte had a narrow, pulvinate base that gave rise to several unbranched axes, most of which terminate in an apical cystocarp. Branches were polystromatic with a central axis of large cells surrounded by up to five layers of smaller cells. The inner layer of elongated cortical cells were spherical near the mature carposporophyte (Fig. 3.2E). The mature carposporophytes were approximately 430-530 µm in diameter and surrounded by an approximately five cell thick pericarp (62 µm; n = 9). The carposporophyte had a single central fusion cell that gave rise to rows of gonimoblast filaments. Carpospores were born in short chains of approximately four ovoid carpospores (19 x 10 µm; Fig. 3.2F).

The tetrasporophyte grew from a rounded base approximately 500 µm in diameter. The base produced multiple simple fusiform branches that rarely branch again (Figs 3.2A, G). Branches had scattered stichidia on their surfaces. The stichidial branch was around 654 x 207 µm (n = 2) in size (Fig. 3.2G) with two to three inner layers of elongated cells and scattered globose tetrasporangia on the surface (Fig. 3.2H). Tetrasporangia were tetrahedrally divided approximately 32 x 40 µm (n = 10; Fig. 3.2I).

Comparision to host species and other parasites on congeneric species

The parasite shared carpospores borne in chains, from a central fusion cell and tetrahedrally divided tetraspores with its host species (*P. novae-zelandiae*) and two other *Phycodrys* species (*P. adamsiae*, *P. franiae* Showe M.Lin *et* W.A.Nelson), but differed in most other characters (Appendix 3.10). The new parasite was similar to other parasites (*Asterocolax denticulatus* (Tokida) Feldmann *et* Feldm.-Maz., *Asterocolax gardneri* (Setch.) Feldmann *et* Feldm.-Maz.) on *Phycodrys* spp., with similar thalli size and pigmentation, tetrahedrally divided tetraspores shattered over the surface, apical cystocarp that were born on branches. It differed in geographical distribution and host species (Appendix 3.11).

3.4.2 Parasite on *Cladhymenia oblongifolia*

The concatenated data set (1613 bp) of *cox*1 and LSU rRNA contained eight samples of two parasite samples and their hosts, an uninfected *C. oblongifolia* and two other *Cladhymenia* species (individual gene data sets were similar – Appendices 3.4, 3.5). This data set supported the shared origin of the parasite and its host *Cladhymenia oblongifolia* with strong support (Fig. 3.3). *Cladhymenia coronata* (Lindauer *et* Setch.) P.Saenger and *Cladhymenia lyalli* Harvey were distinct from *C. oblongifolia* and its parasite.

The partial *rbc*L data set (537 bp) contained taxa representative of all three *Cladhymenia* species in New Zealand. All samples of *C. oblongifolia* grouped with high support (Fig. 3.4). The parasite grouped with *C. lyalli* with high support, and not *C. oblongifolia* as with the previous markers, and both appeared to be sister to *C. coronata*, but this relationship was only supported in the ML analysis.
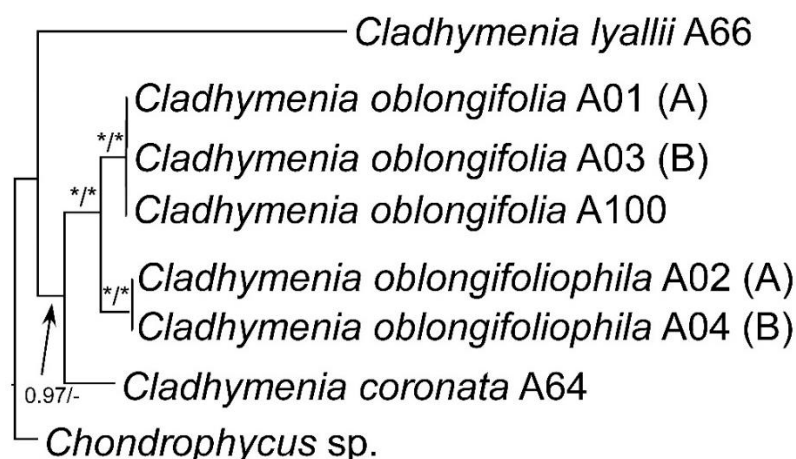
Fig. 3.3. Bayesian topology of concatenated *cox*1 and LSU rRNA sequence data set for *Cladhymenia oblongifolia*, its parasite *C. oblongifoliophila* and two other *Cladhymenia* species: *C. coronata* and *C. lyallii*. Host and parasite from the same host plant are highlighted by capital letters in brackets (A, B). Details of collections in Appendix 3.1. Asterisks indicate posterior probability of 1.00 and bootstrap values of 100%. Values < 85% ML bootstrap not shown. Outgroup was *Chondrophycus* sp.

The phylogenetic data of the parasite growing on *C. oblongifolia* showed two different patterns. Mitochondrial (*cox*1) and nuclei (LSU rRNA) data showed a shared ancestry of the parasite and host. The plastid marker showed a common ancestor between the parasite plastid and plastids of *C. lyallii*.
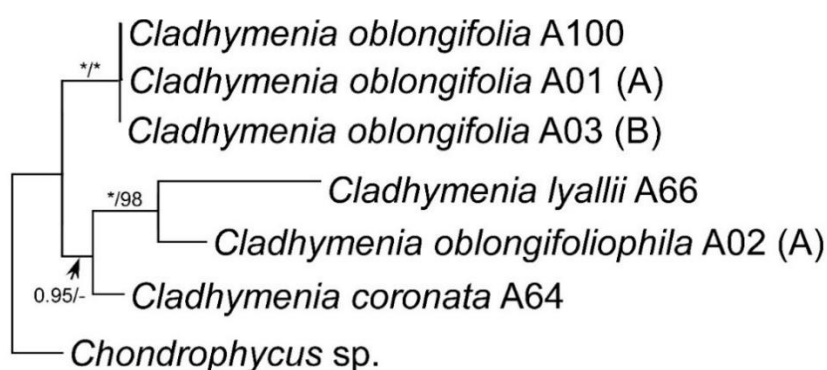
Fig. 3.4. Bayesian topology of *rbc*L relationships for *Cladhymenia oblongifolia*, its parasite *C. oblongifoliophila* and two other *Cladhymenia* species: *C. coronata* and *C. lyallii*. Parasite and host combination is highlighted by capital letter in bracket (A). Details of collections in Appendix 3.1. Asterisks indicate posterior probability of 1.00 and bootstrap value of 100%. Values < 85% ML bootstrap not shown. Outgroup used was *Chondrophycus* sp.

Our phylogenetic data, plus no records of parasites on *Cladhymenia*, indicated that this parasite is new and belongs within the genus *Cladhymenia*. It is described here as a new species.

Cladhymenia oblongifoliophila M.Preuss *et* Zuccarello *sp. nov.*
Figs 3.5A-I

DIAGNOSIS: Thalli unpigmented, 2 mm across, with either smooth spheres or one roundish cushion. Dioecious gametophytes. Carposporophyte approximately 520-570 µm, surrounded by a pericarp, no ostiole. Carposporangia, 55-100 x 14-24 µm, long and clavate to lachrymiform. Spermatangia unknown. Tetrasporangia 45-55 µm across, tetrahedrally divided, formed in branches. Parasitic on *Cladhymenia oblongifolia.*

HOLOTYPE: WELT A033496, collected 21 September 2015, deposited in Museum of New Zealand Tongarewa (Te Papa).

GENBANK ACCESSION NUMBERS: *cox*1: MF319141; LSU: MF319145; *rbc*L: MF319151.

ISOTYPE: WELT A033496, collected 21 September 2015, deposited in Museum of New Zealand Tongarewa (Te Papa).

TYPE LOCALITY: 41°43.667'S, 174°12.917'E.; drift; Marfells Beach, South Island, New Zealand.

ETYMOLOGY: *oblongifoliophila* refers to the preference of the parasite to grow on *Cladhymenia oblongifolia*.

DISTRIBUTION: Te Papa herbarium collections contained 91 specimens of *Cladhymenia oblongifolia* and on eight of these parasites were observed (Appendix 3.12). The parasite was found from the north (36°57'S) to the south of the North Island (41°21'S) and on the Chatham Islands (latitude = 44°16'S). The parasite is not common and has a patchy distribution.
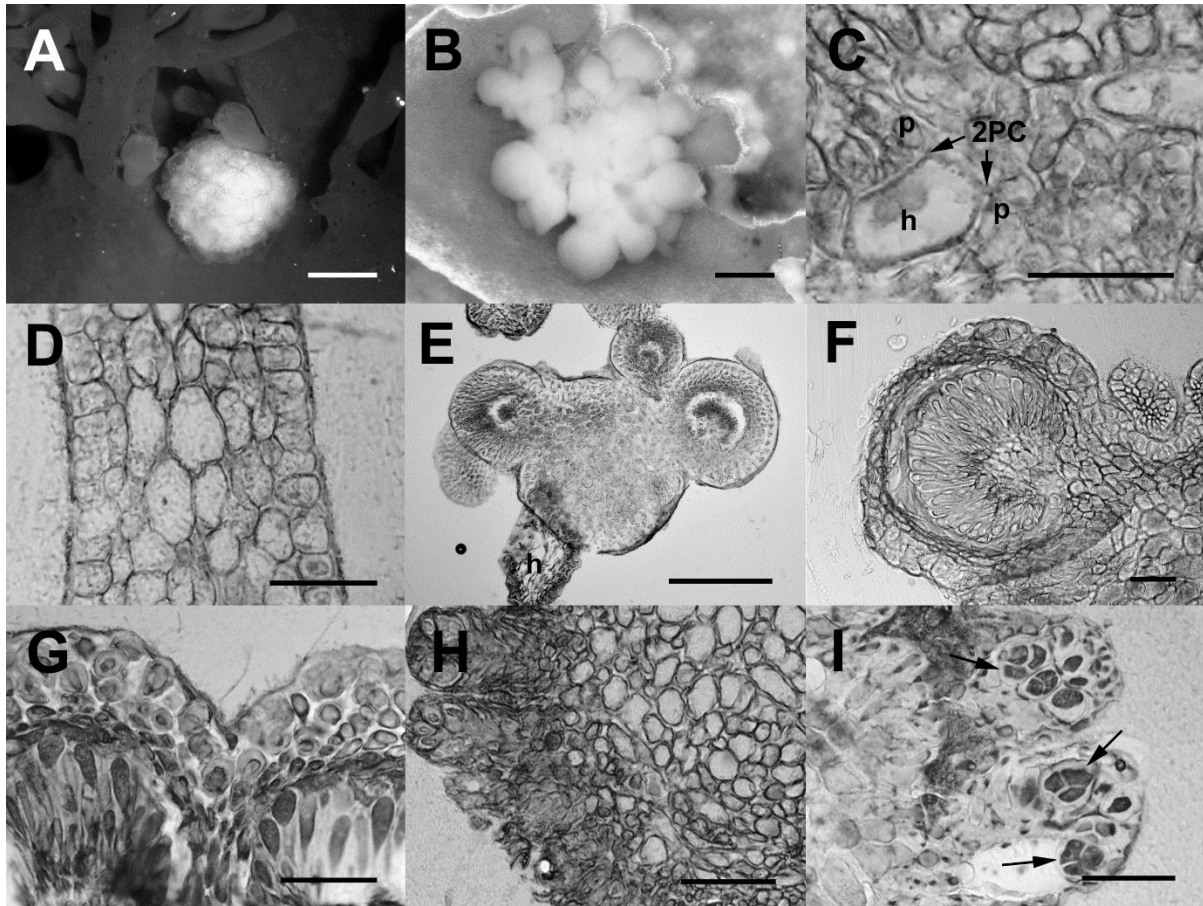
Figs 3.5A-I. Vegetative and reproductive structures of *Cladhymenia olongifoliophila* and its host *Cladhymenia oblongifolia*. **Fig. 3.5A.** Tetrasporophytic parasite thallus growing between the lateral proliferations of its host. Scale bar = 1 mm. **Fig. 3.5B.** Parasitic female gametophyte, with enlarged cystocarps, on host. Scale bar = 550 µm. **Fig. 3.5C.** Contact area between parasite and host cells. Parasite cell (p) forms secondary pit connection (2PC; arrow) with host cell (h). Scale bar = 100 µm. **Fig. 3.5D.** Internal anatomy of uninfected host, *C. oblongifolia*, not disrupted by parasite penetration and used as guide for distinguishing between parasite and host cells in the contact area. Scale bar = 100 µm. **Fig. 3.5E.** Cross section of female *C. oblongifoliophila* with multiple cystocarps. Scale bar = 500 µm. **Fig. 3.5F.** Close-up of cystocarp, showing thick ostiole-less pericarp and carposporophyte. Scale bar = 100 µm. **Fig. 3.5G.** Close-up of pericarp and elongated carpospores. Scale bar = 100 µm. **Fig. 3.5H.** Tetrasporophytic thallus, showing internal anatomy and cluster of tetrasporangia. Scale bar = 200 µm. **Fig. 3.5I.** Tetrasporic clusters with tetrahedrally divided tetraspores (arrows). Scale bar = 100 µm.

Habitat and vegetative morphology

One host plant had over 20 parasites growing on the blade edges and marginal proliferations (Fig. 3.5A). The parasite was found in spring (September, November), summer (January, February) and autumn months (March, April) in New Zealand (Appendices 3.1, 3.12).

The parasite thallus was not pigmented, approximately 2 mm in diameter (Fig. 3.5B). The base of the parasite penetrated deeply into the host thallus. Host and parasite cells were intermixed in the contact area. Secondary pit connections were found between small parasite cells and larger host cells in the contact area (Fig. 3.5C). The vegetative structure of the host *C. oblongifolia* consists of five inner layers of large cells, an outer layer of smaller epidermal cells and a cuticle (Fig. 3.5D).

Reproductive morphology

Female gametophytes and tetrasporophytes were observed. Thalli bear either one rough roundish cushion (tetrasporophyte; Fig. 3.5A) or many smooth spheres of different size (female gametophyte; Fig. 3.5B). All observed parasites were reproductive, but males were not found. Female gametophytes were found on tetrasporophytic host plants.

Mature female gametophytes had circa 30 cystocarps, these were approximately 520-570 µm in diameter (Fig. 3.5E). Pericarp had 5-7 cell layers, approximately 100 µm thick, without an ostiole (Fig. 3.5F). Carposporangia were clavate to lachrymiform, 55-100 x 14-24 µm (Fig. 3.5G).

Internally the tetrasporophytes consisted of many round to oval large cells of different sizes (Fig. 3.5H). The tetrasporophytes formed small clusters on their surface, which contained tetrasporangia. Branches were 300 µm long by 150 µm wide. Tetrasporangia were approximately 45-55 µm in diameter and tetrahedrally divided (Fig. 3.5I).

The parasite shared the location of tetrahedrally divided tetraspores and the location of cystocarps with its host *C. oblongifolia* but differed in thallus size and pigmentation (Appendix 3.13).

4.2.3 Parasite on *Blastophyllis calliblepharoides* and *B. hombroniana*

Individual trees of *cox*1, LSU rRNA and *rbc*L (Appendices 3.6-3.8) showed that the parasites closest relative is *Judithia delicatissima* (R.E.Norris) D'Archino *et* Showe M.Lin with high support for *cox*1 and *rbc*L and good support for LSU rRNA. The congruent results in all three markers supported a concatenated data set for a more robust phylogeny.

The concatenated data set (*cox*1, LSU rRNA and *rbc*L) contained 44 taxa and was 4827 bp long with representatives of the two host species and their parasites. This data set showed strong support for the shared origin of the parasite and *Judithia delicatissima*. Both host species, *Blastophyllis calliblepharoides* and *B. hombroniana*, grouped together with high support (Fig. 3.6) and were not closely related to their parasites, but this relationship was not well supported.

The phylogenetic data for this parasite with markers of the three different genomes supported a shared ancestry of the parasite with *Judithia delicatissima*. *Callocolax neglectus* described on *Callophyllis laciniata* (Huds.) Kütz. (Batters 1895) from Europe was once recorded on *Blastophyllis hombroniana* (as *Callophyllis hombroniana)* from New Zealand (Cotton 1907) but most New Zealand *Callophyllis* spp. were shown to be different genera within the Kallymeniaceae (D'Archino *et al.* 2016; 2017) and the shared ancestry with endemic *Judithia* suggested that this parasite is most likely a new parasite species.
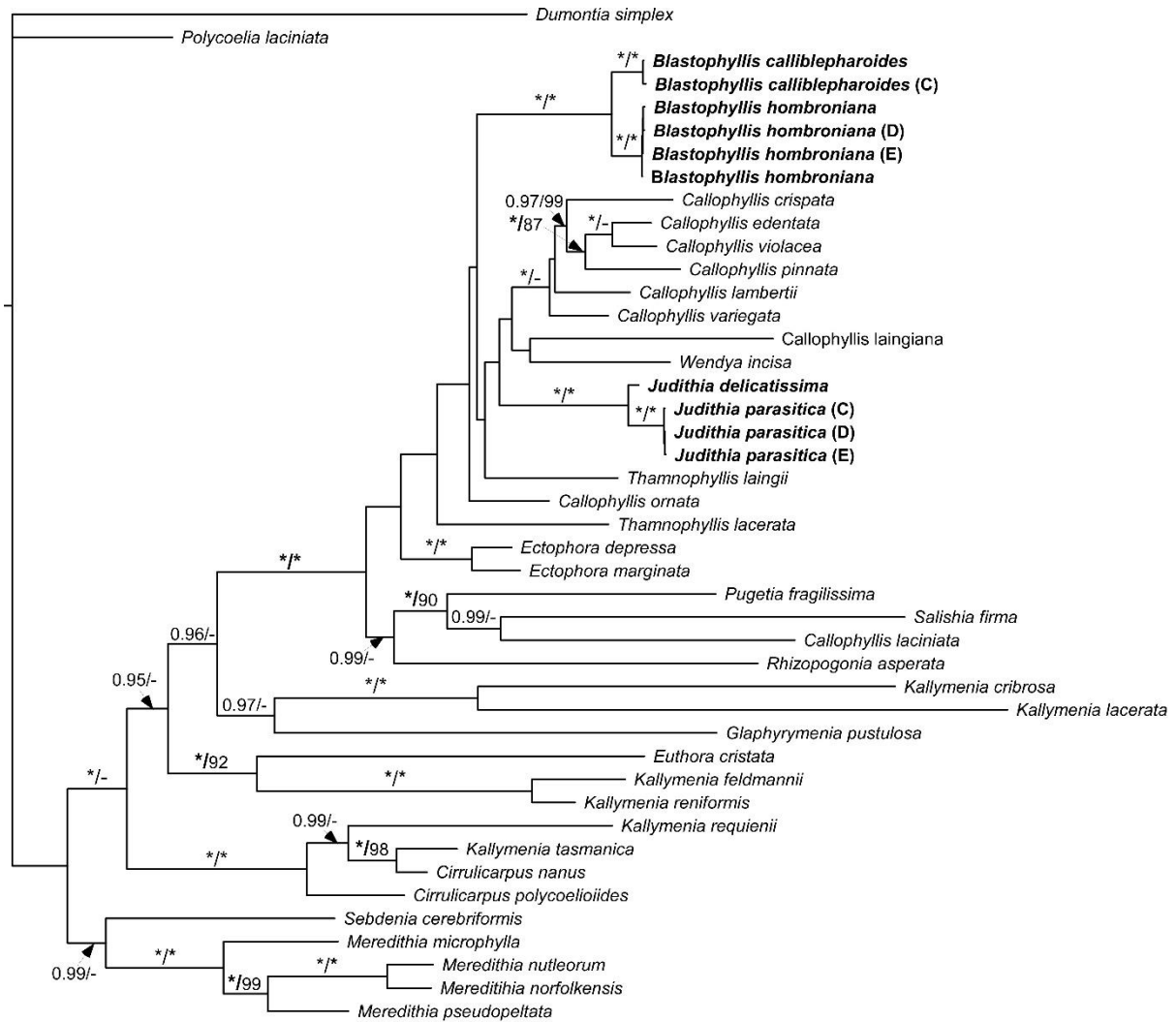
Fig. 3.6. Bayesian topology of concatenated *cox*1, *rbc*L and LSU rRNA sequence data for the parasite *Judithia parasitica* and both hosts *Blastophyllis calliblepharoides* and *B. hombroniana* plus other representative within the Kallymeniaceae. Parasite and host combinations are highlighted by capital letters in brackets (C-E). New (Appendix 3.1) and GenBank samples (Appendix 3.3) were combined. Asterisks indicates posterior probability value of 1.00 and bootstrap value of 100%. Values < 0.85 posterior probability and < 85% ML bootstrap not shown. Outgroups *Dumontia simplex* and *Polycoelia laciniata* were removed to facilitate presentation.

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Judithia parasitica **M.Preuss** *et* **Zuccarello** *sp. nov.*

Figs 3.7A-F

DIAGNOSIS: Thalli pigmented (pale red), less than 1 mm across, with wide base and multiple simple branches. Female and male gametophytes unknown. Tetrasporangia 26 x 13 µm, cruciate divided, scattered on the surface of branches. Parasitic on *Blastophyllis calliblepharoides* and *Blastophyllis hombroniana*.

GENBANK ACCESSION NUMBERS: *cox*1: MF319180; LSU: MF319130; *rbc*L: MF319137.

HOLOTYPE: WELT A033495, collected 18 April 2012, deposited in Museum of New Zealand Te Papa Tongarewa.

ISOTYPE: WELT A033495, collected 18 April 2012, deposited in Museum of New Zealand Te Papa Tongarewa.

TYPE LOCALITY: 41°20.5'S, 174°48.634'E; drift; Moa Point, Wellington, New Zealand.

ETYMOLOGY: *parasitica* (Latin = parasitic) refers to the parasitic lifestyle of this alga.

DISTRIBUTION: Te Papa collections contained 44 specimens of *B. calliblepharoides* and parasites were observed on one specimen from Snares Island (48°01'S), a subantarctic island of New Zealand. On 17 of the 45 specimens of *B. hombroniana* parasites were found. The specimens were from Bank Peninsula (43°45'S) on the South Island (46°36'S), on Stewart Island and on the Auckland Islands (50°30'S), a subantarctic island of New Zealand (Appendix 3.14).

Habitat and vegetative morphology

Judithia parasitica grows on *Blastophyllis calliblepharoides* (previously *Callophyllis calliblepharoides*) and *Blastophyllis hombroniana* (previously *Callophyllis hombroniana*). The position of the parasite and its abundance on the two hosts appeared similar. The hosts had up to a few hundred parasites growing mainly on the edges of the main axis or branches (Fig. 3.7A). The parasite on *B. calliblepharoides* was found in autumn (April), summer (December),

and on *B. hombroniana* in summer (December, January, February), autumn (March), winter (July, August) and spring (October, November) in New Zealand (Appendices 3.1, 3.14).

Thalli of *J. parasitica* were light red, with an average size of less than 1 mm (350-670 µm length to 700-890 µm in width). The parasite had a single, widely spreading base covering the host surface that did not penetrate deeply into the host thallus (Fig. 3.7B). Parasite cells formed secondary pit connection with the top layer of cells (epidermal or sub-epidermal) of the host (Fig. 3.7C). Parasite cells within the parasite thallus were highly connected to each other by either primary or secondary pit connections (Fig. 3.7D).
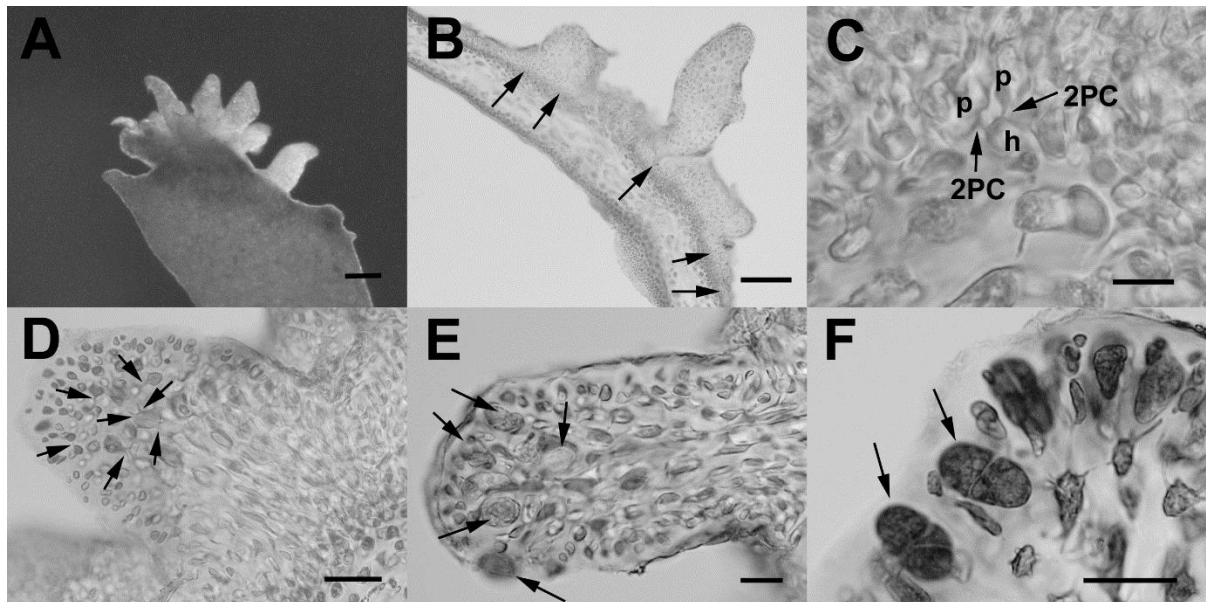
Figs 3.7A-F. Vegetative and reproductive structures of *Judithia parasitica* growing on its host *Blastophyllis hombroniana*. **Fig. 3.7A.** Tetrasporophytic parasite on the edge of the host blade. Scale bar = 200 µm. **Fig. 3.7B.** Cross section of parasite thallus with wide base (arrows) growing over the host thallus. Scale bar = 10 µm. **Fig. 3.7C.** Parasite cells (p) with secondary pit connection (2PC; arrows) to pigmented host cells (h). Scale bar = 20 µm. **Fig. 3.7D.** Parasite cells are highly connected with each other by primary and secondary pit connections (arrows). Scale bar = 100 µm. **Fig. 3.7E.** Longitudinal section of tetrasporic branch with tetrasporangia (arrows) scattered over the surface. Scale bar = 20 µm. **Fig. 3.7F.** Close-up of cruciately divided tetrasporangia (arrows). Scale bar = 20 µm.

Reproductive morphology

Tetrasporophytes were observed. Thalli bore multiple simple branches of different lengths with roundish tips. All observed parasite were either tetrasporophytic or non-reproductive, female and male gametophytes were not found.

The base of the tetrasporophyte produced multiple branches with inner elongated large cells and outer roundish small cells. Branches had tetrasporangia scattered on the surface (Fig. 3.7E). Tetrasporangia were cruciately divided, approximately 13 x 26 µm (n = 6; Fig. 3.7F).

Comparison the parasite and its closest relative Judithia delicatissima

Judithia parasitica sp. nov. shared scattered cruciately divided tetrasporangia of similar size with *J. delicatissima*. The parasite differed is overall thallus size and branching (Appendix 3.15).

3.5 Discussion

This study describes three new red algal parasites from New Zealand that can be distinguished by their host specificity, growth form and reproductive structures. Our phylogeny indicates that the parasites share a common origin, in two cases, with their host genera (*Phycodrys*, *Cladhymenia*) or to a non-host genus (*Judithia*) that is in the same family (Kallymeniaceae) as the host genus (*Blastophyllis*).

A previous study hypothesized three different evolutionary scenarios of red algal parasite origin (Goff *et al.* 1996). First, some parasites (e.g., *Bostrychiocolax australis* Zuccarello *et* J.A.West, *Gardneriella tuberifera* Kylin, *Rhodophyllis parasitica*) evolved from their hosts and solely infect this host species (Goff *et al.* 1996; Zuccarello *et al.* 2004; Preuss & Zuccarello 2014). Second, parasites (e.g., *Faucheocolax attenuata* Setch.) evolved and grow on one host species but also parasitize a second, closely related host species (Goff *et al.* 1996). Third, some parasites (e.g., *Plocamiocolax pulvinata* Setch.) evolved on one species, but now parasitize a secondary host and were lost from the original host species (Goff *et al.* 1996). Our data reflect the first and third scenario and reveals a possible fourth scenario.

In the parasite *Cladhymenia oblongifoliophila* mitochondrial and nuclear markers indicate that the parasite shares an origin with its host *C. oblongifolia,* whereas the plastid marker (*rbc*L) shows that its closest relative is the apparently non-host species *C. lyallii*. There are several possibilities to explain the genetic patterns observed: 1) the parasite evolved from a common ancestor with *C. oblongifolia* and then switched host to *C. lyallii*, where it acquired plastids, as has been shown in *Gracilaria babae* (Ng *et al.* 2014), and subsequently switched back to *C. oblongifolia* and was lost from *C. lyallii*; 2) the species, sharing a common ancestry with *C. oblongifolia* became a parasite on *C. lyallii* and acquired its plastid from this host, it then became a parasite of *C. oblongifolia* but was lost from *C. lyallii*; and 3) the species, sharing a common ancestry with *C. oblongifolia,* became a parasite on *C. lyallii* from which it acquired plastids and subsequently became a parasite of *C. oblongifolia* but has not been observed on *C. lyallii.*

Phycodrys novae-zelandiophila is an example of a parasite that is genetically nearly indistinguishable from its host and found only on this host species. Genetic distances are higher within the host than between parasite and host. Other parasites have been recorded on *Phycodrys* species from other parts of the world (*Asterocolax denticulatus*, *Asterocolax gardneri*, *Choreocolax rabenhorstii*). The common origin of *P. novae-zelandiophila* with its host, and the close phylogenetic relationship of other *Phycodrys* parasites to their hosts (Goff *et al.* 1997) suggest that parasites have evolved multiple time in this genus. Why parasites have evolved so many times in some genera is not yet known.

Judithia parasitica growing on two *Blastophyllis* spp. is another possible example of host switching with extinction on the original host species (Goff *et al.* 1996). All molecular markers indicate that *J. parasitica*'s closest relative is *J. delicatissima* and neither is closely related to the two hosts in *Blastophyllis*. *Judithia parasitica* either evolved on a shared common ancestor of *Judithia* as a parasite or as a free-living organism and became parasitic or switched hosts either to both species of *Blastophyllis* or to the common ancestor of these two species. Host switching to distantly related hosts is not common. One example is *Harveyella mirabilis* (Rhodomelaceae) which also parasitizes *Gonimophyllum skottsbergii* Setch. (Delesseriaceae) (Zuccarello *et al.* 2004).

Our study, and previous studies of different red algal parasites, showed that parasite and host are often sister-species (Goff *et al.* 1996; Goff *et al.* 1997; Zuccarello *et al.* 2004; Kurihara *et al.* 2010; Preuss & Zuccarello 2014). Several parasitic relationships follow Emery's rule, originally developed for insects, that states that parasites are their hosts' closest relative (Emery 1909). These parasites evolved either by sympatric speciation from their host (Bourke & Franks 1991) or were derived from two allopatrically non-parasitic species, one of which parasitize the other during secondary contact (Lowe *et al.* 2002). Emery's rule has been proposed for red algal parasites (Setchell 1918; Goff *et al.* 1997).

Morphological characters of the three parasites are congruent with their phylogenetic relationships and confirm them as red algal parasites. All three parasites were small, with reduced or no pigmentation and formed secondary pit connections to their hosts cells (Goff 1982; Wynne & Scott 1989; Chapter 2). These criteria are the basis for many determinations of species as parasitic; nutrient status and detriment to the host have been seldom investigated (Kremer 1983; Apt 1984a; Goff 1976; 1982; Martin & Pocock 1953).

Our data support the placement of our parasite species in the genus of its closest relative and maintains a taxonomy based on monophyly. Modern classification should reflect phylogenetic relationships (de Queiroz & Gauthier 1992) and we support the idea that the origin of these parasites should be reflected in their taxonomy. This may require that the circumscription of a genus that has been demonstrated to contain a parasitic species be modified to include it ("and the parasites derived from it"), as previously suggested (Preuss & Zuccarello 2014).

A common taxonomic problem of red algal parasites is that names are applied to parasites found on hosts from distant areas or within the same host genus. *Callocolax neglectus* growing on *Callophyllis laciniata* was described from Europe (Batters 1895) but recorded on *Callophyllis hombroniana* (Cotton 1907) in New Zealand. No molecular data are available for *Callocolax* from the north Atlantic. Another example is *Dawsoniocolax bostrychiae* (A.B.Joly *et* Yam.-Tomita) A.B.Joly *et* Yam.-Tomita growing on *Bostrychia radicans* (Mont.) Mont. in Brazil (Joly & Yamaguishi-Tomita 1969) which was later recorded on *Bostrychia radicans* in Australia (West & Calumpong 1988), but phylogenetic and developmental studies showed that the Australian parasite is distinct (*Bostrychiocolax australis*, Zuccarello *et al.* 1994a). These two examples make it obvious that careful morphological and anatomical observations, in addition to molecular data, of host and parasite are necessary to distinguish species.

In summary, we describe these three parasites as new species: *Cladhymenia oblongifoliophila* sp. nov. (Ceramiales), *Phycodrys novae-zelandiophila* sp. nov. (Ceramiales), and *Judithia parasitica* sp. nov. (Gigartinales) based on morphological and molecular evidence. The number of red algal parasites known from New Zealand has increased but further studies into this intriguing group are needed to understand their diversity, classification and evolutionary relationships with their hosts.

Chapter Four

Development of the new red algal parasites *Vertebrata aterrimophila* sp. nov. (Rhodomelaceae, Ceramiales) from New Zealand

4.1 Abstract

Parasitic red algae grow only on other red algae and have over 120 described species. Developmental studies in red algal parasites are few, although they have shown that secondary pit connections formed between parasite and host and proposed that this was an important process in successful parasitism. Furthermore, it was recorded that the transfer of parasite nuclei by these secondary pit connections led to different host cell effects. We used developmental studies to reconstruct early stages and any host cell effects of a parasite on *Vertebrata aterrima*. A mitochondrial marker (*cox*1) and morphological observations (light-and fluorescence microscopy) were used to describe this new red algal parasite as *Vertebrata aterrimophila* sp. nov. Early developmental stages show that a parasite spores connects via secondary pit connections with a pericentral host cell after cuticle penetration. Developmental observations revealed a unique connection cell that grows into a "trunk-like" structure. Host cell transformation after infection by the parasite included an apparent increases in both carbohydrate concentrations and nuclear size, as well as structural changes of infected host cells. Analyses of molecular phylogenies and reproductive structures indicate that the closest relative of *V. aterrimophila* is its host, *V. aterrima*. Our study shows a novel developmental parasite stage ("trunk-like" cell) and highlights the need for further developmental studies to investigate the range of developmental patterns and host effects in parasitic red algae.

4.2 Introduction

Parasitic red algae growing only on other red algae undergo unique development processes from spore attachment to reproductive maturity. While parasitic red algae are taxonomically quite diverse only a few studies have carefully examined parasite development, especially the early stages of infection and the cellular effects of infection on host cells (Chapter 2). An understanding of the diversity of these developmental processes is needed if any patterns, and evolutionary implications, are to be drawn.

Early on, in the study of these organisms, morphological characters were used to describe parasites that were closely related to their host (either same tribe or family) as "adelphoparasites" and those distantly related to their hosts as "alloparasites" (Feldmann & Feldmann 1958, Goff 1982). Since then, phylogenetic studies showed that the terms "adelphoparasites" and "alloparasites" are an extreme oversimplification as there is a range of different degrees of relatedness between parasites and hosts (Zuccarello *et al.* 2004; Kurihara *et al.* 2010; Chapter 3). Close relationships between parasites and hosts range from low to no genetic marker variation (e.g., *Rhodophyllis parasitica* M.Preuss *et* Zuccarello; Preuss & Zuccarello 2014) to parasites being sister to host species and nested within the host genus (e.g., *Gracilariophila oryzoides* Setch. *et* H.L.Wilson; Goff *et al.* 1996). Distant relationships are also found between parasite and host ranging from parasites grouped in the same family as the host (e.g., *Ululania stellata* Apt *et* Schlech; Kurihara et al. 2010) to ones in a different family, but the same order, as the hosts (e.g., *Holmsella pachyderma* (Reinsch) Sturch; Zuccarello *et al.* 2004). Regardless, the terms "adelphoparasites" and "alloparasites" have been continuously used in red algal parasites without reference to the extent of phylogenetic relationships.

In general, parasite spores attach and penetrate the host cuticle, by a spore infection peg, fusing with an epidermal or subepidermal host cell (Goff & Coleman 1987). In some parasites the germination tube tip fuses, via a secondary pit connection with the epidermal host cell, e.g. *Dawsoniocolax bostrychiae* (A.B.Joly *et* Yam.-Tomita) A.B.Joly *et* Yam.-Tomita. Sometimes this germination tube tip expands to form the first parasite cell within the host thallus before fusion, e.g. *Bostrychiocolax australis* Zuccarello *et* J.A.West (Zuccarello & West 1994a). In other parasites, the germination tube divides a few times while growing into the host thallus before connecting to subepidermal host cells, e.g. *Harveyella mirabilis* (Reinsch) F.Schmitz *et* Reinke (Goff 1976). This fusion between host and parasite is through a conjunctor cell, which

leads to a secondary pit connection between parasite and host cells (Goff & Coleman 1985) and is an imporant process in successful parasitism (Zuccarello *et al.* 2004; Chapter 2).

Secondary pit connection formation also transfers parasite organelles (e.g., nuclei, mitochondria) into host cells, and this is thought to lead to "control" of host cells by parasite nuclei, through a process called cellular transformation (Goff & Coleman 1987; Salomaki & Lane 2014). The first demonstration of host nuclear transfer was in the parasite *Leachiella pacifica* Kugrens, which transferred parasite nuclei into the host cells of *Polysiphonia confusa* Hollenb. These parasite nuclei did not divide or undergo DNA synthesis in the host cell (Goff & Coleman 1984; 1985). Another example was the transfer of parasite nuclei of *Gracilariophila oryzoides* into the host cells of *Gracilariopsis andersonii* (Grunow) E.Y.Dawson. These parasite underwent DNA synthesis and divided in host cells (Goff & Zuccarello 1994). Infected host cells containing parasite nuclei always showed some degree of morphological and developmental changes, including increases in starch concentration and nuclear ploidy level, plus the infection spread to surrounding host cells in some species (Goff & Coleman 1987; Goff & Zuccarello 1994).

After fusion of host and parasite cells, parasite growth can be superficial or endophytic. Superficial development is only known from the parasite *Dawsoniocolax bostrychiae*, where all growth is external to the host (Zuccarello & West 1994a). The endophytic growth in all other parasites is either by parasite cells spreading through the host thallus, such as in the parasite *Leachiella pacifica* Kugrens (Goff & Coleman 1987), or through spreading of the infection through infected host cells infecting neighboring cells (Goff & Zuccarello 1994). Often uninfected host cells, parasite cells and infected heterokaryotic cells within the host thallus get pushed upwards forming the mass of the reproductively mature parasite thallus (Goff & Coleman 1987; Goff & Zuccarello 1994).

The Ceramiales is the largest red algal order within the Florideophyceae (Yang *et al.* 2016) with the highest diversity of red algal parasites (Chapter 2). They occur in four families: Ceramiaceae, Daysaceae, Delesseriaceae and Rhodomelaceae (Salomaki & Lane 2014; Chapter 2). Most parasites in the Ceramiales are partially pigmented and grow on only one host species within the same order (Chapter 2). The family Rhodomelaceae contains many red algal parasites. The parasite *Choreocolax polysiphonia* Reinsch grows on *Vertebrata lanosa* (L.) T.A.Chr. (Reinsch 1875) and the parasite *Leachiella pacifica* grows on *Polysiphonia paniculata*

(Mont.) J.N.Norris (as *P. confusa*, Goff & Coleman 1985) and *Polysiphonia hendryi* N.L.Gardner (Kugrens 1982; Zuccarello *et al*. 2004). Both were first placed in the family Choreocolacaceae (order Gigartinales) (Sturch 1926), along with *Harveyella mirabilis*. Later, a phylogenetic study showed these parasites had a distant relationship to their hosts but are in the Rhodomelaceae (Zuccarello *et al*. 2004).

In this study, we describe the development and phylogenetic placement of a new red algal parasite species from New Zealand growing on *Vertebrata aterrima* (Hook.f. *et* Harv.) Kuntze. This study highlights a unique development structure in the parasite and adds to our understanding of variation in parasite development.

4.3 Materials and Methods

Samples of *Vertebrata aterrima*, and its parasite, were collected as drift at Castlepoint (40°54′08″S, 176°13′43″E) or growing as an epiphyte on *Carpophyllum maschalocarpum* (Turner) Grev. at Moa Point, Wellington, New Zealand (41°20′30″ S, 174°48′38″ E) from spring 2015 to spring 2016. All specimens were either pressed as herbarium vouchers, dried in silica gel, fixed in 2% glutaraldehyde in phosphate buffer (0.1M, pH 6.8) in 50% seawater or cultured in containers with sterile seawater.

For developmental experiments, approximately 200 uninfected hosts were infected by spores released from reproductively mature parasites collected in the field. Mature parasites were placed on uninfected host plants floating on a Nitex screen (400 µm mesh, Dynamic Aqua-Supply Ltd., Canada) in sterile seawater (salinity approximately 33) for one day and then removed. Hosts were removed from the Nitex screen and moved into sterile seawater. Infected host samples were cultured at approximately 15°C by 12 hours day (14.5-4.5 µmol photons m^{-2} s^{-1} 126 constant fluorescent) and night cycle. Hosts were fixed at regular time intervals and three samples of every experiment were grown for 2-3 weeks to determine successful infection of host.

For morphological and developmental analyses, samples were either embedded in resin following Preuss & Zuccarello (2014) or prepared for squashing following Goff & Coleman (1984). For squash preparations, samples were softened in saturated chloral hydrate, transferred to slides coated with Haupt's solution (Haupt 1930) and squashed with carefully applied

pressure of a soft rubber. Coverslips were removed in liquid nitrogen, then the samples were fully immersed in 70% ethanol and air dried until staining. Microscopic slides were stained with either 1.0 µg mL^{-1} DAPI in McIlvaine buffer (pH 4.1), 1% acidified aniline blue or 1% toluidine blue. Samples were examined using a microscope (Olympus AX-70, Tokyo, Japan) with an integrated camera (Olympus DP-70) and images were taking using Olympus cellSens software.

Reproductive observations of parasitic gametophytes on tetrasporophytic host plants and tetrasporophytic parasites on host gametophytes were used to confirm the outgrowth was an independent alga and not a host proliferation or bacterial infection.

For phylogenetic analyses, mature parasite thalli were selected. DNA was extracted following a modified CTAB protocol (Zuccarello & Lokhorst 2005) and PCR amplified using the primers, GazF1 (TCA ACA AAT CAT AAA GAT ATT GG, Saunders 2005) and Mam2R (GTA TTA AAA TTW CKA TCW GTT A, Mamoozadeh & Freshwater 2011) for partial *cox*1. PCR conditions consisted of an initial denaturing step at 94°C for 5 min, followed by 35 cycles each 94°C for 1 min, 45°C for 1 min, and 72°C for 1 min, and a final extension step at 72°C for 10 min. Successful amplifications were purified using ExoSAP-IT (USB product; Affymetrix, Santa Clara, CA, USA) and commercially sequenced (Macrogen Inc., Seoul, Korea). Amplifiation of nuclear and plastid markers were unsuccessful. Sequences of the forward and reverse strands were assembled using Geneious 8.0.5 (http://www.geneious.com, Kearse *et al.* 2012) and edited sequences were aligned using MAFFT alignment using default settings. Taxon sampling (Appendix 4.1) for phylogenetic analyses was selected following Díaz-Tapia *et al.* (2017). Bayesian analysis was performed with MrBayes v.3.2.5 (Ronquist & Huelsenbeck 2003) and maximum-likelihood trees (ML) with RAxML 7.2.8 (Stamatakis 2006) following Chapter 3.

All herbarium samples of *V. aterrima* (as *Polysiphonia aterrima*) at the Museum of New Zealand Te Papa Tongarewa in Wellington were searched for parasites under a dissecting microscope and observed parasites were recorded.

4.4 Results

This study describes a new red algal parasite from New Zealand with a unique developmental structure. Our phylogenetic analysis indicates that the parasite shares a close relationship with its host.

Developmental observations

Released parasite spores were pigmented and spherical (~10-20 µm in diameter) and attached to the host cuticle between host cells. The parasite spore developed a germination tube of up to 30 µm in length (Fig. 4.1A). Germination only occurrs in the presence of the host. The germination tube connected to a pericentral host cell through a secondary pit connection (Fig. 4.1B). After the formation of a secondary pit connection between host and parasite, a "connection" cell developed from the germination tube and the parasite grew superficially from this cell by cell division (Fig. 4.1C). Further secondary pit connections between the parasite "connection" cell and several host cells were formed as the parasite grew (Fig. 4.1D). The growing parasite "connection" cell developed several branches that connected to the same or other host cells (Fig. 4.1E). The "connection" cell grew to be the largest parasite cell and was easily recognizable (Fig. 4.1F), sometimes two "connection" cells were present (Fig. 4.1G) and these cells developed into a "trunk-like" structure in mature parasite thalli (Fig. 4.1H). Parasite cells were multinucleate, and highly connected between parasite cells (Fig. 4.1I). Infected host cells contained many nuclei, which were almost double in size (5-10 µm diameter) in comparison to host nuclei in neighbouring non-infected host cells and parasite nuclei (3-5 µm diameter, Figs 4.1J-K). Parasite nuclei were not distinguishable these heterokaryotic cells. After infection, cytological changes in the infected host cells were observed and included an apparent increase in carbohydrates (Fig. 4.1K). Infected host cells were more susceptible to plasmolysis, suggesting structural changes in these cells (Fig. 4.1M). The infection of the parasite was highly localized and superficial, and no parasite cells were ever observed deeper in the tissue (Figs 4.1F-H, 4.1M). The infection did not spread to surrounding host cells, i.e. host cell was not directly connected to parasite cells (Figs 4.1H, 4.1M).
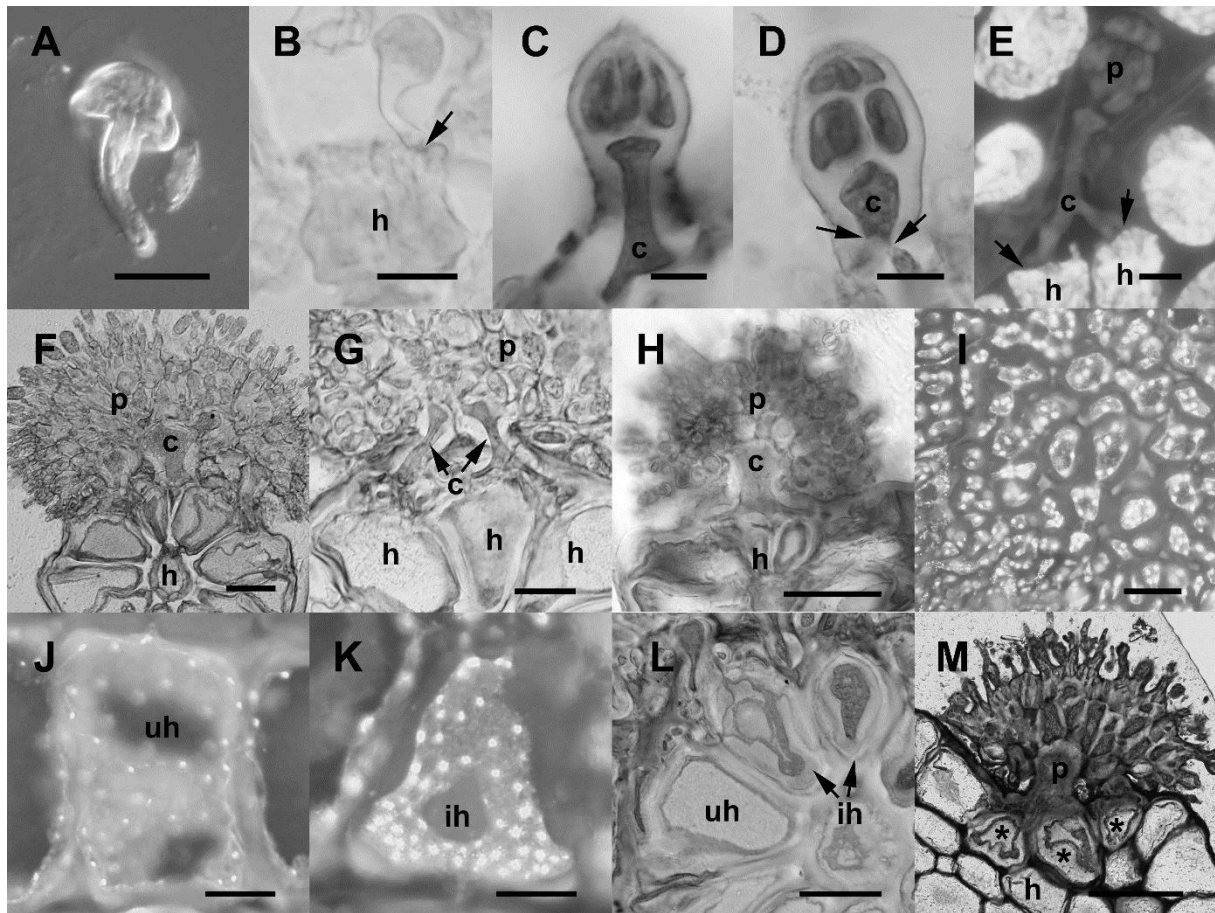
Figs 4.1A-M. Development of *Vertebrata aterrimophila* on its host *Vertebrata aterrima*. **Fig. 4.1A.** Phase contrast of germinated parasite spore, unattached to host, showing germination tube. **Fig. 4.1B.** Light microscopy of germinated parasite spore which connects through a secondary pit connection (arrow) to a host cell (h). **Fig. 4.1C.** Early stages of developing parasite with connection cell (c). Stained with aniline blue. **Fig. 4.1D.** Parasite growing on the surface and two secondary pit connections to host cells (arrow), one out of plane of focus. **Fig. 4.1E.** Autofluorescence shows non-autofluorescing parasite body (p) with large and branched connection cell (c), connecting to two host cells (arrow), plus bright autofluorescing host cells (h). **Fig. 4.1F.** Parasite (p) thallus, and cross section of host thallus, with large connection cell (c). Stained with aniline blue. **Fig. 4.1G.** In some parasites (p) two connection cells (c) were observed. Aniline blue staining. **Fig. 4.1H.** A mature connection cell (c), and developing parasite, resembles a 'trunk'-like connecting to various pericentral cells. **Fig. 4.1I.** Multi-nucleate parasite cells highly connected to each other. Stained with DAPI. **Fig. 4.1J.** Host nuclei in uninfected host cells (uh). Stained with DAPI. **Fig. 4.1K.** Host nuclei in infected host cell (ih), from same plant as Fig. 4.1J. Stained with DAPI. **Fig. 4.1L.** Infected host cells (ih) appear to have higher carbohydrate concentrations (indicated by darker aniline blue staining) than uninfected host cells (uh). **Fig. 4.1M.** Infected host cells (asterisks) are more susceptible to plasmolysis and show structural changes insides cells, stained with toluidine blue. Scale bars: Figs 4.1A-B, 4.1E, 20 µm, Figs 4.1C-D, 10 µm, Figs 4.1F, 4.1H, 4.1L, 100 µm, Figs 4.1G, 4.1I-4.1K, 50 µm, Fig. 4.1M, 200 µm.

Phylogenic results

Partial *cox*1 sequences (726 bp) were obtained for six samples of *Vertebrata aterrima* and five of its parasite. Pairwise distances within parasites were 0-0.7%, and between hosts 0-1.4%, and between host and parasite 7.2-8.5%. All parasites grouped together and were sister to their host *V. aterrima* within the genus *Vertebrata*and this close relationship between parasite and host was strongly supported (Fig. 4.2). Our data showed that this was a new parasite and needed to be formally described.

Vertebrata aterrimophila **M.Preuss** *et* **Zuccarello** *sp. nov.*
Figs 4.3A-K

DIAGNOSIS: Thalli unpigmented to pigmented (dark brownish), 587-1500 µm in diameter with easily recognizable reproductive forms. Gametophytes dioecious. Cystocarps ovoid with ostioles and tear-drop shaped carpospores (49-82 µm x 17-23 µm). Spermatangial branches without sterile apical cells. Tetrasporangia are tetrahedrally divided (33-43 µm x 30-38 µm) and are spirally arranged in branches. Parasitic on *Vertebrata aterrima* (Hook.f. *et* Harv.) Kuntze.
*Cox*1 GenBank Accession numbers: MH670282-MH670284.

HOLOTYPE: WELT A033493, collected as a parasite on *Vertebrata aterrima* from Moa Point, Wellington, New Zealand (41°20′30″ S, 174°48′38″ E); coll. Maren Preuss, 14/09/2015, deposited in Museum of New Zealand Te Papa Tongarewa.

ISOTYPE: WELT A033493, deposited in Museum of New Zealand Te Papa Tongarewa.

TYPE LOCALITY: Moa Point, North Island, New Zealand.

ETYMOLOGY: The name *aterrimophila* refers to the parasites' affinity to its host *Vertebrata aterrima*.

Habitat

Vertebrata aterrimophila usually grows between branches of *Vertebrata aterrima* and was observed in Wellington all year round. Most infected host thalli are highly parasitized with more than 100 parasites covering the whole thallus (Fig. 4.3A).
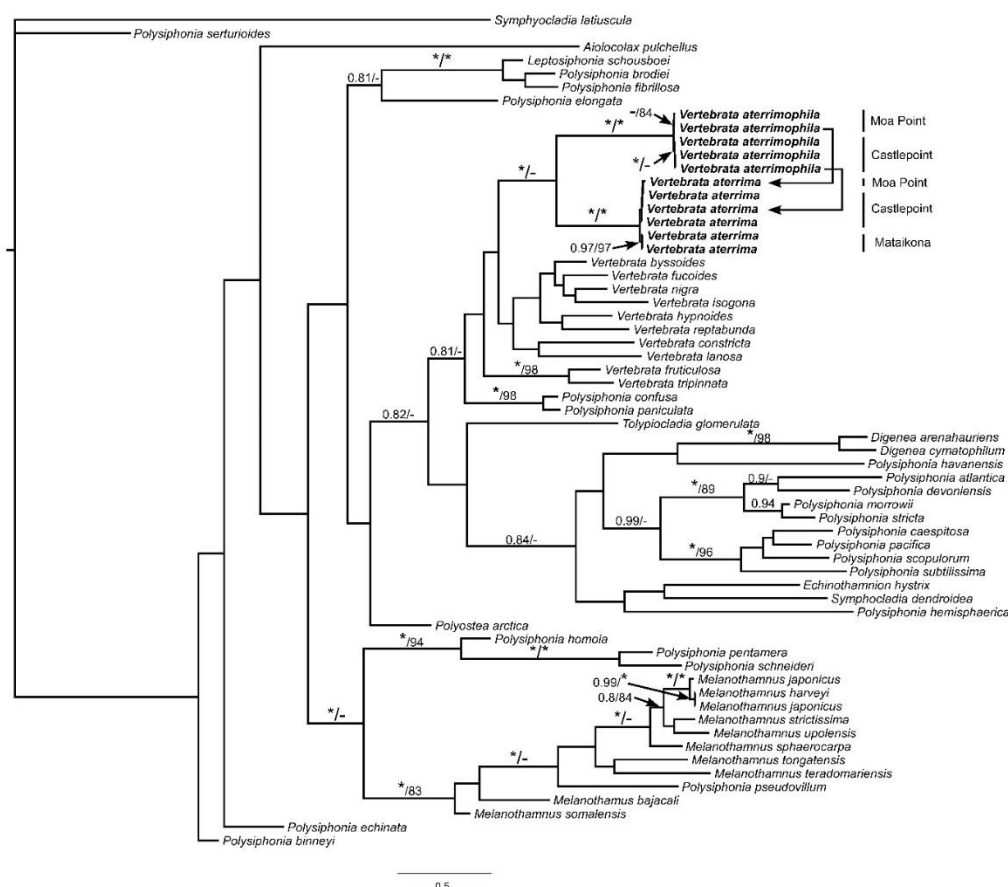
Fig. 4.2. Bayesian topology of partial *cox*1 of *Vertebrata* species. *Vertebrata aterrima* and its parasite *Vertebrata aterrimophila* in bold. *Symphyocladia latiuscula* used as outgroup. Parasite and host combinations are connected by arrows and sampling locations given. Asterisks indicate posterior probability value of 1.00 /bootstrap values of 100%. Values < 0.80 posterior probability and < 80% ML bootstrap not shown and indicated with a dash.

Pigmentation and autofluorescence.

Parasites thalli can be pigmented or unpigmented and no clear pattern by location, size of parasite or reproductive stages was apparent. Small, not fully mature pigmented parasites (~150 µm width) show faint autofluorescence (Fig. 4.3B).

Distribution

Te Papa collections contained 31 specimens of *V. aterrima* and seven of those had parasites on them. The parasite was recorded from Pouawa (38° 36' S) on the east coast to Wellington (41° 20' S) in the North Island and in the Marlborough Sounds (40° 50' S) on the South Island (Appendix 4.2).
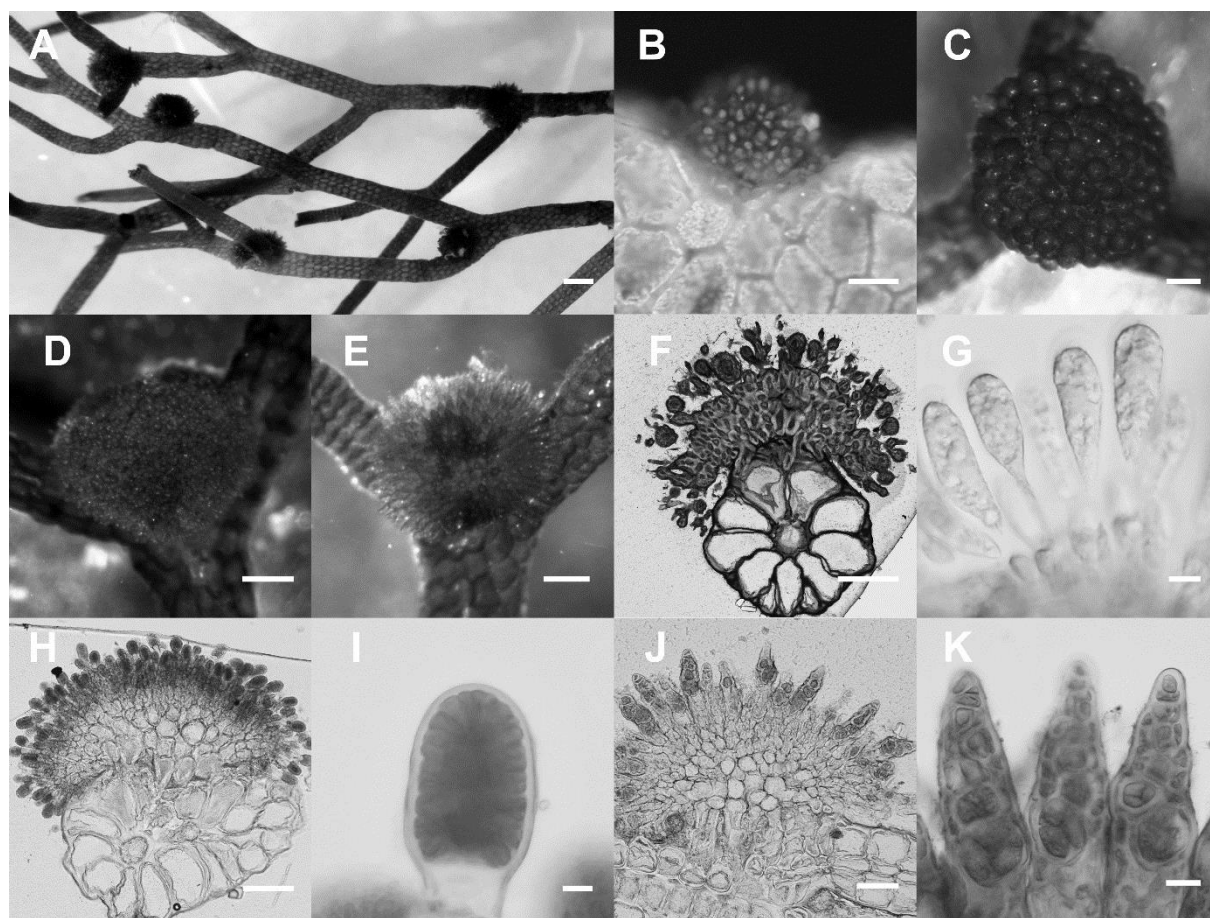
Figs 4.3A-K. Vegetative and reproductive structures of *Vertebrata aterrimophila* on its host *Vertebrata aterrima*. **Fig.** 4.**3A.** Highly infected host thallus, parasites often found in host branch angles. **Fig. 4.3B.** Slight autofluorescence in a young parasite was detectable. **Fig. 4.3C.** Female gametophytes with many smooth spheres (cystocarps) of similar size. **Fig. 4.3D.** Male gametophyte of a roundish cushion with roundish tips (spermatangial stichidia). **Fig. 4.3E.** Tetrasporophyte with pointy branch tips (tetrasporangial stichidia). **Fig. 4.3F.** Female gametophyte with cystocarps over its surface. Resin-embedded transverse section. **Fig. 4.3G.** Elongated carpospores.Squash-preparastion. **Fig. 4.3H.** Male gametophytes covered with spermatangial branches. Resin-embedded transverse section. **Fig. 4.3I.** Spermatangial stichidum with clusters of spermatia and lacking sterile apical cells. Squash-preparation. **Fig. 4.3J.** Tetrasporophyte is covered with acute tetrasporoangial stichidia. Squash-preparation. **Fig. 4.3K.** Tetrasporangium contain tetrahedrally divided tetraspores in a spiral arrangement. Scale bars: Fig. 4.3A, 400 µm, Fig. 4.3B, 50 µm, Figs 4.3CF, 4.3H, 200 µm, Figs 4.3G, 4.3I, 10 µm, Fig. 4.3J, 100 µm, Fig. 4.3K, 20 µm.

Reproductive morphology

Male, female and tetrasporophytes were observed. The parasite was dioecious. The parasite consisted of either many smooth spheres of similar sizes (female gametophyte, Fig. 4.3C) or one rough roundish cushion with roundish branches (male gametophyte, Fig. 4.3D) or elongate branches emanating from the thallus (tetrasporophyte; Fig. 4.3E). Gametophytic parasites were observed growing on tetrasporophytic hosts and tetrasporophytic parasites were observed growing on gametophytic hosts.

The entire surface of mature female gametophytes is covered with ovoid, ostiolate cystocarps (Fig. 4.3F), approximately 160-245 µm in diameter (n=5, Fig. 4.3F). Carposporophytes produced elongated tear-drop shaped carpospores, 49-82 µm x 17-23 µm (n=10, Fig. 4.3G).

Mature male gametophytes were covered with spermatangial branches (Fig. 4.3H) of approximately 47-102 µm x 37-68 µm and were lacking sterile apical cells (Fig. 4.3I).

Sporophytic parasites were covered by tetrasporangial stichidia of approximately 188-218 µm in length and 31-37µm in diameter (Fig. 4.3J). Tetrasporangial stichidia contained spirally arranged pigmented tetrahedrally divided tetrasporangia approximately 33-43 µm x 30-38 µm (Fig. 4.3K).

4.5 Discussion

Our study showed some unqiue developmental structures of a novel parasite, and in conjuction with its phylogenetic relationship to its host *Vertebrata aterrima*, support its recognition as a new parasite.

The unique developmental characteristic of *V. aterrimophila* is a prominent "trunk-like" cell from which all parasite connections form, via secondary pit connections, to host cells. Most parasites have a rhizoidal filament fusing with underlying host cells (Nonomura 1979; Goff & Coleman 1987; Goff & Zuccarello 1994). Other parasites have several parasite cells within the host thallus which fuse with several host cells (Goff & Coleman 1985). Only the parasite *Dawsoniocolax bostrychiae* uses the initial germination tube, and later rhizoidal filaments, derived from the external parasite body, to connect to underlying host cells (Zuccarello & West

1994a). The differences in when and which cell initially connects (e.g., internal parasite cell, germiantion tube, rhizoidal filaments), via secondary pit connections, to host cells varies greatly between the few species studied and further studies might reveal if other parasites have such a prominent connection cell or even new developmental pathways.

From the currently known developmental patterns, *V. aterrimophila* and *D. bostrychiae* are the only two parasites growing mainly superficially, which leads to other similarities between these two parasites (Zuccarello & West 1994a). For example, host cells are not found immersed in either of these two parasite thalli (our study, Zuccarello & West 1994a). All other red algal parasites grow at least partially endophytically, often with deep penetration by parasite cells leading to embedded host cells in parasite thalli (Goff 1976; Nonomura 1979; Goff & Coleman 1987; Goff & Zuccarello 1994). Shared developmental patterns between parasites might led to classifications based on these pattern that overrate similarities.

The only similarity between the development of all red algal parasites are secondary pit connections to their hosts during their early development, but these connections differ in their impacts on host cells. Secondary pit connections are used to transfer parasite nuclei into the host cell (Goff & Coleman 1985) and infected host cells with parasite nuclei are altered ("host cell transformation") (Goff & Coleman 1985; Goff & Zuccarello 1994; Chapter 2). Host cell transformation varies from few host cell changes caused by the parasites *Bostrychiocolax australis*, *Dawsoniocolax bostrychiae, Leachiella pacifica* and *Vertebrata aterrimophila* (our study, Goff & Coleman 1985, Zuccarello & West 1994a) to extreme host cell changes (e.g., heterokaryon syncytium) caused by the parasites *Gracilariophila oryzoides* and *Gardneriella tuberifera* (Goff & Zuccarello 1994). After nuclei transfer morphological and physiological changes were observed in host cells including an increase of carbohydrates (Nonomura 1979; Goff & Coleman 1987; Goff & Zuccarello 1994), cell size 'hypertrophy' (Goff & Coleman 1987; Zuccarello & West 1994a), and increased cell wall thickness (Nonomura 1979; Goff & Coleman 1987). In our study, we observed apparent increases in carbohydrate concentrations and nuclei size and infected host cells seems to be more susceptible to plasmolysis indicated by structural changes.

Previous developmental studies were able to distinguish between transferred parasite and host nuclei in host cells. Differences in relative DNA contents were used to distinguish transferred parasite nuclei and host nuclei in infected host cells (Goff & Coleman 1987). In the case of the parasite *Bostrychiocolax australis*, transferred parasite nuclei in host cells differed in size from host nuclei in infected host cells (Zuccarello & West 1994a). In our study, all observed nuclei in infected host cells increased in size, and parasite and host nuclei could not be distinguished from each other. We do not know if the transferred parasite nuclei might increase in size in host cells, or if only a few parasite nuclei are being transferred and the parasite nuclei overlooked.

Mitochondrial marker (*cox*1) shows that the parasite *Vertebrata aterrimophila* is genetically similar to, and shares a common origin with, its host *V. aterrima*. Many red algal parasites in the Ceramiales show a close host-parasite relationship (Goff *et al.* 1997; Zuccarello *et al.* 2004; Kurihara *et al.* 2010; Chapter 3). Previous morphological descriptions placed many parasites in independently parasitic genera (Kraft & Abbott 2002, Kim & Cho 2010), due to their obvious morphological differences from their hosts (e.g., small size). Phylogenetic analyses showed that most parasites should be placed in the genus of the parasites closest relative, often its host (Ng *et al.* 2014; Preuss & Zuccarello 2014; Chapter 3). While many vegetative characters of the host genus are not found in the parasite (e.g., rhizoid type, pericentral cells), the reproductive characters support the molecular placement of the parasite within the host genus *Vertebrata*.

Based on the few known developmental studies a link was made between development pattern and parasite-host relationships.In closely related parasites ("adelphoparasites") there is greater host cell transformation, including spread to neighbouring cells via infected host cells, than there is between more distantly related parasite-host combinations ("alloparasites") (Blouin & Lane 2012, Salomaki & Lane 2014, Freese & Lane 2017). This simplified classification of parasites as having "adelphoparasites" and "alloparasites" developmental patterns does not hold true. In *V. aterrimophila*, minimal host cell changes were observed eventhough they are closely related, similar to other parasites that are closely related to their hosts such as *Bostrychiocolax australis* (Zuccarello & West 1994a) and *Janczewskia morimotoi* (Nonomura 1979). The phylogenetic relationship of these parasites to their hosts does not reflect host cell transformation and these hypotheses may be oversimplifications that do not reflect the variation of developments. Further studies combining developmental studies and phylogeny, especially of parasites that parasitize other orders within the Florideophyceae, are needed and additional knowledge about these parasites might help us understand their success and evolutionary trends.

Red algal parasites are classified as parasites, but host nutrient dependency has rarely been demonstrated. The majority of red algal parasites are pigmented (Chapter 2) and *V. aterrimophila* is another example of a pigmented parasite. Our study showed that *V. aterrimophila* sometimes demonstrated faint auto-fluorescence, which might indicate a degree of photosynthetic ability. Nutrient transport from a host to the unpigmented parasite has been reported (Evans *et al.* 1973; Harlin 1973; Goff 1979). The degree of pigmentation and nutrient dependency was correlated in parasitic plants, where unpigmented species are fully host nutrient dependent (Westwood *et al.* 2010) and pigmented parasitic plants show a decreased host nutrient dependency, and in some cases even the ability to photosynthesis independently (Tesitel *et al.* 2010). The few studies in red algal parasites have shown that parasites seem to gain nutrients from their hosts (Evans *et al.* 1973; Harlin 1973; Goff 1979) but the extent of host nutrient dependency needs further investigation including quantifying the amount of nutrient gained by the parasite, the impact of the lost nutrients on the host, and the ability of pigmented parasites to photosynthesize. This information will help to establish the case for parasitic status.

Parasitic species are thought to have patchy distributions, which may be associated with different factors. Generally, the majority of potential host populations within a species are uninfected and there are a few highly infected host populations (Poulin 2013). Patchy distribution in parasites can be influenced by host susceptibility (Poulin 2013), and variation in host susceptibility to red algal parasite infection is known in hosts (Zuccarello & West 1994a; b). Patchy distribution was also observed in the parasite *V. aterrimophila* with one local host population being infected and only a few other infected hosts collected or observed on vouchers. The majority of red algal parasites only grow on one or two host species (Chapter 2) but it is not completely known why different populations within a host species are being parasitized and others are not.

In summary, this developmental study documents a new early developmental pathway for a red algal parasite with a localized infection, superficial growth, and a prominent 'trunk-like' cell. Developmental patterns in red algal parasites are varied as are the phylogenetic relationships to their hosts. Further studies are needed before any generalization can be made. In particular, the parasites' nutritional requirements and nutritional independence from their hosts needs further study.

Chapter Five

High mutation rates in a non-photosynthetic plastid hides phylogenetic relationships in the red algal parasite *Pterocladiophila hemisphaerica*

5.1 Abstract

Red algal parasites are a poorly studied group but are often closely related to their hosts from which they presumably evolved directly, or share recent common ancestor. Parasite host switching is known based on phylogenetic parasite and host relationship, but only within the same algal order. We investigated the pigmented red algal parasite *Pterocladiophila hemisphaerica*, which grows on *Pterocladia lucida* (Gelidiales) and is currently placed in the Gracilariales. Whole organelle genomes (mitochondria, plastid) and a complete nuclear ribosomal cistron were assembled and annotated from Illumina sequencing. Compared to other red algae, the parasite had a similar mitochondrial genome structure, but a highly reduced plastid genome of 68,701 bp, making it the smallest known red algal plastid genome. All genes for photosynthesis and many other functions (e.g, ATP synthesis, biosynthetic processes, cytochrome complex assembly) were missing in the parasite plastid genome. Mitochondrial (mt) and plastid (cp) genome phylogenies placed *Pterocladiophila hemisphaerica* on long branches, either as sister to Ceramiales (mt) or Gracilariales (cp). Further analyses, filtering non-elevated plastid genes grouped the parasite either as sister to the Gracilariales (mt) or Gelidiales (cp) on shorter branches but without support. Nuclear phylogeny grouped the parasite *P. hemisphaerica* as sister to the Gelidiales and other red algal orders and was the only phylogenetic relationship with support, indicating that the parasite might have evolved on one of these red algal orders. Large data sets of genes and genomes, under differential selection pressures, could lead to incorrect relationships if not analysed carefully and checked with other biological data.

5.2 Introduction

High-throughput-sequencing (HTS) has become a useful tool to resolve phylogenetic relationships (e.g., Díaz-Tapia *et al.* 2017; Verbruggen *et al.* 2017). Incorrect phylogenetic relationships can be produced even when larger data sets (e.g., HTS data) are being used (Shen *et al.* 2017). Some incorrect relationships can be influenced by long branch attraction (LBA), where fast-evolving taxa group together without reflecting their phylogenetic relationship (Felsenstein 1978; Bergsten 2005), for example, parasitic taxa are 'attracted' to unrelated taxa and not their closest free living relatives (Morin 2000; Evans *et al.* 2008). LBA was first demonstrated theoretically (Felsenstein 1978) and later shown in several studies using amino acid sequences (e.g., Moreira *et al.* 2000; Springer *et al.* 2001; Brinkmann *et al.* 2005); nucleotides (e.g., Yamamoto *et al.* 2001; Li *et al.* 2014); individual genes (e.g., Berger *et al.* 2003; Busse *et al.* 2003; Gómez *et al.* 2011); whole organelle genomes (e.g., Li *et al.* 2014) plus different inference methods (Yamamoto *et al.* 2001; Busse *et al.* 2003). It has been shown that filtering genes by clock-likeness can mitigate LBA (Doyle *et al.* 2015) but this method is not commonly used.

Parasitism is the most common form of symbiosis (de Vargas *et al.* 2015). Red algal parasites, red algae parasitic on other red algae, are highly diverse, with over 123 species (Chapter 2) and many independent transitions to the parasitic life style (Goff *et al.* 1996; Salomaki & Lane 2014; Blouin & Lane 2016; Chapter 3). While parasitic red algae are diverse, they are poorly studied.

Red algal parasites mostly infect members of the same family (Goff *et al.* 1996; Preuss & Zuccarello 2014; Chapter 2) or occasionally different families within the same order, such as *Harveyella mirabilis* (Reinsch) F.Schmitz *et* Reinke (Rhodomelaceae, Ceramiales) growing on *Gonimophyllum skottsbergii* Setch. (Delesseriaceae, Ceramiales) (Zuccarello *et al.* 2004). The close relationships between many red algal parasites and their hosts led to the proposition that red algal parasites evolved from their host (Setchell 1918; Goff *et al.* 1997). Phylogenetic analyses demonstrated that some parasites and their host are more closely related to each other than to other species in the same host genus (Goff *et al.* 1997; Zuccarello *et al.* 2004; Chapter 3), whereas other parasites are more distantly related to their host species, possibly due to host switching (Zuccarello *et al.* 2004; Kurihara *et al.* 2010; Chapter 3).

Red algal parasites exhibit reduced and unique morphological characters and therefore parasite origin and taxonomic placement can only accurately be determined by molecular methods. The parasite *Benzaitenia yenoshimensis* Yendo was grouped with different genera (*Bostrychia*, *Laurencia*, *Levringiella*) in the Rhodomelaceae (Kylin 1956; Morril 1976a) until nuclear rDNA analysis showed a close relationship of *B. yenoshimensis* with its host in the Chondrieae (Kurihara *et al.* 2010). The taxonomic placement of many other parasites has been changed based on molecular phylogenetic data (e.g., Zuccarello *et al.* 2004).

The pigmented red algal parasite *Pterocladiophila hemisphaerica* K.C.Fan *et* Papenf. grows on *Pterocladia lucida* (R.Brown *ex* Turner) J.Agardh in New Zealand (Fan & Papenfuss 1959; Chapter 2) which is currently split into three cryptic species in New Zealand (Boo *et al.* 2015). The parasite was first placed tentatively in the Cryptonemiales (now split into: Ceramiales, Corallinales, Gigartinales (majority), Halymeniales and *Hildenbrandia*), presumably because the parasite has zonately divided tetrasporangia (Fan & Papenfuss 1959). Later, *P. hemisphaerica* was transferred into the Gracilariales based on shared characters with the two parasites *Holmsella* and *Gelidiocolax*, which were simultaneously placed into a parasitic family, Pterocladiophilaceae (Fredericq & Hommersand 1990).

Red algal parasites exhibit a unique organelle transfer mechanism of infection through secondary pit connections (Goff & Coleman 1984; 1985), leading to cells containing components of both cell types ("heterokaryons"). Some studies suggested that the heterokaryon transformed into a parasite cell, reducing host nuclei but keeping host plastids (Goff & Zuccarello 1994; Goff & Coleman 1995). Newly formed parasite cells would then produce reproductive structures that contained parasite nuclei but host plastids (Goff & Coleman 1995). While evidence suggested that parasites only contained host plastids (Goff & Coleman 1995; Zuccarello *et al.* 2004), a reduced plastid of 90,243 bp, i.e. lacking all photosynthetic genes, was found in the parasite *Choreocolax polysiphoniae* Reinsch (Salomaki *et al.* 2015), the so called 'ghost plastid'. Currently, it is unknown if a highly reduced plastid is present in other red algal parasites.

This is the first study investigating the phylogenetic relationship of a red algal parasite using organelle genomes. HTS data were used to investigate if *Pterocladiophila hemisphaerica* has a highly reduced plastid genome. Organelle (plastid, mitochondria) genomes were compared between host and parasite to characterise their gene and functional similarities. Organelle

phylogenies were also compared to nuclear ribosomal RNA relationships to determine the relationships produced by these three genetic regions.

5.3 Materials and Methods

Specimens of *Pterocladia lucida* and its parasite *Pterocladiophila hemisphaerica* were collected from shore (drift) at Akitio Beach (40° 37' 25" S, 176° 24' 39" E) in November 2011 and Kairakau Beach (39° 56' 30" S, 176° 55' 50" E) in May 2013, or by scuba in August 2016 at Princess Bay, Wellington, New Zealand (41° 20' 46" S, 174° 47' 26" E). Drift specimens were dried in silica gel and scuba collections were freshly ground in liquid nitrogen and used for genomic sequencing.

Parasite pustules were cut off at the base with as much distance from the host parasite contact area as possible. All samples were extracted using a modified CTAB protocol (Zuccarello & Lokhorst 2005). Extracted DNA was used to amplify partial *cox*1, LSU and SSU rDNA following established PCR conditions, purification and sequencing (Chapter 3). *cox*1 sequences were used to identify clades of *Pterocladia* host species in New Zealand (Boo *et al.* 2015).

Library preparation and sequencing for the parasite *Pterocladiophila hemisphaerica* (n=~100) and one uninfected specimen of *Pterocladia lucida* (host, n=1) were performed separately using Illumina TruSeq DNA nano by Macrogen Inc. (Seoul, Korea). Libraries of 350 bp were sequenced using a HiSeq 2500 with read lengths of 101 bp and paired ends. Sequenced reads were trimmed with CLC Genomic Workbench 7.5.1 (CLC bio, Aarhus, Denmark) with a quality threshold of 0.05. *De novo* assembly in CLC and SPAdes 3.8.1 (Nurk *et al.* 2013) were performed using automatic k-mer size and default parameters. Plastid, mitochondrial and nuclear contigs were identified with blastx searches against a custom-build database containing known Florideophyte genes. Long contigs identified as mtDNA and cpDNA were imported into Geneious 8.0.5 (Kearse *et al.* 2012). Different assemblers gave similar results but showed slight differences in lengths and further analysis was continued with SPAdes assemblies. Organelle genome circularity was manually checked by mapping 1000 bp of the start and end sequences of the SPAdes contigs against the CLC scaffold.

Gene prediction was carried out in MFannot (http://megasun.bch.umontreal.ca/cgi-bin/mfannot/mfannotInterface.pl) and tRNA prediction in ARAGORN (http://130.235.46.10./ARAGORN/), manually checked and annotated in Geneious. Open reading frames (ORF) were used to identify missing genes and were manually annotated. Previously partial amplified nuclear genes of *Pterocladiophila hemisphaerica* and *Pterocladia lucida* were blast searched against contigs identifying whole SSU rDNA and LSU rDNA sequences and confirming identical overlapping sequences. RNAmmer Prediction server (Lagesen *et al.* 2007) was used to predict the beginning and end of genes. Biological functions of protein coding genes were determined in UniProt (http://uniprot.org) and conserved domains blasted against the NCBI site (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

Progressive Mauve alignment was used to compare plastid genomes using the full alignment option with default seed weight and automatically determined locally collinear blocks (LCB) score (Darling *et al.* 2004).

Taxon selection for phylogenetic analysis was based on available data for mitochondrial and plastid genomes in the subclass Rhodymeniophycidae with the Corallinales as outgroup, following Verbruggen *et al.* (2010) (Appendix 5.1). All protein coding genes were translated into amino acid sequences. MAFFT alignments v1.3.3 in Geneious were used for nuclear rRNA genes and translatorX v1.1 (Abascal *et al.* 2010) for mitochondrial and plastid protein-coding genes. Filtered genes were trimmed using the best automated method in trimAl (http://trimal.cgenomics.org) for nuclear rRNA genes or GBlocks (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) for organelle protein coding genes.

IQ-Tree (Trifinopoulos *et al.* 2016) was used to construct maximum-likelihood (ML) trees, for trimmed nuclear rRNA using automated substitution models (28S: TVMe+I+G4, 18S: TIM3+F+I+G4) and 1000 ultrafast bootstraps. All amino acid alignments of concatenated mitochondrial and plastid genes were run in RAxML (Stamatakis 2006) to construct maximum-likelihood (ML) trees, using a cpRev model and bootstrap. Phylogenetic analyses for *cox*1 were performed following Chapter 3.

The inconsistent phylogenetic position of *P. hemisphaerica* and the observation of long branches led to further analyses in which genes with elevated rates of evolution in *P. hemisphaerica* were removed. Elevated gene rates in *P. hemisphaerica* were calculated by using the ratio of uncorrected distances between the outgroup (*Calliarthron*) and *P. hemisphaerica* and between an ingroup (mitochondria: *Schimmelmannia*, plastid: *Caloglossa*) and *P. hemisphaerica*. Any genes exceeding a pre-defined threshold (mitochondria: 0.9, plastid: 1.2) were removed. The red algal parasite *Choreocolax polysiphoniae* was also removed to avoid long branch attraction by this taxon. RAxML was used to infer ML trees on filtered data sets using cpRev model and 100 bootstrap.

5.4 Results

Plastid genome

The circular mapping plastid genome of *Pterocladiophila hemisphaerica* is highly reduced, consisting of only 68,701 bp (Fig. 5.1). The plastid contains only 70 genes without any photosynthesis and ATP synthesis genes (Fig. 5.1, Table 5.1) but many genes for genetic systems, metabolism, ribosomal proteins and transport are still present (Appendix 5.2). The plastid genome is densely packed with only 13% non-coding regions and all protein coding genes (67.5-85.7% AT content), rRNAs (63.8-67.5%) and tRNAs (47.3-73.0%) show an A-T bias. The rRNA 5S gene (rrn5) is also missing from the parasite plastid genome (Appendix 5.2). The parasite plastid contains several ORFs not found in the host or other red algae: orf114 has a ribosomal protein L22 conserved domain, orf151 with a N-terminal reserve transcriptase domain and orf407 is without any conserved domains. The host, *Pterocladia lucida*, has a standard red algal plastid genome size (176,635 bp) and organization (Appendix 5.3), and shares many genes with other free-living red algae (n=184, Fig. 5.1). In comparison to its host, the plastid genome of *Pterocladiophila hemisphaerica* has fewer protein coding genes, tRNAs and rRNAs (Fig. 5.1, Appendix 5.3).

The parasites *Pterocladiophila hemisphaerica* and *Choreocolax polysiphoniae* have in common a highly reduced plastid genome, but it is more reduced in *P. hemisphaerica* (68,701 versus 90,243 bp) with a core of shared genes (n=56) and a few unique genes (Fig. 5.1). Among the 10 genes that *P. hemisphaerica* shares with the two free-living species (*Pterocladia lucida*, *Vertebrata lanosa* (L.) T.A.Chr.) are genes for transport and fatty acid biosynthesis whereas the

9 genes found in the parasite *C. polysiphoniae* and the two free-living species are genes for ATP synthesis, biosynthetic processes and RNA processing. *P. hemisphaerica* and its host *Pterocladia lucida* share one gene for ribonuclease and *C. polysiphoniae* and its host *Vertebrata lanosa* share one gene for translation (Fig. 5.1). In comparison to their host species and each other, there is significant gene rearrangement in the plastid genomes of the parasites (Appendix 5.4).
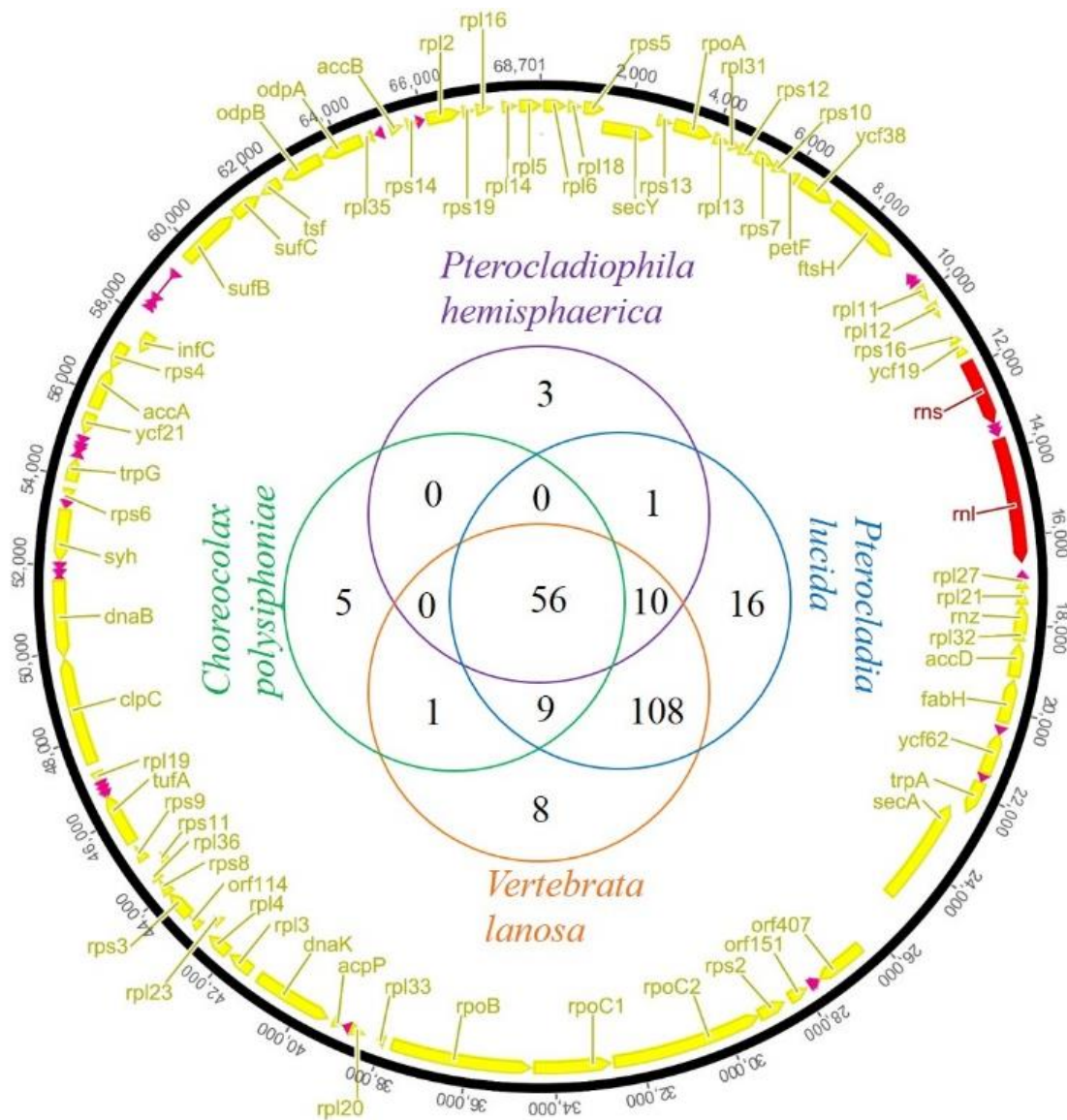
Fig. 5.1. The plastid genome of the parasite *Pterocladiophila hemisphaerica* with 70 protein coding genes (yellow), 2 rRNA's (red) and 26 tRNA's (pink). Venn diagram represents the unique and shared genes between the parasite *Pterocladiophila hemisphaerica* and its host *Pterocladia lucida* and the parasite *Choreocolax polysiphoniae* and its host *Vertebrata lanosa*.

Table 5.1. Whole plastid genome size in the parasite *Pterocladiophila hemisphaerica* and its host *Pterocladia lucida*, plus the parasite *Choreocolax polysiphoniae* and its host *Vertebrata lanosa* (Salomaki *et al.* 2015). Length, AT content, number of protein coding genes, tRNA's, rRNAs and total number of genes. - = missing data.

	pDNA size (bp)	AT content (%)	Protein coding genes	tRNA	rRNA	Total
Pterocladiophila hemisphaerica	68,701	74.2	70	26	2	98
Pterocladia lucida	176,635	70.3	200	30	3	233
Choreocolax polysiphoniae	90,243	79.5	71	24	3	98
Vertebrata lanosa	167,158	70.0	192	27	3	222

Mitochondrial genome

The circular mapping mitochondrial genome of *Pterocladiophila hemisphaerica* is 25,486 bp long (Appendix 5.5). The mitochondrial genome contains 24 protein coding genes and is similar to the mitochondrial genome of its host *Pterocladia lucida* (cryptic species Group II, Table 5.2, Appendix 5.5-5.7). The parasite mitochondrial genome is extremely densely packed with less than 8% non-coding regions and a high A-T content in all protein coding (70.7-89.1% AT content), rRNAs (59.5-73.7%) and tRNAs (71.7-79.1%) genes.

Table 5.2. Whole mitochondrial genomes of *Pterocladiophila hemisphaerica* and *Pterocladia lucida*. Length, AT content, number of protein coding genes, tRNAs, rRNAs and total number of genes.

	mtDNA size (bp)	AT content (%)	Protein coding genes	tRNA	rRNA	Total
Pterocladiophila hemisphaerica	25,486	77.5%	24	24	3	51
Pterocladia lucida	25,257	70.4%	24	23	3	50

Phylogenetic relationships of Pterocladiophila hemisphaerica

Nuclear DNA

The concatenated nuclear alignment (LSU and SSU rDNA) contained 226 taxa and was 3,611 bp long containing *Pterocladiophila hemisphaerica* and *Pterocladia lucida* and representatives of other Florideophyceae. A strongly supported ML topology showed *Pterocladiophila hemisphaerica* as sister to the Gelidiales and other red algal orders (Fig. 5.2).
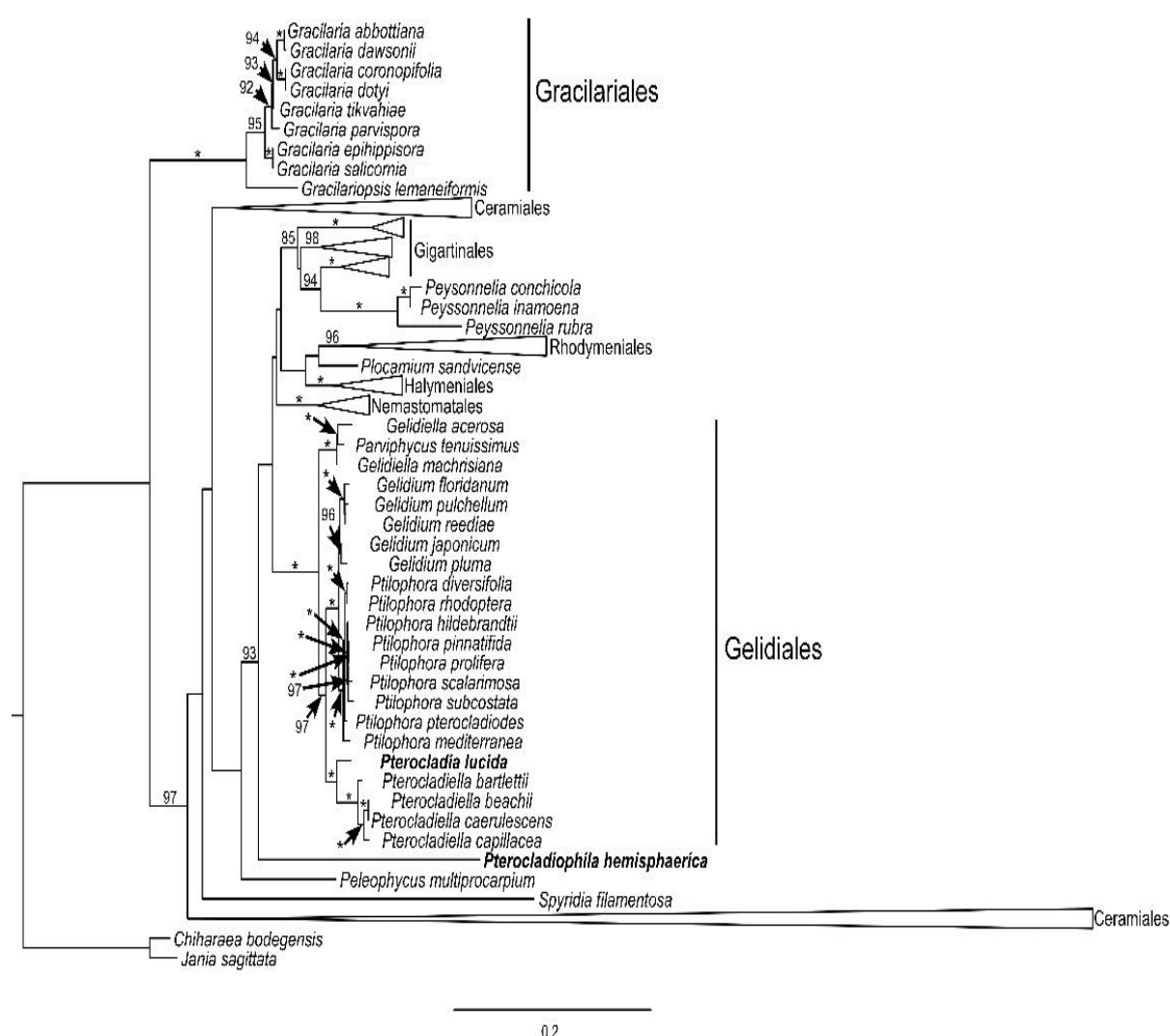
Fig. 5.2. ML topology of concatenated LSU and SSU rDNA sequence data set for the parasite *Pterocladiophila hemisphaerica* and its host *Pterocladia lucida* plus representatives of Gelidiales, Ceramiales, Gracilariales and other related taxa from GenBank (Appendix 5.1). *Jania sagittata* and *Chiharaea bodegensis* were used as outgroups. Asterisks indicate ultrafast ML bootstrap values of 100%. Values <85% ultrafast ML bootstrap not shown. *P. hemisphaerica* groups as sister to the Gelidiales and other red algal orders.

mtDNA

The concatenated mitochondrial data set contained 43 taxa and was 6301 amino acids long with all protein coding genes included. ML topology showed an unsupported relationship for *Pterocladiophila hemisphaerica* on a long branch as sister to the Ceramiales (Fig. 5.3).

After the removal of mitochondrial genes with elevated rates, the remaining data set consisted of 7 genes (1,846 amino acids) and 43 taxa. *P. hemisphaerica* grouped in an unsupported relationship sister to the Gracilariales (Fig. 5.4).
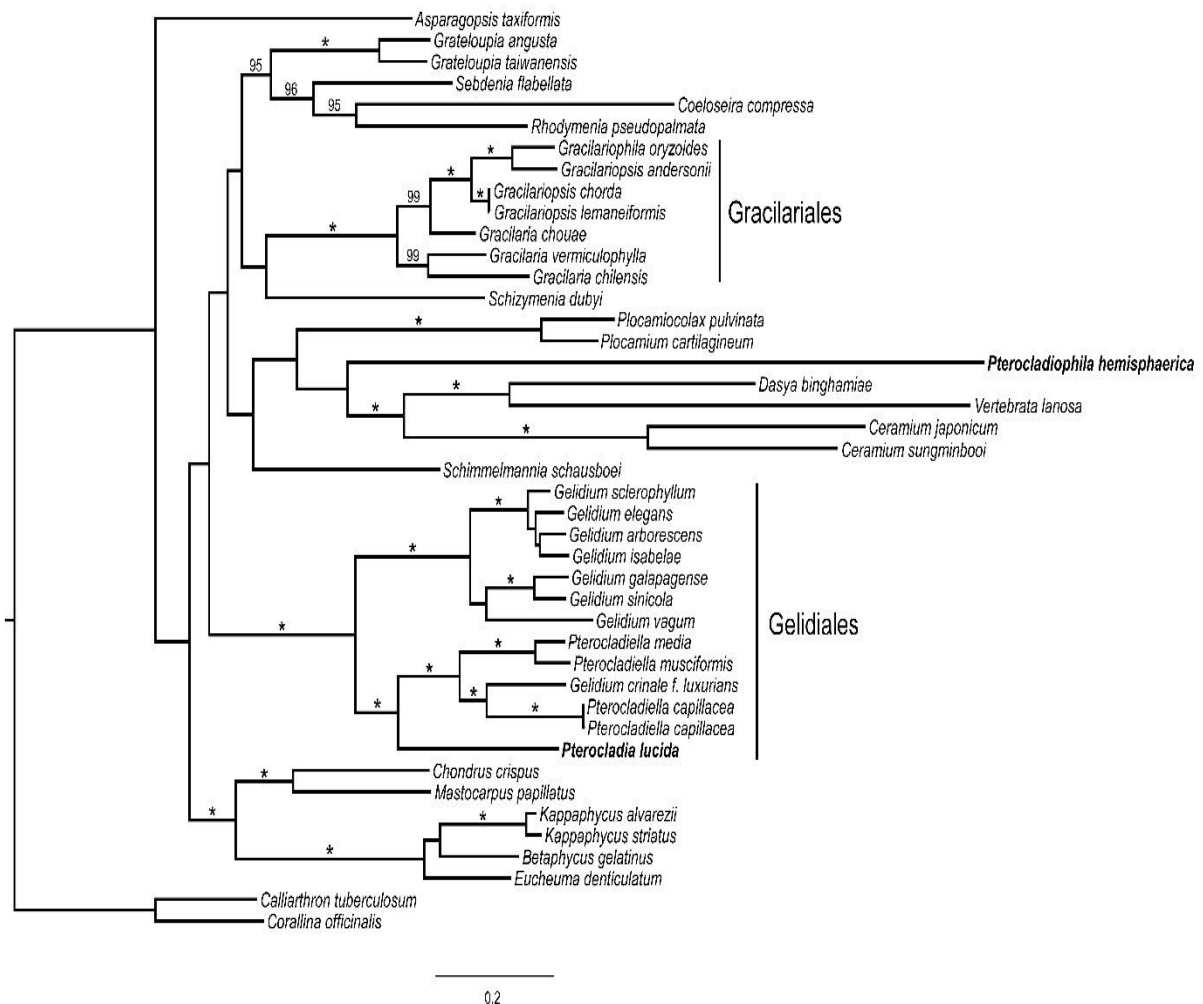
Fig. 5.3. ML topology of all concatenated mitochondrial protein coding genes of the parasite *Pterocladiophila hemisphaerica* with its host *Pterocladia lucida* plus representatives of other red algal taxa including members of the Gelidiales and Gracilariales. *Calliarthron tuberculosum* and *Corallina officinallis* were used as outgroups. Asterisks indicate fast ML bootstrap values of 100%. Values <85% fast ML bootstrap not shown. *Pterocladiophila hemisphaerica* groups unsupported as sister to the Ceramiales on a long branch.

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cpDNA

The concatenated plastid data set contained 82 taxa and was 55,461 amino acids long containing *Pterocladiophila hemisphaerica*. The ML topology showed an unsupported relationship of *Pterocladiophila hemisphaerica,* on a very long branch, as the sister lineage of Gracilariales (Fig. 5.5).

After the removal of plastid genes with elevated rates, the remaining data set consisted of a total of 158 genes with only 8 genes found in *Pterocladiophila hemisphaerica* (38,657 amino acids) and 82 taxa. In phylogenetic analyses of this data set, *Pterocladiophila hemisphaerica* grouped in an unsupported relationship sister to the Gelidiales (Fig. 5.6).
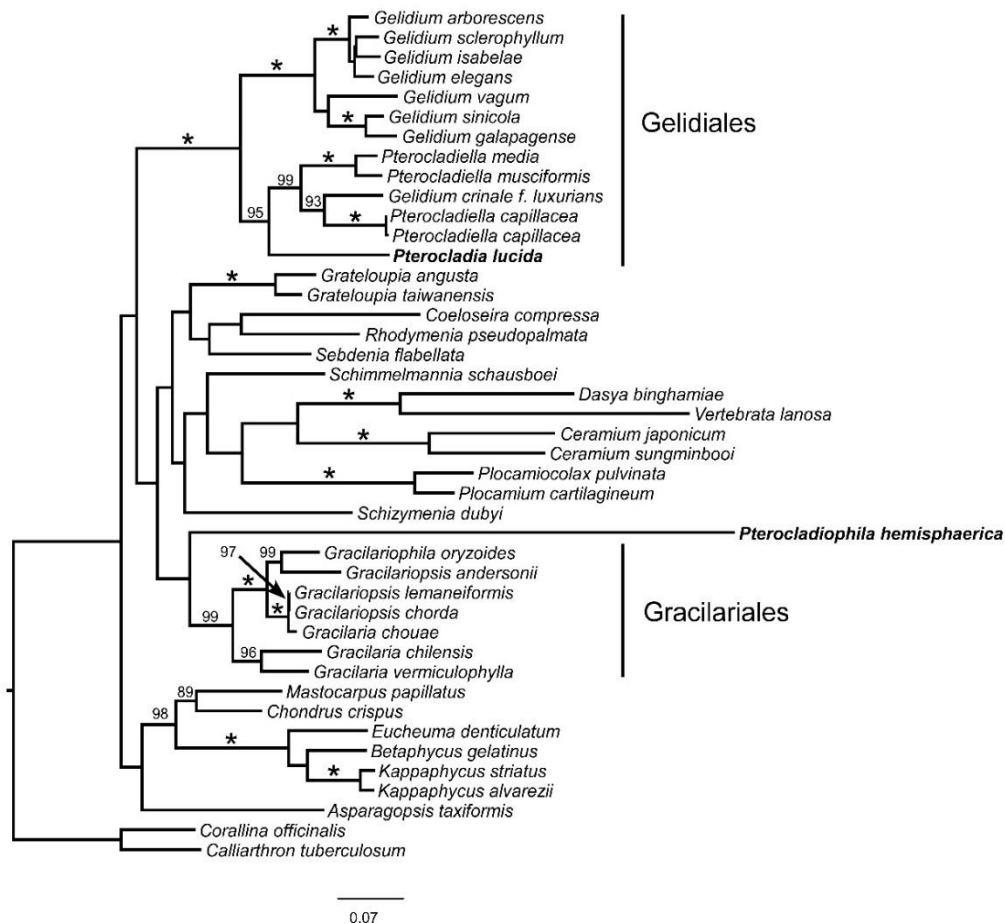
Fig. 5.4. ML topology of 7 trimmed mitochondrial genes without elevated rates of the parasite *Pterocladiophila hemisphaerica* shared with its host *Pterocladia lucida* plus representatives of other red algae taxa including members of the Gelidiales, Gracilariales and Ceramiales. *Calliarthron tuberculosum* and *Corallina officinallis* were used as outgroups. Asterisks indicate ML bootstrap values of 100%. Values <85% ML bootstrap not shown. *Pterocladiophila hemisphaerica* groups unsupported as a sister to the Gracilariales.

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Host organelle genomes in parasite data set

Host mtDNA, nDNA and cpDNA were identified within the HTS data of the parasite tissue. The overlap and resolution were high enough to assemble and annotate whole plastid and mitochondrial genomes of the host. Host organelle genomes sequenced separately, were almost identical to host contigs derived from parasite HTS data (3 bp difference each).
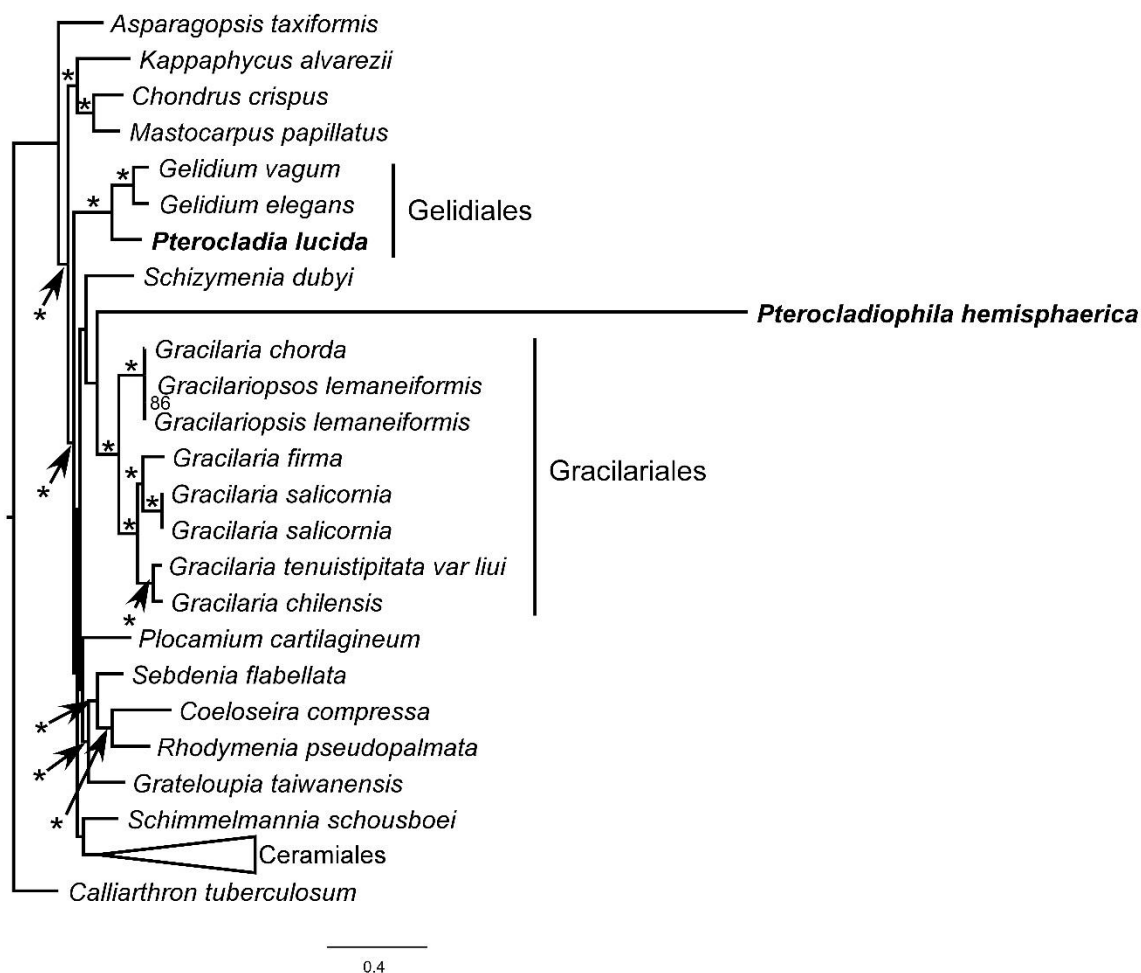
Fig. 5.5. ML topology of all concatenated plastid genes of the parasite *Pterocladiophila hemisphaerica* shared with its host *Pterocladia lucida* plus representatives of other red algal taxa including member of the Gelidiales, Gracilariales and Ceramiales. *Calliarthron tuberculosum* was used as an outgroup. Asterisks indicate ML bootstrap values of 100%. Values <85% ML bootstrap not shown. *Pterocladiophila hemisphaerica* groups is an unsupported position as sister to the Gracilariales on a long branch.

Host specificity of Pterocladiophila hemisphaerica

Parasites were collected from two host populations (Akitio Beach and Kairakau) belonging to different cryptic species of *Pterocladia lucida* (Boo *et al.* 2015; Appendix 5.8).
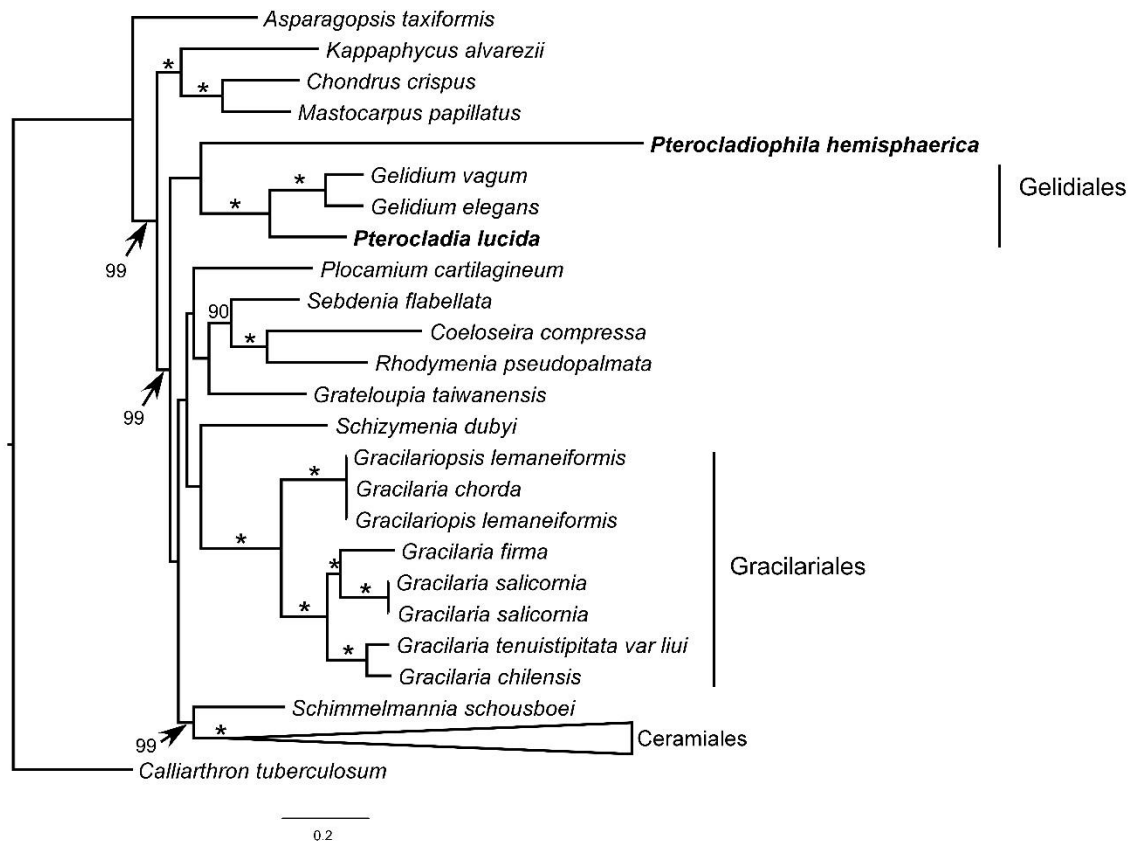
Fig. 5.6. ML topology of a total of 158 trimmed genes with only 8 plastid genes without elevated rates of the parasite *Pterocladiophila hemisphaerica* shared with its host *Pterocladia lucida* plus representatives of other red algae taxa including members of the Gelidiales, Gracilariales and Ceramiales. *Calliarthron tuberculosum* was used as an outgroup. Asterisks indicate ML bootstrap values of 100%. Values <85% ML bootstrap not shown. *Pterocladiophila hemisphaerica* groups unsupported as a sister to the Gelidiales.

In summary, the parasite *P. hemisphaerica* has a highly reduced plastid genome and a similar mitochondrial genome in comparison to its host. Nuclear rDNA phylogenetic relationships indicate that *P. hemisphaerica* does not belong to the Gracilariales but more likely to the Gelidiales (possibly sister to all included Gelidiales species and other included red algal orders). Organelle genome data sets show different phylogenetic relationship of the parasite without support. The parasite is always on long branches but removal of genes with elevated rates increase branch length.

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5.5 Discussion

This is the first study investigating plastid and mitochondrial genomes between parasite and host, and is only the second report of a full red algal parasite plastid genome. For the first time, phylogenetic relationships of a red algal parasites and their host were studied using complete organelle data sets, and our results highlight the importance of careful interpretation of phylogenetic analyses that can be affected by long branch attraction.

The evolution from a free-living ancestor to a parasite has led to a highly reduced plastid genome in *P. hemisphaerica*. This has been found in only one other red algal parasite, *Choreocolax polysiphoniae* (Salomaki *et al.* 2015), but is a characteristic of many unpigmented parasitic plants (Bungard 2004; Krause 2008; Bellot *et al.* 2016). While reduced, the plastid genomes of *P. hemisphaerica* and *C. polysiphoniae* share the majority of protein coding genes, with a similar complement of genes lost, mostly photosynthesis-related genes. Studies of parasitic plants with different degrees of photosynthetic ability showed that rearrangements and gene deletion similarities and difference can be traced between taxa (Wicke *et al.* 2013; Ravin *et al.* 2016; Frailey *et al.* 2018). Our study showed that it is possible to successfully reconstruct host organelles from a 'parasite' data set and this technique might help to increase sampling. Host cells are often embedded in parasite thalli (Goff 1976; Goff & Coleman 1987; Preuss & Zuccarello 2014; Chapter 3) plus the parasite may contain heterokaryotic cells containing both host and parasite genomes (Goff & Coleman 1995; Blouin & Lane 2016). Increased taxon sampling of red algal parasites, with different relationships to their hosts, will show if there is any pattern in gene loss/rearrangement in red algal parasite evolution.

A reduced plastid genome is not surprising in an unpigmented parasite like *C. polysiphoniae* but a highly reduced plastid genome is found in pigmented *P. hemisphaerica*. This indicates that pigmented parasite cells rely on host plastids for pigment production and possibly photosynthesis. Whether these host plastids are supplied with protein subunits from parasite nuclear-encoded genes or rely on host nuclear genes, residing in heterokaryotic cells (Zuccarello & West 1994a; Goff & Coleman 1995; Blouin & Lane 2016), is unknown. The evolutionary distance between the parasite, sister to all Gelidiales, and its *Pterocladia* host, may make protein interactions in oligomers problematic, as has been shown during hybrid breakdown between species crosses due to mitochondria-nuclear incompatibilities or plastid-nuclear incompatibilities (Ellison & Burton 2008; Zeng *et al.* 2016).

The mitochondrial genome of *P. hemisphaerica* is highly conserved in size, architecture and gene number, similar to its host and other Florideophyceae (Yang *et al.* 2015; Salomaki & Lane 2017). Reduction of mitochondrial genomes is known for some parasitic taxa such as the apicomplexan *Plasmodium* (Feagin 2000), parasitic plants (Skippington *et al.* 2015) and the diplomonad *Giardia intestinalis* (Jedelský *et al.* 2011); these studies stand in contrast to the full complement of genes in the mitochondrial genome of *P. hemisphaerica*. The conservation of mitochondrial genome architecture between *P. hemisphaerica* and other red algae would indicate that they are under similar evolutionary constraints, in contrast to the plastid. And yet our analysis of full mitochondrial data sets does show elevated mutation rates, and contrasting phylogenetic placement, between the parasite and nearly all other red algal taxa. Whether this is due to selective changes during parasitism, e.g., associated with lower energy requirements in heterokaryon cells, and/or changes in error rates from suboptimal oligomer repair complexes, is not known.

Currently, the parasite *Pterocladiophila hemisphaerica* is placed with two other parasite genera (*Holmsella* and *Gelidiocolax*) in the Gracilariales as these parasites share the morphological characteristics of a 2-celled carpogonial branch, straight spermatangial chains and transverse divisions of spermatangial parent cells (Fredericq & Hommersand 1990). The placement of *Holmsella* spp. within the Gracilariales was confirmed with a nuclear DNA marker (Zuccarello *et al.* 2004). Our nuclear data set indicates that *P. hemisphaerica* is not part of the Gracilariales but shares a sister relationship with several orders, including the Gelidiales (order of host species). All known red algal parasites infect only species in the same order (Goff 1982; Goff *et al.* 1996; Zuccarello *et al.* 2004; Kurihara *et al.* 2010; Chapter 3) and this would support the placement of *P. hemisphaerica* with the Gelidiales. This distance between parasite and host could be due to an early divergence of the parasitic lineage before present day Gelidiales diversification, suggesting that this could be an ancient parasitism. Further studies of the complete nuclear genomes may support the placement of the parasite with the Gelidiales.

Organelle genome data could not resolve the taxonomic position of *P. hemisphaerica* and always placed the parasite on unsupported long branches with a variety of red algal orders. Our plastid data set grouped *P. hemisphaerica* as an unsupported sister with the Gracilariales on a long branchand after removal of plastid genes with elevates rates grouped the parasite still unsupported with the Gelidiales. The full mitochondrial data set grouped *P. hemisphaerica*

unsupported with the Ceramiales on a long branch and also after removal of mitochondrial genes with elevated rates. The lack of resolution in both organelle data sets, influenced by spurious signals, even with filtered genes, demonstrates that phylogenies cannot always provide reliable placement of these red algal parasites

Our study shows that phylogenetic results from whole organelle genome data sets need to be carefully interpreted. Determining parasite origin from genomes that have high evolutionary rates or are under different selection regimes, could lead to incorrect relationships. The unique morphological characters of *P. hemisphaerica* have always caused uncertainty in its taxonomic placement (Fan & Papenfuss 1959), and its placement in the Gracilariales and family Pterocladiophilaceae, was mostly due to general characters and the fact that other parasites were placed there (Fredericq & Hommersand 1990). Our nuclear data indicates that the parasite did not evolve in the Gracilariales but its taxonomic placement is still uncertain. *P. hemisphaerica* might have evolved in the Gelidiales, one of the other red algal orders such as Gigartinales, or in an early divergent lineage before the present day Gelidiales and/or of the other red algal orders.

Chapter Six

Comparative studies of photosynthetic capacity in three pigmented red algal parasites using chlorophyll *a* concentrations and PAM fluorometry

6.1 Abstract

Over 100 species of red algae have been described as parasites on other red algae, but the majority show some degree of pigmentation. This raises the question of their parasitic status, especially their abilities to photosynthesize and their dependence on their host for fixed carbon. Are they considered parasites only based on morphological characters, for example, reduced size and secondary pit connection to the host? Translocation of nutrients from host to parasite have been shown for very few red algal parasites, and these were mostly unpigmented. This study investigated three pigmented red algal parasites (*Rhodophyllis parasitica*, *Vertebrata aterrimophila* and *Pterocladiophila hemisphaerica*) from New Zealand. We quantified their chlorophyll *a* content and also measured their PSII capacity using PAM fluorometry. All three parasites contained chlorophyll *a*. The parasites *Rhodophyllis parasitica* and *Vertebrata aterrimophila* were not able to photosynthesize and must therefore be fully nutriential dependent on their host. The parasite *Pterocladiophila hemisphaerica* was able to photosynthesize independently, but based on molecular characteristics we suggest that it relies on the host plastid to carry out photosynthesis. Our results support the parasitic status of all three species and highlights the necessity of more studies investigating the differences in host dependency in red algal parasites.

6.2 Introduction

Determining the symbiotic status of organisms (e.g., commensalism, mutualism, parasitism) or endo- and epiphytism, is challenging. In a parasitic relationship, one organism benefits while the other organism is harmed (Price 1980), but this can change during the course of the symbiotic interaction (Neuhauser & Fargione 2004). Parasites and endo-/epiphytes can both be host specific (Goff 1982; González & Goff 1989; Reif *et al.* 2005; Gauna & Parodi 2008) and therefore rely on the host for habitat, even though endo-/epiphytes are able to grow separately from the host in culture (González & Goff 1989; Notoya & Miyashita 1999; Gauna & Pant; Pant & Thapa 2012). In some cases, parasitic plants are also able to be cultivated without their hosts (Furuhashi 1991). Unpigmented algae can be considered parasites, as their only source of nutrition is from their host, but to distinguish parasitism from other symbiotic relationships, a negative host effect also needs to be shown. The classification of pigmented algae, which appear to have host dependency is even more problematic.

Parasitism has been invoked in many red algae, with over 100 parasite species described (Chapter 2). The majority of red algal parasites are taxonomically closely related to their host species (Goff 1982) with a continuum to distantly related host species (Zuccarello *et al.* 2004; Blouin & Lane 2012; Chapter 3). Parasites can have either a plastid captured from their hosts (Goff & Coleman 1995; Goff *et al.* 1996), their own plastid comparable in size and genetic composition with other non-parasitic red algae, or a highly reduced plastid genome with few or no photosynthesis gene remaining (Salomaki *et al.* 2015).

Early description of these organisms classified them as parasites (Reinsch 1875; Schmitz & Falkenberg 1897). Later, the characteristics of reduced size, deep host penetration and reduced pigmentation, were used for morphological descriptions of their parasitic status (Setchell 1918). More recently the presence and absence of secondary pit connections between parasite and host cells was considered an important character to infer parasitic status (Chapter 2). A majority of red algal parasites are also pigmented (Chapter 2) and this pigmentation calls into question their parasitic status.

Negative effects on host cells and host fitness by parasite infection is only known in a few red algal parasite species. These negative effects range from degradative changes in infected host cells (Goff 1982; Apt 1984a), loss of cell cycle regulation (Goff 1976; Goff & Coleman 1985) and infection spreading to surrounding host cells (Goff & Coleman 1995). Studies showing nutrient transfer to red algal parasites are rather limited and focus mainly on unpigmented species. Experiments with $^{14}CO_2$ showed nutrient translocation from host to parasites (Harlin 1973; Callow *et al.* 1979). The translocation of nutrients was also shown into the parasite *Harveyella mirabilis* (Reinsch) F.Schmitz *et* Reinke from the cortical host cell via the contact area between host and parasite thalli (Goff 1979; Kremer 1983).

Pulse amplitude modulated fluorometry (PAM) is a non-invasive tool to relate chlorophyll fluorescence to photosynthesis (Parkhill *et al.* 2001; Murchie & Lawson 2013). Estimates of optimal photochemical efficiency of PSII (effective quantum yield, $\Delta F/Fm'$, light adapted) and photosynthesis potential of PSII (maximum quantum yield, Fv/Fm, dark adapted) are commonly used to show photoinhibition and stress (Kromkamp & Forster 2003; Murchie & Lawson 2013). Red algae in culture commonly have Fv/Fm values of around 0.5-0.6 (e.g., Figueroa *et al.* 1997; Bischof *et al.* 2000; Lüder *et al.* 2001; Liu & Pang 2010). Photosynthetic ability has been demonstrated by measuring Fv/Fm in pigmented parasitic land plants (Strong 2000; van der Kooij *et al.* 2000) but not in red algal parasites.

The majority of red algal parasites in New Zealand are pigmented (10 of the 13 described species). The parasite *Rhodophyllis parasitica* M.Preuss *et* Zuccarello is lightly pigmented and found on its closest relative *Rhodophyllis membranacea* (Harv.) Hook.f. *et* Harv. (Preuss & Zuccarello 2014), *Vertebrata aterrimophila* M.Preuss *et* Zuccarello is unpigmented to dark brown and found on the host *Vertebrata aterrima* (Hook.f. *et* Harv.) Kuntze (Chapter 4), and *Pterocladiophila hemisphaerica* K.C.Fan *et* Papenf. is dark red and found on its host *Pterocladia lucida* (R.Br.) J.Agardh (Fan & Papenfuss 1959).

This study investigates the chlorophyll *a* concentration as well as Fv/Fm and $\Delta F/Fm'$ quantum yield of PSII in these three pigmented parasites and their hosts to investigate their ability to photosynthesize away from their hosts and to provide more understanding of their parasitic status.

6.3 Materials and Methods

Specimens of *Rhodophyllis parasitica* were collected in January, *Vertebrata aterrimophila* in September 2017 at Moa Point (41° 20' 30" S, 174° 48' 38" E) and *Pterocladiophila hemisphaerica* in June 2017 at Princess Bay (41° 20' 46" S, 174° 47' 26" E) from shore or by SCUBA in Wellington, New Zealand. Fresh specimens were transported in an ice chest in seawater to the laboratory and sorted.

Wet weight of algal tissue was determined by measurement of 1.5 ml tubes with and without blotted algal tissue. Tissue was then ground in 0.5 ml of 100% ethanol in 1.5 ml tubes and transferred to glass tube with 9.5 ml of 100% ethanol. Glass tubes were fully covered with aluminium foil and left for 24 hours in the dark and at 4°C. 3 ml of each sample was measured twice (300-650nm) with an AU-10 Fluorometer (Turner Designs, Sunnyvale, California). A second measurement was done after adding 250 µl of 1M HCl. The difference between the two measurements gives the chlorophyll *a* concentration (Strickland & Parsons 1972).

Parasites of similar size were removed from the surface of the host using a razor blade. The parasite and one piece of uninfected host tissue were placed separately in 6-well plates with sterile seawater (salinity approximately 33). Triplicates of removed parasites and uninfected host were used to measure Fv/Fm and ΔF/Fm' yield of photosystem II at 540 nm using a Multi-Color-PAM (Walz, Effentrich, Germany). The first measurements were taken directly after the parasite was removed from its host (Day 0, 0h, light adapted) and second set of measurements after an overnight dark acclimation period (Day 1, 0h, dark adapted). The third set of measurements were in light at different time intervals (Day 1, 2h, 4h, 6h, 8h) after the dark adapted measurement (light acclimated). All light experiments were performed at 14.5-4.5 µmol photons m^{-2} s^{-1} constant fluorescent light (Spectro sense 2+, Skye, Wales, UK) and 15±1°C. The uninfected host was used as a control following the same procedures.

Statistical analyses of chlorophyll *a* concentration per g and ΔF/Fm' over time involved performing linear mixed effects models using R version 3.2.5 software (R Core Team 2016) and the nlme package (Pinheiro *et al.* 2017). The final model was determined via backwards selection, and significant differences in ΔF/Fm' over time were determined using planned comparisons.

6.4 Results

Fluorometry showed chlorophyll *a* concentrations were significantly different for all host and parasite combinations ($P = 0.0335$). All three parasites have less chlorophyll *a* per g than their host species (Fig. 6.1).

On Day 0, after parasite removal from host, ΔF/Fm' (light adapted) was not measurable in the parasites *Rhodophyllis parasitica* and *Vertebrata aterrimophila*, and was 0.37 ± 0.01 (mean ± S.E.) in *Pterocladiophila hemisphaerica*, whereas ΔF/Fm' in the hosts was 0.44 ± 0.01 in *Rhodophyllis membranacea*, 0.35 ± 0.05 in *Vertebrata aterrima* and 0.45 ± 0.03 in *Pterocladia lucida* (Fig. 6.2).
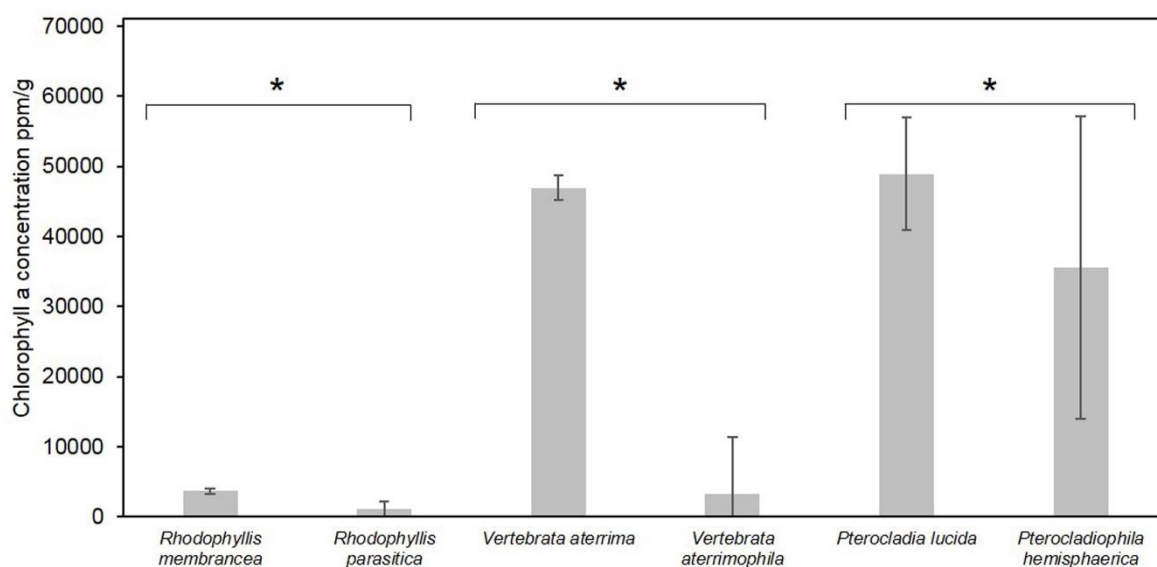
Fig. 6.1. Chlorophyll *a* concentration in parts per millions (ppm) per g in three parasites and their hosts. *Rhodophyllis membranacea* (host) and *Rhodophyllis parasitica* (parasite), *Vertebrata aterrima* (host) and *Vertebrata aterrimophila* (parasite), *Pterocladia lucida* (host) and *Pterocladiophila hemisphaerica* (parasite). Values are means ± S.E. (n=3). Significant differences (asterisk) are found between species and host and parasite combinations ($P = 0.0335$).

On Day 1 (time 0h), Fv/Fm (dark adapted) was not measurable in *R. parasitica* and *V. aterrimophila*, and 0.35 ± 0.02 in *Pterocladiophila hemisphaerica*; and 0.28 ± 0.04 in *R. membranacea*, 0.23 ± 0.06 in *V. aterrima* and 0.36 ± 0.03 in *P. lucida*.

Over an 8h period, $\Delta F/Fm'$ (light adapted) continued to be undetectable in *R. parasitica* and *V. aterrimophila* and was between 0.3-0.4 in their host species (*R. membranacea*, *V. aterrima*, respectively) (Fig. 6.2, Appendix 6.1). Planned comparisons showed $\Delta F/Fm'$ was significantly different between *R. parasitica* and its host ($P < 0.0001$) and *V. aterrimophila* and its host ($P < 0.0001$). $\Delta F/Fm'$ in the parasite *Pterocladiophila hemisphaerica* and its host *Pterocladia lucida* were between 0.35-0.25 and planned comparisons showed $\Delta F/Fm'$ was not significantly different between the parasite and its host ($P = 0.923$) (Fig. 6.2). The overall linear mixed effects model showed significant differences between parasite and hosts ($F_{1,6} = 148.2886$, $P < 0.0001$), between species ($F_{2,6} = 10.5595$, $P = 0.0108$) and between species, host and parasite ($F_{2,6} = 31.6903$, $P = 0.0006$), and no significant differences with time ($F_{1,53} = 0.00643$, $P = 0.9364$).
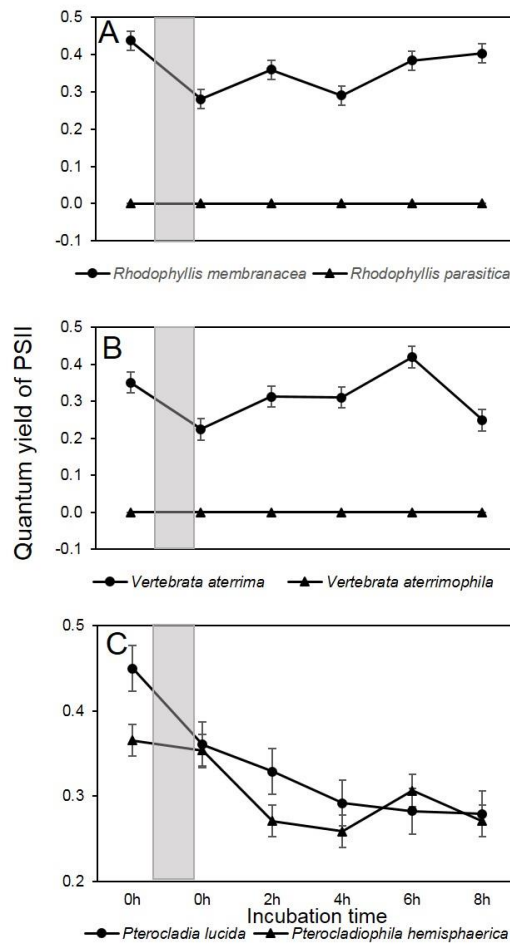
Fig. 6.1A-C. $\Delta F/Fm'$ (Day0, 0h, first points), Fv/Fm (Day1, 0h, dark acclimated), and $\Delta F/Fm'$ values over time (Day1, 2h, 4h, 6h, 8h) in three parasites and their hosts: **A.** *Rhodophyllis membranacea* (host) and *Rhodophyllis parasitica* (parasite), **B.** *Vertebrata aterrima* (host) and *Vertebrata aterrimophila*, **C.** *Pterocladia lucida* (host) and *Pterocladiophila hemisphaerica* (parasite). Values are means ± S.E. (n=3). Significant differences are found between *R. parasitica* and its host *R. membranacea* ($P < 0.0001$) and *V. aterrimophila* and its host *V. aterrima* ($P < 0.0001$). Grey shadow indicates overnight dark acclimation period.

6.5 Discussion

Our study shows that all three pigmented parasites contain chlorophyll *a* but only the parasite *Pterocladiophila hemisphaerica* was able to photosynthesize independently, whereas the parasites *Rhodophyllis parasitica* and *Vertebrata aterrimophila* have lost their photosynthetic ability. *R. parasitica* and *V. aterrimophila* are therefore classified as parasites as they must rely fully on photosynthates from their hosts. The low chlorophyll *a* concentration might indicate the gradually loss of pigments in these parasites, or is caused by embedded host cells in the tissues of some parasitic taxa such as *Rhodophyllis parasitica* (Preuss & Zuccarello 2014). Loss of photosynthetic ability is known from parasitic land plants, without any chlorophyll, such as species of *Orobranche*, but not in species that contain chlorophyll (e.g., *Cuscuta*, Westwood *et. al.* 2010). Red algal species contain additional photosynthetic pigments, such as phycobiliproteins (Hurd et al. 2014), and the presence of these pigments should be investigated in future research.

The parasite *Pterocladiophila hemisphaerica* has a higher chlorophyll *a* concentration than the other parasites and ΔF/Fm' and Fv/Fm of PSII could be measured, demonstrating that *P. hemisphaerica* is able to photosynthesize independently. Photosynthetic ability is not proof that the parasite is self-sufficient and does not gain any photosynthates from the host. Several pigmented parasitic land plants are also able to photosynthesize independently but still gain most of the nutrients from their host (Tesitel *et al.* 2010). Translocation experiments are needed to fully understand the nutrient dependence of *P. hemisphaerica*. While it can photosynthesize *P. hemisphaerica* does show several morphological characteristics that have been used to support its 'parasite' designation, especially secondary pit connection between host and parasite cells (Evans *et al.* 1978), a crucial character to determine parasitic status (Goff & Coleman 1985; Wynne & Scott 1989; Chapter 2).

The photosynthetic ability of *P. hemisphaerica* is unexpected as its plastid genome is highly reduced, with no genes for photosynthetic processes (Chapter 5). This suggests that the parasite either has all photosynthetic genes in its nuclear genome or uses the host plastids. Nuclear genomes of red algal parasites have not been sequenced, but it is likely that the parasite uses host plastids, as host plastid in parasite cells ("organelle capture") have been shown in other red algal parasites (Goff & Coleman 1995). Host plastids have been found in the parasites: *Plocamiocolax pulvinata* Setch., *Gracilariophila oryzoides* Setch. *et* H.L.Wilson, and

Gardneriella tuberifera Kylin (Goff & Coleman 1995). These parasites are unpigmented, and the function of the host plastids, if any, needs further investigation.

Pterocladiophila hemisphaerica might have a similar host dependency as the parasite *Choreocolax polysiphoniae* Reinsch in which photosynthetic ability was assumed when CO_2 fixation increased over time in unattached specimens (Callow *et al.* 1979). *C. polysiphoniae* also has a plastid with a reduced genome (Salomaki *et al.* 2015). We suggest that some parasites with reduced plastid genomes may retain the host plastids in their cells to use for photosynthetic processes. The nuclear-derived plastid proteins for plastid function either come from host nuclei in heterokaryotic cells, or from parasite nuclear RNA transcripts. This second scenario would require interactions of parasite nuclear-derived-plastid proteins with host plastid genome-derived proteins to produce functional plastids. Sequencing of the transcriptome of the parasite and host would address these possibilities.

Fv/Fm values were similar between all host species (*Rhodophyllis membranacea*, *Vertebrata aterrima* and *Pterocladia lucida*) and the parasite *Pterocladiophila hemisphaerica*, but were much lower than in other red algae (usually around 0.6, Figueroa *et al.* 1997; Lüder *et al.* 2001). The lower values of Fv/Fm may demonstrate stress or photoinhibition (Park *et al.* 2002; Mallick & Mohn 2003) or downregulation of photosynthesis (Groom & Baker 1992; Velez-Ramirez *et al.* 2017) in our experiments. The similarities of the Fv/Fm values in our study (when measurable) and similar values to ΔF/Fm' suggests an influence of our culture conditions.

In summary, pigmented red algal parasites can be as dependent on host photosynthates as unpigmented parasites. Other pigmented red algal parasites have the ability to photosynthesize independently, but their degree of host nutritional dependency needs further studies. Host dependency in red algal parasites cannot be determined by degree of pigmentation and needs individual assessment.

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Chapter Seven

General Discussion

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7.1 Findings

My PhD thesis contributes significantly to red algal parasite knowledge with five main findings. Firstly, many parasitic species have been described but our general knowledge of these parasites and parasitic process are still poorly studied. I summarized data of all known parasites and produced a comprehensive review of the current state of knowledge. Secondly, I performed phylogenetic analyses that revealed contrasting evolutionary relationships of three new red algal parasites: *Cladhymenia oblongifoliophila*, *Phycodrys novae-zelandiophila* and *Judithia parasitica* from New Zealand. Thirdly, I studied the development of the new parasite *Vertebrata aterrimophila*, discovering a different development from most previously reported, with localised infection and few changes inside the infected host cells. Fourthly, I sequenced and assembled the organelle genomes of the red algal parasite *Pterocladiophila hemisphaerica*. This parasite has a reduced non-photosynthetic plastid genome, which makes determining its phylogenetic relationships problematic, but careful analysis places it as sister to its host order, the Gelidiales. Lastly, I compared the photosynthetic ability of three pigmented red algal parasites.

My PhD research clearly demonstrated that there are many aspects of these parasites that we do not know nor fully understand (Chapter 2), and the many new findings in this thesis add to our current knowledge of parasites, including parasite and host relationships (Chapters 3-5), development (Chapter 4), genome evolution (Chapter 5) and physiology (Chapter 6). This increases knowledge of the diversity of red algal parasites, and New Zealand macroalgae, by describing four new species (Chapters 3-4). One unexpected discovery was the photosynthetic ability revealed in the pigmented red algal parasite *Pterocladiophila hemisphaerica* (Chapter 6) that has a reduced non-photosynthetic plastid genome (Chapter 5).

7.2 Diversity and evolution in parasites

Diversity of red algal parasites was estimated to be between 100-121 species (Goff 1982; Blouin & Lane 2012; Salomaki & Lane 2014; Blouin & Lane 2016). This study showed that 120 species have been described in the literature (Chapter 2) with several newly described species from this study adding to the current recorded diversity (Chapters 3-4). Understanding red algal parasite diversity will help us to understand their success within red algae, and makes the choice of study organisms more varied. Future research should investigate their diversity in New Zealand and around the world. New Zealand has many more red algal parasites, that were either collected during my field work or observed in the Te Papa herbarium, that have not been formally described. A combined study using extensive fieldwork around New Zealand, including scuba and herbarium investigations, is required to discover more of these undescribed species, which should be morphologically and phylogenetically (using mitochondrial, nuclear and plastid markers) investigated to determine the parasite's origin and placement.

Phylogenetic studies have become a common tool to identify new parasite species (Sekimoto *et al.* 2009; Skovgaard & Salomonsen 2009), investigate their origin (Litaker *et al.* 1999; Skovgaard *et al.* 2007; Barkman *et al.* 2008) and host switching (Fraser & Waters 2013; Pelser *et al.* 2016). Molecular data are still rather limited for red algal parasites with only 27% of all red algal parasites having been sequenced (Chapter 2). Phylogenetic analyses of the four new red algal parasites: (*Cladhymenia oblongifoliophila*, *Phycodrys novae-zelandiophila*, *Judithia parasitica* and *Vertebrata aterrimophila*) show contrasting patterns of phylogenetic relationships by using a range of markers from all three genomes (cpDNA: *rbc*L, nDNA: actin, LSU rRNA; mtDNA: *cox*1) (Chapters 3-4). Current phylogenies are clearly limited by the availability of sequences online (GenBank) as well as a consequence of taxon sampling. Future research should sequence as many of these described species and phylogenies using markers of all three genomes (mitochondrial, plastid, nuclear), and sequence data should also be included in new species descriptions to assist with understanding the parasite relationships with its host and the parasite's taxonomic placement.

The few phylogenetic studies that have been conducted on red algal parasites often only looked at either genes in the nuclear genome (Goff *et al.* 1996; 1997; Zuccarello *et al.* 2004) or genes from the nuclear and mitochondrial genome (Kurihara *et al.* 2010) and rarely at genes from all three genomes (Preuss & Zuccarello 2014). This study showed that phylogenetic data from all three genomes are available for only 2.5% of all species (Chapter 2). In some cases, genes from three genomes can show the same phylogenetic relationships, as shown in *Judithia parasitica* and *Phycodrys novae-zelandiophila*. (Chapter 3). However, in other cases, mitochondrial and nuclear genes show a pattern that differs from the plastid genes, e.g., *Cladhymenia oblongifoliophila*, indicating one mitochondrial and nuclear origin (host species) and one plastid origin (another *Cladhymenia* species) (Chapter 3). My results demonstrate the importance of studying all three genomes to understand parasite evolutionary history. In cases where there is a close phylogenetic relationship between parasite and host, it is often hard to get variable markers that show any difference between parasite and host. An alternative might be the use of uncommon markers (e.g., actin) or designing new primers for more variable genes or genomic regions (introns, spacers). Another alternative could be the use of single nucleotide polymorphisms (SNPs) and microsatellites for studying population pattern within species and within parasites and hosts. The small thallus size and small population size have to be carefully considered for obtaining the necessary amount of DNA and also how many parasites within and between populations can be collected and compared.

Evolutionary rates can differ between genomes of parasites and free-living taxa (Bromham *et al.* 2013). These differences in evolutionary rates might lead to long branch attraction (LBA), where fast evolving taxa group together but which does not reflect their phylogenetic relationship (Bergsten 2005). Our study showed the parasite *Pterocladiophila hemisphaerica* on very long branches in the mitochondrial and plastid data sets giving conflicting phylogenies for the parasite (Chapter 6). In the end, to avoid LBA, our study used non-elevated rates in plastid genes of the parasite and showed the origin of the parasite as sister to its host order, which aligns with our current knowledge of these parasites (Chapter 6). The robustness of using genes with non-elevated rates should be tested with other data sets. LBA might be caused by different factors, e.g., elevated mutation rates, and interpretation of the analyses needs to address these factors. LBA can be a serious problem, and is found in an increasing number of data sets, often hidden under different names, e.g., model misperfection (Bergsten 2005): this study demonstrates that parasite data sets can also be influenced by LBA.

7.3 Organelle genome evolution

Organelle genomes are extremely limited in red algal parasites (Chapter 2). The mitochondrial genomes of the parasites *Gracilariophila oryzoides* and *Plocamiocolax pulvinata* are similar in size, gene content, order and arrangement to other non-parasitic red algae (Hancock *et al.* 2010). Our study shows a similar mitochondrial genome in size, gene content, order and arrangement of the parasite *Pterocladiophila hemisphaerica* and its host *Pterocladia lucida* (Chapter 5). In contrast, the plastid genome of the parasite *Choreocolax polysiphonia* is highly reduced without photosynthetic genes (Salomaki *et al.* 2015). Our study showed that this reduced plastid, without photosynthetic genes, is also found in the red algal parasite *Pterocladiophila hemisphaerica* but both parasite plastids differ in their gene arrangements and, to some degree, gene content (Chapter 5).

Reduced non-photosynthetic plastid genomes are often associated with parasitic plants and algae (Wolfe *et al.* 1992; Wilson *et al.* 1996; de Konig & Keeling 2006; Cusimano & Wicke 2016) and this study shows that in a pigmented red algal parasite a reduced non-photosynthetic plastid genome can be found (Chapter 6). Even though reduced plastid genomes are common, the underlying mechanisms (e.g., patterns of gene deletion) are poorly studied (Cusimano & Wicke 2006). Future research should focus on sequencing a range of red algal parasites with different relatedness to their hosts (and therefore possibly different ages of parasite origin) and determine, by plastid characterization, if the gene deletion processes can be reconstructed. Parasites that are closely related to their hosts might have some photosynthetic genes as it is unlikely that all genes get transferred to the nuclear or deleted at once. Ideally, taxon sampling should be on countries where a rich parasite flora has been reported e.g., USA (26 species), South Africa (13 species), Australia (11 species) (Chapter 2) and New Zealand (13 species) (Chapters 3-4).

Host nutrient dependency is rarely discussed in red algal parasites and this is the first study demonstrating differences in photosynthetic ability in pigmented red algal parasites (Chapter 6). Interestingly, the photosynthetic ability in the pigmented red algal parasite *Pterocladiophila hemisphaerica* (Chapter 6) with the reduced plastid (Chapter 5) must be either due to the use of the host plastid in parasite cells, or that the parasite has transferred all missing photosynthetic genes into the nuclear genome (Chapter 6). Currently, no nuclear genomes are sequenced in red algal parasites and this lack of knowledge is one limitation to fully understand their host nutrient

dependency. Some parasitic nuclear genomes (e.g., in *Plasmodium*, *Microsporidia*) have undergone deletion or compaction processes (Keeling & Slamovits 2005). Parasitic plants with reduced plastids have transferred plastid genes into the nuclear genome either as full-length (possible functional) or nearly full-length genes (Cusimano & Wicke 2016). Generally, sequenced nuclear genomes of parasites are limited in number, with a focus on parasitic species of human importance (e.g., medical, veterinary) and this may lead to bias in the generalisations that are being derived from these data (Poulin & Randhawa 2015). Nuclear sequencing is needed to clarify the photosynthetic ability of *P. hemisphaerica* and to understand nuclear genome organization (e.g., size, gene numbers, missing genes) in comparison to other red algae and the parasite's closest relatives. Transcriptome data would also be useful to look at in *Pterocladiophila hemisphaerica* or other red algal parasites to determine which genes are important (up-regulated) for its lifestyle. Furthermore, comparing differences in gene expression in infected and uninfected host species should show the impact of the parasite on its hosts' transcriptome and how infected host species adapt when being parasitized.

7.4 Is there enough evidence to label red algal parasites as parasites?

Red algal parasites have been labelled as parasites from the earliest studies published (Richard 1891; Schmitz & Falkenberg 1897; Setchell 1914) before any benefit for the parasite (e.g., transfer of nutrients from host to parasite; Evans *et al.* 1973) and any harm for the host (e.g., cellular changes within infected host cells; Goff 1976) were demonstrated. Further evidence of negative impacts on the host is still limited (Chapter 2). This study shows clearly that pigmented parasites can still be totally nutrient dependent on their hosts while other pigmented parasites are able to photosynthesize independently (Chapter 6). An apparent increase in carbohydrate concentrations, nuclei size increase and structural changes in infected host cells of *Vertebrata aterrima* were demonstrated (Chapter 4). At the moment, there is evidence that these parasites gain some nutrients from their hosts and have mostly limited impact on the host itself, which might reflect a more commensalistic relationship (benefit for one organism and no positive or negative effect for the other organism) rather than parasitic relationship. Future research should focus on studying the impact of these parasites on their hosts, and more extensive data should help to clarify if it is appropriate to classify these organisms as parasites. Comparing reproductive output or photosynthetic ability of infected and uninfected host species, e.g., for *Vertebrata aterrima*, would be one approach.

7.5 Pigmentation in parasites

The majority of red algal parasites are pigmented (Chapter 2) and to our knowledge this is the first study looking at chlorophyll concentrations in parasites (Chapter 6). The methods to determine chlorophyll concentrations are simple and do not give any information on other different pigments present. High-performance liquid chromatography (HPLC) was previously used to determine different chlorophyll and carotenoid groups in red algae (Schubert *et al.* 2006; Heriyato *et al.* 2015). In plant parasites, HPLC showed that the parasitic plant *Cuscuta reflexa* uses lutein instead of neoxanthin in its light harvesting complex (Bungard *et al.* 1999). Comparing parasitic plants to non-parasitic plants showed that parasites have a lower chlorophyll *a* to chlorophyll *b* ratio (Esteban *et al.* 2015). HPLC would be a useful tool to compare red algal parasites with different degrees of pigmentation and their hosts and other free-living red algae. Pigment similarity might be used to show close parasite and host relationships and any changes should be due to the parasitic lifestyle.

7.6 Distribution pattern of parasites

Distribution of red algal parasites is still poorly understood and more data is needed (Chapter 2). This study used field collections and herbarium specimens to derive information about the distribution of the four parasites: *Cladhymenia oblongifoliophila*, *Judithia parasitica*, *Phycodrys novae-zelandiophila* and *Vertebrata aterrimophila* (Chapters 3-4) in New Zealand. Generally all four species were rather patchy in their distribution and during fieldwork many uninfected host individuals were observed. Future research should study the distribution of the parasite within the range of its host. The parasite *Vertebrata aterrimophila* would be a good choice because the host (*Vertebrata aterrima*) is found on *Carpophyllum maschalocarpum* and *Cystophora* spp., which makes the possible parasite sites easier to find.

7.7 Summary

In conclusion, red algal parasites provide a rich opportunity to increase understanding in parasitism given their high diversity, different host and parasite relationships, and the different degree of pigmentation they exhibit. Taxonomic studies will be a helpful tool to understand how diverse these parasites are with continuously describing new species and phylogenetic analyses to reveal the number of parasite species of the same or closely related host species. Furthermore this additional taxonomic data will help to understand which specific families and genera are most parasitized. Studying reduced plastid genomes in red algal parasites will make a significant contribution to understanding gene deletion processes over time and the adaptive genetic changes occurring in the transition from free-living to parasitic organisms. Further investigations of host nutrient dependency will improve understanding of the advantages the parasitic life style confers for these species.

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Appendices

Appendix 2.1. Synoptic list of described species of red algal parasites, arranged by order (listed alphabetically) and family. Parasites are characterized by: Host it has been described on; Year of original description; Parasitic character (up = unpigmented, p = pigmented, rm = reduced morphology mentioned, 2PC = secondary pit connections between parasite and host reported, pe = penetration into the host thallus reported; and which reproductive structures were described: M = male, F = female, T = sporophyte); Type locality; Distribution and Rank [+ = red algal parasite characters are met (i.e. 2PC and sporophyte and gametophyte present); +/o = species could be a parasite but characters are missing (i.e. either no 2PC or no sporophyte or no gametophyte reported); o = species could be a parasite but many characters are missing (i.e. missing 2PC and either no sporophyte or gametophyte); o/- = not enough information for informative decision about the species, could be an outgrowth of the host or an epiphyte]. Parasitic species with synonyms are indicated with #. All references are given in Supplemental Appendix 2.2.

Parasite	Host	Year	up	p	rm	2PC	pe	M	F	T	Type locality	Distribution	Rank	Reference
Ceramiales														
Ceramiaceae														
Centrocerocolax ubatubensis A.B.Joly	*Centroceras clavulatum* (C.Agardh) Mont., *Ceramium tenerrimum* (G.Martens) Okamura, *Ceramium* spp.	1965	x	x	x	x	x	x	x	x	Sao Paulo, Brazil	Brazil, Canary Is., Curaçao, Mexico, Venezuela	+	Joly 1966; Stegenga & Vroman 1987; Haroun *et al.* 2002; García & Gómez 2004; Mateo-Cid *et al.* 2006
Episporium centroceratis K.Möbius	*Centroceras clavulatum* (C.Agardh) Mont.	1885	x		x	x	x	x	x	Dirk Hartog Island, Western Australia	Australia, East Africa, South Africa, Tanzania	+	Pocock 1956 ; Womersley 1996; Coppejans *et al.* 2000a;

											Type locality	Distribution		References
														Coppejans *et al.* 2000b
Spyridiocolax capixabus A.B.Joly *et* E.C.Oliveira	*Spyridia aculeata* var. *disticha* Børgesen	1966	x		x			x	x	x	x	Praia de Peracanga, Espirito Santo State, Brazil	Brazil	+/o	Joly & de Oliveira 1966; de Oliveira-Filho 1969
Syringocolax macroblepharis Reinsch	*Gelidium amansii* (J.V.Lamour.) J.V.Lamour., *Plocamium cartilagineum* (L.) Gaillon	1875		x	x			x	x	x	x	Cape Agulhas, South Africa	South Africa	+/o	Reinsch 1875; Martin & Pocock 1953; Kylin 1956
Dasyaceae														
Colacodasya australica Womersley	*Dasya clavigera* (Womersley) Parson	1998		x	x	x		x	x	x	x	Port Elliot, South Australia	Australia	+	Womersley 1998
Colacodasya californica Hollenb.	*Heterosiphonia erecta* N.L.Gardner	1970		x	x			x	x	x		Laguna Beach, Orange County, California, USA	USA	+/o	Hollenberg 1970
Colacodasya inconspicua (Reinsch) F.Schmitz#	*Heterosiphonia berkeleyi* Mont., *Heterosiphonia* spp., *Polysiphonia anisogona* Hook.f. *et* Harv., *Polysiphonia* spp.	1888		x	x			x	x	x	x	-	Argentina, Campbell Is., Chile, Kerguelen Is., Falkland Is., South Georgia	+/o	Reinsch 1890; Kylin & Skottsberg 1919; Kylin 1956; Papenfuss 1964; Ramírez & Santelices 1991

Delesseriaceae

											Locality	Distribution		References
Apoglossocolax pusilla Maggs *et* Hommers.	*Apoglossum ruscifolium* (Turner) J.Agardh	1993		x	x	x	x	x	x	x	Old Harry Rocks, Dorset, UK	Ireland, Spain, UK	+	Maggs & Hommersand 1993; Wynne 2013
Asterocolax denticulatus (Tokida) Feldmann *et* Feldm.-Maz.#	*Phycodrys fimbriata* (Kuntze) Kylin	1934			x		x	x	x	x	Robben Island, Sakhalin Islands, Russia	Russia	+/o	Tokida 1934; Goff *et al.* 1997; Wynne 2013
Asterocolax erythroglossi Feldmann *et* Feldm.-Maz.	*Erythroglossum laciniatum* (Lightfoot) Maggs *et* Hommers., *Erythroglossum sandrianum* (Kütz.) Kylin	1951		x	x	x	x	x	x	x	Brest, France	British Isles to northwest of France	+	Feldmann & Feldmann 1958; Maggs & Hommersand 1993; Goff *et al.* 1997; Wynne 2013
Asterocolax gardneri (Setch.) Feldmann *et* Feldm.-Maz.#	*Anisocladella pacifica* Kylin, *Phycodrys isabelliae* R.E.Norris *et* M.J.Wynne, *Phycodrys setchellii* Skottsb., *Nienburgia andersoniana* (J.Agardh) Kylin, *Polyneura latissimi* (Harv.) Kylin	1923	x	x	x	x	x	x	x	x	Cavallo, Marin County, USA	USA	+	Setchell 1923; Wagner 1954; Kremer 1986; Goff *et al.* 1997, Wynne 2013

Species	Host	Year									Type locality	Distribution	Sex	References
Asterocolax hypophyllophilus M.JWynne	*Mikamiella ruprechtiana* (Zinova) M.J.Wynne	1970	x	x	x			x	x	x	Makarius Point, Amchitka Island, Aleutian Islands	Aleutian Is.	+/o	Wynne 1970; Goff *et al.* 1997
Gonimocolax australis (Skottsb.) Kylin#	*Myriogramme livida* (Hook.f. *et* Harv.) Kylin, *Schizoseris* spp.	1919		x	x		x	x	x	x	Falkland Islands	Falkland Is.	+/o	Kylin & Skottsberg 1919; Kylin 1956; Wynne 2013
Gonimocolax corymbosus Baardseth	*Schizoseris dichotoma* (Hook.f. *et* Harv.) Kylin, *Schizoseris multifoliata* Baardseth	1941		x	x	x	x	x	x	x	Seal Bay, Tristan da Cunha	Nightingale Is., Tristan da Cunha	+	Baardseth 1941; Kylin 1956; Wynne 2013
Gonimocolax roscoffensis Feldmann *et* Feldm.-Maz	*Drachiella spectabilis* J.Ernst *et* Feldmann	1961		x	x		x		x		Roscoff, France	North Atlantic of Europe	o	Feldmann & Feldmann 1961; Guiry 1997
Gonimophyllum africanum M.T.Martin *et* Pocock	*Acrosorium maculatum* (Sonder ex Kütz.) Papenf., *Acrosorium* spp., *Botryoglossum platycarpus* (Turner) Kütz., *Botryocarpa prolifera* Grev., Delesseriaceae spp., *Neuroglossum binderianium* Kütz.	1953		x	x			x	x	x	Cove Rock, near East London, South Africa	Brazil, South Africa, Uruguay	+/o	Martin & Pocock 1953; Maggs & Hommersand 1993; Wynne 2013

Gonimophyllum buffhamii Batters	Acrosorium ciliolatum (Harv.) Kylin, Cryptopleura ramosa (Huds.) L.Newton	1892	x	x	x	x	x	x	x	-	British Isles to Spain	+	Batters 1892; Wagner 1954; Kylin 1956; Maggs & Hommersand 1993; Wynne 2013
Gonimophyllum insulare F.S.Wagner	Hymenena semicostata (J.Agardh) Kylin	1954		x		x	x	x	x	Half Moon Bay, Stewart Island, New Zealand	Argentina, New Zealand	+/o	Wagner 1954; Wynne 2013
Gonimophyllum skottsbergii Setch.	Cryptopleura crispa Kylin, Hymenena flabelligera (J.Agardh) Kylin, Cryptopleura ruprechtianum (J.Agardh) Kylin	1923		x		x	x	x	x	Lands End, San Francisco, California, USA	North America	+/o	Setchell 1923; Wagner 1954; Zuccarello et al. 2004
Phitycolax inconspicua M.J.Wynne et F.J.Scott	Phitymophora amansioides (Sonder) Womersley	1989	x	x	x	x	x	x	x	Ile de Amsterdam, Indian Ocean	Indian Ocean	+	Wynne & Scott 1989; Wynne 2013
Polycoryne compacta Zinova	Myriogramme kerguelensis Levring	1963		x				x	x	Kergules Islands, Indian Ocean	Indian Ocean	+/o	Zinova 1963; Papenfuss 1964; Goff 1982; Wynne 2013
Polycoryne radiata Skottsb.	Nitophyllum sp.	1919		x		x		x	x	South Georgia	South Georgia,	+/o	Kylin & Skottsberg

											Locality	Distribution		References
												Macquarie Is.		1919; Papenfuss 1964; Wynne 2013
Sorellocolax stellaris T.Yoshida *et* Mikami	*Sorella repens* (Okamura) Hollenb.	1996	x	x		x	x	x	x	Onagawa, Honshu, Japan	China, Japan	+/o	Yoshida & Mikami 1996; Wynne 2013
Rhodomelaceae														
Aiolocolax pulchella Pocock	*Polysiphonia atlantica* Kapraun *et* J.N.Norris, *Polysiphonia caespitosa* (Pocock) Hollenb., *Polysiphonia devoniensis* Maggs *et* Hommers.	1956	x		x	x	x	x	x	Muizenberg, South Africa	Canary Is., Namibia, Portugal, South Africa, Spain	+	Pocock 1956; Pérez-Cirera *et al.* 1989; John *et al.* 2004; Araújo *et al.* 2009; Diaz-Tapia & Bárbara 2013
Antarctocolax lambii Skottsb.	*Picconiella plumosa* (Kylin) G.De Toni	1953	x	x		x	x	x	x	Melchior Islands, Palmer Archipelago, Antarctica	Antarctica	+/o	Skottsberg 1953; Hommersand *et al.* 2009
Benzaitenia yenoshimensis Yendo	*Chondria crassicaulis* Harv., *Chondria* spp., *Laurencia* spp., *Palisada peniculata* (Kütz.) Cassano, Sentíes, Gil-Rodriquez *et* M.T.Fujii	1913	x	x	x		x	x	x	Japan	Japan, Korea	+	Kylin 1956; Morrill 1976a; Kim *et al.* 2008

Species	Host	Year	1	2	3	4	5	6	7	Type locality	Distribution	Symbol	Reference
Bostrychiocolax australis Zuccarello *et* J.A.West	*'Bostrychia radicans'* (Mont.) Mont.	1994	x	x	x	x	x	x	x	Florence Bay, Magnetic Island, Queensland, Australia	Australia	+	Zuccarello & West 1994a
Chamaethamnion pocockiae R.E.Norris	*Kentrophora natalensis* (J.Agardh) S.M.Wilson *et* Kraft	1988		x			x	x	x	Palm Beach, near Port Edward, Natal, South Africa	South Africa	+/o	Norris 1988; Womersley 2003
Chamaethamnion schizandra Falkenb.	*Polysiphonia decipiens* Mont., *Micropeuce feredayae* (Harv.) Kylin ex Silva	1897	x	x		x	x	x	x	-	Argentina, Australia	+/o	Schmitz & Falkenberg 1897; Womersley 2003
Choreocolax americanus Reinsch	*Lophura* spp.	1875		x		x				-	USA	o/-	Reinsch 1875
Choreocolax destructor Reinsch	*Chondracanthus teedei* (Mertens) Kütz.	1875		x		x				-	Adriatic Sea	o/-	Reinsch 1875
Choreocolax polysiphoniae Reinsch	*Cystoclonium purpureum* (Hudson) Batters, *Neosiphonia confusa* (Hollenb.) J.N.Norris, *Vertebrata lanosa* (L.) T.A.Chr.	1875	x	x	x	x	x	x	x	-	North Atlantic Ocean	+	Reinsch 1875; Goff & Coleman 1985; Setchell 1918; Zuccarello *et al.* 2004

Species	Host	Year									Type locality	Distribution		References
Choreocolax rabenhorstii Reinsch	*Phycodrys rubens* (L.) Batters	1875		x		x					Gloucester, Massachusetts, USA	USA	o/-	Reinsch 1875
Choreocolax rhodymeniae Reinsch	*Palmaria decipiens* (Reinsch) Ricker, *Palmaria georgica* (Reinsch) R.W.Ricker	1888		x							South Georgia	South Georgia	o/-	Reinsch 1890; Papenfuss 1964; Edelstein 1972
Choreocolax tumidus Reinsch	*Ceramium* spp., *Ceramium virgatum* Roth *Cystoclonium purpureum*	1875	x	x		x					West Gloucester, Massachusetts, USA	English Channel, USA	o/-	Reinsch 1875; Setchell 1918; Lyle 1920
Colacopsis lophurellae Kylin	*Lophurella hookeriana* (J.Agardh) Falkenb.	1919		x			x	x	x	x	Tierra del Fuego, Argentina and Falkland Islands	Argentina, Campbell Is., Falkland Is., New Zealand	+/o	Kylin & Skottsberg 1919; Kylin 1956; Dalen & Nelson 2013
Colacopsis pulvinata (F.Schmitz) G.De Toni#	*Osmundaria serrata* (Suhr) J. Agardh	1897	x	x			x	x	x	x	Southeast Africa	Southeast Africa, South Africa	+/o	Kylin 1956; Norris 1988
Colacopsis smitheniae R.E.Norris	*Aneurianna nozawae* (Norris) L.E.Philipps	1988		x	x	x	x	x	x	x	Jesser Point, Sodwana, Natal, South Africa	South Africa	+	Norris 1988
Colacopsis velutina (M.T.Martin *et* Pocock) R.E.Norris#	*Rhodomelopsis africana* Pocock	1953	x	x	x	x	x	x	x	x	Riet River, Three Sisters, South Africa	Kerguelen Is., South Africa	+	Martin & Pocock 1953;

Species	Host	Year									Type locality	Distribution		References
														Fan 1963; Norris 1988
Dawsoniocolax bostrychiae (A.B.Joly *et* Yam.-Tomita) Joly *et* Yam.-Tomita#	'*Bostrychia calliptera*' (Mont.) Mont., '*Bostrychia montagnei*' Harv., '*Bostrychia radicans*' (Mont.) Mont.	1970	x		x	x	x	x	x	x	Sao Paulo, Brazil	Brazil	+	Joly & Yamaguishi-Tomita 1969; Guimaraes 1993; Zuccarello *et al.* 2004
Dipterocolax fernandezianus J.Morrill	*Dipterosiphonia parva* (Dickie) Skottsb. *et* Levring	1977		x	x	x	x	x	x		Juan Fernández Islands, Chile	Chile	+/o	Morrill 1977
Harveyella mirabilis (Reinsch) F.Schmitz *et* Reinke#	*Gonimophyllum skottsbergii* Setch., *Odonthalia floccosa* (Esper) Falkenb., *Odonthalia washingtoniensis* Kylin, *Rhodomela confervoides* (Huds.) P.C.Silva	1875	x	x	x	x	x	x	x	x	Bohuslän, Sweden	Arctic, North Atlantic Coast, North Pacific Coast	+	Reinsch 1875; Kylin 1956; Goff & Cole 1975; Edelstein & McLachlan 1977; Lee 1980; Wetherbee *et al.* 1984; López-Rodriquez *et al.* 2003;

Taxon	Basionym / synonyms	Year	c1	c2	c3	c4	c5	c6	c7	c8	Type locality	Distribution	Sexuality	References
														Zuccarello *et al.* 2004
Janczewskia gardneri Setch. *et* Guernsey#	*Laurencia gardneri* Hollenb., *Osmundea spectabilis* (K.W.Postels *et* Rupr.) Nam, *Osmundea pinnatifida* (Huds.) Stackh.	1914	x	x	x	x	x	x	x	−		Argentina, Canada, Chile, USA	+	Setchell 1914; Kugrens 1974; Court 1980; Goff 1982; Goff & Coleman 1987; Nonomura & West 1981
Janczewskia hawaiiana Apt	*Laurencia nidifica* J.Agardh, *Laurencia mcdermidiae* I.A.Abbott	1987	x	x	x	x	x	x	x		Kawaikui Beach Park, Aina, Haina, Oahu Island, Hawaii, USA	USA	+	Apt 1987; Kurihara *et al.* 2010
Janczewskia lappacea Setch.	*Chondria nidifica* Harv.	1914	x		x	x	x	x	x		San Pedro, Southern California, USA	USA	+	Setchell 1914; Nonomura & West 1981
Janczewskia meridionalis M.T.Martin *et* Pocock	*Laurencia flexuosa* Kütz., *Laurencia natalensis* Kylin	1953	x		x			x	x	x	Riet River, the Tree Sisters, South Africa	South Africa	+/o	Martin & Pocock 1953
Janczewskia moriformis Setch.	*Chondria atropurpurea* Harv., *Laurencia translucida* M.T.Fujii *et* Cordeiro-Marina,	1914	x	x	x	x	x	x	x	x	Santa Monica, California, USA	Brazil, USA	+	Setchell 1914; Setchell 1918; Fujii & Toyota 1999

		Year									Location	Region	Sign	References
	Palisada flagellifera (J.Agardh) K.W.Nam													
Janczewskia morimotoi Tokida#	*Laurencia nipponica* Yamada	1947	x	x	x	x	x	x	x	x	Japan	Sea of Japan	+	Tokida 1947; Nonomura & West 1981
Janczewskia ramiformis C.F.Chang *et* B.M.Xia	*Laurencia okamurae* Yamada	1978		x	x			x	x	x	Shilaoren, Shanddong Province, China	China	+/o	Chang & Xia 1978
Janczewskia solmsii Guernsey	*Laurencia subopposita* (J.Agardh) Setch.	1914	x		x		x	x	x	x	California, USA	USA	+/o	Setchell 1914; Setchell 1918
Janczewskia tasmanica Falkenb.#	*Laurencia forsteri* (Mertens ex Turner) Grev., *Laurencia heteroclada* Harv., *Laurencia* spp.	1897	x		x	x	x	x	x	x	Tasmania, Australia	Australia	+	Setchell 1914; Setchell 1918; Womersley 2003
Janczewskia teysmannii Weber Bosse	*Acanthophora spicifera* (M.Vahl) Børgesen	1923			x		x		x		Strait de Bali, Indonesia	Indonesia	o/-	Weber-van Bosse 1923
Janczewskia verruciformis Solms	*Laurencia obtusa* (Huds.) J.V.Lamour.	1877	x	x	x	x	x	x	x	x	Mediterranean Sea	Adriatic Sea, Canary Is., Mediter-ranean Sea	+	Setchell 1914; Feldmann & Feldmann 1958; Haroun *et al.* 2002
Jantinella sinicola (Setch. *et* N.L.Gardner) Kylin#	*Chondria acrorhizophora* Setch. *et* N.L.Gardner,	1924			x		x	x	x	x	Eureka, La Paz, California, USA	North Pacific Ocean	+	Setchell & Gardner 1924; Setchell &

Taxon	Host	Year									Locality	Country	+/o	References
	Chondria clarionensis Setch. et N.L.Gardner													Gardner 1930; Kylin 1941
Jantinella verruciformis (Setch. *et* M.E.McFadden) Kylin#	*Chondria* spp., *Mychodea episcopalis* J.Agardh	1911	x		x	x	x	x	x	x	San Pedro, California, USA	USA	+	McFadden 1911; Morrill 1976b
Laurenciocolax polysporus Zinova *et* Perest.	*Laurencia caspica* Zinova *et* Zaberzhinskaya	1964		x		x	x	x	x		Caspian Sea, Russia	Russia	+/o	Zinova 1967
Leachiella pacifica Kugrens	*Neosiphonia paniculata* (Mont.) J.N.Norris, *Polysiphonia hendryi* N.L.Gardner, *Pterosiphonia bipinnata* (Postels *et* Rupr.) Falkenb., *Pterosiphonia dendroidea* (Mont.) Falkenb., *Pterosiphonia* spp.	1982	x		x	x	x	x	x	x	Cattle Point, San Juan Island, Washington, USA	Japan, USA	+	Kugrens 1982; Matsumoto & Yoshida 1991; Zuccarello *et al.* 2004
Levringiella gardneri (Setch.) Kylin#	*Pterosiphonia baileyi* (Harv.) Falkenb.	1923	x	x	x	x	x	x	x	x	Santa Monica, California, USA	USA	+	Setchell 1923; Kylin 1956; Kugrens & West 1973; Goff 1982
Levringiella microscopica (Levring) Kylin#	*Pterosiphonia* spp.	1941		x		x	x	x	x		Juan Fernandez Island, Chile	Chile	+/o	Levring 1941; Kylin 1956

Parasite	Host	Year								Locality	Region	+/o	Reference
Meridiocolax bracteata J.M.Noble *et* Kraft	*Polysiphonia sparsa* (Setch.) Hollenb.	1983		x	x	x	x	x	x	Ned's Beach, Lord Howe Island, Australia	Australia	+	Noble & Kraft 1983
Meridiocolax narcissus Morrill	*Neosiphonia ferulacea* (Suhr ex J.Agardh) S.M.Guim. *et* M.T.Fujii	1976	x	x	x		x	x	x	Key West Florida, USA	USA	+	Morrill 1976c
Meridiocolax polysiphoniae (E.C.Oliveira *et* Ugadim) Morrill#	*Polysiphonia denudata* (Dillwyn) Grev. ex Harv.	1973		x		x	x	x	x	Brazil	Brazil	+/o	de Oliveira & Ugadim 1973; Noble & Kraft 1983
Microcolax africanus M.T.Martin *et* Pocock	*Streblocladia tenuissima* Pocock	1953	x	x		x	x	x	x	Cove Rock, near East London, South Africa	South Africa	+/o	Martin & Pocock 1953
Microcolax botryocarpa (Hook.f. *et* Harv.) F.Schmitz#	*Streblocladia glomerulata* (Mont.) Papenf., *Streblocladia neglecta* F.Schmitz	1845	x	x			x	x	x	Auckland Islands, New Zealand	Auckland Is., Campbell Is.	+/o	Harvey & Hooker 1845; Schmitz & Falkenberg 1897; Kylin 1956; Goff 1982
Neotenophycus ichthyosteus Kraft *et* I.A.Abbott	*Neosiphonia poko* (Hollenb.) I.A.Abbott	2002		x	x	x	x	x	x	Johnston Island, Johnston Atoll	Pacific Is.	+	Kraft & Abbott 2002

Onychocolax polysiphoniae Pocock	*Polysiphonia incompta* Harv.	1956		x		x	x	x	x	The Kowie, beyond Salt Vlei, South Africa	South Africa	+/o	Pocock 1956
Sporoglossum lophurellae Kylin	*Lophurella hookeriana* (J.Agardh) Falkenb.	1919		x			x	x	x	Falkland Islands	Argentina, Campbell Is., Chile, Falkland Is.	+/o	Kylin & Skottsberg 1919; Papenfuss 1964; Ramírez & Santelices 1991
Symphyocolax koreana M.S.Kim	*Symphyocladia latiuscula* (Harv.) Yamada	2010	x	x	x		x	x	x	Molundae, Busan, Korea	Korea	+	Kim & Cho 2010
Stromatocarpus parasiticus Falkenb.	*Placophora monocarpa* (Mont.) Papenf., *Polysiphonia virgata* (C.Agardh) Spreng.	1897	x	x	x	x	x	x	x	-	South Africa	+	Schmitz & Falkenberg 1897; Martin & Pocock 1953; Kylin 1956
Trichidium pedicellatum J.M.Noble *et* Kraft	*Lophocladia kuetzingii* (Kuntze) P.C.Silva	1983	x	x	x	x	x	x	x	Port Denison, Western Australia	Australia	+	Noble & Kraft 1983
Tylocolax microcarpus F.Schmitz	*Lenormandia spectabilis* Sonder	1897		x		x	x	x	x	South Coast Australia	Australia	+/o	Schmitz & Falkenberg 1897;

												Origin	Distribution		Reference
													Womersley 2003		
Ululania stellata Apt *et* Schlech	*Acanthophora pacifica* (Setch.) Kraft, *Acanthophora spicifera*	1998	x		x	x	x	x	x	x	Oahu, Hawaii, USA	USA	+	Apt & Schlech 1998
Corallinales															
Corallinaceae															
Ezo epiyessoense W.H.Adey, T.Masaki *et* Akioka	*Lithophyllum crouaniorium* Foslie, *Lithophyllum yessoense* Foslie, *Titanoderma pustulatum* (J.V.Lamour.) Nägeli	1974	x	x	x	x	x	x	x	x	Hokkaido, Japan	Japan, UK	+	Adey *et al.* 1974; Chamberlain 1988; Chamberlain 1999
Masakiella bossiellae (Klochkova) Guiry *et* Selivanova#	*Bossiella* sp.	2007		x	x		x	x			Sea of Japan	Sea of Japan	o	Guiry & Selivanova 2007
Hapalidiaceaeae															
Choreonema thuretii (Bornet) F.Schmitz#	*Jania micrarthrodia* J.V.Lamour., *Jania rosea* (Lam.) Decaisne, *Jania rubens* (L.) J.V.Lamour., *Jania tenella* (Kütz.) Grunow, *Jania verrucosa* J.V.Lamour.	1889	x	x	x		x	x	x	x	Pointe de Querqueville, France	Australia, Ecuador, Galapagos Is., Japan, Mediter-ranean Sea, Mexico,	+/o	Kylin 1956; Pocock 1956; Zinova 1964; Woelkerling 1987; Womersley 1996;

											New Zealand, North Atlantic, South Africa, Russia		Broadwater & LaPointe 1997; Broadwater *et al.* 2002; Dalen & Nelson 2013
Epulo multipedes R.A.Towns. *et* Huisman	*Jania verrucosa* J.V.Lamour.	2004	x	x		x	x	x	x	Long Reef Point, New South Wales, Australia	Australia	+/o	Townsend & Huisman 2004
Kvaleya epilaeve W.H.Adey *et* Sperapani	*Phymatolithon laeve* (Rosenv.) Düwel *et* Wegeberg	1971	x	x		x	x	x	x	Trömsöy, Norway	Canada, Iceland, Norway, USA	+/o	Adey & Sperapani 1971

Gigartinales
Cystocloniaceae

Hypneocolax stellaris Børgesen	*Hypnea cornuta* (Kütz.) J.Agardh, *Hypnea musciformis* (Wulfen) J.V.Lamour., *Hypnea ramentacea* (C.Agardh) J.Agardh, *Hypnea valentiae*	1920	x	x	x	x	x	x	x	Lime Tree Bay, St. Croix, USA	Canary Is., Colombia, Mexico, USA, Venezuela	+	Børgesen 1920; Albornoz & Ganesan 1994; Haroun *et al.* 2002; Lipkin & Silva 2002;

Parasite	Host	Year									Locality	Distribution	+/o/-	References
	(Turner) Montag., *Hypnea variabilis* Okamura													Diaz-Pulido & Diaz-Ruiz 2003; Robledo *et al.* 2003
Hypneocolax stellaris f. orientalis Weber Bosse#	*Hypnea* spp., *Hypnea filiformis* (Harv.) Womersley	1928	x	x	x	x		x	x	x	Aru Island, Indonesia	Australia, Indo-Pacific	+	Kylin 1956; Womersley 1994
Rhodophyllis parasitica M.Preuss *et* Zuccarello	*Rhodophyllis membranacea* (Harv.) Hook.f. *et* Harv.	2014		x	x	x	x	x	x	x	Houghton Bay, Wellington, New Zealand	New Zealand	+	Preuss & Zuccarello 2014
Kallymeniaceae														
Callocolax acicularis M.J.Wynne *et* J.N.Heine	*Callophyllis rhynchocarpa* Rupr.	1992		x	x		x	x	x	x	St. Matthew Island, Alaska, USA	USA	+/o	Wynne & Heine 1992
Callocolax fungiformis Kylin#	*Callophyllis edentata* Kylin, *Callophyllis heanophylla* Setch., *Callophyllis flabellulata* Harv., *Callophyllis pinnata* Setch. *et* Swezy	1925	x	x	x		x	x	x	x	Friday Harbor Lab, Washington, USA	USA	+/o	Dawson 1945; Abbott & Hollenberg 1992; Wynne & Heine 1992
Callocolax japonica Tsugi nom. inval.	*Callophyllis* spp.	-									-	Japan	o/-	Goff 1982; Wynne & Heine 1992

Callocolax neglectus F.Schmitz ex Batters	*Callophyllis hombroniana* (Mont.) Kütz., *Callophyllis laciniata* (Huds.) Kütz.	1895	x	x	x		x		x	x	-		North Atlantic Coast, New Zealand	+/o	Batters 1895; Cotton 1907; Kylin 1930; Wynne & Heine 1992; Guiry 1996
Phyllophoraceae															
Coccotylus hartzii (Rosenv.) L.Le Gall *et* G.W.Saunders#	*Coccotylus* truncatus (Pall.) M.J.Wynne *et* J.N.Heine	1898		x	x	x	x	x	x	x		Greenland	Arctic, North Atlantic Coast	+/o	Rosenvinge 1931; Newroth & Taylor 1968; Evans *et al.* 1978; Le Gall & Saunders 2010
Solieriaceae															
Gardneriella tuberifera Kylin	*Agardhiella coulteri* (Harv.) Setch., *Sarcodiotheca gaudichaudii* (Mont.) P.W.Gabrielson	1941	x		x	x	x	x	x	x	-		USA	+	Kylin 1956; Goff 1981; Goff & Hommersand 1982; Goff & Zuccarello 1994
Tikvahiella candida Kraft *et* P.W.Gabrielson#	*Solieria robusta* (Grev.) Kylin	1983	x		x	x	x	x	x	x	Port Phillip Bay, Victoria, Australia		Australia	+	Goff 1982; Kraft &

| | | | | | | | | | | | Gabrielson 1983 |

Gracilariales

Gracilariaceae

Species	Host / associated taxa	Year								Locality	Distribution	Sign	Reference
Gracilaria babae (H. Yamam.) P.K.Ng, P.E.Lim *et* Phang#	*Gracilaria salicornia* (C.Agardh) E.Y.Dawson, *Hydropuntia* sp.	1986	x	x		x	x	x		Okinoerabu Island, Kagoshima Prefecture, Japan	Japan, Malaysia, Papua New Guinea, Thailand	+/o	Yamamoto 1986; Coppejans & Millar 2000; Kongkittayapun & Chirapart 2011; Ng *et al.* 2014
Gracilariophila oryzoides Setch. *et* H.L.Wilson#	*Gracilaria multipartita* (Clem.-Munoz) Harv., *Gracilariopsois andersonnii* (Kylin) E.Y.Dawson, *Gracilariopsis confervoides* Rmiki, Y. Lemoine, R.Kling *et* Cabioch, *Gracilariopsis lemaneiformis* (Bory de Saint-Vincent) E.Y.Dawson, Acleto *et* Foldvik	1910	x	x	x	x	x	x	x	Fort Point, Monterey, California, USA	Canada, USA	+	Wilson 1990; Norris & Wynne 1969 '1968'; Fredericq *et al.* 1989; Gerung & Yamamoto 2002

Species	Host	Year								Type locality	Distribution	+/o	References
Gracilariophila gardneri Setch.	*Gracilaria textorii* var. *cunninghamii* (Farl.) E.Y.Dawson	1923	x	x		x		x	x	Santa Monica, California, USA	USA, Venezuela	+/o	Setchell 1923; Kylin 1956; Ganesan 1970
Pterocladiophilaceae													
Gelidiocolax christianae Feldmann *et* Feldm.-Maz.	*Gelidium spathulatum* (Kütz.) Bornet	1963		x	x		x	x	x	Banyuls, France	Mediter-ranean Sea	+	Feldmann & Feldmann 1963; Ganesan 1970; Seoane-Camba 1996
Gelidiocolax deformans Seoane-Camba	*Gelidium cantabricum* Seoane-Camba, *Gelidium corneum* (Huds.) J.V.Lamour.	1982		x	x	x			x	-	Mediter-ranean Sea	+/o	Seoane-Camba 1982; Seoane-Camba 1996
Gelidiocolax desikacharyi Ganesan	*Gelidium floridanum* W.R.Taylor	1973	x	x			x	x	x	Margarita, Venezuela	Venezuela	+/o	Ganesan 1970; Goff 1982; Ouahi 1993
Gelidiocolax lyndae R.E.Norris	*Kentrophora natalensis* (J.Agardh) S.M.Wilson *et* Kraft	1988	x	x		x	x	x	x	Uvongo, Natal, South Africa	South Africa	+/o	Norris 1988
Gelidiocolax mammillatus K.C.Fan *et* Papenf.	*Pterocladiella capillacea* (S.G.Gmel.) Santel. *et* Hommers.	1959		x	x	x	x	x	x	Hanauma Bay, Oahu, Hawaiian Island, USA	USA	+	Fan & Papenfuss 1959; Evans *et al.* 1978; Ouahi 1993

Species	Host	Year								Locality	Distribution		References
Gelidiocolax margaritoides (M.T.Martin *et* Pocock) K.C.Fan *et* Papenf.#	*Gelidium pulchellum* (Turner) Kütz., *Ptilophora pinnatifida* J.Agardh	1953	x	x	x	x	x	x	x	Kleinemonde, South Africa	Portugal, Spain, South Africa	+	Martin & Pocock 1953; Fan & Papenfuss 1959; Seoane-Camba & Cortadellas 1998; Araújo *et al.* 2009
Gelidiocolax microsphaericus N.L.Gardner	*Gelidium coulteri* Harv., *Gelidium nudifrons* N.L.Gardner, *Gelidium pulchrum* N.L.Gardner, *Gelidium pusillum* (Stack.) Le Jol., *Gelidium robustum* (N.L.Gardner) Hollenb. *et* I.A.Abbott	1927	x	x		x	x	x	x	Balboa Beach, California, USA	Canary Is., USA	+/o	Kylin 1956; Fan & Papenfuss 1959; Evans *et al.* 1978; Ouahi 1993; Haroun *et al.* 2002
Gelidiocolax pustulatus E.C.Oliveira *et* Yonesh.	*Pterocladiella capillacea*	1984	x	x	x	x	x	x	x	Cabo Frio Island, Brazil	Brazil	+	Yoneshigue & de Oliveira 1984
Gelidiocolax suhriae (M.T.Martin *et* Pocock) K.C.Fan *et* Papenf.#	*Gelidium vittatum* (L.) Kütz.	1953		x	x		x	x	x	Blaauwberg, South Africa	South Africa	+/o	Martin & Pocock 1953; Fan & Papenfuss 1959

											Locality	Country		Reference
Gelidiocolax verruculatus Ouahi *et* Najiim nom. inval.	*Gelidum attenuatum* (Turner) Thur., *Gelidium pulchellum* (Turner) Kütz., *Gelidium pusillum, Pterocladiella capillacea*	-	x		x	x	x	x	x	x	-	Morocco	+	Ouahi 1993
Holmsella pachyderma (Reinsch) Sturch#	*Gracilaria gracilis* (Stackh.) Steentoft, L.M.Irvine *et* Farnham, *Gracilariopsis longissima* (S.G.Gmel.) Steentoft, L.M.Irvine *et* Farnham, *Gracilariopsis* spp.	1875	x		x	x	x	x	x	x	-	Ireland, Spain, UK	+	Fredericq & Hommersand 1990; Womersley 1996; Zuccarello *et al.* 2004
Holmsella australis J.M.Noble *et* Kraft	*Gracilaria cliftonii* Withell, A.Millar *et* Kraft	1983	x		x	x	x	x	x	x	Flinders, Victoria, Australia	Australia	+	Wetherbee & Quirk 1982; Noble & Kraft 1983
Pterocladiophila hemisphaerica K.C.Fan *et* Papenf.	*Pterocladiella bartlettii* (W.R.Taylor) Santel., *Pterocladia lucida* (R.Brown ex Turner) J.Agardh	1959		x	x	x	x	x	x	x	Island Bay, Wellington, New Zealand	Caribbean, New Zealand	+	Fan & Papenfuss 1959; Evans *et al.* 1978; Stegenga & Vroman 1986

Halymeniales

Halymeniaceae

Species	Host	Year									Type locality	Distribution		Reference
Grateloupiocolax colombiana Schnetter *et* Bula-Meyer	*Grateloupia filicina* (J.V.Lamour.) C.Agardh	1983		x	x		x	x	x	x	Ensenada de Concha, Colombia	Colombia	+/o	Schnetter *et al.* 1983
Kintokiocolax aggregato-ceranthus Tak.Tanaka *et* Nozawa	*Grateloupia angusta* (Okamura) Kawaguchi *et* H.W.Wang	1960		x	x		x		x	x	Hananose, Kagashima Prefecture, Japan	Japan, Korea	+/o	Tanaka & Nozawa 1960; Yang & Kim 2015
Palmariales														
Palmariaceae														
Neohalosacciocolax aleutica I.K.Lee *et* Kurogi	*Halosaccion minjaii* I.K.Lee	1978			x	x	x	x	x	x	Massacre Bay, Attu Island, Aleutian Islands	Aleutian Is.	+	Lee & Kurogi 1978
Rhodophysema kjellmanii G.W.Saunders *et* Clayden#	*Devaleraea ramentacea* (L.) Guiry, *Palmaria palmata* (L.) F.Weber *et* D.Mohr	1959		x	x		x	x		x	Wreck of the Ithaca, Manitoba, Canada	Arctic, North Atlantic Ocean, North Pacific Ocean	+/o	Edelstein 1972; Jonsson & Chesnoy 1988; Wynne & Heine 1992; Saunders & Clayden 2010
Plocamiales														
Plocamiaceae														
Plocamiocolax pulvinata Setch.	*Plocamium cartilagineum* (L.) P.S.Dixon	1923	x	x	x				x	x	Carmel Bay, California, USA	Canada, USA	+/o	Setchell 1923; Saunders &

Taxon	Host	Year								Location	Country	Sign	Reference
													Lehmkuhl 2005
Plocamiocolax papenfussianus M.T.Martin *et* Pocock	*Plocamium corallorhiza* (Turner) Hook.f. *et* Harv.	1953	x	x	x	x	x	x	x	False Bay, South Africa	South Africa	+	Martin & Pocock 1953
Rhodymeniales													
Champiaceae													
Champiocolax lobatus Womersley	*Champia viridis* C.Agardh	1996		x	x		x	x	x	x	Warrnambool, Victoria, Australia	Australia	+/o	Womersley 1998
Champiocolax sarae Bula-Meyer	*Champia* compressa Harv., *Champia parvula* (C.Agardh) Harv., *Champia* salicornoides Harv.	1985		x	x	x	x	x	x	x	Chengue Inlet, Caribbean Coast of Colombia	Colombia	+	Bula-Meyer 1985
Faucheaceae													
Faucheocolax attenuata Setch.	*Gloiocladia laciniata* (J.Agardh) N.Sánchez *et* Rodríguez-Prieto, *Gloiocladia fryeana* (Setch.) N.Sánchez *et* Rodríguez-Prieto	1923	x	x	x		x		x	x	Carmel Bay, California, USA	USA	+/o	Setchell 1923; Sparling 1957
Gloiocolax novae-zelandiae Sparling	*Gloioderma saccata* (J.Agardh) R.E.Norris	1957		x	x	x	x		x	x	Eastbourne, New Zealand	New Zealand	+	Sparling 1957
Rhodymeniaceae													

Species	Host	Year								Locality	Country	Symbol	References
Rhodymeniocolax botryoideus Setch.	*Rhodymenia* sp., *Rhodymenia pacifica* Kylin	1923	x	x	x	x	x	x	x	Whites Point, San Pedro, California, USA	USA	+	Setchell 1923; Sparling 1957; Womersley 1996
Rhodymeniocolax mediterraneus Vergés, Izquierdo *et* M.Verlaque	*Rhodymenia ardissonei* (Kuntze) Feldmann	2005	x	x	x	x	x	x	x	Cala St. Francesc, Blanes, Spain	France, Spain	+	Vergés *et al.* 2005
Incertae sedis													
Gracilariocolax deformans (Weber Bosse) Gerung *et* H.Yamam.	*Gracilaria canaliculata* Sonder, *Gracilaria salicornia*	1928	x	x			x	x	x	Sula Besi, Sula Islands, Indonesia	China, Indonesia	+/o	Weber-van Bosse 1928; Chang & Xia 1978; Gerung & Yamamoto 2002
Gracilariocolax henriettae Weber Bosse#	*Gracilaria hauckii* P.C.Silva	1928	x	x			x	x	x	Nusa Kembangan, Indonesia	Indonesia	+/o	Weber-van Bosse 1928
Gracilariocolax infidelis (Weber Bosse) Gerung *et* H.Yamam.#	*Gracilaria canaliculata, Gracilaria minor* (Sond.) Durair., *Gracilaria salicornia*	1928	x	x			x	x	x	Tual, Kai Islands, Indonesia	China, Indonesia, Thailand	+/o	Chang & Xia 1978; Terada *et al.* 1999; Gerung & Yamamoto 2002

Species	Host	Year						Type locality	Distribution		References
Gracilariocolax setchellii (Weber Bosse) Gerung *et* H.Yamam.#	*Gracilaria canaliculata, Gracilaria salicornia*	1928	x x		x	x	-		China, Indonesia	+/o	Weber-van Bosse 1928; Chang & Xia 1978; Gerung & Yamamoto 2002
Gracilariocolax setchellii var. aggregata (Weber Bosse) Gerung *et* H.Yamam.#	*Gracilaria minor* (Sond.) Durair.	1928	x x		x			Flores and Java, Indonesia	Indonesia	o	Weber-van Bosse 1928; Gerung & Yamamoto 2002
Gracilariocolax sibogae (Weber Bosse) Gerung *et* H.Yamam.#	*Gracilaria arcuata* Zanardini, *Gracilaria canaliculata, Gracilaria dura* (C.Agardh) J.Agardh	1928	x x		x x			Donggala, Sulawesi Island, Indonesia	Indonesia, Eritrea	o	Weber-van Bosse 1928; Gerung & Yamamoto 2002; Lipkin & Silva 2002
Scagelonema parasiticum R.E.Norris et M.J.Wynne[1]	*Antithamnion defectum* Kylin	1969		x x x		x		Whidbey Island, Washington, USA	USA	+/o	Norris & Wynne 1969 '1969'

[1] *Scagelonema* was formerly included in the Ceramiaceae but its position is currently uncertain.

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Appendix 3.1. Samples used for molecular and morphological analysis of parasites, hosts species and species related to hosts collected around New Zealand. No. refers to sample extraction number used for sequencing and associated GenBank Accession numbers.

Species	No.	GenBank Accession no.	Date	Location	Coordinates	Collector
Blastophyllis calliblepharoides	A17	MF319122[b] MF319133[c] MF319171[a]	18.04.2012	Moa Point, Wellington	41°20.5'S, 174°48.634'E	G. C. Zuccarello
	621	MF319170[a]	26.11.2011	Kaka Point, Otago Peninsula, South Island	46°23.183'S, 169°46.933'E	W. A. Nelson
Blastophyllis hombroniana	A72	MF319123[b] MF319135[c] MF319173[a]	22.01.2014	Ringaringa Beach, Stewart Island	46°54.3'S, 168°8.567'E	W. A. Nelson
	A93	-	25.02.2016	Nugget Point, South Island	46°26.883'S, 169°49.017'E	M. Preuss
	A95	MF319172[a]	26.02.2016	Aramoana, South Island	45°46.717'S, 170°42.217'E	M. Preuss
	587	MF319134[c]	25.10.2010	Marfells Beach, South Island	41°43.667'S, 174°12.917'E	R. D'Archino & W. A. Nelson
Callophyllis laingiana A.Millar	07	MF319124[b] MF319174[a]	02.03.2006	Moturoa/Rangiatea, Bay of Islands, North Island	35°12'S, 174°5.733'E	R. D'Archino, S. Miller & J. Forman
Callophyllis ornata (Mont.) Kütz.	237	MF319126[b] MF319176[a]	03.03.2009	Chase Head, Pearl Island, Port Pegasus, Stewart Island	47°12.783'S, 167°41.1'E	R. D'Archino

Species	Code	Accession	Date	Location	Coordinates	Collector
Cladhymenia coronata	A64	MF319142[a] MF319146[b] MF319152[c]	12.12.2012	Horseshoe Bay, Stewart Island	46°52.433'S, 168°7.6'E	C. Hepburn
Cladhymenia lyallii	A66	MF319143[a] MF319147[b] MF319150[c]	13.04.2013	Catton's Cave, Rosemary Island, Princes Islands, Three Kings Islands/Manawatāwhi	34°10.933'S, 172°3.383'E	R. D'Archino
Cladhymenia oblongifolia	A01	MF319138[a] MF319148[c]	11.11.2014	Marfells Beach, South Island	41°43.667'S, 174°12.917'E	M. Preuss
	A03	MF319140[a] MF319144[b] MF319149[c]	11.11.2014	Marfells Beach, South Island	41°43.667'S, 174°12.917'E	M. Preuss
	A100	MF319139[a]	18.03.2016	Owenga house, Chatham Island	44°1.208'S, 176°22.767'W	M. Preuss
Ectophora depressa J.Agardh	26	MF319127[b] MF319177[a]	31.01.2006	Waitangi wharf, Chatham Island	43°56.716'S, 176°33.633'W	W. A. Nelson
Ectophora marginata D'Archino *et* W.A.Nelson	046	MF319128[b] MF319178[a]	06.04.2006	Karikari Bay, Northland, North Island	34°52.683'S, 173°22.833'E	D. Freeman & N. Shears
Judithia delicatissima (R.E.Norris) D'Archino *et* Showe M.Lin	113	MF319129[b] MF319179[a]	07.12.2006	Marfells Beach, South Island	41°43.667'S, 174°12.917'E	W. A. Nelson & K. Neill
Phycodrys adamsiae	A91	MF319158[d] MF319169[c]	30.05.2011	Ranfurly Bank, Hicks Bay, North Island	37°32.733'S, 178°53.55'E	-

	A92	-	01.06.2011	Ranfurly Bank, Hicks Bay, North Island	37°32.733'S, 178°53.55'E	-
Phycodrys novae-zelandiae	A05	MF319153[a] MF319159[d] MF319164[e] MF319167[c]	11.11.2014	Marfells Beach, South Island	41°43.667'S, 174°12.917'E	M. Preuss
	A07	MF319154[a] MF319161[d]	11.11.2014	Marfells Beach, South Island	41°43.667'S, 174°12.917'E	M. Preuss
	A27	MF319156[a] MF319162[d] MF319168[c]	02.02.2015	Akitio Beach, South Island	40°37.417'S, 176°24.65'E	M. Preuss
	A78	MF319163[d]	21.09.2015	Marfells Beach, South Island	41°43.667'S, 174°12.917'E	M. Preuss
	A84	-	21.09.2015	Marfells Beach, South Island	41°43.667'S, 174°12.917'E	M. Preuss
	A103	-	19.02.2016	Princess Bay, Wellington, North Island	41°20.767'S, 174°47.433'E	M. Preuss
Rhizopogonia asperata (Harv.) Kylin	187	MF319131[b] MF319181[a]	08.11.2006	Evans Bay, Wellington, North Island	41°18.683'S, 174°47.8'E	W. A. Nelson
Thamnophyllis laingii (J.Agardh) R.E.Norris	117	MF319132[b] MF319182[a]	12.07.1998	Brighton, Dunedin, South Island	45°56.833'S, 170°20.067'E	W. A. Nelson
Wendya incisa D'Archino *et* Showe M.Lin	282	MF319125[b] MF319175[a]	07.10.2010	Mataikona, North Island	40°47.3'S, 176°16.033'E	W. A. Nelson & R. D'Archino

Parasites:

Cladhymenia oblongifoliophila on *Cladhymenia oblongifolia*	A02	MF319141[a] MF319145[b] MF319151[c]	11.11.2014	Marfells Beach, South Island, NZ	41°43.667'S, 174°12.917'E	M. Preuss
	A04	-	11.11.2014	Marfells Beach, South Island, NZ	41°43.667'S, 174°12.917'E	M. Preuss
Judithia parasitica on *Blastophyllis calliblepharoides*	A18	MF319130[b] MF319137[c] MF319180[a]	18.04.2012	Moa Point, Wellington, NZ	41°20.5'S, 174°48.634'E	G. C. Zuccarello
Judithia parasitica on *Blastophyllis hombroniana*	A73	-	22.01.2014	Ringaringa Beach, Stewart Island, NZ	46°54.3'S, 168°8.567'E	W. A. Nelson
	A96	MF319136[c]	26.02.2016	Aramoana, South Island, NZ	45°46.717'S, 170°42.217'E	M. Preuss
Phycodrys novae-zelandiophila on *Phycodrys novae-zelandiae*	A06	MF319164[e]	11.11.2014	Marfells Beach, South Island, NZ	41°43.667'S, 174°12.917'E	M. Preuss
	A08	MF319155[a]	11.11.2014	Marfells Beach, South Island, NZ	41°43.667'S, 174°12.917'E	M. Preuss
	A28	MF319157[a] MF319160[d] MF319166[c]	02.02.2015	Akitio Beach, South Island, NZ	40°37.417'S, 176°24.65'E	M. Preuss

[a]*cox*1 [b]LSU [c]*rbc*L [d]actin [e]SSU

Appendix 3.2. Primers used for PCR amplifying and sequencing.

Primer name	Primer sequence	Reference
Actin		
Act1.f1	GCC CGC GGT TGT CAT YGA CAA TGG	(Kamiya *et al.* 2011)
Act1.r1	GCS GCR ATA ACC TTA ATC TTC AT	(Kamiya *et al.* 2011)
***Cox*1**		
GazF1	TCA ACA AAT CAT AAA GAT ATT GG	(Saunders 2005)
GazR1	ACT TCT GGA TGT CCA AAA AAY CA	(Saunders 2005)
LSU		
X.LSU.f	GAT GAC CCG CTG AAT TTA AG	(Harper & Saunders 2001)
X.LSU.r	AGC GCC ATC CAT TTT YAG GG	(Harper & Saunders 2001)
Y.LSU.f	GCA GGA CGG TGG CCA TGG AAG T	(Harper & Saunders 2001)
Y.LSU.r	CAG AGC ACT GGG CAG AAA TCA C	(Harper & Saunders 2001)
Z.LSU.f	GCA ACG GGC AAA GGG AAT CCG	(Harper & Saunders 2001)
Z.LSU.r	TGA TAG GAA GAG CCG ACA TCG A	(Harper & Saunders 2001)
SSU		
GO4	CAG AGG TGA AAT TCT TGG AT	(Harper & Saunders 2001)
JO4	AAA CCT TGT TAC GAC TTC TCC	(Harper & Saunders 2001)
***rbc*L**		
F8	GGT GAA TTC CAT ACG CTA AAA TG	(Wang *et al.* 2000)
F145	CAA CCA GGW GTA GAT CCA GTA GAA GC	(Kim *et al.* 2010)
R753	GCT CTT TCA TAC ATA TCT TCC	(Freshwater & Rueness 1994)

Appendix 3.3. List of species used in molecular analyses and their GenBank Accession numbers for *cox*1, *rbc*L and LSU sequences.

Species	GenBank Accession numbers		
	*cox*1	*rbc*L	LSU rRNA
Blastophyllis calliblepharoides		HM587174	HM587201
Blastophyllis hombroniana			HM587205
Callophyllis cristata Okamura	KM675349	KR231932	KR231920
Callophyllis edentata Kylin	JX034268	KC130228	AY171604
Callophyllis laciniata (Huds.) Kütz.	JF903294	KF280968	JF833333
Callophyllis laingiana A.Millar		HM587176	JX296178
Callophyllis lambertii (Turner) Kütz.	HM917637	HQ910509	JX296155
Callophyllis ornata (Mont.) Kütz.		HM587180	HM587214
Callophyllis pinnata Setch. *et* Swezy	JX034365	AY294397	AY171608
Callophyllis variegata (Bory) Kütz.	JX034431	KF280964	HM587220
Callophyllis violacea J.Agardh	JX034438	CVU04191	JX296161
Chondrophycus sp.	HQ423050	FJ785310	KX145615
Cirrulicarpus nanus (J.Agardh) Womersley	KF280934	KF280981	KF280956
Cirrulicarpus polycoelioides (J.Agardh) Womersley	HM915947	KF280972	JX296142
Dumontia simplex Cotton	AY971153	KT310711	JN403052
Ectophora depressa J.Agardh		GQ376535	JN543696
Ectophora marginata		HM587177	HM587212
Euthora cristata (C.Agardh) J.Agardh	GU140145	JX969805	KF280993
Glaphyrymenia pustulosa J.Agardh	KC157606	KF280988	JX296147
Judithia delicatissima D'Archino *et* W.A.Nelson		KR231930	JN543699
Kallymenia cribrosa Harv.	KF280930	EU349216	KF280953
Kallymenia feldmannii Codomier	KJ083054	EU543487	KJ083095
Kallymenia lacerata Feldmann	KJ083056	KJ083103	KJ086096
Kallymenia tasmanica Harv.	HM917780	KC157624	KF280954
Kallymenia reniformis (Turner) J.Agardh	KJ960795	KJ404065	KJ083098
Kallymenia requienii (J.Agardh) J.Agardh	KJ083091	KJ083106	KJ083099
Meredithia microphylla (J.Agardh) J.Agardh	KJ083093	KC157626	KC157656

Meredithia norfolkensis G.W.Saunders *et* C.W.Schneid.	KF280922	KF280971	KF280949
Meredithia nutleorum G.W.Saunders *et* C.W.Schneid.	KF280921	KF280969	KF280948
Meredithia pseudopeltata G.W.Saunders *et* C.W.Schneid.	KF280929	KF280984	KF280959
Polycoelia laciniata J.Agardh	KT307606	KF280983	KF280958
Pugetia fragilissima Kylin	HQ919395	KR231931	AY171614
Rhizopogonia asperata (Harv.) Kylin		HM587196	JN543700
Salishia firma (Kylin) Clarkston *et* G.W.Saunders	JF903349	HQ910506	JF833329
Sebdenia cerebriformis N'Yeurt *et* Payri	KU568457	KU568458	KU568459
Thamnophyllis lacerata Womersley *et* R.E.Norris	KF280931	KF280979	JX296176
Thamnophyllis laingii (J.Agardh) R.E.Norris		HM587198	JX543698
Wendya incisa		KR2331927	KR231921
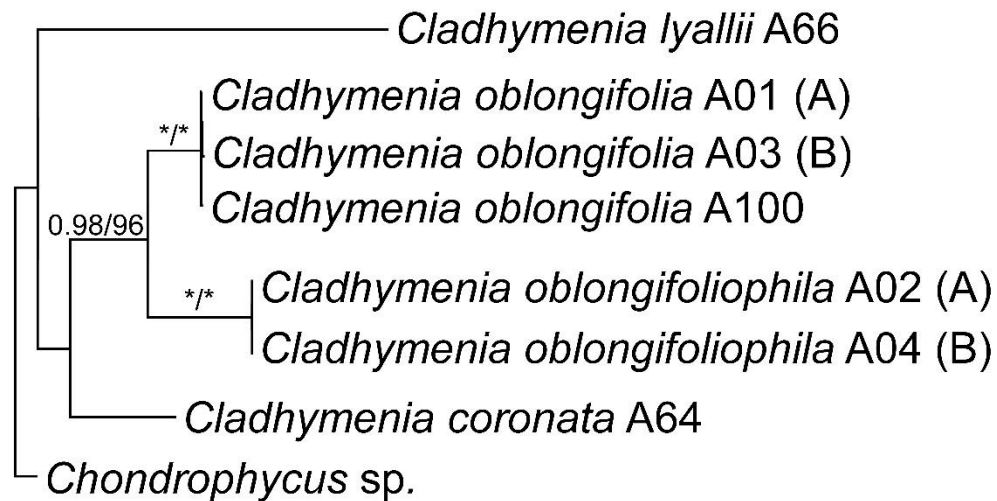
Appendix 3.4. Bayesian topology of *cox*1 sequence data for *Cladhymenia oblongiofolia* and its parasite *Cladhymenia oblongifoliophila* and two other species of *Cladhymenia*: *C. coronata* and *C. lyallii.* Sequences with numbers indicate new sequences (Appendix 3.1) and capital letters in brackets (A-B) indicate parasite and host combinations. Asterisks indicate posterior probability of 1.00 and ML bootstrap value of 100%. Outgroup used was *Chondrophycus* sp. from GenBank (Appendix 3.3).
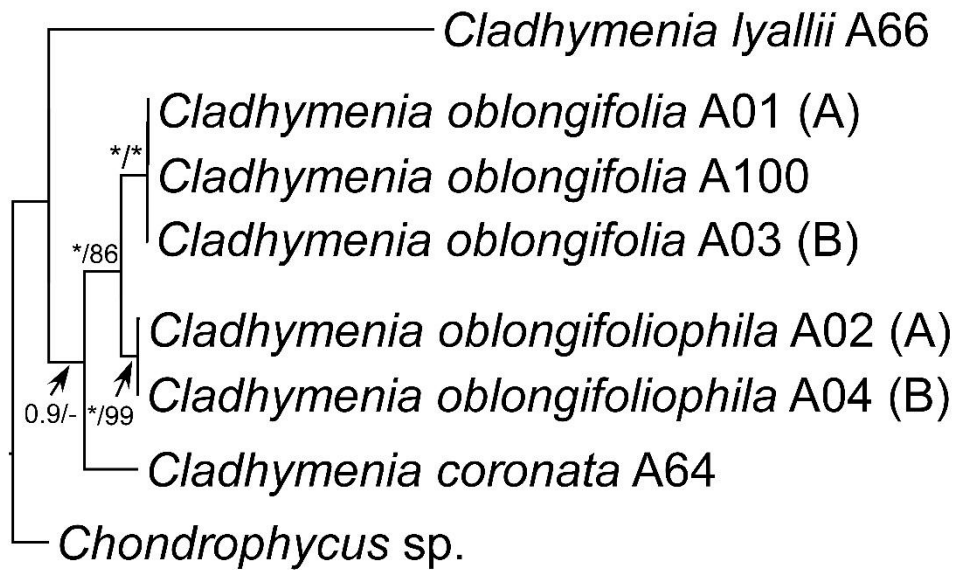
Appendix 3.5. Bayesian topology of LSU rRNA sequence data for *Cladhymenia oblongifolia* and its parasite *Cladhymenia oblongifoliophila* and two other species of *Cladhymenia*: *C. coronata* and *C. lyallii*. Sequences with numbers indicate new sequences (Appendix 3.1) and capital letters in brackets (A-B) indicate parasite and host combinations. Asterisks indicate posterior probability of 1.00 for MrBayes and bootstrap value of 100 of RAxML. Outgroup used was *Chondrophycus* sp. from GenBank (Appendix 3.3).
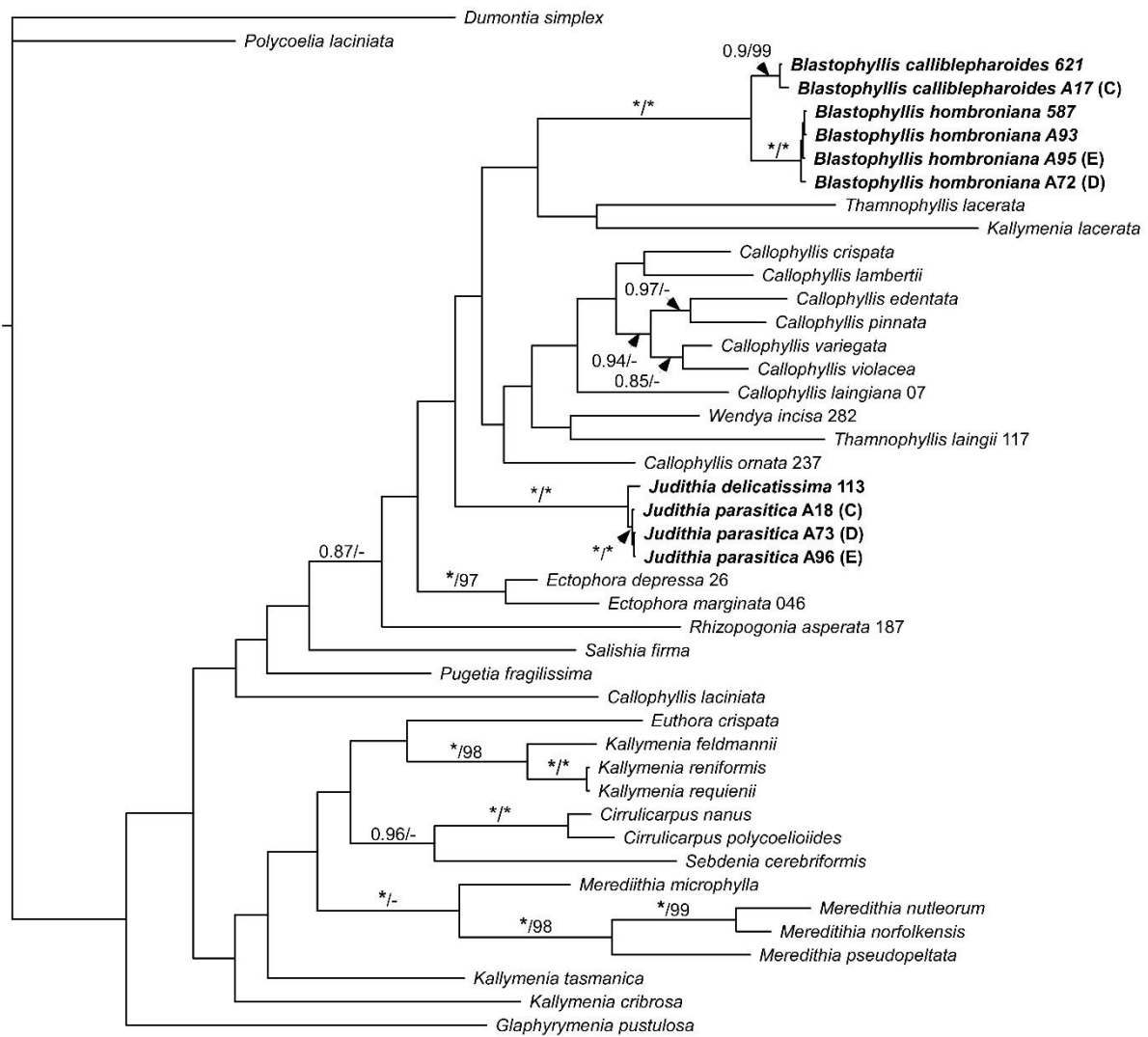
Appendix 3.6. Bayesian topology of *cox*1 phylogenetic analysis for *Judithia parasitica* and its two host species *Blastophyllis calliblepharoides* and *Blastophyllis hombroniana* and other representative within the Kallymeniaceae. Parasite and host combinations are indicated by capital letters in brackets (C-E). Sequences with extraction numbers indicate new sequences (see Appendix 3.1 for collection information) and samples without numbers were downloaded from GenBank (Appendix 3.3). Asterisks indicate posterior probability of 1.00 and ML bootstrap value of 100%. Values < 0.85 posterior probability or < 85% ML bootstrap not shown. Outgroup used were *Dumontia simplex* and *Polycoelia laciniata.*
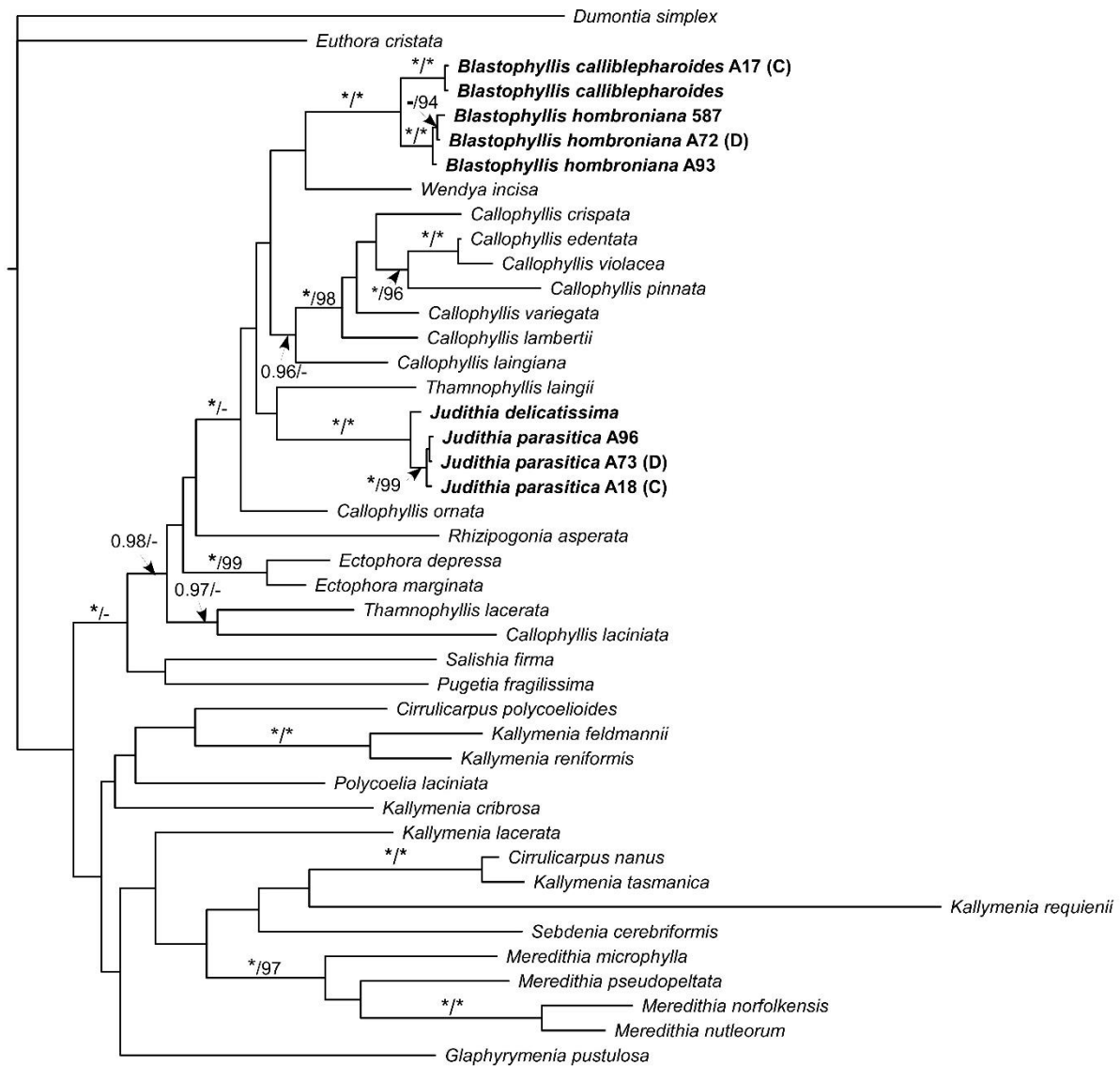
Appendix 3.7. Bayesian topology of *rbc*L data for *Judithia parasitica* and its two host species *Blastophyllis calliblepharoides* and *Blastophyllis hombroniana* and other representative within the Kallymeniaceae. Parasite and host combinations are indicated by capital letters in brackets (C-D). Sequences with numbers indicate new sequences (see Appendix 3.1 for collection information) and samples without numbers were downloaded from GenBank (Appendix 3.3). Asterisks indicate posterior probability of 1.00 and bootstrap value of 100%. Values < 0.85 posterior probability or < 85% ML bootstrap not shown. Outgroup used was *Dumontia simplex*.
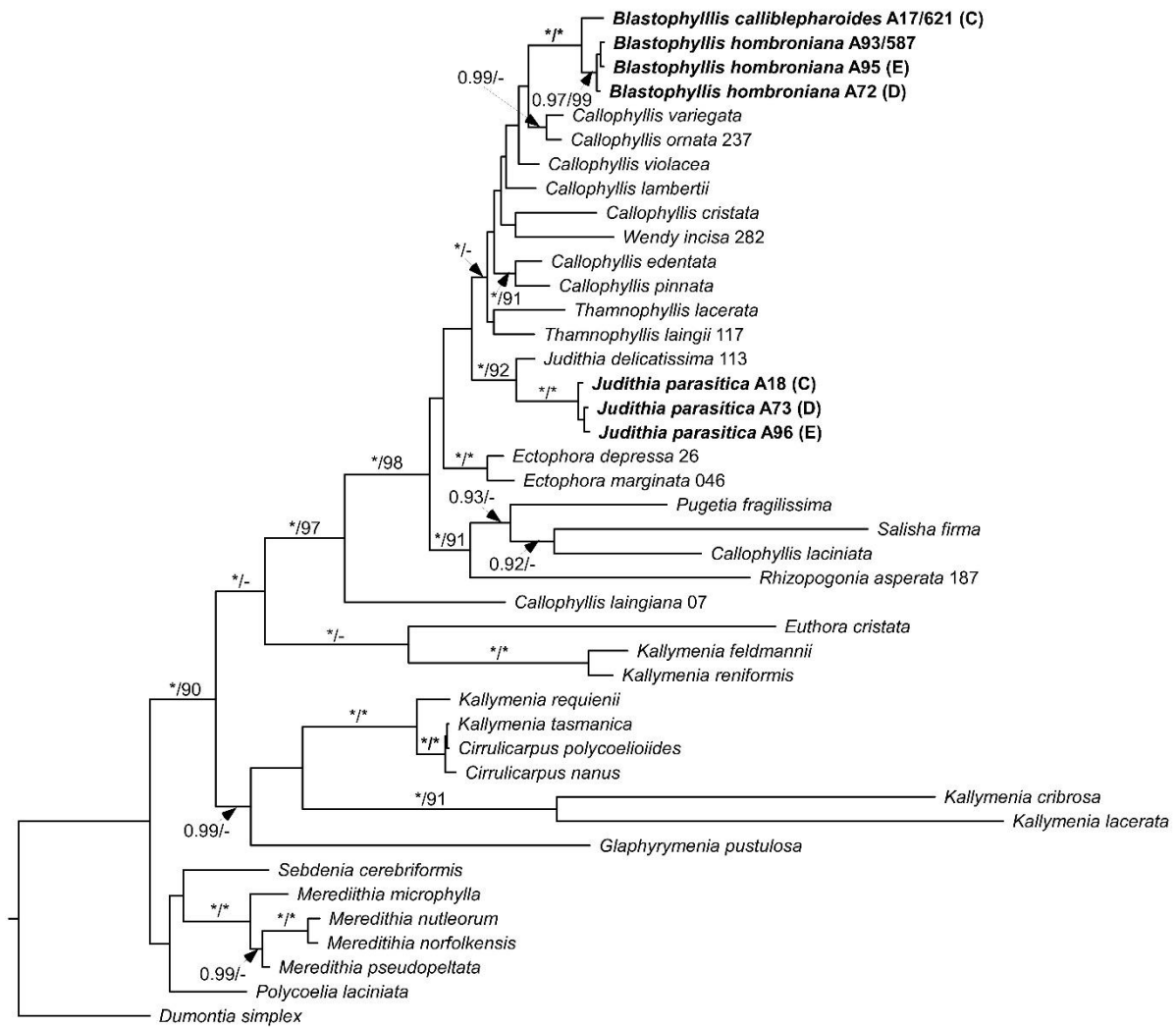
Appendix 3.8. Bayesian topology of LSU rRNA sequence data for the parasite *Judithia parasitica* and its two host species *Blastophyllis calliblepharoides* and *Blastophyllis hombroniana* and other representative within the Kallymeniaceae. Parasite and host combinations are indicated by capital letters in brackets (C-E). Sequences with numbers indicate new sequences (see Appendix 3.1 for collection information) and samples without numbers were downloaded from GenBank (Appendix 3.3) and combined. Sequences with several numbers are combined to represent one species. Asterisk indicate posterior probability of 1.00 and bootstrap value of 100%. Values < 0.85 posterior probability or < 85% ML bootstrap value not shown. Outgroup used was *Dumontia simplex*.

Appendix 3.9. WELT vouchers of *Phycodrys novae-zelandiophila* on its host *Phycodrys novae-zealandiae* with red algal parasites from New Zealand, arranged north to south.

WELT voucher no.	Collection date	Location	Coordinates	Collector
A2939a/b	07.03.1970	Mataikona, East Wairarapa, North Island	40°47'S, 176°16'E	N. M. Adams
A4273	28.02.1971	Ngakawa, south of Castlepoint, North Island	40°56'S, 176°12'E	N. M. Adams & E. Harris
A026386	28.11.2001	Lyall Bay, Wellington, North Island	41°21'S, 174°48'E	W. A. Nelson
A19043	26.04.1990	Port Underwood, Horahora Kakahu Island, South Island	41°19'S, 174°08'E	C. Duffy
A8234a	13.11.1973	Off old Kaikoura wharf, South Island	42°25'S, 173°42'E	G. Fenwick
A024384	29.09.2007	Otago Harbour, Waipuna Bay, Te Ngaru, South Island	45°47'S, 170°40'E	K. Neill
A6952	23.11.1971	Aquarium Street, Portobello, Otago Harbour, South Island	45°50'S, 170°37'E	E. J. Batham
A2979	26.01.1970	Paterson Inlet, Stewart Island	46°55'S, 168°05'E	P. Cresswell

Appendix 3.10. Comparison of vegetative and reproductive structures of the parasite *Phycodrys novae-zelandiophila*, its host (*Phycodrys novae-zelandiae*) and two other *Phycodrys* species from New Zealand: *P. adamsiae* and *P. franiae.*

	Phycodrys novae-zelandiophila sp. nov.	*Phycodrys novae-zelandiae*	*Phycodrys franiae*	*Phycodrys adamsiae*
Thallus				
Size	1-2 x 1-2 mm	8-15 x 3-5 cm	4-11 x 4-12 cm	3-8 x 1-8 cm
Growth form	Upright, several single branches	Blades	Blades	Blades
Pigmentation	Light red	Rose-pink to dark red	Rose-pink to dark red	Brownish to dark red
Reproductive structures				
Gametophyte	Dioecious	Dioecious	Dioecious	Dioecious
Carposporophyte				
Size in diameter	430-530 µm	650-900 µm	600-950 µm	680-850 µm
Carpospores	Born in short chains	In terminal clusters	Born in short chains	Born in short chains
Central Fusion cell	Yes	Yes	Yes	Yes
Tetrasporangia				
Location	Scattered on the surface in stichidia	Both sides of fertile blade	In fertile blade	In marginal bladelets
Form	Tetrahedrally divided	Tetrahedrally divided	Tetrahedrally divided	Tetrahedrally divided
Size	32 x 40 µm in diameter	55 x 65 µm in diameter	45 x 60 µm in diameter	35-45 x 55-60 µm in diameter
Reference	This study	Lin & Nelson 2009	Lin & Nelson 2009	Lin & Nelson 2009

Appendix 3.11. Comparison of vegetative and reproductive structures between *Phycodrys novae-zelandiae* and two different parasites of *Phycodrys*[1]: (*Asterocolax denticulatus* and *Asterocolax gardneri*) * = indicates that more host species are known but from different genera.

	Asterocolax denticulatus	*Asterocolax gardneri*	*Phycodrys novae-zelandiophila* sp. nov.
Host(s)	*Phycodrys fimbriata* (Kuntze) Kylin	*Phycodrys isabelliae* R.E. Norris & M.J. Wynne, *Phycodrys setchellii* Skottsb.*	*Phycodrys novae-zelandiae*
Distribution	Tyuleny Island (as Robben Island), Russia	California, USA	New Zealand
Thallus (width)	-	2-3 mm	1-2 mm
Pigmentation	-	Yes	Yes
Tetrasporophyte			
Division	Tetrad or obliquely cruciate	-	Tetrahedrally
Shape	Oblong-obovate	-	Globose
Location	Scattered over the surface	Scattered over the surface	Scattered over the surface
Size	-	-	40 x 32 µm
Female gametophyte			
Cystocarp size	500 x 840 µm in diameter	-	430 x 530µm in diameter
Cystocarps on branch	One, rarely two	One, born in chains	One, born in chains
References	Tokida 1934	Setchell 1923; Wagner 1954	This study

[1]Exclusion of *Choreocolax rabenhorstii* growing on *Phycodrys rubens* (L.) Batters for lack of morphological data.

Appendix 3.12. Te Papa voucher specimens of *Cladhymenia oblongifolia* with *Cladhymenia oblongifoliophila* in New Zealand, arranged from north to south.

WELT voucher	Collection date	Location	Coordinates	Collector
A13737a, b	29.09.1973	Piha, Auckland, North Island	36°57'S, 174°28'E	C. H. Hay
A17564	22.04.1984	Maketu Bay, Bay of Plenty, North Island	37°45'S, 176°28'E	W. A. Nelson
A025857	26.02.1993	Marlborough Sounds, D'Urville Island, South Island	40°48'S, 173°47'E	W. A. Nelson
A4332	18.03.1971	Lyall Bay, Wellington, North Island	41°21'S, 174°48'E	A. N. Baker
A17109a, b, c	30.01.1973	Mangere Island, Chatham Islands	44°16'S, 176°18'W	C. H. Hay

Appendix 3.13. Comparison of *Cladhymenia oblongifolia* and its parasite *Cladhymenia oblongifoliophila.*

	Cladhymenia oblongifoliophila sp. nov.	*Cladhymenia oblongifolia*
Thallus		
Size	2 mm in diameter	30 cm high
Colouration	Unpigmented	Pinkish red with yellow cast
Female gametophytes		
Location	On branches	On proliferations
Tetrasporangia		
Form	Tetrahedrally divided	Tetrahedrally divided
Location	On branches	On branches
Reference	This study	Nelson 2013

Appendix 3.14. Te Papa voucher specimens of *Blastophyllis calliblepharoides* and *Blastophyllis hombroniana* with the parasite *Judithia parasitica* from New Zealand, arranged north to south.

Host species	WELT voucher number	Collection date	Location	Coordinates	Collector
Blastophyllis calliblepharoides	A11368b/c	23.12.1976	North side of Boat Harbour, Snares Island	48°01'S, 166°36'E	C. D. Fenwick
Blastophyllis hombroniana	A1262		Banks Peninsula, South Island	43°45'S, 72°55'E	Berggren
	A2833		Timaru, South Island	44°24'S, 171°16'E	
	A11547	10.02.1981	Shag Point, Bay south of Boat Harbour, North Otago, South Island	45°28'S, 170°50'E	N. M. Adams
	A2832		Dunedin, South Island	45°53'S , 170°31'E	
	A02126	07.1998	Brighton, Otago, South Island	45°57'S, 170°20'E	W. A. Nelson
	A024349	08.12.2008	Brighton, Otago, South Island	45°57'S, 170°20'E	R. D'Archino
	A028700	12.03.1998	Brighton, Otago, South Island	45°57'S, 170°20'E	J. Broom
	A028701	12.03.1998	Brighton, Otago, South Island	45°57'S, 170°20'E	J. Broom
	A12937	03.03.1982	South end Tautuku Beach, South East Otago, South Island	46°36'S, 169°26'E	C. Hay & P. Hay
	A7530	22.11.1959	Ringaringa, Stewart Island	46°54'S, 168°8'E	E. A. Willa
	A029648	06.10.1994	Ringaringa, Stewart Island	46°54'S, 168°8'E	W. A. Nelson
	A029649	06.10.1994	Ringaringa, Stewart Island	46°54'S, 168°8'E	W. A. Nelson
	A029650	06.10.1994	Ringaringa, Stewart Island	46°54'S, 168°8'E	W. A. Nelson
	A029651	06.10.1994	Ringaringa, Stewart Island	46°54'S, 168°8'E	W. A. Nelson

A029786	06.10.1994	Ringaringa, Stewart Island	46°54'S, 168°8'E	W. A. Nelson
A9457	01.1975	Sandy Beach, Enderby Island, Auckland Islands	50°30'S, 166°17'E	J. C. Yaldwyn
A16585	02.1985	Sandy Beach, Enderby Island, Auckland Islands	50°30'S, 166°17'E	J. C. Yaldwyn

Appendix 3.15. Comparison of *Judithia parasitica* and its closest relative *Judithia delicatissima.*

	Judithia parasitica sp. nov.	*Judithia delicatissima*
Thallus		
Size	1 x 1 mm	12-21 x 4-10 cm
Branching	One time	One or more times
Pigmentation	Light reddish	Light rose-red
Tetrasporangia		
Form	Cruciate	Cruciate
Location	Scattered in branches	Scattered outer cortex
Size	26 x 13 µm	25-28 x 18-20 µm
Reference	This study	D'Archino *et al.* 2016

Appendix 3.16. References for Appendices 3.2, 3.5, 3.6, 3.8 and 3.10 (not included in the main text).

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Harper, J. T. & Saunders, G. W.2001. The application of sequences of the ribosomal cistron to the systematics and classification of the florideophyte red algae (Florideophyceae, Rhodophyta). *Cah. Biol. Mar.* **42**:25-38.

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Kamiya, M., West, J. A. & Hara, Y. 2011. Induction of apomixis by outcrossing between genetically divergent entities of *Caloglossa leprieurii* (Ceramiales, Rhodophyta) and evidence of hybrid apomicts in nature. *J. Phycol.* **47**:753-762.

Kim, M. S., Kim, S. Y. & Nelson, W. A. 2010. *Symphyocladia lithophila* sp. nov. (Rhodomelaceae, Ceramiales), a new Korean red algal species based on morphology and rbcL sequences. *Bot. Mar.* **53**:233-241.

Nelson, W. A. 2013. *New Zealand Seaweeds.* Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand.

Wang, H. W., Kawaguchi, S. Horiguchi, T. & Masuda, M. 2000. Reinstatement of *Grateloupia catenata* (Rhodophyta, Halymeniaceae) on the basis of morphology and *rbc*L sequences. *Phycologia* **39**:228-237.

Appendix 4.1. Samples used for phylogenetic analysis.

	*cox*1
Aiolocolax pulchellus Pocock	KF671160
Digenea arenahauriens C.W.Schneider, Hamzeh *et* G.W.Saunders	MG648076
Digenea cymatophilum (R.E.Norris) Díaz-Tapia *et* Maggs	HQ422981
Echinothamnion hystrix (Hook.f. *et* Harv.) Kylin	KU564426
Leptosiphonia schousboei (Thur.) Kylin	KF671176
Melanothamnus bajacali (Hollenb.) Díaz-Tapia *et* Maggs	HM573526
Melanothamnus harveyi (Bailey) Díaz-Tapia *et* Maggs	KJ202082
Melanothamnus japonicus (Harv.) Díaz-Tapia *et* Maggs	KM894048
	KX265515
Melanothamnus pseudovillum (Hollenb.) Díaz-Tapia *et* Maggs	HM573524
Melanothamnus somalensis Bornet *et* Falkenb.	KU564334
Melanothamnus sphaerocarpa (Børgesen) Díaz-Tapia *et* Maggs	KX265541
Melanothamnus strictissima (Hook.f. *et* Harv.) Díaz-Tapia *et* Maggs	HM573534
Melanothamnus teradomariensis (Noda) Díaz-Tapia *et* Maggs	KX265517
Melanothamnus tongatensis (Harv. ex Kütz.) Díaz-Tapia *et* Maggs	HM573518
Melanothamnus upolensis (Grunow) Díaz-Tapia *et* Maggs	HQ422784
Polyostea arctica (J.Agardh) Savoie *et* G.W.Saunders	JX571980
Polysiphonia atlantica Kapraun *et* J.N.Norris	HM573539
Polysiphonia caespitosa (Pocock) Hollenb.	KF671181
Polysiphonia binneyi Harv.	KY656536
Polysiphonia brodiei (Dillwyn) Spreng.	KJ961047
Polysiphonia confusa Hollenb.	KR080578
Polysiphonia devoniensis Maggs *et* Hommers.	KF671186
Polysiphonia echinata Harv.	HM573559
Polysiphonia elongata (Huds.) Spreng.	KJ961050
Polysiphonia fibrillosa (Dillwyn) Spreng.	KJ961052
Polysiphonia havanensis Mont.	HM573522
Polysiphonia hemisphaerica Aresch.	HQ412544
Polysiphonia homoia Setch. *et* N.L.Gardner	HM573507
Polysiphonia morrowii Harv.	HM573540
Polysiphonia pacifica Hollenb.	KM254964
Polysiphonia paniculata Mont.	KR090577

Polysiphonia pentamera Hollenb.	HM573510
Polysiphonia schneideri B.Stuercke *et* Freshwater	HM573514
Polysiphonia scopulorum Harv.	HM573535
Polysiphonia sertularioides (Gratel.) J.Agardh	HM573519
Polysiphonia stricta (K.Mert ex Dillwyn) Grev.	KJ961053
Polysiphonia subtilissima Mont.	JX294916
Symphyocladia latiuscula (Harv.) Yamada	KC782862
Symphyocladia dendroidea (Mont.) Savoie *et* G.W.Saunders	KU564383
Tolypiocladia glomerulata (C.Agardh) F.Schmitz	HQ423106
Vertebrata aterrima	HM573536
	HM573537
	MH670285
	MH670286
Vertebrata aterrimophila	MH670282
	MH670283
	MH670284
Vertebrata byssoides (Gooden. *et* Woodw.) Kuntze	KJ960354
Vertebrata constricta (Womersley) Díaz-Tapia *et* Maggs	HM573542
Vertebrata fruticulosa (Wulfen) Kuntze	KJ960346
Vertebrata fucoides (Huds.) Kuntze	HM5734496
Vertebrata hypnoides (Welw.) Kuntze	KF671184
Vertebrata isogona (Harv.) Díaz-Tapia *et* Maggs	HM573541
Vertebrata lanosa (L.) T.A.Chr.	KX687880
Vertebrata nigra (Huds.) Díaz-Tapia *et* Maggs	KC130873
Vertebrata tripinnata (Harv.) Kuntze	KC130871
Vertebrata reptabunda (Suhr) Díaz-Tapia *et* Maggs	KF671184

Appendix 4.2. Te Papa vouchers of *Vertebrata aterrimophila* on its host *Vertebrata aterrima* from New Zealand, arranged north to south.

Te Papa voucher	Collection date	Location	Collectors
A022101	06.10.1991	Pouawa, East Coast	W. A. Nelson
A19890	05.10.1991	Mahia Peninsula	W. A. Nelson
A19887	05.10.1991	Mahia Peninsula, East of Aurora Point	W. A. Nelson
A3773a	24.07.1970	Island Bay, Wellington	N. M. Adams
A025251	12.06.1998	Island Bay, Wellington	W. A. Nelson
A16028	19.03.1984	Kairākau, Hawkes Bay	W. A. Nelson
A19089	06.04.1990	Middle Trio Island, Trio Islands	C. Duffy

Appendix 5.1. Sequences and whole organelle genomes plus associated GenBank Accession numbers used for molecular analysis of parasite, host species and representatives within the Florideophytes. Sequences and whole organelle genomes newly obtained during this study are highlighted in bold.

Species	*cox*1	cp genome	mt genome	SSU rDNA	LSU rDNA
Acanthophora pacifica (Setch.) Kraft				HQ421679	
Acanthophora spicifera (M.Vahl) Børgesen				HQ422025	
Acrosorium ciliolatum (Harv.) Kylin		MF101411			MF093911
Acrosorium yendoi Yamada				KC795858	
Acrosymphyton purpuriferum (J.Agardh) G.Sjöstedt					
Aglaothamnion boergesenii (Aponte *et* D.L.Ballant.) L'Hardy-Halos *et* Rueness				HQ422268	
Aglaothamnion cordatum (Børgesen) Feldm.-Maz.				HQ422402	
Aglaothamnion halliae (Collins) Aponte, D.L.Ballant. *et* J.N.Norris					DQ022771
Ahnfeltiopsis chnoosporoides (T.Tanaka *et* Pham-Hoàng Hô) Masuda				KU640338	
Ahnfeltiopsis concinna (J.Agardh) P.C.Silva *et* DeCew				HQ422135	
Ahnfeltiopsis devoniensis (Grev.) P.C.Silva *et* DeCew				KU640342	
Ahnfeltiopsis fastigata J.A.Lewis *et* Womersley				KU640344	
Ahnfeltiopsis flabelliformis (Harv.) Masuda				HQ422144	
Ahnfeltiopsis linearis (C.Agardh) P.C.Silva *et* DeCew				KU640353	
Ahnfeltiopsis paradoxa (Suringar) Masuda				KU640354	
Ahnfeltiopsis pusilla (Mont.) P.C.Silva *et* DeCew				KU640356	
Ahnfeltiopsis pygmaea (J.Agardh) P.C.Silva *et* DeCew				KU640357	

Species				
Amansia fimbrifolia (R.E.Norris) L.E.Phillips			HQ422093	
Amansia glomerata C.Agardh			HQ421684	AF251512
Antithamnion antillanum Børgesen			HQ422561	
Antithamnion decipiens (J.Agardh) Athanas.			HQ422454	
Antithamnionella spiirographidis (Schiffn.) E.M.Woll.				DQ022761
Antithamnion erucacladellum R.E.Norris			HQ422565	
Antithamnion sparsum Tokida				AY168238
Asparagopsis taxiformis (Delile) Trevis.	NC031148	KJ398158		
Asterfilopsis centralis M.S.Calderon *et* S.M.Boo			KU640365	
Asterfilopsis disciplinalis (Bory) M.S.Calderon *et* S.M.Boo			KU640360	
Asterfilopsis furcellata (C.Agardh) P.C.Silva *et* DeCew			KU640362	
Asterfilopsis piurana M.S.Calderon *et* S.M.Boo			KU640363	
Besa catenata (Yendo) M.S.Calderon *et* S.M.Boo			KU749583	
Besa divaricata (Holmes) M.S.Calderon *et* S.M.Boo			HQ421815	
			KU749585	
Besa leptophylla (J.Agardh) M.S.Calderon *et* K.A.Miller			KU640351	
Betaphycus gelatinus (Esper) Doty ex P.C.Silva		MF680514		
Bostrychia moritziana (Sond. ex Kütz.) J.Agardh	MF101419			
Bostrychia simpliciuscula Harv. ex J.Agardh	MF101421			
Bostrychia tenella (J.V.Lamour.) J.Agardh	MF101417			
Botryocladia occidentalis (Børgesen) Kylin			KT154741	

Botryocladia pyriformis (Børgesen) Kylin			KT154739		
Botryocladia skottsbergii (Børgesen) Levring			HQ422497		
Botryocladia spinulifera W.R.Taylor *et* I.A.Abbott					EU670591
Botryocladia wynnei D.L.Ballant.					EU670589
Bryothamnion seaforthii (Turner) Kütz.	NC021075				
Calliarthron tubercolosum (Postels *et* Rupr.) E.Y.Dawson	NC021075	NC027061			
Callithamnion corymnosum (Sm.) Lyngb.			KC795866		
Caloglossa adhaerens R.J.King *et* Puttock			AF522199		
Caloglossa beccarii (Zanardini) De Toni	MF101422		AF522208	MF093916	
Caloglossa bengalensis (G.Martens) R.J.King *et* Puttock			AF522210		
Caloglossa continua (Okamura) R.J.King *et* Puttock			AF5522212		
Caloglossa intermedia M.Kamiya *et* J.A.West	MF101418			MF093917	
Caloglossa leprieurii (Mont.) G.Martens			AF522204		
			AF522217		
Caloglossa monosticha M.Kamiya	MF101416		AF522213		
				MF093918	
Caloglossa ogasawaraensis Okamura			AF522239	AF251514	
Caloglossa postiae M.Kamiya *et* R.J.King			AF522242		
Caloglossa rotundata M.Kamiya			AF522248		
Caloglossa saigonensis Tanaka *et* Pham-Hoàng Hô			AF522244		
Caloglossa stipitata E.Post			AF522247		

Ceramium affine Setch. *et* N.L.Gardner				AF460859
Ceramium codii (H.Richards) Feldm.-Maz.			HQ421967	
Ceramium diaphanum (Lightf.) Roth			KC795860	DQ022760
Ceramium dumosertum R.E.Norris *et* I.A.Abbott			HQ421685	
Ceramium hyalacanthum (Kütz.) Sond.			HQ422526	
Ceramium japonicum Okamura	NC031174	KJ398159	KC795870	
Ceramium kondoi Yendo			KC795848	
Ceramium nakamurae E.Y.Dawson			HQ421934	
Ceramium sungminbooi Hughey *et* G.H.Boo	NC031211	KU145004		
Ceramium tenerrimum (G.Martens) Okamura			KC795867	AF460867
Ceramium womersleyi R.E.Norris *et* I.A.Abbott			HQ422536	
Ceratodictyon scoparium (Mont. *et* Millardet) R.E.Norris			HQ422496	
Champia harveyana D.L.Ballant. *et* C.Lozada-Troche				FJ212289
Champia parvula (C.Agardh) Harv.			HQ422011	
Champia vieillardii Kütz.			HQ422541	FJ212290
Chiharaea bodegensis H.W.Johans.			KC157588	KC157576
Chondracanthus acucularis (Roth) Fredericq			HQ421761	
Chondracanthus intermedius (Suringar) Hommers.			KU640368	
Chondracanthus tenellus (Harv.) Hommers.			HQ422443	
Chondria crassicaulis Harv.			KC795859	
Chondria dangeardii E.Y.Dawson			HQ422160	

Species				
Chondria spp.	MF101429			
	MF101431			
	MF101451			
Chondrophycus cartilagineus (Yamada) Garbary *et* J.T.Harper			HQ421772	
Chondrophycus dotyi (Y.Saito) K.W.Nam			HQ421698	
Chondrophycus succisus (Cribb) K.W.Nam			HQ422366	
Chondrophycus undulatus (Yamada) Garbary *et* J.T.Harper			HQ421943	
Chondrus crispus Stackh.	NC020795	NC001677	KU640369	DQ317002
Chondrus ocellatus Holmes			HQ421762	DQ316985
Chrysymenia brownii (Harv.) De Toni			KT154734	
Chrysymenia kaernbachii Grunow			HQ422492	
Chrysymenia littleriana J.N.Norris *et* D.L.Ballant.			KT154728	
Chrysymenia nodulosa J.N.Norris *et* D.L.Ballant.			KT154724	
Chrysymenia ornata (J.Agardh) Kylin			KT154735	
Chrysymenia planifrons (Melvill) J.Agardh			KT154725	
Chrysymenia pseudoventricosa W.E.Schmidt, Gurgel *et* Fredericq			KT154727	
Chrysymenia ventricosa (J.V.Lamour.) J.Agardh			KT154732	
Cliftonaea pectinata (Harv.) Harv.	MF101450			
Coelarthrum cliftonii (Harv.) Kylin			HQ421847	EU670595
Coeloseira compressa Hollenb.	NC030338	KU053956		
Coelothrix irregularis (Harv.) Børgesen			HQ422007	FJ173068

Corallina officinalis L.			KU641510		
Corallophila huysmansii (Weber Bosse) R.E.Norris				HQ421935	
Cryptonemia yendoi Weber Bosse				HQ422439	
Cubiculosporum koronicarpis Kraft				HQ421763	
Dasya anastomosans (Weber Bosse) M.J.Wynne				HQ422407	
Dasya binghamiae A.Millar		NC031161	KX247283		
Dasya corymbifera J.Agardh				HQ422118	
Dasya iridescens (Schlech) A.Millar *et* I.A.Abbott				HQ422246	
Dasya kristeniae I.A.Abbott				HQ421889	
Dasya murrayana I.A.Abbott *et* A.Millar				HQ422216	
Dasya naccarioides Harv.		MF101436			
Dasyclonium flaccidium (Harv.) Kylin		MF101455			
Dictyomenia sonderi Harv.		MF101455			
Digenea simplex (Wulfen) C.Agardh		MF101465			
Diplothamnion jolyi C.Hoek				HQ422542	
Dipterocladia arabiensis M.J.Wynne *et* Y.S.D.M.de Jong		MF101408			
Dipterosiphonia australica Womersley		NC035288			
Dudresnaya hawaiiensis R.K.S.Lee				HQ421771	
Eucheuma denticulatum (Burm.f.) Collins *et* Herv.			MF680515		
Euptilocladia magruderi I.A.Abbott *et* R.E.Norris				HQ422348	
Gelidiella acerosa (Forssk.) Feldmann *et* Hamel	HM102421			GAU60342	AF296518

Species			
Gelidiella machrisiana E.Y.Dawson			HQ421741	
Gelidium arborescens N.L.Gardner		KX427228		
Gelidium crinale f. luxurians		KX427229		
Gelidium elegans Kütz.	NC029858	KF290995		
Gelidium floridanum W.R.Taylor			AF296510	GFU60351
Gelidium galapagense W.R.Taylor		KX427230		
Gelidium isabelae W.R.Taylor		KX427231		
Gelidium japonicum (Harv.) Okamura			AF521185	AB017667
Gelidium pacificum Okamura	HM629871			
Gelidium pluma Bornet ex Loomis			HQ422413	
Gelidium pulchellum (Turner) Kütz.			AF296509	
Gelidium reediae Loomis			HQ421956	
Gelidium sclerophyllum W.R.Taylor		KX427232		
Gelidium sinicola N.L.Gardner		KX427233		
Gelidium vagum Okamura	NC029859	KC875854		
Gelinaria ulvoidea Sond.			GQ471910	
Gibsmithia dotyi Kraft *et* R.W.Ricker			HQ421756	
Gibsmithia hawaiiensis Doty			HQ422508	
Gloiocladia iyoensis (Okamura) R.E.Norris			HQ422184	
Gracilaria abbottiana M.D.Hoyle			HQ422425	
Gracilaria chilensis C.J.Bird, McLachlan *et* E.C.Oliveira	NC029860	KP728466		

Species				
Gracilaria chouae J.F.Zhang *et* B.M.Xia		MF351970		
Gracilaria coronopifolia J.Agardh			HQ421829	
Gracilaira dawsonii M.D.Hoyle			HQ422426	
Gracilaria dotyi M.D.Hoyle			HQ421977	
Gracilaria epihippisora M.D.Hoyle			HQ422428	
Gracilaria firma C.F.Chang *et* B.M.Xia	NC033877			
Gracilaria parvispora I.A.Abbott			HQ422206	
Gracilaria salicornia (C.Agardh) E.Y.Dawson	KF861575		HQ422218	KT897251
	NC023785			
Gracilaria tenuistipitata C.F.Chang *et* B.M.Xia	AY673996			
Gracilaria tikvahiae McLachland			HQ422432	GRCEARA
Gracilaria vermiculophylla (Ohmi) Papenf.		KJ526626		
Gracilariophila oryzoides Setch. *et* H.L.Wilson		HQ586059		
Gracilariopsis andersonii (Grunow) E.Y.Dawson		HQ586060		
Gracilariopsis chorda (Holmes) Ohmi	KX284722	KC875851		
Gracilariopsis lemaneiformis (Bory de Saint-Vincent) E.Y.Dawson	NC029644	JQ071938	HQ422429	KC577234
	KP330491			
Grateloupia angusta (Okamura) Kawag. *et* H.W.Wang		KC875853		
Grateloupia catenata Yendo			HQ422450	
Grateloupia filicina (J.V.Lamour.) C.Agardh			HQ422213	
Grateloupia hawaiiana E.Y.Dawson			HQ422300	

Species			
Grateloupia ovata Womersley *et* J.A.Lewis		GQ471911	
Grateloupia phuquocensis Tanaka *et* Pham-Hoàng Hô		HQ421682	
Grateloupia taiwanensis Showe M.Lin *et* H.Y.Liang	KC894740	KM999231	
Gredgaria maugeana Womersley	MF101446		
Griffithsia heteromorpha Kütz.		HQ422062	
Griffithsia schousboei Mont.		HQ421680	
Griffithsia subcylindrica Okamura		HQ421691	
Gymnogongrus crenulatus (Turner) J.Agardh		KU640371	
Gymnogongrus griffithsiae (Turner) Mart.		KU640372	
Gymnogongrus guadalupensis E.Y.Dawson		KU640373	
Gymnothamnion elegans (Schousb. ex C.Agardh) J.Agardh		HQ422562	
Halichrysis coalescens (Farl.) R.E.Norris *et* A.Millar		HQ421699	
Halymenia floresii (Clemente) C.Agardh		GQ471912	
Halymenia formosa Harv. ex Kütz.		HQ422182	
Halymenia maculata J.Agardh		GQ471913	
Halymenia plana Zanardini		GQ471914	HPU33133
Halymenia pseudofloresii Collins *et* M.Howe		GQ471915	
Herposiphonia versicolor (Hook.f. *et* Harv.) Reinbold	MF101434		
Heterosiphonia crispella (C.Agardh) M.J.Wynne		HQ422436	
Heterosiphonia japonica Yendo		KC795855	
Hypnea cervicornis J.Agardh		HQ421782	

Hypnea chordacea Kütz.			HQ421792
Hypnea musciformis (Wulfen) J.V.Lamour.			HQ421809
Hypnea nidifica J.Agardh			HQ421816
Hypnea pannosa J.Agardh			HQ421823
Hypnea spinella (C.Agardh) Kütz.			HQ421827
Hypnea valentiae (Turner) Mont.			HQ421828
Hypneocolax stellaris Børgesen			HQ422549
Janczewskia hawaiiana Apt			HQ422189
Jania sagittata (J.V.Lamour.) Blainv.			KC157591 KC157580
Kallymenia sessilis Okamura			HQ421882
Kallymenia thompsonii I.A.Abbott *et* McDermid			HQ422445
Kappaphycus alvarezii (Doty) Doty ex P.C.Silva	NC036637	KU885455	
Kappaphycus striatus (F.Schmitz) Doty ex P.C.Silva		KF833365	
Kuetzingia canaliculata (Grev.) Sond.	MF101449		
Laurencia brachyclados Pilg.			HQ422341
Laurencia decumbens Kütz.			HQ421722
Laurencia galtsoffii M.Howe			HQ421781
Laurencia majuscula (Harv.) A.H.S.Lucas			HQ421712
Laurencia mcdermidiae I.A.Abbott			HQ421713
Laurencia nidifica J.Agardh			HQ421726
Laurencia nipponica Yamada			KC795864

Species				
Laurencia obtusa (Huds.) J.V.Lamour.			KC795869	
Laurencia sp.	LN833431			
Laurencieae sp.	MF101412			
Laurencia tenera C.K.Tseng			HQ422278	
Laurenciella marilzae (Gil-Rodríguez, Sentíes, Díaz-Larrea, Cassano *et* M.T.Fujii) Gil-Rodríguez, Sentíes, Díaz-Larrea, Cassano *et* M.T.Fujii	MF101410			
Lejolisia pacifica Itono			HQ421689	
Leveillea jungermannioides (K.Hering *et* G.Martens) Harv.			HQ422288	
Lomentaria hakodatensis Yendo			HQ422104	
Lophocladia kipukaia Schlech			HQ422350	
Lophocladia kuetzingii (Kuntze) P.C.Silva	MF101448			
Martensia flabelliformis Harv. ex J.Agardh			HQ421860	
Martensia fragilis Harv.			HQ422500	
Mastocarpus papillatus (C.Agardh) Kütz.	NC031167	KX525587		
Mazzaella japonica (Mikami) Hommers.			KU640374	
Mazaella volans (C.Agardh) Fredericq			HQ421873	
Melaconema minimum Hollenb.			HQ422545	
Melanothamnus ferulacea (Suhr ex J.Agardh) Díaz-Tapia *et* Maggs				HM560645
Melanothamnus harveyi (J.W.Bailey) Díaz-Tapia *et* Maggs	MF101437			
Melanothamnus japonicus (Harv.) Díaz-Tapia *et* Maggs			KC795854	AB219908
Melanothamnus upolensis (Grunow) Díaz-Tapia *et* Maggs			HQ421932	

Membranoptera platyphylla (Setch. *et* N.L.Gardner) Kylin	NC032041	
Membranoptera tenuis Kylin	NC032399	
Membranoptera weeksiae Setch. *et* N.L.Gardner	NC032396	
Millerella pannosa (Feldmann) G.H.Boo *et* L.Le Gall		AF308799
Monosporus indicus Børgesen		HQ422554
Neorhodomela munita (Perest.) Masuda		KC795863
Neosiphonia tepida (Hollenb.) S.M.Guim. *et* M.T.Fujii		HQ421963
Ophidocladus simpliciuscula (P.Crouan *et* H.Crouan) Falkenb.	MF101440	
Osmundaria fimbriata (J.V.Lamour.) R.E.Norris	MF101415	
Osmundaria obtusiloba (C.Agardh) R.E.Norris		HQ422217
Pachymenia lusoria (Grev.) J.Agardh		GQ471917
Pachymenia orbicularis (Zanardini) Setch. *et* N.L.Gardner		GQ471918
Palisada crustiformans (McDermid) A.R.Sherwood, Kurihara *et* K.W.Nam		HQ421721
Palisada parvipapillata (C.K.Tseng) K.W.Nam		HQ422036
Palisada sp.	MF101453	
Palisada yamadana (M.Howe) K.W.Nam		HQ421780
Peleophycus multiprocarpium I.A.Abbott		HQ421875
Perikladosporon percurrens (E.Y.Dawson) Athanas.		HQ422566
Periphykon beckeri Weber Bosse	MF101413	
Peyssonnelia conchicola Picc. *et* Grunow		HQ421876

Peyssonnelia inamoena Pilg.		HQ421886	
Peyssonnelia rubra (Grev.) J.Agardh		HQ421916	
Phycodrys radicosa (Okamura) Yamada *et* Inagaki		KC795861	
Platoma ardreanum Kraft *et* I.A.Abbott		HQ421896	
Platysiphonia delicata (Clemente) Cremades	MF101409		
Plocamiocolax pulvinatus Setch.	HQ586061		
Plocamium cartilagineum (L.) P.S.Dixon	KX284727	KJ398160	
Plocamium sandvicense J.Agardh		HQ422471	
Polyopes hakalauensis (Tilden) I.A.Abbott		HQ422038	
Polyopes tasmanicus (Womersley *et* J.A.Lewis) Kawag. *et* J.A.Lewis		GQ471919	
Polysiphonia binneyi Harv.			HM560636
Polysiphonia brodiei (Dillwyn) Spreng.	MF101425		
Polysiphonia elongata (Hudson) Spreng.	MF101427		
Polysiphonia howei Hollenb.		HQ422015	
Polysiphonia infestans Harv.	MF101432		
Polysiphonia schneideri B.Stuercke *et* D.W.Freshwater	MF101454		
Polysiphonia scopulorum Harv.	MF101438		
Polysiphonia senticulosa Harv.		KC795862	AB219907
Polysiphonia sertularioides (Grateloup) J.Agardh	MF101423		
Polysiphonia spp.	MF101414		
	MF101456		

Species					
Polysiphonia stricta (Mertens ex Dillwyn) Grev.	MF101428				
Predaea laciniosa Kraft				HQ422487	
Predaea weldii Kraft *et* I.A.Abbott				HQ422488	
Pterocladia lucida Group I	XXXXXX				
	KT443928				
	KT443932				
	KT443933				
	KT443936				
	KT443937				
Pterocladia lucida Group II	XXXXXX	XXXXXX		XXXXXX	XXXXXX
	KT443939				
	KT443942				
	KT443943				
	KT443946				
Pterocladia lucida Group III	KT443950				
Pterocladiella bartlettii (W.R.Taylor) Santel.				AF296515	EF191192
Pterocladiella beachiae Freshwater				AF296514	
Pterocladiella caerulescens (Kütz.) Santel. Hommers.				AF296513	AB031301
Pterocladiella capillacea (S.G.Gmel.) Santel. *et* Hommers.	HM629885		KX427235	AF308797	AB017672
			KX427237		
Pterocladiella media (E.Y.Dawson) G.H.Boo *et* K.A.Miller			KX427234		

Species				
Pterocladiella musciformis (W.R.Taylor) G.H.Boo *et* K.A.Miller		KX427236		
Pterocladiophila hemisphaerica	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Pterothamnion yezoense (Inagaki) Athanas. *et* Kraft			KC795865	
Ptilophora diversifolia (Suhr) Papenf.			AF521182	
Ptilophora hildebrantii (Hauck) R.E.Norris			AF521178	
Ptilophora mediterranea (H.Huvé) R.E.Norris			AF521179	
Ptilophora pinnatifida J.Agardh			AF521180	PPU60345
Ptilophora prolifera (Harv.) J.Agardh			AF296511	
Ptilophora pterocladiodes Andriam.			AF521181	
Ptilophora rhodoptera R.E.Norris			AF521183	
Ptilophora scalaramosa (Kraft) R.E.Norris			AF296512	EF191195
Ptilophora subcostata (Okamura) R.E.Norris				PSU60348
Rhodolachne decussata M.J.Wynne			HQ422564	
Rhodomela confervoides (Huds.) P.C.Silva	MF101424			
Rhodymenia californica Kylin			KT154743	
Rhodymenia corallina (Bory) Grev.			KT154742	
Rhodymenia pseudopalmata (J.V.Lamour.) P.C.Silva	KX284709	KC875852		
Schimmelmannia schousboei (J.Agardh) J.Agardh	KX284711	KJ398162		
Schizymenia dubyi (Chauvin ex Duby) J.Agardh	KX284712	KJ398163		
Schottera koreana M.S.Calderon, T.H.Seo *et* S.M.Boo			KU749587	
Schottera nicaeensis (J.V.Lamour. ex Duby) Guiry *et* Hollenb.			KU640376	SNU33137

Sebdenia flabellata (J.Agardh) P.G.Parkinson	KX284713	KJ398164	
Sonderella linearis (Harv.) F.Schmitz	MF101445		
Spirocladia barodensis Børgesen		HQ422437	
Spyridia filamentosa (Wulfen) Harv.	MF101441	HQ422400	MG680743
Symphyocladia dendroidea (Mont.) Savoie *et* G.W.Saunders	MF101420		
Symphyocladia latiuscula (Harv.) Yamada		KC795850	
Taenioma perpusillum (J.Agardh) J.Agardh	MF101447	AF522249	MF093957
Tayloriella dictyurus (J.Agardh) Kylin		HQ422440	
Thaumatella adunca (J.Agardh) M.J.Parsons *et* Womersley	MF101447		
Thuretia quercifolia Desne.	MF101442		
Tolypiocladia glomerulata (C.Agardh) F.Schmitz	MF101467	HQ422440	MF093960
Tsengiella spinulosa J.F.Zhang *et* B.M.Xia		KC795856	
Ululania stellata Apt *et* Schlech		HQ422063	
Vertebrata australis (C.Agardh) Kuntze	MF101439		
Vertebrata isogona (Harv.) Díaz-Tapia *et* Maggs	MF101433		
Vertebrata lanosa	KP308097	NC032003	
Vertebrata thuyoides (Harv.) Kuntze	MF101426		
Wrangelia elegantissima R.E.Norris		HQ422251	

Appendix 5.2. Plastid protein coding genes, tRNA and rRNA in alphabetical order by functional group with gene length in bp and AT content in percentage in *Pterocladiophila hemisphaerica* and *Pterocladia lucida*. - = missing in the plastid genome.

	Pterocladiophila hemisphaerica		*Pterocladia lucida*	
	Length (bp)	AT content (%)	Length (bp)	AT content (%)
Protein coding genes				
ATP synthesis coupled proton transport				
*atp*A	-		1512	65.3
*atp*B	-		1419	64.6
*atp*D	-		555	74.1
*atp*E	-		405	67.9
*atp*F	-		534	68.5
*atp*G	-		480	69.8
*atp*H	-		249	59.0
*atp*I	-		741	68.2
Acyl carrier protein				
*acp*P	258	79.5	258	65.1
Biosynthetic processes				
*arg*B	-		855	70.1
*car*A[2]	-		1173	71.4
*glt*B[2]	-		4596	66.7
*ilv*B	-		1770	66.8
*ilv*H	-		534	69.7
*moe*B	-		1089	73.9
*thi*G	-		819	66.4
Cell division				
*fts*H[7]	1803	74.4	1881	64.6
Cell redox homeostasis				
*bas*1	-		600	69.2
Cytochrome complex assembly				
*ccs*A	-		924	71.3

*ccs*1	-		1317	74.9
*dsb*D[3]	-		723	73.3
DNA replication				
*dna*B	1773	78.0	1803	76.2
Fatty acid biosynthesis				
*acc*A	981	70.9	972	66.7
*acc*B	342	73.7	465	72.3
*acc*D	816	71.4	864	67.8
*fab*H	1011	71.8	1005	67.6
Glycolytic processes				
*odp*A	996	68.7	1029	66.5
*odp*B	984	68.5	978	67.4
*pgm*A[7]	-		1536	70.4
Histidyl-tRNA aminoacylation				
syh	1251	73.8	1245	72.2
Iron-sulfur cluster transfer				
orf114	345	74.8	-	
*pet*F	297	69.0	297	65.3
*suf*B	1449	72.6	1458	69.0
*suf*C	741	75.8	753	71.0
Metabolic processes				
*clp*C	2466	69.2	2472	64.9
*trp*G	582	71.1	573	71.0
*trx*A[8]	-		333	66.1
Oxidation-reduction processes				
*ftr*B	-		351	66.7
Phenylalanyl-tRNA amnioacylation				
*syf*B	-		2082	75.1
Photosynthetic processes				
*apc*A[1,3]	-		486	63.4
*apc*B[1,3]	-		486	65.8

*apc*D[1,3]	-	486	68.9
*apc*E[1,3]	-	2652	68.9
*apc*F	-	510	72.2
*chl*I	-	1062	65.7
*cbb*X	-	897	67.3
*cpc*A[1,3]	-	489	64.0
*cpc*B[1,3]	-	519	62.8
*cpc*G	-	696	68.1
*cpe*A[1,3]	-	495	62.8
*cpe*B[1,3]	-	534	62.4
*pbs*A[10]	-	696	70.5
*pet*A[3]	-	957	70.0
*pet*B[11]	-	648	65.6
*pet*D	-	483	63.4
*pet*G[3]	-	114	63.2
*pet*J	-	324	67.3
*pet*L[3]	-	96	75.0
*pet*M[3]	-	99	69.7
*pet*N[3]	-	90	58.9
*pre*A[9]	-	972	70.5
*psa*A[1]	-	2259	63.8
*psa*B[1]	-	2205	64.1
*psa*C	-	246	58.9
*psa*D	-	426	66.2
*psa*E	-	186	71.5
*psa*F	-	558	66.1
*psa*I	-	111	68.5
*psa*J	-	129	74.4
*psa*K	-	276	65.9
*psa*L	-	453	64.9
*psa*M	-	93	73.1
*psb*A[1,6]	-	1083	62.4
*psb*B[1]	-	1530	60.8
*psb*C[1]	-	1449	61.0

*psb*D[1]	-		1056	61.3
*psb*E	-		255	63.4
*psb*F	-		135	67.4
*psb*H	-		204	69.6
*psb*I	-		117	75.2
*psb*J	-		120	62.5
*psb*K	-		138	66.7
*psb*L	-		117	70.9
*psb*N	-		132	70.5
*psb*T	-		96	71.9
*psb*V	-		486	67.9
*psb*W	-		348	66.7
*psb*X	-		120	69.2
*psb*Y	-		105	61.9
*psb*Z	-		192	70.3
*ycf*3	-		522	69.5
*ycf*4	-		546	70.9
*ycf*12	-		105	71.4
*ycf*54	-		312	73.1
*ycf*59[9]	-		1050	70.6
Phycobilisome degradation protein				
*nbl*A	-		156	77.6
Protein biosynthesis				
*inf*B	-		2115	71.8
*tuf*A	1230	68.9	1227	63.8
Protein chromophore linkage				
*ycf*17	-		144	66.0
Protein folding				
*dna*K	1848	70.7	1881	66.7
Protein-phycocyanobilin linkage				
orf149	-		450	77.1

*ycf*58	-		207	80.7
Protein refolding				
*gro*EL	-		1593	66.7
Protein transport				
*sec*Y	1212	76.8	1236	71.0
Proton transport				
*cem*A	-		837	71.9
Reductive pentose-phosphate cycle				
*rbc*L	-		1467	63.4
*rbc*S	-		417	65.2
*thi*S	-		216	75.5
Regulation of transcription				
dfr	-		1944	73.5
*omp*R[12,13]	-		735	66.9
Ribonuclease				
rnz	684	79.1	672	73.7
RNA processing				
rne	-		1488	73.3
Translation				
*inf*C	525	77.5	543	70.9
*rpl*1	-		702	66.4
*rpl*2	822	67.5	828	62.8
*rpl*3	633	74.2	621	67.3
*rpl*4	597	75.9	651	72.5
*rpl*5	501	73.7	540	71.5
*rpl*6	504	72.2	537	70.0
*rpl*9	-		474	75.3
*rpl*11	411	74.7	426	64.8
*rpl*12	375	76.0	396	68.2
*rpl*13	405	76.8	441	71.2
*rpl*14	369	73.2	369	65.0
*rpl*16	408	71.1	405	63.2
*rpl*18	315	77.5	318	70.4

*rpl*19	294	76.2	357	74.2
*rpl*20	339	74.9	345	71.9
*rpl*21	339	81.1	315	71.4
*rpl*23	237	85.7	315	73.3
*rpl*24	-		255	74.5
*rpl*27	339	81.1	255	67.8
*rpl*28	-		192	72.9
*rpl*29	-		201	77.6
*rpl*31	219	72.6	210	67.1
*rpl*32	144	70.8	177	68.9
*rpl*33	186	80.6	201	71.1
*rpl*34	-		138	66.7
*rpl*35	195	74.4	198	73.2
*rpl*36	114	77.2	114	67.5
*rps*1	-		786	72.9
*rps*2	678	74.9	684	68.1
*rps*3	633	74.2	654	66.5
*rps*4	615	74.3	606	68.5
*rps*5	486	71.6	522	67.0
*rps*6	285	79.3	312	77.2
*rps*7	462	75.5	471	67.9
*rps*8	-		399	76.9
*rps*9	426	72.1	414	64.3
*rps*10	333	77.5	312	67.0
*rps*11	363	72.2	390	64.1
*rps*12	366	67.5	375	64.8
*rps*13	348	76.1	381	66.7
*rps*14	294	73.5	303	67.0
*rps*16	240	76.2	255	72.2
*rps*17	-		237	72.6
*rps*18	-		213	71.4
*rps*19	282	72.0	279	70.6
*rps*20	-		267	74.9

*ycf*65	-		300	70.7
Transcription				
*lys*R	-		951	64.4
*ntc*A	-		657	78.5
*rpo*A	909	75.8	936	67.6
*rpo*B	3246	73.4	3417	67.5
*rpo*C1	1785	71.3	1890	66.2
*rpo*C2	3474	75.8	3657	70.0
*ycf*29[12]	-		657	70.0
*ycf*61	-		234	72.2
Transport				
*sec*A[4,5]	2589	79.6	2640	71.7
*ycf*38	846	77.2	837	71.7
*ycf*63	-		696	71.1
tRNA 5'-leader removal				
*rnp*B	-		339	69.3
tRNA processing				
*ycf*62	963	79.8	813	74.5
Tryptophan synthase				
*trp*A	783	73.9	795	70.4
Uncharacterized proteins				
orf105	-		318	77.4
orf110	-		333	71.8
orf151	456	81.6	-	
orf181	-		546	76.2
orf257	-		774	79.6
orf395	-		1188	79.2
orf407	1224	80.8	-	
orf491	-		1476	76.5
orf623	-		1872	79.3
*tat*C	-		717	74.2
tsf	501	72.9	657	69.7
*ycf*19	297	76.1	291	74.2
*ycf*20	-		234	67.5

*ycf*21	528	77.8	564	75.9
*ycf*22	-		471	75.2
*ycf*23	-		801	71.8
*ycf*33	-		198	76.3
*ycf*34	-		207	75.8
*ycf*35	-		384	71.1
*ycf*36	-		495	73.3
*ycf*37	-		447	78.7
*ycf*39	-		969	70.9
*ycf4*1	-		321	74.1
*ycf*45	-		1707	67.7
*ycf*46	-		1467	69.9
ycf52	540	68.7	540	64.3
*ycf*53	-		675	67.4
*ycf*55	-		993	75.5
*ycf*60	-		525	73.0
rRNA				
rrn5	-		118	54.2
rnl	2894	63.8	2866	54.3
rns	1535	62.5	1475	50.3
tRNA				
Ala (TGC)	74	55.4	73	41.1
Arg (ACG)	75	57.3	74	39.2
Arg (CCG)	-		73	54.8
Arg (CCG)	-		95	42.1
Arg (TCT)	75	65.3	75	56.0
Asn (GTT)	72	59.7	74	48.6
Asp (GTC)	74	60.8	74	41.9
Cys (GCA)	72	62.5	73	52.1
Glu (TTC)	75	54.7	75	42.7
Gln (TTG)	74	59.5	74	41.9
Gly (GCC)	73	56.2	72	45.8
Gly (TCC)	73	71.2	73	50.7
His (GTG)	75	50.7	74	45.9

Ile (GAT)	77	62.7	74	47.3
Leu (CAA)	-		82	18.0
Leu (TAA)	82	65.9	86	53.5
Leu (TAG)	85	69.4	83	53.0
Lys (TTT)	72	56.9	72	45.8
Met (CAT)	74	47.3	74	40.5
Met (CAT)	76	69.7	74	44.6
Met (CAT)	89	48.3	-	
Phe (GAA)	73	54.8	73	47.9
Pro (TGG)	76	52.6	75	42.7
Ser (TGA)	88	63.6	86	50.0
Ser (GCT)	91	71.4	90	53.3
Thr (GGT)	-		74	60.8
Thr (TGT)	75	66.7	73	56.2
Trp (CCA)	74	73.0	75	56.0
Tyr (GTA)	81	60.5	82	39.0
Val (GAC)	-		74	58.1
Val (TAC)	74	67.6	72	38.9

[1]Protein chromophore linkage, [2]Metabolic processes, [3]Oxidation-reduction processes, [4]Protein import, [5]Protein targeting, [6]Reponse to herbicide, [7]Catabolic processes, [8]Cell redox homeostasis, [9]Biosynthetic processes, [10]Heme oxidation, [11]Respiratory electron transport chain, [12]Signal transduction system, [13]Transcription

Appendix 5.3. The plastid genome of *Pterocladia lucida* with 200 protein coding genes (yellow), three rRNA's (red) and 30 tRNA's (pink).
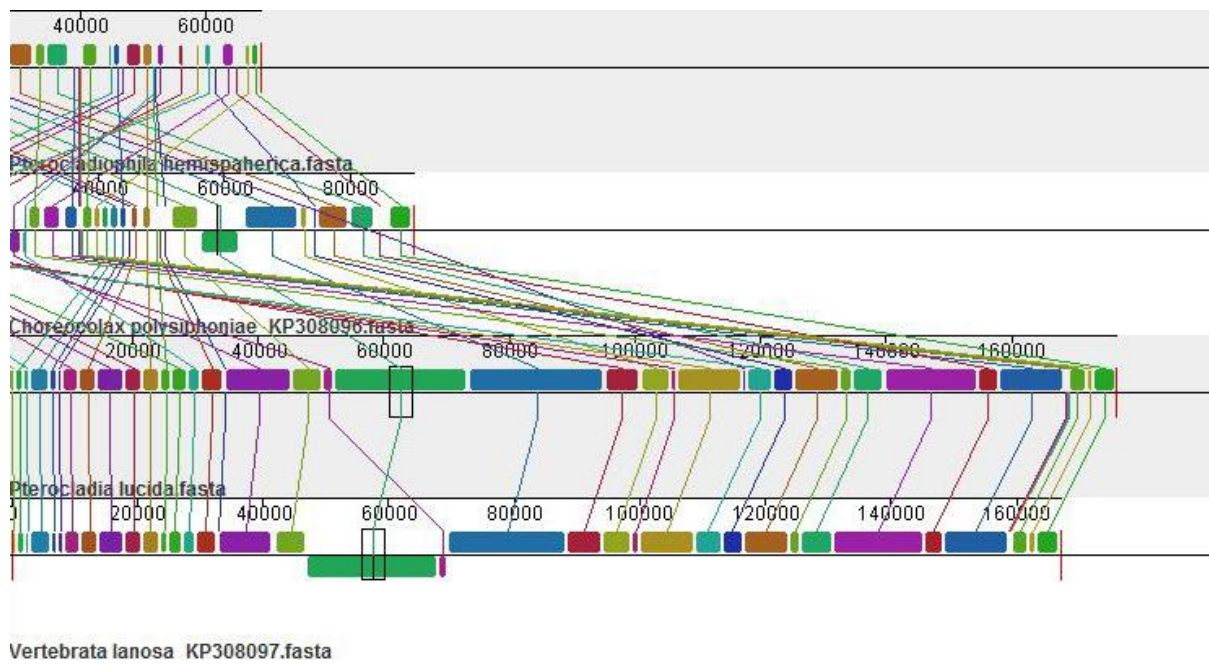
Appendix 5.4. Progressive Mauve alignment of *Pterocladiophila hemisphaerica*, its host *Pterocladia lucida*, the parasite *Choreocolax polysiphoniae* and its host *Vertebrata lanosa*. The parasites show highly reduced organelle genomes.
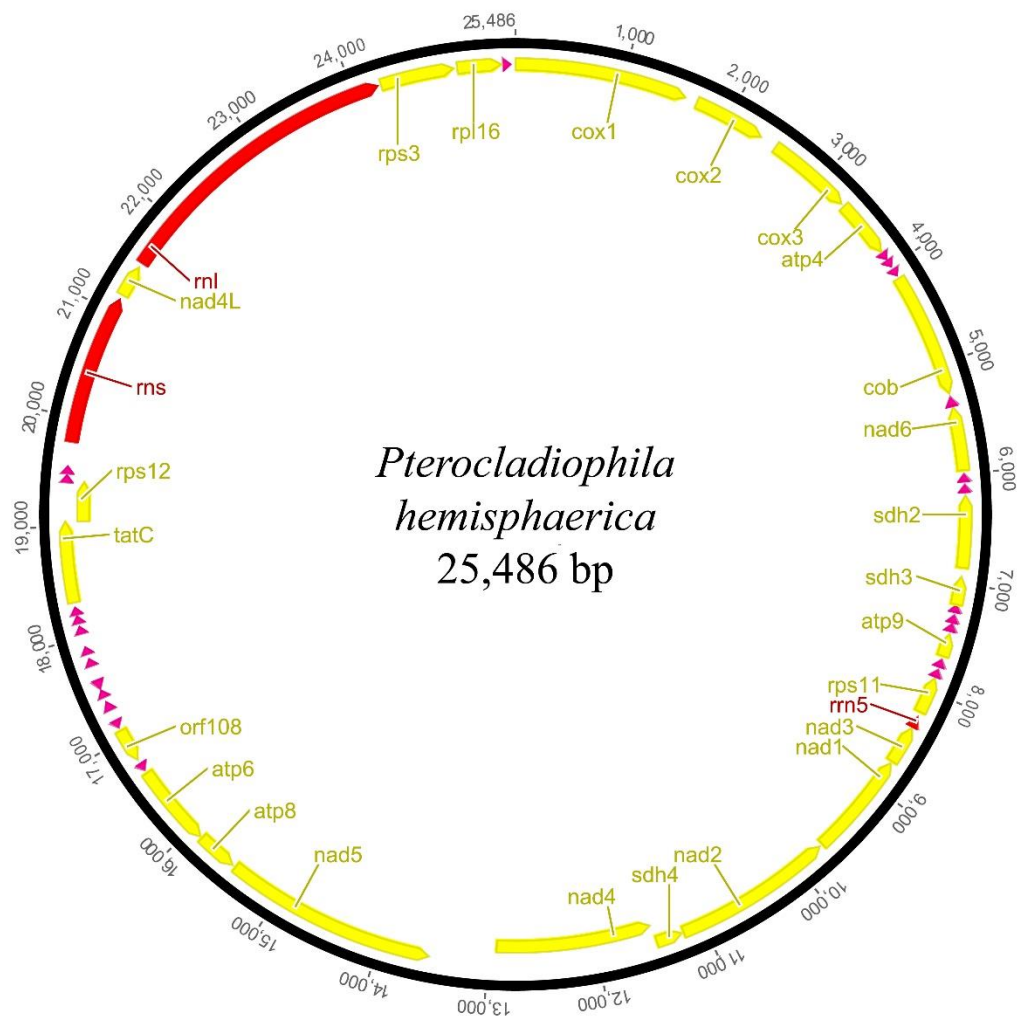
Appendix 5.5. The mitochondrial genome of the parasite *Pterocladiophila hemisphaerica* with 24 protein coding genes (yellow), three rRNA's (red) and 24 tRNA's (pink).
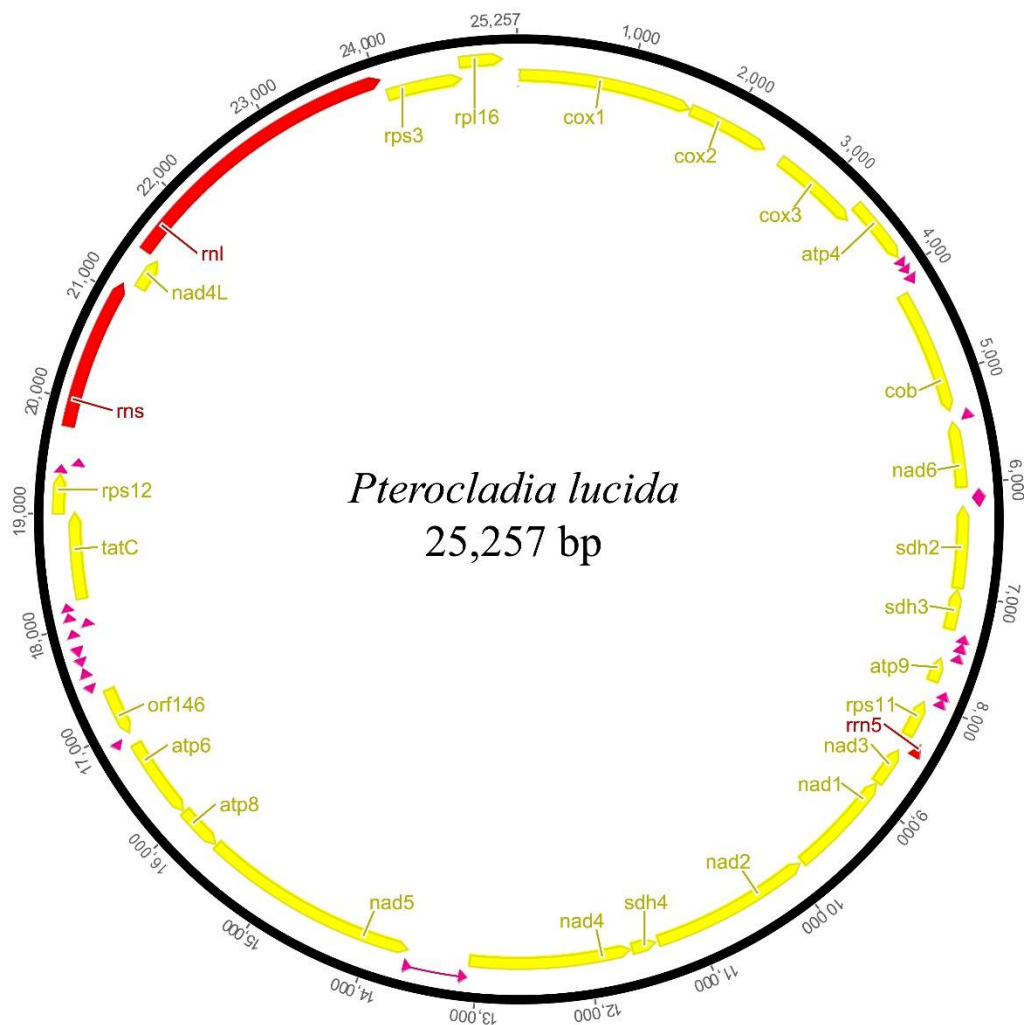
Appendix 5.6. The mitochondrial genome of *Pterocladia lucida* with 24 protein coding genes (yellow), three rRNA's (red) and 23 tRNA's (pink).

Appendix 5.7. Mitochondrial protein coding genes, tRNA and rRNA in alphabetical order by functional group with gene length in bp and AT content in percentage in *Pterocladiophila hemisphaerica* and *Pterocladia lucida*.

	Pterocladiophila hemisphaerica		*Pterocladia lucida*	
	Length (bp)	AT content (%)	Length (bp)	AT content (%)
Protein coding genes				
ATP synthesis coupled proton transport				
*atp*4	543	89.1	600	79.2
*atp*6	762	77.6	759	70.4
*atp*8	384	84.9	414	75.6
*atp*9[1]	231	68.4	231	64.9
*nad*1	975	75.5	984	69.9
*nad*2	1482	81.8	1482	73.9
*nad*3	366	77.6	366	71.9
*nad*4	1479	77.9	1479	70.7
*nad*4L	306	79.7	306	72.2
*nad*5	1962	76.7	1995	69.2
*nad*6	591	82.4	609	73.7
Electron transport chain				
cob	1152	76.0	1143	69.5
*cox*1[2,3]	1578	70.7	1599	65.9
*cox*2	672	75.1	777	68.7
*cox*3	810	75.3	819	65.5
Translation				
*rpl*16	417	83.2	399	71.4
*rps*3	693	82.4	702	74.1
*rps*11	357	81.2	354	77.1
*rps*12	384	70.8	366	70.4
Tricarboxylic acid cycle				
*sdh*2	675	77.8	750	70.9
*sdh*3	273	83.9	369	76.7
*sdh*4	246	87.0	243	77.4
Uncharacterized protein				

*tat*C	744	86.6	810	76.9
ORF108	327	84.1		
ORF146	-		441	76.2
rRNA				
rnl	2726	76.2	2591	70.6
rns	1380	71.7	1353	65.0
rrn5	115	79.1	120	76.7
tRNA				
Ala (TGC)	74	71.6	76	67.1
Asn (GTT)	-		75	60.0
Arg (ACG)	75	70.7	76	68.4
Arg (TCT)	76	73.7	74	68.9
	76	73.7	-	
Cys (GCA)	73	71.2	71	64.8
Gln (TTG)	76	65.8	72	58.3
Glu (TTC)	74	68.7	74	60.8
Gly (GCC)	74	66.2	75	60.0
Gly (TCC)	77	71.4	75	58.7
His (GTG)	75	64.0	75	49.3
Ile (GAT)	-		73	57.5
Leu (TAA)	84	66.7	86	58.1
Leu (TAG)	83	73.5	84	65.5
Lys (TTT)	77	72.7	75	69.3
Met (CAT)	71	71.8	75	61.3
	74	70.3	73	64.4
Phe (GAA)	74	68.9	73	54.8
Pro (TGG)	74	64.9	75	61.3
SeC (TCA)	76	64.5	75	56.0
Ser (TGA)	85	70.6	84	64.3
Ser (GCT)	90	64.4	86	58.1
Tyr (GTA)	87	65.5	84	61.9
	87	65.5	-	
Val (GTC)	74	59.5	-	
Val (TAC)	72	70.8	73	71.2

[1]ATP hydrolysis coupled proton transport, [2]aerobic respiration, [3]oxidative phosphorylation
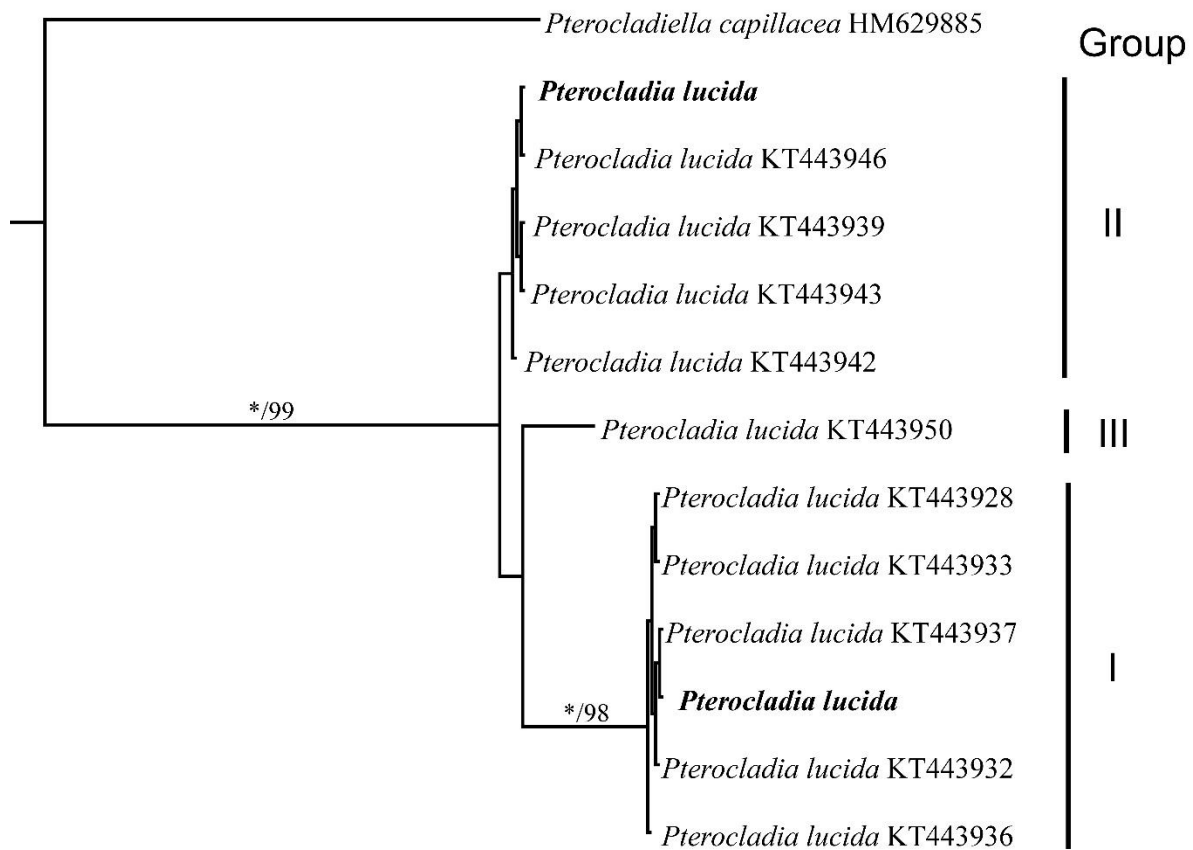
Appendix 5.8. Bayesian topology of partial *cox*1 of two *Pterocladia lucida* samples infected with *Pterocladiophila hemisphaerica*, plus representatives of the three cryptic species of *Pterocladia lucida* and *Pterocladiella capillacea* with GenBank Accession numbers (Appendix 5.1). Outgroups *Gelidiella acerosa* and *Gelidium pacificum* were removed to facilitate presentation. Asterisks indicate posterior probability value of 1.00 / ultrafast bootstrap values of 100%. Values <0.85 posterior probability and <85% ultrafast ML bootstrap not shown.

Appendix 6.1. Measurements of ΔF/Fm' (Day 0, 0h and Day 1, 2-8h) and Fv/Fm (Day1, 0h) in three parasites (*Rhodophyllis parasitica*, *Vertebrata aterrimophila*, *Pterocladiophila hemisphaerica*) and their hosts (*Rhodophyllis membranacea*, *Vertebrata aterrima*, *Pterocladia lucida*).

	Day 0 (0h)	Day 1 (0h)	Day 1 (2h)	Day 1 (4h)	Day 1 (6h)	Day 1 (8h)
Rhodophyllis parasitica	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
Rhodophyllis membrancaea	0.408	0.173	0.369	0.258	0.280	0.314
	0.445	0.333	0.367	0.152	0.445	0.446
	0.462	0.337	0.344	0.460	0.429	0.451
Vertebrata aterrimophila	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
Vertebrata aterrima	0.405	0.345	0.381	0.280	0.693	0.252
	0.413	0.225	0.255	0.389	0.328	0.240
	0.235	0.104	0.302	0.262	0.238	0.256
Pterocladiophila hemisphaerica	0.370	0.308	0.187	0.212	0.231	0.247
	0.350	0.379	0.359	0.344	0.354	0.295
	0.376	0.374	0.267	0.221	0.335	0.271
Pterocladia lucida	0.462	0.326	0.321	0.352	0.152	0.227
	0.383	0.332	0.324	0.262	0.386	0.272
	0.504	0.424	0.341	0.261	0.310	0.339