

Investigation of the red algal parasites
Rhodophyllis parasitica sp. nov. and
Pterocladiophila hemisphaerica
from New Zealand

BY
MAREN PREUSS

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Abstract

Red algal parasites are common within red algae and most parasites are closely related to their host. Red algal parasites can switch hosts and their development is unique. Red algal parasites are poorly known in New Zealand. There are only four parasites described in New Zealand and those are based on morphological characteristics. This thesis focuses on the two red algal parasites *Rhodophyllis parasitica* sp. nov. and *Pterocladiophila hemisphaerica* from New Zealand. First, development and phylogeny and distribution of an undescribed red algal parasite growing on *Rhodophyllis membranacea* was investigated. Microscopy, molecular markers (ITS2, *cox1*, *cox2*-3 spacer) and phylogenetic analysis, and herbarium sampling were used to address these questions. The parasite, described as *Rhodophyllis parasitica* sp. nov. shows a close relationship of all genomes to *Rhodophyllis membranacea*, which suggests that the parasite evolved from its hosts. The parasite is widely distributed throughout New Zealand. The second parasite, *Pterocladiophila hemisphaerica* was grouped taxonomically, based on morphology, in the order Gracilariales and parasitizes *Pterocladia lucida* in the order Gelidiales. Molecular marker were used to reveal the relationship of *Pterocladiophila hemisphaerica* to its host: if the parasite is grouped in the Gracilariales or the Gelidiales; if host switches might have occurred; and if *atp8* is present in the parasite. Nuclear DNA (SSU rRNA, LSU rRNA), mitochondrial (*cox1*) and plastid regions (*rbcL-rbcS* spacer) from the parasite were sequenced and phylogenetic analysis performed. New primer were designed to amplify *atp8* and genetic analysis performed. *Pterocladiophila hemisphaerica* evolved in the Florideophytes but neither in the Gracilariales or Gelidiales and the parasite possibly switched hosts at least two times, which was shown by three different origins of chloroplast, mitochondria and nuclear DNA. *Atp8* in the parasite is present but probably a pseudogene. *Rhodophyllis parasitica* sp. nov. is the first described red algal parasite species in New Zealand in 55 years and *Pterocladiophila hemisphaerica* is the first parasite with organelles and nuclei with different histories of origin.

Abbreviations

1° PC	Primary pit connections
2° PC	Secondary pit connections
<i>Atp8</i>	Adenosintriphosphate 8
BSA	Bovine serum albumin
<i>Cox1</i>	Cytochrome c oxidase subunit I
CTAB	Cetyltrimethylammoniumbromide
DAPI	4',6-Diamidin-2-phenylindole
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleosidetriphosphate
EDTA	Ethylenediaminetetraacetic acid
ITS	rRNA internal transcribed spacers
H	Host
HCl	Hydrogen chloride
LSU	Large subunit ribosomal RNA
ML	Maximum likelihood
N	Number of samples / sequences
NaCl	Sodium chloride
ORF	Open reading frame
P	Parasite
PCR	Polymerase chain reaction
PVP	Polyvinylpyrrolidone
RFLP	Restriction fragment length polymorphism

RNA	Ribonucleic acid
rRNA	ribosomal RNA
RuBisCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
<i>Sdhc</i>	Succinate dehydrogenase complex subunit C
SSU	Small subunit ribosomal RNA
<i>Sp.</i>	Species (singular)
<i>Spp.</i>	Species (plural)
TE	Tris-EDTA solution / buffer
Te Papa	Museum of New Zealand Te Papa Tongarewa
Tris	Tris(hydroxymethyl)-aminomethane

The two chapters of my Master's thesis are written as independent papers and therefore there may be some repetitions in the introduction and discussion.

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1. Introduction

1.1 Symbiosis

Symbiosis is the general term for an intimate living together of dissimilar organisms, first described by De Bary in 1879 (Bary, 1879). Symbiosis can be characterized by, in most cases, the smaller organism being the symbiont and the larger organism the host (Graham & Wilcox, 1999). Ectosymbiosis defines a symbiont growing outside of the host or within close proximity, and endosymbiosis defines a symbiont that is living inside of the host (Smith & Douglas, 1987). Furthermore, endosymbionts can occur within host cells (intracellular) (Ball *et al.*, 2011) or in the host tissue between host cells (extracellular) (Smith & Douglas, 1987). An obligate symbiont depends on the host to survive and reproduce and a facultative symbiont can survive and reproduce without a host (Koga *et al.*, 2003). Another feature is host specificity, which describes the degree of relationship between host and symbiont and therefore symbionts with high specificity occur only on one, or a few, species (Smith & Douglas, 1987).

Symbiosis can also be divided into commensalism, mutualism and parasitism. Commensalism describes an interaction where one organism neither harms nor benefits another organism (Paracer & Ahmadjian, 2000). It is presumed that some species of beetles, mites and moths gain shelter without harming or benefiting a sloth (Gilmore *et al.*, 2001). Mutualism describes an interaction where both organisms benefit (Paracer & Ahmadjian, 2000). There are several mutualistic relationships between bacteria and protozoa in human intestine and animal guts (Bäckhed *et al.*, 2005). Another example are prokaryotic (bacteria) and eukaryotic (flagellates, yeasts) organism in termite guts

(Konig *et al.*, 2013). The enzymes to degrade lignocellulose are essential for the survival of termites and are provided by those symbionts, which depend on the termite gut as habitat (Ohkuma, 2008). Parasitism defines a relationship where one organism benefit from another organism by harming it (Paracer & Ahmadjian, 2000). There are several parasitic human pathogens which can cause infectious diseases (Daszak *et al.*, 2000). *Plasmodium sp.*, which causes malaria, is transferred by mosquitoes into blood (Gueirard *et al.*, 2010) which causes nonspecific detrimental symptoms (Trampuz *et al.*, 2003).

Many symbioses can't be strictly classified as commensalism, mutualism or parasitism (Roossinck, 2011) as the interaction can change over time due to changes in environment (Neuhäuser & Fargione, 2004). Normally, mycorrhizae are a mutualistic relationship between a host plant and a symbiotic fungus, but the interaction can change to a parasitic interaction with increasing soil fertility (Neuhäuser & Fargione, 2004).

The understanding of the diversity of parasites can help science in two main areas. First, parasites can be used to study host specificity and diversification rate and evolutionary processes because parasitism has arisen several times independently. Second, they are also important for the understanding of medical and veterinary science as many parasites have switched their animal host to a human host (Poulin & Morand, 2000).

One example is the switch of the parasite *Plasmodium falciparum* Welch, 1897 from an avian host to a human host (Waters *et al.*, 1991). The understanding of the host-switches in parasites could help to control infectious diseases and virulence (Poulin & Morand, 2000). Symbiotic relationships also help organisms adapt in environment with poor nutrition (Muscatine & Porter, 1977). For example, in corals the symbiotic

dinoflagellates share their photosynthetic products with the host (Porter, 1976), which is essential because the surrounding waters have a low productivity (Stoddart, 1969).

Finally, mycorrhizae are an important symbiosis in plants, which could have been around since plants colonized land (Pirozynski & Malloch, 1975). The arbuscular mycorrhizae formed between a plant and a fungi within the phylum Glomeromycota occurring in 70-90% of all land plants (Schussler *et al.*, 2001). The fungi supplies the host with water and nutrients and receives up to 20% of the plant-fixed carbon (Parniske, 2008).

1.2 Origin of Rhodophytes

The superkingdom Archaeplastida contain three lineages: Rhodophyta (red algae), Chloroplastida (green algae, land plants) and Glauco phyta (Adl *et al.*, 2005). All three lineages share a plastid which is derived from a primary endosymbiosis approximately 1.5 billion years ago (Yoon *et al.*, 2004). The plastid originated from an up-take of a cyanobacterium by a heterotrophic eukaryote (Reyes-Prieto *et al.*, 2007). This plastid gave the eukaryote the ability to convert light into chemical energy through photosynthesis (Ball *et al.*, 2011).

1.3 Rhodophyta (red algae)

Rhodophyta contains over 6400 described species (<http://www.algaebase.org/>) among 834 genera (Schneider & Wynne, 2007). Most Rhodophyta are marine but a few live in freshwater (Graham & Wilcox, 1999). Rhodophyta lack centrioles and flagella in all life history stages (Yoon *et al.*, 2006). The chloroplasts are surrounded by a double membrane and contain unstacked thylakoids (van den Hoek *et al.*, 1996). Rhodophyta

have chlorophyll *a* but no chlorophyll *b* or *c* (Yoon *et al.*, 2006). The unstacked thylakoids contain allophycocyanin, phycocyanin and phycoerythrin (van den Hoek *et al.*, 1996) in phycobilisomes. Phycoerythrin is a red pigment and gives the algae their characteristic colour (Graham & Wilcox, 1999). Floridean starch is the most important carbohydrate storage product and it is found in the cytoplasm (van den Hoek *et al.*, 1996). Sexual reproduction is in most cases isomorphic or heteromorphic diplohaplontic and gametic fusion is always oogamous (van den Hoek *et al.*, 1996).

Most Rhodophyta have three life-history stages: a carposporophyte, a tetrasporophyte and a gametophyte. The tetrasporophyte produces haploid tetraspores through meiosis, which can become female or male gametophytes or are bisexual. The male gametophyte produces spermatia (sperm) and the female gametophyte produces a carpogonium (egg). The released spermatia can fertilize the carpogonium, which stays attached to the female gametophyte (Flint *et al.*, 1992). The fertilized carpogonium (zygote) is diploid and grows into a diploid carposporophyte on the female. The carposporophyte produces non-flagellate carpospores, which are released and develop into the free-living tetrasporophyte (Saunders & Hommersand, 2004).

1.4 Taxonomy and Systematic of Rhodophyta

Traditionally, Rhodophyta were divided into two classes; the Florideophyceae and the Bangiophyceae (Saunders & Hommersand, 2004). The Bangiophyceae contained the six orders: Bangiales, Cyanidiales, Compsopogonales, Erythropeltidales, Porphyridiales and Rhodochaetales (Freshwater *et al.*, 1994). Recent studies showed that a monophyletic group is formed by the Florideophyceae and Bangiales (Oliveira & Bhattacharya, 2000, Yoon *et al.*, 2002).

Yoon (2006) used a multigene analysis to show that the order Rhodophyta consists of seven lineages, which he decided to make into seven classes: Cyanidiophyceae, Bangiophyceae, Compsopogonophyceae, Florideophyceae, Porphyridiophyceae, Rhodellophyceae and Styloematophyceae. Cyanidiophyceae is the sister class to the six remaining lineages (Yoon *et al.*, 2010).

1.5 Importance of red algae

Red algae are abundant and species-rich (Graham & Wilcox, 1999). Their distribution is from the Arctic to Antarctic and includes temperate as well as tropical locations (van den Hoek *et al.*, 1996). Red algae are also associated with mangroves and coral reef (Graham & Wilcox, 1999). Coralline, calcareous, red algae consolidate and stabilize the reef structure because they form a hard crust over the soft limestone. This is especially important for areas with high wave exposure (Björk *et al.*, 1995). Red algae are also commercially important. In 2010 the total value of farmed algae were estimated at 5.7 billion US\$ and the most common farmed red algae were *Kappaphycus spp.*, *Gracilaria spp.* and *Pyropia spp.* (FAO, 2012). The use of red algae differ and especially in Asian countries red algae are used for direct human consumption like nori, made from *Pyropia sp.* (Wikfors & Ohno, 2001).

The extracellular matrices of red algae contain sulfated polysaccharides, which are extracted and purified as agar, agarose and carrageenan. Red algal are the only source of these polysaccharides because the compounds are too complex for industrial synthesis. These carbohydrates are used for cell-culture media, nucleic acid research and food processing (Graham & Wilcox, 1999). Another important area is medical research and treatment, where red algae are used for the development of new drugs,

antiviral substances and antifouling compounds (Smit, 2004).

1.6 Primary and Secondary pit connections

Primary pit connections are formed during cytokinesis between daughter cells (Graham & Wilcox, 1999) and are the result of incomplete septum formation (Blouin & Lane, 2012).

The incomplete septum formation leaves a pore in the central region with a tubular membrane around it. Then a homogenously granular protein mass is deposited around the tubular membrane, which afterwards disappears. This leaves a structural linkage between adjacent mature cells (Graham & Wilcox, 1999).

The primary pit (1° PC) connection is not really an intercellular connection comparable to the plasmodesmata in certain brown algae, green algae and land plants (Pueschel, 1990). Secondary pit connections (2° PC) are formed by cell fusion between non-daughter cells (Blouin & Lane, 2012). 2° PC are produced when cells divide unequally (Graham & Wilcox, 1999). The smaller cell, called a conjunctive cell, fuses with a non-daughter cell (Wetherbee & Quirk, 1982). The nucleus, and other cell organelles, of the conjunctive cell moves into the non-daughter cell (Goff & Coleman, 1985).

1.7 Red algal parasites

In the beginning of the 19th century red algal parasites were first closer studied and red algae were characterized as parasites by Setchell (1918). The characteristics used to describe red algal parasites were: 1) the penetration of the parasite beyond the superficial cells of the host; 2) reduction of the thallus, and 3) the loss of color (Setchell,

1918). Over 100 species in 50 genera are described as red algal parasites within the Florideophyceae but there are no descriptions of red algal parasites within the Bangiophyceae (Goff, 1982). The majority occur in the orders of Gigartinales, Cryptonemiales and Ceramiales (Evans *et al.*, 1978). Red algal parasites can be divided into “adelphoparasites” and “alloparasites” (Goff, 1982). “Adelphoparasites” are taxonomically within in the same family or tribe as their host based on morphological characters (Blouin & Lane, 2012) and include 80% of all described red algal parasites and are closely related to their host (Goff, 1982). “Adelphoparasites” have a high host-specificity and in most cases they can only infect one species (Goff, 1982). One example is the red algal parasite *Dawsonicolax bostrychiae* (Joly et Yamaguishi-Tomita) Joly et Yamaguishi-Tomita, which can only grow on *Bostrychia radicans* (Montagne) Montagne (Zuccarello & West, 1994a). “Alloparasites” include the remaining 20% of described red algal parasites and are not closely related to their host (Goff, 1982). “Alloparasites” grow on distantly related hosts (Blouin & Lane, 2012).

The classification in “adelphoparasite” and “alloparasite” are still used even though it is suggest that there is a correlation between newly evolved parasites, which are closely related to their hosts and parasites not closely related to their hosts. It was proposed that all red algal parasites evolved from their host, which evolved further over time until they switched their host (Zuccarello *et al.*, 2004).

1.8 Development of red algal parasites

All described red algal parasites where found to have secondary pit connections to their hosts (Evans *et al.*, 1978) and these are essential for early development of the red algal parasites (Zuccarello *et al.*, 2004). Secondary pit connections are used to

connect the parasite cells with the host cells (Goff & Zuccarello, 1994) and are used to transfer parasite nuclei and other organelles (mitochondria, plastids) into a host cell (Goff & Coleman, 1995).

The development of red algal parasites is unique. Parasite spores attach to their host and germinate (Goff & Coleman, 1984). This produces an infection peg and the infection peg fuses with an epidermal or subepidermal cell of the host (Goff & Zuccarello, 1994). The nucleus of the parasite enters the cytoplasm of the host cell via a 2nd PC and either divides (Goff & Coleman, 1984) or not in the host cytoplasm (Goff & Zuccarello, 1994). The replicated nuclei can be transferred by secondary pit connections to other host cells and spread the infection (Goff & Coleman, 1995).

Host cellular transformation describes the process of the red algal parasites controlling their hosts (Goff & Coleman, 1987). There are two examples of host cellular transformation (Graham & Wilcox, 1999). The red algal parasite *Leachiella pacifica* Kugrens transfers its nucleus through secondary pit connection into the host cells of *Polysiphonia confusa* Hollenberg (Goff & Zuccarello, 1994). The parasite nuclei does not divide or undergo DNA synthesis in the host cytoplasm (Goff & Coleman, 1984). The red algal parasite *Gracilariphila oryzoides* Setchell et Wilson transfers its nucleus into the host cells of *Gracilaropsis andersonii* (Grunow) Dawson (Goff & Zuccarello, 1994). The nuclei of the parasite undergoes DNA synthesis and divides in the host cells (Goff & Coleman, 1984). Those host cells, which received parasite DNA through 2° PC are called infected cells and they undergo unusual developmental processes (Goff & Coleman, 1995). The infection can spread either by 2° PC or by dissolution of primary pit connection of the host until a tumor like mass of “transformed” host cells is formed,

which contain parasite DNA (Goff & Coleman, 1995).

1.9 Molecular markers

Molecular markers are used in phylogenetic studies to study interspecific or intraspecific evolutionary relationships (Chenuil, 2006). There are five molecular markers used in the phylogeny of red algal parasites: cytochrome c oxidase subunit I (*coxI*) (Kurihara *et al.*, 2010), rRNA internal transcribed spacers (ITS) (Goff *et al.*, 1997), restriction fragment length polymorphism (RFLP) (Goff & Coleman, 1995), small subunit ribosomal RNA (SSU) (Kurihara *et al.*, 2010, Zuccarello *et al.*, 2004) and large subunit ribosomal RNA (LSU) (Kurihara *et al.*, 2010).

Gene sequences of the plastid between host and parasite are identical and suggest that the parasite retained the chloroplast of its host (Goff & Coleman, 1995). Mitochondrial and nuclear DNA are different between host and parasite which suggest that the parasite keeps its own mitochondria and nucleus (Goff *et al.*, 1996). RFLP pattern of mitochondrial and nuclear DNA showed those differences between host and parasite (Goff & Coleman, 1995). Mitochondrial and nuclear DNA can show the phylogenetic relationship between host and parasite (Goff & Coleman, 1995).

Goff (1997) used ITS to show that the red algal parasite species within the genus *Asterocolax* are polyphyletic. Furthermore ITS is a useful molecular marker to study the evolutionary relationships between host and parasite because it is highly variable and it is assumed that the nucleus of the parasite doesn't recombine with the host during cellular transformations (Goff *et al.*, 1997).

SSU was used to study the relationship between host and parasite within the *Choreocolacaceae* and showed that all studied red algal parasite are in the same family

as their hosts (Zuccarello *et al.*, 2004).

Kurihara (2010) used *cox1*, SSU and LSU to show that *Benzaitenia yenoshimensis* Yendo and *Janczewskia morimotoi* Tokida are closely related to their hosts. In addition, *Ululania stellata* Apt et Schlech and *Janczewskia hawaiiana* Apt are not sister-species to their host and probably arose from other species (Kurihara *et al.*, 2010).

Hancock (2010) sequenced the mitochondrial genome of the red algal parasite *Gracilariophila oryzoides* and its host *Gracilaria andersonii*, both genomes are similar to each other but *atp8* and *sdhc* are pseudogenes in the parasite (Hancock *et al.*, 2010).

1.10 Red algal parasites in New Zealand

There are four described red algal parasites in New Zealand. The alloparasite *Pterocladiophila hemisphaerica* Fan et Papenfuss was found on the North Island in Wellington and on the South Island in Kaikoura parasitizing *Pterocladia lucida* (R. Brown ex Turner) J. Agardh. The adelphoparasite *Gloiocolax novae-zelandiae* Sparling grows on *Gloioderma saccatum* (J. Agardh) Kylin. The adelphoparasite *Gonimophyllum buffhamii* Batters grows on *Hymenena semicostata* (J. Agardh) Kylin. The adelphoparasite *Microcolax botryocarpa* (Hooker et Harvey) Schmitz et Falkenberg was found on the North Island in Auckland grown on *Streblocladia glomerulata* (Montagne) Papenfuss (Goff, 1982).

1.11 General goals

Red algal parasite diversity is poorly known. There are still many undescribed species and most studies focused only on a few morphological characteristics. As mentioned previously only a few red algal parasites are taxonomically described in New Zealand. There are no current studies on the phylogeny of red algal parasites in New Zealand, plus the development of red algal parasitism had only been described in a few species. Further studies are important to get a better understanding and insight into the evolution of parasites. Therefore this study focuses on one undescribed species and one described species from New Zealand. This study will describe the morphology and developmental processes on their host of the undescribed red algal parasites and the evolutionary relationships between parasite and host of both red algal parasites.

Chapter 2: *Rhodophyllis parasitica* (Cystocloniaceae, Gigartinales, Rhophyta) sp. nov. from New Zealand

2.1 Introduction

Red algal parasites are common on other red algae and the majority of species descriptions are based on morphology (Goff, 1982). The molecular diversity of red algal parasite has been studied in only a few species, and new species are still being described (Ng *et al.*, 2013). Red algal parasites are described in several orders within the Florideophyceae (Kurihara *et al.*, 2010). One of the orders, where red algal parasites are well-represented, is the Gigartinales (Evans *et al.*, 1978), which contains several parasitic genera: *Coccotylus* (Kützing, 1843), *Callocolax* (Batters, 1895), *Hypneocolax* (Børgesen, 1920), *Gardneriella* (Kylin, 1941).

The morphological characteristics used to describe red algal parasites are: 1) the penetration of the parasite beyond the superficial cells of the host; 2) reduction of the thallus; and 3) the loss of colour (Setchell, 1918). Another important character are the presence of secondary pit connections (2° PC) formed between host and parasite (Goff, 1982). 2° PC were observed in all described red algal parasites studied (Goff & Coleman, 1995, Goff & Zuccarello, 1994, Kim & Cho, 2010, Zuccarello & West, 1994b).

The taxonomy of red algal parasites is mostly based on morphology, and this is true for the few described red algal parasites from New Zealand (Goff, 1982). There have been very few molecular studies of red algal parasites but these are helpful to determine the evolutionary relationship between host and parasite (Zuccarello *et al.*,

2004). Gene sequences of plastid DNA showed that they are identical between host and parasite, which suggests that red algal parasite retain the host chloroplast (Goff & Coleman, 1995). Comparison of mitochondria and nuclear sequences showed differences between the red algal parasite *Plocamicolax pulvinata* Setchell, *Gracilariophila oryzoides* Setchell et Wilson, *Gardneriella tubifera* Kylin and their respective hosts *Plocamium cartilagineum* (Linnaeus) Dixon, *Gracilaropsis lemaneiformis* (Bory de Saint-Vincent) Dawson, Acleto et Foldvik and *Sarcodiotheca gaudichaudii* (Montagne) Gabrielson, which suggest that the red algal parasite retains its own mitochondria und nucleus (Goff & Coleman, 1995).

The distribution of red algal parasites are unknown and incomplete. There are no studies in which the distribution of parasites has been carefully documented. Distribution patterns could be useful to better understand parasite range (is parasitic distribution the same as the host?), tolerances (can the parasite tolerate the same water temperatures of its host?) and host switching (do parasite switch host if the host is rare?).

This study will combine morphological and phylogenetic data to study an undescribed red algal parasites from New Zealand growing on *Rhodophyllis membranacea* (Harvey) Hooker et Harvey. This study will address the following research questions: Does the suspected parasite fulfill the characters of a red algal parasite? What are the differences and similarities in morphological characters between host and parasite? What is the distribution of the parasite in New Zealand? What is the phylogenetic relationship between host and parasite?

2.2 Material and Methods

2.2.1 Sampling

Rhodophyllis membranacea and its parasite were collected from 2012 to 2013 at different locations in New Zealand. A list of all collected samples and locations is shown in Table 2.1. All host and parasite tissue was collected as drift on the beach. Only tissue without any sign of degradation was collected. All tissue was collected and transported in plastic bags to the lab.

2.2.2 Fixation & Embedding

Parasite tissue with surrounding host tissue were cut out under a dissecting microscope (Olympus SZ61) using a scalpel blade and transferred in containers with sterile sea water. The tissue samples were fixed overnight in 2% glutaraldehyde in phosphate buffer (0.1 M, pH 6.8) in 50% sea water. Then the tissue was washed three times at intervals of 10 minutes with phosphate buffer (0.1 M, pH 6.8) in 50% sea water. The tissue was dehydrated in an ascending ethanol-water-series of 20%, 40% and 60% ethanol and three times with 70% ethanol for 15 minutes each. The fixed samples were stored in 70% ethanol at 4 °C until embedding in JB-4. The tissue was transferred in a mixed series of ascending infiltration solution (JB-4 Solution A-Monomer, Benzoyl Peroxide) and descending ethanol series. The tissue was transferred once every 15 minutes to a solution of 75% ethanol and 25% infiltration solution, 50% ethanol and 50% infiltration solution, 25% ethanol and 75% infiltration solution and 10% ethanol and 90% infiltration solution and three times in 100% infiltration solution. Then embedding stubs were filled with embedding solution (infiltration solution, JB-4

solution plus B-Accelerator) and the tissue transferred with tweezers in those stubs. The embedding stubs were fully filled with embedding solution. Then the embedding stubs were covered with parafilm and stored in a desiccator until hardened.

Table 2.1. Collected samples of *Rhodophyllis membranacea* and its parasites from different locations in New Zealand.

Species	Date	Location	Coordinates	Collector
<i>Rhodophyllis membranacea</i> (ASH037)	27.01.08	Thompson Sound	S 45° 13' 20" E 166° 58' 20"	C. Hepburn D. Richards
<i>Rhodophyllis membranacea</i> + parasite	27.02.12	Houghton Bay	S 41° 20' 33" E 174° 47' 6"	P. Northcote
<i>Rhodophyllis membranacea</i> + parasite	04.03.13	Moa Point	S 41° 20' 30" E 174° 48' 38"	M. Preuß
<i>Rhodophyllis membranacea</i> + parasite	08.03.13	Moa Point	S 41° 20' 30" E 174° 48' 38"	M. Preuß
<i>Rhodophyllis membranacea</i> + parasite	13.03.13	Cape Palliser	S 41° 36' 48" E 175° 17' 30"	M. Preuß
<i>Rhodophyllis membranacea</i> + parasite	05.04.13	Moa Point	S 41° 20' 30" E 174° 48' 38"	M. Preuß
<i>Rhodophyllis membranacea</i> + parasite	26.04.13	Moa Point	S 41° 20' 30" E 174° 48' 38"	M. Preuß
<i>Rhodophyllis membranacea</i> + parasite	19.05.13	Blackhead	S 40° 10' 9" E 176° 49' 37"	M. Preuß
<i>Rhodophyllis membranacea</i> + parasite	20.05.13	Akitio Beach	S 40° 36' 53" E 176° 24' 53"	M. Preuß
<i>Rhodophyllis membranacea</i> + parasite	20.05.13	Between Sandy Beach and Mataikona	S 40° 36' 53" E 176° 15' 8"	M. Preuß

2.2.3 Sectioning

The embedded tissue was sectioned with a diamond blade (Delaware Diamond Knives) on a microtome (American Optical Corporation, 860 Model). The tissue was first trimmed with a razor blade to fit in the sample holder of the microtome. Sections were 10 µm thick. The sections were carefully transferred with tweezers to the surface of distilled water in a beaker and transferred on a microscopic slide after they had fully spread in the water. The microscopic slide was then dried and stored in a slide folder.

2.2.4 Staining and microscopy

All microscopic slides were either stained with 1.0 µg/ml DAPI in McIlvaine buffer (pH 4.1) or 1% Aniline blue. DAPI was used to stain nuclei and Aniline blue for general structures in the tissue.

For DAPI staining, microscopic slides were stained in a coplin jars with 1.0 µg/ml DAPI for 5 minutes and rinsed carefully with distilled water. For Aniline blue staining, microscopic slides were stained in coplin jars with 1% Aniline blue for 5 minutes, rinsed carefully with distilled water and fixed in 2% HCl for 5 minutes, then air-dried.

The microscopic slides were examined under a fluorescence microscope (Olympus AX-70) with integrated camera (Olympus DP-70) and images were taken using either DP Controller (Olympus software) imaging software or Stream Enterprise (Olympus software).

2.2.5 Permanent slides

The microscopic slides with Aniline blue stained tissue were covered with mounting medium (Depex mounting medium Gurr, Leuven, Belgium) and carefully covered with a cover slip. The mounting medium hardened within a day.

2.2.6 DNA extraction

DNA from fresh, or silica gel dried samples, was extracted either with 5% Chelex or using a modified CTAB procedure. A list of all samples used can be found in Table 2.2.

Table 2.2. List of all samples of *Rhodophyllis membranacea* and its parasite used in molecular analysis. No.= Code used for sample. Date, location, coordinates and host source of collection also shown. Host source described the host from which the parasite was removed.

Species	No.	Date	Location	Coordinates	Host source
<i>Rhodophyllis membranacea</i>	12H	27.01.08	Thompson Sound	S 45° 13' 20" E 166° 58' 20"	-
<i>Rhodophyllis membranacea</i>	2H	27.02.12	Houghton Bay	S 41° 20' 33" E 174° 47' 6"	-
<i>Rhodophyllis membranacea</i>	3H	27.02.12	Houghton Bay	S 41° 20' 33" E 174° 47' 6"	-
<i>Rhodophyllis membranacea</i>	4H	27.02.12	Houghton Bay	S 41° 20' 33" E 174° 47' 6"	-
<i>Rhodophyllis membranacea</i>	5H	27.02.12	Houghton Bay	S 41° 20' 33" E 174° 47' 6"	-
<i>Rhodophyllis membranacea</i>	8H	04.03.13	Moa Point	S 41° 20' 30" E 174° 48' 38"	-
<i>Rhodophyllis membranacea</i>	9H	08.03.13	Moa Point	S 41° 20' 30" E 174° 48' 38"	-
<i>Rhodophyllis membranacea</i>	14H	13.03.13	Cape Palliser	S 41° 36' 48" E 175° 17' 30"	-
<i>Rhodophyllis membranacea</i>	15H	13.03.13	Cape Palliser	S 41° 36' 48" E 175° 17' 30"	-

Species	No.	Date	Location	Coordinates	Host source
<i>Rhodophyllis membranacea</i>	39H	20.05.13	Akitio Beach	S 40° 36' 53" E 176° 24' 53"	-
<i>Rhodophyllis membranacea</i>	40H	20.05.13	Akitio Beach	S 40° 36' 53" E 176° 24' 53"	-
<i>Rhodophyllis membranacea</i>	42H	20.05.13	Akitio Beach	S 40° 36' 53" E 176° 24' 53"	-
<i>Rhodophyllis membranacea</i>	43H	20.05.13	Between Sandy Beach and Mataikona	S 40° 36' 53" E 176° 15' 8"	-
<i>Rhodophyllis membranacea</i>	44H	19.05.13	Blackhead	S 40° 10' 9" E 176° 49' 37"	-
<i>Rhodophyllis parasite</i>	2P	27.02.12	Houghton Bay	S 41° 20' 33" E 174° 47' 6"	2H
<i>Rhodophyllis parasite</i>	4P	27.02.12	Houghton Bay	S 41° 20' 33" E 174° 47' 6"	4H
<i>Rhodophyllis parasite</i>	5P	27.02.12	Houghton Bay	S 41° 20' 33" E 174° 47' 6"	5H
<i>Rhodophyllis parasite</i>	8P	04.03.13	Moa Point	S 41° 20' 30" E 174° 48' 38"	8H
<i>Rhodophyllis parasite</i>	9P	08.03.13	Moa Point	S 41° 20' 30" E 174° 48' 38"	9H
<i>Rhodophyllis parasite</i>	13P	13.03.13	Cape Palliser	S 41° 36' 48" E 175° 17' 30"	13H
<i>Rhodophyllis parasite</i>	14P	13.03.13	Cape Palliser	S 41° 36' 48" E 175° 17' 30"	14H
<i>Rhodophyllis parasite</i>	40P	20.05.13	Akitio Beach	S 40° 36' 53" E 176° 24' 53"	40H
<i>Rhodophyllis parasite</i>	42P	20.05.13	Akitio Beach	S 40° 36' 53" E 176° 24' 53"	42H
<i>Rhodophyllis parasite</i>	43P	20.05.13	Between Sandy Beach and Mataikona	S 40° 36' 53" E 176° 15' 8"	43H
<i>Rhodophyllis parasite</i>	44P	19.05.13	Blackhead	S 40° 10' 9" E 176° 49' 37"	44H

For Chelex extraction, 100 µl Chelex solution (5% Chelex-100, Bio-Rad, Hercules, California, USA; in 10 mM Tris pH 8.0) was added to a small amount of tissue in a 1.5 mL tube. The tissue was ground until no more tissue was visible and the solution became coloured. The tissue was heated to 100 °C for 10 minutes and centrifuged (Eppendorf) at full speed for 10 minutes. The supernatant was transferred

carefully in a new tube and stored at -20 °C.

For CTAB extraction, 500 µl of a CTAB buffer (2% CTAB, 0.1 M Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 1% PVP) plus 50 µg RNase A and 80 µg Proteinase K were added to 1.5 ml tube with a piece of tissue and ground after the tissue was softened. The grinded tissue in the tube was heated to 60 °C for one hour. An equal volume of 24:1 chloroform:isoamyl alcohol solution was added, mixed and centrifuged at the highest speed for 5 minutes. The supernatant was transferred in a new tube. Then an equal volume of 24:1 chloroform: isoamyl alcohol was added, mixed and centrifuged at full speed for 5 minutes. The supernatant was transferred to a new tube. An equal volume of 100% isopropanol was added to the tube, waited for 30 minutes and centrifuged at the highest speed for 20 minutes. The supernatant was transferred in a new tube, 500 µl of 70% ethanol was then added and the solution centrifuged for 5 minutes. The supernatant was decanted and the pellet air-dried. 50 µl of 0.1xTE buffer (10 mM Tris-HCl, (pH 8.0), 1 mM EDTA) was added to the dry pellet. The extracted samples were stored at -20 °C.

2.2.7 Primers

Two mitochondrial markers (*cox1* and *cox2-3* spacer) and one nuclear marker (ITS2) were used for *Rhodophyllum membranacea* and its parasites. The forward primer GazF1 (TCA ACA AAT CAT AAA GAT ATT GG) and reverse primer GazR1 (ACT TCT GGA TGT CCA AAA AAY CA) were used to amplify the 5 prime end of *cox1* (Saunders, 2005). The forward primer *cox2F* (GTA CCW TCT TTD RGR RKD AAA TGT GAT GG) and reverse primer *cox3R* (GGA TCT ACW AGA TGR AAW GGA

TGT C) were used to amplify the *cox2-3* spacer (Zuccarello *et al.*, 1999). The forward primer ITS3 (GCA TCG ATG AAG AAC GCA GC) and reverse primer ITS4 (TCC TCC GCT TAT TGA TAT GC) were used to amplify ITS2 (White *et al.*, 1990).

2.2.8 Primer Design for specific ITS2 primer

The general ITS2 primers (above) were inefficient in the amplification and specific primer were designed using successful amplified *Rhodophyllis membranacea* and parasite sequences. Two forward primers ITS3F1 (CGA ATG CGA TAT GTA ATG) and ITS3F2 (AAT CAT CGA ATT TTT GAA CG) and one reverse primer ITS4R1 (CAA AAC GNT TCC CTC TCC TC) were created in Geneious (Biomatters Ltd., New Zealand) using the Primer3 software. Primer were manufactured by IDT (Integrated DNA Technologies, Inc.).

2.2.9 PCR

Amplification reaction were performed in a 30 µl reaction of the final concentrations: 1x buffer (Biotherm), 0.2 mM dNTP's, 2.5 mM MgCl₂, 0.25% BSA (BSA, Sigma), 25 pmol of each primer, 1 U *Taq* polymerase (New England BioLabs, Inc.), and 1 µl of template DNA. The PCR were performed using a MJR PTC100 Thermal Cycler. PCR was carried out with an initial denaturation at 94 °C for 5 minutes, followed by 36 cycles of 94°/45°/72° for 1 minute each and a final step 72 °C for 5 minutes. The PCR products were electrophoresed using 4 µl of amplified template and 1 µl Gel Loading Buffer (65% (w/v) Sucrose, 10 mM Tris-HCl (pH 7.5), 10 mM EDTA, 0.3% (w/v) Bromophenol blue) in a 1% agarose gel (0.5x TRIS-Borate-EDTA buffer).

The length of the amplified product was determined by running a molecular ladder (Hyperladder II, Bioline) on every gel. Afterwards the agarose gel was stained with ethidium bromide (0.01 µl/ml) for 15 minutes and examined in an UV chamber (Uvitech). Successful amplifications were purified using Exo SAP-IT (Affymetrix, USB product, USA). 0.5 µl Exo SAP-IT was added to approximately 25 µl of PCR product. The tubes were heated to 37 °C for 30 minutes and afterwards incubated at 80 °C for 15 minutes. The purified samples were sequenced commercially (Macrogen Inc, Korea).

2.2.10 Alignment and haplotype networks

Forward and reverse sequences were assembled and edited in Geneious (Biomatters Ltd., USA). All sequences were verified by matching a red algae species using Blast search. The edited sequences were aligned using MAFFT alignment using the default parameters. The alignment was checked and realigned by eye. The alignment of *cox1* and ITS2 from host and parasite was used to calculate haplotype networks using TCS 1.21 (Clement *et al.*, 2000), due to the similarity between host and parasite samples phylogenetic analysis was not needed.

2.2.11 Distribution

On 8th of April 2013, all herbarium samples of *Rhodophyllum membranacea* were provided by Jenn Dalen at the Museum of New Zealand Te Papa Tongarewa (Te Papa) in Wellington. All samples were searched for parasites under a dissecting microscope. Te Papa voucher, collection date, location and collector of all samples with observed parasites were recorded.

2.3 Results

2.3.1 Morphology of the *Rhodophyllis* parasite

Habitat and seasonality. The red algal parasite grows on *Rhodophyllis membranacea* and was found from January to May. The surface of the host can have from one to over 20 parasites at a time. The parasite can be found close to the edges and tips of the host plant. The parasites never grow close to each other and vary in size.

Thallus. The parasite is coloured (light reddish) and less pigmented than the host, which makes the parasite easy to spot (Fig. 2.1A). The parasite thallus is on average 2.5 x 3 mm in size. The thallus is mostly bipinnately branched and the surface of the branches are smooth. The parasite has a single base attached to the host surface (stipe) that branches distally. The branches are terete and not flattened as in *Rhodophyllis membranacea*. The branch tips are blunt (Fig. 2.1B).

Male and female reproductive structures can be differentiated. Spermatangia are found on the surface on the thallus (Fig 2.1C). Carposporophytes form round structures on the branches (Fig. 2.1D).

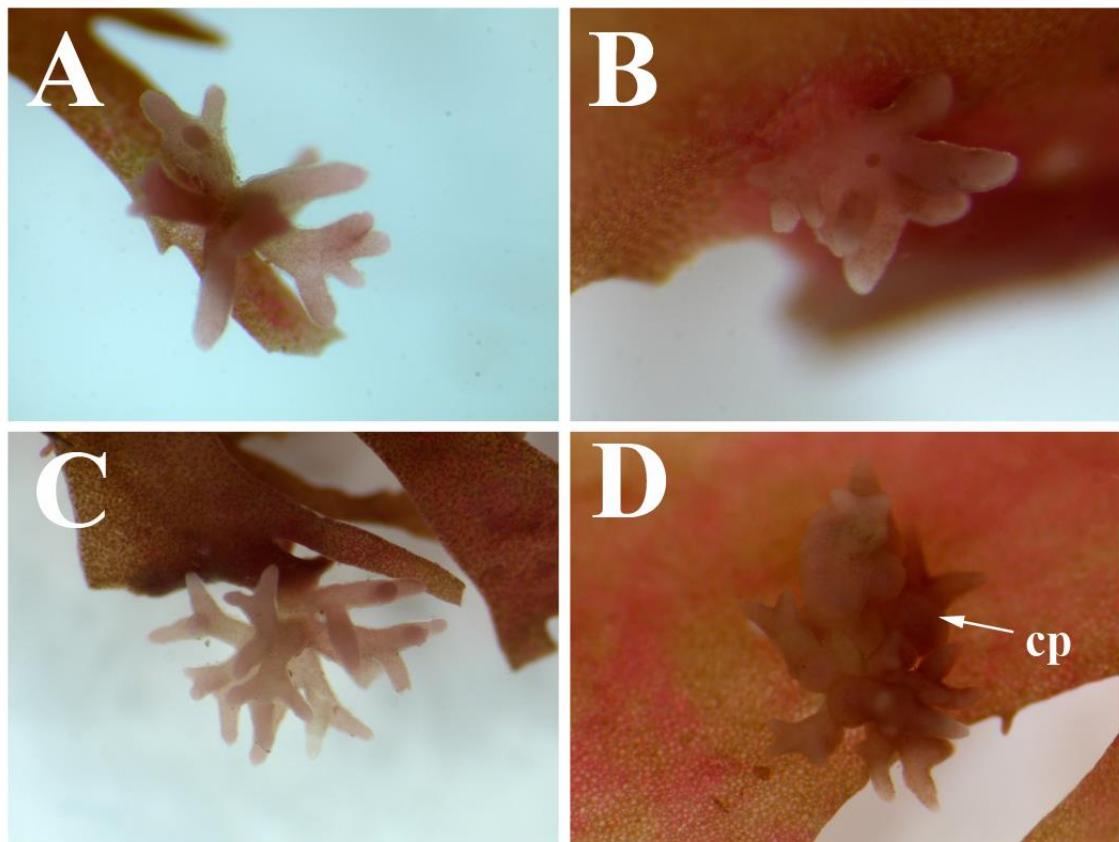


Figure 2.1A-D. *Rhodophyllis* parasite thallus on its host *Rhodophyllis membranacea*. Fig. 2.1A-B. Vegetative structure of the *Rhodophyllis* parasite. Fig 2.1A. The parasite is bipinnately branched and less pigmented than its host. Fig. 2.1B. The parasite has a single base attached to the host tissue. Fig. 2.1C-2.1D. Monocious gametophytes of *Rhodophyllis* parasite. Fig. 2.1C. Male gametophyte. Fig. 2.1D. Arrowhead indicates carpospores (cp) structure within the branches of a female parasite.

Vegetative structure. The thallus of the parasite is composed in an irregular massive cell network (Fig. 2.2A). The area, which connects the host and parasite, has elongated cells of various lengths and smaller cells. This area also contains embedded host cells in the parasite cells and vice versa. The cortical cells of the host seem to be bigger in this area than the surrounding host tissue (Fig. 2.2B). The parasite cells form primary pit connection with other parasitic cells and secondary pit connect with host cells (Fig.

2.2C). Primary pit connections can be found between smaller parasite cells in the center of the parasite thallus and the branch tips. Secondary pit connections can be found between large cortical host cells and small parasitic cells in the contact area. The small cells cannot be host cells as the host has a very regular cell distribution pattern (Fig. 2.2D). The vegetative structure of *Rhodophyllis membranacea* consists of one or two inner layers of large cortical cells, an outer layer of smaller epidermal cells and a cuticle. DAPI-staining showed highly fluorescent parasitic nuclei in the host and parasite contact area. The parasite thallus further away from the host surface consists of pigmented round cells and elongated cells.

Development. The normal cell layer structure of *Rhodophyllis membranacea* is disrupted by the infection of the parasite. The disruption of parasite filaments is localised and those parasite filaments are a long way from where the parasite is found (Fig. 2.2E). DAPI-staining revealed an accumulation of parasite nuclei in rhizoidal parasite cells in the host, which are probably parasite nuclei due to the high number of nuclei in the cells and host thalli don't have this layer of filamentous cells (Fig. 2.2F).

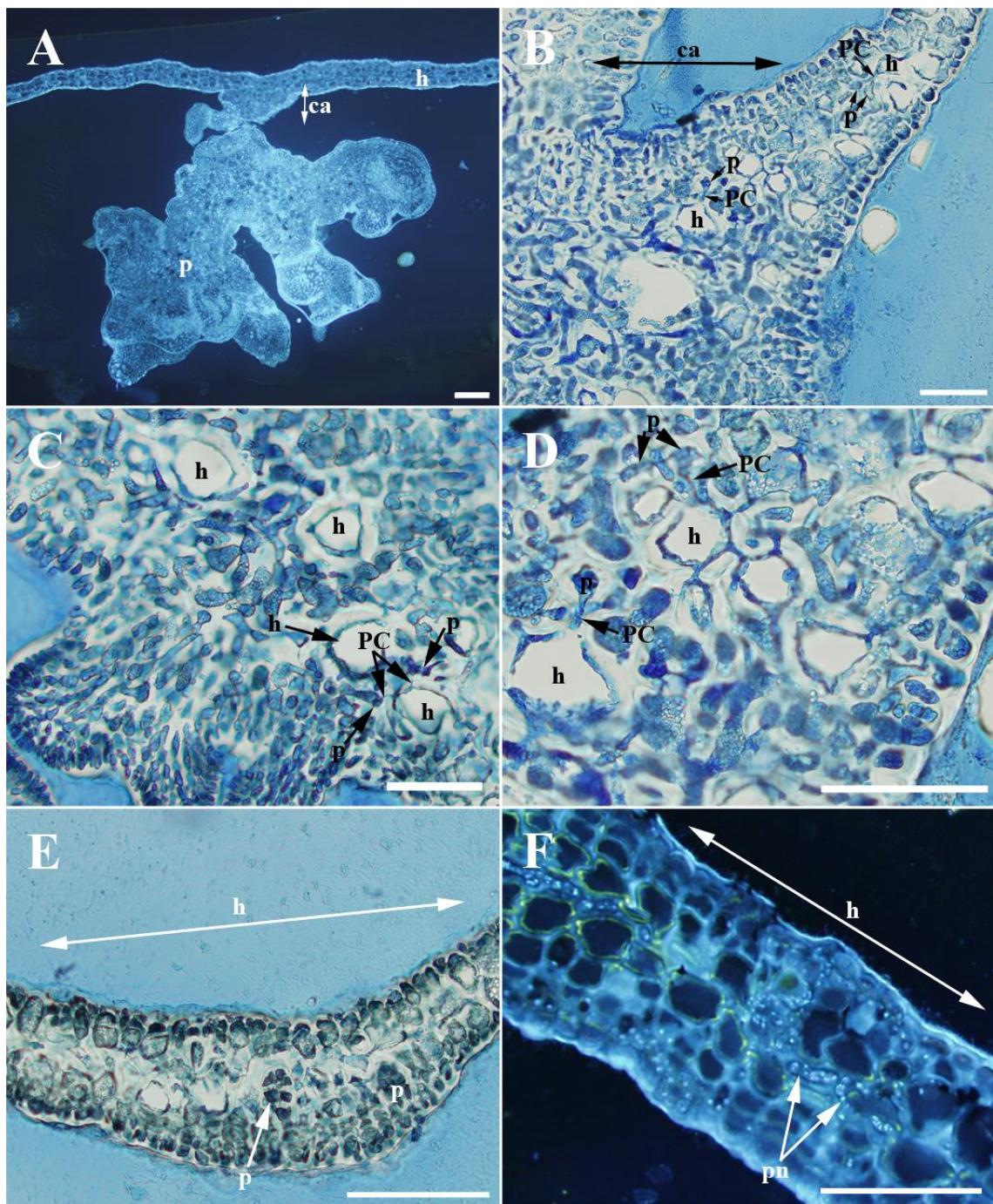


Figure 2.2A-F. Vegetative structure of *Rhodophyllis* parasite. Fig. 2.2A. DAPI-staining of vegetative structure of *Rhodophyllis membranacea* (h) and its parasite (p). The parasite penetrates deep beyond the superficial layer of the host cells. Host and parasite cells can be both found in the contact area between host and parasite (ca). Arrowhead indicates contact area between host and parasite (ca). Scale =250 μm . Fig. 2.2B. Aniline blue staining of contact area between host and

parasite (ca). Parasite disrupts the cell layer structure of the host. The parasite forms secondary pit connection (PC) with *Rhodophyllis membranacea* (h). Arrowhead indicates contact area between host and parasite (ca). Scale = 100 μm . Fig 2.2C-D. Contact area between host (h) and parasite (p) stained with Aniline blue. Parasite forms secondary pit connection (PC) with its host. Arrowheads indicate the parasite cells (p), host cells (h) or secondary pit connections (PC). Scale = 50 μm (Fig. 2.2C) and 100 μm (Fig. 2.2D). Fig. 2.2E. Vegetative structure of host cell layers after infection by parasite stained with Aniline blue. Parasite cells (p) grow in the host thallus (h) and disrupt the cell layer structure of the host. Arrowhead indicates parasite cells (p) or the host thallus (h). Scale = 100 μm . Fig. 2.2F. DAPI staining of vegetative structure of *Rhodophyllis membranacea* (h) with parasite nuclei (pn) in parasite rhizoidal cells in the host. Arrowhead indicate parasite nuclei (pn) or host thallus. Scale = 100 μm .

Reproductive structures. Carposporophytes, spermatangia and tetrasporangia were observed. The parasite has monoeious gametophytes (Fig. 2.3A).

Carposporophyte. The carposporophyte contains an accumulation of carpospores. The carpospores are oval shaped with an average size of 20 μm ($n=5$) and are pigmented. Cystocarps are marginal in the branches of the parasite (Fig. 2.3B). Carposporophytes are 230 x 330 μm ($n=3$) and surrounded by a pericarp. The pericarp is approximately 5-10 cells thick (200 μm ; $n=3$) (Fig. 2.3C).

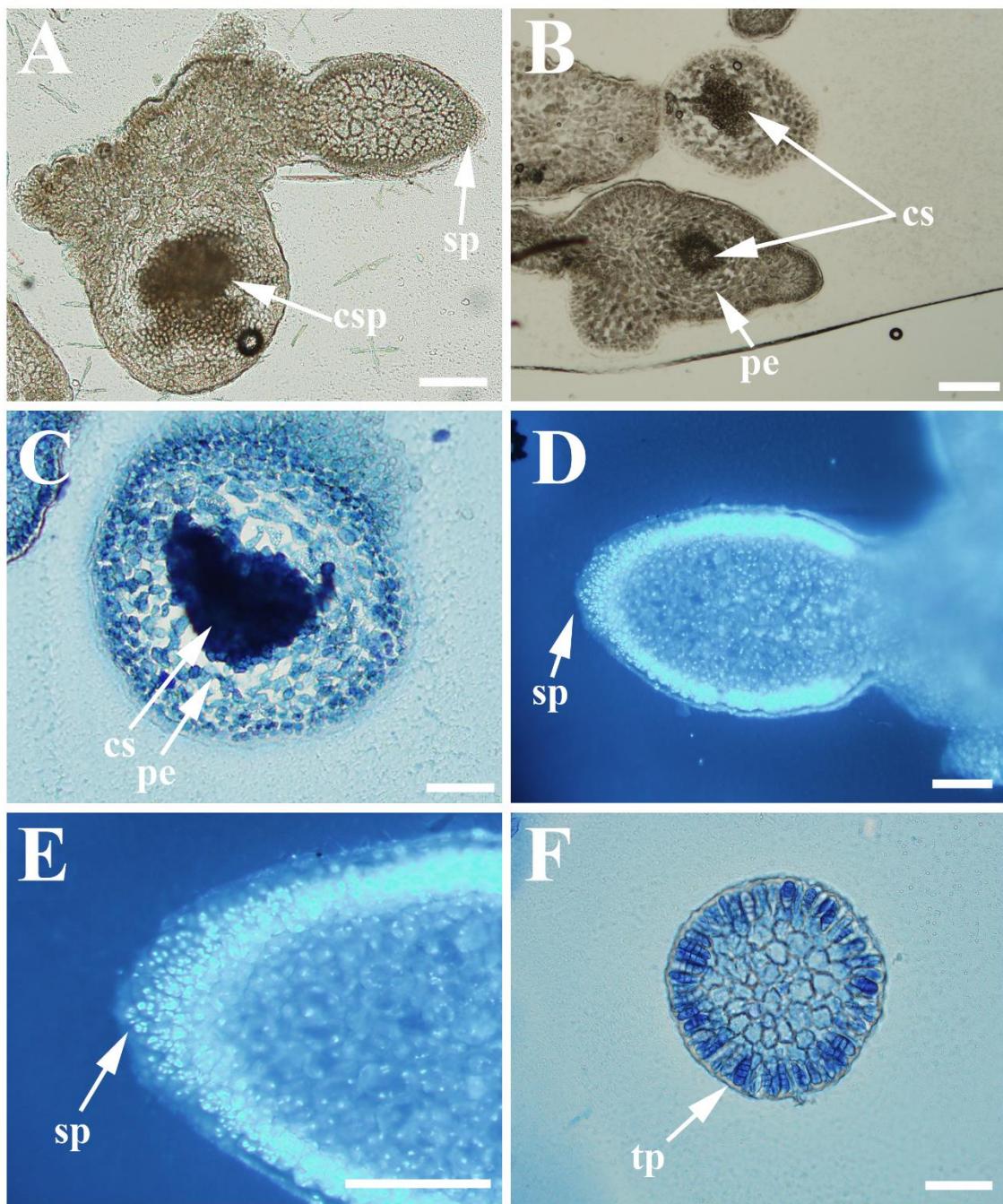


Figure 2.3A-F. Reproductive structure of the *Rhodophyllis* parasite. Fig 2.3A. Monoeious gametophyte with spermatangia (sp) and carposporophyte (csp). Unstained. Scale = 200 µm. Fig. 2.3B-C. Unstained (B) and Aniline blue stained (C) carposporophyte containing an accumulation of carospores (cs) and surrounded by a pericarp (pe). Scale = 100 µm (Fig. 2.3B) and scale = 200 µm (Fig. 2.3C). Fig. 2.3D. DAPI-staining of dense patches of spermatangia (sp) on the parasite surface.

Scale = 100 µm. Fig. 2.3E. Great magnification of DAPI -stained spermatangia. Spermatia visible (sp). Scale = 100 µm. Fig. 2.3F. Aniline blue stained tetrasporangia (ts) of the parasite. Tetrasporangium contains four zonately divided tetraspores. Scale = 100 µm.

Males. Spermatangia are in dense patches on the surface of the parasite thallus (Fig. 2.3D). Spermatia are less than 10 µm in diameter and are round. DAPI-staining shows a high concentration of DNA in the spermatia (Fig. 2.3E).

Tetrasporophyte. Tetrasporangia are scattered and numerous on the surface. Each tetrasporangium contains four tetraspores, which are zonately divided (Fig. 2.3F). Tetrasporangia are pigmented and an average size from 48 x 20 µm (n=10).

2.3.2. Comparison of morphological characters between host and parasite

Rhodophyllis membranacea and its parasite differ in: 1) size (thallus and reproductive structure), 2) cell structure and 3) sexuality. Both have the shape and the location of their reproductive structures in common (Table 2.3).

Table 2.3. Comparison of vegetative and reproductive structures between *Rhodophyllis membranacea* and its parasite.

	<i>Rhodophyllis membranacea</i>	<i>Rhodophyllis parasite</i>
Reference	(Chapman, 1969, Nelson, 2013, Womersley, 1994)	This study
Thallus		
Size	6-15 cm long	2.5 x 3 mm
Branches	Flattened	Terete
Cells Layers	Two type of cell layers (2 layers of large cortical cells and one layer of small epidermal cells)	Cells scattered of various sizes (small, large and elongated cells)
Reproductive structures		
Sexuality	Dioecious	Monoecious
Carpospores		
Shape	Ovoid	Ovoid
Size	15-25 µm (diameter)	20 µm (diameter)
Tetrasporangia		
Location	Scattered	Scattered
Form	Zonately divided	Zonately divided
Size	20-35 µm diameter	20µm diameter
Spermatangia		
Location	Scattered on surface	Scattered on surface
Form	Superficial patches	Superficial patches
Spermatia		
Size	-	approx. 10 µm

2.3.3 Phylogeny of *Rhodophyllis* parasite

ITS2. The ITS2 alignment contained 22 samples of collected *Rhodophyllis membranacea* and parasite samples (11 hosts, 11 parasites) and had a length of 512 base pairs (Appendix 1). Three ITS2 ribotypes were found within samples: A1, A2 and A3 and differ by up to 2 base pairs. Lineage A1 contains all ITS2 sequences of *Rhodophyllis membranacea* (n=11) and one parasite sequence. Lineage A2 and A3 contain only parasite sequences. Lineage A3 is the most common ribotype within the

parasite ($n=7$) (Fig. 2.4A). The two parasite ribotypes A2 and A3 were each found in the same collection location and therefore are not correlate with location.

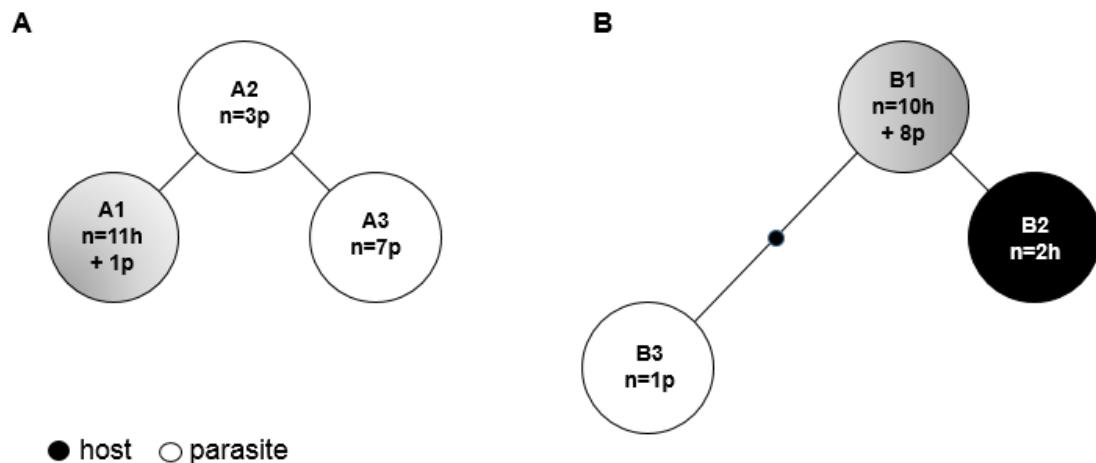


Figure 2.4A-B. Networks of *Rhodophyllis membranacea* and its parasite sequence data. Fig. 2.4A.

ITS2 ribotype network with three lineages represented (A1, A2, A3). Fig. 2.4B. *cox1* haplotype network with three lineages represented (B1, B2, B3). Line is one base pair difference. Small black circle is missing haplotype. Number of samples (n) divided in host (h) and parasite sequences (p). Grey area shows lineage, which is found in parasite and host.

Cox1. The *cox1* alignment contained 21 samples of *Rhodophyllis membranacea* and parasite samples (12 hosts, 9 parasites) and had a length of 676 base pairs (Appendix 2). Three haplotypes were found within the *cox1* sequences of *Rhodophyllis membranacea* and its parasite: B1, B2 and B3 and differ by up to 3 base pairs. Lineage B1 contains most ($n=18$) host and parasite sequences (10 host and 8 parasite). Lineage B2 contains two host sequences. Lineage B3 contains one parasite sequences (Fig. 2.4B).

Cox2-3 spacer. The *cox2-3* spacer alignment contained 15 samples of *Rhodophyllis*

membranacea and parasite samples (10 hosts, 5 parasites) and had a length of 356 base pairs. All *cox2-3* spacer sequences of *Rhodophyllis membranacea* and its parasite are identical except one parasite sample, which shows one base pair difference (Appendix 3).

2.3.4 Distribution of the *Rhodophyllis* parasite

The Te Papa herbarium collection of *Rhodophyllis membranacea* contained over 60 specimens and 29 of those had red algal parasites on them. A list of all samples with date of collection and collector is shown in Table 2.4. The parasite is widespread and can be found throughout New Zealand. Samples with parasites can be found on the subantarctic islands (Auckland Islands) and Stewart Island to the islands north of the North Island (Three Kings Islands). The parasite can be found in collections year round except in June and July.

Table 2.4. Te Papa vouchers of *Rhodophyllis membranacea* with red algal parasites on them.

Te Papa voucher	Collection date	Location	Collector
A1573	31.01.1968	Houghton Bay, Wellington	N. M. Adams
A2686	10.04.1969	Karehana Bay, Plimmerton	E. Harris
A4151	07.01.1971	Lyall Bay, Wellington	N. M. Adams
A4240	15.02.1971	Kaikoura Peninsula	Baker
A7201	20.02.1972	Port Pegasus, Stewart Island	G. Moreland
A7200	28.02.1972	Port Pegasus, Stewart Island	N. M. Adams
A10189	16.09.1978	Port Ross, Auckland Island	C. H. Hay
A10423	23.10.1978	Great Island, Three King Island	H. Choat
A14765	14.02.1980	Preservation Inlet, Fiordland	-
A11577	08.02.1981	Aorere Point, North Otago	N. M. Adams
A11580	10.02.1981	Shag Point, North Otago	N. M. Adams
A13411	25.02.1982	Between Lyall Bay & Houghton Bay, Wellington	W. Nelson
A14250	02.12.1983	T449 Bushett's Shoal	C. H. Hay
A14215	05.12.1983	7459 Cape Campbell Scuba Street	C. H. Hay
A14206	14.12.1983	Chetwode Island (Nukuwaiata)	C. H. Hay
A22508	11.05.1986	Preservation Inlet, Gulches Head	G. Scringer
A25901	-	Doubtful Sound, Fiordland	S. Wing
A18456a	04.03.1987	Point Gap Bay, Chatham Island	D. Schiel
A18459b	04.03.1987	Chatham Island	W. Nelson
A026481	27.03.1987	Karorio stream mouth, Wellington	C. H. Hay
A18630a	11.08.1987	Chetwode Island (Nukuwaiata)	C. H. Hay
A19277	28.03.1990	Fighting Bay, Marlborough	C. H. Hay
A19048	26.04.1990	Horahora, Kakahu Island	C. Duffy
A21013	10.11.1994	Dusky Sound, Lama Island	E. Villouta
A029799	01.09.1996	Kaikoura Peninsula	W. Nelson & G. Knight
A22424	08.02.1999	Smoothwater Bay, South Westland	Neale
A022772	20.02.2000	Dagg Sound, Four Fanthom Bank	F. Smith
A022771	23.02.2000	Chalky Inlet, Edwardson Sound	C. Mundy
A024446	22.02.2007	Tarakena Bay, Wellington	K. Neill

2.4 Discussion

Morphological analysis revealed a close similarity between *Rhodophyllis membranacea* and its parasite. The parasite is small, less pigmented than its host and secondary pit connections were formed between parasite and host cells. Morphological analysis showed also that the parasite has most of the characteristics used to define red algal parasite, which are 1) reduced size; 2) reduced pigmentation; 3) presence of both gametophyte and sporophytes on the same host plant; and 4) formation of secondary pit connections between host and parasite cells (Wynne & Scott, 1989). Due to the morphological and genetic similarity of red algal parasite and host it is suggested that the parasite evolved from its host. The red algal parasite is widespread throughout New Zealand.

2.4.1 Morphology of *Rhodophyllis* parasite

The parasite penetrates beyond the superficial cells of *Rhodophyllis membranacea*, has a reduced thallus and is less pigmented than its host. The deep penetration of the parasite is correlated with the disruption of host cell layers. This has been observed in other red algal parasite-host symbioses (Fujii & Toyota, 1999, Goff & Coleman, 1984, Noble & Kraft, 1983). Examples of the disruption of cell layers in the host by parasites, are the red algal parasite *Neohalosacciocolax aleutica* Lee et Kurogi, which contains cortical cells of its host *Halosaccion minjaii* Lee after penetration and disruption of the host (Lee & Kurogi, 1978) and *Neotenophycus ichthyosteus* Kraft et Abbott, which inserts its basal cells progressively into the host tissue of *Neosiphonia poko* (Hollenberg) Abbott (Kraft & Abbott, 2002).

The parasite is less pigmented than its hosts *Rhodophyllis membranacea*. Studies showed that the parasite *Choreocolax polysiphoniae* Reinsch, which varies in pigmentation, could still do photosynthesis after being removed from its host (Callow *et al.*, 1979). Measuring the parasite photosynthesis after removing it from its host *Rhodophyllis membranacea* could show if the parasite is able to grow by itself and if not, this would suggest that the parasite probably depends on its host.

Another character used in the description of red algal parasites, were the presence of sporophyte and gametophyte of the parasite on the same host plant (Wynne & Scott, 1989). At this point of the study, there are only slides of gametophytes and sporophytes collected from the same location but it is not possible to tell if they are from the same host plant. Further investigation of this character needs to be done to fulfil all previous mentioned characters in the description of new red algal parasites.

Another important character to describe red algal parasites were the observations of 2° PC between *Rhodophyllis membranacea* and its parasite. Secondary pit connections between host and parasite were observed in all described red algal parasites (Apt & Schlech, 1998, Goff & Zuccarello, 1994, Kim & Cho, 2010, Kraft & Abbott, 2002). An attached parasite spore on a host surface penetrates into the host via an infection peg, which fuses with either an epidermal cell or with the underlying cortical cell (Goff & Coleman, 1987). The red algal parasite *Sorellocolax stellaris* Yoshida et Mikami uses its infections peg to fuse with an epidermal cell of its host *Sorella repens* (Okamura) Hollenberg (Yoshida & Mikami, 1996). In contrast, the red algal parasite *Dawsonicolax bostrychiae* (Joly et Yamaguishi-Tomita) Joly et Yamaguishi-Tomita uses its infection peg to fuse with the underlying cell layer of the host *Bostrychia*

radians (Lamouroux) J. Agardh (Zuccarello & West, 1994b). This suggests that secondary pit connections are an essential feature not only for the early developmental stages of the parasite (Zuccarello *et al.*, 2004) but are also essential for parasite-host development as it is suggested that the transfer of nutrients to the parasite is only possible through physiological changes in infected host cells connected to parasite cells via 2° PC (Goff & Coleman, 1985, Zuccarello & West, 1994b).

2° pit connections allow the parasite to transfer nuclei and cytoplasmic organelles to host cells and the nuclei in some red algal parasites are able to divide in the host cytoplasm (Goff & Coleman, 1984). In other cases the parasite nuclei do not divide, e.g. *Bostrychiocolax australis* Zuccarello et West (Zuccarello & West, 1994b). 2° pit connections appear to give the parasite control over the host cells (Goff & Coleman, 1987) and the parasite changes the metabolism within the infected host cells and transforms the morphology of host cells (Goff & Zuccarello, 1994). Even though 2° pit connections are observed between the parasite and *Rhodophyllis membranacea*, changes in host cell morphology were not observed.

Mistakes have been made in describing red algal parasites where growths caused by other non-algal ‘pathogens’ have been misidentified as parasites (Künzenbach & Brucker, 1960). *Lobocolax deformans* was described by Howe (1914) as a red algal parasite but it is known to be an bacterial infection on *Prionitis lanceolata* (Harvey) Harvey (Ashen & Goff, 1998). *Catenellocolax leeuwenii* was described by Weber-van Bosse (1928) as a red algal parasite on *Catenella impudia* (Montagne) J. Agardh but this is doubtful as *Catenella nipae* Zanardini forms similar galls, which are caused by fungal infections (Zuccarello, 2008).

Red algal parasite descriptions are difficult because some species are rare or small in size. The red algal parasite *Tylocolax microcarpus* Schmidt on *Lenormandia spectabilis* Sonder, seems to be one of those rare species because it was only recorded once (Schmitz & Falkenberg, 1897). One example for small size is the parasite *Aiocolax pulchella* Pocock growing on *Falkenbergiella caespitosa* Pocock with a maximum size of 202-375 µm (Pocock, 1956). A careful examination and description of red algal parasites is important due to the fact that some parasites are quite small or rare and for a good description of a parasite it is important to collect as many morphological characters as possible. I showed that *Rhodophyllis membranacea* and its parasite share many morphological and reproduction features but the parasite has still enough differences from *Rhodophyllis membranacea* to be recognised as its own species. The parasite differs from its host in: 1) size of thallus and reproductive structures; 2) sexuality; and 3) number of cell layers. The reduction of the thallus is a well-known character for red algal parasites (Setchell, 1918, Wynne & Scott, 1989). The difference in sexuality is already known in other red algal parasites (Goff, 1982). The red algal parasite *Champiocolax sarae* Bula-Meyer is monoecious and its host species *Champia parvula* (C. Agardh) Harvey, *Champia salicornioides* Harvey and *Champia compressa* Harvey are dioecious (Bula-Meyer, 1985). A monoecious gametophyte can be an advantage for the parasite because of the ability to reproduce without finding individuals of the opposite sex (Les, 1991).

The morphological and reproductive characters as well as genetic data are quite similar between *Rhodophyllis membranacea* and its parasite. This similarity suggests that the parasite evolved from its host. This close relationship between the parasite to its

host is not surprising as 80% of all described red algal parasites are closely related to their hosts (Goff, 1982).

Rhodophyllis membranacea is in the family Cystocloniaceae. In this order, there is another red algal parasite, *Hypneocolax stellaris* Børgesen, which grows on its taxonomically closely related host *Hypnea musciformis* (Wulfen) Lamouroux (Børgesen, 1920). The family itself has currently 93 taxonomically described species (Guiry & Guiry, 2013).

2.4.2 Phylogeny of *Rhodophyllis* parasite

Nuclear (ITS2) and mitochondrial (*cox1*, *cox2-3* spacer) markers showed that parasite and host are closely related to each other. Due to the very close genetic similarity it is suggested that the parasite is newly evolved from its host. The close relationship between host and parasite was shown by using ITS data in the red algal parasite *Asterocolax gardneri* (Setchell) Feldmann et Feldmann, which is more closely related to the North Pacific host *Polyneura latissima* (Harvey) Kylin than to the North Atlantic *Asterocolax* and suggests a polyphyletic origin of the parasite (Goff *et al.*, 1997). SSU and ITS data revealed that the red algal parasite *Faucheocolax attenuata* Setchell is more closely related to its host *Fauchea laciniata* J. Agardh than it is to its other host *Fauchea fryeana* Setchell, which suggest that the parasite evolved on *Fauchea laciniata* and switched host to another closely related species (Goff *et al.*, 1996). SSU data from the red algal parasite *Harveyella mirabilis* (Reinsch) Schmitz et Reinke revealed that the parasite has a single origin but switched hosts several times and grows on distantly related species: *Rhodomela confervoides* (Hudson) Silva, *Odonthalia*

washingtoniensis Kylin and *Gonimophyllum skottsbergii* Setchell (Zuccarello *et al.*, 2004).

The three ITS2 ribotypes and *cox1* haplotypes each found in this study weren't exclusive between host and parasite. Identical sequences could be due to host contamination or due to close similarity between parasite and host. Host contamination was lowered by using parasite tissue from areas further away from the base of the parasite without embedded host cells. Host contamination is unlikely but can't be excluded. Close similarity due to ancestral sequences could be tested by separating only parasite tissue, for example freshly released parasite spores (Goff *et al.*, 1997). As previous mentioned the morphological and reproductive characters are quite similar so genetic similarity is expected and this would support our results.

The *cox2-3* spacer sequences between host and parasite were identical except for one sequence, which was one base pair different. Other studies showed that the *cox2-3* spacer is a highly variable marker (Saunders & Moore, 2013). The identical *cox2-3* spacer sequences would suggest that the parasite is recently new evolved from its host. The recent new evolution of the parasite is also supported by the other sequences (*cox1* and ITS) and the morphological data.

2.4.3 Distribution of *Rhodophyllis* parasite

The red algal parasite on *Rhodophyllis membranacea* is widespread and can be found almost all year throughout New Zealand. The earliest herbarium sample of *Rhodophyllis membranacea* and its parasites from Te Papa was collected in 1968 but the parasite was not described. Only two or three of the vouchers from Te Papa mentioned

parasites at all. As previously mentioned the small size is the reason for red algal parasites to be overlooked. Diversity of red algal parasites in the world is probably higher than expected and new red algal parasite species are still being described (Kim & Cho, 2010). There are probably many undescribed red algal parasite species in New Zealand because New Zealand is known for its high endemism and biodiversity (Gordon *et al.*, 2010). Herbarium samples can be helpful to determine distribution patterns over time and space and biodiversity (Nelson *et al.*, 2013). In the case of red algal parasites, herbarium samples are helpful to determine the distribution of the parasite and its host and find collection locations in which parasite and host could be found. Distribution pattern of red algal parasites and their host are generally unknown because the literature only lists sampling locations used in their studies. A careful examination of herbarium vouchers could help to describe new red algal parasites.

Rhodophyllis membranacea is also known from Australia (Womersley, 1994). It would be interesting to check herbarium samples of *Rhodophyllis membranacea* from Australian museums for the parasite to extend the distribution of the parasite.

2.4.4 Placement and naming of the *Rhodophyllis* parasite

The red algal parasite evolved from its host *Rhodophyllis membranacea*. Phylogenetic analysis revealed that the red algal parasite and its host *Rhodophyllis membranacea* are closely related to each other. Morphological and reproductive structures are also quite similar between host and parasite. Those results suggest that the parasite evolved from its host. There are five described species in the genus *Rhodophyllis* in New Zealand: *Rhodophyllis acanthocarpa* (Harvey) J. Agardh,

Rhodophyllis centrocarpa (Montagne) Wynne, *Rhodophyllis gunnii* (Harvey) Harvey, *Rhodophyllis lacerata* Harvey and *Rhodophyllis membranacea* (Cotton, 1908). The red algal parasite is easily distinguishable from those species due to its small size and parasitic lifestyle on *Rhodophyllis membranacea*. There are no known red algal parasites in the genus *Rhodophyllis*. For those reasons, this algae should be considered as the new species *Rhodophyllis parasitica* sp. nov.

The morphological characters of *Rhodophyllis parasitica* such as protruding and oval carposporophytes with an aggregation of oval carospores and scattered tetrasporangium supports the placement in the genus *Rhodophyllis* (Kützing, 1847). The tetraspores are also known to be zonately divided as in all *Rhodophyllis* species (Cotton, 1908). Another shared character between the parasite and *Rhodophyllis* species (*Rhodophyllis acanthocarpa*, *Rhodophyllis gunnii* and *Rhodophyllis membranacea*) is spermatangia, which are scattered in patches on the surface (Chapman, 1969, Womersley, 1994).

Red algal parasites were always placed in a new genus since their first description (Fan & Papenfuss, 1959, Lee & Kurogi, 1978, Wynne & Scott, 1989). Red algal parasites have special morphological characters like their small size and reduced pigmentation due to their parasitic lifestyle but most parasites still have similar morphology to their host and thereby to the host genus (Goff, 1982). These similarities suggest that they should be grouped in the same genus as the host, but they are not. Genetic methods introduced modern classification that are used to produce monophyletic genera based on the assumption that the genes and tree-building programs reflect the species relationship and paraphyly and polyphyly are not to be accepted in

phylogenetic classification (Zuccarello, 2011). Modern phylogenetic analyses revealed that parasite and host are often closer related to each other than to species in the same genus but new described parasites are still grouped in a new genus (Kim & Cho, 2010). These new parasitic genera are often, based on molecular sequences, nested within the host genus (Kurihara *et al.*, 2010, Ng *et al.*, 2013, Saunders *et al.*, 2004). For example, the red algal parasites *Bostrychiocolax australis* and *Dawsoniocolax bostrychiae* are nested within the host genus, thus making the host genus *Bostrychia* paraphyletic (Zuccarello *et al.*, 2004). The paraphyletic genus is after strictly modern classification methods not acceptable and this would lead to the question if it's not easier to add the parasite characters to the host genus to maintain the monophyly than to always describe a new genus?

Rhodophyllis parasitica is considered part of the genus *Rhodophyllis* to maintain the monophyly of the genus. A revision is necessary of either all morphological characters in the genus *Rhodophyllis* or the characters of the genus are maintained with additional characters of the parasite because there appears to be only one.

***Rhodophyllis parasitica* Preuß et Zuccarello sp. nov.**

Diagnosis: thalli coloured (light reddish), size 2.5 x 3 mm, branches bipinnately and terete with smooth surface and blunt branch tips. Monoeicous gametophyte. Carposporophyte 230 x 330 µm, surrounded by pericarp, contain oval carpospores. Carpospores 20 µm in diameter, pigmented, marginal in branches. Spermatangia 10 µm in diameter, dense patches on thallus. Tetrasporangia scattered on surface, zonately divided, pigmented, 48 x 20 µm. Parasitic on *Rhodophyllis membranacea*.

Type locality: RPPZ0212; S 41 20 33, E 174 47 6; growing on *Rhodophyllis*

membranacea, collected as drift on 27.02.2012 at Houghton Bay, New Zealand.

Etymology: *parasitica* refers to the parasitic lifestyle of the red algae. *Rhodophyllis parasitica* is neither a legitimate scientific name nor synonym of species within in the genus *Rhodophyllis* based on known literature.

2.4.5 Future work on *Rhodophyllis parasitica*

Rhodophyllis parasitica could be an interesting organism to test if the parasite infects other closely related species in the same genus. Parasites have different evolutionary histories to their hosts, where some red algal parasites evolved from their host and only infect the host, other red algal parasite evolved and parasitizes on its host and also parasitizes on other closely related species (Goff *et al.*, 1996). Culture experiments of *Rhodophyllis parasitica* and the four other species of *Rhodophyllis* in New Zealand (Cotton, 1908) and species in other closely related genera (*Calliblepharis*, *Craspedocarpus*, *Hypnea*) could be used to determine the host-specificity of the parasite. Due to the close phylogenetic similarity between *Rhodophyllis parasitica* and its host *Rhodophyllis membranacea*, it would be expected that if the parasite can infect other species they have to be closely related to the parasite. The infection of closely related species was shown in the experimental studies of the red algal parasite *Bostrychiocolax australis*, which has a broad host range and could not only parasitize its closely related host species *Bostrychia radicans* (Montagne) Montagne but also the closely related taxa: *Bostrychia moritziana* (Sonder) J. Agardh and *Bostrychia kelanensis* Grunow (Zuccarello & West, 1994a, Zuccarello *et al.*, 2004). Surprisingly, experimental studies in the red algal *Leachiella pacifica* Kugrens showed that the

parasites growing on *Polysiphonia* can not infect *Pterosiphonia* and vice versa (Zuccarello & West, 1994c), which suggests the parasite developed special host specific characters.

Chapter 3: A phylogenetic study of the red algal parasite

Pterocladiophila hemisphaerica

3.1 Introduction

Red algal parasites are common on other red algae and make up approximately 10% of all described red algal genera (Goff, 1982). Red algal parasites are successful because they form secondary pit connection with their hosts (Goff & Coleman, 1987) and these are essential for early parasite development (Zuccarello *et al.*, 2004). Secondary pit connections have been observed in all red algal parasite and host combinations (Apt & Schlech, 1998, Evans *et al.*, 1978, Goff & Coleman, 1984, Kim & Cho, 2010, Norris, 1988, Wetherbee & Quirk, 1982) and are used to transfer parasite nuclei and other organelles (mitochondria, plastids) from the parasite into a host cell (Goff & Coleman, 1995).

80% of red algal parasites are called “adelphoparasites” and are taxonomically closely related to their hosts (Goff, 1982). “Adelphoparasites” are often more closely related to their hosts than their host is to other species in the same genus (Goff *et al.*, 1996). The minority of red algal parasites are called “alloparasites” and are taxonomically not closely related to their hosts (Goff, 1982). One example is the red algal parasite *Harveyella mirabilis* (Reinsch) Schmitz et Reinke in which small subunit ribosomal RNA (SSU) data showed that the parasite has a single origin but switched hosts several time and grows on the distantly related species: *Rhodomela confervoides* (Hudson) Silva, *Odonthalia washingtoniensis* Kylin and *Gonimophyllum skottsbergii* Setchell (Zuccarello *et al.*, 2004). The terms “adelphoparasite” and “alloparasites” are

still used but controversial as there is a continual gradation of different levels of relatedness between parasites and their hosts, and it is hypothesised that all parasite originated from a closely related species but this relationship can be changed with host switching (Zuccarello *et al.*, 2004).

Host-switching is known in red algal parasites and has been confirmed genetically using ITS data in the red algal parasite *Plocamiocolax pulvinata* Setchell, who is more closely related to *Plocamium violaceum* Farlow than to its host *Plocamium cartilagineum* (Linnaeus) Dixon, which suggests that the parasite evolved on *Plocamium violaceum* and switched hosts to *Plocamium cartilagineum* (Goff *et al.*, 1996).

The taxonomy of red algal parasites is mostly based on morphology, and this includes the few described red algal parasites from New Zealand (Goff, 1982). Often however the relationship between host and parasite can not be resolved based solely on morphological characters because host and parasite are very different and diagnostic characters are too conserved (Zuccarello *et al.*, 2004).

There have been few molecular studies of red algal parasites but these are a helpful tool to understand the genomic changes in parasites (Hancock *et al.*, 2010) and the evolutionary relationships between host and parasite (Zuccarello *et al.*, 2004). Gene sequences of plastid DNA showed that plastids are identical between host and parasite, which suggests that the red algal parasite retains the host chloroplast (Goff & Coleman, 1995). Nuclear markers (SSU, large subunit ribosomal RNA) and mitochondrial marker (Cytochrome c oxidase subunit I) showed differences between host and parasite sequences (Kurihara *et al.*, 2010). The genetic difference suggest that the red algal

parasite keeps its own mitochondria and nucleus and this can be used to determine the parasites origin (Goff & Coleman, 1995).

Atp8 is one of the essential coding genes for components of ATP synthase (Blouin & Lane, 2012). Sequencing of the whole mitochondrial genome in the red algal parasite *Plocamiocolax pulvinata* revealed that the *atp8* gene is missing and the mitochondrial genome in the red algal parasite *Gracilariphila oryzoides* Setchell et Wilson showed that in this species it is a pseudogene because the sequences showed a frameshift mutation and premature stop codon (Hancock *et al.*, 2010).

This study focuses on *Pterocladiophila hemisphaerica* Fan et Papenfuss from New Zealand, which parasitize *Pterocladia lucida* (Brown) J. Agardh (order Gelidiales) and was first described in 1959 by Fan and Papenfuss. The parasite thallus looks like a hemispherical solid tubercle with an uneven surface, which can be 0.4-1 mm wide and 0.4-0.8 mm high. The branching is irregular and the parasite cells penetrate deep into the host tissue. The cystocarps, spermatangia and tetrasporangia are formed in conceptacles. The carpogonial branch is two-celled, spermatangia are produced in chains and tetrasporangia divided zonately. *Pterocladiophila hemisphaerica* was placed in a new family Pterocladiophilaceae in the Cryptonemiales (Fan & Papenfuss, 1959). Later, Pterocladiophilaceae including the parasites *Holmsella* and *Gelidiocolax* were transferred into the Gracilariales because it was proposed that *Holmsella* belonged to the Gracilariales (Fredericq & Hommersand, 1990). Phylogenetic analysis of nuclear genes confirmed that *Holmsella pachyderma* (Reinsch) Sturch is grouped in the Gracilariales (Zuccarello *et al.*, 2004).

This study will address the following questions: What are the evolutionary

relationships of the red algal parasite *Pterocladiophila hemisphaerica* to its host *Pterocladia lucida*? Is the red algal parasite part of the Gracilariales or does it belong to the Gelidiales like the host? Has host-switching occurred? Is the *atp8* gene missing or a pseudogene in *Pterocladiophila hemisphaerica*?

3.2 Material and Methods

3.2.1 Sampling

Samples of *Pterocladia lucida* and its parasite *Pterocladiophila hemisphaerica* were collected in 2011 and 2013 at different locations in New Zealand. A list of all collected samples and locations is shown in Table 3.1. All host and parasite tissue was collected as drift on the beach. Only tissue without any sign of degradation was collected. All tissue was collected and transported in plastic bags to the lab.

3.2.2 DNA extraction

DNA from fresh or silica gel dried samples were extracted either with 5% Chelex or using a modified CTAB procedure outlined in Chapter 2 (2.2.6 DNA extraction).

Table 3.1. All samples of *Pterocladia lucida* and its parasite *Pterocladiophila hemisphaerica* used in molecular analysis. No.= Code used in the chapter. Date and location, with coordinates, and collector of collection also shown. Host source described the host from which the parasite was removed.

Species	No.	Date	Location	Coordinates	Collector	Host source
<i>Pterocladia lucida</i>	45H	19.05.13	Kairakau Beach	S 39° 58' 18" E 176° 54' 53.7	M. Preuß	-
<i>Pterocladia lucida</i>	46H	19.05.13	Aramonan a	S 40° 8' 51" E 176° 50' 27"	M. Preuß	-
<i>Pterocladia lucida</i>	G214	27.11.11	Akitio Beach	S 40° 36' 53" E 176° 24' 53"	J. Zuccarello	-
<i>Pterocladiophila hemisphaerica</i>	45P	19.05.13	Kairakau Beach	S 39° 58' 18" E 176° 54' 53"	M. Preuß	45H
<i>Pterocladiophila hemisphaerica</i>	45P.2	19.05.13	Kairakau Beach	S 39° 58' 18" E 176° 54' 53"	M. Preuß	45H
<i>Pterocladiophila hemisphaerica</i>	G215	27.11.11	Akitio Beach	S 40° 36' 53" E 176° 24' 53"	J. Zuccarello	G214
<i>Pterocladiophila hemisphaerica</i>	G215.2	27.11.11	Akitio Beach	S 40° 36' 53" E 176° 24' 53"	J. Zuccarello	G214

3.2.3 Primer

One chloroplast marker (Ribulose-1,5-bisphosphate carboxylase/oxygenase = RuBisCO), one mitochondrial marker (*cox1*) and two nuclear markers (LSU, SSU) were used. The forward primer- ori.pts.for (TGT GGA CCT CTA CAA ACA GC) and the reverse primer ori.pts.rev (CCC CAT AGT TCC CAA T) were used to amplify the RUBISCO spacer (Maggs *et al.*, 1992). The forward primer GazF1 (TCA ACA AAT CAT AAA GAT ATT GG) and the reverse primer GazR1 (ACT TCT GGA TGT CCA AAA AAY CA) were used to amplify *cox1* (Saunders, 2005). The forward primer Z.LSU.for (GCA ACG GGC AAA GGG AAT CCG) and the reverse primer Z.LSU.rev

(TGA TAG GAA GAG CCG ACA TCG A) were used to amplify partial sequences of LSU (Harper & Saunders, 2001). Most of SSU was amplified using three combinations of forward (GO1, GO4, GO6) and reverse (GO8, JO4, 18S rev) primers (Saunders & Kraft, 1994). The forward GO1 (CAC CTG GTT GAT CCT GCC AG) and the reverse primer GO8 (GAA CGG CCA TGC ACC ACC ACC) primer combination was used to amplify the first part of the SSU sequence. The forward GO4 (CAG AGG TGA AAT TCT TGG AT) and the reverse primer JO4 (AAA CCT TGT TAC GAC TTC TCC) were used to amplify the middle part of the SSU sequence. The forward primer GO6 (GTT GGT GGT GCA TGG CCG CCT TC) and the reverse primer 18S rev (CCC GGG ATC CAA GCT TGA TCC TTC TGC AGG TTC ACC TAC) were used to amplify the last part of the SSU sequence.

3.2.4 Primer Design

Mitochondrial genomes were used to design new primer to check the presence of the *atp8* gene. Four whole mitochondrial genomes of *Chondrus crispus* (NC_001677), *Gracilariphila oryzoides* (HQ586059), *Gracilaropsis andersonii* (NC_014772) and *Plocamiocolax pulvinata* (NC_014773) were downloaded into Geneious. The contiguous gene region of *atp6-atp8-nad5* was removed and aligned. Forward primer were designed to start in the 3-prime end of *atp6* and reverse primer in the 5-prime end of *nad5* gene to capture the whole intervening *atp8* gene. Three new forward ATP6_F1 (CAA GCT TAY GTT TTT ACV TTA TTA AC), ATP6_F2 (CCT TAY AGY TTT ACW GTT AC) and ATP6_F3 (GCA AAT ATG ACD TCW GGY CAY AC) and two new reverse primer NAD5_R1 (CTT TWA WTG CSG ATT GAT TAG C) and NAD5_R2 (GTT AAW AAT AAW ATA AKT AAA TAC) were designed by hand. All

primer were tested individually in Geneious to ensure primer binding. Primer were produced by IDT (Integrated DNA Technologies, Inc.). All primers were successfully used in the amplification.

3.2.5 PCR-Gel

PCR and sequencing followed procedure following outlined in Chapter 2 (PCR-Gel 2.2.9).

3.2.6 Assembly and alignment

Forward and reverse sequences were individually assembled and edited. Difficult to read bases were deleted at the end and bases, which had two different base pair peaks were changed using the nucleotide ambiguity code (IUPAC). Only good readable sequences were used. All edited sequences were Blast searched for closest match and all sequences downloaded from one marker were aligned using MAFFT alignment (Katoh *et al.*, 2002) in Geneious. The alignment for SSU and LSU were checked by eye and changed if necessary.

DNA sequences from Genbank were downloaded to add to the aligned dataset. The sequences were selected by three different criteria: 1) species in the same genus, 2) top ten blast search hits and 3) one representative of a family within the Florideophyceae. A combined list of all sequences used from Genbank are shown in Appendix 4.

The *cox1* alignment contained sequences from *Pterocladia lucida*, *Pterocladiophila hemisphaerica*, different *Pterocladiella* species (Genbank) and *Acanthopeltis longiramulosa* Lee et Kim (Genbank) as an outgroup.

The partial LSU alignment contained sequences from *Pterocladia lucida*, *Pterocladophila hemisphaerica*, species in the order Gracilariales and Gelidiales, the top ten blast search host for the parasite (Table 3.2) and *Caloglossa stipitata* Post (Ceramiales) as an outgroup.

Table 3.2. List of top ten species from Blast search hits of the *Pterocladophila hemisphaerica* LSU sequence. All samples were downloaded from Genbank. Taxonomic Order, Genbank Accession No. and E-value of the Blast hit are shown.

Species	Order	Genbank Accession No.	E-value
<i>Dilsea integra</i> (Kjellman) Rosenvinge	Gigartinales	FJ848966	1.96e ⁻¹⁶⁹
<i>Farlowia mollis</i> (Harvey et Bailey) Farlow et Setchell	Gigartinales	GU176299	196e ⁻¹⁶⁹
<i>Furcellaria lumbricalis</i> (Hudson) Lamouroux	Gigartinales	HM600853	1.96e ⁻¹⁶⁹
<i>Gainia mollis</i> Moe	Gigartinales	GQ406351	1.96e ⁻¹⁶⁹
<i>Hyalosiphonia caespitosa</i> Okamura	Gigartinales	JN403057	1.96e ⁻¹⁶⁹
<i>Lesleigha</i> sp.	Sebdeniales	JN602193	2.52e ⁻¹⁷³
<i>Neodilsea borealis</i> (Abbott) Lindstrom	Gigartinales	EF033610	1.96e ⁻¹⁶⁹
<i>Neodilsea natashae</i> Lindstrom	Gigartinales	JF928825	1.96e ⁻¹⁶⁹
<i>Predaea kraftiana</i> Millar et Guiry	Nemastomatales	EF033618	2.52e ⁻¹⁷⁰
<i>Sphaerococcus coronopifolius</i> Stackhouse	Gigartinales	FJ84974	1.96e ⁻¹⁶⁹

For the SSU alignment, sequences from *Pterocladia lucida*, *Pterocladophila hemisphaerica* and species from different families within the Florideophyceae were used. SSU alignment of Florideophyceae were obtained from Prof. Olivier DeClerck and modified as needed. *Pyropia tenera* (Bangiophyceae) was used as outgroup.

3.2.7 Phylogenetic analysis

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 with all three codons partitioned for *cox1* but not for LSU and SSU and the GTR+gamma model and 500 non-parametric bootstrap replicates (Felsenstein, 1985).

Further Maximum-likelihood trees were constructed using PHYML (Guindon & Gascuel, 2003). PHYML analyses were performed using Geneious (GTR model) with 100 non-parametric bootstrapping pseudoreplicates.

Bayesian inference was performed with MrBayes v.3.1.2 (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.

Bootstrap support from RAxML and PHYML and Bayesian posterior probability was added to the constructed RAxML Maximum-likelihood tree to get better supported for our branches using different algorithm.

3.2.8 Analysis of *atp8* gene

The edited sequences were searched for open reading frames (ORF) using the genetic code for mold and protozoan mitochondria and a minimum size of 100 base pairs in Geneious. Blastx in Genbank was used to search the protein database for matches for the found open reading frames. The *atp8* gene in *Pterocladiophila hemisphaerica*, *Pterocladia lucida* and *Gelidium vagum* were aligned using MAFFT and pairwise distance calculated. The *atp8* protein sequence of *Gelidium vagum*

(YP_008963212) was downloaded from Genbank.

3.3 Results

3.3.1 Chloroplast

The alignment of the RuBisCO spacer sequences of *Pterocladia lucida* and *Pterocladophila hemisphaerica* was 316 base pairs (Appendix 5). We sequenced two host-parasite combinations from two locations in New Zealand: Kairakau Beach and Akitio Beach. Host and parasite from the same location have identical RuBisCO spacers. Host and parasite combinations were one base pair different in the spacer region between these two locations in New Zealand (Fig. 3.1).

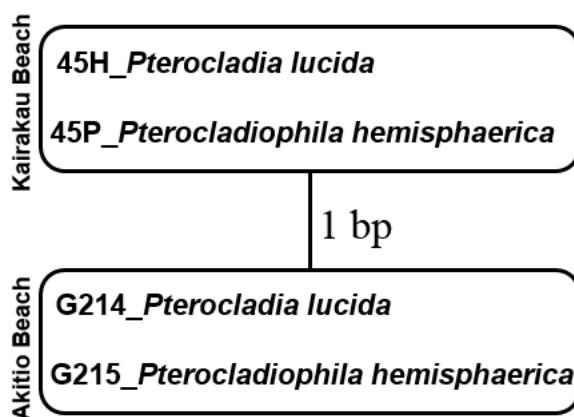


Figure 3.1. Two host-parasite combinations of *Pterocladia lucida* and *Pterocladophila hemisphaerica* from two different locations in New Zealand: Kairakau Beach and Akitio Beach. Samples of host-parasite combinations from the same location have identical RuBisCO spacer and the samples between locations are different by one base pair.

3.3.2 Cox1

The alignment of partial *cox1* gene sequences was 542 base pairs long (Appendix 6). The *cox1* data set resolved three groups: one containing the host *Pterocladia lucida*, the second *Pterocladiella* species (*Pterocladiella caerulescens*, *Pterocladiella capillacea*, *Pterocladiella psammophila*) and the last containing one *Pterocladiella capillacea* sequence (HQ422696) and the parasite *Pterocladiophila hemisphaerica* (moderate support). The *Pterocladiella capillacea* sequence HQ422696 is from Hawaii (USA) and the other *Pterocladiella capillacea* sequence (HM629885) is from South Korea. Both samples of the host *Pterocladia lucida* form a well-supported group. Most of the *Pterocladiella* species form a moderate supported group (Fig. 3.2).

3.3.3 LSU

The alignment of the LSU gene sequences is 920 base pairs long (Appendix 7). The partial LSU data resolved three groups (Fig. 3.3): one containing members of the order Gracilariales (*Gracilaria spp.* and *Melanthalia sp.*) with strong support. The second group contains the parasite *Pterocladiophila hemisphaerica* and its 10 closest blast search result but this collection of samples has no bootstrap or Bayesian posterior probability support. The last group containing members of the order Gelidiales (*Gelidiella*, *Gelidium*, *Pterocladia* and *Pterocladiella*) is negligible supported, especially with PHYML analysis. The parasite *Pterocladiophila hemisphaerica* groups with *Predaea kraftiana* (order: Nemastomatales) but without support. *Pterocladiophila hemisphaerica* groups neither with species in the Gelidiales or Gracilariales.

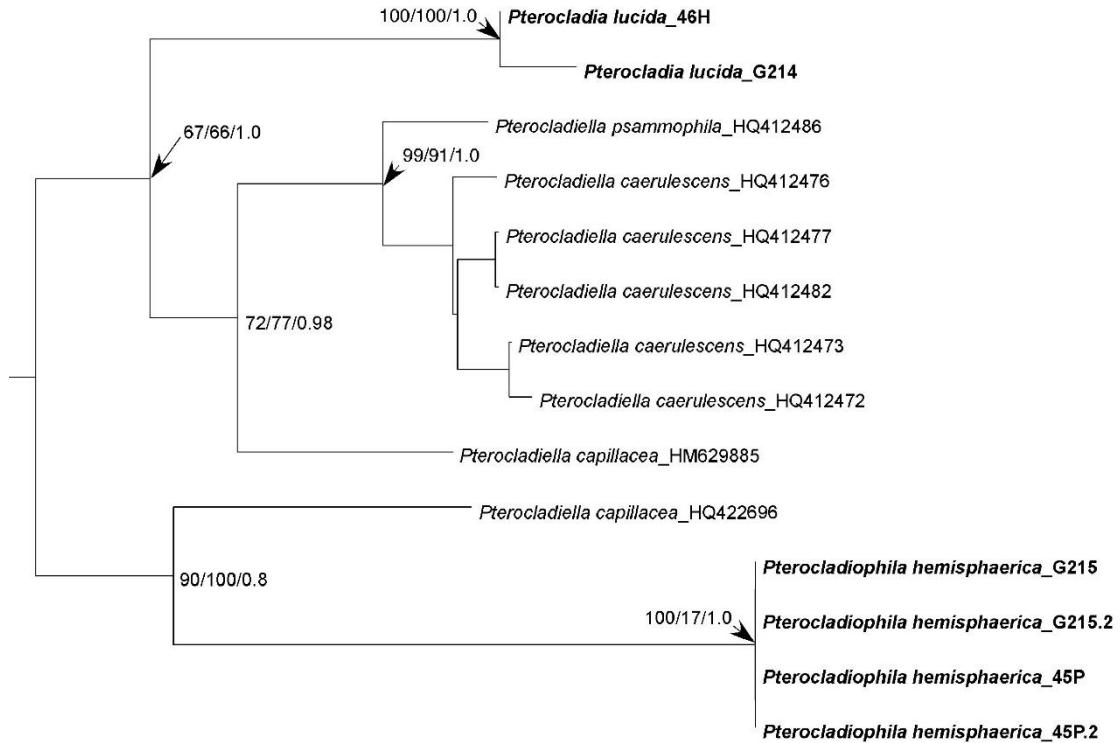


Figure 3.1. ML reconstruction of relationships based on *cox1* sequences of *Pterocladiophila hemisphaerica*, *Pterocladia lucida* and downloaded Genbank samples of *Pterocladiella* species.
Branches show Bootstrap support by RAxML and PHYLIP, and Bayesian posterior probabilities.
Species are followed by their sample number or Genbank accession number. Outgroup removed for clarity.

3.3.4 SSU

The alignment of the SSU sequence was 1525 base pairs long (Appendix 8). The SSU data resolved several groups within the Florideophyceae. We will focus on *Pterocladiophila hemisphaerica* and its relationship to species in the Gracilariales and Gelidiales. *Pterocladiophila hemisphaerica* is not part of the well-supported group of species in the Gelidiales. Our *Pterocladia lucida* sequences is grouped with the other Gelidiales. The order Gracilariales is also well supported. All three samples of

Pterocladiophila hemisphaerica group together and are well supported.

Pterocladiophila hemisphaerica groups with *Trematocarpus fragilis* but with poor support (Fig. 3.4).

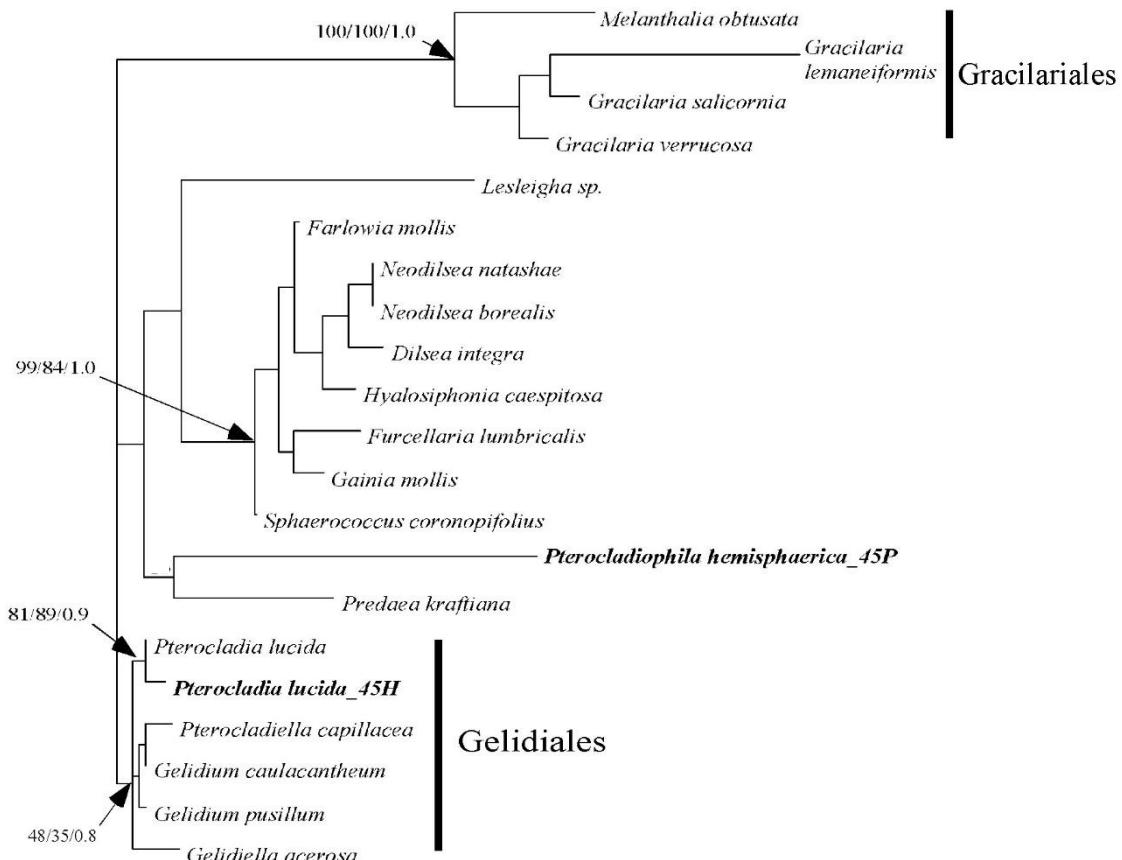


Figure 3.2. ML reconstruction of *Pterocladiophila hemisphaerica*, *Pterocladia lucida* and downloaded Genbank samples of species in the Gelidiales (*Gelidiella acerosa*, *Gelidium caulacantheum*, *Gelidium pusillum*, *Pterocladia lucida*, *Pterocladiella capillacea*) species in Gracilariales (*Gracilaria lemaneiformis*, *Gracilaria salicornia*, *Gracilaria verrucosa*, *Melanthalia obtusata*) and top ten blast search for the parasite sample (*Dilsea integra*, *Farlowia mollis*, *Furcellaria lumbricalis*, *Gainia mollis*, *Hyalosiphonia caespitosa*, *Lesleigha sp.*, *Neodilsea borealis*, *Neodilsea natashae*, *Predaea kraftiana*, *Sphaerococcus coronopifolius*) based on LSU data. Branches show Bootstrap support by RAxML and PHYML, and Bayesian posterior probability. Species are followed by their sample number. Outgroup was removed for clarity.

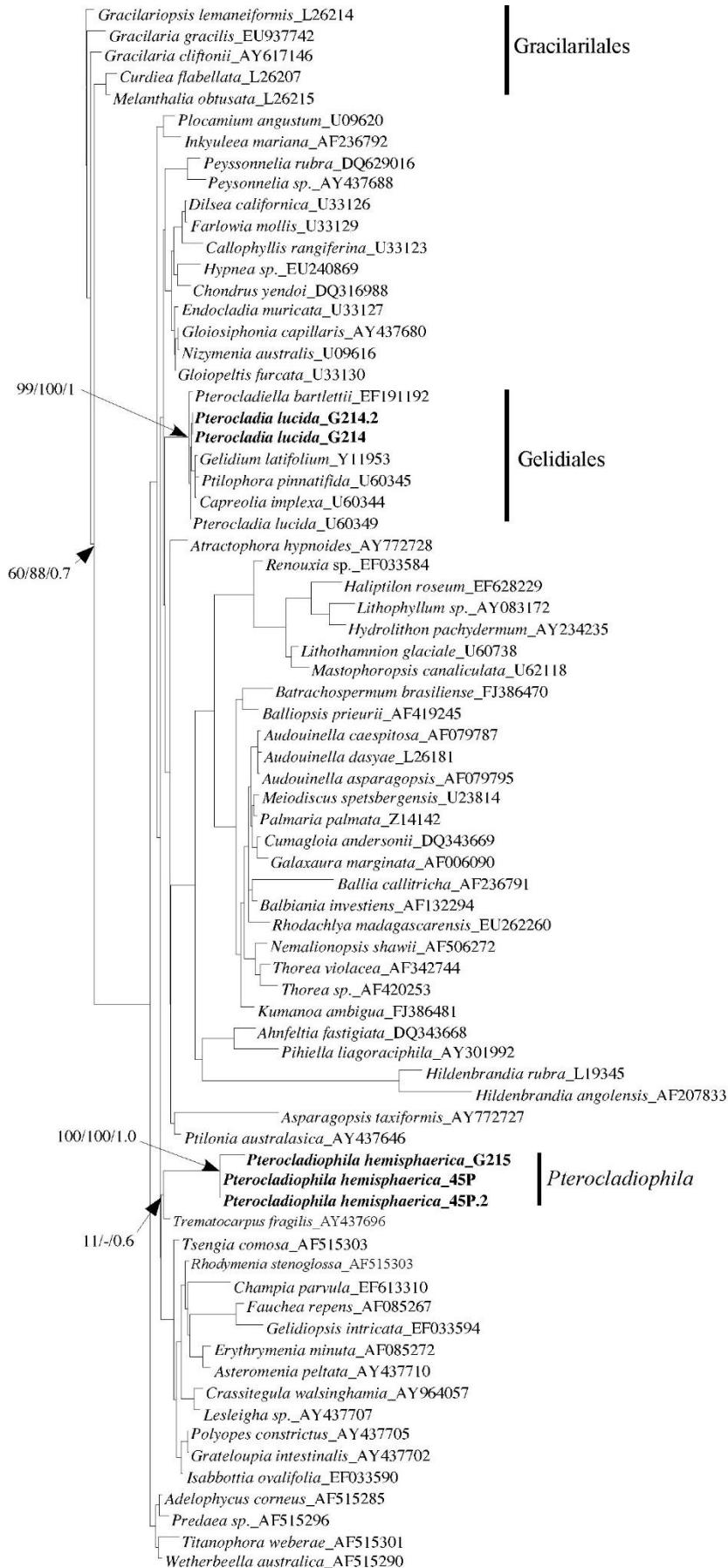


Figure 3.3. ML reconstruction of SSU sequences of member of the Florideophyceae, including *Pterocladiophila hemisphaerica*, *Pterocladia lucida* and species downloaded by Genbank. Branches show Bootstrap support by RAxML and PHYML and Bayesian posterior probabilities. Species are followed by their sample number or Genbank accession number. Outgroup was removed for clarity.

3.3.5 *Atp8*

A combination of the forward ATP6F3 and reverse NAD5R2 primer amplified three *Pterocladia lucida* (45H, 46H, G214) and one *Pterocladiophila hemisphaerica* sequence (45P). The edited parasite sequence was 615 base pairs long and one open reading was best matched by Blastx to the *atp8* protein from *Gracilariopsis andersonii* (YP_004062191). The reading frame was from position 209 to 592 and was 383 base pairs long (Appendix 9). The amino acid sequence of the *atp8* sequence of *Pterocladiophila hemisphaerica* are shown in Fig. 3.5.

1 10 20 30 40 50
| | | | | |
MPQLDRRIIIIFNFVFWFVLFYIFIYIYMLYYIILPQLLKSMIIRSKILLNHK
KEREDLWNQNINNNIKQFYIFFTYKCLVIKTKLATTINSQKNLKKIIVDKQ
ISYSIKNLFIYCNKHLCKSIIIFYPKNK*

Figure 3.4. Amino acid sequence of the *atp8* sequence of *Pterocladiophila hemisphaerica*.

All edited host sequence contain one open reading frame that matched the *atp8* protein from *Gracilariopsis andersonii*. The open reading frame of *atp8* was 414 base pairs long in the 45H and 46H sample and 402 base pair in the G214 *Pterocladia lucida* sample (Appendix 10).

The alignment of the translated amino acid sequences of the *atp8* protein of *Pterocladia lucida*, *Pterocladiophila hemisphaerica* and *Gelidium vagum* showed that the parasite sequence is quite different from the host. One *Pterocladia lucida* sequence (G214), the *Gelidium vagum* and *Pterocladiophila hemisphaerica* have deletion at the end of their sequence but the parasite also shows a deletion in the middle of its sequence (Fig. 3.6).

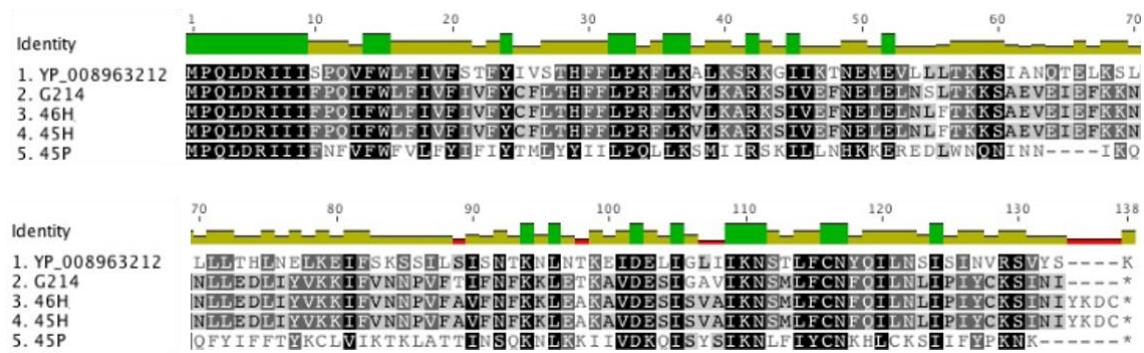


Figure 3.5. *Atp8* amino acid alignment of *Gelidium vagum* (YP_008963232), *Pterocladia lucida* (45H, 46 H, G214) and *Pterocladiophila hemisphaerica* (45P). Similarity is highlighted.

Pairwise distance between the *atp8* amino acid sequence show that the sequence of *Pterocladiophila hemisphaerica* are closer to *Gelidium vagum* than to the *Pterocladia lucida* sequence (Tab. 3.3).

Table 3.3. Pairwise distance between the *atp8* amino acid sequence of *Gelidium vagum*, *Pterocladia lucida* and *Pterocladiophila hemisphaerica*.

	<i>G. vagum</i>	<i>P. lucida</i> (45H)	<i>P. lucida</i> (46H)	<i>P. lucida</i> (G214)	<i>Pt. hemisphaerica</i>
<i>Gelidium vagum</i>	-				
<i>Pterocladia lucida</i> (45H)	0.63	-			
<i>Pterocladia lucida</i> (46H)	0.63	0.00	-		
<i>Pterocladia lucida</i> (G214)	0.59	0.06	0.06	-	
<i>Pterocladiophila hemisphaerica</i>	1.34	1.24	1.24	1.88	-

3.4 Discussion

Phylogenetic analysis revealed a complex evolutionary relationship between *Pterocladiophila hemisphaerica* and its host *Pterocladia lucida*. Phylogenetic analysis showed that *Pterocladiophila hemisphaerica* has identical RuBisCO spacer with its host *Pterocladia lucida*, similar mitochondrial genes to *Pterocladiella capillacea* and nuclear sequences within the Florideophyceae but the nuclear sequences of the parasite neither groups in the Gelidiales or the Gracilariales. The *atp8* gene in *Pterocladiophila hemisphaerica* seems to be a pseudogene.

The identical RuBisCO spacer sequence between host and parasite suggest that *Pterocladiophila hemisphaerica* contains the chloroplast of its host. *Pterocladiophila hemisphaerica* has mitochondrial sequence closer to one sample of *Pterocladiella capillacea* from Hawaii than to its host *Pterocladia lucida* suggesting that *Pterocladiella capillacea* or a related alga was a previous host. Nuclear markers indicated that *Pterocladiophila hemisphaerica* originated from a different red algal

order from either the Gelidiales, which contains its host, or Gracilariales, where the parasite has been placed when the family Pterocladiophiloceae was moved.

3.4.1 Chloroplast

The identical gene sequences of the chloroplast between host and parasite from the same location and a slight different between host and parasite sequences from different locations in New Zealand, suggest that either only the host chloroplasts were amplified or that the parasite and host share the same chloroplasts. Identical chloroplasts between host and parasite have been found in the parasite *Plocamiocolax pulvinata* Setchell and its host *Plocamium cartilagineum* (Linneaus) Dixon using RFLP pattern (Goff & Coleman, 1995). The identical chloroplasts between host and parasite could be explained by the retention of the chloroplasts by the parasite cell after parasitizing a particular host.

Why does the parasite retain the chloroplast from the host and is it important for the parasite or is it just a coincidence? The easiest explanation for gaining the chloroplast from the host would be photosynthesis, which could be an essential function for the parasite. The red algal parasite *Choreocolax polysiphonae* Reinsch increases the fixation of CO₂ when the parasite is detached from its host *Polysiphona lanosa* (Linnaeus) Tandy (Callow *et al.*, 1979). No pigmented or non-pigmented red algal parasites are known to be without chloroplasts (Goff, 1982) suggesting a function of these plastids. Other functions of chloroplasts besides photosynthesis are amino acid biosynthesis (Singh & Matthews, 1994), fatty acid synthesis (Wang & Benning, 2012), pyrimidine synthesis (Kafer & Thornburg, 1999) and immune response (Nomura *et al.*,

2012). Due to the fact that identical chloroplasts are found between other red algal parasites and their hosts (Goff & Coleman, 1995) it suggests that it is unlikely to be a coincidence. It is likely that the parasite needs particular fatty acids and can not function without them.

3.4.2 Mitochondria

Cox1 gene sequences showed that the red algal parasite *Pterocladiophila hemisphaerica* is more closely related to one *Pterocladiella capillacea* sample (HQ422696) from Hawaii than to its host *Pterocladia lucida*, which suggests that the Hawaiian *Pterocladiella capillacea* or a related algae is a former host before the parasite switched to *Pterocladia lucida*. The *cox1* sequence of the parasite shares a common origin with the Hawaiian *Pterocladiella capillacea* sample to the exclusion of its own host or anyone else so far sequenced. While we tried we were unable to collect and sequence any *Pterocladiella capillacea* from New Zealand. The parasite had to somehow gain the mitochondria from the Hawaiian *Pterocladiella capillacea* to show a common origin of the *cox1* gene. A likely explanation would be that *Pterocladiophila hemisphaerica* gained it from a former host like *Pterocladiella capillacea* or something related to it. This would indicate that the parasite switched hosts, assuming that the Hawaiian *Pterocladiella* is a former host. Host-switching seems to be a natural process in the evolution of parasitism (Jackson, 1999) and it is also known in some red algal parasites (Goff *et al.*, 1996, Zuccarello *et al.*, 2004). One example for host-switching is the red algal parasite *Ululania stellata* Apt et Schlech, where mitochondria (*cox1*) and nuclear (LSU) are more closely related to *Chondria* species than to its host *Acanthophora pacifica* (Setchell) Kraft, which suggest that *Ululania stellata* shares a

common origin with a *Chondria* species, and then switched to an unrelated host (Kurihara *et al.*, 2010).

In red algal parasites there are examples known where the parasite switched hosts but remained on the host of origin or switched hosts and is not growing on the host of origin anymore. One example for the parasite no longer growing on the host of origin is *Faucheocolax attenuata* Setchell, which grows on *Fauchea frysiana* Setchell but is more closely related to *Fauchea laciniata* J. Agardh (Goff *et al.*, 1996). One example for parasitizing two species is *Holmsella pachyderma* (Reinsch) Sturz, which grows on two species in the same family: *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine et Farnham and *Gracilaria longissima* (Gmelin) Steentoft, Irvine et Farnham but is closer related to *Gracilaria gracilis* (Zuccarello *et al.*, 2004). Our study shows that *Pterocladiophila hemisphaerica* grows neither on its host of origin or on *Pterocladiella capillacea* but grows on *Pterocladia lucida* and indicates at least two host-switches of the parasite. The possible two host-switches of the parasite could indicate an old age of the origin of the parasite. The complex history of *Pterocladiophila hemisphaerica* is the first known case of three different origin of mitochondria, nucleus and chloroplast and this also shows that our knowledge of red algal parasites is still quite limited.

The sequence of *Pterocladiella capillacea* which groups with the parasite comes from Hawaii. While the other sequences of *Pterocladiella capillacea*, which groups with all other *Pterocladiella* species comes from South Korea. A misidentification of one or the other *Pterocladiella capillacea* would explain why they are not grouped together. The misidentification of the Hawaiian *Pterocladiella* sample would falsify the

common origin of the mitochondria between *Pterocladiella capillacea* and *Pterocladiophila hemisphaerica*.

Pterocladiella capillacea is also known from New Zealand and it would be interesting to see what the relationship between the parasite and other *Pterocladiella* samples are. I tried to collected samples of *Pterocladiella capillacea* from New Zealand but was unsuccessful because *Pterocladia lucida* and *Pterocladiella capillacea* are morphological quite similar and hard to distinguish.

3.4.3 Nuclear DNA

Partial LSU and SSU showed that the red algal parasites *Pterocladiophila hemisphaerica* is neither grouped in the Gracilariales or with its hosts order, the Gelidiales. The origin of the parasite could not be resolved. The parasite nuclear sequences always grouped in the Florideophyceae but even when the parasite grouped with another species it was poorly supported. Neither using top ten Blast searches nor using one representative from every family in the Florideophyceae could resolve the nuclear origin of the parasite.

The taxonomic history of *Pterocladiophila hemisphaerica* is complex. The description of the red algal parasite *Pterocladiophila hemisphaerica* led to the description of the new family Pterocladiophilaceae, and this was placed in the Cryptonemiales (Fan & Papenfuss, 1959). Later, *Holmsella* and *Gelidiocolax* were added to the family but the family was then grouped in the Gracilariales because of the similarities of *Holmsella pachyderma* to the Gracilariales. The family was regrouped in the order Gracilariales, including *Pterocladiophila hemisphaerica* (Fredericq & Hommersand, 1990). SSU data confirmed that *Holmsella pachyderma* is closely related

to species in the Gracilariales (Zuccarello *et al.*, 2004). It seems rather hasty to transfer a whole family because of the similarity of morphological characters between one red algal parasite and its host species. Our data shows that *Pterocladiophila hemisphaerica* is not a part of the Gracilariales. The family Pterocladiophilaceae has to be removed from the Gracilariales and a new family described for *Holmsella*. The family Pterocladiophilaceae was created by the morphological characters of *Pterocladiophila hemisphaerica* (Fan & Papenfuss, 1959) and the family name must remain with this entity. A new family for *Holmsella* is recommended. Phylogenetic data is needed to determine the relationships of *Gelidioloax* to the other red algal parasites placed in the family Pterocladiophilaceae.

The origin of *Pterocladiophila hemisphaerica* is still unknown but it is clear that the parasite is not part of the Gelidiales. The nuclear DNA of the parasite has a different origin than the mitochondria. A better resolved phylogeny based on nuclear DNA could reveal from where *Pterocladiophila hemisphaerica* evolved.

3.4.4 *Atp8*

The *atp8* gene could be amplified with our designed primers and *atp8* seems to be present in the parasite *Pterocladiophila hemisphaerica* but is probably a pseudogene.

Is our open reading frame the *atp8* gene? Our primers were designed to amplify the flanking regions between *atp6* and *nad5*. Open reading frames, which matched the *atp6* and *nad5* genes, were found in the 45H and 46H *Pterocladia lucida* sequences and in the *Pterocladiophila hemisphaerica* sequence. The amplified host sequences had different lengths and the minimum identified size of the open reading frame of 100 base

pairs would explain why *atp6* and *nad5* wasn't found in all host sequences. Next, whole sequencing of the mitochondria in red algae showed that all genes are collinear (Burger *et al.*, 1999, Hancock *et al.*, 2010, Hwang *et al.*, 2013, Leblanc *et al.*, 1995, Ohta *et al.*, 1998, Yang *et al.*, 2013, Zhang *et al.*, 2012). Therefore we would expect *atp8* between *atp6* and *nad5*. The Blast search from our *atp8* protein sequences matched it to *atp8* protein sequences in other algal species. Lastly, the *atp8* gene in *Pterocladiophila hemisphaerica* is 383 base pairs long but in *Pterocladia lucida* (45H, 46H) it is 414 base pairs and in the other *Pterocladia lucida* sequence (G214) 402 base pairs long, which is a similar size of the *atp8* in *Pyropia tenera* (402 base pairs, (Hwang *et al.*, 2013), *Gracilariopsis lemaneiformis* (405 base pairs, (Zhang *et al.*, 2012) and *Gelidium vagum* (405 base pairs, (Yang *et al.*, 2013). The differences in the length of the *atp8* sequences could be explained by mutations, deletions or insertion in the mitochondria genome. The finding of the flanking regions, the collinear genes in algal mitochondria, the blast search to other *atp8* in other red algae and the similar length of the sequences suggest that the found open reading frame is the *atp8* gene in *Pterocladiophila hemisphaerica*.

A previous paper (Hancock *et al.*, 2010) indicates that *atp8* is non-functional in other parasites, e. g. in *Gracilariphila oryzoides*. The *Pterocladiophila hemisphaerica* gene is shorter than in its host, so the question is, is it functional in this parasite? There are two reasons to suggest that *atp8* in *Pterocladiophila hemisphaerica* is non-functional: 1) there is a deletion of four amino acid in a conserved region in the middle of the parasite sequence; and 2) there are fewer conserved amino acid regions in *Pterocladiophila hemisphaerica* when compared to the sequences of *Pterocladia lucida* and *Gelidium vagum*. Overall, *atp8* in *Pterocladiophila hemisphaerica* is present but it

is probably a pseudogene because of the deletion in a conserved region and fewer conserved regions.

3.4.5 Phylogenetic studies in red algal parasites

A possible scenario to explain the origin and results of the organelles is that *Pterocladiophila hemisphaerica* originated on an unknown red algal species, and this relationship is revealed by nuclear DNA sequences. It retained a mitochondria after switching hosts to *Pterocladiella capillacea* and then gained the host chloroplast when it switched host to *Pterocladia lucida*.

This is the first study using nuclear (SSU, LSU) and mitochondrial marker (*cox1*) to show that the parasite is not closely related to its host and in another family. The evolutionary history of *Pterocladiophila hemisphaerica* is the first known case with at least two host-switches. The complex evolutionary history of the parasite suggests that at some point there was a close relationship to its host when the parasite originated but because of host-switching the close relationship between the parasite and its host of origin changed and therefore there isn't a clear distinction of "adelphoparasite" and "alloparsite", which was already indicated by other studies (Goff *et al.*, 1996, Zuccarello *et al.*, 2004). Interestingly, there are more red algal parasites that are closely related to their host than red algal parasites which are not closely related to their hosts (Goff *et al.*, 1997, Goff & Coleman, 1995, Goff *et al.*, 1996, Kurihara *et al.*, 2010, Ng *et al.*, 2013, Zuccarello *et al.*, 2004). Is host-switching in red algal parasite to another order the exception and most red algal parasite stay on closely related species? This question could probably only be answered with further phylogenetic studies in red algal parasites as only a small subset have been studied so far.

4. Discussion

This study has four main findings: 1) the description of a new red algal parasite *Rhodophyllis parasitica* sp. nov. which is closely related to its host *Rhodophyllis membranacea*; 2) the evolutionary relationships of red algal parasite *Pterocladiophila hemisphaerica* showing it evolved in the Florideophyceae but it originated neither from the Gracilariales or Gelidiales; 3) the host switching of *Pterocladiophila hemisphaerica* at least two times, shown by three different origins of its chloroplast, mitochondria and nuclear DNA; and 4) *atp8* is present in *Pterocladia lucida* and *Pterocladiophila hemisphaerica*, but *atp8* in *Pterocladiophila hemisphaerica* is probably a pseudogene.

The red algal parasite *Rhodophyllis parasitica* sp. nov. is morphologically and genetically similar to its host *Rhodophyllis membranacea*, which suggests that the parasite evolved recently from its host. *Rhodophyllis parasitica* is easily distinguishable from the described *Rhodophyllis* species in New Zealand but shares morphological characters with *Rhodophyllis*. The monophyly of the genus *Rhodophyllis* can only be maintained when the parasite is grouped in it.

Pterocladiophila hemisphaerica is placed in the family Pterocladiophilaceae (Fan & Papenfuss, 1959) which is presently part of the order Gracilariales (Fredericq & Hommersand, 1990). This is not supported by our data. Another member of the Pterocladiophilaceae is the red algal parasite *Holmsella pachyderma* (Reinsch) Sturch, which is closely related to species in the Gracilariales (Zuccarello *et al.*, 2004). The different relationships of the two red algal parasites make it clear that the family Pterocladiophilaceae needs to be reevaluated and probably split.

The relatively newly evolved red algal parasite *Rhodophyllis parasitica*

compared to the more anciently evolved parasites *Pterocladiophila hemisphaerica* shows the complexity of diversity in the evolution of parasitic red algae. Some parasites are relatively young and very similar to their hosts. One example for a relatively young parasite is *Congracilaria babae* Yamamoto growing on *Gracilaria salicornis* Yamamoto, where host and parasite nuclear DNA are more closely related to each other than to other species in the Gracilariales (Ng *et al.*, 2013). Some parasites are older and more distantly related to their hosts. This is shown by the red algal parasite *Plocamiocolax pulvinata* Setchell growing on *Plocamium cartilagineum* (Linnaeus) Dixon while the parasite is more closely related to *Plocamium violaceum* Farlow, which suggests that the parasite evolved on *Plocamium violaceum*, radiated to its current host and now is not found on *Plocamium violaceum* anymore (Goff *et al.*, 1996). Then there are parasites like *Pterocladiophila hemisphaerica* that are not even in the same order as their hosts.

Even though *atp8* is an essential coding gene for the ATP synthase, it is assumed to be a pseudogene in the parasite *Gracilariphila oryzoides* Setchell et Wilson and is even missing in the parasite *Plocamiocolax pulvinata* (Hancock *et al.*, 2010). The *atp8* is present in *Pterocladiophila hemisphaerica* but we suggest that it is a pseudogene also.

This study adds to the knowledge of red algal and red algal parasite diversity in New Zealand. It's the first study with a phylogenetic analysis of red algal parasites in New Zealand and one of a few studies to described development of red algal parasites. *Rhodophyllis parasitica* sp. nov. is the first described red algal parasite species in New Zealand in 55 years and *Pterocladiophila hemisphaerica* is the first parasite with organelles and nuclei with different histories of origin.

Future research in red algae parasite is important to get a better understanding of red algal parasite evolution. What are the genomic consequences of becoming parasitic? Whole genome sequencing could address this question (Schuster, 2008). Comparisons of whole genomes between different parasites and their hosts could help to understand which genes are lost or expanded and which organelles are reduced after a red algae becomes parasitic. Another research project could be the study of the development in different parasitic red algae species in New Zealand. There are only a few developmental studies on red algal parasites and further research would give an insight in the different infection mechanism and show the similarities and differences in parasite infection, cell transformation and changes in the infected hosts between parasitic species. Another possibility is the further study of the phylogenetic relationships of the other red algal parasites and their hosts in New Zealand? There are also several more known but undescribed red algal parasites in New Zealand, for example on *Polysiphonia atterima* Greville, *Callophyllis calliblepharoides* J. Agardh and *Sarcodia sp.*

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Appendices

Appendix 1

ITS alignment of *Rhodophyllis membranacea* (2H, 3H, 4H, 5H, 9H, 12H, 14H, 15H, 40H, 43H, 44H) and it's parasite (2P, 3P, 4P, 5P, 9P, 13P, 14P, 40P, 42P, 43P, 44P)

	1	10	20	30	40	50	60
2H	--AT-CGAATTGCAGAACCCGTGAATCATCGAATTTTGAACGCAAGTTGCGCTCGCGGG						
2P	--ATGCGAATTGCAGRACCCGTGAATCATCGAATTTTGAACGCMAGTTGCGCTCGCGGG						
3H	TAATGCGAATTGCAGAACCCGTGAATCATCGAATTTTGAACGCAAGTTGCGCTCGCGGG						
3P	-AATGCGAATTGCAGRAMCCGTGAATCATCGAWTTTTG-ACGCAAGTTGCGCTCGCGGG						
4H	TAATGCGAATTGCAGAACCCGTGAATCATCGAATTTTGAACGCAAGTTGCGCTCGCGGG						
4P	TAATGCGAATTGCAGAACCCGTGAATCATCGAATTTTGAACGCAAGTTGCGCTCGCGGG						
5H	TAATGCGAATTGCAGAACCCGTGAATCATCGAATTTTGAACGCAAGTTGCGCTCGCGGG						
5P	TAATGCGAATTGCAGAACCCGTGAATCATCGAATTTTGAACGCAAGTTGCGCTCGCGGG						
9H	TAATGCGAATTGCAGAACCCGTGAATCATCGAATTTTGAACGCAAGTTGCGCTCGCGGG						
9P	---TGCAGATTGCAGAACCCGTGAATCATCGAATTTTGAACGCAAGTTGCGCTCGCGGG						
12H	TAATGCGAATTGCAGAACCCGTGAATCATCGAATTTTGAACGCAAGTTGCGCTCGCGGG						
13P	-----						
14H	TAAT-CGAATT-CAGAACCCG-GAATCATCGAA-TTTTGAACGCAAGTTGCGCTCGCGGG						
14P	T-ATG-GAATTGCAGAACCCGTGAATCATCGAATTTTGAACGC-AGTTGCGCTCGCGGG						
15H	TAATGCGAATTGCAGAACCCGTGAATCATCGAATTTTGAACGCAAGTTGCGCTCGCGGG						
40H	TAATGCGAATTGCAGAACCCGTGAATCATCGAATTTTGAACGCAAGTTGCGCTCGCGGG						
40P	TAATGCGAACTGCAGCMCCCGTGAATCATCGAATTTTGAACGCAAGYTGCGCTCGCGGG						
42P	TAATGAGAATTGCAGMMCCCGTGTATCATCGAATTTTGNACGCAAGTTGCGCTCGCGGG						
43H	TAATGCGAATTGCAGAACCCGTGAATCATCGAATTTTGAACGCAAGTTGCGCTCGCGGG						
43P	-----						
44H	-AAT-CGAATTGCAGAACCCGTGAATCATCGAATTTTGAACGCAAGTTGCGCTCGCGGG						
44P	-----						

	61	70	80	90	100	110	120
2H	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
2P	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
3H	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
3P	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
4H	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
4P	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
5H	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
5P	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
9H	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
9P	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
12H	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
13P	-----						
14H	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
14P	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
15H	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
40H	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
40P	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
42P	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
43H	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
43P	-----						
44H	TAATCCTGCGAGCATGTCTGTTGAGTGTCCCGTCTTGAGAAATTGGCGCTGTTGACG						
44P	-----						

	121	130	140	150	160	170	180
2H	CGCTCTTCGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
2P	CGCTCTTGGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
3H	CGCTCTTCGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
3P	CGCTCTTCGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
4H	CGCTCTTCGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
4P	CGCTCTTGGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
5H	CGCTCTTCGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
5P	CGCTCTTGGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
9H	CGCTCTTCGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
9P	CGCTCTTGGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
12H	CGCTCTTCRGAGTGTGGCSNCGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
13P	-----						
14H	CGCTCTTCGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
14P	CGCTCTTGGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
15H	CGCTCTTCGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
40H	CGCTCTTCGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
40P	CGCTCTTGGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
42P	CGCTCTTGGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
43H	CGCTCTTCGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
43P	-----						
44H	CGCTCTTCGGAGTGTGGCGACGCGCAGCTGATGGCGTCTTTGGCTCTTCGCAAG						
44P	-----						

	181	190	200	210	220	230	240
2H	GGAGAGTGAAGAATAATTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
2P	GGAGAGTGAAGAATAAGTTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
3H	GGAGAGTGAAGAATAATTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
3P	GGAGAGTGAAGAATAAKTTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
4H	GGAGAGTGAAGAATAATTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
4P	GGAGAGTGAAGAATAAGTTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
5H	GGAGAGTGAAGAATAATTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
5P	GGAGAGTGAAGAATAAGTTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
9H	GGAGAGTGAAGAATAATTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
9P	GGAGAGTGAAGAATAAGTTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
12H	GGAGAGTGAAGAATAATTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
13P	-----						
14H	GGAGAGTGAAGAATAATTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
14P	GGAGAGTGAAGAATAAGTTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
15H	GGAGAGTGAAGAATAATTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
40H	GGAGAGTGAAGAATAATTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
40P	GGAGAGTGAAGAATAAGTTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
42P	GGAGAGTGAAGAATAAGTTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
43H	GGAGAGTGAAGAATAATTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
43P	-----						
44H	GGAGAGTGAAGAATAATTGTCCGTGTGGCTTGTCCCTTATCGGGGTGAGGC GTGCGGT						
44P	-----						

	241	250	260	270	280	290	300
2H	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
2P	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
3H	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
3P	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
4H	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
4P	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
5H	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
5P	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
9H	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
9P	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
12H	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
13P	-----						
14H	CGCCCGAAGAAAAGCTCGCTGAATTGTT-GAAAACGGCATGTTTTGTGTGTTATG						
14P	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
15H	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
40H	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
40P	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
42P	CGCCCGAARAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
43H	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
43P	-----						
44H	CGCCCGAAGAAAAGCTCGCTGAATTGTTGGAAAACGGCATGTTTTGTGTGTTATG						
44P	-----						

	301	310	320	330	340	350	360
2H	TCACCTTGAGGGCTGT	CATGTACTTGGGATT	TGGGTGCAAACCT	CTTGAATCGAC	---		
2P	TCACCTTGAGGGCTGT	CA-GTACTTGGGATT	TGGGTGCAAACCT	CTTGAATCGACT	TT		
3H	TCACCTTGAGGGCTGT	CATGTACTTGGGATT	TGGGTGCAAACCT	CTTGAATCGAC	---		
3P	TCACCTTGAGGGCTGT	CATGTACTTGGGATT	TGGGNCGAAACCT	CTTGAATCGAC	---		
4H	TCACCTTGAGGGCTGT	CATGTACTTGGGATT	TGGGTGCAAACCT	CTTGAATCGAC	---		
4P	TCACCTTGAGGGCTGT	CA-GTACTTGGGATT	TGGGTGCAAACCT	CTTGAATCGACT	TT		
5H	TCACCTTGAGGGCTGT	CATGTACTTGGGATT	TGGGTGCAAACCT	CTTGAATCGAC	---		
5P	TCACCTTGAGGGCTGT	CA-GTACTTGGGATT	TGGGTGCAAACCT	CTTGAATCGACT	TT		
9H	TCACCTTGAGGGCTGT	CATGTACTTGGGATT	TGGGTGCAAACCT	CTTGAATCGAC	---		
9P	TCACCTTGAGGGCTGT	CA-GTACTTGGGATT	TGGGTGCAAACCT	CTTGAATCGACT	TT		
12H	TCACCTTGAGGGCTGT	CATGTACTTGGGATT	TGGGTGCAAACCT	CYTGAATCGAC	---		
13P	-----						
14H	TCACCTTGAGGGCTGT	CATGTACTTGGGATT	TGGGTGCAAACCT	CTTGAATCGAC	---		
14P	TCACCTTGAGGGCTGT	CA-GTACTTGGGATT	TGGGTGCAAACCT	CTTGAATCGACT	TT		
15H	TCACCTTGAGGGCTGT	CATGTACTTGGGATT	TGGGTGCAAACCT	CTTGAATCGAC	---		
40H	TCACCTTGAGGGCTGT	CATGTACTTGGGATT	TGGGTGCAAACCT	CTTGAATCGAC	---		
40P	TCACCTTGAGGGCTGT	CACGTNCTTGGGATT	TGGGNCGAAACCT	CTTGAATCGAC	---		
42P	TCACCTTGAGGGCTGT	CAWGTACKTKGGANT	TGGGRCAACCCTYWG	GAAYCRMY	---		
43H	TCACCTTGAGGGCTGT	CATGTACTTGGGATT	TGGGTGCAAACCT	CTTGAATCGAC	---		
43P	-----T						
44H	TCACCTTGAGGGCTGT	CATGTACTTGGGATT	TGGGTGCAAACCT	CTTGAATCGAC	---		
44P	-----TT						

	361	370	380	390	400	410	420
2H	-TTGTTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGGG	
2P	TTTTTGGTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
3H	-TTGTTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
3P	-TTGTTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
4H	-TTGTTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
4P	TTTTTGGTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
5H	-TTGTTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
5P	TTTTTGGTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
9H	-TTGTTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
9P	TTTTTGGTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
12H	-TTGTTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGRC	GGTCTTCCGAG	TCGG	
13P	-TTTTTGGTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
14H	-TTGTTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
14P	TTTTTGGTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
15H	-TTGTTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
40H	-TTGTTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
40P	-TTTTTGGTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
42P	-TTTTTGGTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
43H	-TTGTTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
43P	TTTTTGGTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
44H	-TTGTTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	
44P	TTTTTGGTTGT	GATAGAGT	GGGAGCTGCCATT	CCTCTGGGACC	GGTCTTCCGAG	TCGG	

	421	430	440	450	460	470	480
2H	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
2P	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
3H	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
3P	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
4H	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
4P	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
5H	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
5P	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
9H	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
9P	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
12H	GATTACTTCTGTGTGACTGTCGATCGTARCGYAWARTGTTSCYCGCGGGAMCSGGAT						
13P	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
14H	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
14P	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
15H	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
40H	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
40P	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
42P	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
43H	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
43P	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
44H	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						
44P	GATTACTTCTGTGTGACTGTCGATCGTAACGTATAAGTGTGCTCGCGGGGAACGGGAT						

	481	490	500	510
2H	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
2P	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
3H	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
3P	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
4H	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
4P	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
5H	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
5P	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
9H	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
9P	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
12H	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
13P	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
14H	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
14P	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
15H	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
40H	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
40P	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
42P	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
43H	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
43P	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
44H	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			
44P	TTGATTTTGATGAGGGGGTTGTGATTCTAGA			

Appendix 2

Cox1 alignment of *Rhodophyllis membranacea* (4H, 5H, 8H, 9H, 12H, 14H, 15H, 39H, 40H, 42H, 43H, 44H) and its parasite (5P, 8P, 9P, 13P, 14P, 40P, 42P, 43P, 44P)

	1	10	20	30	40	50	60
4H	AAGATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTTAGGGGTTGTA					
5H	AAGATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTTAGGGGTTGTA					
5P	AAGATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTTAGGGGTTGTA					
8H	AAGATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTTAGGGGTTGTA					
8P	AAGATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTTAGGGGTTGTA					
9H	AAGATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTTAGGGGTTGTA					
9P	AAGATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTTAGGGGTTGTA					
12H						TAGGGGTTGTA	
13P							
14H	---ATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTAGGGGTTGTA					
14P	AAGATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTAGGGGTTGTA					
15H							GGGTTGTA
39H	---ATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTAGGGGTTGTA					
40H	--GATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTAGGGGTTGTA					
40P	AAGATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTAGGGGTTGTA					
42H	GAGATATTGGTACTTTATTRAATT	AKRGTCCTTCTGGGTTAGGGGTTSTA					
42P	AAGATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTAGGGGTTGTA					
43H	AAGATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTAGGGGTTGTA					
43P	AAGATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTAGGGGTTGTA					
44H	-AGATATTGGTACTTTATTTAATT	TTTGCGCTTTCTGGGTTAGGGGTTGTA					
44P	AAAGATATTGAAC	TTATTTAATTGCGCTTTCTGGGTTAGGGGTTGTA					

	61	70	80	90	100	110	120
4H	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
5H	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
5P	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
8H	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
8P	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
9H	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
9P	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
12H	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
13P	---CTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
14H	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
14P	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
15H	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
39H	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
40H	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
40P	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
42H	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
42P	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
43H	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
43P	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
44H	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						
44P	TGTCTATGTTAATACGTATGGAATTAGCTAACCCAGGTAATCATTACTTTAGGTAATC						

	121	130	140	150	160	170	180
4H	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
5H	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
5P	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
8H	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
8P	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
9H	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
9P	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
12H	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
13P	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
14H	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
14P	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
15H	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
39H	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
40H	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
40P	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
42H	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
42P	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
43H	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
43P	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
44H	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						
44P	ACCAAGTATATAATGTTCTTATTACAGCTCATGCTTTTAATGATATTTTATGGTAA						

	181	190	200	210	220	230	240
4H	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
5H	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
5P	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
8H	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
8P	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
9H	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
9P	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
12H	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
13P	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
14H	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
14P	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
15H	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
39H	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
40H	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
40P	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
42H	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
42P	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
43H	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
43P	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
44H	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						
44P	TGCCTGTAATGATTGGAGGTTTGGTAATTGGTTAGTCCTATAATGATAGGAAGCCCGG						

	241	250	260	270	280	290	300
4H	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
5H	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
5P	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
8H	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
8P	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
9H	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
9P	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
12H	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
13P	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
14H	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
14P	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
15H	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
39H	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
40H	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
40P	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
42H	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
42P	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
43H	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
43P	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
44H	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						
44P	ATATGGCATTTCCTAGATTAATAATATCTTTTGATTATTACCCCCGTCTTATGTT						

	301	310	320	330	340	350	360
4H	T	A	T	A	C	T	G
5H	T	A	T	A	C	T	G
5P	T	A	T	A	C	T	G
8H	T	A	T	A	C	T	G
8P	T	A	T	A	C	T	G
9H	T	A	T	A	C	T	G
9P	T	A	T	A	C	T	G
12H	T	A	T	A	C	T	G
13P	T	A	T	A	C	T	G
14H	T	A	T	A	C	T	G
14P	T	A	T	A	C	T	G
15H	T	A	T	A	C	T	G
39H	T	A	T	A	C	T	G
40H	T	A	T	A	C	T	G
40P	T	A	T	A	C	T	G
42H	T	A	T	A	C	T	G
42P	T	A	T	A	C	T	G
43H	T	A	T	A	C	T	G
43P	T	A	T	A	C	T	G
44H	T	A	T	A	C	T	G
44P	T	A	T	A	C	T	G

	361	370	380	390	400	410	420
4H	C	T	T	A	G	T	T
5H	C	T	T	A	G	T	T
5P	C	T	T	A	G	T	T
8H	C	T	T	A	G	T	T
8P	C	T	T	A	G	T	T
9H	C	T	T	A	G	T	T
9P	C	T	T	A	G	T	T
12H	C	T	T	A	G	T	T
13P	C	T	T	A	G	T	T
14H	C	T	T	A	G	T	T
14P	C	T	T	A	G	T	T
15H	C	T	T	A	G	T	T
39H	C	T	T	A	G	T	T
40H	C	T	T	A	G	T	T
40P	C	T	T	A	G	T	T
42H	C	T	T	A	G	T	T
42P	C	T	T	A	G	T	T
43H	C	T	T	A	G	T	T
43P	C	T	T	A	G	T	T
44H	C	T	T	A	G	T	T
44P	C	T	T	A	G	T	T

	421	430	440	450	460	470	480
4H	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
5H	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
5P	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
8H	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
8P	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
9H	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
9P	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
12H	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
13P	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
14H	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
14P	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
15H	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
39H	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
40H	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
40P	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
42H	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
42P	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
43H	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
43P	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
44H	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA
44P	ATTTATCAGGGGCTTCTTC	A	TTT	TAGGTGCTGTAA	ATT	TTA	TACTATATTAAATA

	481	490	500	510	520	530	540
4H	TGCGAAGCCCTGGTCAA	AGTATGTACAGAA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
5H	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
5P	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
8H	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
8P	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
9H	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
9P	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
12H	TGCGAAGCCCTGGTCAA	AGTATGTACAGAA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
13P	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
14H	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
14P	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
15H	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
39H	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
40H	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
40P	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
42H	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
42P	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
43H	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
43P	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
44H	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA
44P	TGCGAAGCCCTGGTCAA	AGTATGTATAGA	ATAC	CTTATT	TGTATGATCTATT	CTTGT	TA

	541	550	560	570	580	590	600
4H	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
5H	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
5P	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
8H	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
8P	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
9H	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
9P	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
12H	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
13P	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
14H	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
14P	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
15H	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
39H	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
40H	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
40P	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
42H	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
42P	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
43H	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
43P	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
44H	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						
44P	CAGCTTTTATTACTATTAGCGGTACCTGTTTAGCAGGTGCTATTACAATGTTATTAA						

	601	610	620	630	640	650	660
4H	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
5H	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
5P	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
8H	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
8P	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
9H	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
9P	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
12H	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
13P	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
14H	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
14P	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
15H	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
39H	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCT-----						
40H	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
40P	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
42H	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
42P	CAGACCGAAATTTAATACAG-----						
43H	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
43P	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGAGTC-----						
44H	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						
44P	CAGACCGAAATTTAATACAGCTTTTTGATCCTGCTGGGGGAGGAGATCCTGTTTAT						

	661	670
4H	ATCAACATTTATTTG	
5H	ATCAACATTTATTTG	
5P	ATCAACATTTATTTG	
8H	ATCAACATTTATTTG	
8P	ATCAACATTTATTTG	
9H	ATCAACATTTATTTG	
9P	ATCAACATTTATTTG	
12H	ATCAACATTTATTTG	
13P	ATCAACATTTATTTG	
14H	ATCAACATTTATTTG	
14P	ATCAACATTTATTTG	
15H	ATCAACATTTATTTG	
39H	-----	
40H	ATCAACATTTATTTG	
40P	ATCAACATTTATTTG	
42H	ATCAACATTTATTTG	
42P	-----	
43H	ATCAACATTTATTTG	
43P	-----	
44H	ATCAACATTTATTTG	
44P	ATCAACATTTATTTG	

Appendix 3

Cox2-3 alignment of *Rhodophyllis membranacea* (2H, 3H, 4H, 5H, 6H, 8H, 9H, 13H, 14H, 15H) and its parasite (5P, 6P, 8P, 10P, 13P)

	1	10	20	30	40	50	60
2H	AAATGTGATGCTATA	CCGGGAAGATTA	AATCAAAC	TCTTATT	TGTAAA	ACGTTCA	GGT
3H	AAATGTGATGCTATA	CCGGGAAGATTA	AATCAAAC	TCTTATT	TGTAAA	ACGTTCA	GGT
4H	-----						
5H	AAATGTGATGCTATA	CCGGGAAGATTA	AATCAAAC	TCTTATT	TGTAAA	ACGTTCA	GGT
5P	AAATGTGATGCTATA	CCGGGAAGATTA	AATCAAAC	TCTTATT	TGTAAA	ACGTTCA	GGT
6H	AAATGTGATGCTATA	CCGGGAAGATTA	AATCAAAC	TCTTATT	TGTAAA	ACGTTCA	GGT
6P	AAATGTGATGCTATA	CCGGGAAGATTA	AATCAAAC	TCTTATT	TGTAAA	ACGTTCA	GGT
8H	AAATGTGATGCTATA	CCGGGAAGATTA	AATCAAAC	TCTTATT	TGTAAA	ACGTTCA	GGT
8P	AAATGTGATGCTATA	CCGGGAAGATTA	AATCAAAC	TCTTATT	TGTAAA	ACGTTCA	GGT
9H	AAATGTGATGCTATA	CCGGGAAGATTA	AATCAAAC	TCTTATT	TGTAAA	ACGTTCA	GGT
10P	AAATGTGATGCTATA	CCGGGAAGATTA	AATCAAAC	TCTTATT	TGTAAA	ACGTTCA	GGT
13H	AAATGTGATGCTATA	CCGGGAAGATTA	AATCAAAC	TCTTATT	TGTAAA	ACGTTCA	GGT
13P	AAATGTGATGCTATA	CCGGGAAGATTA	AATCAAAC	TCTTATT	TGTAAA	ACGTTCA	GGT
14H	AAATGTGATGCTATA	CCGGGAAGATTA	AATCAAAC	TCTTATT	TGTAAA	ACGTTCA	GGT
15H	AAATGTGATGCTATA	CCGGGAAGATTA	AATCAAAC	TCTTATT	TGTAAA	ACGTTCA	GGT
	61	70	80	90	100	110	120
2H	ATTTATTATGGTCAAT	GTA	TGTAGTGAA	ATATGTGGT	ATTAA	CCATGGGTTA	TGCCTATAGTT
3H	ATTTATTATGGTCAAT	GTA	TGTAGTGAA	ATATGTGGT	ATTAA	CCATGGGTTA	TGCCTATAGTT
4H	-----						
5H	ATTTATTATGGTCAAT	GTA	TGTAGTGAA	ATATGTGGT	ATTAA	CCATGGGTTA	TGCCTATAGTT
5P	ATTTATTATGGTCAAT	GTA	TGTAGTGAA	ATATGTGGT	ATTAA	CCATGGGTTA	TGCCTATAGTT
6H	ATTTATTATGGTCAAT	GTA	TGTAGTGAA	ATATGTGGT	ATTAA	CCATGGGTTA	TGCCTATAGTT
6P	ATTTATTATGGTCAAT	GTA	TGTAGTGAA	ATATGTGGT	ATTAA	CCATGGGTTA	TGCCTATAGTT
8H	ATTTATTATGGTCAAT	GTA	TGTAGTGAA	ATATGTGGT	ATTAA	CCATGGGTTA	TGCCTATAGTT
8P	ATTTATTATGGTCAAT	GTA	TGTAGTGAA	ATATGTGGT	ATTAA	CCATGGGTTA	TGCCTATAGTT
9H	ATTTATTATGGTCAAT	GTA	TGTAGTGAA	ATATGTGGT	ATTAA	CCATGGGTTA	TGCCTATAGTT
10P	ATTTATTATGGTCAAT	GTA	TGTAGTGAA	ATATGTGGT	ATTAA	CCATGGGTTA	TGCCTATAGTT
13H	ATTTATTATGGTCAAT	GTA	TGTAGTGAA	ATATGTGGT	ATTAA	CCATGGGTTA	TGCCTATAGTT
13P	ATTTATTATGGTCAAT	GTA	TGTAGTGAA	ATATGTGGT	ATTAA	CCATGGGTTA	TGCCTATAGTT
14H	ATTTATTATGGTCAAT	GTA	TGTAGTGAA	ATATGTGGT	ATTAA	CCATGGGTTA	TGCCTATAGTT
15H	ATTTATTATGGTCAAT	GTA	TGTAGTGAA	ATATGTGGT	ATTAA	CCATGGGTTA	TGCCTATAGTT

	121	130	140	150	160	170	180
2H	GTTGAAGCTATTACATTACCAATTACATAAAATTGAATATCTAATAAAAATAAACGAATAA						
3H	GTTGAAGCTATTACATTACCAATTACATAAAATTGAATATCTAATAAAAATAAACGAATAA						
4H	GTTGAAGCTATTACATTACCAATTACATAAAATTGAATATCTAATAAAAATAAACGAATAA						
5H	GTTGAAGCTATTACATTACCAATTACATAAAATTGAATATCTAATAAAAATAAACGAATAA						
5P	GTTGAAGCTATTACATTACCAATTACATAAAATTGAATATCTAATAAAAATAAACGAATAA						
6H	GTTGAAGCTATTACATTACCAATTACATAAAATTGAATATCTAATAAAAATAAACGAATAA						
6P	GTTGAAGCTATTACATTACCAATTACATAAAATTGAATATCTAATAAAAATAAACGAATAA						
8P	GTTGAAGCTATTACATTACCAATTACATAAAATTGAATATCTAATAAAAATAAACGAATAA						
8H	GTTGAAGCTATTACATTACCAATTACATAAAATTGAATATCTAATAAAAATAAACGAATAA						
9H	GTTGAAGCTATTACATTACCAATTACATAAAATTGAATATCTAATAAAAATAAACGAATAA						
10P	GTTGAAGCTATTACATTACCAATTACATAAAATTGAATATCTAATAAAAATAAACGAATAA						
13H	GTTGAAGCTATTACATTACCAATTACATAAAATTGAATATCTAATAAAAATAAACGAATAA						
13P	GTTGAAGCTATTACATTACCAATTACATAAAATTGAATATCTAATAAAAATAAACGAATAA						
14H	GTTGAAGCTATTACATTACCAATTACATAAAATTGAATATCTAATAAAAATAAACGAATAA						
15H	GTTGAAGCTATTACATTACCAATTACATAAAATTGAATATCTAATAAAAATAAACGAATAA						
	181	190	200	210	220	230	240
2H	TTTATGAAACTTCTTATTTCATAAATTTTTATTGGTTATTATTTTTAGTACTATT						
3H	TTTATGAAACTTCTTATTTCATAAATTTTTATTGGTTATTATTTTTAGTACTATT						
4H	TTTATGAAACTTCTTATTTCATAAATTTTTAWTGGTTATTATTTTTAGTACTATT						
5H	TTTATGAAACTTCTTATTTCATAAATTTTTATTGGTTATTATTTTTAGTACTATT						
5P	TTTATGAAACTTCTTATTTCATAAATTTTTATTGGTTATTATTTTTAGTACTATT						
6H	TTTATGAAACTTCTTATTTCATAAATTTTTATTGGTTATTATTTTTAGTACTATT						
6P	TTTATGAAACTTCTTATTTCATAAATTTTTATTGGTTATTATTTTTAGTACTATT						
8H	TTTATGAAACTTCTTATTTCATAAATTTTTATTGGTTATTATTTTTAGTACTATT						
8P	TTTATGAAACTTCTTATTTCATAAATTTTTATTGGTTATTATTTTTAGTACTATT						
9H	TTTATGAAACTTCTTATTTCATAAATTTTTATTGGTTATTATTTTTAGTACTATT						
10P	TTTATGAAACTTCTTATTTCATAAATTTTTATTGGTTATTATTTTTAGTACTATT						
13H	TTTATGAAACTTCTTATTTCATAAATTTTTATTGGTTATTATTTTTAGTACTATT						
13P	TTTATGAAACTTCTTATTTCATAAATTTTTATTGGTTATTATTTTTAGTACTATT						
14H	TTTATGAAACTTCTTATTTCATAAATTTTTATTGGTTATTATTTTTAGTACTATT						
15H	TTTATGAAACTTCTTATTTCATAAATTTTTATTGGTTATTATTTTTAGTACTATT						

	241	250	260	270	280	290	300
2H	TAATTTAAATT TATAAATAATAATT TATTAAATT TTTTATAAGTTTAAAAAA						
3H	TAATTTAAATT TATAAATAATAATT TATTAAATT TTTTATAAGTTTAAAAAA						
4H	TAATTTAAATT TATAAATAATAATT TATTAAATT TTTTATAAGTTTAAAAAA						
5H	TAATTTAAATT TATAAATAATAATT TATTAAATT TTTTATAAGTTTAAAAAA						
5P	TAATTTAAATT TATAAATAATAATT TATTAAATT TTTTATAAGTTTAAAAAA						
6P	TAATTTAAATT TTTTATAAATAATAATT TATTAAATT TTTTATAAGTTTAAAAAA						
6H	TAATTTAAATT TTTTATAAATAATAATT TATTAAATT TTTTATAAGTTTAAAAAA						
8H	TAATTTAAATT TTTTATAAATAATAATT TATTAAATT TTTTATAAGTTTAAAAAA						
8P	TAATTTAAATT TTTTATAAATAATAATT TATTAAATT TTTTATAAGTTTAAAAAA						
9H	TAATTTAAATT TTTTATAAATAATAATT TATTAAATT TTTTATAAGTTTAAAAAA						
10P	TAATTTAAATT TTTTATAAATAATAATT TATTAAATT TTTTATAAGTTTAAAAAA						
13H	TAATTTAAATT TTTTATAAATAATAATT TATTAAATT TTTTATAAGTTTAAAAAA						
13P	TAATTTAAATT TTTTATAAATAATAATT TATTAAATT TTTTATAAGTTTAAAAAA						
14H	TAATTTAAATT TTTTATAAATAATAATT TATTAAATT TTTTATAAGTTTAAAAAA						
15H	TAATTTAAATT TTTTATAAATAATAATT TATTAAATT TTTTATAAGTTTAAAAAA						

	301	310	320	330	340	350
2H	AAAAAGTTAACACATGTTTATCCGAATTCTAAATATATTCAAAGACATCC					
3H	AAAAAGTTAACACATGTTTATCCGAATTCTAAATATATTCAAAGACATCC					
4H	AAAAAGTTAACACATGTTTATCCGAATTCTAAATATATTCAAAGACATCC					
5H	AAAAAGTTAACACATGTTTATCCGAATTCTAAATATATTCAAAGACATCC					
5P	AAAAAGTTAACACATGTTTATCCGAATTCTAAATATATTCAAAGACATCC					
6P	AAAAAGTTAACACATGTTTATCCGAATTCTAAATATATTCAAAGACATCC					
6H	AAAAAGTTAACACATGTTTATCCGAATTCTAAATATATTCAAAGACATCC					
8H	AAAAAGTTAACACATGTTTATCCGAATTCTAAATATATTCAAAGACATCC					
8P	AAAAAGTTAACACATGTTTATCCGAATTCTAAATATATTCAAAGACATCC					
9H	AAAAAGTTAACACATGTTTATCCGAATTCTAAATATATTCAAAGACATCC					
10P	AAAAAGTTAACACATGTTTATCCGAATTCTAAATATATTCAAAGACATCC					
13H	AAAAAGTTAACACATGTTTATCCGAATTCTAAATATATTCAAAGACATCC					
13P	AAAAAGTTAACACATGTTTATCCGAATTCTAAATATATTCAAAGACATCC					
14H	AAAAAGTTAACACATGTTTATCCGAATTCTAAATATATTCAAAGACATCC					
15H	AAAAAGTTAACACATGTTTATCCGAATTCTAAATATATTCAAAGACATCC					

Appendix 4

Table A4.1. Downloaded sequences from Genbank used in all phylogenetic analysis. Species name and Genbank Accession Number is also shown.

Species	Genbank Accession No.
<i>Acanthopeltis longiramulosa</i> Lee et Kim	HM629884
<i>Adelophycus corneus</i> (J. Agardh) Kraft	AF515285
<i>Ahnfeltia fastigiata</i> (Endlicher) Makienko	DQ343668
<i>Asteromenia peltata</i> Huisman et Millar	AY437710
<i>Asparagopsis taxiformis</i> (Delile) Trevisan de Saint-Léon	AY772727
<i>Atractophora hypnoides</i> Crouan et Crouan	AY772728
<i>Audouinella asparagopsis</i> (Chemin) Dixon	AF079795
<i>Audouinella caespitosa</i> (J. Agardh) Dixon	AF079787
<i>Audouinella dasyae</i> (Collins) Woelkerling	L26181
<i>Balbiania investiens</i> (Lenormand) Sirodot	AF132294
<i>Ballia callitricha</i> (C. Agardh) Kützing	AF236791
<i>Balliosis prieurii</i> (Kützing) Saunders et Necchi	AF419245
<i>Batrachospermum brasiliense</i> Necchi	FJ386470
<i>Callophyllis rangiferina</i> (Brown) Womersley	U33123
<i>Caloglossa stipitata</i> Post	AF522247
<i>Capreolia implexa</i> Guiry et Womersley	U60344
<i>Champia parvula</i> (C. Agardh) Harvey	EF613310
<i>Chondrus yendoi</i> Yamada et Mikami	DQ316988
<i>Crassitegula walsinghamii</i> Schneider, Lane et Saunders	AY964057
<i>Cumagloia andersonii</i> (Farlow) Setchell et Gardner	DQ343669
<i>Curdiea flabellata</i> Chapman	L26207
<i>Dilsea californica</i> (J. Agardh) Kuntze	U33126
<i>Dilsea integra</i> (Kjellman) Rosenvinge	FJ848966
<i>Endocladia muricata</i> (Endlicher) J. Agardh	U33127
<i>Erythrymenia minuta</i> Kylin	AF085272
<i>Farlowia mollis</i> (Harvey et Bailey) Farlow et Setchell	GU176299
<i>Farlowia mollis</i>	U33129
<i>Fauchea repens</i> (C. Agardh) Montagne et Bory de Saint-Vincent	AF085267
<i>Furcellaria lumbricalis</i> (Hudson) Lamouroux	GQ406350
<i>Galaxaura marginata</i> (Ellis et Solander) Lamouroux	AF006090
<i>Gainia mollis</i> Moe	GQ406351
<i>Gelidiella acerosa</i> (Forsskål) Feldmann et Hamel	AF039551
<i>Gelidiopsis intricata</i> (C. Agardh) Vickers	EF033594
<i>Gelidium caulacanthicum</i> J. Agardh	AF039544
<i>Gelidium latifolium</i> Bornet	Y11953
<i>Gelidium pusillum</i> (Stackhouse) Le Jolis	AF039542
<i>Gloiopektis capillaris</i> Suringar	AY437680

<i>Gloiopelets furcata</i> (Postels et Ruprecht) J. Agardh	U33130
<i>Gracilaria cliftonii</i> Withel, Millar et Kraft	AY617146
<i>Gracilaria gracilis</i> (Stackhouse) Steentoft	EU937742
<i>Gracilaria lemameiformis</i> (Bory de Saint-Vincent) Greville	JN609255
<i>Gracilaria salicornia</i> (C. Agardh) Dawson	EF033615
<i>Gracilaria verrucosa</i> (Hudson) Papenfuss	Y11508
<i>Gracilariopsis lemameiformis</i> (Bory de Saint-Vincent) Dawson, Acleto et Foldvik	L26214
<i>Gratelouphia intestinalis</i> (Harvey) Setchell et Parkinson	AY437702
<i>Haliptilon roseum</i> (Lamarck) Garbary et Johansen	EF628229
<i>Hildenbrandia angolensis</i> Welwitsch et West	AF207833
<i>Hildenbrandia rubra</i> (Somerfelt) Meneghini	L19345
<i>Hyalosiphonia caespitosa</i> Okamura	JN403057
<i>Hydrolithon pachydermum</i> (Foslie) Bailey, Gabel et Freshwater	AY234235
<i>Hypnea</i> sp.	EU240869
<i>Inkyuleea mariana</i> (Harvey) Choi, Kraft et Saunders	AF236792
<i>Isabbottia ovalifolia</i> (Kylin) Balakrishnan	EF033590
<i>Kumanoa ambigua</i> (Montagne) Entwistle	FJ386481
<i>Lesleigha</i> sp.	AY437707
<i>Lesleigha</i> sp.	JN602193
<i>Lithophyllum</i> sp.	AY083172
<i>Lithothamnion glaciale</i> Kjellman	U60738
<i>Mastophoropsis canaliculata</i> (Harvey) Woelkerling	U62118
<i>Meiodiscus spetsbergensis</i> (Kjellman) Saunders et McLachlan	U23814
<i>Melanthalia obtusata</i> (Labillardière) J. Agardh	DQ343692
<i>Melanthalia obtusata</i>	L26215
<i>Nemalionopsis shawii</i> Howard et Parker	AF506272
<i>Neodilsea borealis</i> (Abbott) Lindstrom	EF033610
<i>Neodilsea natashae</i> Lindstrom	JF928825
<i>Nizymenia australis</i> Sonder	U09616
<i>Palmaria palmata</i> (Linnaeus) Weber et Mohr	Z14142
<i>Peyssonnelia</i> sp.	AY437688
<i>Peyssonnelia rubra</i> (Greville) J. Agardh	DQ629016
<i>Pihella liagoraciphila</i> Huisman, Sherwood et Abbott	AY301992
<i>Plocamium angustum</i> (J. Agarth) Hooker et Harvey	U09620
<i>Polyopes constrictus</i> (Turner) J. Agardh	AY437705
<i>Pyropia tenera</i> Kjellman	AB101442
<i>Predaea kraftiana</i> Millar et Guiry	EF033618
<i>Predaea kraftiana</i>	AF515296
<i>Pterocladia lucida</i> (Brown) J. Agardh	AF419118
<i>Pterocladia lucida</i>	U60349
<i>Pterocladia bartlettii</i> (Taylor) Santelices	EF191192

<i>Pterocladiella caerulescens</i> (Kützing) Santelices et Hommersand	HQ412472
<i>Pterocladiella caerulescens</i>	HQ412473
<i>Pterocladiella caerulescens</i>	HQ412476
<i>Pterocladiella caerulescens</i>	HQ412477
<i>Pterocladiella caerulescens</i>	HQ412482
<i>Pterocladiella capillacea</i> (Gmelin) Santelices et Hommersand	HM629885
<i>Pterocladiella capillacea</i>	HQ422696
<i>Pterocladiella capillacea</i>	AF039549
<i>Pterocladiella psammophila</i> Tronchin et Freshwater	HQ412486
<i>Ptilonia australasica</i> Harvey	AY437646
<i>Ptilophora pinnatifida</i> J. Agardh	U60345
<i>Renouxia</i> sp.	EF033584
<i>Rhodachlyia madagascarensis</i> West, Scott, West, Karsten, Clayden et Saunders	EU262260
<i>Rhodymenia stenoglossa</i> J. Agardh	AF515303
<i>Sphaerococcus coronopifolius</i> Stackhouse	FJ848974
<i>Thorea</i> sp.	AF42053
<i>Thorea violacea</i> Bory de Saint-Vincent	AF342744
<i>Titanophora weberae</i> Børgesen	AF515301
<i>Trematocarpus fragilis</i> (C. Agardh) De Toni	AY437696
<i>Tsengia comosa</i> (Harvey) Womersley et Kraft	AF515303
<i>Wetherbeella australica</i> (Womersley et Kraft) Saunders et Kraft	AF515290

Appendix 5

RuBisCO spacer alignment of *Pterocladia lucida* (45H, 6214) and
Pterocladiophila hemisphaerica (45P, G215)

	1	10	20	30	40	50	60
45H	CAAACAGCTT	AGACTTG	GAAAGATATA	ACATTCA	ATTACACTT	CTACAGATA	CAGCT
45P	-----						
G214	CAAACAGCTT	AGACTTG	GAAAGATATA	ACATTCA	ATTACACTT	CTACAGATA	CAGCT
G215	CAAACAGCTT	AGACTTG	GAAAGATATA	ACATTCA	ATTACACTT	CTACAGATA	CAGCT
	61	70	80	90	100	110	120
45H	GACTTCGTAGAA	ACTCCA	ACAGCAA	ACGTATAA	ATAATT	TATATCGTAC	GCTTATAAAAG
45P	-----						
G214	GACTTCGTAGAA	ACTCCA	ACAGCAA	ACGTATAA	ATAATT	TATATCGTAC	GCTTATAAAAG
G215	GACTTCGTAGAA	ACTCCA	ACAGCAA	ACGTATAA	ATAATT	TATATCGTAC	GCTTATAAAAG
	121	130	140	150	160	170	180
45H	CTTTTCTTAAA	ATTCCA	ACATCTG	CTTAATATAA	ATAAGGAGT	ATATCATAGT	GAGA
45P	-----	RCRTCYGCY	TAMATATARAT	WAGGAGT	ATATCATADKGAGA		
G214	CTTTTCTTAAA	ATTCCA	ACATCTG	CTTAATATAA	ATAAGGAGT	ATATCATAGT	GAGA
G215	CTTTTCTTAAA	ATTCCA	ACATCTG	CTTAATATAA	ATAAGGAGT	ATATCATAGT	GAGA
	181	190	200	210	220	230	240
45H	CTAACACAAGGA	ACTTCTCG	TTCCCTAC	CTGATTAA	CTGACGA	ACAAATTAA	ATAAACAG
45P	CTAACACAAGGA	ACTTYTCG	TTCCCTAC	CTGATTAA	CTGACGA	ACAAATTAA	ATAAACWG
G214	CTAACACAAGGA	ACTTCTCG	TTCCCTAC	CTGATTAA	CTGACGA	ACAAATTAA	ATAAACAG
G215	CTAACACAAGGA	ACTTCTCG	TTCCCTAC	CTGATTAA	CTGACGA	ACAAATTAA	ATAAACAG
	241	250	260	270	280	290	300
45H	ATTGCATACG	CAGTATCT	CAAATTGGT	CAATTAA	ATATAGA	ACATA	CAGAAGATCCTCAT
45P	ATTGCATACG	CAGNATCT	CAAATTGGY	CAATTAA	ATATAGA	ACATA	CAGAAGATCCTCAT
G214	ATTGCATACG	CAGTATCT	CAAATTGGT	CAATTAA	ATATAGA	ACATA	CAGAAGATCCTCAT
G215	ATTGCATACG	CAGTATCT	CAAATTGGT	CAATTAA	ATATAGA	ACATA	CAGAAGATCCTCAT

	301	310
45H	CCACGTAATAATTATT	
45P	CCACGTAATAATTATT	
G214	CCACGCAATAATTATT	
G215	CCACGCAATAATTATT	

Appendix 6

Cox1 alignment of *Acanthopeltis longiramulosa* (HM629884), *Pterocladiella caeruleescens* (HQ412472, HQ412473, HQ412476, HQ412477, HQ412482), *Pterocladiella capillacea* (HM629885, HQ422696), *Pterocladiella psammophila* (HQ412486), *Pterocladia lucida* (45H, 6214) and *Pterocladiophila hemisphaerica* (45P, 45P.2, G215, G215.2)

	1	10	20	30	40	50	60
45P	TATAATGTTTAATAACTGCACATGCATTGGATGATATTGGTTATGCCAGTT						
45P.2	TATAATGTTTAATAACTGCACATGCATTGGATGATATTGGTTATGCCAGTT						
46H	TACAATGTCTTGATTACTGCCATGCATTTAATGATTTTATGGTTATGCCTGTT						
G214	TACAATGTCTTGATTACCGCTCATGCATTTAATGATTTTATGGTTATGCCTGTT						
G215	TATAATGTTTAATAACTGCACATGCATTGGATGATATTGGTTATGCCAGTT						
G215.2	TATAATGTTTAATAACTGCACATGCATTGGATGATATTGGTTATGCCAGTT						
HM629884	TACAATGTATTAAATTACAGCTACGCTTTGGATGATTTTATGGTAATGCCGGTT						
HM629885	TATAATGTTTAATTACTGCTCATGCATTTAATGATATTTCATGGTATGCCTGTA						
HQ412472	TATAATGTCTTGATTACTGCACATGCATTGGATGATATTGGTTATGCCTGTT						
HQ412473	TATAATGTCTTGATTACTGCACATGCATTGGATGATATTGGTTATGCCTGTT						
HQ412476	TATAATGTTTAATTACTGCACATGCATTGGATGATATTGGTTATGCCTGTT						
HQ412477	TATAATGTTTAATTACTGCACATGCATTGGATGATATTGGTTATGCCTGTT						
HQ412482	TATAATGTTTGATTACTGCACATGCATTGGATGATATTGGTTATGCCTGTT						
HQ412486	TATAACGTTTAATTACTGCACACGCATTGGATGATATTTCATGGTATGCCAGTT						
HQ422696	TATAATGTTTAATTACAGCCCACGCATTGGATGATATTGGTTATGCCCGTT						

	61	70	80	90	100	110	120
45P	TTAATAGGTGGATTGGTAATTGGTAATTCCAATTATGATAGGAAGTCCAGATATGGCG						
45P.2	TTAATAGGTGGATTGGTAATTGGTAATTCCAATTATGATAGGAAGTCCAGATATGGCG						
46H	TTAATTGGTGGTTGGTAATTGGTAGTACCCATAATGATTGGTAGTCCTGATATGGCT						
G214	TTAATTGGTGGTTGGTAATTGGTAGTACCCATAATGATCGGTAGCCTGATATGGCT						
G215	TTAATAGGTGGATTGGTAATTGGTAATTCCAATTATGATAGGAAGTCCAGATATGGCG						
G215.2	TTAATAGGTGGATTGGTAATTGGTAATTCCAATTATGATAGGAAGTCCAGATATGGCG						
HM629884	TTAATAGGAGGTTGGAAATTGGTAGTACCAATAATGATAGGTAGTCGGATATGGCT						
HM629885	TTAATTGGGGATTGGAAATTGATTAGTACCCATAATGATAGGTAGTCCTGATATGGCA						
HQ412472	CTTATAGGGGCTTGGAAATTGATTAGTCCTATTATGATAGGTAGCCTGATATGGCA						
HQ412473	CTTATAGGAGGCTTGGAAATTGATTAGTCCTATTATGATAGGTAGTCCTGATATGGCA						
HQ412476	CTTATAGGAGGTTGGAAATTGATTAGTCCTATTATGATAGGTAGTCCTGATATGGCA						
HQ412477	CTTATAGGAGGTTGGAAATTGATTAGTCCTATTATGATAGGTAGTCCTGATATGGCA						
HQ412482	CTTATAGGAGGTTGGAAATTGATTAGTCCTATTATGATAGGTAGTCCTGATATGGCA						
HQ412486	CTTATAGGTGGTTGGAAATTGATTGGTCCTATTATGATTGGTAGTCCTGATATGGCG						
HQ422696	ATGATTGGGGTTCGGCAATTGATTAGTCCTATTATGATAGGTAGCCTGATATGGCT						

	121	130	140	150	160	170	180
45P	TTTCCACGACTAAATAATTTCATTTGATTATTGCCTCCTTCTTAGGATTATTATTA						
45P.2	TTTCCACGACTAAATAATTTCATTTGATTATTGCCTCCTTCTTAGGATTATTATTA						
46H	TTCCCTCGTTAATAAACATCTCATTGATTACTCCACCTCTCTGTCTTTATTA						
G214	TTCCCTCGTTAATAAACGTTCATTGATTACTCCACCTCTCTGTCTTTATTA						
G215	TTTCCACGACTAAATAATTTCATTTGATTATTGCCTCCTTCTTAGGATTATTATTA						
G215.2	TTTCCACGACTAAATAATTTCATTTGATTATTGCCTCCTTCTTAGGATTATTATTA						
HM629884	TTTCCTCGTTGAATAACATCTCCTTTGATTACTTCCCCCTCTTATGTTACTTTA						
HM629885	TTCCCCCGTCTAAATAATATTCGTTGGTATTACCTCCATCGTTATGTTATTGTTA						
HQ412472	TTTCCTCGTTGAATAATATTCATTGATTACTGCCACCATTATGTTACTATTG						
HQ412473	TTTCCTCGTTGAATAATATTCATTGATTACTGCCACCATTATGTTACTATTG						
HQ412476	TTTCCTCGTTGAATAATATTCATTGATTACTGCCACCATTATGTTACTATTG						
HQ412477	TTTCCTCGTTAATAATATTCATTGATTATTGCCGCCATTATGTTACTATTAA						
HQ412482	TTTCCTCGTTAATAATATTCATTGATTATTGCCGCCATTATGTTACTATTAA						
HQ412486	TTTCCTCGTTGAATAACATTTCATTGATTACTGCCACCATTATGTTACTATTG						
HQ422696	TTTCCAAGACTAAATAATATTCCTTTGATTATTACCTCCATCATTGTGTTATTATTA						
	181	190	200	210	220	230	240
45P	CTTCCCGCATTTATTGAAGTAGGAGTAGGGACCGGATGAACGTATATCCGCCATTAAGT						
45P.2	CTTCCCGCATTTATTGAAGTAGGAGTAGGGACCGGATGAACGTATATCCGCCATTAAGT						
46H	ACTTCTGCATTAGTAGAAGTTGGGTAGGAACAGGGTGAACAGTTATCCTCCTTAAGT						
G214	ACTTCTGCATTAGTAGAAGTTGGGTAGGGACAGGGTGAACAGTTACCCCTCCTTAAGT						
G215	CTTCCCGATTATTGAAGTAGGAGTAGGGACCGGATGAACGTATATCCGCCATTAAGT						
G215.2	CTTCCCGATTATTGAAGTAGGAGTAGGGACCGGATGAACGTATATCCGCCATTAAGT						
HM629884	ACTTCGGCAATTGTAGAAGTAGGTGTTGGTACTGGTGAACGTGTATCCACCTCTAAGT						
HM629885	ACATCTGCATTGGTTGAAGTAGGTGTTGGAACCTGGTGAACAGTTATCCACCCCTTAAGT						
HQ412472	ACATCTGCATTGGTTGAAGTAGGTGTTGGAACGGGTTGAACGTCTACCCCCCTTAAGC						
HQ412473	ACATCTGCATTAGTTGAAGTAGGTGTTGGAACGGGTTGAACGTCTACCCCCCTTAAGC						
HQ412476	ACATCTGCATTAGTTGAAGTAGGTGTTGGAACGGGTTGAACGTCTACCCCCCTTAAGC						
HQ412477	ACATCTGCATTAGTTGAAGTAGGTGTTGGAACGGGTTGAACGTCTACCCCCCTTAAGC						
HQ412482	ACATCTGCATTAGTTGAAGTAGGTGTTGGAACGGGTTGAACGTCTACCCCCCTTAAGC						
HQ412486	ACATCTGCATTAGTTGAAGTAGGTGTTGGAACGGGTTGAACGTCTACCCCCCTTAAGC						
HQ422696	GCTTCTGCTGTAGTAGAAGTTGGTAGGTACAGGATGAACGTATACCCACCATTGAGT						
	241	250	260	270	280	290	300
45P	GCTATTCAAAGTCATTCAAGCGGATCAGTCGATTTAGCTATATTTAGTTGCATTTATCA						
45P2	GCTATTCAAAGTCATTCAAGCGGATCAGTCGATTTAGCTATATTTAGTTGCATTTATCA						
46H	TCAATTCAAAGCCATTCTGGAGGAGCCGTTGATTTAGCTATATTTAGTTACACATCTCA						
G214	TCAATTCAAAGTCATTCTGGAGGAGCTATTGATTTAGCTATATTTAGCTTACACATCTCA						
G215	GCTATTCAAAGTCATTCAAGCGGATCAGTCGATTTAGCTATATTTAGTTGCATTTATCA						
G215.2	GCTATTCAAAGTCATTCAAGCGGATCAGTCGATTTAGCTATATTTAGTTGCATTTATCA						
HM629884	TCGATTCAAAGTCATTCAAGCGGATCAGTCGATTTAGCTATATTTAGTTGCATTTATCA						
HM629885	TCAATTCAAAGCCATTCTGGGGGCCGTTGATTTAGCTATATTTAGTTGCATATTTCG						
HQ412472	TCGATTCAAAGTCATTCAAGCGGATCAGTCGATTTAGCTATATTTAGTTGCATATTCTCA						
HQ412473	TCAATTCAAAGTCATTCAAGCGGATCAGTCGATTTAGCTATATTTAGTTGCATATTCTCA						
HQ412476	TCAATTCAAAGTCATTCAAGCGGATCAGTCGATTTAGCTATATTTAGTTGCATATTCTCA						
HQ412477	TCAATTCAAAGTCATTCAAGCGGATCAGTCGATTTAGCTATATTTAGTTGCATATTCTCA						
HQ412482	TCAATTCAAAGTCATTCAAGCGGATCAGTCGATTTAGCTATATTTAGTTGCATATTCTCA						
HQ412486	TCAATTCAAAGCCACTCAGGTGGGCCGTTGATTTAGCTATATTTAGCTTACACATATCTCA						
HQ422696	TCAATTCAAAGTCATTCTGGAGGTGCCGTAGATTAGCTATATTTAGCTTACACATATCTCA						

	301	310	320	330	340	350	360
45P	GGAATTCTCAATTAGGAGCTATTAATTATCACACGATTAAATATGAGAAAT						
45P.2	GGAATTCTCAATTAGGAGCTATTAATTATCACACGATTAAATATGAGAAAT						
46H	GGAGCGTCATCAATTCTAGGTGCAGTTAATTATCACACAATTAAATATGCGTAAT						
G214	GGAGCGTCATCAATTCTAGGCAGTTAATTATCACACAATTAAATATGCGTAAT						
G215	GGAATTCTCAATTAGGAGCTATTAATTATCACACGATTAAATATGAGAAAT						
G215.2	GGAATTCTCAATTAGGAGCTATTAATTATCACACGATTAAATATGAGAAAT						
HM629884	GGGGCTCCTCAATCCTTGGTGCAATTAAATTATTCAACTATTGAAATATGCGAAC						
HM629885	GGAGCTTCATCAATTAGGTGCCAAATTATTCAACTATTCTAACATGCGTAAT						
HQ412472	GGTGCTTCATCTATTAGGTGCTGTTAATTATCTACTATTGAAATATGCGTAAT						
HQ412473	GGTGCTTCATCTATTAGGTGCTGTTAATTATCTACTATTGAAATATGCGTAAT						
HQ412476	GGTGCTTCATCTATTAGGAGCTGTTAATTATCTACTATTGAAATATGCGTAAT						
HQ412477	GGTGCTTCATCTATTAGGTGCTGTTAATTATCTACTATTGAAATATGCGTAAT						
HQ412482	GGTGCTTCATCTATTAGGTGCTGTTAATTATCTACTATTGAAATATGCGTAAT						
HQ412486	GGTGCTTCATCTATTAGGTGCCAAATTATTCAACTATTAAATATGCGTAAT						
HQ422696	GGTGCTTCATCTATTAGGTGCCATCAATTATCACACTATTAAATATGCGTAAT						
	361	370	380	390	400	410	420
45P	CCAGGACAAACAATGTATCGAATTCCCTTATTGTTGATCAATTGTAACAGCATT						
45P.2	CCAGGACAAACAATGTATCGAATTCCCTTATTGTTGATCAATTGTAACAGCATT						
46H	CCAGGACAGACAATGTACAGGATGCCCTTTGTCTGATCAATTGTAACAGCTTT						
G214	CCAGGACAGACAATGTAGGATGCCCTTTGTTGATCGATTGTAACAGCTTT						
G215	CCAGGACAAACAATGTATCGAATTCCCTTATTGTTGATCAATTGTAACAGCATT						
G215.2	CCAGGACAAACAATGTATCGAATTCCCTTATTGTTGATCAATTGTAACAGCATT						
HM629884	CCTGGCAAACAATGTACCGAATGCCATTATTGTTGATCAATTGTAACAGCTTT						
HM629885	CCTGGCAAACAATGTAGAATGCCATTATTGTTGATCAATTGTAACAGCTTT						
HQ412472	CCGGGTCAAACCATGTACAGAATGCCATTATTGTTGATCGATTGTAACAGCTTT						
HQ412473	CCGGGTCAAACCATGTACAGAATGCCATTATTGTTGATCGATTGTAACAGCTTT						
HQ412476	CCAGGTCAAACCATGTAGAATGCCATTATTGTTGATCAATTGTAACAGCTTT						
HQ412477	CCAGGTCAAACATGTACAGAATGCCATTATTGTTGATCAATTGTAACAGCTTT						
HQ412482	CCAGGTCAAACATGTACAGAATGCCATTATTGTTGATCAATTGTAACAGCTTT						
HQ412486	CCCGGTCAAACATGTACAGAATGCCACTATTGTTGATCAATTGTAACAGCTTT						
HQ422696	CCAGGCCAAATATGTACCAAATGCCACTATTGTTGGTCAATTGTAACAGCATT						
	421	430	440	450	460	470	480
45P	TTATTATTAGCTGTCCTGTATTAGCAGGTGCTATTACAATGCTTTAACTGATCGT						
45P.2	TTATTATTAGCTGTCCTGTATTAGCAGGTGCTATTACAATGCTTTAACTGATCGT						
46H	TTACTTTATTAGCGGTCCCCGTTAGCAGGAGCTATCACAAATTGTTACTAACAGATCGA						
G214	TTACTCTATTAGCGGTCCCCGTTAGCAGGAGCTATCACAAATTGTTACTAACAGATCGA						
G215	TTATTATTAGCTGTCCTGTATTAGCAGGTGCTATTACAATGCTTTAACTGATCGT						
G215.2	TTATTATTAGCTGTCCTGTATTAGCAGGTGCTATTACAATGCTTTAACTGATCGT						
HM629884	TTGTTATTAGCTGTACCAGTTAGCTGGAGCTATTACAATTGTTACTAACAGATCGT						
HM629885	TTACTACTATTAGCAGTACCAGTTAGCTGGTGCTATTACTATGTTACTAACGATAGA						
HQ412472	CTGCTATTAGCAGTACCGGTTGGCTGGCAATCACTATGTTACTAACGATAGG						
HQ412473	CTGCTATTAGCAGTACCGAGTTAGCTGGTGCAATCACTATGTTACTAACGATAGA						
HQ412476	TTATTATTAGCAGTACCGAGTTAGCTGGTGCAATTACTATGTTACTAACGATAGG						
HQ412477	CTATTATTAGCGGTACCACTAGTTAGCTGGTGCAATTACTATGTTACTAACGATAGG						
HQ412482	CTATTATTAGCGGTACCACTAGTTAGCTGGTGCAATTACTATGTTACTAACGATAGG						
HQ412486	TTACTACTATTGGCGGTACCACTAGCTGGCGCAATTACTATGTTACTAACGATAGA						
HQ422696	TTATTACTATTAGCTGTACCACTAGCAGGTGCTATTACAATGCTTTAACAGATCGT						

	481	490	500	510	520	530	540
45P	AATTTAATACAGCATTGGTGGAGATCCTGTTTATATCAACAT						
45P.2	AATTTAATACAGCATTGGTGGAGATCCTGTTTATATCAACAT						
46H	AATTTAATACCTCATTGGTGGAGATCCTGACTTTATCAACAT						
G214	AATTTAATACCTCATTGGTGGAGATCCTGACTTTACCAACAT						
G215	AATTTAATACAGCATTGGTGGAGATCCTGTTTATATCAACAT						
G215.2	AATTTAATACAGCATTGGTGGAGATCCTGTTTATATCAACAT						
HM629884	AATTTAATACATCATTTCGACCCAGCTGGTGGAGGTGATCCCGTACTGTATCAGCAT						
HM629885	AATTTAATACATCGTTCTTGACCCCGCAGGTGGAGGTGATCCAGTACTATATCAGCAT						
HQ412472	AATTTAATACATCATTGGTGGAGGTGGTGATCCAGCAGGAGGTGGTACTTTATCAACAC						
HQ412473	AATTTAATACATCATTGGTGGAGGTGGTGATCCAGCAGGAGGTGGTACTTTATCAACAC						
HQ412476	AATTTAATACATCATTGGTGGAGGTGGTGATCCGGTACTTTATCAACAC						
HQ412477	AATTTAATACATCATTGGTGGAGGTGGTGATCCGGTACTTTACCAACAC						
HQ412482	AATTTAATACATCATTGGTGGAGGTGGTGATCCGGTACTTTACCAACAC						
HQ412486	AATTTAATACATCATTGGTGGAGGTGGTGATCCGGTACTTTATCAACAT						
HQ422696	AATTTAATACATCTTTT-----						

	540
45P	TTA
45P.2	TTA
46H	TTA
G214	TTA
G215	TTA
G215.2	TTA
HM629884	TTG
HM629885	TTA
HQ412472	TTA
HQ412473	TTA
HQ412476	TTA
HQ412477	TTA
HQ412482	TTA
HQ412486	TTA
HQ422696	---

Appendix 7

LSU alignment of *Pterocladia lucida* (45H), *Pterocladophila hemisphaerica* (45P) and downloaded Genbank sequences: *Caloglossa stipitata* (AF522247), *Dilsea integra* (FJ848966), *Farlowia mollis* (GU176299), *Furcellaria lumbricalis* (GQ406350), *Gainia mollis* (GQ406351), *Gelidiella acerosa* (AF039551), *Gelidium caulacanthum* (AF039544), *Gelidium pusillum* (AF039542), *Gracilaria lemaneiformis* (JN609255), *Gracilaria salicornia* (EF033615), *Gracilaria verrucosa* (Y11508), *Lesleigha* sp. (JN602193), *Hyalosiphonia caespitosa* (JN403057), *Melanthalia obtusata* (DQ343692), *Neodilsea borealis* (EF033610), *Neodilsea natashae* (JF928825), *Predaea kraftiana* (EF033618), *Pterocladia lucida* (AF419118), *Pterocladella capillacea* (AF039549), *Sphaerococcus coronopifolius* (FJ848974).

	1	10	20	30	40	50	60
45H	-----			GGGCAAAGGGATCCGACTGTTAATT			
45P	-----			-----	ATCCGACTG-TTAATT		
AF039542	CGCCCACAGCATGCAGCAA	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT			
AF039544	CGCCCACAGCATGCAGCAA	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT			
AF039549	CGCCCACAGCATGCAGCAA	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT			
AF039551	CGCCCACAGCATGCAGCAA	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT			
AF419118	CGCCCACAGCATGCAGCAA	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT			
AF522247	CGCCCAACA	CATGCAGCAA	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT		
DQ343692	CGCCCACAGAATGCAGC	GA	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT		
EF033610	CGCCCACAGCATGCAGCAG	A	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT		
EF033615	CGCTCACAGCATGCAGCGA	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT			
EF033618	CGCCCACAGCATGCAGCAA	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT			
FJ848966	CGCCCACAGCATGCAGCAG	A	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT		
FJ848974	CGCCCACAGCATGCAGCAG	A	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT		
GQ406350	CGCCCACAGCATGCAGCAG	A	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT		
GQ406351	CGCCCACAGCATGCAGCAG	A	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT		
GU176299	CGCCCACAGCATGCAGCAG	A	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT		
JF928825	CGCCCACAGCATGCAGCAG	A	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT		
JN403057	CGCCCACAGCATGCAGCAG	A	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT		
JN609255	CGCTCACAGCATGCAGCAA	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT			
JN602193	CGCCCACAGCATGCAGCAG	A	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT		
Y11508	CGCTCACAGCATGCAGCGA	ACTAGAAC	TGCAACGGCAAAGGGAA	CTCGACTGTTAATT			

	61	70	80	90	100	110	120
45H	AAAACAAAGCATTGCGAGGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
45P	AAAACAAAGCATTGCGAAGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
AF039542	AAAACAAAGCATTGCGAGGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
AF039544	AAAACAAAGCATTGCGAGGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
AF039549	AAAACAAAGCATTGCGAGGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
AF039551	AAAACAAAGCAACCGAGGGCCGTGGCCGGTGTGACGCGTTGTGATTCTGCTCAGTGC						
AF419118	AAAACAAAGCATTGCGAGGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
AF522247	AAAACAAAGCATTGCGACGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
DQ343692	AAAACAAAGCATTGCGAGGGCCGGGACGGTGTGACGCAATGTGATTCTGCTCAGTGC						
EF033610	AAAACAAAGCATTGCGAAGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
EF033615	AAAACAAAGCATTGCGAGGCCGGGACGGTGTGACGCAATGTGATTCTGCTCAGTGC						
EF033618	AAAACAAAGCATTGCGAGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
FJ848966	AAAACAAAGCATTGCGAAGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
FJ848974	AAAACAAAGCATTGCGAAGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
GQ406350	AAAACAAAGCATTGCGAAGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
GQ406351	AAAACAAAGCATTGCGAAGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
GU176299	AAAACAAAGCATTGCGAAGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
JF928825	AAAACAAAGCATTGCGAAGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
JN403057	AAAACAAAGCATTGCGAAGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
JN609255	AAAAC-AAGCATTGCGA-GGCCGGGACGGTGTGACGCAATGTGATTCTGCTCAGTGC						
JN602193	AAAACAAAGCATTGCGAGGGCCGTGGCCGGTGTGACGCAATGTGATTCTGCTCAGTGC						
Y11508	AAAACAAAGCATTGCGAGGGCCGGGACGGTGTGACGCAATGTGATTCTGCTCAGTGC						

	121	130	140	150	160	170	180
45H	TCTGAATGTCAAAGTGAAGAGCTTCATCAAGCGCGSGTAAACAGCAGGGAGTAACATATGA						
45P	TCTGAATGTCAAAGTGAAGAAATTCAACCSAGCGCGGGTMAACAGCAGGGAGTAACATATGA						
AF039542	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
AF039544	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
AF039549	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
AF039551	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
AF419118	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
AF522247	TCTGAATGTCAAAGTGAAGAGATTCAATAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
DQ343692	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
EF033610	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
EF033615	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
EF033618	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
FJ848966	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
FJ848974	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
GQ406350	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
GQ406351	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
GU176299	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
JF928825	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
JN403057	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
JN609255	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
JN602193	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						
Y11508	TCTGAATGTCAAAGTGAAGAGATTCAATCAAGCGCGGGTAAACAGCAGGGAGTAACATATGA						

	181	190	200	210	220	230	240
45H	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTCTGACGCCATGAAYGGATTAAC						
45P	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
AF039542	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
AF039544	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
AF039549	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
AF039551	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
AF419118	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
AF522247	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
DQ343692	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
EF033610	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
EF033615	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
EF033618	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
FJ848966	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
FJ848974	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
GQ406350	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
GQ406351	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
GU176299	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
JF928825	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
JN403057	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
JN602193	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
JN609255	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						
Y11508	CTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCCATGAATGGATTAAC						

	241	250	260	270	280	290	300
45H	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
45P	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
AF039542	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
AF039544	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
AF039549	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
AF039551	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
AF419118	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
AF522247	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGGGGAACGGGCTCGGC						
DQ343692	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGGGGAACGGGCCCGC						
EF033610	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
EF033615	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGGGGAACGGGCCCGC						
EF033618	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
FJ848966	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
FJ848974	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
GQ406350	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
GQ406351	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
GU176299	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
JF928825	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
JN403057	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
JN609255	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
JN602193	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGAGGGAACGGGCTCGGC						
Y11508	GAGATTCTACTGTCCCTATCTGTTCTAGCGAAACCACAGCCGGGGAACGGGCCCGC						

	301	310	320	330	340	350	360
45H	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
45P	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
AF039542	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
AF039544	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
AF039549	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
AF039551	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
AF419118	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
AF522247	AAAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAGAACGTGAAACGA						
DQ343692	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGGAGCAA						
EF033610	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
EF033615	AAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGGAGCAA						
EF033618	AAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
FJ848966	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
FJ848974	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
GQ406350	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
GQ406351	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
GU176299	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
JF928825	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
JN403057	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
JN609255	AAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGGAGCAA						
JN602193	CAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGAAGCAA						
Y11508	GAAATCGGCGGGAAAGAAGACCTGTTGAGCTTACTCTATTCTAATTGTGGAGCAA						

	361	370	380	390	400	410	420
45H	GTTGGGAGGTGTAGCATAGGTGGGAG-----CGCT-----TGCGCGTCCG-						
45P	GTTGGGAGGTGTAGCATAGGTGGGAG-----AGTT-----TAACCGTAAGGT						
AF039542	GTTGGGAGGTGTAGCATAGGTGGGAG-----CGCT-----TGCGCGTCCG-						
AF039544	GTTGGGAGGTGTAGCATAGGTGGGAG-----CGCT-----TGCGCGTCCG-						
AF039549	GTTGGGAGGTGTAGCATAGGTGGGAG-----CGCT-----TGCGCGTCCG-						
AF039551	GTTGGGAGGTGTAGCATAGGTGGGAG-----CGCT-----TGCGCGTCCG-						
AF419118	GTTGGGAGGTGTAGCATAGGTGGGAG-----CGCT-----TGCGCGTCCG-						
AF522247	GTTAGGAGGTGTAGCATAGGTGGGAG-----AGCTT-----TGCTCGTCAG-						
DQ343692	GTTGGGAGGTGTAGCATAGGTGGGAGACCAA-----TTTT-----TGGTAAAAG-						
EF033610	GTTGGGAGGTGTAGCATAGGTGGGAG-----CGCT-----TGCGCGTCCG-						
EF033615	GTTGGGAGGTGTAGCATAGGTGGGAG-----ACTTT-----TTGTCGTCAAG-						
EF033618	GTTGGGAGGTGTAGCATAGGTGGGAG-----TGTTCGCTTCGGCGTGCACGGCCG-						
FJ848966	GTTGGGAGGTGTAGCATAGGTGGGAG-----CGCT-----TGCGCGTCCG-						
FJ848974	GTTGGGAGGTGTAGCATAGGTGGGAG-----CGCT-----TGCGCGACCGG-						
GQ406350	GTTGGGAGGTGTAGCATAGGTGGGAG-----CGCT-----TGCGCAACCGG-						
GQ406351	GTTGGGAGGTGTAGCATAGGTGGGAG-----CGCT-----TGCGCGTCCG-						
GU176299	GTTGGGAGGTGTAGCATAGGTGGGAG-----CGCT-----TGCGCGTCCG-						
JF928825	GTTGGGAGGTGTAGCATAGGTGGGAG-----CGCT-----TGCGCGTCCG-						
JN403057	GTTGGGAGGTGTAGCATAGGTGGGAG-----CGCT-----TGCGCGTCCG-						
JN602193	GCTGGGAGGTGTAGCATAGGTGGGAGACTCTCTGCTTCGGGGGATTGCGAACG-						
JN609255	GTTGGGAGGTGTAGCATAGGTGGGAG-----ACTTT-----ACGTCGCCAG--						
Y11508	GTTGGGAGGTGTAGCATAGGTGGGAG-----ACCT-----CGGTCGTCAAG--						

	421	430	440	450	460	470	480
45H	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACTAGGTACGT						
45P	ATGATATTAGCAAACTTGAAATACCACTACTCCCAGTCTGCTTACTTACTGGATAAGT						
AF039542	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACTAGGTAAAGC						
AF039544	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACTAGGTAAAGC						
AF039549	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACTAGGTTAGT						
AF039551	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACTAGGTAAATT						
AF419118	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACTAGGTACGT						
AF522247	-----TGAAATACCACTACTCCCTAATCTCGTTTACTTACCACTGGCGT						
DQ343692	-----TGAAATACCACTACTCCCAATCTTGCTTACTTACTAGGCTGGT						
EF033610	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACTGGGTTGGT						
EF033615	-----TGAAATACCACTACTCCCAATCTTGCTTACTTACTAGGCAGGG						
EF033618	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACGGGTTGGT						
FJ848966	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACGGGTTGGT						
FJ848974	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACGGGTTGGT						
GQ406350	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACGGGTTGGT						
GQ406351	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACGGGTTGGT						
GU176299	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACGGGTTGGT						
JF928825	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACGGGTTGGT						
JN403057	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACGGGTTGGT						
JN602193	-----TGAAATACCACTACTCCCAGTCTGCTTACTTACGGGTTGGT						
JN609255	-----TGAAATACCACTACTCCCAATCTTGCTTACTTACTAGGCAGGT						
Y11508	-----TGAAATACCACTACTCCCAATCTTGCTTACTTACTAGGCTGGT						

	481	490	500	510	520	530	540
45H	GTGGATGAGTC-----TTACGAC-TTCATTTAG						
45P	GTGGAATGGGCAGCACGTGGCGGCCGTTAAAAGCCACGCGTGTGCGCCTTATATTTAA						
AF039542	GTGGATGAGTC-----TTACGAC-TTCATTTAG						
AF039544	GTGGATGAGTC-----TTACGAC-TTCATTTAG						
AF039549	GTGGATGAGTC-----TTACGAC-TTCATTTAG						
AF039551	GTGGATGAGTT-----TTACGAC-TTGATTTAG						
AF419118	GTGGATGAGTC-----TTACGAC-TTCATTTAG						
AF522247	GTGGATGAATT-----GAACCTCATTTTCAG						
DQ343692	GTGGATGGGTT-----GTAGCATTGTTGCAAT-CTTGTGTTTG						
EF033610	GTGGATGAGGC-----ATTACGCT-TCTTTCTGG						
EF033615	GTGGATGGGTT-----GTCAAATCTTGGCAAT-CTTTTTTTGG						
EF033618	GTGGATGGGAT-----TTACGTC-CCTTTTTGG						
FJ848966	GTGGATGAAGC-----ATTACGCT-TCTTTTTGG						
FJ848974	GTGGATGAGTC-----TTACGAC-TCTATTCTAG						
GQ406350	GTGGATGAGCC-----TTACGGC-TCTATTCTGG						
GQ406351	GTGGATCAGCC-----TTACGGCTTTATTCTGG						
GU176299	GTGGATGAGCC-----TTACGGC-TCTATTCTGG						
JF928825	GTGGATGAGGC-----ATTACGCT-TCTTTCTGG						
JN403057	GTGGATGAGGC-----TATTTACGCC-TCTTTCTGG						
JN602193	GTGGAGTTGTC-----TTAACAGAC-TTTATTCTGG						
JN609255	GTGGATGGGTT-----CAGTTCTGATTCTTTTTGG						
Y11508	GTGGTGGATT-----GTT-----ATTTTGGCAAT-CTTTTTTTTG						

	541	550	560	570	580	590	600
45H	AATCAAACCAG			CTTGCTG			
45P	ACCCAAACGCA			ATTTGACTTGTGGTGGGTGCGTCTGCCCGTA			
AF039542	AACCAAACCAG			CTCGCTG			
AF039544	AACCAAACCAG			CTTGCTG			
AF039549	AACCAAACCAG			TTTACTG			
AF039551	AACCAAACCAG			CTTGCTG			
AF419118	AATCAAACCAG			CTTGCTG			
AF522247	AACCTAACACA			CCTGTAAACCG-TG			
DQ343692	AGCCAAGCTAT			GTGTGGTATTACGTACAGAGCAA			
EF033610	AACCAATGTGA			GATTTATTTTCG			
EF033615	AGCCAAGCCAT			GTCGGAATCCTCGGATTGGAGTAG			
EF033618	AACCAAATTG			TGACTTGTACAA			
FJ848966	AACCAATGCGG			GATTTATTTCTTCG			
FJ848974	AACCAATGCAA			TTTTTTG			
GQ406350	AACCAACGTGC			TTTTGCG			
GQ406351	AACCAATGCACATTAAATTATTTTTCCGTG						
GU176299	AACCAATGCAA			AATTGTTTTTTTG			
JF928825	AACCAATGTGA			GATTTATTTTCG			
JN403057	AACCAATGCAAA			AACTCGTTTCTG			
JN602193	AATCAACTCGG			CGCGGCAAGCGCTG			
JN609255	AGCCAAGCCGG			TTTTTCTCGGGAAAGACC-ATAG			
Y11508	AGCCAAGCCAT			GTCAGAACCTCGGATACTGAGTAG			

	601	610	620	630	640	650	660
45H				GTGACCACAGCCTAGCACACATTAGGAGGG			
45P	GAATGTCCCGATCCATGACGACGATGCCGTGACCACGGTCCAGAACACATTAGGAGGG			GTGACCACAGCCTAGCACACATTAGGAGGG			
AF039542				GTAAACCACAGCCTAGCACACATTAGGAGGG			
AF039544				GTGACCACAGCCTAGCACACATTAGGAGGG			
AF039549				GTAAACCACAGCCTAGCACACATTAGGAGGG			
AF039551				GTGACCACAGCCTAGCACACATTAGGAGGG			
AF419118				GTGACCACAGCCTAGCACACATTAGGAGGG			
AF522247				TGGATCTCGCGTGGACACTTTAGGAGGG			
DQ343692				GTGACCACAGCCTAGGACAGATTAGGAGGG			
EF033610				CAGACCACAGCCCAGCACACATTAGGAGGG			
EF033615				GTGACCACAGCCTAGGACAGACTTAGGAGGG			
EF033618				ATGACCACAGCCCCGTACACATTAGGAGGG			
FJ848966				CAGACCACAGCCCAGCACACATTAGGAGGG			
FJ848974				CAGACCACAGCCCAGCACACATTAGGAGGG			
GQ406350				CGGACCACAGCCCAGCACACATTAGGAGGG			
GQ406351				CAGACCACAGCCCAGCACACATTAGGAGGG			
GU176299				CAGACCACAGCCCAGCACACATTAGGAGGG			
JF928825				CAGACCACAGCCCAGCACACATTAGGAGGG			
JN403057				ACGACCACAGCCCAGCACACATTAGGAGGG			
JN602193				GTGACCACAGCCTAGGACAGATTAGGAGGG			
JN609255				GTGACCACAGCCTAGGACAGATTAGGAGGG			
Y11508				GTGACCACAGCCTAGGACAGATTAGGAGGG			

	661	670	680	690	700	710	720
45H	AGTTGGCTGGGGCGGCACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
45P	AGTTGGCTGGGGCGGTACATCTTCTACAAAACAACGAAGGTGTCCAAAGATAAGCTAG						
AF039542	AGTTGGCTGGGGCGGCACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
AF039544	AGTTGGCTGGGGCGGCACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
AF039549	AGTTGGCTGGGGCGGCACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
AF039551	AGTTGGCTGGGGCGGCACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
AF419118	AGTTGGCTGGGGCGGCACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
AF522247	AGTTGGCTGGGGCGGCACATTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
DQ343692	AGTTGGCTGGGGCGGTACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
EF033610	AGTTGGCTGGGGCGGCACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
EF033615	AGTTGGCTGGGGCGGTACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
EF033618	AGTTGGCTGGGGCGGTACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
FJ848966	AGTTGGCTGGGGCGGCACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
FJ848974	AGTTGGCTGGGGCGGCACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
GQ406350	AGTTGGCTGGGGCGGCACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
GQ406351	AGTTGGCTGGGGCGGCACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
GU176299	AGTTGGCTGGGGCGGCACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
JF928825	AGTTGGCTGGGGCGGCACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
JN403057	AGTTGGCTGGGGCGGCACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
JN602193	AGTTGGCTGGGGCGGTACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
JN609255	AGTTGGCTGGGGCGGTACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						
Y11508	AGTTGGCTGGGGCGGTACATCTTCTACAAGACAACGAAGGTGTCCAAAGATAAGCTAG						

	721	730	740	750	760	770	780
45H	TGAGAACAGAAATCTCACGTAGCGCAAAAGGGTAAAGCTTGTGTTATTTGATTTTAG						
45P	TGAGAACAGAAATCTCATGTAGCGCAAAAGGGTAAAGCTTGTGTTGATTCGATTTAG						
AF039542	TGAGAACAGAAATCTCACGTAGCGCAAAAGGGTAAAGCTTGTGTTATTTGATTTAG						
AF039544	TGAGAACAGAAATCTCACGTAGCGCAAAAGGGTAAAGCTTGTGTTATTTGATTTAG						
AF039549	TGAGAACAGAAATCTCACGTAGCGCAAAAGGGTAAAGCTTGTGTTATTTGATTTAG						
AF039551	TGAGAACAGAAATCTCACGTAGCGCAAAAGGGTAAAGCTTGTGTTATTTGATTTAG						
AF419118	TGAGAACAGAAATCTCACGTAGCGCAAAAGGGTAAAGCTTGTGTTATTTGATTTAG						
AF522247	TGAGAACAGAAATCTCACGTAGCGCAAAAGGGTAAAGCTTGTGTTGATTTGATTTAG						
DQ343692	TGAGAACAGAAATCTCACGTAGCGAAAAGGGCTAAAGCTTGTGTTGATTTGATTTAG						
EF033610	TGAGAACAGAAATCTCACGTAGCGAAAAGGGCTAAAGCTTGTGTTGATTTGATTTAG						
EF033615	TGAGAACAGAAATCTCACGTAGCGAAAAGGGCTAAAGCTTGTGTTGATTCGATTTAG						
EF033618	TGAGAACAGAAATCTCACGTAGCGAAAAGGGCTAAAGCTTGTGTTGATTTGATTTAG						
FJ848966	TGAGAACAGAAATCTCACGTAGCGAAAAGGGCTAAAGCTTGTGTTGATTTGATTTAG						
FJ848974	TGAGAACAGAAATCTCACGTAGCGAAAAGGGCTAAAGCTTGTGTTGATTTGATTTAG						
GQ406350	TGAGAACAGAAATCTCACGTAGCGAAAAGGGCTAAAGCTCGTTGATTTGATTTAG						
GQ406351	TGAGAACAGAAATCTCACGTAGCGAAAAGGGCTAAAGCTTGTGTTGATTTGATTTAG						
GU176299	TGAGAACAGAAATCTCACGTAGCGAAAAGGGCTAAAGCTTGTGTTGATTTGATTTAG						
JF928825	TGAGAACAGAAATCTCACGTAGCGAAAAGGGCTAAAGCTTGTGTTGATTTGATTTAG						
JN403057	TGAGAACAGAAATCTCACGTAGCGAAAAGGGCTAAAGCTTGTGTTGATTTGATTTAG						
JN602193	TGAGAACAGAAATCTCACGTAGCGAAAAGGGCTAAAGCTTGTGTTGATTTGATTTAG						
JN609255	TGAGAACAGAAATCTCACGTAGCGAAAAGGGCTAAAGCTTGTGTTGATTTGATTTAG						
Y11508	TGAGAACAGAAATCTCACGTAGCGAAAAGGGCTAAAGCTTGTGTTGATTTGATTTAG						

	781	790	800	810	820	830	840
45H	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
45P	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
AF039542	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
AF039544	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
AF039549	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
AF039551	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
AF419118	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
AF522247	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTTGAGTTTCGAGACTTGAGC						
DQ343692	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCATGATTGAGATTGAAGC						
EF033610	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
EF033615	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
EF033618	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
FJ848966	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
FJ848974	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
GQ406350	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
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JF928825	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
JN403057	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
JN602193	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
JN609255	TGCGAGTCGAAAACCGCGAAAGCGTGGCTATCGATCCTTCACGATTGAGATTGAAGC						
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	841	850	860	870	880	890	900
45H	GAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						
45P	GAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						
AF039542	GAGAGGTGTCAGAAAAGT-----						
AF039544	GAGAGGTGTCAGAAAAGT-ACCACAGG-----						
AF039549	GAGAGGTGTCAGAAAAGT-----						
AF039551	GAGAGGTGTCAGAAAAGTTACCACAGGGATAAC-----						
AF419118	GAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						
AF522247	AAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						
DQ343692	GAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						
EF033610	GAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						
EF033615	GAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						
EF033618	GAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						
FJ848966	GAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						
FJ848974	GAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						
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GU176299	GAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						
JF928825	GAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						
JN403057	GAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						
JN602193	GAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						
JN609255	GAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						
Y11508	GAGAGGTGTCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGGCCAAGCGTTCATAG						

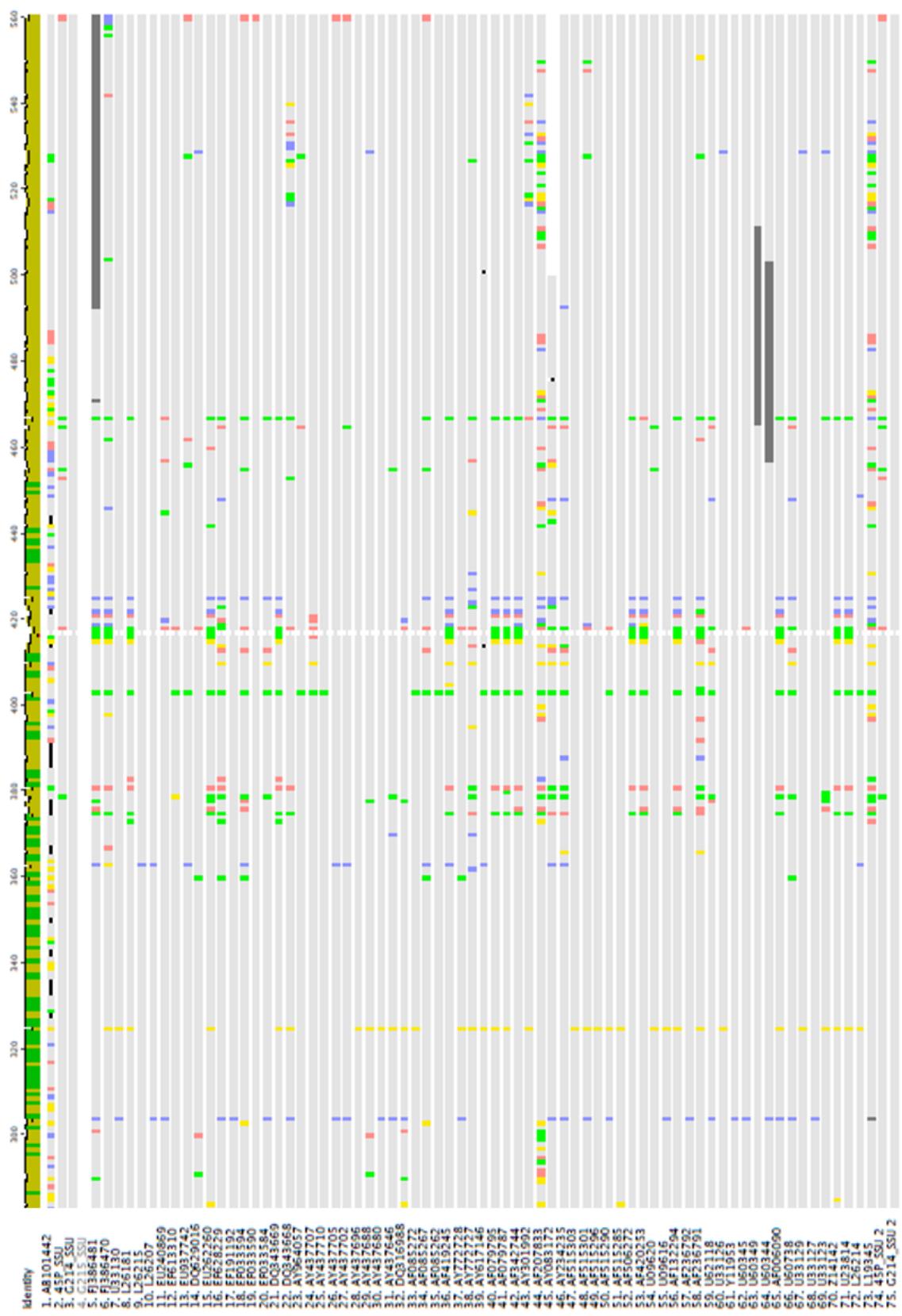
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45P	CGACGTCGCTTTTGATCCT		
AF039542	-----		
AF039544	-----		
AF039549	-----		
AF039551	-----		
AF419118	CGACGTCGCTTTTGATCCT		
AF522247	CGACGTCGCTT-----		
DQ343692	CGACGTCGCTTTTGATCCT		
EF033610	CGACGTCGCTTTTGATCCT		
EF033615	CGACGTCGCTTTTGATCCT		
EF033618	CGACGTCGCTTTTGATCCT		
FJ848966	CGACGTCGCTTTTGATCCT		
FJ848974	CGACGTCGCTTTTGATCCT		
GQ406350	CGACGTCGCTTTTGATCCT		
GQ406351	CGACGTCGCTTTTGATCCT		
GU176299	CGACGTCGCTTTTGATCCT		
JF928825	CGACGTCGCTTTTGATCCT		
JN403057	CGACGTCGCTTTTGATCCT		
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Y11508	CGACGTCGCTTTTGATCCT		

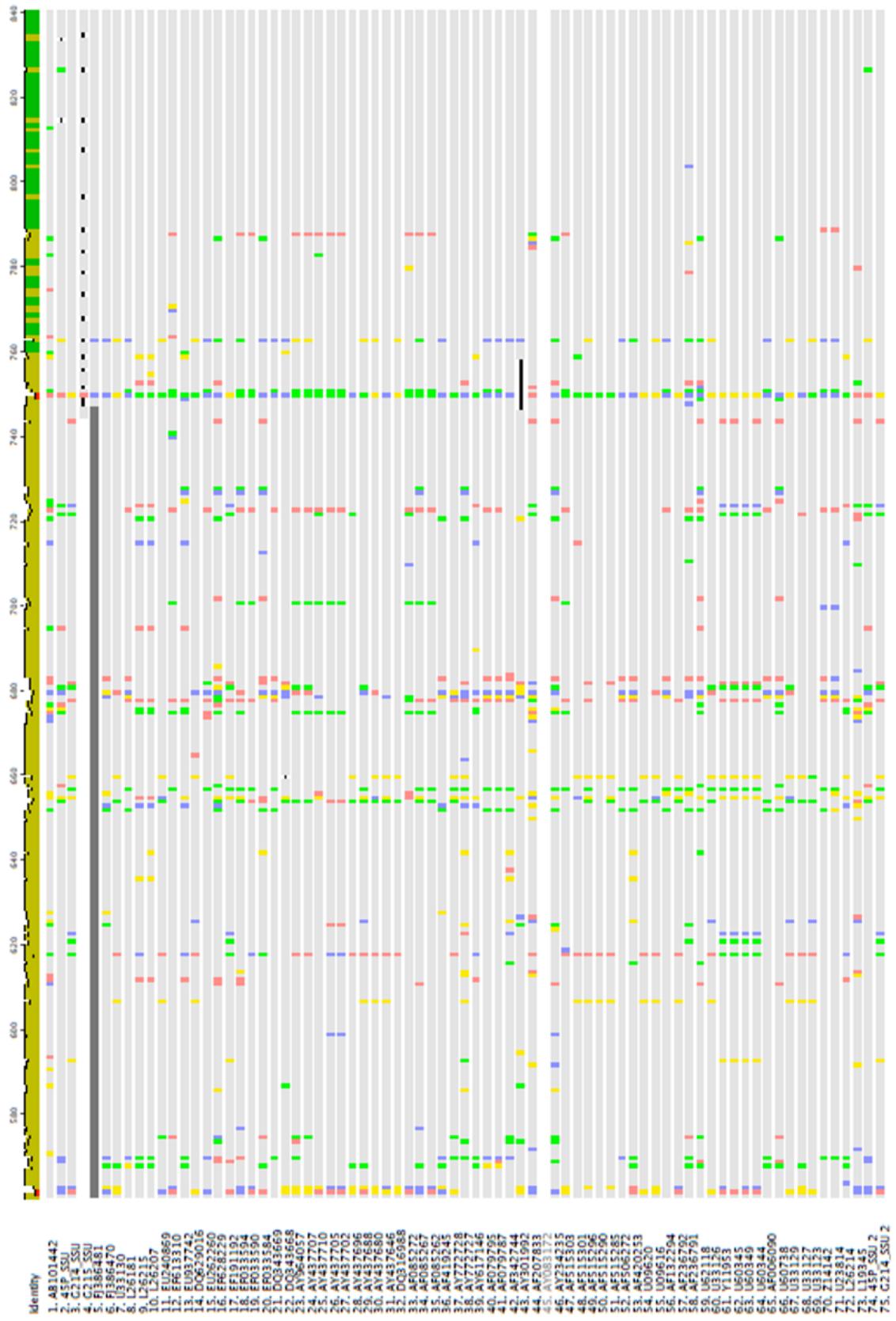
Appendix 8

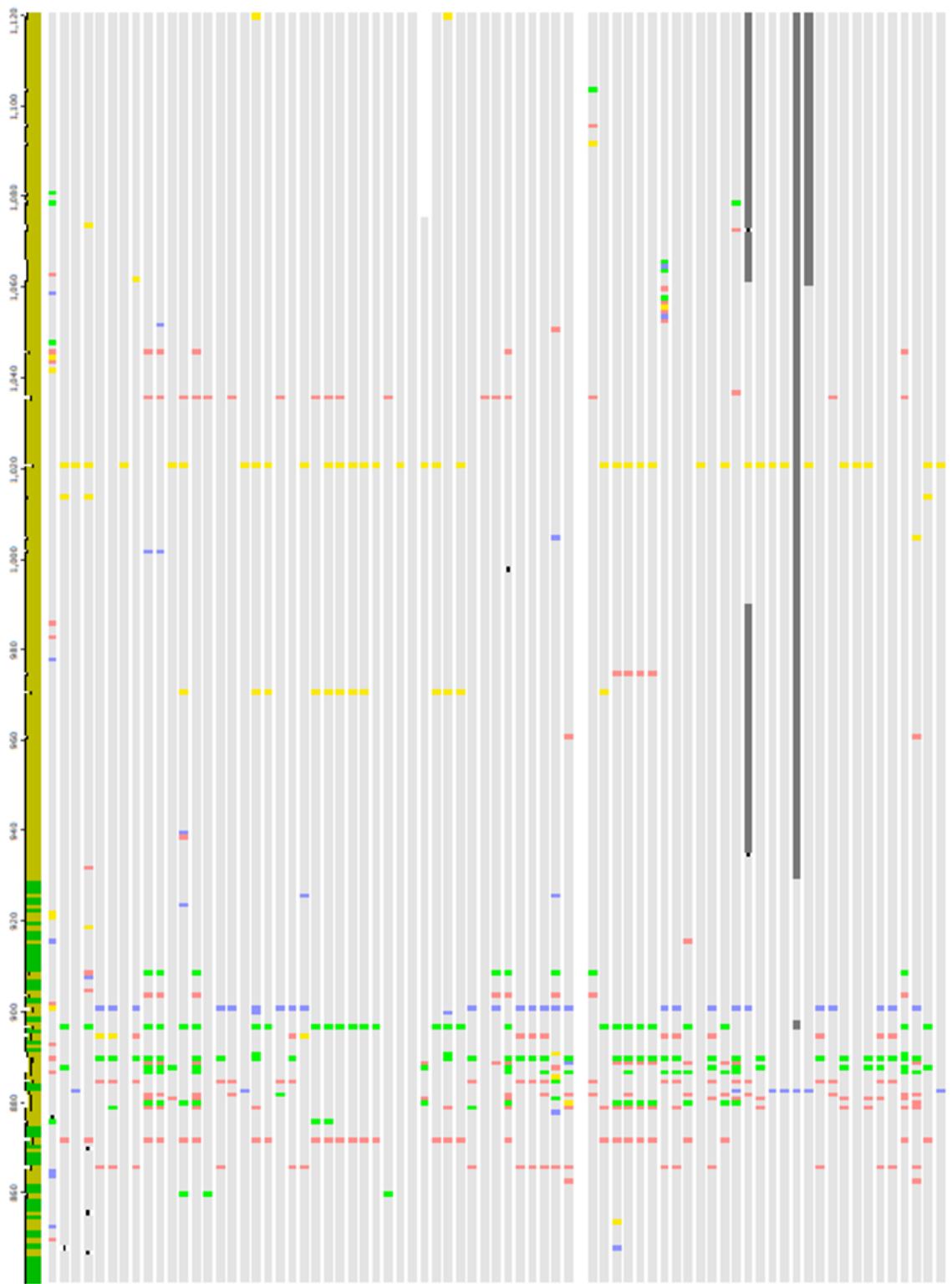
SSU alignment of *Adelophycus corneus* (AF515285), *Ahnfeltia fastigiata* (DQ343668), *Asparagopsis taxiformis* (AY772727), *Asteromenia peltata* (AY437710), *Atractophora hypnoides* (AY772728), *Audouinella asparagopsis* (AF079795), *Audouinella caespitosa* (AF079787), *Audouinella dasyae* (L26181), *Balbiana investiens* (AF132294), *Ballia callitricha* (AF236791), *Balliopsis prieurii* (AF419245), *Batrachospermum brasiliense* (FJ386470), *Callophyllis rangiferina* (U33123), *Capreolia implexa* (U60345), *Champia parvula* (EF613310), *Chondrus yendoi* (DQ316988), *Crassitegula walsinghamia* (AY964057), *Cumagloia andersonii* (DQ343669), *Curdiea flabellata* (L26207), *Dilsea californica* (U33126), *Endocladia muricata* (U33127), *Erythrymenia minuta* (AF085272), *Farlowia mollis* (U33129), *Fauchea repens* (AF085267), *Galaxaura marginata* (AF006090), *Gelidium latifolium* (Y11953), *Gelidiopsis intricata* (EF033594), *Gloiopeltis furcata* (U33130), *Gloiosiphonia capillaris* (AY437680), *Gracilaria cliftonii* (AY617146), *Gracilaria gracilis* (EU937742), *Gracilariopsis lemaneinformis* (L26214), *Grateloupia intestinalis* (AY437702), *Haliptilon roseum* (EF628229), *Hildenbrandia angolensis* (AF207833), *Hildenbrandia rubra* (L19345), *Hydrolithon pachydermum* (AY234235), *Hypnea* sp. (EU240869), *Inkyuleea marina* (AF236792), *Isabbottia ovalifolia* (EF033590), *Kumanoa ambigua* (FJ386481), *Lesleigha* sp. (AY437707), *Lithophyllum* sp. (AY083172), *Lithothamnion glaciale* (U60738), *Mastophoropsis canaliculata* (U62118), *Meiodiscus spetsbergensis* (U23814), *Melanthalia obtusata* (L26215), *Nemalionopsis shawii* (AF506272), *Nizymenia australis* (U09616), *Palmaria palmata* (Z14142), *Peyssonnelia* sp. (AY437688), *Peyssonnelia rubra* (DQ629016), *Pichiella liagoraciphia* (AY301992), *Plocamium angustum* (U09620), *Polyopes constrictus*

(AY437705), *Pyropia tenera* (AB101442), *Pterocladia lucida* (G214, G214.2, U60349), *Pterocladiella bartlettii* (EF191192), *Pterocladiophila hemisphaerica* (45P, 45P.2, G215), *Ptilonia australasica* (AY437646), *Ptilophora pinnatifida* (U60345), *Predaea* sp. (AF515296), *Renouxia* sp. (EF033584), *Rhodachlyya madagascarensis* (EU262260), *Rhodymenia stenoglossa* (AF515303), *Thorea* sp. (AF420253), *Thorea violacea* (AF342744), *Titanophora weberae* (AF515301), *Trematocarpus fragilis* (AY437696) and *Wetherbeella australica* (AF55290). Base pair differences are highlighted.

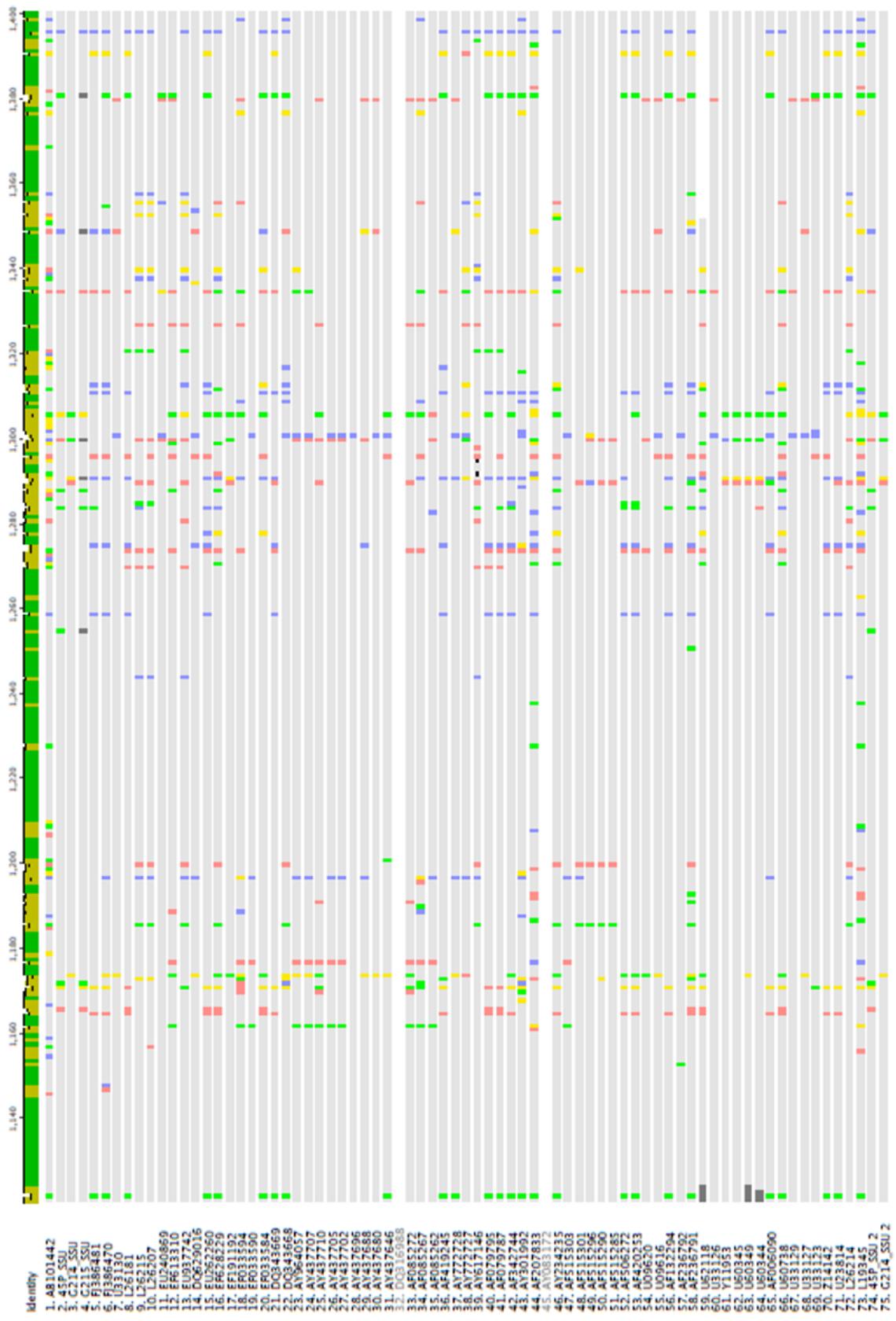


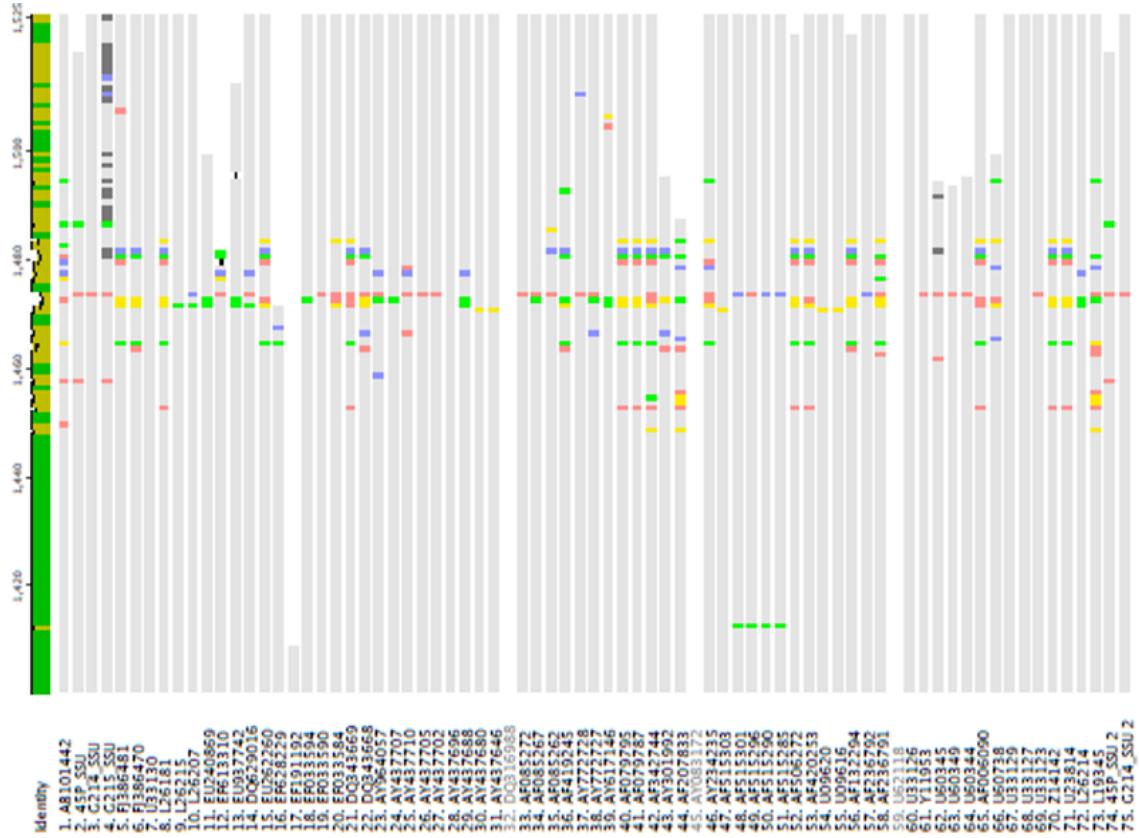






Identity
 1. A8101442
 2. 45P_SSU
 3. C214_SSU
 4. G336481
 5. G336470
 6. L26130
 7. L26215
 8. L26181
 9. L26207
 10. EU20859
 11. EU20850
 12. EU63310
 13. DUG93774
 14. DUG93774
 15. DUG93774
 16. DUG93774
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 71. DUG93774
 72. DUG93774
 73. DUG93774
 74. 45P_SSU_2
 75. C214_SSU_2





Appendix 9

Gene sequence for the *atp8* gene in *Pterocladiophila hemisphaerica* (45P). The open reading frame for the *atp6* gene is indicated in yellow and for the *atp8* gene in blue.

1	10	20	30	40	50
TCAGGCCACACATTGTTAAAAATTATTGCTGGATTGCCTGAACAATGTT					
ATCAGCAGGAGGAATTTAACATTATTATATGTTATTCCCTTAAGTTAT					
TATTTATTTAATTGGGTTAGAATTGCGATTGCTGGATTACAAGCTTAT					
GTATTTACATTATTGACTGTATTATTAAAAGATGTTAGATTTCA					
TTAAGTTTATGCCACAACTAGATCGTATTATAATTTCATTGTTATT					
TGATTGTTTATTATATTTTACTATGCTATATTATATTAT					
TTGCCACAGTTAAAAAGTATGATAATACGCTCTAAATATTGTTAA					
ATCATAAAAAAGAACGAGAAGATTATGAAATCAAATATAAAATATA					
AAACAATTATTTATTTTACTTATAAAATGTTAGTAATAAAACTAA					
ATTAGCAACAATTCTCAAAAAATTAAAAATTATAGTAG					
ATAAACAAATAAGTTAGTATAAAAATTATTATTATTGTAATAAA					
CATTTATGTAAGTCTATAATTTTATCCAAAAATAAAATTGACTA					
AAAAATGTATTTACT					

Appendix 10

Alignment of the gene sequence for the *atp8* gene in *Pterocladius lucida* (45H, 46H, G214). The open reading frame for the *atp6* gene is indicated in yellow, for the *atp8* gene in blue and for the *nad5* gene in green.

	1	10	20	30	40	50	60
45H	-	-	-	-	-	-	T
46H	TGCAACTTACTTAAATCATCGCTGGATTKGCTTGAACA			ATGCTTTCTGCAGGTGGTTAT			
G214	-	-	-	-	-	-	
	61	70	80	90	100	110	120
45H	TGGCTGTTTCATATAAACCTCTAGGACTTTAGTAGC	ACTAACAGGTCTTGAATTAG					
46H	TGGCTGTTTCATATAAACCTCTAGGACTTTAGTAGC	ACTAACAGGTCTTGAATTAG					
G214	-	-	-	-	-	-	
	121	130	140	150	160	170	180
45H	CTATAGCTGGTCTTCAAGCATATGTATTACACTGTTA	ACTTGTATTATTGAATGACG					
46H	CTATAGCTGGTCTTCAAGCATATGTATTACACTGTTA	ACTTGTATTATTGAATGACG					
G214	-	-	-	ACTGTATTATTGAATGACG	-	-	
	181	190	200	210	220	230	240
45H	TGTTAGATTTACATTA	AAAATGCCCAATTAGACCGTATAATT	ATTTACCTCCGCAGATT				
46H	TGTTAGATTTACATTA	AAAATGCCCAATTAGACCGTATAATT	ATTTACCTCCGCAGATT				
G214	TGTTAGATTTACATTA	AAAATGCCCAATTAGACGTATAATT	ATTTACCTCCGCAGATT				
	241	250	260	270	280	290	300
45H	TTGACTTTTATTGTTTATTGTTTATTGTTTATTGTTT	TAACCCATT	TTCTACCTAG				
46H	TTGACTTTTATTGTTTATTGTTTATTGTTTATTGTTT	TAACCCATT	TTCTACCTAG				
G214	TTGACTTTTATTGTTTATTGTTTATTGTTTATTGTTT	TAACCT	TTCTACCTAG				
	301	310	320	330	340	350	360
45H	ATTCTTAAAGTATTGAAAGCTAGAAAGAGTATAGT	GAATT	TAATGAATTAGAGTTGAA				
46H	ATTCTTAAAGTATTGAAAGCTAGAAAGAGTATAGT	GAATT	TAATGAATTAGAGTTGAA				
G214	ATTCTTAAAGTATTGAAAGCTAGAAAGAGTATAGT	GAATT	TAATGAATTAGAGTTAAA				

361	370	380	390	400	410	420	
45H	TTTGTTCACGAAAAATCTGCAGAGGTTGAGATTGAATTAAAGAAGAACCTTTAGAGGA						
46H	TTTGTTCACGAAAAATCTGCAGAGGTTGAGATTGAATTAAAGAAGAACCTTTAGAGGA						
G214	TCGCTTACGAAAAATCTGCAGAGGTAGAAATTGAATTAAAGAAGAACCTTTAGAGGA						
421	430	440	450	460	470	480	
45H	TTTAATTATGTGAAGAAAATATTGTAAATAATCCGGTATTGCCGTTTTAATTAA						
46H	TTTAATTATGTGAAGAAAATATTGTAAATAATCCGGTATTGCCGTTTTAATTAA						
G214	TTTAATTATGTGAAGAAAATATTGTAAATAATCCAGTATTCACTATTAAATTAA						
481	490	500	510	520	530	540	
45H	AAAATTAGAACGCTAAAGCTGTAGATGAATCAATAAGTGTAGCTATAAAAATTCTATGTT						
46H	AAAATTAGAACGCTAAAGCTGTAGATGAATCAATAAGTGTAGCTATAAAAATTCTATGTT						
G214	AAAATTAGAAACTAAAGCTGTAGACGAATCAATAGGTGCAGTTATAAAAATTCTATGTT						
541	550	560	570	580	590	600	
45H	ATTCTGCAATTCCAAATACTAAATTAAACCTATCTATTGCAAATCTATTAAATATATA						
46H	ATTCTGCAATTCCAAATACTAAATTAAACCTATCTATTGCAAATCTATTAAATATATA						
G214	ATTCTGCAATTCCAAATACTAAATTAAACCTATCTACTGCAAATCTATTAA--ATATA						
601	610	620	630	640	650	660	
45H	TAAAGACTGTTAATGTAATAACATGTATATTACTGTAATAATTGCCCCTAACCGTTCA						
46H	TAAAGACTGTTAATGTAATAACATGTATATTACTGTAATAATTGCCCCTAACCGTTCA						
G214	TAAAGACTGTTAATGTAATAACATGTATATTACTGTAATAATTGCCCCTAACCGTTCA						
661	670	680	690	700	710	720	
45H	CTAATTCTGGATTGGGGTCGTTATTGGGATATTATGGAGCAAGCATCTTCGACT						
46H	CTAATTCTGGATTGGGGTCGTTATTGGGATATTATGGAGCAAGCATCTTCGACT						
G214	TTAATTCTGGATTGGGGTCGTTATTGGGATATTATGGAGCAAGCATCTTCGACT						
721	730	740	750	760	770	780	
45H	GTTTGTGTCAACTATCTTTTATGTCTATCTTATTGAGGTGGGTTTG						
46H	GTTTGTGTCAACTATCTTTTATGTCTATCTTATTGAGGTGGGTTTG						
G214	GCTTGTGTCACTATCTTTTATGTCTATCTTATTGAGGTGGGTTTG						
781	790	800	810	820	830	840	
45H	AATACAGTTGTAATATCATTAGTACCTTGATTTACTGTAGGGACATTACGGTTAAT						
46H	AATACAGTTGTAATATCATTAGTACCTTGATTTACTGTAGGGACATTACGGTTAAT						
G214	AATATAGTTGCAATATCATTAGTACCTTGATTTACTGTAGGGACCTTACGGTTAAT						

	841	850	860	870	880	890	900	
45H	TGAAGTTTTATTGATTCTGTAACGTGCTGTAATGTTAATTGTTAGTAACATCGATTCA							
46H	TGAAGTTTTATTGATTCTGTAACGTGCTGTAATGTTAATTGTTAGTAACATCGATTCA							
G214	TGAAGTTTTATTGATTCTGTAACCGCTGTAATGTTAATTGTTAGTAACATCGATTCA							
	901	910	920	930	940	950	960	
45H	ACATTAGTCCATTTTATTCAATTAGTTACATGCAATCTGATCCACATTACCCACGTTT							
46H	ACATTAGTCCATTTTATTCAATTAGTTATGCAATCTGATCCACATTACCCACGTTT							
G214	ACATTAGTTCATTTTACTCAATTAGCTATGCAATCTGATCCACATTACCCACGTTT							
	961	970	980	990	1000	1010	1020	
45H	ATGGCTTATTTAGAAATTTTACCTTTTATGCTAGTATTAGTAACTGCGGATAATGTT							
46H	ATGGCTTATTTAGAAATTTTACCTTTTATGCTAGTATTAGTAACTGCGGATAATGTT							
G214	ATGGCTTATTTAGAAATTTTACCTTTTATGCTCGTATTAGTAACTGCGGATAATGTT							
	1021	1030	1040	1050	1060	1070		
45H	GTACAGATGTTTTAGGGTGAGAAGGCGTAGGG-----							
46H	GTACAGATGTTTTAGGGTGAGAAGGCGTAGGGTTAGCATCTTATT-----							
G214	GTGCAGATGTTTTAGGGTGAGAAGGTGTAGGGTAGCATCTTATTATTGA							