Research Article

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What's in a name? Monophyly of genera in the red algae: *Rhodophyllis parasitica* sp. nov. (Gigartinales, Rhodophyta); a new red algal parasite from New Zealand

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Red algal parasites are common within red algae and are mostly closely related to their hosts, but have a reduced habit. In the past, red algal parasites, due to their reduced morphology, have been given distinct generic names, even though they are often phylogenetically nested in their host's genus. This is a problem nomenclaturally for maintenance of a taxonomy based on monophyly. This study investigates the morphology, genetic variation and distribution of an undescribed red algal parasite growing on its host *Rhodophyllis membranacea*, widely distributed throughout New Zealand. Microscopy, molecular markers (plastid, mitochondrial, nuclear), and herbarium investigation were used to investigate this species. The parasite is widely distributed throughout New Zealand. All molecular markers clearly show that the parasite is almost identical to the host, even though morphologically quite distinct from members of the host genus. We believe that to maintain monophyly of *Rhodophyllis* the parasite should be described as a new species of *Rhodophyllis*, *Rhodophyllis parasitica* sp. nov. We also recommend that in order to maintain generic monophyly most red algal parasite genera should also be transferred to their host genus.

Key Words: *cox*1; *cox*2-3 spacer; Gigartinales; ITS2; monophyly; New Zealand; red algal parasites; *Rhodophyllis membranacea*; *Rhodophyllis parasitica* sp. nov.; RuBisCO spacer

INTRODUCTION

Red algal parasites are common on other red algae and have been described from several orders within the Florideophyceae (Goff 1982), a few molecular-based phylogenetic studies have focused on a handful of species (Goff et al. 1997, Zuccarello et al. 2004, Ng et al. 2013), with new species still being described (Kim and Cho 2010).

The morphological characteristics traditionally used to determine if a red alga is a parasite include: 1) the penetration of the parasite beyond the superficial cells of the host (Setchell 1918); 2) reduction of the parasite thallus; and 3) the loss of colour (Wynne and Scott 1989). Another important character are the presence of secondary pit connections (2nd PC) formed between parasite and host cells (Goff 1982). 2nd PC have been observed in nearly all described red algal parasites studied (e.g., Fredericq and Hommersand 1990, Goff and Zuccarello 1994, Goff and Coleman 1995).

Molecular studies are helpful in determining the evolutionary relationship between host and parasite (Zuccarello et al. 2004). Gene sequences of plastid DNA have shown that they are mostly identical between hosts and parasites, which suggests that red algal parasite retain the host plastids (Goff and Coleman 1995). Comparison of mitochondrial and nuclear sequences between hosts and

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parasites has shown differences between them, which suggests that the red algal parasite retains its own mitochondria and nuclei (Goff and Coleman 1995).

Several studies have shown that red algal parasites are often more closely related to their host than the hosts are to other species in the host genus (e.g., Goff et al. 1996, Kurihara et al. 2010). In all these cases, a distinct genus name for the parasite has been retained, rendering the host genus paraphyletic. A strict monophyletic (holophyletic) classification system is supported by many systematists but maintaining paraphyly (for evolutionarily innovative lineages) also has backers. The arguments about maintaining strict holophly is on-going (Hörandl and Stuessy 2010) and may depend on the purposes of a classification system.

There are four reported red algal parasites in New Zealand. *Pterocladiophila hemisphaerica* Fan & Papenfuss (1959) was found on the North Island in Wellington and on the South Island in Kaikoura parasitizing *Pterocladia lucida* (R. Brown *ex* Turner) J. Agardh. Other parasites have also been reported from New Zealand: *Gloiocolax novae-zelandiae* Sparling on *Gloioderma saccatum* J. Agardh; *Gonimophyllum buffhamii* Batters on *Hymenena semicostata* (J. Agardh) Kylin and *Microcolax botryocarpa* (J. D. Hooker et Harvey) F. Schmitz & Falkenberg on *Streblocladia glomerulata* (Montagne) Papenfuss (Nelson 2012) but little specific information is available on these species.

We describe a new red algal parasite from New Zealand growing on *Rhodophyllis membranacea* (Harvey) J. D. Hooker et Harvey, and discuss the monophyly of red algal genera that have parasites associated with them.

MATERIALS AND METHODS

Rhodophyllis membranacea and its parasite were collected as drift from subtidal samples around New Zealand (Table 1). Only host thalli without any sign of degradation was collected.

Microscopy

Parasite tissue with surrounding host tissue was removed, fixed overnight in 2% glutaraldehyde in phosphate buffer (0.1 M, pH 6.8) in 50% seawater. The tissue was then washed three times at intervals of 10 min with phosphate buffer (0.1 M, pH 6.8) in 50% seawater. The tissue was dehydrated in an ascending ethanol-water series of 20, 40, and 60% ethanol and three times with 70% ethanol for 15 min each. The fixed samples were stored in 70% ethanol at 4°C until embedding in glycol methacrylate (JB-4; Sigma-Aldrich, St. Louis, MO, USA) following the manufacturers instructions. The embedded stubs were covered with parafilm and stored in a desiccator until hardened. The embedded tissue was sectioned on a microtome (860 Model; American Optical Corporation, Southbridge, MA, USA). Sections were 10 µm thick and carefully transferred from the surface of distilled water to a microscopic slide after they had fully spread in the water. Slide were then dried and stored.

All microscopic slides were either stained with 1.0 µg mL^{-1} DAPI in McIlvaine buffer (pH 4.1) or 1% acidified aniline blue solution. DAPI was used to stain nuclei and Aniline blue was used as a general cytological stain. The microscopic slides were examined under a fluorescence microscope (Olympus AX-70) with integrated camera (Olympus DP-70; Olympus, Tokyo, Japan) and images were taken using either DP Controller (Olympus) imaging software or Stream Enterprise software (Olympus).

DNA from fresh, or silica gel dried samples, was extracted with 5% Chelex following Zuccarello et al. (1999) (Table 1). Two mitochondrial markers (*cox*1 and *cox*2-3 spacer), one nuclear marker (ITS2) and one plastid marker (RuBisCo spacer) were used. The forward GazF1 and reverse GazR1 primers were used to amplify the 5′ end of *cox*1 (Saunders 2005). The forward primer *cox*2F and reverse primer *cox*3R were initially used to amplify the *cox*2-3 spacer (Zuccarello et al. 1999). The primers ITS3 and ITS4 were used to amplify the ITS2 region (White et al. 1990). The general ITS2 primers (above) were inefficient (low polymerase chain reaction [PCR] success) so specific primer were newly designed using successfully amplified *R. membranacea* and parasite sequences. Two forward primers ITS3F1 (5′-CGAATGCGATATGTAATG) and ITS3F2 (5′-AATCATCGAATTTTTGAACG) and one reverse primer ITS4R1 (5′-CAAAACGNTTCCCTCTCCTC) were created in Geneious (Biomatters Ltd., Auckland, New Zealand) using the Primer3 software. The forward and reverse primers were used to amplify the RuBisCo spacer (Maggs et al. 1992).

Amplification reaction (30 µL) were performed with the following final concentrations: 1× buffer (BioTherm; Genecraft, Ludinghausen, Germany), 0.2 mM dNTP's, 2.5 mM $MgCl_2$, 0.04% BSA (Sigma), 0.25 pmol of each primer, 1 U *Taq* polymerase (New England BioLabs, Inc., Ipswich, MA, USA), and 1 µL of template DNA. PCR was carried out with an initial denaturation at 94°C for 5 min, followed by 36 cycles of 94°C / 45°C / 72°C for 1 min each and a final step 72°C for 5 min. The PCR products were confirmed

using agarose gel electrophoresis. Successful amplifications were purified using ExoSAP-IT following manufactures instructions (USB product; Affymetrix, Santa Clara, CA, USA) and sequenced commercially (Macrogen Inc., Seoul, Korea). Unique haplotpyes are deposited in GenBank (KM407518-KM407529).

Forward and reverse sequences were assembled and edited in Geneious. The edited sequences were aligned using MAFFT alignment using the default parameters. The alignment was checked and realigned by eye. The alignment of *cox*1 and ITS2 from hosts and parasites was used to calculate haplotype networks using TCS 1.21 (Clement et al. 2000).

All herbarium samples of *R. membranacea* at the Museum of New Zealand Te Papa Tongarewa in Wellington were searched for parasites under a dissecting microscope and observed parasites were recorded.

RESULTS

Morphology of the *Rhodophyllis* parasite

Habitat and seasonality. The red algal parasite growing on *R. membranacea* was found from January to May. The host can have between one and 20 parasites often close to the edges and tips of the host plant.

Thallus. The parasite (Fig. 1A) is less pigmented than the host (light reddish), which makes the parasite easy to spot. The parasite thallus is on average 2.5 × 3 mm in size and consists of a radiating cluster of terete, smooth branches that often terminate bipinnately or remain unbranched (Fig. 1A). The parasite has a single base attached to the host surface and a short stipe that branches distally (Fig. 1B). The branches are not flattened as in *R. membranacea*, and the branch tips are blunt. Spermatan-

Table 1. List of all samples of *Rhodophyllis membranacea* and its parasite collected and used in molecular analysis

Species	No.	Date	Location	Coordinates	Collector	Host source
Rhodophyllis membranacea	12H	Jan 27, 2008	Thompson Sound	45°13′20″ S, 166°58′20″ E	C. Hepburn, D. Richards	-
	F943	Apr 17, 2010	Moa Point	41°20′30″ S, 174°48′38″ E	W. Grant	-
	F945	Apr 17, 2010	Moa Point	41°20′30″ S, 174°48′38″ E	W. Grant	-
	F947	Apr 17, 2010	Moa Point	41°20′30″ S, 174°48′38″ E	W. Grant	-
	2H	Feb 27, 2012	Houghton Bay	41°20′33″ S, 174°47′06″ E	P. Northcote	-
	3H	Feb 27, 2012	Houghton Bay	41°20′33″ S, 174°47′06″ E	P. Northcote	-
	4H	Feb 27, 2012	Houghton Bay	41°20′33″ S, 174°47′06″ E	P. Northcote	-
	5H	Feb 27, 2012	Houghton Bay	41°20′33″ S, 174°47′06″ E	P. Northcote	-
	8H	Mar 4, 2013	Moa Point	41°20′30″ S, 174°48′38″ E	M. Preuss	-
	9H	Mar 8, 2013	Moa Point	41°20′30″ S, 174°48′38″ E	M. Preuss	-
	14H	Mar 13, 2013	Cape Palliser	41°36′48″ S, 175°17′30″ E	M. Preuss	-
	15H	Mar 13, 2013	Cape Palliser	41°36′48″ S, 175°17′30″ E	M. Preuss	-
	44H	May 19, 2013	Blackhead	40°10′09″ S, 176°49′37″ E	M. Preuss	-
	39H	May 20, 2013	Akitio Beach	40°36′53″ S, 176°24′53″ E	M. Preuss	-
	40H	May 20, 2013	Akitio Beach	40°36′53″ S, 176°24′53″ E	M. Preuss	-
	42H	May 20, 2013	Akitio Beach	40°36′53″ S, 176°24′53″ E	M. Preuss	-
	43H	May 20, 2013	Between Sandy Beach and Mataikona	40°36′53″ S, 176°15′08″ E	M. Preuss	-
Rhodophyllis parasite	F944	Apr 17, 2010	Moa Point	41°20′30″ S, 174°48′38″ E	W. Grant	F945
	2P	Feb 27, 2012	Houghton Bay	41°20′33″ S, 174°47′06″ E	P. Northcote	2H
	4P	Feb 27, 2012	Houghton Bay	41°20′33″ S, 174°47′06″ E	P. Northcote	4H
	5P	Feb 27, 2012	Houghton Bay	41°20′33″ S, 174°47′06″ E	P. Northcote	5H
	8P	Mar 4, 2013	Moa Point	41°20′30″ S, 174°48′03″ E	M. Preuss	8H
	9P	Mar 8, 2013	Moa Point	41°20′30″ S, 174°48′38″ E	M. Preuss	9H
	13P	Mar 13, 2013	Cape Palliser	41°36′48″ S, 175°17′30″ E	M. Preuss	13H
	14P	Mar 13, 2013	Cape Palliser	41°36′48″ S, 175°17′30″ E	M. Preuss	14H
	40P	May 20, 2013	Akitio Beach	40°36′53″ S, 176°24′53″ E	M. Preuss	40H
	42P	May 20, 2013	Akitio Beach	40°36′53″ S, 176°24′53″ E	M. Preuss	42H

Date, location, coordinates, collector, and host source of collection also shown. Host source described the host from which the parasite was removed.
No., code used for sample.

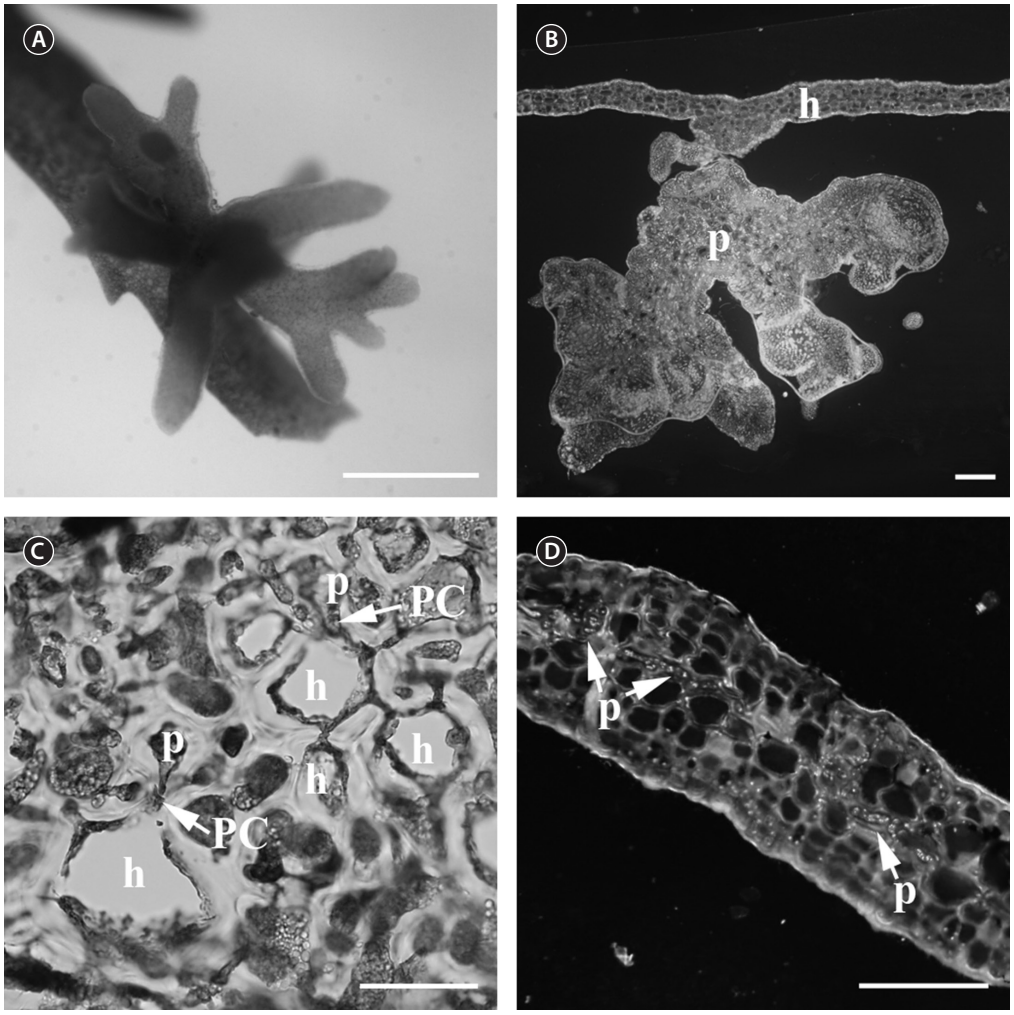

Fig. 1. Vegetative structure of the *Rhodophyllis* parasite. (A) The parasite is bipinnately branched and less pigmented than its host. (B) DAPI-staining of *Rhodophyllis membranacea* (h) and its parasite (p). The parasite penetrates deep beyond the superficial layer of the host cells and is attached by a short stipe to the host. (C) Contact area between host and parasite stained with Aniline blue. Parasite (p) forms 2nd pit connections (PC) with its host (h). (D) DAPI-staining of vegetative structure of *R. membranacea* with parasite nuclei in the parasite rhizoidal cells (p) visible between round host cells. Scale bars represent: A, 1 mm; B, 250 μm; C, 50 μm; D, 200 μm.

gia and tetrasporangia are scattered on the thallus surface. Carposporophytes form roundish structures on the branches.

Vegetative structures. The thallus area, which connects the host and parasite cells, consists of elongated cells of various sizes. This contact area also contains embedded host cells between parasite cells. The parasite cells form secondary pit connections with host cells (Fig. 1C). 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. The vegetative structure of *R. membranacea* consists of one or two inner layers of large cortical cells, an outer layer of smaller epidermal cells and a cuticle. The parasite thallus further away from the host surface consists of pigmented round and elongated cells. The normal cell layer structure of *R. membranacea* is disrupted by the infection of the parasite. The disruption caused by parasite filaments is localised, but parasite filaments are also found further from the contact area (Fig. 1D). DAPI-staining revealed a large number of parasite nuclei in rhizoidal parasite cells within the host tissue (Fig. 1D).

Reproductive structures. Carposporophytes, spermatangia and tetrasporangia were observed. The parasite has bisexual gametophytes (Fig. 2A).

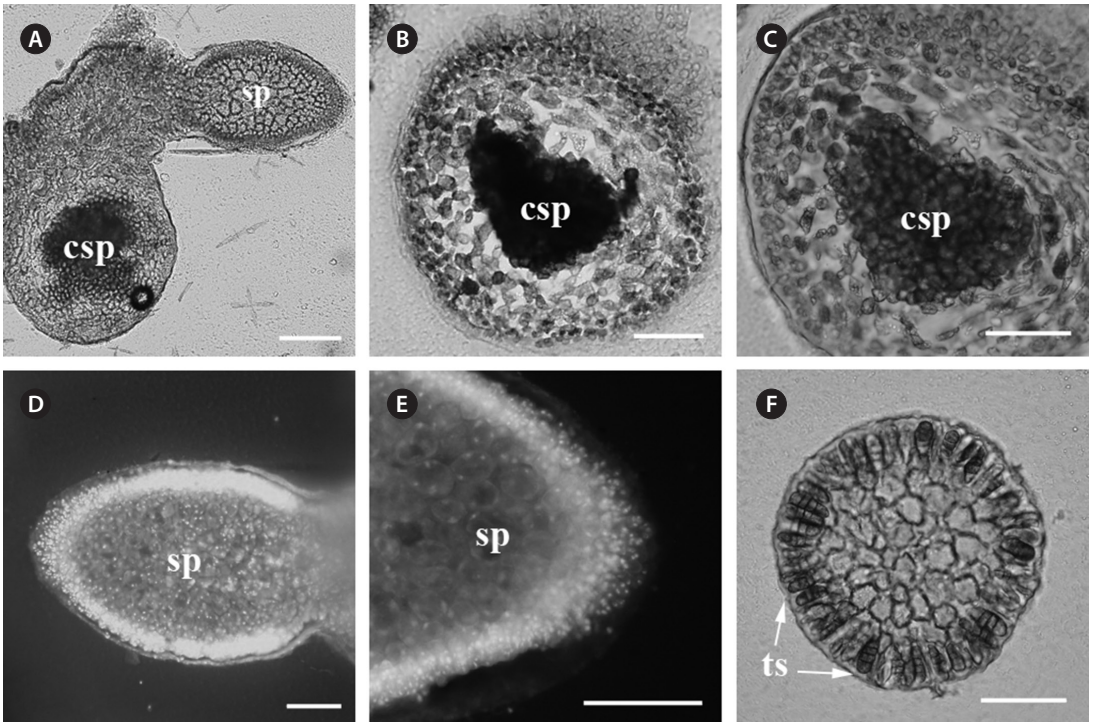

Fig. 2. Reproductive structure of the *Rhodophyllis* parasite. (A) Bisexual gametophyte with spermatangia (sp) and carposporophyte (csp). Unstained section. (B & C) Aniline blue stained (B) and unstained (C) carposporophyte (csp) containing an accumulation of carpospores and surrounded by a pericarp. (D) DAPI-staining of dense patches of spermatangia on the parasite surface. (E) Higher magnification of DAPI-stained spermatangia. Spermatia visible at periphery. (F) Aniline blue stained tetrasporangia (ts) of the parasite. Tetrasponrangium contains four zonately divided tetraspores. Scale bars represent: A, 200 µm; B-D & F, 100 µm; E, 50 µm.

Carposporophyte. The mature carposporophyte (Fig. 2A-C) contains oval carposporangia with an average size of 20 µm (n = 5), and are located marginally on the branches of the parasite (Fig. 2A). Carposporophytes are 230 × 330 µm (n = 3) and surrounded by a pericarp. The pericarp is approximately 5-10 cells thick (200 µm; n = 3) (Fig. 2C).

Male gametophyte. Spermatangia are in dense patches on the surface of the parasite thallus (Fig. 2D). Spermatia are round and less than 10 µm in diameter (Fig. 2E).

Tetrasporophyte. Tetrasporangia are scattered and numerous on the surface. Tetrasporangia are zonately divided (Fig. 2F). Tetrasporangia are pigmented and of an average size of 48 × 20 µm (n = 10).

Comparison of morphological characters between host and parasite

R. membranacea and its parasite differ in the size and shape of the thallus, reproductive structures, and individual cells. They share the shape and location of their reproductive structures (Table 2).

Phylogeny of *Rhodophyllis* parasite

ITS2. The 512 basepairs (bp) ITS2 alignment contained 22 samples of *R. membranacea* and parasite samples (11 hosts and 11 parasites). Three ITS2 ribotypes were found that differed by at most 2 bp (A1, A2, and A3). Ribotype A1 contained all sequences of *R. membranacea* (n = 11) and one parasite sequence. Ribotype A2 and A3 included only parasite sequences. Lineage A3 is the most common ribotype among the parasite sequences (n = 7) (Fig. 3A). Ribotypes A2 and A3 were each found at Cape Palliser (Table 1) and therefore are not correlated with location.

***Cox*1.** The *cox*1 alignment contained 21 samples of *R. membranacea* and parasite samples (12 hosts and 9 parasites) and was 676 bp in length. Three haplotypes were found that differed by at most 3 bp: B1, B2, and B3. Haplotype B1 contained most host and parasite sequences (10 hosts and 8 parasites) while haplotype B2 contained two host sequences and haplotype B3 contained one parasite sequence (Fig. 3B).

***Cox*2-3 spacer.** The *cox*2-3 spacer alignment contained 15 samples of *R. membranacea* and parasite samples (10

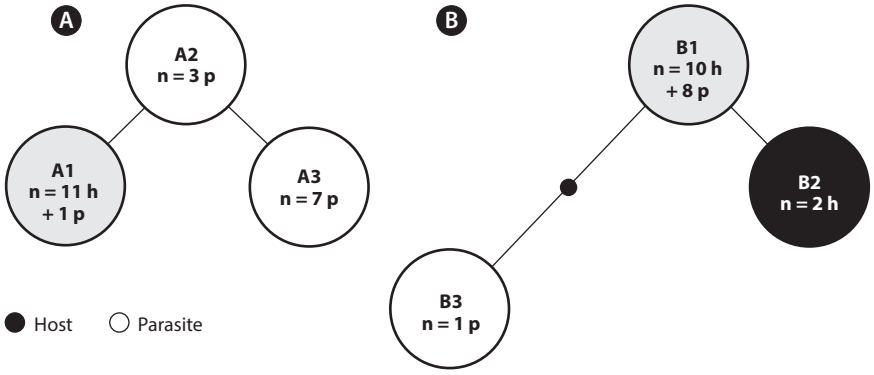

Fig. 3. DNA sequence networks of *Rhodophyllis membranacea* and its parasite. (A) ITS2 ribotype network with three different ribotypes represented (A1, A2, A3). (B) *Cox*1 haplotype network with three different haplotpyes represented (B1, B2, B3). Line is one base pair difference. Small black circle is missing haplotype. Number of samples (n) divided into host (h) and parasite (p) sequences.

hosts and 5 parasites) and was 356 bp in length. All *cox*2-3 spacer sequences of *R. membranacea* and its parasite were identical except one parasite sample, which showed 1 bp difference.

RuBisCo spacer. The RuBisCo spacer alignment contained 4 samples of *R. membranacea* and parasite samples (3 hosts and 1 parasite) and had a length of 355 bp. All RuBisCo sequences of host and parasite are identical.

Distribution of the *Rhodophyllis* parasite

The Te Papa herbarium collection contained over 60 specimens of *Rhodophyllis membrancaea* and 29 of those had red algal parasites on them (Table 3). The parasite is widespread and can be found throughout New Zealand. Samples with parasites can be found on the subantarctic islands (Auckland Islands) and from Stewart Island in the south to the Three Kings Islands in the north. The parasite can be found in collections year-round except for June and July.

Table 2. Comparison of vegetative and reproductive structures between *Rhodophyllis membranacea* and its parasite

	Rhodophyllis membranacea	*Rhodophyllis parasitica*
Thallus		
Size	6-15 cm long	2.5 × 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		
Carpospores		
Shape	Ovoid	Ovoid
Size	15-25 μm diameter	20 μm diameter
Tetrasporangia		
Location	Scattered	Scattered
Form	Zonately divided	Zonately divded
Size	20-35 μm diameter	20 μm diameter
Spermatangia		
Location	Scattered on surface	Scattered on surface
Form	Superficial patches	Superficial patches
Spermatia		
Size	-	Approx. 10 μm
Reference	Chapman (1979), Womersley (1994), Nelson (2013)	Present study

DISCUSSION

Our genetic evidence clearly shows that this parasite is closely related to its host. It is almost indistinguishable genetically with the markers used that have been used to distinguish species in other red algae (e.g., *cox*1) (Le Gall and Saunders 2010, Saunders and Moore 2013). Morphologically the parasite is distinct from its host, and all other species in the host genus, mostly due to its reduced size. Morphological analysis showed that the parasite has most of the characteristics used to define red algal parasites, which are 1) reduced size; 2) reduced pigmentation; and 3) formation of secondary pit connections between host and parasite cells. These fulfil criteria used previously to identify algal species as parasitic (Goff 1982, Wynne and Scott 1989). As no parasites have been described on the genus *Rhodophyllis*, and this parasite is morphologically clearly distinct from other species of the genus, we describe it as a new species of *Rhodophyllis*, *Rhodophyllis parasitica* sp. nov.

Since the level of genetic similarity between the parasite and host is less than expected between two species of red algae, it is likely that the parasite is more closely related to its host than *R. membranacea* is to other four species in the genus, *Rhodophyllis acanthocarpa* (Harvey) J. Agardh, *R. centrocarpa* (Montagne) Wynne, *R. gunnii* (Harvey) Harvey, and *R. lacerata* Harvey (Cotton 1908), even if we have not collected these other species for molecular study. Interspecific variation of *cox*1 in red algae is usually at least >25 bp (Robba et al. 2006, Geraldino et al. 2009, Cassano et al. 2012). For example 29 bp difference were found between sister species of *Euthora* (Clarkston and Saunders 2010) and 40 bp between species of *Gracilaria* (Yang et al. 2007). These values are all much greater than what we see between host and parasite (1-2 bp).

Due to the morphological and genetic similarity of red algal parasite and host, it has been suggested that the parasite evolved from its host (Goff et al. 1997, Blouin and Lane 2012, Ng et al. 2013). For example, the parasite *Congracilaria babae* Yamamoto is more closely related to its host *Gracilaria salicornia* (C. Agardh) Dawson than the

Table 3. Te Papa vouchers of *Rhodophyllis membranacea* with red algal parasites on them

Te Papa voucher	Collection date	Location	Collector
A1573	Jan 31, 1968	Houghton Bay, Wellington	N. M. Adams
A2686	Apr 10, 1969	Karehana Bay, Plimmerton	E. Harris
A4151	Jan 7, 1971	Lyall Bay, Wellington	N. M. Adams
A4240	Feb 15, 1971	Kaikoura Peninsula	Baker
A7201	Feb 20, 1972	Port Pegasus, Stewart Island	G. Moreland
A7200	Feb 28, 1972	Port Pegasus, Stewart Island	N. M. Adams
A10189	Sep 16, 1978	Port Ross, Auckland Island	C. H. Hay
A10423	Oct 23, 1978	Great Island, Three King Island	H. Choat
A14765	Feb 14, 1980	Preservation Inlet, Fiordland	-
A11577	Feb 8, 1981	Aorere Point, North Otago	N. M. Adams
A11580	Feb 10, 1981	Shag Point, North Otago	N. M. Adams
A13411	Feb 25, 1982	Between Lyall Bay and Houghton Bay, Wellington	W. Nelson
A14250	Dec 2, 1983	T449 Bushett's Shoal	C. H. Hay
A14215	Dec 5, 1983	7459 Cape Campbell Scuba Street	C. H. Hay
A14206	Dec 14, 1983	Chetwode Island (Nukuwaita)	C. H. Hay
A22508	May 11, 1986	Preservation Inlet, Gulches Head	G. Scringer
A25901	-	Doubtful Sound, Fiordland	S. Wing
A18456a	Mar 4, 1987	Point Gap Bay, Chatham Island	D. Schiel
A18459b	Mar 4, 1987	Chatham Island	W. Nelson
A026481	Mar 27, 1987	Karorio stream mouth, Wellington	C. H. Hay
A18630a	Aug 11, 1987	Chetwode Island (Nukuwaita)	C. H. Hay
A19277	Mar 28, 1990	Fighting Bay, Marlborough	C. H. Hay
A19048	Apr 26, 1990	Horahora, Kakahu Island	C. Duffy
A21013	Nov 10, 1994	Dusky Sound, Lama Island	E. Villouta
A029799	Sep 1, 1996	Kaikoura Peninsula	W. Nelson & G. Knight
A22424	Feb 8, 1999	Smoothwater Bay, South Westland	Neale
A022772	Feb 20, 2000	Dagg Sound, Four Fanthom Bank	F. Smith
A022771	Feb 23, 2000	Chalky Inlet, Edwardson Sound	C. Mundy
A024446	Feb 22, 2007	Tarakena Bay, Wellington	K. Neill

host is to conspecifics based on mitochondrial markers (Ng et al. 2013). The parasite *Janczewskia morimotoi* Tokida is identical to its host *Laurencia nipponica* Yamada based on rRNA and *cox*1 genes (Kurihara et al. 2010). SSU and ITS data revealed that the red algal parasite *Faucheocolax attenuata* Setchell is more closely related to one of its host *Fauchea laciniata* J. Agardh than it is to its other (*Fauchea fryeana* Setchell), which suggests that the parasite evolved on *Fauchea laciniata* and switched host to another closely related species (Goff et al. 1996).

Red algal parasites have traditionally been placed in a separate genus from their host since their first description (Fan and Papenfuss 1959, Lee and Kurogi 1978). Red algal parasites have special morphological characters such as their small size and reduced pigmentation due to their parasitic lifestyle, but reproductive characters suggested their close similarity to their host in many cases (Goff 1982). These morphological similarities suggested that parasites should be classified at least within the same family as their host. Molecular methods have improved our understanding of evolutionary relationships in organisms. Phylogenetic nomenclature proposes that modern classification should reflect phylogenetic relationships and not support paraphyly (De Queiroz and Gauthier 1994) even suggesting the removal of nomenclatural ranks. Often an emphasis on monophyly as a criterion for rank assignment takes precedence over paraphyly that could highlight evolutionary novelty (Hörandl 2007, Hörandl and Stuessy 2010). The novel origin of these parasites from their hosts, an evolutionary relationship that is common in red algal parasites but unknown in other parasitisms, should be reflected in the taxonomy over the changes in morphology due to the parasitic mode. The incorporation of the parasites into the host genus also changes the morphological circumscription of the host genus. We feel that this is not a serious issue as the genus can be amended to include "and the parasites derived from it," but this needs to be tested as some parasites are not so closely related to their hosts (Zuccarello et al. 2004). The first example to follow this practice is the transfer of *Congracilaria babae* Yamamoto to *Gracilaria babae* (Yamamoto) P. -K. Ng et al. as it is nested in the host genus (Ng et al. 2014). This is the first time a new species of red algal parasite has been described and placed in the hosts genus.

Another character used in the description of red algal parasites was the presence of sporophyte and gametophyte of the parasite on the same host plant (Wynne and Scott 1989). At this point of the study, while gametophytes and sporophytes were collected from the same location we did not note if they are from the same host plant. Further investigation of this character needs to be done to fulfil designation of all previous mentioned characters of red algal parasites.

The parasite penetrates beyond the superficial cells of *R. membranacea*. 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 and Guimarães 1999). This *Rhodophyllis* parasite also produces 2nd PC between host and parasite cells, which have been observed in all described red algal parasites to date (Goff and Zuccarello 1994, Kraft and Abbott 2002). This suggests that 2nd pit connections are an essential feature not only of the early developmental stages of the parasite (Zuccarello et al. 2004) but are also essential for parasite-host development (Goff and Coleman 1985, Zuccarello and West 1994). 2nd pit connections allow the parasite to transfer nuclei and cytoplasmic organelles to host cells (Goff and Coleman 1984) and appear to give the parasite control over the host cells (Goff and Coleman 1987); the parasite changes the metabolism within the infected host cells and transforms the morphology of host cells (Goff and Zuccarello 1994). Even though 2nd pit connections were observed between the parasite and *R. membranacea*, changes in host cell morphology were not observed.

The three ITS2 ribotypes and *cox*1 haplotypes each found in this study were not exclusive to hosts or parasites. Identical sequences could be due to host contamination or due to close phylogenetic similarity between parasites and hosts. The potential for host contamination was minimized by using parasite tissue from areas further away from the base of the parasite and presumably without embedded host cells. Host contamination is unlikely but cannot be excluded. It is also possible that due to the close relationship of the host and the parasite (i.e., recent divergence) lineage sorting has not occurred between the two entities.

The red algal parasite of *R. membranacea* is widespread and can be found almost all year throughout New Zealand. The earliest herbarium sample of *R. membranacea* and its parasites f was collected in 1968 but the parasite was not described. Only two or three of the herbarium vouchers examined mentioned parasites at all. The small size may be one reason the parasite was overlooked. The diversity of red algal parasites in the world is probably higher than expected and new red algal parasite species are still being described (Kim and Cho 2010). There are probably many undescribed red algal parasite species in New Zealand as New Zealand is known for its high ende-

mism and biodiversity (Gordon et al. 2010). Although distribution patterns of red algal parasites on their host are generally unknown, herbarium samples can be helpful in determining distribution patterns over time and space (Nelson et al. 2013). For example, *R. membranacea* is also know from Australia (Womersley 1994). It would be interesting to check herbarium samples of *R. membranacea* from Australian museums for the parasite to extend the distribution of the parasite.

We describe this parasite on *R. membranacea* for the first time.

Rhodophyllis parasitica Preuss et Zuccarello sp. nov.

Diagnosis. Thalli coloured (light reddish), size 2.5 × 3 mm, branches mostly irregular to bipinnately branched, and terete with smooth surface and blunt branch tips. Bisexual gametophyte. Carposporophyte 230 × 330 µm, surrounded by pericarp, containing oval carposporangia. Carposporangia 20 µm in diameter, located marginally on branches. Spermatangia 10 µm in diameter, in dense patches on thallus surface. Tetrasporangia scattered on surface, zonately divided, 48 × 20 µm. Parasitic on *Rhodophyllis membranacea* (Harvey) J. D. Hooker and Harvey.

Type locality. RPPZ0212; 41°20′33″ S, 174°47′6″ E; growing on *R. membranacea*, collected as drift on Feb 27, 2012 at Houghton Bay, New Zealand.

Holotype. WELT A032962; collected by M. Preuss.

GenBank accession numbers. COI: KM407523; ITS2: KM407520; *cox* 2-3 spacer: KM407523; RuBisCO spacer: KM407518.

Etymology. The specific epithet refers to the parasitic lifestyle of this red alga.

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