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

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

Key Words: Biodiversity, Ceramiales, Emery's rule, Gigartinales, Monophyletic taxonomy, Parasitism, Phylogenetics, Plastid capture, Rhodophyta, Speciation

INTRODUCTION

Red algal parasites, a poorly studied polyphyletic category with many unique features, are found exclusively on red algal species in eight orders within the Florideophyceae (Blouin & Lane 2015; Preuss *et al*. 2017). Approximately 120 species have been described world-wide but their diversity is probably severely underestimated due to their small size and patchy distribution (Preuss *et al*. 2017). Four key characters are used to identify red algae as parasitic: (1) reduced size, (2) lack of or reduced pigmentation, (3) formation of secondary pit connection between parasite and host cells and (4) both gametophytic and sporophytic parasite life stages on the same host stage (Wynne & Scott 1989). In the past, similarities in reproductive structures were used to indicate a close taxonomic relationship ('adelphoparasites') or more distant relationship ('alloparasites') between parasite and host combinations (Goff 1982); whereas, more recent phylogenetic data indicate a continuum of relatedness between hosts and parasites (e.g. Zuccarello *et al*. 2004; Blouin & Lane 2012).

The close relationship between most red algal parasites and their hosts led to a hypothesis that these parasites evolved directly from their hosts (Setchell 1918), consistent with the entomological concept known as 'Emery's rule' (Emery 1909). Later molecular evidence supported Emery's rule (Goff *et al*. 1997) but also revealed varied phylogenetic relationships. Several studies showed that some parasites are more closely related to their hosts than the host is to other

species in the same genus (Goff *et al*. 1997; Zuccarello *et al*. 2004; Preuss & Zuccarello 2014), while parasites with multiple hosts in different genera [e.g. *Harveyella mirabilis* (Reinsch) F.Schmitz & Reinke] have undergone host switching (Zuccarello *et al*. 2004; Kurihara *et al*. 2010). Previous studies indicated that the plastid was mobile between hosts and parasites with the parasite 'capturing' the host plastid (Goff & Coleman 1995; Goff *et al*. 1996), a phenomenon that was not seen with the mitochondria (Goff & Coleman 1995). This led to varied relationships between hosts and parasites using plastid sequence data and could be used to indicate parasite origins and host switching. For example, parasites can have similar, occasionally nearly identical, plastid gene sequences to the host (e.g. *Rhodophyllis parasitica* M.Preuss & Zuccarello; Preuss & Zuccarello 2014), matching the relationships of the nuclear and mitochondrial markers, indicating a recent evolution from the host species. Parasites can have plastids more closely related to another species of host from the host they are found on [e.g. *Gracilaria babae* (H.Yamamoto) P.K.Ng, P.E.Lim & Phang; Ng *et al*. 2014], indicating that the parasite acquired its plastids from a previous host. Recent studies have also shown that parasites can have a highly reduced plastid genome relative to that of the host (e.g. *Choreocolax polysiphoniae* Reinsch; Salomaki e*t al*. 2015), possibly indicating a long history of parasitism.

Previously, newly described parasites were grouped into independent parasitic genera (e.g. Kraft & Abbott 2002; Townsend & Huisman 2004; Vergés *et al*. 2005; Kim & Cho 2010). Several phylogenetic studies have now shown that parasites and hosts are often closely related to each other, using nuclear and mitochondrial markers, and parasites have

origins within the host genus but still distinct parasite generic names were retained (e.g. Goff *et al.* 1996; Kurihara *et al.* 2010). Newer studies support a strictly monophyletic scheme reflecting the integration of the parasites into the host genus based on phylogenetic support (Ng *et al.* 2014; Preuss & Zuccarello 2014).

Of the 120 recognised red algal parasite species, 10 are currently known from New Zealand (Preuss *et al.* 2017). Five species were described from New Zealand, and the others were recorded for New Zealand but described from other parts of the world. The five described parasites from New Zealand are *Colacopsis lophurellae* Kylin, *Gloiocolax novae-zelandiae* Sparling, *Gonimophyllum insulare* F.S.Wagner, *Pterocladiophila hemisphaerica* K.C.Fan & Papenfuss and *Rhodophyllis parasitica*. The five remaining species are *Callocolax neglectus* F.Schmitz & Batters, *Choreonema thuretii* (Bornet) F.Schmitz, *Colacodasya inconspicua* (Reinsch) F.Schmitz, *Microcolax botryocarpa* (Hooker f. & Harvey) F.Schmitz and *Sporoglossum lophurellae* Kylin. Molecular data are available only for *Rhodophyllis parasitica* (Preuss & Zuccarello 2014).

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

MATERIAL AND METHODS

Samples were collected mostly as drift around New Zealand (Table S1). All specimens were pressed as herbarium vouchers, dried in silica gel or fixed in 2% glutaraldehyde in phosphate buffer (0.1 M, pH 6.8) in 50% seawater.

For anatomical observations, sections were either embedded in resin following Preuss & Zuccarello (2014) or hand sectioning with a razor blade. Sections were stained with 1% acidified aniline blue in either water or 50% karo syrup (ACH Food Co., Englewood Cliffs, New Jersey USA). Samples were examined using Olympus AX-70 and Olympus BX53 microscopes (Tokyo, Japan) with integrated cameras (Olympus DP-70, Olympus SC100), and images were captured using Olympus CellSens Software (2017).

DNA was extracted either using 5% Chelex following Zuccarello *et al.* (1999) or following a modified cetyl trimethyl ammonium bromide protocol (Zuccarello & Lokhorst 2005). Mitochondrial (*cox*1), nuclear [actin, large subunit (LSU) rDNA, small subunit (SSU) rDNA] and plastid (*rbc*L) markers were used for analysis (Table S2). Polymerase chain reaction (PCR) conditions for actin amplification were as follows: initial denaturation at 94°C for 5 min, followed by nine cycles of 94°C/55°C/72°C for 1 min each, followed by 29 cycles of 94°C for 30 s, 45°C and 72°C for 1 min and a final step at 72°C for 10 min. PCR conditions for all other genes were carried out with an initial denaturation at 94°C for 5 min, followed by 36 cycles of 94°C/45°C/72°C for 1 min each and a final step at 72°C for 5 min. Successful amplifications were purified using ExoSAP-

IT following manufacturer's instructions (USB product; Affymetrix, Santa Clara, California USA) and commercially sequenced (Macrogen Inc., Seoul, South Korea).

New sequences were assembled and edited in Geneious 8.0.5 (http://www.geneious.com, Kearse *et al.* 2012). Genbank sequences were added to the alignments following D'Archino *et al.* (2017) or using the closest Basic Local Alignment Search Tool search hits (Table S3). Multiple Alignment using Fast Fourier Transform alignments implemented in Geneious were used and modified by eye. Bayesian inference was performed with MrBayes v.3.2.5 (Ronquist & Huelsenbeck 2003). Analyses consisted of two independent simultaneous runs of one cold and three incrementally heated chains, and 3×10^6 generations with sampling every 1000 generations. A 'burn-in' of 5×10^5 generations was used, and 25,000 trees were saved to make the consensus tree. RAxML 7.2.8 (Stamatakis 2006) was used to construct maximum-likelihood (ML) trees to show the most likely tree from the data set. RAxML was performed using the General Time Reversible + gamma model and 500 non-parametric bootstrap replicates (Felsenstein 1985). RAxML and Bayesian inference was performed with all three codons partitioned for *cox*1 and *rbc*L. Phylogenies of *cox*1, LSU and *rbc*L sequences of the parasite growing on *Blastophyllis* spp. and of *cox*1 and LSU sequences of the parasite growing on *Cladhymenia* sp. were congruent (Supplementary Figs S1–S5), and the data sets were concatenated (with partition for LSU and partitioned codons for *cox*1 and *rbc*L) for a more robust phylogeny.

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

Unique sequences were deposited in Genbank (MF319122–MF319182).

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

RESULTS

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

Parasite on *Phycodrys novae-zelandiae*

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

Partial *cox*1 sequences (623 bp) were obtained for six samples of *Phycodrys novae-zelandiae* and three of its parasites. Genetic distances within *P. novae-zelandiae* ranged between 0.16 and 0.64% (1 and 4 bp), and between the parasite and host 0.0 and 1.12% (0 and 9 bp) and up to 0.8% (5 bp) between parasite specimens. Four haplotypes were found: C1–
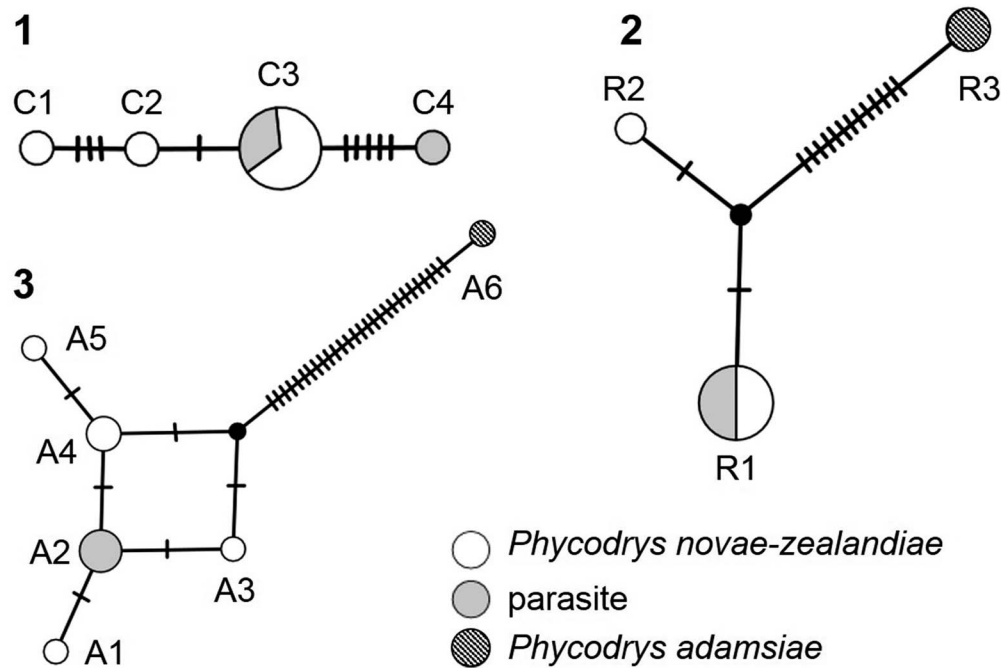
Figs 1–3. DNA sequence networks of *Phycodrys novae-zelandiae*, its parasite *Phycodrys novae-zelandiaephila* and *Phycodrys adamsiae*.
  Fig. 1. *Cox*1 haplotype network with four different haplotypes (C1–C4).
  Fig. 2. *Rbc*L haplotype network with three different haplotypes (R1–R3).
  Fig. 3. Actin haplotype network with six haplotypes (A1–A6). Small dark circle represents missing intermediates, lines = one mutational step. Parasite, host and *P. adamsiae* haplotypes highlighted in white, gray and hatched, respectively.

C4. Five hosts and two parasites had Haplotype C3, while C1 and C2 were represented in one host specimen each. C4 was represented in one parasite specimen (Fig. 1).

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

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

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

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

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Phycodrys novae-zelandiaephila
M.Preuss & Zuccarello *sp. nov.*
Figs 4–12

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

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

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

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

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

ETYMOLOGY: *novae-zelandiaephila* refers to the parasite's affinity to its host *Phycodrys novae-zelandiae*.

DISTRIBUTION: The collection at Te Papa contained 52 specimens of *Phycodrys novae-zelandiae* of which red algal parasites were observed on nine. The parasite was found from Mataikona (40°47′S) on the North Island to Stewart Island (46°55′S), south of the South Island (Table S4).

Habitat and vegetative morphology

Phycodrys novae-zelandiaephila grew on blades of *Phycodrys novae-zelandiae*, which had over 20 parasites on one blade, usually growing on the veins of the host (Fig. 4). *Phycodrys novae-zelandiaephila* was found in spring (September, November), summer (January, February) and autumn (March, April) (Table S1, S4).
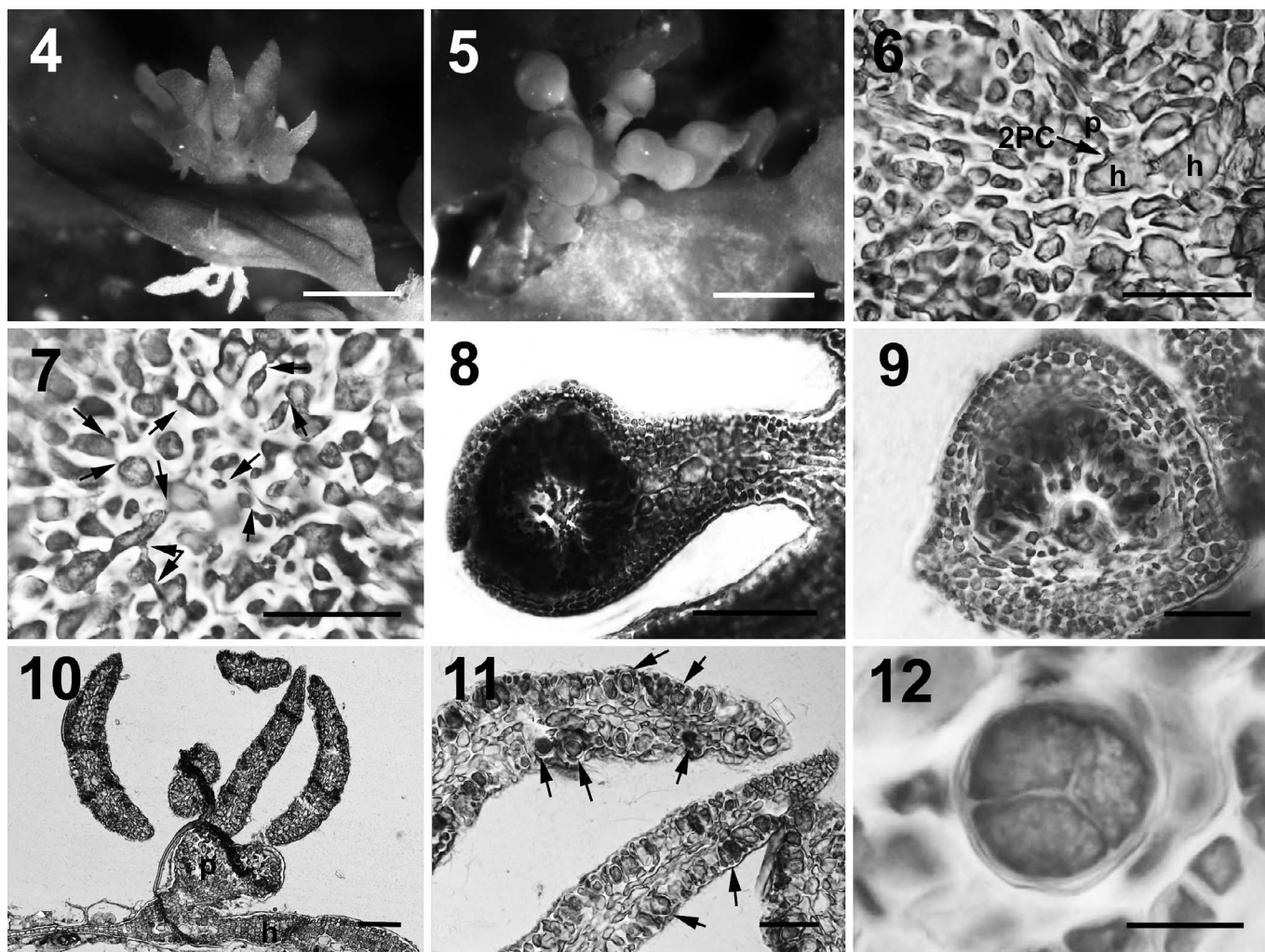
Figs 4–12. Vegetative and reproductive structures of *Phycodrys novae-zelandiaephila sp. nov.* on its host *Phycodrys novae-zelandiae*.
　　Fig. 4. Habit of a tetrasporophytic parasite growing on the central vein of its host. Scale bar = 1 mm.
　　Fig. 5. Habit of cystocarpic gametophyte growing on host thallus. Scale bar = 1 mm.
　　Fig. 6. Contact area between parasite and host. Parasite cell (p) forms secondary pit connection (2PC; arrow) with host cell (h). Scale bar = 100 μm.
　　Fig. 7. Parasite cells are highly connected with each other. Arrows indicate pit connections. Scale bar = 100 μm.
　　Fig. 8. Branch with mature cystocarp of parasite. Central fusion cell visible. Scale bar = 250 μm.
　　Fig. 9. Close-up of cystocarp of parasite, showing pericarp of approximately five cell layers. Scale bar = 100 μm.
　　Fig. 10. Cross section of parasite (p) tetrasporangial stichidia on its hosts (h). Scale bar = 200 μm.
　　Fig. 11. Tetraspores scattered on the surface of the tetrasporangial stichidia, tetrasporangia indicated by arrows. Scale bar = 100 μm.
　　Fig. 12. Mature tetrahedrally divided tetrasporangium. Scale bar = 20 μm.

The thallus was light red, 1–2 mm in size (Figs 4, 5). It had a single base that penetrated and disrupted the cell layers of the host. Host cells were embedded between parasite tissue in the contact area. Secondary pit connections were found between large host cells and smaller parasitic cells in the contact area (Fig. 6). The cells within the main body of the parasite thallus were highly connected, by either primary or secondary pit connections (Fig. 7).

Reproductive morphology

Female gametophytes and tetrasporophytes were observed. Thalli bear branches with either fusiform stichidia bearing tetraspores (Fig. 4) or apical, rounded cystocarps (Fig. 5). All observed parasites were reproductive but males were not found, suggesting dioecious gametophytes.

The female gametophyte had a narrow, pulvinate base that gave rise to several unbranched axes, most of which terminate in an apical cystocarp. Branches were polystromatic with a central axis of large cells surrounded by up to five layers of smaller cells. The inner layer of elongated cortical cells was spherical near the mature carposporophyte (Fig. 8). The mature carposporophytes were approximately 430–530 μm in diameter and surrounded by an approximately five cell thick pericarp (62 μm; *n* = 9). The carposporophyte had a single central fusion cell that gave rise to rows of gonimoblast filaments. Carpospores were
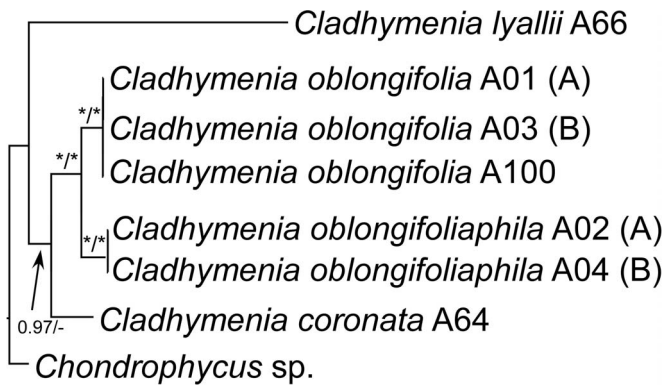
Fig. 13. Bayesian topology of concatenated *cox*1 and LSU rRNA sequence dataset for *Cladhymenia oblongifolia*, its parasite *Cladhymenia oblongifoliaphila* and two other *Cladhymenia* species: *C. coronata* and *C. lyallii*. Host and parasite from the same host plant are highlighted by capital letters in parentheses (A, B). Details of collections in Table S1. Asterisks indicate posterior probability of 1.00 and bootstrap values of 100%. Values <85% ML bootstrap not shown. Outgroup was *Chondrophycus* sp.

borne in short chains of approximately four ovoid carpospores (19 × 10 μm; Fig. 9).

The tetrasporophyte grew from a rounded base approximately 500 μm in diameter. The base produced multiple simple fusiform branches that rarely branch again (Figs 4, 10). Branches had scattered stichidia on their surfaces. The stichidial branch was around 654 × 207 μm (*n* = 2) in size (Fig. 10) with two to three inner layers of elongated cells and scattered globose tetrasporangia on the surface (Fig. 11). Tetrasporangia were tetrahedrally divided approximately 32 × 40 μm (*n* = 10; Fig. 12).

Comparison to host species and other parasites on congeneric host species

The parasite shared carpospores borne in chains, from a central fusion cell and tetrahedrally divided tetraspores with its host species (*Phycodrys novae-zelandiae*) and two other *Phycodrys* species (*Phycodrys adamsiae*, *Phycodrys franiae* Showe M.Lin & W.A.Nelson) but differed in most other characters (Table S5). The new parasite was similar to other parasites [*Asterocolax denticulatus* (Tokida) Feldmann & Feldmann-Maz., *Asterocolax gardneri* (Setchell) Feldmann & Feldmann-Maz.] on *Phycodrys* spp., with similar thalli size and pigmentation, tetrahedrally divided tetraspores shattered over the surface, and apical cystocarp that were borne on branches. It differed in geographical distribution and host species (Table S6).

Parasite on *Cladhymenia oblongifolia*

The concatenated data set (1613 bp) of *cox*1 and LSU rRNA contained eight samples of two parasite samples and their hosts, an uninfected *Cladhymenia oblongifolia* and two other *Cladhymenia* species (individual gene data sets were similar – supplementary data Figs 1, 2). This dataset supported the shared origin of the parasite and its host *C. oblongifolia* with strong support (Fig. 13). *Cladhymenia coronata* (Lindauer & Setchell) P.Saenger and *Cladhymenia lyallii* Harvey were distinct from *C. oblongifolia* and its parasite.
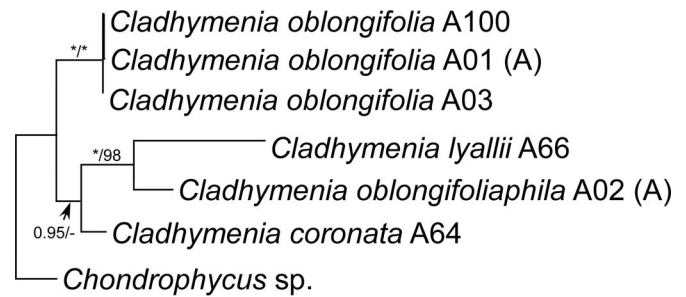

Fig. 14. Bayesian topology of *rbc*L relationships for *Cladhymenia oblongifolia*, its parasite *Cladhymenia oblongifoliaphila* and two other *Cladhymenia* species: *C. coronata* and *C. lyallii*. Parasite and host combination is highlighted by capital letter in parentheses (A). Details of collections in Table S1. Asterisks indicate posterior probability of 1.00 and bootstrap value of 100%. Values <85% ML bootstrap not shown. Outgroup used was *Chondrophycus* sp.

The partial *rbc*L data set (537 bp) contained taxa representative of all three *Cladhymenia* species in New Zealand. All samples of *Cladhymenia oblongifolia* grouped with high support (Fig. 14). The parasite grouped with *Cladhymenia lyallii* with high support, and not *C. oblongifolia* as with the previous markers, and both appeared to be sister to *Cladhymenia coronata* but this relationship was only supported in the ML analysis.

The phylogenetic data of the parasite growing on *Cladhymenia oblongifolia* showed two different patterns. Mitochondrial (*cox*1) and nuclear (LSU rRNA) data showed a shared ancestry of the parasite and host. The plastid marker showed a common ancestor between the parasite plastid and plastids of *Cladhymenia lyallii*.

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

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Cladhymenia oblongifoliaphila
M.Preuss & Zuccarello *sp. nov.*
Figs 15–23

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

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

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

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

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

ETYMOLOGY: *oblongifoliaphila* refers to the preference of the parasite to grow on *Cladhymenia oblongifolia*.
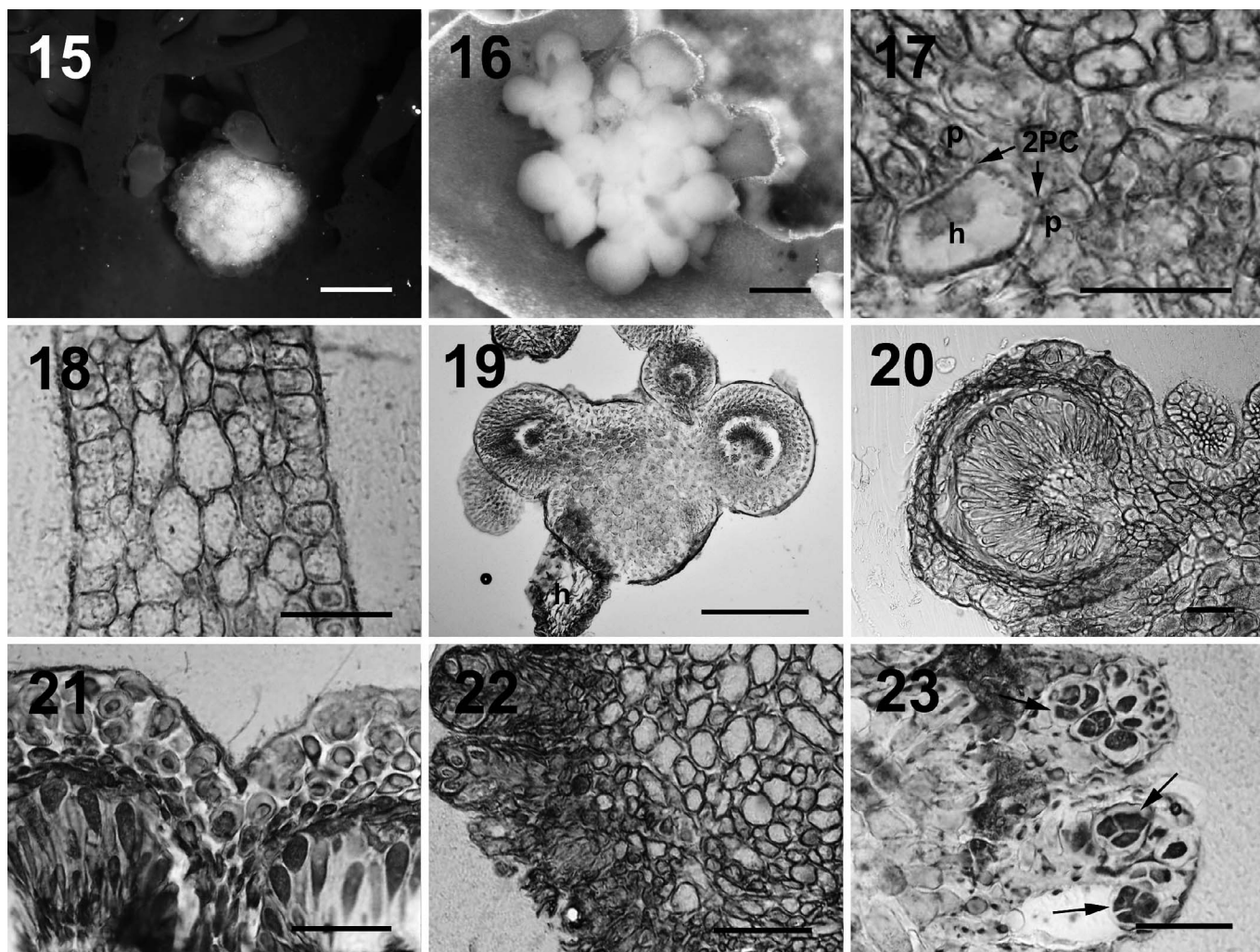
Figs 15–23. Vegetative and reproductive structures of *Cladhymenia oblongifoliaphila* and its host *Cladhymenia oblongifolia*.

Fig. 15. Tetrasporophytic parasite thallus growing between the lateral proliferations of its host. Scale bar = 1 mm.

Fig. 16. Parasitic female gametophyte, with enlarged cystocarps, on host. Scale bar = 550 μm.

Fig. 17. Contact area between parasite and host cells. Parasite cell (p) forms secondary pit connection (2PC; arrow) with host cell (h). Scale bar = 100 μm.

Fig. 18. Internal anatomy of uninfected host, *Cladhymenia oblongifolia*, not disrupted by parasite penetration and used as guide for distinguishing between parasite and host cells in the contact area. Scale bar = 100 μm.

Fig. 19. Cross section of female *Cladhymenia oblongifoliaphila* with multiple cystocarps. Scale bar = 500 μm.

Fig. 20. Close-up of cystocarp, showing thick ostiole-less pericarp and carposporophyte. Scale bar = 100 μm.

Fig. 21. Close-up of pericarp and elongated carpospores. Scale bar = 100 μm.

Fig. 22. Tetrasporophytic thallus, showing internal anatomy and cluster of tetrasporangia. Scale bar = 200 μm.

Fig. 23. Tetrasporic clusters with tetrahedrally divided tetraspores (arrows). Scale bar = 100 μm.

DISTRIBUTION: Te Papa herbarium collections contained 91 specimens of *Cladhymenia oblongifolia*, and on eight of these, parasites were observed (Table S7). The parasite was found from the north (36°57′S) to the south of the North Island (41°21′S) and on the Chatham Islands (latitude = 44°16′S). The parasite is not common and has a patchy distribution.

Habitat and vegetative morphology

One host plant had over 20 parasites growing on the blade edges and marginal proliferations (Fig. 15). The parasite was found in spring (September, November), summer (January, February) and autumn months (March, April) in New Zealand (Tables S1 and S7).

The parasite thallus was not pigmented, approximately 2 mm in diameter (Fig. 16). The base of the parasite penetrated deeply into the host thallus. Host and parasite cells were intermixed in the contact area. Secondary pit connections were found between small parasite cells and larger host cells in the contact area (Fig. 17). The vegetative structure of the host *Cladhymenia oblongifolia* consists of five inner layers of large cells, an outer layer of smaller epidermal cells and a cuticle (Fig. 18).

Reproductive morphology

Female gametophytes and tetrasporophytes were observed. Thalli bear either one rough roundish cushion (tetraspor-

ophyte; Fig. 15) or many smooth spheres of different size (female gametophyte; Fig. 16). All observed parasites were reproductive but males were not found. Female gametophytes were found on tetrasporophytic host plants.

Mature female gametophytes had *c*. 30 cystocarps, these were approximately 520–570 μm in diameter (Fig. 19). Pericarp had five to seven cell layers, approximately 100 μm thick, without an ostiole (Fig. 20). Carposporangia were clavate to lachrymiform, 55–100 × 14–24 μm (Fig. 21).

Internally the tetrasporophytes consisted of many round to oval large cells of different sizes (Fig. 22). The tetrasporophytes formed small clusters on their surface, which contained tetrasporangia. Branches were 300 μm long by 150 μm wide. Tetrasporangia were approximately 45–55 μm in diameter and tetrahedrally divided (Fig. 23).

Comparison between host and parasite

The parasite shared the location of tetrahedrally divided tetraspores and the location of cystocarps with its host *Cladhymenia oblongifolia* but differed in thallus size and pigmentation (Table S8).

Parasite on *Blastophyllis calliblepharoides* and *Blastophyllis hombroniana*

Individual trees of *cox*1, LSU rRNA and *rbc*L (Supplementary Figs S3–S5) showed that the parasite's closest relative is *Judithia delicatissima* (R.E.Norris) D'Archino & Showe M.Lin with high support for *cox*1 and *rbc*L and good support for LSU rRNA. The congruent results in all three markers supported a concatenated data set for a more robust phylogeny.

The concatenated data set (*cox*1, LSU rRNA and *rbc*L) contained 44 taxa and was 4827 bp long with representatives of the two host species and their parasites. This dataset showed strong support for the shared origin of the parasite and *Judithia delicatissima*. Both host species, *Blastophyllis calliblepharoides* and *Blastophyllis hombroniana*, grouped together with high support (Fig. 24) and were not closely related to their parasites but this relationship was not well supported.

The phylogenetic data for this parasite with markers of the three different genomes supported a shared ancestry of the parasite with *Judithia delicatissima*. *Callocolax neglectus* described on *Callophyllis laciniata* (Hudson) Kützing (Batters 1895) from Europe was once recorded on *Blastophyllis hombroniana* (as *Callophyllis hombroniana*) from New Zealand (Cotton 1907) but most New Zealand *Callophyllis* spp. were shown to be different genera within the Kallymeniaceae (D'Archino *et al.* 2016, 2017), and the shared ancestry with endemic *Judithia* suggested that this parasite is most likely a new parasite species.

Judithia parasitica M.Preuss & Zuccarello *sp. nov.*
Figs 25–30

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

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

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

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

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

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

DISTRIBUTION: Te Papa collections contained 44 specimens of *Blastophyllis calliblepharoides*, and parasites were observed on one specimen from Snares Island (48°01′S), a subantarctic island of New Zealand. On 17 of the 45 specimens of *Blastophyllis hombroniana* parasites were found. The specimens were from Bank Peninsula (43°45′S) on the South Island (46°36′S), on Stewart Island and on the Auckland Islands (50°30′S), a subantarctic island of New Zealand (Table S9).

Habitat and vegetative morphology

Judithia parasitica grows on *Blastophyllis calliblepharoides* (previously *Callophyllis calliblepharoides*) and *Blastophyllis hombroniana* (previously *Callophyllis hombroniana*). The position of the parasite and its abundance on the two hosts appeared similar. The hosts had up to a few hundred parasites growing mainly on the edges of the main axes or branches (Fig. 25). The parasite on *B. calliblepharoides* was found in autumn (April), summer (December), and on *B. hombroniana* in summer (December, January, February), autumn (March), winter (July, August) and spring (October, November) in New Zealand (Table S1, S9).

Thalli of *Judithia parasitica* were light red, with an average size of less than 1 mm (350–670 μm length to 700–890 μm in width). The parasite had a single, widely spreading base covering the host surface that did not penetrate deeply into the host thallus (Fig. 26). Parasite cells formed secondary pit connections with the top layer of cells (epidermal or subepidermal) of the host (Fig. 27). Parasite cells within the parasite thallus were highly connected to each other by either primary or secondary pit connections (Fig. 28).

Reproductive morphology

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

The base of the tetrasporophyte produced multiple branches with inner elongated large cells and outer roundish small cells. Branches had tetrasporangia scattered on the surface (Fig. 29). Tetrasporangia were cruciately divided, approximately 13 × 26 μm ($n = 6$; Fig. 30).

Comparison of the parasite and its closest relative *Judithia delicatissima*

Judithia parasitica sp. nov. shared scattered cruciately divided tetrasporangia of similar size with *Judithia delicatissima*. The
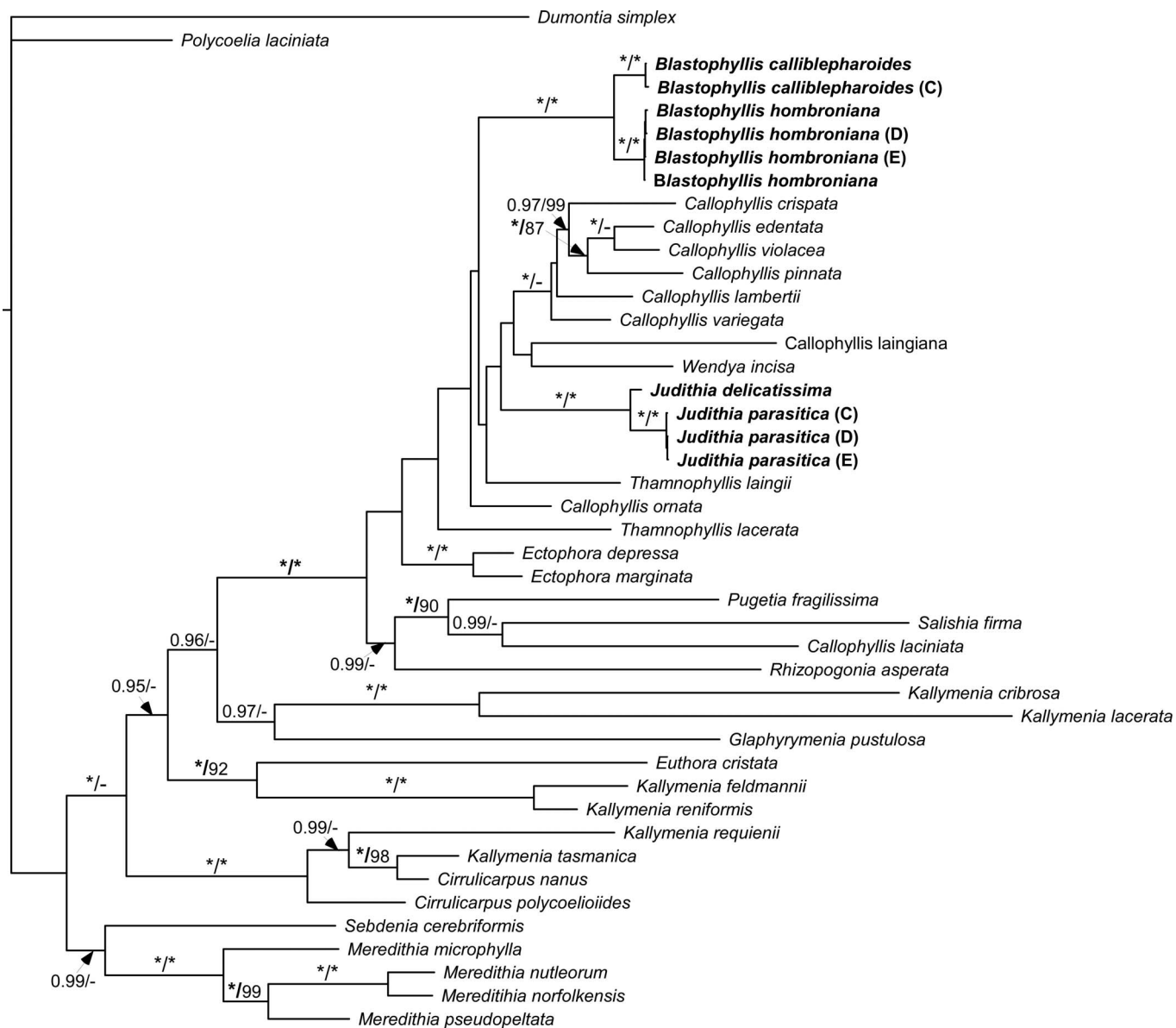
Fig. 24. Bayesian topology of concatenated *cox*1, *rbc*L and LSU rRNA sequence data for the parasite *Judithia parasitica* and both hosts *Blastophyllis calliblepharoides* and *Blastophyllis hombroniana* plus other representative within the Kallymeniaceae. Parasite and host combinations are highlighted by capital letters in parentheses (C–E). New (Table S1) and Genbank samples (Table S3) were combined. Asterisks indicate posterior probability value of 1.00 and bootstrap value of 100%. Values <0.85 posterior probability and <85% ML bootstrap not shown. Outgroups *Dumontia simplex* Cotton and *Polycoelia laciniata* J.Agardh were removed to facilitate presentation.

parasite differed is overall thallus size and branching (Table S10).

DISCUSSION

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

A previous study hypothesized three different evolutionary scenarios of red algal parasite origin (Goff *et al*. 1996). First, some parasites (e.g. *Bostrychiocolax australis* Zuccarello & J.A.West, *Gardneriella tuberifera* Kylin, *Rhodophyllis parasitica*) evolved from their hosts and solely infect this host species (Goff *et al*. 1996; Zuccarello *et al*. 2004; Preuss & Zuccarello 2014). Second, parasites (e.g. *Faucheocolax attenuata* Setchell) evolved and grow on one host species but also parasitize a second, closely related host species (Goff *et al*. 1996). Third, some parasites (e.g. *Plocamiocolax pulvinata* Setchell) evolved on one species but now parasitize a secondary host and were lost from the original host species (Goff *et al*. 1996). Our data reflect the first and third scenario and reveal a possible fourth scenario.
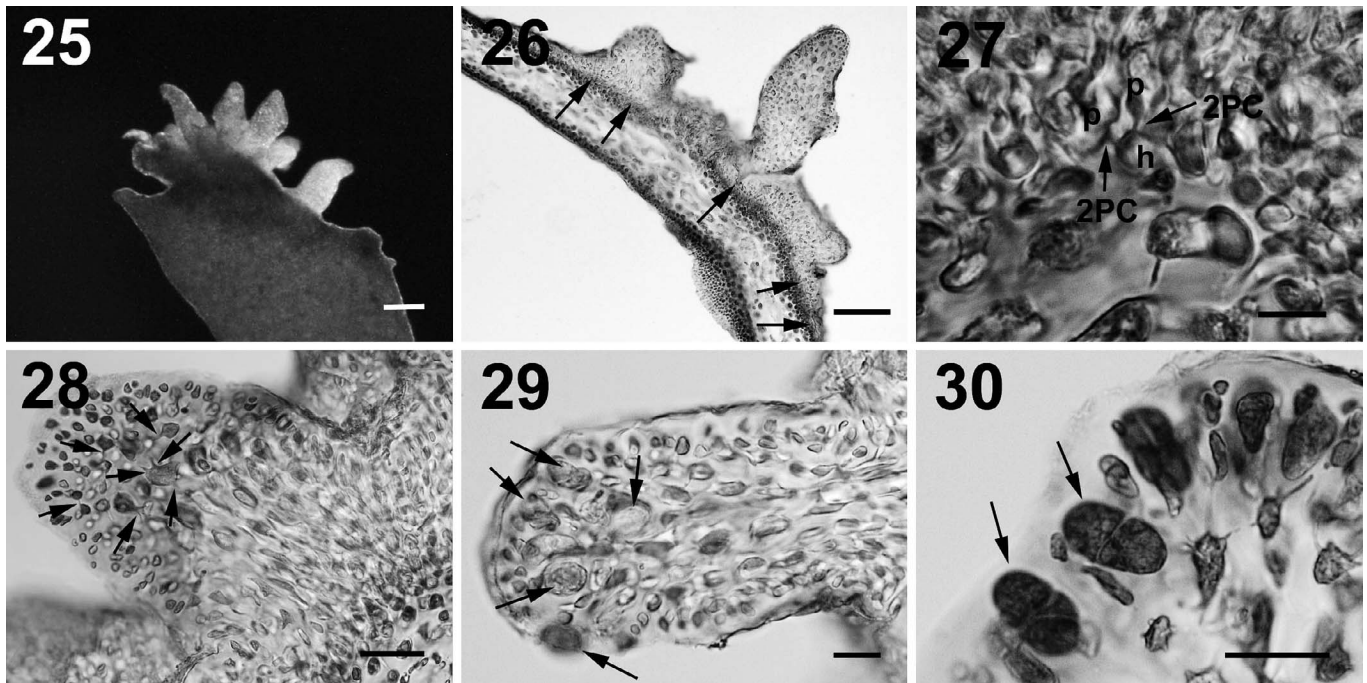
Figs 25–30. Vegetative and reproductive structures of *Judithia parasitica* growing on its host *Blastophyllis hombroniana*.
Fig. 25. Tetrasporophytic parasite on the edge of the host blade. Scale bar = 200 μm.
Fig. 26. Cross section of parasite thallus with wide base (arrows) growing over the host thallus. Scale bar = 10 μm.
Fig. 27. Parasite cells (p) with secondary pit connection (2PC; arrows) to pigmented host cells (h). Scale bar = 20 μm.
Fig. 28. Parasite cells are highly connected with each other by primary and secondary pit connections (arrows). Scale bar = 100 μm.
Fig. 29. Longitudinal section of tetrasporic branch with tetrasporangia (arrows) scattered over the surface. Scale bar = 20 μm.
Fig. 30. Close-up of cruciately divided tetrasporangia (arrows). Scale bar = 20 μm.

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

Phycodrys novae-zelandiaephila is an example of a parasite that is genetically nearly indistinguishable from its host and found only on this host species. Genetic distances are higher within the host than between parasite and host. Other parasites have been recorded on *Phycodrys* species from other parts of the world (*Asterocolax denticulatus*, *Asterocolax gardneri*, *Choreocolax rabenhorstii* Reinsch). The common origin of *P. novae-zelandiaephila* with its host, and the close phylogenetic relationship of other *Phycodrys* parasites to their hosts (Goff *et al*. 1997), suggests that

parasites have evolved multiple times in this genus. Why parasites have evolved so many times in some genera is not yet known.

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

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

Morphological characters of the three parasites are congruent with their phylogenetic relationships and confirm them as red algal parasites. All three parasites were small, with reduced or no pigmentation and formed secondary pit connections to their host's cells (Goff 1982; Wynne & Scott 1989; Preuss *et al.* 2017). These criteria are the basis for many determinations of species as parasitic; nutrient status and detriment to the host have been seldom investigated (Martin & Pocock 1953; Goff 1976, 1982; Kremer 1983; Apt 1984).

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

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

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

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found online at http://dx.doi.org/10.2216/17-36.1.s1.

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Corrigendum

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

Two of the described species names are not in accordance with the International Code of Nomenclature for algae, fungi, and plants (Art 60.G.1, Orthography)

(1) removing the case ending of the genitive singular (Latin *-ae*, *-i*, *-us*, *-is*; transcribed Greek*-ou*, *-os*, *-es*, *-as*, *-ous* and its equivalent *-eos*) and
(2) before a consonant, adding a connecting vowel (*-i-* for Latin elements, *-o-* for Greek elements)

and the correct names are proposed below.

Cladhymenia oblongifoliophila **M.Preuss & Zucccarello** *sp. nov.*
The diagnosis is provided in *Phycologia* 57: 13 (2018) and Figures 13–23.

Phycodrys novae-zelandiophila **M.Preuss & Zuccarello** *sp. nov.*
The diagnosis is in *Phycologia* 57: 11 (2018) and Figures 1–12.

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Queries for phya-57-04-13

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