

doi.org/10.3114/fuse.2021.07.11

Sirolopidium bryopsidis, a parasite of green algae, is probably conspecific with *Pontisma lagenidioides*, a parasite of red algae

A.T. Buaya¹, B. Scholz², M. Thines^{1,3,4*}

¹Senckenberg Biodiversity and Climate Research Center, Senckenberganlage 25, D-60325 Frankfurt am Main, Germany

²BioPol ehf, Marine Biotechnology, Einbúastíg 2, 545 Skagaströnd, Iceland

³Goethe-University Frankfurt am Main, Department of Biological Sciences, Institute of Ecology, Evolution and Diversity, Max-von-Laue Str. 13, D-60438 Frankfurt am Main, Germany

⁴LOEWE Centre for Translational Biodiversity Genomics, Georg-Voigt-Str. 14-16, D-60325 Frankfurt am Main, Germany

*Corresponding author: m.thines@thines-lab.eu

Key words:

chlorophyte algae
early-diverging
new taxa
Oomycota
Petersenia
phylogeny
Pontismataceae
Sirolopidiaceae
taxonomy
type species

Abstract: The genus *Sirolopidium* (*Sirolopidiaceae*) of the *Oomycota* includes several species of holocarpic obligate aquatic parasites. These organisms are widely occurring in marine and freshwater habitats, mostly infecting filamentous green algae. Presently, all species are only known from their morphology and descriptive life cycle traits. None of the seven species classified in *Sirolopidium*, including the type species, *S. bryopsidis*, has been rediscovered and studied for their molecular phylogeny, so far. Originally, the genus was established to accommodate all parasites of filamentous marine green algae. In the past few decades, however, *Sirolopidium* has undergone multiple taxonomic revisions and several species parasitic in other host groups were added to the genus. While the phylogeny of the marine rhodophyte- and phaeophyte-infecting genera *Pontisma* and *Eurychasma*, respectively, has only been resolved recently, the taxonomic placement of the chlorophyte-infecting genus *Sirolopidium* remained unresolved. In the present study, we report the phylogenetic placement of *Sirolopidium bryopsidis* infecting the filamentous marine green algae *Capsosiphon fulvescens* sampled from Skagaströnd in Northwest Iceland. Phylogenetic reconstructions revealed that *S. bryopsidis* is either conspecific or at least very closely related to the type species of *Pontisma*, *Po. lagenidioides*. Consequently, the type species of genus *Sirolopidium*, *S. bryopsidis*, is reclassified to *Pontisma*. Further infection trials are needed to determine if *Po. bryopsidis* and *Po. lagenidioides* are conspecific or closely related. In either case, the apparently recent host jump from red to green algae is remarkable, as it opens the possibility for radiation in a largely divergent eukaryotic lineage.

Citation: Buaya AT, Scholz B, Thines M (2021). *Sirolopidium bryopsidis*, a parasite of green algae, is probably conspecific with *Pontisma lagenidioides*, a parasite of red algae. *Fungal Systematics and Evolution* 7: 223–231. doi: 10.3114/fuse.2021.07.11

Received: 18 January 2021; **Accepted:** 22 February 2021; **Effectively published online:** 5 March 2021

Corresponding editor: P.W. Crous

INTRODUCTION

Holocarpic oomycete parasites of macroalgae are diverse and have been reported from various hosts in different aquatic habitats (oceans, brackish water, rivers, lakes, ponds and swamps) (Sparrow 1960, Beakes & Thines 2017, Buaya & Thines 2020b). In the marine environment, these obligate endoparasites mostly infect *Rhodophyte* algae (e.g. *Callithamnion corymbosum*, *Ceramium rubrum*), but some can also be found on phaeophytes (e.g. *Ectocarpus granulosus*, *Pylaiella littoralis*) or chlorophytes (e.g. *Bryopsis plumosa*, *Cladophora japonica*) (Karling 1942, Sparrow 1960). Specifically, these organisms were classified into the early diverging oomycete genera *Anislopidium* (Karling 1943), *Ectrogella* (Zopf 1884), *Eurychasma* (Magnus 1905), *Eurychasmidium* (Sparrow 1936), *Pontisma* (Petersen 1905), *Petersenia* (Sparrow 1934), and *Sirolopidium* (Petersen 1905). Most species have been reported from cool temperate regions. Some of these pathogens (e.g. *Pontisma porphyrae*, *Po. bostrychiae*) are known to be destructive pathogens of several

economically important macroalgae (e.g. *Porphyra*, *Pyropia*) in marine aquaculture (Sekimoto *et al.* 2008b, 2009, Kwak *et al.* 2017, Buaya & Thines 2020b).

Over the past few decades several studies were conducted that included oomycetes infecting marine macroalgae. These were focused on various topics, such as new species descriptions (Sekimoto *et al.* 2008b, 2009, Klochkova *et al.* 2016, 2017, Kwak *et al.* 2017, Badis *et al.* 2019), phylogenetic relationships (Küpper *et al.* 2006, Sekimoto *et al.* 2008a, Fletcher *et al.* 2015, Gachon *et al.* 2017, Buaya *et al.* 2019c), environmental sequencing (Garvetto *et al.* 2018, Hassett *et al.* 2019), host range determination (Raghukumar 1986, Müller *et al.* 1999, West *et al.* 2006, Strittmatter *et al.* 2009, Gachon *et al.* 2009, Klochkova *et al.* 2012), and host-parasite culture establishment (Müller *et al.* 2008, Gachon *et al.* 2017). These developments consequently resulted in the additions of half a dozen novel species (*Po. bostrychiae*, *Po. heterosiphoniae*, *Po. muelleri*, *Po. palmariae*, *Po. porphyrae*, *Po. pyropiae*), and the phylogenetic placement of several rediscovered and important species (*A. rosenvingei*,

A. ectocarpii, *Eu. dicksonii*, *Po. lagenidioides*) (Sekimoto et al. 2008a, b, 2009, Klochkova et al. 2016, 2017, Gachon et al. 2017, Badis et al. 2019, Buaya et al. 2019c). However, knowledge on the biology and ecology of these organisms remains limited despite these advancements and their potential as serious pathogens to many economically important algae in aquaculture (Strittmatter et al. 2009, Buaya & Thines 2020b).

Currently the phylogenetic affinities of most macroalgae-infecting species remain unresolved, since several of the previously described species have not been investigated for molecular phylogeny, including several key and type species (e.g. *A. sphacellarum*, *E. tumefaciens*, *Pe. lobata*, *S. bryopsisidis*) (Petersen 1905, Sparrow 1934, 1936). Other unsequenced taxa include marine species from the genera *Anisolpidium*, *Ectrogella*, *Eurychasma*, *Eurychasmidium*, *Pontisma*, *Petersenia*, and *Sirolopidium* (Petersen 1905, Dangeard 1912, Jokl 1916, Karling 1943, Sparrow 1943, 1969, Feldmann & Feldmann 1955, 1967, Whittick & South 1972, Van der Meer & Pueschel 1985, Dick 2001). Presently only 11 species (*A. rosenvingei*, *A. ectocarpii*, *Po. feldmanii*, *Po. bostrychiae*, *Po. heterosiphoniae*, *Po. muelleri*, *Po. palmariae*, *Po. porphyrae* and *Po. pyropiae*) (Sekimoto et al. 2008b, 2009, Klochkova et al. 2016, 2017, Gachon et al. 2017, Badis et al. 2019), including two type species (*Eu. dicksonii*, *Po. lagenidioides*) (Sekimoto et al. 2008a, Buaya et al. 2019c) have been investigated for their molecular phylogenetic relationships.

The genus *Pontisma* with its type species *Po. lagenidioides*, contains the largest monophyletic assemblage of marine parasites of red algae and was shown not to belong to the *Olpidiopsidales* (Buaya et al. 2019c). Although two genera infecting phaeophyte algae, *Anisolpidium* and *Eurychasma*, have also been investigated for their molecular phylogeny (Sekimoto et al. 2008a, Gachon et al. 2017), the phylogeny of another key genus, *Sirolopidium*, remains unresolved. The type species of the genus *Sirolopidium*, *S. bryopsisidis*, is known to commonly occur in marine filamentous green algae (e.g. *Bryopsis plumosa*, *Cladophora japonica*) in temperate zones (Petersen 1905, Sparrow 1936, Kobayashi & Ookubo 1953), but has not been reported recently.

Sirolopidium bryopsisidis was originally classified by de Bruyne (1890) as *Olpidium bryopsisidis*, and was initially thought to have fungus-like uniflagellate zoospores. However, Petersen (1905) discovered that the zoospores of the parasite are actually biflagellate, and subsequently reclassified the organism as *Sirolopidium bryopsisidis*, a placement taken up by later authors (Karling 1942, Sparrow 1942, 1960). Sparrow (1942, 1960) classified the genus *Sirolopidium* in its own family, *Sirolopidiaceae*, accepting the concept of Petersen (1909), who placed it in the *Lagenidiales*. Because of similarities in morphology and life cycle traits (e.g. thallus, discharge tube, and zoospore formation), Karling (1942) tentatively transferred the type species of genus *Pontisma*, *Po. lagenidioides* to *Sirolopidium*, and also included *Petersenia* in *Sirolopidiaceae*. Later, Sparrow (1960) excluded *Petersenia* from the *Sirolopidiaceae* (Sparrow 1960). Dick (2001) did a major revision of the taxonomic framework for holocarpic oomycetes, and regrouped the marine parasites in accordance with their host type, placing *Pontisma* and *Sirolopidium* in the families *Pontismataceae* (together with *Petersenia*) and *Sirolopidiaceae*, respectively. The taxonomic framework of Dick (2001) bears similarities to the early classification of Petersen (1909), which also divided the parasites of marine macroalgae according to their respective host type.

The taxonomy of the genus *Sirolopidium* remains unsettled, however, as none of its species has been rediscovered and investigated for their molecular phylogeny. Without these data, the relationships to other marine genera infecting macroalgae, especially *Pontisma* and *Petersenia* cannot be resolved, and the relationships of *S. andreei*, *S. glenodiniumum*, *S. globosum*, *S. marinum*, *S. paradoxum*, *S. salinum* and *S. zoophthorum* (Dangeard 1888, Petersen 1905, Dangeard 1912, Dangeard 1932, Vishniac 1955, Anastasiou & Churchland 1968, Dick 2001) to the type species, *S. bryopsisidis*, remain obscure.

As a first step towards resolving the phylogeny of the genus *Sirolopidium* and its type species *S. bryopsisidis*, efforts were made to isolate the organism from marine habitats of Iceland, where the species had been reported previously (Johnson 1968). While screening for biotrophic oomycetes from filamentous green algae (e.g. *Capsosiphon fulvescens*, *Cladophora* spp.) sampled during autumn of 2019 at the coast of Skagaströnd in Northwest Iceland, endobiotic holocarpic oomycetes with features resembling *S. bryopsisidis* were observed, enabling investigation of its life cycle traits and molecular phylogenetic affinities, which are reported in the current study.

MATERIALS AND METHODS

Collection, isolation and characterisation

The filamentous marine green algae *Capsosiphon fulvescens* was found on 11 September 2019 in Skagaströnd, Northwest Iceland (65°50'00.7"N 20°19'25.6"W). Samples were collected along the rocky intertidal zone by plucking the algae from their substrate and subsequent immersion in seawater in 1 L plastic bottles. Afterwards, samples were transferred into several 15 mL Petri-dishes containing seawater and screened for the presence of *Sirolopidium bryopsisidis* using an inverted compound light microscope (AE31, Motic, Xiamen). Infected algal filaments were carefully removed using a sterile forceps and scalpel (3C-SA, Rubis, Switzerland), rinsed multiple times in autoclaved seawater using a 10 µL micropipette (Brandt, Germany), and selected segments were immersed in 500 µL RNAlater solution (Invitrogen, Thermo Fisher, Lithuania) or 70 % ethanol (VWR, France) for subsequent DNA extraction or archiving, respectively. Approximately 60 pieces of infected filaments were collected for these purposes. A specimen preserved in 70 % ethanol was deposited in the herbarium collection of the Senckenberg Museum of Natural History, Cryptogams Section, Frankfurt am Main (accession: FR-0046127). Other infected filaments were mounted on microscopic slides after removal from the bulk sample using autoclaved seawater for morphological characterisation and life cycle observations as described earlier (Buaya et al. 2019c). For this a compound light microscope (Imager2, Carl Zeiss, Göttingen, Germany) with DIC was used, and photographs were taken using a Zeiss Axiocam MRc5 camera (Carl Zeiss, Göttingen, Germany). In addition, cellulose presence in the thallus wall of the parasite was tested using zinc iodine chloride (Carl Roth GmbH, Germany) solution. The identity of the green algae that hosted the pathogen was determined by light microscopy and 18S sequence barcoding. Axenic host and parasite dual culture of the isolated endoparasitoid was attempted using Guillard's f/2 medium (Guillard & Ryther 1962) and Provasoli enriched Seawater Medium (Provasoli 1968) as previously described (Buaya et al. 2019b, 2020a). Line drawings were done on the basis of multiple photographic images with different focus depths.

DNA extraction, PCR and molecular phylogeny

DNA extraction of *S. bryopsidis* was performed using an innuPREP Plant DNA extraction Kit (Analytik Jena, Germany). Isolates were centrifuged at 19 000 *g* for 2 min at 22 °C to concentrate the cells. Subsequently, the RNAlater or 70 % ethanol solution was carefully removed using 1 000 µL pipette tips, and 400 µL SLS buffer from the extraction kit was added. About 100 mg of sterile 0.1 mm Silica Glass Beads (Carl Roth GmbH, Germany) and 60–100 pieces of 1 mm metal beads (KRS Seigert GmbH, Immelborn) were added to the 2 mL tubes (Sarstedt, Germany). Afterwards, the sample was homogenised at maximum frequency (25 Hz) for 25 min in a Retsch Mixer Mill MM 200 (Retsch GmbH, Germany). From this step onwards, the instructions provided with the kit were followed. The PCR amplification of the nuclear-encoded ribosomal small subunit (nrSSU, 18S) was performed as described in Buaya *et al.* (2019a) using Mango-Taq DNA Polymerase (Bioline, UK) and the 18S primer pair EUK422-445 and EUK1422-1440_R (Wang *et al.* 2014). Amplicons were sent for sequencing to the laboratory centre of the Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main, Germany) using the primers used in the PCR. In addition, direct PCRs were also done on the isolated samples as described in Buaya *et al.* (2020a). Sequences obtained from *S. bryopsidis* were assembled using Geneious v. 5.6, added to the dataset of Buaya *et al.* (2020a) and aligned using MUSCLE (Edgar 2004) as implemented in MEGA v. 6 (Tamura *et al.* 2011) using a gap opening penalty of -600 and a gap extension penalty of -4. Phylogenetic analyses were done using MEGA v. 6 for Minimum Evolution analysis with 1 000 bootstrap replicates, and on the TrEase webserver (<http://thines-lab.senckenberg.de/trease/>), using RAxML (Stamatakis 2014) for Maximum Likelihood inference, also with 1 000 bootstrap repeats. Bayesian inference was also done on the TrEase webserver, which implements MrBayes v. 3.2 (Ronquist *et al.* 2012), running 10 M generations and discarding the first 3 M generations as burnin to assure sampling only from the stationary phase for calculating posterior probabilities. The partial nrSSU sequence obtained in this study was deposited in GenBank under the accession number MW489460.

RESULTS

Morphology and life cycle

The morphology and life cycle development of *S. bryopsidis* isolated in the present study was documented using several infected filaments of *Capsosiphon fulvescens* are in line with previous descriptions of Petersen (1905), Sparrow (1934, 1936), and Aleem (1950, 1953). The life cycle of the parasite started with the germination of an encysted zoospore on the outer surface of its algal host, developing a thin penetration tube that pierced through the cellulosic wall and delivers the parasite into the host cytoplasm. Once established, the parasite thallus grew rapidly, causing gradual degradation of the host chloroplasts, reducing them into dark green or golden green coloured residues deposited at the centre or periphery of the infested cell. At an early stage of development, the pathogen formed a rather thin, hyphal-like thallus (Figs 1A, 2A). While

the thallus was in its hyphal stage, the host cytoplasm and the chloroplasts gradually disintegrated. Then, the thallus branched and became more irregular in shape, with globular droplets and a coarse inner structure (Fig. 2B). At this stage, the thallus was surrounded by a smooth, thin, colourless wall. After this phase of growth, the thallus developed constrictions at thinner parts of the hyphae, resulting in multiple, irregular compartments that further developed independently (Figs 1B, C, 2C). Subsequently, the globular droplets gradually disappeared, condensing into scattered vacuoles, and the colourless wall became slightly thickened as the thallus matured and the connection between the segments was cut. Normally, at maturity a single host cell contained one to six (but sometimes more than ten) elongated tubular thallus segments that were ovoid or irregularly cylindrical in shape (10–80 µm long, 9–26 µm diam). At this stage, the thallus segments were surrounded by a smooth, colourless but clearly visible wall. Once the thallus became fully mature, each thallus segment developed a single, elongated and narrowly cylindrical discharge tube of variable length (up to 180 µm or more) that extended through the host wall into the surrounding medium but sometimes also entered into an adjacent, healthy algal cell. The protoplasmic contents of the sporangia became coarser at this stage, as it differentiated into narrowly pyriform biflagellate zoospores (4–9 µm long, 2–3 µm wide) with refractive granules on the anterior end. The zoospores matured within the thallus before emerging through the discharge tube and swimming away from the orifice for a short distance in random directions for a few minutes. After this, they settled down and became immotile. Rarely, a few of the zoospores did not successfully pass through the discharge tube and remained within the sporangia. However, mostly all zoospores eventually escaped, leaving the empty thallus segments behind (Figs 1D, 2D). The entire parasite thallus tested positive for the presence of cellulose, as evidenced by a strong violet to blueish colour after staining with a solution of zinc iodine chloride. Resting spores were not observed.

Infections of the parasite were observed to be predominantly localised in senescent or moribund portions of the host algae being weakly aggressive. However, sometimes also cells in young filaments showed infections, but none were observed in the gametophytes and other reproductive structures of the host algae.

About 20 % of the *C. fulvescens* thalli collected during autumn of 2019 from the intertidal zone of the coast at Skagaströnd were observed to be parasitized by *S. bryopsidis*. After the collected samples were further incubated for 3–4 wk with the addition of *f/2* medium or Provasoli enriched Seawater Medium at controlled conditions as previously reported (Buaya *et al.* 2020a), *C. fulvescens* filaments infected with *S. bryopsidis* significantly increased in all samples. The infections could be maintained for up to 4 mo and were observed to be dependent on the density of old *C. fulvescens* filaments. Other filamentous algae *e.g.* *Bryopsis* spp. and *Cladophora* spp., which are also reported as host of *S. bryopsidis*, were co-occurring with infected *C. fulvescens*, but did not show infection. Attempts to establish a stable dual culture containing the host and parasite were made using Guillard's *f/2* medium and Provasoli enriched Seawater Medium. However, once the axenic culture *C. fulvescens* was successfully established in Provasoli enriched Seawater Medium, the parasite had already disappeared from the samples.

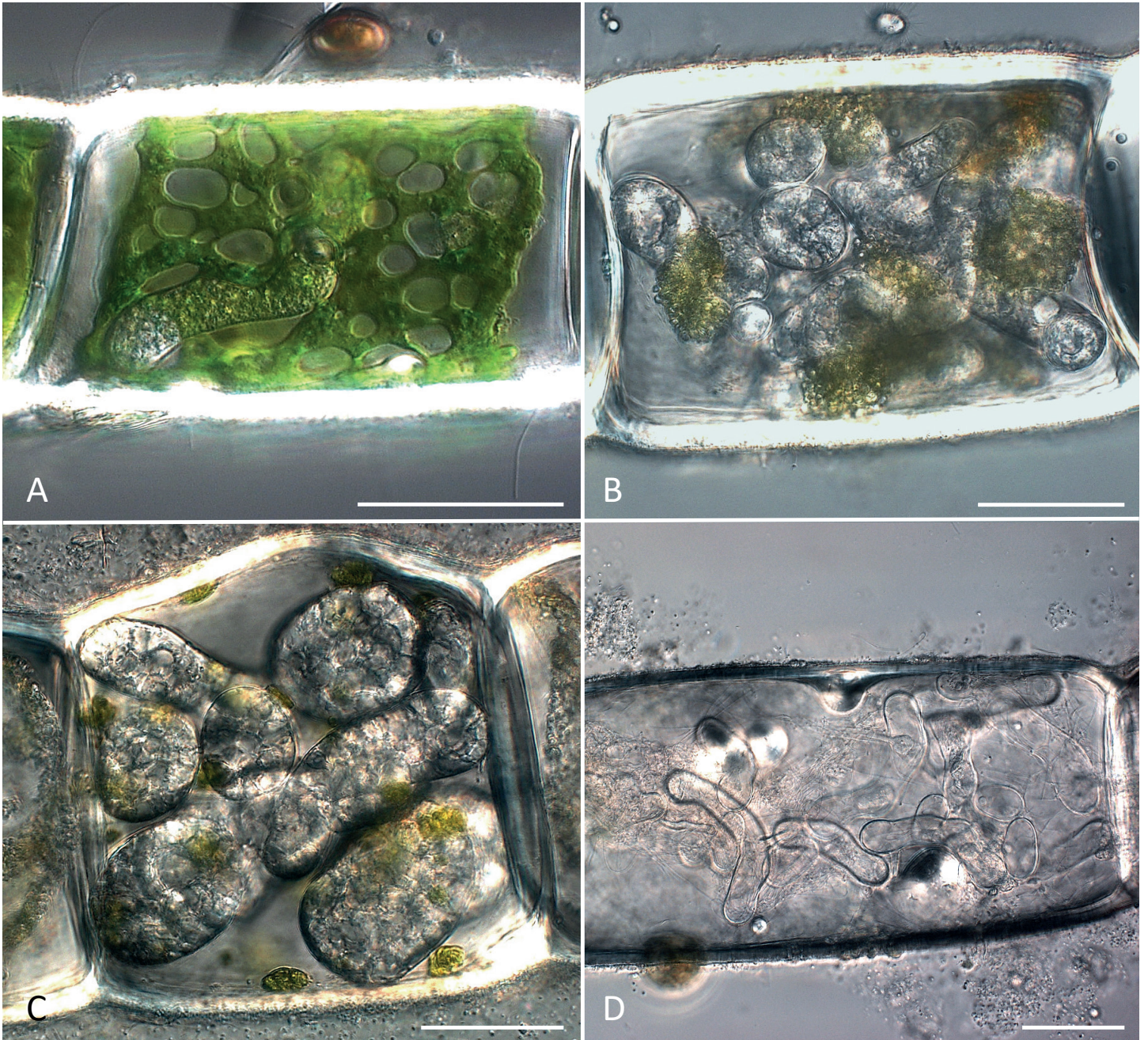


Fig. 1. Micrographs of *Sirolpidium bryopsidis* thallus development at various stages on *Capsosiphon fulvescens* (DIC). **A.** Developing thallus of *S. bryopsidis* at an early stage of infection, in which the chloroplasts of the host chloroplast are not fully disintegrated. **B.** Thallus elongation and start of segment formation. **C.** Segmented thallus close to maturity. **D.** Empty thallus segments after spore release. Scale bars = 50 μ m.

Phylogeny

In the phylogenetic reconstructions inferred from partial nrSSU (18S) sequences (Fig. 3), *S. bryopsidis* grouped together with the type species of *Pontisma*, *Po. lagenidioides*, with maximum support in all analyses, and with no genetic distance, being identical over the alignment length in their sequences. The lineage consisting of *S. bryopsidis* and *Po. lagenidioides* clustered with other members of the rhodophyte-infecting order *Pontismatales* that were previously classified in the genus *Olpidiopsis* (Buaya et al. 2019c) without support. The *Pontismatales* were inferred as the sister group to the *Anisolpidiales*, with the phaeophyte-infecting species of *Anisolpidium*, and the diatom-infecting genus *Diatomophthora*. Similar to previous studies, the relationships of the early-diverging oomycete lineages remained poorly resolved.

However, with the exception of the *Pontismatales*, all order-level clades, corresponding to *Haliphthorales*, *Anisolpidiales*, *Haptoglossales*, *Eurychasmatales*, *Olpidiopsidiales*, and *Miraculales* received strong support for their monophyly in at least one of the phylogenetic analyses.

TAXONOMY

Based on life cycle traits, morphology and phylogenetic inference, *S. bryopsidis* is closely affiliated with the type species of *Pontisma*, *Po. lagenidioides*. Because the full DNA sequence contained a SNP between the two species, it cannot be excluded that the two species are independent. Thus, *S. bryopsidis* is reclassified to the genus *Pontisma*. Further studies, involving

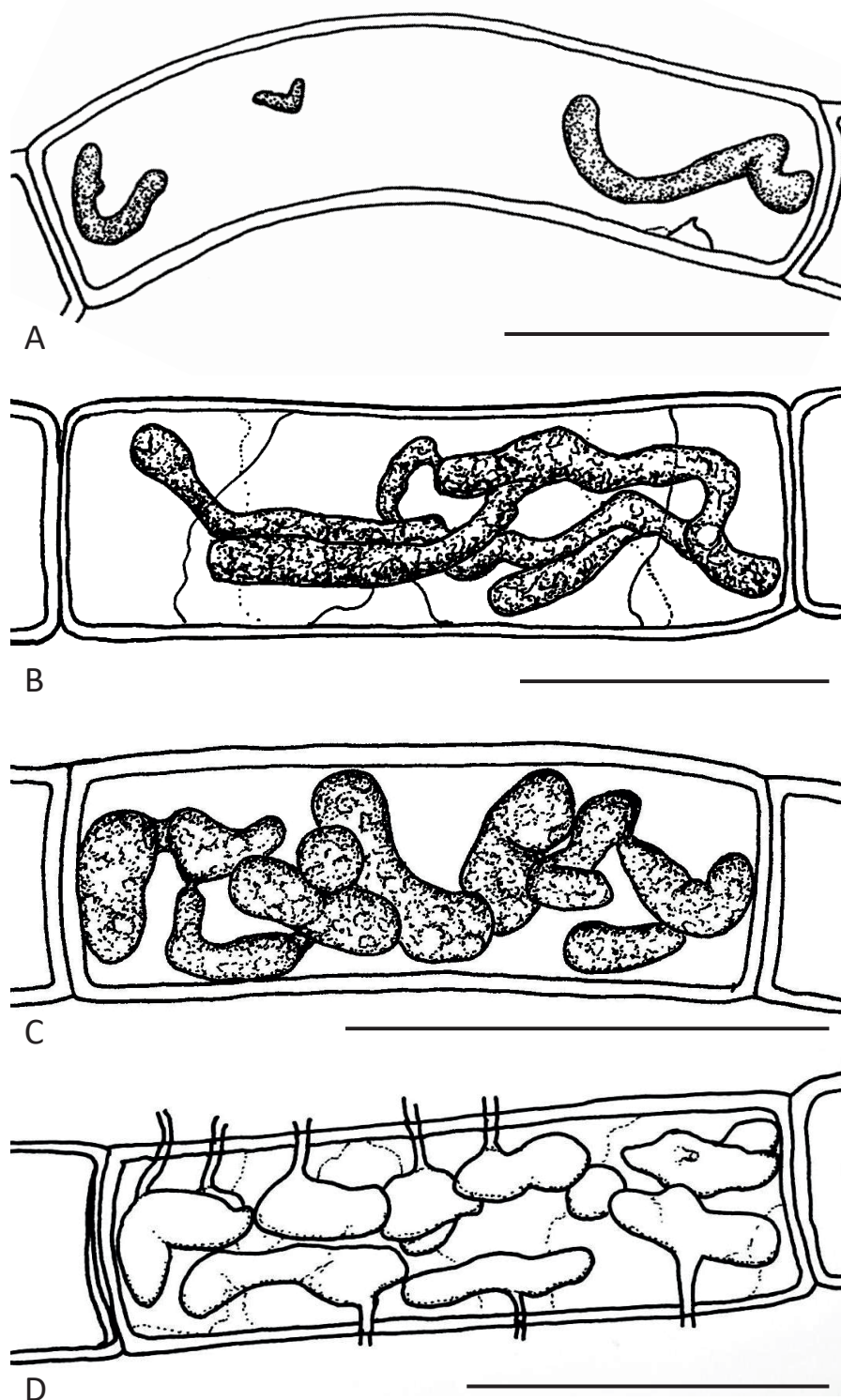


Fig. 2. Line drawings of *Sirolopidium bryopsidis* thallus developmental stages. **A.** Early stage of thallus formation, hyphal phase. **B.** Early stage of thallus differentiation, with beginning segmentation. **C.** Late stage of thallus fragmentation, close to maturation. **D.** Empty thallus segments after spore discharge. Scale bars = 100 μ m.

cross-infection experiments and sequencing of variable loci from several specimens will be necessary to evaluate if *S. bryopsidis* and *Po. lagenidioides* are conspecific, or if additional species infecting green algae exist.

Pontisma bryopsidis (de Bruyne) Buaya & Thines, *comb. nov.* MycoBank MB838524.

Basionym: *Olpidium bryopsidis* de Bruyne, *Arch. De Biol.* **10:** 85. 1890.

Synonym: *Sirolopidium bryopsidis* (de Bruyne) H.E. Petersen, *Overs. K. danske Vidensk. Selsk. Forh.* **5:** 480. 1905.

As the *Miraculaceae* have been consistently resolved as an independent early-diverging lineage with no clear affinities to any described order, the order *Miraculales* is introduced to accommodate them.

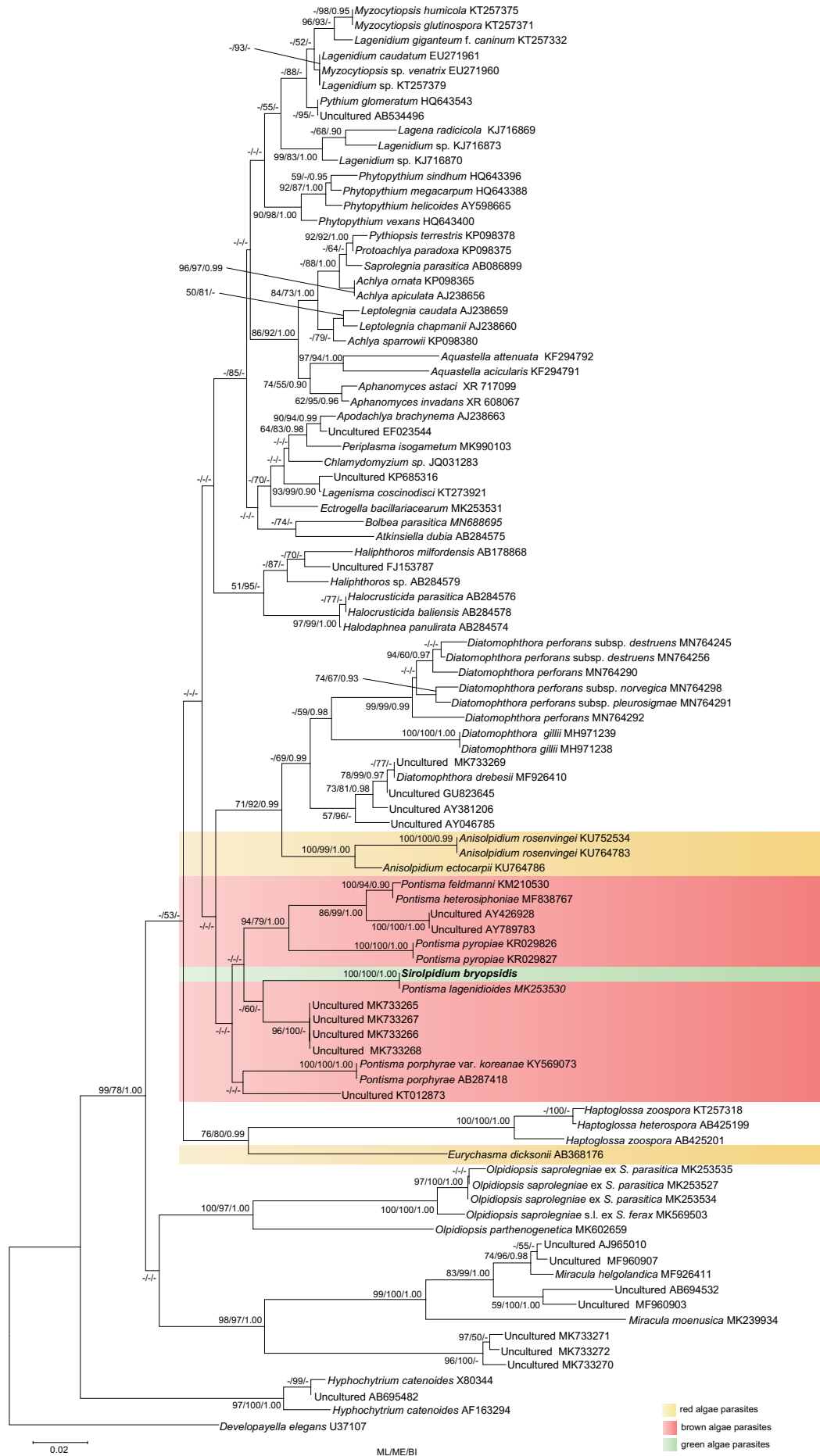


Fig. 3. Molecular Phylogeny derived from Minimum Evolution analysis based on partial nrSSU sequences. Numbers on branches denote support in Maximum Likelihood (ML), Minimum Evolution (ME), and Bayesian analyses (BA), in the respective order. A dash “-” indicates less than 50 % bootstrap support for the presented topology in ML and MN, and less than 0.9 posterior probability in BA for the present or a conflicting topology.

Miraculales Buaya & Thines, **ord. nov.** MycoBank MB838525.

Description: *Straminipila*, *Oomycota*. Thallus in members of the *Bacillariophyta*, holocarpic, simple, subglobose to limoniform, thin-walled, evanescent. Germ tube short, often with a thickened base. Zoospores biflagellate, immediately dispersing after release. Resting spores not known.

Type species: *Miracula helgolandica* Buaya *et al.*, *Mycol. Prog.* **16**: 1048. 2017.

DISCUSSION

Most species of the early-diverging oomycete lineages are obligate endoparasites of micro- and macroalgae (Beakes & Thines 2017, Buaya & Thines 2020b). In the marine realm, most parasites of macro-algae are classified in six genera (*Anisolpidium*, *Eurychasma*, *Eurychasmidium*, *Pontisma*, *Petersenia* and *Sirolopidium*), and infect a wide range of rhodophyte (*e.g.* *Ceramium*, *Callithamnion*), phaeophyte (*e.g.* *Ectocarpus*, *Pylaiella*), and chlorophyte algae (*e.g.* *Bryopsis*, *Cladophora*) (Magnus 1905, Petersen 1905, Sparrow 1934, 1936, Karling 1943).

In the past decades, half a dozen new species from marine red algae were introduced (*Pontisma bostrychia*, *Po. heterosiphoniae*, *Po. muelleri*, *Po. palmariae*, *Po. porphyrae*, *Po. pyropiae*), mostly based on phylogeny and host range (Sekimoto *et al.* 2008b, 2009, Klochkova *et al.* 2016, 2017, Badis *et al.* 2019). The recently revised genus *Pontisma*, with its type species *Po. lagenidioides*, thus constitutes the largest monophyletic assemblage of early-diverging oomycetes (Buaya *et al.* 2019c). Within the genus *Pontisma*, only *Po. lagenidioides* is known to form larger hyphal thalli, but the thallus segments in that species develop in a very similar manner as compared to the species in which the thallus remains smaller (Buaya *et al.* 2019c).

Some species of other genera parasitizing marine macroalgae have recently been recollected, including the two type species *Eurychasma dicksonii* and *Po. lagenidioides* (Sekimoto *et al.* 2008a, Fletcher *et al.* 2015, Gachon *et al.* 2017, Buaya *et al.* 2019c). The type species of the genera *Anisolpidium* (*A. sphacellarum*), *Eurychasmidium* (*Eu. tumefaciens*), and *Petersenia* (*Pe. lobata*), still remain to be recollected (Buaya & Thines 2020b).

Also, for the genus *Sirolopidium*, the only oomycete genus reported to infect marine filamentous green algae, molecular phylogenetic information had been lacking, despite the widespread nature of its type species, *S. bryopsidis* (Sparrow 1960, Johnson 1967). The genus was introduced by Petersen (1905), who observed that the zoospores of the species were actually oomycete-like and biflagellate, contrary to the original description of the species (de Bruyne 1890). While Petersen (1909) assigned the species into its own family, *Sirolopidiaceae*, in the *Lagenidiineae* together with three other families (*Lagenidiaceae*, *Pontismataceae*, *Pseudolpidiaceae*), other authors have suggested various other classifications for *Pontisma* and *Sirolopidium*. Karling (1942) suggested that the type species of the two genera were congeneric, while Dick (2001) again placed them into separate families. In this study, the view of Karling (1942) is confirmed, with the two species being highly similar both morphologically and in terms of their phylogeny.

That no infections were observed in other potential hosts of *Po. bryopsidis* in this investigation might hint at the presence of host specific lineages. However, this situation and the question of whether *Po. lagenidioides* and *Po. bryopsidis* should be considered conspecific can only be clarified by sequencing more variable loci on several specimens from both green and red algae and by performing infection experiments. However, these could not be done in the present study, as attempts to establish an axenic dual culture failed.

After the description of *S. bryopsidis*, several other species were added to or described in *Sirolopidium*. *Sirolopidium zoophthorum* is a reportedly cultivable facultative biotrophic parasite of marine molluscs with a morphology and life cycle characteristics similar to *S. bryopsidis*. It was the first species to be added to the genus (Vishniac 1955). Other species subsequently added include *S. andreei* (Lagerheim 1899, Dick 2001), *S. glenodinium* (Dangeard 1888, Dick 2001), *S. globosum* (Anastasiou & Churchland 1968), *S. marinum* (Dangeard 1912, Dick 2001), *S. paradoxum* (Petersen 1905, Dick 2001), and *S. salinum* (Dangeard 1932, Dick 2001). To *Pontisma* the so far unsequenced species *Po. antithamnionis* (Whittick & South 1972), *Po. dangeardii* (Feldmann & Feldmann 1967), *Po. inhabilis* (Petersen 1905), and *Po. magnusii* (Feldmann & Feldmann 1955) were added. The host range of these two genera would thus include aquatic fungi (*S. globosum*, *S. paradoxum*), dinoflagellates (*S. glenodinium*), invertebrate animals (*S. zoophthorum*, *Po. dangeardii*), apart from marine and freshwater algae (*S. andreei*, *S. marinum*, *S. salinum*, *Po. antithamnionis*, *Po. inhabilis* and *Po. magnusii*) (Karling 1942, Sparrow 1960, Dick 2001). As these species show divergent morphological and lifecycle traits and parasitize highly divergent hosts across four different kingdoms (*Animalia*, *Mycota*, *Alveolata*, *Chlorobionta*), it can be expected that several of these organisms are not taxonomically related to *Pontisma*. However, until sequence data become available for these organisms, their phylogenetic affinities have to be considered unresolved. Furthermore, the concept of Dick (2001) who placed all marine parasites of green macroalgae in *Sirolopidium* (but also *S. glenodinium* in a dinoflagellate), needs further scrutiny to clarify if these species are distinct from *Po. bryopsidis*, or belong to other genera.

ACKNOWLEDGEMENTS

ATB is thankful to KAAD for funding a three-year fellowship. MT gratefully acknowledges a DAAD travel award that seeded the collaboration leading to the present study.

Conflict of interest: The authors declare that there is no conflict of interest.

REFERENCES

- Aleem AA (1950). Phycomycetes marins parasites de diatomées et d'algues. *Comptes Rendus de l'Académie des Sciences* **231**: 713–715.
- Aleem AA (1953). Marine fungi from the West-Coast of Sweden. *Arkiv för Botanik* **3**: 1–33.
- Anastasiou CJ, Churchland LM (1968). An *Olpidiopsis* parasitic on marine habitats. *Syesis* **1**: 81–85.
- Badis Y, Klochkova TA, Strittmatter M, *et al.* (2019). Novel species of the oomycete *Olpidiopsis* potentially threaten European red algal

- cultivation. *Journal of Applied Phycology* **31**: 1239–1250.
- Beakes GW, Thines M (2017). *Hyphochytriomycota* and *Oomycota*. In: Handbook of the Protists (Archibald JM, Simpson AGB, Slamovits CH, eds). Springer Verlag, Germany: 435–505.
- Buaya AT, Ploch S, Thines M (2019a). Rediscovery and phylogenetic placement of *Olpidiopsis gillii* (de Wildeman) Friedmann, a holocarpic oomycete parasitoid of freshwater diatoms. *Mycoscience* **60**: 141–146.
- Buaya AT, Kraberg A, Thines M (2019b). Dual culture of the oomycete *Lagenisma coscinodisci* Drebes and *Coscinodiscus* diatoms as a model for plankton/parasite interactions. *Helgoland Marine Research* **73**: 92.
- Buaya AT, Ploch S, Inaba S, et al. (2019c). Holocarpic oomycetes parasitoids of red algae are not *Olpidiopsis*. *Fungal Systematics and Evolution* **4**: 21–31.
- Buaya AT, Ploch S, Kraberg A, et al. (2020a). Phylogeny and cultivation of the holocarpic oomycete *Diatomophthora perforans* comb. nov., an endoparasitoid of marine diatoms. *Mycological Progress* **19**: 441–454.
- Buaya AT, Thines M (2020b). An overview on the biology and phylogeny of the early-diverging oomycetes. *Philippine Journal of Systematic Biology* **14**: 1–20.
- Dangeard PA (1888). Les péridiniens et leurs parasites. *Journal de Botanique* **2**: 126–132.
- Dangeard PA (1912). Recherches sur quelques algues nouvelles ou peu connues. *Le Botaniste* **12**: 1–21.
- Dangeard PA (1932). Observations sur la famille des *Labyrinthulees* et sur quelques autres parasites des *Cladophora*. *Le Botaniste* **12**: 1–21.
- de Bruyne C (1890). Monadines et chytriacees, parasites des algues du Golfe de Naples. *Archives de Biology* **10**: 43–104.
- Dick MW (2001). *Straminipilous Fungi*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Feldmann J, Feldmann G (1955). Observations sur quelques *Phycomycetes* marins nouveaux ou peu connus. *Revue Mycologique* **20**: 231–251.
- Feldmann J, Feldmann G (1967). Le parasitisme d'un *Olpidiopsis* sur le *Radicilingua reptans* (Kylin) Papenfuss. *Botaniste* **50**: 189–203.
- Fletcher KA, Žuljević A, Tsigoti A, et al. (2015). New record and phylogenetic affinities of the oomycete *Olpidiopsis feldmanni* infecting *Asparagopsis* sp. (*Rhodophyta*). *Diseases of Aquatic Organisms* **117**: 45–57.
- Gachon CMM, Strittmatter M, Kleinteich J, et al. (2009). Detection of differential host susceptibility to the marine oomycete pathogen *Eurychasma dicksonii* by Real-Time PCR: not all algae are equal. *Applied and Environmental Microbiology* **75**: 322–328.
- Gachon CMM, Strittmatter M, Badis Y, et al. (2017). Pathogens of brown algae: culture studies of *Anisulpidium ectocarpii* and *A. rosenvingei* reveal that the *Anisulpidiales* are uniflagellated oomycetes. *European Journal of Phycology* **52**: 133–148.
- Garvetto A, Nézan E, Badis Y, et al. (2018). Novel widespread marine oomycetes parasitising diatoms, including the toxic genus *Pseudonitzschia*: genetic, morphological, and ecological characterisation. *Frontiers in Microbiology* **9**: 2918.
- Guillard RR, Ryther JH (1962). Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Canadian Journal of Microbiology* **8**: 229–239.
- Hassett BT, Thines M, Buaya A, et al. (2019). A glimpse into the biogeography, seasonality, and ecological functions of arctic marine *Oomycota*. *IMA Fungus* **10**: 6.
- Johnson TW (1968). Aquatic fungi of Iceland: introduction and preliminary account. *Journal of the Elisha Mitchell Scientific Society* **84**: 179–183.
- Jokl M (1916). Eine neue Meereschytridinee: *Pleotrachelus ectocarpii*, nov. spec. *Österreichische botanische Zeitschrift* **66**: 267–272.
- Karling JS (1942). The simple holocarpic biflagellate *Phycomycetes*. JS Karling, USA.
- Karling JS (1943). The life history of *Anisulpidium ectocarpii* gen. nov. et sp. nov., and a synopsis and classification of other fungi with anteriorly uniflagellate zoospores. *American Journal of Botany* **30**: 637–648.
- Klochkova TA, Shim JB, Hwang MS, et al. (2012). Host-parasite interactions and host species susceptibility of the marine oomycete parasite, *Olpidiopsis* sp., from Korea that infects red algae. *Journal of Applied Phycology* **24**: 135–144.
- Klochkova TA, Shin Y, Moon KH, et al. (2016). New species of unicellular obligate parasite, *Olpidiopsis pyropiae* sp. nov. that plagues *Pyropia* sea farms in Korea. *Journal of Applied Phycology* **27**: 73–83.
- Klochkova TA, Kwak MS, Kim GH (2017). A new endoparasite *Olpidiopsis heterosiphoniae* sp. nov. that infects red algae in Korea. *Algal Research* **28**: 264–269.
- Kobayashi Y, Ookubo M (1953). Studies on marine *Phycomycetes*. *Bulletin of National Science Museum Tokyo* **33**: 53–65.
- Küpper FC, Maier I, Müller DG, et al. (2006). Phylogenetic affinities of two eukaryotic pathogens of marine macroalgae, *Eurychasma dicksonii* (Wright) Magnus and *Chytridium polysiphoniae* Cohn. *Cryptogamie Algologie* **27**: 165–184.
- Kwak MS, Klochkova TA, Jeong S, et al. (2017). *Olpidiopsis porphyrae* var. *koreanae*, an endemic endoparasite infecting cultivated *Pyropia yezoensis* in Korea. *Journal of Applied Phycology* **29**: 2003–2012.
- Lagerheim G (1899). Om växt- och djurlämningarna i Andrées polarboj. *Svenska Sällskapet för Antropologi och Geografi* **19**: 425–443.
- Magnus P (1905). Über die Gattung, zu der *Rhizophydium dicksonii* Wright gehört. *Hedwigia* **44**: 347–349.
- Müller DG, Küpper FC, Küpper H (1999). Infection experiments reveal broad host ranges of *Eurychasma dicksonii* (*Oomycota*) and *Chytridium polysiphoniae* (*Chytridiomycota*), two eukaryotic parasites of marine brown algae (*Phaeophyceae*). *Phycological Research* **47**: 217–223.
- Müller DG, Gachon CMM, Küpper FC (2008). Axenic clonal cultures of filamentous brown algae: Initiation and maintenance. *Cahiers de Biologie Marine* **49**: 59–65.
- Petersen HE (1909). Studier over Ferskvands-Phycomyceter. *Botaniske Tidsskrift* **29**: 345–440.
- Petersen HE (1905). Contributions a la connaissance des *Phycomycetes* marins (*Chytridineae* Fischer). *Oversigt over det Kongelige Danske videnskabernes selskabs forhandling* **5**: 439–188.
- Provasoli L (1968). Media and prospects for the cultivation of marine algae. *Proceedings of the US-Japan Conference*, Hakone, 12–15 September 1966: 63–75.
- Raghukumar C (1986). Fungal parasites of the marine green algae, *Cladophora* and *Rhizoclonium*. *Botanica Marina* **29**: 289–297.
- Ronquist F, Teslenko M, van der Mark P, et al. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.
- Sekimoto S, Beakes GW, Gachon CMM, et al. (2008a). The development, ultrastructural cytology, and molecular phylogeny of the basal oomycete *Eurychasma dicksonii*, infecting the filamentous phaeophyte algae *Ectocarpus siliculosus* and *Pylaiella littoralis*. *Protist* **159**: 299–318.
- Sekimoto S, Yokoo K, Kawamura Y, et al. (2008b). Taxonomy, molecular phylogeny, and ultrastructural morphology of *Olpidiopsis porphyrae* sp. nov. (*Oomycetes*, *stramenopiles*), a unicellular obligate endoparasite of *Porphyra* spp. (*Bangiales*, *Rhodophyta*). *Mycological Research* **112**: 361–374.
- Sekimoto S, Kochkova TA, West JA, et al. (2009). *Olpidiopsis bostychieae*: A new species endoparasitic oomycete that infects *Bostrychia* and

- other red algae. *Phycologia* **48**: 460–472.
- Sparrow FK (1934). Observations on marine *Phycomycetes* collected in Denmark. *Dansk Botanisk Arkiv Udgivet af Dansk Botanisk Forening* **8**: 1–24.
- Sparrow FK (1936). Biological observations on the marine fungi of Woods Hole waters. *Biological Bulletin* **70**: 236–263.
- Sparrow FK (1943). *The aquatic Phycomycetes, exclusive of the Saprolegniaceae and Pythium*. University of Michigan Press, USA.
- Sparrow FK (1960). *Aquatic Phycomycetes*. The University of Michigan Press, Ann Arbor, USA.
- Sparrow FK (1969). Zoosporic marine fungi of the Pacific Northwest (U.S.A). *Archives of Microbiology* **66**: 129–146.
- Stamatakis A (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.
- Strittmatter M, Gachon CMM, Küpper FC (2009). Ecology of lower oomycetes. In: *Oomycete Genetics and Genomics: Diversity, Interactions, and Research Tools* (Lamour K & Kamoun S, eds). Wiley-Blackwell, New Jersey: 1–24.
- Tamura K, Peterson D, Peterson N, *et al.* (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**: 2731–2739.
- Van der Meer CM, Pueschel JP (1985). Ultrastructure of the fungus *Petersenia palmariae* (Oomycetes) parasitic on the alga *Palmaria mollis* (Rhodophyceae). *Canadian Journal of Botany* **63**: 409–418.
- Vishniac HS (1955). The morphology and nutrition of a new species of *Sirolopidium*. *Mycologia* **47**: 633–645.
- Wang Y, Tian RM, Gao ZM, *et al.* (2014). Optimal eukaryotic 18S and universal 16S/18S ribosomal RNA primers and their application in a study of symbiosis. *PLoS ONE* **9**: e90053.
- West JA, Klochkova TA, Kim GH, *et al.* (2006). *Olpidiopsis* sp., an oomycete from Madagascar that infects *Bostrychia* and other red algae: host species susceptibility. *Phycological Research* **54**: 72–85.
- Whittick A, South GR (1972). *Olpidiopsis antithamnionis* n. sp. (Oomycetes, *Olpidiopsidaceae*), a parasite of *Antithamnion floccosum* (O. F. Müll.) Kleen from Newfoundland. *Archiv für Mikrobiologie* **82**: 353–360.
- Zopf W (1884). Zur Kenntniss der Phycomyceten. I. Zur Morphologie und Biologie der Ancylisteen und Chytridiaceen, zugleich ein Beitrag zur Phytopathologie. *Abhandlungen der Kaiserlichen Leopoldinisch-Carolinischen Deutschen Akademie der Naturforscher* **47**: 141–236.