Taxonomy and phylogeny of *Aphanomycopsis bacillariacearum*, a holocarpic oomycete parasitoid of the freshwater diatom genus *Pinnularia*

Anthony T. Buaya¹ · Bettina Scholz² · Marco Thines^{1,3}

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Abstract

Investigations into simple holocarpic oomycetes are challenging, because of the obligate biotrophic nature of many lineages and the periodic presence in their hosts. Thus, despite recent efforts, still, the majority of species described remains to be investigated for their phylogenetic relationships. One of these species is *Aphanomycopsis bacillariacearum*, the type species of the genus *Aphanomycopsis*. Species of *Aphanomycopsis* are endobiotic holocarpic parasites of diverse hosts (e.g., diatoms, desmids, dinoflagellates). All species classified in this genus were assigned to it based on the presence of branching hyphae and the formation of two generations of zoospores, of which the first one is not motile. Originally, *Aphanomycopsis* with its type species, *A. bacillariacearum*, had been classified in the *Saprolegniaceae*. However, the genus has undergone multiple taxonomic reassignments (to *Ectrogellaceae*, *Lagenidiaceae*, and *Leptolegnielleaceae*) in the past. To settle the taxonomy and investigate the phylogenetic placement of *Aphanomycopsis*, efforts were undertaken to isolate *A. bacillariacearum* from its original host, *Pinnularia viridis* and infer its phylogenetic placement based on nrSSU (18S) sequences. By targeted isolation, the diatom parasitoid was rediscovered from Heiðarvatn lake, Höskuldssstaðir, Iceland. Phylogenetic reconstruction shows that *A. bacillariacearum* from *Pinnularia viridis* is embedded within the *Saprolegniales*, and largely unrelated to both diatom-infecting oomycetes in the *Leptomitales* (*Ectrogella*, *Lagenisma*) and those placed within the early-diverging lineages (*Miracula*, *Diatomophthora*) of the *Oomycota*.

Keywords *Aphanomycopsis* · Oomycetes · Diatom parasites · Taxonomy · Phylogeny

Introduction

Protist pathogens of photosynthetic eukaryotes are widely distributed in aquatic environments (Chambouve et al. 2019; Markussen Bjorbækmo et al. 2019; Hassett et al. 2019). These biotrophic parasites and parasitoids are diverse and

infect wide varieties of hosts, especially marine and freshwater diatoms (Kühn 1998; Scholz et al. 2016; Guinder et al. 2017; Chambouve et al. 2019). However, the biology and ecological importance of these inconspicuous organisms are still widely unknown and understudied despite their abundance also during diatom blooms (Hanic et al. 2009; Thines et al. 2015; Hassett et al. 2019). Many of the oomycete parasitoids of freshwater diatoms were documented in studies from more than half a century ago (Karling 1942; Sparrow 1960) and were classified into various genera, mainly *Aphanomycopsis* (Scherffel 1925), *Ectrogella* (Zopf 1884), *Lagenidium* (Zopf 1878), and *Olpidiopsis* (Cornu 1872). Interest in these endobiotic parasitoids has resurged recently, with studies focusing on systematics (Buaya et al. 2017; Buaya and Thines 2019; Buaya and Thines 2020a; Buaya et al. 2020), axenic dual culture of host and parasite (Buaya et al. 2019b; Buaya et al. 2020), and environmental sequencing (Garvetto et al. 2018; Hassett et al. 2019). Despite these

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✉ Marco Thines
m.thines@thines-lab.eu

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

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

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

advances, the taxonomic affinities of most oomycete diatom parasitoids remain unresolved because of lacking sequence data, including also the type species of *Aphanomycopsis*, *Aphanomycopsis bacillariacearum* (Buaya and Thines 2020c). *Aphanomycopsis* was described by Scherffel in 1925 as a monotypic genus, characterized by non-septate holocarpic thalli, an aphanomyces-like pattern of zoospore development, and the production of multiple resting spores without apparent sexual organs. The species recurs seasonally in large freshwater diatoms, e.g., *Pinnularia* and *Nitzschia* (Scherffel 1925; Friedmann 1953; Kadlubowska 1970). The genus *Aphanomycopsis* has been subsequently emended and multiple holocarpic species with divergent hyphal morphology and host ranges were added (Tokunaga 1934; Canter 1949; Friedmann 1952; Martin 1975; Karling 1968; Boltovskoy 1984; Canter and Heaney 1984; Dick 2001), rendering the genus rather heterogeneous, despite its small size. In line with the heterogeneous nature of the genus, it was placed into various families over time, including *Ectrogellaceae*, *Lagenidiaceae*, *Leptolegniellaceae*, and *Saprolegniaceae* (Scherffel 1925; Tokunaga 1934; Karling 1942; Sparrow 1942; Dick 1971).

In order to resolve the phylogeny of the type species of *Aphanomycopsis*, attempts were made to isolate this organism from different freshwater habitats. While screening for holocarpic oomycete parasites of freshwater diatoms sampled from Heiðharvatn at Hoskuldssstaðhir in Iceland in the autumn of 2019, the species was rediscovered, and it was the aim of this study to document its life cycle and clarify its phylogenetic placement.

Materials and methods

Collection, isolation, and characterization

Diatom samples were collected from the lake Heiðharvatn, Hoskuldssstaðhir, Northeast Iceland (64° 54' 12.54" N 14° 35' 29.29" W). *Pinnularia viridis* infected with oomycetes were collected in September 2019, using 20 µm mesh plankton nets (Hydro-Bios, Kiel, Germany), horizontally towed. About 10 mL of phytoplankton concentrates were diluted, poured into several 15 mL petri-dishes, and screened for oomycete-infected diatoms using an inverted compound light microscope (Type AE31, Motic, Xiamen). Infected diatoms were individually picked using a 10 µL micropipette (Brandt, Germany), rinsed multiple times in autoclaved deionized water, and transferred to 2 mL tubes containing 0.5 mL RNeasy lysis solution (Invitrogen, Thermo Fisher, Lithuania) or 70% ethanol (VWR, France) for subsequent DNA extraction. Approximately 50 diatoms infected with *Aphanomycopsis* were collected this way for DNA extraction. Samples preserved in 70% ethanol were deposited in the herbarium

collection of the Senckenberg Museum of Natural History (Herbarium Senckenbergianum, FR), Cryptogams Section, Frankfurt am Main, accession number FR-0046138. Morphological characterization of the parasite was done as described earlier (Buaya et al. 2020) using a compound light microscope (Imager2, Carl Zeiss Göttingen, Germany) with DIC, and photographs were taken using a Zeiss AxioCam MRc5 (Carl Zeiss, Göttingen, Germany). In addition, cellulose presence in the thallus wall of the parasitoids was tested using zinc iodine chloride (Carl Roth GmbH, Germany) solution. The identity of the diatom host was determined by light microscopy and 18S rRNA gene sequence barcoding as outlined for the oomycetes below. Axenic host and parasite dual culture of the isolated parasitoid were also attempted using different enrichment media, e.g., Guillard's f/2 medium (Guillard and Ryther 1962), DAM medium (Gagneux-Moreaux et al. 2007), freshwater DM medium (Beakes et al. 1988), and WC medium (Guillard and Lorenzen 1972). However, all cultivation trials of *P. viridis* were unsuccessful, as were efforts to cultivate the parasitoid using common media, e.g., MEA (malt extract agar), PDA (potato dextrose agar), SAM (standard agar medium), and PYG (peptone yeast glucose agar).

DNA extraction, PCR amplification, and phylogenetic analyses

DNA extraction was performed using an innuPREP Plant DNA extraction Kit (Analytik Jena, Germany), as previously described (Buaya et al. 2017). Initially, isolates were centrifuged at 19 000 g for 2 min at 22 °C to concentrate the cells. Subsequently, RNeasy lysis solution was carefully removed using 1000 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) were added into each 2 mL tubes (Sarstedt, Germany) and homogenized at 25 Hz for 25 min in a Retsch Mixer Mill MM 200 (Retsch GmbH, Germany). The PCR amplification of the nuclear encoding small subunit (nrSSU) was performed as described in Buaya et al. (2019a) using Mango-Taq DNA Polymerase (Bioline, UK) but with the 18S primer pair EUK422-445 and EUK1422-1440_R (Wang et al. 2014). Positive amplicons were sent for sequencing to the laboratory center of the Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main (SBiK-F, Frankfurt, Germany) using the 18S primer pairs used in the PCR. In addition, direct PCRs were also done as described in Buaya et al. (2019a). To obtain high-quality nrSSU sequences of the oomycete infecting *P. viridis*, PCR amplicons were also cloned. Initially, the PCR amplicons were purified using magnetic bead-based nucleic acid purification (AMPure XP, Beckman Coulter Inc., USA) as previously described (Buaya et al. 2020). Subsequently, PCR amplicons were diluted by a factor of

ten, and the mixture was cloned into competent *Escherichia coli* using a StrataClone TA cloning kit (Agilent Technologies, Santa Clara, United States) following instructions of the manufacturer. Single bacterial colonies were picked into 20 µL molecular grade water (Life Technologies, USA) and colony PCR was carried out with the Mango DNA Polymerase using M13-F and M13-R plasmid primers with amplification conditions set to an initial denaturation at 96 °C for 10 min, 36 cycles at 96 °C for 20 s, 56 °C for 20 s, and 72 °C for 60 s, and concluding with a final elongation at 72 °C for 10 min. Amplicons were sent for sequencing to the laboratory center of the Senckenberg Biodiversity and Climate Research Centre (Frankfurt am Main, Germany) using M13 (M13-F, M13-R), T3, and T7 plasmid primers. The resulting sequences obtained were edited using Geneious (version 5.6), and the assembled sequences of *A. bacillariacearum* were aligned together with reference sequences of various members of the *Saprolegniales* (Table 1), using mafft, version 7 (Katoh and Standley 2013), employing the Q-INS-I algorithm. Minimum evolution phylogenetic inference with 500 bootstrap replicates was computed using MEGA7 (Kumar et al. 2016) with pairwise deletion, the Tamura-Nei substitution model, and all other parameters set to default. Other phylogenetic analyses were done on the TrEase webserver (<http://thines-lab.senckenberg.de/trease/>) using RAxML (Stamatakis 2014) for maximum likelihood inference with 1000 bootstrap replicates. Bayesian inference was done on the same server using MrBayes, version 3.2 (Ronquist et al. 2012) and 5 million generations, while other parameters were set to default. The consensus partial 18S (nrSSU) sequence obtained in this study was deposited in GenBank under the accession number MW307772.

Results

Parasite screening and detection

Diatoms collected during autumn 2019 from Heiðharvatn at Hoskuldssstaðhir on Iceland were detected to contain *Pinnularia viridis* with biotrophic oomycete infections. About 10% of the *P. viridis* individuals screened from Heiðharvatn were infected by *Aphanomycopsis bacillariacearum*. All samples were further incubated for 3–4 weeks with addition of f/2 medium and kept at controlled conditions as previously described (Buaya et al. 2019b). This allowed further propagation of the host diatom to increase chances of re-isolating the target pathogen. These samples were screened daily, and after 3–4 weeks, *P. viridis* reappeared in all samples. Infections were observed to increase in parallel to the abundance of *P. viridis* cells, but disappeared again quickly, lasting only 1–2 weeks, after devastating almost all *P. viridis* individuals present. *Epithemia*

turgida, *Cymbella gastroides*, *Nitzschia sigmoidea*, and *Synedra* spp., which were also reported to be host for *A. bacillariacearum* (Karling 1942; Sparrow 1960), were co-occurring with the infected individuals of *P. viridis*, but no individuals of these species were detected to be parasitized throughout the examination period. Attempts to establish a stable dual culture containing host and parasite were unsuccessful.

Morphology and lifecycle observations

The development of the parasitoid isolated in this study, from the start of infection until zoospore release, was documented using several specimens of infected *P. viridis*. The morphology and life cycle of the *P. viridis* parasitoid (Fig. 1a–j) agrees well with previous descriptions of *Aphanomycopsis bacillariacearum* (Scherffel 1925; Friedmann 1953).

The life cycle of this parasite started when an encysted spore attached on the outer surface of the host frustule germinated (Fig. 1a), producing a very fine, needle-like tube, penetrating at the girdle bands, and growing into the protoplasm usually at the central nodule, close to the nucleus. Once established, the thallus rapidly grew, causing gradual degradation of the host phaeoplasts that normally fill the entire cell in girdle view, reducing them into golden green or light brown to slightly chestnut colored, elongated or fragmented residues. These were lining at the center (girdle view) (Fig. 1a) or at the periphery of the girdle bands (valve view), while the parasitoid extended to the polar nodules (Fig. 1b). Subsequently, the host protoplasm began to disintegrate around the undifferentiated thalli which contained scattered globular droplets and were surrounded by a thin, colorless wall (Fig. 1b, c). The droplets gradually disappeared as the thalli elongated, and the colorless thallus wall slightly thickened. Normally, a single host contained one unbranched or branched non-septate holocarpic hyphal thallus of 4–12 µm in diameter (Fig. 1d), but occasionally 2–3 that were tightly compacted within the frustule and variable in length were also observed. The thalli did not cause hypertrophy nor disintegrate the valves. As the thalli matured, one to several slightly tapering slender discharge tubes began to form per thallus. Exit tubes were normally 180–240 µm long, with a diameter of 4–8 µm, and with a thickened base (Fig. 1f). After some time, the content of the sporangia slowly moved into the tip of the discharge tube, forming a circular mass of aplanospores at the orifice. Subsequently, these rapidly differentiated into primary cysts of 8–12 µm in diameter in variable numbers (Fig. 1g). After encystation, a short period of quiescence followed. Subsequently, each of the cysts developed a single discharge papilla of 3–5 µm in diameter that forms in parallel with the development of the secondary zoospores. The cysts then germinated almost simultaneously to produce biflagellate secondary zoospores, which were 10–12 µm long and 7–8 µm

Table 1 Sequence data used in this study

Genus	Species	GenBank accession #	Citation
<i>Pythiopsis</i>	<i>intermedia</i>	KP098377	Steciow et al. (2014)
<i>Pythiopsis</i>	<i>terrestris</i>	KP098379	Steciow et al. (2014)
<i>Protoachlya</i>	<i>paradoxa</i>	KP098375	Steciow et al. (2014)
<i>Pythiopsis</i>	<i>cymosa</i>	AJ238657	Dick et al. (1999)
<i>Pythiopsis</i>	<i>humphreyana</i>	KP098376	Steciow et al. (2014)
<i>Saprolegnia</i>	<i>parasitica</i>	XR001099850	Jiang et al. (2013)
<i>Saprolegnia</i>	<i>parasitica</i>	AB086899	Khomvilai et al. (2005)
<i>Achlya</i>	<i>apiculata</i>	AJ238656	Dick et al. (1999)
<i>Newbya</i>	<i>dichotoma</i>	KP098364	Steciow et al. (2014)
<i>Achlya</i>	<i>ornata</i>	KP098365	Steciow et al. (2014)
<i>Aplanes</i>	<i>treleaseanus</i>	KP098363	Steciow et al. (2014)
<i>Thraustotheca</i>	<i>clavata</i>	KP098372	Steciow et al. (2014)
<i>Achlya</i>	<i>bisexualis</i>	M32705	Gunderson et al. (1987)
<i>Achlya</i>	<i>debaryana</i>	KP098371	Steciow et al. (2014)
<i>Thraustotheca</i>	<i>clavata</i>	KP098373	Steciow et al. (2014)
<i>Leptolegnia</i>	<i>caudata</i>	KP098368	Steciow et al. (2014)
<i>Leptolegnia</i>	<i>chapmanii</i>	AJ238661	Dick et al. (1999)
<i>Achlya</i>	<i>sparrowii</i>	KP098380	Steciow et al. (2014)
<i>Leptolegnia</i>	sp.	AJ238662	Dick et al. (1999)
<i>Aphanomyces</i>	sp.	FJ794896	Wolinska et al. (2009)
<i>Aphanomyces</i>	sp.	FJ794897	Wolinska et al. (2009)
<i>Aphanomyces</i>	<i>astaci</i>	XR717099	Unpublished
<i>Aphanomyces</i>	<i>invadans</i>	DQ403202	Sosa et al. (2007)
<i>Aphanomyces</i>	<i>invadans</i>	XR608067	Unpublished
<i>Aphanomycopsis</i>	<i>bacillacearum</i>	MW307772	This study
<i>Aquastella</i>	<i>attenuata</i>	KF294792	Molloy et al. (2014)
<i>Aquastella</i>	<i>acicularis</i>	KF294791	Molloy et al. (2014)
<i>Uncultured</i>		KP685316	Jiang et al. (2016)
<i>Lagenisma</i>	<i>coscinodisci</i>	KT273921	Thines et al. (2015)
<i>Chlamydomyzium</i>	sp.	EU271965	Beakes et al. (2006)
<i>Blastulidium</i>	<i>paedophthorum</i>	KR869808	Duffy et al. (2015)
<i>Apodachlya</i>	<i>brachynema</i>	AJ238663	Dick et al. 1999
<i>Chlamydomyzium</i>	sp.	JQ031283	Beakes et al. (2014)
<i>Atkinsiella</i>	<i>dubia</i>	AB284575	Muraosa et al. (2009)
<i>Bolbea</i>	<i>parasitica</i>	MN688695	Buaya and Thines (2020b)
<i>Myzocytiopsis</i>	<i>humicola</i>	KT257375	Unpublished
<i>Myzocytiopsis</i>	<i>glutinospora</i>	KT257371	Unpublished
<i>Myzocytiopsis</i>	<i>venatrix</i>	EU271960	Beakes et al. (2006)
<i>Lagenidium</i>	<i>caudatum</i>	EU271961	Beakes et al. (2006)
<i>Lagenidium</i>	<i>giganteum</i>	KT257332	Unpublished
<i>Pythium</i>	<i>glomeratum</i>	HQ643543	Robideau et al. (2011)
<i>Phytopythium</i>	<i>megacarpum</i>	HQ643388	Robideau et al. (2011)
<i>Phytopythium</i>	<i>vexans</i>	HQ643400	Robideau et al. (2011)
<i>Halocrusticida</i>	<i>parasitica</i>	AB284576	Muraosa et al. (2009)
<i>Halocrusticida</i>	<i>baliensis</i>	AB284578	Muraosa et al. (2009)
<i>Halodaphnea</i>	<i>panulirata</i>	AB284574	Muraosa et al. (2009)
<i>Haliphthoros</i>	<i>milfordensis</i>	AB178868	Sekimoto et al. (2007)
<i>Haliphthoros</i>	sp.	AB284579	Muraosa et al. (2009)
<i>Pontisma</i>	<i>lagenidioides</i>	MK253530	Buaya et al. (2020)
<i>Diatomophthora</i>	<i>gillii</i>	MH971238	Buaya et al. (2017)
<i>Diatomophthora</i>	<i>gillii</i>	MH971239	Buaya et al. (2017)
<i>Diatomophthora</i>	<i>drebesii</i>	MF926410	Thines et al. (2015)
<i>Anisopididium</i>	<i>ectocarpii</i>	KU764786	Gachon et al. (2015)

in diameter (Fig. 1h, red arrows). These swam in a zigzag pattern for a few minutes before coming to a rest. One to several oospores were observed to develop within some thalli

similar to *Chlamydomyzium*. The oospores were spherical to broadly ovoid, 20–30 µm in diameter, and surrounded by a smooth, colourless wall (Fig. 1e), which was thinner than in

Fig. 1 DIC light micrographs of *Aphanomycopsis bacillariacearum* at different life cycle stages on *Pinnularia viridis*. **a** Early infection of *P. viridis*, with developing endobiotic holocarpic thalli. An encysted zoospore is marked by a red arrow; **b, c** elongating thallus at early (**b**) and intermediate stage (**c**) of developing thallus surrounded by light brown-green colored phaeoplast residues; **d** highly branched non-septate mature thallus; **e** multiple spherical resting spores (oospores) with eccentric globules and refractive droplets surrounded by a smooth,

colourless, thick wall; **f** elongating discharge tube; **g** fully formed mass of primary aplanospores at the orifice of the discharge tube; **h** mature zoospores emerging individually from cysts, squeezing through the discharge papillae (red arrows) in achlya-like pattern; **i** cluster of empty zoospores cysts (red arrow); **j** zinc iodine stained empty thallus of *A. bacillariacearum* with a pronounced *Spreizapparat* on the base of the discharge tube (red arrow). Bar = 50 µm in all pictures

Saprolegniaceae. The oospores usually contained eccentric globules or tiny refractive droplets, and probably developed parthenogenetically. Oospore germination was not observed. The entire parasitoid thallus and especially the thickened section of the discharge tube base were 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 (Fig. 1j).

Phylogenetic placement

In the phylogenetic reconstructions inferred from partial nrSSU rRNA gene sequences (Fig. 2), *Aphanomycopsis bacillariacearum* was resolved as member of the *Saprolegniales* together with *Aquastella*, *Aphanomyces*, and various members of the *Saprolegniaceae*. It formed the sister to *Aphanomyces* in Minimum Evolution, while no support for this or an alternative placement was obtained in Maximum Likelihood and Bayesian Inference. However, the placement of *A. bacillariacearum* in *Saprolegniales* received low support in Minimum Evolution and Maximum Likelihood analyses, but maximum support in Bayesian Inference.

Discussion

Oomycete parasites of diatoms are diverse, belonging to six different genera—*Aphanomycopsis* (Scherff 1925), *Diatomophthora* (Buaya and Thines 2020a), *Ectrogella* (Zopf 1884), *Lagenidium* (Zopf 1878), *Lagenisma* (Drebes 1966), and *Miracula* (Buaya et al. 2017). So far, only a handful of these holocarpic endoparasitoids have sequence data available (e.g., *D. drebesii*, *E. bacillariacearum*, *L. coscinodisci*), rendering the phylogenetic placement of most species unresolved and their taxonomic assignment unconfirmed (Buaya and Thines 2020c). This includes five species of *Ectrogella* (*E. monostoma*, *E. gomphonematis*, *E. eunotiae*, *E. licmophorae*, *E. eurychasmoides*), three species of *Lagenidium* (*L. cyclotellae*, *L. brachystomum*, *L. enecans*), and *A. bacillariacearum* (Zopf 1884; Scherff 1925; Friedmann 1952; Feldmann and Feldmann 1955).

However, *A. bacillariacearum* remains the only genus type among diatom-infecting genera that has not been investigated in terms of molecular phylogeny. This species is widespread in freshwater environments and has been reported from a variety of pennate diatoms (*Pinnularia viridis*, *Epithemia turgida*, *Cymbella gastroides*, *Nitzschia sigmoidea*, *Synedra*

Fig. 2 Molecular phylogeny based on Minimum Evolution analysis inferred from partial nrSSU rRNA gene sequences. Numbers on branches denote bootstrap values from Minimum Evolution, Maximum Likelihood, and Bayesian Inference, in the respective order. A dash “-” indicates less than 60% bootstrap support or less than 0.8 posterior probability for the respective node

spp.) as potential hosts (Scherffell 1925; Sparrow 1933; Sparrow 1935; Sparrow 1936; Friedmann 1953).

The genus *Aphanomycopsis* was introduced by Scherffell (1925), as a monotypic genus to accommodate his newly described holocarpic diatom parasitoid *A. bacillariacearum*.

This genus was originally placed in the *Saprolegniaceae*, because of similarities to *Ectrogella* and *Aphanomyces* in terms of morphology and zoosporogenesis (Scherffell 1925). Subsequent investigators accepted this taxonomic assignment (e.g., Sparrow 1933). However, Tokunaga (1934) disagreed

and reassigned the organism into *Lagenidiaceae*, based on his isolates having a septate thallus, no *Spreizapparat* on the discharge tube base, and a resting spore formation similar to *Lagenidium*. This placement was not accepted by Coker and Matthews (1937), who included *Aphanomycopsis* in *Ectrogellaceae*. Karling (1942) discussed the findings of Tokunaga (1934) and accepted a provisional placement of *Aphanomycopsis* in the *Ectrogellaceae* together with *Ectrogella*, *Eurychasma*, and *Eurychasmidium*, as he concluded that Tokunaga (1934) had probably seen an organism different from the one of Scherffel (1925). Similarly, Sparrow (1942, 1943) placed *Aphanomycopsis* and subsequently also *Pythiella* (Sparrow 1960) in *Ectrogellaceae*. Later, Karling added two plant saprophytic and keratinophilic species, *A. saprophytica* and *A. punctata* (Karling 1968) to the genus. Dick (1971) placed *Aphanomycopsis* into the new family *Leptolegniellaceae* and included four species (*A. bacillariacearum*, *A. desmidiella*, *A. saprophytica*, and *A. punctata*) in the genus. Karling (1981) followed the assignment of Dick (1971), accepting *Aphanomycopsis* in *Leptolegniellaceae*. Dick (2001) recognized the six genera *Aphanodictyon*, *Brevilegniella*, *Leptolegniella*, *Nematophthora*, *Aphanomycopsis*, and *Cornumyces* in *Leptolegniellaceae*. However, he regarded the taxonomic placement of *Aphanomycopsis* as uncertain, due to the lack of a separable endospore membrane, which is a distinctive character of the *Leptolegniellaceae sensu stricto* (Dick 2001).

The isolate investigated in this study agrees well with the original description of Scherffel (1925), in terms of both morphology and diatom host. To fix the application of the name, Fig. 42 of Tafel 2 drawn by Scherffel for the original description of *A. bacillariacearum* in Archiv für Protistenkunde 52 (1925) on page 14 is designated as lectotype here (MBT395398), and the specimen collected in September 2019 in Iceland by A.T. Buaya and M. Thines deposited in the Herbarium Senckenbergianum under the accession number FR-0046138 is designated as epitype here (MBT395399). The observation that *A. bacillariacearum* produces broadly unbranched to branched, nonseptate thalli with a thick-walled *Spreizapparat* like *Ectrogella bacillariacearum*, but unlike *Lagenidium*, with zoospore production resembling *Aphanomyces* (Scherffel 1925; Sparrow 1933, 1960), is confirmed in this study and support its placement in *Saprolegniales*. In line with this, the phylogenetic reconstruction of this study revealed *Aphanomycopsis* as a member of the order *Saprolegniales*, with affinities to the genus *Aphanomyces*. Thus, it might be considered a member of the *Verrucalvaceae*, but support for the grouping with *Aphanomyces* was low, necessitating multigene phylogenies for testing this hypothesis. Considering the significant variation in thallus arrangement (eucarpic and holocarpic), lifestyles (saprotrophic, necrotrophic, and obligate biotrophic), and the various host groups observed (plants, animals, and

straminipilous organisms) in the species with aphanomyces-like spore production (Karling 1968, Gaulin et al. 2007; Diéguez-Urbeondo et al. 2009), a detailed investigation of these organisms might give important insights into thallus evolution and host specificity in oomycetes.

The sexual cycle of *A. bacillariacearum* is not yet fully understood, but the species is known to produce hyaline resting spores that have a thinner wall than members of the *Saprolegniaceae*, and which probably originate from parthenogenesis (Scherffel 1925; Dick 2001). Thus, it is highly likely that the specimens from Japan investigated by Tokunaga (1934) did not belong to *A. bacillariacearum*. Probably, his isolate, the isolate of West and West (1906) from the desmid algae *Pleurotaenium ehrenbergii*, and Friedmann's (1952) isolate from the diatom *Pinnularia viridis*, which also contains transverse thallus septations correspond to a novel group of peronosporalean oomycetes.

In the absence of sequence data for other members of *Aphanomycopsis sensu* Dick (2001), it is uncertain, if the generic placement is correct for *Aphanomycopsis entophyta* (Dick 2001), *A. saprophyticus* (Karling 1968), *A. punctatus* (Karling 1968), *A. cryptica* (Canter and Heaney 1984), and *A. peridiniella* (Boltovskoy 1984), as well as *A. desmidiella* (Canter 1949) and *A. sexualis* (Martin 1975). The collective host range of these species includes diatoms (*A. bacillariacearum*), filamentous algae (*A. entophyta*), desmid algae (*A. desmidiella*), dinoflagellates (*A. cryptica*, *A. peridiniella*), insects (*A. sexualis*), and extends to keratinophilic (*A. punctatus*) and plant saprophytic species (*A. saprophyticus*) (Sparrow 1960; Dick 2001). Given this diverse range of hosts and substrates, as well as differences in zoospore formation, it is highly probable not all are closely related to *Aphanomycopsis* and that the genus will need substantial revision in the future.

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Authors' contributions ATB and MT conceived the study; ATB, BS, and MT conducted fieldwork; ATB carried out the experiments; ATB and MT analyzed the data and generated the figures; ATB and MT wrote the manuscript, with contributions from BS.

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Data availability Sequence data have been deposited in GenBank under the accession number MW307772. The epitype of *Aphanomycopsis*

bacillariacearum, which is part of the same gathering that was used to produce the sequence data, has been deposited in the Herbarium Senckenbergianum (FR) under the accession number FR-0046138.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

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