FUSE

Fungal Systematics and Evolution

Volume 12
DECEMBER 2023
PAGES 247–254

doi.org/10.3114/fuse.2023.12.12

Ducellieriales ord. nov. and evidence for a novel clade of endobiotic pollen-infecting "lagenidiaceous" *Peronosporomycetes*

A.T. Buaya[1,2*], M. Thines[1,2]

[1]Goethe-Universität Frankfurt am Main, Department of Biological Sciences, Institute of Ecology, Evolution and Diversity, Max-von-Laue Str. 13, D-60438 Frankfurt am Main, Germany
[2]Senckenberg Biodiversity and Climate Research Centre, Senckenberganlage 25, D-60325 Frankfurt am Main, Germany

*Corresponding author: anthony.buaya@senckenberg.de

Key words:
biotrophic oomycetes
chlorophyte algae
Ducellieria
new order
Oomycetes
phylogeny
xanthophyte algae

Abstract: The genus *Ducellieria* (*Ducellieriaceae*) contains three species (*D. chodatii, D. tricuspidata, D. corcontica*), and a single variety (*D. chodatii* var. *armata*) of obligate endobiotic pollen parasites. These organisms have been first assigned to the green alga genus *Coelastrum*, as they form very similar spherical structures, but the observation of heterokont zoospores has led to their reclassification to the phylum *Oomycota*. However, despite their widespread nature, these organisms are only known from their descriptive morphology, and life cycle traits of some species still remain incompletely known. Only the type species, *D. chodatii*, has been rediscovered several times, but the phylogeny of the genus remains unresolved, since none of its species has been studied for their molecular phylogeny. At present the genus is still included in some algal databases. To clarify the evolutionary affiliation of *Ducellieria*, efforts were undertaken to isolate *D. chodatii* from pollen grains, to infer its phylogenetic placement based on nrSSU sequences. By targeted isolation, the pollen endoparasitoid was rediscovered from three lakes in Germany (Mummelsee, Okertalsperre, Knappensee). Apart from the typical coelastrum-like spheroids, oomycetes sporulating directly from pollen grains in a lagenidium-like fashion were observed, and molecular sequences of both types of oomycetes were obtained. Phylogenetic reconstruction revealed that coelastrum-like and lagenidium-like forms are unrelated, with the former embedded within the deep branching early-diverging lineages, and the later stage forming a distinct clade in *Peronosporales*. Consequently, the life cycle of *D. chodatii* needs careful revision using single-spore isolates of the species, to infer if previous lifecycle reconstructions that involve various different thallus types are stages of a single species or potentially of several ones.

Citation: Buaya AT, Thines M (2023). *Ducellieriales ord. nov.* and evidence for a novel clade of endobiotic pollen-infecting "lagenidiaceous" *Peronosporomycetes*. *Fungal Systematics and Evolution* **12**: 247–254. doi: 10.3114/fuse.2023.12.12
Received: 12 July 2023; **Accepted:** 31 August 2023; **Effectively published online:** 19 October 2023
Corresponding editor: P.W. Crous

INTRODUCTION

Freshwater holocarpic oomycetes are diverse and widespread (Karling 1942, Sparrow 1960, Dick 2001, Buaya & Thines 2020). They are mostly obligate biotrophic endobiotic parasites, infecting various hosts, including diatoms (Sparrow 1960, Buaya *et al*. 2019a, Buaya & Thines 2019b, 2020, 2021, Thines & Buaya 2022), filamentous macroalgae (Zopf 1884, de Wildeman 1893, 1896, 1897, Scherffel 1925), cyanobacteria (Ingold 1949), plants (Zopf 1887, Karling 1981), oomycetes (Cornu 1872, Barrett 1912, McLarty 1941, Whiffen 1942, 1946, Sparrow 1950, Buaya *et al*. 2019d), and invertebrate animals (Sparrow 1939, Drechsler 1940, Karling 1944, Barron 1980, Glockling & Beakes 2000). Most of the species belong to the early-diverging lineages of oomycetes (*e.g. Diatomophthora, Olpidiopsis*), while others are in the crown clades of *Saprolegniomycetes* (*e.g. Aphanomycopsis, Ectrogella*) and *Peronosporomycetes* (*e.g. Lagena, Lagenidium*) (Karling 1942, Sparrow 1960, Dick 2001, Buaya & Thines 2000). But despite recent progress, the taxonomic affiliations of most freshwater holocarpic oomycetes remain obscure, since many species have not been investigated with respect to their molecular phylogeny (Beakes & Thines 2017, Buaya & Thines 2020).

In particular, the pollen-parasitic oomycetes, of which less than a dozen species have been reported, remain unresolved, despite their widespread occurrence and potential ecological role for carbon cycling in freshwater environments (Kagami *et al*. 2014, Masigol *et al*. 2019). Records of pollen inhabiting oomycetes are known from both gymnosperm and angiosperm plants, and almost none of them have been rediscovered, to date (Sparrow 1960). These include two varieties classified in the genus *Lagenidium*, (*L. pygmaeum* var. *pygmaeum, L. pygmaeum* var. *pygmaeoides*) (Zopf 1887, Karling 1981), one each from *Aphanomycopsis* (*A. saprophytica*) (Karling 1968), and *Anisolpidium* (*A. saprobium*) (Karling 1968), a handful of enigmatic species classified into genus *Ducellieria* (*D. chodatii, D. corcontica, D. tricuspidata*) (Teiling 1957, Kusel Fetzmann & Nouak 1981, Hesse *et al.* 1989, Matula 1980), and the

provisional genus *Lagenidicopsis* (*L. arctica*) (Artemchuk 1972). Dick (2001) subsequently reclassified some of these parasites, and reassigned *L. pygmaeum* to *Cornumyces*, *L. pygmaeum* var. *pygmaeoides* to *Pleocystidium*, *A. saprophytica* to *Hyphochytrium*, and introduced the family *Ducellieriaceae* to accommodate the pine pollen pathogen *D. chodatii* within *Oomycota*. However, there are no molecular data supporting the placement of these species, including also the type species of the genus *Ducellieria*, *D. chodatii*, which is still sometimes listed as a member of the yellow-green algae family *Botryochloridaceae* of the class *Xanthophyceae* (Teiling 1957, Coute 1984) in algae databases (*e.g.* AlgaeBase) and papers in phycology (Wehr & Sheath 2003, Ettl 2009, Godínez Ortega 2017).

The genus *Ducellieria* was described by Teiling in 1957. It currently comprises three species, its types species, *Ducellieria chodatii*, and with two additional species, *D. corcontica* and *D. tricuspidata* (Matula 1980), along with a variety, *D. chodatii* var. *armatum*. In addition, one species, *D. bicuspidata*, has not been validly described, as no type specimen had been indicated (Oblinger 2005). In any case, the description provided does not contain enough detail to infer if it can be considered a member of the genus. *Ducellieria* is primarily characterized by forming colourless, multicellular, coelastrum-like spherical aggregates, connected by hollow spines, and outward-directed spines (Kusel Fetzmann & Nouak 1981, Hesse *et al.* 1989). The spherical aggregate is formed at the tip of a discharge tube from an endobiotic non-septate holocarpic thallus (Kusel Fetzmann & Nouak 1981, Hesse *et al*. 1989, Stoyneva *et al.* 2013). *Ducellieria chodatii* recurs seasonally in parallel to the peak of the pollen bloom of *Pinus* and *Picea* species, but has only rarely been reported (Kusel Fetzmann & Nouak 1981, Hesse, Kusel Fetzmann & Carniel 1989, Gorbulin 2012, Stoyneva *et al.* 2013, Bancsó 2023).

Ducellieria with its type species, *D. chodatii*, has been reclassified multiple times into different taxonomic lineages. Originally described as *Coelastrum chodatii* by Ducellier (1915) in the green algae family *Chlorophyceae* (Ducellier 1915, Bourelly 1968, Komarek & Fott 1983), the parasite has been reclassified into *Ducellieria chodatii* by Teiling (1957) with its own genus in the yellow-green algae family *Xanthophyceae* containing three species (*D. chodatii, D. corcontica, D. tricuspidata*) (Teiling 1957, Matula 1980), and one variety, *D. chodatii* var. *armata* (Teiling 1957), of which only *D. chodatii* has been proven to be an oomycete by electron microscopy (Hesse *et al.* 1989). Subsequently, a detailed re-examination of its life cycle and cellular ultrastructure confirmed its identification as an oomycete, and it was initially placed in the *Saprolegniales* (Hesse *et al.* 1989). Dick (2001) reclassified the species into its own family (*Ducellieriaceae*), provisionally assigning it to the *Leptomitales*, but excluding *D. tricuspidata* and *D. chodatii* var. *armata*.

In order to resolve the phylogeny of the type species of *Ducellieria*, attempts were made to isolate this oomycete from different freshwater environments and geographic locations in Germany. While screening for holocarpic oomycete parasites from pollen grains from the lakes Mummelsee, Okertalsperre, and Knappensee, the species was rediscovered, and it was the aim of this study to clarify its phylogenetic placement.

MATERIALS AND METHODS

Isolation, characterisation, and host and parasite dual culture attempts

Dense pollen films (predominantly from *Pinus sylvestris* and *Picea abies*) were collected from shores of the lake Mummelsee, in the state of Baden-Württemberg, Southwest Germany (48°35’53.1”N, 8°12’03.9”E), the reservoir Okertalsperre in the state of Sachsen-Anhalt, Central Germany (51°51’02.9”N, 10°27’33.0”E), the lake Knappensee (50°26’27.3”N, 8°53’55.2”E), and the pond Forbachteich (50°22’47.2”N, 8°36’45.1”E) in the state of Hessen, Central Germany. Pollen clusters with living aggregates of *Ducellieria chodatii* were collected in June 2020 from Mummelsee, Knappensee, and Forbachteich, and in June 2021 from Mummelsee and Okertalsperre, by directly collecting masses of floating pollen grains using multiple 50 mL sterile plastic tubes (Sarstedt, Nümbrecht, Germany). About 10 mL of pollen concentrates were poured onto several Petri dishes (150 × 20 mm) (Sarstedt, Nümbrecht, Germany) on the day of collection, and 100 mL of autoclaved distilled water were added to dilute the samples. Subsequently, samples were screened for *D. chodatii* using an inverted compound light microscope (AE31, Motic, Xiamen, China) and were individually picked using a 10 µL pipette (Brandt, Wertheim, Germany), rinsed multiple times in autoclaved deionized water, and transferred to 2 mL tubes containing 0.5 mL RNA*Later* solution (Invitrogen, Thermo Fisher, Lithuania) or 70 % ethanol (VWR, France) for subsequent DNA extraction. Approximately 100 spherical coelastrum-like aggregates, and pine pollen grains with endobiotic oomycete thalli were collected in this manner 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 numbers (Forbachteich 2020 isolate FR-0046136, Knappensee 2020 isolate FR-0046137) for pollen grains with endobiotic thalli, and (Mummelsee 2021 isolate FR-0046135, Okertalsperre 2021 isolate FR-0046156) for coelastrum-like spheroids). Morphological characterisation of the parasite was done as described earlier (Buaya *et al*. 2022) 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), or an SLR digital camera (EOS 500D, Canon, Tokyo Japan) for life cycle observations, mounted on the inverted compound light microscope. To inhibit motility of freshly released zoospores for microscopic photography, small amounts of Sorbitol (Carl Roth GmbH, Karlsruhe, Germany) solution (2 mg/mL) were added to the slides.

Parasite cultures with pollen grains were also attempted using living spherical aggregates of *D. chodatii* and pine pollen directly collected from pine trees (*P. sylvestris*) in 15 mL Petri dishes (60 × 15mm), with the addition of 50 µg/mL ampicillin or 100 µg/mL rifampicin (Carl Roth GmbH, Karlsruhe, Germany) and 50 mg/L Benomyl (Edgington *et al*. 1971) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) to control unwanted microbial contaminants. Culture replicates were incubated in a climate chamber (CMP 6010, Conviron, Canada) at 16 °C and 12 °C, 14 h in light and in 10 h darkness (1 000 lux, Narva, bio-vital, Germany), respectively, per day cycle. To these cultures, new pine pollen and autoclaved water were added regularly. However, long term culture trials to obtain single spore cultures from living spherical aggregates of *D. chodatii* were unsuccessful.

DNA extraction, PCR amplification, and phylogenetic analyses

DNA extraction was performed using an innuPREP Plant DNA extraction Kit (analytikjena, Jena, Germany), as previously described (Buaya *et al*. 2017) on isolated living spherical coelastrum-like aggregates and endobiotic oomycete thalli. Initially, the collected thalli were centrifuged at maximum speed (19 000 *g*) for 2 min at 22 °C to concentrate the cells. Subsequently, RNA*Later* or 70 % ethanol 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, Karlsruhe, Germany) were added into each 2 mL tube (Sarstedt, Nümbrecht, Germany) and the samples were homogenized at 25 Hz for 25 min in a Retsch Mixer Mill MM 200 (Retsch GmbH, Haan, Germany). DNA extraction of all samples was conducted following the manufacturer's instructions of the innuPREP Plant DNA extraction Kit. The PCR amplification of the partial nuclear encoding small subunit (18S; nrSSU) was performed as described in Buaya *et al*. (2019a) using Mango*Taq*™ DNA Polymerase (Bioline, London, UK) with the 18S primer pair EUK422-445 and EUK1422-1440_R (Wang *et al*. 2014) on all samples. Subsequently, all positive amplicons were sent for sequencing to the laboratory centre of the Senckenberg Biodiversity and Climate Research Centre, Frankfurt am Main (SBiK-F, Frankfurt, Germany) using the 18S primers used in the PCR. In addition, direct PCRs (putting cells of the oomycete directly into PCR tubes without prior DNA extraction) using 18S primers (Wang *et al*. 2014) were also done as described in Buaya *et al*. (2019a). To obtain high quality nrSSU sequence data for *D. chodatii* coelastrum-like aggregates, PCR amplicons were cloned into competent *Escherichia coli* (Agilent Technologies, Santa Clara, United States) 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*Taq*™ 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 centre of the Senckenberg Biodiversity and Climate Research Centre (Frankfurt am Main, Germany) using M13 (M13-F, M13-R), T7 and T3 plasmid primers.

The resulting sequences were edited using Geneious v. 5.6, and the assembled sequences of *D. chodatii* were aligned together with sequences of various members of the *Pythiales, Saprolegniales,* and early-diverging oomycete lineages, using MAFFT v. 7 (Katoh & Stanley 2013), employing the Q-INS-i algorithm. The final alignment can be found on FigShare (doi: 10.6084/m9.figshare.24185379). Minimum Evolution phylogenetic inference with 1 000 bootstrap replicates was computed using MEGAX (Kumar *et al*. 2018) with pairwise deletion, the Tamura-Nei substitution model, and all other parameters set to default. Phylogenetic analyses using the GTR model were done on the TrEase webserver (http://thines-lab.senckenberg.de/trease/, Mishra *et al*. 2023) using RAxML v. 8 (Stamatakis 2014) for Maximum Likelihood inference with 1 000 bootstrap replicates. Bayesian inference was done on the same server using MrBayes v. 3.2 (Ronquist *et al*. 2012) and 5 M generations, while other parameters were set to default. Partial nrSSU sequences obtained in this study were deposited in GenBank under the accession numbers given in the phylogenetic tree.

RESULTS

Screening and observation

Floating pollen collected during spring of 2020 and 2021 from Mummelsee, Knappensee, and Okertalsperre in Germany were found to contain abundant *Pinus sylvestris* and *Picea abies* pollen grains, living spherical coelastrum-like aggregates of *Ducellieria chodatii*, and endobiotic oomycete thalli in the pollen grains. Spherical aggregates of *D. chodatii* were highly abundant on pollen samples from Mummelsee and Okertalsperre, but very rare in samples from Knappensee, while pollen from all sites contained dense clusters of endobiotic oomycete thalli and epibiotic thalli of monocentric fungi (*e.g. Chytridiomycota*), as well as filamentous oomycetes (*e.g. Aphanomyces*, *Lagenidium*, *Pythium*). All samples were further incubated for 2–4 wk under controlled conditions, with the addition of freshly collected pollen of *Pinus sylvestris.* Every week, about 50 mL of autoclaved deionised water and pine pollen were added to prevent desiccation and to provide continuous substrate for *D. chodatii*. These culture plates were screened daily, and after more than a week, spherical coelastrum-like aggregates of *D. chodatii* became more abundant, especially on Mummelsee samples. Two weeks after this, the amount of coelastrum-like aggregates declined rapidly and finally they disappeared from all culture plates. In all samples both colonial and single-celled varieties of algae were common. During zoospores release, some protists become attracted to the newly released mass of zoospores, centrally located within the cage of empty aggregates of pentagonal or triangular shaped cells (as seen from above or the side, respectively). Often, the cage provided an effective barrier against the external predators, protecting the newly released zoospores, before they attained full motility. However, sometimes other unicellular eukaryotes penetrated into the cage after multiple attempts, but frequently remained trapped, and sometimes died within the cage.

Axenic host and pathogen dual cultures of *D. chodatii* were attempted using freshly collected *Pinus sylvestris* pollen. For this living spherical coelastrum-like aggregates were isolated and inoculated into freshly collected and autoclaved pollen grains of *Pinus sylvestris*. Subsequently, cultures were inspected daily for 1 wk for the presence of the pathogen and production of coelastrum-like spherical aggregates. However, diverse microbial contaminants, especially filamentous oomycetes *e.g. Aphanomyces, Lagenidium*, *Pythium* and fungal organisms *e.g.* chytrids, filamentous ascomycetes, and yeasts, quickly colonised all the substrates, despite application of antibiotics, and no coelastrum-like aggregates of *D. chodatii* were observed after more than 2 wk of incubation in multiple attempts. In addition, growth of the pathogen was neither observed on autoclaved pollen grains nor on pollen grains treated with freezing at -80 °C after more than 2 wk of incubation.

While the development of the endobiotic thalli (Fig. 1A) seemed to show features of several genera, including *Aphanomyces*, *Ducellieria*, and *Lagenidium*, the development of the coelastrum-like form (Fig. 1A, B) was always in line with previous descriptions of *D. chodatii* (Kusel-Fetzmann & Nouak 1981, Kusel-Fetzmann & Carniel 1984, Hesse *et al*. 1989, Stoyneva *et al*. 2013). As we assume that multiple species of oomycetes were causing the endobiotic thalli in this study, only the coelastrum-like form is described here. The number of cells in a spherical aggregate varied, likely reflecting the nutritional

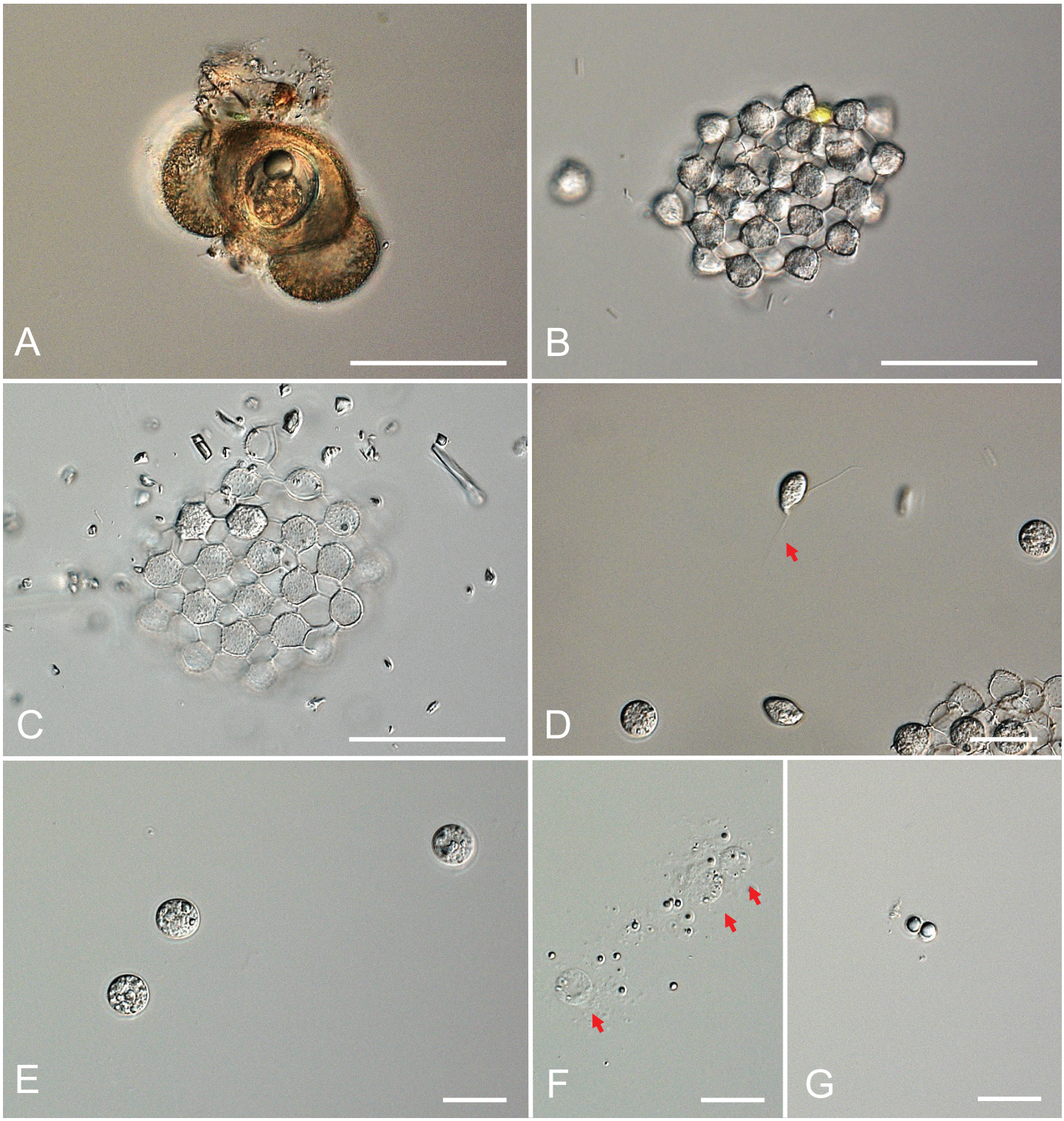

Fig. 1. DIC light micrographs of parasites of coniferous pollen. **A.** Representative of an endobiotic parasite thallus within a *Pinus* sp. pollen grain. **B–G.** Development of the coelastrum-like state of *Ducellieria chodatii*, at different stages **B.** Fully developed spherical aggregate. **C.** Spherical aggregate after most zoospore have been released. **D.** Biflagellate zoospores (red arrow pointing to the forward-directed tinsel flagellum). **E.** Zoospores resting after encystation. **F.** Empty cysts with scattered granular globules (**F, G**) after disintegration. Scale bars: A–C = 50 µm; D–G = 20 µm.

state of the thallus from which they were formed, and, thus, probably depending on the size of the pollen grains consumed. *Pinus* pollen grains, which are much smaller than those of *Picea*, usually produced smaller aggregates. The hollow coelastrum-like aggregates from *Picea* pollen grains usually consisted of more than 20 cells, with a diameter of about 50–80 µm (Fig. 1B, C). The warty to papillate individual cells usually measured 10–15 µm diam, as seen from above, each cell forming 4–6 connections to neighbouring cells. Cells in aggregates were hyaline, at maturity contained fine granules with mixtures of larger droplets (Fig. 1B). In conventional light microscopy, the spore ball sometimes appears light yellow or faint yellow green. After some time, each cell of the aggregate developed a single zoospore almost simultaneously, which escaped into the inner side of the sphere. The newly released spores usually remained in the hollow centre of the sphere for a while, initially weakly

moving inside the sphere. Subsequently, zoospore motility became more intense, and after 10–15 min zoospores swam away in an irregular motion, escaping through from the cage formed by the cell connections, leaving an empty spherical aggregate behind (Fig. 1C). The zoospores were about 8–13 µm long and 7–10 µm diam, broadly limoniform to pyriform or reniform, biflagellate with two subapically inserted flagella, of which one was forward-directed (Fig. 1D, red arrow). The heterokont zoospores usually swam for several minutes before they settled down, becoming aflagellate and spherical in shape, surrounded by thin cell wall (Fig. 1E). Sometimes, the spherical resting cysts fragmented into minute spherical globules (Fig. 1F, G) after several minutes leaving behind empty cysts (Fig. 1F, red arrows).

Molecular phylogeny

In the phylogenetic reconstructions inferred from partial nrSSU sequences (Fig. 2), the coelastrum-like form of *Ducellieria chodatii* and the endobiotic thalli belonged to different oomycete lineages, in line with the observation that in the samples obtained most endobiotic thalli showed a lagenidium-like spore discharge. *Ducellieria chodatii* coelastrum-like isolates had identical sequences and formed a monophyletic group with maximum support. This group was resolved as a member of the early-diverging oomycete lineages, forming separate, deep-branching clade. The clade was grouped with *Olpidiopsidales* and *Miraculales*, but without support. The lagenidium-like thalli were imbedded within the crown oomycete order *Peronosporales* with lacking to weak support and no clear affinities to any of the genera of the *Peronosporales* sequenced so far.

TAXONOMY

Based on the life-cycle traits and phylogenetic placement, *Ducellieria chodatii* is a *bona fide* member of the early-diverging oomycete lineages. Thus, the provisional placement of the parasitoid in the *Leptomitales* by Dick (2001) cannot be upheld, and the assignment is revised in the present study by reclassifying *D. chodatii* to an order of its own.

Ducellieriales A.T. Buaya & Thines, ***ord. nov.*** MycoBank MB 849453.

Obligate biotrophic parasite of *Pinus* and *Picea* pollen grains, thallus holocarpic, forming hollow sphere at discharge, which at maturity forms a multicellular hollow spherical aggregate, individual cells with a thin, colourless wall, ornamented with warts and spines directed outwards; zoospores single per individual cell, biflagellate and heterokont.

Type genus: *Ducellieria* Teiling

Type species: *Ducellieria chodatii* (F. Ducell.) Teiling, *Svensk bot. Tidskr*. **51**: 209. 1957.

The typification of Dick (2001) was made by choice of an iconotype, the figures in Hesse *et al*. (1989). Thus, no molecular phylogenetic investigation of the type can be done to clarify if morphologically similar and divergent species are conspecific. Therefore, FR-0046135 (**Germany**, Baden-Württemberg, Black Forest, Mummelsee, 14 Jun. 2021, *M. Thines,* ex type partial nrSSU sequence deposited in GenBank under thre accession number OR282458) deposited in the Herbarium Senckenbergianum (FR), is here designated as an **epitype** (MBT 10014079).

DISCUSSION

Holocarpic parasites of plant pollen are widespread in freshwater aquatic environments and ecologically important for carbon cycling (Karling 1942, Sparrow 1960, Dick 2001). Despite their ubiquitous nature, none of these organisms have been investigated for molecular phylogeny, leaving their taxonomic placement largely unresolved (Beakes & Thines 2017, Buaya & Thines 2020). Arguably the most enigmatic of these organisms belong to the genus *Ducellieria* (*D. chodatii*, *D. chodatii* var. *armata, D. corcontica, D. tricuspidata*), which forms a unique coelastrum-like sporangial state that has led to a classification of the pathogen in green and later golden-green algae (Ducellier 1915, Teiling 1957, Karling 1968, Artemchuk 1972, Matula 1980).

Originally, the type species of the genus was classified as *Coelastrum chodatii* in the *Chlorophyceae* by Ducellier (1915). It was later transferred to a new genus in the *Xanthophyceae*, *Ducellieria*, by Teiling (1957). However, the placement of *D. chodatii* among algae was questioned by subsequent investigators (Kusel-Fetzmann & Nouak 1981, Kusel-Fetzmann & Carniel 1984) due to the lack of plastids, absence of solid connection between cells (unlike *Coelastrum*), and other traits of its complex life cycle evidently divergent from algae (*e.g*. the formation of a holocarpic thallus). After detailed re-examination of its life cycle and cellular ultrastructure, its affiliation to the *Oomycota* was confirmed by Hesse *et al*. (1989). The pathogen was initially thought to be a member of the *Saprolegniales* (Kusel-Fetzmann & Nouak 1981). However, Dick (2001) reclassified the species to the order *Leptomitales* in separate family (*Ducellieriaceae*), excluding other related species originally included by Teiling (1957), due to the difference in the structure of connections between cells (*D. chodatii* var. *armata*), or incomplete life cycle observations by the original author (*D. tricuspidata*).

The isolate investigated in the present study agrees well with the description of previous investigators (Kusel Fetzmann & Nouak 1981, Hesse *et al*. 1989, Stoyneva *et al*. 2013), in terms of morphology and parasitism to coniferous plant pollen. However, it is noteworthy that the coelastrum-like forms co-occurred with a widespread lagendium-like form and several other pollen parasites of both chytrids and oomycetes (*e.g*. an aphanomyces-like pathogen). The possibility of confusing *D. chodatii* with other species has already been pointed out before (Kusel Fetzmann & Nouak 1981, Kusel Fetzmann & Carniel 1984, Hesse *et al*. 1989, Stoyneva *et al*. 2013), but it also seems possible, that forms attributed to the life cycle of *D. chodatii* might actually represent other oomycetes or fungi, since drifting gymnosperm pollen harbours a great diversity of fungi and oomycetes (Sparrow 1960, Sparrow 1968, Czeczuga & Muszyńska 2004, Wurzbacher *et al*. 2014, Van den Wyngaert *et al*. 2022), as we have also observed in all our samples. Due to the cage-like nature of the aggregates it seems possible that even isolations of single spheroids might contain additional organisms able to infect pollen grains. Thus, until dual cultures using single zoospore isolates confirm the various stages previously reported, only cycle “A” and “B” from Hesse *et al*. (1989) featuring the coelastrum-like phase and the fact that thalli can develop within pollen grains can be considered doubtless, while variations of the endobiotic stage

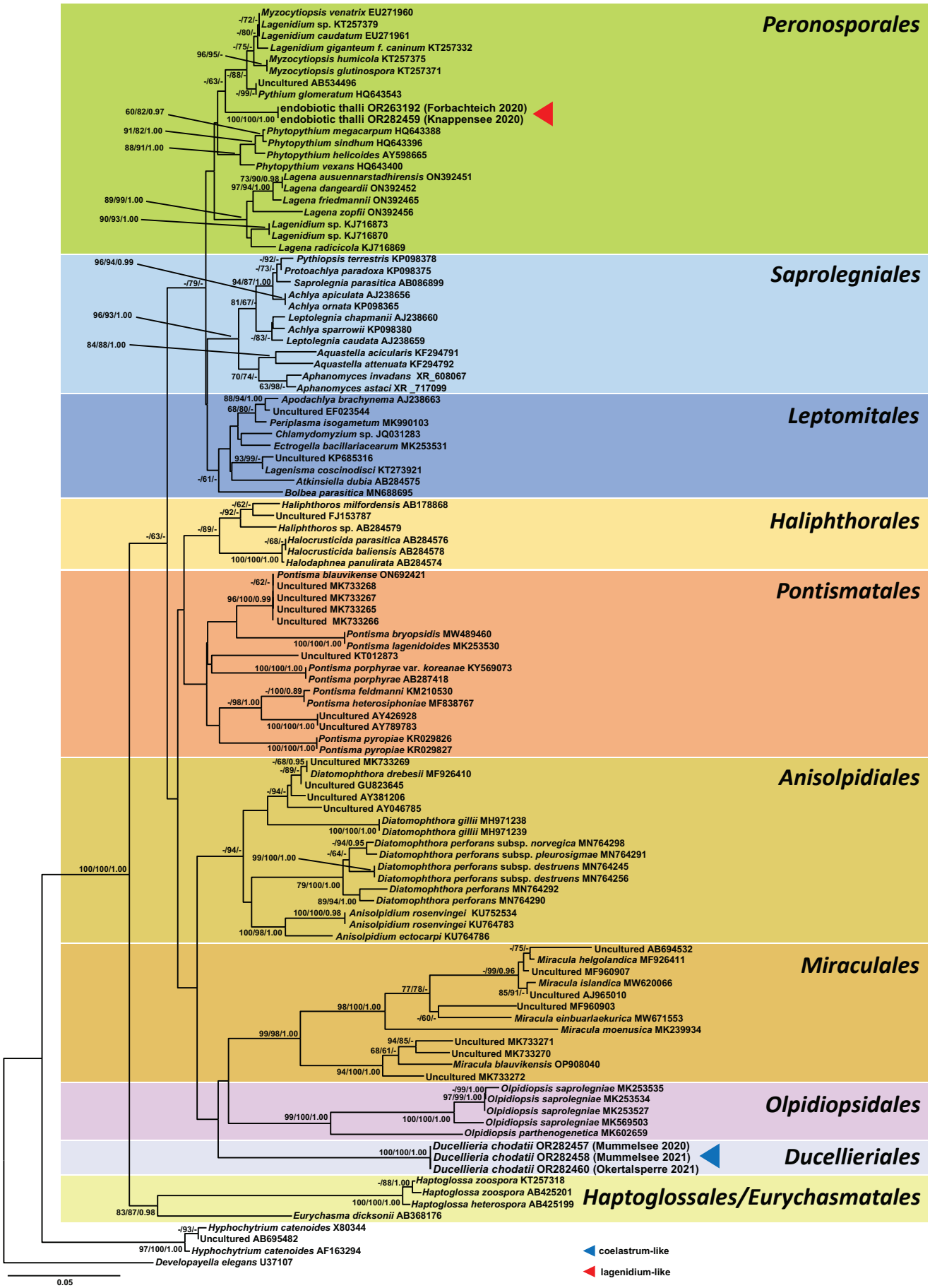

Fig. 2. Molecular phylogeny using Minimum Evolution inferred from partial nrSSU sequences. The numbers at the branches denote bootstrap support values from Maximum Likelihood and Minimum Evolution analyses, in respective order. A hyphen (-) indicates less than 60 % bootstrap support or a conflicting topology. The third number refers to posterior probabilities equal to or greater than 0.95 from the Bayesian phylogenetic inference. Also in this case, a hyphen (-) sign denotes lack of support for the presented or an alternate topology.

and the additional stages (A1, B1, D1) found by Stoyneva (2013) should be confirmed with single spore isolates. This might not always be easy, as it is conceivable, that the formation of these stages depends on environmental conditions. Also, the resting stage reported (*e.g.* Hesse *et al.* 1989) should be considered as not conclusively belonging to *D. chodatii*, as Hesse *et al.* (1989) did not succeed to trigger the formation of coelastrum-like forms from these. The exclusion of *D. tricuspidata* (basionym: *Coelastrum tricuspidatum*), and *D. chodatii* var. *armata* (basionym: *Coelastrum augustae* var. *armata*) from *Ducellieria* by Dick (2001) also requires re-evaluation. Considering the similarities with respect to the formation of a coelastrum-like stage, these species are probably better retained in the genus until a re-evaluation of their life cycle and molecular phylogenetic reconstructions can clarify their evolutionary affinities.

ACKNOWLEDGEMENTS

ATB and MT are thankful to Stefan Wötzel and Tetyana Tsykun for pollen collection at Mummelsee Lake, Baden-Württemberg, Germany in June 2020, and to José Luis Godínez-Ortega for pollen collection at the Zempoala Lagoon Mexico in 2021, even though *Ducellieria* could not be retrieved from there.

Conflict of interest: M. Thines is a senior editor of the journal. However, he was not involved in the handling or review process of this manuscript.

REFERENCES

Artemchuk JA (1972). The fungi of the White Sea. III New Phycomycetes, discovered in the Great Salma Strait of the Kandalakshial Bay. *Veröffentlichungen des Instituts fur Meeresforschung in Bremerhaven* **13**: 231–237.

Bancsó S (2023). Adatok a *Ducellieria chodatii* nyugat-magyarországi előfordulásához. *Kitaibelia* **28**: 3–10.

Barrett JT (1912). Development and sexuality of some species of *Olpidiopsis* (Cornu) Fischer. *Annals of Botany London* **26**: 209–238.

Barron GL (1980). A new *Haptoglossa* attacking rotifers by rapid injection of an infective sporidium. *Mycologia* **72**: 1186–1194.

Beakes GW, Thines M (2017). *Hyphochytriomycota and Oomycota*. In: *Handbook of the Protists* (Archibald JM, Simpson AGB, eds). Springer Verlag, Germany: 435–505.

Bourelly P (1968). Les algues d'eau douce. Inition á la systématique. II. Les algues jaunes et brunes. *Boubée ed. Paris*: 1–438.

Buaya AT, Ploch S, Hanic L, *et al*. (2017). Phylogeny of *Miracula helgolandica gen. et sp. nov.* and *Olpidiopsis drebesii sp. nov*. two basal oomycete parasitoids of marine diatoms, with notes on the taxonomy of *Ectrogella*-like species. *Mycological Progress* **16**: 1041–1050.

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, Thines M (2019b). *Miracula moenusica*, a new member of the holocarpic parasitoid genus from the invasive freshwater diatom *Pleurosira laevis*. *Fungal Systematics and Evolution* **3**: 19–33.

Buaya AT, Kraberg A, Thines M (2019c). 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* (2019d). Holocarpic oomycete parasitoids of red algae are not *Olpidiopsis*. *Fungal Systematics and Evolution* **4**: 21–31.

Buaya AT, Thines M (2020). An overview on the biology and phylogeny of the early-diverging oomycetes. *Philippine Journal of Systematic Biology* **14**: 1–20.

Buaya AT, Thines M (2021). *Miracula einbuarlaekurica sp. nov.*, a new holocarpic endoparasitoid species from pennate freshwater diatoms in Iceland. *Mycology* **13**: 1–9.

Buaya AT, Tsai I, Thines M (2022). *Pontisma blauvikense sp. nov.* the first member of the early-diverging oomycete genus *Pontisma* parasitizing brown algae. *Journal of Eukaryotic Microbiology* **70**: e12957.

Choi YJ, Beakes G, Glocking S, *et al.* (2015). Towards a universal barcode of oomycetes – a comparison of the *cox1* and *cox2* loci. *Molecular Ecology Resources* **15**: 1275–1288.

Cobelas MA (1984). Catalogo de las algas continentales Españolas. II. *Craspedophyceae*, *Cryptophyceae*, *Chrysophyceae*, *Dinophyceae*, *Euglenophyceae*, *Haptophyceae*, *Phaeophyceae*, *Rhodophyceae*, *Xanthophyceae*. *Acta Botánica Malacitana* **9**: 27–40.

Cornu M (1872). Monographie des Saprolégniées, étude physiologique et systematique. *Annales des Sciences Naturelles Botanique* **15**: 1–198.

Coute A (1984). Premiéres observations au M.E.T. et au M.E.B. sur la cytologie de *Ducellieria chodatii* (Ducell.) Teiling (*Xanthophyceae*, *Mischococcoales*, *Chlorobotrydaceae*). *Nova Hedwigia* **39**: 651–662.

Czeczuga B, Muszyńska E (2004). Aquatic zoosporic fungi from baited spores of cryptogams. *Fungal Diversity* **16**: 11–22.

de Wildeman E (1893). Notes mycologiques I. *Annales de la Société Belge de Microscopie* **17**: 5–30.

de Wildeman E (1896). Notes mycologiques VII. *Annales de la Société Belge de Microscopie* **20**: 21–64.

de Wildeman E (1897). Notes mycologiques IX. *Annales de la Société Belge de Microscopie* **21**: 3–31.

Dick MW (2001). *Straminipilous Fungi*. Kluwer, Netherlands.

Drechsler C (1940). Three fungi destructive to free-living terricolous nematodes. *Journal of the Washington Academy of Sciences* **30**: 240–254.

Ducellier F (1915). Note sur un nouveau *Coelastrum*. *Bulletin de la Sociéte Botanique de Genéve, 2. Serie* **7**: 73–74.

Edgington LV, Khew KL, Barron GL (1971). Fungitoxic spectrum of benzimidazole compounds. *Phytopathology* **61**: 42–44.

Ettl H (2009). *Xanthophyceae*. In: *Suwasserflora von Mitteleuropa* (Ettl H, Gerloff J, Heynig H, eds). Fischer, Stuttgart, Germany: 1–530.

Glockling SL, Beakes GW (2000). An ultrastructural study of sporidium formation during infection of a rhabditid nematode by large gun cells of *Haptoglossa heteromorpha*. *Journal of Invertebrate Pathology* **76**: 208–215.

Godínez-Ortega JL, Oliva-Martínez MG, Escobar-Oliva MA, *et al.* (2017). Diversidad algal del Parque Nacional Lagunas de Zempoala, México, excepto diatomeas. *Hidrobiológica* **27**: 45–58.

Gorbulin OS (2012). Ecological and biological characteristics of *Xanthophyta* of Ukraine inland water bodies. *The Journal of V.N. Karazin Kharkiv National University. Series: Biology* **15**: 50–66.

Hesse M, Kusel-Fetzmann E, Carniel K (1989). Life cycle and ultrastructure of *Ducellieria chodatii* (*Oomycetes*). *Plant Systematics and Evolution* **165**: 1–15.

Hudspeth DSS, Nadler SA, Hudspeth MES (2000). A *cox*2 molecular phylogeny of the *Peronosporomycetes*. *Mycologia* **92**: 674–684.

Ingold CT (1949). On the genus *Resticularia*. *Annals of Botany. London. N.S.* **13**: 435–443.

Kagami M, Miki T, Takimoto G (2014). Mycoloop: chytrids in aquatic food webs. *Frontiers in Microbiology* **5**: 1–9.

Karling JS (1942). *The simple holocarpic biflagellate Phycomycetes*. JS Karling, USA.

Karling JS (1944). New lagenidiaceous parasites of rotifers from Brazil. *Lloydia (Cincinnati)* **7**: 328–342.

Karling JS (1968). Zoosporic fungi of Oceania. II. Two saprophytic species of *Aphanomycopsis*. *Mycologia* **60**: 271–284.

Karling JS (1981). Some zoosporic fungi from soils of Sri Lanka. *Nova Hedwigia* **35**: 107–116.

Katoh K, Standley DM (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.

Komarek J, Fott B (1983). *Chlorophyceae* (Grünalgen); Ordnung: *Chlorococcales.* In: *Die Binnengewässer 16. Das Phytoplankton des Süßwassers* (Huber Pestalozzi, ed). Schweizerbartsche Verlagsbuchhandlung, Stuttgart, Germany: 1–1044.

Kumar S, Stecher G, Li M, *et al.* (2018). MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution* **35**: 1547–1549.

Kusel-Fetzmann E-L, Nouak H (1981). *Ducellieria chodatii* - Alge oder Pilz? *Plant Systematics and Evolution* **138**: 199–207.

Kusel-Fetzmann E-L, Carniel K (1984). Beiträge zur Biologie und Ultrastruktur von *Ducellieria Chodatii*. *Mitteilungsband d. Tagung der Deutschen Botanischen Gesellschaft in Wien, Kurzfassung der Beiträge*: 107.

Masigol H, Khodaparast SA, Woodhouse JN, *et al.* (2019). The contrasting roles of aquatic fungi and oomycetes in the degradation and transformation of polymeric organic matter. *Limnology and Oceanography* **64**: 2662–2678.

Matula J (1980). *Ducellieria chodatii* (Ducel.) Tell. and *D. corcontica* Mat. *nova sp.* (*Xanthophyceae*) found in a peat bog in the Karkonosze Mts. *Fragmenta Floristica et Geobotanica* **26**: 349–353.

McLarty DA (1941). Studies in the family *Woroninaceae*. I. Discussion of a new species including a consideration of the genera *Pseudolpidium* and *Olpidiopsis*. *Bulletin of the Torrey Botanical Club* **68**: 49–66.

Mishra B, Ploch S, Weiland C, Thines M (2023). The TrEase web service – inferring phylogenetic trees with ease. *Mycological Progress* **22**: 81.

Oblinger H (2005). Über die Kleinlebewelt in den Hochmoorgewässem des Haspelmoores und des Mödishofer Moores. *Berichte des naturwissenschaftlichen Vereins für Schwaben, Augsburg* **109**: 124–133.

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* **6**: 539–542.

Scherffel A (1925). Endophytische Phycomycetenparasiten der Bacillariaceen und einige neue Monadinen. Ein Beitrag zur Phylogenie der Oomyceten (Schröter). *Archiv für Protistenkunde* **52**: 1–141.

Sparrow FK (1939). A new species of *Lagenidium* parasitic in rotifer eggs. *Mycologia* **31**: 527–532.

Sparrow FK (1950). Some Cuban *Phycomycetes*. *Journal of the Washington Academy of Sciences* **40**: 50–55.

Sparrow FK (1960). *Aquatic Phycomycetes*. *The University of Michigan Press*, Ann Arbor Michigan, USA.

Sparrow FK (1968). Remarks on the *Thraustochytriaceae*. *Veröffentlichungen des Instituts for Meeresforschung in Bremerhaven, Sonderband* **3**: 7–18.

Stamatakis A (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.

Stoyneva MP, Uziunov BA, Gärtner G (2013). First record of the parasitic oomycete *Ducellieria chodatii* from Pirin Mts (Bulgaria) with notes on its taxonomy, life cycle and ecology. *Sydowia* **65**: 1–12.

Teiling E (1957). Some little known Swedish phytoplankters. *Svensk Botanisk Tidskrift* **51**: 207–222.

Thines M, Buaya AT (2022). *Lagena* - an overlooked oomycete genus with a wide range of hosts. *Mycological Progress* **21**: 66.

Van den Wyngaert S, Ganzert L, Seto K, *et al.* (2022). Seasonality of parasitic and saprotrophic zoosporic fungi: linking sequence data to ecological traits. *ISME Journal* **16**: 2242–2254.

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.

Wehr J, Sheath RG (2003). *Freshwater Algae of North America: Ecology and Classification*. Academic Press.

Whiffen AJ (1942). A discussion of some species of *Olpidiopsis* and *Pseudolpidium*. *American Journal of Botany* **29**: 607–611.

Whiffen A J (1946). Two new terricolous phycomycetes belonging to the genera *Lagenidium* and *Blastocladiella. Journal of the Elisha Mitchell Scientific Society* **62**: 54–58.

Wurzbacher C, Rösel S, Rychla A, *et al.* (2014). Importance of saprotrophic freshwater fungi for pollen degradation. *PLoS ONE* **9**: e94643.

Zopf W (1884). Zur Kenntniss der Phycomyceten. I. Zur Morphologie und Biologie der Ancylisteen und Chytridiaceen. *Novorum actorum Academia Caesareae Leopoldinae-Carolinae Germanicae Naturae Curiosorum* **47**: 143–236.

Zopf W (1887). Über einige niedere Algenpilze (Phycomyceten) und eine neue Methode ihre Keime aus den Wasser zu isolieren. *Abhandlungen der naturforschenden Gesellschaft zu Halle* **17**: 77–107.