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"Ectrogella" Parasitoids of the Diatom *Licmophora* sp. Are Polyphyletic

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ABSTRACT

The diatom genera *Licmophora* and *Fragilaria* are frequent epiphytes on marine macroalgae, and can be infected by intracellular parasitoids traditionally assigned to the oomycete genus *Ectrogella*. Much debate and uncertainty remains about the taxonomy of these oomycetes, not least due to their morphological and developmental plasticity. Here, we used single-cell techniques to obtain partial sequences of the parasitoids 18S and *cox2* genes. The former falls into two recently-identified clades of *Pseudo-nitzschia* parasites temporarily named OOM_1_2 and OOM_2, closely related to the genera of brown and red algal pathogens *Anisolpidium* and *Olpidiopsis*. A third group of sequences falls at the base of the red algal parasites assigned to *Olpidiopsis*. In one instance, two oomycete parasitoids seemed to co-exist in a single diatom cell; this co-occurrence of distinct parasitoid taxa not only within a population of diatom epiphytes, but also within the same host cell, possibly explains the ongoing confusion in the taxonomy of these parasitoids. We demonstrate the polyphyly of *Licmophora* parasitoids previously assigned to *Ectrogella* (*sensu* Sparrow, 1960), and show that parasites of red algae assigned to the genus *Olpidiopsis* are most likely not monophyletic. We conclude that combining single-cell microscopy and molecular methods is necessary for their full characterisation.

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Keywords
Anisolpidium; Ectrogella; Olpidiopsis; Peronosporomycota; single-cell

PARASITES play a key role in phytoplankton ecological succession and in their host population dynamics (Chambouvet *et al.*, 2008). Recent studies in marine and freshwater systems, especially when molecular barcoding has been used, have consistently highlighted the importance and continuing lack of knowledge of parasitic relationships in structuring pelagic microbial communities (Lepère *et al.*, 2008; de Vargas *et al.*, 2015; Lima-Mendez *et al.*, 2015). Planktonic diatoms are no exception to this pattern, and the most frequently described parasites of diatoms are Chytridiomycota (Fungi) and Oomycota (Stramenopila; Ibelings *et al.*, 2004; Hanic *et al.*, 2009; Gutiérrez *et al.*, 2016; Taylor and Cunliffe, 2016). Although less extensively studied, recent studies have shown that benthic and epiphytic diatoms are similarly subject to infection by parasites (Scholz *et al.*, 2014, 2016). An abundant body of literature dating back from the 19th century describes the presence of intracellular oomycetes infecting diatoms and mostly assigns them to the genus *Ectrogella*, which has traditionally been included in the Saprolegniales (Sparrow 1960). The original description of the genus corresponds to *Ectrogella bacillariacearum* infecting a freshwater *Synedra*; it stresses its spherical shape, lack of pigmentation, intracellular coenocytic habit and describes its zoosporogenesis. In this paper, a particular feature of *Ectrogella* is the presence of several discharge tubes in the bigger sporangia, and the uniflagellate, monoplanetic zoospores (Zopf, 1884). This original description, however, conflicts with a second account given by Scherffel (1925), who stressed that zoospores emerging from sporangia were in fact biflagellate and diplanetic (see detailed review and illustration in Garvetto *et al.*, 2018). Afterwards, several *Ectrogella* species were defined but in most cases important criteria, such as zoosporogenesis, were not observed; on the other hand, additional criteria absent from the original description were added whilst the presence of sexual reproduction or resting spores was reported for other species in the genus (Scherffel, 1925). Thus, generally speaking, *Ectrogella* species are described as intracellular, unbranched, obligate biotrophic, holocarpic parasites. In the late stage of infection, the mature zoosporangium occupies the whole cell and disintegrated diatom plastids can be seen surrounding it. In their syntheses, Sparrow (1960) and Karling (1981) recognised eight species of *Ectrogella*; seven of them infecting diatoms, with only three in marine hosts: *E. licmophorae* (diplanetic with spores encysting at the mouth of the discharge tube, i.e. achlyoid), *E. perforans* (monoplanetic with spores swarming directly away from the sporangium, i.e. saprolegnoid) and *E. eurychasmoides* (diplanetic with primary spores encysting within the sporangium, i.e. eurychasmoid). In 2001, and without much more evidence at hand, Dick proposed to synonymise all the freshwater species of *Ectrogella* into *E. bacillariacearum*, to the exception of *E. monostoma*, which was synonymised with *Aphanomycopsis bacillariacearum* (Dick, 2001). Thus, he reduced the number of extant marine species to two, by including *E. licmophorae* into *E. perforans*, and keeping *E. eurychasmoides* as a "doubtfully distinct" species (Table 1), whereas Johnson (1966) argued to synonymise it with *E. perforans* and keep *E. licmophorae* a separate entity. Recently, molecular data have started to clarify the phylogenetic relationships between these parasitoids of diatoms and other intracellular parasites of brown and red algae. The genus of brown algal pathogens *Anisolpidium* (and the order Anisolpidiales) was transferred from the hyphochytrids into the oomycetes (Gachon *et al.*, 2017). Several species of red algal pathogens assigned to the genus *Olpidiopsis* were sequenced (Sekimoto *et al.*, 2008, 2009; Klochkova *et al.*, 2016; Kwak *et al.*, 2017; Badis *et al.*, 2018). However, the recent

epitypification of the genus *Olpidiopsis*, due to the molecular description of *Olpidiopsis saprolegniae*, ascertained that these red algal pathogens do not belong to the same clade. The same authors also sequenced and defined an epitype for the red algal pathogen *Pontisma lagenioides*, and thus tentatively reassigned all other *Olpidiopsis* pathogens of red algae to the genus *Pontisma* despite the lack of strong phylogenetic support (Buaya *et al.*, 2019a). To date, all red algal pathogens characterised molecularly cluster in three closely related clades; however, their monophyly and exact relatedness with *Pontisma*, *Anisolpidium* and the pathogen of freshwater diatoms *Olpidiopsis gillii* (Buaya *et al.*, 2019b) remain to be established. Garvetto *et al.*, (2018) recently identified novel pathogens of the marine diatoms *Pseudo-nitzschia* and *Melosira*; but in the light of the doubtful typification of *Ectrogella* and the unresolved relationships between different clades, they adopted a transient nomenclature OOM_1 (containing three subclades OOM_1_1, OOM_1_2 and OOM_1_3) and OOM_2. In parallel, Buaya *et al.*, (2017) described the species *Olpidiopsis drebesii*, which falls in the OOM_2 clade, and defined a novel monogeneric family, the Miraculaceae. The latter contains the genus *Miracula*, with two species *M. helgolandica* (Buaya *et al.*, 2017) and *M. moenusica* (Buaya and Thines, 2019), both belonging to the subclade OOM_1_1. Among the hosts of *E. eurychasmoides*, *E. licmophorae*, and *E. perforans* are the diatoms *Licmophora* sp. and *Fragilaria* sp. (Table 1). *Licmophora* is an almost ubiquitous marine, colonial, mostly epiphytic diatom with wedge-shaped cells in girdle view; while *Fragilaria* is a filament-forming diatom which can be planktonic, benthic, free living in colonies or epiphytic. *E. perforans*, has been reported to cause epidemics outbreaks affecting 30% of *Fragilaria* and up to an astonishing 99% of *Licmophora* populations in the USA (Sparrow, 1969). In the early 1980s, Raghu Kumar successfully cultivated a pathogen of *Licmophora* also identified as *E. perforans* (Raghu Kumar, 1978), and described its ultrastructure (Raghu Kumar, 1980b, 1980a). Interestingly however, Johnson (1966) highlighted the variability of the morphological criteria used to delimit species amongst *Ectrogella* parasitoids of *Licmophora* (namely, *L. abbreviata* and *L. gracilis*). He mentioned the co-existence in field-collected materials of several modes of zoospore discharge and concluded that the morphological features used to define each species were unreliably polymorphic. Apparently, he did not envisage that several parasites species may co-exist in a same sample. With the above in mind, we developed single-cell approaches based on individual whole genome amplification and subsequent selection of suitable molecular markers, in order to investigate species boundaries within the genus *Ectrogella* and similar parasitoid genera.

MATERIALS AND METHODS

Sampling and single cell isolation

On the 11.07.2017 and 27.07.2017, the macroalgae *Laminaria digitata* and *Ulva* sp. were sampled at Clachan Bridge (56°19'03.2"N 5°34'59.7"W; Isle of Seil, United Kingdom) and kept in seawater at 10°C under constant air supply, with a 12h light-dark cycle. Algal fragments or their epiphytic community were screened for the presence of infected diatoms by bright field optical microscopy (Axioskop 2 plus, Zeiss, Oberkochen, Germany). Upon microscopic inspection of the epiphytic microbial community growing on *Ulva* sp. and *Laminaria digitata*, cells and chains of *Licmophora* sp. and *Fragilaria* sp. containing endobiotic thalli were recorded. The samples were further stained with 1% Calcofluor White (CW) for 5 minutes in the dark, to facilitate the detection of oomycete infective structures. Pictures were taken with an AxioCam HRc coupled to the AxioVision software (Zeiss, Oberkochen, Germany, version 4.7.1). The most developed thalli were stained with CW and some exhibited discharge tubes, strongly suggestive of an infection by *Ectrogella*-like parasites. Samples containing infected diatoms were then transferred into sterile Petri dishes

with sterile filtered seawater and infected cells were isolated through mouth pipetting under an inverse microscope. Single cells (for *Licmophora* sp.) or single colonial chains (for *Fragilaria* sp.) were washed four times by sequential transfer in sterile seawater drops, transferred into sterile Eppendorf-tubes, and stored at -20°C until further handling. This procedure resulted in fourteen single cell isolates. Isolate names provide information on the infected diatom host (Inf = infected; Lic = *Licmophora* and Fr = *Fragilaria*), sampling date (the code S10 refers to samples collected on the 27 of July, whilst the absence of it refers to samples from the 11 of July) and ordinal number of the single cell isolate (SC = single cell / single chain).

Single-cell whole genome amplification, PCR and sequencing

Multiple displacement amplification (MDA) was carried out using the REPLI-g® Single Cell Kit (Qiagen, Hilden, Germany). Briefly: 4 µL PBS buffer and 3 µL lysis buffer were added to single cells. After 10 minutes of incubation at 65°C, the lysis was stopped with 3 µL of Stop Solution. 40 µL of master mix (containing φ-29 polymerase) were added to each single cell and samples were incubated for 8 hours at 30°C, before polymerase inactivation by heating for 3 minutes at 65°C. MDA material was then kept at -80°C for long term storage. Aliquots of MDA-amplified material were diluted 1:100 (V:V) in autoclaved Milli-Q water (Merck Millipore) and 2 µL were used as a template for downstream targeted PCRs. Each PCR contained 25 µL of master mix solution (Taq PCR Mastermix, Qiagen, Hilden, Germany), 1 µL (0.2 ng/µL) of each primer and 20 µL of autoclaved Milli-Q water. Primers and PCR conditions used in this study are detailed in Supplementary Information 1, including specific primers designed to discriminate two oomycetes co-infecting a single *Licmophora* sp. cell. In order to rule out cross-contamination in the co-infected sample, both 18S rDNA and *cox2* sequences were repeatedly retrieved from different dilution of the MDA product and after all reagents and laboratory consumables had been replaced. PCR products were checked by gel electrophoresis. When present, bands of different length were excised from the gel and purified with the X-tracta Gel Extraction Tool (Sigma-Aldrich, St. Louis, Missouri, USA). If a single amplification product was detected, the purification was performed using the Thermo Scientific GeneJET Gel Extraction and DNA Cleanup Micro Kit, and PCR products were Sanger-sequenced (GATC Biotech AG, Köln, Germany). Overall, marker genes were successfully amplified for seven of the single cell isolates.

Phylogenetic reconstruction

Sequences were quality-controlled, trimmed and assembled in contigs using Geneious 6.1.8 (Kearse *et al.*, 2012). The dataset of 18S rDNA sequences of oomycetes used in this study is amended from Garvetto *et al.* (2018) in order to include novel sequences of parasitoids of diatoms. Alignments were carried out using the MAFFT (Katoh *et al.*, 2002) algorithm implemented in Geneious 6.1.8, manually checked and curated with Gblocks 0.91b (Castresana, 2000). Substitution models were assessed through ModelFinder (Kalyaanamoorthy *et al.*, 2017) in IQ-TREE 1.5.5 (Nguyen *et al.*, 2015), resulting in TN+R4. IQ-TREE 1.5.5 was also used for the phylogenetic reconstruction, which was computed with a Maximum Likelihood method using the Ultrafast bootstrap approximation (UFBoot, 1000 replicates) test of phylogeny (Minh *et al.*, 2013). Aiming at backing up the results from the 18S rDNA, a second phylogenetic reconstruction was computed (as described above) based on the aminoacid sequence of the mitochondrial marker *cox2*, under the substitution model mtZOA+F+G4 (Rota-Stabelli *et al.*, 2009).

RESULTS AND DISCUSSION

Observed morphological features

Different morphological features were observed in the oomycetes present in the environmental samples (Fig. 1 A-B) containing the isolated single cells (Fig. 1 C-I, Supporting Table 2), which are in line with descriptions of *Ectrogella perforans* infecting *Licmophora* (Sparrow, 1960) and *Fragilaria* (Sparrow, 1969). In *Fragilaria* sp. parasitic thalli developed in an ellipsoidal shape occupying the central portion of the cell; generally confined between two collapsed plastids (Fig.1 C; Fig.2 B). In one instance a big central vacuole was observed in one thallus, whereas multiple thalli in the same diatom cell were often observed (Fig.2 B). CW staining highlighted the presence of broadly conical discharge tubes (Fig.2 C, arrowhead) as well as more elongated ones (Fig.2 D, arrowheads). Hollow spore cysts have been observed within empty sporangia (Fig.2 C, arrows), although clusters of spore cysts at the mouth of the discharge tube were also observed (Fig.2 E). Both these zoospore behaviours have been reported for the *Fragilaria islandica* infected by *E. perforans* in Sparrow material (1969). In parasites of *Licmophora* sp. thalli were mainly spherical in shape and closely surrounded by red-brown degraded plastids when developing (Fig.1 G, H; Fig.2 G). At maturity, the sporangial cell wall was weakly stained by CW (Fig.2 G, inlet). Empty sporangia observed in our sample were less regular in shape and bore up to four broadly conical discharge tubes (Fig.2 H, I; arrowheads). These sporangial features are consistent with the descriptions of lenticular/spherical or irregular/saccate sporangia reported for *Licmophora* infected by *E. perforans* in Johnson (1966). In few cases spores could be observed within the sporangium, but in no instance we were able to observe their flagellation. Spores are spherical and seem to surround a central vacuole when within the sporangium (Fig.2 J). Empty spore cysts could be observed both within and outside the sporangium (Fig.2 H, I; arrows). Despite the lack of first-hand experience of spore discharge behaviour, our observations of empty spore cysts are consistent with observations made by Raghu Kumar (1980b) on *E. perforans* in culture, where zoospores were observed to either escape before encysting or to encyst within the sporangium or at the mouth of the discharge tube. Discharge tubes with a circular or broadly ellipsoidal outline were observed to coexist in the same sporangium, incorporated in a thickened area of the sporangial wall (Fig. 2K, L).

Co-existence of two taxonomically distinct oomycetes in a single host cell

Single cell (or chain) whole genome amplification followed by PCR allowed to gather genetic markers used to phylogenetically place the *Ectrogella*-like organisms detected and described above. Two distinct oomycete 18S rDNA and *cox2* sequences were amplified from the cell InfLicSC2 (noted InfLicSC2-a and InfLicSC2-b in Fig. 3-4; Supplementary Information 2). The 18S rDNA InfLicSC2-b was very closely related to *Olpidiopsis drebesii* within the OOM_2 clade whereas InfLicSC2-a was identical with the 18S rDNA sequences retrieved from InfLicSC1, InfLicSC3, InfLicSC4 (Fig. 3). *cox2* data were in full agreement with results from the 18S rDNA, though less easily resolved due to the paucity of reference sequences for the OOM_2 clade (Fig. 4). Bearing in mind that some oomycetes affiliated to the genus *Olpidiopsis* are themselves intracellular parasites of oomycetes (e.g. the type of the genus, *Olpidiopsis saprolegniae*) and that hyperparasites (i.e. parasitic organisms whose host is itself a parasite) have been reported in endocellular holocarpic oomycetes (e.g. *Pythiella besseyi* in *Olpidiopsis schenkiana* Sparrow and Ellison, 1949; Sparrow, 1960), we next investigated the possibility of a cryptic hyperparasitism of the *Ectrogella* parasite: we reasoned that if a hyperparasite was present, its sequence should always be associated to its oomycete host. Therefore, two specific reverse primers were designed to target either of the two rDNA sequences (18S_SC1spe_R1 and 18S_SC1spe_R2 for the 18S rDNA of

InfLicSC1 and 18S_SC2spe_R3 and 18S_SC2spe_R4 for the 18S rDNA of InfLicSC2-b, Supplementary Information 1). Those were used in direct and nested PCRs on the MDA-amplified InfLicSC1, InfLicSC2, InfLicSC3, and InfLicSC4. Overall, only one oomycete sequence was retrieved from each isolated cell, except in InfLicSC2. On the other hand, the InfLicSC2-b 18S and *cox2* sequences have never been obtained in our lab apart from this sample, limiting the risk of an external contamination of the InfLicSC2 isolate by foreign DNA. Finally, a close re-inspection of our photographs highlighted the possible presence of two thalli inside the host diatom cell (Fig. 1F). Therefore, we conclude that InfLicSC2 was concurrently infected by two taxonomically distinct oomycete parasitoids; our data do not give us any reason to suspect a non-random association between them, such as a hyperparasitic relationship.

Molecular phylogeny of the parasitoids infecting *Licmophora* sp. and *Fragilaria* sp.
Overall three distinct 18S rDNA sequences were retrieved. The 18S rDNA of InfLicSC1 (identical to InfLicSC2-a, InfLicSC3, InfLicSC4) diverges early and without bootstrap support from the group of *Olpidiopsis* parasites of red seaweeds, together with *Olpidiopsis pyropiae*, a pathogen of *Pyropia* sp. recently discovered in Korean laver farms (Klochkova *et al.*, 2016) and *Pontisma lagenidioides*, recently isolated from *Ceramium rubrum* (Buaya *et al.*, 2019a). The 18S rDNA InfLicSC2-b is closely related to *Olpidiopsis drebesii* within the OOM_2 clade. Finally, the 18S rDNA S10InfFrLic and S10InfLicSC (identical to S10InfLicSC6) fall within the OOM_1_2 subclade, the only morphologically known member of which is a parasitoid of *Pseudo-nitzschia fraudulenta* (Garvetto *et al.*, 2018; Fig. 3). Likewise, three distinct *cox2* sequences were retrieved. Their position in the phylogenetic tree is consistent with the 18S rDNA data, despite a generally lower resolution of the tree (Fig. 4). The latter is due to the limited availability of reference sequences in the alignment, and their shorter length, a well- known limitation of *cox2*-based oomycete phylogenies (Badis *et al.*, 2018).

Parasitoids of *Licmophora* are polyphyletic
Here, we show that parasitoids of *Licmophora* sp. fall into three distinct taxonomic entities: all of them belong to the "early diverging oomycetes" (*sensu* Beakes *et al.*, 2012), a poorly known group that contains the Haliphthorales, Anisolpidiales, Pontismatales, Olpidiopsidales (*sensu* Badis *et al.*, 2018), Eurychasmales and Haptoglossales (Garvetto *et al.* 2018; Buaya *et al.*, 2019a; Fig.3). However, these three parasitoid taxa of *Licmophora* are polyphyletic, and may co-occur in nature. The implications of our findings are several-fold. Firstly, we demonstrate that the genus *Ectrogella* is polyphyletic. Unfortunately, evidence is lacking to link either of our sequences to the type of the genus, the freshwater *E. bacilliacearum* (Zopf, 1884). In the light of our results, *Ectrogella* may thus correspond either to the subclade OOM_1_2, the clade OOM_1, the clade OOM_2, encompass the branch retrieved with the parasitoid InfLicSC1 or fall in a clade yet to be sequenced (Fig.3). Therefore, we suggest to retain the temporary nomenclature OOM_1 and OOM_2 (Garvetto *et al.*, 2018), compliant with the EukRef guidelines (Berney *et al.*, 2017) until the typification of *Ectrogella* is clarified and enough information is available to formally describe each taxon. Secondly, we demonstrate that the *Olpidiopsis* parasites of red algae are unlikely to be monophyletic, and that our diatom parasitoids are distinct from the freshwater parasitoid of *Pleurosigma* sp., *Olpidiopsis gilli* (Buaya *et al.*, 2019b). Support is lacking to link either of the "*pyropiae*", "*porphyrae*" or *"bostrychiae"* lineages (*sensu* Badis et al, 2018) to the genus type, *Pontisma lagenidioides*, therefore the re-assignation of all red algal *Olpidiopsis* pathogens to the genus *Pontisma* proposed by Buaya *et al.* (2019a) remains unconfirmed. Overall, until type identities and phylogenetic placement are ascertained, we feel that caution should be applied

before updating the nomenclature of these groups. Finally, the natural co-existence of three phylogenetically distinct parasitoids infecting *Licmophora* in the same sample means that all morphological observations conducted thus far on diatom parasitoids, to the exception perhaps of the ultrastructural work by Raghu-Kumar (1980a, 1980b) may have been performed on a mix of several species. This co-existence may explain to some extent the ongoing disagreement between respected taxonomists about species delimitation in *Ectrogella*; it also means that a very careful appraisal of the literature is required to link existing taxonomic descriptions with novel molecular data. Although useful, the limited morphological evidence that we have gathered on a limited number of cells is insufficient to tackle this question. In the future, only single cell approaches, hopefully combined with the laboratory cultivation of clonal isolates will enable to resolve the taxonomy of these parasitoids.

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FIGURE LEGENDS

Table 1. Summary of the taxonomic treatment and molecular data available for oomycete parasites of diatoms. Species described as or later assigned to *Ectrogella* are shaded in grey. Habitats are defined as M=marine, F=freshwater and B=brackish waters.

Fig. 1. Epiphytic diatom populations and single cell (chain) isolates molecularly characterised in this study. (A-B) Epiphytic diatom community growing on *Ulva* sp. and *Laminaria digitata*, without (A) or with (B) calcofluor-white staining (blue in epifluorescence). Oomycete thalli are visible in *Licmophora* sp. and *Fragilaria* sp. (C) Isolate **S10InfFrLic**: chain of *Fragilaria* sp. heavily infected by endobiotic parasites. Grey ellipsoidal sporangia are surrounded by collapsed phaeoplasts. (D) Isolate **S10InfLicSC6**: single infected cell of *Licmophora* sp.; the parasite cytoplasm is visible as a grey-blue mass surrounded by the host phaeoplasts. (E) Isolate **S10InfLicSC**: Grey ellipsoidal mature sporangium within a specimen of *Licmophora* sp. (F) Isolate **InfLicSC2**: *Licmophora* sp. co-infected by two parasites, for which the putative thalli are indicated by asterisks. Note the different dimensions and the highly vacuolised thallus on the left hand side. Isolates **InfLicSC3** (G) and **InfLicSC1** (H): spherical mature sporangia within two *Licmophora* sp. cells. In both cases the host cytoplasm has been completely consumed and the collapsed phaeoplasts surround the sporangium. (I) Isolate **InfLicSC4**: spore release from an infected *Licmophora* sp. cell. Note the absence of flagellation and the *Achlya*-like spore release. The spore mass and the carefully rinsed sporangium constitute the biological material for this isolate. Scale bars = 10 μm. Whenever present, asterisks point to parasitic oomycete thalli.

Fig. 2 Morphological features observed for oomycete parasites infecting *Fragilaria* and *Licmophora*. (A) Healthy and (B) infected *Fragilaria* sp. chains, the infected chain shows developing parasitic thalli and empty sporangia (asterisks). (C) DIC and CW epifluorescence overlaid picture featuring a growing thallus (left) and three empty sporangia. Note the thickened discharge tubes (arrowheads) and empty spore cysts (arrows). CW epifluorescence (D) and DIC (E) pictures of an infected *Fragilaria* sp. chain in apical view. Note the thick-walled discharge tubes (arrowheads) and a cluster of released spores at the mouth of the discharge tube at the top of the picture. (F) Healthy uninfected *Licmophora* sp. cell. (G) Spherical mature sporangium surrounded by collapsed red-brown chloroplasts. Note the sporangia wall reacted positively to CW (inlet). (H-I) The same empty sporangium featured in two different focal planes showing four discharge tubes (arrowheads) and empty spore cysts both within and outside the sporangium (arrows). (J) Spherical spores as observed within the sporangium. Note a big vacuole seems to occupy the centre of the sporangium. (K) A mature sporangium in *Licmophora* sp. is filled with zoospores and shows an opened discharge tube (arrowhead). (L) The same sporangium marked with CW and observed in epifluorescent microscopy highlights a second discharge tube (arrowheads) in a thickened area of the sporangial wall.

Fig. 3 Maximum-likelihood reconstruction (1,000 Ultra-Fast bootstrap) of oomycetes (grey area) phylogeny based on the 18S rDNA gene sequence. Isolate names in bold indicate parasites sequenced in this study and in smaller font share a > 99% sequence identity with the represented sequence and have been therefore omitted from the alignment. Well-supported (> 95% UFBoot support) nodes highlighted in red may correspond to *Ectrogella*, in the light of our findings.

Fig. 4 Maximum-likelihood phylogenetic tree reconstruction (1,000 Ultra-Fast bootstrap) using amino acid sequences for the mitochondrial genetic marker *cox2* (196 positions) of the parasites of *Licmophora* sp. and *Fragilaria* sp. (isolate names in bold) within the oomycetes. Isolate names in brackets and in smaller font share a > 99% sequence identity with the represented sequence and have been therefore omitted from the alignment.

Supporting Information

Table S1. List of primers and PCR conditions used in this study.

Table S2. Summary of metadata for the single-cell isolated and analysed in this study.

Table 1. Summary of the taxonomic treatment and molecular data available for oomycete parasites of diatoms. Species described as or later assigned to Ectrogella are shaded in grey. Habitats are defined as M=marine, F=freshwater and B=brackish waters.

Traditional taxonomic treatment						
Taxonomic authority	**in Sparrow 1960**		**in Dick 2001**		**Habitat**	**Hosts**
Zopf 1884	*Ectrogella*	*bacillariacearum*	*Ectrogella*	*bacillariacearum*	F	*Synedra lunularis, S. ulna, S. capitata, Meridion circulare, Nitzschia sigmoidea, Gomphonema sp., Pinnularia sp.*
Friedmann 1952	*Ectrogella*	*eunotiae*	*Ectrogella*	*bacillariacearum*	F	*Eunotia arcus*
Feldmann 1955	*Ectrogella*	*eurychasmoides*	*Ectrogella*	*eurychasmoides*	M	*Licmophora lyngbyei*
Scherffel 1925	*Ectrogella*	*gomphonematis*	*Ectrogella*	*bacillariacearum*	F	*Gomphonema micropus*
Scherffel 1925	*Ectrogella*	*licmophorae*	*Ectrogella*	*perforans*	M	*Licmophora sp.*
Petersen 1905	*Ectrogella*	*perforans*	*Ectrogella*	*perforans*	M (F,B)	*Licmophora lyngbyei, L. abbreviata, L. flagellata, L. gracilis, Synedra ulna, S. tabulata, Striatella unipunctata, Podocystis adriatica,Thalassionema nitzsachioides, Lauderia borealis, Fragilaria islandica*
Scherffel 1925	*Ectrogella*	*monostoma*	*Aphanomycopsis*	*bacillariacearum*	F	*Synedra ulna,Pinnularia sp.*
Scherffel 1925	*Aphanomycopsis*	*bacillariacearum*	*Aphanomycopsis*	*bacillariacearum*	F	*Pinnularia viridis, Epithemia turgida, Cymbella gastroides, Nitzschia sigmoidea, Synedra sp., Surirella sp., Navicula sp.*
Zopf 1884	*Lagenidium*	*enecans*	*Lagenidium*	*enecans*	F	*Amphora ovalis, Cocconema lanceolatum, Cymatopleura solea, Cymbella cistula, C. gastroides, Navicula cuspidata, Pinnularia sp., P. viridis, Stauroneis phoenicentron*
Scherffel 1925	*Lagenidium*	*cyclotellae*	*Ectrogella*	*sp.*	F	*Cyclotella kutzingiana*
Scherffel 1925	*Lagenidium*	*brachystomum*	*Ectrogella*	*sp.*	F	*Synedra sp., S. ulna, Cymbella cymbiformis var. parva, Gomphonema constrictum, Nitzschia linearis*
(de Wilderman) Friedman 1952	*Olpidiopsis*	*gillii*	*Ectrogella*	*bacillariacearum*	F (M)	*Pleurosigma attenuatum, Cocconema lanceolatum, Nitzschia sigmoidea, Gyrosigma attenuatum, G. acuminatum*
Available molecular data						
Reference	**Genus/Group**	**Species/Single-cell Id**	**18S rDNA**	***cox2***	**Habitat**	**Hosts**
Thines et al. 2015	*Lagenisma*	*coscinodiscii*	KT273921	//	M	*Coscinodiscus wailesii*
Buaya & Thines 2017	*Olpidiopsis*	*drebesii*	MF926410	//	M	*Rhizosolenia imbricata*
Buaya & Thines 2017	*Miracula*	*helgolandica*	MF926411	//	M	*Pseudo-nitzschia pungens*
Garvetto et al. 2018	OOM_1_1	10-044	MF960901	//	M	*Pseudo-nitzschia australis*
Garvetto et al. 2018	OOM_1_1	10-045	MF960902	//	M	*Pseudo-nitzschia australis*
Garvetto et al. 2018	OOM_1_1	Ect6para	MF960903	MG787100	M	*Pseudo-nitzschia australis*
Garvetto et al. 2018	OOM_1_1	Melo1para	MF960907	MF960909	M (B)	*Melosira cf. nummuloides*
Garvetto et al. 2018	OOM_1_2	13-374	MF960905	//	M	*Pseudo-nitzschia fraudulenta*
Garvetto et al. 2018	OOM_1_3	14-236	MF960906	//	M	*Pseudo-nitzschia pungens*
Garvetto et al. 2018	OOM_2	12-150	MF960904	//	M	*Pseudo-nitzschia cf. plurisecta*
Buaya & Thines 2019	*Miracula*	*moenusica*	MK239934	//	F	*Pleurosira laevis*
Buaya, Ploch and Thines 2019	*Olpidiopsis*	*gillii*	MH971239	//	F	*Gyrosigma acuminatum*
Buaya, Ploch and Thines 2019	*Olpidiopsis*	*gillii*	MH971238	//	F	*Gyrosigma acuminatum*