Fibrophrys columna gen. nov., sp. nov: a member of the family Amphifilidae

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Abstract

A novel *Diplophrys*-like organism, *Fibrophrys columna*, was isolated from Hiuchigaike Pond in Japan. *F. columna* showed a nearly orbicular or broadly elliptical cell shape and has fine filamentous, branching ectoplasmic elements emanating from both polar ends of the cell. Cells also contain orange, amber, or colorless lipid bodies. Although its whole cell morphology resembles that of the genus *Diplophrys*, *Fibrophrys* is clearly distinct from *Diplophrys* on the basis of 18S rDNA sequences. Molecular phylogenetic analysis showed a close relationship of *F. columna* with *Amphifila marina*, and its sequence is similar to many environmental stramenopile sequences. The cells of *F. columna* measured 5.0–8.3 × 5.6–10.3 μm and sometimes possessed hernia-like prongs instead of filamentous ectoplasmic elements. An axis-like electron-dense body was observed in the mitochondria. We also studied the ultrastructure of another *Fibrophrys* strain, *Fibrophrys* sp. E-1, which is different from the type strain of *F. columna*. A ladder-like pattern was recognized in the outer part of unidentified cytoplasmic membranes connected with the mitochondria. The unidentified cytoplasmic membranes were connected to the nuclear, lipid body, and mitochondrial outer membranes. We propose a new genus, *Fibrophrys*, and a new species, *F. columna*, based on these ultrastructural and molecular features.

Keywords: Amphifilidae, *Diplophrys*, *Fibrophrys* gen. nov., *Fibrophrys columna* sp. nov., Phylogeny

Introduction

The Labyrinthulomycetes, a class of mainly marine protists, is a member of the stramenopiles [Dick 2001 (as "Straminipila"); Patterson 1989] and is characterized by the following features: rhizoid-like ectoplasmic net elements produced by a unique organelle, the bothrosome (sagenogen, sagenogenetosome) (Moss 1980; Perkins 1972; Porter 1972), biflagellate zoospores possessing an anterior flagellum with tripartite tubular mastigonemes (Kazama 1973), and multilamellate cell walls composed of Golgi body-derived scales (Alderman et al. 1974; Darley et al. 1973). This class includes three orders and one superfamily: Thraustochytrida Sparrow 1973, Labyrinthulida Doflein 1901, Amphitremida Poche 1913, and Amphifiloidea Cavalier-Smith 2012. The Amphifiloidea superfamily comprises two families: Amphifilidae Cavalier-Smith 2012 and Sorodiplophryidae Cavalier-Smith 2012.

Amphifila marina, the type species of Amphifilidae, was first described as *Diplophrys marina* (Dykstra and Porter 1984) based exclusively on its morphology. Recently, molecular phylogenetic analysis revealed that *D. marina* belongs to a phylogenetic group distinctly different from other *Diplophrys* species. Consequently, *D. marina* was transferred to a new genus, *Amphifila*, Amphifilidae (Anderson and Cavalier-Smith 2012). The Amphifilidae is currently composed of only one marine species, *A.*

marina. However, based on molecular phylogeny, many environmental DNA sequences obtained from freshwater and terrestrial sampling sites across several regions, including Asia (Kojima 2009), Europe (Lara 2011; Slapeta 2005; Zettler 2002), America (Richard 2005), and Antarctica (Nakai 2012), have been found to belong to this family. Furthermore, related sequences were obtained from extreme environments such as suboxic ponds (Slapeta 2005), rivers with a low pH and high concentrations of heavy metals (Zettler 2002), and glacial ponds in Antarctica (Nakai 2012). These reports have revealed wide ecological distribution of related organisms. However, because there are no reports of successful isolation or available cultures for the members of this family, except *A. marina*, their morphological features remain unclear.

This study is the first report of the isolation and establishment of a stable culture of members of the Amphifilidae family obtained from freshwater habitats. We describe a new genus and a new species isolated from Hiuchigaike Pond, Ibaraki Prefecture, Japan, and specify the morphological characteristics and molecular phylogenetic position of this new genus based on microscopy and 18S rDNA sequence comparisons.

Materials and Methods

Sample collection and cultivation

Fibrophrys columna was isolated from freshwater samples collected from Hiuchigaike Pond, Ibaraki Prefecture, Japan in July 2011. Another strain, *Fibrophrys* sp. E-1, was isolated from the freshwater samples collected from Lake Echigo, Hokkaido Prefecture, Japan in July 2012. Both samples were collected from surface water using a sampling bottle.

A clonal culture of each strain was established using a single-cell isolation technique with micropipettes. Autoclaved distilled water and commercially available dried water fleas for aquarium fish were used as the growth medium. We added 5–10 individual dried water fleas to 5 ml of distilled water and autoclaved the mixture at 120°C for 20 min. The cultures were maintained in test tubes at room temperature under a shade.

Morphological observations

For light microscopy, a Leica DM2500 microscope (Leica Microsystems KK, Tokyo, Japan) and an Olympus IX71 microscope (Olympus Corporation, Tokyo, Japan) equipped with Nomarski differential interference contrast optics were used.

For scanning electron microscopy, cultured samples were mounted on glass plates

coated with poly-L-lysine and fixed at 4°C for 2 h in 5% glutaraldehyde. After rinsing with 0.2 M sodium cacodylate buffer (pH 7.2) several times, the prefixed samples were then fixed in 1% osmium tetroxide (OsO_4) for 30 min. The samples were then dehydrated through a graded ethanol series (50, 75, 90, 95, and 100%) by incubating the samples at each concentration for 15 min, followed by the substitution of 100% ethanol with dehydrated *t*-butyl alcohol. The specimens were then freeze-dried using a VFD-21S freeze drier (Shinku Device Co., Ltd., Ibaraki, Japan) and mounted on specimen stubs. Next, the specimens were coated with platinum/palladium using an E102 ion sputter (Hitachi, Ltd., Tokyo, Japan) and observed using a JSM-6330F field emission scanning electron microscope (JEOL, Ltd., Tokyo, Japan).

For whole-mount images, the cells were exposed to 4% OsO_4 fumes for 5 min, followed by washing in distilled water. The cells were stained for 3 min with 4% uranyl acetate and then viewed using a Hitachi H-7650 transmission electron microscope (TEM; Hitachi, Ltd., Tokyo, Japan).

For thin sectioning, vegetative cells were exposed to 1% OsO_4 fumes for 3 min. The cells were then fixed in a solution containing 2.5% glutaraldehyde, 2% OsO_4, 4.5% sucrose, and 0.1 M cacodylate buffer at pH 7.0 for 90 min under refrigeration (4°C, in darkness), followed by washing in the same buffer three times for 10 min each. The cells

were successively dehydrated in 30, 50, 70, 90, 95, and 100% acetone by incubating the cells at each concentration for 10 min under refrigeration, followed by incubating twice in an acetone–propylene oxide (PO) mixture and pure PO for 10 min each. The dehydrated pellet was embedded in an agar low-viscosity resin (LV Resin, VH1 and VH2 Hardener, and LV Accelerator; Agar Scientific, Stansted, Essex, UK), and a 1:1 mixture of PO and the resin was prepared. The resin was polymerized for 12 h at 70°C.

Thin sections were cut with an ultramicrotome (EM UC7; Leica Camera AG, Wetzlar, Germany) and stained for 5 min with 4% uranyl acetate, followed by incubation with Sato's lead citrate (Sato 1968) for 5 min. The sections were viewed with the Hitachi H-7650 TEM.

Molecular phylogenetic analyses

To amplify the 18S rDNA of the strains obtained, single cells of each strain were isolated with micropipettes using a single-cell isolation technique and were transferred into polymerase chain reaction (PCR) tubes with autoclaved distilled water. The tubes were first stored overnight at room temperature to digest the engulfed feed and then placed in a freezer at −20°C overnight to break the cell membranes. 18S rDNA was amplified by PCR with the primer pairs described by Nakayama et al. (1998). In the first

round of PCR, primers SR1 and SR12 were used. The obtained PCR products were amplified again using the following primer pairs: SR1 and SR5; SR4 and SR9; and SR8 and SR12. Nonspecific PCR products were detected electrophoretically, and specific PCR products were purified using the QIAquick® Gel Extraction Kit (Qiagen, Venlo, Limburg, Netherlands). The purified PCR products were sequenced with a BigDye Terminator v. 1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using a 3130 Genetic Analyzer (Applied Biosystems). The other sequences used were obtained from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/, retrieved April 1, 2016) and automatically aligned with CLUSTAL X, version 1.81, using default parameters (Thompson et al. 1997; ftp://ftp.ebi.ac.uk/pub/software/clustalw2/, retrieved April 1, 2016). For phylogenetic analyses, ambiguously aligned regions were manually adjusted or deleted using the BioEdit Sequence Alignment Editor, version 7.0.9.0 (Hall 1999), and finally, 1,274 base pairs (bp) of 18S rDNA were used for the analyses.

Phylogenetic trees were constructed based on a 1,274-bp alignment using both maximum-likelihood (ML) and Bayesian approaches, with three sequences of Alveolata used as the outgroup. We used PHYLIP version 3.69 (Felsenstein 2005) for the ML method and MrBayes 3.2.1 (Ronquist et al. 2012) for the Bayesian analysis. For the ML analysis, the Jones–Taylor–Thornton (JTT) + G model with global rearrangement was

used. For the Bayesian analysis, the GTR + I + G models were selected using MrModeltest 2.3 (Nylander 2004; https://github.com/nylander/MrModeltest2, retrieved April 1, 2016). The stability of the relationships was assessed using bootstrap analyses based on 100 resamplings. The Bayesian analysis was run for 1,000,000 generations, with a sampling frequency of every 100th generation. All other settings were retained at their default values.

Results

Taxonomic treatments

Based on the morphological examinations and the results of molecular phylogenetic analyses of 18S rDNA sequences, we describe a new genus of the family Amphifilidae, *Fibrophrys* gen. nov., and a new species, *F. columna* sp. nov.

Taxonomic descriptions

Fibrophrys gen. nov.

The cell shape is nearly orbicular or broadly elliptical, asymmetric to the axis connecting the two antipolar ends. Fine filamentous, branching ectoplasmic elements emanate from both polar ends of the cell and are spread evenly, similar to a fibrous root system of a plant. An internal membrane system exists in the filamentous ectoplasmic elements. In the body of the organism, generally one (but up to five) orange, amber, or colorless lipid bodies are immersed. In colonies, cells maintain an equal distance using the ectoplasmic elements and rarely make direct contact with each other. Gliding motility is not observed, and cells move over a limited distance.

Taxonomic summary: Chromalveolata, Stramenopiles, Labyrinthulomycetes (Labyrinthulea), Amphifiloidea, Amphifilidae.

Type species: *Fibrophrys columna*.

***Fibrophrys columna* sp. nov.**

The cells measure 5.0–8.3 × 5.6–10.3 μm. Sometimes, instead of the ectoplasmic elements, hernia-like prongs emanate from the cells. An axis-like electron-dense body exists in the mitochondria.

Type material: Holotype: EM block.

Type habitat/locality: Hiuchigaike Pond, Ibaraki Prefecture, Japan (36.202516°N, 140.087326°E).

Etymology: the specific epithet “columna” means pillar, referring to the electron-dense body-like pillar evident in mitochondria on examination by TEM.

Gene sequence: AB856528 as Amphifilidae sp. H-1 gene for 18S ribosomal RNA, partial sequence (Takahashi et al. 2014).

General morphology

The morphology of the members of the genus *Fibrophrys* was orbicular or broadly elliptical in shape. The cells contained refractive bodies, a single nucleus, a contractile vacuole, and ectoplasmic elements emanating from the poles (Figs. 1, 2). Generally, one

(but up to five) refractive body was observed in each cell. The cells measured 5.0–8.3 × 5.6–10.3 μm for *F. columna* and 2.4–6.1 × 3.4–7.2 μm for *Fibrophrys* sp. E-1. Gliding motility was not observed in either species. Instead, *Fibrophrys* moved like a moored body within a loose colony. Although individual cells sometimes gathered around water fleas, single cells moving out of colonies were not observed. This implies that *Fibrophrys* exhibits some chemotactic properties in order to feed and can move separately.

The ectoplasmic elements were branching but not anastomosing and evenly spread (Fig. 3A). Although it was difficult to recognize the ectoplasmic elements by optical microscopy, *Fibrophrys* cells maintained an equal distance from each other in colonies using their ectoplasmic elements and rarely made close contact with each other (Figs 1A, 2A). Sometimes, globular protrusions of the ectoplasmic elements were evident (Fig. 1A, upper left).

Ultrastructural observations

By examination of thin sections using TEM, we observed the nucleus, mitochondria, lipid bodies, Golgi bodies, and a complex membrane system, which we termed “the unidentified membrane system” (Figs. 1B, C, 2B–D). The ectoplasmic elements contained ribosome-free cytoplasm and tubular internal membrane system elements in

both species (Fig. 3B). Many layers of ectoplasmic elements surrounded the surface of water fleas (Fig. 3C). Bothrosomes and bothrosome-like bodies were not observed.

Both *Fibrophrys* species possessed mitochondria containing distinctive cristae with short, stubby branches (Figs. 1B, 2D). In the mitochondria of *F. columna*, an axis-like electron-dense body was observed, which was not evident in *Fibrophrys* sp. E-1. In the latter strain, a ladder-like pattern was observed between the cytoplasmic membrane and mitochondrial outer membrane, close to mitochondria. In addition, to some extent, the distribution of certain organelles in *Fibrophrys* cells was fixed. The Golgi body was situated close to the nucleus, and many small vesicles, which seemed to be a *cis*-Golgi network, were observed between these organelles in both species. The unidentified cytoplasmic membranes were connected to the nuclear membrane, lipid membrane, and mitochondrial outer membrane in *Fibrophrys* sp. E-1 (Fig. 2B–D), but not in *F. columna*. The unidentified cytoplasmic membranes and the neighboring endoplasmic reticulum were ribosome-free in both species.

Molecular phylogenetic analyses

Phylogenetic analyses based on 18S rDNA gene sequences revealed that *F. columna* and the other *Fibrophrys* species were new members of the class Labyrinthulomycetes and

belonged to the same clade, Amphifiloidea (Fig. 4). The whole topology of the phylogenetic tree was similar to those reported previously (Anderson and Cavalier-Smith 2012; Leander and Porter 2001; Takahashi et al. 2014). Our analysis revealed a close phylogenetic relationship among the sequences of *F. columna*, *Fibrophrys* sp. E-1 (LC096096), *A. marina*, and many environmental sequences in the family Amphifilidae. It also revealed significant independence of the organisms from two representative clades of the Labyrinthulomycetes, Labyrinthulida and Amphitremida. Although the relationship between Amphifiloidea, to which the genus *Fibrophrys* belongs, and Thraustochytrida is still unclear (Anderson and Cavalier-Smith 2012; Gomaa et al. 2013; Takahashi et al. 2014), Amphifiloidea may be a separate group of Thraustochytrida.

Discussion

The light microscopic appearance of the *Fibrophrys* species resembles that of *Diplophrys archeri*, *D. parva*, *D. mutabilis*, *A. marina*, and of the vegetative cells of *Sorodiplophrys stercorea* (Anderson and Cavalier-Smith 2012; Dykstra and Olive 1975; Dykstra and Porter 1984). These organisms are nearly orbicular or broadly elliptical in shape, and the cells feature refractive bodies, a contractile vacuole, and ectoplasmic elements emanating from the poles.

In the mitochondria of *F. columna*, an axis-like electron-dense body was observed (Fig. 1B). This electron-dense body was not visible in *Fibrophrys* sp. E-1 and has not been reported in any other species of the Labyrinthulomycetes. In *Fibrophrys* sp. E-1, a ladder-like pattern consisting of unidentified cytoplasmic membranes connected to the mitochondria was evident. This ladder-like pattern resembles that of the rumposome of chytrids, which is connected to the lipid body and associated with the flagellar apparatus and plasma membrane in some chytrid species (Dorword 1982). Because the ladder-like pattern seen in *Fibrophrys* sp. E-1 exists between the mitochondria and unidentified cytoplasmic membranes, no robust topological similarity was recognized with the rumposome.

Fibrophrys resembles *A. marina*, the type species of the Amphifilidae family. The

most apparent difference between the genera *Fibrophrys* and *Amphifila* is their habitat. The habitat of *A. marina* is, as its name suggests, a marine environment. *A. marina* is a heterotrophic protist associated with marine vascular plants such as *Spartina alterniflora* and *Zostera marina* (Porter 1972). On the other hand, the *Fibrophrys* species were isolated from freshwater habitats, an inland pond, and a lake. The second difference is the motility of the cells. *A. marina* exhibits gliding motility on substrates, while *F. columna* and *Fibrophrys* sp. E-1 did not show this type of motility. *F. columna* usually floated and moved like a moored ship. The third difference is the presence or absence of the unidentified membrane system and internal membranous tubes within ectoplasmic elements. Both of these peculiar structures are evident in *F. columna* and *Fibrophrys* sp. E-1 (Figs. 1C 2B–D, 3B, C), but neither is present in *A. marina* (Dykstra and Porter 1984). Based on these differences, we conclude that the organisms belong to separate genera.

In light microscopy images, the vegetative cells of *S. stercorea* resemble *Fibrophrys* cells in terms of their morphology and structure of organelles, such as the unidentified cytoplasmic membranes and internal membrane systems (Dykstra and Olive 1975). However, the aggregative behavior, terrestrial habitat, and complex life cycle (which includes a sorocarp) of *Sorodiplophrys* are sufficient to distinguish it from *F. columna* (Table 1). The independence of the genus *Sorodiplophrys* should be confirmed based on

molecular data when the DNA sequence of *Sorodiplophrys* becomes available. In a recent study, a new strain of *Sorodiplophrys* has been established, and its 18S rDNA sequence has been determined (Tice et al. 2016). In the phylogenetic trees, *S. stercorea* is closely related to *A. marina*. The above study is currently under review/in press and will provide insight not only into *Sorodiplophrys* but also into the Labyrinthulomycetes in their entirety.

F. columna also resembles *D. archeri, D. parva*, and *D. mutabilis.* We compared the *Fibrophrys* and *Diplophrys* species as shown in Table 1. The average size of *D. archeri*, the type species of the genus *Diplophrys*, was reported to be 1/2,000 inch, i.e., 12.7 μm (Anderson and Cavalier-Smith 2012; Baker 1868). This cell size is larger than that of *F. columna*. In TEM images, *D. parva* can be seen to possess a bothrosome-like electron-dense body in the base of its ectoplasmic elements (Anderson and Cavalier-Smith 2012). In the ectoplasmic elements of *D. mutabilis*, internal membrane systems are more developed than in those of *Fibrophrys* (Fig. 3B; Takahashi et al. 2014). Moreover, *D. mutabilis* shows gliding motility, whereas *Fibrophrys* does not. Based on these morphological differences, *Fibrophrys* and *Diplophrys* are distinguishable and should be considered two independent genera.

The definitions of the families Diplophryidae (Takahashi et al. 2014) and

Amphifilidae (Anderson and Cavalier-Smith 2012) remain unclear. As shown in Table 1, there is no definitive behavior or ultrastructure that would allow us to distinguish between Diplophryidae and Amphifilidae. At present, they can only be separated by phylogenetic analysis. Furthermore, the morphological differentiation between *Diplophrys* and freshwater Amphifilidae performed in some past studies (e.g., Anderson and Cavalier-Smith 2012) is insufficient because no cultured strain of freshwater Amphifilidae was available to the public. For example, Baker (1868) stated that "in the body of the organism (*D. archeri*) is immersed an oil-like refractive globule of an orange or amber color;" however, there are up to 10 lipid bodies present in *D. parva* and *D. mutabilis*. On the contrary, generally only one lipid body is present in *Fibrophrys*. In addition, as shown in Table 1, *D. archeri*, *D. parva*, and *F. columna* do not show gliding motility, but *D. mutabilis* does. *F. columna* matches the description of *D. archeri*, except for the cell size. To definitively distinguish these species, the election of a neotype of *D. archeri* and the establishment of a cultured strain that satisfies all definitions are necessary.

Furthermore, *Elaeorhanis cincta*, a filopodial amoeba with debris on its cell surface, has been suggested to be closely related to *Diplophrys*-like species (Patterson 1996). The organisms share filopodia, an oil-like refractive body of an orange or amber color, and some other morphological features. Although *Elaeorhanis* and *Diplophrys*-like species

are easily distinguished by the presence or absence of a debris layer, they may be closely related species or simply different ecotypes of the same species of the genus *Diplophrys*. No strains or sequence data are currently available for *Elaeorhanis*, although the genus is common in freshwater habitats. A detailed comparison between *Elaeorhanis* and *Diplophrys*-like organisms is required to resolve this issue.

As mentioned earlier, *Sorodiplophrys* is closely related to the Amphifilidae family. This is consistent with the findings of Anderson and Cavalier-Smith (2012) who demonstrated a relationship between Sorodiplophryidae and Amphifilidae and determined that they comprise the Amphifiloidea superfamily. One of the two distinct, deep-branching soil lineages in the Amphifilidae clade, namely, Soil, US, not Eimeriidae (Fig. 4; Lesaulnier 2008), is related to the dung-dwelling *Sorodiplophrys* (Tice et al. 2016). Because the organisms belonging to Amphifilidae were isolated from soil samples moistened with distilled water obtained from the campus of University of Tsukuba, Tsukuba, Japan (data not shown), some of the registered environmental sequences obtained from soil may belong to the genus *Sorodiplophrys* or neighboring genera. This implies that some freshwater members of the Amphifilidae family exhibit potentially high resistance to desiccation and exist in soil. This may be one reason why environmental sequences derived from soil are polyphyletic (Fig. 4).

Based on our phylogenetic tree, the monophyletic group including *F. columna* is composed of sequences obtained from freshwater habitats. This clade also includes sequences from samples collected from a suboxic pond in Paris (Slapeta 2005). Our culture of *F. columna* is maintained in a medium with many bacteria, which includes water fleas as an organic substance and smells a little rotten, implying that it is probably suboxic. Accordingly, this species exhibits tolerance to low-oxygen conditions. Based on these findings and morphological features, we propose that this monophyletic group be named *Fibrophrys*.

Although the type species of the family Amphifilidae is a marine species, the preference for marine habitats seems rare among members of the Amphifiloidea superfamily because no environmental sequences of Amphifiloidea, derived from marine habitats, are currently available.

As shown in the phylogenetic tree, there are many clades without cultured strains associated with diverse environments, such as the Rio Tinto, which has a pH of 2 and contains much higher concentrations of heavy metals than typically found in fresh waters (Zetter 2002). To fully appreciate the diversity of members of the Amphifiloidea superfamily, more cultured strains are necessary.

Conflicts of Interest

No conflicts of interest declared.

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Figure Legends

Fig. 1. (**A-C**) Light micrograph and transmission electron microscopy images of *Fibrophrys columna*

A. Colonial cells connected through ectoplasmic elements (white arrowheads). Cells contain refractive lipid bodies (arrows) and contractile vacuoles (arrowheads). Sometimes, globular protrusions of the ectoplasmic elements are visible (upper left window).

B. Section near the cell surface. An electron-dense body-like pillar is evident in the mitochondria (arrowheads) in the transmission electron microscopy images. L: lipid body, M: mitochondria,

C. Diametric section of a spherical cell. An electron-dense structure resembling a pillar is observed in the mitochondria (arrowheads). G, Golgi body; L, lipid body; M, mitochondria; N, nucleus; U, unidentified cytoplasmic membranes; V, vacuole.

Fig. 2. (**A–D**)

Light micrograph and transmission electron microscopy images of *Fibrophrys* sp. E-1.

A. Colonial cells. The cells contain refractive bodies (arrow). Some bacteria and dust are also visible.

B. Unidentified cytoplasmic membranes and mitochondria. A ladder-like pattern is visible between the cytoplasmic membrane and mitochondrial outer membrane (arrowheads). L, lipid body; M, mitochondria; U, Unidentified cytoplasmic membranes.

C. Lipid body, mitochondria, nucleus and unidentified cytoplasmic membranes connected by the endoplasmic reticulum. A ladder-like pattern is evident, bordered by mitochondria (arrowheads).

D. Diametric section of a spherical cell. G, Golgi body; L, lipid body; M, mitochondria; N, nucleus; U, unidentified cytoplasmic membranes; V, vacuole.

Fig. 3. (**A–C**) Scanning and transmission electron microscopy images of *Fibrophrys*.

A. Scanning electron microscopy image of *Fibrophrys columna.* Some bacteria (arrowheads) are attached to the ectoplasmic elements (arrows).

B. Section of the basal part of an ectoplasmic element of *Fibrophrys* sp. E-1. Internal membrane systems are running in the ectoplasmic element (arrows).

C. Section of distal parts of the ectoplasmic elements surrounding the surface of the substrate. Left side: water flea substrate. Right side: cell body of *Fibrophrys* sp. E-1. Internal membrane systems are running in the ectoplasmic element (arrows). C, cell; S, substrate.

Fig. 4. Phylogenetic tree based on alignment of 1,274 base pairs of 18S ribosomal DNA sequences, constructed using the maximum-likelihood method. The Bayesian approach resulted in the same topology (Supplementary File). Support values at each node are presented for the maximum-likelihood/Bayesian approaches. Bootstrap values larger than 50% and posterior probabilities larger than 0.80 are shown. Smaller values are represented by "–."

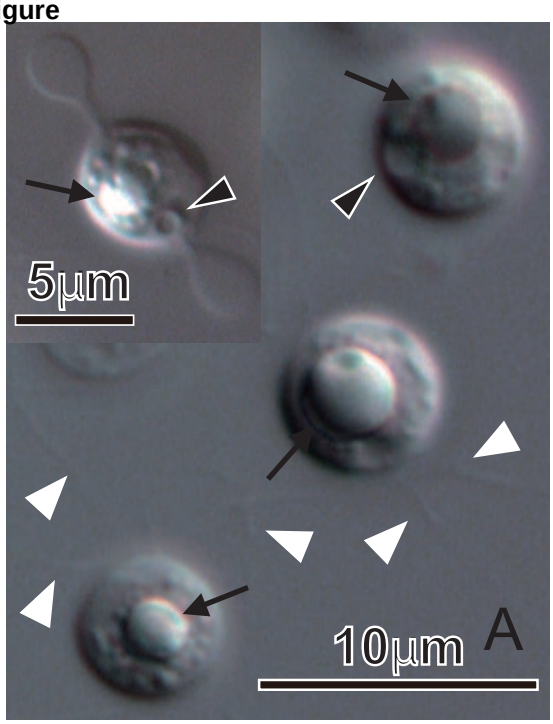
Table 1. Comparative summary of the characteristics of *Fibrophrys columna* and related organisms.

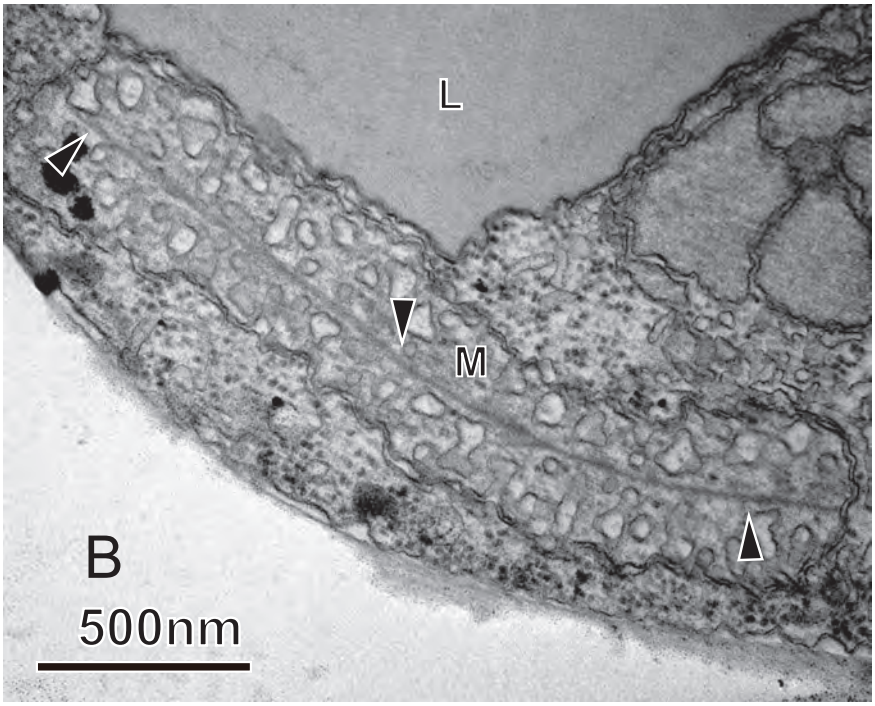
?: Question mark indicates that the corresponding organ-like microstructures are observed, but with less certainty.

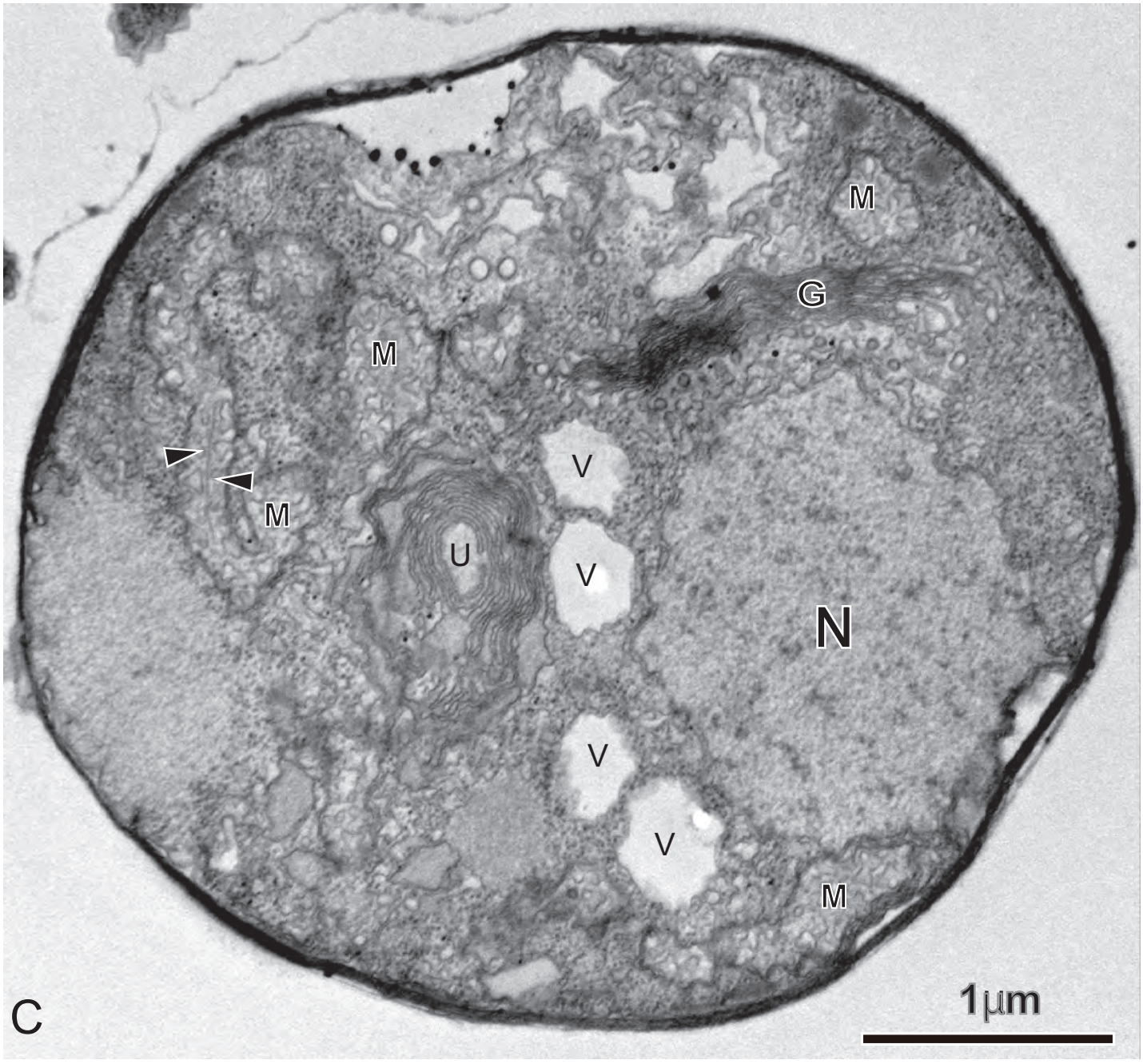
*The characteristic is not specified but has been reported in other species of the same genus.

**In this table, "aggregation" refers to the active aggregation of free-moving individuals. Aggregation as a result of cell division of aplanatic cells, which is observed in some species, is treated as negative ("–").

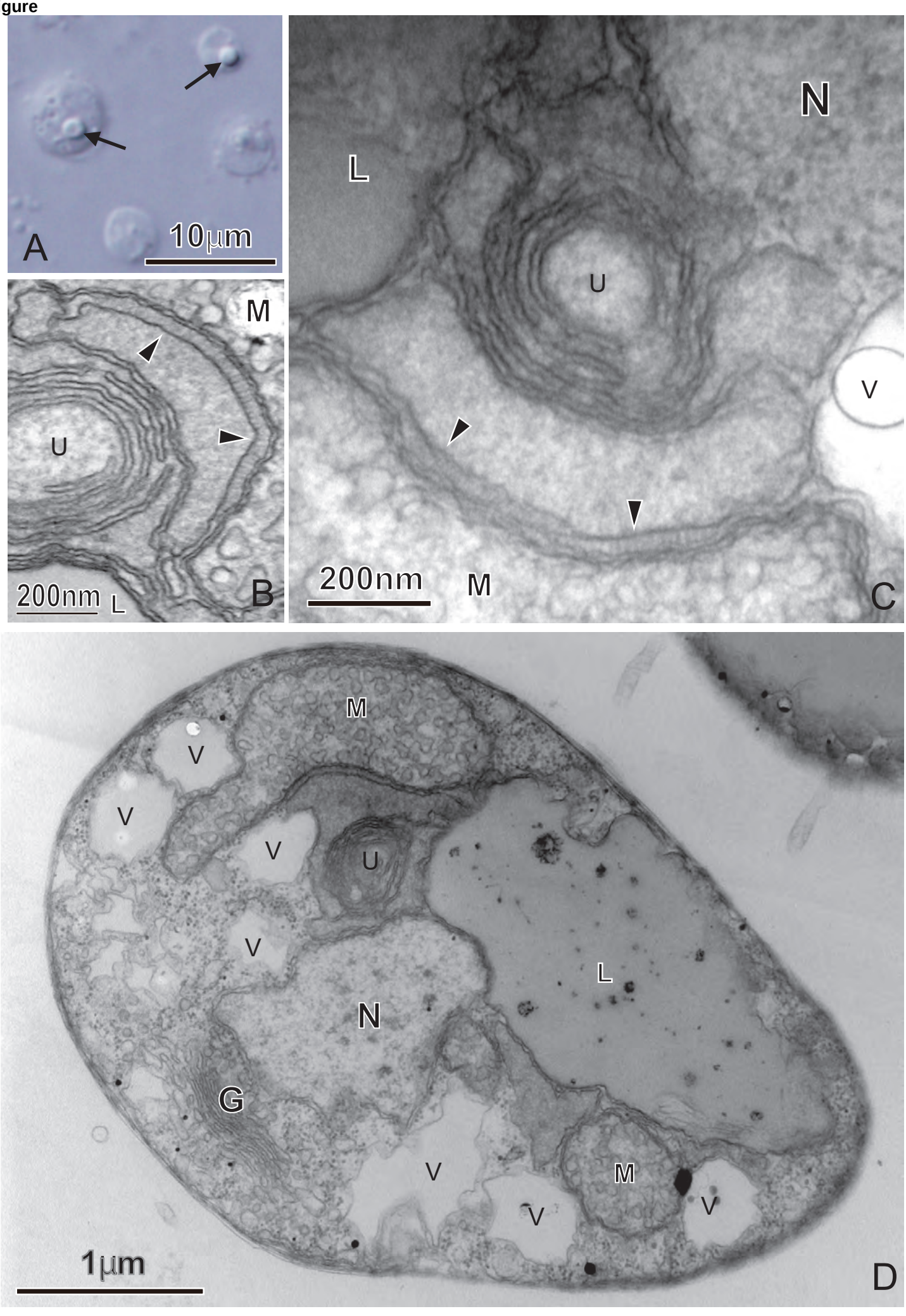
Figure







Figure



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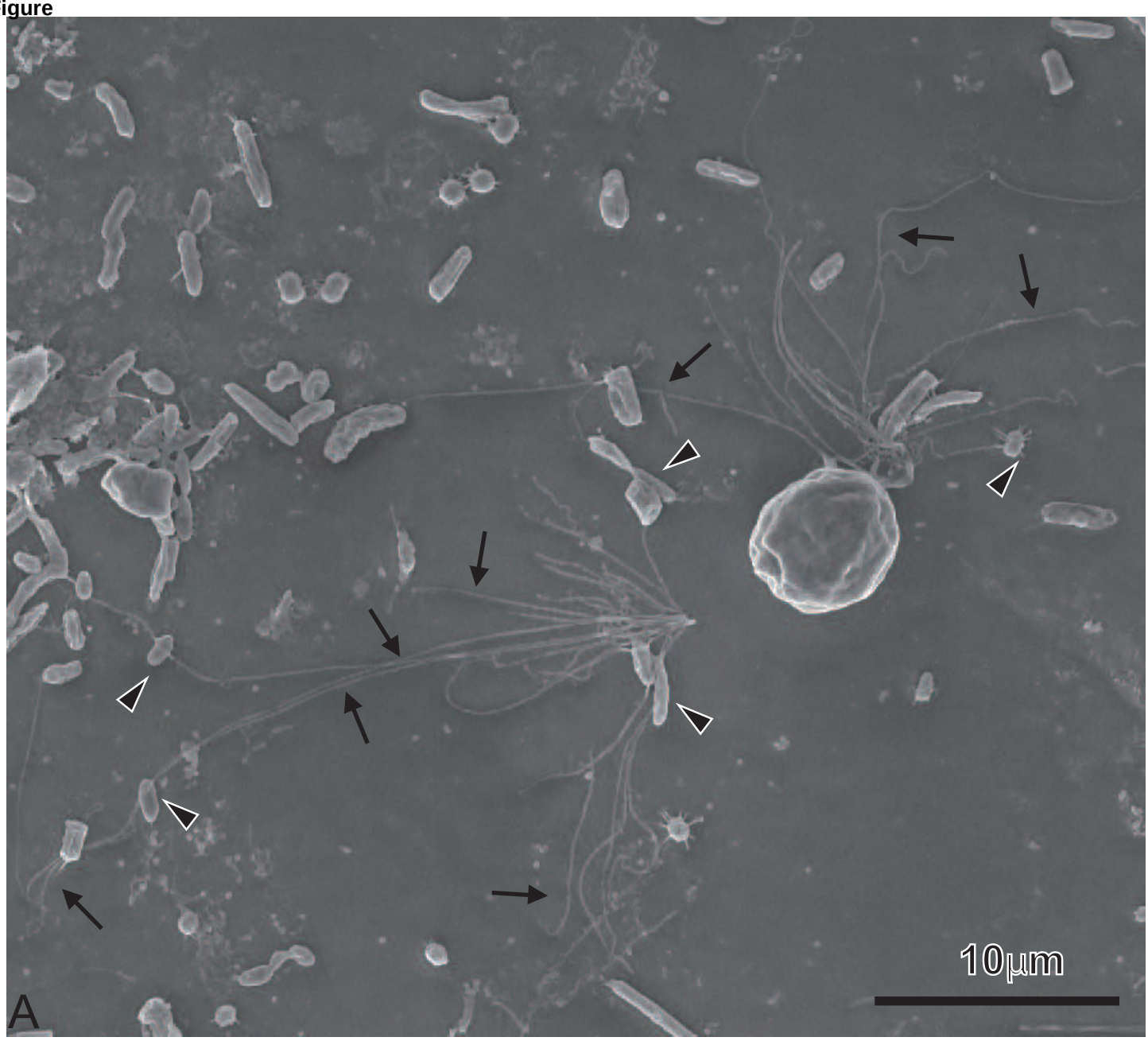
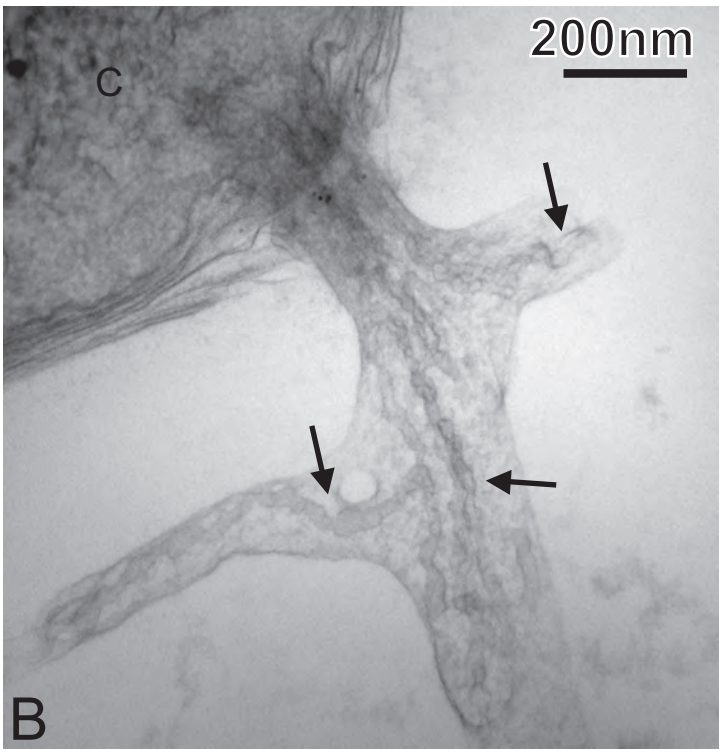
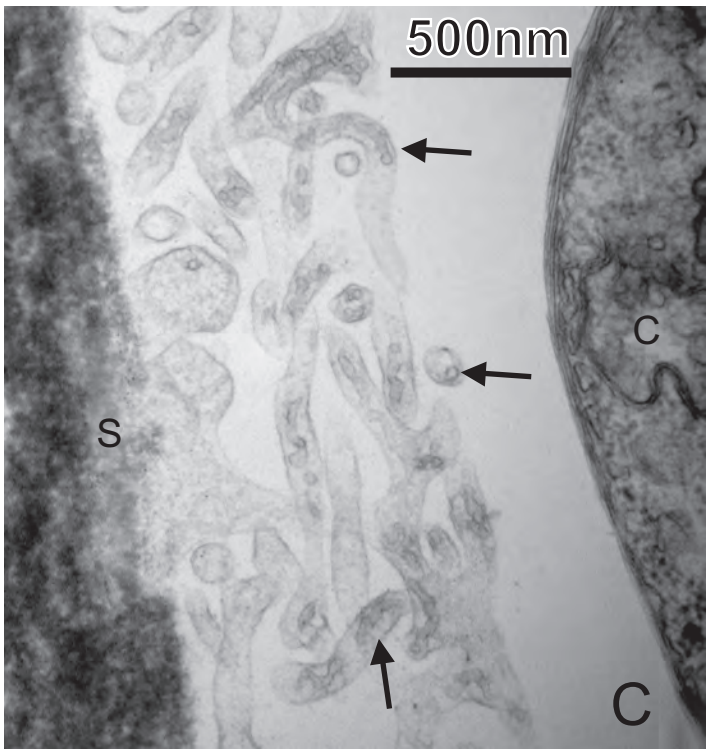
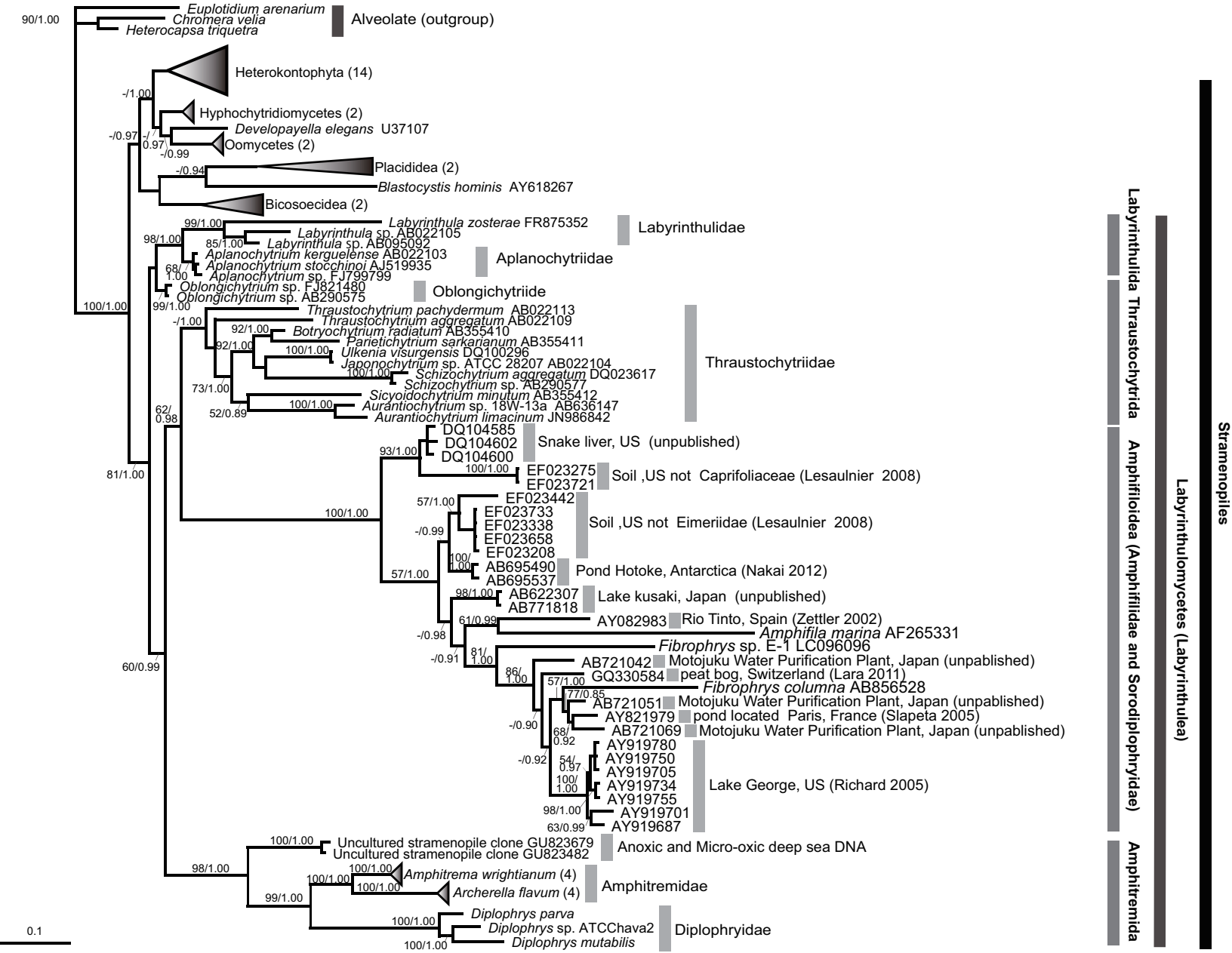






Figure


Euplotidium arenarium
Chromera velia
Heterocapsa triquetra
Alveolate (outgroup)
Heterokontophyta (14)
Hyphochytridiomycetes (2)
Developayella elegans U37107
Oomycetes (2)
Placididea (2)
Blastocystis hominis AY618267
Bicosoecidea (2)
Labyrinthula zosterae FR875352
Labyrinthula sp. AB022105
Labyrinthula sp. AB095092
Labyrinthulidae
Aplanochytrium kerguelense AB022103
Aplanochytrium stocchinoi AJ519935
Aplanochytrium sp. FJ799799
Aplanochytriidae
Oblongichytrium sp. FJ821480
Oblongichytrium sp. AB290575
Oblongichytriide
Thraustochytrium pachydermum AB022113
Thraustochytrium aggregatum AB022109
Botryochytrium radiatum AB355410
Parietichytrium sarkarianum AB355411
Ulkenia visurgensis DQ100296
Japonochytrium sp. ATCC 28207 AB022104
Schizochytrium aggregatum DQ023617
Schizochytrium sp. AB290577
Sicyoidochytrium minutum AB355412
Aurantiochytrium sp. 18W-13a AB636147
Aurantiochytrium limacinum JN986842
Thraustochytriidae
DQ104585
DQ104602
DQ104600
Snake liver, US (unpublished)
EF023275
EF023721
Soil ,US not Caprifoliaceae (Lesaulnier 2008)
EF023442
EF023733
EF023338
EF023658
EF023208
Soil ,US not Eimeriidae (Lesaulnier 2008)
AB695490
AB695537
Pond Hotoke, Antarctica (Nakai 2012)
AB622307
AB771818
Lake kusaki, Japan (unpublished)
AY082983
Rio Tinto, Spain (Zettler 2002)
Amphifila marina AF265331
Fibrophrys sp. E-1 LC096096
AB721042
Motojuku Water Purification Plant, Japan (unpablished)
GQ330584
peat bog, Switzerland (Lara 2011)
Fibrophrys columna AB856528
AB721051
Motojuku Water Purification Plant, Japan (unpublished)
AY821979
pond located Paris, France (Slapeta 2005)
AB721069
Motojuku Water Purification Plant, Japan (unpublished)
AY919780
AY919750
AY919705
AY919734
AY919755
AY919701
AY919687
Lake George, US (Richard 2005)
Uncultured stramenopile clone GU823679
Uncultured stramenopile clone GU823482
Anoxic and Micro-oxic deep sea DNA
Amphitrema wrightianum (4)
Archerella flavum (4)
Amphitremidae
Diplophrys parva
Diplophrys sp. ATCChava2
Diplophrys mutabilis
Diplophryidae
Labyrinthulida Thraustochytrida
Amphifiloidea (Amphifiliidae and Sorodiplophryidae)
Amphitremida
Labyrinthulomycetes (Labyrinthulea)
Stramenopiles
0.1

Table

Table 1

Genus/species (sources)	Cell size (μm)	habitat	gliding motility	unidentified cytoplasmic membranes	Internl membrane system	aggregation (**)	sagenogenetosome (= bothrosome, sagenogen)
Fibrophrys columna (This study)	5.0 – 8.3 × 5.6 – 10.3	freshwater	−	+	+	−	−
Amphifila marina Dykstra et Porter, 1984 （Dykstra And Porter 1984)	3.7 - 5.9 × 5.1 - 8.5	marine	+	−	−	−	−
Sorodiplophrys stercorea (Cienkowski) Olive et Dykstra, 1975 （Dykstra And Olive 1975)	2.4 - 4.8 × 4.8 - 9.6	terrestrial	+	+	+	+	−
Diplophrys archeri Barker, 1868 (Anderson and Cavalier-smith 2012, Barker 1868, Patterson 1996)	12.7 in diameter	freshwater	−	No data	No data	−	No data
Diplophrys parva Anderson et Cavarier-smith, 2012 (Anderson and Cavarier-smith 2012)	6.5 ± 0.08 × 5.5 ± 0.06; mean ± SE	freshwater	−	++	−	−	+?
Diplophrys mutabilis Takahashi et al. 2014 （Takahashi et al. 2014）	3.1 - 8.3 × 3.4 - 10.3	freshwater	+	++	++	−	−
Elaeorhanis cincta Greeff, 1873 （Lee 2000, Patterson 1996）	10 - 20 in diameter	freshwater	No data	No data	No data	No data	No data
Labyrinthula zosterae Muehlstein et Porter, 1991 (Muehlstein and Porter 1991)	15.5 - 19.5 × 3.5 - 5.0	marine	+	−	+* （Perkins 1972)	+	+
Aplanochytrium stocchinoi Morro et al. 2003 (Morro et al. 2003)	4 - 8 in diameter	marine	+	−	No data	−	+* (Watanabe 2012)
Schizochytrium aggregatum Goldstein et Belsky, 1964 (Goldstein and Belsky 1964)	6 - 12 in diameter	marine	−	−	+* （Perkins 1972)	−	+