Trachyrhizium urniformis n. g., n. sp., a novel marine filose thecate amoeba related to a cercozoan environmental clade (novel clade 4)

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ABSTRACT

A novel cercozoan filose thecate amoeba, *Trachyrhizium urniformis* n. g., n. sp., was isolated from a marine sediment sample collected at Agenashiku Island, Okinawa, Japan. We performed light and electron microscopic observations, and a molecular phylogenetic analysis using the small subunit ribosomal RNA gene of the isolate. Cells of *T. urniformis* are spherical in shape and are covered by a thin theca possessing a wide rounded aperture. Branching and occasionally anastomosing filopodia with small granules emerge from the aperture. The granules are transported in the filopodia bidirectionally. Transmission electron microscopy showed that cells of *T. urniformis*

possess nucleus with permanently condensed chromatin, Golgi apparatuses, microbodies, mitochondria with tubular cristae, and extrusomes. Several morphological and ultrastructural features of *T. urniformis* (the presence of thecae and nucleus with permanently condensed chromatin) show similarities with those of Thecofilosea. In a phylogenetic analysis, *T. urniformis* included in Thecofilosea with weak statistical supports and formed a clade with two sequences that constitutes a cercozoan environmental clade, novel clade 4. Based on morphological and ultrastructural information and the results of the phylogenetic analysis, we propose *T. urniformis* as a new member of class Thecofilosea.

Keywords

Cercozoa; phylogeny; SSU rRNA gene; Thecofilosea; ultrastructure

PHYLUM Cercozoa is a large assemblage of unicellular eukaryotes identified by result of molecular phylogenetic analyses (Cavalier-Smith 1998; Cavalier-Smith and Chao 1997). Cercozoa is widely distributed in various environments such as marine, freshwater, and soil and consists of protists with diverse morphologies and nutritional behaviors, such as phagotrophic amoebae (e.g., *Gromia*, *Euglypha* and *Vampyrella*) and amoeboflagellates (e.g., *Cercomonas*, *Thaumatomonas*), photosynthetic amoebae (e.g., *Chlorarachnion*, *Paulinella*, and parasites of land plants (e.g., *Plasmodiophora*), animals (e.g., *Haplosporidium* and *Mikrocytos*), and algae (e.g., *Phagomyxa*) (Bass et al. 2009; Cavalier-Smith 1998; Cavalier-Smith and Chao 2003; Adl et al. 2012). However environmental DNA survey revealed that Cercozoa still includes many environmental clades (novel clades) that have not morphologically identified (Bass and Cavalier-Smith 2004). To date, over 20 novel clades have been reported (Bass et al. 2009; Bass and Cavalier-Smith 2004), but only a few members of these environmental clades were identified (novel clade 7 = *Agitata tremulans* [= *Cercobodo agilis*]; novel clade 8 = *Platyreta germanica*; novel clade 11 = *Tremula longifila*; and novel clade endo-3 = *Paradinium poucheti*) (Bass and Cavalier-Smith 2004; Bass et al. 2005; Bass et al. 2009; Howe et al. 2011a). To understand diversity and evolution of Cercozoa, morphological and ultrastructural characterizations of these environmental clades are required.

Testate and thecate filose amoebae have been described for more than 150 years, and there are many species and genera with variations in cell size, habitat, and morphology of the thecae or tests (Cash et al. 1915; De Saedeleer 1934; Meisterfeld 2002). Cercozoa includes many testate and thecate filose amoebae and they are classified into several subgroups (e.g. Euglyphida, Pseudodifflugiidae, and Rhizaspididae). However, most of them lack molecular data, and therefore their phylogenetic positions remain uncertain.

In this study, we successfully established a culture of novel cercozoan thecate

filose amoeba isolated from marine sediment sample collected from Agenashiku Island, Okinawa, Japan. A molecular phylogenetic analysis shows that the new amoeba forms a clade with two environmental sequences of novel clade 4 (Bass and Cavalier-Smith 2004). We also performed light and electron microscopic observations on the new amoeba. Based on these data, we discuss the taxonomic position of the new amoeba, and the morphology and lifestyle of novel clade 4 organisms.

MATERIALS AND METHODS

Culture establishment

Marine sediments samples were collected from the northwest side of Agenashiku Island (latitude = 26.2177 °N, longitude = 127.2927 °E), Okinawa, Japan, on January 12, 2011. The samples were incubated at 20 °C under a 14-h light/10-h dark cycle in ESM medium (Kasai et al. 2009). Cells of *Trachyrhizium urniformis* n. g., n. sp. that fed on pennate diatoms were found in the incubated sample. An individual *T. urniformis* cell was isolated by micropipetting. The isolated cell was incubated in ESM medium with a culture of *Nitzschia* sp. (strain E06) at 20 °C under a 14-h light/10-h dark cycle. An established culture of *T. urniformis* (strain SRT104) was maintained in ESM medium with the strain E06 or a culture of *Nitzschia* sp. (strain E09 [= NIES- 3877]) at 18 °C under a 14-h light/10-h dark cycle.

Light and Electron microscopy

Living cells of strain SRT104 were observed in glass-bottomed dishes using an Olympus IX71 inverted microscope (Olympus, Tokyo, Japan) equipped with an Olympus DP73 CCD camera.

Specimen for transmission electron microscopy (TEM) was prepared as follow: a suspension of cultivated cells was pre-fixed using an equal amount of 4% (v/v)

glutaraldehyde and 0.02% OsO_4 in natural seawater for 1 h at room temperature. Fixed cells were centrifuged and the resultant pellet was washed three times with 0.2 M sodium cacodylate buffer (SCB; pH 7.2). Cells were post-fixed using 1% (v/v) OsO_4 with 0.1 M SCB for 30 min at 4 °C. Cells were dehydrated in a graded ethanol series beginning at 30% and ending at 100% (v/v). After dehydration, cells were placed in 1:1 mixture of 100% ethanol and acetone for 10 min, followed by two 10 min intervals in acetone. Resin replacement was performed using a 1:1 mixture of acetone and Agar Low Viscosity Resin R1078 (Agar Scientific Ltd., Stansted, England) for 30 min, followed by pure resin for 2 h. Resin was polymerized by heating at 60 °C for 12 h. Ultrathin sections were prepared on a Reichert Ultracut S ultramicrotome (Leica, Vienna, Austria), and then double-stained with 2% (w/v) uranyl acetate and lead citrate (Hanaichi et al. 1986; Sato 1968), and observed using a Hitachi H-7650 electron microscope (Hitachi High-Technologies Corp., Tokyo, Japan) equipped with a Veleta TEM CCD camera (Olympus, Tokyo, Japan).

DNA extraction and polymerase chain reaction (PCR)

Cells in the culture medium were collected by centrifugation and total DNA was extracted from cells using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Small subunit (SSU) rRNA of strain SRT104 was amplified by the polymerase chain reaction (PCR) using the forward and reverse primers 18F and 18R, respectively (Yabuki et al. 2010). Amplifications consisted of 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 min, and extension at 72 °C for 2 min. An additional extension for 4 min at 72 °C was performed at the end of the reaction. Amplified DNA fragments were purified after gel electrophoreses using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany), and then cloned into the p-GEM®-T Easy Vector (Promega, Tokyo, Japan). Three independent clones were completely sequenced using a internal primer nest18sF2

(5'-GGTTCGATTCCGGAGAGGG-3') by a 3130 Genetic Analyzer (Applied Biosystems, CA, USA) with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA). The SSU rDNA sequence of strain SRT104 was deposited as LC125926, in GenBank.

Sequence alignment and phylogenetic analysis

The SSU rRNA gene sequence of strain SRT104 was added to our alignment set of cercozoan SSU rRNA genes. Sequences of the alignment set were automatically aligned with MAFFT (Katoh and Toh 2008), and then edited manually with SeaView (Galtier et al. 1996). For the phylogenetic analysis, ambiguously aligned regions were manually deleted from each alignment. Finally, we prepared the SSU rDNA alignment (1,615 positions). The alignment files used in the analysis are available upon request. The maximum likelihood (ML) tree was heuristically searched using RAxML v.7.4.4 (Stamatakis 2006) under the GTR+Γ model. Tree searches started with 20 randomized maximum-parsimony trees, and the highest log likelihood (lnL) was selected as the ML tree. An ML bootstrap analysis (1000 replicates) was conducted under the GTR+Γ model. A Bayesian analysis was run using MrBayes v. 3.2.2 (Ronquist and Huelsenbeck 2003) with the GTR +Γ model. One cold and three heated chains (Markov chain Monte Carlo at default chain temperatures were run for 5×10^6 generations, sampling lnL values and trees at 100-generation intervals. The first 1×10^6 generations had average standard deviation of split frequencies (ASDSF) values greater than 0.01 were discarded as "burn-in." Bayesian posterior probabilities (BPP) and branch lengths were calculated from the remaining trees.

RESULTS

Light microscopy

Cells of *Trachyrhizium urniformis* n. g., n. sp. were fully covered by a thin smooth theca except for the single, wide aperture (2.3–4.4 μm in diameter) (Fig. 1A–C). Thecae were round or broadly ellipsoidal, and 7.5–17.6 μm in length (12.0 μm in average, n=36) (Fig. 1A–C).A large nucleus was located at the opposite side of the aperture (Fig. 1A) and many bacteria, diatoms, and debris were observed near the aperture (Fig. 1C, D, H). Thin filopodia emerged from the aperture and extended radially (Fig. 1E). The filopodia were well branched and occasionally anastomosed, containing small granules (Fig. 1E, F, H). The granules were transported within the filopodia bidirectionally (movie S1). Aggregated colonies were frequently observed in aged cultures (Fig. 1G, H). The colonies occasionally reached a maximum diameter of 40 μm.

Transmission electron microscopy

Cells were covered with a thin smooth bilayered theca except for the single aperture (Fig. 2A, B). The theca consisted of an electron dense outer layer, and a slightly ambiguous and less dense inner layer (Fig. 2C). Cells had a nucleus with a conspicuous nucleolus and permanently condensed chromatin (Fig. 2A, B, F). Several Golgi apparatuses were located around the nucleus (Fig. 2D). Rounded mitochondrial profiles with tubular cristae and microbodies were scattered throughout the cell (Fig. 2D, E). Cells occasionally included lipid globules and food vacuoles that contain the digested remnants of diatoms (Fig. 2F). Slender extrusomes covered with a single membrane were observed mainly in the pseudopodia and occasionally in the cell body (Fig. 3). Extrusomes were 0.4–0.5 μm in length and 0.15–0.2 μm in width, and consisted of a spherical cap structure and an electron dense cylinder (Fig. 3B). The cylinder included a less dense core approximately 40 nm in diameter (Fig. 3B, C).

Molecular phylogenetic analysis

We sequenced 1766 bp of SSU rRNA gene sequence of *T. urniformis*. Our SSU rRNA

gene tree showed that *T. urniformis* was included in the cercozoan subphylum Filosa and formed a clade with two environmental sequences, AY620348 and AY620314, that make up novel clade 4 of Bass and Cavalier-Smith (2004), with high statistical support (BP = 100%, BPP = 1.00) (Fig. 4). The clade including *T. urniformis* and the environmental sequences of novel clade 4 formed the sister group to another clade consisting of several thecofiloseans (Cryomonadida, Ebriida, Matazida, and the genus *Botuliforma*), although statistical supports were weak (Fig. 4).

DISCUSSION

Morphological and ultrastructural comparison between *T. urniformis* and other thecate or testate filose amoebae

Our phylogenetic tree using SSU rRNA genes showed that *Trachyrhizium urniformis* n. g., n. sp. forms a weakly supported clade with several thecofiloseans, and a robust clade with two environmental sequences that make up novel clade 4 (Bass and Cavalier-smith 2004). Although *T. urniformis* was placed at an independent phylogenetic position, there are several thecate and testate filose amoebae that have similar morphologies to *T. urniformis*. Here, we compare the morphology of *T. urniformis* with other filose testate or thecate amoebae to clarify its taxonomic position.

Filose thecate or testate amoebae have been described for more than 150 years, and there are many species and genera with variations in cell size, habitat, and morphology of the thecae or tests (Cash et al. 1915; De Saedeleer 1934; Meisterfeld 2002). These amoebae are recognized as paraphyletic and most of them are now placed in separate classes of Cercozoa (e.g., Imbricatea, Thecofilosea, and possibly Granofilosea), stramenopiles (Labyrinthulea), or treated as incertae sedis (Adl et al. 2012; Bass et al. 2009; Gomaa et al. 2013; Howe et al. 2011a). Thecofilosea mainly consists of non-granular filose amoebae with non-siliceous thecae or tests, and some thecofilosean genus such as *Rhogostoma*, *Lecythium*, and *Chlamydophrys* have smooth

thecae without debris and particles on their surfaces like that of *T. urniformis*. *Rhogostoma* is a genus of small filose testate amoebae living in marine and freshwater environments. Members of *Rhogostoma* resemble *T. urniformis* in cell size, and in their possession of branching, and sometimes anastomosing pseudopodia; however, *Rhogostoma* cells have crack-like apertures instead of round apertures (Myl'nikova and Myl'nikov 2012; Howe et al. 2011a). *Chlamydophrys* and *Lecythium* are tectofilosid filose thecate amoebae with an oval test and circular aperture. *Chlamydophrys* is clearly different from *T. urniformis* in lacking granules in the filopodia, and by possessing gaps between the cytoplasm and test (De Saedeleer 1934; Howe et al. 2011a; Meisterfeld 2002). *Lecythium* is different from *T. urniformis* in having non-granular filopodia without anastomosing. Most species of *Lecythium* also can be distinguished from *T. urniformis* based on size (i.e., they are larger) and by living in freshwater habitats (Kudo 1954; Meisterfeld 2002). Although another species, *L. minutum*, lives in marine environments and has smaller cell (9.3–11 μm), neither granules nor anastomose were observed in its filopodia like other *Lecythium* species (De Saedeleer 1934).

In our molecular phylogenetic analysis, *T. urniformis* was included in a thecofilosean clade with weak statistical support and formed a sister lineage to novel clade 4. Novel clade 4 also appeared to be one of the basal branches of Thecofilosea in previous phylogenetic analyses (Bass et al. 2005; Howe et al. 2011a). *Trachyrhizium urniformis* has a thin, bilayered, and smooth theca. In Cercozoa, extracellular thecae are observed in Thecofilosea and Metromonadea. Metromonadean flagellates have a delicate mono- or bilayered theca covered with fibrous material (Myl'nikov et al. 1999; Myl'nikova and Myl'nikov 2011). The theca of *T. urniformis* is rigid, electron dense, and lacks fibrous materials, features clearly different from those possessed by metromonadeans. The thecae of thecofiloseans are basically rigid and consist of single or multiple layers. The thickness, number of the layers, and presence or absence of fibrous materials on the theca vary among species or genera (e.g., Hargraves 2002;

Hoppenrath and Leander 2006; Thomsen et al. 1991). Bilayered smooth and rigid thecae are also reported in *Mataza hastifera* and *Cryothecomonas vesiculata*; however, the thecae of these flagellates are different from *T. urniformis* in the density and thickness of each layer (Yabuki and Ishida 2011; Thomsen et al. 1991). In conclusion, the theca of *T. urniformis* is structurally more similar to those of thecofiloseans than to those of metromonadeans, which corroborates the phylogenetic position of *T. urniformis*.

Nucleus with permanently condensed chromatin is widely reported in various thecofilosean species (e.g., *Cryothecomonas*, *Protaspa*, *Ebria*, and *Mataza*) (e.g. Hargraves 2002; Hoppenrath and Leander 2006, Myl'nikova and Myl'nikov 2012; Thomsen et al. 1991; Yabuki and Ishida 2011). On the other hand, this ultrastructural characteristic is rarely observed in other cercozoan groups, with some exceptions (Kies 1974; Shiratori et al. 2014). Permanently condensed chromatin was also observed in the nucleus of *T. urniformis*, suggesting a close relationship between *T. urniformis* and other thecofiloseans.

Trachyrhizium urniformis has filose branching pseudopodia like most other thecofiloseans (e.g., *Botuliforma*, *Protaspa*, *Rhogostoma*, and *Ventrifissura*) (Chantangsi and Leander 2010a; Hoppenrath and Leander 2006; Myl'nikova and Myl'nikov 2012). However, the filopodia of *T. urniformis* are different from that of other thecofiloseans in having granules that are actively transported in the filopodia bidirectionally. Based on the transmission electron microscopic observations of the filopodia, the granules appear to be extrusomes. Although bidirectional streaming of granules in pseudopodia are characteristics of Foraminifera (Hausmann et al. 2003), it is relatively rare in Cercozoa. Granular filose or reticulose pseudopodia are widely reported in Granofilosea (e.g., *Limnofila*, *Massisteria*, and *Mesofila*); however, bidirectional streaming of the granules has only been reported in *Reticulamoeba* (Bass et al. 2009, 2012; Patterson and Fenchel 1990).

Extrusomes are widely observed in Cercozoa and vary in size and morphology.

In Thecofilosea, highly elongated extrusomes have been reported in several flagellates (e.g., *Cryothecomonas*, *Mataza*, and *Protaspa*), but the shorter bipartite extrusomes possessed by *T. urniformis* have never been reported (Hoppenrath and Leander 2006; Schnepf and Kühn 2000; Yabuki and Ishida 2011). On the other hand, somewhat similar extrusomes have been reported in Thaumatomonadida (*Thaumatomonas* spp.) and Chlorarachnea (*Bigelowiella natans* and *Chlorarachnion reptans*) despite their distant phylogenetic positions (Hibberd and Norris 1984; Karpov and Zhukov 1993; Moestrup and Sengco 2001; Myl'nikov and Myl'nikova 2012).

As mentioned above, *T. urniformis* has rigid thecae and nucleus with permanently condensed chromatin, which are characteristics shared with other thecofiloseans. Our phylogenetic analysis also suggests the monophyly of *T. urniformis* and several thecofilosean groups, although with weak statistical support. Therefore, we propose to include *T. urniformis* within Thecofilosea. On the other hand, our microscopic observations showed that *T. urniformis* has granular filopodia and shorter extrusomes. These features have never been reported in Thecofilosea, suggesting there is hidden morphological and ultrastructural diversity within Thecofilosea.

Putative morphology of novel clade 4 and taxonomic studies on eukaryovorous Cercozoa

Our study examined the morphology and ultrastructure of *T. urniformis* that is closely related to novel clade 4. Novel clade 4 was initially recognized by an environmental DNA survey focusing on the phylum Cercozoa (Bass et al. 2004) and there was no morphological information so far. Interestingly, both environmental sequences of novel clade 4 (i.e., AY620348 and AY620314) were collected from marine sediments, as was *T. urniformis*, suggesting that members of novel clade 4 may possess similar morphologies and lifestyles such as being algae-feeding filose thecate amoebae.

Recently, Dumack et al. (2016) succeeded to establish a culture of a new

species of poorly studied testate amoeba *Lecythium* (*L. terrestris*) and revealed phylogenetic placement of the genus. Interestingly, *L. terrestris* is eukaryovorous (feeds on various fungi and algae) like *T. urniformis*, which probably the reason why they had not been cultivated. Actually, culture-based taxonomic studies on Cercozoa have mainly focused on bacterivorous species (e.g., Bass et al. 2009; Howe et al. 2009, 2011b; Scoble and Cavalier-Smith 2014) than eukaryovorous species. However, previous studies on eukaryovorous cercozoans showed that they represent deep branches or novel lineages within Cercozoa (e.g., Chantangsi and Leander 2010a, b; Hess and Melkonian 2013; Shiratori and Ishida 2014). Further taxonomic studies focusing on eukaryovorous cercozoans will help for understanding the diversity of Cercozoa.

Taxonomic Treatment

Class Thecofilosea Cavalier-Smith 2003

Trachyrhizium Shiratori and Ishida, n. g.

Description: Marine filose amoebae with thin smooth organic thecae. Filopodia thin, branching, and occasionally anastomosing, including small granules with bidirectional movement. Thecae consisting of two layers. Extrusomes present. Mitochondria with tubular cristae. Presence of Golgi apparatuses and microbodies.

Type species: *Trachyrhizium urniformis*

Etymology: The genus name "*Trachyrhizium*" derived from Latin *Trachy* (rough) and *rhizium* (root), refering to the granular pseudopodia of the type species. *Trachyrhizium* is considered to be neuter.

Trachyrhizium urniformis Shiratori and Ishida, n. sp.

Description. Cells spherical, 7.5–17.6 μm in diameter. Theca spherical with one wide circular aperture. Theca bilayered with an electron dense outer layer, and a slightly ambiguous and less dense inner layer. Feeding on diatoms. Extrusomes 0.4–0.5 μm in

length and 0.15–0.2 μm in width, consisting of a spherical cap structure and electron dense cylinder that includes a less dense core.

Hapantotype: One microscope slide (TNS-AL-58920-s) deposited in the herbarium of the National Museum of Nature and Science (TNS), Tokyo, Japan.

Paratype: One EM block (TNS-58920-b) deposited in the TNS. These cells are derived from the same sample as the holotype.

DNA sequence: Small subunit ribosomal DNA, LC125926.

Type locality: Marine sediments collected from northwest side of Agenashiku Island, Okinawa, Japan. (latitude = 26.2177 °N, longitude = 127.2927 °E).

Collection date: January 12, 2011.

Authentic culture: The strain SRT104 was used to describe this species, and is deposited in and maintained by the National Institute for Environmental Sciences, Tokyo, Japan, as NIES-3876.

Etymology: The specific epithet "*urniformis*" (urn-shaped) refers to the cell shape of this organism.

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FIGURE LEGENDS

Fig. 1. Differential interference contrast (DIC) micrographs of living cells of *Trachyrhizium urniformis* n. g., n. sp. B, bacteria; N, nucleus. Arrows indicate the theca. Arrowheads indicate the wide aperture. Double arrowheads indicate granules in

filopodia. Thin arrows indicate anastomosing filopodia. Asterisks indicate diatoms. **A.** Individual cell (side view) indicating the large nucleus (top) and the wide aperture (bottom). **B.** Individual cell (top view) showing the wide aperture. **C, D.** Cells with debris, bacteria, and/or diatoms near the apertures. **E.** Individual cell with branching and anastomosing filopodia. **F.** High-magnification view of pseudopodia with granules. **G.** Colony of cells. **H.** Colony of cells with diatoms and bacteria. Scale bar: A–C, H = 10 μm; D–G = 20 μm

Fig. 2. Transmission electron micrographs of *Trachyrhizium urniformis* n. g., n. sp. FV, food vacuole; G, Golgi apparatus; IT, inner layer of theca; L, lipid globules; M, mitochondria; MB, microbody; N, nucleus; n, nucleolus; OT, outer layer of theca. **A.** Approximate longitudinal section of the cell. Scale bar = 2 μm. **B.** Approximate transverse section of the cell. Scale bar = 2 μm. **C.** High-magnification view of the theca. Scale bar = 500 nm. **D.** High-magnification view of the Golgi apparatus and mitochondria. Scale bar = 1 μm. **E.** High-magnification view of microbodies. Scale bar = 1 μm. **E.** High-magnification view of lipid globules and food vacuoles. Scale bar = 2 μm.

Fig. 3. Transmission electron micrographs of *Trachyrhizium urniformis* n. g., n. sp. E, extrusome. Double arrows indicate the less dense core of the cylinder. Triple arrowheads indicate the spherical cap structure. **A.** Pseudopodium with several extrusomes. Scale bar = 1 μm. **B.** High-magnification view of the longitudinal section of an extrusome. Scale bar = 200 nm. **C.** High-magnification view of the transverse section of an extrusome. Scale bar = 200 nm.

Fig. 4. Maximum likelihood phylogeny of 96 cercozoan small subunit ribosomal RNA gene sequences (1,615 bp). Environmental sequences are labeled with accession

numbers. Bootstrap support values ≥ 50% are shown. Nodes supported by Bayesian posterior probabilities ≥ 0.95 are indicated in bold.

SUPPORTING INFORMATION

FIGURE LEGEND

Movie S1. Branching filopodia of *Trachyrhizium urniformis* n. g., n. sp.

Movie S2. Filopodia of *Trachyrhizium urniformis* n. g., n. sp., showing granules that transported bidirectionally.

Fig. 1

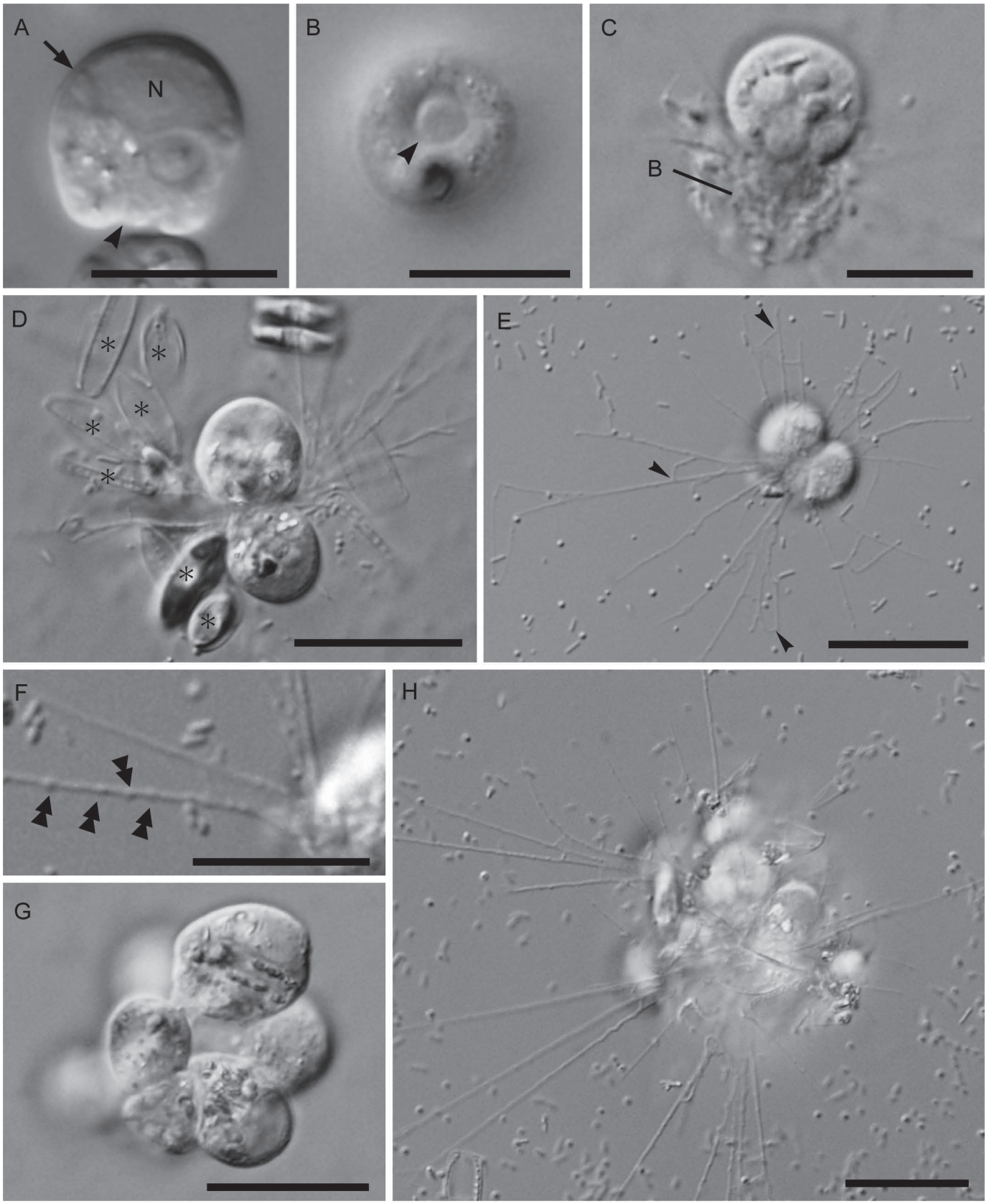


Fig. 2

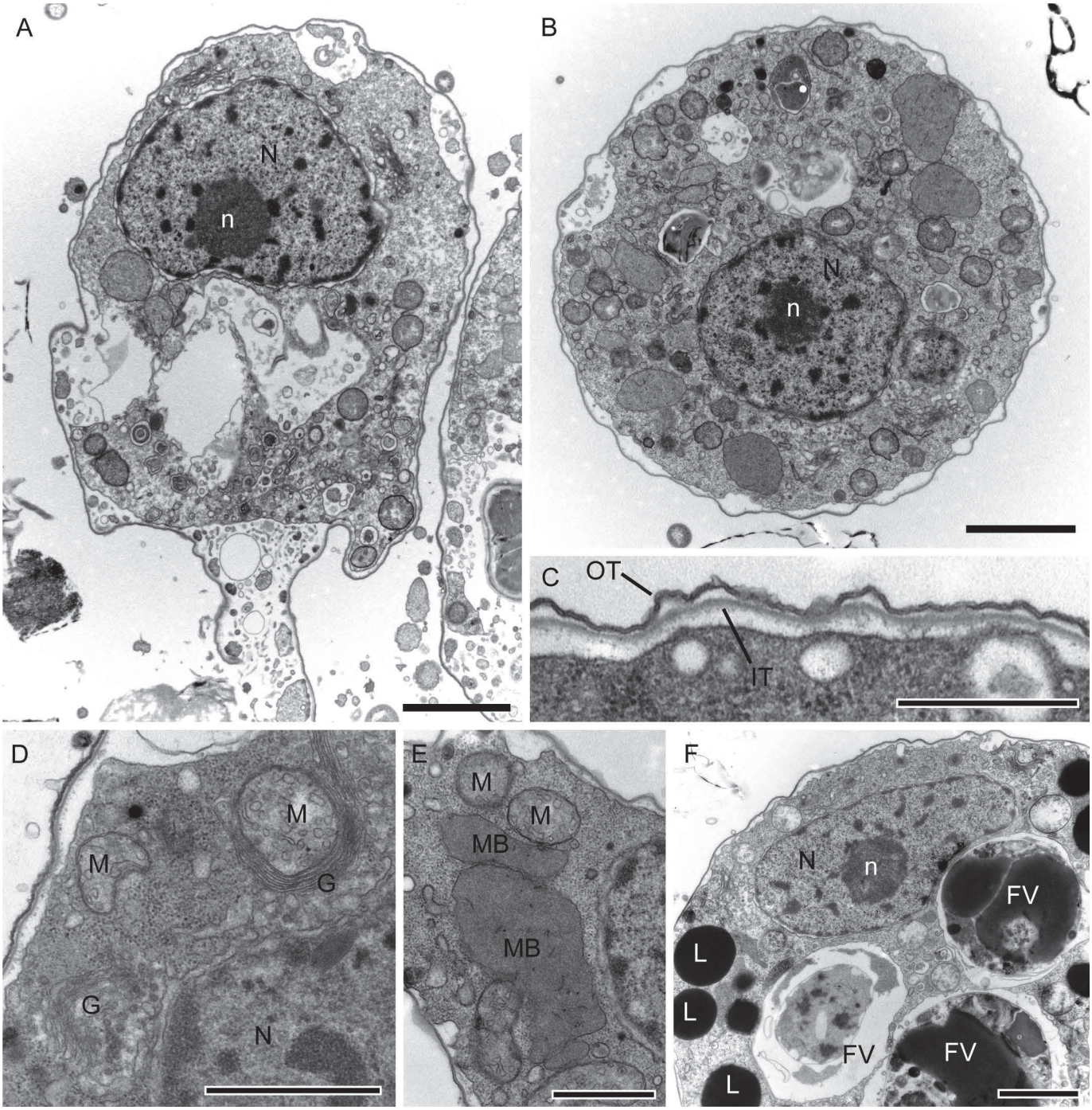


Fig. 3

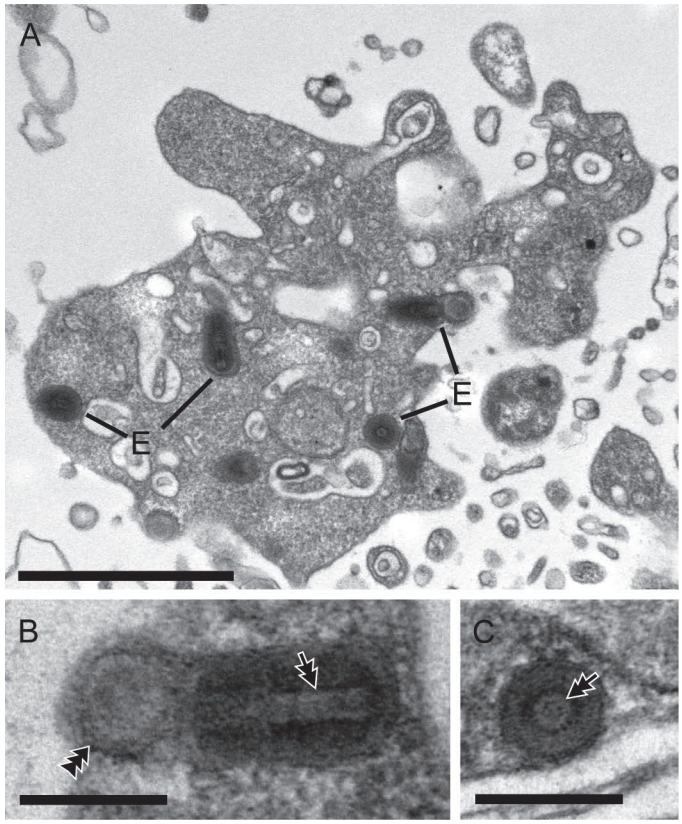
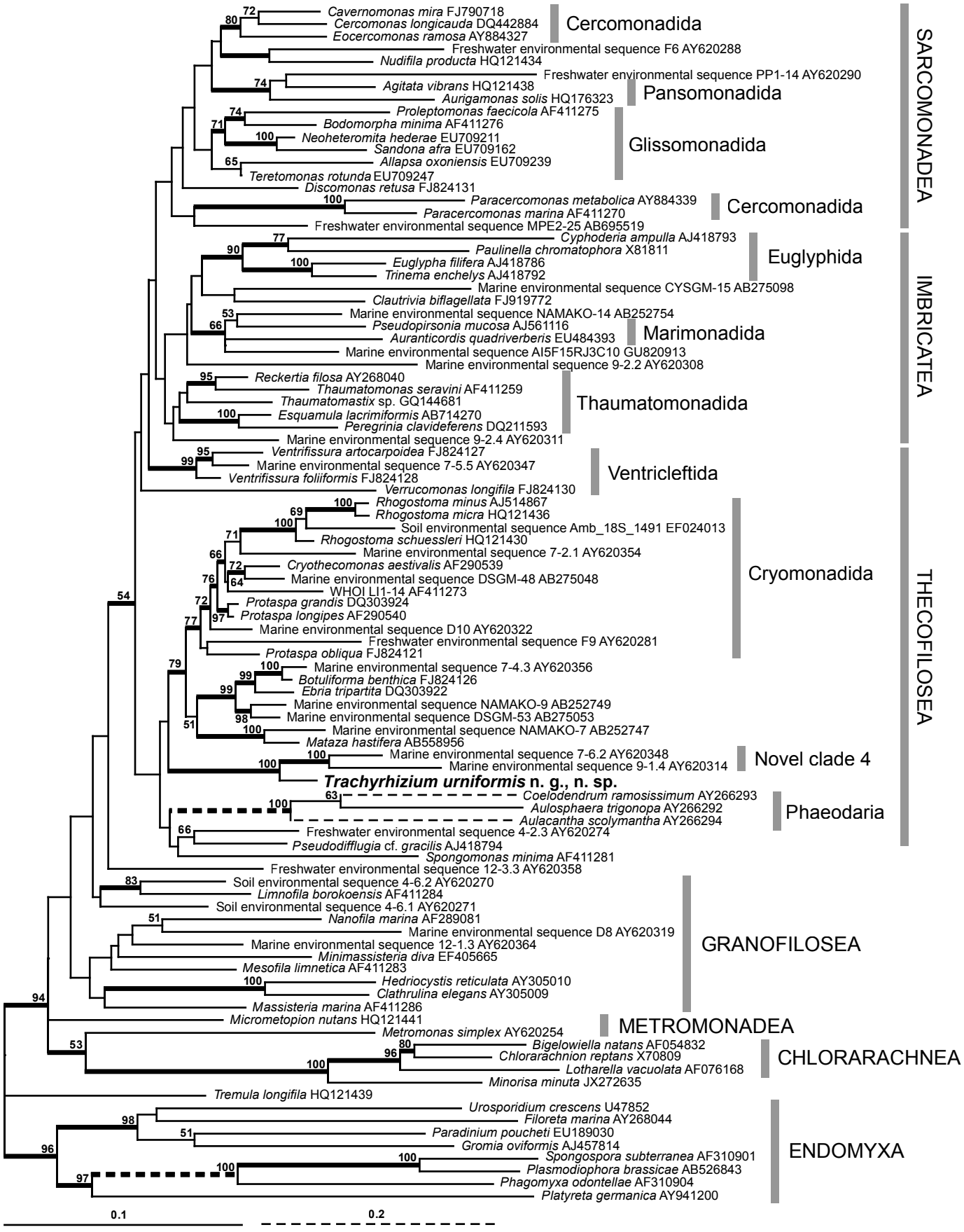


Fig. 4


Cavernomonas mira FJ790718
Cercomonas longicauda DQ442884
Eocercomonas ramosa AY884327
Cercomonadida
Freshwater environmental sequence F6 AY620288
Nudifila producta HQ121434
Freshwater environmental sequence PP1-14 AY620290
Agitata vibrans HQ121438
Aurigamonas solis HQ176323
Pansomonadida
Proleptomonas faecicola AF411275
Bodomorpha minima AF411276
Neoheteromita hederae EU709211
Sandona afra EU709162
Allapsa oxoniensis EU709239
Teretomonas rotunda EU709247
Glissomonadida
Discomonas retusa FJ824131
Paracercomonas metabolica AY884339
Paracercomonas marina AF411270
Cercomonadida
Freshwater environmental sequence MPE2-25 AB695519
SARCOMONADEA
Cyphoderia ampulla AJ418793
Paulinella chromatophora X81811
Euglypha filifera AJ418786
Trinema enchelys AJ418792
Euglyphida
Marine environmental sequence CYSGM-15 AB275098
Clautrivia biflagellata FJ919772
Marine environmental sequence NAMAKO-14 AB252754
Pseudopirsonia mucosa AJ561116
Auranticordis quadriverberis EU484393
Marimonadida
Marine environmental sequence AI5F15RJ3C10 GU820913
Marine environmental sequence 9-2.2 AY620308
Reckertia filosa AY268040
Thaumatomonas seravini AF411259
Thaumatomastix sp. GQ144681
Esquamula lacrimiformis AB714270
Peregrinia clavideferens DQ211593
Thaumatomonadida
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IMBRICATEA
Ventrifissura artocarpoidea FJ824127
Marine environmental sequence 7-5.5 AY620347
Ventrifissura foliiformis FJ824128
Verrucomonas longifila FJ824130
Ventricleftida
Rhogostoma minus AJ514867
Rhogostoma micra HQ121436
Soil environmental sequence Amb_18S_1491 EF024013
Rhogostoma schuessleri HQ121430
Marine environmental sequence 7-2.1 AY620354
Cryothecomonas aestivalis AF290539
Marine environmental sequence DSGM-48 AB275048
WHOI LI1-14 AF411273
Protaspa grandis DQ303924
Protaspa longipes AF290540
Marine environmental sequence D10 AY620322
Freshwater environmental sequence F9 AY620281
Protaspa obliqua FJ824121
Cryomonadida
Marine environmental sequence 7-4.3 AY620356
Botuliforma benthica FJ824126
Ebria tripartita DQ303922
Marine environmental sequence NAMAKO-9 AB252749
Marine environmental sequence DSGM-53 AB275053
Marine environmental sequence NAMAKO-7 AB252747
Mataza hastifera AB558956
Marine environmental sequence 7-6.2 AY620348
Marine environmental sequence 9-1.4 AY620314
Novel clade 4
Trachyrhizium urniformis n. g., n. sp.
Coelodendrum ramosissimum AY266293
Aulosphaera trigonopa AY266292
Aulacantha scolymantha AY266294
Phaeodaria
Freshwater environmental sequence 4-2.3 AY620274
Pseudodifflugia cf. gracilis AJ418794
Spongomonas minima AF411281
Freshwater environmental sequence 12-3.3 AY620358
THECOFILOSEA
Soil environmental sequence 4-6.2 AY620270
Limnofila borokoensis AF411284
Soil environmental sequence 4-6.1 AY620271
Nanofila marina AF289081
Marine environmental sequence D8 AY620319
Marine environmental sequence 12-1.3 AY620364
Minimassisteria diva EF405665
Mesofila limnetica AF411283
Hedriocystis reticulata AY305010
Clathrulina elegans AY305009
Massisteria marina AF411286
GRANOFILOSEA
Micrometopion nutans HQ121441
METROMONADEA
Metromonas simplex AY620254
Bigelowiella natans AF054832
Chlorarachnion reptans X70809
Lotharella vacuolata AF076168
CHLORARACHNEA
Minorisa minuta JX272635
Tremula longifila HQ121439
Urosporidium crescens U47852
Filoreta marina AY268044
Paradinium poucheti EU189030
Gromia oviformis AJ457814
Spongospora subterranea AF310901
Plasmodiophora brassicae AB526843
Phagomyxa odontellae AF310904
Platyreta germanica AY941200
ENDOMYXA
0.1
0.2