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Daniel J.G. Lahr
Smith College

Gabriela M. Kubik
Smith College

Anastasia L. Gant
Smith College

Jessica Grant
Smith College

O. Roger Anderson
Lamont-Doherty Earth Observatory

See next page for additional authors

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Morphological Description of *Telaepolella tubasferens* n. g. n. sp., Isolate ATCC© 50593™, a Filose Amoeba in the Gracilipodida, Amoebozoa

Daniel J. G. LAHR[1,2]*, Gabriela M. KUBIK[1]*, Anastasia L. GANT[1], Jessica GRANT[1], O. Roger ANDERSON[3] and Laura A. KATZ[1,2]

[1]Department of Biological Sciences, Smith College, Northampton, MA, USA; [2]Program in Organismic and Evolutionary Biology, University of Massachusetts, Amherst, MA, USA; [3]Biology, Lamont-Doherty Earth Observatory of Columbia University, Palisades, New York; * D. J. G. Lahr and G. M. Kubik contributed equally to this work

Abstract. We describe the amoeboid isolate ATCC© 50593™ as a new taxon, *Telaepolella tubasferens* n. g. n. sp. This multinucleated amoeba has filose pseudopods and is superficially similar to members of the vampyrellids (Rhizaria) such as *Arachnula impatiens* Cienkowski, 1876, which was the original identification upon deposition. However, previous multigene analyses place this taxon within the Gracilipodida Lahr and Katz 2011 in the Amoebozoa. Here, we document the morphology of this organism at multiple life history stages and provide data underlying the description as a new taxon. We demonstrate that *T. tubasferens* is distinct from *Arachnula* and other rhizarians (*Theratromyxa*, *Leptophrys*) in a suite of morphological characters such as general body shape, relative size of pseudopods, distinction of ecto- and endoplasmic regions, and visibility of nuclei in non-stained cells (an important diagnostic character). Although Amoebozoa taxa generally have lobose pseudopods, genera in Gracilipodida such as *Flamella* and *Filamoeba* as well as several organisms previously classified as protosteloid amoebae (e.g. schizoplasmodiis, cavosteliids and Stemonitales) present filose pseudopodia. Thus, classification of amoeboid organisms merely by filose-lobose distinction must be reconsidered.

Key words: Filose pseudopod, Amoebozoa, Gracilipodida.

INTRODUCTION

Early classifications combined all amoeboid organisms into a single evolutionary group named Sarcodina (Pawlowski 2008). Within the Sarcodina, species were further divided based on the morphology of their pseudopodia. For instance, the Actinopodea consisted of the organisms that had pseudopods supported by an internal 'skeletal' organization. The Rhizopodea was, by contrast, made up of organisms with 'soft' pseudopods (Bovee 1985). Within each, the organisms were further subdivided following other pseudopodial characteristics: the Rhizopodea consisted of eight classes, including Lobosea, which comprised the classical lobose amoebae such as *Chaos carolinensis*, and the Granuloreticulosea, which contained the Foraminifera with anastomosing pseudopods capable of bidirectional streaming (Pawlowski 2008).

Address for correspondence: Laura A. Katz, Department of Biological Sciences, Smith College, 44 College Lane, Northampton, MA 01063, USA; E-mail: lkatz@smith.edu

The innovation of ultrastructural analyses and more recently molecular phylogenetics radically altered our understanding of relationships among amoebae (Cavalier-Smith *et al.* 2004, Page 1987, Patterson 1999). Today, amoebae that used to be classified within the Sarcodina are spread out all over the eukaryotic tree of life and appear in more than 30 of the ~ 75 major eukaryotic lineages (Patterson 1999). The bulk of amoebae fall in two major clades, Rhizaria and Amoebozoa, which are often differentiated by the presence of filose and lobose pseudopods, respectively (Adl *et al.* 2012, Parfrey *et al.* 2006, Pawlowski and Burki 2009). The isolate ATCC© 50593™, originally identified as the vampyrellid (Rhizaria) genus *Arachnula* sp. is an exception as multigene sequence analyses places this filose amoeba well within the Amoebozoa (Lahr *et al.* 2011a, Tekle *et al.* 2007).

Here, we characterized the life history of the isolate ATCC© 50593™ by light and electron microscopy to document the morphology in a detailed manner. Based on these analyzes, we propose a new taxon, *Telaepolella tubasferens* n. g. n. sp. within Amoebozoa. Further, we compare morphological features of the isolate to other members of Amoebozoa, especially those in Gracilipodida that include the closest relatives of *T. tubasferens,* and to amoebae with similar morphologies among the Rhizaria. We also discuss the implications of the presence of this group capable of producing mostly filose pseudopods within Amoebozoa.

METHODS

Cultures

Isolate ATCC© 50593™, which was deposited and identified by T. K. Sawyer, was acquired from ATCC© on two occasions and results were consistent across isolates. Cultures were kept at room temperature on a shelf, with natural ambient light exposure. The isolate used in this study, was kept in 25 cm^2 standard culture flasks with autoclaved and micropore-filtered (Whatman Grade 1 filters, 8 µm pore size) pond water and was fed bacterialized cerophyl following the protocol described in Lahr *et al.* (2011b), briefly, bacteria (we used a non-pathogenic *E. coli* lineage) are inoculated into a cerophyl solution and grown for 4 h at 36°C in a shaker. The bacterialized cerophyl is inoculated into previously sterilized pondwater in a 1:100 solution.

Light microscopy

To observe the various life history stages, we used Phase Contrast, Hoffman Modulation Contrast and Differential Interference Contrast microscopy at 200 × and 400 × magnification variously in three microscopes: Olympus BX51 upright microscope, Olympus CKX31 and T1-Sm Nikon inverted microscope. Images were taken with an Olympus DP70 camera or a Canon PowerShot A640 digital camera.

Fluorescence microscopy

Fluorescence imaging was used on live cells. The nuclei of the cells were stained with 4',6-diamidino-2-phenylindole [DAPI]. Amoebae were grown on coverslips in a 6-well plate in pond water. Staining was accomplished by adding 3 µl of stock 5 µg/ml DAPI solution into the well and allowed to incubate in the dark for one hour. Wells were washed with pond water for 20 min. before inverting the coverslip onto a glass slide. These amoebae are extremely sensitive to UV light, and when exposed would very quickly disintegrate. Our method enabled enough time to visually inspect the amoebae, but not enough time to capture images of high-quality.

Transmission electron microscopy

Cultures of ATCC© 50593™ (originally designated as *Arachnula*) were fixed for transmission electron microscopy as previously published (Anderson *et al*. 1997). A cell suspension (at 25°C) contained in a 15-ml graduated conical centrifuge tube was mixed with an equal volume of 4% TEM-grade glutaraldehyde in 0.2 M cacodylate buffer (pH 7.2) to yield a final fixative of 2% (w/v). The fixed cells were placed in an ice bath after 20 min. at 3°C, the glutaraldehyde-fixed cells were gently spun down to form a pellet, supernatant was removed by aspiration, and 2 ml of 2% cold osmium tetroxide solution in 0.2 M cacodylate buffer (pH 7.2) were added and the pellet thoroughly dispersed in the fixative. After 1-h post-fixation at 3°C, the cells were again pelleted and the supernatant removed. The cells were enrobed in 0.4% solidified agar, and small cubes (~ 1 mm) were cut from the agar block, washed in distilled water, dehydrated in a graded acetone/aqueous series, infiltrated with and embedded in low viscosity epon (Energy Beam Sciences, Agawam, MA), and polymerized at 75°C for 12–18 h. Ultrathin sections were cut with a Porter-Blum MT-2 ultramicrotome (Sorvall, Norwalk, CT) using a diamond knife, collected on uncoated copper grids, post-stained with Reynold's lead citrate, and observed with a Philips TEM-201 transmission electron microscope (Einthoven, Netherlands) operated at 60 kV accelerating voltage.

RESULTS

By maintaining the isolate in long-term culture (about five months), we observed different life history stages and the transformations between forms (Fig. 1). As described in detail below, there were two main forms: 1) the trophic amoeboid form, highly variable in size and often with fine protruding pseudopods and 2) cysts of many different types, including spherical, irregularly shaped, with thin or thick outer layers, and with layers differing in composition.

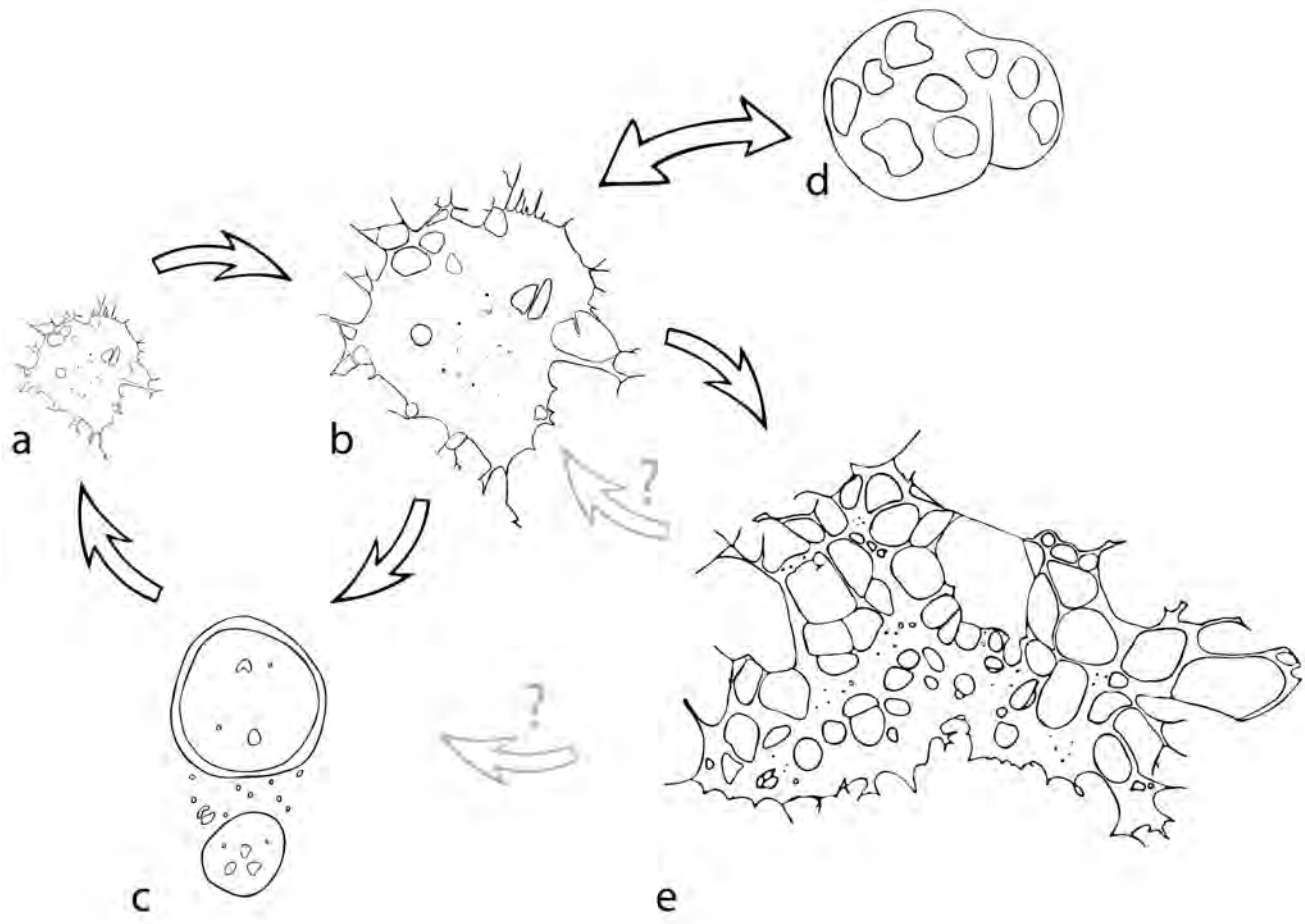

Fig. 1. Life history diagram of *T. tubasferens*. **a** – small trophic form (50-100 μm); **b** – larger trophic form (100–300 μm); **c** – cyst; **d** – floating form; **e** – larger multinucleated form (up to 500 μm). Steps in gray indicated with a question mark have not been observed, but are hypothesized to happen. Image drawn by GK and DL.

Amoeboid form

The amoeboid form varies considerably in size, gross morphology and number of nuclei (Figs 2A, 3). There are small (~ 50 μm at widest breadth, Fig. 2B–F) and medium trophic forms (100–300 μm, Fig. 2G–K) plus much larger, more stationary forms (300–500 μm, Fig. 2L). At any one time, the sizes of most amoebae in culture are fairly similar, so variation appears to occur as the culture ages. Excysting amoebae, observed on ~ 10 separate occasions, are small and have as few as four nuclei. Medium-sized amoebae are characterized by being roughly 100–300 μm and usually with one endoplasmic area where all nuclei and vesicles are together (Figs 2G–I, 3F–M). This contrasts with other stages that have multiple clumps of nuclei and vesicles throughout (e.g. Figs 2J–L, 3A, E). Larger trophic forms were normally around 500 μm, and generally possessed more than 100 nuclei. We counted as many as 132 nuclei in one organism after DAPI staining and fluorescence imaging (not shown). Generally, larger cells contained more nuclei.

The larger, highly nucleated amoeboid forms may have occurred either by growth or plasmogamy. Under these scenarios, either: (1) the amoebae multiplies nuclei while increasing cytoplasmic mass, or (2) small amoebae fuse together to form one larger amoeba, as is common in many amoebozoan lineages such as the leptomyxids and arcellinids (Lahr *et al.* 2011c). We have observed what we interpret as plasmogamy in *T. tubasferens* at least twice, independently, but the two occasions involved few amoebae that alone would not be sufficient to generate the very large multinucleated amoebae observed.

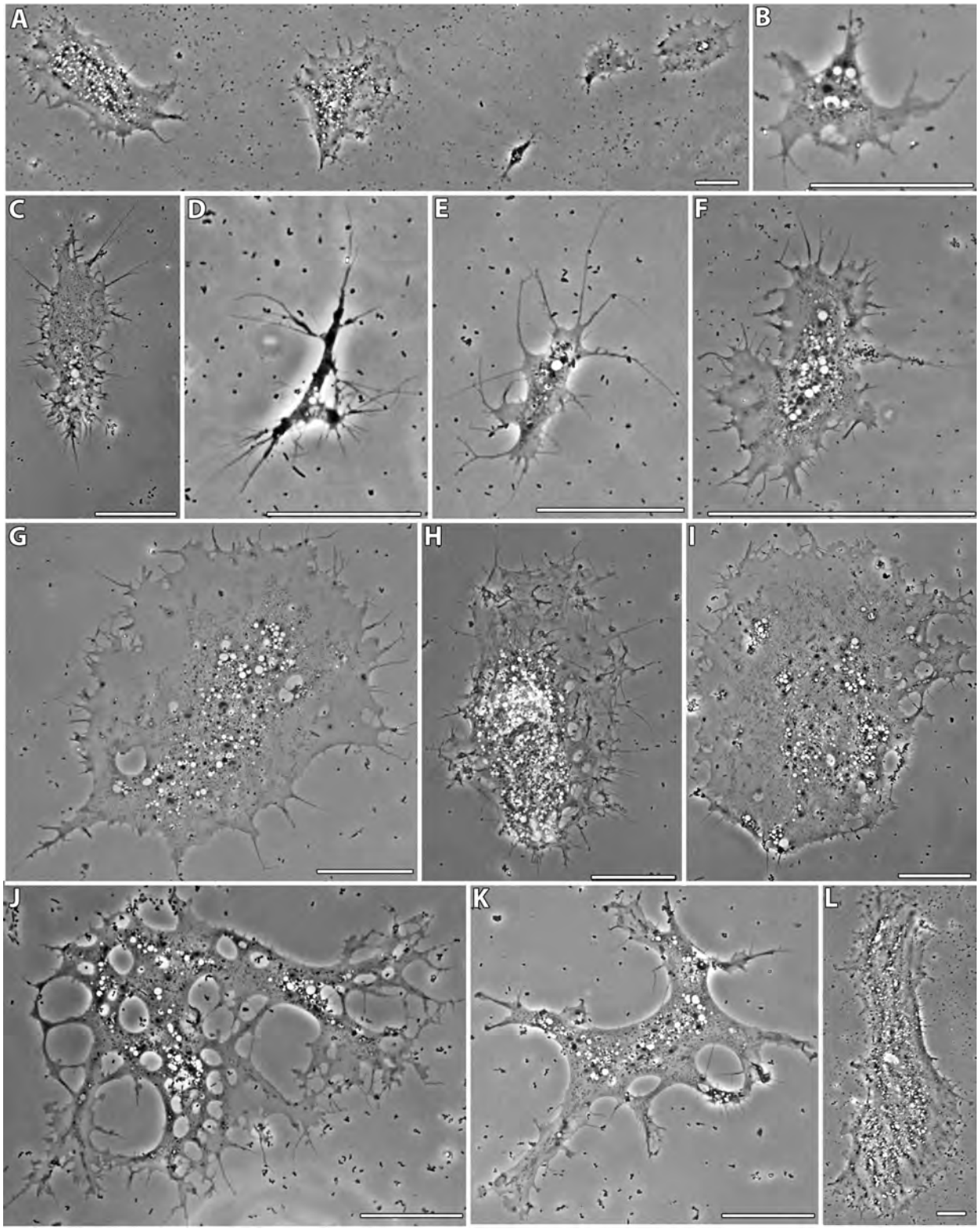

Fig. 2. Phase contrast micrographs of *T. tubasferens* trophic forms. **A** – contrasting size of five individuals within a monoeukaryotic culture; **B** – a small tetranucleated individual; **C** – medium sized individual with a large anterior region; **D**, **E**, **F** – different morphologies of small sized individuals; **G**, **H**, **I** – different morphologies of large, multinucleated individuals, without extensive branching; **J**, **K** – typical extensively branched large individual, showing many gaps within the cytoplasm; **L** – a large individual. Scale bar for all images: 50 μm.

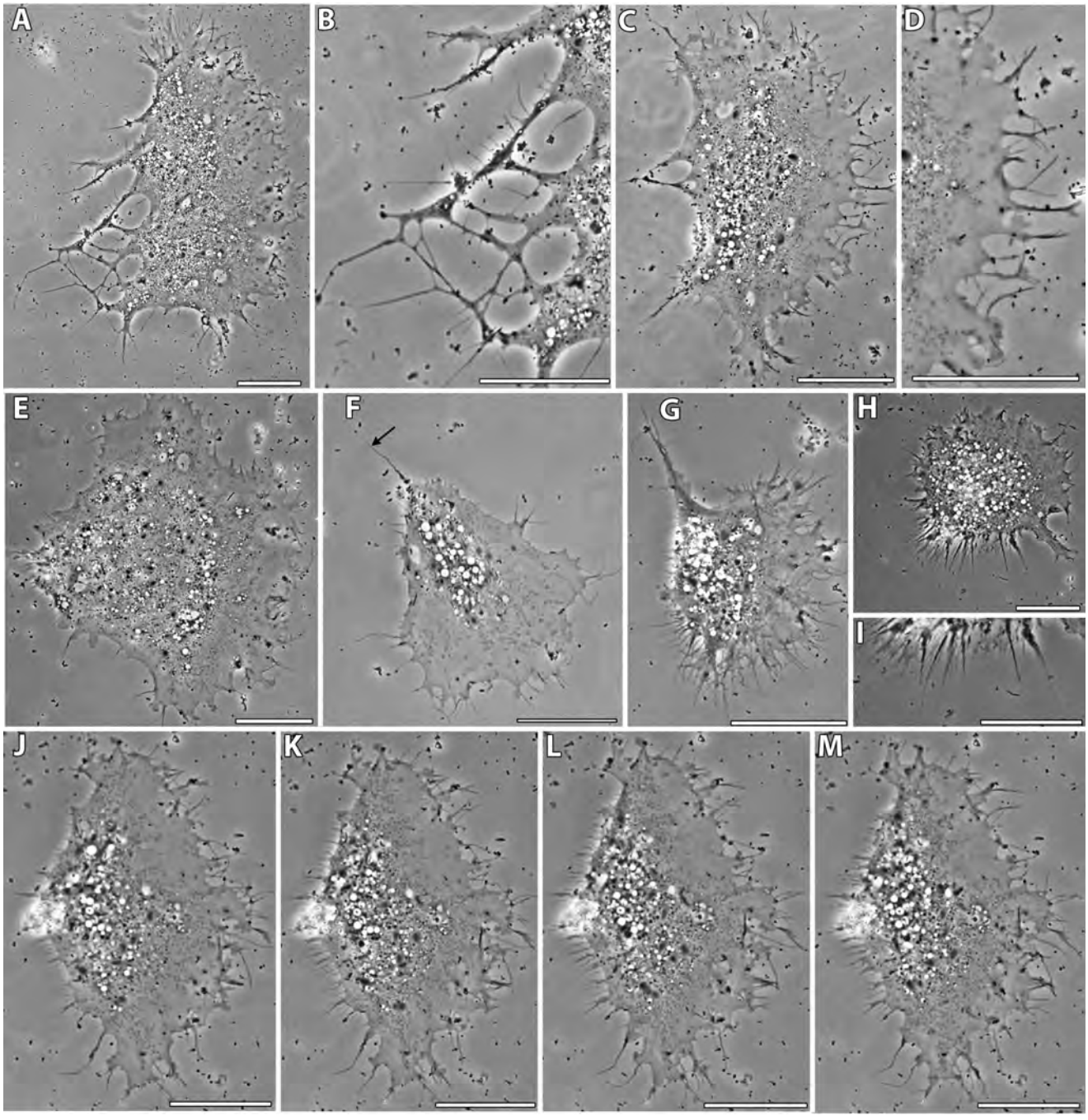

Fig. 3. Phase contrast micrographs of *T. tubasferens*, large locomotive forms. **A** – a large multinucleated individual in active locomotion; **B** – a detail of the fine, filose pseudopods at the trailing region of the individual in A; **C** – a distinct large individual in active locomotion; **D** – a detail of the anterior hyaline margin of the individual in C; **E** – a different morphology of large individual, at beginning of locomotion; **F** – a typical trailing pseudopod at the uroidal region, with corkscrew aspect; **G** – an individual with a very high number of filose pseudopods protruded at the anterior region; **H**, **I** – a locomotive individual with a high number of pseudopods at the trailing region; **J**, **K**, **L**, **M** – a sequential documentation of a locomotive amoeba, to demonstrate the aspect of the trailing and anterior regions during active movement. Scale bar for all images: 50 μm.

During locomotion, the anterior region of the amoebae stretches out into a very thin, broad fan-like hyaloplasm (Fig. 3). At the edges, few long and many smaller pseudopodia are present (Fig. 3). The posterior end, as it is pulled forward, detaches its extended pseudopodia from the substrate and then fully reabsorbs the pseudopodia back into the cell or leaves them trailing behind, in a characteristic corkscrew shape (Fig. 3B, F, I). When a pseudopod is retracted, sometimes it takes on an angled, crooked aspect. Pseudopodia at the tail end (Fig. 3B) are longer and more tapered than those in front, which are shorter and have smoother tips (Fig. 3D). In contrast to the relatively slow speed of pseudopodial protrusion and general locomotion, the endoplasm always maintains very rapid cytoplasmic flow in multiple directions, sometimes going towards the edges of the cell, sometimes in the reverse direction. Multiple flows can occur simultaneously, with certain areas flowing inwards while adjacent areas flow outward. In all cases, the flow speed, observable by movement of granules and other inclusions, is very dynamic. We also saw pseudopodia on the dorsal side of the amoeba (Fig. 2H) at times when the amoeba was active, such as during cell fusion. These pseudopodia generally hover and drift above as dark extensions when observed under phase contrast.

Occasionally, amoebae are found to be stationary and in a flattened form that are either branched like a network with many lacunae in the hyalo- and endoplasm (Fig. 2J), or have a continuous hyaline layer without many gaps (Fig. 2G). The isolate has rapid cytoplasmic streaming whether during active locomotion or while relatively stationary, consisting of an endoplasm provided with dark nuclei, contractile vesicles, and granular inclusions. Inside and surrounding the endoplasm are small dark vesicles that extend into the interior edge of the ectoplasm. A posterior tail end is not always distinct.

Amoebae would at times attach themselves to clusters of assorted materials – such as organic fibers and bacterial clumps. Larger individuals were able to engulf and carry these clusters inside the cell before releasing them, though we could not evaluate whether clumps were altered in any way by amoebae. Attachment to these clusters was seen in all different types of the amoeboid form.

Fine structure of the amoeboid forms (Fig. 4) reveals a nucleus with undulating margin (similar to the variations in the perimeter of nuclei observed by light microscopy) and contain an irregularly shaped, somewhat denser nucleolus (Fig. 4A). Mitochondria (Fig. 4B) are tubulocristate and contain occasional dense granules, either solitary or clumped. The occasional dense mitochondrial granules are also observed in other Amoebozoa, including many slime mold members (e.g. Anderson 1992, Suzuki *et al.* 1980). Slender, filose, subpseudopodia (Fig. 4B) extend from the uncoated plasma membrane, and become more enlarged and numerous in invaginated regions of the cell where apparently food particles are ingested (Fig. 4C). Food vacuoles within the cytoplasm contained predominantly bacteria. "Trumpet-shaped" tapered bundles of fine filaments (Fig. 4D) contain a relatively dense broadened attachment plate (Fig. 4E), c. 0.4 μm in width. A thin, somewhat more electron dense layer lines the periphery of the bundles; and also is apparent in cross-sections, that are oval or circular in shape. Because the bundles are tapered at the distal end opposite the attachment plate, it is not possible to determine the entire length in ultrathin sections. However, the observed lengths vary between 1 to 2 μm. The "trumpet-shaped" bodies are scattered at varying angles to one another throughout the cytoplasm, but in this preparation occur most prominently near the periphery of the amoeba. They were observed only occasionally in ultrathin sections. Their function is unknown.

Floating form

Telaepolella tubasferens is regularly seen as a floating form (Fig. 5). We observed that medium-sized amoebae entered into floating form more commonly after 2–3 days in a newly established culture. In preparation for floating form, the trophic amoeba gradually produces vacuoles in the endoplasmic region, while at the same time reducing cytoplasmic flow (Fig. 5A). These vacuoles have a distinct refractive appearance compared to ordinary vacuoles (Fig. 5A). For about twenty minutes, the amoeba remains floating, unattached from the substrate and filled with vacuoles. Initially the form is roughly spherical (Fig. 5B) while after about 20 min. hemispherical bulges (Fig. 5C) and longer protrusions (Fig. 5D) erupt. It is interesting to point out that most large amoebozoans have similarly bulbous floating forms (Page 1987). Total size varies from 25 × 22.5–65 × 40 μm, with median of 35 × 30 μm (n = 13). The amoeba settles on a surface by setting down small conical protrusions and progressively attaches to the substrate completely. As the amoeba lands, the distinctive floating vacuoles are still visible in the cytoplasm; but within five minutes, they shrink

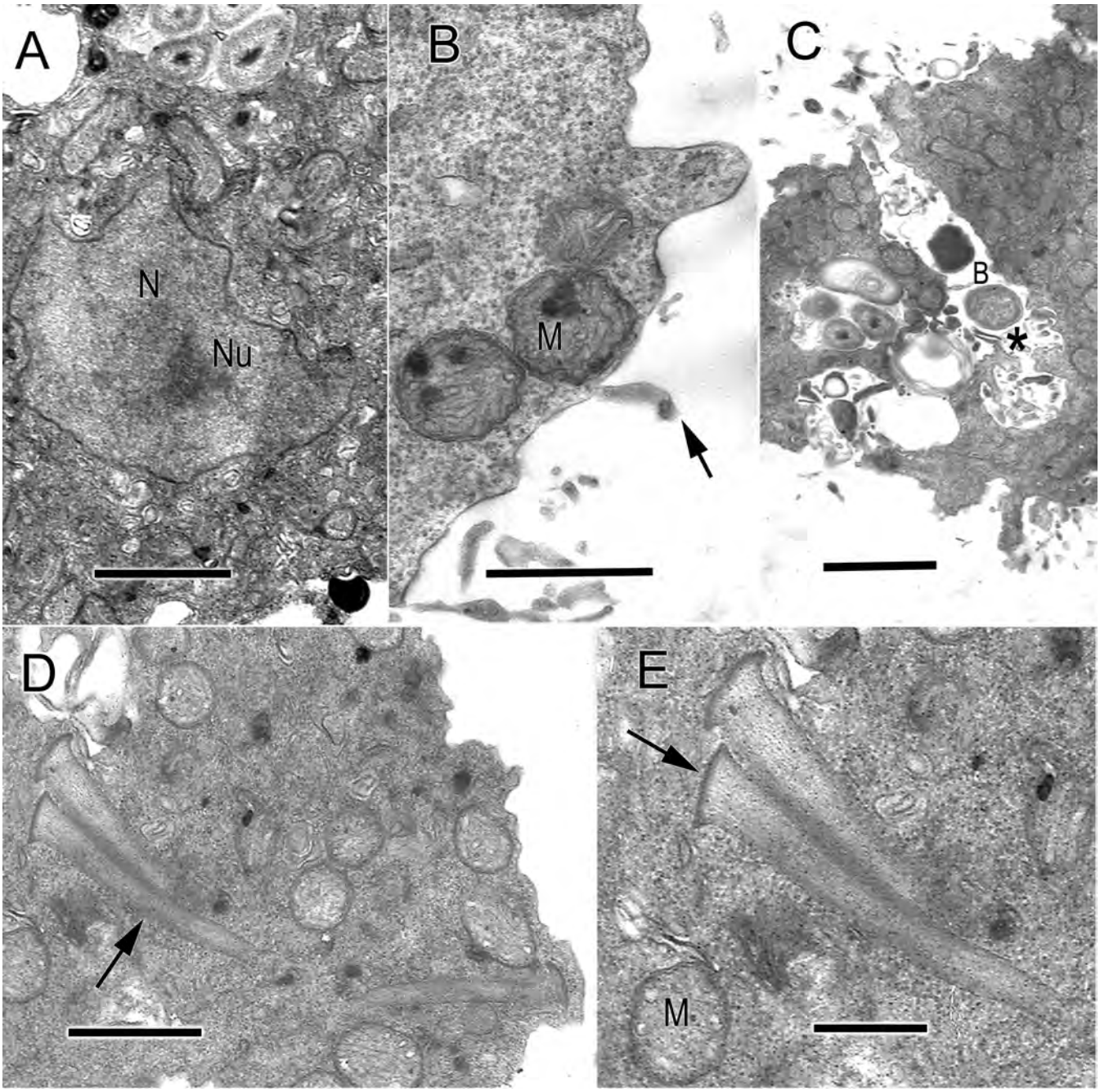

Fig. 4. Fine structure of amoeboid forms. **A** – a nucleus (N) with undulating margin and somewhat irregularly shaped nucleolus (Nu); **B** – mitochondria (M) with tubular cristae, and the uncoated peripheral plasma membrane with villous-like protrusions (arrow); **C** – an apparent feeding invagination of the cell contains bacteria (B) and an enlarged arrangement of plasma membrane protrusions (asterisk); **D** – "trumpet-shaped" tapered assembly of microtubules (arrow) distributed in the cytoplasm; **E** – an enlarged view of the microtubular assembly showing arrangement of the electron dense broadened attachment plates (arrow) where the microtubules terminate. Scale bar in A: 2 μm, B: 0.5 μm, C: 1 μm and D, E: 0.3 μm.

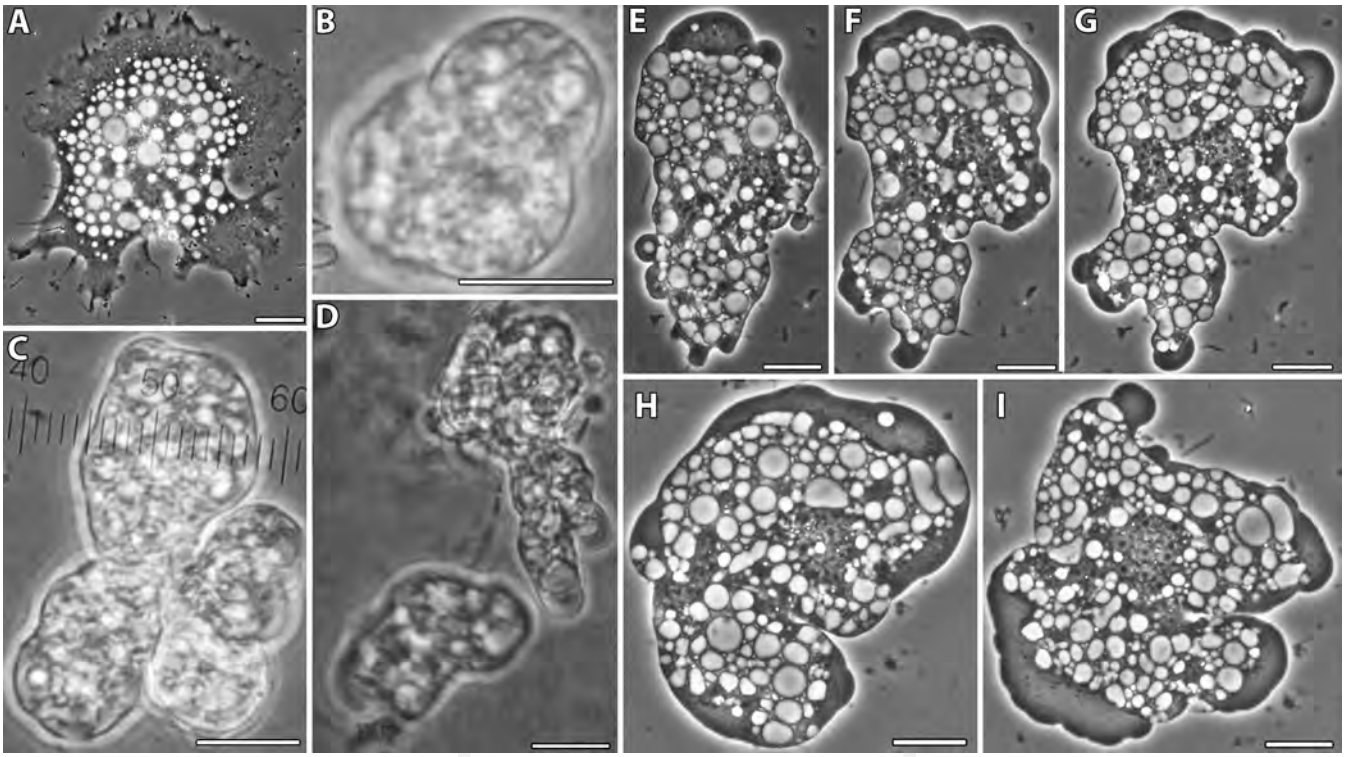

Fig. 5. Phase contrast micrographs of floating form of *T. tubasferens*. **A** – immediately before floating; **B**, **C**, **D** – actively floating. In C, there are three separate floaters, which united in the water column, apparently the cytoplasm has not joined. In D there are two separate floating forms. **E–I** – phase contrast micrographs of a floating *T. tubasferens* purposely flattened for observation. These are sequential images of the initial moments after forceful flattening. The amoeba clearly shows lobose pseudopods (all images) and the nuclei gathered at the center, especially visible in I. Scale bar for all images: 20 μm.

and disappear. After landing, the amoeba generally resumes active locomotion.

We identified larger floating stages, with as many as two or three clearly separate floating entities (Fig. 5C). At times, these appeared to be fusing or splitting. When one floating form made contact with the substrate and transformed into amoeboid form, the other one or two forms would shortly settle nearby and transform as well. In the two instances that we observed this phenomenon, plasmogamy (i.e. cytoplasmic fusion) of the two or more amoebae occurred within an hour of settling. We have not stained these preparations, and hence cannot affirm whether karyogamy occurs.

We were able to capture and prepare an individual in floating form before it actually transformed into the locomotive form (Fig. 5E–I). This perspective reveals nuclei gathered at the center (Fig. 5E–G). Many of the vacuoles are not perfectly spherical, appearing ovoid or reniform (Fig. 5H, I). The most interesting aspect of this particular isolate/culture is that cytoplasmic protrusions are lobose and rounded, more amoebozoan-like than in the locomotive form.

Cysts

We observed both spherical and irregularly-shaped cysts. Under phase contrast cysts appear yellow with a brownish core (Fig. 6A–G). The most common cysts were spherical (Fig. 6A), but irregularly shaped cysts were always seen in cultures as well (Fig. 6B, D). Irregular cysts were larger and darker than the spherical cysts, and they appeared to have thinner and/or fewer layers of cyst walls. Cysts often appeared together in the same mucoid envelope, though cysts within the same mucoid envelope did not necessarily excyst simultaneously. One hypothesis is that amoebae start encystation by releasing a mucoid envelope and then enter plasmotomy (multiple fission) to make multiple daughter cells that immediately encyst. This is consistent with our ob-

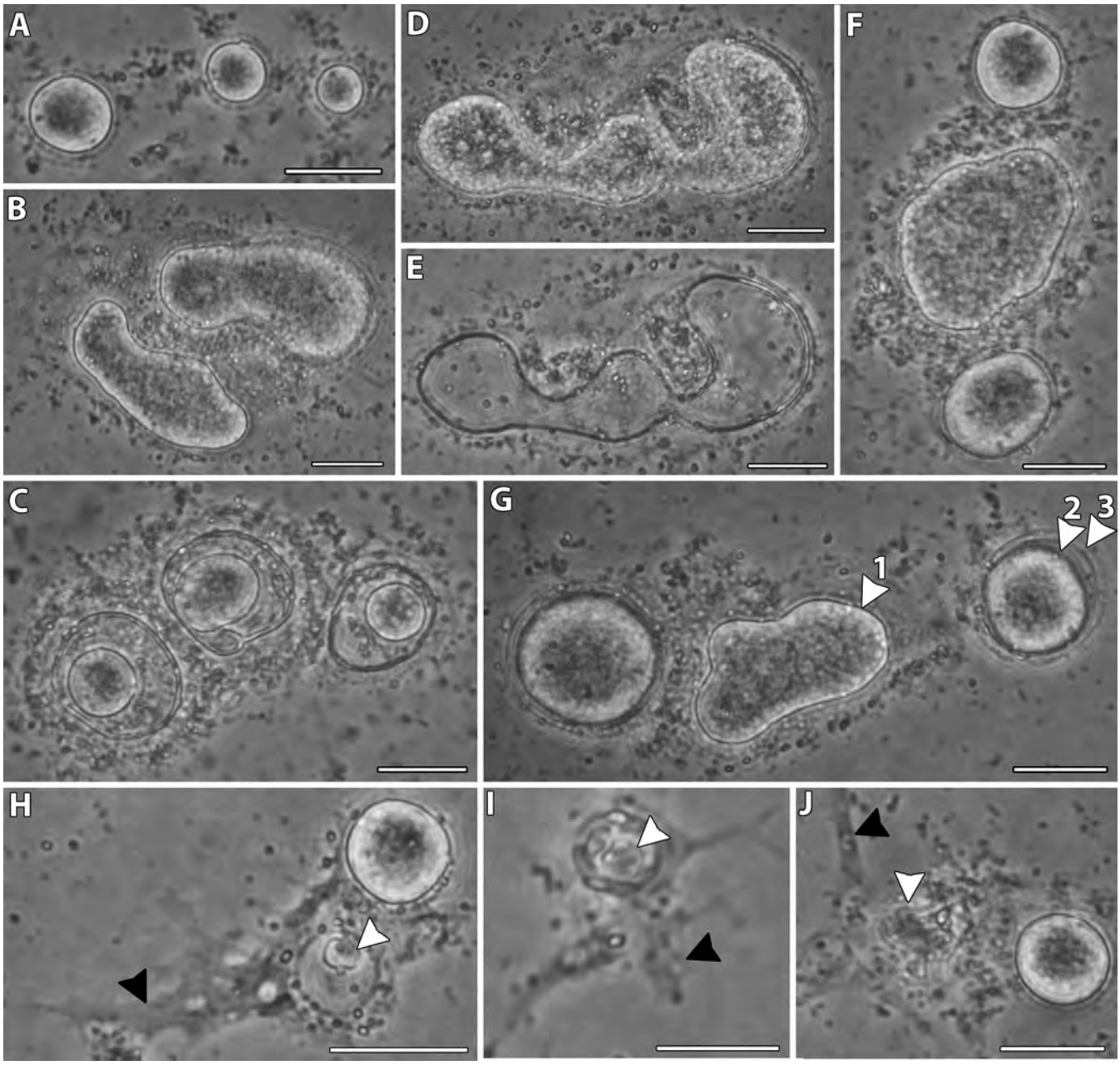

Fig. 6. Phase contrast micrographs of *T. tubasferens* cysts. **A** – typical round, thin-walled cysts; **B** – typical irregular cysts, walls are even thinner than in spherical cysts; **C** – thick-walled, spherical cysts, note that besides the wall being thicker, there is a space between the cell and the cyst wall; **D**, **E** – an irregular shaped cyst from which the amoeba excysted successfully; **F**, **G** – a typical combination of cysts that come from a single amoeba, one irregular cyst surrounded by two thin or thick walled cysts (1 indicates an irregular cyst, 2 indicates the inner part of a thick walled cyst and 3 indicates the wall); **H**, **I** and **J** – amoebae exiting their cysts, cysts are indicated by the white arrow, amoebae are indicated by the black arrow. Scale bar for all images: 20 μm.

servations on a few occasions (Fig. 7). A single mucoid envelope could contain many different combinations of cysts: one or many spherical cysts; or one to two irregular cysts; or a spherical and an irregular cyst; or an irregular cyst between two spherical cysts (Fig. 6F, G). Spherical cysts appear to maintain viability for a longer time, thus we have been able to start cultures from spherical cysts after at least six months. Another mor-

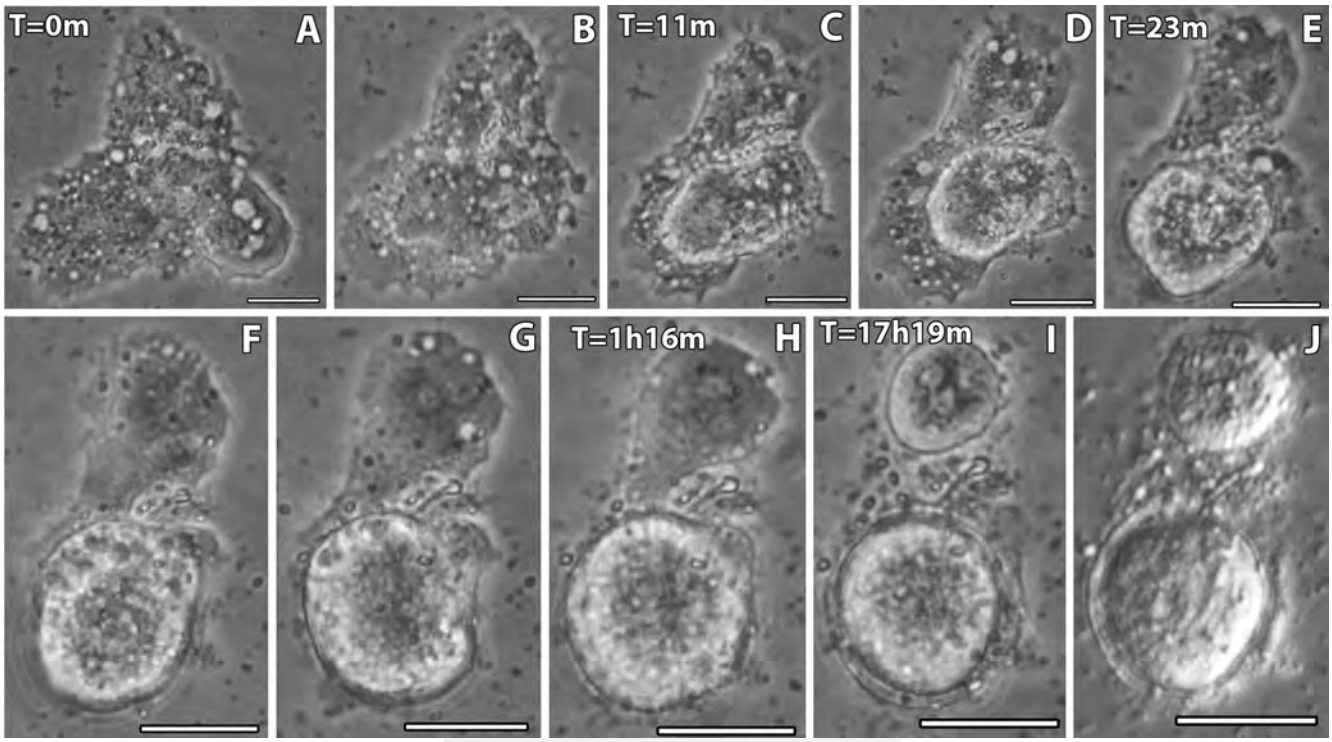

Fig. 7. Sequential phase contrast micrographs of encysting *T. tubasferens*. The individual was observed at irregular intervals. **A–H** – formation of a first, larger cyst; **I–J** – formation of second, smaller cyst; phase contrast in I, Hoffmann modulation contrast in J. Scale bar for all images: 20 µm.

photype of cyst can appear after about a week and a half after subculturing and seemed to be short-lived. This morphotype had a thick-wall, but in comparison with other thick-walled cysts there was more space between the cell and the cyst wall (Fig. 6C). We observed excystment of both thick- and thin-walled, spherical and irregular-shaped cysts. The cyst walls show no obvious excystation pores, so presumably the amoebae are able to exit the cyst by digesting the cyst walls.

Fine structure of an early stage of a spherical cyst (Fig. 8A) shows an electron dense inner layer with a closely apposed, finely fibrous, less-dense outer layer enclosed in a mucoid envelope. The dense inner layers in perceivably mature cysts (Fig. 8B), identified as more mature due to the more electron dense and contracted cytoplasm, were crenulated and the fibrous outer-layer of the wall is more thickened peripherally, with a thin less electron-dense fibrous layer proximal to the crenulated dense inner layer of the cyst wall. The mucoid layer became substantially thicker and contained bacteria and other particles within hyaline regions.

DISCUSSION

The isolate ATCC© 50593™ has been the subject of some controversy in recent literature (Bass *et al.* 2009, Kudryavtsev *et al.* 2009, Pawlowski and Burki 2009, Tekle *et al.* 2007). Originally identified by the depositor as *Arachnula,* a molecular characterization of the isolate placed it as an Amoebozoa (Tekle *et al.* 2007). Further, to exclude the possibility that the Tekle *et al*. (2007) analyses dealt with a contaminating organism, we reordered, subcultured and reanalyzed the SSU rDNA from the ATCC isolate and obtained identical sequences, confirming the taxonomic position of ATCC© 50593™ as a member of the Gracilipodida (Lahr *et al.* 2011a). This was considered a surprising placement at the time, since *Arachnula* is a vampyrellid genus that is believed to group within the Rhizaria. Later on, Bass *et al*. (2009) isolated an organism with morphological characters consistent with the original description of *Arachnula,* and this isolate fell within the Rhizaria.

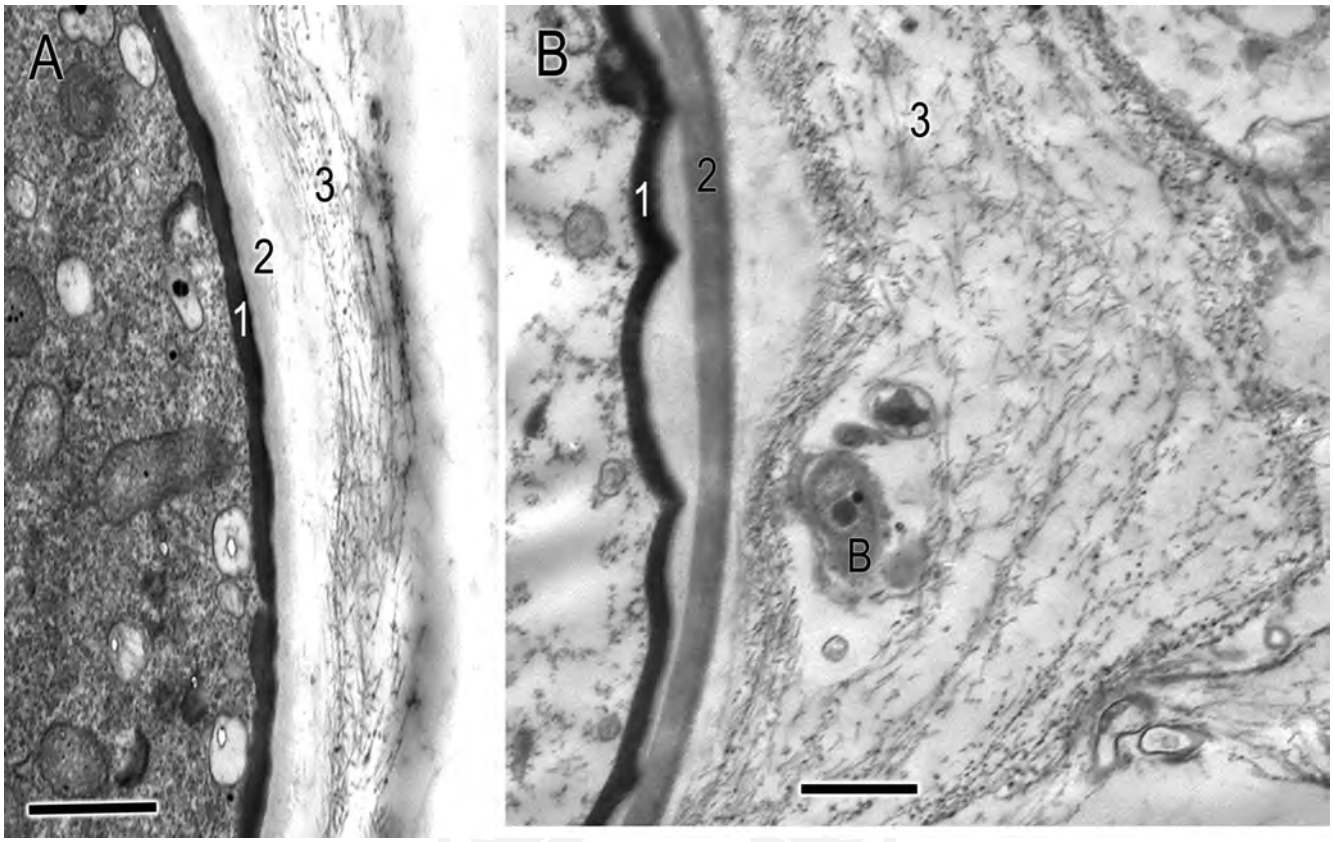

Fig. 8. Fine structure of the cyst wall. **A** – an early stage of cyst wall development shows the electron dense inner layer (1) and closely apposed finely fibrillar outer layer (2) surrounded by a mucoid thicker layer (3); **B** – a more mature cyst wall with undulating electron dense inner layer (1), thickened fibrous outer layer (2) consisting of a proximal finely fibrillar layer and a more distal denser layer, surrounded by a much thickened mucoid layer (3) containing organic debris particles and occasional bacteria (B). Scale bars in A, B: 1 μm.

However, the sequence obtained by Bass *et al.* (2009) is identical to a sequence obtained from an organism characterized as *Theratromyxa weberi* by R. M. Sayre (Sayre 1973) and later deposited at ATCC© by T. K. Sawyer with accession number 50200™ (Parfrey *et al.* 2010). Unfortunately, the organism used in Bass *et al.* (2009) was not maintained in culture, hence we cannot directly compare it morphologically to either ATCC isolate, so the identity of the organism reported in Bass *et al.* (2009) remains elusive. Hence, it is unclear whether the genus *Arachnula* described by Cienkowski (1876) has been examined using molecular tools or modern microscopes.

The isolate ATCC© 50593™, described here as *Telaepolella tubasferens*, is morphologically distinct from *Arachnula* in many aspects. *Arachnula* as described by Cienkowski (1876) and Dobell (1913) is a large, slender amoeboid organism, with fine reticulating pseudopods. The pseudopods of *Arachnula* generally arise from fan-shaped portions of the cell, these are connected to each other by slender tubular projections (see Fig. 18 in Cienkowski 1876). *Telaeopolella* trophic form, when in comparable size to *Arachnula*, is a single fan-shaped amoeboid cell, not with multiple fan-shaped portions aiming different directions. Moreover, the fine pseudopodia that arise from the edge of the hyaloplasm in *Telaepolella* are much shorter relative to body size when compared to those of *Arachnula*: *Telaepolella* pseudopods are about 1/10 to 1/5 of the body length, while *Arachnula*'s are around 1–2 × longer than the fan-shaped portion from which they stem (Cienkowski 1876). Cienkowski (1876) also makes emphatic mention to the dynamic movements performed by *Arachnula*, in page 28 roughly translated to: "In no Rhizopoda

I have seen the pseudopodia as vigorously on all sides bend, merge and emerge again as *Arachnula*." He goes on to mention that pseudopods vigorously and quickly feel the substrate around, making the organism crawl actively. *Telaepolella* on the other hand crawls very slowly, pseudopodial activity being relatively slow and pseudopods apparently have a tactile function rather than locomotive like in *Arachnula*. Rapid granular flow of pseudopods is easily recognizable in both organisms. Perhaps the most striking character distinguishing these genera is that the multiple nuclei of *Telaepolella tubasferens* are large enough to be visible without any staining (e.g. Fig. 2I). The nuclei are varied in shape, including somewhat elongated forms and those with crenulated perimeters as observed by light and electron microscopy (e.g. different forms are visible in the center of the cell in Fig. 5I and in Fig. 4). The original description of the genus *Arachnula* makes specific reference to the difficulty of recognizing nuclei with conventional microscopy techniques as Cienkowski's original description states: "Zellkerne habe ich nicht auffinden können" (p. 29), roughly translated to "I was not able to detect nuclei".

There are additional vampyrellid genera (i.e. related to *Arachnula*) that can be differentiated from *Telaepolella* (Table 1). These all fall into the morphological designation II of Hess *et al.* (2012): the expanded morphotype. Of special mention are *Theratromyxa* sp., and *Leptophrys* sp. *Theratromyxa* is a genus of soil-dwelling amoeboid organism that can feed on nematodes (Zwillenberg 1953). They do not present a pronounced differentiation of endo- and ectoplasma, as can be seen in *Telaepolella* (Sayre 1973). The floating form presents itself as a rounded sphere with fine radiating pseudopodia (Sayre *et al.* 1989), as opposed to *Telaepolella* which creates a lobose floating form without radiating pseudopods. The cell is also able to expand itself and form fan-shaped regions from which pseudopodia protrude (Fig. 10 in Sayre *et al.* 1989). Nuclei are similarly inconspicuous as reported for *Arachnula*, not easily visible without staining and high-magnification (see stained Fig. 4 in Sayre 1973). *Theratromyxa* appears to be very similar in several aspects (feeding, general morphology, size) to *Arachnula*, and there is a great deal of uncertainty whether this organism is related to other vampyrellids (Hess *et al.* 2012). *Leptophrys* may present itself in similar fan-shape to *Telaepolella*, but again here, the fine pseudopodia stemming from the hyaloplasm in *Leptophrys* are relatively larger than those in *Telaepolella* (Hertwig *et al.* 1874, Zopf 1885). *Leptophrys* is also capable of separating portions of its cell body to move in different directions while remaining connected by a thin cytoplasmic strand (Fig. 4F in Hess *et al.* 2012), which does not happen in *Telaepolella*. Similarly to *Arachnula* and *Theratromyxa*, *Leptophrys* has very small and inconspicuous nuclei which are difficult to see in low magnification (Hess *et al.* 2012).

The isolate ATCC© 50593™ is more similar to genera within Gracilipodida: *Flammella* and *Filamoeba* than to vampyrellids or other Rhizaria. Multiple nuclei are also clearly visible in *Flamella*, which usually exists as a crawling form that presents thin pseudopods

Table 1. Comparative morphology of the amoebozoan *Telaepolella* and similar rhizarian genera in locomotive form.

	Telaepolella	*Arachnula*	*Theratromyxa*	*Leptophrys*
General morphology	large, fan-shaped	spreading, with two or more fan-shaped regions (Hess morphotype II)	spreading, does not make fan-shaped regions often (Hess morphotype II)	spreading, sometimes with two or more fan-shaped regions (Hess morphotype II), but more often just fan-shaped
Cytoplasm	clear separation in ecto- and endoplasm	no pronounced separation between ecto- and endoplasm	no pronounced separation between ecto- and endoplasm	clear separation in ecto- and endoplasm
Pseudopods	filose, arising from hyaline region, relatively small (1/10 to 1/5 of body length)	filose, arising from fan-shaped portions of cell, large (up to 2 × size of cell)	filose, thicker than in *Arachnula*	filose, arising from hyaline portion, can be as long as 1 × length of body, can get longer
Dynamics of pseudopodial movement	slow, tactile, rarely used for locomotion	quick movements, several fusions and separations, used for locomotion	quick movements, locomotive	quick movements, locomotive
Nuclei	multiple, readily visible in low magnification	multiple, only visible in high-magnification or with staining	multiple, only visible in high-magnification or with staining	multiple, visible in high-magnification of compressed dying cells, or with staining

extending from a fan shaped region, with trailing adhesive filaments (Kudryavtsev *et al.* 2009). Moreover, the medium sized cells of *T. tubasferens* (Fig. 3F) are morphologically akin to *Flamella lacustris* (Michel and Smirnov 1999). However, one noteworthy difference is that *T. tubasferens* takes up a very vacuolized appearance, and a flattened form with no definite direction. *Flamella lacustris,* in contrast, always maintains an anterior hyaline region and a pronounced adhesive uroid (Michel and Smirnov 1999). Additionally, *Flamella* species tend to be bi- or tetranucleated, whereas *Telaepolella* tends to produce more nuclei (although bi- and tetranucleated forms are common). Amoebae of the gracilipodid genus *Filamoeba* present a more disparate morphology compared to *Telaepolella* than *Flamella*. Despite both *Telaepolella* and *Filamoeba* being capable of producing thin, filose pseudopods, in *Filamoeba* these are produced directly from the cell body, without the characteristic hyaline regions from where the pseudopods emerge in *T. tubasferens* (Dykova *et al.* 2005). Additionally, *Filamoeba* usually has a single, difficult to see nucleus (Dykova *et al.* 2005), while *T. tubasferens* is provided with multiple, clearly visible nuclei. Further characteristics that separate *T. tubasferens* from these closely related taxa are the multiple types of cysts and the unique trumpet-shaped internal cytoplasmic structures, occasionally observed in ultrathin sections. The presence of elongated, filamentous or trichocyst-like bodies has also been reported in other Amoebozoa, e.g. *Vexillifera armata* (including a filamentous core of longitudinally oriented filaments in long pseudopodia) (Page 1979), but their cytoplasmic origin and function also are unknown.

Telaepolella tubasferens represents an additional member of the Gracilipodida, a lineage that produces filose pseudopods among the generally lobose Amoebozoa. Additional amoebozoans which produce fine, anastomosing pseudopodia are plasmodial stages in schizoplasmodiids (Lindley *et al.* 2006, Spiegel 1990), solitary amoebae and plasmodia in the cavosteliids (Spiegel 1990), as well as the thin ectoplasmic aphanoplasmodium in the Stemonitales (Pope *et al.* 2006). These observations undermine the idea that Amoebozoa and Rhizaria can be characterized by a general morphology of the pseudopod. Frederick C. Page (1967) already hinted at this in his extensive experience, mentioning while characterizing *Filamoeba* as a member of the Amoebida: "Today it should be obvious that the distinction between lobose and filose may often be a microscopical rather than a biological distinction, since 'pointed' tips would probably always be found to be rounded with adequate magnification."

Molecular genetic techniques often group together organisms that seem morphologically disparate, as the inclusion of filopodia forming organisms within the Amoebozoa. However, detailed observation and comparison of morphological characters, coupled with diverse taxonomic sampling, may support relationships at less inclusive levels, as shown here for the Gracilipodida.

Taxonomic Appendix

Amoebozoa Luhe 1903
Gracilipodida Lahr and Katz 2011

Telaepolella n. g.

Diagnosis: Multi-nucleated freshwater naked amoeba with fine, sometimes branching pseudopodia and rapid cytoplasmic movement. Fan-shaped hyaloplasm with shorter pseudopodia and thinner, longer trailing pseudopodia during locomotion. Trumpet-shaped microtubular structures of unknown function distributed at varying angles throughout the cytoplasm, and particularly near the periphery. Produces hyaline cysts.

Etymology: *Telaepolella* in reference to the dynamically moving cytoplasm, which resembles the busy network of roads in large cities Gr. *Telae* – net, *polis* – city, plus the diminutive feminine suffix *ella*.

Type-species: *Telaepolella tubasferens*.

Telaepolella tubasferens n. sp.

Diagnosis: With characteristics of the genus. Locomotive form usually 50–300 μm at widest breadth. Capable of producing a large multinucleated form up to 500 μm with hundreds of nuclei. Stationary form is flat with thin hyaline sheet that may be broad and wide or slender with many lacunae. Presence of floating form. Produces at least two types of cysts: round with regular outline and non-round with irregular shape. Round cysts may have thin or thick walls. Round, thin-walled cysts remain viable at least for six months.

Etymology: *tubasferens*. Gr. *tuba* – trumpet, horn; *ferens* – bearer, in reference to the trumpet-shaped, microtubular tapered structures present in the cytoplasm.

Type material: The mono-eukaryotic culture deposited at ATCC©, number 50593™.

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