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Correct identification of species makes the amoebozoan rRNA tree congruent with morphology for the order Leptomyxida Page 1987; with description of *Acramoeba dendroida* n. g., n. sp., originally misidentified as '*Gephyramoeba* sp.'

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Abstract

Morphological identification of protists remains an expert task, especially for little known and poorly described species. Culture collections normally accept organisms under the name provided by depositors and are not responsible for identification. Uncritical acceptance of these names by molecular phylogeneticists may result in serious errors of interpretation of phylogenetic trees based on DNA sequences, making them appear more incongruent with morphology than they really are. Several cases of misidentification in a major culture collection have recently been reported. Here we provide evidence for misidentifications of two more gymnamoebae. The first concerns "Gephyramoeba sp." ATCC 50654; it is not *Gephyramoeba*, a leptomyxid with lobose pseudopods, but a hitherto undescribed branching amoeba with fine, filamentous subpseudopods named here *Acramoeba dendroida* gen. et sp. nov. We also sequenced 18S rRNA of Page's strain of *Rhizamoeba saxonica* (CCAP 1570/2) and show that it is the most deeply branching leptomyxid and is not phylogenetically close to 'Rhizamoeba saxonica' ATCC 50742, which was misidentified. Correcting these misidentifications improves the congruence between morphological diversity of Amoebozoa and their rRNA-based phylogenies, both for Leptomyxida and for the *Acramoeba* part of the tree. On morphological grounds we transfer Gephyramoebidae from Varipodida back to Leptomyxida and remove *Flamella* from Leptomyxida; sequences are needed to confirm these two revisions.

Keywords: *Rhizamoeba*; *Gephyramoeba*; *Acramoeba*; Amoebozoan phylogeny; Systematics; Identification

Introduction

Morphological identification of amoeboid protists is a difficult task, requiring expert skills and knowledge. Fewer than half of the approximately 600 species of naked amoebae named in the literature were properly

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described; no type material was preserved for most. Re-isolation of such species requires careful analysis of all available data; otherwise application of a name may be arbitrary or wrong. If an ancient name is applied, an adequate description should be provided, and new type material (neotype) should be established (e.g. Baldock and Baker 1980; Kudryavtsev 2000, 2004; Page and Baldock 1980; Smirnov 2002; Smirnov and Goodkov 1998).

The American Type Culture Collection (ATCC) held two strains of amoeboid protists identified as "Gephyramoeba sp.". This genus was established for the only species – *Gephyramoeba delicatula* by Goodey (1914); no culture was kept since then. Pussard and Pons (1976) probably reisolated this species, but their strain also was lost. By its morphological characters, *Gephyramoeba* belongs to the lobosean order Leptomyxida (Page 1987), but when "Gephyramoeba sp." strain ATCC 50654 was sequenced, it appeared very distant from all other leptomyxids in the molecular tree (Amaral-Zettler et al. 2000). No morphological data on that strain were presented to support its correct identification. The strange position of that "Gephyramoeba" sequence in the phylogenetic tree long attracted our attention. Being unable to find any morphological support for such a position (Bolivar et al. 2001; Fahrni et al. 2003; Smirnov et al. 2005), we investigated strain ATCC 50654 and found that it was clearly misidentified, having completely different pseudopodial morphology from that originally described for *Gephyramoeba*. Therefore we describe it here as a new genus and species, *Acramoeba dendroida*.

The strain sequenced by Amaral-Zettler et al. (2000) as *Rhizamoeba* sp. ATCC 50742, and currently held by ATCC as *R. saxonica* oddly groups within *Paraflabellula* (Cavalier-Smith et al. 2004). We sequenced 18S rRNA from the original *R. saxonica* strain CCAP 1570/2 and find that it differs in sequence from, and does not group with, the ATCC strain, which must be described in the future as a new species following proper morphological study. We show that correcting these misidentifications makes amoebozoan evolution more comprehensible.

Material and methods

The ATCC strain 50654 isolated by T. Sawyer in 1991 from a pond near Grand River, Grand Haven, MI, USA was obtained under the name "Gephyramoeba sp." as a cryopreserved cyst, recovered and maintained according to ATCC instructions in Sonneborn's Paramecium medium. CCAP strain 1570/2 *Rhizamoeba saxonica*, isolated by F. Page in 1973 from an estuary mouth, West Mersea, Essex, England was obtained and maintained on MY75S agar (Page 1983) under room conditions.

Extraction of DNA using guanidine thiocyanate was followed by isopropyl alcohol precipitation (Maniatis et al. 1982). To collect cells, amoebae and food bacteria were gently scraped from the agar surface with a disposable plastic scraper to form an aggregate, to the top of which ~100 µl of guanidine thiocyanate was added, mixed briefly, and immediately transferred by Pasteur pipette to an Eppendorf tube. SSU genes were amplified using universal eukaryotic primers RibA (direct; 5′> acc tgg ttg atc ctg cca gt <3′) and F (reversed; 5′> ga tcc atc tgc agg ttc acc tac <3′) (Cavalier-Smith et al. 1995; Pawlowski 2000). Positive amplification products were purified using a GFX PCR Purification Kit (Amersham Biosciences) and sequenced using an ABI-PRISM Big Dye Terminator Cycle Sequencing Kit. Sequences were aligned manually to our database of over 350 amoebozoan 18S rRNAs; 1555 reliably aligned nucleotide positions and 95 broadly representative amoebozoan sequences were selected for initial phylogenetic analysis using 25 opisthokont and rhizarian sequences as outgroups. Analyses were carried out both for this relatively comprehensive large data set of 120 sequences and for a somewhat pruned but Amoebozoa-wide smaller data set of 96 sequences and 1574 positions to test the position of *Acramoeba* more thoroughly, as well as for a set of 28 sequences and 1578 positions for Lobosea alone, rooted as in the larger tree, to establish the position of *Rhizamoeba saxonica*.

Phylogenetic trees were constructed by distance and maximum likelihood methods using BioNJ as implemented in PAUP (Swofford 2000) and TREEFINDER (Jobb 2006), applying the GTR substitution model with gamma corrections for intersite variation and corrections for invariant sites (parameter values were chosen for BioNJ by Modeltest and for ML by the Treefinder algorithm using four gamma rate categories plus invariant sites and the GTR substitution model). Because maximum likelihood methods do not search tree space efficiently and tend to get stuck in local minima (definitely the case for our larger data sets), we used about 15 different starting trees in addition to the initial default tree. New starting trees were made first by deliberately changing the positions of key taxa on the initial ML tree using Treeview PPC (Page 1996) and then by generating 20 randomly modified trees using Treefinder from the highest likelihood ML tree that was thus produced. For the larger data sets the resulting trees differed in some aspects of topology and in likelihood. That with the highest likelihood was chosen as the best. For the ML trees approximate support values for clades were given by the RELL method as implemented in Treefinder with 100 replicates. For BioNJ bootstrap analysis used 1000 resamplings. Parsimony methods were not used as they would be too unreliable given the very marked differences in evolutionary rates among amoebozoan rRNA genes and

their inability to correct for intersite variation, which is essential for yielding reliable trees from highly divergent rRNA sequences.

Results and discussion

'Gephyramoeba sp.' ATCC 50654 is neither a *Gephyramoeba* nor a lobose amoeba

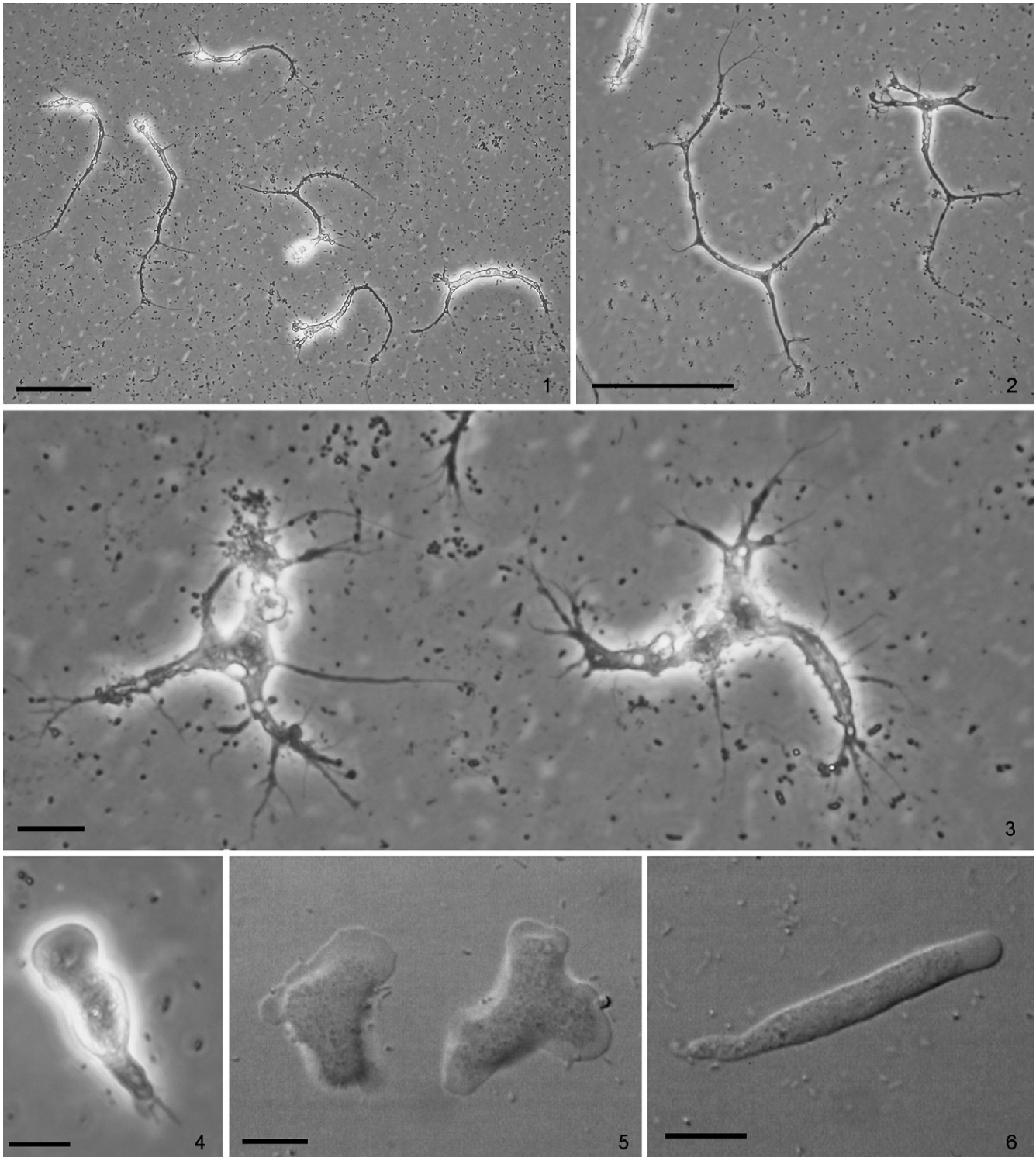
After defreezing, the amoebae started to excyst in 2 days, and by the 14th day the entire bottom of the Petri dish was colonized by trophozoites. They were of irregular shape, branched, with long arm-like pseudopodia (Figs 1, 2). These pseudopodia branched, forming a tree-like pattern and subsequently decreased in width, ending with fine, hair-like hyaline subpseudopodia. Sometimes small islands of hyaloplasm were seen at the sites of branching (Fig. 3). Separate subpseudopodia could be formed from any part of the cell as well. Trophozoites had very low mobility; virtually no visible movement of the main cell body was detectable; cytoplasmic flows in pseudopodia were hardly seen and only subpseudopodial activity was visible. These subpseudopodia very rarely fused, and in shape and structure resembled the pseudopodia of certain cercomonads, e.g. *Cercomonas cometa*, (see Hollande 1942; Myl'nikov 1986, 2000; Myl'nikov and Karpov 2004). Some of them contained large refractive inclusions moving inside the cytoplasm, but the general pattern was very different from the typical granuloreticulopodial network of foraminifera and the movement of these inclusions was much slower. The size of the cell (across the longest dimension) varied from 50 to 350 μm, depending on its shape. The organism was uninucleate, with a single-vesicular nucleus 5–6 μm in diameter. Cysts were rounded, with a single, smooth wall, 25–35 μm in diameter. During 6 months of observation we saw only trophozoites and cysts in culture and nothing resembling plasmodia or flagella.

The 18S rRNA gene sequence of the present isolate was virtually identical to that deposited by Amaral-Zettler et al. (2000) under GenBank number AF293897, which proves identity of the strains. Hence we conclude that we have re-investigated the very same strain sequenced by Amaral-Zettler et al. (2000) under the incorrect name "Gephyramoeba sp." ATCC 50654, and not a contaminant of the culture.

The only known species of the genus *Gephyramoeba* – *G. delicatula* – was described by Goodey (1914) and documented with line drawings and photographs of the stained preparations. Pussard and Pons (1976) investigated a strain that fitted Goodey's description and provided LM photographs, description of mitosis and other details of its morphology. Both these descriptions showed that *G. delicatula* is a flattened, branched amoeboid organism "producing long narrow arms 3 or 4 μm across" (Goodey 1914, p. 95). Its cytoplasm is "stretching out in a thin sheet at the end of some of the pseudopodial arms" (Goodey 1914, p. 92), which is well exemplified in the line drawings (Goodey 1914, Figs 14, 15) and in his photographs of stained preparations (Goodey 1914, Figs 20, 21). The same characters were documented by Pussard and Pons (1976, p. 353) and the presence of thin rounded sheets of cytoplasm at the tip of these arms is well visible in their photographs. No subpseudopodia or projections of any kind at the end of these "arms" are drawn or photographed or noted in the text of either paper (op. cit.); the note that "the character of its pseudopodia… give it affinities with the members of the Reticulosa" (Goodey 1914, p. 95) should not mislead, because he classified there all genera of leptomyxids he described; further comparison of his species with *Arachnula*, *Biomyxa*, *Gymnophrys* (now known to be Cercozoa) and other similar organisms (op. cit. pp. 96–99) makes it clear that Goodey considered the reticulate or branched body shape of his organisms as a primary criterion, without distinction of the pseudopodial form. To clarify this, Page (1987) used the term "loboreticulopodia" to recognize the specific, anastomosing and fusing lobose pseudopodia formed at the frontal area of a moving *Leptomyxa reticulata*.

All these characters substantially differ from the ATCC 50654 strain. The only obvious feature shared by ATCC 50654 and Goodey's *Gephyramoeba* is a branched body form, but that character is not even phylum specific, being found in both Amoebozoa and Cercozoa. Hence, we have to conclude that ATCC 50654 was misidentified, and that no species of *Gephyramoeba* has been sequenced yet.

The present strain cannot be clearly assigned to any named species, genus or family. It is evidently different from naked foraminifera, like *Reticulomyxa filosa*, because it has no fine granules in its pseudopodia and a much less developed pseudopodial network, which is far less mobile than in *Reticulomyxa* (Nauss 1949) and has a very different appearance. It cannot be reliably associated with any known naked amoeba species of unclear systematic position that possess reticulopodia-like pseudopodia (Rogerson and Patterson 2002) and is clearly distinct both morphologically and in sequence from biomyxid Cercozoa (Cavalier-Smith and Chao 2003). We cannot be certain that its entire life cycle has been traced, because an organism may show only part of a cycle in culture. However, so far we must accept that this organism has neither flagellate nor plasmodial stages. We describe it here as a new species, *Acramoeba dendroida* and designate ATCC strain 50654 as type culture, which may encourage further study of this interesting protist.



Figs 1–6. LM images of *Acramoeba dendroida* ATCC 50654 (1–3) and *Rhizamoeba saxonica* CCAP 1570/2 (4–6). Amoebae were photographed in liquid medium; each culture was incubated in liquid medium not less than 7 days prior to photographing. Scale bar is 100 μm in 1–2 and 10 μm in 3–6.

The phylogenetic position of *Acramoeba* is unclear. It robustly groups inside Amoebozoa, and never shows any tendency to group with Cercozoa (Fig. 7) despite some similarity in morphology to certain cercozoans; however it does not closely resemble any neighboring organisms on the tree in morphology and detailed pseudopodial pattern. In our best maximum likelihood trees it is sister to *Filamoeba* plus an array of quite

divergent environmental DNA sequences (Fig. 7), although on some preliminary ML and on BioNJ trees, all of lower likelihood, it was sister to *Multicilia* instead (with very low bootstrap support of 35% using BioNJ). Its exact position within the variosean region of phylogenetic trees is thus unstable, depending on the algorithm used, as it always groups in a generally poorly resolved part of the tree (Cavalier-Smith et al. 2004; Fahrni et al. 2003; Nikolaev et al. 2006; Smirnov et al. 2005). If our ML tree in Fig. 7 is correct, then the order Varipodida (*Filamoeba* plus environmental relatives and *Acramoeba*) is holophyletic. Our trees have a better representation of dictyostelids than previous Amoebozoa-wide trees, and include a protostelid for the first time as well as three key environmental sequences related to *Filamoeba*. This better taxon sampling may be why Mycetozoa and Varipodida both appear monophyletic in Fig. 7 in contrast to previous trees where they are typically intermingled (Cavalier-Smith et al. 2004; Nikolaev et al. 2006). On the distance tree *Planoprotostelium* was sister to dictyostelids (50% support) but Myxogastria grouped not with them but with other long branches; this apparent polyphyly of Mycetozoa may be a long-branch artifact to which BioNJ seems somewhat more prone than does maximum likelihood.

None of the listed organisms seems to be a close relative of *Acramoeba*, because of substantial differences in cell organization. The pointed projections of *Filamoeba* and dictyostelids are actually subpseudopodia arising from a proximal lamellipodium, (those of *Dictyostelium* are often incorrectly called pseudopods). They do not closely resemble the pseudopodial pattern of *Acramoeba*. Despite these significant differences Varipodida now appear more uniform in morphology than originally thought (Cavalier-Smith et al. 2004). The pseudopodial patterns among Varipodida are clearly very different from the lobose pseudopodia of all leptomyxids (including *Gephyramoeba*) and other Lobosea. *Acramoeba*'s morphology and pseudopodial pattern is very similar to the poorly known organism called "big freshwater plasmodium" (see Canter-Lund and Lund 1995, p. 273), but in contrast with that organism, it never shows plasmodial organization of the body. Morphologically and phylogenetically closer relatives therefore remain to be discovered and sequenced.

By its body shape and pseudopodial morphology *Acramoeba* clearly does not fit any existing amoebozoan family; so we establish the new family Acramoebidae for it, placed within the order Varipodida sensu Cavalier-Smith et al. (2004). Given that Arcellinida are probably 760 My old (Cavalier-Smith 2006) the deep divergence of *Acramoeba* and Filamoebidae on our trees must represent several hundred million years of independent evolution. Because of misidentification of strain ATCC 50654, the family Gephyramoebidae Pussard et Pons, 1976 was grouped with Filamoebidae as the order Varipodida (Cavalier-Smith et al. 2004). In the light of our present results it must be removed from Varipodida. The genus *Gephyramoeba* was left incertae sedis by Smirnov et al. (2005), but it is now reasonable to return it to Leptomyxida, according to the system of Page (1987), which for this order is not yet disproved by any molecular trees.

Diagnoses

Acramoebidae fam. n. Highly branched, flattened naked amoeboid organisms, with very slender, pointed, sometimes branched hyaline subpseudopodia never forming a network, lacking granules; lacking cilia or flagella. Type genus *Acramoeba* gen. n.

***Acramoeba* gen. n**. With diagnosis of the family. Type species: *Acramoeba dendroida*.

Acramoeba dendroida sp. n. Flattened, expanded and branched organism, up to 300 μm across; minimally mobile. Pseudopodia long, arm like; fine, ending up with fine, hyaline, non-granulated subpseudopodia. Uninucleate, vesicular nucleus 5–6 μm in diameter. Cysts rounded, single-walled, 25–35 μm in diameter. Simple life cycle consists of cyst and trophozoite stages.

Etymology: Acr- Gk pointed, because of the pointed pseudopods; + amoeba; dendroida from English dendroid, meaning tree-like because of branched body form (Gk dendros tree).

Type strain: ATCC 50654. Type location: pond near Grand River, Grand Haven, MI. Freshwater. Type sequence AF293897.

"Rhizamoeba saxonica" ATCC 50742 is a new species of Leptomyxida

The CCAP 1570/2 strain of *Rhizamoeba saxonica* is not formally designated as a type strain, but it is the same strain as that isolated by Fred Page in 1973 and described in 1974 (Page 1974); stained preparation No 1973:4:1:13 held in the Natural History Museum (London, UK) done from this very strain was designated as the type slide for *R. saxonica*. Hence, in the opinion of Page who named the species, the CCAP strain 1570/2 was the original *Rhizamoeba saxonica*; provided that this culture has been maintained since then without contamination, mislabeling or significant evolutionary change, it must be accepted as more accurately representing the species than any other. There are no reasons to doubt this, as this strain shows all characteristic features of *R. saxonica* noted by Page (1974, 1983), including the presence of adhesive uroidal filaments, occasional eruptions of the frontal hyaloplasm and alterations of the locomotive form from flattened, flabellate to limax, worm-shaped (Figs 4–6).

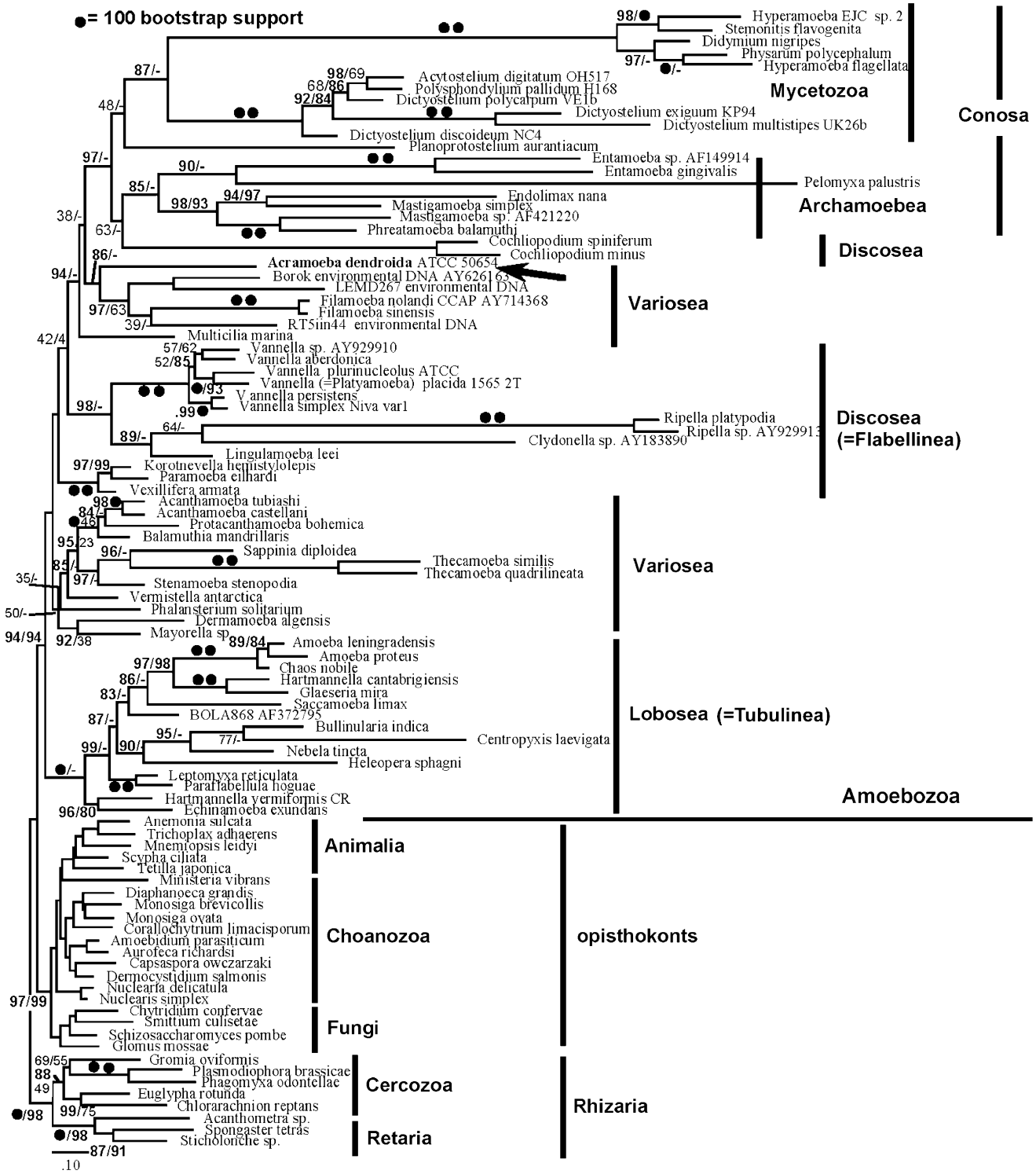


Fig. 7. The best maximum likelihood tree found for 71 amoebozoan sequences using 1574 nucleotide positions of the 18S rRNA gene, with 25 opisthokonts and Rhizaria as outgroup (log likelihood: −59928.71). The arrow indicates *Acramoeba*. The tree is rooted between the bikont Rhizaria and opisthokonts plus Amoebozoa (collectively unikonts) in accordance with the evidence that the eukaryote root lies between unikonts and bikonts (Richards and Cavalier-Smith, 2005; Stechmann and Cavalier-Smith, 2003). The figures and blobs at the nodes are RELL approximate bootstrap percentages for this ML tree (left or upper) and bootstrap percentages for the corresponding BioNJ tree for this data set (right or lower). Gamma intersite variation (4 rates) and invariant sites GTR substitution models were used: for ML using Treefinder alpha = 0.4562304 and theta 1.263948e-05; for distance $i = 0.181498$ and alpha = 0.652573. Bootstrap percentages within opisthokonts are omitted for clarity. Black blobs indicate 100% bootstrap support; dashes are used for clades not found in the distance tree because a few long branches moved dramatically. Names of higher taxa follow Cavalier-Smith et al. (2004) and, in brackets where different, also Smirnov et al. (2005).

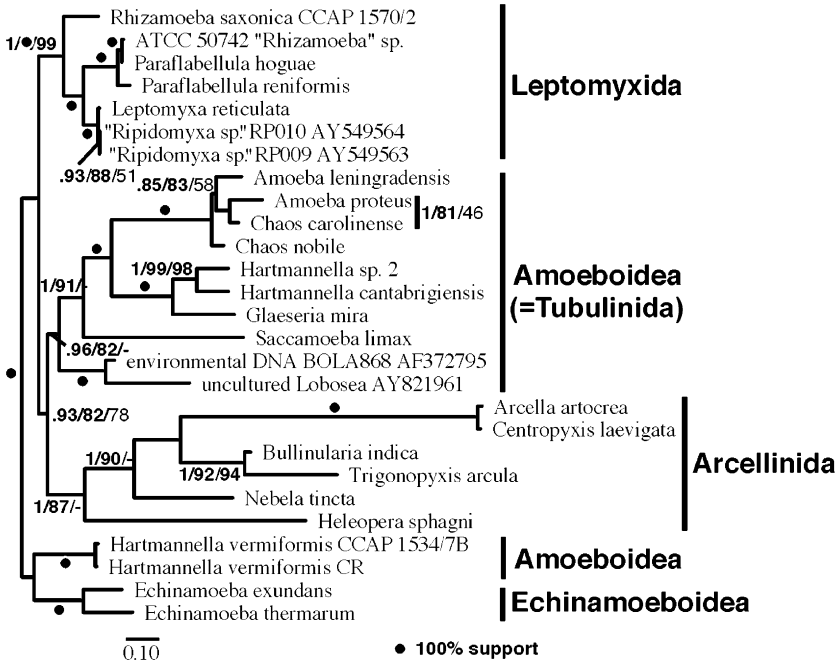


Fig. 8. Bayesian tree for 27 sequences of Lobosea using 1578 nucleotide positions of the 18S rRNA gene, using a GTR covarion, correlated sites, and gamma intersite variation (four rates) substitution model. Six runs totaling >12 million generations gave identical trees; the first 20% of trees were discarded as burn in. The tree is rooted as robustly shown in the Amoebozoa-wide tree of Fig. 7. Support values for bipartitions are for Bayesian posterior probability (left), RELL approximate bootstrap percentages for the corresponding ML tree (middle), and bootstrap percentages for the corresponding BioNJ tree for this dataset (lower). When both bootstrap percentages were 100 and the posterior probability was 1 only a single blob is shown. For the non-Bayesian trees gamma intersite variation (four rates) and invariant sites GTR substitution models were also used: for ML alpha = 4573735, theta = 1.263948e-05. Dashes are used for clades not found in the distance tree (because *Saccamoeba* moved to within Arcellinida, clearly a long branch artefact). The scale bar indicates the substitutions per site. Names of higher taxa follow Cavalier-Smith et al. (2004) and, in brackets where different, also Smirnov et al. (2005).

The cells preserved on the type slide (examined by AS) show the same characteristic variation in body shape. We sequenced the 18S rRNA gene of this Page strain (CCAP 1570/2) and found that it differs substantially from the sequence deposited by Amaral-Zettler et al. (2000) under GenBank number AY121847, and very clearly fails to group with it on the rRNA tree (Fig. 8). Hence, the ATCC 50742 strain, isolated by T. Sawyer from sediment at the mouth of Pocomoke River, MD in 1998; originally deposited as *Rhizamoeba* sp. and sequenced by Amaral-Zettler et al. (2000) under that name but now held in ATCC as *Rhizamoeba saxonica* must instead be recognized as a new species; in the absence of any microscopic data on this strain we cannot name it in the present paper.

Rhizamoeba saxonica (CCAP 1570/2) does not group in our trees with "Rhizamoeba sp." of Amaral-Zettler et al. (2000); moreover *R. saxonica* occupies a rather distant position from it as the most divergent leptomyxid yet sequenced, being excluded from all other leptomyxid subclades with very robust bootstrap support by both methods (Fig. 8) and with all taxon samples. This suggests that even the generic identification of the ATCC 50742 strain may need revision. Its robust grouping with *Paraflabellula hoguae* in our tree and in all previous ones (Amaral-Zettler et al. 2000; Bolivar et al. 2001; Cavalier-Smith et al. 2004; Fahrni et al. 2003; Kudryavtsev et al. 2005; Smirnov et al. 2005), with *P. reniformis* being their sister, may mean that actually it is related to *Paraflabellula*.

Two sequences of "Ripidomyxa sp." are available from GenBank (Fig. 8). Chakraborty and Pussard (1985) established this monotypic genus for a *Rhizamoeba*-like species; Page (1988) transferred its single species to the genus *Rhizamoeba*, as *R. australiensis* (Chakraborty and Pussard 1985) Page 1988. Since the genus *Ripidomyxa* was monotypic, this generic name should not be applied any more (or neotype must be established). There are no published data on "Ripidomyxa sp."; according to the GenBank annotation the sequence results from the Ph.D. thesis of Dr. Hewett Melissa Kim. A brief description and two photographs provided in this thesis (available via Australian Digital Theses Program (ADT), http://www.library.unisa.edu.au/adtroot/public/adt-SUSA-20082006-163911/index.html) show a species resembling *Rhizamoeba* in non-directed movement. Hence, supposed members

of the genus *Rhizamoeba* (*R. saxonica* CCAP 1570/2; "Rhizamoeba sp." ATCC 50742 and "Ripidomyxa sp.") are dispersed on the phylogenetic tree. The genus *Rhizamoeba* is defined in such a way that virtually any leptomyxid with a temporary monopodial locomotive form may be recognized as its member (Page 1972), because other morphological features of this genus (adhesive uroidal filaments; occasional cytoplasmic eruptions) are shared by most leptomyxids. We cannot exclude that in future, with accumulation of molecular data, it may need splitting into several independent genera. In the light of this, *Rhizamoeba saxonica* with its unique subsurface structures (collosomes) (Page 1980) and specific alteration of the locomotive form from flabellate to narrow worm-like, with higher length/breadth ratio and much less clavate shape than in all other *Rhizamoeba* species (illustrated in Figs 5, 6), may well be a candidate for a separate genus.

Flamella species are not leptomyxids

Another uncertainty over leptomyxid taxonomy concerns the genus *Flamella*, which Page (1988) could not firmly place in a family or order (though he suspected it might have affinities with the genus *Hyalodiscus*), but which was assigned to Leptomyxida by Michel and Smirnov (1999) based on the fan-shaped appearance of amoebae and presence of an adhesive uroid in locomotive cells. However, further studies indicated that the pattern of amoeboid movement in *Flamella* (independent cytoplasmic streams in locomotive cells) contradicts the concept of Tubulinea (Smirnov et al. 2005); *Flamella* amoebae never show a monopodial locomotive form (Michel and Smirnov 1999). Hence, we now exclude *Flamella* from Leptomyxida, leaving it incertae sedis within Amoebozoa until its position is clarified. Now that misidentification of "Gephyramoeba ATCC 50654" is corrected, and *Flamella* also excluded, the order Leptomyxida Page 1987 (or superfamily Leptomyxoidea Cavalier-Smith, Chao and Oates, 2004) is a solid, monophyletic group both at the morphological and the molecular level (Fig. 8).

Concluding remarks

The present study stresses again the importance of careful morphological identification of organisms used for molecular studies. A "trust but check" approach is especially important in molecular phylogeny, where the species is represented by a sequence only, as taxonomic identification cannot be checked "a posteriori". Unfortunately, the widespread practice now is that molecular biologists accept cultures under the name provided. This may result in confusing errors, as happened with "Gephyramoeba sp." and "Rhizamoeba sp.", as shown here, as well as with the amoeboflagellate sequenced as a strain of "Mastigamoeba invertens" from ATCC (Stiller et al. 1998; Stiller and Hall 1999), and now known as *Breviata anathema*: it does not even belong to Amoebozoa (Walker et al. 2006). Among zooflagellates we previously showed by light microscopy that the ATCC *Cercomonas longicauda* and several other *Cercomonas* were previously misidentified (Karpov et al. 2006) and that about four ATCC heterokont strains had similarly been misidentified (Cavalier-Smith and Chao 2006); we have discovered further examples of this among Cercozoa that we have yet to publish, including one CCAP culture. All these corrections indicate that (1) proper taxonomic identification is of critical importance in molecular studies, (2) no identification should be accepted without critical analysis, and (3) if possible, sequenced cultures should be deposited in culture collections and/or light microscope documentation should be prepared to give more confidence in identifications. In many cases electron micrographs or at least embeddings to allow subsequent ultrastructural studies would also be desirable. These precautions are particularly important for organisms whose sequences occupy "strange" positions in phylogenetic trees. While some degree of trust of expert identifications is appropriate, in some taxonomic groups there are very few, if any, real experts and a highly critical mistrust and careful checking of identifications is essential. We have experienced several cases where preliminary identifications made by an expert whom we respect very highly have turned out to be wrong. Further problems are that contaminant sequences are surprisingly often wrongly attributed to the organism bearing the name of the culture or that investigators may mix up the names of sequences when submitting them to GenBank or submit the same sequence under different names (for examples see Cavalier-Smith and Chao 2006).

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