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The Testate Lobose Amoebae (Order Arcellinida Kent, 1880) Finally Find their Home within Amoebozoa

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Testate lobose amoebae (order Arcellinida Kent, 1880) are common in all aquatic and terrestrial habitats, yet they are one of the last higher taxa of unicellular eukaryotes that has not found its place in the tree of life. The morphological approach did not allow to ascertain the evolutionary origin of the group or to prove its monophyly. To solve these challenging problems, we analyzed partial small-subunit ribosomal RNA (SSU rRNA) genes of seven testate lobose amoebae from two out of the three suborders and seven out of the 13 families belonging to the Arcellinida. Our data support the monophyly of the order and clearly establish its position among Amoebozoa, as a sister-group to the clade comprising families Amoebidae and Hartmannellidae. Complete SSU rRNA gene sequences from two species and a partial actin sequence from one species confirm this position. Our phylogenetic analyses including representatives of all sequenced lineages of lobose amoebae suggest that a rigid test appeared only once during the evolution of the Amoebozoa, and allow reinterpretation of some morphological characters used in the systematics of Arcellinida.

Key words: testate amoebae; phylogeny; evolution; SSU rRNA gene; actin; Amoebozoa.

Introduction

The testate lobose amoebae (order Arcellinida Kent, 1880) are common in a wide range of moist and freshwater habitats. These small amoebae feed mostly on bacteria, algae, and fungi. The larger species also prey on other protozoans and small metazoans (Heal 1963; Mast and Root 1916). The Arcellinida are distinguished by their tests, comprising a single aperture and composed of

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either secreted proteinaceous material or agglutinated. Proteinaceous tests can be flexible, with rigid sheets of fibrous material or with regularly arranged hollow building units that form an areolate surface. Agglutinated tests can be either calcareous or siliceous (Ogden 1990; Ogden and Ellison 1988). Dictyosomes are involved in the secretion of the organic building units and the cement. Mitochondria have branched tubular cristae. Contractile vacuoles are present. Arcellinida have either endolobopodia that are granular or completely hyaline, or ectolobopodia that are generally fingerlike and in some species can anastomose (reticulolobopodia) (Bonnet 1961, 1963). Under unfavorable environmental conditions, most testate lobose amoebae produce cysts.

Classification of the Arcellinida is based mainly on characters of the test. Kudo (1954) presents the arcellinids as two unrelated families, Arcellinidae (with a membranous shell) and Difflugiidae (shell with foreign bodies, platelets, or scales). A more recent classification (Meisterfeld 2002) considers Arcellinida as a monophyletic order divided into three suborders: Arcellinina (membranous test, digitate pseudopodia), Difflugiina (test rigid with mineral particles, digitate pseudopodia), and Phryganellina (test with siliceous material, pseudopodia conical).

Testate lobose amoebae were for a long time considered as part of the Testacea (Rhizopoda), a taxon uniting all amoeboid protists that are enveloped by a single-chambered shell (Kudo 1954). In the first edition of the “Illustrated Guide to Protozoa” (Bovee 1985), the Testacea are not treated as a monophyletic taxon. The shape of pseudopodia (lobose or filose) is considered as an important taxonomic feature and testate lobose amoebae are placed within the class Lobosea Carpenter, 1861; while the testate filose amoebae are placed within the class Filosea Leidy, 1879. In the second edition of the “Illustrated Guide to the Protozoa” (Meisterfeld 2002), the Arcellinida are treated as a group of amoebae of uncertain affinities, because their evolutionary origins are unclear and molecular data are awaited to solve the problem.

Molecular data have already helped to resolve the phylogenetic status of testate filose amoebae which form at least three independent lineages within the recently defined super-group Rhizaria (Burki et al. 2002; Nikolaev et al. 2003, 2004; Wylezich et al. 2002). In contrast, the Arcellinida is one of the last widespread and well-known groups of eukaryotes that is not represented in molecular databases. To test the monophyly of Arcellinida, determine their position in the eukaryotic tree, and to resolve their internal relationships, we obtained partial small-subunit ribosomal RNA (SSU rRNA) gene sequences from representatives of two out of the three suborders and seven out of the 13 families of Arcellinida. We show that testate lobose amoebae are monophyletic and belong to the Amoebozoa, and confirm this result using complete SSU rRNA gene sequences from two species, and a partial actin sequence from one species.

Results

Seven species of testate lobose amoebae (*Arcella artocrea, Bullinularia indica, Centropyxis laevigata, Heleopera sphagni, Hyalosphenia papilio, Nebela tincta var. major, and Trigonopyxis arcula*) were examined (Fig. 1, Table 1). The partial SSU rRNA gene tree (Fig. 2) shows that the seven species form a monophyletic group within Amoebozoa. Monophyly of the Arcellinida is supported with both MrBayes and Maximum Likelihood analyses. The relationships within Arcellinida are relatively well resolved. *Heleopela sphagni* branches at the base of the testate lobose amoebae with strong support (PP of 1.00 and BV of 82%). The next branch is comprised of *H. papilio and N. tincta var. major*, which group together with high support (PP of 1.00 and BV of 100%). The four remaining species groups together with moderate support (PP of 0.82 and BV of 64%) and are divided in two highly supported (PP of 1.00 and BV of 100%) clusters: *T. arcula*+*B. indica* and *A. artocrea*+*C. laevigata*.

Analysis of complete SSU rRNA sequences (Fig. 3), including two arcellinid species (*H. sphagni and C. laevigata*), further supports the monophyly of Arcellinida, recovered here with strong support (PP of 0.97 and BV of 83%). In both SSU rRNA gene trees (Figs 2, 3), the testate lobose amoebae branch within a highly supported clade (PP of 1.00, BV of 94—100%) comprising the genus *Echinamoeba*, the order Leptomyxida, and the families Hartmannellidae and Amoebidae. This clade was named Tubulina in a recent classification of lobose amoebae (Smirnov et al. 2005), and we follow this classification here. Within Tubulina, a close relationship was recovered between testate lobose amoebae, and the families Amoebidae and Hartmannellidae. Support for this relation is moderate in the partial SSU rRNA analysis (PP of 0.63 and BV of 68%; Fig. 2), but it is high using complete SSU rRNA sequences