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Polyphyly in the Thecate Amoeba Genus *Lecythium* (Chlamydophryidae, Tectofilosida, Cercozoa), Redescription of its Type Species *L. hyalinum*, Description of *L. jennyae* sp. nov. and the Establishment of *Fisculla* gen. nov. and Fiscullidae fam. nov.

Kenneth Dumack[1], **Paul Mausbach**, **Mona Hegmann**, and **Michael Bonkowski**

University of Cologne, Department of Terrestrial Ecology, Institute for Zoology, Zülpicher Str. 47b, 50674 Köln, Germany

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Although testate amoebae have attracted great interest of protistologists for more than a century, some groups, especially those with a hyaline, organic test (=theca) are still poorly known. One of those is the genus *Lecythium* Hertwig et Lesser, 1874. Only recently *Lecythium* spp. were characterized by morphological and molecular means, but data on the type species *Lecythium hyalinum* Hertwig et Lesser, 1874, was still lacking. In this study, we screened for *L. hyalinum* in freshwater samples of Germany and the Netherlands. Four different isolates of *L. hyalinum* and one novel species were cultured and characterized by light microscopy. Phylogenetic analyses based on the small ribosomal subunit (SSU) RNA gene show that the genus *Lecythium* forms two robust clades, one forming a sister group to the Rhizaspididae/Pseudodifflugiidae clade (Tectofilosida), the other branching within 'Novel Clade 4' (Tectofilosida). We untangle this polyphyly by establishing *Fisculla* gen. nov. and the Fiscullidae fam. nov. for the former of these two clades.

Key words: *Chlamydophrys*; protist; algivorous; testate amoebae; ectocytobiotic bacteria.

Introduction

Since their discovery, testate amoebae have been of considerable interest to protistologists and ecologists. They are one of the few protist groups with often very clear and easily identifiable morphological traits, thus representing useful model organisms for protist ecology and evolution. The specific ways in which their tests are constructed have been intensively studied and were used for testate amoeba identification and taxonomy. Research solely based on morphology however led to complex taxonomical concepts that were often

[1]Corresponding author; fax +49-221-470-5038
e-mail kenneth.dumack@uni-koeln.de (K. Dumack).

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contradictory and thus frequently changed (Cash et al. 1915; de Saedeleer 1934). With phylogenetic approaches a widely accepted consensus has been achieved. It has been shown that testate amoebae are polyphyletic and belong to different phyla, like Amoebozoa, Stramenopiles and Cercozoa (Cavalier-Smith 1998a,b; Kosakyan et al. 2016; Nikolaev et al. 2005). The phylum Cercozoa, established by Cavalier-Smith (1998a,b) is highly diverse in morphology and ecology. The Cercozoa consist predominantly of naked amoebae, flagellates and amoeboflagellates (Bass et al. 2009; Dumack et al. 2016a; Hess and Melkonian 2013; Howe et al. 2011). Nestling between these are several distinct testate amoeba lineages: e.g. the order Euglyphida with tests made out of siliceous plates (Cavalier-Smith 1998a,b; Wylezich et al. 2002) and the classes Cryomonadida and Tectofilosida, both in the order Thecofilosea, comprising amoebae with organic and agglutinated tests (Dumack et al. 2016b,c; Dumack et al. 2017; Howe et al. 2011; Wylezich et al. 2002).

The Tectofilosida, established by Cavalier-Smith and Chao (2003), contain up to now the testate amoeba families Rhizaspididae, Pseudodifflugiidae and Chlamydophryidae (Dumack et al. 2016b,c, 2017; Wylezich et al. 2002). Knowledge about the Chlamydophryidae is scarce. De Saedeleer (1934) established the family Chlamydophryidae as a subfamily of the Gromiidae, to accommodate genera such as *Lecythium* and *Chlamydophrys*, thus containing amoebae with thin hyaline tests, but until recently no molecular data was available. Dumack et al. (2016b,c, 2017) shed some light on such thecate amoebae by culturing and describing several strains of *Lecythium*-like amoebae with spherical organic tests and longitudinal division. They showed that these isolates grouped as a sister clade to the Rhizaspididae/Pseudodifflugiidae clade.

Nevertheless the type species, *Lecythium hyalinum* Hertwig et Lesser, 1874, could not yet be sequenced, leaving the possibility open that the genus *Lecythium* might be polyphyletic. We therefore decided to screen samples for *L. hyalinum*, isolate it and perform genetic and morphological analyses to clarify the existing taxonomy.

We isolated four strains of *Lecythium hyalinum* from Germany and the Netherlands, sequenced their SSU sequence and conducted phylogenetic analyses. We show its phylogenetic placement in the Cercozoa and discuss the polyphyly of the genus *Lecythium*. Additionally, we isolated another *Lecythium*-like amoeba. After morphological observations and intensive literature research, we are convinced that this species is new to science, and we herein give it a formal description.

Results

Sampling and Culturing

We isolated four different strains of *Lecythium hyalinum*-like amoebae (strains KD1010, KD1011, KD1012 and KD1013) from Germany and the Netherlands (Table 1). Three cultures of *Lecythium hyalinum* were not stable and were therefore lost during the study (KD1010, KD1011 and KD1012). Strains of *Lecythium* were highly sensitive to the composition of culture media and food organisms. Strains of *L. hyalinum* were only viable in WC-Medium (Guillard and Lorenzen 1972), whereas *Lecythium jennyae* sp. nov. (strain KD1014) was only cultivable in Waris-H (McFadden and Melkonian 1986). *Lecythium hyalinum* cultures fed only with the green alga *Characium* sp. (or with a combination of *Nitzschia communis* and *Characium* sp.) as food source suddenly collapsed after few weeks of culturing. Only the last isolated *L. hyalinum* (strain KD1013) which was cultured with a combination of *Nitzschia communis* (CCAC 5737B), *Characium* sp., *Nitzschia amphibia* (CCAC 5733B) and *Pinnularia* sp. (CCAC 0222B) was culturable over the whole time of our analyses (~5 months). *Lecythium jennyae* sp. nov. (strain KD1014) was cultured with *Nitzschia amphibia* (CCAC 5733B), *Characium* sp. and *Pinnularia* sp. (CCAC 0222B).

Morphological Observations

Lecythium hyalinum

All isolates of *L. hyalinum* (strains KD1010, KD1011, KD1012 and KD1013) were of similar morphology (Figs 1A, 2A, 3A ; strain KD1011 is not shown due to the early extinction of the culture). The amoebae bore a thin and hyaline theca 18–50 μm in length and 24–61 μm in width (KD1010: length 27.5 ± 6.7 μm, width 33.7 ± 9.1 μm ($n = 21$); KD1012: length 35.25 ± 7.6 μm, width 40.2 ± 5.8 μm ($n = 3$); KD1013: length 26.22 ± 3.2 μm, width 30.7 ± 3.5 μm ($n = 12$); KD1011 length 30.7 μm, width 32.0 μm ($n = 1$)) of roundish or slightly curved shape and radial symmetry. Around the aperture of the cells, small wrinkles and folds in the theca could be seen. These wrinkles and folds occurred most often in starving cells, thus we assume the theca shriveled inwards when cells shrank during starvation (Figs 1E, 2E; for individuals without wrinkles or folds see: