Transfer of the Thecate Amoeba *Lecythium mutabilis* to a Novel Genus *Omnivora* (Fiscullidae, Thecofilosea, Cercozoa)

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**ABSTRACT**

Thecofilosea is a class in Cercozoa (Rhizaria) comprising mainly freshwater-inhabiting algivores. Recently, numerous isolates of thecofilosean amoebae have been cultured and were characterized by an integrated morphological and molecular approach. As attempts to establish a culture of *Lecythium mutabilis* repeatedly failed, it was not yet investigated by molecular means. We isolated single cells of *L. mutabilis* directly from their habitat and successfully sequenced the V4 region of their SSU rDNA. Phylogenetic analyses showed that *L. mutabilis* is not directly related to the genus *Lecythium* and instead branches within the Fiscullidae (Tectofilosida, Thecofilosea). Accordingly, we transfer the species *L. mutabilis* to a novel genus *Omnivora* gen. nov.

**Keywords**

Algivory, Chlamydophryidae; *Pamphagus*; Tectofilosida; testate amoebae.

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**TESTATE amoebae** have been of considerable interest to protistologists and ecologists since their discovery. The specific ways in which their tests are constructed have been intensively studied and are still important characters for testate amoebae identification and taxonomy in relation to pseudopodial morphology (Meisterfeld 2002). However, amoebae with a theca, a flexible, transparent, usually colourless shell, have comparatively few morphological characters and thus largely evaded the attention of protistologists.

Recently, numerous isolates of thecofilosean amoebae, or amoebae that were previously assigned to the Thecofilosea, have been cultured and were characterized by an integrated morphological and molecular approach (Dumack et al. 2016a,b, 2017a,b,c,d, 2018a,b; Shiratori and Ishida 2016). These studies have shown that the trait of attaching or embedding xenosomes into the theca is polyphyletic within Thecofilosea and thus largely unsuitable for taxonomic assignments (Dumack et al. 2017d). Meanwhile, phylogenetic analyses based on SSU rDNA resulted in the establishment of a stable taxonomic framework for Thecofilosea despite their morphologic similarity. Due to the limited low-value morphological characters, major revisions of their taxonomy are inevitable.

De Saedeleer (1934) established the family Chlamydophryidae to accommodate thecate amoeba genera such as *Lecythium* and *Chlamydophrys*. The phylogenetic position of the type species of the genus *Lecythium*, *Lecythium hyalinum* (Hertwig and Lesser 1874), could recently be assigned to the Tectofilosida (Thecofilosea, Cercozoa), but still several species previously assigned to the Chlamydophryidae group elsewhere (Dumack et al. 2017c). Meanwhile, molecular data could be obtained for other genera in the Chlamydophryidae; *Trachyrhizium*, *Lecythium* and *Diaphoropodon* (Dumack et al. 2017b,d; Shiratori and Ishida 2016).

Attempts by us to establish a culture of *Lecythium mutabilis* (Bailey, 1853) repeatedly failed, and thus, it was not yet investigated by molecular means (Dumack et al. 2017b). Bailey (1853) described *L. mutabilis* under the name of *Pamphagus mutabilis* to the nowadays obsolete group Rhizopoda. *Pamphagus* is an invalid genus name for these amoebae, because it was already in use for a metazoan genus, Cash et al. (1915) pointed out that Schoute-den (1906 after Cash et al. 1915) proposed to use the genus name *Lecythium* which is still in use today. *Pamphagus* is an invalid genus name for these amoebae, because it was already in use for a metazoan genus, Cash et al. (1915) pointed out that Schouteden (1906 after Cash et al. 1915) proposed to use the genus name *Lecythium* which is still in use today. Morphological identification of thecofilosean species is very difficult and already led to confusion in the past. Leidy (1879) did not recognize *Lecythium mutabilis* and redescribed the species under the new name, *L. avidus*. However, Hoogenraad and de Groot (1940) and we argue that the descriptions refer to the same species (see Dumack
et al. 2016a,b). Here, we present the first molecular data of *Lecythium mutabilis* and an updated phylogenetic analysis of the Thecofilosea which led us to transfer the species to a newly erected genus *Omnivora* gen. nov.

**MATERIALS AND METHODS**

**Sampling and amoeba identification**

Two hundred to five hundred ml of substrates were repeatedly collected from the sediment of an urban pond behind the greenhouses of the Biocenter building of the University of Cologne (50.925279, 6.935951) between August and November 2018. Subsamples of five ml were transferred into a Petri dish. The samples were repeatedly observed with a light microscope (Nikon Eclipse TS100; Ph1; 40X, 100X, 200X and 400X magnification). Single cells were isolated, and each transferred into a well of a 24-well-plate filled with Waris-H + Si (McFadden and Melkonian 1986). Photos of single cells were taken with Nikon Eclipse TE2000-E (Ph1, up to 400X magnification) and Nikon Eclipse 90i (DIC, up to 600X magnification).

**DNA extraction, amplification and sequencing**

Single cells were starved for up to five days and then transferred with approx. 1 μl medium into a PCR-tube containing 4 μl ddH2O. Directly after isolation, 4.6 μl PCR mixture was added. The mix included 1.7 μl ddH2O, 1 μl Thermo Scientific Dream Taq Green Buffer, 1 μl of 10 μM forward and reverse primers each, 0.2 μl 10 μM dNTPs and 0.1 μl DreamTaq polymerase (Thermo Fisher Scientific, Dreieich, Germany). The sequences were obtained in two subsequent amplifications. After an initial PCR, a semi-nested or nested reamplification was performed using 1 μl of the first PCR product as template.

The primers used for this study were EukA, EukB, 590F, 1300R, 616F_Eocerco, 963R_Cerco and 947R_Cerco (Fiore-Donno et al. 2017; Medlin et al. 1988; Quintela-Alonso et al. 2011). However, all attempted PCRs involving the primers EukA and EukB did not lead to successful amplification of SSU rDNA of *Omnivora mutabilis* (see Results). The SSU rDNA could successfully be amplified with the primer combinations 616F_Eocerco and 963R_Cerco in the first amplification and a subsequent reamplification with 616F_Eocerco in combination with 947R_Cerco. Eight μl of the PCR products were purified by adding 0.15 μl of exonuclease, 0.9 μl FastAP and 1.95 μl ddH2O and heating the mixture for 30 min at 37 °C, and subsequently for 20 min at 85 °C. The Big dye Terminator Cycle sequencing Kit (Thermo Fisher Scientific, Dreieich, Germany) and an ABI PRISM automatic sequencer were used for the sequencing.

**Phylogenetic analysis**

To create a data set for phylogenetic analysis, representative sequences belonging to all major lineages of the Cercozoa with focus on Thecofilosea and Imbricatea were downloaded and manually aligned in SeaView (V4.5.3, Gouy et al. 2010); Endomyxa were added as the out-group. The data were adjusted and an alignment of 92 sequences and 1,754 aligned sites of which 45.90% were invariant was used for phylogenetical analysis. Phylogenetic trees were inferred with the GTR + I + G model in RAxML (Stamatakis 2014) with 200 random taxon additions for the maximum likelihood tree search and a 200 replicate bootstrap analysis and MrBayes (Altekar et al. 2004; Ronquist and Huelsenbeck 2003). The Bayesian analysis was set up with a sampling of every 100 and a diagnosis of every 500 trees and 25% of burn-in of 2.5 M generations. The sequences were submitted to the NCBI database under the accession numbers: MN504643 and MN504644.

**RESULTS**

**Light microscopic observations**

In total, 50 cells were observed, measured and subjected to molecular analyses. All observed individuals showed a similar morphology, with an average length of 231 ± 90.09 μm and width of 146 ± 52.74 μm, with a length/width ratio of 1.61 ± 0.35. The cells were usually droplet-shaped or pyriform, but sometimes deformed depending on the shape of the ingested material (Fig. 1). The aperture was located at the broadest part of the cell, here referred to as basal, and surrounded by a large number of folds (Fig. 2D). Cells were rarely attached with the basal side to the surface of the Petri dishes or glass slides, instead, they laid most often on their lateral side and did rarely move at all. The apical end often exhibited a cylindrical tip with several vacuoles located inside (Fig. 2F). Ingested inorganic particles and food organisms, like algae, occurred all over the cell body. Partially starved individuals showed numerous large vacuoles and lipid droplets that were distributed throughout the cell (Fig. 1A, 2E, F). The nucleus was roundish and of the oval shape, while it could rarely be identified in well-fed cells, starving cells revealed its presence between the centre of the cell and its apical end (Fig. 2A). Inside the nucleus, several granular structures resembling a lamellar nucleolus or several nucleoli (Fig. 2B) could be observed. The filopodia, although rarely formed, branched and anastomosed (Fig. 2G). Division or cysts were not observed.

**Molecular analyses**

From a total of 50 attempted PCRs to obtain molecular data, by far most PCRs resulted in negative results as indicated by gel electrophoresis, or more rarely, in the amplification of putatively ingested eukaryotes. All attempts using commonly used primers EukA and EukB in combination with each other or with other primers did not lead to successful amplification of thecofilosean SSU rDNA sequences (see Materials and Methods section). Only the two attempted PCRs conducted with the primer combinations 616F_Eocerco and 963R_Cerco in the first amplification and 616F_Eocerco and 947R_Cerco in a subsequent
amplification led to successfully amplified thecofilosean SSU rDNA sequences. We obtained two sequences of 339 nucleotides length from two different individuals which were identical to each other.

The two sequences branched next to the genus Fisculla in the Fiscullidae (Tectofilosida, Thecofilosea) with a high support of 94% bootstrap values and 1.0 posterior probability.

DISCUSSION

Morphological analysis

Bailey (1853) described L. mutabilis as P. mutabilis, a filose amoeba as he stated without a shell, but made out of a flexible, hyaline matter, which was filled with many different particles, for example sand, diatoms and green algae. Bailey probably did not recognize the theca due to it being very thin, flexible and hyaline. Leidy (1879) complemented Bailey’s description of P. mutabilis and noticed that the cell was covered by a shell. Figures 1 and 2 clearly show the common organic theca of many thecofilosean taxa.

In accordance with our observations, Bailey (1853) described the shape of the amoeba as pyriform. From his drawings, a size of $100 \pm 37 \mu m$ in length and $43 \pm 11 \mu m$ in width and a length/width ratio of $2.46 \pm 1.65$ can be determined, which is smaller than our observations although not clearly disjunct. Our observations show a large variety in...
cell sizes, also due to their very flexible cells. Ingested materials, especially large pennate diatoms lead to the deformation of the cell shape as noted by us and others (Bailey 1853; Leidy 1879, Dujardin 1852 after Leidy 1879; Siemensma 2019). The sizes for this species reported by Leidy (1879) are well in the range of our observations. Due to the large variation in size that comes with the ingested food particles and longitudinal division of Thecofilosea, we do not see this size difference as a discriminating factor.

Bailey did not mention the nucleus, and it is hard to observe in well-fed organisms. However, Bailey (1853) emphasized on the amoeba’s “tranquillity” when he described the movement of *L. mutabilis*. A trait that was obvious to us too.

Bailey (1853) stressed the algivory of this amoeba as it was clearly filled with different algal species. Algivory is common in thecofiloseans (Dumack et al. 2018b; Seppey et al. 2017) and as seen in Fig. 1, 2 all cells contained a large variety of algae.

**Figure 2** Cellular features of *Omnivora mutabilis*. Scale bars indicate 10 µm. **A.** Roundish nucleus inside the cell. **B.** Nucleus in detail, nucleolar structures within highlighted. **C.** Aperture from which filopodia emerge. **D.** Folds in the theca around the aperture. **E.** The theca with underlying vacuoles and lipid droplets. **F1** and **F2.** Apical end of the cell with vacuoles inside the theca in two different focus layers. **G.** Filopodia emerging out of the aperture. *ap* = aperture; *fd* = folds; *fi* = filopodia; *gr* = granula; *ld* = lipid drops; *no* = nucleolus; *nu* = nucleus; *th* = theca; *va* = vacuoles.
Figure 3  SSU rDNA phylogeny of filosa with selected Endomyxa as out-group with focus on the Thecofilosa. Shown is the maximum likelihood tree obtained by the RAxML GTR + I + G analyses including 92 sequences and using 1,754 aligned sites of which 45.90% were invariant. The support levels of the maximum likelihood and the Bayesian analysis are shown on the respective branches (ML/BI) if support was over 50% bootstrap. Bold lines indicate a bootstrap value ≥ 95%. Support values below 50% bootstrap are omitted. The here studied amoebae are shown in bold. Thecofilosean thecate amoebae are marked.
Molecular Characterization of Lecythium mutabilis

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Taken together, our observations are in accordance with the descriptions of Bailey (1853) and Leidy (1879), which leads to the conclusion that our sampled individuals correspond to the species Bailey (1853) named P. mutabilis.

Historical analysis and the erection of Omnivora gen. nov.

Our phylogenetic analysis shows an affiliation of Lecythium mutabilis to the Fiscullidae as it branches at its base, the type species of the genus Lecythium however groups elsewhere in Thecofilosea and thus Lecythium is not the suitable genus name under which this species should be referred to. Lecythium mutabilis exhibits the characters unifying the Fiscullidae, but we decided to erect a novel genus to accommodate L. mutabilis and not assign it to the genus Fisculla based on the following morphological differences: species of the genus Fisculla are all more or less spherical, whereas L. mutabilis is elongated; L. mutabilis shows striking difference in behaviour, as it is extraordinarily inert and does not grow under culture conditions suitable for Fisculla, furthermore, L. mutabilis is much larger than all other described Fiscullidae and due to the habit to ingest anorganic material it cannot be confused with Fisculla. The question arises which name the novel genus should bear. Bailey was mentioned as a candidate genus name to replace the invalid genus name Pamphagus (Shouteden 1906, after Cash et al. 1915). Bailey would be an obvious choice to rename this novel genus in order to accommodate L. mutabilis (Bailey, 1853), and we would have considered it to accommodate L. mutabilis if not already in use by the International Code of Zoological Nomenclature. Grote (1896) introduced this genus name in 1896 (often referenced as 1895, but actually printed in 1896) to accommodate species of insects. Therefore, we erect the novel genus name Omnivora, referring to the habit of L. mutabilis to ingest next to algal prey also anorganic material-like sand grains and other items. From now on, we will refer to this species under the novel name O. mutabilis. Although we did not yet observe fusion of cells in O. mutabilis, its morphology does not contradict the diagnosis given for the Fiscullidae. Longitudinal division has been reported by Bailey (1853) and Siemensma (2019). Due to its close relation to the genus Fisculla, we assign the genus Omnivora to the Fiscullidae.

TAXONOMIC ACTIONS

Fiscullidae DUMACK, MAUSBACH et BONKOWSKI 2017

Omnivora Dumack, gen. nov.

Diagnosis: Cells exhibit characters as Fiscullidae, DUMACK, MAUSBACH et BONKOWSKI 2017. Cells pyriform or droplet-shaped if not deterred by large ingested food particles. Cells strikingly inert. Cells ingest large food particles and diverse inorganic materials, for example sand particles.

Etymology: Omnivora (feminine)—derived from the Latin words omnis (=all/everything) and vorax (=voracious); the created name was feminized since most shelled amoeba taxa are by tradition feminine. The name refers to the peculiar habit of Omnivora mutabilis to ingest inorganic material.

Type Species: Omnivora mutabilis comb. nov.

Basionym: Pamphagus mutabilis Bailey, 1853.

Remarks: The genus Omnivora is so far monotypic. Limnic, Eukaryovorous, mainly consuming algae.

ZooBank registration number: urn:lsid:zoobank.org:act:B74859F9-3440-4531-9CE2-B7A691B51CFB

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