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Novel Cultured Protists Identify Deep-branching Environmental DNA Clades of Cercozoa: New Genera *Tremula*, *Micrometopion*, *Minimassisteria*, *Nudifila*, *Peregrinia*

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We describe three new orders of filosan Cercozoa, five new deep-branching genera, eight new species of *Thaumatomonas*, *Reckertia*, *Spongomonas*, *Rhogostoma*, *Agitata*, *Neoheteromita* and *Paracercomonas*, sequence their 18S rDNA, and construct 18S rDNA trees for 148 Cercozoa. Our phylogeny indicates that Filosa were ancestrally gliding flagellates; non-flagellate filose amoebae evolved from them five times independently. The new genera are more closely related to environmental DNA sequences than cultured organisms. *Tremula longifila*, a zooflagellate glider on both flagella (unlike other Cercozoa), is the most divergent filosan (Tremulida ord. n.). *Micrometopion nutans* is a eukaryote-eating gliding zooflagellate like *Metopion* and *Metromonas*. *Minimassisteria diva* is a widespread trimorphic marine amoeboflagellate granofilosan. *Peregrinia clavideferens*, a non-testate, scale-bearing, filose amoeba, branches deeply in Thaumatomonadida, which are probably sisters to Spongomonadida. *Nudifila producta* is a filose amoeboflagellate related to *Clautriavia* and Marimonadida (ord. n., e.g. *Pseudopirsonia, Auranticordis*). We substantially revise Imbricatea, now including Spongomonadida, and Thecofilosea to include Phaeodaria. Thecofilosea and Imbricatea and Thecofilosea are sisters, both arguably ancestrally rigid gliding flagellates with ventral pseudopod-emitting grooves. Scale-free *Ovulinata parva* is sister to *Paulinella*, so imbricate silica scales can be lost. Internal hollow silica skeletons evolved twice in Thecofilosea (Ebriida, Phaeodaria) or were multiply lost. *Protaspa* replaces preoccupied '*Protaspis*'.

Key words: 18S rDNA phylogeny; *Peregrinia*; *Protaspa*; *Rhogostoma*; *Tremula*; Ventricleftida.

Introduction

Cercozoa is a diverse, increasingly well-characterised phylum of protists (Cavalier-Smith 1998a,b) founded mainly on the basis of 18S rDNA

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phylogenies that revealed a phylogenetic relationship between a variety of gliding zooflagellates and filose amoebae and the plasmodiophorid plant parasites (Cavalier-Smith 1996/7; Cavalier-Smith and Chao 1997). Its unity was later confirmed by actin trees (Keeling 2001), molecular synapomorphies (Archibald and Keeling 2004; Archibald et al. 2003; Bass et al. 2005; Cavalier-Smith 2002; Cavalier-Smith and Chao 2003a), morphological synapomorphies (Cavalier-Smith et al. 2008a, 2009), and combined 28S and 18S rDNA phylogenetic analyses (Moreira et al. 2007). Together with Amoebozoa, Cercozoa have by far the greatest biomass of any protist phyla in soil (Urich et al. 2008) and are also abundant in every marine habitat, and so must be quantitatively significant players in the carbon and mineral cycles by preying on bacteria and other protists. Cercozoa probably diversified from an early phagotrophic, rhizopodial biflagellate (Cavalier-Smith and Chao 2003b) to contain a vast array of phenotypes (Bass et al. 2009a,b; Cavalier-Smith 1998a,b), all but overwriting and obscuring ancestral morphological characters.

This diversity of cercozoan body forms includes filose testate amoebae (both scale-bearers like *Euglypha* and non-silicified ones like *Pseudodifflugia*), amoeboflagellate algae (e.g. *Chlorarachnion*), zooflagellates with pseudopodia and siliceous scales (e.g. *Allas*), diverse parasites (e.g. Ascetosporea, the syncytial *Plasmodiophora, Pseudopirsonia*), naked reticulose amoebae (e.g. *Filoreta*), testate reticulose amoebae (*Gromia*), colonial flagellates (e.g. some *Spongomonas*), planktonic swimming flagellates (e.g. *Ebria*), numerous small gliding flagellates (e.g. *Sandona*, *Bodomorpha*, *Allapsa*, *Cavernomonas*), helioflagellates (e.g. *Aurigamonas*), soft-bodied amoeboflagellates (e.g. *Agitata, Cercomonas, Eocercomonas* and *Paracercomonas*), and thecate or scaly amoeboflagellates that emit ventral pseudopods from a marked groove or cleft (e.g. Thaumatomonadida, '*Protaspis*' (here renamed *Protaspa*), *Ventrifissura*), and even protists with axopodia (desmothoracids and phaeodarians). Recent studies show that many hitherto completely unknown organisms as well as those of previously uncertain affinity are actually Cercozoa (e.g. Bass et al. 2009a,b; Burki et al. 2002; Chantangsi et al. 2008; Chantangsi and Leander 2010a,b; Hoppenrath and Leander 2006a,b; Kühn et al. 2000; Longet et al. 2004; Vickerman et al. 2005). Despite this great expansion of the group, phylum-specific environmental gene libraries reveal that many Cercozoa remain to be described (Bass and Cavalier-Smith 2004; Bass et al. 2009a,b; Howe et al. 2009). Targeted surveys within major cercozoan groups have shown some genera, notably the bacterivorous *Cercomonas* (Bass et al. 2009b) and *Sandona* (Howe et al. 2009), to be astoundingly speciose and genetically diverse. Others, such as predators on other eukaryotes like *Aurigamonas* and *Metopion* and the divergent flagellates *Sainouron* (Cavalier-Smith et al. 2008a) and *Helkesimastix* (Cavalier-Smith et al. 2009), are relatively rarely detected in either environmental or culture surveys, although they may be more common than current indications suggest.

This paper uses light microscopy, electron microscopy, 18S rDNA sequencing and phylogenetics to describe species representing five new genera: the basal filosan *Tremula longifila*, and four divergent but later branching filosans: *Micrometopion nutans*; *Minimassisteria diva*; *Nudifila producta*; and *Peregrinia clavideferens*. We also describe eight new species in existing genera: 1) a new solitary spongomonad, *Spongomonas solitaria;* 2) a novel group B2 paracercomonad (Bass et al. 2009b), *Paracercomonas baikali*; 3) a new gliding pansomonad, *Agitata vibrans* (and make the new combination *Agitata agilis*); 4) the first clearly marine glissomonad, *Neoheteromita tolerans*; 5) three thaumatomonads: *Reckertia filosa*, recognizing the genus *Reckertia* Conrad, 1920 as distinct from *Thaumatomastix; Thaumatomonas oxoniensis*; and *Ts vancouveri*; 6) a filose thecate amoeba, *Rhogostoma micra*. Where possible we use ITS2 rDNA sequences to barcode the new species at comparably high resolution and describe scale ultrastructure for the four new thaumatomonads. We also identify a novel scale type in *Allas* that distinguishes it more clearly from *Thaumatomonas* and revise existing taxonomy where necessary using the new morphological and phylogenetic data and critical evaluation of the literature, establishing three new orders and three families and several new combinations for species previously incorrectly assigned to genera. Finally we discuss evolutionary and taxonomic implications of our trees, the most comprehensive so far for Cercozoa, which particularly illuminate the evolution of Thecofilosea and Imbricatea and the nature of the earliest filosan lineages.

Results

Phylogenetic Analyses

Figure 1 shows the phylogenetic positions of the new isolates described below. The most striking phylogenetic novelties are the deep position of the