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Phylogeny and Systematics of Leptomyxid Amoebae (Amoebozoa, Tubulinea, Leptomyxida)

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We describe four new species of *Flabellula*, *Leptomyxa* and *Rhizamoeba* and publish new SSU rRNA gene and actin gene sequences of leptomyxids. Using these data we provide the most comprehensive SSU phylogeny of leptomyxids to date. Based on the analyses of morphological data and results of the SSU rRNA gene phylogeny we suggest changes in the systematics of the order Leptomyxida (Amoebozoa: Lobosa: Tubulinea). We propose to merge the genera *Flabellula* and *Paraflabellula* (the genus *Flabellula* remains valid by priority rule). The genus *Rhizamoeba* is evidently polyphyletic in all phylogenetic trees; we suggest retaining the generic name *Rhizamoeba* for the group unifying *R. saxonica, R.matisi* n. sp. and *R. polyura*, the latter remains the type species of the genus *Rhizamoeba.* Based on molecular and morphological evidence we move all remaining *Rhizamoeba* species to the genus *Leptomyxa.* New family Rhizamoebidae is established here in order to avoid paraphyly of the family Leptomyxidae. With the suggested changes both molecular and morphological systems of the order Leptomyxida are now fully congruent to each other.

Key words: Protists; amoeba; phylogeny; systematics; morphology; ultrastructure.

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Introduction

General Characteristics of Leptomyxida

Amoebae of the order Leptomyxida, according to the classification scheme by Smirnov and Cavalier-Smith (Smirnov et al. 2011) belong to the phylum Amoebozoa, subphylum Lobosa, class Tubulinea. This taxon unifies flattened, expanded or reticulate amoeboid protists forming adhesive uroidal structures and possessing no differentiated glycocalyx (Page 1987). The main characters of leptomyxid amoebae, providing a general impression on this group of protists, are summarized in Figure 1.

By their morphology leptomyxids are lobose amoebae – organisms characterized by wide, smooth, non-anastomosing cytoplasmic projections (lobopodia), driven by an actomyosin cytoskeleton (Smirnov 2008). An exception is the genus *Leptomyxa*, where lobose cytoplasmic projections in the frontal part of the expanded, ramose cell may anastomose and thus were named loboreticulopodia (Page 1987). All leptomyxids are able to form adhesive uroidal structures; this is a synapomorphy of this group. All species of Leptomyxida can alter their locomotive morphology from flattened, expanded to tubular, limax-like. This is a morphological basis for the inclusion of the order Leptomyxida in the class Tubulinea (Smirnov et al. 2005, 2011). All leptomyxids possess a simple life cycle, which includes a subsequent alteration between the cyst and the trophozoite stage. Cysts are solitary; formation of fruiting bodies of any kind was never described for leptomyxids. Some species show a complex cysts structure (e.g. *Leptomyxa* may form several endocysts covered with the shared external cyst wall). Leptomyxids populate freshwater, marine and terrestrial habitats; some species of the genera *Leptomyxa* and the species *Gephyramoeba delicatula* seem to be specifically soil-dwelling. Sex is not known in this group of protists. Amoebae of this order currently are divided into three families – Leptomyxidae, Flabellulidae and Gephyramoebidae. Together these three families comprise 20 valid species of amoeboid organisms (Smirnov et al. 2011).

The main sources of data on Leptomyxida remain keys by Page (1983, 1988, 1991) and a set of papers by Pussard and Pons (1976a, b, c); the systematics and diversity of the taxon were briefly covered by Smirnov and Goodkov (2000) and Smirnov (2008, 2012). However, there is not a single comprehensive review considering taxonomy and diversity of leptomyxids and covering soil freshwater and marine species. Literary data are scarce and dispersed among numerous publications; some of these papers are hardly available. The present paper represents an attempt by a community of researchers to summarize data on morphology and taxonomy of these organisms and revise their system, including new relevant information. We start this paper with the review of the systematics and taxonomy of leptomyxid amoebae, with special attention to the complex taxonomic perturbations of genera and families and changing concepts of Leptomyxida. Then we characterize the main leptomyxid taxa, provide new data on a number of known leptomyxid species and present descriptions of several new species. Next we show results of comprehensive phylogenetic analyses and discuss the molecular phylogeny of leptomyxid amoebae. We suggest a revision of leptomyxid taxa and outline a new system of this group, provide revised diagnoses of taxa and a checklist of valid species of Leptomyxida, as well as information on poorly described strains that potentially belong to this group. The suggested changes of the system of the order Leptomyxida will ease the morphological identification of amoebae possessing adhesive uroidal structures, making morphological and molecular systems of this order fully congruent to each other.

Discovery and Taxonomic history of the Order Leptomyxida

The first representatives of the order Leptomyxida were found by Goodey (1915; note: often erroneously dated 1914) who described three species – *Leptomyxa reticulata*, *L. flabellata* and *Gephyramoeba delicatula*. These species were placed following the classification scheme of amoeboid protists proposed by Minchin (1912) in the order Amoebea, suborder Reticulosa according to the definition: “With filose or reticulose pseudopodia, without shell” (Minchin 1912; p. 217). It is now evident that Goodey (1915) considered the ramose body of *Leptomyxa* and *Gephyramoeba* as a primary criterion: although the suborder Lobosa is recognized in Minchin’s (1912) classification, it was not considered by Goodey as a proper place for the species he described. However, one must keep in mind that at that time part of the suborder Lobosa comprising naked forms – “Lobosa Nuda” was “furnished by the species of the genus *Amoeba* and allied forms only” (Michin 1912, p. 219), while Goodey (1915, p. 94) noted that “the genus *Amoeba* is notoriously a mixed and, one might almost say, a chaotic one; scarcely any of the standard works on Protozoa agreeing as to