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Abstract

Members of the Archamoebae comprise free-living and endobiotic amoeboid flagellates, amoeboflagellates, and amoebae, with distinctive hyaline cytoplasm and bulging pseudopodia. They live in anoxic or microoxic habitats and are anaerobes, lacking typical mitochondria, as well as Golgi stacks, plastids, and normal peroxisomal microbodies. They have a distinctive flagellar apparatus present in all flagellated members of the group. Life cycles of individual species can include flagellates, amoebae of various sizes, and cysts. In recent years, the group has been divided into five separate families, Mastigamoebidae, Entamoebidae, Pelomyxidae, Tricholimacidae, and Rhizomastixidae, whose interrelationships have not been completely resolved. Here, we clarify the composition of these groups and the circumscription of genera in the Archamoebae.

Keywords

Archamoebae • Pelobiont • Amoebozoa • *Mastigamoeba*, *Pelomyxa*, *Entamoeba*

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Summary Classification

- Archamoebae
- Pelobiontida
- Mastigamoebina
- Mastigamoebidae (*Mastigamoeba*, *Iodamoeba*, *Endolimax*)
- Rhizomastixidae (*Rhizomastix*)
- Pelomyxina
- Pelomyxidae (*Pelomyxa*, *Mastigella*)
- Entamoebida
- Entamoebidae (*Entamoeba*)

[Archamoebae incertae sedis: *Mastigina*, Tricholimacidae (*Tricholimax*), *Endamoeba*]

Introduction

General Characteristics

The Archamoebae is a group of amoebae, amoeboid flagellates, and amoeboflagellates (i.e., organisms with both flagellates and amoebae in their life cycle), with distinctive hyaline cytoplasm and bulging pseudopodia (Fig. 1). They comprise three

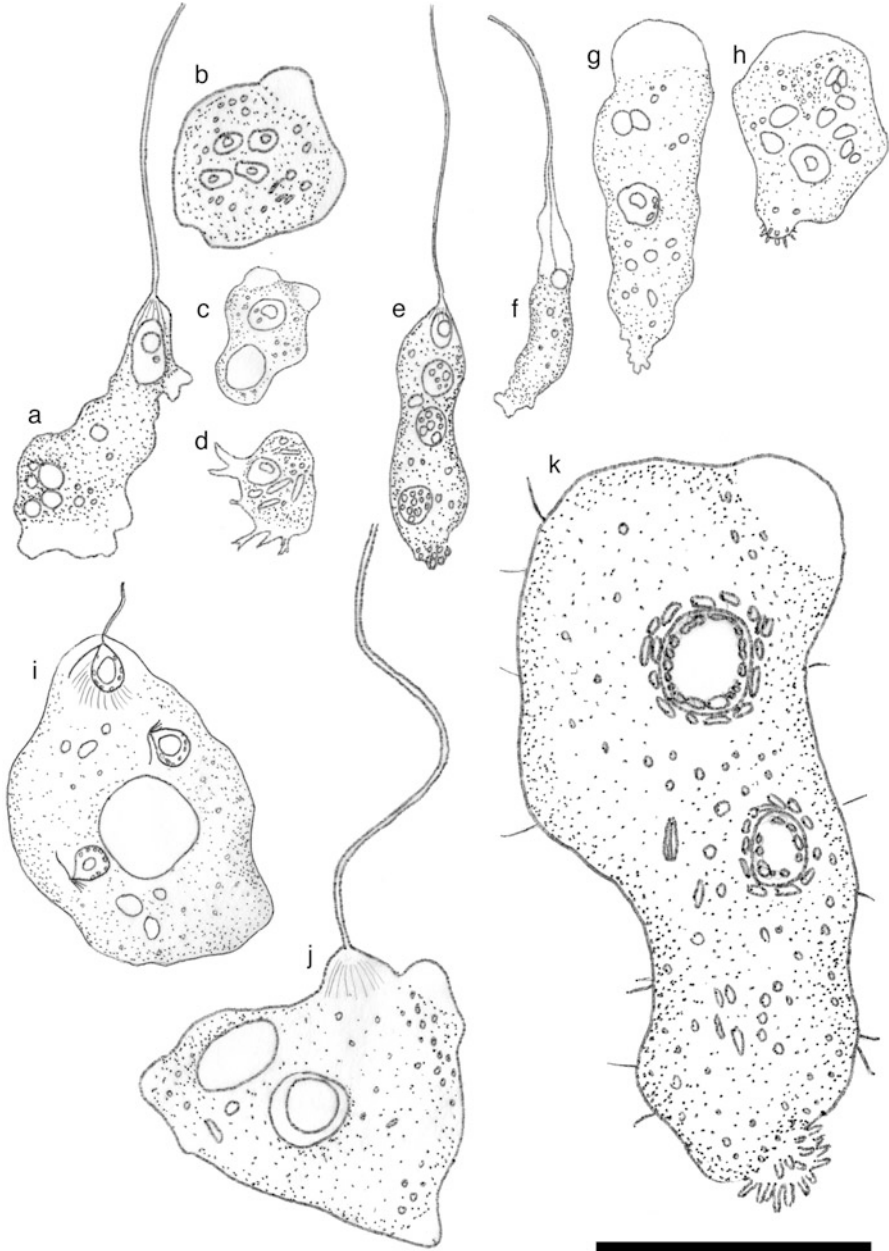


Fig. 1 General appearances of genera in the Archamoebae. (a) In the dominant flagellated trophic stage, *Mastigamoeba* has a microtubular cone that connects the flagellar base to the nucleus. (b) *Mastigamoeba* also typically has amoeboid stages, which may be multinucleate. (c) *Iodamoeba* contains small aflagellate amoebae with hyaline lobopodia. (d) *Endolimax* forms small aflagellate amoebae with variable pseudopodia similar to those in *Mastigamoeba*. (e) *Mastigina* contains

lineages that have collectively been referred to as pelobionts: mastigamoebids, pelomyxids, and *Tricholimax*, as well as *Rhizomastix* and entamoebids. All Archamoebae are anaerobic (some more aerotolerant than others) and lack normal mitochondria (which are reduced to remnants), Golgi stacks, plastids, and normal peroxisomal microbodies. Many contain endosymbiotic bacteria and archaea. Flagellated taxa have a distinctive cytoskeleton.

Mastigamoebids currently include *Mastigamoeba*, *Endolimax*, and *Iodamoeba*: these include mostly free-living amoeboid flagellates as well as endobiotic amoebae; many have distinctively hyaline cytoplasm and move with pseudopodia when attached to a substrate. In flagellate mastigamoebids, the flagellum is functional and has a conventional 9 + 2 microtubular arrangement, but there is a distinctively “languid” or slow flagellar beat arising from the lack of outer dynein arms in the flagellar axoneme. The base of the flagellum gives rise to a cone of microtubules that connect to the nucleus. Molecular phylogenies divide mastigamoebids into two clades, “A,” containing large species with a broad flagellar apparatus (e.g., *Mastigamoeba balamuthi*), and “B,” containing small species with a narrow flagellar apparatus and trailing pseudopodia (e.g., *Mastigamoeba simplex*) as well as *Endolimax* and *Iodamoeba* (Ptáčková et al. 2013; Pánek et al. 2016). Members of *Endolimax* and *Iodamoeba* have entirely lost the flagellar apparatus and were historically classified within entamoebids. They have only recently been transferred to mastigamoebids on the basis of molecular-phylogenetic analyses (Cavalier-Smith et al. 2004; Ptáčková et al. 2013; Stensvold et al. 2012; Poulsen and Stensvold 2016). Historically, the flagellated *Mastigella* has usually been thought of as a mastigamoebid, but it is more closely affiliated with *Pelomyxa* (Zadrobílková et al. 2015).

Pelomyxids include members of *Mastigella* and *Pelomyxa*. *Mastigella* includes amoebae and flagellated amoebae where the base of the flagellum gives rise to a microtubular cone that is not connected to the nucleus. *Pelomyxa* includes large (up to several millimeters) amoebae, with nonfunctional, short flagella that have a disordered microtubular arrangement and a microtubular cone that is unconnected to the nucleus or nuclei. Recent phylogenetic analyses have shown that *Mastigella* is closely related to *Pelomyxa* (Zadrobílková et al. 2015).

Rhizomastix was recently placed in the Archamoebae (Čepička 2011; Ptáčková et al. 2013). It includes amoeboid flagellates with a single anterior flagellum, which live



Fig. 1 (continued) amoeboid flagellates with a limax appearance and no lateral pseudopodia. **(f)** *Rhizomastix* contains small amoeboid flagellates that have a microtubular rhizostyle connecting the flagellar base to the nucleus. **(g)** *Entamoeba* contains amoebae with hyaline lobopodia and granular cytoplasm. **(h)** *Endamoeba* includes amoebae with a characteristic pattern of nuclear chromatin and no strong distinction between hyaline and granular areas of cytoplasm. **(i)** *Tricholimax* includes a single species of multinucleate amoeboid flagellate with a short, nonfunctional flagellum. **(j)** *Mastigella* contains amoeboid flagellates where the flagellar base is not connected to the nucleus by the cone of microtubules; the dominant trophic stage may be an aflagellate amoeba. **(k)** *Pelomyxa* contains large amoebae with multiple nuclei and very short, nonfunctional flagella. Scale bar = 20 μm

endobiotically in the intestines of insects and amphibians or are free-living in freshwater sediments. In *Rhizomastix* the cone of microtubules arising from the base of the flagellum has been modified into a tapering microtubular tube, the “rhizostyle.”

Entamoebids are aflagellated, mostly endobiotic amoebae that were, until relatively recently, classified among other amoebae and taxonomically separated from the pelobionts. They currently formally include the genus *Entamoeba* (historically also *Endamoeba*, *Endolimax*, and *Iodamoeba*) and have in the past also included several genera of currently uncertain phylogenetic position, such as *Schizamoeba*, *Hydramoeba*, and *Malpighamoeba*. Their morphological similarities to pelobionts were recognized several decades ago (Cavalier-Smith 1983, 1987a, b) and since confirmed with molecular phylogenetics. Most entamoebids are probably harmless commensals of the digestive tract in invertebrates or vertebrates, including humans (e.g., *Entamoeba moshkovskii*; Heredia et al. 2012); a recent description reports a free-living or commensal marine species (Shiratori and Ishida 2016). The most important is the parasitic *Entamoeba histolytica*, which causes the dangerous amebic dysentery of humans.

Mastigina is a poorly known genus, with few sightings and no molecular data, currently classified as *incertae sedis* (Pánek et al. 2016) though likely to be a member of the mastigamoebids. It has many similarities to *Mastigamoeba* but has a limax body shape where pseudopodia emerge only at the anterior or posterior ends. Its identity has historically been confused with that of *Tricholimax* (see section “Systematics and Taxonomy”, below).

Tricholimax hylae, a large multinucleate amoeba with a short, nonfunctional flagellum, is endobiotic in the hindgut of frog tadpoles. The phylogenetic placement of *Tricholimax* is unknown, in the absence of molecular data, so it is classified as Archamoebae incertae sedis (Pánek et al. 2016); nonetheless, it shows considerable similarity to *Mastigella* and *Pelomyxa*. Its identity has historically been confused with that of *Mastigina*.

Endamoeba is a poorly known genus; it includes aflagellate Archamoebae found in insects. Its morphological similarity to *Entamoeba* suggests it is likely to be a member of Entamoebidae, but it is currently classified as Archamoebae incertae sedis in the absence of molecular data (Pánek et al. 2016).

History of Knowledge and Literature

Taxonomic History of the Archamoebae as a Group

The name Archamoebae was introduced and used by Cavalier-Smith (1983, 1987a, b) and Cavalier-Smith et al. (2004) to group the pelomyxids, entamoebae, and mastigamoebids. The grouping of entamoebae, pelomyxids, and mastigamoebids was later supported by molecular phylogenetic analyses with complex evolutionary models (Cavalier-Smith et al. 2004; Edgcomb et al. 2002; Kudryavtsev et al. 2005; Milyutina et al. 2001; Nikolaev et al. 2006; Pánek et al. 2016; Ptáčková et al. 2013; Stensvold et al. 2012). The taxonomic concept of Archamoebae has been used at

ranks of infraphylum and class (Cavalier-Smith 1998, 2013; Cavalier-Smith et al. 2004) and has been compositionally unstable (Cavalier-Smith 1991, 1997; Cavalier-Smith and Chao 1995). It is currently ranked as a class, Archamoebae Cavalier-Smith, 1983 (Pánek et al. 2016).

In its recent incarnations (e.g., Cavalier-Smith 2013; Ptáčková et al. 2013; Zadrožilková et al. 2015, 2016), Archamoebae has included four main clades, the entamoebae, pelomyxids, mastigamoebids, and *Rhizomastix*, with *Tricholimax* sometimes treated as a fifth clade (Cavalier-Smith 2013) or regarded as incertae sedis but probably part of the pelomyxids, as discussed above (Frolov 2011; Pánek et al. 2016; Ptáčková et al. 2013; Zadrožilková et al. 2016). Relationships between clades have recently been resolved using multigene phylogenetics, dividing the group clearly into entamoebids (*Entamoeba*) and pelobionts (pelomyxids; mastigamoebids and *Rhizomastix*), and the high-level taxonomy has been updated to reflect this, as presented below here (Pánek et al. 2016).

Flagellated mastigamoebids and pelomyxids have historically been considered together as “pelobionts.” The Order Pelobiontida was originally introduced to include only the genus *Pelomyxa* (Page 1976, 1987) and has occasionally been used at other ranks, e.g., Class Pelobionta (Krylov et al. 1980). Its trophic form being a large amoeba, *Pelomyxa* was until recently more usually classified with lobose amoebae (e.g., Bovee 1972; Bütschli 1880; Chatton 1925, 1953; Page 1976; Reichenow 1952; Siemensa 1987) but with increasing recognition that it was distinct from other large amoebae (Whatley and Chapman-Andresen 1990). Griffin (1988) revised the Order Pelobiontida to include *Mastigamoeba*, *Mastigella*, *Mastigina*, and *Dinamoeba*, on the basis of the ultrastructural evidence for flagella in *Pelomyxa* (Griffin 1979, 1988). Cavalier-Smith (1987a, b) created a different concept of pelobionts consisting of pelomyxids and entamoebids. However, this composition of the pelobionts has only rarely been used (e.g., Cavalier-Smith et al. 2004). The term “pelobiont” has since been used to encompass mastigamoebids and pelomyxids to the exclusion of entamoebae (Pánek et al. 2016).

The name Entamoebae was created to group aflagellate Archamoebae (*Entamoeba*, *Endamoeba*, *Endolimax*, and *Iodamoeba*). However, *Endolimax* and *Iodamoeba* have been removed from entamoebids, *Endamoeba* is currently regarded as Archamoebae incertae sedis, and the loss of the flagellum has occurred at least twice independently in Archamoebae. Thus, the name Entamoebae is confusing and should ideally only be used in the future with specific clarification as to its composition, noting that this is different from that of recent years.

History of Genera in the Archamoebae

Mastigamoeba was the first genus created to house species with a flagellum and an amoeboid body, with a hyaline cytoplasm unlike that of other superficially similar taxa such as the cercozoans (Kent 1880; Klebs 1892; Schulze 1875b; Stokes 1886, 1888, 1890). Frenzel (1897) then created *Mastigella* as a vehicle for species with similar characteristics but no (direct) connection between the nucleus and the

flagellum and *Mastigina* for flagellated species that had a limax shape and few lateral pseudopodia. These three genera have historically been described collectively as “mastigamoebids” (i.e., hyaline amoeboid flagellates), though the composition of this informal group has recently changed (see “[General Characteristics](#)”, above). Subsequent revision of *Mastigina* from Goldschmidt (1907a) added an apical, spherical nucleus as a distinguishing criterion, as well as fountain-flow cytoplasm, though this is not present in all described species of *Mastigina* (Frenzel 1897) and is present in species of other genera that had already been described at that time, e.g., *Pelomyxa palustris*, *Mastigamoeba aspera*, and *Tricholimax hylae* (Frenzel 1897; Greeff 1874; Leidy 1879; Schulze 1875a, b). This had the effect of narrowing the circumscription of *Mastigamoeba* to include only hyaline amoeboid flagellates with a connection between the flagellum and the nucleus, with lateral pseudopodia, and with elongated nuclei. *Tricholimax hylae* (the sole species of *Tricholimax*) was then also treated as belonging to *Mastigina* (Brugerolle 1982; Brugerolle and Patterson 2000; Collin 1913; Goldschmidt 1907a; Wickerham and Page 1970), leading to some confusion about the distinguishing criteria of *Mastigina* (Frolov 2011).

Through the twentieth century, over 200 nominal species of *Mastigamoeba* and *Mastigella* were created on the basis of shape and size, pseudopodial form, and contractile vacuole number and location (e.g., Goldschmidt 1907a, Lemmermann 1914; see list of names in Ptáčková et al. 2013). Early studies of life cycles (Goldschmidt 1907a, b) and more recent culture-based studies (Bernard et al. 2000; Chystjakova et al. 2012; Ptáčková et al. 2013; Simpson et al. 1997; Walker et al. 2001; Zadrožilková et al. 2015) have shown that a single individual over time can express a very wide range of size, mode of amoeboid movement, gliding, swimming, and/or pseudopodial form, meaning that many previously described species are difficult to recognize with any confidence. However, recent molecular phylogenetic work has demonstrated that the ephemeral characteristics previously used to distinguish species can be used successfully as taxonomic characters, given a sufficiently detailed description (Ptáčková et al. 2013; Zadrožilková et al. 2015, 2016). Molecular phylogenetics has also shown that there are two main clades within *Mastigamoeba*, which have different ultrastructural characteristics (Pánek et al. 2016; see below).

What are now the type species of *Endolimax* and *Iodamoeba* were described in 1917 and 1912, respectively, as two *Entamoeba* species from humans (originally *Entamoeba nana* and *Entamoeba buetschlii*, respectively). *Endolimax* was created in 1917 (Kuenen and Swellengrebel 1917), and *Iodamoeba* in 1919 (Dobell 1919). Species have subsequently been assigned to each genus mostly based on morphology, with some debate about the degree of host specificity leading to fluctuating numbers of nominal species. Recent molecular phylogenetic studies suggest that the diversity encompassed by these descriptions is highly inconsistent from one nominal species to the next. Both genera are in need of revision (Stensvold et al. 2012; Poulsen and Stensvold 2016).

Species description in *Pelomyxa* has followed a similar pattern to that of *Mastigamoeba*. The amoeba now known as *Pelomyxa palustris* was first found by Greeff in 1870 and was named *Pelobius* (a name already occupied by an insect); it

was subsequently redescribed in more detail under its present name, *Pelomyxa palustris* (Greeff 1874). Greeff recognized that this “new, large freshwater rhizopod” was distinctively different from members of the genus *Amoeba* and emphasized the great morphological variability of the species. Leidy (1879) was also aware of this variability and suggested that the different forms of *Pelomyxa* might all be shown later to be different stages in the life cycle of the same species. During the twentieth century, various authors assigned numerous species to *Pelomyxa* (e.g., Penard 1902), but the prevailing view by the end of the twentieth century was that there was one or a few highly polymorphic species (Whatley and Chapman-Andresen 1990). Subsequently, as studies have employed light and electron microscopy and molecular phylogenetics, the number of species has begun to increase again (Berdieva et al. 2015; Chystjakova and Frolov 2011; Chystjakova et al. 2014; Frolov et al. 2004, 2005a, b, 2006, 2011; Ptáčková et al. 2013). The concept of *Pelomyxa* as a flagellated amoeba is relatively recent (Griffin 1979, 1988), but its similarities to mastigamoebids have long been noted (Bütschli 1880; Goldschmidt 1907a; Kudo 1939; Page 1970; Penard 1936; Schulze 1875b).

The genus *Endamoeba* was created in 1879 by Leidy for his newly described species, *Endamoeba blattae*, from cockroaches. Without noticing this, Casagrandi and Barbagallo (1895) created the genus *Entamoeba* and transferred into it the human parasitic species known previously as *Amoeba coli*. Since the names *Entamoeba* and *Endamoeba* are very similar, they were often confused, leading to their homonymization and the formal suppression of *Entamoeba* in 1928. The human pathogen *Entamoeba histolytica* can be, therefore, found under name *Endamoeba histolytica* in the older literature. This concept was, however, challenged by many authors (e.g., Kirby 1945) who argued that *E. blattae* and *E. coli* were not congeneric. The *Entamoeba/Endamoeba* problem was settled in 1954 when the International Commission on Zoological Nomenclature refuted the homonymization. Since that time, *Entamoeba* and *Endamoeba* have universally been considered distinct genera. The phylogenetic placement of *Endamoeba* remains unknown: although it is assumed to be related to *Entamoeba*, the cases of *Endolimax* and *Iodamoeba* now being placed in Mastigamoebidae suggest that *Endamoeba*'s position should be regarded as *incertae sedis* for now.

Since *Entamoeba histolytica* is an important human parasite, it is by far the best-known member of the Archamoebae. It was first reported by Lösch in 1875 and has since been intensively studied (see Martínez-Palomo (1993) and Wenyon (1926) for pertinent citations). The research into cell biology and biochemistry of *Entamoeba* was greatly facilitated when methods of its axenic culture became available (Diamond 1961). *Entamoeba histolytica* was one of the first putatively amitochondriate eukaryotes whose mitochondrial derivative (mitosome in this case) was discovered (Tovar et al. 1999). The genome sequence of *E. histolytica* was published more than 10 years ago (Loftus et al. 2005). Genome sequences of several other *Entamoeba* species are currently available (<http://amoebadb.org/amoeba/>). Several hundred papers on *E. histolytica* have been published annually during the last few years.

The genus *Rhizomastix* was described in 1911 by Alexeieff for his species *Rhizomastix gracilis* from the intestines of an axolotl. *Rhizomastix gracilis* was soon found also in larvae of crane flies (Mackinnon 1913), and its morphology was studied in detail by Ludwig (1946). Although a few additional *Rhizomastix* species have been described from insects and amphibians (see Čepička (2011) and Zadrožilková et al. (2016) for pertinent references), the phylogenetic position of this genus remained unclear until the recent molecular phylogenetic study of Ptáčková et al. (2013), which showed that *Rhizomastix* belongs to Archamoebae. The diversity of the genus has recently been studied using cultures and molecular phylogenetics (Zadrožilková et al. 2016; Pánek et al. 2016).

Practical Importance

Endobiotic Taxa Most described endobiotic Archamoebae are poorly known, and their relationships with their hosts have not been determined. Nevertheless, most species are probably harmless commensals.

The situation is much clearer in case of the few human symbionts. Several species have been reported from the intestine of humans (*Entamoeba histolytica*, *E. coli*, *E. dispar*, *E. polecki*, *E. hartmanni*, *E. moshkovskii*, *Endolimax nana*, and *Iodamoeba buetschlii*) and oral cavity (*Entamoeba gingivalis*). Although most of them are considered non-pathogenic, *Entamoeba histolytica* is an important human pathogen, which is responsible for 500 million new cases and 100,000 deaths annually. The disease caused by *E. histolytica* is called amebiasis or amebic dysentery and is distributed worldwide, though most cases are reported in developing countries. Amebiasis is also commonly diagnosed in travelers returning from tropical countries. The infection by *E. histolytica* usually occurs by ingestion of cysts with food or water. The primary site of infection is the large intestine where the amoebae may invade the mucosa and cause colitis that itself may be lethal. Occasionally, the amoebae spread via blood to various internal organs (usually the liver), and extra-intestinal amebiasis (with significant mortality) develops. For more information on amebiasis, see Martínez-Palomo (1993) and Haque et al. (2003).

Entamoeba dispar is morphologically indistinguishable from *E. histolytica* and was historically considered its non-pathogenic variant. It has been, however, shown that the two organisms represent separate species (see Diamond and Clark 1993). The pathogenicity of *E. moshkovskii* is currently under debate (Heredia et al. 2012). *Entamoeba invadens* is known to cause severe reptilian amebiasis (see Reavill and Schmidt 2010).

Endolimax has been considered to be pathogenic by some authors, possibly causing diarrhea, intestinal inflammation, polyarthritis, or urticaria, but evidence for this is inconclusive (Poulsen and Stensvold 2016).

Free-Living Taxa Pelobionts and rhizomastixids are of unknown practical importance. While they have a worldwide distribution, they are not known to contribute to

any specific agricultural or pathogenic processes. They have historically been regarded as extremely “primitive” or deep-branching eukaryotes but are no longer regarded as such (see “[Evolutionary History](#)”, below).

Habitats and Ecology

Occurrence/Distribution in Nature

Free-living pelobionts and rhizomastixids are found in the upper layers of mud or sand, mostly in freshwater rivers, lakes, bogs, or pits, and usually in stagnant or near-stagnant water, which creates a low-oxygen environment. The ideal place to find them is among undisturbed algal growth at the water-sediment interface, in shallow low-oxygen ponds (about 10–20% atmospheric oxygen) with relatively low pH (Bernard et al. 2000; Chystjakova et al. 2012; Frolov 2011; Ptáčková et al. 2013; Whatley and Chapman-Andresen 1990). Several papers have described them from sewage treatment works (e.g., Lackey 1923, 1932). Some taxa have been also described from low-oxygen intertidal marine sediments (Bernard et al. 2000; Bovee and Sawyer 1979; Larsen and Patterson 1990; Lee and Patterson 2000; Page 1983; Simpson et al. 1997; Shiratori and Ishida 2016; Zadrožilková et al. 2015).

Pelobionts have been described from all over the world (e.g., Larsen and Patterson 1990; Lee and Patterson 2000). The majority of reports in the literature are from temperate Europe and North America, due to the intensity of study in these locations in the last 200 years.

Endobiotic Archamoebae are widely distributed among various vertebrates and invertebrates. However, host specificity of particular species is only poorly understood and has been partially elucidated only in human parasites. For example, humans seem to be the primary host of *Entamoeba histolytica* though it has also been isolated from nonhuman primates and dogs. Similarly, *Entamoeba coli*, another species from the intestines of humans, can infect also nonhuman primates, dogs, and marsupials (see Thompson and Smith 2011). *Entamoeba moshkovskii* is most probably both endobiotic and free-living (Heredia et al. 2012; our observations).

Characterization and Recognition: Light-Microscopical Features

Recognizing Archamoebae

Most of the nominal species among Archamoebae have been described from a few cells seen on a single occasion (e.g., Calaway and Lackey 1962; Lackey 1923; Larsen and Patterson 1990; Penard 1902, 1909; Stokes 1886, 1888, 1890; Skvortzkov and Noda 1976). Many species can exist as flagellates, uninucleate amoebae, multinucleate amoebae, and cysts, a situation referred to as polymorphism.

Also, individual amoebae and flagellates change their appearance and are therefore pleomorphic. As most well-studied pelobionts are both polymorphic and pleomorphic, it would be tempting to assume that only static taxonomic characters that transcend any ephemeral changes can be used to distinguish species, such as the spines on the outside of *Mastigina setosa* (Goldschmidt 1907a), the extranucleolar “dot” in *Mastigamoeba punctachora* (Walker et al. 2001), or the doubled nucleus in *Mastigamoeba schizophrenia* (Simpson et al. 1997). But recent molecular work combined with observations of cultures (Ptáčková et al. 2013; Zadrožilková et al. 2015, 2016) confirms that – while transcendent characters make distinctions easier – ephemeral characters such as pseudopodial shape do correlate with molecular phylogenetic differences. Under these circumstances, it is important to not only observe variation in size and shape, flagellar length, speed of swimming, cytoplasmic movement, or separation into inner and outer layers but also to observe what percentage of the time characters are expressed.

General Appearance of the Cell

Archamoebae are chiefly recognized by being obviously amoeboid or amoeboid flagellates but are differentiable from other, similar taxa (e.g., myxomycete or protostelid swimmers, breviate, cercozoans, lobose amoebae) by having hyaline (clear) cytoplasm, which gives rise to “eruptive” or “bulging” hemispherical pseudopodia, usually at the anterior end of a moving cell (Fig. 1). The range of pseudopodial shapes reported in the literature also includes other morphologies – lateral or trailing pseudopodia may be rarely fine and filose or, more often, tapering finger shaped, broadly conical, or broadly lobate (e.g., Chystjakova et al. 2012; Frolov 2011; Frolov et al. 2004, 2006; Ptáčková et al. 2013; Zadrožilková et al. 2015, 2016). A uroid is frequently present at the posterior end of the cell; though in some taxa, particularly *Mastigamoeba simplex* and other members of the “Mastigamoebidae B” clade, and some *Mastigina* species, trailing pseudopodia are present instead (e.g., Goldschmidt 1907a; Pánek et al. 2016; Walker et al. 2001). *Rhizomastix* displays various pseudopodial shapes (Čepička 2011; Ptáčková et al. 2013; Zadrožilková et al. 2016). *Pelomyxa* has larger cells, and short flagella, but has many of the amoeboid characteristics seen in *Mastigamoeba*, as do members of *Mastigella* (Zadrožilková et al. 2015). Cells of *Entamoeba* spp. move by eruptive, clear lobopodia, though trailing filaments may be rarely formed. Although *Endolimax* is superficially similar to *Entamoeba*, its cells often form short tapering pseudopodia, similar to those seen in some species of *Mastigamoeba* (see Figs. 1, 3, and 4).

In some archamoebae, the cytoplasm is arranged in a distinct separation between “endoplasm” (interior of the cell body, where organelles are located) and the “ectoplasm” – a clear peripheral layer of cytoplasm, directly underneath the plasma membrane, which remains distinct from the agglomeration of nuclei, vacuoles, and endomembrane system in the center of the cell. This is the layer from which eruptive

pseudopodia form, into which the central cell contents then roll. The differentiation of ectoplasm has been described in mastigamoebids, including *Mastigamoeba scholaia* (Klug 1936), *Mastigella nitens* (Penard 1909), and *Mastigina setosa* (Goldschmidt 1907a; Skibbe and Zöllfel 1991).

The ectoplasm may also be involved in “fountain flow” cytoplasmic streaming, as the outer part of the cytoplasm that runs backward, relative to the forward motion of the middle of the cell. Cytoplasmic streaming has mostly been described in *Pelomyxa* (Blochmann 1894; Hollande 1945; Rhumbler 1905; inter alia) but has also been reported in *Mastigamoeba aspera* (Chystjakova et al. 2012; Schulze 1875b), *Tricholimax hylae* (Becker 1928; Brugerolle 1982; Collin 1913; Frenzel 1897), and *Mastigina setosa* (Goldschmidt 1907a; Skibbe and Zöllfel 1991).

Polymorphism and Life Cycle

Polymorphism is well-documented and a defining feature in pelobiont Archamoebae, as well as being necessary for completion of the life cycle in parasitic entamoebae. Life cycles have also been studied in several species. However, the actual cytological processes accompanying changes from one form to another remain mostly unknown, except in *Entamoeba* (Frolov 2011).

Recent descriptions of members of *Mastigamoeba*, *Rhizomastix*, and *Mastigella* have usually employed laboratory culture conditions that do not permit a study of the life cycle under normal environmental conditions. The general picture is that mastigamoebid Archamoebae can produce some or all of the following stages: amoeboid flagellates, aflagellate amoebae, large multinucleate amoebae, and cysts. All of these forms have been documented in detailed studies of *Mastigamoeba balamuthi* (Chavez et al. 1986; Pánek et al. 2016) and *M. schizophrenia* (Simpson et al. 1997), while flagellates and amoebae have been documented in *M. aspera* (Chystjakova et al. 2012), *M. simplex* (Bernard et al. 2000), *M. punctachora* (Bernard et al. 2000), *M. lenta*, *M. abducta*, *M. guttula*, *M. errans*, and *Rhizomastix libera* (Ptáčková et al. 2013); *Mastigella erinacea* and *Mastigella rubiformis* (Zadrobílková et al. 2015); flagellates and cysts in *Mastigella nitens* (Frolov 2011), *Rhizomastix bicornata*, *R. tipulae*, and *R. elongata* (Zadrobílková et al. 2016); and flagellates, amoebae, and cysts in *R. vacuolata* (Zadrobílková et al. 2016).

Earlier studies of members of *Mastigamoeba* and *Mastigella* (e.g., Frenzel 1897; Goldschmidt 1907a; Schulze 1875b) employed microcosms rather than typical modern laboratory culture conditions, mimicking normal environmental conditions and allowing some observations of succession of polymorphism, from which life cycle details could be inferred. Flagellates reproducing by mitosis, aflagellate amoebae, and large, multinucleate division stages giving rise to uninucleate cells were documented in *Mastigella vitrea* and *Mastigina setosa* (Goldschmidt 1907a, b).

A series of studies of *Pelomyxa* species has also employed microcosms as a culture method, permitting extensive observation of the life cycle of natural populations (Whatley and Chapman-Andresen 1990; Frolov 2011). *Pelomyxa palustris* progresses in spring from cysts containing four nuclei, to small binucleate amoebae (100 µm long), then to larger multinucleate amoebae with or without endosymbionts, and then in summer to large elongate multinucleate amoebae (up to 5 mm long) with 30–60 nuclei, endosymbionts, flagella, and a posterior uroid. In autumn, they progress to large, spherical amoeboid cells with bacteria around the thousands of nuclei; these giant cells then divide by plasmotomy to form cysts or small amoebae over winter (Frolov 2011; Frolov et al. 2007; Schirch 1914; Whatley and Chapman-Andresen 1990). Other life-cycle descriptions exist for *P. corona* (Frolov et al. 2004), *P. gruberi* (Frolov et al. 2006), *P. binucleata* (Frolov et al. 2005a; Frolov 2011), *P. flava* (Frolov et al. 2011), and *P. paradoxa* (Chystjakova et al. 2014).

Cysts

The structure of the cyst has been best characterized in *Entamoeba invadens* (see Samuelson and Robbins 2011). The cyst wall is composed mainly from chitin and various glycoproteins. Cysts of particular *Entamoeba* species are rounded and differ in the number of nuclei present, e.g., the uninucleate *E. polecki*; *E. histolytica*, *E. hartmanni*, and *E. marina* with four nuclei; and *E. coli* and *E. muris* with up to eight nuclei (recently summarized in Shiratori and Ishida 2016). During the encystation of *Entamoeba* trophozoites, so-called chromatoid bodies are formed in the cytoplasm. These are elongated bars with rounded ends and are formed by ribosomes arranged in helices. The chromatoid bodies disappear from the older cysts (Rosenbaum and Wittner 1970; Schuster 1990).

Apart from *Entamoeba* spp., cysts with various numbers of nuclei have been documented in some other members of Archamoebae: uninucleate cysts in *Mastigamoeba balamuthi* (Chavez et al. 1986), *M. punctachora* (Bernard et al. 2000), *M. schizophrenia* (Simpson et al. 1997), and *Iodamoeba buetschlii*, binucleate cysts in *Rhizomastix* spp. (Čepička 2011; Zadbílková et al. 2016), and four-nucleated cysts in *Endolimax* spp. and *Pelomyxa palustris* (Frolov 2011; Frolov et al. 2007; Griffin 1988; Ptáčková et al. 2013; Whatley and Chapman-Andresen 1990). The cysts of *Endamoeba* are multinucleate. *Tricholimax* varies between two and four nuclei according to the age of the cyst (Collin 1913). The composition of the cyst wall of non-*Entamoeba* Archamoebae is still unknown. In *Pelomyxa*, the cyst has a central vacuole containing endosymbionts, and the cyst wall has three layers. The laminated structure of cyst walls would be better revealed by cryofixation techniques, which have not yet been employed, than by chemical fixation, which has been used hitherto except on cysts of *Rhizomastix* (Zadbílková et al. 2016). The cyst of *Iodamoeba buetschlii* contains a characteristic glycogen-containing inclusion in the cytoplasm (see Fig. 5).

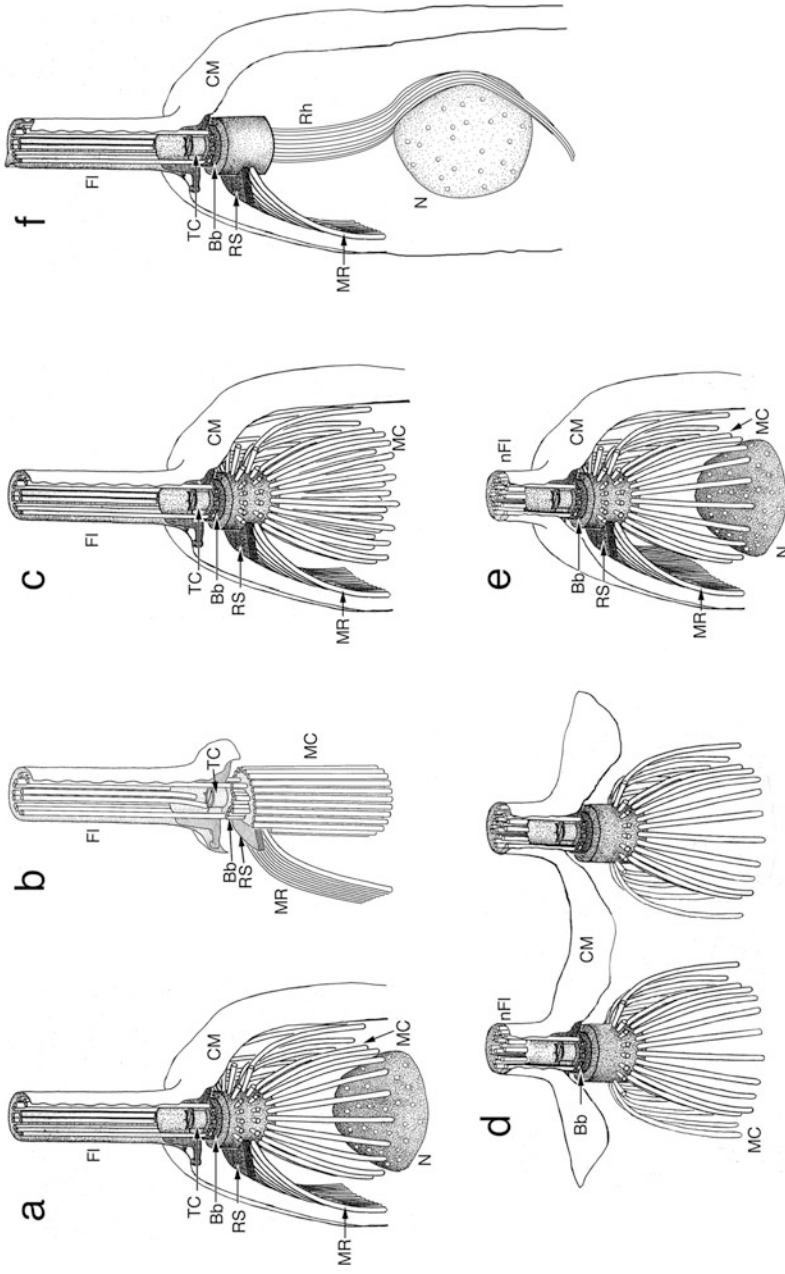


Fig. 2 Flagellar apparatus ultrastructure defines the identities of genera of flagellated Archamoebae. The features common to all flagellated Archamoebae include a single basal body (*Bb*), from which arises a flagellum (*FI*) and a cone (*MC*) or rhizostyle (*Rh*) of microtubules, and (in most taxa) a

Ultrastructure

Flagellar Cytoskeleton

Flagella in Archamoebae appear to have degenerated in several groups independently, either to structures with dysfunctional axonemes or to the complete absence of a flagellar apparatus (discussed in Zadrožilková et al. 2015). Members of *Entamoeba*, *Endamoeba*, *Endolimax*, and *Iodamoeba* have no flagellar apparatus (El-Hashimi and Pitman 1970; Morris 1936; Rosenbaum and Wittner 1970; Zaman et al. 1998, 2000) and are not referred to further in the sections below.

Flagellated Archamoebae have a relatively simple microtubular cytoskeleton (see Fig. 2). Among the flagellated taxa that have been studied by TEM (see Table 1), the flagellar apparatus consists of one or more “monokinetids” – single, flagellated basal bodies – giving rise proximally to a cone or cylinder of microtubules, as well as (in most taxa) a lateral root of microtubules with a bilaminar “root sheet” at the origin of the root on its distal face. Members of *Mastigamoeba*, *Mastigella*, *Mastigina*, *Tricholimax*, and *Rhizomastix* typically have only one flagellum per cell, though the type species of *Mastigella*, *M. polymastix*, has up to four flagella per cell (Frenzel 1897) and *Tricholimax* has multiple nuclei, each with its own attached (internal) kinetid (Becker 1925; Brugerolle 1982, 1991; Collin 1913). The flagellar apparatus in *Mastigamoeba* varies between a wide distribution of microtubules, seen in Mastigamoebidae “A” (as defined in Ptáčková et al. 2013, Pánek et al. 2016; see also Table 1 and Figs. 2a and 3), and a very narrow single layer of microtubules, seen in members of Mastigamoebidae “B” (as defined in Ptáčková et al. 2013; Pánek et al. 2016; see also Table 1 and Figs. 2b and 3).



Fig. 2 (continued) transitional cylinder (*TC*). In most cells, the flagellar apparatus is usually directed perpendicularly to the cell membrane (*CM*), but the cone of microtubules can sometimes run parallel to it. (a) *Mastigamoeba* has a microtubular cone (*MC*) that connects to the nucleus; a microtubular root (*MR*) also arises from the basal body, immediately proximal to a bilaminar root sheet (*RS*); typically there is only one monokinetid flagellum per cell. This shows a representative flagellar apparatus from the group “Mastigamoebidae A” where the cone of microtubules (*MC*) arises laterally from both the sides and the base of the basal body; the flagellar transition zone is long. *Fl* flagellar axoneme, *TC* transition zone cylinder, *Bb* basal body. *RS* bilaminar root sheet, *MR* microtubular root. (b) *Mastigamoeba* showing a representative of group “B” where the cone of microtubules (*MC*) arises longitudinally from near the base of the basal body, and the flagellar transition zone (*TZ*) is short and contains no extra elements. (c) *Mastigella* has a microtubular cone that does not connect to the nucleus. There may be up to four long flagella per cell in some species. (d) *Pelomyxa* has numerous nonfunctional, short, monokinetid flagella (*nFl*) with “n + n” arrangement of microtubules. There is no obvious microtubular root or root sheet. (e) *Tricholimax* has a flagellar apparatus similar to that of *Mastigamoeba*, but it has nonfunctional “n + n” flagella (*nFl*), and it frequently has several nonflagellated monokinetids connected to nuclei in the cytoplasm. (f) *Rhizomastix* has a rhizostyle (*Rh*) – a tapering bundle of microtubules that extends from the base of the basal body, to wrap around the nucleus. Its flagellum has two vanes, which may contribute to its fast, jerky swimming movement. Scale bar = 250 nm (Figures (a) and (c) redrawn with modifications from Walker et al. 2001, and Figure (b) reproduced from Walker et al. 2001 with permission from Elsevier)

Table 1 Flagellar apparatus details for taxa described by TEM. Further details are provided in the taxonomic section. Where a value is given as “?”, there is no data for that feature in a published account. Where a value is given as “(?)”, the interpretation of data here differs from that given in the published account

Larger group/species	Flagellum	9 + 2 axoneme	Dynein arms missing	Transition zone length	Transitional dense column or spiral	Transition zone cylinder	Cartwheel in base of Bb	Bb length	Lateral root of MT	Bilaminar root sheet	MT of cone emerging from sides of Bb	MT of cone emerging from Bb around Bb	Electron-dense material around Bb	Electron-dense MTOC below Bb	References
Rhizomastixidae															
<i>Rhizomastix elongata</i>	Long (1 × cell length)	?	?	“Short”	Spiral	Yes	?	250 nm	Yes	No	No	Rhizostyle	No	Dense material at base	Zadobilková et al. (2015)
<i>Rhizomastix libera</i>	Long (1 × cell), with vanes	9 + 2	?	“Short”	?	Yes (?)	?	250 nm	Yes	No	No	Rhizostyle	No	Dense material at base	Pláčková et al. (2013)
Mastigamoebidae “A”															
<i>Mastigamoeba aspera</i>	Long (>2 × cell)	9 + 2	?	“Short”	?	?	?	250 nm	Yes	?	Many of cone MT at sides	Most of cone	No	Triangle	Chystjakova et al. (2012)
<i>Mastigamoeba balamuthi</i>	Long (>2 × cell)	9 + 2	Outer ones	700 nm	DC	Yes	?	250 nm	Yes	Yes	Most of cone arises at sides	Few	No	No	Chavez et al. (1986), Brugerolle (1991), Pánek et al. (2016)
<i>Mastigamoeba punctachora</i>	Long (>2 × cell)	9 + 2	Outer ones	1000 nm	DC	Yes	No	250 nm	Yes	Yes	Most of cone arises at sides	Few	No	Possibly?	Bernard et al. (2000), Walker et al. (2001)
<i>Mastigamoeba schizophrenia</i>	Long (>2 × cell)	9 + 2	Outer ones	700 nm	DC	Yes	Yes	250 nm	Yes	Yes	Single layer arises at base, lateral	No	No	No	Simpson et al. (1997)
<i>Mastigamoeba</i> sp.	Long (>2 × cell)	9 + 2	?	700 nm	Spiral + central filament	Yes	?	250 nm	Yes	Yes	Single layer arises at base, lateral	No	No	Ring	Brugerolle (1991)
Mastigamoebidae “B”															
<i>Mastigamoeba simplex</i>	Long (>2 × cell)	9 + 2	Outer ones	200 nm	No	Yes	Yes	250 nm	Yes	Yes	Single longitudinal-axis layer	No	No	No	Walker et al. (2001)
<i>Mastigamoeba guttula</i>	Long (>2 × cell)	9 + 2	?	200 nm	No	Yes	?	250 nm	Yes	Yes	Single longitudinal-axis layer arises at base	No	No	No	Pláčková et al. (2013), Pánek et al. (2016)

Pelomyxididae													
<i>Mastigella commutans</i>	Long (>2 × cell)	9 + 2	Outer ones	200 nm	No	Yes	No	250 nm	Yes	Yes	No	No	Walker et al. (2001)
<i>Mastigella rubiformis</i>	Long (>1 × cell)	9 + 2	Outer ones	200 nm	?	?	?	250 nm	Yes	Yes	No	No	Zadrobilková et al. (2015)
<i>Pelomyxa palustris</i> (short group ¹)	Short (ca. 10 µm)	n + n	All arms	200 nm	No (?)	Yes	?	250 nm	No (?)	?	No	Ring	Seravin and Goodkov (1987a, b, d), Griffin (1988), Goodkov and Seravin (1991), Frolov (2011), Ptáčková et al. (2013)
<i>Pelomyxa belevskii</i> (short group)	Short (ca. 10 µm)	?	All arms	?	?	?	?	500 nm	?	?	No	Probably	Frolov (2011), Ptáčková et al. (2013)
<i>Pelomyxa hinculeta</i> (short group)	Short (ca. 10 µm)	n + n	All arms	200 nm	No	Yes	No	200 nm	?	?	No	Ring	Frolov et al. (2005a), Frolov (2011)
<i>Pelomyxa coronata</i> (?) (group)	?	?	All arms	?	?	?	?	?	?	?	?	?	Frolov et al. (2004)
<i>Pelomyxa flava</i> (long group)	Short (ca. 10 µm)	n + n	All arms	250 nm (?)	Spiral + central filament	No (?)	Yes (?)	700 nm	Yes	Yes	No	No	Frolov et al. (2011)
<i>Pelomyxa graberi</i> (long group)	Short (ca. 10 µm)	2n + n	All arms	400 nm	DC	Yes	?	600 nm (?)	Yes (?)	Yes, 1/3	Yes	No	Frolov et al. (2006), Frolov (2011)
<i>Pelomyxa stagnalis</i> (short group)	?	n + n	All arms	200 nm	No	No (?)	?	150 nm	?	?	No	?	Chystjakova and Frolov (2011); Ptáčková et al. (2013)
<i>Pelomyxa paradoxa</i> (intermediate form)	Medium (20–50 µm)	?	?	?	?	?	?	200 nm	Yes	Yes (?)	Yes	No	Chystjakova et al. (2014)
<i>Pelomyxa prima</i> (long group)	Short (ca. 10 µm)	?	All arms	?	?	?	?	700 nm	?	?	Yes	Triangle	Frolov et al. (2005b), Frolov (2011)

(continued)

Table 1 (continued)

Larger group/ species	Flagellum	9 + 2 axoneme	Dynein arms missing	Transition zone length	Transitional dense column or spiral	Transition zone cylinder	Cartwheel in base of Bb	Bb length	Lateral root of MT	Bilaminar root sheet	MT of cone emerging from Bb base	Electron- dense material around Bb	Electron- dense MTOC below Bb	References
<i>Pelomyxa secundata</i> (?) group)	None seen	?	?	?	?	?	?	?	?	?	?	?	?	Berdieva et al. (2015)
<i>Pelomyxa schiedii</i> (probable short group)	Short (ca. 5 µm)	9 + 1	All arms	?	?	?	?	200 nm	Yes	?	?	No?	?	Zadrobilková et al. (2015)
<i>Pelomyxa terita</i> (short group)	Short (ca. 10 µm)	?	All arms	?	?	?	?	600 nm	Yes (?)	?	Yes (?)	?	?	Frolov (2011)
Incertae sedis														
<i>Tricholimnax hylaе</i>	Short (ca. 10 µm)	2n + n	All arms	<400 nm	No	Yes (?)	?	500 nm	Yes	Yes	Many	No	Yes	Brugerolle (1982, 1991) (as <i>Mastigina hylaе</i>)

Bb single basal body, MT microtubules, MTOC microtubular organizing center

"Short group," "intermediate form," and "long group" refers to the categorization by basal body length and microtubular cone type, in Chyşjakova et al. (2014)

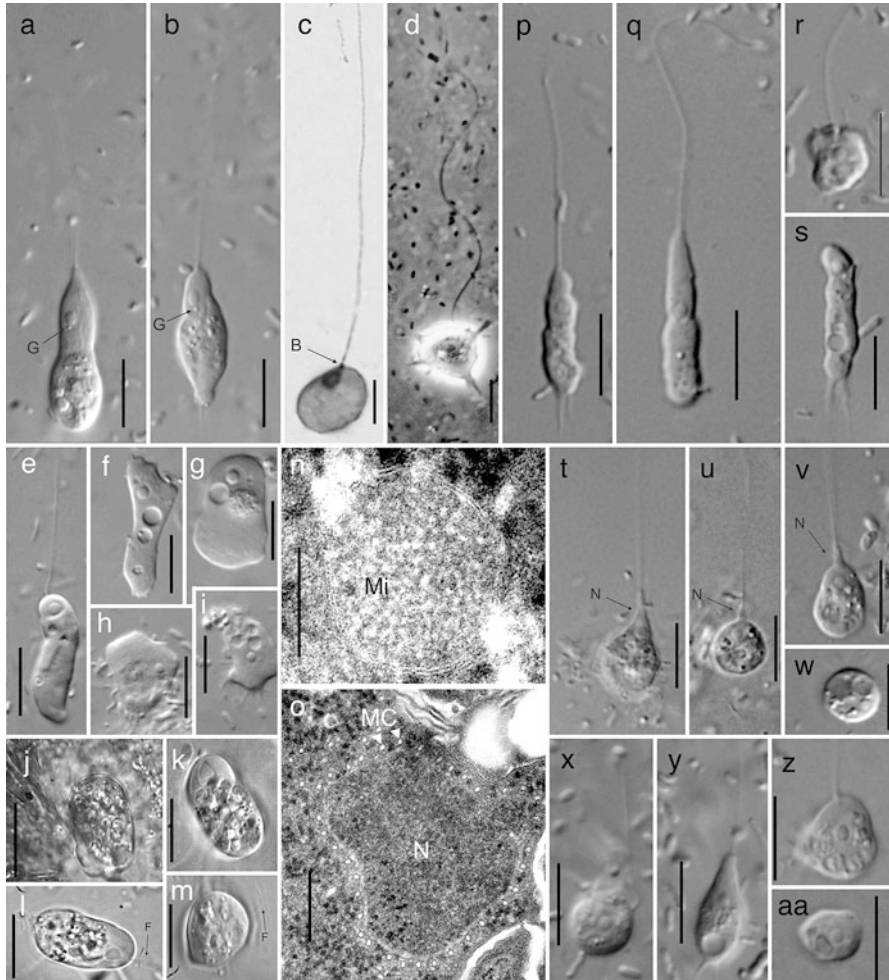


Fig. 3 *Mastigamoeba*. All images are differential interference contrast light microscopy (DIC), unless otherwise specified. **(a–o): Representatives of Mastigamoebidae A.** **(a–c)** *Mastigamoeba punctachora*, G = extranucleolar granule. **(c)** Bright field microscopy with protargol staining; B = basal body, immediately adjacent to the stained nucleus. **(d)** *Mastigamoeba balamuthi* swimming cell, phase contrast LM. **(e–i)** *Mastigamoeba errans*. In e, gliding flagellate cell; f–i, aflagellate cells. **(j–m)** *Mastigamoeba* sp. ex *Pelomyxa belevskii*. F = flagellum. In j, bright field light microscopy. **(n)** *M. punctachora* transmission electron microscopy. Double-membraned, mitochondrion-related organelle (Mi). **(o)** *M. punctachora* TEM. Nucleus (N) surrounded by microtubules of the cone (MC) cut in transverse section. **(p–aa): Representatives of Mastigamoebidae B.** **(p–s)** *Mastigamoeba simplex*. In p–r, gliding flagellates; in s, aflagellate cell. **(t–w)** *Mastigamoeba scholaia*. In t, u, gliding cells; in v, gliding aflagellate cell; in w, aflagellate cell. N = neck. **(x–aa)** *Mastigamoeba guttula*. In x–z, flagellates; in aa, aflagellate cell. Scale bars in a–c, e–i, p–aa = 10 µm; in d = 20 µm, in j–m = 50 µm, in n, o = 200 nm (Figures a–c, e–m, and p–aa all reproduced from Ptáčková et al. 2013 with permission from Elsevier)

Flagella are usually positioned at the anterior end of the cell and used in movement, though particularly amoeboid cells in *Mastigella* may move predominantly by pseudopodia, with the poorly functional flagellum pointing in a different direction (Frenzel 1897; Frolov 2011; Goldschmidt 1907a; Walker et al. 2001, Zadrožilková et al. 2015). The flagellum of *Mastigella* is generally slower beating and less motile than that seen in *Mastigamoeba* and may be an intermediate stage that gave rise to the extreme flagellar reduction seen in *Pelomyxa* (discussed in Zadrožilková et al. 2015).

Giant amoeboid cells of *Pelomyxa* have numerous separate monokinetids, bearing (usually) short, nonfunctional flagella with a disorganized axoneme. Typically, a microtubular cone is formed, but this varies between a dense cone and a single layer of microtubules, with or without a lateral root (Fig. 2d; see Table 1 for further details). The number of monokinetids varies between ca. 50 and ca. 700 in *P. palustris* (Griffin 1988). Likewise, their density varies more than tenfold (Griffin 1988), and their abundance and distribution over the cell's sides or uroid also varies (e.g., compare *P. flava*, *P. paradoxa*, and *P. stagnalis*; Chystjakova and Frolov 2011; Chystjakova et al. 2014; Frolov et al. 2011). Whether basal bodies form flagella or merely “buds” can vary widely through the life cycle (e.g., *P. paradoxa* vs. *P. gruberi*; Chystjakova et al. 2014; Frolov et al. 2006). Flagella are typically not found on anterior pseudopodia in *Pelomyxa* (Frolov 2011) and have not been seen at all in *P. secunda* (Berdieva et al. 2015).

For further details of the flagellar cytoskeleton, see Fig. 2 and Table 1.

Nuclei and Cell Division

Numbers of nuclei are one of the main features that distinguish “pelomyxid” Archamoebae from “mastigamoebid” or “entamoebid” Archamoebae. In many pelomyxids, the usual state is cells with tens or hundreds of nuclei, whereas in other Archamoebae, the usual state is one or few nuclei, though stages with tens of nuclei may exist.

A uninucleate flagellate is the main trophic form in *Mastigamoeba*, some members of *Mastigella*, *Mastigina*, and *Rhizomastix*. Reproduction of uninucleate flagellates by mitosis has been documented only in *Mastigella* and *Mastigina* (Goldschmidt 1907a, b). The nuclei of *Mastigamoeba schizophrenia* appear in “pairs” of two adpressed nuclei, but the extent of differentiation within these pairs is unknown (Simpson et al. 1997). Some “flagellate” taxa may typically have more than one nucleus, such as *Tricholimax hylae*, which has 1–6 (Becker 1925; Brugerolle 1982, 1991; Collin 1913; Frenzel 1897), and members of *Mastigella* (Zadrožilková et al. 2015).

As discussed above, a few “flagellate” species also have distinct, large amoeba stages with more than one nucleus: *Mastigamoeba schizophrenia* has 2–10 (i.e., 1–5 pairs; Simpson et al. 1997); *M. punctachora* has 1–8 (Bernard et al. 2000); and *M. aspera* has two or more nuclei in its amoeba form, which would previously have

been described as a different species, *Dinamoeba mirabilis* (see Chystjakova et al. 2012). In at least one such species, *Mastigamoeba balamuthi*, the multinucleate amoebae (with on average 15 nuclei but up to 46), are the dominant trophic stage, reproducing while multinucleate by mitosis and subsequent plasmotomy (i.e., cytokinesis not preceded by mitosis), which results in large and asymmetric numbers of nuclei in daughter cells.

“Budding” plasmotomy, from multinucleate amoebae to uninucleate flagellates, only happens rarely and only under specific trophic conditions (Chavez et al. 1986). Production of small uninucleate flagellates, from a large, rounded amoeboid cell that is filled with smaller cells, has also been described from *Mastigina setosa* and *Mastigella vitrea* (Goldschmidt 1907a, b); whether this is a form of plasmotomy or more akin to sporogony is unclear. It resembles the processes described in *Pelomyxa palustris* (Frolov 2011; Whatley and Chapman-Andresen 1990).

In *Pelomyxa*, the main described trophic “adult” form is a large, multinucleate amoeba, which also has flagella (see above). This “flagellated” trophic form, typically a cylindrical amoeba, may in some cases later transform into an immotile, rounded amoeboid form with hundreds of nuclei (e.g., *P. corona*, *P. gruberi*; Frolov 2011; Frolov et al. 2004, 2006). It is unclear whether this rounded form is homologous to the large multinucleate amoebae seen in *Mastigamoeba schizophrenia* (Simpson et al. 1997) and *M. balamuthi* (Pánek et al. 2016) or to the large rounded forms that divide by plasmotomy in *Mastigella vitrea* and *Mastigina setosa* (Goldschmidt 1907a), as they are only rarely seen and have not been studied by electron microscopy. Species of *Pelomyxa* can be divided into taxa that have hundreds of nuclei in the adult motile form (e.g., *P. palustris*, *P. corona*, *P. prima*; Frolov 2011; Whatley and Chapman-Andresen 1990) and taxa with tens (e.g., *P. belevskii*, *P. stagnalis*; Frolov 2011; Ptáčková et al. 2013) or one or two nuclei in mature cells (e.g., *P. binucleata*, *P. flava*, *P. paradoxa*, *P. schiedti*; Chystjakova et al. 2014; Frolov 2011; Frolov et al. 2005a; Zadrožilková et al. 2015). Division from multinucleate adult cells to smaller juvenile cells is typically by unequal plasmotomy (Frolov 2011; Ptáčková et al. 2013; Schulze 1875a; Whatley and Chapman-Andresen 1990).

The trophozoites of *Entamoeba* spp. are uninucleate, and no multinucleate trophic forms are usually created. Mitosis of *Entamoeba histolytica* was studied, for example, by Solis and Barrios (1991) and by Chávez-Munguía et al. (2006).

Nuclear Chromatin

Patterns of nuclear chromatin appear to distinguish different species in Archamoebae, though there is not a clear taxonomic pattern above species level. Chromatin patterns can be a peripheral arrangement of small clumps of chromatin around the edge of the nucleus or a single large nucleolus or a few chromatin clumps joined together in the center of the nucleus (described further in Frolov 2011). Peripheral arrangements are common in *Entamoeba*, pelomyxids, and *Rhizomastix* (Čepička 2011; Chystjakova et al. 2014; Frolov 2011; Frolov et al. 2004, 2005a, b,

2006; Ptáčková et al. 2013; Zadrobílková et al. 2015, 2016) and are also present in “*Mastigina trichophora*,” *Mastigella eilhardi*, and *Mastigella nitens* (Frolov 2011; Penard 1909; Zadrobílková et al. 2015).

Nuclear chromatin is used as a diagnostic feature in *Entamoeba* species, with *E. histolytica*, *E. dispar*, and *E. marina* showing a fine peripheral ring with a small central dot, *E. hartmanni* showing a large central nucleolus and thick peripheral clumps of chromatin, and *E. coli* showing peripheral clumps only (Shiratori and Ishida 2016). The majority of members of *Mastigamoeba* have large nucleoli, as do *Tricholimax hylae*, *Pelomyxa palustris*, *P. gruberi*, *P. stagnalis*, and *Rhizomastix* species. In both *Iodamoeba* and *Endamoeba*, the nucleolus is large and central, and there is no peripheral ring of chromatin (see section “[Systematics and Taxonomy](#)”, below).

There are cajal-body-like stacked inclusions in the central nucleolus in *Pelomyxa stagnalis* (Chystjakova and Frolov 2011; Ptáčková et al. 2013), and *P. belevskii* has a loose stacked arrangement of “vermiform bodies” of chromatin around the edge of the nucleus, similar in size to the inclusion in *P. stagnalis* (Ptáčková et al. 2013).

Cell Coverings

As reviewed in Frolov (2011), most pelobionts are covered in a thin layer of amorphous or filamentous organic material. The extracellular layer may be irregularly, thinly distributed over the cell surface (e.g., Brugerolle 1982; Chavez et al. 1986; Simpson et al. 1997; Walker et al. 2001), often being thickest at the posterior end of the cell, particularly in pelomyxids (Frolov 2011). Finely filamentous cell coverings may be present, with filaments apparently perpendicular to the cell surface (e.g., *Pelomyxa palustris*, *P. gruberi*, *Mastigella nitens*: Frolov 2011) or parallel to the surface in the case of very thick coverings, which can reach up to 1 μm thickness (e.g., *P. flava*, *Mastigamoeba aspera*; Chystjakova et al. 2012; Frolov 2011; Frolov et al. 2011). These fine extracellular layers are rarely preserved well with chemical fixation for electron microscopy; cryofixation would be appropriate for further study.

Numerous species in pelobionts have been described with spines on the outside of the cell, with varying degrees of packing, from the dense, highly refringent layer of very tightly packed spines in *Mastigina chlamys* (Frenzel 1897; Seckt 1922), through regularly arranged but obviously discrete spines in *Mastigina setosa* (Goldschmidt 1907a; Skibbe and Zöllffel 1991), to irregular and loosely packed spicules in *Mastigamoeba trichophora* (Lauterborn 1901), *Mastigamoeba pilosa* (Schoudeten 1907), and *Mastigina spicata* (Penard 1909). Frolov (2011) shows electron micrographs of “*Mastigina trichophora*” where hollow spicules are each placed in “boat-shaped” scales, which are packed in a tight layer over the extracellular glycocalyx. It is unclear whether these spines are made only of organic material (Frolov 2011) or are mineralized as well, as seen in the euglenid *Trachelomonas* (Conforti et al. 1994; Preisig 1994).

Mitochondrial Remnants

Like other anaerobic groups in the eukaryotes, Archamoebae are “secondarily amitochondriate.” In almost all cases, such anaerobic eukaryotes retain reduced mitochondrion-related organelles (MROs) which have usually lost DNA, genome maintenance proteins, and a typical ATP-producing electron transport chain. They often still produce ATP by partial anaerobic oxidation of pyruvate (hydrogenosomes), but some have lost energy metabolic function entirely (mitosomes), preserving only a few other functions (such as aspects of mitochondrial type iron-sulfur cluster assembly machinery) and acquiring the ability to import ATP (Chan et al. 2005; Maguire and Richards 2014).

Both kinds of double-membrane-bound MROs have been described from Archamoebae (Barberà et al. 2007; Hampl and Simpson 2008). Mitochondrial *cpn60* function has been demonstrated in *Entamoeba histolytica*, and localized, to the mitosomes in *E. histolytica* (Chan et al. 2005; Clark and Roger 1995; Leon-Avila and Tovar 2004; Tovar et al. 1999), and to hydrogenosome-like organelles in *Mastigamoeba balamuthi* (Gill et al. 2007; Hampl and Simpson 2008). Iron-sulfur cluster assembly proteins are closely related to those of epsilon proteobacteria both in *Mastigamoeba balamuthi* (Gill et al. 2007) and *Entamoeba histolytica* (Maralíkova et al. 2010; van der Giezen et al. 2005), implying a single lateral transfer event where mitochondrial Fe:S assembly appears to have been lost entirely and replaced with an Fe:S assembly system that is not homologous to the standard mitochondrial system and is now cytosolic (Nývltová et al. 2013). The acquisition of alternatives to normal mitochondrial pyruvate dehydrogenase may have happened more than once, with *M. balamuthi* and *E. histolytica* each possessing copies of pyruvate ferredoxin/oxidoreductase (Embley and Martin 2006; Gill et al. 2007); but *M. balamuthi* also possesses a pyruvate formate lyase that was laterally transferred from a firmicute (Stairs et al. 2011). Mitosomes are extremely abundant in *E. histolytica* implying that some functions are yet to be uncovered in detail (Aguilera et al. 2008) – an example being the synthesis of sulfur compounds required for encystation (Mi-ichi et al. 2015). The MROs of Archamoebae are discussed in the context of other MROs by Maguire and Richards (2014).

Ultrastructural reports also exist for double-membrane-bound organelles in *Mastigamoeba simplex*, *Mastigamoeba punctachora*, and *Mastigella commutans* (Walker et al. 2001), *Mastigella rubiformis*, *M. ineffigiata* (Zadrobílková et al. 2015), *Endolimax piscium* (Constenla et al. 2013), *Rhizomastix libera* (Ptáčková et al. 2013), *R. elongata* (Zadrobílková et al. 2016), and *Pelomyxa palustris* (Seravin and Goodkov 1987c). As previously discussed (Zadrobílková et al. 2015), there is a notable lack of reports of MROs in EM studies of *Pelomyxa*. This may be because of the difficulty of serially sectioning a large amoeba, a misidentification of MROs as symbionts, or, alternatively, the real absence of MROs in all examined species of *Pelomyxa* except *P. palustris*.

Golgi

Canonical Golgi dictyosomes have not been reported in the Archamoebae so far, though related elements of the endomembrane system have been shown to be functionally present in *Entamoeba histolytica* (Bredeston et al. 2005) and *Mastigamoeba balamuthi* (Dacks et al. 2004), with all “core” functions being present (Mowbrey and Dacks 2009). However, methods for detecting endomembrane system function and Golgi dictyosomes are diverse, and many eukaryotes do not possess canonical Golgi stacks that are easy to recognize by transmission electron microscopy. Tantalizing reports exist suggesting that *Entamoeba* may possess Golgi stacks that are only visible with the application of cryofixation techniques (Chavez-Munguia et al. 2000; Ghosh et al. 2000). A similar situation may hold in pelobionts: a few reports exist of Golgi-like stacks of membranes, but in each case, these may be poorly fixed endoplasmic reticulum, and further ultrastructural work, using different techniques, is required. The multi-membrane organelle seen in *Rhizomastix libera* (Ptáčková et al. 2013) is positioned close to the flagellar base, as per the Golgi dictyosomes of other superficially similar amoeboid taxa with flagella (e.g., Heiss et al. 2013; Walker et al. 2003), but the published preservation of *Rhizomastix* is not sufficiently good for any clear identification of the organelle, and it is not seen in *R. elongata* under better fixation conditions (Zadrobílková et al. 2016). Similar, though bigger and more organized stacked structures have been reported from *Pelomyxa palustris* (Seravin and Goodkov 1987b) and *P. corona* (Frolov et al. 2004) as well as reticulate multimembranous organelles from *P. flava* (Frolov et al. 2011). These have subsequently been interpreted as endoplasmic reticulum (Frolov 2011), as have reports of stacked structures in *Mastigamoeba schizophrenia* (Simpson et al. 1997), *M. punctachora* (Walker et al. 2001), and *Rhizomastix elongata* (Zadrobílková et al. 2016).

Peroxisomes

While Archamoebae have traditionally been considered to lack peroxisomes (Žárský and Tachezy 2015), an unpublished thesis reports peroxisomal proteins in both *Mastigamoeba balamuthi* and *Entamoeba histolytica* (Žárský 2012), which would be consistent with secondary loss of peroxisomal function in Archamoebae.

Symbionts

The best-known symbionts of Archamoebae are the three morphologies of prokaryotes that surround the nuclei of *Pelomyxa* species (Berdieva et al. 2015; Chystjakova et al. 2014; Frolov 2011; Frolov et al. 2006; Whatley 1976; Whatley and Chapman-Andresen 1990). There is one large bacterium with a distinctive axial cleft and up to two smaller methanogenic archaea, one of which is known as *Methanobacterium formicicum* DSM3637 (Frolov et al. 2004, 2005a, b, 2006, 2011; Griffin 1988; van Bruggen et al. 1988; Whatley 1976). *Methanobacterium*

formicum DSM3637 was isolated from *P. palustris* (van Bruggen et al. 1983, 1988), and a draft of its genome has been published (Gutierrez 2012). Endobiotic prokaryotes also exist in *Mastigella* species (Frolov 2011; van Bruggen et al. 1985; Walker et al. 2001; Zdrobilková et al. 2015) and *Rhizomastix libera* (Ptáčková et al. 2013). The organic layer covering *Mastigamoeba aspera* also contains numerous small, rod-shaped prokaryotic ectobionts of uncertain identity (Chystjakova et al. 2012; Goldschmidt 1907a; Kudo 1950; Lemmermann 1914; Page 1970; Penard 1902, 1909, 1936; Schulze 1875b; Siemensma 1987).

The physiological role played by these endo- and ecto-symbionts has not been clearly demonstrated, but numerous cases exist of symbiosis between methanogenic archaea (which presumably derive hydrogen from within their hosts) and anaerobic eukaryotes with mitochondria that have been reduced to hydrogenosomes (Embley et al. 1995; Fenchel and Finlay 1995; Martin and Müller 1998; van Bruggen et al. 1983; van Hoek et al. 2000).

Endobiotic mastigamoebids have also been described as emerging from *Pelomyxa* (Greiff 1874; Hollande 1945; Whatley and Chapman-Andresen 1990); recent observations including sequence data confirm that this is not a life-cycle stage of *Pelomyxa* (Ptáčková et al. 2013).

Systematics and Taxonomy

Class Archamoebae Cavalier-Smith, 1983

Anaerobic/microaerophilic Amoebozoa with reduced mitochondria. May exist as amoebae, amoebflagellates, or cysts. Ancestrally with a single apical flagellum, arising from a single basal body that gives rise proximally to a microtubular cone and laterally to a microtubular root arising immediately proximal to a double-layered “root sheet” sitting perpendicular to the axis of the basal body. Secondarily aflagellate or multiflagellate, with disordered flagellar axoneme, or with no flagellar apparatus. Amoeboid movement with eruptive lobopodia. Free-living or endobiotic.

Order Pelobiontida Page, 1976

For the current composition, see Pánek et al. (2016). The clade including mastigamoebids, pelomyxids, and rhizomastixids; using the branch-based definition introduced and discussed by Pánek et al. (2016), the clade consisting of *Mastigella eilhardii* Bürger 1905 and all organisms that share a more recent common ancestor with *M. eilhardii* than with *Entamoeba histolytica* Schaudinn 1903.

Suborder Mastigamoebina Frenzel, 1897

Rank changed from order to suborder by Pánek et al. (2016). The clade including Mastigamoebidae and Rhizomastixidae; using the node-based definition introduced and discussed by Pánek et al. (2016), the least-inclusive clade consisting of *Mastigamoeba balamuthi* (Chávez et al. 1986) Simpson et al. 1997, *Mastigamoeba abducta* Ptáčková et al. 2013, and *Rhizomastix libera* Ptáčková et al. 2013.

Family Mastigamoebidae Goldschmidt, 1907

Archamoebae with trophozoites which are uninucleate to multinucleate, with single motile anterior flagellum associated with microtubular cone, or aflagellate. Amoebae flattened, amoeboid movement slow, typically with multiple pseudopodia. Free-living or endobiotic. Current composition as per Ptáčková et al. (2013).

Mastigamoeba Schulze, 1875 (Fig. 3)

Mastigamoeba contains amoeboid cells where the flagellated basal body and the anterior nucleus are immediately adjacent to each other during movement. The basal body and nucleus are joined by a cone of microtubules.

Circumscription Archamoebae with a uniflagellated trophic stage, in which the nucleus and flagellum are connected by a cone of microtubules that arises from the base and sides of the single (flagellated) basal body; a cylinder is present in the transition zone of the flagellum. A single root of microtubules arises from the side of the basal body, and the root has a bilaminar “root sheet” on its anterior edge (Fig. 2a, b). Basal bodies usually have nine triplets of microtubules, but *M. schizophrenia* has nine doublets (Simpson et al. 1997). The flagellum has a conventional eukaryotic “9 + 2” arrangement of microtubules but lacks outer dynein arms, giving rise to a distinctively languid flagellar beat. The flagellates may, at least in some species, transform to amoebae with one, few, or many nuclei. Both flagellate and amoeboid forms may transform into cysts. Nuclei are usually single, but are paired in *M. schizophrenia*, and have vesicular nucleoli. In *M. punctachora*, the nucleus contains a small extranucleolar “dot” of chromatin (Bernard et al. 2000). The outside of the cell is usually naked, but in *M. aspera*, there are ectobiotic bacteria (Chystjakova et al. 2012) and spined species have been described. Cells have been found in soils and freshwater and marine habitats. Phylogenetic analyses divide this genus into two separate groups (as defined in Ptáčková et al. 2013; Pánek et al. 2016): “A” with a broad multilayered microtubular cone radiating laterally from the sides of the basal body, a long flagellar transition zone, and, in some taxa, a transitional column or spiral, microtubules arising from the base of the basal body or an MTOC immediately proximal to the basal body (Fig. 2a; Table 1) and “B” with a single-layered microtubular cone extending posteriorly from sides the basal body, and a short transition zone, and none of the extra features seen in some members of “A” (Fig. 2b; Table 1).” There is little morphological variation at the light microscopical level in “B” (Fig. 3).

Type Species *Mastigamoeba aspera* Schulze, 1875

(See Chystjakova et al. 2012 for a recent description and discussion of synonymy with *Dinamoeba*; discussed further in Ptáčková et al. 2013)

Remarks Based on recent phylogenetic analyses (Stensvold et al. 2012; Ptáčková et al. 2013), the flagellum-lacking entamoebid genera *Endolimax* and *Iodamoeba* form an internal branch of *Mastigamoeba*, making the latter genus paraphyletic. Recent analyses also divide *Mastigamoeba* into “A” and “B” clades, as discussed

above. The full scope and character of *Mastigamoeba* is uncertain, as recent phylogenetic analyses do not include most of the previously described taxa in *Mastigamoeba*, and we lack electron microscopical data for many taxa, as well as molecular data on the phylogenetic position of *M. aspera*, the type species. The degree of overlap between *Mastigamoeba*, *Mastigina*, and *Tricholimax* has long been unclear, leading to confused and inconsistent taxonomy of some species (Frenzel 1897; Frolov 2011; Goldschmidt 1907a; Lemmermann 1914). Other genera that fall within the circumscription of *Mastigamoeba* include *Dinamoeba* Leidy, 1874 (*D. mirabilis* has long been regarded as a synonym of *M. aspera* – as most recently discussed in Chystjakova et al. 2012; Ptáčková et al. 2013) and *Phreatamoeba* Chávez et al. 1986 (transferred to *Mastigamoeba* by Simpson et al. 1997).

Endolimax Kuenen and Swellengrebel, 1917 (Fig. 4)

Endolimax contains aflagellate uninucleate amoebae reminiscent of *Entamoeba*. All are intestinal symbionts of various insects and vertebrates including humans.

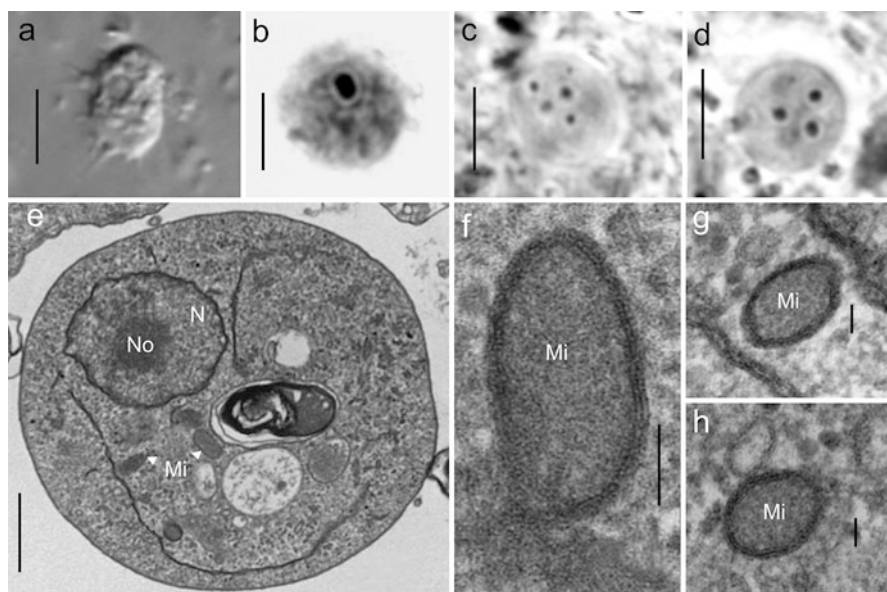


Fig. 4 *Endolimax*. (a) *Endolimax nana* live trophozoite (amoeba) showing characteristic finger-shaped pseudopodia and a single nucleus with a vesicular nucleolus. Differential interference contrast light microscopy. (b–d) *E. nana* prepared slides, stained with iron hematoxylin, bright field light microscopy: (b) trophozoite, showing a large spherical nucleolus in the nucleus. (c, d) Cysts with four nuclei. (e–h) Transmission electron micrographs of *Endolimax piscium* from Constenla et al. 2013: (e) Whole cell, showing nucleus (N) with central nucleolus (No), and mitochondrion-related organelles (Mi, arrowheads). (f–h) Mitochondrion-related organelles (Mi), with double membranes. Scale bars in a, c, d = 5 μ m; in b = 10 μ m, in e = 500 nm, in f, g, h = 50 nm (Micrographs in e, f, g, h reprinted from *Journal of Fish Diseases* 37, Constenla et al., *Endolimax piscium* sp. nov. (Amoebozoa), causative agent of systemic granulomatous disease of cultured sole, *Solea senegalensis* Kaup, pp. 229–240, Copyright (2013), with permission from John Wiley & Sons)

Circumscription Trophozoites are aflagellate amoebae measuring up to 20 μm . Actively moving cells form a single broad, hyaline, and eruptive lobopodium. Morphologically, *Endolimax* closely resembles *Entamoeba*, except that the nucleus of *Endolimax* does not contain peripheral heterochromatin (Figs. 4 and 9). There is a single large central nucleolus in the nucleus. Nucleoli in nuclei of cysts are often elongate. Cysts are rounded or elongate, with one to eight nuclei (usually four; rarely many). Approximately 20 *Endolimax* species have been described, all being commensals of the lower intestine of various animals (e.g., *E. nana* from humans, *E. caviae* from rodents, *E. gregariniformis* from birds, *E. clevelandi* from reptiles, *E. ranarum* from amphibians, *E. leptocoridis*, and *E. termitis* from insects). *Endolimax piscium* was reported from lesion in the muscle tissue of a fish (Costenla et al. 2013).

Type Species *Endolimax nana* (Wenyon and O'Connor 1917) Kuenen and Swellengrebel, 1917 (basonym *Entamoeba nana* Wenyon and O'Connor 1917).

Remarks Because it is aflagellate, *Endolimax* was traditionally considered a member of Entamoebidae. Based on SSU rRNA phylogeny, Cavalier-Smith et al. (2004) removed *Endolimax* from Entamoebidae and created the family Endolimacidae for it. Since *Endolimax* forms an internal branch of *Mastigamoeba*, Ptáčková et al. (2013) transferred it to Mastigamoebidae. The genus as a whole is relatively understudied (Poulsen and Stensvold 2016).

Iodamoeba Dobell, 1919 (Fig. 5)

Iodamoeba contains aflagellate uninucleate amoebae reminiscent of *Entamoeba* and *Endolimax* that are intestinal commensals of vertebrates.

Circumscription Trophozoites are aflagellate amoebae measuring up to 20 μm . The cells move slowly by hyaline lobopodia. The nucleus contains a single large nucleolus surrounded by globules, though these do not lie directly beneath the nuclear membrane as in *Entamoeba*. Cysts are often irregularly shaped and possess a single nucleus with an eccentric nucleolus. Typically, a large glycogen granule is present in the mature cyst. Four *Iodamoeba* species have been described from lower intestines of mammals (e.g., *I. buetschlii* from humans) and reptiles (*I. testudinis*).

Type Species *Iodamoeba buetschlii* (Prowazek 1912) Dobell, 1919 (basonym *Entamoeba buetschlii* Prowazek 1912).

Remarks Because it is aflagellate, *Iodamoeba* was traditionally considered a member of Entamoebidae. On the basis of recent molecular phylogenetic work (Stensvold et al. 2012; Ptáčková et al. 2013), it was recently removed from Entamoebidae and transferred to Mastigamoebidae together with *Endolimax* (Ptáčková et al. 2013).

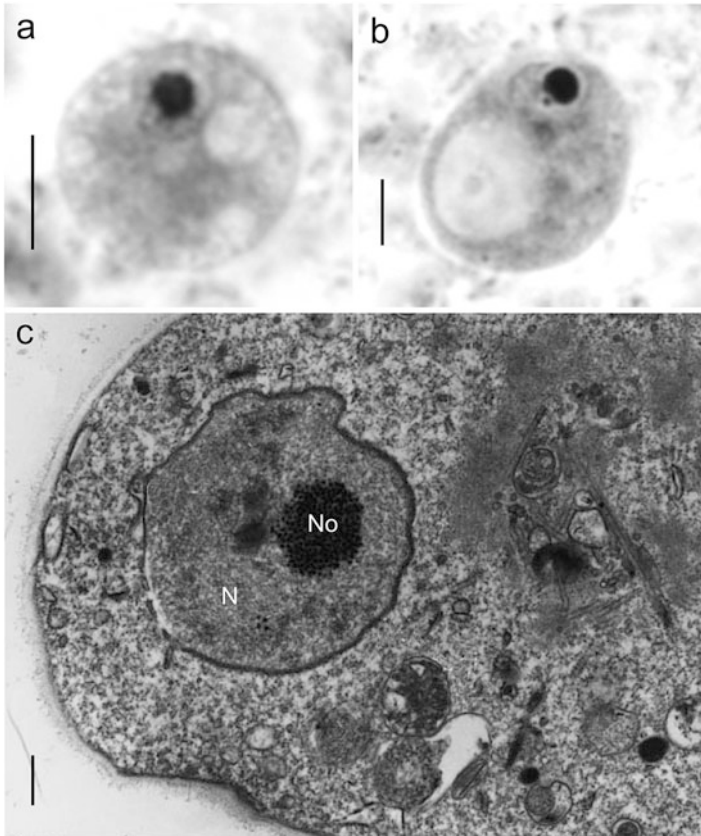


Fig. 5 *Iodamoeba* (a) Trophozoite of *Iodamoeba buetschlii* showing nucleus with central nucleolus and granules of chromatin; prepared slide, stained with iron-hematoxylin. (b) Cyst of *I. buetschlii* with a single vacuole; prepared slide, stained with iron-hematoxylin. (c) Transmission electron micrograph of cyst, showing the structure of nucleolar material (*No*) and chromatin in the nucleus (*N*). Scale bar in a = 5 μm , in b = 10 μm , in c = 1 μm (Micrograph in c reprinted from *Parasitology Research* 84, Zaman et al., *Ultrastructure of the Iodamoeba bütschlii* cyst, pp. 421–422, Copyright (1998), with permission from Springer)

Family Rhizomastixidae Ptáčková et al. 2013

Amoeboflagellate Archamoebae. Trophozoites with single anterior flagellum. Microtubular cone modified into the “rhizostyle.” Amoeboid movement slow. See below and Ptáčková et al. (2013), for discussion of the *nomen nudum* Rhizomastixidae.

Rhizomastix Alexeieff, 1911 (Fig. 6)

Rhizomastix contains amoeboid uniflagellate or biflagellate species whose microtubular cone has been modified into a tube that extends through the cell. Most species are endobiotic, but two (and possibly a third) free-living species have been described as well.

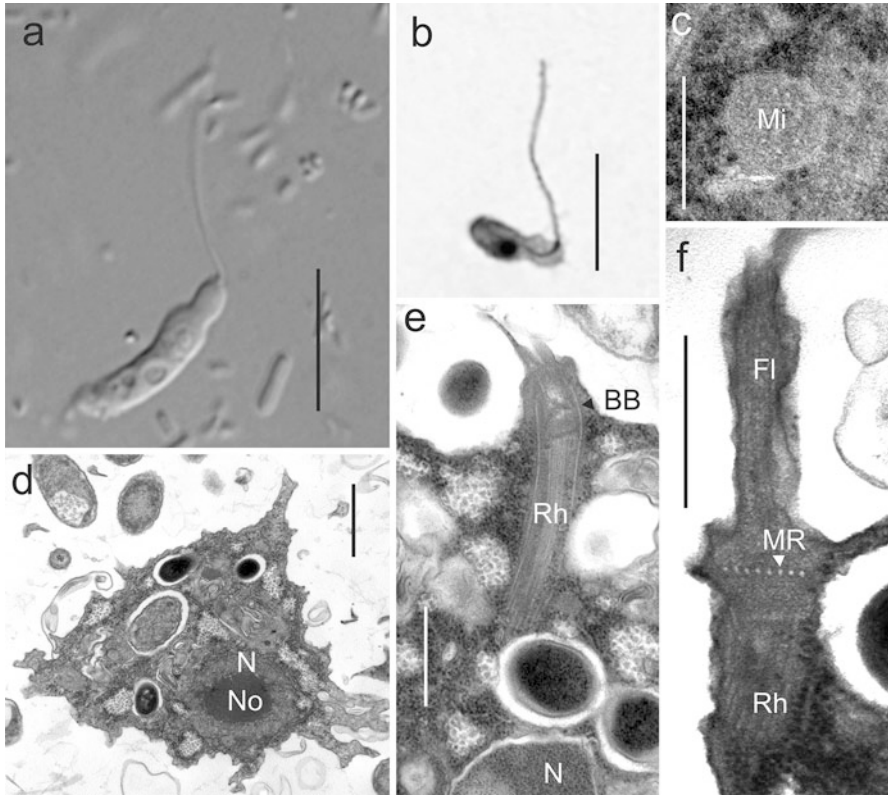


Fig. 6 *Rhizomastix*. (a–f) *Rhizomastix libera*. (a) Light micrograph of flagellate showing central, rounded nucleus and nucleolus and posterior food vacuoles (DIC). (b) Protargol-stained cell showing the rhizostyle connecting the anterior flagellum to the central nucleus. (c–f) Transmission electron microscopy. (c) Mitochondrion-related organelle (*Mi*) with double membrane. (d) Whole cell, showing large nucleus (*N*) and nucleolus (*No*) and food vacuoles. (e, f) Details of the flagellar apparatus showing the flagellum (*Fl*), basal body (*BB*) with proximally extending microtubular rhizostyle (*Rh*), and laterally extending microtubular root (*MR*). *N* nucleus. Scale bar in a, b = 10 μm , in c, f = 200 nm, in d = 1 μm , and in e = 500 nm (Figures c, d, e, and f reprinted from *Protist* 164, E. Ptáčková et al., *Evolution of Archamoebae: Morphological and Molecular Evidence for Pelobionts Including Rhizomastix, Entamoeba, Iodamoeba, and Endolimax*, Pp. 380–410, Copyright (2013), with permission from Elsevier)

Circumscription Archamoebae with a uniflagellate (biflagellate in *R. biflagellata*) trophic stage. A single-layered cylindrical bundle of microtubules, the “rhizostyle,” extends from the bottom edge of the basal body through the cell. A second microtubular element, arising from the base of the basal body and running parallel to the rhizostyle, may also be present. A single-layered root of microtubules arises from the side of the basal body (Fig. 2f). Fine structure of the basal body is unknown; a cylinder is present in the transition zone of the flagellum. The flagellum has a conventional eukaryotic “9 + 2” arrangement of microtubules, but it is unclear

whether it lacks outer dynein arms. Flagellar movement is relatively fast in comparison with *Mastigamoeba*. The nucleus is similar to that of *Entamoeba*, having a single central (large) nucleolus and peripheral heterochromatin granules at least in some species. The flagellates may transform to uninucleate amoebae. Binucleate cysts have been reported in some species. Sixteen *Rhizomastix* species have been described. Most of them are intestinal commensals of vertebrates and insects (e.g., *R. gracilis*, *R. biflagellata*, and *R. bicoronata*), three are described as free-living (*R. libera*, *R. borealis*, *R. varia*); and some are of uncertain status (*R. elongata*) (Ptáčková et al. 2013; Zadrožilková et al. 2016).

Type Species *Rhizomastix gracilis* Alexeieff, 1911.

Remarks The genus *Rhizomastix* was recently transferred into the Archamoebae and a new family Rhizomastixidae established to accommodate it (Ptáčková et al. 2013). *Rhizomastix* was classified with *Mastigamoeba* and *Mastigella* by Kudo (1939, 1977); Čepička (2011) suggested it might be related to pelobionts; its position was clarified on the basis of molecular and ultrastructural data (Ptáčková et al. 2013; Zadrožilková et al. 2016). The name Rhizomastigidae has historically been used for today's Mastigamoebidae (e.g., Bütschli 1880, 1884; Calkins 1901; Lepš 1965; Reichenow 1952). The name was created by Bütschli (1884) as Rhizomastigina and later standardized to Rhizomastigidae by Calkins (1901); however, as it was not based on and often did not include *Rhizomastix*, Rhizomastigidae is regarded by some as a *nomen nudum* (Loeblich and Tappan 1961). The composition of Rhizomastigidae has typically been very confused (e.g., Cavalier-Smith and Scoble 2013), leading Ptáčková et al. (2013) to create Rhizomastixidae as the family containing *Rhizomastix*.

Suborder Pelomyxina Starobogatov, 1980

See Pánek et al. (2016) for current composition. The clade containing *Pelomyxa* and *Mastigella*; using the branch-based definition introduced and discussed in Pánek et al. (2016), the clade containing *Pelomyxa palustris* Greeff, 1874, and all organisms sharing a more recent common ancestor with *P. palustris* than with *Mastigamoeba balamuthi* (Chávez et al. 1986) Simpson et al. 1997.

Family Pelomyxidae Schulze, 1877

Anaerobic or microaerophilic flagellated amoebae with slow-beating monokinetid or immobile polykinetids. See Zadrožilková et al. 2015 for current composition.

Pelomyxa Greeff, 1874 (Fig. 7)

Pelomyxa was originally described as a large multinucleate amoeba, with a division of the cytoplasm into an inner layer containing organelles displaying fountain-flow movement and a clear hyaline outer layer from which pseudopodia can “roll” out and with a posterior uroid attaching the amoeba to the substrate. Later reports extended the description to refer to prokaryotes that coexist endosymbiotically in the cell (van Bruggen et al. 1988) and to non-motile flagella (Frolov 2011; Griffin 1979, 1988; Seravin and Goodkov 1987a).

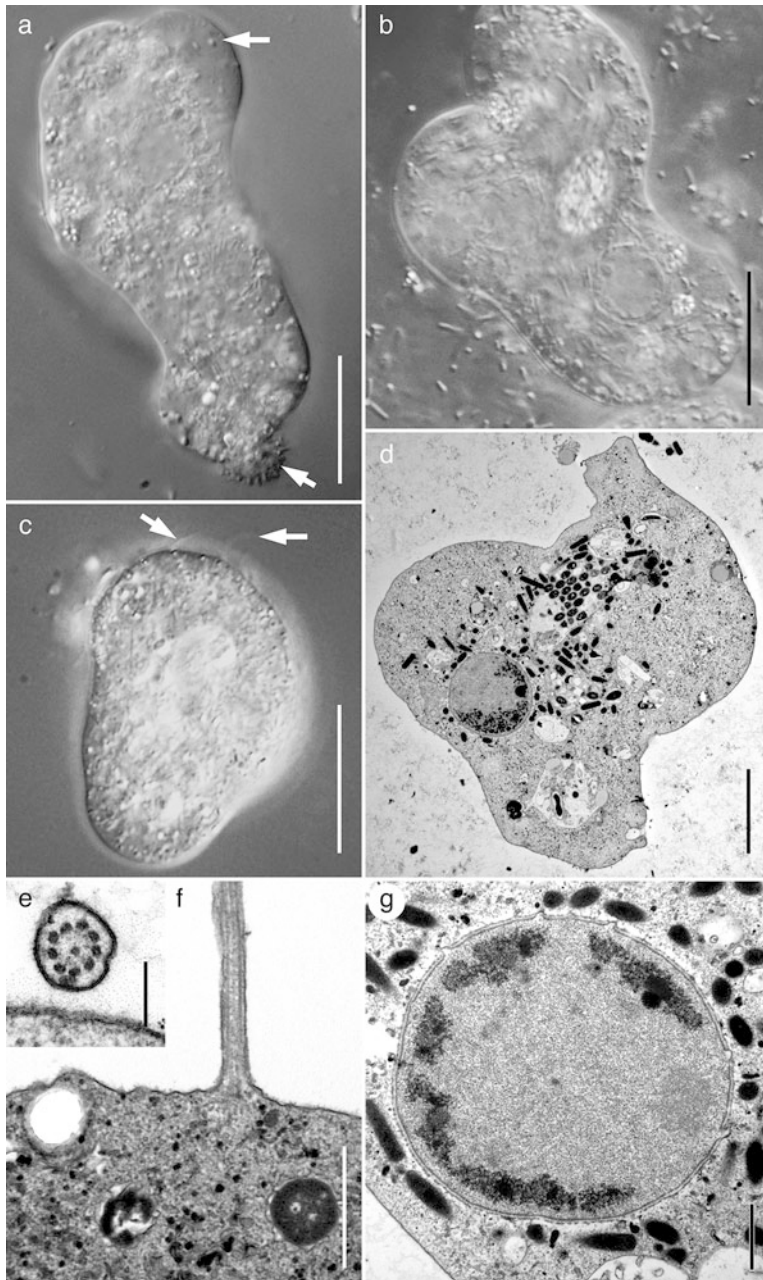


Fig. 7 *Pelomyxa*. (a–g) *Pelomyxa schiedti*, strain SKADARSKE in (a, b), strain TIWI in (c), strain WACT07 in (d–g). (a–c) Differential interference contrast showing cells filled with granules and endosymbionts, distinctive nuclear structure (in b), leading pseudopodium (in a, arrow upper right), a bulbous, villous uroid-like area (in a, arrow lower right), and multiple immobile poorly

Circumscription *Pelomyxa* is distinguished by the form most usually encountered: a large multinucleate amoeba, with many inactive flagella. It includes polymorphic species, with a large multinucleate amoeboid feeding form up to 5000 μm long, with poorly motile or non-motile flagella that insert into a clear “hyaline” outer cytoplasmic layer, which encloses an inner layer filled with organelles, with endosymbiotic bacteria, and sometimes with refringent cytoplasmic granules (sand); movement is directed by fountain-flow cytoplasm and an anterior monopodium. The posterior uroid is active in food uptake. Cysts about 100 μm in diameter are known from at least one species. *Pelomyxa* species can be divided into two groups on the basis of the organization of the flagellar apparatus: the first group is characterized by a long basal body and very numerous radiating microtubules, while the second is characterized by a short basal body associated with a very reduced number of radiating microtubules. There is at least one intermediate state between these groups (Chystjakova et al. 2014). Cells have many flagella, each arising from a single basal body; each basal body gives rise proximally to a cone of microtubules, which has not been shown to extend to the nucleus (though several species do have microtubules surrounding the nucleus). The transition zone of each flagellum contains a transitional column. Flagella are with or (in most cases) without a 9 + 2 arrangement of microtubules in flagellar axoneme; the pattern of microtubules may be unstable in individuals in some species, particularly in the central pair or group of axonemal microtubules. The flagella insert into the hyaline outer cytoplasmic layer, which is devoid of organelles (ER, nuclei, vacuoles). Nuclei show distinctive, diagnostic patterns of chromatin condensation and may be surrounded by endosymbiotic bacteria. Morphological and molecular data suggest that *Pelomyxa* may have evolved from *Mastigella* by nuclear and flagellar multiplication (Zadrobílková et al. 2015).

Type Species *Pelomyxa palustris* Greeff, 1874.

Remarks *Pelomyxa* has been widely reported (e.g., Greeff 1874; Griffin 1988; Grüber 1885; Schirch 1914; Schulze 1875a; Whatley and Chapman-Andresen 1990; Frolov 2011). *Pelomyxa palustris* is currently relatively poorly known, and descriptions of it (e.g., Griffin 1988; Whatley and Chapman-Andresen 1990) probably encompass the variation of multiple species (Frolov et al. 2004; Goodkov et al. 2004). On the other hand, very few of the light microscopy-based species



Fig. 7 (continued) visible flagella (in c). (d–g) Transmission electron microscopy; (d) section through the cell showing the amoeboid body, single nucleus, and endosymbionts. (e) Transverse section of the flagellum with aberrant arrangement of microtubules. (f) Longitudinal section of the flagellar apparatus. (g) Detail of the nucleus, showing peripheral chromatin and small nucleolus. Scale bars in a–c = 20 μm ; d = 5 μm ; e = 200 nm; f and g = 1 μm (Figures a–g reprinted from *Protist* 164, E. Ptáčková et al., *Evolution of Archamoebae: Morphological and Molecular Evidence for Pelobionts Including Rhizomastix, Entamoeba, Iodamoeba, and Endolimax*, pp. 380–410, Copyright (2013), with permission from Elsevier)

descriptions currently available can be differentiated from the life cycle described by Whatley and Chapman-Andresen (1990). All the previous accounts of species probably describe real variation; however it is not currently possible to assign most of this variation to different species in any consistent or clear way, on the basis of the published descriptions. Species are currently distinguished mainly on characteristic patterns of nuclear chromatin, the thickness of the outer cytoplasmic layer, and the flagellar apparatus (Berdieva et al. 2015; Chystjakova and Frolov 2011; Chystjakova et al. 2014; Frolov 2011; Frolov et al. 2005a, b, 2006; Griffin 1979, 1988; Seravin and Goodkov 1987a; Ptáčková et al. 2013; Zadrožilková et al. 2015).

Mastigella Frenzel, 1897 (Fig. 8)

Mastigella contains amoeboid cells with a flagellated basal body with a cone of microtubules, but there is no connection between the cone and the nucleus. Endosymbiotic prokaryotes are present in several species.

Circumscription Archamoebae with a uniflagellated trophic stage, in which the nucleus is not connected to a cone of microtubules that arises from the base and sides of the single (flagellated) basal body; a cylinder is present in the transition zone of the flagellum. A single root of microtubules arises from the side of the basal body, and the root has a bilaminar sheet on its anterior edge. Basal bodies have nine triplets of microtubules. The flagellum has a conventional eukaryotic “9 + 2” arrangement of microtubules but lacks outer dynein arms. The flagellum may contribute to cell movement, but its beating is typically slower and less effective than that seen in *Mastigamoeba*. The flagellates may, at least in some species, transform to amoebae with one, few, or many nuclei. The nuclei may contain a distinctive distribution of chromatin; endosymbiotic bacteria may be present; the outside of the cell may be covered with irregular spines. Both flagellate and amoeboid forms may transform into cysts. Cells are from 3 to 150 µm long with one to four flagella up to 150 µm long. Reported from anoxic or low-oxygen freshwater or marine sediments (Walker et al. 2001; Zadrožilková et al. 2015).

Type Species *Mastigella polymastix* Frenzel, 1897.

Remarks *Mastigella* was introduced to describe an amoeboid flagellate with multiple long flagella extended from the cell body on small “necks” that wandered over the cell body and were not attached to the nucleus (Frenzel 1897). Subsequently, the concept of the genus changed to one of amoeboid flagellates without a connection between the flagellum and nucleus (Goldschmidt 1907a, b; Lemmermann 1914). Following from Goldschmidt’s (1907a, b) informal group “Mastigamöben,” *Mastigamoeba* and *Mastigella* were grouped as the Mastigamoebidae by Chatton (Chatton 1925; Kudo 1939, 1977) and have traditionally been thought of as sister taxa. This was initially supported by phylogenies that included the GenBank sequence AF421219: it had been ascribed to *Mastigella commutans* but belongs to *Mastigamoeba punctachora* (Ptáčková et al. 2013), meaning that these phylogenies

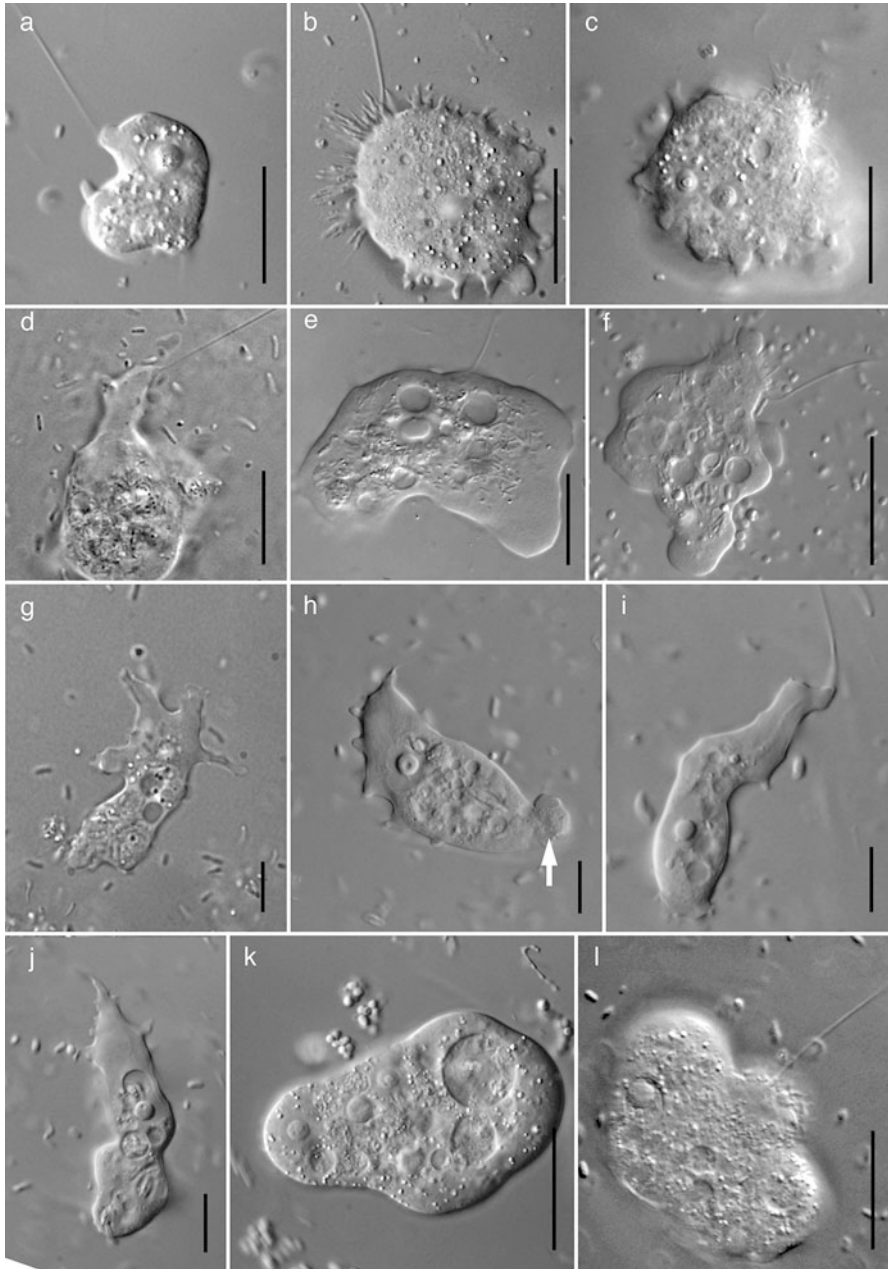


Fig. 8 *Mastigella*. (a–l) Differential interference contrast. (a–c) *Mastigella erinacea* strain TOLEDO, showing binucleate cells with distinctive “fried-egg” nucleus and granular nucleolus, endosymbiotic bacteria, and highly variable villous, lobate or finger-shaped pseudopodia. (d, e) *Mastigella ineffigiata* strain OLB6AN, showing “shapeless” morphology and conspicuous

are silent on the placement of *Mastigella*. However, cell form and movement, nuclear structure, and the presence of endosymbiotic prokaryotes resembling methanogenic archaea (Frolov 2011; van Bruggen et al. 1985; Walker et al. 2001; Zadrožilková et al. 2015) all suggest a relationship of *Mastigella* with *Pelomyxa*. This hypothesis was supported by recent phylogenetic analyses where *Pelomyxa* forms an internal branch of *Mastigella*, while *Mastigamoeba* is phylogenetically more distant (Zadrožilková et al. 2015). Other nominal genera that fall within the circumscription of *Mastigella* include *Limulina* Frenzel, 1897; *Micromastix* Frenzel, 1897 (Frenzel 1897) and *Mastigamoebula* Fantham, 1919 (Fantham 1919).

Order Entamoebida Cavalier-Smith, 1993

Using the branch-based definition introduced by and discussed in Pánek et al. (2016), the clade consisting of *Entamoeba histolytica* Schaudinn, 1903 and all organisms that share a more recent common ancestor with *E. histolytica* than with *Mastigella eilhardi* Bürger, 1905.

Family Entamoebidae Chatton, 1925

Aflagellate Archamoebae. Flagellar apparatus completely reduced. Amoeboid movement typically monopodial and relatively fast. See Cavalier-Smith 1993 for current composition.

Entamoeba Casagrandi and Barbagallo, 1895 (Fig. 9)

Entamoeba contains most of the species of aflagellate Archamoebae. They are usually intestinal commensals of various animals, though at least three are likely to be free-living. A few species, including *E. histolytica* from humans, are pathogenic for their hosts.

Circumscription Trophozoites are aflagellate, uninucleate amoebae measuring up to 60 μm . The flagellar apparatus of *Entamoeba* is completely absent, and no cytoplasmic microtubules can be found in nondividing cells. Actively moving cells usually crawl using a single or several eruptive lobopodia; the hyaline lobopodia are clearly distinguishable from the granuloplasm. Uroidal filopodia have been rarely observed (Martínez-Palomo 1993). Nuclear structure is distinctive,



Fig. 8 (continued) endosymbionts. (f) *Mastigella rubiformis* strain HRAAN, showing cells with hyaline area, distinctive “*Pelomyxa*-like” nucleus, and prominent endosymbiotic bacteria. (g, h, j) *Mastigella eilhardi* strain ATCC 50342, showing pseudopodial variation; nucleus with “hollow,” “donut-shaped” nucleolus; and endosymbiotic bacteria, with a posterior uroid (arrow). (i) *M. eilhardi* strain GO7 showing its characteristic swanlike long “neck” and posterior villous pseudopodia. (k) *Mastigella erinacea* strain KORISSION, showing binucleate or quadrinucleate cells with distinctive “fried-egg” nucleus with a granular nucleolus and villous pseudopodia from which the flagellum originates in some cells. Scale bars in a–f, i, k, l = 20 μm ; g, h, j = 10 μm (Figures a–l reprinted from Protist 164, E. Ptáčková et al., *Evolution of Archamoebae: Morphological and Molecular Evidence for Pelobionts Including Rhizomastix, Entamoeba, Iodamoeba, and Endolimax*, pp. 380–410, Copyright (2013), with permission from Elsevier)

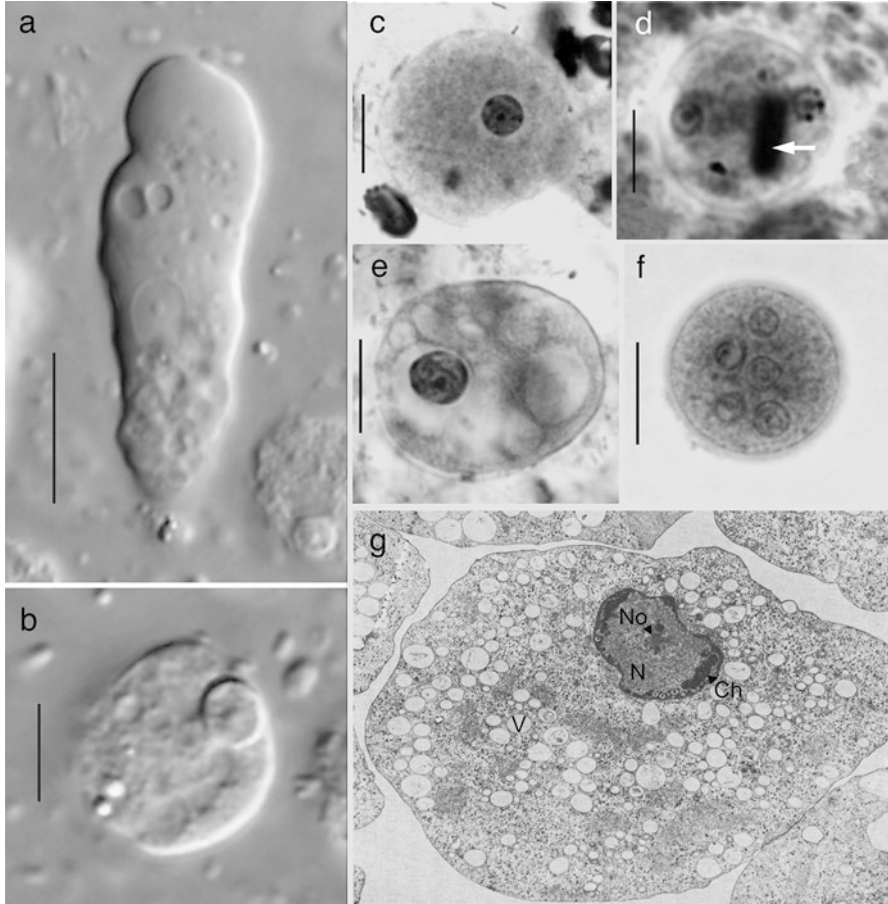


Fig. 9 *Entamoeba*. (a) *Entamoeba moshkovskii* free-living trophozoite amoeba showing an anterior hyaline pseudopodium and central nucleus with the characteristic “dot” nucleolus (DIC). (b) *E. moshkovskii* resting cell, showing the nucleus with its characteristic “dot” nucleolus and peripheral chromatin (DIC). (c, d) Parasitic *Entamoeba histolytica* trophozoite (c) and immature cyst (d) with two visible nuclei and chromatoid body (arrow); prepared slide material, stained with iron-hematoxylin to show nuclei with a thick ring of peripheral chromatin and a small nucleolus. (e, f) Parasitic *Entamoeba coli* trophozoite (e) and cyst (f) with eight nuclei (five visible in the focal plane of the photograph); prepared slide material, stained with iron-hematoxylin to show nuclei with large chromatin granules. (g) Transmission electron micrograph of *E. histolytica*, showing numerous vacuoles (*V*) and the characteristic arrangement of nuclear chromatin (*Ch*) and nucleolus (*No*) in the nucleus (*N*). Scale bar in a, c, d, e, f = 10 μ m; in b = 5 μ m (Figure in g reprinted from *Parasitic Protozoa* Volume 3, 2nd Edition, J.P. Kreier & J.R. Baker, Chapter 3: Parasitic amebas of the intestinal tract., pp. 65–141, Copyright (1993), with permission from Elsevier)

with a single small, central nucleolus and conspicuous peripheral granules of heterochromatin (Fig. 9). The number and size of the granules differ between particular *Entamoeba* species. A cyst stage is documented for many species (see

above). The number of nuclei in the cyst is an evolutionarily important feature (Silberman et al. 1999). Cysts have been lost in *E. gingivalis*, which is found in the oral cavity of humans and transferred between hosts via saliva.

Many *Entamoeba* species have been described. The vast majority live as intestinal endobionts of various animals (e.g., *E. histolytica*, *E. dispar*, and *E. coli* from humans, *E. muris* and *E. cobayae* from rodents, *E. gallinarum* from birds, *E. invadens* from reptiles, *E. ranarum* from amphibians, *E. gadi* from fish, *E. aulastomi* from leeches, and *E. minchini* from insects). Some species are pathogenic, the best known being *E. histolytica*. Further information on endobiotic and parasitic species is given in the “[Practical Importance](#)” section.

Three *Entamoeba* species may be facultatively free-living. *Entamoeba moshkovskii* is relatively well known and has been repeatedly isolated from wastewaters, anoxic sediments, as well as from human stool (see Heredia et al. 2012; our observations). The second species, *E. ecuadoriensis*, has been isolated only once from sewage water (Clark and Diamond 1997). The third, *E. marina*, was isolated from marine tidal flats (Shiratori and Ishida 2016).

Type Species *Entamoeba coli* Grassi, 1879, Casagrandi and Barbagallo 1895 (basionym *Amoeba coli* Grassi 1879).

Remarks There was some uncertainty regarding the names of genera *Entamoeba* and *Endamoeba* during the first half of the twentieth century (see section “[History of Genera in the Archamoebae](#)”). *Entamoeba histolytica* can be, therefore, found under the name *Endamoeba histolytica* in the older literature. Since the 1950s, the concept of *Entamoeba* is stable, and *Endamoeba* is now considered a separate genus (e.g., Patterson et al. 2000). *Entamoeba* constitutes the family Entamoebidae. Historically, the other aflagellate Archamoebae, *Endolimax* and *Iodamoeba*, were classified within Entamoebidae as well but were recently removed from it (Cavalier-Smith et al. 2004; Ptáčková et al. 2013). *Endamoeba* is considered here as Archamoebae *incertae sedis*.

Archamoebae *Incertae Sedis*

Mastigina Frenzel, 1897 (Fig. 10)

Mastigina contains limax-shape amoeboid cells with a flagellum and no lateral pseudopodia and with a connection between the base of the flagellum and the rounded nucleus. Its microtubular ultrastructure has not been studied by electron microscopy.

Circumscription Archamoebae with a uniflagellated trophic stage, with limax amoeboid shape and no lateral pseudopodia, and in which the round nucleus is connected to the base of the flagellum, which has the distinctively languid flagellar beat typical of other pelobionts. One species, *Mastigina setosa*, has fountain-flow cytoplasmic movement (Goldschmidt 1907a). The outside of the cell is covered with

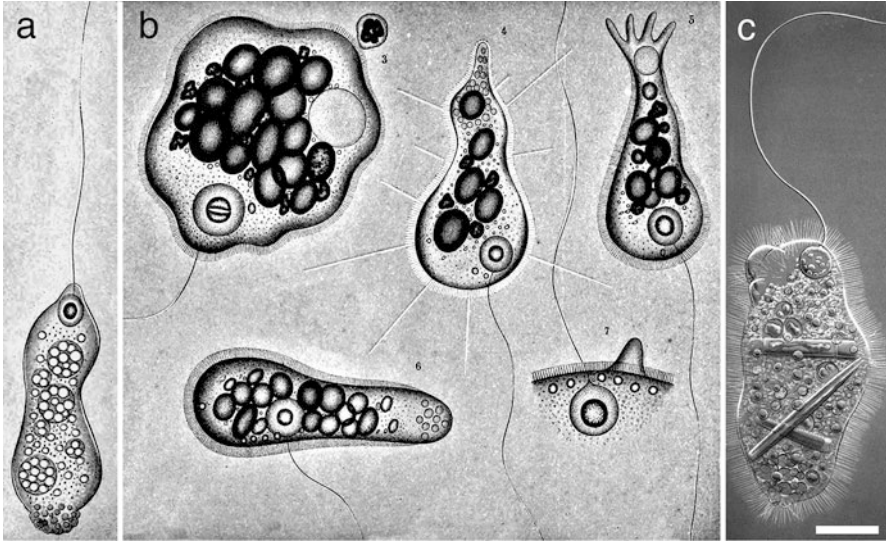


Fig. 10 *Mastigina*. (a) Drawing of one of the two original species of *Mastigina*, *M. paramylon*, showing numerous starch granules inside the cell, connection between the long flagellum and the nucleus (with central vesicular nucleolus), and the lack of any lateral pseudopodia. (b) The type species of *Mastigina*, *M. chlamys*, showing a dense layer of short, refringent spines and in one case longer spines interspersed with the short ones, a connection between the long flagellum and the nucleus (with central nucleolus), and pseudopodia arising only at the anterior and posterior of the cell, not laterally. (c) *Mastigina setosa*, showing similar characteristics to *M. chlamys* but longer, less dense spines. Scale bar in a = 5 μm ; in b, c = 20 μm (Drawings in a and b reprinted from *Untersuchungen über die mikroskopische Fauna Argentiniens. Erster Teil: Die Protozoen. I und II, Abteilung: die Rhizopoden und Helioamoeben*, J. Frenzel, (1897), Erwin Nägele, Stuttgart; with no known copyright restrictions. Drawing in c reprinted from *Archiv für Protistenkunde Supplement 1*, R. Goldschmidt, *Lebensgeschichte der Mastigamöben Mastigella vitrea* n.sp. u. *Mastigina setosa* n.sp., pp. 83–165, Copyright (1907), with permission from Elsevier)

closely packed spines in two species, *Mastigina chlamys* and *M. setosa* (Frenzel 1897; Goldschmidt 1907a; Skibbe and Zölffel 1991). Reported from anoxic or low-oxygen freshwater sediments.

Type Species *Mastigina chlamys* Frenzel, 1897.

Remarks *Mastigina* was not clearly distinguished from other pelobiont genera when it was introduced, in the original descriptions of *M. chlamys* and *M. paramylon* (Frenzel 1897). Goldschmidt (1907a) defined the genus on the basis of limax body shape with no lateral pseudopodia, round apical nucleus connected closely to the base of the flagellum (as opposed to elongated or drop-shaped, sometimes appearing removed from the base of the flagellum, in *Mastigamoeba*), characters that are adopted here. Goldschmidt (1907a) also regarded *Tricholimax hylae* as belonging to *Mastigina*, leading to later confusion about the characters

displayed by *Mastigina* species: he used fountain-flow cytoplasmic movement as a defining feature of *Mastigina*, despite it being present only in *M. setosa* and *T. hylae* and despite the two species in the original description of *Mastigina* not displaying it. Ultrastructural studies of *Tricholimax hylae* have been carried out under the name *Mastigina hylae* (Brugerolle 1982, 1991), and summaries of the genus have subsequently relied largely on descriptions of *T. hylae* (Griffin 1988; Brugerolle and Patterson 2000).

Perhaps on the basis that fountain-flow cytoplasm is also present in *Mastigamoeba aspera* (Chystjakova et al. 2012; Schulze 1875b), Lemmermann (1914) in turn regarded *Mastigina* as a junior synonym of *Mastigamoeba*, leading to further confusion about the distribution of fountain-flow cytoplasm and spines in pelobionts. Frolov (2011) redefined *Mastigina* as containing only taxa with spines on the surface, disregarding the criteria used by Frenzel (1897) and Goldschmidt (1907a). Although 50% of the species currently assigned to *Mastigina* do have spines, 50% do not, and there are also members of *Mastigamoeba* and *Mastigella* with spines.

In the absence of clear ultrastructural and phylogenetic data on any of these species, we see no reason to reject Goldschmidt's circumscription of the genus.

Family Tricholimacidae Cavalier-Smith, 2013

Monotypic family with the characteristics of the sole species. Using the diagnosis of Cavalier-Smith (2013), uniciliate (i.e., uniflagellate) endosymbiotic anaerobes with inactive cilium (i.e., flagellum) with numerous irregularly arranged doublets; no obvious transition zone cylinder; dense microtubule nucleating center attached to the proximal side of the single centriole (with doublets not triplets) nucleates (a) multilayered cone of microtubules that surround nucleus and (b) lateral rhizostyle, a broad band of microtubules with numerous underlying singlet and bundled microtubules.

Tricholimax Frenzel, 1897 (Fig. 11)

This monotypic genus has been described from the guts of amphibians. Cells have a limax shape with fountain-flow cytoplasmic movement and without lateral pseudopodia, and the anterior nucleus is connected to the short non-motile flagellum, which lacks normal 9 + 2 organization of the microtubular axoneme.

Circumscription The one species, *Tricholimax hylae*, exists mostly as flagellates measuring 50–135 μm . Flagellated amoeboid and cyst stages (up to 30 μm in diameter with up to four nuclei) may form. Flagellated cells have one to six nuclei, one of which is connected to the emergent flagellum. In the flagellar apparatus, there is a cone of microtubules arising from base and sides of the single basal body; these enclose the nucleus and connect to the nuclear envelope by microfibrils. A single root of 32 microtubules arises from the side of basal body, and there is a bilaminar sheet associated with the anterior edge. There is an “n + n” arrangement of microtubules in the flagellar axoneme, though no dynein arms are visible. There may be

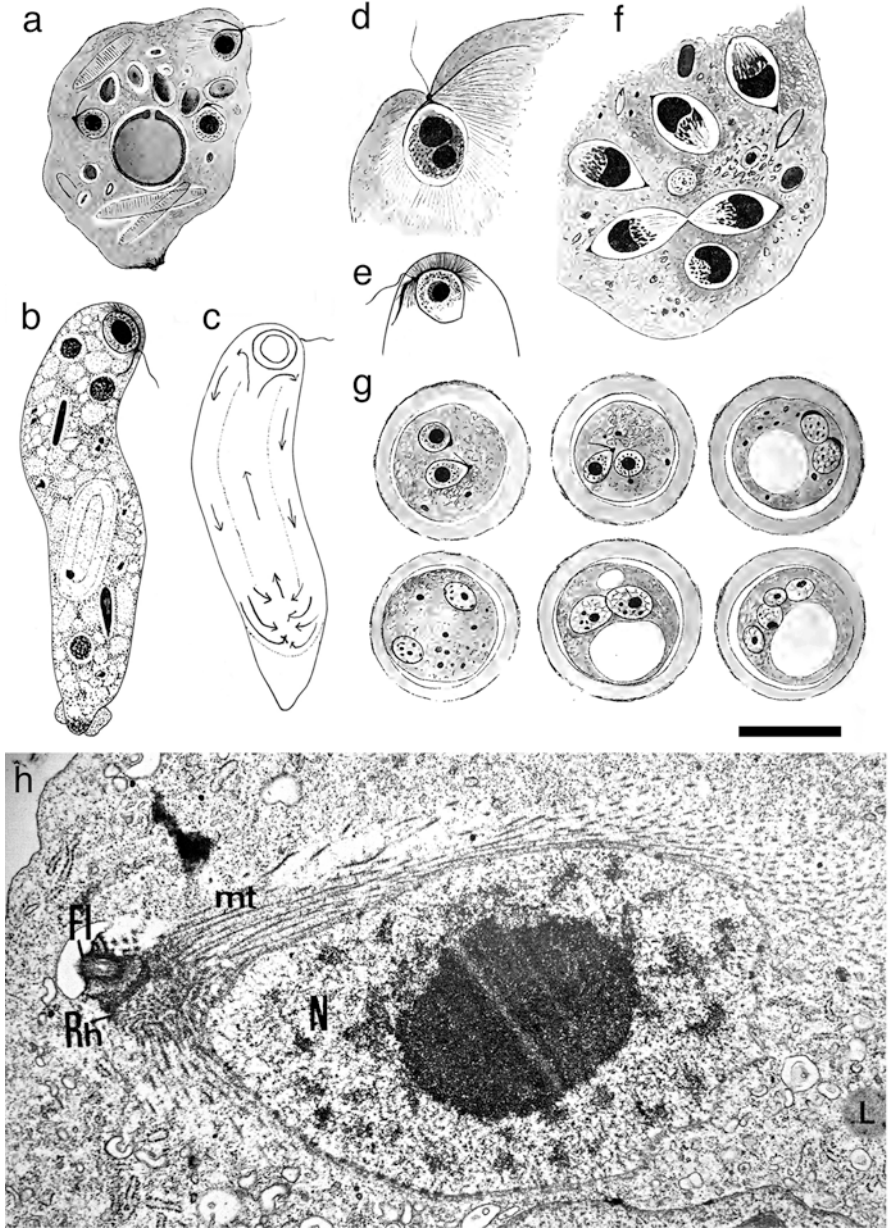


Fig. 11 *Tricholimax hylae*. (a–b) Drawings of whole cells showing the microtubular connection between the short, nonfunctional flagellum and the large apical nucleus with its central nucleolus. Accessory nuclei, with attached kinetids, are visible in the cytoplasm in **a**, along with numerous food vacuoles. (c) Diagram showing the direction of fountain-flow cytoplasmic streaming, where the posterior uroid is not included. (d, e) The microtubular cone and lateral root (which are clearly visible under the light microscope, even without DIC optics). There is some variation in the

reduced internal basal bodies with cones of microtubules attached to extra internal nuclei (Brugerolle 1982, 1991).

Type Species *Tricholimax hylae* Frenzel, 1897.

Remarks This genus can be distinguished from *Mastigina* because of the atypical organization of the single non-motile flagellum. It has been widely described (Frenzel 1897; Goldschmidt 1907b; Collin 1913; Lemmermann 1914; Becker 1928; Sassuchin 1928; Ivanic 1936; Chatton 1953; Brugerolle 1982, 1991; Griffin 1988). The occurrence of the “n + n” flagellar organization and fountain-flow cytoplasm both here and in *Pelomyxa* has resulted in the placement of both genera in the Pelomyxidae, though this circumscription is now widened to also include *Mastigella*. No molecular phylogenetic information exists for *Tricholimax*, so its formal placement in the Pelomyxidae remains premature.

Endamoeba Leidy, 1879 (Fig. 12)

Poorly known genus containing aflagellate Archamoebae found in insects.

Circumscription Trophozoites measure up to 120 µm. They are aflagellate and move slowly by one or several lobopodia. Unlike in *Entamoeba*, there is no marked distinction between hyaloplasm and granuloplasm (Wenyon 1926). The nucleus of *Endamoeba* has a typical structure dissimilar from that of *Entamoeba* and other aflagellate Archamoebae (Wenyon 1926; compare Fig. 12 with Figs. 4, 5, and 9). Several species have been described from the hindgut of insects, such as *Endamoeba blattae* from cockroaches and *E. disparata* from termites.

Type Species *Endamoeba blattae* Bütschli, 1878 Leidy 1879 (basionym *Amoeba blattae* Bütschli, 1878).

Remarks Probable member of Entamoebidae. This classification is, however, tentative, since no sequence data from *Endamoeba* are currently available.



Fig. 11 (continued) presence of a single or a double nucleolus. (f) Nuclei dividing, showing a connection between the posterior poles of the nuclei, with anterior kinetids present. (g) Six cyst stages, showing variation in the number of nuclei from two to four, and gradual development of a central vacuole. (h) Transmission electron micrograph showing the flagellum (*Fl*), single basal body with microtubular root (*Rh*), and dense cone of microtubules (*mt*) connecting to the nucleus (*N*). Scale bar: in a, b, c = 30 µm, in d, e, f = 10 µm, in g = 20 µm, in h = 3 µm (Drawings in a, d, f, and g reprinted from *Archives de Zoologie expérimentale et générale* 51, B. Collin, Sur un ensemble de protistes parasites des batraciens (Note préliminaire). Pp. 59–76 (1913) with no known copyright restrictions. Drawings in b, c, e reprinted from *Journal of Parasitology* 11, E. R. Becker, The morphology of *Mastigina hylae* (Frenzel) from the intestine of the tadpole. Pp. 213–216, Copyright (1925) with permission from Allen Press. Micrograph in h reprinted, from *Protistologica* 18, G. Brugerolle, *Caractères ultrastructuraux d'une mastigamibe: Mastigina hylae* (Frenzel), pp. 227–235, Copyright (1982), with permission from Elsevier)

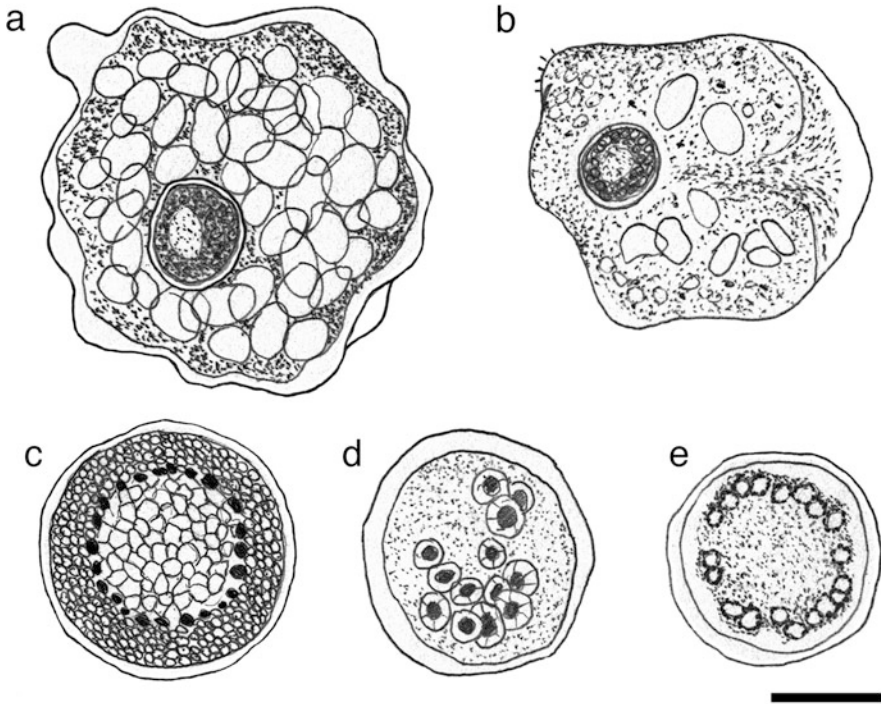


Fig. 12 *Endamoeba* (a–e); *Endamoeba blattae*, redrawn from Kudo (1960). (a, b) Trophozoites, showing the distinctive arrangement of chromatin in the nucleus and eruptive pseudopodia. Drawn from live material. (c) Nucleus, showing the distinctive ring of chromatin. (d) Multinucleate cyst, as seen when stained with iron-hematoxylin, showing changed nuclear structure, relative to (e) Cyst, showing distinctive ring of chromatin in the nuclei. Scale bar in a, b = 10 μm , in c = 4 μm , in d, e = 5 μm . Pictures redrawn from Kudo (1960)

Maintenance and Cultivation

Various free-living Archamoebae from the genera *Mastigamoeba*, *Mastigella*, and *Rhizomastix* can be isolated and maintained in xenic cultures with bacteria. Sonneborn's *Paramecium* medium (cereal grass infusion medium, ATCC medium 802; see www.atcc.org) is suitable for freshwater strains (Ptáčková et al. 2013). Seawater 802 medium (ATCC medium 1525; see www.atcc.org) can be used for the cultivation of marine pelobionts (Zadrobílková et al. 2015). The strains grow well at room temperature in sealed 15 ml tubes with 10 ml of medium and transfers every 1–3 weeks or in sealed tissue flasks filled with medium and transfers every 1–2 months. Although the Archamoebae are anaerobic and die when exposed to oxygen for a longer period, no special precautions are necessary, since bacteria present in the culture quickly consume the oxygen. Most strains grow well also under anoxic conditions.

Members of the genera *Entamoeba* (including the free-living *Entamoeba moshkovskii*) and *Endolimax* can be isolated and maintained in xenic cultures as well. Various media developed for cultivation of intestinal flagellates and amoebae, both biphasic (e.g., LE medium, Robinson's biphasic medium) and monophasic (TYSGM-9 medium, Robinson's monophasic medium) can be used (see Clark and Diamond 2002). Culture conditions depend on the origin of the organisms – strains from invertebrates and poikilotherm vertebrates are cultivated at room temperature with transfers once a week to once a month; strains from homoiotherms should be cultivated at 37 °C with transfers approximately twice a week.

Only a few species of *Entamoeba* and *Mastigamoeba balamuthi* have been successfully axenized. The process of isolation and axenization of *Entamoeba histolytica* is described in Clark and Diamond (2002). For maintenance, several complex media, such as TYI-S-33, YI-S, and LYI-S-2 (ATCC medium 2154), can be used (for preparation, see Clark and Diamond 2002 and www.atcc.org). Apart from *Entamoeba* spp., only a single strain of *Mastigamoeba balamuthi* has been axenized (Chávez et al. 1986). Chávez et al. (1986) described the process of the axenization in a detail. *Mastigamoeba balamuthi* can be maintained in PYGC (Chávez et al. 1986) or LYI-S-2 (see above) media.

So far, only one successful long-term cultivation attempt of *Pelomyxa* spp. has been published (Zadrobílková et al. 2015). Cells of *Pelomyxa* spp. can be maintained alive for several months in hermetically closed flasks filled with fresh water and sediments, or with Lozina-Lozinskii medium supplemented with boiled detritus (see Chystjakova and Frolov 2011; Frolov et al. 2011).

Cultures of Archamoebae can be cryopreserved in the presence of 5% dimethyl sulfoxide using a standard procedure and stored in liquid nitrogen. Detailed protocols for cryopreservation of *Entamoeba* cultures are described in Diamond (1995) and Samarawickrema et al. (2001).

Evolutionary History

Phylogeny and Evolution

Separately and together, members of the Archamoebae have been considered to be some of the most deep-branching eukaryotes, on the basis of lacking mitochondria and Golgi dictyosomes, and having a “simple” or nonexistent flagellar apparatus. Using the argument that some or all of these traits are primitive, it was suggested that the pelobionts represent a very early stage in the evolution of eukaryotes (Brugerolle 1993; Cavalier-Smith 1983; Griffin 1979, 1988; Margulis 1970; Patterson 1994; Patterson and Sogin 1992; Whatley 1976; Whatley and Chapman-Andresen 1990). This evolutionary significance was in the context of an evolutionary paradigm known as the Archezoa hypothesis (Cavalier-Smith 1983), where “amitochondriate” protists represented basal eukaryotic lineages that had diverged before the acquisition of the mitochondrial endosymbiont and other key eukaryotic innovations (e.g., introns, Golgi dictyosomes, peroxisomes).

The Archezoa hypothesis was supported by molecular phylogenetic trees of eukaryotes consisting of a “crown” (animals, plants, fungi, and many algae) and a ladderlike sequential divergence of amitochondriate parasites from the base (Sogin 1991; Sogin and Silberman 1998).

In the late 1990s, developments in phylogenetic methods and the identification of mitochondriate remnant organelles led to the rejection of the Archezoa hypothesis and the crown/base view of eukaryotic relationships (Roger 1999). The development of evolutionary models incorporating heterogeneity among taxa (of evolutionary rate or of base composition) led to the realization that systematic phylogenetic error, such as “long branch attraction,” had had a major effect on the positioning of “archezoan” organisms in the tree (Dacks et al. 2002; Holder and Lewis 2003; Philippe and Germot 2000). The finding of gene sequences of

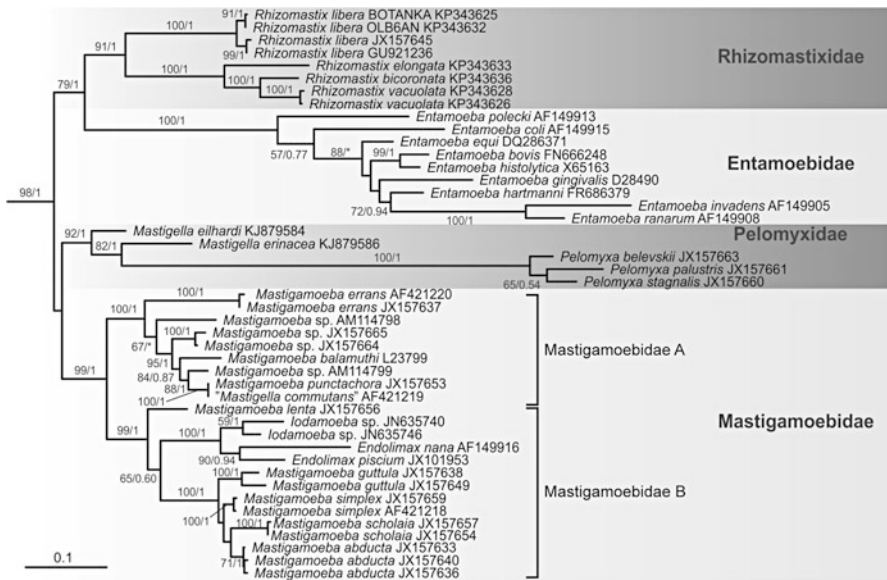


Fig. 13 Phylogenetic tree of Archamoebae based on SSU rRNA genes, showing generic relationships within families. *Mastigamoeba* is divided into two main clades, corresponding to “Mastigamoebidae A,” with 100% ML bootstrap support and Bayesian posterior probability of 1, and “Mastigamoebidae B,” with 99% ML bootstrap support and Bayesian posterior probability of 1. Note that the sequence labelled “*Mastigella commutans*” is thought to be from *Mastigamoeba punctachora* rather than *M. commutans* (see Zadrobílková et al. 2015). Support for relationships between families is not recovered from SSU rRNA data alone, requiring a multigene approach (Pánek et al. 2016). Sequences were aligned using the G-INS-I algorithm with default settings, on the MAFFT7 server (<http://mafft.cbrc.jp/alignment/server/>). The resulting alignment was manually edited to remove ambiguously aligned sites; the final dataset contained 1263 nucleotide positions. A maximum-likelihood phylogenetic tree was constructed in RAXML 7.2.3 (Stamatakis 2006) using the GTRGAMMAI model; the values at the branches represent statistical support in maximum likelihood (RAXML, 1000 pseudoreplicates)/Bayesian posterior probability (MrBayes, GTR + Γ + I + covarion model). Support values below 50/0.90 are not shown or are represented by an asterisk (*)

mitochondrial origin (*cpn60*, *HSP70*, *IscU*) and the localization of these gene products (by immuno-microscopy) to double-membrane-bounded organelles (Bui et al. 1996; Clark and Roger 1995; Roger et al. 1996, 1998; Tovar et al. 2003) have led to the identification of mitochondrion-derived organelles (i.e., hydrogenosomes and mitosomes) in almost all of the proposed “amitochondriate” organisms (van der Giezen 2009).

The identification of mitochondrial-remnant organelles (discussed above) and comparisons of ribosomal DNA sequences (Cavalier-Smith 1997; Hinkle et al. 1994; Milyutina et al. 2001; Morin and Mignot 1995; Silberman et al. 1999; Stiller and Hall 1999) and other genes (Arisue et al. 2002; Bapteste et al. 2002; Hannaert et al. 2000) now suggest that the Archamoebae are derived within the mitochondriate taxon Amoebozoa and are not deep-branching eukaryotes (Cavalier-Smith 1997, 1998; Edgcomb et al. 2002; Simpson and Roger 2004).

As discussed above, systematics within the Archamoebae is currently undergoing considerable flux, while many gene and protein sequences are obtained for many more representatives of the group. So far this has clarified that endobiotic taxa are distributed among the free-living taxa, showing that parasitism has clearly evolved multiple times in the Archamoebae (Pánek et al. 2016; Ptáčková et al. 2013; Stensvold et al. 2012; Zadrobílková et al. 2015, 2016). Obtaining multiple sequences from a single taxon can improve the robustness of placement of particular taxa, both at species level and at higher levels – for example the placement of *Pelomyxa* has long been unstable because the original sequence obtained was highly divergent (Milyutina et al. 2001; Ptáčková et al. 2013). Further sequences from *Mastigella*, *Tricholimax*, and *Mastigina* will be key to resolving relationships among the genera of the Archamoebae; and sequences from *Mastigamoeba aspera* would permit certainty in the proposed split of *Mastigamoeba* discussed above (Pánek et al. 2016) (Fig. 13).

Acknowledgments We gratefully acknowledge the input from Professor David J. Patterson on knowledge of free-living taxa and the input on knowledge of parasitic taxa from Professor RNDr. Jaroslav Kulda, who also provided the prepared slide material in the plates. This work was supported by a grant from the Czech Science Foundation (project GA14-14105S).

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