Levin, L. A. 1994. Ecology and paleoecology of xenophyophores. *In*: Miller, W., (ed.), *Recent Advances in Deep-Sea Paleoecology. Palaios* Theme Issue.

Levin, L. A. & Gooday, A. J. 1992. Possible roles for xenophyophores in deepsea carbon cycling. *In*: Rowe, G. T. & Pariente, V. (eds.), *Deep-Sea Food Chains and the Global Carbon Cycle.* Kluwer Academic Publishers, Dordrecht, The Netherlands. pp 93-104

Levin, L. L. & Thomas, C. L. 1988. The ecology of xenophyophores (Protista) on east Pacific seamounts. *Deep-Sea Res.,* 35:2003-2027.

Lewis, K. B. 1966. A giant foraminifer: a new species of *Syringammina* from the New Zealand region. *N. Z. J. Sci.,* 9:114-123.

Maybury, C. A. & Evans, K. R. A new interpretation of certain Pennsylvanian phylloid algae as shallow water xenophyophores. *Lethaia* (in press)

Mullineaux, L. 1987. Organisms living on manganese nodules and crusts: distribution and abundance at three North Pacific sites. *Deep-Sea Res.,* 34:165-184.

Rice, A. L., Aldred, R. G., Billett, D. S. M. & Thurston, M. H. 1979. The combined use of an epibenthic sledge and a deep-sea camera to give quantitative relevance to macro-benthos samples. *Ambio Special Report,* **6**:59-72.

Schroder, C. J. 1986. Deep-water arenaceous Foraminifera in the Northwest India Ocean. *Canadian Technical Report of Hydrography and Ocean Sciences,* 71:1-191.

Schroder, C. J., Medioli, F. S. & Scott, D. B. 1989. Fragile abyssal foraminifera (including new Komokiacea) from the Nares Abyssal Plain. *Micropaleontology N.Y.,* 35:10-48.

Schulze, F. E. 1906. Die Xenophyophoren der 'Siboga'-Expedition. *Siboga-Exped.* Monographie IV:1-18, pls I-III.

Schulze, F. E. 1907a. Die Xenophyophoren, eine besondere Gruppe der Rhizopoden. Wiss. *Ergebn. dt. Tiefsee-Exped.,* 11:1-55, pls I-VIII.

Schulze, F. E. 1907b. Die Xenophyophora. *Bull Mus. Comp. Zool. Harv.,* 51:205-229.

Swinbanks, D. D. 1982. *PaleodicLyon:* The traces of infaunal xenophyophores? *Science*, 218:47-49.

Tendal, O. S. 1972. A monograph of the Xenophyophora. *Galathea Report,* 12:7-103, pls 1-17.

Tendal, O.S. 1975. A new xenophyophore (Rhizopoda, Protozoa), living on solid substratum, and its significance. *Deep-Sea Res.*, 22:45-48.

Tendal, O. S. 1980a. Xenophyophores from the French expeditions "INCAL" and "BIOVEMA" in the Atlantic Ocean. *Cah. Biol. Mar.,* 21:303-306.

Tendal, O. S. 1980b. *Stannophyllum setosum* sp. n., a remarkable xenophyophore (Rhizopoda, Protozoa) from the eastern Pacific. *Cah. Biol. Mar.*, 21:383-385.

Tendal, O. S. 1989. Phylum Xenophyophora. *In*: Margulis, L., Corliss, J. O., Melkonian, M. & Chapman, D. J. (eds.), *Handbook of the Protoctista.* Jones and Bartlett Publishers, Boston. pp 135-138

Tendal, O. S. & Lewis, K. B. 1978. New Zealand xenophyophores: upper bathyal distribution, photographs of growth position, and a new species. N.Z. *J. Mar. Freshwat. Res.,* 12:197-203.

Tendal, O. S., Swinbanks, D. D. & Shirayama, Y. 1982. A new infaunal xenophyophore (Xenophyophorea, Protozoa) with notes on its ecology and possible trace fossil analogues. *Oceanol. Acta,* 5:325-329.

Tendal, O. S. & Gooday, A. J. 1981. Xenophyophoria (Rhizopoda, Protozoa) in bottom photographs from the bathyal and abyssal NE Atlantic. *Oceanol. Acta,* 4:415-422.

*Supported partly by the European Community under contracts 0037C(EDB) and MAS2-CT92-0

ORDER PELOBIONTIDA Page, 1976

by

GUY BRUGEROLLE and DAVID PATTERSON

The order Pelobiontida includes four genera of mostly free-living heterotrophic protists. They are usually found in anaerobic or microaerophilic habitats such as stagnant water which is rich in organic matter. They occur in freshwater and marine habitats. One species is endocommensal. The most usual trophic form in all but *Pelomyxa* is a flagellated cell, but amoeboid and encysted forms are known from some species and may be characteristic of all species. Flagellated cells have one or many flagella and move by flagellar

motion and/or by (a sometimes distinctive type of) amoeboid streaming.

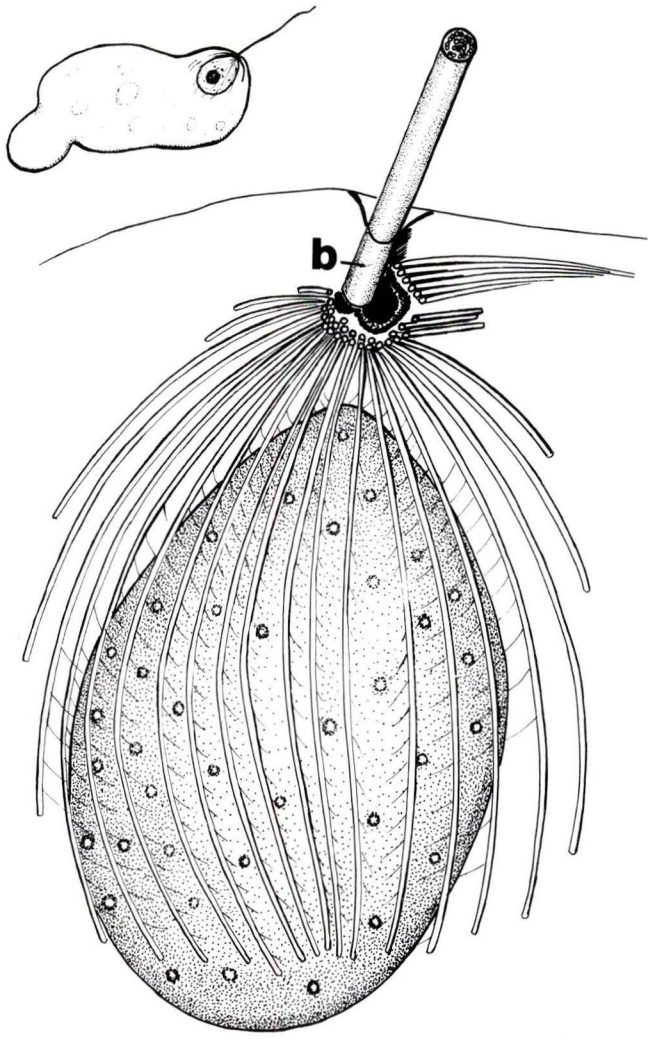

Fig. 1. Basic organization of a mastigamoebid (from Brugerolle, 1991a). Upper left: typical uniflagellated cell. The single flagellum/basal body (b) is connected to the nucleus by a cone of microtubules. The microtubules arise from a microtubule-organizing-center structure that is attached to the base of the basal body. A lateral band of microtubules extends towards the cell surface.

The order has been more clearly circumscribed after the discovery of flagella in *Pelomyxa* by Griffin (1988) and as a result of ultrastructural studies of various species (Brugerolle 1991a, 1991b; Mylnikov, 1991; Simpson et al., 1997). Light and electron microscopic studies have shown that flagella/basal bodies occur singly. In some cases the flagella have an 'n+n' substructure rather than the usual '9+2' arrangements of microtubules, and there may also be abnormalities in the basal body. Flagella are often attached to the nucleus by a cone or cape of microtubules radiating from the basal body (Figs. 1, 2a,b,c). The microtubules arise from a region at the base of or around the basal body. This has been demonstrated in *Mastigina* (Brugerolle, 1982), *Pelomyxa* (Griffin, 1988), *Mastigamoeba* (Brugerolle, 1991a, 1991b; Simpson et al., 1997), and *Mastigella* (A. G. B. Simpson and C. Bernard, unpubl. observ.). The cytoplasm is rich in microfilaments, and cytoplasmic movements may lead to locomotion (especially in *Mastigina* and *Pelomyxa*) or to the ingestion of food. Most species move by the slow, undulating actions of the flagella.

The endoplasmic reticulum is sometimes concentrated in large stacks of cisternae. There are no dictyosomes nor mitochondria; glycogen is used as a reserve material. Cells contain one to many nuclei. These contain large nucleoli. Mitosis involves an intranuclear spindle; the nucleoli are persistent and divide, but without ultrastructural studies of division it is unclear if the nuclear envelope is persistent or if it breaks down.

Because of the absence of mitochondria and Golgi apparatus and because of the simplicity of the mastigont system, pelobionts have been regarded as an early offshoot in eukaryotic evolution and have also been classified as Archeozoa (Cavalier-Smith, 1991). The term Archezoa was previously used by Perty and Haeckel with different meanings and therefore is not adopted here. The primitive status of the Pelobionts is not supported by current molecular studies (Hinkle et al., 1994). General reviews of the group are found in Lemmermann, 1914 (species

diversity), Mylnikov, 1991 (generic diversity), and Brugerolle, 1991a, 1991b (ultrastructure).

KEY CHARACTERS

All pelobionts have an amoeboid body which may give rise to pseudopodia and have one (or more, e.g. in *Pelomyxa*) flagella. The body gives rise to blunt pseudopodia, the form of which may have some diagnostic value—although several species are known to have variable pseudopodial form. Amoeboid motion occurs in *Mastigina* and *Pelomyxa*. This motion is distinctive with the cytoplasm flowing along the center of the body and spreading out, fountain-like, at the anterior end. The posterior end of the cell has a bulbous or filose uroid, and food particles may be ingested here.

In most cells of genera other than *Pelomyxa* a flagellum is located anteriorly in moving cells. The relationship between the nucleus and flagellum is used to segregate the genera *Mastigamoeba* and *Mastigella*—the latter genus being said to have a greater relative difference between the two organelles. The flagellum is often very long and beats languidly, typically with a single wave passing from base to tip—the beat pattern is distinctive.

Members of this genus may be confused with some other taxa (e.g. *Cercomonas*), and electron microscopy is desirable to confirm that the organism is a pelobiont. Taxa are assigned to genus depending on the number of flagella, the connections between flagella and nuclei, and the extent of amoeboid motion.

KEY TO FAMILIES AND GENERA

1. Amoeboid, one large pseudopod, posterior **uroid**, one to many nuclei, flagella numerous, but hard to see..... ***Pelomyxa***
1'. With prominent apical flagellum when gliding/ swimming (some cells may have more than one flagellum) 2

2. With marked cytoplasmic streaming; one species endocommensal..... ***Mastigina***
2'. Cytoplasmic streaming not marked 3

3. Flagellum closely linked to the conical anterior nucleus ..... ***Mastigamoeba***
3'. Flagellum and nucleus distant from each other, cytoskeletal material linking the two visible or not visible..... ***Mastigella***

FAMILY PELOMYXIDAE Schulze, 1877

Flagellated monopodial amoebae, thickly cylindrical during amoeboid motion, sometimes with fountain streaming and with food being taken up posteriorly. Cell with one to many nuclei with associated flagella (**karyomastigonts**). Two genera.

Genus ***Pelomyxa*** Greeff, 1874

Free-living from anaerobic or micro-aerophilic habitats, cells amoeboid reaching 3 mm long with a large, anterior pseudopod and a posterior uroid. With one to several nuclei. Short flagella have been demonstrated at some stages of the life cycle. Cytoplasm contains 2 to several "glycogen bodies" as large as the nuclei (10 µm). A complex life cycle has been described. In spring, cysts release small binucleate amoebae, which grow and become multinucleate and acquire **endosymbiotic** bacteria. They become elongate with a posterior uroid, and flagella are evident at this stage. The cells later become spherical, and endosymbiotic bacteria congregate around the nuclei. These cells fragment into rosettes giving rise either to cysts in winter or to small amoebae which may undergo another cycle of development (Whatley and Chapman-Andresen, 1990). Electron-microscopical studies have shown that the basal bodies and flagella are connected to a cone of microtubules. The microtubules are not in contact with the nuclei when the nuclei are surrounded by endosymbiotic bacteria. Three types of bacteria have been observed: one is a large, cylindrical bacterium with an internal cleft, the other is a long rod-shaped bacterium, the third is a **methanogen** (Stumm and Zwart,

1986; Griffin, 1988; Whatley and Chapman-Andresen, 1990). Many species have been assigned to this genus, but all may be assignable to the type species *P. palustris* Greeff, 1874.

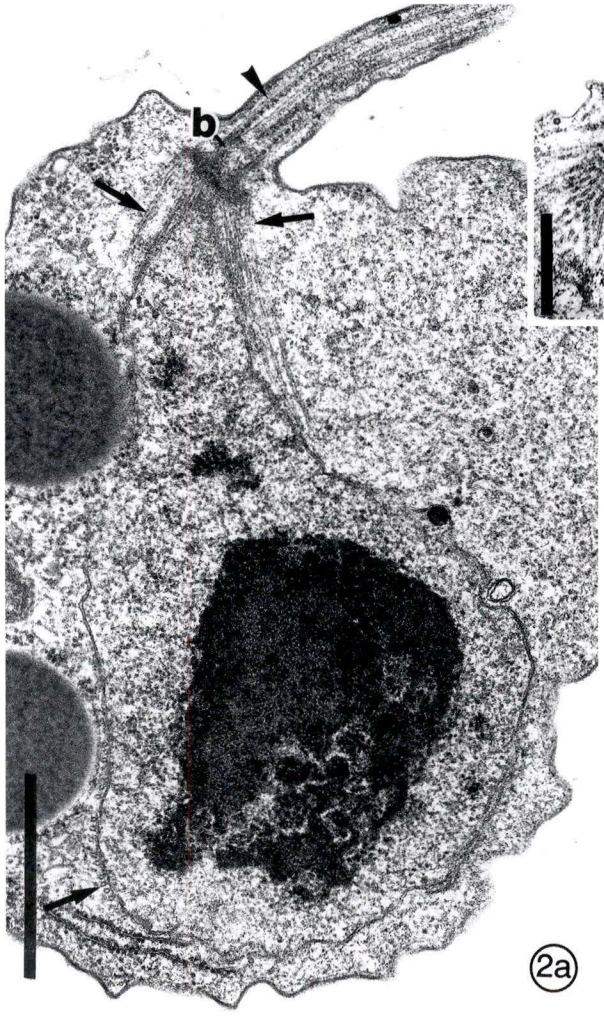

Fig. 2a. Section of *Mastigamoeba* showing the cone of microtubules (arrows) arising from the basal body (b). These microtubules link the flagellum to the nucleus. Note the helix-like structure around the axoneme of the flagellum (arrowhead) and the large nucleolus. Bar=1 μm.

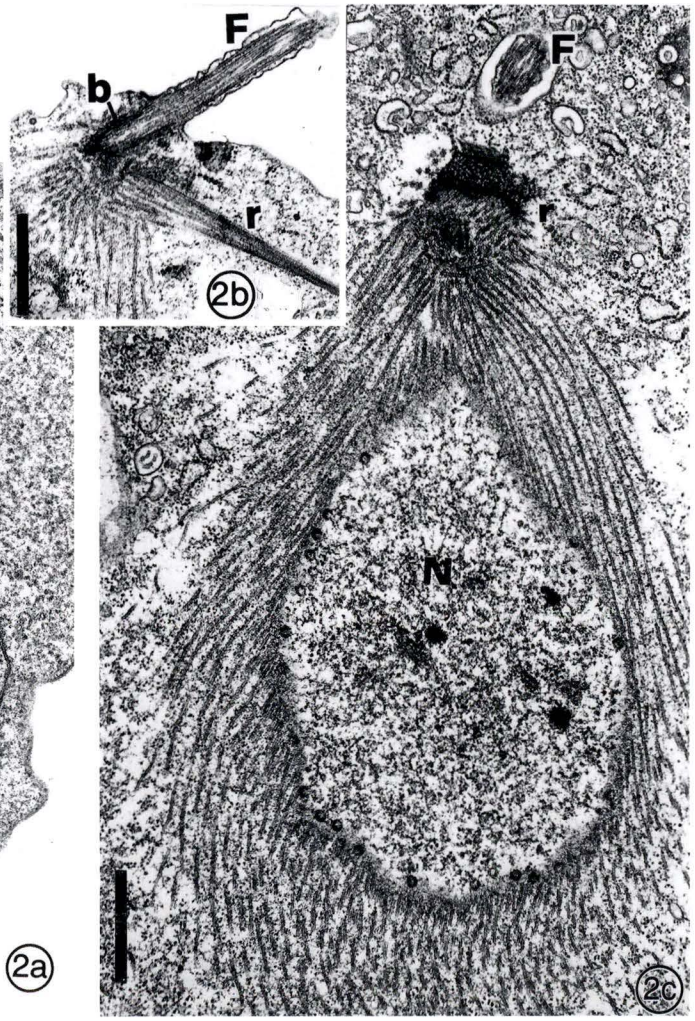

Fig. 2b,c. In *Mastigella hylae*, the microtubules of the cape covering the nucleus (N) are numerous; they arise from a MTOC at the base of the basal body (b) and some form a lateral ribbon (r). The flagellum (F) is composed of microtubules not arranged in a 9+2 configuration. Bar=1 μm. (from Brugerolle, 1991a)

Genus ***Mastigina*** Frenzel, 1897

Amoeboid monopodial cells with one flagellum, long or short, active or inactive, and always attached to an anterior nucleus. There is no posterior uroid, and there are several nuclei in one species. The nucleus has a large, central **endosome**, and mitosis includes division of the endosome. *Mastigina hylae,* which is endocommensal in intestine of toads, has solitary basal bodies connected to the nucleus by a cape or cone of microtubules (Figs. 2b,c and Fig. 5). The single flagellum may not have the usual 9+2 arrangement of microtubules in the axoneme. A lateral ribbon of microtubules extends from the

basal body to the cell surface. Secondary nuclei, which occur in large cells, bear one basal body without a flagellum and have a few associated microtubules. Some species may have a **polymorphic life cycle** (Goldschmidt, 1907). Electron microscopy has been carried out by Brugerolle (1982, 1991a). About 6 species have been reported. Refs. Brugerolle, 1982, 1991a; Collin, 1913; Goldschmidt, 1907; Penard, 1909.

TYPE SPECIES: *Mastigina chlamys* Frenzel, 1897

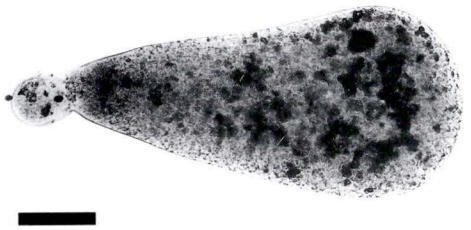

Fig. 3. *Pelomyxa palustris,* showing the anterior, large pseudopod and the small, posterior uroid. Bar=250 µm. (courtesy of C. K. Stumm)

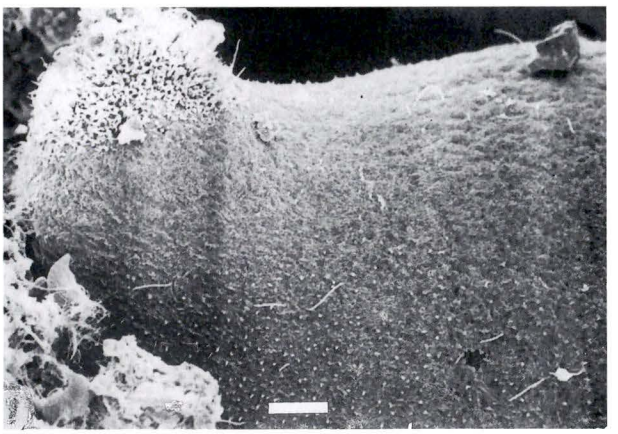

Fig. 4. *Pelomyxa palustris* showing flagella on the surface and the posterior uroid. Bar=10 µm. (from Griffin, 1988)

FAMILY MASTIGAMOEBIDAE Goldschmidt, 1907

Flagellated cells with or without simple or branched, hyaline pseudopodia during amoeboid motion. Swimming is erratic with the single, languidly beating flagellum directed forward. Species with one or several nuclei, with a life cycle which may include amoebae without flagella, and cysts.

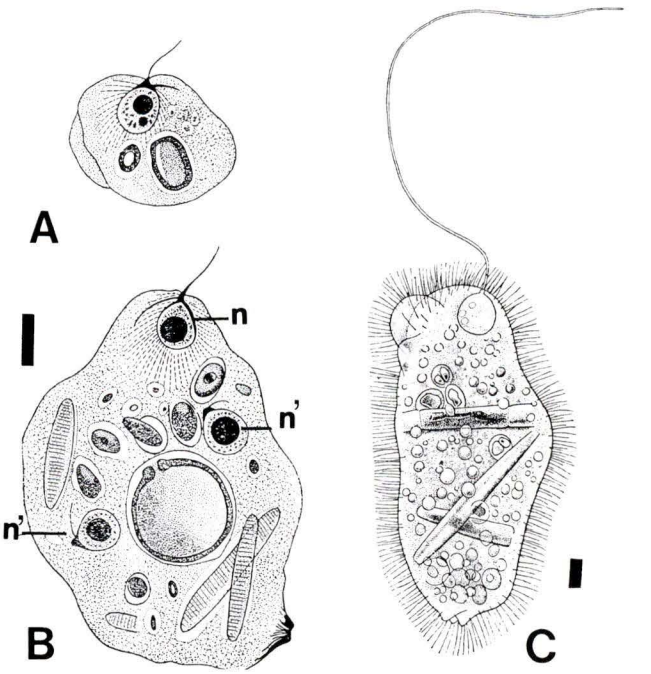

Fig. 5. A. *Mastigina hylae* with one nucleus and associated flagellum. B. With two secondary nuclei (n') without flagella. Bar=10 µm. (after Collin, 1913) C. *Mastigina setosa* (after Goldsdhmidt, 1907). Bar=10 µm.

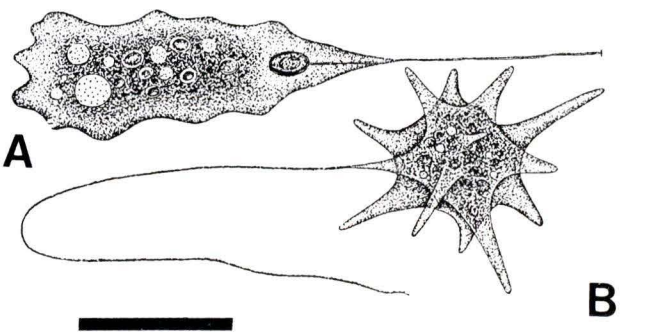

Fig. 6. *Mastigamoeba scholaia* with flagella connected to the nucleus; cell swimming (A), gliding (B). Bar = 10 µm. (from Klug 1936).

Genus ***Mastigamoeba*** Schulze, 1875

Amoeboid cells with a flagellated basal body located immediately adjacent to the anterior nucleus. The nucleus may be pulled out anteriorly to have a conical appearance (Fig. 6). The connection may be seen with the light microscope. The flagellum is long and directed forward, the pseudopodia are simple, branched,

or absent. The cells may swim or glide. Life cycles may include amoeboid organisms, multinucleated cells, or cysts (Simpson et al., 1997). Electron-microscopical studies (e.g. Brugerolle et al., 1991a) have shown that a cone of microtubules connects the short basal body to the nucleus (Fig. 2a) and that there is a ribbon of microtubules extending from the basal body into the cytoplasm. The flagellum has the conventional 9+2 arrangement of microtubules within the axoneme, but variations on the normal organization of the basal body have been reported (Simpson et al., 1997). The monotypic genus *Phreatamoeba* was erected by Chavez et al. (1986) for organisms with a complex life cycle. *Phreatamoeba* cannot be distinguished from *Mastigamoeba* and thus is a synonym of *Mastigamoeba*, (Simpson et al., 1997). *Dinamoeba mirabilis* Leidy, 1874, the type species of that genus, also appears to be an incomplete description of *Mastigamoeba aspera* (Simpson et al., 1997). With about 40 nominal species, many of which will prove to be incomplete descriptions of other species. References: Brugerolle, 1991a; Klug, 1936; Lemmermann, 1914; Penard, 1909; Schulze, 1875; Simpson et al., 1997.
TYPE SPECIES: *Mastigamoeba aspera* Schulze, 1875

Genus ***Mastigella*** Frenzel, 1897

Amoeboid cells with an apical flagellum (Fig. 7) and the nucleus removed some distance from the flagellum. Reportedly without direct connection between the two, although unpublished ultrastructural studies indicate that connections are present. The cells are naked but usually with a distinct cortical region or periplast, and some species may have adhering bacteria. Pseudopodia are simple or absent and there may be one 1 or many contractile vacuoles. Swimming is disorderly, cells may glide but rarely. One species at least described with aflagellated amoebae and cyst in life cycle. The distinction between this genus and *Mastigamoeba* is probably not sustainable.

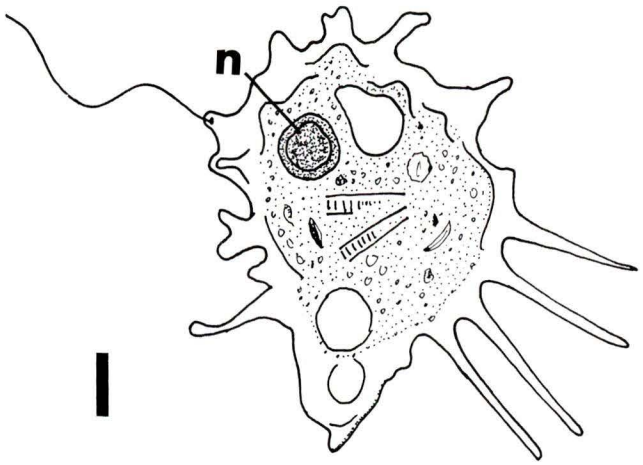

Fig. 7. *Mastigella nitens* with a flagellum not directly connected to the nucleus (n). Bar=10 µm. (after Penard, 1909)

With about 150 nominal species, many of which have been reported by Skvortzkov and Noda (1975) and are probably not distinct species. Published ultrastructure is restricted to an examination of symbiotic methanogens. Refs. Goldschmidt, 1907; Klug, 1936; Lemmermann, 1914; Penard, 1909; Skvortzkov and Noda 1975; van Bruggen et al., 1986.
TYPE SPECIES: *Mastigella polymastix* Frenzel, 1897

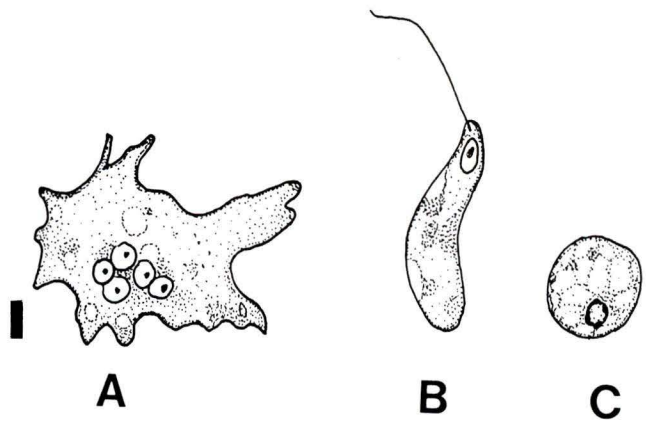

Fig. 8. *Mastigamoeba balamuthi*, multinucleated amoeboid stage (A), flagellate (B), and cyst (C). Bar=10 µm. (after Chavez et al., 1986)

Genus ***Phreatamoeba***
Chavez, Balamuth & Gong, 1986 (Fig. 8)

See *Mastigamoeba*.

References

Brugerolle, G. 1982. Caractères ultrastructuraux d'une mastigamibe: *Mastigina hylae* (Frenzel). *Protistologica*, 18:227-235.

Brugerolle, G. 1991a. Flagellar and cytoskeletal systems in amitochondrial flagellates: Archamoebae, Metamonada and Parabasala. *Protoplasma*, 164:70-90.

Brugerolle, G. 1991b. Cell organization in free-living amitochondriate heterotrophic flagellates. *In*: Patterson, D. J. & Larsen, J. (eds.), *The Biology of Free-living Heterotrophic Flagellates*. The Systematics Association, Clarendon Press, Oxford, 45:133-158.

Chavez, L. A., Balamuth, W. & Gong, T. 1986. A light and electron microscopical study of a new, polymorphic free-living amoeba, *Phreatamoeba balamuthi* n. g., n. sp. *J. Protozool.*, 33:397-404.

Cavalier-Smith, T. 1991. Cell diversification in heterotrophic flagellates. *In*: Patterson, D. J. & Larsen, J. (eds.), *The Biology of Free-living Heterotrophic Flagellates*. The Systematic Association, Clarendon Press, Oxford, 45:113-131.

Collin, B. 1913. Sur un ensemble de protistes parasites des Batraciens. *Arch. Zool. Exp. Gén.* (Notes Rev.), 52:67-76.

Goldschmidt, R. 1907. Lebensgeschichte der mastigamöben *Mastigella vitrea* n. sp. u. *Mastigina setosa* n. sp. *Arch. Protistenkd.*, 1: (suppl.1) 83-168.

Griffin, J. L. 1988. Fine structure and taxonomic position of the giant amoeboid flagellate *Pelomyxa palustris*. *J. Protozool.*, 35:300-315.

Hinkle, G., Leipe, D. D., Nerad, T. A. & Sogin, M. L. 1994. The unusually long small subunit ribosomal RNA of *Phreatamoeba balamuthi*. *Nucl. Acids Res.*, 22:465-469.

Klug, G. 1936. Neue oder wenig bekannte Arten der Gattung *Mastigamoeba*, *Mastigella*, *Cercobodo*, *Tetramitus* und *Trigonomonas*. *Arch. Protistenkd.*, 87:97-116.

Lemmermann, E. 1914. Flagellata I. *In*: Pascher, A. (ed.), *Die Süsswaterflora Deutschlands, Osterreichs und der Schweiz*, vol. 1., G. Fisher, Jena. pp 30-51

Mylnikov, A. P. 1991. Diversity of flagellates without mitochondria. *In*: Patterson, D. J. & Larsen, J. (eds.), *The Biology of Free-living Heterotrophic Flagellates*. The Systematic Association, Clarendon Press, Oxford. pp 149-158

Penard, E. 1909. Sur quelques mastigamibes des environs de Genève. *Rev. Suisse Zool.*, 17:405-440.

Schulze, F. E. 1875. Rhizopodienstudien. *Arch. Mikrosk. Anat.*, 11:583-596.

Simpson, A. G. B., Bernard, C., Fenchel, T. & Patterson, D. J. 1997. The organisation of *Mastigamoeba schizophrenia* n. sp.: more evidence of ultrastructural idiosyncrasy and simplicity in pelobiont protists. *Eur. J. Protistol.*, 33:87-98.

Skvortzkov, B. V. & Noda, M. 1975. Flagellates of clean and polluted waters. V. A short description of colourless flagellates with one swimming flagellum— genus *Mastigella* (Frenzel) nob (Rhizomastigaceae) reported during 1962 - 1975 in many parts of the world and especially from subtropics in Brasil, South America. *In*: *Prof. Mitsuzo Noda commemorative publication on his retirement from the Faculty of Science, Niigata Universit*. Niigata University. pp 25-66

Stumm, C. K. & Zwart, K. B. 1986. Symbiosis of protozoa with hydrogen utilizing methanogens. *Microb. Sci.*, 3:100-105.

van Bruggen, J. J. A., Stumm, C. K., Zwart, K. B. & Vogels, G. D. 1985. Endosymbiotic methanogenic bacteria of the sapropelic amoeba *Mastigella*. *FEMS Microb. Ecol.*, 31:187-192.

Whatley, J. M. & Chapman-Andresen, C. 1990. Phylum Karyoblastea. *In*: Margulis, L., Corliss, J. O., Melkonian, M. & Chapman, D. J. (eds.), *Handbook of Protoctista*. Jones & Bartlett, Boston. pp 167-185