CLASS HETEROLOBOSEA

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This grouping of the schizopyrenid amoebae and amoeboflagellates and the acrasid (cellular) slime moulds was created by Page and Blanton (1985) on the basis of ultrastructural characteristics (see also Page, 1985). Initially, the schizopyrenid organisms were thought of as being predominantly amoeboid with some species having distributive flagellated stages. Several taxa have been added that are known only as flagellates (Fenchel and Patterson, 1986; Larsen and Patterson, 1990). As the flagellated state probably evolved only once in eukaryotes, the ancestors of the schizopyrenids must have been flagellated organisms. The group contains some parasitic organisms. Divisions among some genera are subtle and often require examination of life cycle characteristics. These characteristics may only be explored with any confidence using pure cultures. Note that some species are facultative pathogens. The group has been reviewed by Page (1988) and Page and Siemensma (1991) and the genera with flagellates in Patterson and Larsen (1991). The organism referred to as a vahlkampfiid by Larsson et al. (1992) is not accepted as such here. This account concentrates on those characteristics which can be determined by light microscopy of living cells. Details on techniques for isolation and culturing these flagellates are given in Page (1988) and in Patterson and Larsen (1991). Primary characters are (i) the number of phenotypes in the life cycle (amoebae, flagellates, cysts), (ii) the nature of the cysts (with or without excystment pores and gelatinous coat), and (iii) the flagellated forms (number of flagella, with or without cytostome, and curving ridge near flagella). Family level distinctions depend on the aspect or the behavior of the nucleus during mitosis.

CLASS HETEROLOBOSEA
Page & Blanton, 1985

Protists with flattened cristae in mitochondria, mitochondria associated with endoplasmic reticulum, dictyosomes not well developed, nuclear division **promitotic** (persistent nucleoli and endonuclear division spindle). Amoebae with eruptive pseudopodia, cell length usually less than 65 µm. Species may exhibit amoeboid and/or flagellated and/or encysted forms. Some taxa (acrasids) produce stalked fruiting bodies in which encysted cells are referred to as spores. With two orders, Schizopyrenida and Acrasida.

The Schizopyrenids contain facultative pathogens. The group is abundant, but any cells should be regarded initially as potentially pathogenic until full identification has been carried out.

ORDER SCHIZOPYRENIDA Singh, 1952

Heterolobosea without **fruiting bodies**. With two families (Vahlkampfiidae and Gruberellidae).

FAMILY VAHLKAMPFIIDAE Jollos, 1917

Schizopyrenids in which the nucleolus forms two **polar masses** during mitosis (**promitosis**). Amoeboid form usually cylindrical and usually uninucleate. Most species with a flagellated stage, one genus (*Percolomonas*, *incertae sedis*) so far not known to have an amoeboid stage. *Vahlkampfia* lacks a flagellated stage.

KEY TO GENERA OF THE FAMILY VAHLKAMPFIIDAE

To determine generic identities it is necessary to establish pure cultures of each isolate. The Heterolobosea can have amoeboid and/or flagellated and/or encysted stages in their life cyle, and the presence or absence and appearance of these are essential diagnostic criteria. In practice this may be difficult since, depending upon culture conditions used, some isolates may fail to display all stages in the laboratory. Moreover, older amoeboid cultures frequently lose their ability to form flagellates or cysts. Viewing several protozoa in a culture is required to detail the features of a

'typical' vahlkampfiid. For example, some flagellates have four flagella, but occasionally three. For the purposes of this key, the typical form would be four. A phenotypically diverse group of protists which are being studied by molecular phylogeneneticists (e.g. Hinkle and Sogin, 1993). One genus, *Lyromonas*, is amitochondriate and requires TEM for identification.

1. With mitochondria 2
1'. Without mitochondria ..... ***Lyromonas***

2. Without flagellate stage 3
2'. With a flagellate stage (or known only as a flagellate) 4

3. Amoebae always uninucleate ..... ***Vahlkampfia***
3'. Amoebae form multinucleate plasmodia on agar ..... ***Pseudovahlkampfia***

4 . With two flagella 5
4'. With more than two flagella 9

5. With anterior beak-like rostrum ..... ***Paratetramitus***
5'. Without beak-like rostrum 6

6. With cytostome 7
6'. Without cytostome 8

7. Cytostome obvious, as deep groove ..... ***Heteramoeba***
7'. Cytostome indistinct, as circular apical depression ..... ***Adelphamoeba***

8. Cysts with pores ..... ***Naegleria***
8'. Cysts without pores ..... ***Didascalus***

9. With three flagella ..... ***Trimastigamoeba***
9'. With four or more flagella 10

10. With four flagella 11
10'. With four sets of four flagella ..... ***Psalteriomonas***

11. Cysts with pores ..... ***Willaertia***
11'. Cysts without pores 12

12. With cytostomal groove 13
12'. Without cytostomal groove ..... ***Tetramastigamoeba***

13. With lip-like anterior rostrum... ***Tetramitus***
13'. Without anterior rostrum..... ***Percolomonas***

Genus ***Lyromonas*** Cavalier-Smith, 1993

Amitochondriate genus with one species described as *Psalteriomonas vulgaris* (Broers et al. 1993), the flagellated stage only is known. Closely related to *Tetramitus*.

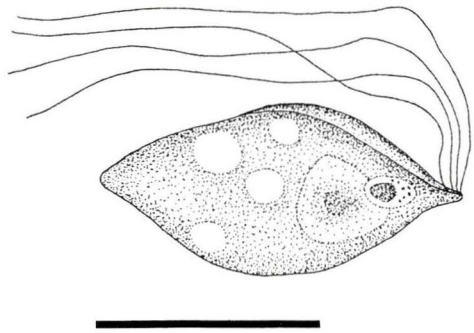

Fig. 1. *Lyromonas vulgaris* (after Broers et al.). Bar=5 µm.

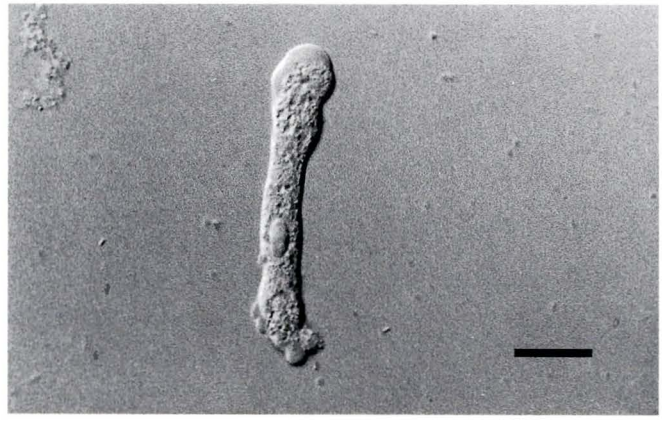

Fig. 2. *Vahlkampfia ustiana*. Active cell. Bar=10 µm

Genus ***Vahlkampfia*** Chatton & LaLung-Bonnaire, 1912 (Fig. 2)

Heterolobosea without flagellated stage described in life cycle. Cysts with or without gelatinous coat. One species only reported to have excystment pores. Many species (Page 1988; Page and Siemensma).

Genus ***Pseudovahlkampfia*** Sawyer, 1980 (Fig. 3)

Uninucleate amoeboid locomotive cells 18-30 µm long with the tendency to form multinucleate plasmodia up to 245 µm when cultured on agar. A vahlkampfiid without described flagellate stage. Monotypic genus. Marine, commensal in digestive tract and on gills of crabs (Bovee and Sawyer, 1979).

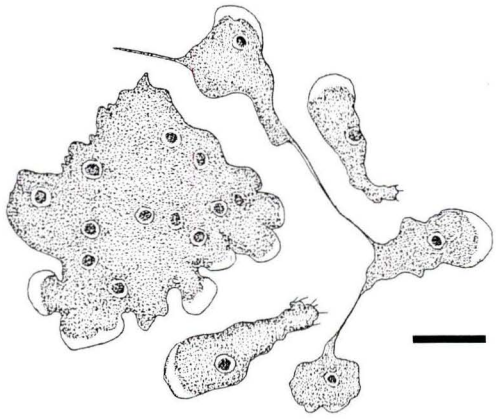

Fig. 3. Different forms of *Pseudovahlkampfia emersoni* (after Sawyer). Bar=10 µm.

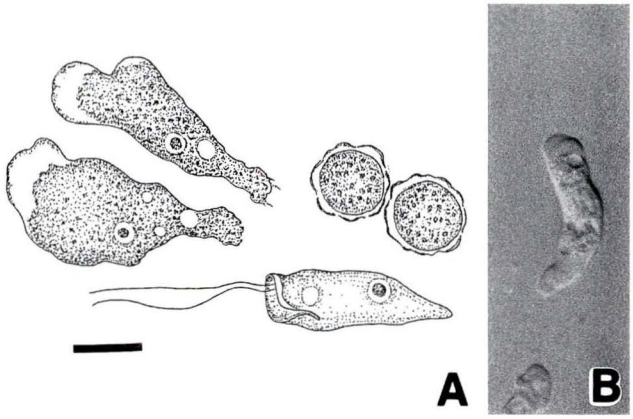

Fig. 4. *Paratetramitus jugosus*. (A) Line drawings of flagellate, cysts, and amoeboid stages. (after Page). (B) Bocomotive amoeboid cells. Bar=10 µm.

Genus ***Paratetramitus*** Darbyshire, Page & Goodfellow, 1976 (Fig. 4)

Monotypic vahlkampfiid genus, with cyst, amoeba, and flagellate phenotypes. Flagellates measure 15-20 µm, usually with 2 flagella but up to 6 reported; an anterior curved lip is present but ingestion not observed in original study. Flagellates may divide. *Paratetramitus* is most similar to *Heteramoeba*, being distinguished principally by chromatin distribution in the nucleus. Cyst walls without excystment pores (Darbyshire et al., 1976).

Genus ***Heteramoeba*** Droop, 1962 (Fig. 5)

The flagellated form is large (30 µm), 2 flagella, an elongate cytostome curving around the anterior of the cell and forming a groove. Nucleus with peripheral chromatin. Probably feeds and divides as a flagellate. One species. This genus is most like *Paratetramitus* from which it can be distinguished by peripheral location of chromatin material. Cysts without pores, excystment through a weak region of wall (Page, 1983). Marine.

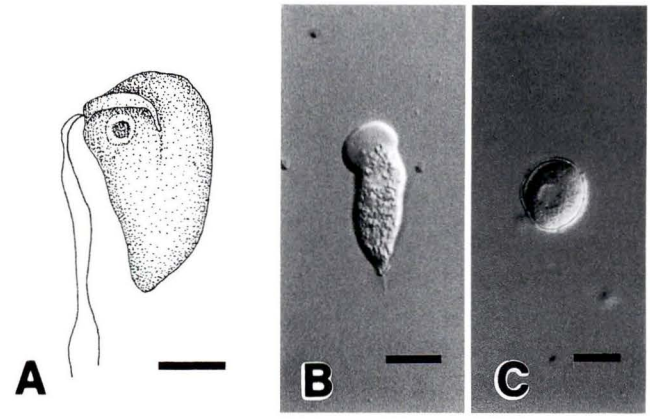

Fig. 5. *Heteramoeba clara*. (A) Flagellate stage. (B) Locomotive amoeba. (C) cyst. (after Page) Bars=10 µm.

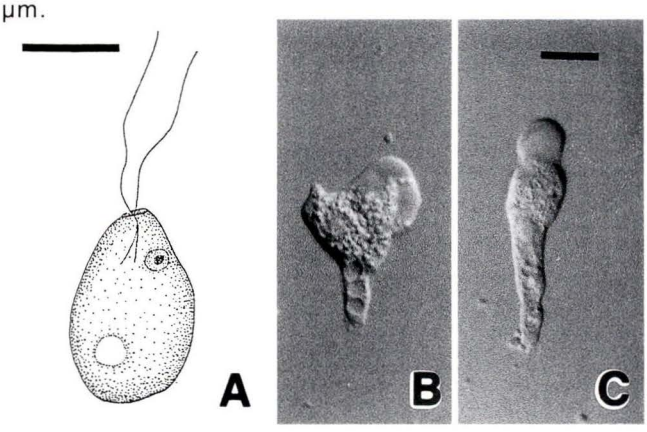

Fig. 6. *Adelphamoeba galeacystis*. (A) Flagellate stage (after Page) and (B, C) amoeboid stages. Bars=10 µm.

Genus ***Adelphamoeba***
Napolitano, Wall & Ganz, 1970 (Fig. 6)

Monospecific vahlkampfiid genus, with cyst, amoeboid, and flagellated stages. Flagellate about 10-20 µm long, 2 anterior flagella insering apically, with circular cytostome adjacent to flagellar insertion, feeds on bacteria. Cysts without pores, and no gelatinous sheath. Found in soil.

Genus ***Naegleria*** Aléxéieff, 1912 (Fig. 7)

Vahlkampfiid with cyst, amoeboid, and flagellated forms. Flagellate with 2 anteriorly inserting flagella, without cytostome, and without division. Cysts with plugged pores. Includes pathogenic species.

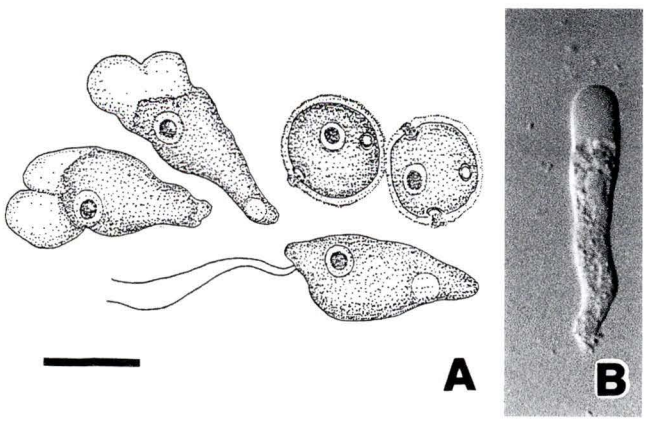

Fig. 7. *Naegleria gruberi* (A) Flagellate, cyst, and amoeboid stages (after Page). Note the plugged pores in the cysts. (B) N. jadini, active amoeboid stage. Bar=10 µm.

Genus **Didascalus** Singh, 1952 (Fig. 8)

Vahlkampfiid with amoeba, cyst, and biflagellated stage resembling the flagellate stage of *Naegleria*, the genus being distinguished by lack of excystment pores in cyst wall and by the presence of a mucus sheath around the cyst. Monotypic genus found in soil.

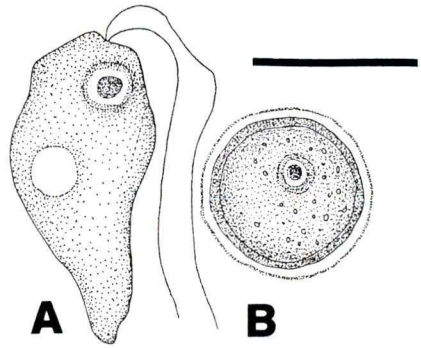

Fig. 8. *Didascalus thorntoni*. (A) Flagellate stage and (B) cyst (after Singh). Bar=10 µm.

Genus ***Trimastigamoeba*** Whitmore, 1911 (Fig. 9)

Vahlkampfiid with cysts, amoeba, and flagellate stage. Flagellate measures 16-22 µm. According to the original description normally with 3 flagella inserting in a deep depression, but no mouth—although the flagellated form has been reported as dividing. Cyst with pores. The nature of the taxon is unclear but probably related to the vahlkampfiids (Page, 1988).

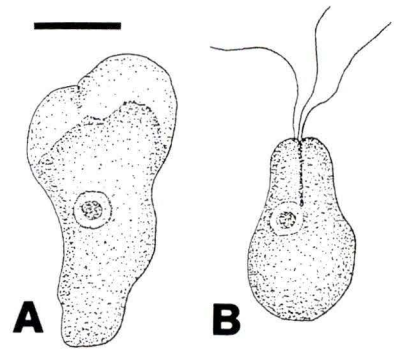

Fig. 9. *Trimatigamoeba philippenensis*. (A) Amoeboid stage. (B) Flagellated stage. Bar=10 µm.

Genus ***Psalteriomonas***
Broers, Stumm, Vogels & Brugerolle, 1990 (Fig. 10)

Vahlkampfiid, the flagellated stage of which has 4 nuclei and 4 sets of 4 flagella, giving rise to mononucleated amoeboid stage. From anoxic habitats. With methanogenic bacteria. Type species, *P. lanterna* (Broers et al., 1990). This genus is probably most closely related to *Tetramitus* because of similarly complex

microtubular and fibrous roots derived from the flagellar basal bodies.

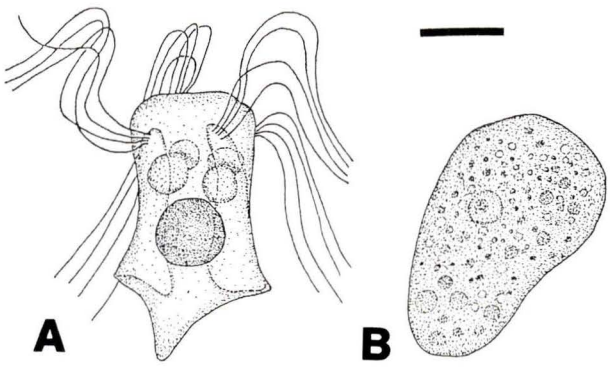

Fig. 10. *Psalteriomonas lanterna*. (A) Flagellate with prominent central globule and (B) amoeboid stage (after Broers et al.). Bar=10 µm.

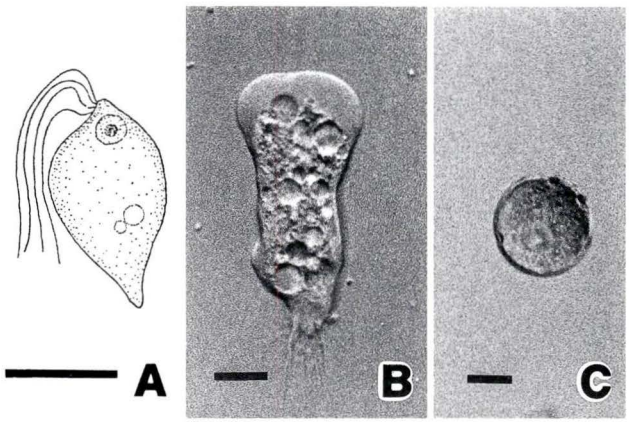

Fig. 11. *Willaertia magna*. (A) Flagellate, (B) locomotive amoeboid cell, (C) cysts with plugged pores. Bars=10 µm.

Genus ***Willaertia***
de Jonckheere, Dive, Pussard & Vickerman, 1984
(Fig. 11)

Vahlkampfiid with amoeboid stage, multi-pored cyst, and flagellated stage which can divide. Flagellate 19-20 µm in length with 4 flagella and no cytostome. Synonymy with *Protonaegleria* (Michel and Raether, 1985) suggested by Page (1988). *Protonaegleria* was distinguished by having a flagellated stage in the life cycle, but this was subsequently confirmed for *Willaertia* (Robinson et al., 1989). There is a rhizoplast and curving microtubular rootlets as in *Tetramitus* (Michel et al., 1987). Division of flagellate reported in Page (1988). Distinguishable from *Tetramastigamoeba* by having plugged pores in the cyst.

Genus ***Tetramastigamoeba***
Singh & Hanumaiah, 1977 (Fig. 12)

Monospecific genus of vahlkampfiids; with amoeboid and flagellated stages, plus cyst without pores. Flagellate with 4 flagella and no cytostome or ridge; the flagellates divide. Page (1988) doubts that the cyst is without pores. If pores are present, then this is the same as *Willaertia* and *Protonaegleria*, and *Tetramastigamoeba* is the senior synomym.

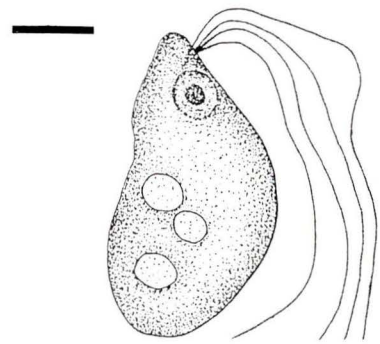

Fig. 12. *Tetramastigamoeba hoarei* (after Page). Bar=10 µm.

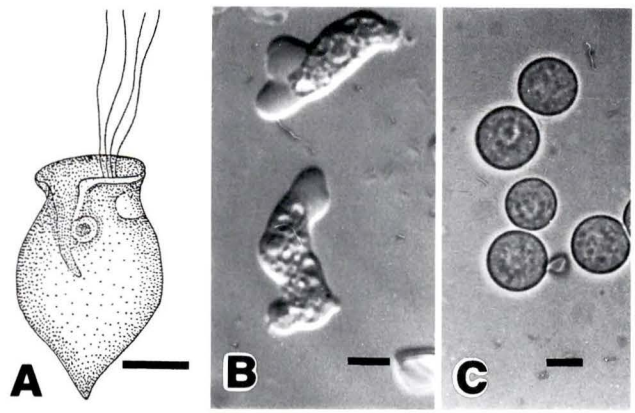

Fig. 13. *Tetramitus rostratus*. (A) Flagellate (after Page). (B) Amoeboid cells. (C) Cysts without pores. Bars=5 µm.

Genus ***Tetramitus*** Perty, 1852 (Fig. 13)

With 4 equal flagella arising in a flattened slit at the front end of the cell, surrounded by a distinct anterior lip-like rostrum. Substantial fibrous root associated with flagellar bases. Cysts without pores. Many of the flagellates assigned to this genus are likely to be more closely related to *Percolomonas cosmopolitus* than to *T. rostratus*, the type of this genus. Reported from soil, freshwater, and feces.

Genus ***Percolomonas*** Fenchel & Patterson, 1986. *incertae sedis* (Fig. 14)

Protists known only as flagellates; with 4 flagella, usually 1 used for adhesion, others for feeding on small suspended particles which are ingested within a ventral grove. Ultrastructural features suggest affinities with the Heterolobosea (Fenchel and Patterson, 1986).

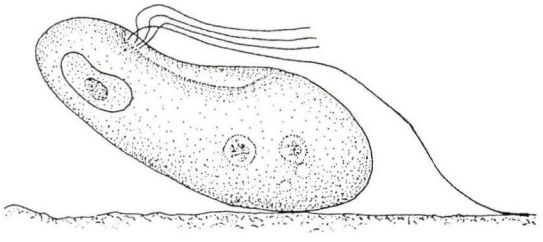

Fig. 14. *Percolomonas cosmopolitus*, attached (after Fenchel and Patterson). Bar=5 µm.

FAMILY GRUBERELLIDAE Page & Blanton, 1985

Nucleolus disintegrates during mitosis, amoeboid form flattened or limax and often multinucleate. No flagellated stage known. Amoebae may have fine **subpseudopodia.**

KEY TO THE GENERA OF THE FAMILY GRUBERELLIDAE

1. Normally uninucleate; from soil or freshwater ..... ***Stachyamoeba***
1'. Normally multinucleate, marine.. ***Gruberella***

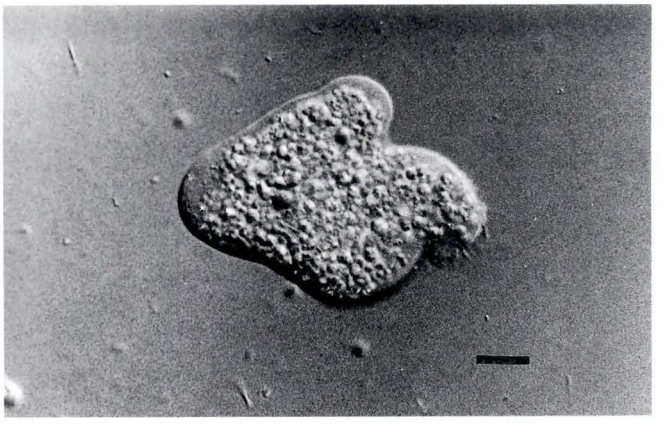

Fig. 15. *Gruberella flavescens*. Bar=10 µm.

Genus ***Gruberella*** Gruber, 1889 (Fig. 15)

Marine amoeba with rapidly moving locomotive form as a thick cylinder around 60 µm in length. Produces eruptive hyaline bulges. Multinucleate with around 6 nuclei but up to 37 per cell. Posterior adhesive filaments a common feature. At the ultrastructural level surface coat as a thin glycocalyx. Monotypic genus. *Euhyperamoeba*, a **limax** marine amoeba, is similar to *Gruberella* (see Chapter on Lobosea).

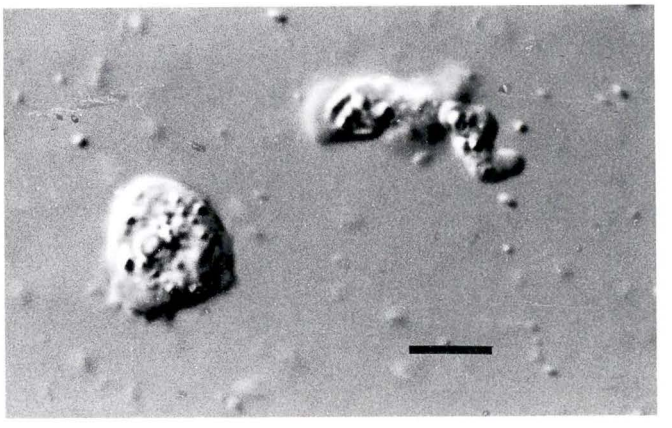

Fig. 16. *Stachyamoeba lipophora*, limax and flattened forms. Bar=10 µm.

Genus ***Stachyamoeba*** Page, 1975 (Fig. 16)

Amoeboid form commonly flattened during locomotion, sometimes fan-shaped, occasionally limax. Pseudopodia eruptive; some may have a few, short, fine subpseudopodia. Cytoplasm with single

nucleus with parietally arranged nucleolar material. Often with at least one prominent lipid inclusion. Cysts spherical with crenulated wall, but without excystment pores. Monotypic genus found in soil.

GENERA WITH UNCERTAIN FAMILIAL POSITION

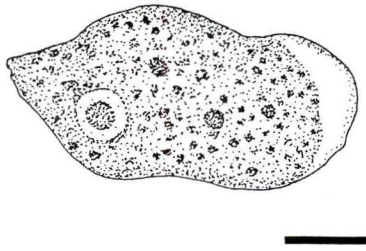

Fig. 17. *Pernina chaumonti* (after Kadiri et al.). Bar=10 µm.

Genus ***Pernina*** Kadiri, Joyon & Pussard, 1992 (Fig. 17)

Locomotive amoeba **limax** with rapid, eruptive monopodial locomotion. Lobed pseudopodia produced from anterior hyaloplasm. Posterior uroidal filaments common. Usually uninucleate, nucleus with a conspicuous central nucleolus. At the ultrastructural level, mitochondria with flattened cristae and cell surface without coating. Forms spherical cysts with double walls, with pores and plugs. This strain possesses characters intermediate between the families Gruberellidae and Vahlkampfiidae (Kadiri et al., 1992). Monotypic genus, marine. Type species: P. chaumonti Kadiri, Joyon & Pussard, 1992.

Genus ***Rosculus*** Hawes, 1963 (Fig. 18)

Protists known only as an amoeboid stage, reportedly with discoid mitochondrial cristae (Page, 1988). Morphologically very similar to *Guttulinopsis* (subclass Guttulinia) but fruiting bodies have not been seen in culture. Produces smooth, oval or spherical cysts. Monotypic genus. From freshwater, forest litter, and the rectum of *Natrix natrix*.

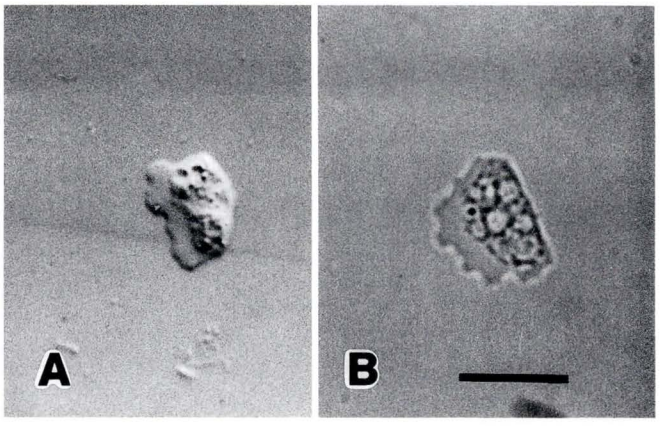

Fig. 18. *Rosculus ithacus*. Locomotive cell by interference contrast (A) and phase contrast (B) microscopy. Bar=10 µm. Courtesy of Dr. F. C. Page and CCAP.

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ORDER CRYPTOMONADIDA Senn, 1900

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The cryptomonads are a relatively small but distinct taxonomic group of unicellular flagellates that are cosmopolitan in distribution and are important primary producers in both freshwater and marine habitats (Gillott, 1990; Klaveness 1988). Cryptomonads are common in many phytoplankton samples, but because their cells are extremely delicate, they rupture when fixatives are added or when temperatures are elevated; their numbers generally are underestimated in preserved samples. In sub-ice habitats and in early spring and late fall populations of temperate lakes and reservoirs, they often become dominant. In fact, the structural diversity discovered by using specialized electron-microscopic techniques (Hill, 1990, 1991b; Hill and Wetherbee, 1986, 1988, 1989, 1990; Kugrens and Lee, 1987, 1991, Kugrens et al., 1986a, 1987; Lee and Kugrens, 1986) strongly suggest that the numbers of species in this group of organisms probably has been significantly underestimated (Andersen, 1992).

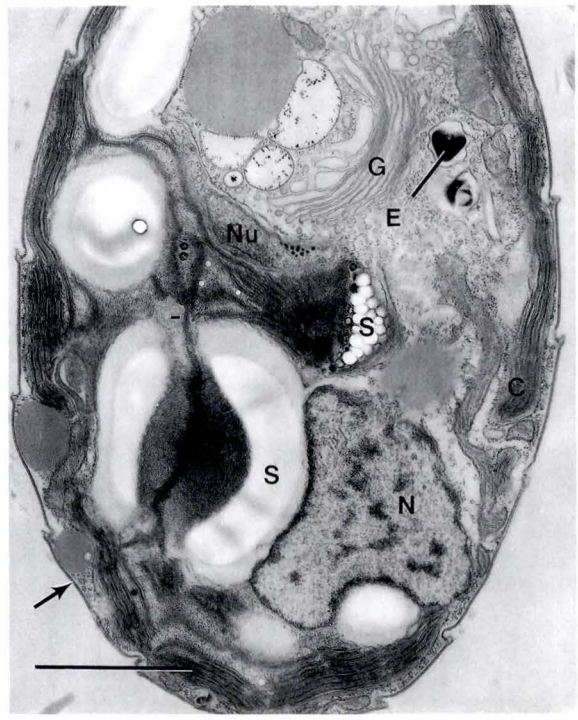

Fig 1. TEM of *Chroomonas coerulea* showing the general features of a cryptomonad cell and the relative locations of cellular structures. The periplast plates impart a serated appearance to the periplast (arrow). Important cell structures include a chloroplast (C) with a pyrenoid (Py) traversed by a pair of thylakoids and a stigma (S); a nucleus (N); a nucleomorph (Nm); a golgi apparatus (G); ejectosomes (E); and starch in the periplastidal space. Scale=1 μm.