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Taxonomy and Phylogeny of Heliozoa. I. The Order Desmothoracida Hertwig et Lesser, 1874

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Summary. The order Desmothoracida Hertwig et and Lesser, 1874 is reviewed. Five genera and 10 species are recognised: *Clathrulina* Cienkowski, 1867 [*C. elegans* Cienkowski, 1867 and *C. smaragdea* (Entz, 1877) comb. n.], *Hedriocystis* Hertwig et Lesser, 1874 [*H. pellucida* Hertwig et Lesser, 1874, *H. minor* Siemensma, 1991 and *H. zhadani* sp. n.], *Penardiophrys* gen. n. [*P. reticulata* (Penard, 1904) comb. n. and *P. spinifera* (Brown, 1918) comb. n.], *Cienkowskya* Schaudinn, 1896 [*C. mereschkovckii* (Cienkowski, 1881) Schaudinn, 1896 and *C. brachypous* (De Saedeleer, 1930) comb. n.] and *Actinosphaeridium* Zacharias, 1893 [*A. pedatus* Zacharias, 1893]. The generic names *Elaster* Grimm, 1872 and *Orbulinella* Entz, 1877 are synonymised with *Clathrulina*, and *Monomastigocystis* De Saedeleer, 1930 with *Cienkowskya*. The genus *Cienkowskya* differs from *Hedriocystis* by an irregular shape of the capsule, and by its attachment to the substrate by a short conical basal region of the capsule rather than by a differentiated tubular stalk as in *Hedriocystis*. The 2 species of *Hedriocystis* having a capsule wall composed of polygonal facets are transferred to a new genus *Penardiophrys*. The genus *Actinosphaeridium* includes a single freshwater species with branching granule-bearing filopods and the cell body surrounded by a mucus sheath located at the top of a tubular stalk; and is here regarded as a primitive desmothoracid. The first marine *Hedriocystis* - *H. zhadani* sp. n. - is described. The stalked heliozoan genus *Servetia* Poche, 1913 is not included within desmothoracids. Diagnoses of all desmothoracid taxa and a key to10 species are included. We should look for phylogenetic roots of desmothoracids among heterotrophic biflagellates; cercomonads and gymnosphaerid heliozoa are considered as the most closely related groups to them.

Key words: *Actinosphaeridium*, *Cienkowskya*, *Clathrulina*, Desmothoracida, *Elaster*, fauna, *Hedriocystis zhadani* sp. n., heliozoa, *Orbulinella*, *Penardiophrys* gen. n., phylogeny, *Servetia*, taxonomy.

INTRODUCTION

Recently, we (Mikrjukov 1998, 1999 b, 2000 a, b, c; Mikrjukov *et al.* 2000) have presented evidence in favour of the opinion postulated by Tregouboff (1953),

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Smith and Patterson (1986) and Patterson (1988, 1994, 1999) that the Heliozoa Haeckel, 1866 are polyphyletic. The Heliozoa is one of the most long-lived of the traditional protozoan taxa. It can be found in the majority of recent systems of protists, protoctists and protozoa (Lee *et al.* 1985, Karpov 1990, Margulis *et al.* 1990, Corliss 1994, Hausmann and Hülsmann 1996, Cavalier-Smith 1998, Kussakin and Drozdov 1998). Heliozoa seem to be comprised of 7-8 mainly distantly related taxa (Mikrjukov, 1999 b, 2000 a). The heliozoa are therefore

to be thought of as members of an adaptive group of spherically symmetrical protists with radiating, granule-bearing axopods and lacking any intracellular inorganic skeleton and a central capsule (in contrast with another polyphyletic axopodiate group - the radiolaria). An organic or inorganic skeleton (if present) is always extracellular (Mikrjukov 1998, 2000 a, b).

The Desmothoracida Hertwig et Lesser, 1874 appeared in the earliest schemes of classification of heliozoa (Bütschli, 1882; Schaudinn, 1896). The diagnosis and the principle composition of the desmothoracids have remained broadly unchanged despite the disappearance of heliozoa as a taxonomic group in recent systems of protists (Lee *et al.* 2000, Mikrjukov 2000 a). The taxonomy of various heliozoan taxa is now being reviewed, but this process has yet to extend to the desmothoracids (Roijackers and Siemensma 1988; Siemensma and Roijackers 1988 a, b; Siemensma 1991; Mikrjukov 1995 a; 1996 a, b; 1997; 1999 c).

The order Desmothoracida includes heliozoa enclosed in a (usually) stalked capsule perforated by openings of a various size. Granule bearing, filopods extend out of these perforations. Sometimes these pseudopodia branch off. Cienkowski (1867) was the first to describe a desmothoracid with his account of *Clathrulina elegans*. He noted that only one from of the products of the cell fission remains inside the capsule to remain a heliozoon, whilst the second one transforms into a distributive biflagellate swarmer cell, which leaves the capsule through one of the openings in the capsule wall. Further investigations (Foulke 1885, Penard 1904, Deflandre 1926, Hoogenraad 1927, Valkanov 1928, De Saedeleer 1930, Bardele 1972, Brugerolle 1985, Siemensma 1991, Young *et al.* 1995) have confirmed the polymorphic nature of the life cycle of desmothoracids (Figs. 1 C-F, K-P, 2 D-G). This includes uni- or biflagellate swarmers, which transform after the settling into an amoebo-flagellate, then into an amoeba, then into a naked heliozoon. The latter acquires its final appearance with the production of a mucous material of the capsule from the whole cell surface. Similar material makes up the non-cellular, tubular stalk and this is produced by substantial, stalk-moulding pseudopodium. After the production of the material, the stalk-moulding pseudopodium withdraws into the body mass, and the whole mucous material becomes more solid and acquires a microfibrillar structure. The unusual general appearance of desmothoracids and the production of flagellated swarmers led some authors to refer to desmothoracids as "pseudoheliozoa" (Tregouboff 1953, Rainer 1968, Bardele

1972). The capsule of the most commonly occurring species, *Clathrulina elegans* is the only known palaeonthological (Oligocene) record of heliozoa (Deflandre 1953). All the desmothoracid genera are normally placed within a single family, Clathrulinidae Claus, 1874, but the group has been ranked up to the level of the order.

Electron microscopical studies of the fine structure of *Clathrulina elegans* by Bardele (1971, 1972) and of *Hedriocystis pellucida* by Brugerolle (1985) have added to our knowledge on this group. They have shown that: (i) a the capsule wall has a microfibrillar organic appearance; (ii) their extrusosomes correspond to kinetocysts but their structure does not resemble that of centrohelids (Mikrjukov 1995 b); (iii) microtubules in the pseudopods are not ordered in regular axonemal arrays and on this basis the pseudopods do not comply with the definition of being axopodia. The fine structure of flagellated swarmers is not well understood but (iv) flagella begin to develop from a pair of orthogonally oriented elongated kinetosomes; (v) the kinetosomes are connected with the front of the nucleus by either an electron-dense matrix (in *Clathrulina*) or by a striated rhizoplast (in *Hedriocystis*).

Desmothoracids are traditionally considered to be freshwater group of protozoa (Siemensma 1991, the taxonomic appendix below). The order has never included a large number of species. The desmothoracids have a worldwide distribution, are commonly encountered, but there is a great variability in the size, shape and ornamentation of the skeleton. Previous workers (Penard 1904, Rainer 1968, Febvre-Chevalier 1985, Siemensma 1991) have used taxonomic criteria differently, such that there is dispute as to the composition of the group. According to Rainer (1968), the order Desmothoracida is composed of 4 genera and 8 species: *Clathrulina* Cienkowski, 1867, with 2 species, *Hedriocystis* Hertwig et Lesser, 1874, with 2 species, and the monotypic genera *Monomastigocystis* De Saedeleer, 1930 and *Orbulinella* Entz, 1877. Febvre-Chevalier (1985) follows the Rainer's taxonomic perspective but considers only generic taxa. Siemensma (1991) ignores *Orbulinella*, regards *Monomastigocystis* as a junior synonym of *Hedriocystis*, describes *H. minor*, and regards *C. cienkowskyi* as a junior synonym of the type-species; thus concluding there are 2 genera and 7 species. This uncertainty as to the composition of the group has prompted this review.

The ecology of heliozoa is studied very insufficiently, and these organisms are almost always ignored in all
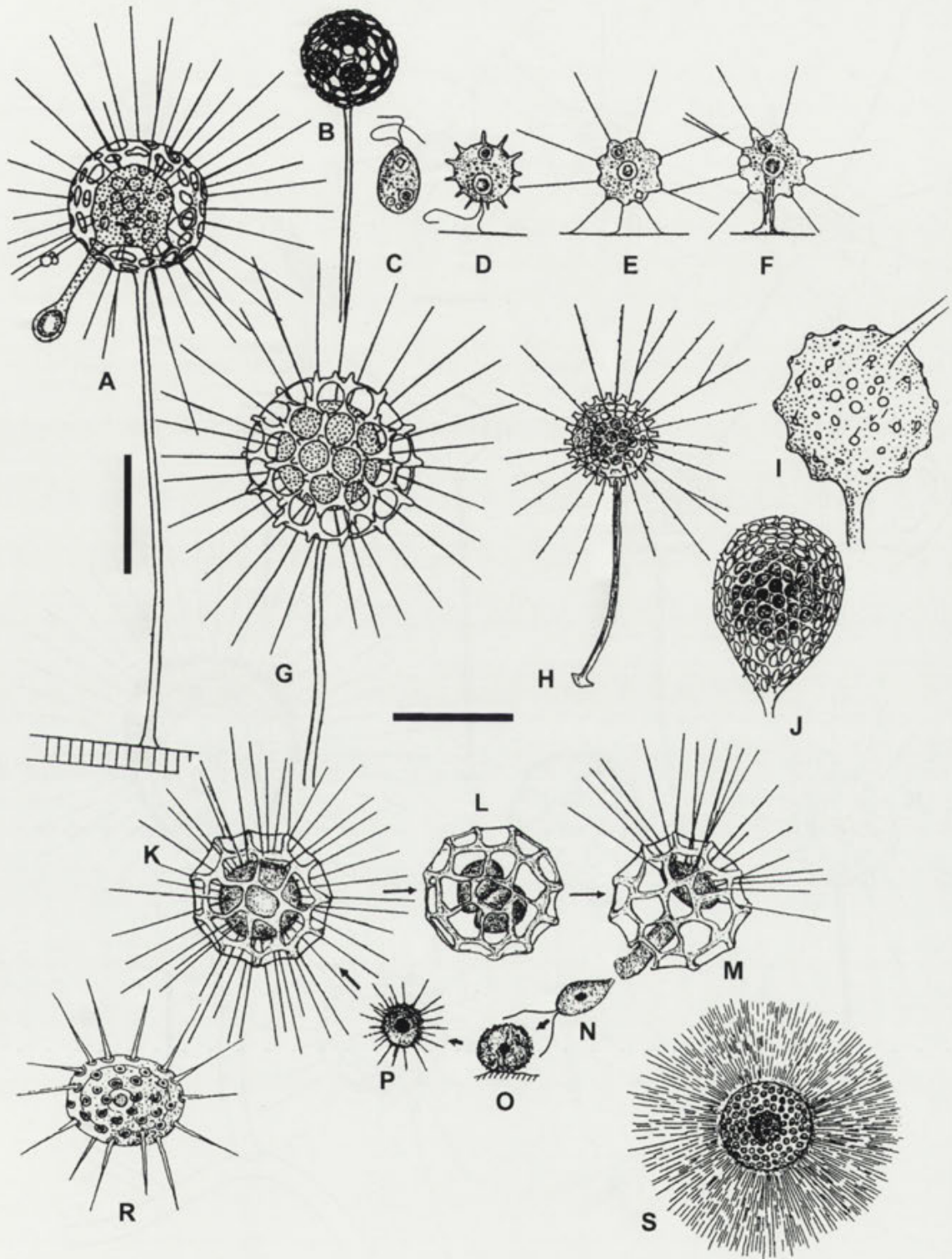
Figs. 1 A-S. Genus *Clathrulina* Cienkowski, 1867. **A, B** - the type-species, *C. elegans* and its capsule with 4 cysts (from Cienkowski 1867); **C-F** - the life cycle of *C. elegans* (from Siemensma 1991): a biflagellate swarmer (**C**), an amoebo-flagellate (**D**), transitional stages of the transformation to heliozoon (**E, F**); **G** - *C. cienkowskyi* (from Mereschkowsky 1877); **H** - the organism drawn as *C. fragilis* (from Febvre-Chevalier 1990); **I** - *C. stuhlmanni* (from Schaudinn 1897); **J** - *C. cienkowskyi* ssp. *ovalis* (from von Daday 1905); **K-P** - the life cycle of a stalkless form of *C. elegans* (from Young *et al.* 1995); **R** - *C. smaragdea* (from Entz sen. 1877); **S** - the organism described as *Elaster greeffi* (from Grimm 1872). Scale bars - 50 µm
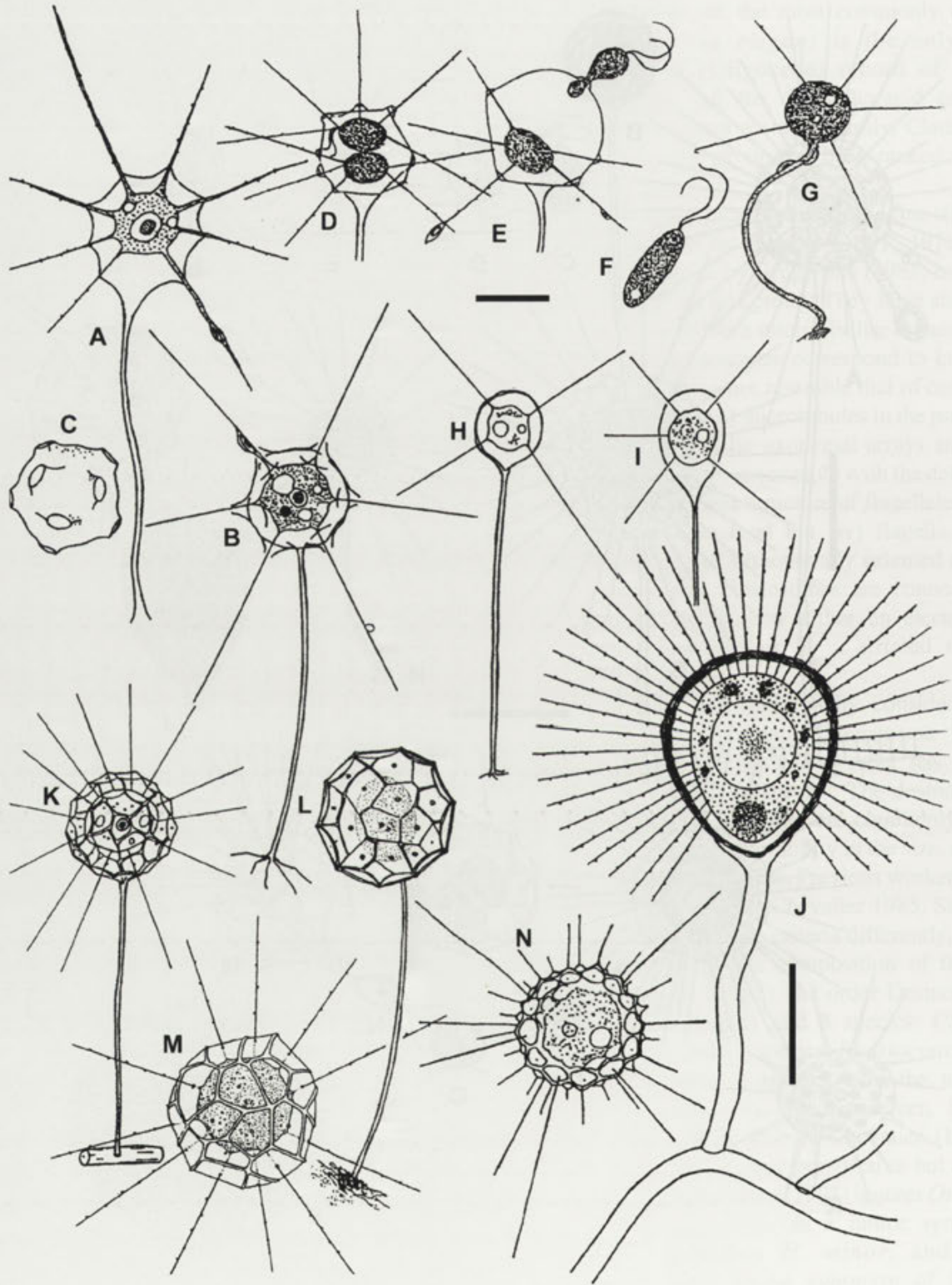
Figs. 2 A-N. Genera *Hedriocystis* Hertwig et Lesser, 1874 (**A-J**) and *Penardiophrys* gen. n. (**K-N**). **A** - the type species, *H. pellucida* (from Hertwig and Lesser 1874); **B, C** - *H. pellucida* and the shape of its capsule (from Siemensma 1991); **D-G** - the life cycle of *H. pellucida* (from Hoogenraad 1927); **H, I** - *H. minor* (from Siemensma 1991); **J** - *H. zhadani* sp. n.; **K** - the type-species, *P. reticulata* (from Penard 1904); **L, M** - *P. reticulata*, the structure of the capsule, and a stalkless form (from Siemensma 1991); **N** - *P. spinifera* (from Brown 1918). Scale bars - 50 μm in **J**, and 20 μm - in others
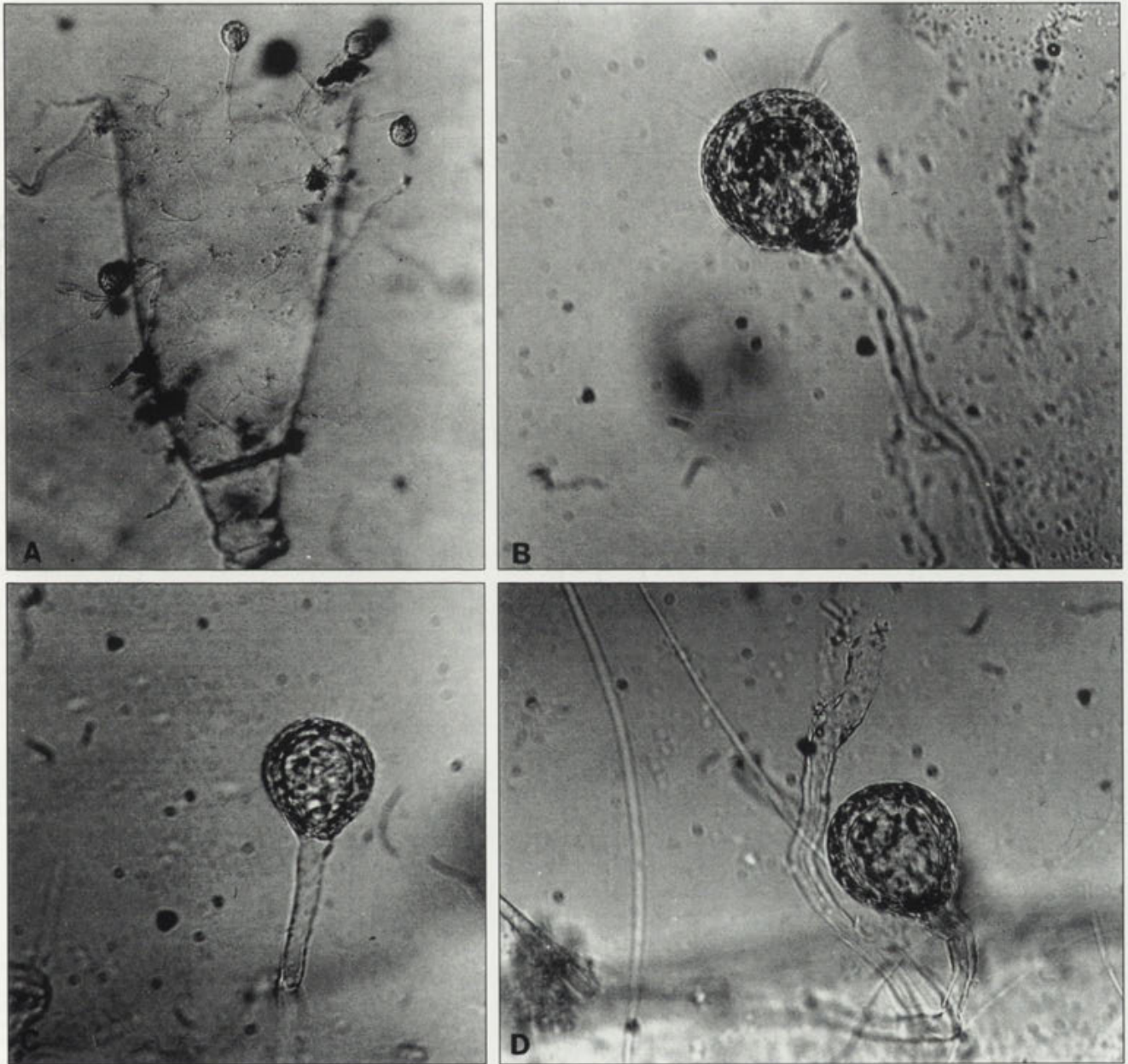
Figs. 3 A-D. *Hedriocystis zhadani* sp. n. (light microscopy). **A** - a colony at the hydrotheca of *Obelia*, x 100; **B-D** - various views of separate specimens (a thick capsule wall formed by an amorphous material is seen), x 400

recent reviews of the ecology of protists (Fenchel 1987). The desmothoracid heliozoa do not represent an exception from this rule. Old authors reported these organisms feeding on algae and ciliates (Cienkowski 1867, 1881; Hoogenraad 1927; Valkanov 1928). In laboratory culture by Bardele (1972) studied the ultrastructure of *Clathrulina*, this organism was maintained by using an algae, *Chlorogonium elongatum* as food; bacteria were used by Brugerole (1985) for maintaining of *Hedriocystis*

pellucida. In our recent review of heliozoa as a component of microbenthos (Mikrjukov 2000 b) we regard heliozoa as a group of passive benthic consumers with a food capturing apparatus represented by a large radial system of axopods with granules paralyzing and gluing a great spectrum of various small motile preys in a large volume of water. We believe the desmothoracids as heliozoa attached by a stalk to bottom sediments could combine a predatory habit of feeding by a small mobile
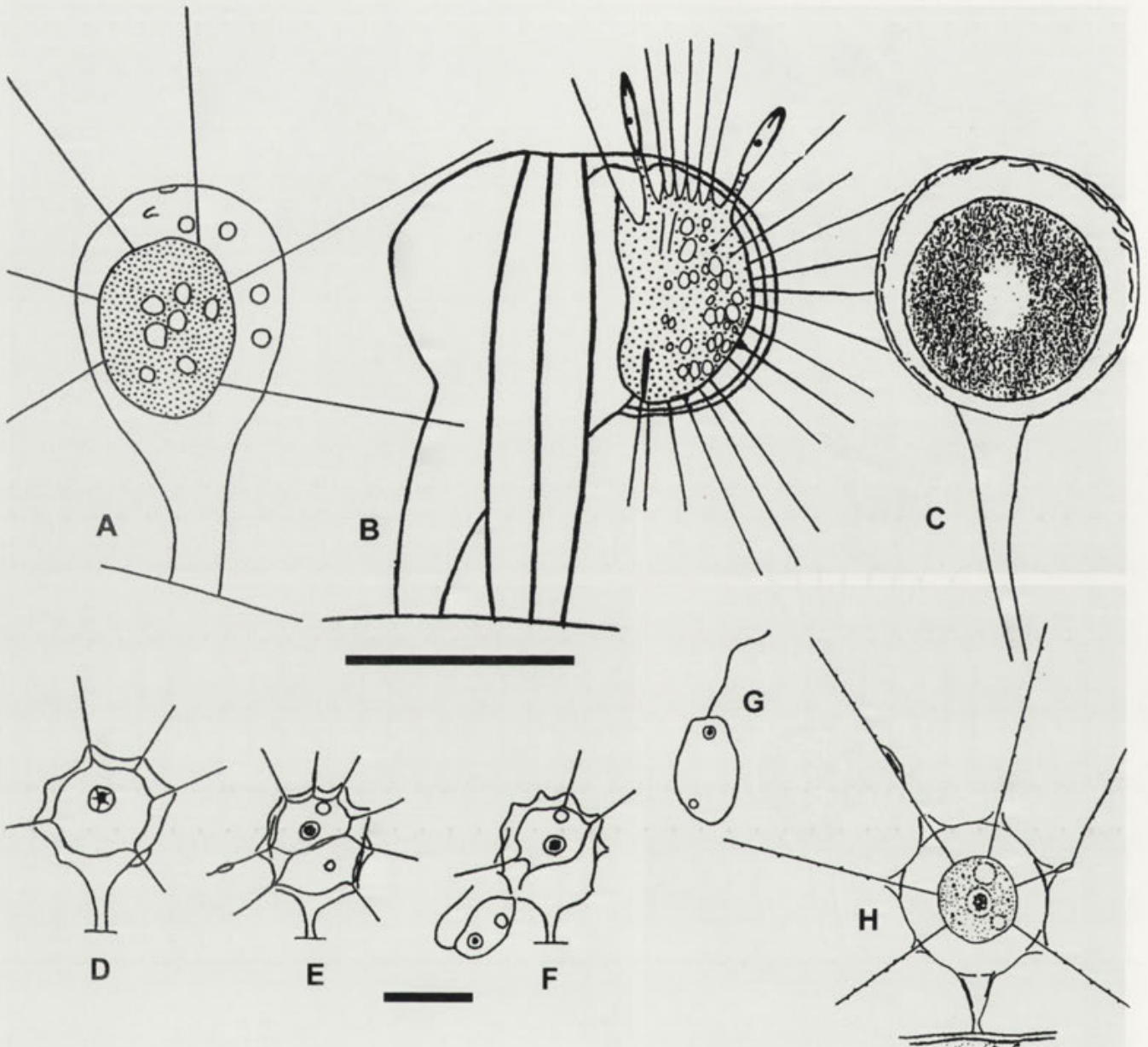
Figs. 4 A-H. Genus *Cienkowskya* Schaudinn, 1896. **A-C** - the type-species, *C. mereschkovckii* (**A, B**) and its cyst (from Cienkowski 1881); **D-F** - the life cycle of *C. brachypous* (from De Saedeleer 1930); **G, H** - a mature specimen and a uniflagellate zoospore of *C. brachypous* (from Siemensma 1991). Scale bars - 50 μm in **A-C**, and 20 μm - in **D-H**

prey with a sedimentation catching and accumulation of organic matter by the upper directed axopods. Desmothoracids are mainly a freshwater group; they are more frequently reported in a littoral zone of small ponds and marshes (Rainer 1968, Siemensma 1991). However Brown (1918) described *Hedriocystis spinifera* from the wed moss.

DISCUSSION OF THE TAXA

Genus *Clathrulina* Cienkowski, 1867

Clathrulina elegans Cienkowski, 1867 was the first desmothoracid to be described. It was reported from brackish water bays in Southern Russia, near Odessa.
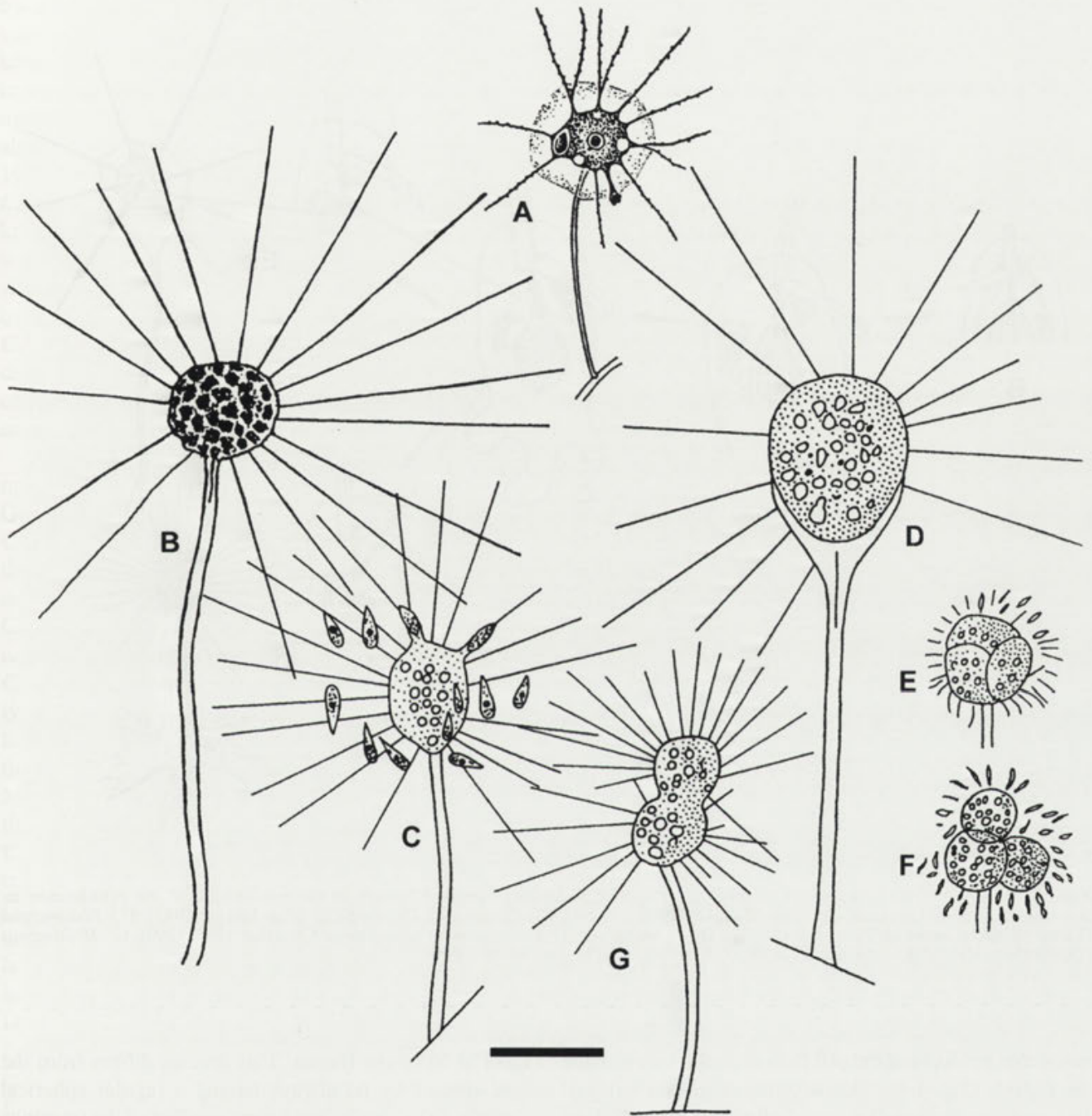
Figs. 5 A-G. Genera *Actinosphaeridium* Zacharias, 1893 (**A**) and *Servetia* Poche, 1913 (**B-G**). **A** - *Actinosphaeridium pedatus* (from Penard 1903); **B** - *Servetia borealis* (from Mereschkowsky 1877); **C-G** - mature and dividing specimens of *S. borealis* (from Cienkowski 1881). Scale bars - 20 μm

Two years later, Archer (1869) described *Podosphaera haeckeliana* Archer, 1869 which indistinguishable from *Clathrulina elegans* and is regarded hence as a junior synonym of it. The original description of *C. elegans*

includes reference to a lattice-like (with large pores) capsule at the top of a hollow tubular stalk and not close to the cell body. Numerous filopods radiating through pores are sometimes branching (Fig. 1 A). Cienkowski
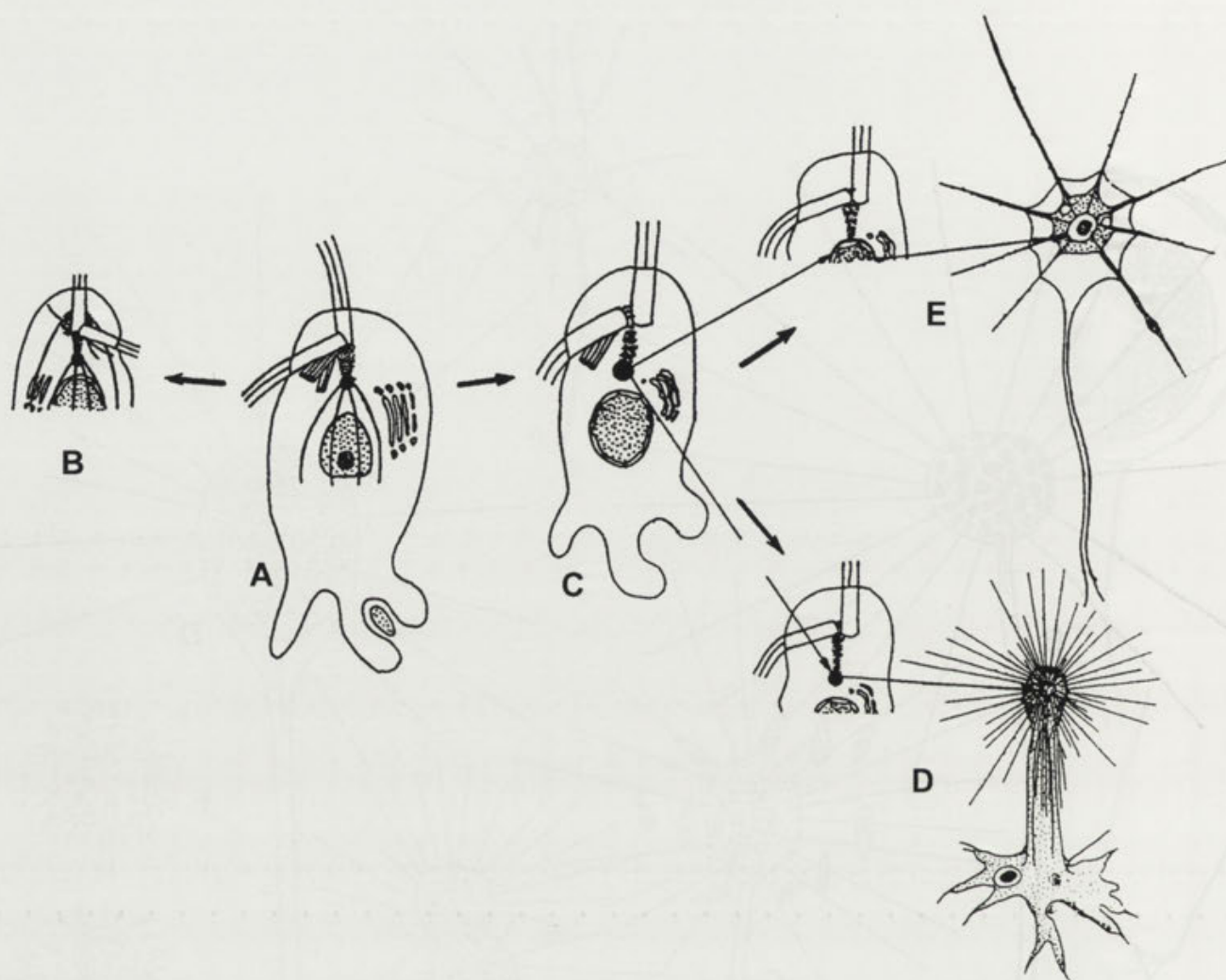
Figs. 6 A-E. A possible comparative morphological scheme illustrating common features in the construction of the cytoskeleton in: **A** - *Cercomonas* (Cercomonadida) (after Mylnikov 1986 a, 1989); **B** - *Cavostelium* (Protostelida) (after Spiegel 1981); **C** - *Bodomorpha* (Cercomonadida) (after Mylnikov 1984, 1989); **D** - *Actinocoryne* (Gymnosphaerida) (after Febvre-Chevalier 1985, 1990); **E** - *Hedriocystis* (Desmothoracida) (after Hertwig and Lesser 1874, micrographs by Brugerolle, 1985)

noted that products of the cell fission could encyst inside the capsule (Fig. 1 B); the excysting stage is a biflagellate swarmer leaving the capsule through the pore [our current understanding of the polymorphism in this species is indicated in Figs. 1 C-F, after Siemensma (1991)]. The original description lacks comments on the shape or the ornamentation of the capsule. The structure of the capsule of this species is described using scanning electron microscopy by Croome (1987).

A decade after Cienkowski's description of *C. elegans*, Mereschkowsky described *C. cienkowskyi* Mereschkowsky, 1877 (Fig. 1 G) from the Onezhskoe

Lake in Northern Russia. This species differs from the type-species by (i) always having a regular spherical capsule, (ii) a regular hexagonal spacing of the openings in the capsule wall and (iii) the presence of papillary protrusions (knobs) between the pores in the capsule. Recently, Febvre-Chevalier (1990) included in her review of heliozoa, a figure of the organism named *C. fragilis* (Fig. 1 H). There is no indication of the authority, nor any description or remarks to the figure. This does not meet the criteria for a validly published species. Finally, Schaudinn described *C. stuhlmanni* Schaudinn, 1897 from the Eastern Africa which differs

by having a massive capsule perforated by pores which vary considerably in size and placed at the tops of small knob-like protrusions (Fig. 1 I). *C. stuhlmanni* forms colonies when flagellated swarmers to settle on the mother specimens [we should note that this feature is also reported in *C. elegans* (Rainer 1968, Siemensma 1991)]. Thus criteria used in distinguishing species *Clathrulina* are consequently: size, ornamentation by knobs, and relative position of openings in the capsule wall. Some authors attach taxonomic significance to the shape of the capsule i.e. whether it is spherical or oval. Von Daday (1885) described a subspecies, *C. cienkowskyi* ssp. *ovalis* von Daday, 1885 which differs from the type subspecies only by the oval shape of the capsule (Fig. 1 J); later Deflandre (1926) considered *C. ovalis* von Daday, 1885 as a separate species.

Recently, our observations of enriched cultures of mangrouve sediments in Cape Tribulation (Northern Qeensland, Australia) containing numerous cells of *Clathrulina* (Patterson *et al.* in preparation) have shown that both the spherical and oval shape of the capsule may occur within the same cell culture of *C. elegans*. Consequently, the oval shape of the capsule cannot be used as a taxonomic character in *Clathrulina*. We treat *C. ovalis* von Daday, 1885 as a junior synonym of the type-species *C. elegans*. In cultures from Cape Tribulation we have also observed a wide spectrum of transitional forms from those identical to organisms drawn by Mereschkowsky (1877) as of *C. cienkowskyi* up to those identical to Schaudinn's (1897) figures of *C. stuhlmanni*. Indeed, on occasions, a sequence of transitional forms (*C. cienkowskyi* - *C. elegans* - *C. stuhlmanni*) was observed in a colony of 5-7 specimens. Young cells still forming (transparent) capsules have smaller openings that are not uniform in size and less regularly distributed and so resembles *C. stuhlmanni*. During the maturation of specimens, the openings in their capsule wall become broader and rounder and mature specimens are more similar to *C. elegans* and *C. cienkowskyi*. Thus we do not consider the shape of the capsule, its size and the ornamentation by knobs and openings (i.e. the relative position of openings) as reliable taxonomic criteria in *Clathrulina*. *C. cienkowskyi* Mereschkowsky, 1877 and *C. stuhlmanni* Schaudinn, 1897 are consequently regarded as junior synonyms of *C. elegans* Cienkowski, 1867.

Another problem in the taxonomy of *Clathrulina* is report of unstalked forms of *C. elegans* first reported by Penard (1905) from Loch Ness in Scotland. This was recently confirmed by Young *et al.* (1995) who gave a careful description of the life cycle of stalkless *C. elegans* (Figs. 1 K-P) showing that it is identical to that in stalked forms (Figs. 1 C-F). We, like Penard (1905) do not regard the absence of a stalk as significant taxonomic character; Young *et al.* (1995) called these organisms in the title of their paper as a "stalkless sub-species of *C. elegans*" although they did not propose neither its description nor the taxonomic name for it. The stalkless populations of *C. elegans* appear to develop in some locations. This issue is linked to the validity of 2 monotypic genera, *Elaster* Grimm, 1872 and *Orbulinella* Entz, 1877. *Elaster greeffi* Grimm, 1872 (Fig. 1 S) is a freshwater species and it has been described once. We regard it as indistinguishable from a stalkless form of *C. elegans* with which it is synonymised. *Orbulinella smaragdea* Entz, 1877 (Fig. 1 R) was described from brackish and fresh waters (Entz sen. 1877, Francé 1897, Entz jun. 1906, Labbé 1924); this species is described as being (i) enclosed by a siliceous (but this needs to be verified) capsule, (ii) having broad massive bases of pseudopods and probably without granules, and (iii) bearing complex, inverted, conical pores in the capsule wall. This organism is of uncertain taxonomic position and needs a careful further investigation. It is here treated as an always unstalked species of *Clathrulina* - *C. smaragdea* (Entz, 1877) comb. n.

Genus *Hedriocystis* Hertwig et Lesser, 1874

Hedriocystis differs from *Clathrulina* by the minute size of pores in the capsule wall although even though the diameter of openings varies strongly in *Clathrulina*. The type-species, *H. pellucida* Hertwig et Lesser, 1874 (Figs. 2 A-G) is able to produce biflagellate swarmers (Figs. 2 E, F). The genus can be divided into 2 groups of species: those with a homogeneous mucous capsule (Figs. 2 A-J), and species with a capsule wall composed of jointed polygonal facets with raised edges and with each facet bearing a single pore (Figs. 2 K-N). This difference separates a group of species, we propose a new genus *Penardiophrys* gen. n. with the diagnosis given below to accommodate those species. Species with a homogeneous capsule (i.e. *Hedriocystis sensu nova*) are distinguished from each other by the ornamentation of pores in the capsule wall - a character that is unreliable in *Clathrulina*. The pores may be placed at the tops of conical knobs (*H. pellucida* - Fig. 2 C) or may be simple perforations in the capsule wall (*H. minor* Siemensma, 1991 - Figs. 2 H, I). The description of the

first marine member of the order - *H. zhadani* Mikrjukov, sp. n. (Figs. 2 J, 3) is given below. Despite its large size, this species also has simple minute pores.

Genus *Penardiophrys* Mikrjukov, gen. n.

The new genus includes 2 species previously assigned to *Hedriocystis* with a complex capsule wall composed of jointed polygonal facets. They are the type-species, *P. reticulata* (Penard, 1904) comb. n. (Figs. 2 K, L), and *P. spinifera* (Brown, 1918) comb. n. The latter species was recorded once from wet moss in Scotland. It differs from *P. reticulata* by the presence of spines radiating from the capsule wall from sites of the joint of facets (Fig. 2 N). *P. spinifera* is described as an unstalked species; Siemensma (1991) reports the occurrence of stalkless forms in *P. reticulata* (Fig. 2 M) too. The diagnosis of a new genus is given below.

Genus *Cienkowskya* Schaudinn, 1896

The history of this genus is confused because different stalked marine heliozoa have been misidentified *C. mereschkovckii* (Cienkowski, 1881) Schaudinn, 1896. Villeneuve (1937) studied mitosis in *Polyplacocystis pedunculata* (Mikrjukov, 1994) Mikrjukov, 1996 b but referred to it as *C. mereschkovckii*. This mistake was repeated by Jones (1974) who also described the colonial forms as a new species of *Cienkowskya* - *C. arborescens* Jones, 1974. Later Febvre-Chevalier and Febvre (1984) and Mikrjukov (1994) studied another stalked marine centrohelid, *Heterophrys minutus* (Walton, 1905) Mikrjukov, 2000 b also referring to it as *C. mereschkovckii*. These issues are discussed else-ᵂʰ⁻ːre (Mikrjukov 1996 b, 2000 b). Schaudinn (1896) introduced *Cienkowskya* to replace the original *Wagneria*, which was preoccupied by a genus of ciliates. He did not observe this organism that has never been recorded since its original discovery in the White Sea. The original line drawings by Cienkowski (Figs. 4 A-C) show that *Cienkowskya* is not a centroheliozoon but is a desmothoracid because a stalked mucous capsule perforated by minute pores through which the pseudopodia extend surrounds it. It encysts inside the capsule by forming a single cyst with an organic wall (Fig. 4 C); "Cienkowski's" *Cienkowskya* lacks the centrohelid characters such as the presence of the centroplast and of fine stiff arms with prominent extrusomes. Of the desmothoracids, *Cienkowskya* mostly resembles *Hedriocystis sensu nova* but differs by a gradual narrowing of the basal part of the capsule into a short conical stalk (in *Hedriocystis*, the skeleton is sharply differentiated into a capsule and a long, tubular stalk).

Taking *Cienkowskya* in the present sense i.e. as including desmothoracid heliozoa with a homogeneous mucous capsule tapering into a short conical stalk, we need to consider *Monomastigocystis brachypous* De Saedeleer, 1930. This species forms uniflagellate swarmers (Figs. 4 D-H). Siemensma (1991) transferred the single species, *M. brachypous* to *Hedriocystis*. *Monomastigocystis* De Saedeleer, 1930 is here regarded as a junior synonym of *Cienkowskya* Schaudinn, 1896, and hence *C. brachypous* (De Saedeleer, 1930) comb. n. is the second, freshwater species of this genus. It has an irregularly shaped capsule that narrows gradually to a short conical stalk (Figs. 4 D, G). It differs from *C. mereschkovckii* by being substantially smaller (the diameter of the capsule is 35-42 and 10-15 μm respectively) and has pores of the capsule wall placed at the tips of conical knobs as in *H. pellucida*.

Genus *Actinosphaeridium* Zacharias, 1893

A stalked protozoon was described by Zacharias as *Actinosphaeridium pedatus* Zacharias, 1893, and later referred by Penard as *Nuclearia caulescens* Penard, 1903 (Fig. 5 A). Penard (1904) considered these organisms as synonyms incorrectly using the junior name, *N. caulescens*. This organism does not belong to nucleariid filose amoebae, a well defined group of discicristate filopodiate protists, which never have extrusive organelles or a stalk (Patterson 1984, Patterson *et al.* 1987, Mikrjukov 1999 c) because it has granular pseudopodia. *A. pedatus* has a central nucleus, branching granule-bearing filopods, and a long tubular stalk. In contrast with the desmothoracids, the cell body of this organism is surrounded by a substantial mucous sheath but not by a capsule. As skeleton material of desmothoracids is initially excreted as mucus (Bardele 1972) we regard this species as a desmothoracid for which the correct generic vehicle is *Actinosphaeridium* Zacharias, 1893. We regard the organism described by Penard as indistinguishable from *A. pedatus* Zacharias, 1893. There is still some uncertainty as to the placement of this taxon. However a careful study of the fine structure and (possibly) the life cycle of this organism would help in establishing its position. Zacharias (1893) reported but did not figure that siliceous (?!) plates cover the cell body during the cyst formation. This is a characteristic of actinophryids (Patterson and Thomp-

son 1981) whereas the cyst wall of desmothoracids is organic (Cienkowski 1867, Bardele 1972, Young *et al.* 1995).

Stalked heliozoa of *incertae sedis*: *Servetia* Poche, 1913, *Microsol* Dons, 1918, and *Actinolophus* Schulze, 1874

As in the case of *Cienkowskya*, Poche (1913) (the author of the generic name *Servetia*) had not observed this organism initially described by Mereschkowsky (1877) from the same site and biotope with *C. mereschkovckii* (clumps of filamentous algae at the upper sublittoral zone at the Solovetzkaya biostation at the White Sea) as *Haeckelina borealis* Mereschkowsky, 1877 also using the preoccupied generic name. *Servetia* contains one species, *S. borealis* (Mereschkowsky, 1877) Poche, 1913. This is a marine stalked heliozoon with a long mucous stalk. It was later reported from the same location by Cienkowski (1881). The Cienkowski's description is more careful but his figures (Figs. 5 C, D) differ significantly from those of Mereschkowsky (Fig. 5 B). We doubt if these two protozoologists studied the same organism. Cienkowski (1881) reported that the apical part of the stalk might broaden in some specimens forming a pad which envelopes the basal part of the cell (Fig. 5 D). A capsule entirely enclosing the whole cell body has not been reported. Reproduction is said to include schizogony into 3 cells (Cienkowski 1881) (Figs. 5 E, F) but this needs to be verified. Cienkowski's *S. borealis* can also reproduce by binary fission (Fig. 5 G). Because of the unusual nature of reproduction and the absence of the capsule, we do not include the genus *Servetia* Poche, 1913 in the order Desmothoracida; but rather treat this heliozoon as a member of Protista *incertae sedis*.

Rainer (1968) regarded another stalked marine heliozoon - *Microsol borealis* Dons, 1918 - as a junior synonym of *Servetia borealis* and even used the line drawing of *Microsol* for illustration of *Servetia* in his monograph. *M. borealis* resembles a disturbed specimen of a centroheliozoon *Heterophrys minutus* (Walton, 1905) Mikrjukov, 2000 b (initially wrongly described as a member of a probably gymnosphaerid genus *Actinolophus* - *A. pedatus* Walton, 1905), which has a long mucus stalk, spines and a mucus sheath. The original figures of *M. borealis* do not show any internal cell structures but demonstrate a substantial mucous

sheath around the cell, pseudopods without granules and about as long as the cell is wide. These resemble the fine spicules of *H. minutus,* and we consider the generic name *Microsol* Dons, 1918 as a junior synonym of *Heterophrys* Archer, 1869, and *M. borealis* Dons, 1918 as that of *H. minutus* (Walton, 1905) Mikrjukov, 2000 b. Thus the genus *Microsol* Dons, 1918 is not a member of Desmothoracida.

On this basis, 5 genera and 10 species of desmothoracids are recognised.

DIAGNOSES AND DESCRIPTIONS OF THE TAXA

Order DESMOTHORACIDA Hertwig et Lesser, 1874
Diagnosis: heliozoa with the cell body surounded by a perforated organic capsule or (occasionally) a mucous coat; axonemes terminate at the nuclear envelope.
Composition: 5 genera within a single family Clathrulinidae Claus, 1874

Family Clathrulinidae Claus, 1874

Diagnosis: heliozoa with the cell body surrounded by a perforated organic capsule or (occasionally) a mucous coat; axonemes terminate at the nuclear envelope.
Composition: 5 genera.

Genus *Clathrulina* Cienkowski, 1867

Syn.: *Podosphaera* Archer, 1869; *Elaster* Grimm, 1872; *Orbulinella* Entz, 1877
Diagnosis: desmothoracids with a lattice-like capsule perforated by large openings.
Composition: 2 species.
Type-species: *C. elegans* Cienkowski, 1867

C. elegans Cienkowski, 1867

Syn.: *Podosphaera haeckeliana* Archer, 1869; *Elaster greeffi* Grimm, 1872; *C. cienkowskyi* Mereschkowsky, 1877; *C. cienkowskyi* ssp. *ovalis* Daday, 1885; *C. stuhlmanni* Schaudinn, 1897; *C. ovalis* Daday, 1885.
Diagnosis: species of *Clathrulina* with the cell body surrounded by a lattice-like capsule with simple pores of equal or various size; filopods do not taper.
Description: cell body enclosed by spherical, ovoid or bottle-shaped capsule, 26-102 µm in diameter, which usually have a hollow, non-cytoplasmic stalk, 30-308 µm long, 1-4 µm wide; populations of stalkless forms have

been reported. Capsule wall perforated by circular, oval or polygonal openings of various sizes, and hence capsules have a lattice-like appearance. Filopods are fine, sometimes branching, with numerous granules. Swarmers biflagellate. Encystment inside capsule, with formation of 2-8 cysts with organic wall.

Habitat: freshwater.

Occurrence: Southern Russia (Cienkowski 1867), Northern Russia (Mereschkowsky 1877), Germany (Greeff 1869, Hertwig and Lesser 1874, Rainer 1968, Bardele 1972), Switzerland (Penard 1904), Great Britain (Archer 1869, Wailes 1921), Ireland (Wailes and Penard 1911), France (Febvre-Chevalier 1990), the Netherlands (Hoogenraad and De Groot 1940, Siemensma 1981), Sweden (Siemensma 1991), Hungary (von Daday 1885), Bulgaria (Valkanov 1928), Czechoslovakia (Stepanek 1952), Spitzbergen (Penard 1903), Estonia (Jacobson 1928), Eastern Africa (Schaudinn 1897), USA (Leidy 1879, Foulke 1885), Paraguay (von Daday 1905), Venezuela (Deflandre 1926), Australia (Whitelegge 1891, Schewiakoff 1893, Croome 1987). Stalkless forms: Germany (Grimm 1872), Scotland (Penard 1905, Young *et al.* 1995)

C. smaragdea (Entz, 1877) comb. n.

Basionym: *Orbulinella smaragdea* Entz, 1877
Syn.: *Orbulinella salina* Labbé, 1924
Diagnosis: body mass enclosed in a massive, ovoid capsule, about 30 μm in diameter, with inverted, conical pores through which filopods measuring 30-40 μm long extend. The cell body is greenish or colourless. Pseudopods do not branch, with broadened bases; no granules on pseudopodia.

Habitat: brackish and fresh waters.

Occurrence: Hungary, fresh water lake Balaton (Francé 1897, Entz jun. 1906), Bulgaria (Entz sen. 1877) and France (Labbé 1924) brackish water ponds.

Genus *Actinosphaeridium* Zacharias, 1893

Diagnosis: desmothoracids surrounded by a mucous coat.
Composition: monotypic.
Type-species: *A. pedatus* Zacharias, 1893

A. pedatus Zacharias, 1893

Syn.: *Nuclearia caulescens* Penard, 1903
Diagnosis: cell body of irregular shape, 16-23 μm in diameter, with regularly radiating, granule-bearing, branching filopods, 1.5-2 times longer the cell diameter. Heavy mucous cell coat about 6 μm thick. Stalk tubular, about 40 μm long. Several contractile vacuoles.

Habitat: freshwater.
Occurrence: Germany (Zacharias 1893), Spitzbergen (Penard 1903).

Genus *Hedriocystis* Hertwig et Lesser, 1874

Diagnosis: desmothoracids enclosed by homogeneous mucous capsule perforated by minute pores, and body sharply differentiated from tubular stalk.
Composition: 3 species.
Type-species: *H. pellucida* Hertwig et Lesser, 1874

H. pellucida Hertwig et Lesser, 1874

Diagnosis: capsule oval, clear to yellow, 16.8-30 μm in diameter, with pores at tips of conical knobs. Filopodia not numerous (6-15). Several contractile vacuoles at periphery. Biflagellate swarmers.

Habitat: freshwater.
Occurrence: Germany (Hertwig and Lesser 1874, Rainer 1968), Switzerland (Penard 1904), the Netherlands (Hoogenraad 1927, Siemensma 1981), France (Brugerolle 1985), Sweden (Siemensma 1991), Finland (Levander 1894), Bulgaria (Valkanov 1928), Qeensland Australia (Patterson *et al.*, in prep.)

H. minor Siemensma, 1991

Diagnosis: capsule skittle-shaped, clear, 8-10 μm in diameter, tapering in basal part. A few minute pores without conical knobs. Stalk about 30 μm long.

Habitat: freshwater.
Occurrence: the Netherlands (Siemensma 1991).

H. zhadani sp. n.

Diagnosis: marine *Hedriocystis*, with ovoid homogeneous capsule about 50 μm in diameter; pores without conical knobs. Massive stalk about one fifth of the capsule diameter and 2-3 times the capsule diameter in length.
Description: the ovoid homogeneous transparent capsule is about 50 μm in diameter. It is without conical knobs. The cell body, 25-40 μm in diameter, gives rise to numerous filopods extending in all directions. The nucleus occupies a central position. Filopods are about as long as the capsule is wide, they bear numerous granules. The stalk is very massive, up to 8 μm in diameter and usually exceeds 2 or 3 times the diameter of the capsule. No contractile vacuoles were observed. Often form colonies on organic substrates or algae, possibly resulting from the absence of the swarmer.
Type locality: marine, N.A. Pertzov White Sea Biological Station of Moscow State University (Velikaya

Salma Strait, Kandalaksha Bay, 66° 31' N, 33° 07' E).
Type micrograph: Fig. 3 B.
Etymology: the species name is given in honour of
Dr. D.G. Zhadan (Moscow State University).
Occurrence: the White Sea.

Genus *Penardiophrys* gen. n.

Diagnosis: desmothoracids enclosed by capsule com-
posed of jointed polygonal facets; each facet bears a
minute pore.
Composition: 2 species.
Type-species: *P. reticulata* (Penard, 1904) comb. n.

P. reticulata (Penard, 1904) comb. n.

Basionym: *Hedriocystis reticulata* Penard, 1904
Diagnosis: cell body round, about 12 µm in diameter.
Capsule round, clear to yellow, with numerous minute
pores, each in a polygonal facet with raised edges. Stalk
about 2 times longer than the capsule diameter.
Habitat: freshwater, mainly marshy pools.
Occurrence: Switzerland (Penard 1904), Scotland (Brown
1911), the Netherlands (Siemensma 1981)

P. spinifera (Brown, 1918) comb. n.

Basionym: *Hedriocystis spinifera* Brown, 1918
Diagnosis: stalkless *Penardiophrys* with minute, transpar-
ent, thin, colourless to pale yellow, spherical capsule, 8-
12 µm in diameter. Facets with 5 or 6 sides, with raised
borders from the junctions of which arise many slender
spines. The body is spherical, nearly filling the capsule.
Habitat: freshwater, in wet moss.
Occurrence: Scotland (Brown 1918).

Genus *Cienkowskya* Schaudinn, 1896

Syn.: *Wagneria* Cienkowski, 1881; *Monomastigocystis*
De Saedeleer, 1930
Diagnosis: desmothoracids enclosed by a capsule perfo-
rated by minute pores, and body gradually narrowing to
form a conical stalk.
Composition: 2 species.
Type-species: *C. mereschkovckii* (Cienkowski, 1881)
Schaudinn, 1896.

C. mereschkovckii (Cienkowski, 1881) Schaudinn, 1896

Basionym: *Wagneria mereschkovckii* Cienkowski, 1881
Diagnosis: capsule is transparent, irregular in shape, 35-42

µm in diameter, with conical stalk about 45 µm long. Cell
body about 30 µm in diameter, gives rise to several
filopods extending through simple pores. Encystment
inside the capsule by a single cyst.
Habitat: marine.
Occurrence: the White Sea (Cienkowski 1881).

C. brachypous (De Saedeleer, 1930) comb. n.

Basionym: *Monomastigocystis brachypous* De Saedeleer,
1874
Diagnosis: capsule irregularly polygonal, 10-15 µm in
diameter, with short basal stalk. The cell body rounded,
8-10 µm in diameter. Several filopods extend through
pores at tops of conical knobs. Uniflagellate swarmers.
Habitat: freshwater.
Occurrence: Belgium (De Saedeleer 1930), the Nether-
lands (Siemensma 1981)

KEY TO DESMOTHORACID GENERA AND SPECIES

1. Surrounded by a mucous coat.......................................
...................................*Actinosphaeridium pedatus*
1' Surrounded by a perforated capsule.........................2
2. Capsule is with large openings, normally lattice-like....
....*Clathrulina* 5
2' Capsule is non-lattice-like, penetrated by pores or
small openings...3
3. Capsule gradually narrows to form a short conical
stalk *Cienkowskya*......................................6
3' Capsule is sharply differentiated from a long tube like
stalk...4
4. Capsule is smooth.............................*Hedriocystis* 7
4' Capsule wall is composed of jointed polygonal facets
....*Penardiophrys* 9
5. Capsule usually stalked, with simple pores; filopods
fine, branching, granule-bearing filopods.....................
....*Cl. elegans*
5' Capsule always stalkless, has complex inverted coni
cal pores; pseudopods with broad bases, unbranching,
without granules.................................*Cl. smaragdea*
6. Capsule 35-42 µm in diameter; marine.......................
....*Ci. mereschkovckii*
6' Capsule 10-15 µm in diameter; freshwater..............
....*Ci. brachypous*

7. Capsule rounded, pores at the tops of conical knobs...
....*H. pellucida*
7' Capsule ovoid or bottle-shaped, no conical knobs.....8
8. Capsule ovoid, about 50 μm in diameter; marine......
....*H. zhadani*
8' Capsule bottle-shaped, 8-10 μm in diameter; freshwa-
ter..*H. minor*
9. Capsule usually stalked; without radial spines…...........
....*P. reticulata*
9' Capsule stalkless, with some radial spines…....…..........…
....*P. spinifera*

PHYLOGENY

Presently we regard heliozoa as passive benthic predators, which capture their food by the radiating axopods. The characteristic body form of heliozoa has arisen in many branches of the eukaryotic evolutionary tree making the heliozoa to be an adaptive group (a grade) but not a monophyletic group (a clade). It is composed of 7 natural taxa (Mikrjukov 1998, 2000 a, b) - Centrohelida Kühn, 1926, Actinophryida Hartmann, 1913, Desmothoracida Hertwig et Lesser, 1874, Gymnosphaerida Poche, 1913, and 3 taxa of helioflagellates - Ciliophryida Febvre-Chevalier, 1985, Pedinellales Zimmermann *et al.*, 1984 (partly, i.e. only mixotrophic and heterotrophic members), and Dimorphida Siemensma, 1991.

The relationship of desmothoracids to other protists in the general system of Protista is far from resolved. Suggestions by some authors (Karpov 1990; Cavalier-Smith 1993, 1996/97, 1998; Kussakin and Drozdov 1998) that the heliozoa with the central nucleus should be united (as the Pedinellomorpha, or Actinophryidea, or Nucleohelea, etc.) would place together organisms with different patterns of ultrastructural organization and hence are likely to be a polyphyletic taxon (Patterson 1994, Mikrjukov 2000 a). There are molecular data on pedinellids and ciliophryids (Cavalier-Smith *et al.* 1995, Cavalier-Smith and Chao 1996) but no further data, which would help to establish the relatedness or non-relatedness of heliozoa as a whole. The flagellated forms (helioflagellates) and flagellated stages (swarmers of desmothoracids and gametes of gymnosphaerids) may prove to be more informative in establishing relatedness because more characters are available.

A comparative analysis of the general organization of biflagellate amoebo-flagellates of the order Cercomonadida Poche, 1913 lead us to regard them as derived from a hypothetical common ancestor of protostelid mycetozoa (Karpov and Mylnikov 1997, Karpov 1997), gymnosphaerid and desmothoracid heliozoa (Mikrjukov 2000 a). Some species of the genus *Cercomonas* (e.g. *C. rhacodes* Skuja, 1948) are able to form radial filopodia (Skuja 1948), whilst another member of this order - *Massisteria marina* Larsen et Patterson, 1990 - has reticulopodia which bear numerous granules/extrusomes and are supported by a longitudinal bundle of several microtubules (Larsen and Patterson 1990, Patterson and Fenchel 1990). When disturbed, *Masisteria* cells transform from the radial state with inactive flagella to the biflagellate locomotor phase similar to dimorphid helioflagellates. A desmothoracid, *Hedriocystis pellucida* has kinetocysts-like extrusomes almost identical to those in cercomonads *Bodomorpha* and *Massisteria*, and originating not in the endoplasmic reticulum (ER) (as in centrohelids) but certain from dictyosomes (Mylnikov 1988, Patterson and Fenchel 1990, Mikrjukov 1995 b).

Cercomonads have a pair of orthogonal kinetosomes. Near them is a massive electron dense body which in some species serves as a MTOC nucleating numerous microtubules that form a cone which envelops the nucleus (Fig. 6 A) (Mylnikov 1984; 1986 a, b; 1987; 1989; 1990). The electron-dense body is connected with the bases of kinetosomes by a fibrillar or striated rhizoplast. Flagellated cells of protostelids have a similar organization but the microtubules associated with the kinetosomes form a second (outer) cone (Fig. 6 B) that can also be found in flagellated cells of other mycetozoa (Barr 1981; Spiegel 1981; Spiegel and Feldmann 1985, 1988; Wright *et al.* 1988).

The cercomonad, *Bodomorpha reniformis* lacks both cones (i.e. the inner, true cercomonad cone too) (Fig. 6 C) but has a striated rhizoplast connecting the kinetosomes with a massive electron-dense body (Mylnikov 1984, 1988). *Bodomorpha*-like cercomonads may have given rise to a branch of amoebo-flagellates, in which the separation of amoeboid and flagellated phases has taken place. This branch may include gymnosphaerid and desmothoracid heliozoa; with the plesiomorphic cercomonad-like amoebo-flagellated form in these groups still present as the biflagellated locomotor stage, a swarmer (zoospore) or gamete. According to the verbal descriptions of the ultrastructure of gametes of gymnosphaerid heliozoa, *Gymnosphaera* and *Actinocoryne* [there are no published micrographs: Jones 1976, Febvre-Chevalier 1990], their general organization seems to resemble that of cercomonads with an electron-dense, osmiophilic MTOC connected to a pair of

orthogonally positioning kinetosomes by a fibrillar rhizoplast (Fig. 6 D left). With metamorphosis, the interphase MTOC of gametes is transforms into the axoplast of a heliozoon (Fig. 6 D right). The organization of desmothoracid swarmers could be hypothesised to be a homologue in which the electron-dense material of the osmiophilic interphase MTOC has moved at the surface of the nuclear envelope directly connected with the bases of orthogonally positioning kinetosomes by the striated rhizoplast (in *Hedriocystis* - Fig. 6 E left) or by the fibrillar matrix (in *Clathrulina*); after the transformation into the heliozoon-state, the nuclear envelope becomes the MTOC of axonemes of filopodia (Fig. 6 E- right) (Bardele 1972, Brugerolle 1985).

So we consider desmothoracid and gymnosphaerid heliozoa as groups derived from the mass of heterotrophic biflagellates and related to cercomonads, mycetozoa and to some other taxa.

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ACTA PROTOZOOLOGICA

Taxonomy and Phylogeny of Heliozoa. II. The Order Dimorphida Siemensma, 1991 (Cercomonadea classis n.): Diversity and Relatedness with Cercomonads

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Summary. A taxonomic review of dimorphid helioflagellates, and the analysis of the phylogenetic position of this group among other protists are performed. The generic name *Dimorphella* Valkanov, 1928 is considered as a junior synonym of *Dimorpha* Gruber, 1882, whilst its single member is transferred to *Dimorpha* as the second, most widespread, species of this genus - *D. elegans* (Valkanov, 1928) comb. n. *D. floridanis* Bovee, 1960 is a junior synonym of *D. mutans*. The marine *D. salina* Ruinen, 1938 is not a dimorphid. Three species are considered in *Tetradimorpha* Hsiung, 1927; they are *T. radiata* Hsiung, 1927 (the type-species), *T. tetramastix* (Penard, 1921) Siemensma, 1991, and a new species, *T. pterbica* Mikrjukov et Patterson, sp. n. described from a mangrove swamp in the northern Australia. A brief report and a scheme of the fine structure of *T. pterbica* is adduced. The marine *T. marina* Fenchel *et al.*, 1995 is regarded as a member of a separate, monotypic genus *Pseudodimorpha* gen. n. not belonging to dimorphids; it can be circumscribed as "bi- or tetraflagellate helioflagellates with axopodial axonemes ending at the nucleus". Diagnoses of 2 dimorphid genera and 5 species, and a key to them are given. The analysis of the biology and cell structure suggests that the order Dimorphida Siemensma, 1991 can be placed among amoeboid heterotrophic biflagellates of the phylum Sarcomonada Cavalier-Smith, 1993, stat. n. inside a new class Cercomonadea classis n. together with orders Cercomonadida Poche, 1913, Gymnosphaerida Poche, 1913, and Desmothoracida Hertwig et Lesser, 1874. Members of this new class could be briefly characterized as "tubulocristate heterotrophic amoeboid biflagellates with a clear interphase microtubule organizing centre (MTOC), nucleating cytoplasmic and pseudopodial microtubules, which appears as a separate electron-dense organelle at the forepart of the cell or by a material at the nuclear envelope".

Key words: Cercomonadea classis n., Cercomonadida, *Dimorpha elegans* comb. n., Dimorphida, helioflagellates, heliozoa, heterotrophic flagellates, phylogeny, *Pseudodimorpha* gen. n., taxonomy, *Tetradimorpha pterbica* sp. n.

INTRODUCTION

The term "helioflagellates" is commonly used to designate heliozoa with one or several flagella. Doflein (1916) united the variety of flagellates which produce pseudopodia i.e. the amoebo-flagellates in the order Rhizomastigida Bütschli, 1882 within the sarcodines, and hence this order included such different organisms as dimorphid and actinomonad helioflagellates, *Ciliophrys, Multicilia,* cercomonads, mastigamoebae and so on (Kudo 1954). Since the polyphyletic nature of rhizomastigids was becoming more and more obvious based on ultrastructural data on these organisms

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(Brugerolle 1982; Davidson 1982; Brugerolle and Mignot 1984 a; Larsen 1985; Patterson and Fenchel 1985; Mylnikov 1986 a, b; Mikrjukov and Mylnikov 1998 b) most of authors of more recent systems began to consider helioflagellates as heliozoa (Levine *et al.* 1980, Febvre-Chevalier and Febvre 1984, Lee *et al.* 1985, Margulis *et al.* 1990, Cavalier-Smith 1993, Hausmann and Hülsmann 1996).

The first recorded helioflagellate was a uniflagellate *Ciliophrys infusionum* reported by Cienkowski (1876) from Northern Russian freshwater reservoirs and considered now as a member of a group related to pedinellids (Preisig *et al.* 1991, Cavalier-Smith and Chao 1996, Mikrjukov and Patterson 2000). The first biflagellate helioflagellate was *Dimorpha mutans* (Fig. 1 A) described somewhat later by Gruber (1882).

Dimorphid helioflagellates are now a small group of freshwater amoebo-flagellates which according to a recent review of the heliozoan taxonomy (Siemensma 1991) includes 3 freshwater species united in 2 genera - *Dimorpha* Gruber, 1882 and *Tetradimorpha* Hsiung, 1927, and is considered as a separate heliozoan order Dimorphida Siemensma, 1991. Two marine species were also described by Ruinen (1938) and Fenchel *et al.* (1995). The phylogenetic position of dimorphids in the general system of protists is entirely unclear (Patterson and Zöllfel 1991, Patterson 1999, Patterson *et al.* 2000 b). Dimorphids are free-swimming bi- or tetraflagellates with axopods usually emerging from most of the body surface; axopodial axonemes terminate at an electron-dense fibro-granular structure called the "axoplast" which lies between the nucleus and the basal parts of elongate kinetosomes; flagella lack mastigonemes; extrusomes are kinetocysts with a concentric transverse structure.

Ecology of heliozoa is studied insufficiently. Our recent data (Mikrjukov 1999, 2000 b) and those of others (Tobiesen 1991, Pierce and Coats 1999) on a subbenthic or interstitial nature of these organisms leads us to regard heliozoa as a group of passive benthic consumers with a food capturing apparatus represented by a large radial system of axopods with granules paralysing and gluing a great spectrum of various small motile prey in a large volume of water. Fenchel (1986) reports that a sessile or little mobile mode of life in heterotrophic flagellated protists is usually associated with filter-feeding. Indeed, dimorphid helioflagellates are normally not mobile, and their flagella continue to accomplish weak oscillating movement not resulting in locomotion of the cell but creating a water current through the radiating

axopods as shown in *Ciliophrys* and pedinellid helioflagellates (Schewiakoff 1893, Sleigh 1964, Pedersen *et al.* 1986, Larsen and Patterson 1990); so during the heliozoon state, these helioflagellates combine a predatory habit with filter-feeding (Mikrjukov 2000 b). However when disturbed they withdraw their axopods slowly and transform into a pear-shaped or conical flagellate-state. The transformation takes several minutes; it is well described by Siemensma (1981, 1991) in *Dimorpha elegans* and *Tetradimorpha tetramastix*, respectively, and in the present work in *T. pterbica*. Axopods could either be entirely withdrawn at the flagellate state or only become shorter (Figs. 1 C-F; 2 A, B, E-G). Axopods still continue to withdraw when the cell begins actively to move.

DISCUSSION OF THE TAXA

### Genera *Dimorpha* Gruber, 1882 and *Dimorphella* Valkanov, 1928

The type-species of the genus *Dimorpha - D. mutans* Gruber, 1882 was described by Gruber from freshwaters in Germany as a biflagellate with axopods radiating from the axoplast and emerging from the whole body surface. The original Gruber's figure does not clearly demonstrate the position of the nucleus in the cell. A decade later, the same organism was surely recorded in Germany by Blochmann (1894). A clearly visible nucleus is shown in his figure near the posterior periphery of the cell (Fig. 1 A) but it is surely wrongly labelled as a "food particle".

Again a decade later, Schouteden (1907) recorded another organism differing clearly from the type and the Blochmann's material by an antero-central position of the nucleus, which surrounds the axoplast. In this position, the axoplast is enclosed in a cup-like invagination of the nucleus that hence is penetrated by axopodial axonemes radiating from the axoplast (Fig. 1 F). However, Schouteden (1907) also defined his helioflagellate as *D. mutans* Gruber, 1882. This mistake was followed later by Penard (1921), Pascher (1925), Siemensma (1981) and Brugerolle and Mignot (1984 b). Brugerolle and Mignot (1984 a, b) performed a careful study of the ultrastructure of the "Schouteden's" helioflagellate (also identifying it as *D. mutans*) and showed that (i) the nucleus is indeed perforated by numerous nuclear channels (lined by a double membrane) for insertion of axonemes radiating from the axoplast situated in the
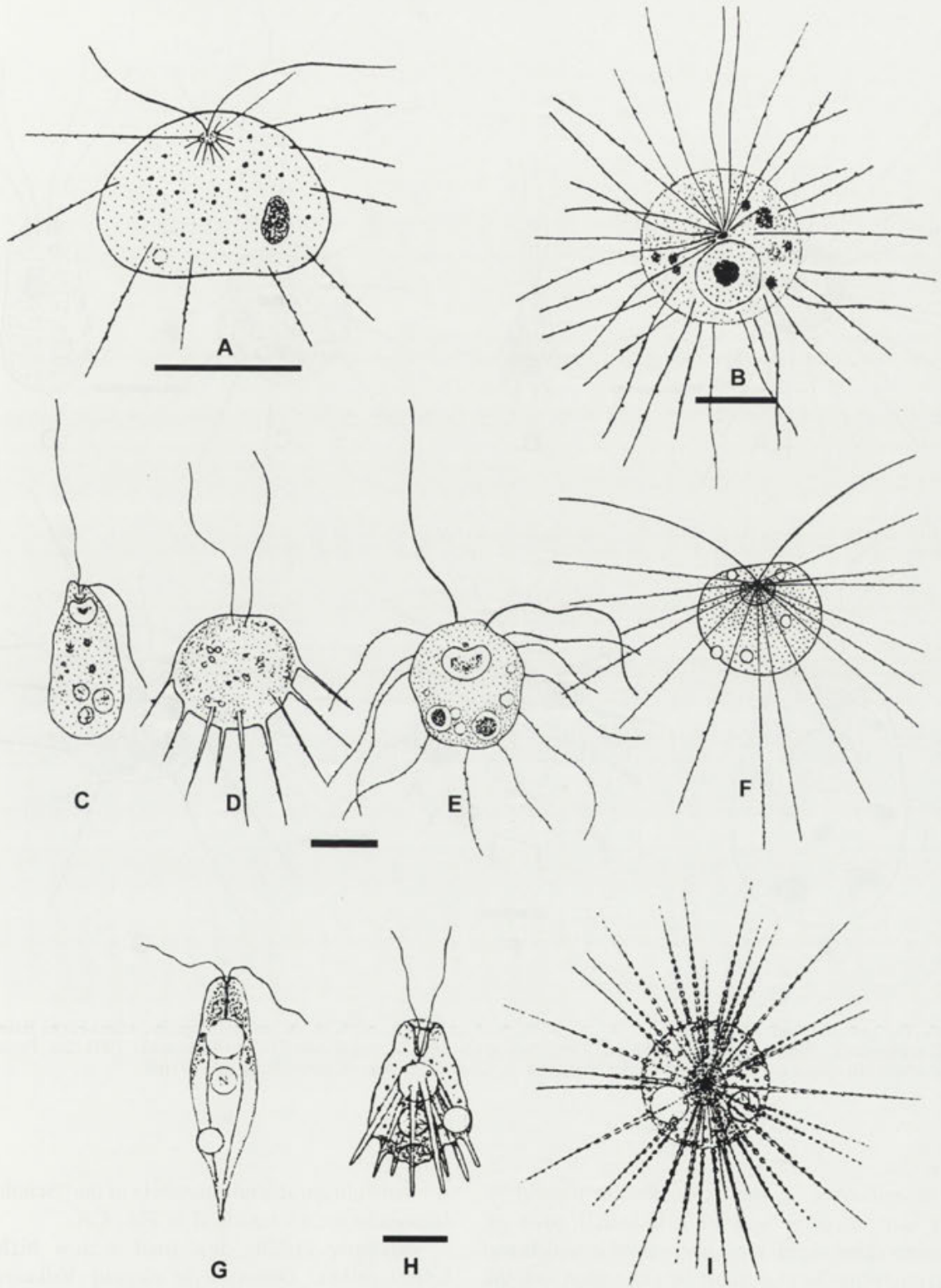
Fig. 1 A-I. The genus *Dimorpha* Gruber, 1882. **A** - *D. mutans* Gruber, 1882 (from Blochmann 1894); **B** - *D. elegans* (Valkanov, 1928) comb. n. (from Valkanov 1928); **C-F** - flagellate-to-heliozoon transformation of *D. elegans* (**C, D** - from Siemensma 1991, **E** - from Brugerolle and Mignot 1984 b, **F** - from Schouteden 1907); **G-I** - flagellate-to-heliozoon transformation of the organism described as *D. floridanis* (from Bovee 1960). Scale bar - 10 μm in **A** and **B**, and 20 μm - in others
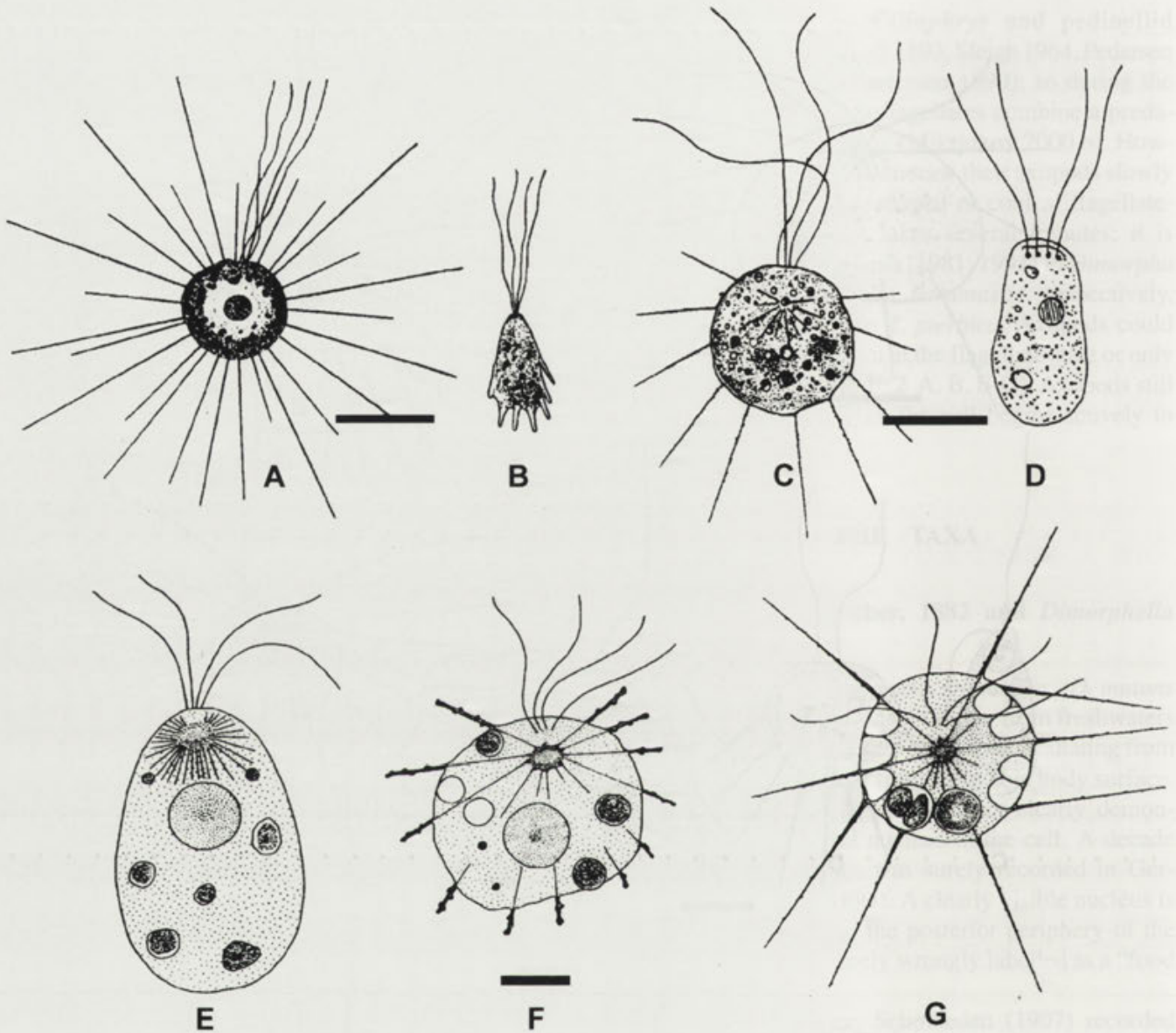
Fig. 2 A-G. The genus *Tetradimorpha* Hsiung, 1927. **A, B** - *T. radiata* Hsiung, 1927, heliozoon and flagellate states (from Hsiung 1927); **C, D** - *T. tetramastix* (Penard, 1921) Siemensma, 1991, heliozoon and flagellate states (from Siemensma 1991 and Penard 1921); **E-G** - *T. pterbica* Mikrjukov et Patterson, sp. n., flagellate, intermediate and heliozoon states. Scale bar - 20 μm

nuclear invagination, (ii) the axonemes are formed by complex lattices composed of orthogonal rows of 4-valent microtubules, (iii) some microtubular rootlets and a striated microfibrillar rhizoplast are connected with the basal apparatus represented by a pair of orthogonal, elongated kinetosomes, (iv) mitochondria have vermiform, tubular cristae, and (v) extrusive organelles are kinetocysts and have a concentric transverse structure.

All main ultrastructural characters of the "Schouteden's" *Dimorpha* are summarized in Fig. 4 A.

Valkanov (1928) described a new biflagellate helioflagellate, *Dimorphella elegans* Valkanov, 1928 (Fig. 1 B) which was wrongly considered by further investigations as a junior synonym of *Dimorpha mutans*. To Valkanov's (1928) point of view, the genus *Dimorphella* differs from *Dimorpha* Gruber, 1882 by
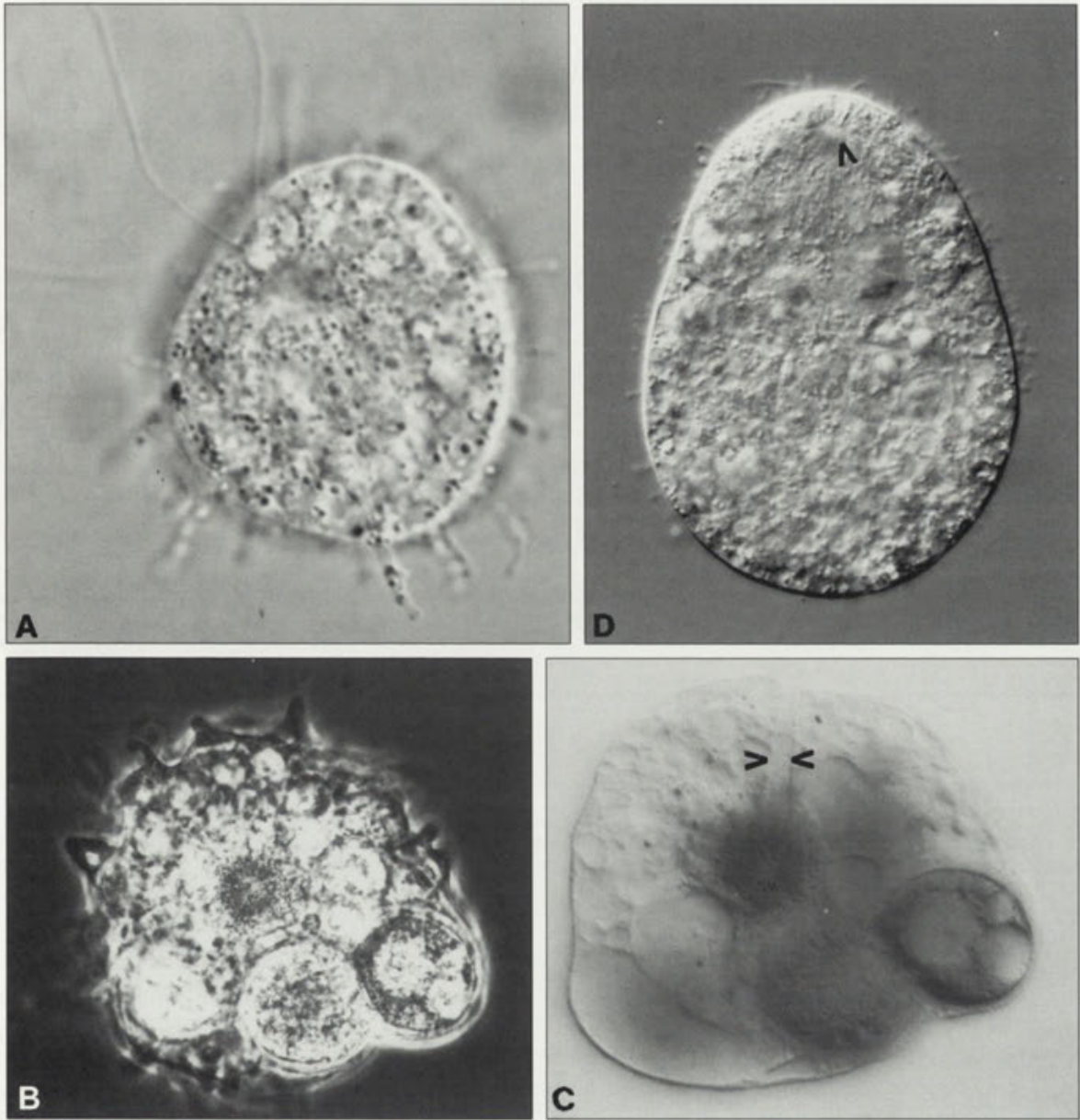
Fig. 3 A-D. Light micrographs of *T. pterbica* sp. n., x 850. **A** - a disturbed cell during the transformation from heliozoon to flagellate (4 flagella and the axoplast are clearly seen); **B** - a cell with a clearly visible heteromorphic axoplast (a cytoplasm is filled with food vacuoles); **C** - a cell with withdrawn axopods (a rhizoplast connecting the axoplast and kinetosomes is clearly seen and indicated by arrows); **D** - a pear-shaped flagellate-state (the position of the axoplast is indicated by an arrow). Micrograph by D.J. Patterson

the central position of the axoplast whilst it is moved to the frontal part of the cell in the latter genus. However the antero-central position of the nucleus which adjoins directly or (possibly) surrounds the axoplast indicates its identity with the helioflagellate firstly taken by Schouteden (1907) as *D. mutans* (Fig. 1 F), and later was reported using this name by Penard (1921), Pascher (1925), Siemensma (1981) and Brugerolle and Mignot (1984 b).

However we think that there are no reasons to take these differences as a base for generic distinctions and hence we consider the organism corresponding to all records listed above as the second species of *Dimorpha - D. elegans* (Valkanov, 1928) comb. n., the most widespread species of *Dimorpha*; the generic name *Dimorphella* Valkanov, 1928 is considered as a junior synonym for *Dimorpha* Gruber, 1882.
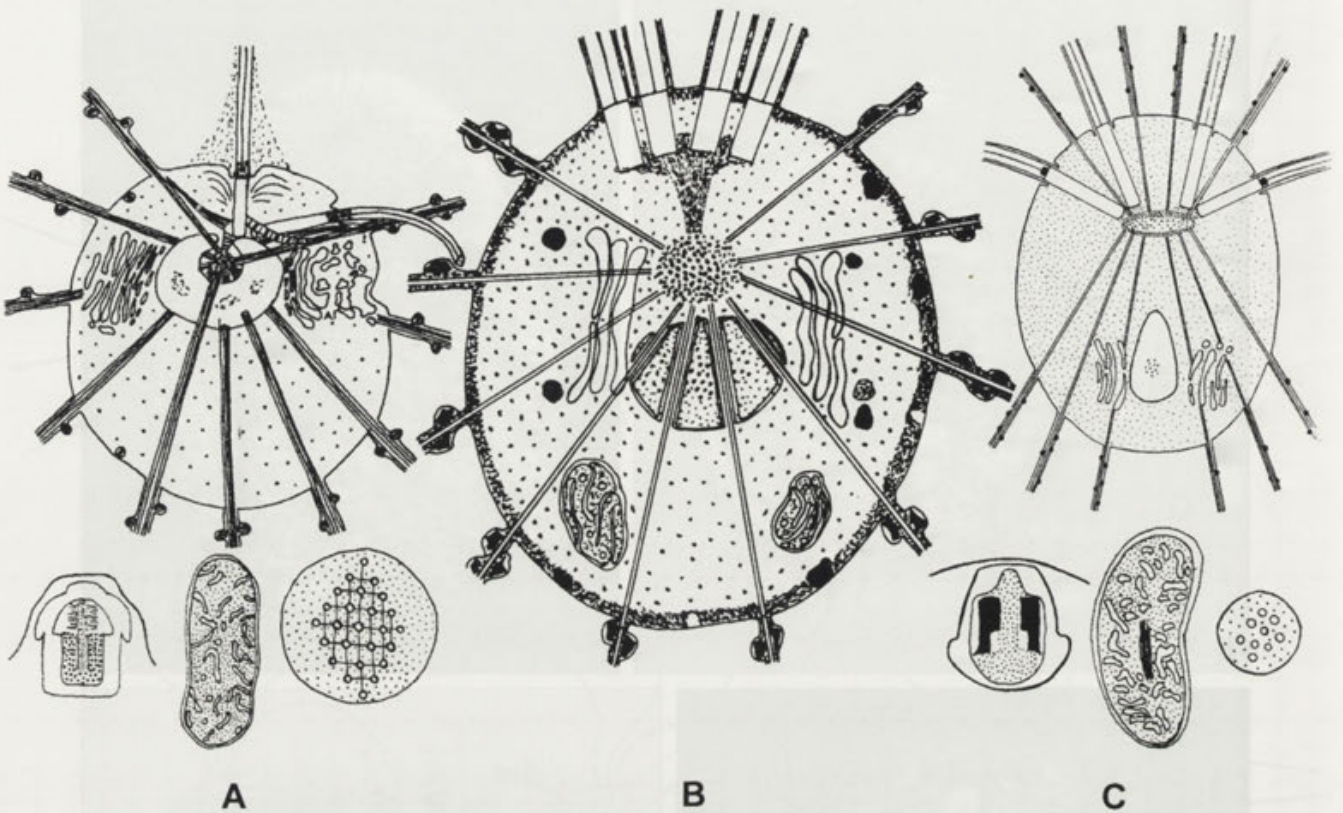
Fig. 4 A-C. A comparative scheme of the ultrathin organization of 3 dimorphids, *Dimorpha elegans* (**A**) (after Brugerolle and Mignot 1984 b); *Tetradimorpha pterbica* sp. n. (**B**) and *T. radiata* (**C**) (after micrographs by Brugerolle and Mignot 1983 b); a structure of kinetocysts, mitochomdria and axonemal lattices is shown in **A** and **C**

Bovee (1960) described another species of *Dimorpha - D. floridanis* Bovee, 1960 taking physiological and environmental characters as the main species distinctions, i.e. a comparative prevalence of a heliozoan or flagellate-state, or reactions to changes in the environment. No morphological basis is presented in the description of this organism. Analysing numerous figures adduced in the work by Bovee (some of them are copied in Figs. 1 G-I) it is important to note the extremely large size of the axoplast and its isolated position relative to the nucleus which occupies an eccentric position in the heliozoon-state and lies subcentrally at the flagellate-state; in both cases it is situated at some distance from the axoplast. According to the latter character of the position of the axoplast in respect to the nucleus, which we consider to be the main one in the species diagnostics in *Dimorpha*, we are prone to regard *D. floridanis* Bovee, 1960 as a junior synonym of the type-species, *D. mutans* Gruber, 1882.

Penard (1921) reported *D. elegans* (as *D. mutans*) and described 2 other species of *Dimorpha* - *D. monomastix* Penard, 1921 and *D. tetramastix* Penard, 1921. We should make a taxonomic remark here because in both Gruber's (1882) and Penard's (1921) sense, the assignment of a species to the genus *Dimorpha* was determined by its tendency to undergo heliozoon/flagellate-transformation and by the presence of the axoplast, but not by the number of flagella. So the description of a tetraflagellated species of *Dimorpha* by Penard (1921) was not a taxonomic mistake at that time, because the genus *Tetradimorpha* Hsiung, 1927 uniting tetraflagellated dimorphids was created 6 years later. Thus following Siemensma (1991) and Patterson and Zöllfel (1991) we regard the latter species as a member of *Tetradimorpha* (see below). The description of *D. monomastix* was a taxonomic mistake because no axoplast was seen by the author in the uniflagellate cells and hence we follow Siemensma (1991) and Mikrjukov
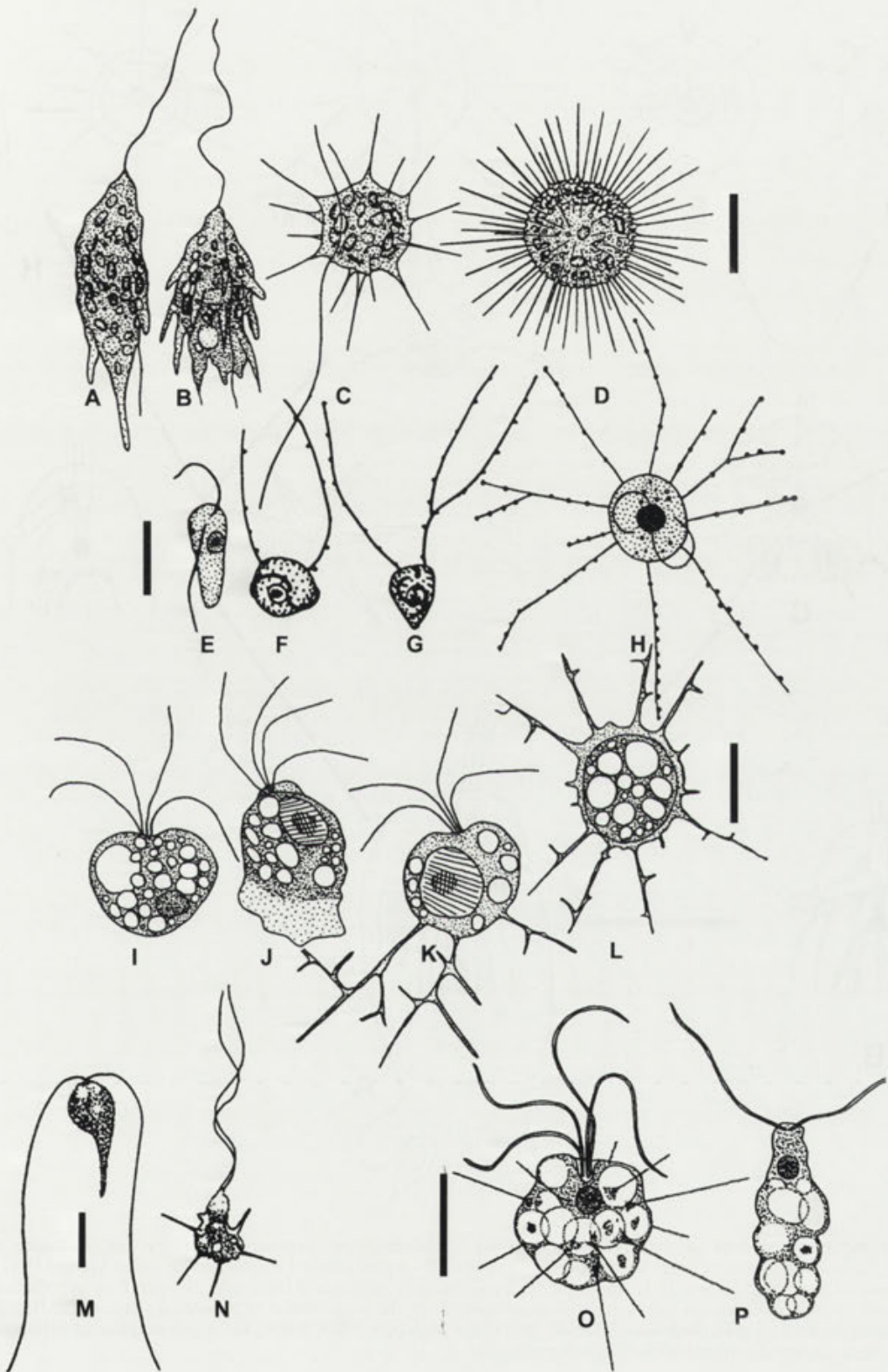
Fig. 5 A-P. Some other amoeboid flagellates discussed in the text. **A-D** - *Cercomonas rhacodes* (from Skuja 1948); **E-H** - *Massisteria marina* (**E-G** - from Patterson and Fenchel 1990; **H** - from Larsen and Patterson 1990), **I-L** - *Quadricilia rotunda* (from Vørs 1992); **M, N** - "*Dimorpha*" *salina* (from Ruinen 1938); **O, P** - *Pseudodimorpha marina* (from Fenchel *et al.* 1995). Scale bar - 10 μm
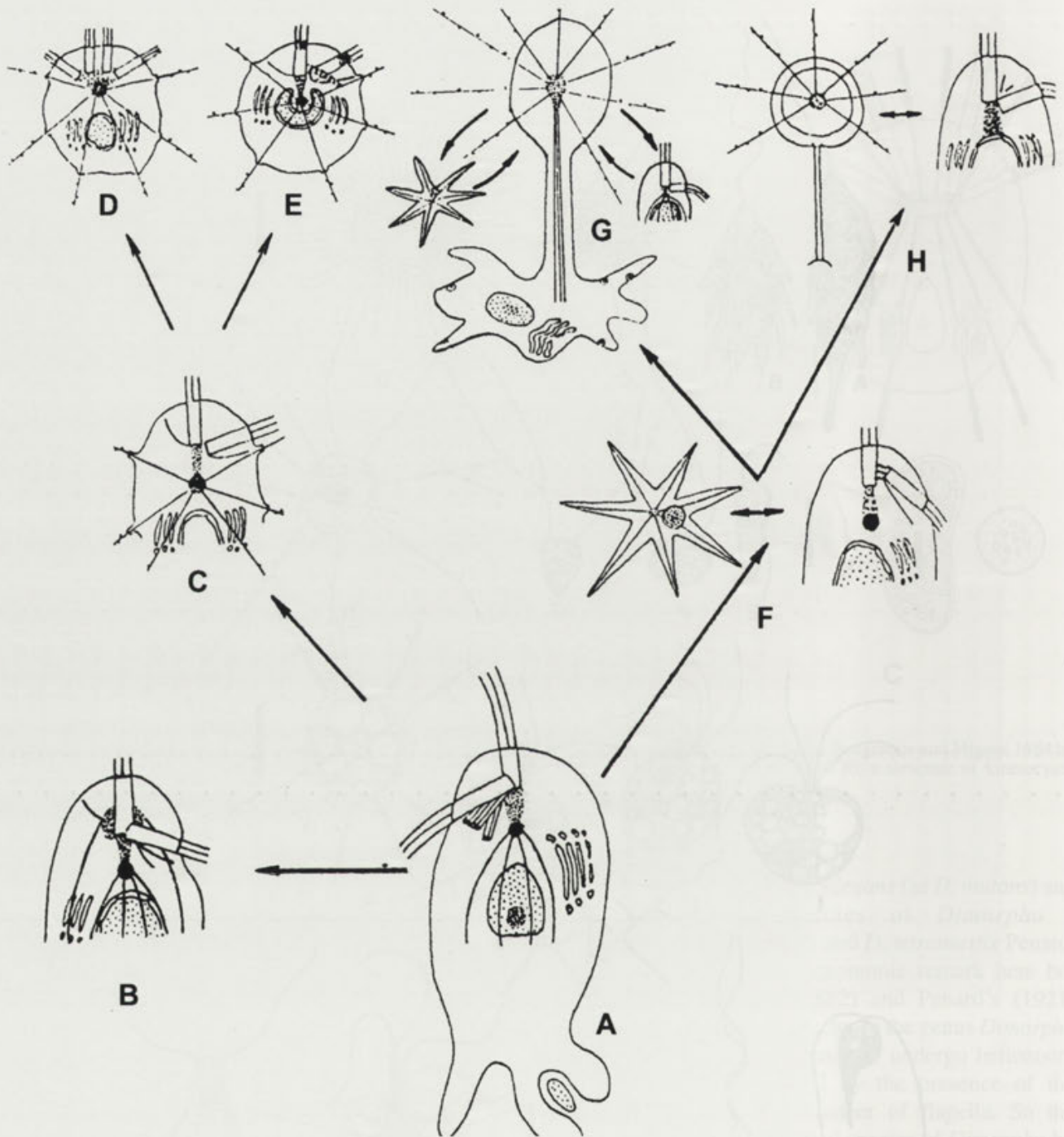
Fig. 6 A-H. A comparative scheme of the organization of the cytoskeleton of members of classes Cercomonadea and Mycetozoa. **A** - a cercomonad, *Cercomonas varians* (after Mylnikov 1986 a, 1989); **B** - a protostelid, *Cavostelium sp.* (after Spiegel 1981); **C** - a common hypothetical ancestor of dimorphid genera; **D, E** - dimorphids, *Tetradimorpha radiata* and *Dimorpha elegans*; **F** - a hypothetical stage at which separation of the life cycle into amoeboid and flagellated states took place; the organization of the cytoskeleton at the flagellate-state (right) corresponds to that in the cercomonad, *Bodomorpha reniformis* (after Mylnikov 1984, 1989); **G** - a gymnosphaerid heliozoon, *Actinocoryne contractilis*; **H** - a desmothoracid heliozoon, *Hedriocystis pellucida*

and Patterson (2000) and regard *Dimorpha monomastix* Penard, 1921 as a junior synonym of a ciliophryid helioflagellate, *Ciliophrys infusionum* Cienkowski, 1876.

Marine organisms previously classified as dimorphids can hardly be considered as belonging to this group. So a presently existing species of the genus, *D. salina*

Ruinen, 1938 (Figs. 5 M, N) described from the Tasman Sea possesses no dimorphid features such as the presence of the axoplast and of granules (extrusomes) in the pseudopods. Moreover the latter are conical, finger-shaped and do not resemble axopods of heliozoa. We think it possibly could be an amoeboid flagellate related to the cercomonads, but in all original figures by Ruinen (1938) (some of them are copied in Figs. 5 M, N) both flagella are isokont whilst cercomonads have normally clearly developed axial and recurrent flagella. Thus, *D. salina* Ruinen, 1938 is not a species of *Dimorpha* Gruber, 1882.

Hence we consider only 2 species of the genus *Dimorpha*: the type-species, *D. mutans* Gruber, 1882, and the second one, *D. elegans* (Valkanov, 1928) comb. n.

Genera *Tetradimorpha* Hsiung, 1927 and *Pseudodimorpha* gen. n.

The genus *Tetradimorpha* differs from *Dimorpha* by the presence of 4 flagella, and includes more species, which however are recorded more occasionally. The type-species, *T. radiata* Hsiung, 1927 has axopods gathered in anterior and posterior collars, and a more robust anterior one surrounds 4 apical isodynamic flagella (Fig. 4 C). A study of its ultrastructure performed by Brugerolle and Mignot (1983 b) has shown that the construction of the axoplast and kinetocysts is significantly similar to that in *D. elegans*. However, in contrast to *Dimorpha elegans*, the axoplast in *T. radiata* is not surrounded by the nucleus and lies freely in the cytoplasm near the bases of kinetosomes, whilst the nucleus occupies a postero-central position in the cell inside the cone formed by axonemes of the posterior collar of axopods. Moreover the rhizoplast is absent and the bases of kinetosomes are connected with the axoplast by a microfibrillar matrix, and axopodial microtubules in *T. radiata* are not gathered in axonemal lattices being connected in 2 or 3 or lie separately. The general ultrastructure of *T. radiata* is summarized schematically in Fig. 4 C.

The second species of *Tetradimorpha* - *T. tetramastix* (Penard, 1921) Siemensma, 1991 was first described as *Dimorpha tetramastix*, i.e. as a tetraflagellated species of *Dimorpha*. However this state does not correspond to the diagnosis of *Dimorpha* Gruber, 1882 in our present understanding of it (see above). Later a description of the genus *Tetradimorpha* by Hsiung (1927) created the situation when tetraflagellated dimorphids were separated into 2 genera. *D. tetramastix* is undoubtedly the first tetraflagellated dimorphid. Recently Siemensma (1991) carefully redescribed this species, and then transferred it to the genus *Tetradimorpha* as the type-species. Thus Siemensma (1991) and Patterson and Zöllfel (1991) consider *T. tetramastix* (Penard, 1921) Siemensma, 1991 as the type-species of the genus *Tetradimorpha* because it was described before *T. radiata* Hsiung, 1927. However we believe that assigning any species transferred from another genus to an already existing one as the type-species of the latter is radically incorrect (even if it was described before the primordial type-species). Hence we insist that the primordial type-species should be *T. radiata* Hsiung, 1927. Nevertheless *T. tetramastix* represents a real species of the genus, and differs from *T. radiata* by a uniform radiation of axopodia from the whole body surface (not gathered in collars), and by the entire withdrawal of axopodia into the cell body during the transformation to a flagellate-state (short axopods remain existing even at the flagellate state in *T. radiata*).

A recently described marine microaerobic species, *T. marina* Fenchel *et al.*, 1995 (Figs. 5 O, P) should not be considered as a member of the genus *Tetradimorpha* Hsiung, 1927, nor a member of the dimorphid helioflagellates as a whole. It has a changeable (2 or 4) number of flagella, and their number does not depend upon the length of time after the last cell division. The anterior part of the cell is occupied by the nucleus; however the presence of the axoplast is not shown, and axonemes of numerous granule-bearing axopods seem to terminate at the nucleus. A careful study of the biology and of the ultrastructure of this unusual protist is necessary. Presently, it can be considered as a member of a separate (non-described) group of helioflagellates (i.e. besides dimorphids, pedinellids and ciliophryids) and as an organism possibly related to another tetraflagellated amoebo-flagellate, *Quadricilia rotunda* (Skuja, 1948) Vørs, 1992 described from the Baltic sediments. It also has alternating amoeboid and amoebo-flagellated states (Figs. 5 I-L). Thus, we should regard *T. marina* Fenchel *et al.*, 1995 as a member of a separate, monotypic genus *Pseudodimorpha* gen. n. not belonging to dimorphids. The formal description is given below in Diagnoses of the taxa.

Finally one additional species of the genus, a giant *Tetradimorpha pterbica* sp. n. (Figs. 2 E-F, 3), discovered in samples taken from a mangrove swamp (S<2‰) in Queensland (Australia) differs from other species of *Tetradimorpha* by a series of characteristics. *T. pterbica* differs from the type-species, *T. radiata* in: (i) being three or more times larger in diameter, (ii) the presence

of numerous axopodia covering the whole body but not gathered in anterior and posterior collars, (iii) flagella that are shorter than the cell diameter, and (iv) a flagellate-state lack remnants of axopods (Fig. 3 D). *T. pterbica* differs from *T. tetramastix* in: (i) a twice larger diameter, (ii) flagella that are shorter than the cell diameter and the length of axopods, whilst comparatively in *T. tetramastix* axopods are shorter and flagella longer, and (iii) massive size of the axoplast. A clear rhizoplast, not recorded in other species of *Tetradimorpha* (but found in *D. elegans*), is seen between the basal part of the flagellar apparatus and the axoplast (Fig. 3 C). So we consider *T. pterbica* as a real, new species of *Tetradimorpha* Hsiung, 1927. The formal description of this species is given below in the taxonomic appendix.

Recently, we (Mikrjukov, unpubl.) performed a brief study of the ultrastructure of *T. pterbica*; the main ultrastructural characteristics of this species are summarized in a scheme (Fig. 4 B). This is the third species of the group studied by electron microscopy. As in the other studied dimorphids, *T. pterbica* has mitochondria with vermiform, tubular cristae typical for dimorphids and a massive axoplast has a microfibrillar core surrounded by a microgranular sheath. As in some strains of *D. elegans*, the nucleus of *T. pterbica,* lying adjacent to the axoplast, is perforated by channels allowing passage of axonemes of the posterior axopods, and the kinetosomes are connected with the axoplast by a rhizoplast, which is nonstriated in this case. As in *T. radiata*, 4 kinetosomes of *T. pterbica* lie at an angle of about 30° and occupy the frontal pole of the cell where a poorly developed flagellar pocket was observed; massive axonemes have a variable number of microtubules which are not gathered in regular lattices. However besides the characters mentioned above, *T. pterbica* has some peculiar features: (i) the plasmalemma (including the axopods) is lined from inside by a mucopolysacharide layer 0.2-0.3 μm thick, i.e. by the pseudopellicle (according to Karpov 1986); (ii) axopods and the cortical cytoplasm contain numerous homogeneous electron-dense bodies of irregular shape, 0.3-0.5 μm in diameter, possibly being the primitive extrusomes (no other extrusome-like bodies are found in clearly granule-bearing axopods of this species). So our study of the fine structure of *T. pterbica* has shown that its structure is very similar to that in *D. elegans* and *T. radiata*, but differs by some peculiar features including the presence of the pseudopellicle in the cortical cytoplasm, and a very primitive structure of extrusomes [both other studied dimorphids have very complex kinetocysts (Figs. 4 A, C)].

Thus in the taxonomy of *Tetradimorpha,* we use the following taxonomic characters: 1) are the axopodia gathered in collars or not, 2) do axopodia entirely withdraw during the transformation to a flagellate-state or not, and 3) a comparative length of flagella, axopodia and the cell diameter (which among these is longer?). The genus *Tetradimorpha* Hsiung, 1927 is comprised of 3 species. Thus, we consider 2 dimorphid genera including 5 species.

DIAGNOSES OF THE TAXA

Order DIMORPHIDA Siemensma, 1991

Diagnosis: helioflagellates with 2 or 4 flagella, and axonemes terminating at the axoplast.
Composition: 2 genera.

Genus Dimorpha Gruber, 1882

Syn.: Dimorphella Valkanov, 1928
Diagnosis: dimorphids with 2 flagella.
Composition: 2 species.
Type-species: *Dimorpha mutans* Gruber, 1882

D. mutans Gruber, 1882

Syn.: *Dimorpha floridanis* Bovee, 1960
Diagnosis: species of *Dimorpha* with the axoplast placed freely at the frontal part of the cell; the nucleus occupies a central or a postero-lateral position.
Habitat: freshwater.
Occurrence: Germany (Gruber 1882, Blochmann 1894), Florida, USA (Bovee 1960).

D. elegans (Valkanov, 1928) comb. n.

Basionym: *Dimorphella elegans* Valkanov, 1928
Diagnosis: species of *Dimorpha* with the axoplast placed closely adjoined to, or in the invagination of the antero-central positioned nucleus.
Habitat: freshwater
Occurrence: Germany (Pascher 1925, Switzerland (Penard 1921), Belgium (Schouteden 1907), Bulgaria (Valkanov 1928), the Netherlands (Siemensma 1981), France (Brugerolle and Mignot 1984 b)

Genus Tetradimorpha Hsiung, 1927

Diagnosis: dimorphids with 4 flagella.
Composition: 3 species.
Type-species: *T. radiata* Hsiung, 1927

T. radiata Hsiung, 1927

Diagnosis: species of *Tetradimorpha* with axopodia grouped in anterior and posterior collars; flagella are longer than the cell diameter. During the transformation into the flagellate-form, axopods shorten but are retained.
Habitat: freshwater.
Occurrence: USA (Hsiung 1927), France (Brugerolle and Mignot 1983 b).

T. pterbica Mikrjukov et Patterson, sp. n.

Diagnosis: species of *Tetradimorpha* with axopods radiating from the whole cell surface; flagella are shorter than the cell diameter and than the length of axopods; axopods are entirely withdrawn at the pear-shaped flagellate-state.
Description: during a heliozoon-state, cells are rounded, 65-95 (mean 80) μm in diameter, covered regularly with long, tapering arms (axopods), one and a half times longer than the cell diameter; arms often radiate in radial but occasionally in tangential directions. Clear granules (extrusomes) are seen along the arms. Four apical flagella, 50-70 μm long, beating languidly but not resulting in essential cell locomotion. Central cytoplasm is denser, brownish, while peripheral zone is often highly vacuolated and forms a thick layer of large vacuoles (which may be absent in some specimens). A massive axoplast, 18-20 μm in diameter, with a clearly visible heteromorphic central part (Fig. 3 B), lies in the anterior part of the cell, in front of a large nucleus, *ca* 23 μm, displacing as well towards the frontal part. When disturbed, the cell withdraws its axopods slowly and transforms into a pear-shaped flagellate-state (Fig. 3 D); the transformation takes about 2 minutes.
Type figures: Fig. 2 G
Type locality: a mangrove swamp (S<2‰) at the environs of the Cape Tribulation Tropical Research Station, Cape Tribulation, Queensland, Australia (16°05'S, 145°27'E) in March 1997.
Habitat: freshwater, mangrove swamp.
Remarks: *Tetradimorpha pterbica* differs from the type-species *T. radiata* Hsiung, 1927 in: (i) being three or more times larger in diameter, (ii) the presence of numerous axopodia covering the whole body but not gathered in anterior and posterior clumps (as in *T. radiata*), (iii) flagella are shorter than the cell diameter, and (iv) its flagellate-state lacks axopods. *T. pterbica* differs from *T. tetramastix* (Penard, 1921) Siemensma, 1991 in: (i) being twice large in diameter, (ii) flagella are

shorter than the cell diameter and axopods lengths, while in *T. tetramastix* axopods are shorter and flagella longer, and (iii) massive size of the axoplast.

T. tetramastix (Penard, 1921) Siemensma, 1991

Basionym: *Dimorpha tetramastix* Penard, 1921.
Diagnosis: species of *Tetradimorpha* with flagella about 1.5 times longer than the cell diameter. Fifteen to 20 axopods arise all over the cell surface. When disturbed, the cell withdraws its axopods and transforms into a conical flagellate.
Habitat: freshwater.
Occurrence: Switzerland (Penard, 1921), the Netherlands (Siemensma, 1981).

FLAGELLATA *incertae sedis*

Genus *Pseudodimorpha* gen. n.

Diagnosis: bi- or tetraflagellate helioflagellates with axopodial axonemes ending at the nucleus.

P. marina (Fenchel *et al.*, 1995) comb. n.

Basionym: *Tetradimorpha marina* Fenchel *et al.*, 1995.
Diagnosis: a spherical cell *ca* 5-20 μm in diameter. A spherical nucleolus is displaced towards a well-developed flagellar pocket from which 2 or 4 flagella arise. About 20 granule-bearing axopodia radiate from the whole body surface. No axoplast is recorded. The cell is able to transform into the drop-shaped flagellated state.
Habitat: marine, microaerobic.
Distribution: Denmark (Fenchel *et al.* 1995).

KEY TO DIMORPHID GENERA AND SPECIES

1 2 flagella Dimorpha..2
1' 4 flagella Tetradimorpha...3
2. Axoplast located in the cup-like invagination of the nucleus or closely adjoined to it..............*D. elegans*
2' Axoplast is not surrounded by the nucleus; the latter lays in central or postero-lateral parts of the cytoplasm..*D. mutans*
3. Axopodia are clustered in anterior and posterior collars..*T. radiata*
3' Axopodia arise uniformly over the cell surface..........4
4. Flagella are shorter than the diameter of the cell..... ....*T. pterbica*
4' Flagella are longer than the diameter of the cell....... ....*T. tetramastix*

PHYLOGENY

A phylogenetic position of dimorphid helioflagellates among other protistan taxa seems to be entirely unclear (Patterson and Zöllfel 1991, Patterson *et al*. 2000 b). Brugerolle and Mignot (1983 a, 1984 a) regard dimorphids as a connective link between the centrohelid heliozoa and a variety of flagellated protists. This position is supported by the presence of the centro- or the axoplast as an axonemal MTOC in both groups, and by the presence of kinetocysts with a concentric cross structure as extrusive organelles. Febvre-Chevalier (1985) even places dimorphid genera among centrohelids.

However this suggestion is very confused for the following reasons. The "pure" centrohelid heliozoa (= Centroheliozoa *sensu* Dürrschmidt and Patterson 1987 a = or Centroplasthelida *sensu* Febvre-Chevalier et Febvre, 1984) are a very well circumscribed group in terms of fine morphology which is well studied by many authors (Hovasse 1965; Davidson 1972; Bardele 1975, 1977; Rieder 1979; Febvre-Chevalier and Febvre 1984; Patterson and Dürrschmidt 1986; Dürrschmidt and Patterson 1987 a, b; Kinoshita *et al*. 1995), and thus looking for any taxa related to it is a little successful now (Mikrjukov and Mylnikov 1998 a, Mikrjukov 2000 a). A comparison of the general organization of dimorphids with that in centroheliozoa shows there is little in common among these groups. Thus, dimorphids (i) have clear tubular (vermiform) mitochondrial cristae (Brugerolle and Mignot 1984 a), (ii) despite a superficial similarity in the structure of extrusomes to centroheliozoa, the prekinetocysts of *Dimorpha* appear adjacent to dictyosomes (Brugerolle and Mignot 1984 b), (iii) finally, the axoplast of dimorphids represents a massive clump of a fibro-granular material without a clearly visible substructure (reviews by Febvre-Chevalier and Febvre 1984, Febvre-Chevalier 1990). On the other hand, the Centroheliozoa (i) have clear flat (ribbon-like) cristae in contrast with tubular cristae of other heliozoa and of the majority of heterotrophic biflagellates (Cavalier-Smith and Chao 1995), (ii) the characteristic complex centrohelidean kinetocysts are budding from the centrally located vesicles of the endoplasmic reticulum (Mikrjukov 1995 a, b), and (iii) the structure of the centrohelidean centroplast is highly conservative, and this organelle is composed of a tripartite disc sandwiched between two, more electron-opaque hemispheres (Kormos 1971, Rieder 1980, Klewer *et al*. 1997). Thus, the centroplast of Centroheliozoa does not correspond in its structure to interphase MTOCs of either dimorphids

or other protistan taxa [electron-dense cytoplasmic organelles organizing interphase microtubules often appear in various non-related groups of protists (Benwitz and Grell 1971, Pons and Pussard 1974, Bennett 1986, Wright *et al*. 1988) and thus this character cannot be used in phylogenetic reconstructions]. Hence, the ultrastructure of Centroheliozoa is absolutely unique, and dimorphid helioflagellates are obviously not related to them.

Febvre-Chevalier and Febvre (1984), Karpov (1990) and Cavalier-Smith (1993, 1996/97) consider heliozoa with a MTOC represented by a centro- or axoplast as a heliozoan or protistan macrotaxon of the rank of an order or a class, and thus unite centrohelids, gymnosphaerids and dimorphids, separating them from heliozoa with a central nucleus (i. e. actinophryids, desmothoracids, ciliophryids and taxopodids) which comprise another group. None of the evidence allows us to suppose a possible position of dimorphids in recent systems of protists by Corliss (1994), Kussakin and Drozdov (1998) and Cavalier-Smith (1998). A careful cluster analysis of heliozoan interrelationships performed by Smith and Patterson (1986) based on a number of morphological and ultrastructural characters indicate another group that seems to be related to dimorphids - the desmothoracid heliozoa. Indeed, both groups are tubulocristate, both have kinetocysts originating in contact with dictyosomes, and moreover some desmothoracids are able to produce flagellated swarmers with 2 orthogonally positioned, elongated kinetosomes giving rise to a pair of heterodynamic flagella (occasionally to a single apical flagellum) (see review by Mikrjukov 2000 c); desmothoracids lack an axoplast. However the present statement on the polyphyletic nature of the taxon "Heliozoa" (Patterson 1994; Mikrjukov 1998, 2000 a) considers only 4 non-related groups of "true heliozoa", i.e. centrohelids, desmothoracids, gymnosphaerids and actinophryids (Mikrjukov *et al*. 2000). Other traditional heliozoa (*sensu lato*) are distributed among filose amoebae (rotosphaerids), stramenopiles (pedinellids and *Ciliophrys*), or flagellates of uncertain position (dimorphids) (Patterson *et al*. 2000 a, b; Mikrjukov 2000 b; Mikrjukov *et al*. 2000; Mikrjukov and Patterson 2000). Thus, we ought to look for taxa related to dimorphids not among various heliozoan groups but among tubulocristate heterotrophic biflagellates as a whole.

A comparative analysis of the general organization of the cytoskeleton of cercomonad amoebo-flagellates studied by Brugerolle and Mignot (1984), Mylnikov (1984;

1986 a, b; 1987; 1990), *etc.* allows us to consider it as an ancestral state not only for that of protostelid zoospores (Karpov 1997, Karpov and Mylnikov 1997) but also to the cell structure of dimorphid helioflagellates (Fig. 6). We note that some species of *Cercomonas* (Figs. 5 A-D) are able to produce radial filopodiate forms (Skuja 1948), and a cercomonad *Massisteria marina* (Figs. 5 E-H) has granule-bearing (with kinetocysts) reticulopodia supported by one or several microtubules (Larsen and Patterson 1990, Patterson and Fenchel 1990).

In both cercomonads and dimorphids, there are 2 heterodynamic flagella (Figs. 6 A, D, E) arising from a pair of orthogonal kinetosomes (the flagellar apparatus is duplicated in *Tetradimorpha*); the centre of the cell is occupied by an interphase MTOC formed by an osmiophilic body in cercomonads, and by a fibro-granular clump - the axoplast in dimorphids; in both cases the MTOC is connected with the bases of kinetosomes by a microfibrillar rhizoplast (Figs. 4 A, B; 6 A, E). In cercomonads, the osmiophilic interphase MTOC gives rise to numerous back directed interphase microtubules forming a cone enveloping the nucleus which is moved to the frontal part of the cell (Fig. 6 A) (Mylnikov 1984; 1986 a, b; 1987; 1989; 1990); Fig. 4 C clearly demonstrates that the nucleus in *T. radiata* also lies inside a cone of back directed microtubules arising from the interphase MTOC (i. e. the axoplast) and composing the axonemes of the posterior collar of axopods. Extrusive organelles of the cercomonad, *Bodomorpha* are also kinetocysts originating in contact with dictyosomes (Mylnikov 1988). Mitosis in both groups is an open orthomitosis, with rhizoplasts in dimorphids (Mignot and Brugerolle 1991) or kinetosomes in cercomonads (Mylnikov 1986 a) as polar structures of the spindle.

Thus, we suppose that dimorphid helioflagellates have a hypothetical cercomonad-type ancestor (Fig. 6 C) which has remained evident as an almost unchanged organization of the cytoskeleton; however microtubules radiating from its interphase MTOC have increased in number and transformed to axonemes representing an internal skeleton of a radial pseudopodial apparatus serving for more extensive phagotrophic feeding. A free organization of microtubules (not ordered in axonemal lattices) in axopods of *Tetradimorpha* spp., together with the location of the axoplast in the cytoplasm, both argue for a more close relation of the genus *Tetradimorpha* to the organization of the ancestral (cercomonad-type) form than of the genus *Dimorpha*.

According to the arguments discussed above, a revision of the taxonomic position of dimorphid helioflagellates

in the general system of Protista suggests the following. We do not consider a lowering of the rank of dimorphids (i. e. the order Dimorphida Siemensma, 1991) to be appropriate because the diversity of the ultrastructural characters among the members is rather high: (i) a free (in *Tetradimorpha*) or complex, "chess-board pattern" (in *Dimorpha*) organization of microtubules in axonemal lattices, (ii) a cytoplasmic or an intranuclear (in the nuclear invagination) position of the axoplast, and (iii) a various composition of kinetosomal rootlets and a possible presence of a rhizoplast. This great diversity of a cell organization of dimorphids led Febvre-Chevalier and Febvre (1984) to separate dimorphids in 2 subtaxa in the rank of suborders: Endonucleaoaxoplasthelina Febvre-Chevalier et Febvre, 1984 (the genus *Dimorpha*) and Exonucleoaxoplasthelina Febvre-Chevalier et Febvre, 1984 (the genus *Tetradimorpha*); however our foregoing analysis of the dimorphid taxonomy leads to a different view because we know now that the type-species of *Dimorpha* - *D. mutans* is characterized by a free position of the axoplast in the cytoplasm.

The position of the order Dimorphida Siemensma, 1991 seems more properly to be among amoeboid heterotrophic biflagellates of the phylum Sarcomonada Cavalier-Smith, 1993, stat. n. inside a new class Cercomonadea classis n. together with amoeboflagellated orders Cercomonadida Poche, 1913, Desmothoracida Hertwig et Lesser, 1874, and Gymnosphaerida Poche, 1913; all listed subtaxa are known as orders in different systems of protists, and hence it determines the class rank of the macrotaxon uniting them. A possible scheme of the evolution of the cytoskeleton in cercomonads, dimorphids, flagellated swarmers of desmothoracids and protostelids, and gametes of gymnosphaerids are shown in Fig. 6. The organization of the genus *Cercomonas* (Fig. 6 A) is taken as the base; the osmiophilic body playing a role of the MTOC of cytoplasmic microtubules is connected with the bases of kinetosomes by a fibrillar, more usually striated rhizoplast.

We can consider the cell organization of cercomonads to be a prototype for that in dimorphids as was mentioned above. We suppose the origin of dimorphids from any cercomonad-like ancestor is indicated by an immutable general plan of the cell construction and a locomotor flagellar apparatus (Fig. 6 C). However, the pseudopodial apparatus serving for phagotrophic feeding has undergone an essential improvement. So it has assumed the appearance of fine radiating axopods serving as a capturing apparatus and promoting the flotation in the

Smith, 1993, stat. n. together with classes Mycetozoea de Bary, 1873, Thaumatomonadea Cavalier-Smith, 1993, stat. n. and Thecomonadea Cavalier-Smith, 1993, stat. n.

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ACTA PROTOZOOLOGICA

Morphology and Morphogenesis of a Spanish Population of *Urosomoida agiliformis* (Ciliophora, Hypotrichida) from a Wastewater Treatment Plant

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Summary. The morphology and morphogenesis of a population of the hypotrichous ciliate *Urosomoida agiliformis* from a wastewater treatment plant with a RBC system are described. This population is compared with some populations of *U. agiliformis* studied previously. The Spanish population shows an infraciliary pattern similar to Austrian and Israeli populations of *Oxytricha longa* studied before. However, the morphogenesis in this case is similar to some soil populations of *U. agiliformis* from alpine pastures in Austria. The morphology and division morphogenesis for this Spanish population are schematised in a computer drawing using symbols. An improved diagnosis for the genus *Urosomoida* is also provided.

Key words: Ciliophora, Hypotrichida, morphogenesis, morphology, *Urosomoida agiliformis*.

INTRODUCTION

Urosomoida agiliformis Foissner 1982 has been recently included both in the Family Parakahliellidae (Eigner 1997) and in the family Oxytrichidae (Berger and Foissner 1997) depending on the interpretation of morphological and morphogenetical data considered.

Morphogenetical criteria have been widely used in the reconstruction of ciliate phylogenies (Corliss 1968). The discussion of how these data might be used in classification of taxa at genus or species level is not new (Ganner *et al.* 1986-87). However, information about the variability of these features in different popu-

lations of the same species is lacking (Foissner and Adam 1983, Ganner *et al.* 1986-87), since these ontogenetic studies are usually based on data from just single populations.

In this study, new data on the morphology and morphogenesis of a Spanish population of *U. agiliformis* are provided. These observations are compared with those from the populations described by Foissner (1982), Foissner and Adam (1983) and Ganner *et al.* (1986-87). This study shows how morphogenetic data might be useful to discern genus or species with very similar morphologies.

MATERIALS AND METHODS

Urosomoida agiliformis was recovered from biofilm samples of a wastewater treatment plant with a rotating biological contactor (RBC) system in Boadilla (Madrid, Spain). The original samples were en-

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riched with wheat grains, and kept at room temperature (14-20 °C). The culture was subsequently maintained in cereal leaf media (Sigma). Cells were studied *in vivo* with bright field and phase contrast microscopy. Infraciliature was studied using the pyridinated silver carbonate method (Fernández-Galiano 1994, Olmo and Téllez 1997a) and protargol method (Wilbert 1975, Foissner 1991). Morphometrical data were obtained from 30 protargol impregnated specimens.

The terminology is based on Corliss (1979), Berger and Foissner (1997) and Eigner (1997).

RESULTS

Morphology of *Urosomoida agiliformis*, Spanish population (Fig. 1, Table 1)

Size *Urosomoida agiliformis in vivo* is about 70-100 x 20-35 μm, ellipsoid, with pointed anterior end and rounded posterior one. Contractile vacuole is located almost at the level of cytostome. Food vacuoles are filled with bacteria.

Buccal area is relatively narrow. Paroral and endoral membranes are in *Oxytricha* pattern (Berger and Foissner 1997, Berger 1999). Adoral zone of membranelles (AZM) with a question mark - shape outline is about 2/3 of the body length in size. Each membranelle has 4 kineties.

Somatic ventral infraciliature has 3 enlarged frontal cirri and 1 buccal cirrus. These are located near the anterior end of the endoral membrane and above the paroral membrane. Frontoventral cirri are disposed in a V-shaped pattern. Postoral ventral cirri are set in a cluster underneath the buccal vortex. Marginal cirri are disposed in 2 rows, on the right and left of the cell respectively. Two pretransverse cirri and 4 transverse cirri are near the end of the marginal rows. The dorsal side of the cell has 4 kineties and 2 caudal cirri.

Nuclear apparatus has 2 ellipsoid macronuclear segments and 1 or 2 micronuclei located near them.

Occurrence and ecology of *U. agiliformis*, Spanish population (Table 2)

The Spanish population of *U. agiliformis* was found in association with other ciliates which typically inhabit RBC wastewater treatment such as: *Acineria incurvata, Amphileptus pleurosigma, Aspidisca cicada, A. lynceus, Carchesium polypinum, Cinetochillum margaritaceum, Chilodonella uncinata, Cyclidium glaucoma, Dexiostoma campylum, Dexiotricha* sp., *Euplotes* sp., *Halteria grandinella, Glaucoma scintillans, Opercularia coarctata, Paramecium aurelia, Paramecium caudatum, Trithigmostoma cucullulus, Trimyema compressum, Uronema nigricans, Vorticella microstoma, Vorticella striata* and *Zoothamnium procerius.*

The RBC system, from where this species was recovered, was clearly compartmentalised. This species was generally found on the latter stages of the biological treatment, where physico-chemical conditions in the

Table 1. Morphometric characterisation of *Urosomoida agiliformis* Spanish population

Character	x̄	M	SD	CV	Min	Max	n
Body, length	71.2	70	7.5	10.6	54	89	30
Body, width	21.5	21	2.7	12.9	17	26	30
Adoral zone of membranelles, length	21.7	22	1.6	7.4	19	24	30
Macronucleus segment, length	16.6	16	2.3	14.0	12	21	30
Macronucleus segment, width	6.8	7	1.4	21.3	5	9	30
Micronuclei, number	2.4	2	0.7	30.2	2	4	30
Adoral membranelles, number	24.9	25	2.7	10.9	20	31	30
Right marginal row, number of cirri	22.1	22	2.1	9.9	18	26	30
Left marginal row, number of cirri	20.8	20	2.2	11.0	17	25	30
Buccal cirri, number	1	1	0	0	1	1	30
Frontal cirri, number	3	3	0	0	3	3	30
Frontoventral cirri, number	4	4	0	0	4	4	30
Postoral ventral cirri, number	2.9	3	0.5	19.9	2	4	30
Pretransverse cirri, number	1.7	2	0.4	25.9	1	2	30
Transverse cirri, number	3.8	4	0.6	16.1	3	5	30
Caudal cirri, number	2	2	0	0	2	2	30
Dorsal kineties, number	4	4	0	0	4	4	30

Measurements in μm. x̄ - mean, M - median, SD - standard deviation, CV - coefficient of variation, Min - minimum, Max - maximum, n - sample size.
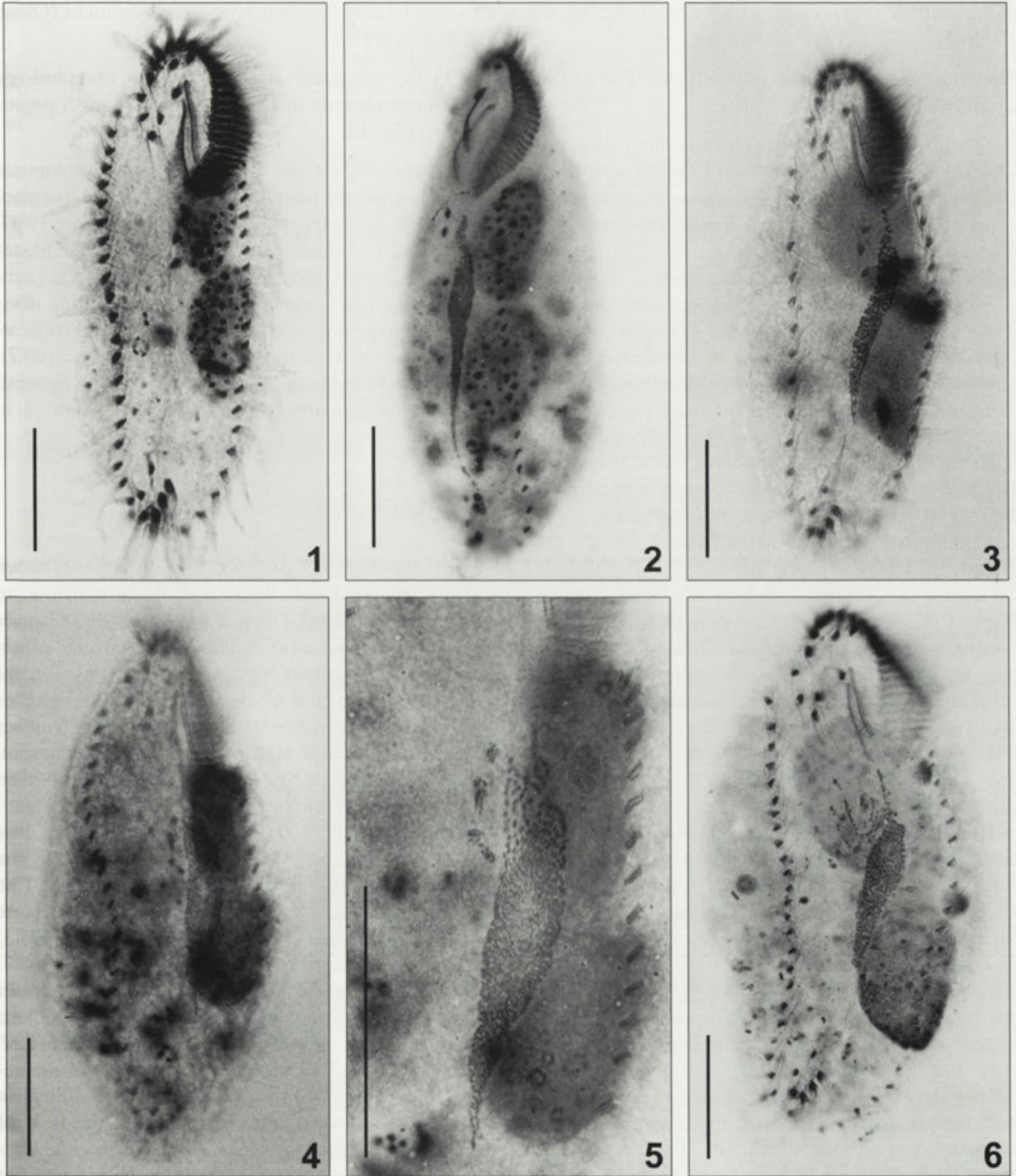
Fiigs. 1-6. Photomicrographs of protargol impregnations (Wilbert 1975) of interphase and dividing stages of *Urosomoida agiliformis* ventral view: **1** - oral and somatic infraciliature of specimens in interphase; **2-3** - early divider developing an oral primordium; **4** - early divider shows a developing oral primordium and disorganisation of two postventral cirri; **5** - detail of the disorganisation of postventral cirri (arrows); **6** -- middle divider showing the opisthe primary primordium. Scale bars - 20 μm

system had already improved compared to the influent (Table 2).

Morphogenesis of Spanish population of *Urosomoida agiliformis* (Figs. 2-12)

Stomatogenesis. The oral primordium originates apokinetally with the proliferation of 2 kinetosome fields; one field close to the left of the anterior postoral ventral cirrus and another above the leftmost transverse cirrus (Fig. 2). The basal bodies of these fields grow together generating a narrow anarchic field (Figs. 3, 4). Further kinetosomal proliferation and membranellar differentiation produce a new AZM for the opisthe (Figs. 6-8). The differentiation of membranelles proceeds towards the posterior area. Meanwhile, the primordium for the undulating membrane separates as a group of loosely arranged pairs of kinetosomes at the right hand side of the differentiating AZM (Figs. 6-7). The parental AZM is retained but the anterior portions of the paroral and endoral membranes reorganise.

Development of cirral primordia. Firstly, the postoral ventral cirri disorganise in the opisthe (Figs. 5-6). At the same time, 3 anlagen evolve from the oral primordium. The fronto-ventral-transverse primordia of the opisthe originate earlier than those of the proter (Fig. 6). Then, there is a disorganisation of the buccal cirrus and the posterior fronto-ventral cirrus in the proter, followed by a development *de novo* of 2 anlagen (Fig. 7). The undulating membranes also disorganise (Fig. 8). Finally, 6 anlagen develop for both proter and opisthe (Figs. 8, 9).

Development of the somatic primordia. Marginal primordia can be observed within the marginal rows in both proter and opisthe (Figs. 8-10). At the same time, 3 anlagen on the dorsal surface migrate along the parental dorsal kineties forwards and backwards (Fig. 9). The 4 dorsal kineties are formed from the right marginal primordia (Fig. 10). This type of dorsal ciliature ontogenesis has been described as type 2 (Foissner and Adam 1983) or *Urosomoida* pattern (Berger and Foissner 1997, Berger 1999).

New cirri differentiation. The frontal cirri originate from anlagen I, II and III while the fronto-ventral cirri originate from anlagen III and IV. The buccal cirrus develops from anlagen II, the postoral ventral cirri from anlagen IV and V and the protransverse ventral cirri from anlagen V and VI. Finally, the transverse cirri arise from anlagen II, IV, V, and VI (Figs. 10-12).

Nuclear apparatus. This population has 2 macro- and 2 micronuclear segments. The divisional pattern observed follows that shown by other hypotrichs (Olmo and Téllez 1997b)

Schematic computer drawing of the morphology and morphogenesis in *U. agiliformis* (Spanish population) (Fig. 13)

Recently, Eigner has used a schematic computer method to show the morphology and divisional morphogenesis of forty-five hypotrichous species (Eigner 1997, 1999). This schematic method with symbols facilitates the observation of interphase morphology and the complex evolution of morphogenetic processes during division supplying "a standardised account of known morphogenetic process in any given group" (Eigner 1997).

The morphology and morphogenesis of the Spanish population of *U. agiliformis* is shown in this fashion in Fig. 13.

DISCUSSION

Urosomoida agiliformis was initially isolated from soil samples of beech wood and alpine meadow in Austria (Foissner 1982, Foissner and Adam 1983, Ganner *et al.* 1986-87) and other studies have recovered afterwards this species from at least four continents (Berger 1999). Now it has been recovered from biofilm samples in a wastewater treatment plant in Spain with other species from a beta- to alphamesosaprobic environment. These results indicate that the cosmopolitan distribution of *U. agiliformis* cannot be doubted.

The population of *U. agiliformis* studied here has slight morphological differences when compared to the populations studied by Ganner *et al.* (1986-87). The Spanish population is biometrically more similar to those described by Foissner (1991), Foissner and Adam (1983) than to those described by Ganner *et al.* (1986-87; populations designed P3 and P4). However, the number of transverse and pretransverse cirri and the nuclear apparatus are similar to those described by Ganner *et al.* (1986-87; populations designed P3 and P4). These results show again the transverse cirri variability in *Urosomoida* and *Oxytricha* and therefore, the lack of reliability this taxonomic character has to separate species in the genus *Urosomoida*, as it has been found for other hypotrichous genus i.e. *Gonostomum* (Foissner 1982, Song 1990, Olmo and Téllez 1997b, Eigner 1999).

In this case, morphogenetic data could be invaluable to identify this species. The morphogenesis in *U. agiliformis* has been studied before in two popula-
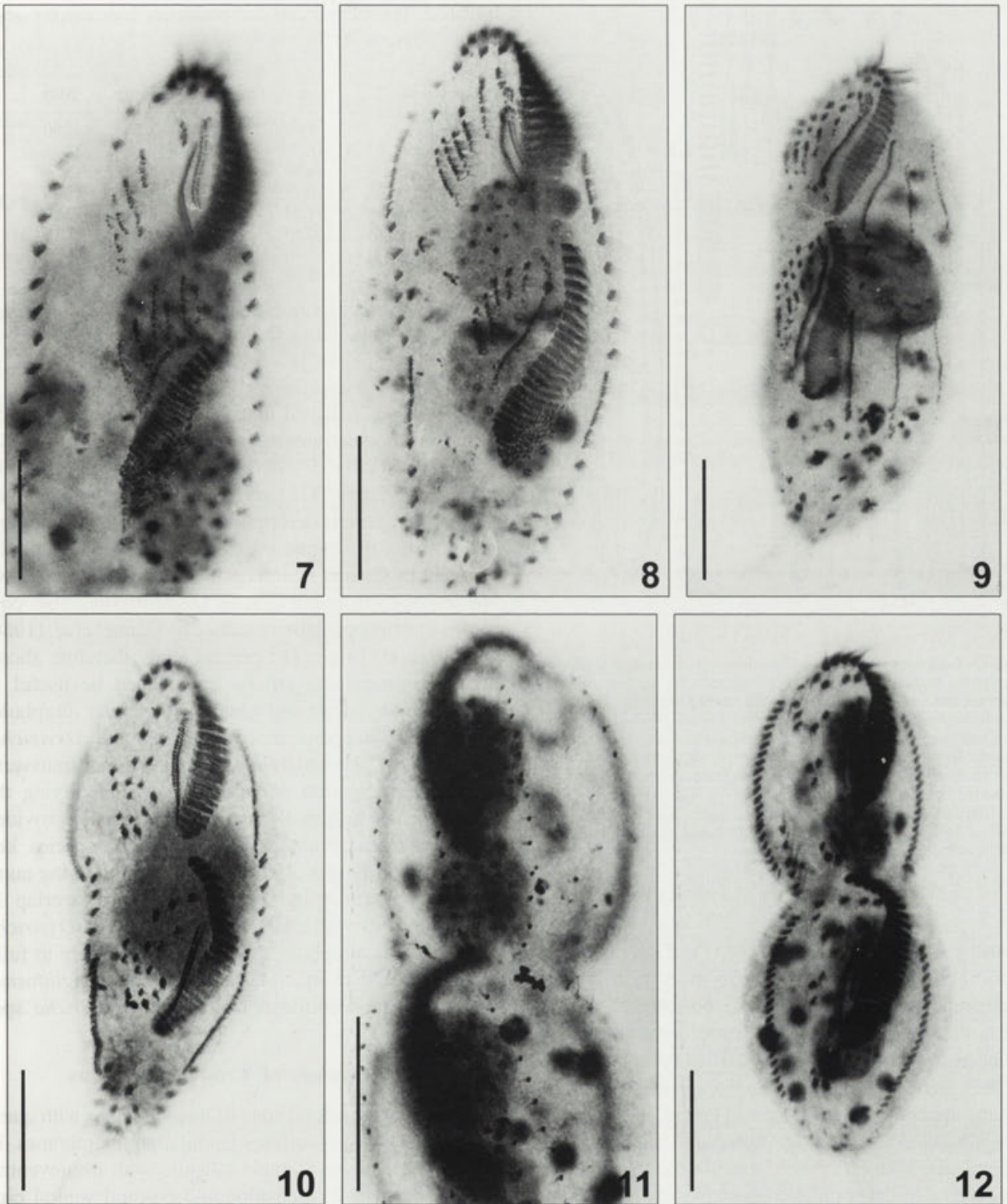
Figs. 7-12. Photomicrographs of protargol impregnation (Wilbert 1975) on dividing *Urosomoida agiliformis*: **7** - middle divider showing formation of anlagen in the proter and six anlagen in the opisthe (arrows show "*de novo*" primordia); **8** - middle divider showing developing primordia of the marginal rows (arrows) and 6 anlagen in both proter and opisthe; **9** - middle divider showing development of two dorsal primordial; **10** - late stage of morphogenesis showing migration of new cirri and formation of one dorsomarginal kinety (arrow); **11** - detail of dorsal infraciliature in a late stage of morphogenesis showing two caudal cirri (arrows) and four dorsal kineties; **12** - late stage of morphogenesis showing migration of new cirri and separation of cells. Scale bars - 20 μm

Table 2. Physical and chemical parameters from samples where *Urosomoida agiliformis* was recorded

Parameters	x	SD	Min	Max	n
ph	7.49	0.19	7.23	7.80	12
Temperature (°C)	16.76	4.04	10.80	22	12
BOD_5(mg/l)	16.69	11.28	5.25	43	11
NH_4^+-N(mg/l)	10.71	5.29	4.35	21.80	10
NO_3^--N (mg/l)	4.23	3.80	0.63	12.30	9
NO_2^--N (mg/l)	0.55	0.29	0.15	1.18	12
DO (mg/l)	3.20	1.07	0.55	4.53	12
PO_4^{-3} (mg/l)	3.90	0.92	2.11	5.67	12

x - mean, SD - standard deviation, Min - minimum, Max- maximum, n - sample size

Fig. 13. Computer sketch of the Spanish population of *U. agiliformis*. Interphase infraciliature and morphogenesis on the left side of the diagram and ventral cirral pattern of a stained specimen on the right. Symbols explained in detail elsewhere (Eigner 1997). Briefly, numbers and lines indicate cirral rows anlagen and are identical in both images. Boxes indicate anlagen and are positioned where the anlagen start to develop. The anterior boxes indicate anlagen for proter, the posterior boxes for opisthe (Eigner 1999). The boxes with number one indicate the neokinetal 1 (N1) anlagen development (Eigner 1997). L1 - left marginal row. RM - right marginal row. Number (1-7) and lines indicate anlagen and cirral rows

tions by Foissner and Adam (1983) and later by Ganner *et al*. (1986-87). Two main differences were found in the comparisons carried out by Ganner *et al*. (1986-87). First, the development of the frontal ventral transverse anlagen, in the populations studied by Foissner and Adam (1983), started a little earlier in the opisthe. While in the strains studied by Ganner *et al*. (1986-87), this process ran simultaneously in both proter and opisthe. Second, the anlagen V and VI extend towards the frontal field in the opisthe of the population described by Ganner *et al*. (1987), while this phenomenon does not occur in those populations described by Foissner and Adam (1983). The Spanish population studied here shows a divisional morphogenesis similar to the populations studied by Foissner and Adam (1983). Recently, Berger and Foissner

(1997) have indicated that, based on morphogenetical data, the populations studied by Ganner *et al*. (1987) were not really *U. agiliformis* but populations of *Oxytricha longa*. This can also be corroborated with the morphogenetic description in *O. longa* by Hemberger (1982) that fits the descriptions of the populations described by Ganner *et al*. (1986-87). The morphogenesis of the Spanish population of *U. agiliformis* also confirms that the populations studied by Ganner *et al*. (1986-87) were *O. longa*. The present study therefore shows that morphogenetic criteria can indeed be useful to differentiate species and genera with similar morphologies, as it happens in *Urosomoida* and *Oxytricha*. Furthermore, the variability in the number of transverse cirri in this Spanish population allows modifying the diagnosis of the genus *Urosomoida* originally provided by Berger and Foissner (1997) and the species key produced by Berger (1999). The variability in the number of transverse and pretransverse cirri overlap in *U. agiliformis* with characteristics of certain *Oxytricha* species, thus morphogenetic data are necessary to fully identify some populations of *Urosomoida agiliformis* and avoid misidentification with other *Oxytricha* species.

Improved diagnosis of *Urosomoida* genus

Ciliates with adoral zone of membranelles with question mark shape outline. Undulating membranes in *Oxytricha* pattern. Somatic ciliature with frontoventral cirri in V-shaped disposition and postoral ventral cirri form a dense cluster underneath buccal vortex. One or two pretransverse ventral cirri and four or less transverse cirri present. Two marginal cirri rows located in rigth and left side of cell respectively. Dorsal somatic ciliature has four dorsal kineties and caudal cirri. Rightmost

ventral cirral rows originate, in both proter and opisthe, by neokinetal 1 anlagen development. Dorsal morphogenesis in *Urosomoida* pattern.

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ACTA
PROTOZOOLOGICA

Morphological Observations on the Life Cycle of *Dermocystidium cyprini* Červinka and Lom, 1974, Parasitic in Carps (*Cyprinus carpio*)

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Summary. The genus *Dermocystidium* Pérez, 1908 has been assigned to the DRIPs clade near the animal-fungal dichotomy in 1996. The life cycle of *Dermocystidium cyprini* Červinka and Lom, 1974, the gill parasite of common carps, includes cysts with lipid-rich plasmodial stages, formation of sporonts by division of the plasmodia, and maturation of spores within the cysts. Motile zoospores appeared a few times in cultures of isolated cysts *in vitro*. Zoospores may develop from the spores or from sporoblasts without formation of the spore stage. Zoospores possibly escape from the cysts through pores in the cyst wall. The cyst wall comprises several strata. During plasmotomy the fibrous stratum of the wall is engulfed to form the walls of different compartments. Cells maturing into zoospores have a prominent inclusion, an eccentric nucleus, a few mitochondria and several membrane-surrounded bodies. The largest body has a quadruple membrane, and the smallest ones have ribosome-like inclusions. The flagellum of the zoospore has a striated rhizoplast penetrating to a ribosome-rich area in the zoospore cytoplasm. The functional kinetosome and rhizoplast are at an angle of 130 to 140° to each other, and a non-functional kinetosome seems to be at a right angle to the rhizoplast. Although the ciliary root with its associated structures of *Dermocystidium cyprini* bears some resemblance to that of some chytridiomycetes, the systematic position of the organism still remains unsettled.

Key words: *Cyprinus carpio*, *Dermocystidium*, development, parasite ultrastructure, zoospore.

Abbreviations: BB - basal body; CV - clear vacuole; D - desmosome; ED - electron dense stratum; ER - endoplasmic reticulum; F - flagellum; G - granular stratum; GF - granular-fibrous stratum; I - inclusion; LD - lipid droplet; MC - mitochondrion; MT - microtubules; MVB - multivesicular body; Nu - nucleus; PC - phagocyte; Pl - plasmodium; Pr - prop; PS - plasmodium shoot; R - ribosomes; TF - transitional fibres; TZ - transitional zone; V - villus, villi; W - cyst wall.

INTRODUCTION

Dermocystidium is a genus of unicellular parasites of fish, or in some cases of amphibians, with a taxo-nomic affinity that awaits complete clarification. Some researchers have assigned them to protists (Garkavi *et al.* 1980, Nash *et al.* 1989), others to lower fungi (Pauley 1967, Allen *et al.* 1968). A recent study allocates the taxon to a novel clade near the animal-fungal divergence (Ragan *et al.* 1996).

A diagnostic feature of the genus is the existence of spherical spores with a refractile body as a stage of the developmental cycle. Data on the life cycle and

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ultramicromorphology are relatively scarce. New data are hard to gain since it has not been possible to observe *Dermocystidium* species in the laboratory throughout the life cycle.

The genus *Dermocystidium* is known to include about twenty species. Lom and Dyková (1992) distinguished three groups of species within *Dermocystidium*: (1) species which form spherical spores with a solid central refractile body in spherical or oval cysts on gills or skin of the hosts (examples: *D. branchialis* Léger, 1914; *D. percae* Reichenbach-Klinke 1950; *D. cyprini* Červinka and Lom, 1974; *D. anguillae* Spangenberg, 1975), (2) species which develop in subdermal tissue form spores that vary greatly in size and have coenocytic, ramified hyphae with thick homogeneous walls (example: *D. koi* Hoshina and Sahara, 1950), (3) partly or completely visceral species which form spores with a large central vacuole instead of a solid body (example: *Dermocystidium* sp. McVicar and Wootten, 1980).

Dermocystidium cyprini is a gill parasite that causes remarkable mortality of fingerlings of common carp (*Cyprinus carpio* L.). Several stages of development inside the cysts attached to the carp gill were mentioned by the authors of the original description (Červinka *et al.* 1974). Developmental stages most commonly seen are: multinuclear plasmodium, plasmodium segmented by septa, sporonts, sporoblasts and ripe spores. It must be noted that this remarkably thorough study of the species was carried out using only light microscopy.

We have determinated carp dermocystidiosis in two Estonian fish farms since 1982 (Kasesalu and Lotman 1995 a,b,c). The parasites have been confirmed as *Dermocystidium cyprini* by J. Lom (personal communication), one of the authors of the original description. Because little is known about the biology, especially the ultrastructure and the developmental cycle of *D. cyprini*, material from these farms was used to obtain more data. The methods used, in addition to light microscopy, were transmission and scanning electron microscopy (TEM and SEM). It has been possible to raise *D. cyprini* in vitro through sporogenesis (Lotman and Kasesalu 1997 a). As the final stage of development of the parasite we have revealed uniflagellate motile zoospores (Lotman and Kasesalu 1997 b). This is interesting because Olson *et al.* (1991) have described a uniflagellate life stage as a transmission agent of *Dermocystidium salmonis* - a species with several remarkable similarities to *D. cyprini*.

Here we present new insights into the developmental cycle of *Dermocystidium cyprini*.

MATERIALS AND METHODS

The main body of data is from material collected in February, March, April and May 1996 and 1997, with some additional material from 1994 and 1995. Three developmental stages of cysts of *D. cyprini* were obtained from the gills of common carp (*Cyprinus carpio*) fingerlings: plasmodia (n = 31, removed in February), dividing plasmodia (n = 35), and early sporogonic stages (n = 40), (removed in April).

Cysts were removed from the gills, examined under a light microscope, washed several times in fresh water, and incubated in Petri dishes in fresh water at 4°C without antibiotics (Olson *et al.* 1991).

Cysts removed from the gills of carp fingerlings as well as mature cysts from the cultures described above were examined using light microscopy, TEM and SEM. For light microscopy, smears of cysts were air-dried, fixed in methanol, and stained with May-Grünwald and Giemsa. Lipids were demonstrated in formalin-fixed smears with Fettrot. The pathological altered carp gill tissue was fixed with 5% formalin or Bouin's fixative and embedded in paraffin wax; sections were stained with haematoxylin and eosin, or treated with PAS reaction, and Grocott's impregnation methods.

Prior to electron microscopy, the cysts or the cultured organisms (after centrifugation) were fixed in 2.5 or 3% glutaraldehyde in 0.1M phosphate or cacodylate buffer (pH 7.2 or 7.4) at 4°C for 24 h. The samples were post-fixed in 2% OsO_4 (in the same buffer) for 1 h. For TEM, the fixed material was dehydrated and embedded in EPON. The ultrathin sections were stained with uranyl acetate and lead citrate prior to examination with JEOL JEM 1200EX in the Electron Microscopy Unit of the Department of Biotechnology, University of Helsinki. For SEM, the dried samples were attached on stubs sputter and coated with gold. They were examined with a TESLA BS-301 scanning electron microscope in Tartu University.

RESULTS

We have found cysts of *Dermocystidium cyprini* (Fig. 1) on the gills of fingerlings and only rarely on the gills of two-year-old common carps from January till May. The following stages of the parasite can be distinguished within cysts:

(1) plasmodium with many nuclei and characteristic patterns of chromatin, as well as large vacuoles that appear ash grey in EM figures;

(2) sporonts as a result of plasmodium division, still containing several nuclei;

(3) uninuclear cells which may develop into spores or zoospores;

(4) spores.

Flagellated zoospores were observed only as the very last stage of development, in culture, in May or early June. Cultured zoospores which had left the cyst were observed only once. During the movement of the zoospores the flagellum is located at the posterior end.
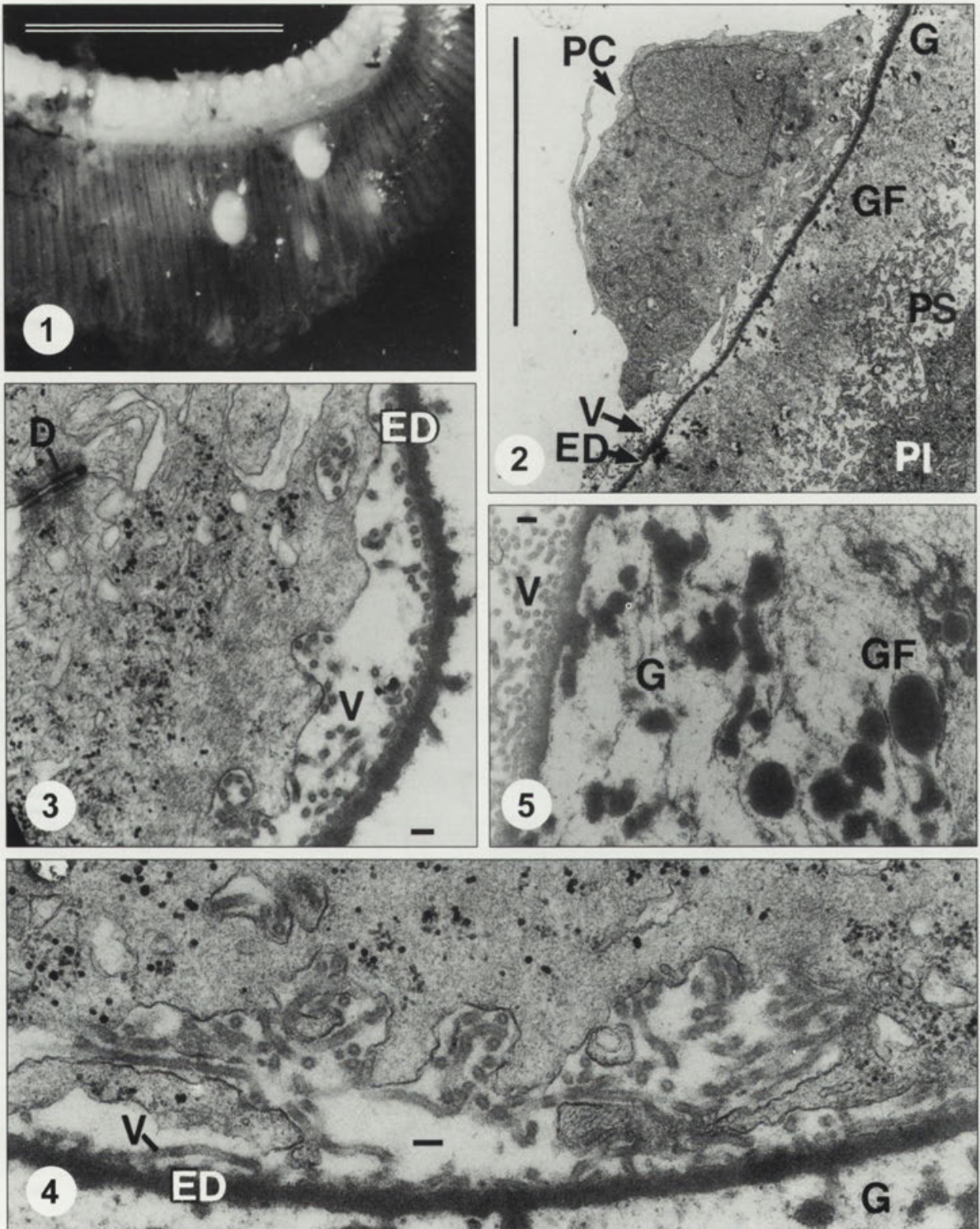
Figs. 1-5. *Dermocystidium cyprini*: **1** - cysts on the gill of a fingerling *Cyprinus carpio*; **2** - cyst wall at plasmodial stage and host phagocyte; **3** - electron dense layer with villi penetrating to host epithelial cells; **4** - villi penetrating to host epithelium, higher magnification; **5** - granular and granular-fibrous layer. D - desmosome, ED - electron dense layer, G - granular layer, GF - granular-fibrous layer, PC - phagocyte, Pl - plasmodium, PS - plasmodium shoots, V – villi. Scale bars - **1** - 10 mm; **2** - 10 μm; **3-5** - 100 nm
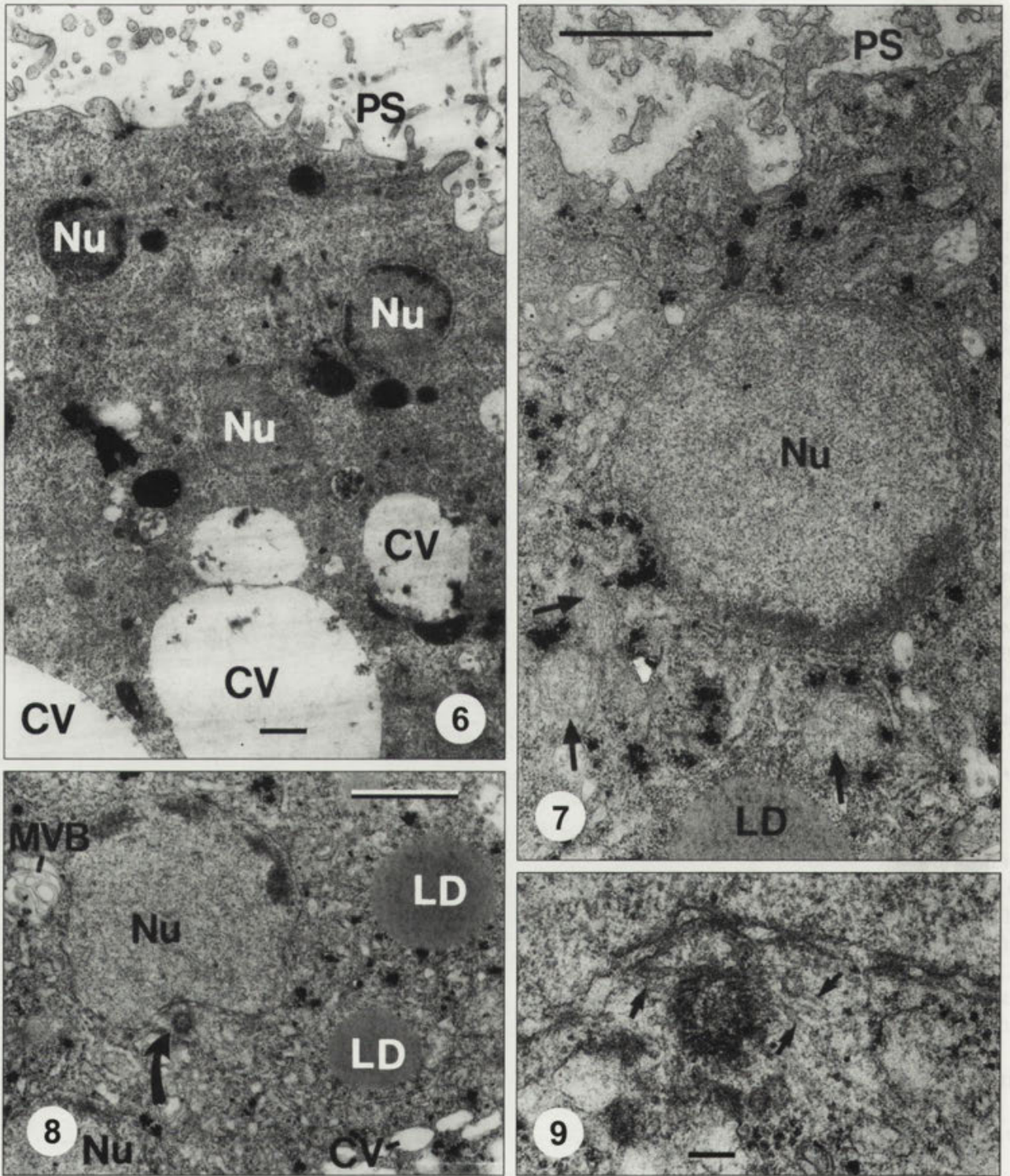
Figs. 6-9. *Dermocystidium cyprini*: **6** - margin of young plasmodium with plasmodium shoots, nuclei and large clear vacuoles; **7** - part of young plasmodium showing concentration of chromatin on one side of nucleus, a few inconspicuous mitochondria (arrows) and a lipid droplet; **8** - centriole (arrow) near a nucleus, a multivesicular body, lipid droplets and small clear vacuoles; **9** - the centriole from Fig. 8 and associated microtubules (arrowheads). CV - clear vacuole, LD - lipid droplet, MVB - multivesicular body, Nu - nucleus, PS - plasmodium shoots. Scale bars - **6-8** - 1 μm; **9** - 100 nm
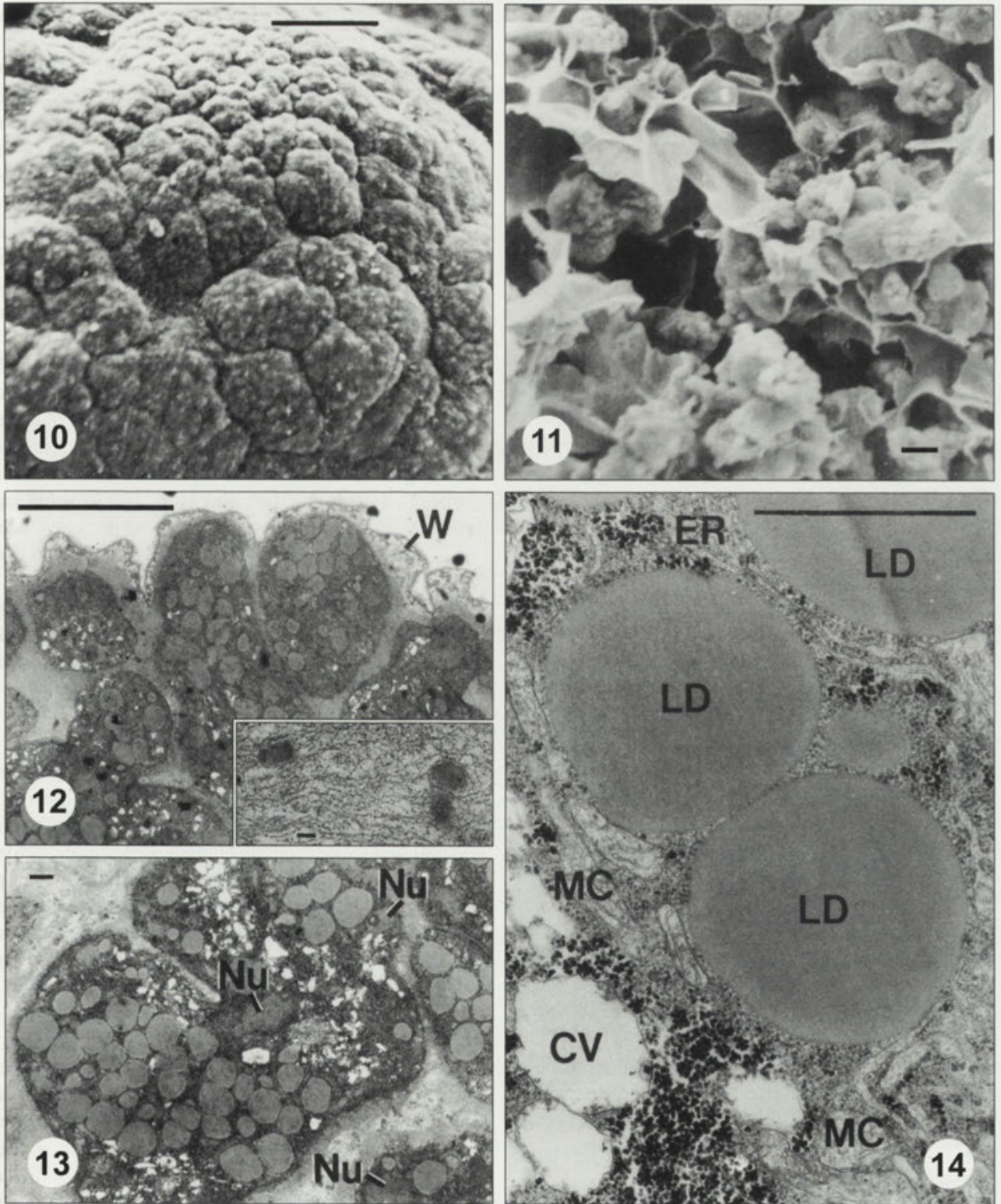
Figs. 10-14. *Dermocystidium cyprini*: **10** - engulfment of cyst surface during fragmentation of plasmodium (SEM); **11** - walls of chambers viewed with SEM; **12** - margin of cyst with dividing plasmodia in chambers bordered by engulfment of the granular-fibrous stratum of the cyst wall; insert - granular-fibrous chamber wall; **13** - division of a plasmodium; **14** - complex of lipid droplets, mitochondria and endoplasmic reticulum; glycogen rosettes and clear vacuoles also visible. CV - clear vacuole, ER - endoplasmic reticulum, MC - mitochondrion, Nu - nucleus, W - cyst wall. Scale bars - **10, 12** - 10 μm; **11,13,14** - 1 μm; **12** insert - 100 nm

Description of the stages

Cysts containing plasmodia

Cysts containing plasmodia were approximately 20 to 500 μm in diameter and, when viewed under a binocular microscope, seemed to contain a finely granulated mass.

Viewed with TEM (Fig. 2), the cyst wall at this stage was fairly thick, and several strata could be distinguished (Figs. 2-5):

(1) Fine tubular villi that were penetrating the gill tissue. The villi could be seen intermingled between the pseudopods of phagocytic cells (Fig. 2) or the edges of epithelial or pillar cells (Figs. 3, 4). The stratum was about 1.2 μm in thickness, but varied at different sites. The longest villus measured was 1 μm. The diameter of the villi was about 40 nm. Cells affected by the villi showed clear signs of degradation: shed membranes and empty cytoplasm.

(2) Thin electron-dense homogeneous layer (Figs. 3-5).

(3) Stratum containing electron-opaque round or elongated granules 100 to 200 nm in diameter and a sparse fibrous substance (Fig. 5). The thickness of the stratum was 1 μm on average. Some plasmodium shoots, always bordered by a membrane, could be found in this stratum.

(4) Granulated, fibrous stratum, 2 μm thick on average. This layer, especially its innermost part, contained many more plasmodium shoots than the previous stratum (Fig. 2).

In the plasmodia of this stage, TEM revealed numerous nuclei, large clear vacuoles and small multivesicular structures, as well as electron-opaque bodies, lipid inclusions and ribosomes (Figs. 6-8). Glycogen and endoplasmic reticulum were sparse. Mitochondria were not clear (Fig. 7), and sometimes they seemed to be degradating. The diameter of nuclei was about 2.2 to 2.5 μm (larger in younger plasmodia), and their chromatin mass showed a marked concentration towards the sides or one side of the nucleus. Sometimes centrioles and microtubules were seen near nuclei, apparently as a sign of nuclear division (Figs. 8, 9). The clear vacuoles became smaller in older plasmodia (cf. Figs. 6, 8).

Development of sporonts

Further development was accompanied by the growth of the cyst and fragmentation of plasmodia into "chambers" of sporogenesis.

During division of the plasmodia, fissures could be seen on the surface of the cyst as a result of partial engulfment of the cyst wall (Fig. 10). The granulated fibrous stratum of the cyst wall is engulfed between the dividing plasmodia, and it forms the walls of the chambers (Figs. 11, 12). In every chamber the multinuclear plasmodia continued to fragment into smaller plasmodia until formation of sporonts (Figs. 12, 13). The process was asynchronous and asymmetric. The division went on simultaneously in the whole plasmodium in 30% of the observations (n=58), and in 70% of the cases it started first in one part of the plasmodium. The granulated fibrous layer which formed the chamber walls was strongly PAS- and Grocott-positive, and colourless in vivo.

As a result of the formation of sporonts, the number of nuclei decreased and the number of mitochondria and ribosomes as well as the volume of glycogen and endoplasmic reticulum increased. In some cases lipid inclusions, mitochondria and endoplasmic reticulum formed a closely tied complex (Fig. 14). In some cases smaller lipid droplets fused to form bigger ones. Membranous condensation and shedding of membranes could be seen on the surface of forming sporonts.

The sporont was ultimately 4 to 6 μm in diameter, had only one nucleus and a great number of lipid droplets (0.5 to 1.5 μm) as well as several empty vacuoles (0.5 to 1 μm).

Sporogony, spores and zoospores

Sporogony means division of sporonts with several nuclei into smaller unicellular forms, accompanied by fusion of lipid inclusions and disappearance of the clear vacuoles. Mature spores contain one large, natively refractile and in EM osmiophilic inclusion and a peripheral nucleus. In cultures, zoospores possibly developed from sporoblasts without spore formation. The groups of cells determined to transform into zoospores were separated from other groups by fibrous matter (insert in Fig.12). The cells had a prominent inclusion and an eccentric nucleus but, except for a thin surface coat, no walls or coverings typical of spores of other species (cf. spores of *Dermocystidium percae*: Reichenbach-Klinke 1950). Close to the nucleus there were membrane-surrounded bodies of various consistency (Figs. 15, 16). In later developmental stages these bodies are located close to the root of the flagellum. Some of the bodies are about 0.6 μm in diameter, contain finely granulated matter, and are surrounded at least on one side by a quadruple membrane, while others are only 0.1 to 0.2 μm in diameter and are surrounded by one membrane. The bodies are usually seen in groups of 2
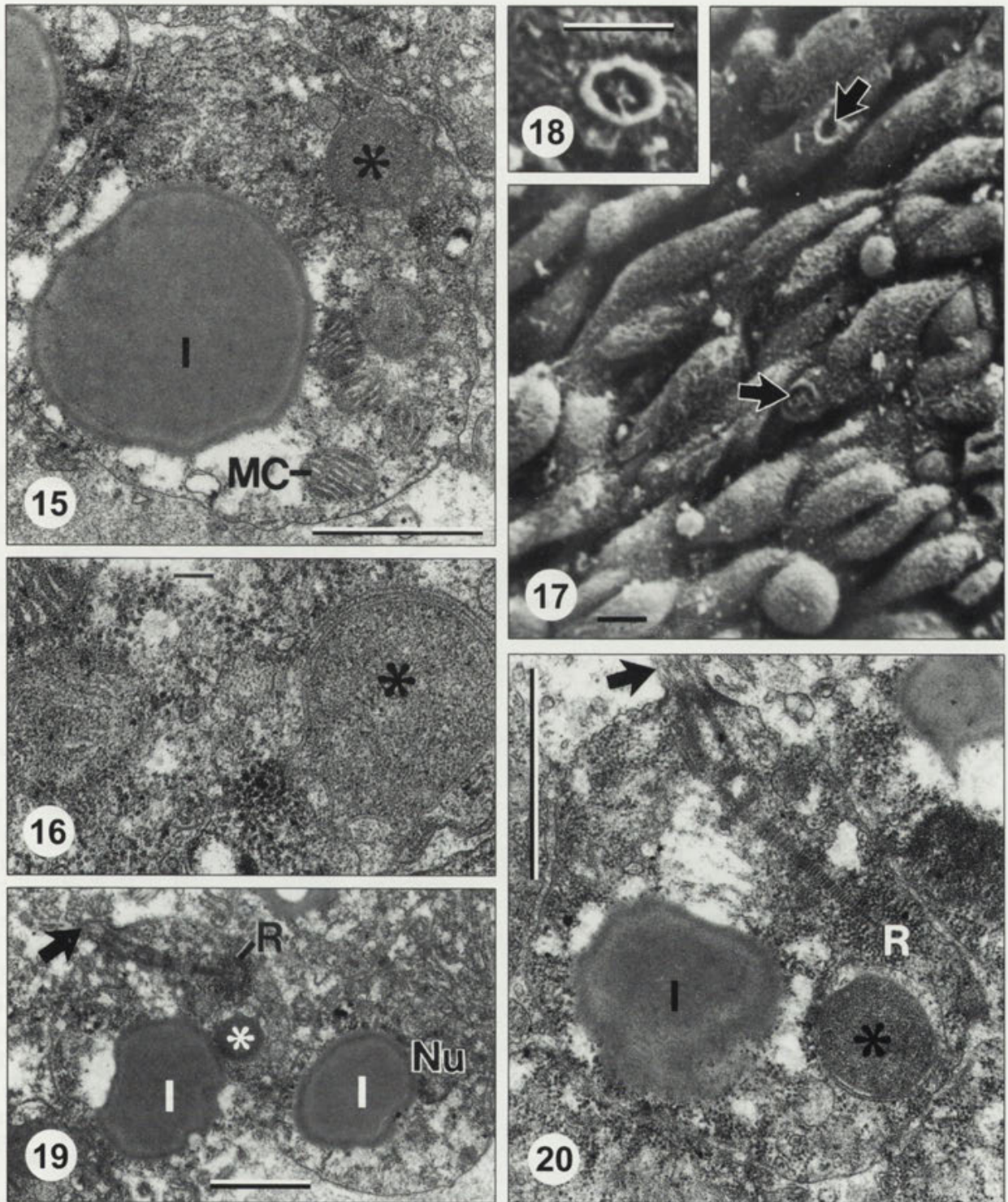
Figs. 15-20. *Dermocystidium cyprini*: **15** - cell determined to develop into a zoospore, with a prominent inclusion, a few mitochondria and membrane-surrounded bodies, the largest of which (asterisk) has a quadruple membrane; **16** - greater magnification from part of the cell in Fig. 15; **17** - cyst wall with pores (arrows) at the time of maturation of zoospores, SEM; **18** - a pore with greater magnification; **19** - two zoospores, one showing the origin of the flagellar root in a ribosome-rich area, the flagellum proper (arrow) and the body with quadruple membrane (asterisk), the other showing the nucleus beside the inclusion; **20** - a zoospore at greater magnification. I - inclusion, MC - mitochondrion, Nu - nucleus R - ribosomes. Scale bars - **15**, **17-20** - 1 μm; **16** - 100 nm
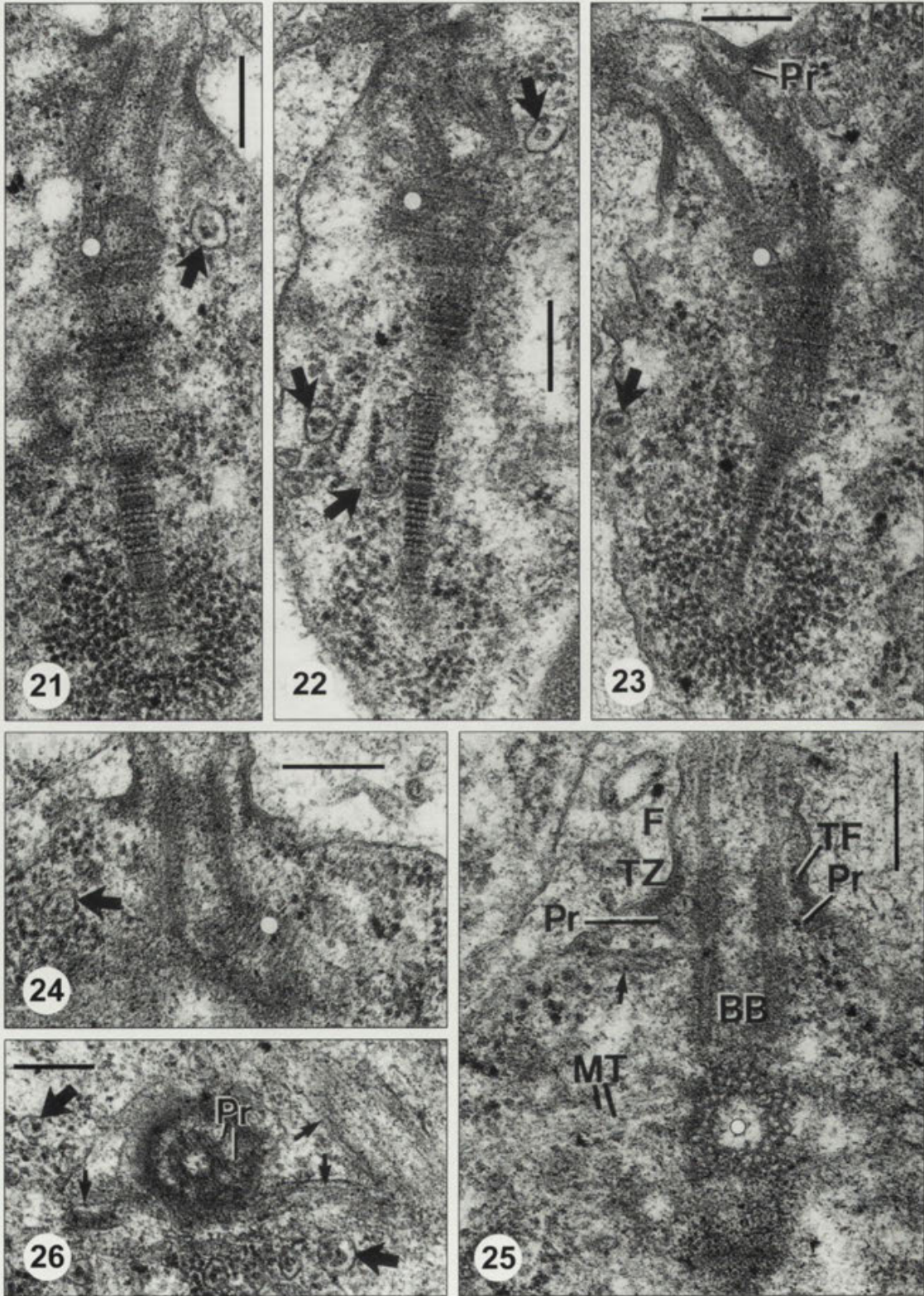
Figs. 21-25. *Dermocystidium cyprini*: **21-25** - longitudinal sections of flagellar basal bodies and roots with associated structures in zoospore cytoplasm, nonfunctional centrioles (vestigial kinetosomes) marked with white dots, small membrane-surrounded bodies with ribosome-like inclusions marked with bold arrows and fibres marked with thin arrow; **26** - basal body (functional kinetosome) with props in cross section, and associated fibres or microtubules in cytoplasm marked with thin arrows. BB - basal body, F - flagellum, Pr - kinetosomal props, TF - transitional fibres, TZ - transitional zone. Scale bars - 200 nm

to 4, some are irregular in shape and contain very electron-opaque fibrous matter. Four to five mitochondria are also seen close to the nucleus.

By the time the zoospores ripen, the cyst wall becomes very thin, and the cells can be distinguished through the wall with SEM (Fig. 17). At this time pores can be seen in the cyst wall (Figs. 17, 18).

The zoospore is characterized by a flagellum (Figs. 19, 20). The length of the flagellum is at least 8 μm and the diameter is 200 nm. The flagellum has a fibrous, striated rhizoplast that unites the functional and non-functional kinetosome (centriole). The rhizoplast apparently grows towards the bodies described above, and it is surrounded by ribosomes.

The functional kinetosome (basal body) and rhizoplast seem to be oriented at an angle of 130 to 140° in relation to each other (Figs. 20-24). The non-functional centriole may be at a right angle to the rhizoplast (Figs. 21-25). There may be transitional fibres and kinetosome props connecting the transitional zone to the flagellar membrane and basal body to the cell membrane. Additional microtubules or fibres are in close association with the kinetosome and non-functional centriole (Figs. 20, 25, 26).

DISCUSSION

The description of the development of *Dermocystidium cyprini* presented above is principally similar to the original description by Červinka *et al.* (1974). Using additional methods, including electron microscopy, new details and stages that are lacking in the original description have been observed and described.

The most important of the new observations is the stage of flagellated zoospore. The flagellum and associated structures have been described. It, however, remained unclear whether the zoospores developed from spores or sporoblasts.

EM has also provided a more thorough description of the ultrastructure of the cyst wall. The layers of the latter, as well as the dynamics of its development, have been described. Differentiations of the role of the layers of the cyst wall and development of pores in the wall have been described. A hypothesis that the zoospores leave the cyst through these pores awaits further study.

These new insights into the ultrastructure and development of *Dermocystidium cyprini* need to be discussed in the light of some new findings concerning the

molecular biology of the genus *Dermocystidium* and their implications for the evolutionary affinities of the taxon. As stated above, the genus has a long and uncertain systematic and evolutionary status. The new findings by Ragan *et al.* (1996), based on statistical analyses of DNA primary structure, suggest that there is an "as-yet-unnamed clade" of eukaryotic protists, containing several parasites of aquatic animals. The clade includes *Dermocystidium*, a 'rosette-agent' of salmonids, a fish pathogen *Ichthyophonus* and a crayfish parasite *Psorospermium*. Ragan *et al* (1996) have assigned it the provisional name 'DRIPs clade'. The analyses demonstrated the clade to diverge near the animal-fungal dichotomy. Probably the clade is the most basic branch of Metazoa, but it is also possible that it diverged immediately prior to the animal-fungal dichotomy.

Flagellated forms exist both among choanoflagellates and chytrids, which are early diverged branches among animals and fungi, respectively. However, existence of a flagellum alone does not convey much information about phylogenetic affinities since it can be characteristic of very distant taxa. It should, however, be noticed that *D. cyprini* and flagellated chytrids and choanoflagellates all have just one, posteriorly located flagellum. It is interesting to note that the zoospores of *D. cyprini* have a fibrous, striated root - a rhizoplast - that has, to the authors' knowledge, thus far been described only among some chytrid species (Lange and Olson 1979; Barr 1980, 1992). Flagella or cilia of metazoan animals also often have striated roots (Lentz 1971, Pekkarinen, unpublished notes).

Flagellated zoospores of similar appearance have previously been described once among *Dermocystidium* species, namely in *D. salmonis* (Olson *et al.* 1991). Moreover, *D. cyprini* and *D. salmonis* are also similar in cyst shape (both are oval), and their spores have a large, solid inclusion. Both species thus belong to the first group of *Dermocystidium* species categorized by Lom and Dyková (1992). Thus far no rhizoplast has been described in the zoospores of *D. salmonis*. Pekkarinen has also seen flagellated zoospores of *Dermocystidium percae* (unpublished results), and possibly the uninuclear forms developed from the spores of *D. granulosum* described by Sterba and Naumann (1970) have been zoospores.

It should be noted that Perkins (1976) described a flagellated stage of *Dermocystidium marinum*. However, the presence of the apical complex in this species led Levine (1978) to include this species in a new phylum Apicomplexa under the generic name *Perkinsus*

(*P. marinus*). The analysis by Ragan *et al.* (1996) referred to above led to placement of *Perkinsus* and other Apicomplexa very far below *Dermocystidium*.

The presence of flagellated zoospores in some *Dermocystidium* species, combined with the data on their evolutionary relationships, can lead to new insights into the early evolution of animals (Lotman 1998). However, more research on both molecular genetics and developmental cycles of *Dermocystidium* species and related organisms is needed.

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ACTA
PROTOZOOLOGICA

Surface Kinetosomes and Disconnected Nuclei of a Calonymphid: Ultrastructure and Evolutionary Significance of *Snyderella tabogae*

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Summary. The karyomastigont cytoskeletal pattern in which a nucleus is connected to motility organelles [1-5 kinetosomes and associated axonemes, i.e., undulipodia (= eukaryotic flagella*), and axostyles] occurs in all early-branching lineages of eukaryotes (mastigamebids, diplomonads, oxymonads, retortamonads and trichomonads). Dispersed throughout the cytoplasm, the 50 or more nuclei of *Snyderella* (Calonymphidae: Trichomonadida) are not attached to the mastigont organelles. Except at the extreme posterior undulipodia cover the surface of the cell. All of the hundreds of undulipodia are arranged in groups of four typical of trichomonad mastigonts, but, since they lack nuclei, are akaryomastigonts. Each four-kinetosome akaryomastigont is connected to a set of axostylar microtubules, which extends toward the cell posterior. The axostyles, that together form a central bundle, like the shaft of an umbrella, position all the akaryomastigonts at the cell periphery. When groups of 20-50 motile akaryomastigonts become independently organized, two, three or more competing anterior conical regions appear. Karyokineses of the multiple nuclei in *Snyderella* are synchronized as in other calonymphids. We suggest that the ancestral character of eukaryotic cell organization is the karyomastigont, i.e., a stable nucleus-kinetid connection. *Snyderella* evolved from more basal calonymphids by severance of the nuclear connection, which led to the lack of karyomastigonts. The akaryomastigonts were retained and reproduced with each cell division.

Key words: akaryomastigont, amitochondriate, archaeprotista, centriole, *Cryptotermes cavifrons,* early mitosis, karyomastigont, kinetosome, symbiosis, termite, trichomonad.

INTRODUCTION

Early diverging lineages of protists determined by 16S rRNA analysis (Sogin 1989, 1991), bear distinctive nuclear-cytoskeletal morphology confirmed in electron micrographs (Brugerolle 1991). The highly conserved complex organelle system, the karyomastigont, consists of a nucleus, microtubule-based motility organelles and other structural components that include proteinaceous fibers and filaments. In trichomonads, when cell division occurs, not only are nuclei reproduced, but the entire complex, the karyomastigont reproduces as well. In most lineages, at karyokinesis the kinetosomes segregate in a semi-conservative fashion near the nucleus. The parent kinetosomes, usually two to six per karyomastigont, split into two groups. The nuclear envelope remains intact in the division process (closed mitoses). The new kinetosomes develop at the poles of a thin

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* We use the term undulipodium rather than flagellum to signify that the structure is different from the bacterial flagellum and that it is the homologous structure known as flagellum, cilium, and sperm tail (Margulis *et al.* 1990).

extranuclear spindle (the paradesmose). As the cell cycle proceeds new kinetosomes form such that half are retained from the previous cell cycle and half are generated in the current cycle in each offspring cell (Honigberg and Brugerolle 1990).

The class Parabasalia includes the order Trichomonadida (monocercomonads, trichomonads, devescovinids, calonymphids) in the phylum Archaeprotista (Margulis and Schwartz 1997). A Golgi complex is associated with the karyomastigont in members of this order. The stacked Golgi cisternae are conspicuously organized around a parabasal fiber. This fiber and the membrane complex comprise "the parabasal body", which confers the taxon name on these amitochondriate protists. The cell anatomy of hypermastigotes, the second order of Parabasalia, is differently organized: the one large central nucleus is dissociated from the many undulipodia such that the karyomastigont concept does not apply.

All five genera of multinucleate trichomonads in the family Calonymphidae are symbionts in the hindgut of about 50 species of kalotermitid termites (Yamin 1979). An evolutionary trend from the basal condition of a single karyomastigont is inferred (Kirby 1949). Cells of three genera contain only karyomastigonts (*Coronympha, Metacoronympha* and *Stephanonympha*). The fourth genus, *Calonympha*, we assess, is more derived. Both karyomastigonts and akaryomastigonts co-exist in the same cell. Akaryomastigonts are the extremely similar four-kinetosome, axostylar and Golgi cytoskeletal units that lack nuclei (Kirby and Margulis 1994). The cells of the most derived genus, *Snyderella*, contain only akaryomastigonts. The nuclei, not organized as karyomastigonts, are suspended freely in the cytoplasm. *Snyderella* is one of the few archaeprotist cells in which the nuclei are not attached to kinetosomes in a karyomastigont-based pattern. This report of the ultrastructure, karyokinesis and cytokinesis of *Snyderella tabogae* from *Cryptotermes cavifrons* extends the light microscopic classical study of Kirby (1929).

MATERIALS AND METHODS

A colony of *Cryptotermes cavifrons* from southern Florida, sent to us by Mark Deyrup, was reared in the laboratory for three years in the southern ground pine, *Pinus pinus*, logs in which it was collected. Termites taken from the wood were grown on cellulose filter paper in Petri dishes. Distilled water was added dropwise to the dishes every two days. The termite guts were punctured in Trager's solution (1934), and the live microorganisms were pipetted onto a slide and covered with a vaseline rimmed coverslip. They remained motile for at least one day.

For fluorescent staining, cells were fixed in 1.0% glutaraldehyde in phosphate buffered saline (PBS). The sample after centrifugation for one minute (9 g) was washed in PBS and stained (30 min) in 1.0 μM SYTOX (Molecular Probes, Eugene, OR).

Hindgut contents were fixed in 0.5% glutaraldehyde in PBS (1 h, 22°C) and stored at 4°C for electron microscopic preparations. They were post-fixed (1 h, 1% OsO_4), washed twice (15 min) in phosphate buffer solution, and dehydrated in an alcohol series (50%, 70%, 80%, 90%, 95% and 100% (3 x, 15 min each). The sample was then immersed in propylene oxide (3 changes, 15 min each) and left overnight in a mix of 1:1 propylene oxide-Spurr to harden at 60°C. The blocks were mounted and sectioned with a glass knife on an MT 2B Porter Blum ultramicrotome. Sections collected on 200-mesh copper grids were stained 5 min in uranyl acetate followed by 5 min in lead citrate and viewed in a Philips electron microscope 410 at 60 kV.

RESULTS

Our description supports Kirby's (1929) establishment of the genus and species *Snyderella tabogae* and extends the host range of the protist to include *Cryptotermes cavifrons*. Typical *S. tabogae* pyriform mastigotes measure 170 (139-195) μm long and 130 (119-159) μm wide (n=20). The transparent *Snyderella* cells, with their tapered anteriors and rounded posteriors, are supported by axostyles, which extend posteriorly from each akaryomastigont to form a central bundle (Fig. 1). Ovoid forms are artifacts of fixation, absent in live material. The akaryomastigonts are at the cell surface, and extend most of the length of the cell (Fig. 2). The posterior lacks akaryomastigonts. Thirty to 80 nuclei are scattered in the cytoplasm except for the anterior region (Fig. 3).

The assemblage of surface akaryomastigonts can be identified at the ultrastructural level (Fig. 4). Each akaryomastigont, including its microtubular axostyle-pelta complex, is composed of 4 kinetosomes, associated fibers, and a Golgi complex (parabasal body). The stack of 15-25 membranes per Golgi complex (about 0.5 μm in thickness) is situated laterally and beneath the kinetosomes. The Golgi cisternae, each about 22±2 nm thick, tend to be concave and smaller at their contact site with the parabasal fiber. They are flat or slightly convex and larger on the distal side. Each akaryomastigont is associated with a single parabasal body connected to the kinetosomes by two parabasal fibers (Figs. 5, 7).

The pelta microtubules surround the akaryomastigont but exclude the Golgi complex and hydrogenosomes
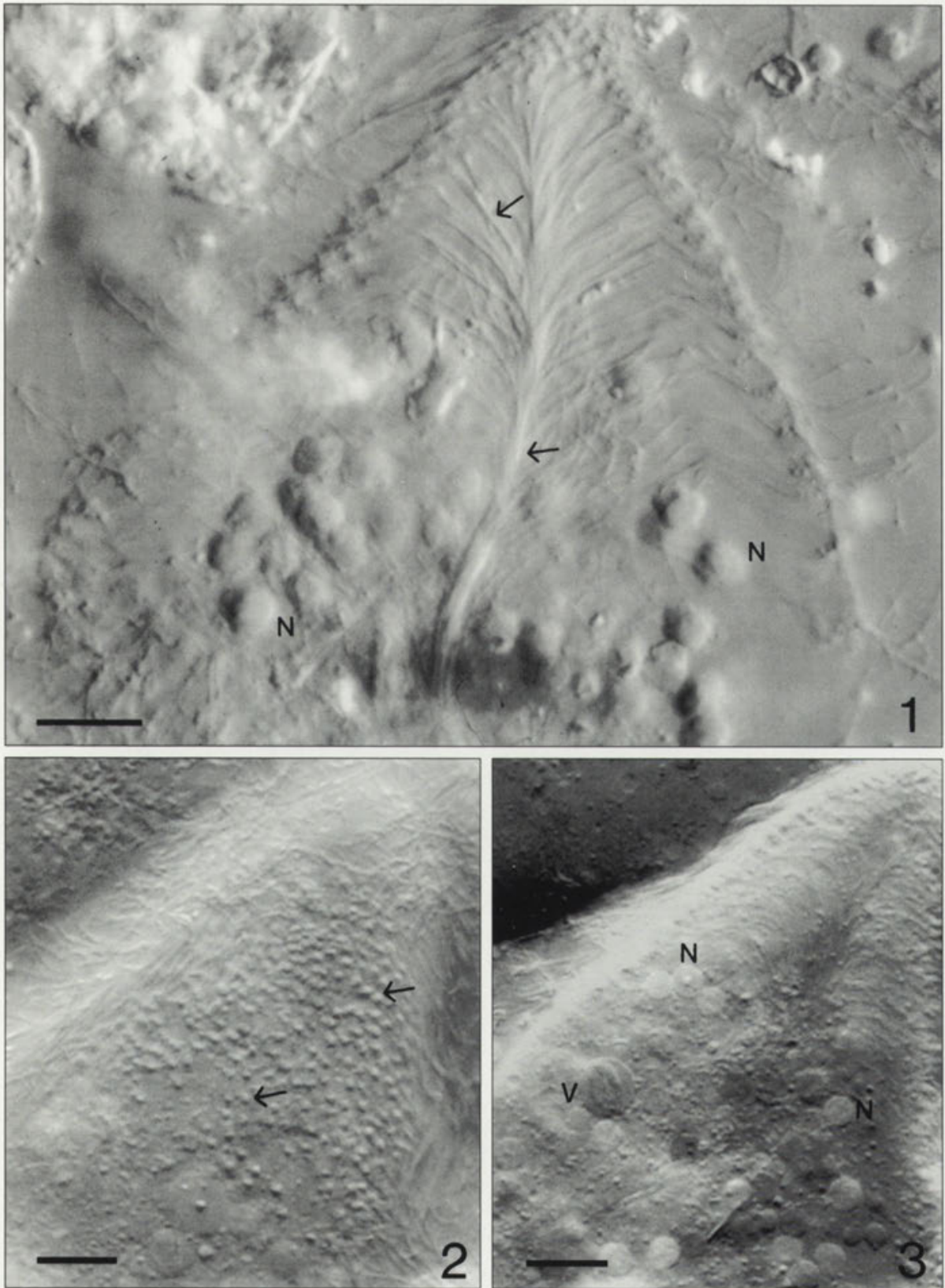
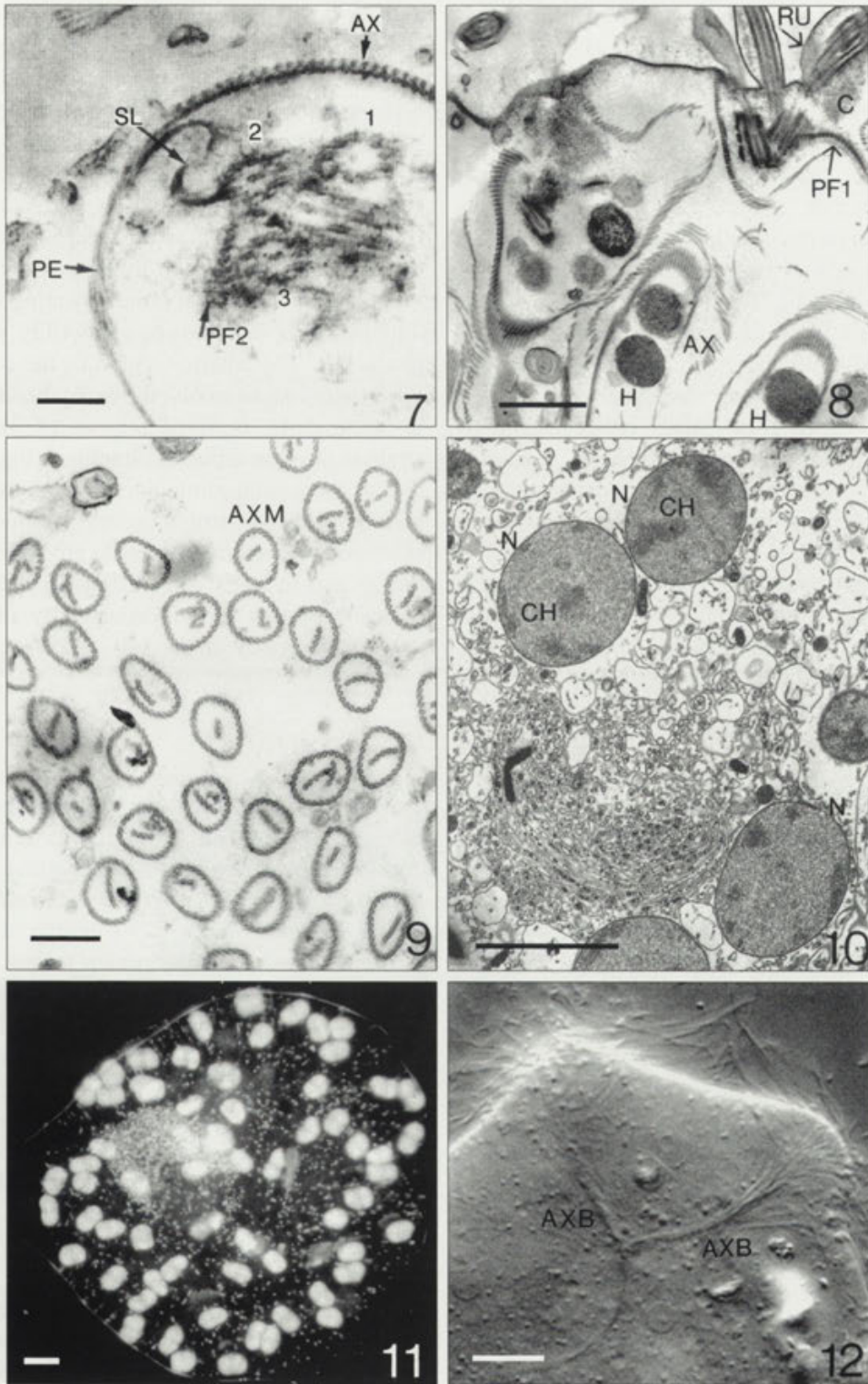**Figs. 1-3**. *Snyderella tabogae* light micrographs. **1** - anterior portion of fixed cell with numerous nuclei (N), some labeled, and "umbrella handle" arrangement of axostyles (arrows). **2** - surface of the anterior region of live cell showing the akaryomastigonts (arrows). **3** - same live cell as Fig. 2, optical plane below the surface, shows nuclei (N) and wood-filled vacuole (V). Scale bars - **1-3** - 10 μm
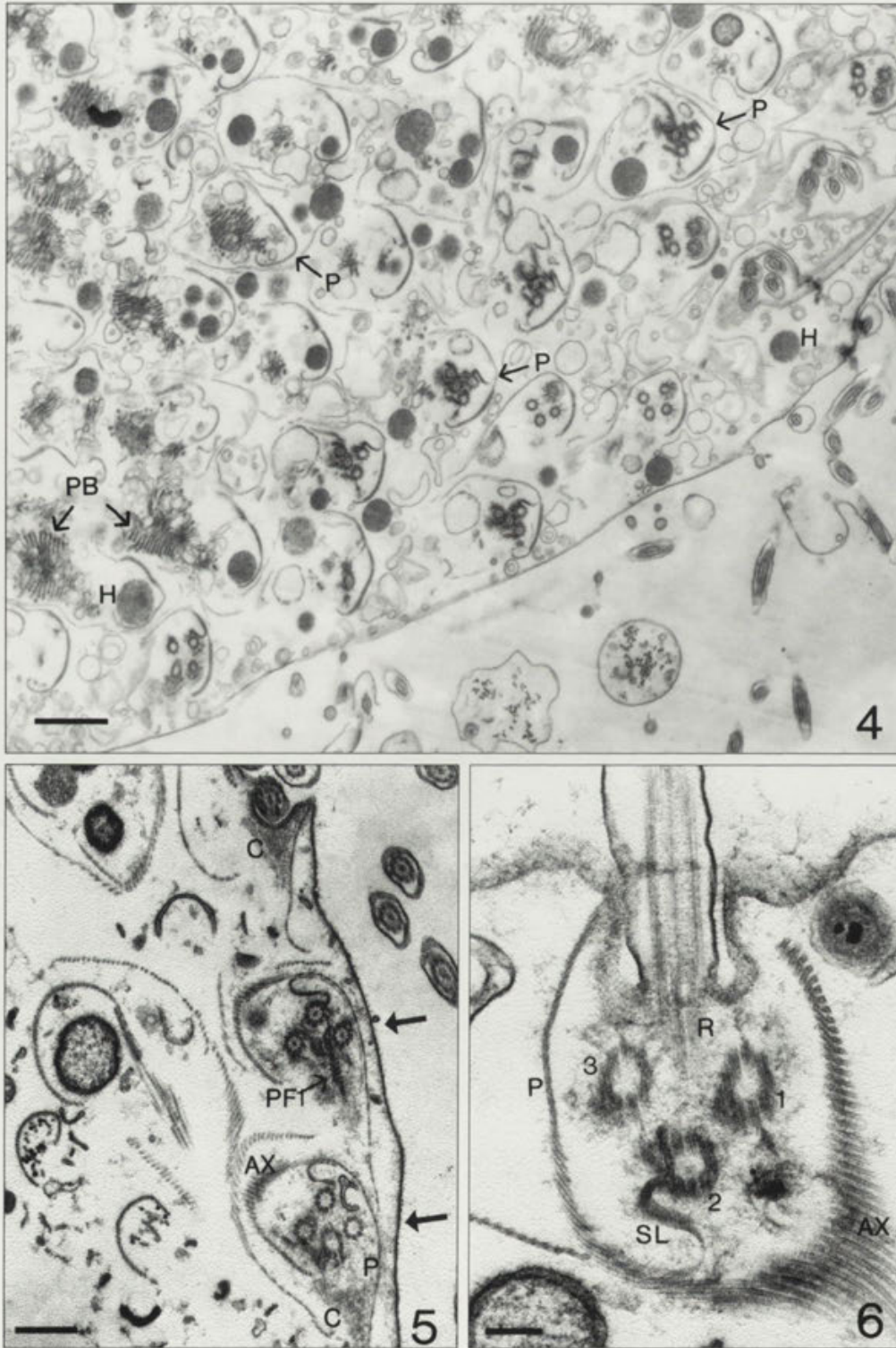
Figs. 4-6. Transmission electron micrographs of *Snyderella tabogae*; 4 - cell cortex that shows more than a dozen akaryomastigonts in antero-posterior just below the cell membrane, longitudinal section. Note hydrogenosomes (H), pelta (P), parabasal body (PB); 5 - akaryomastigonts showing fuzz that may correspond to the cresta remnant (C), parabasal fiber 1 (PF1) and the pelta (P) and axostylar (AX) microtubules; section through two akaryomastigonts (dark arrows); 6 - akaryomastigont in transverse section includes the sigmoid lamella (SL) attached to kinetosome 2, the pelta microtubules (P) and the axostylar microtubules (AX). Scale bars - **4** - 1, **5** - 0.5, **6** - 0.25 μm

Figs. 7-12. **7-10** - transmission electron micrographs, **11-12** - light micrographs of *Snyderella tabogae*; **7** - transverse section through an akaryomastigont; parabasal fiber 2 (PF2) lies perpendicular to the recurrent kinetosome. PE is the pelta; **8** - akaryomastigont structure, cresta remnant (C) underlies the recurrent undulipodium (RU). A pair of hydrogenosomes (H) is associated with an axostyle (AX). Parabasal fiber 1 (PF1) is attached to the recurrent kinetosome; **9** - at the posterior of *Snyderella* the axostyles (AXM), bundled together, have short rows of microtubules in their lumen, as seen in transverse section; **10** - nuclei (N) with clumped chromatin (CH) are not associated with kinetosomes. **11** - karyokineses, synchronous division of more than 60 nuclei after SYTOX-labeling. Small stained structures are probably bacteria. **12** - axostyle bundles (AXB) reveal presence of two competing anterior regions in a live cell. Scale bars - **7**, **9** - 0.25, **8** - 1, **10**, **11** - 5, **12** - 10 μm

(Figs. 4-7). The pelta microtubules that extend from the axostyle contact the plasma membrane (Fig.6). Each akaryomastigont bears three anterior undulipodia and one recurrent undulipodium. The recurrent undulipodium lies at a variable degree angle, generally 45° or less, relative to the other three parallel kinetosomes. Electron dense fuzz, which may be the cresta remnant, underlies the recurrent undulipodium for less than 1 μm (Figs.5, 8). The axonemes arise from a slight concave indentation of the cell surface (Fig. 6). The sigmoid lamella which abuts the transition zone of the axostyle and pelta is always associated with kinetosome 2 (Fig. 6). Two cross-striated parabasal fibers arise from the kinetosomes: parabasal fiber 1 originates between kinetosomes 2 and 3 and is parallel with the recurrent kinetosome (Figs. 5, 8). Parabasal fiber 2 originates between kinetosomes 2 and 3 and is roughly perpendicular to the recurrent kinetosome (Fig. 7).

Some hydrogenosomes, often in pairs or groups lie associated with the axostyles (Fig. 8). Some spherical hydrogenosomes with their amorphous matrices, are bounded by a thick membrane, not visible in others (Fig. 8). Several to many per akaryomastigont may lie beneath the kinetosomes. The multiple axostyles extend centrally and posteriorly form the axostylar bundle. Axostyles, bundled together conspicuously at the cell's posterior, are composed of rolled sheets of 22-35 linked microtubules some of which have been rolled into the lumen of the axostyle (Fig. 9).

Thirty to over 80 spherical nuclei (4-6 μm in diameter), bounded by well-defined envelopes, are scattered throughout the cytoplasm except in the cell's conical anterior where they are absent. Dense chromatin is distributed among homogeneous nucleoplasm both at the periphery and interior of each nucleus (Fig. 10). Squashed *Snyderella* cells yielded nuclei that freely float, and fibers or filaments that attach nuclei to the cytoskeleton or cell membrane were not ever seen.

In those few cells in which dividing nuclei were observed with light microscopic preparations karyokinesis was synchronized (Fig. 11). Late anaphase is equivalent to that in other calonymphids: 2 rounded chromatin masses group at the poles of the thin spindle (the paradesmose) (Dolan 1999). Distinct chromosomes were not observed in live or stained preparations.

Karyokinesis is temporally separated from cytokinesis as seen clearly in live specimens examined for evidence of cell division. While full cytokinesis was not observed, cells often seemed to be in a process of incomplete division in two.

Endonuclear bacteria, common in parabasalids (D'Ambrosio *et al.* 1999), were not seen in over one hundred cells examined with DAPI stain or in electron micrographs (Fig. 10). There was no distinct complement of endocytoplasmic bacteria or of epibiotic bacteria. Spirochetes were attached to the posterior end of the cell, as is common with termite gut symbionts. A large fusiform bacterium, 6-10 μm long and 1-2 μm wide, was often attached by its end to the posterior of the cell.

In addition to the continual beating of thousands of undulipodia a second type of motility was observed in this genus. The anterior region of the cell (replete with 40-50 akaryomastigonts) moves by bending. In contrast, in some cells localized regions of akaryomastigonts strain to move in different directions (Fig. 12). Cells with two or three protruding anterior regions, underlined by bundled akaryomastigonts, are commonly seen. The akaryomastigonts of *Snyderella* are so poorly integrated that groups of 10-20 can entirely detach from the cortex to beat together, but independently of other groups, toward the center of the cell.

DISCUSSION

This first ultrastructural description of *Snyderella tabogae*, although of *Cryptotermes cavifrons*, Florida, conforms to Kirby's (1929) original description of *S. tabogae* from *Cryptotermes longicollis* from Taboga Island, Panama. *Snyderella*'s mastigont system, typical of trichomonads, is quite similar to that of *Calonympha* (Joyon *et al.* 1969). Calonymphids most likely directly descend from devescovinids (Honigberg 1963, Brugerolle 1976, Gunderson *et al.* 1995). Our observations support Kirby's (1949) statement, "In those polymastigote groups (i.e., calonymphids) mastigonts have been duplicated as units, in which the basic pattern has not been altered, and each unit is initially associated with a nucleus. Each unit divides separately; the situation suggests that of a colony of flagellates [=mastigotes] united by a common cytoplasm."

The genus *Snyderella* is unique in the calonymphids: the lack of connection of the nuclei to the mastigont system means only akaryomastigonts (and no karyomastigonts) occur. The similarity of the mastigont systems of *Snyderella*, *Stephanonympha* and *Calonympha* (kinetids, parabasal bodies and the axostyle-pelta complex), argues common ancestry of the group. The family most likely evolved from a basal *Coronympha*-like ancestor with a long cresta and

ungrouped axostyles (Kirby 1949). We think it likely, as did Kirby, that *Snyderella* evolved by dissociation of the mastigont system from the nuclei. The detachment led to the loss of karyomastigonts, and movement of the nuclei from the periphery to the center of the cell. The akaryomastigonts, which were freed up for proliferation of undulipodia (kinetosomes and axonemes), continued to reproduce. The increased number of akaryomastigonts led to greater swimming speed and larger cell surface and volume.

The cresta, while prominent in basal calonymphid genera (Dolan 1999) is reduced or absent in derived ones (Kirby 1949). As in *Stephanonympha nelumbium* (Rösel *et al.* 1996) the typical cresta is not found in *Snyderella tabogae*. The layer of electron dense granular material along the plasma membrane at the attachment of the recurrent undulipodium may be a cresta remnant (C, Fig. 5).

Snyderella tabogae's complex cell division is here described for the first time. Synchronized karyokineses occur in *Snyderella* as they do in other calonymphids. Karyokinesis and cytokinesis are spatially and temporally separated, also typical for calonymphids. The nuclear envelope remains intact as indicated by the rounded offspring nuclei at early and late anaphase. However, we could not determine the relationship between the nuclei and the kinetosomes at mitosis because mitosis was seen so rarely.

A bending motility was observed in this genus. This type of cell bending was seen by the authors in both *Snyderella* and *Calonympha*, where the axostyles, one per mastigont, join to form a thick central bundle. Yet, it was absent in *Coronympha* and *Metacoronympha*, whose axostyles, not collected in a bundle, remain dispersed in the cytoplasm. *Stephanonympha*, whose axostyles are bundled together, does not exhibit this type of motility. In contrast to *Calonympha* and *Snyderella*, *Stephanonympha*'s anterior region is fixed and rounded rather than conical.

Multiple conical anterior regions consisting of protrusions of akaryomastigonts seem to occur after karyokinesis. As the interphase cell becomes more integrated, a single conical anterior region and a rounded posterior appear. These movements most likely lead to division by budding in *Snyderella*; these processes were observed in *Metacoronympha* (Dolan 1999).

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ACTA
PROTOZOOLOGICA

Role of Extracellular Calcium in the Induction of Pinocytosis in *Amoeba proteus* by Na and K Ions

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Summary. Pinocytosis induced in amoebae by Na^+ and K^+ is commonly interpreted as a result of competitive calcium displacing from the cell surface. However, the present experiments showed that pinocytosis induced by these monovalent cations in *Amoeba proteus* does not require net calcium release from the glycocalyx. Ca^{2+} is released from the surface of amoebae during initiation of pinocytosis by the usually applied strong inducer (125 mM KCl) and by the weaker inducer applied in an increased concentration (250 mM NaCl). However, Ca^{2+} remains bound by amoebae at the usual concentration of the weaker inducer (125 mM NaCl) and in the low concentration of the strong one (30 mM KCl). The results also suggest a correlation between Na^+ and K^+ efficiency of inducing pinocytosis and their effects on the Ca equilibrium between the cell surface and the medium.

INTRODUCTION

Pinocytosis in *Amoeba proteus* has been discovered by Mast and Doyle (1934), and for a long time pioneer studies of this phenomenon were carried out on freshwater amoebae (e.g. Holter and Marshall 1954, Chapman-Andresen and Holter 1955, Chapman-Andresen 1962). NaCl and KCl in concentrations about 100 mM were currently used as inducers. The pinocytosis is a common function of most animal cells, how-ever, the cation induced pinocytosis in amoebae has at least one outstanding feature: it clearly is a kind of motor activity requiring development of specialised pinocytotic pseudopodia and cell shape changing into a rosette form; gradients of these changes depend on endoplasmic streaming pattern (Klein and Stockem 1979) and on motor polarity of these cells (Grębecka and Kłopocka 1985). The involvement of submembrane actin cytoskeleton in protrusion of pinocytotic pseudopodia, membrane invagination, formation of pinocytotic channels and endosome separation was demonstrated by electron microscopy (Klein and Stockem 1979), and in living amoebae by video-enhancement (Grębecki 1991).

Therefore, triggering of cation induced pinocytosis in *A. proteus* by entry of extracellular Ca ions is an

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attractive hypothesis. Calcium may enter into this cell not directly from the surrounding fluid, but from the surface coat, the glycocalyx that in freshwater amoebae is extremely well developed, being 100-200 nm thick. Concentration of inorganic cations in glycocalyx was estimated as about 20 times higher than in the medium (Hendil 1971). It was suggested that Na^+ or K^+ induced pinocytosis in amoebae is initiated by inducers binding to negative groups in the glycocalyx and plasma membrane as a result of competitive calcium displacing from these sites, which leads to membrane depolarisation and permeabilisation, and may be followed by entry of extracellular Ca^{2+} into the cell (Brewer and Bell 1970, Brandt and Hendil 1972, Josefsson 1975, Josefsson *et al.* 1975, Gawlitta *et al.* 1980, Taylor *et al.* 1980, Prusch 1986). Alternatively, the increased adsorption of external Na^+ or K^+ to the surface of amoebae may initiate a signal to release endogenous Ca^{2+} from intracellular stores.

More questions arise from observations that the influence of extracellular Ca^{2+} on pinocytosis may differ depending on the kind of inducer. K^+ is a stronger inducer than Na^+, since pinocytosis terminated in NaCl may be reactivated by KCl, but not *vice versa* (Kłopocka and Grębecka 1986). The induction of pinocytosis by K^+ is less dependent on extracellular Ca^{2+} than its induction by Na^+ (Johansson and Josefsson 1984 b). Pinocytosis induced by NaCl requires an optimal $[Ca^{2+}]_e$, hence it is inhibited by removing calcium ions from the medium as well as by their too high concentration (Josefsson 1975, Prusch and Hannafin 1979, Johansson and Josefsson 1984 a, Prusch 1985, Prusch 1986, Stockem and Klein 1988). In contrast, such changes in $[Ca^{2+}]_e$ fail to influence the initiation of pinocytosis induced by KCl (Johansson and Josefsson 1984 a, b). Moreover, Na^+ and K^+ provoke quite different morphological types of pinocytosis in *Amoeba proteus* (Grębecka and Kłopocka 1986).

In the present experiments Ca^{2+} dynamics during induction of pinocytosis by Na^+ or K^+, in two different concentrations of each, was estimated by measuring the radioactivity of amoebae after introduction of $^{45}Ca^{2+}$ simultaneously with the pinocytotic inducers. Obviously, this method cannot falsify the hypothesis of exogenous Ca^{2+} entry into the cell, because it does not discriminate between $[Ca^{2+}]_e$ bound to the cell surface and $[Ca^{2+}]_i$ present in the cytoplasm, but it can verify two other assumptions made in literature: (1) is calcium in fact generally released from the surface of amoeba during initiation of pinocytosis induced by monovalent cations,

and (2) is there any relation between the different efficiency of Na^+ and K^+ as pinocytotic inducers and their effects on Ca equilibrium between the medium and amoebae.

MATERIALS AND METHODS

Amoeba proteus was cultured in standard Pringsheim medium [which contains 0.85 mM $Ca(NO_3)_2$] and fed on *Tetrahymena pyriformis*. The cells were used for experiments 3 days after feeding, when they are most sensitive to the pinocytotic inducers. The pinocytotic activity of these amoebae was controlled on a slide in light microscope before each experiment. Experiments were run in pH 6.8-7.2, at room temperature. For investigating calcium dynamics 30 amoebae per sample with 40 ml of culture medium were transferred into a Godet chamber. Aliquots of pinocytotic inducers were added to the Pringsheim medium as to obtain final concentrations of 125 or 250 mM NaCl and 30 or 125 mM KCl. Changes in the amount of cell-associated Ca^{2+} during pinocytotic induction were assessed by adding 0.9 µCi/sample of $^{45}Ca^{2+}$ (0.1 mCi/ml, Amersham Life Science, Little Chalfont, Buckinghamshire, England). The radioactive calcium was added simultaneously with pinocytotic inducers. Experiments of each type were repeated 3-4 times.

The radioactivity of $^{45}Ca^{2+}$ retained by amoebae was counted after 2, 7 and 12 min from the beginning of experiment. After these incubation periods the reaction was stopped in one of the samples by transferring amoebae with a pipette from the Godet chamber on the filter and washing them with a vacuum pump three times with 10 ml of ice-cold Pringsheim solution (lacking only $^{45}Ca^{2+}$), to remove the radioactive Ca from the medium. The amount of $^{45}Ca^{2+}$ retained by amoebae was counted in Beckman LS 6000TA liquid scintillation counter. The radioactivity of filters treated in the same manner without amoebae were always by two orders of magnitudes lower than in experiments.

The radioactivity of $^{45}Ca^{2+}$ in all measurements was expressed in dpm/sample. The results in the graphs (Fig.1) are presented as percent of $^{45}Ca^{2+}$ radioactivity recorded after 2 min of incubation in the first sample of each series, because this result obtained before the onset of pinocytosis may be considered as a control reference value specific for each particular experiment. As a second type of control, aliquots of Pringsheim medium, with $^{45}Ca^{2+}$ but without pinocytotic inducers, were added to other samples.

RESULTS

The light microscope tests performed before each experiment with pinocytotic inducers have proved that, in the concentrations of NaCl and KCl, which were applied in this study, the pinocytosis was always manifested. The first channels appeared after 2-5 min, the pinocytotic rosette stage was achieved after 5-10 min from application of the stimulus, and the stage of maxi-
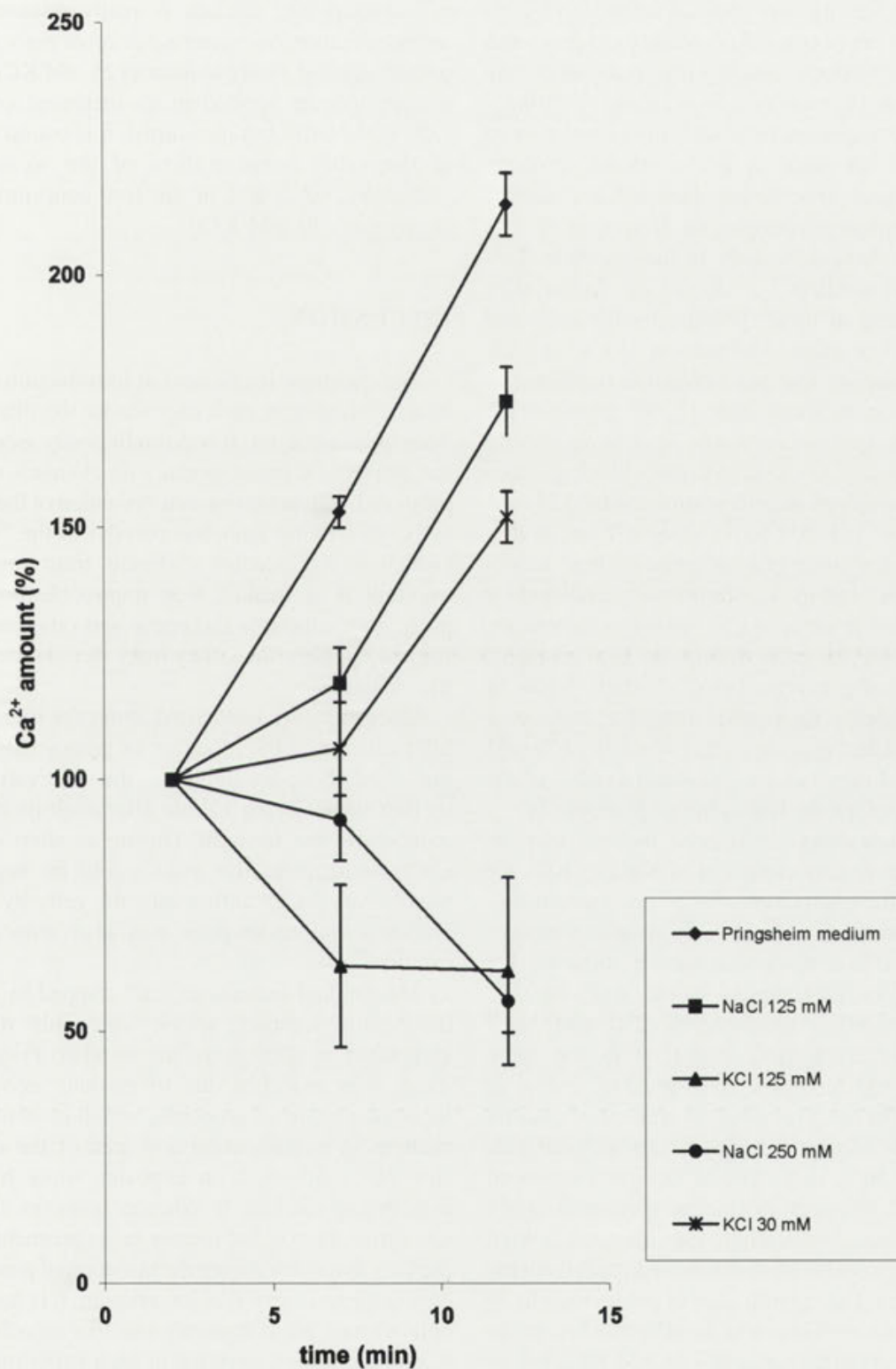
Fig. 1. Time course of changes in the amount of Ca^{2+} associated with the surface of amoebae during the induction of pinocytosis by 125 mM NaCl, 250 mM NaCl, 30 mM KCl, 125 mM KCl and in cells, which were stimulated by adding of Pringsheim culture medium instead of pinocytotic inducer. The diagram shows the average results of each experiment as 100% of dpm incorporated by amoebae during 2 min of incubation in $^{45}Ca^{2+}$ added with the stimulating agents

mal pinocytotic activity was attained after 10-15 min. Later on, the number of channels gradually fell down and the pinocytotic rosettes converted into postpinocytotic forms. The whole phenomenon lasted about 30 min.

Addition of Pringsheim medium without inducers to the control samples acted as a mechanical stimulus, which never induced pinocytosis but immediately caused a transient inhibition of endoplasmic flow, cessation of locomotion and changes in shape of the amoebae. This stage lasted no longer than 2-3 min and was followed by a gradual restoring of motor polarity by the cells and resumption of locomotion. The amount of Ca^{2+} associated to these amoebae was not stable, but increased as a function of time, reaching after 12 min 214% of the initial value (Fig. 1).

Changes in the Ca^{2+} association to pinocytosing amoebae were first measured in cells stimulated by 125 mM NaCl (squares) or 125 mM KCl (triangles), i.e., within the commonly used concentration range of these inducers. As it is shown in Fig. 1, in amoebae stimulated by 125 mM NaCl, the amount of Ca^{2+} bound to the surface or incorporated by the cells during the first phase of pinocytosis gradually increased to 175% after 12 min. In contrast, Ca^{2+} quickly dissociated from the surface of cells investigated during pinocytosis induced by 125 mM KCl, and after 12 min its level fell down to 62% of the value recorded before the beginning of pinocytosis.

Since the pinocytosis in *Amoeba proteus* may be induced by different concentrations of NaCl or KCl, we have measured the association of Ca^{2+} to pinocytosing amoebae in a low concentration of the strong inducer (30 mM KCl) and in a high concentration of the weaker one (250 mM NaCl). Changes in the level of Ca^{2+} associated to amoebae stimulated by 250 mM NaCl strongly differed from those observed in the same inducer at 125 mM (compare corresponding curves in Fig. 1). In 250 mM NaCl (circles) the amount of calcium bound to the cells decreased during pinocytosis and after 12 min it fell to 56%, very close to the effect produced by 125 mM KCl, although the decline was significantly slower. It indicates that sodium ions in such a high concentration are capable of displacing calcium from the surface of amoeba. During initiation of pinocytosis in 30 mM KCl (asterisks) calcium was not displaced from the cell surface like in the presence of 125 mM KCl, but on the contrary, the amount of Ca^{2+} bound to the surface or incorporated by the pinocytosing amoebae gradually increased up to 152% after 12 min. The curve produced in 30 mM KCl became rather similar to that representing the effect of 125 mM NaCl (Fig. 1).

Summarising, calcium is really released from the surface of amoebae during initiation of pinocytosis by the usually applied strong inducer (125 mM KCl) and by the weaker inducer applied in an increased concentration (250 mM NaCl), but in contrast, it is bound by amoebae at the usual concentration of the weaker inducer (125 mM NaCl) and in the low concentration of the strong one (30 mM KCl).

DISCUSSION

The questions formulated in Introduction necessitated testing whether in each experiment the pinocytosis has been induced or not. It was qualitatively ascertained that the pinocytotic pseudopodia with channels were always produced. A quantitative determination of the pinocytotic activity of living amoebae parallel to the $^{45}Ca^{2+}$ assays (which is a procedure different from measuring the eventual fluid intake), was impossible because some pinocytotic channels disappear and other new ones are formed quicker than they may be counted in single individuals.

Since we were concerned about the role of extracellular calcium in the initiation of pinocytosis, the results presented above are limited to the observations made at its first stage, during 12 min after addition of $^{45}Ca^{2+}$ and inducers to the medium. During so short experiments Ca^{2+} release from the glass could be neglected; the possibility of Ca^{2+} influx into the cells by pinocytotic channels and endosomes was also reduced, but not excluded.

The gradual increase of Ca^{2+} trapped by amoebae in the control samples, which were only mechanically stimulated by addition of the standard Pringsheim medium, was probably due to gradual recovery of the locomotor shape of amoebae, which involves important changes in conformation and area of the cell surface, and thus may result in exposing more binding sites available to calcium. It indicates however, that this was not a true control, but merely an experiment comparing the Ca^{2+} dynamics during the initiation of pinocytosis and during the resumption of locomotion. It is not possible to obtain intact but completely inactive amoebae. For that reason the results recorded in each experiment until the pinocytosis began (after 2 min of incubation in an inducing solution) should be taken as reference values.

The radioactivity of amoebae measured in our experiments was probably a product of 3 fractions of Ca^{2+} trapped by the cells: (1) taken in by pinocytosis and

confined to the pinocytotic channels and endosomes, (2) cytoplasmic Ca^{2+} which could enter through calcium channels in plasma membrane, and (3) Ca^{2+} bound to glycocalyx at the cell surface.

The fluid intake during a full pinocytotic cycle was estimated by measuring ^{14}C-glucose and $^{22}Na^+$ ingestion (Chapman-Andresen and Holter 1955; Chapman-Andresen and Dick 1962) as corresponding to the range of 1-10% of the volume of the amoeba. Therefore the endosomal Ca^{2+} fraction is negligible with respect to extracellular calcium content in glycocalyx, but it might be important with respect to the free calcium concentration in the cytoplasm. Nevertheless, this fraction could hardly be suspected to trigger pinocytosis at its initial stage, because it is absent when the first channels are formed, and it remains „external" for the actomyosin system, until it penetrates through the endosomal membranes.

Entry of Ca^{2+}_e to the cytoplasm through the plasma membrane during initiation of pinocytosis in amoebae, has never been directly demonstrated or disproved. It is however known that pinocytosis in *A. proteus* occurs even in the absence of extracellular Ca^{2+}: it was provoked by 52-117 mM Na_2EGTA, i.e., when all Ca^{2+}_e was certainly chelated even in the glycocalyx and $[Na^+]_e$ was raised to 105-235 mM, which induces pinocytosis under normal conditions (Kłopocka and Grębecka 1985). Pinocytosis is not inhibited by Ca-influx antagonists: Verapamil and D 600 (Stockem and Klein 1979), nor by a blocker of calcium channels, protopine hydrochloride (our unpublished observations). These arguments may imply that transient increase of the internal Ca^{2+} concentration during cation induced pinocytosis, measured by free Ca imaging with fura-2 fluorescence (Kłopocka and Pomorski 1996), is not due to the entry of exogenous calcium, but to Ca^{2+} release from intracellular stores.

In *A. proteus* glycocalyx is this Ca-binding structure, which is largest in volume and is most easily accessible to Ca^{2+} from the fluid medium. It seems therefore reasonable to suppose, that in our experiments calcium bound to the cell surface represented at least the major fraction of Ca^{2+} associated to amoebae. It was postulated in the literature that competitive calcium substitution in glycocalyx by monovalent ions mobilises Ca^{2+} entry into the cell, which triggers pinocytosis (see Introduction). Present experiments show that pinocytosis is manifested regardless of Ca^{2+} dissociation from the cell surface (in 250 mM NaCl and 125 mM KCl) or its binding by amoebae (in 125 mM NaCl and 30 mM KCl).

This allows us to oppose to the view, that release and internalisation of calcium from the glycocalyx is necessary for initiating the cation induced pinocytosis in *A. proteus*, and to support instead the hypothesis of endogenous origin of Ca^{2+} that triggers this phenomenon. We don't know, what is the messenger provoking calcium release from the intracellular stores.

On the other hand however, our experiments with different concentrations of a strong inducer of pinocytosis (KCl) and a weaker one (NaCl) suggest a correlation between their efficiency of inducing pinocytosis and their effects on the Ca equilibrium between the cell surface and the medium. We think therefore that although pinocytosis does not necessitate a net calcium release from glycocalyx and it may occur in the complete absence of extracellular calcium, the concentration of this ion in natural milieu of amoeba may modulate the pinocytotic reaction.

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ACTA
PROTOZOOLOGICA

Benthic Freshwater Testate Amoebae Assemblages (Protozoa: Rhizopoda) from Lake Dongting, People's Republic of China, with Description of a New Species from the Genus *Collaripyxidia*

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Summary. A total of 42 testate amoebae species and varieties were identified in sediment samples taken from three stations (L, O, R) around the outlet of Lake Dongting to the ChangJiang (Yangtze) River. From these species, *Collaripyxidia dongtingiensis* sp. n. is new to science and 25 are new to the testate amoebae fauna of the People's Republic of China. The most numerous were representatives of the genus *Centropyxis*, followed by *Difflugia* and *Cyclopyxis*. *Centropyxis aculeata* is the most dominant species in the lake (Station L), while *Plagiopyxis callida* in both the outlet (Station O) and the river (Station R). Each habitat has peculiar testate amoebae assemblage, only three species, *Centropyxis aculeata*, *Centropyxis ecornis* and *Phryganella acropodia* var. *depressa*, have been observed at all habitats. The greatest total abundance and biomass values were observed at the outlet, while the smallest ones in the river. From the lake to the river, both number of species and the Shannon-Weaver diversity index values are decreasing, although the equitability index of the outlet habitat was a little lower than that of the river habitat. The main cause for the decreasing diverse testacean assemblages from the lake to the river may be the unstable environment resulting from high water velocity.

Key words: benthos, China, *Collaripyxidia dongtingiensis* sp. n., faunistics, lake Dongting, testate amoebae.

INTRODUCTION

Testate amoebae fauna from different lake types is very well known. Many workers published papers about the species composition, ecology and biology of testate amoebae in different lake habitats (plankton, benthal, profundal, littoral). For example: Laminger (1971; 1972; 1973 a, b) studied testate amoebae fauna in high mountain lakes in Austria, Green (1965; 1972; 1979; 1986 a, b) and Green *et al.* (1976, 1984) investigated testacean communities in tropical crater and soda lakes, Golemansky (1967, 1994) published papers about testate amoebae in the littoral of the tectonic lakes in Macedonia, Schönborn (1962, 1965, 1966) and Moraczewski (1962, 1967) studied the ecology of testaceans living in sediments in German and Polish lakes, Štěpánek (1967) investigated faunistically a testate amoebae assemblage

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in the benthos of the Vranov Dam in the Czech Republic, Grospietsch (1982) described a testacean community in the thermal lake Héviz in Hungary and Penard (1902) published the first and great work about testate amoebae in the Lac Léman (Switzerland).

Testate amoebae communities in lakes have usually a very large species richness and abundance. These communities are in many cases dominated by members of the genus *Difflugia*, followed by other genera (e.g. *Centropyxis*, *Cyclopyxis*, *Euglypha*, *Trinema*, *Zivkovicia*). Species richness and abundance increased with the enhancement of dead organic matter content in lake sediments.

The outlet of a lake to a river is a kind of special ecosystem. There are quite few studies on protozoans in these habitats. Actually, we know very little about the structure and functioning of such open ecosystems.

Chinese testate amoebae were up to this time not very known. About testate amoebae few studies were published: Bartoš (1963), Fielde (1887 a, b), Gong *et al.* (1990), Lemmermann (1907), Tai (1931), Wang (1923, 1925, 1928), Wang and Nie (1934), Wang (1977), Wang and Min (1987) and Zanyin Gaw (1941). Little is known also on the protozoan communities of Lake Dongting (Song and Xie 1997).

During the observations on the testate amoebae communities from the sediment samples of Lake Dongting, a conspicuous new member of the genus *Collaripyxidia* was met. Up to this time, only one species from this genus was described, *Collaripyxidia stankovici* Živković, 1975, from the Danube River (dam Đerdap) from former Yugoslavia (Živković 1975).

In order to study this lake-river complex ecosystem, the present study intended to compare benthic testacean assemblages of three different ecological habitats around the outlet: lake, outlet of the lake to the river, and river.

MATERIALS AND METHODS

The mesosaprobic Lake Dongting (20°30' - 30°20'N, 111°40' - 113°10'E) is the second largest freshwater lake in the People's Republic of China. It is located in northeastern Hunan Province, south China, on the plain of the middle reaches of the ChangJiang (Yangtze) River. It has a total surface area of 2,740 km², and is 33.5 m above sea level. Average and maximum depths are 6.7 m and 30.8 m, respectively. The volume of the lake is about 17.8 x 10⁹ m³ and the catchment area is 259,430 km². Lake Dongting is divided into three connected parts (East Dongting, South Dongting and West Dongting) and is connected directly to the ChangJiang (Yangtze) River and some other rivers. The lake receives water from the ChangJiang (Yangtze)

River at four inlets (Taiping, Songzhi, Ouci and Fudu), and from outer four smaller rivers (the XiangJiang, Zishui, YuanJiang and Lishui Rivers), and finally the lake water flows into the ChangJiang (Yangtze) River from the only outlet - Chenglinji Channel in the East Dongting Lake (Fig. 1). With these interconnections, lake water flows very fast and exchanges rapidly: average and maximum water flow velocities are 0.53 m. s⁻¹ and 1.52 m s⁻¹, respectively; water of the whole lake exchanges completely once in less than 20 days. Additionally, due to heavy soil erosion at the upper reaches of the rivers, Lake Dongting has received a great sediment load (as many as 1,434 x 10⁸ m³ yr⁻¹ of sand and mud are left in the lake). Sedimentation rate is 3.7 cm yr⁻¹ on the whole lake average. Normally, the lake water contains a lot of silt (about 89.05 mg l⁻¹ on annual average) and looks yellowish.

The outlet of Lake Dongting to the ChangJiang (Yangtze) River has been chosen for sampling sites. From the lake to the river, three sampling stations have been set. One is in the lake (Station L) and represents lake habitat. The second one is directly at the outlet of the lake to the river, but on the lakeside (Station O) and represents the outlet habitat. The third one is near the Station O, but directly in the river (Station R) and represents river habitat. The sketch of the sampling stations is showed in Fig. 2. The main environmental characteristics of the sampling sites are shown in Table 1.

Sediment samples were taken by using a Peterson's crab sampler on September 13, 1996 and were preserved in 4% formaldehyde solution. Testate amoebae assemblages were examined in laboratory under a light microscope.

RESULTS

Description of the testate amoebae assemblages found in Lake Dongting

Altogether 42 species and varieties of the testate amoebae from 14 genera were identified from the sediment samples. From these one is new to science and 27 are newly recorded in the present study for China (Table 2). All of the three testate amoebae assemblages are composed of typical benthic species (e.g. members of the genera *Arcella*, *Difflugia*, *Centropyxis aculeata*, *C. marsupiformis*, *C. spinosa*, *Cucurbitella mespiliformis*) and of ubiquitous species with cosmopolitan distribution (e.g. *Centropyxis aerophila*, *Cyclopyxis kahli*, *Microcorycia flava*, *Phryganella acropodia*, *Plagiopyxis declivis*, *P. callida*, *Trinema enchelys*, *T. lineare*). The testate amoebae species found in Lake Dongting are bacterivorous (e.g. *Trinema enchelys*, *T. lineare*), mycophagous (e.g. *Phryganella acropodia*), algivorous (many *Difflugia* species, *Cucurbitella mespiliformis*), detritivorous (*Hoogenraadia cryptostoma*) or omnivorous and carnivorous. No species found are endemic for the fauna of the People's Republic of China (with exception of *Collaripyxidia dongtingiensis* sp. n.). Occurrence of

Fig. 1. Schematic drawing of the Lake Dongting and ChangJiang (Yangtze) River water system. E - East Dongting Lake, S - South Dongting Lake, W - West Dongting Lake
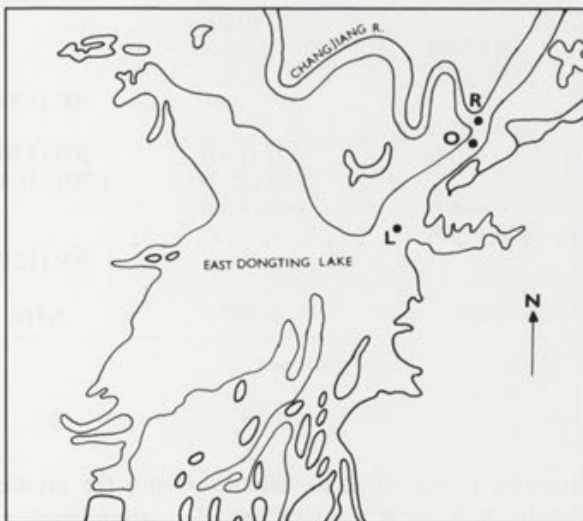

Fig. 2. Sketch-map of the East Dongting Lake showing the positions of three sampling sites, Station L, Station O and Station R

few species is up to this time known from a geographically restricted area (e.g. *Cyclopyxis thomasi* from Congo;

Table 1. Main environmental characteristics of the sampling sites

Water characteristics	Station L	Station O	Station R
Water temperature (°C)	21.6	21.7	20.8
pH	7.69	8.08	7.87
Dissolved oxygen (mg . l⁻¹)	8.31	8.72	7.97
Total phosphorus (ppm)	0.1153	0.1377	0.2058
Total nitrogen (ppm)	2.5830	2.6400	2.6360
SiO_2 (mg . l⁻¹)	4.5778	2.3887	2.7091
Secchi transparency depth (cm)	32	38	30
Content of organic matter (%)	15.77	13.37	9.22

Hoogenraadia cryptostoma from Congo and Malaysia; *Phryganella acropodia* var. *depressa* from Australia and Vietnam). The remaining number of species found in Lake Dongting is known from many countries of the world.

From relative abundance values it can be seen that the outlet (Station O) and the river (Station R) have similar dominant species: *Plagiopyxis callida* (the dominance values are 25.79% and 31.43%, respectively), *Centropyxis aculeata* is the second one (20.06% and 22.18%, respectively), and the third one is *Centropyxis platystoma* (14.33%) in the outlet and *Trinema lineare* (12.94%) in the river, respectively. In the lake (Station L) *Centropyxis aculeata* is the first dominant (18.58%), followed by *Centropyxis ecornis* (10.84%), *Cyclopyxis penardi* (10.84%) and *Phryganella acropodia* (9.29%).

As a whole, representatives of the genus *Centropyxis*, with 23.81% of the species listed were the most numerous, followed by the species of the genera *Difflugia* (21.43%) and *Cyclopyxis* (14.29%). Considering each ecological habitat, the situations are a little different. At the lake habitat (Station L), representatives of the genus *Centropyxis* (35.00%) were the most numerous, followed by *Cyclopyxis* (15.00%) and *Phryganella* (10.00%). At the outlet (Station O) *Difflugia* is the most numerous (33.33%), *Centropyxis* the second (22.22%), followed by *Cyclopyxis* and *Plagiopyxis* (11.11% each). In the river (Station R), *Centropyxis* is the first (26.67%), *Difflugia* the second (20.00%), and *Cyclopyxis* the third (13.33%). (For the occurrence of the remaining genera see Table 3).

Comparing the species composition among the three ecological habitats (lake, outlet, river), there were 3 species only, *Centropyxis aculeata*, *Centropyxis ecornis* and *Phryganella acropodia* var. *depressa* occurring in all of the three habitats (composing only 7.14% of the total species found). As many as 11 species

Table 2. Testate amoebae species and their abundance (ind. g^{-1}) values observed in the sediment samples. The figure in the parenthesis denotes the dominance value, as expressed in percentage (%). Symbol "*" marks the new testate amoebae found for the fauna of the People's Republic of China, symbol "**" denotes the new species for the science

| Species	Station L	Station O	Station R
* *Amphitrema wrightianum* Archer, 1869		20 (0.29)	
* *Arcella megastoma* Penard, 1913			200 (3.70)
Arcella vulgaris Ehrenberg, 1932		100 (1.43)	
* *Arcella vulgaris* var. *undulata* Deflandre, 1928	500 (7.74)		
Assulina muscorum var. *stenostoma* Schönborn, 1964		50 (0.72)	
* *Assulina seminulum* (Ehrenberg, 1848) Leidy, 1879			100 (1.85)
Centropyxis aculeata (Ehrenberg, 1838) Stein, 1857	1,200 (18.58)	1,400 (20.06)	1,200 (22.18)
Centropyxis aerophila Deflandre, 1929	100 (1.55)		
* *Centropyxis aerophila* var. *minuta* Chardez, 1964			10 (0.37)
Centropyxis constricta (Ehrenberg, 1838) Penard 1902	200 (3.10)	500 (7.16)	
Centropyxis ecornis (Ehrenberg, 1841) Leidy, 1870	700 (10.84)	300 (4.30)	500 (9.24)
* *Centropyxis marsupiformis* (Wallich, 1864) Deflandre, 1929	300 (4.64)		
* *Centropyxis marsupiformis* var. *scapula* Decloitre, 1970	50 (0.77)		
* *Centropyxis orbicularis* Deflandre, 1929	100 (1.55)		
Centropyxis platystoma (Penard, 1890) Deflandre, 1929		1,000 (14.33)	
* *Centropyxis spinosa* (Cash, 1905) Deflandre, 1929			100 (1.85)
** *Collaripyxidia dongtingiensis* sp. n.	10 (0.15)		
* *Cucurbitella mespiliformis* Penard, 1902	100 (1.55)		
* *Cyclopyxis ambigua* Bonnet & Thomas, 1960	350 (5.42)		
* *Cyclopyxis arcelloides* (Penard, 1902) Deflandre, 1929	200 (3.10)	100 (1.43)	
Cyclopyxis kahli Deflandre, 1929			200 (3.70)
* *Cyclopyxis kahli* var. *cyclostoma* Bonnet & Thomas, 1960		50 (0.72)	
* *Cyclopyxis penardi* Deflandre, 1929	700 (10.84)		
* *Cyclopyxis thomasi* Štěpánek, 1963			10 (0.18)
* *Difflugia avellana* Penard, 1890		30 (0.43)	
* *Difflugia corona* Wallich, 1864			30 (0.55)
Difflugia elegans Penard, 1890	300 (4.63)	800 (11.46)	
* *Difflugia elegans* var. *teres* Penard, 1899		50 (0.72)	
* *Difflugia globulosa* Dujardin, 1837			300 (5.55)
Difflugia lanceolata Penard, 1890		50 (0.72)	
Difflugia lucida Penard, 1890		10 (0.14)	
Difflugia sarissa Li Sun Tai, 1931			50 (0.92)
* *Difflugia scalpellum* Penard, 1899		20 (0.29)	
* *Hoogenraadia cryptostoma* Gauthier-Lièvre & Thomas, 1958/59	50 (0.77)		
Microcorycia flava (Greeff, 1866) Cockerell, 1911	100 (1.55)		
* *Netzelia tuberculata* (Wallich, 1864) Netzel, 1983			100 (1.85)
* *Phryganella acropodia* (Hertwig & Lesser, 1874) Hopkinson, 1909	600 (9.29)		
* *Phryganella acropodia* var. *depressa* Playfair, 1917	200 (3.10)	100 (1.43)	200 (3.70)
* *Plagiopyxis callida* Penard, 1910		1,800 (25.79)	1,700 (31.42)
* *Plagiopyxis declivis* Thomas, 1955	300 (4.64)	600 (8.60)	
Trinema enchelys (Ehrenberg, 1838) Leidy, 1878	400 (6.19)		
Trinema lineare Penard, 1890			700 (12.94)
Total	6,460	6,980	5,410

were found only in the river, not in the lake and the outlet of the lake to the river, 27 species only in both the lake and the outlet, but not in the river. Comparing the species composition between Station L and Station O, although these two stations are all in the Chenglinji Channel, as many as 11 species only live at the outlet, and 13 species only in the lake.

In terms of the total, the Station O has the greatest, while the Station R has the smallest abundance and biomass value. From the lake to the river, both the number of species and the Shannon-Weaver diversity index values are decreasing. The most diverse taxocoenosis has been found at the Station L (20 species), the least one at the Station R (15 species), although the equitability index at
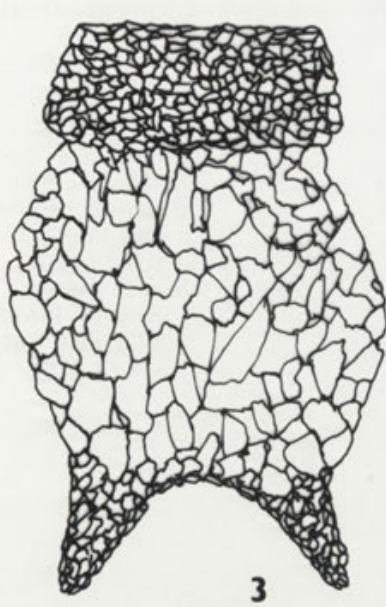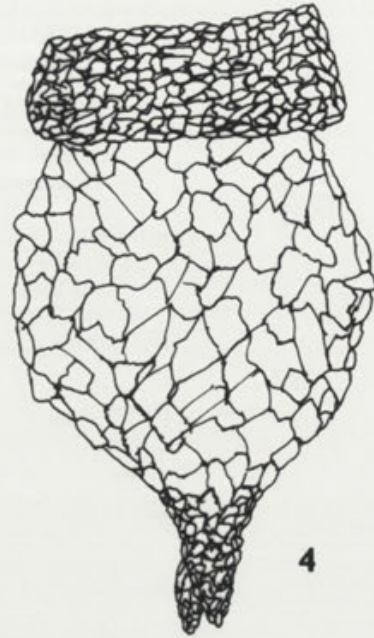
Figs. 3-6. *Collaripyxidia dongtingiensis* sp. n. **3** - general view of the shell; **4** - side view of the shell; **5** - aperture view; **6** - photomicrograph of the typical shell. Scale bar -100 μm

the Station O is a little lower than that of the Station R (for other detailed coenological characteristics see Table 4).

The Jaccard similarity index has shown that the testacea assemblages among the three habitats were obviously different and individual communities have been found

Table 3. Distribution of the testate amoebae species in each genus. The figure in the parenthesis denotes their percentage (%) in the total number of species observed; the genera are listed orderly in the decreasing percentage values

Station L	Station O	Station R
Centropyxis 7 (35.00)	*Difflugia* 6 (33.33)	*Centropyxis* 4 (26.67)
Cyclopyxis 3 (15.00)	*Centropyxis* 4 (22.22)	*Difflugia* 3 (20.00)
Phryganella 2 (10.00)	*Cyclopyxis* 2 (11.11)	*Cyclopyxis* 2 (13.33)
Arcella 1 (5.00)	*Plagiopyxis* 2 (11.11)	*Arcella* 1 (6.67)
Collaripyxidia 1 (5.00)	*Assulina* 1 (5.56)	*Assulina* 1 (6.67)
Cucurbitella 1 (5.00)	*Amphitrema* 1 (5.56)	*Netzelia* 1 (6.67)
Difflugia 1 (5.00)	*Arcella* 1 (5.56)	*Phryganella* 1 (6.67)
Hoogenraadia 1 (5.00)	*Phryganella* 1 (5.56)	*Plagiopyxis* 1 (6.67)
Microcorycia 1 (5.00)		*Trinema* 1 (6.67)
Plagiopyxis 1 (5.00)		
Trinema 1 (5.00)		

Table 4. Some data about the testate amoebae assemblages in the three sampling stations

Character	Station L	Station O	Station L
Total number of species	20	18	15
Number of new species to China	12 (60%)	9 (50%)	9 (60%)
Number of new species to science	1 (5%)	0	0
Total abundance (ind. g⁻¹)	6,460	6,980	5,510
Total biomass (μg g⁻¹)	52.74	57.34	19.64
Shannon-Weaver index of species diversity (H')	2.71	2.12	2.03
Index of equitability (E)	0.90	0.73	0.75

Table 5. The biometrical characteristics of the *Collaripyxidia dongtingiensis* sp. n.

Character	$\bar{x}$	M	max	min	s	V	n
Length of shell	186	187	191	180	3.15	1.69	12
Diameter of shell	78	78	82	71	3.16	4.05	12
Length of collar	25	25	29	22	2.49	9.96	12
Diameter of aperture	56	56	60	52	2.39	4.27	12
Length of spines	25	25	29	20	2.35	9.40	12

Measurements in μm, $\bar{x}$ - arithmetic mean, M - median, max – maximum, min - minimum, s - standard deviation, V - coefficient of variance in %, n - number of specimens

adapting to live there. Additionally, the adjacent stations have slightly more similar testacean communities than the distant ones. That it, the communities of the Stations L and O (Jaccard index 0.226), the Stations O and R (Jaccard index 0.138) are more similar than those of the Stations L and R (Jaccard index 0.090).

Description of *Collaripyxidia dongtingiensis* sp. n. (Figs. 3-6)

Description: in general the shell is ellipsoidal, in cross section circular or almost circular. The shell is divided into two well distinguishable parts. The anterior part is the circular collar with aperture, the posterior part is ellipsoidal "body" with two conspicuous hollow spines equal in shape and size. The posterior part of the shell is covered with relatively large polygonal mineral particles. Collar and spines are covered with fine mineral grains. The shell is greyish or grey-brown in colour. Mineral particles on the "body" of the shell are often bordered with visible thin stripes from the organic cement (brown-yellow in colour). The aperture lies in the anterior part of the shell (in collar). It is large, circular with very fine undulation on its border.

Dimensions (in μm): total length of the shell - 180-191; diameter of the shell - 71-82; length of the collar - 22-29; diameter of the aperture - 52-60; length of spines - 20-29. For detailed biometrical characteristics see Table 5.

Protoplasm colourless, pellucid. In the interior one nucleus, one contractile vacuole and 2 - 4 food vacuoles are visible. Food is composed probably of little diatoms (rests of the diatom frustules were observed in the food vacuoles). Pseudopodia are broad, finger-like (lobopodia-type).

Location: People's Republic of China, Lake Dongting, Chenglinji Channel. Fine sand sediment poor in organic debris. Sampling date September 13, 1996. Leg. Song Biyu.

Ecology: benthic freshwater species.

Type material: one holotype slide and three paratype slides are deposited in the V. Balík collection of the microscopical slides (Institute of Soil Biology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic).

Derivatio nominis: the name of this species is designated after the site of the finding - Lake Dongting.

Differential diagnosis: *Collaripyxida dongtingiensis* sp. n. differs from the previously described *C. stankovici* by the presence of two spines on the posterior part of the shell. Also the dimensions of *C. dongtingiensis* are somewhat larger than in *C. stankovici* (see Table 5). Both of these species resemble to *Centropyxis marsupiformis*. Both of these of the genus *Collaripyxidia* differ from *C. marsupiformis* in a more distinctive collar and in the presence of larger hollow spines (*C. stankovici* - one spine, *C. dongtingiensis* - two spines).

DISCUSSION

The majority of the species found in the present study have previously been reported elsewhere, no other endemic species have been found, with the only exception of one species being new to science (*Collaripyxidia dongtingiensis* sp. n.). Species composition, number of species, dominating species and abundance and biomass values are comparable with the literature data obtained in similar habitats. Both the number of testate amoebae species and the diversity indices of the testate amoebae assemblages in the sandy samples are low, which can be

attributed to extremely inorganic environments. There is very little content of plant debris and amorphous organic detritus in the sediment. Food resources scarcity will surely result in poor testate amoebae assemblages. On the other hand, different sized grains of the sediment, together with continuous sedimentation of sand and mud brought by the water flow, can form complex habitat heterogeneity for testate amoebae, and therefore can explain the rather high evenness components. The Station L, which is located far in the lake has the highest organic matter content (Table 1) and the smallest water flow velocity among the three stations. Relative ample food resources and more stable environments may explain the most diverse testacean community at this habitat. Meanwhile, from the lake to the river, the environments become unstable as the water flow velocity increases, and therefore, less diverse testacean communities occur in the outlet and river habitats.

Conclusions

A total of 42 testate amoebae species and varieties were identified from the sediment samples of the outlet of Lake Dongting to the ChangJiang (Yangtze) River, one of which is new for science and 27 are new to the testate amoebae fauna of the People's Republic of China. The most numerous were representatives of the genus *Centropyxis*, followed by *Difflugia* and *Cyclopyxis*.

The most dominant species was *Plagiopyxis callida* at the outlet and in the river, while *Centropyxis aculeata* in the lake.

The benthic testacean communities in the three habitats were obviously different; the adjacent stations had more similar testacean communities than the distant ones; only three species, *Centropyxis aculeata*, *Centropyxis ecornis* and *Phryganella acropodia* var. *depressa*, were observed at all of the three habitats.

The outlet habitat had the greatest total abundance and biomass values, in the river they were at the smallest.

From the lake to the river, both the numbers of species and the Shannon-Weaver diversity index values were decreasing, although the equitability index at the outlet habitat was a little lower than of the river habitat.

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ACTA
PROTOZOOLOGICA

Description of Two New Species of *Parvicapsula* Shulman, 1953 (Myxosporea: Parvicapsulidae) Parasitic in the Urinary Bladder of Marine Fishes, *Paralichthys olivaceus* and *Kareius bicoloratus*, from the Coast of the Yellow Sea, China

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Summary. Two new species of the genus *Parvicapsula* (Parvicapsulidae), found in the urinary bladder of marine fishes from the coasts off Weihai, Rongcheng and Qingdao (Tsingtao), China, are described: *Parvicapsula kareii* sp. n. from *Kareius bicoloratus* and *Parvicapsula anisocaudata* sp. n. from *Paralichthys olivaceus*. The diagnostic features of *P. kareii* sp. n.: trophozoite monosporous, disporous trisporous; spore broad and short, 13.2 ± 1.5 (11.7 - 15.6) x 7.6 ± 0.8 (7.0 - 8.9) μm in size, lateral line bent and thin; a pair of valves with two small unequal tail-like processes posteriorly; two subspherical polar capsules at anterior end of spore, 3.2 ± 0.1 (3.0 - 3.3) x 2.4 ± 0.3 (2.2 - 2.8) μm in size, coelozic. *P. anisocaudata* sp. n.: trophozoite disporous; spore small spindle-like in lateral view and ellipsoid in frontal view, lateral line curved and thin, cell length (with process) about 18.5 ± 1.9 (16.7 - 22.0), length (without tail) x width x thickness is 12.9 ± 0.9 (12.0 - 14.4) x 4.8 ± 0.2 (4.9 - 5), 4.9 ± 0.11 (4.9 - 5) μm; two asymmetrical thecae valves forming two unequally long tails posteriorly; a pair of pyrimidal polar capsules, which are 2.7 ± 0.4 (2.4 - 3.2) x 2.0 ± 0.2 (1.9 - 2.2) μm in size; coelozic.

Key words: *Kareius bicoloratus*, marine fishes, Myxozoa, *Paralichthys olivaceus, Parvicapsula anisocaudata* sp. n., *Parvicapsula kareii* sp. n., Parvicapsulidae, urinary bladder.

INTRODUCTION

In China, taxonomic studies on myxosporeans are mainly focused on freshwater habitats and around 574 species have been identified and reported in last decades from freshwater fishes (Chen and Ma 1998). Compared with those investigations in freshwater biotopes, the forms from marine fishes remain, however, only poorly studied (Xie and Chen 1988).

Since Shulman (1953) isolated *Parvicapsula asymmetrica* from the marine fish, *Cyclopterus lumpus*, seven *Parvicapsula* have been so far described and named from marine habitats. Three species had been

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found from the lumen of the urinary bladder of fisher: *P. asymmetrica* Shulman, 1953 from *Cyclopterus lumpus, P. unicornis* Kabata, 1962 from *Callionymus lyra* and *P. lobata* (syn. *Ceratomyxa lobata* Evidokimova, 1977) Gaevskaya *et al.,* 1982 from *Austroatherina incisa.* In addition, *P. shulmani* Kovaleva & Gaevskaya, 1981 was isolated from the gall bladder of marine fish *Beryx splendens* whereas *P. hoffmani* Padma-Dorothy & Kalavati, 1993 from the intestinal muscles of *Liza macrolepis, P. renalis* Landsberg, 1993 from the lumen of posterior kidney proximal tubules of *Sciaenops ocellalus* and *P. minibicornis* Kent *et al.,* 1997 from the renal tubules and glomeruli of *Oncorhynchus nerka.* Hoffman (1981) found an unrecognized form of *Parvicapsula* sp. from *Oncorhynchus kisutch* while Lester and Sewell (1989) reported another form of *Parvicapsula* sp. from the kidney of *Chaetodon aureofasciatus* and *Diodon hystrix.*

As notes in most taxonomic studies of myxosporeans, the critical features for the species separation are the shape and dimensions of the spore, arrangement and number of polar capsules, number and shape (with or without process or striation on shell) of thecae valves.

During investigations on the fauna of protozoan parasites from marine fishes off the coasts of the Yellow Sea and the Bohai Bay, some myxosporean parasites were found. Among those, two *Parvicapsula* species (Parvicapsulidae) were observed in the urinary bladder of *Kareius bicoloratu* and *Paralichthys olivaceus* respectively, which are two common maricultural fishes in this area. Compared with the known species of *Parvicapsula,* the two organisms are believed to be new members of this genus.

MATERIALS AND METHODS

The host fishes *Kareius bicoloratus* and *Paralichthys olivaceus* were collected on two occasions in March to July, 1998 and April to June, 1999 from the Yellow Sea off the Weihai and Qingdao coast.

The nucleus and polar capsules were observed from fresh spores in wet mount preparations and were revealed using the Giemsa's staining (Lom and Dykova 1992). The mixture of glycerin-alcohol (ethyl)-formalin (G. A. F.) was employed to show the structure of both mature spores and immature spores as well as the plasmodia (Chen 1981).

Materials were observed and measured at the magnification of 1250 x. The illustrations were drawn with the aid of camera lucida and computer program Photoshop 5.0.

DESCRIPTION

Parvicapsula kareii sp. n. (Figs. 1-8)

Class: Myxosporea Bütschli, 1881
Order: Bivalvulida Shulman, 1953
Family: Parvicapsulidae Shulman, 1953
Genus: *Parvicapsula* Shulman, 1953
Host: *Kareius bicoloratus* (Basilewsky)
Site of infection: urinary bladder cavity.

Geographic distribution: coastal waters off Weihai (37°30, N, 122°06, E), Qingdao (36°08, N, 120°43, E) and Rongcheng (37°18, N, 122°42, E), the Yellow Sea, China.

Incidence: 10/20 (50%)

Pathogenicity: urine appears milky white with small white dots.

Type-specimens: one slide as holotypes (No. wh-19980617) is deposited in the College of Fisheries, Ocean University of Qingdao, Qingdao, China. Paratypes (2 slides) are deposited in the Department of Biology, Chongqing Normal Colloge, Chongqing, China.

Diagnosis: trophozoite monosporous, disporous trisporous; spore broad and short, lateral line bent and thin, a pair of valves with two small unequal tail-like process at posterior end; two subspherical polar capsules at the anterior end of spore, coelozic.

Description: early stage of plasmodium small and irregular in shape (Fig.1), where 8 nuclei were clearly observed. Monosporous, disporous or trisporous plasmodia distinctly recognizable (Figs. 4, 5, 7), which measured 8.8 - 13 x 7.8 - 18.9 μm. Mature spores wider on anterior end (Fig. 2) and peach-shaped in oblique sutural view (Fig. 8), broad ellipsoid in frontal view (Fig. 3). Sutural line weakly sinuous and bent in middle but not twisted on its axis. Two shell valves thin-walled, smooth, unequal tail-like appendiculate respectively (Figs. 2, 3). Spore cavity often separated into two parts, i.e. anterior and posterior part. Two polar capsules subspherical or pyriform, about the same size located anteriorly and arranged side by side (Figs. 2, 3, 6, 8). Posterior part of the cavity partly filled with binucleated, rounded small mass of sporoplasm which is often finely granulated, (Fig. 6). Neither iodinophilous vacuole in the sporoplasm nor mucous envelope around the spore observed.

Measurements (in μm, n = 20) based on spores (treated with G. A. F., from a single host): length of
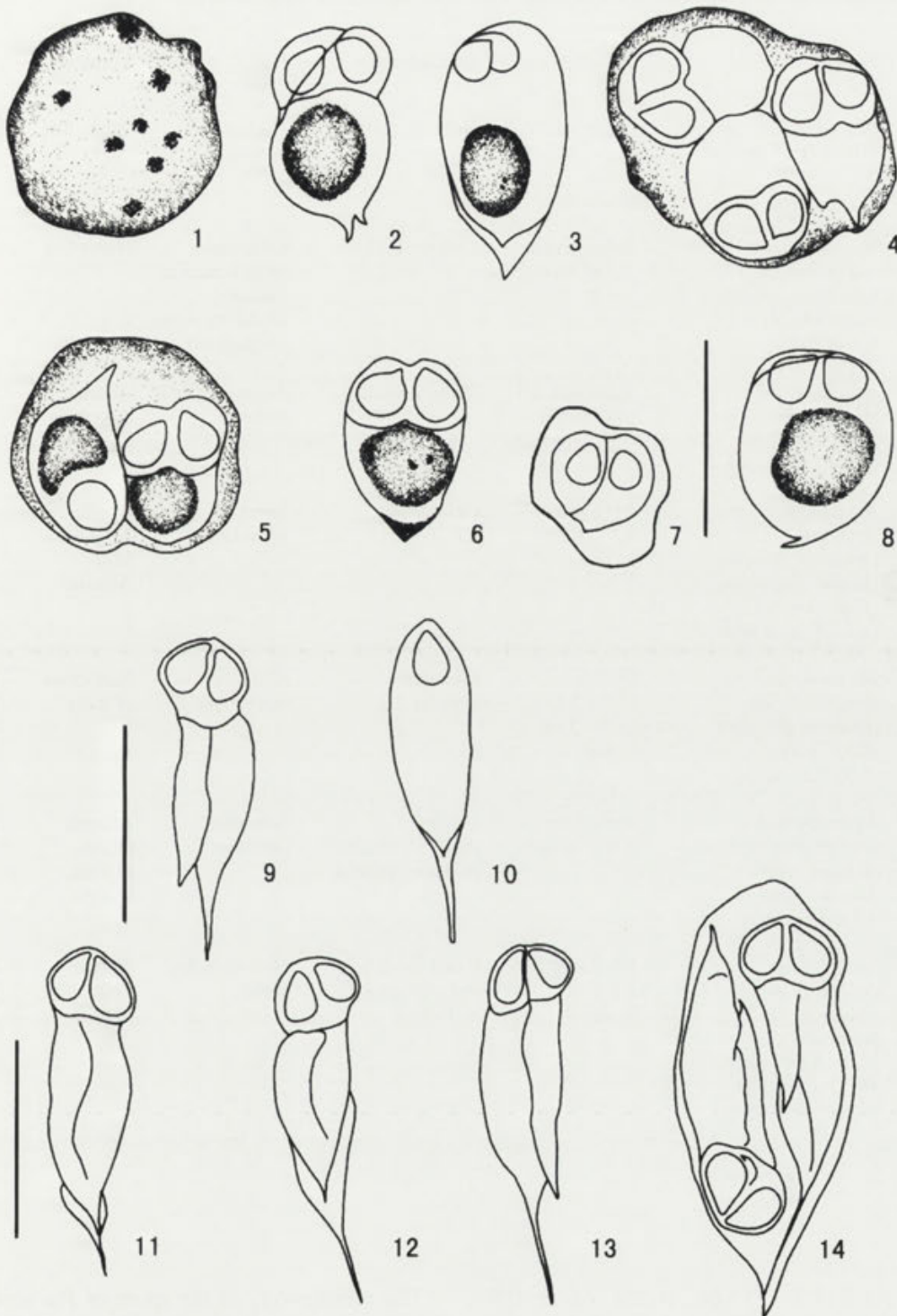
Figs. 1-14. Schematic drawing of plasmodia and spores. **1-8** - *Parvicapsula kareii* sp. n. **1** - early stage of plasmodium with 8 nuclei; **2, 6, 8** - mature spore in lateral view; **3** - mature spore in oblique view; **4** - plasmodium with three spores; **5** - plasmodium with two spores; **7** - plasmodium with one developing spore. **9-14** - *Parvicapsula anisocaudata* sp. n.; **9, 11-13** - mature spore in lateral view; **10** - mature spore in frontal view; **14** - plasmodium with two spores. All of above are from fresh materials. Scale bars - 10 μm

Table 1. Comparative studies of known species of *Parvicapsula* Schulman, 1953, measurements in μm

Parasite	Spore	Polar capsule	Infected organ	Host	Locality	Data source
P. asymmetrica Shulman, 1953	Asymmetrical, smooth, (10 - 17) x 7 in size	Subspherical, (1.5 - 2.5) x 1.5 in diameter	Urinary bladder	*Cyclopterus lumpus*	White Sea	Shulman, 1953
P. unicornis Kabata, 1962	Asymmetrical, one prominent horn-like posterior outgrowth, (7 - 8) x 4 in size	Subspherical, 1.5 in diameter	Urinary bladder	*Cllionymus luza, Limanda limanda, Lepidorhombus whiffiagonis*	North Sea	Kabata, 1962
P. lobata Evdokimova, 1977	Asymmetrical, smooth, (14.0 - 14.7) x (6.3 - 7.0) in size	Spherical, 3.5 in diameter	Urinary bladder	*Austroatherina incisa*	Atlantic, Argentina	Evidokimova 1977
P. schulmani Kovaleva and Gaevskaya, 1981	Asymmetrical, one small pin-like posterior process, grooves on valves, (10.6 - 14.9) x (4.2 - 5.3) in size	Pyriform, 1.3 - 1.5 in size	Gall bladder	*Beryx splendens*	North Atlantic, Mid-Atlantic	Kovaleva and Gaevskaya, 1981
P. hoffmani Padma-Dorothy and Kalavati, 1993	Asymmetrical, one blunt lappet-like posterior projection, (8.5 - 10.3) x (5.6 - 6.5) in size	Pyriform (3.0 - 3.8) x (1.7 - 2.6) in size	Intestinal muscles	*Liza macrolepis*	East coast of India	Padma-Dorothy and Kalavati, 1993
P. renalis Landsberg. 1993	Asymmetrical, smooth, the posterior of spore wider than the anterior, 9.5 x 4.7 in size	Pyriform, 3 x 2 in size	Lumen of posterior kidney proximal tubules	*Sciaenops ocellalus*	Atlantic Ocean, Florida, U.S.A.	Landsberg, 1993
P. minibicornis Kent, Whitaker and Dawe, 1997	Symmetrical, pyriform, two posterior processes, 11.0 x 6.8 in size. Mucous envelope at posterior spore	Pyriform 2.5 x 1.3 in size	Renal tubules and glomeruli of kidney	*Oncorhynchus nerka*	British Columbia, Canada	Kent, Whitaker and Dawe, 1997

spores 13.2 ± 1.5 (11.7 - 15.6), width 7.6 ± 0.8 (7.0 - 8.9); length of polar capsules 3.2 ± 0.1 (3.0 - 3.3), width 2.4 ± 0.3 (2.2 - 2.8).

Comparison: *Parvicapsula kareii* sp. n. is characterized by its broad, short and asymmetrical body shape, thecae valves with unequally long tail-like processes at the posterior end, a pair of subspherical polar capsules as well as the bent and thin suture.

The morphology of the spore of *Parvicapsula kareii* corresponds to that of *P. minibicornis* Kent *et al.*, 1997 having similar size and (in some way) body shape. However, according to the original description (Kent *et al.* 1997), the new species differs by conspicuously asymmetrical body shape (*vs.* symmetrical), two unequally long tail-like process (*vs.* two short similar ones), subspherical polar capsules (*vs.* pyriform) and different organ locations

Table 1. (contd)

P. anisocaudata sp. n.	Asymmetrical, smooth, two spine-like tails, 12.9 ± 0.9 (12.0 - 14.4) x 4.8 ± 0.2 (4.9 - 5) x 4.9 ± 0.1 (4.9 - 5) in size	Pyriform, 2.7 ± 0.4 (2.4 - 3.2) x 2.0 ± 0.2 (1.9 - 2.2) in size	Urinary bladder	*Paralichthys olivaceus*	Yellow Sea, China	Present paper
P. kareii sp. n.	Asymmetrical, smooth, two tail-like processes, L. 13.2 ± 1.53 (11.7 - 15.6) x W. 7.6 ± 0.8 (7.0 - 8.9)	Subspherical, 3.2 ± 0.1 (3.0 - 3.3) x 2.4 ± 0.3 (2.2 - 2.8)	Urinary bladder	*Kareius bicoloratus*	Yellow Sea, China	Present paper

(urinary bladder in *Kareius bicolorutus vs.* renal tubles and glomeruli of kidney in *Oncorhynchus nerka*) (Table 1).

Parvicapsula anisocaudata sp. n. (Figs. 9-14)

Host: *Paralichthys olivaceus* (Temminck *et* Schlegel)
Site of infection: urinary bladder (cavity).
Geographic distribution: off the Qingdao (36°08, N, 120°43, E) coast of the Yellow Sea, China.
Incidence: 3/12 (25%)
Pathogenicity: urine appears milky white with small white dots.

Type-specimens: one slide as holotypes (No. qd-19980428) is deposited in the College of Fisheries, Ocean University of Qingdao, Qingdao, China. Paratypes (2 slides) are deposited in the Department of Biology, Chongqing Normal Colloge, Chongqing, China.

Diagnosis: trophozoite disporous; spore small spindle-like in lateral view and long ellipsoid in frontal view, lateral line curved and thin, two asymmetrical thecae valves forming two non-equally long tails at posterior end, two pyramidal polar capsules.

Description: early stage of plasmodia or trophozoites not seen, but some spore-producing trophozoites (plasmodia) observed, which were usually elongated spindle-shaped with two developing spores. Peripheral layer of hyaline non-granular ectoplasm enclosed the two developing spores (Fig. 14). Trophozoites or plasmodia disporous and measured about 22.8 x 9.8 μm.

Mature spores small spindle-shaped in lateral view (Figs. 9, 11-13) and ellipsoid in frontal view (Figs. 10), suture line curved and thin (Figs. 9, 11-13). Anterior end slightly rounded and posterior end with unequally long (about 5.5 ± 0.8 (4.4 - 6.7) spine-like tails (Figs. 9, 11-13). Two shell valves smooth, asymmetrical (Figs. 9, 10, 12, 13). Two polar capsules pyriform, about the same size, located anterior of spore and arranged side by side, resembling two eyes of a goldfish. Binucleated sporoplasm was located in the posterior end of the spore cavity. Neither iodinophilous vacuole in the sporoplasm and nor mucous envelope around the spore observed.

Measurements (in μm, n = 20) based on spores (treated with G. A. F., from a single host): total length of spore (with process) 18.5 ± 1.9 (16.7 - 22.0), length of spore (without process) 12.9 ± 0.9 (12.0 - 14.4), width 4.8 ± 0.2 (4.9- 5), thickness 4.9 ± 0.1 (4.9 - 5); length of polar capsule 2.8 ± 0.4 (2.4 - 3.2), width 2.0 ± 0.2 (1.9 - 2.2).

Comparison: the most characteristic features of *Parvicapsula anisocaudata* are the long asymmetrical body shape, ellipsoidal thecae valves with spine-like tails, 2 pyriform polar capsules and a waved sutural line.

The new species differs from *P. kareii* described above in that it has a long ellipsoid body shape (*vs.* broad ellipsoid), 2 long spine-like tails (*vs.* 2 short tail-like processes), pyriform polar capsule (*vs.* subspherical) and different ratio of the polar capsule length to the cell length (*ca* 1 : 4.5 *vs.* 1 : 6). In addition, the two parasites are found

in a different host (*Paralichthys olivaceus vs. Kareius bicolorutus*).

There are at least 3 morphologically similar forms, *P. unicornis* Kataba, 1962, *P. shulmani* Kovaleva & Gaevskaya, 1981 and *P. hoffmani* Padma-Dorothy & Kalavati, 1993, which should be compared with our new species. They possess also asymmetrical spore body shape, long extension at the acapsule end of the spore. All those species exhibit, however, only one process at the posterior end and have different body shape (i. e. asymmetrically oval with one horn-like outgrowth by *P. unicornis*, ellipsoidal with a small pin-like process in *P. shulmani*, broadly oval with a blunt lappet-like posterior projection in *P. hoffmani*) (Table 1).

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ACTA
PROTOZOOLOGICA

Remarks on articles by K. Mikrjukov on taxonomy and phylogeny of heliozoa, I and II

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At the dawn of the 21st century, I consider that changing the status of taxa on the basis of compiling documents that refer to genera and/or species reported once and/or described too succinctly is unhelpful and is likely to further confuse a classification already complicated enough. Light, scanning and transmission electron microscopy with their many technical refinements and molecular approaches have become absolutely necessary for assessing taxonomic relationships satisfactorily.

In the article entitled "Taxonomy and phylogeny of heliozoa. I. The order Desmothoracida Hertwig et Lesser, 1874", I have selected the section concerned with the discussion of *Cienkowskya mereschkowski* since it refers to a significant example of a species reported once and described succinctly, that led to further misidentification, synonymy and confusion.

In his paper, K. Mikrjukov considers, that the status of the species *Cienkowskya mereschkovckii*, (Cienkowski, 1881), needs to be re-evaluated. Although,

this species "has never been recorded since its original discovery", K. Mikrjukov recognizes characters of desmothoracids on the basis of Cienkowsky's sketch drawings (Figs. 4 A-C in the present manuscript), and claims that other species were erroneously considered to be this one. It is likely that these errors came from Villeneuve's description and drawing of a spherical heliozoan that he referred to *C. mereschkowskyi* (Cienkowski, 1881) Schaudinn, 1896. This centroheliozoan was borne on a hollow stalk and covered with a mucous coat including tiny siliceous spicules. The cell center was occupied by a dense granule from which axial "filaments" arose and the nucleus was eccentric. Villeneuve's misidentification was perpetuated in Rainer's Fauna „Die Tierwelt Deutschlands" (1988). If I agree with K. Mikrjukov that the species described by Villeneuve (1937), Jones (1974), Febvre-Chevalier and Febvre (1984), and Mikrjukov (1994) were presumably different from that found by Cienkowski, I consider that K. Mikrjukov lacks indisputable criteria for placing the Cienkowsky's species among the desmothoracids. As noted by Mikrjukov himself for *Clathrulina*, I consider that the shape of the capsule, size and ornamentation are not reliable taxonomic criteria, especially when they are mainly based on sketch drawings. In 1996, Mikrjukov

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gave the diagnosis of a species called by him "*H. febvre-chevalierae* sp. nov. (Syn.: *Cienkowskya mereschkowskii* (Cienkowsky, 1881), *sensu* Febvre-Chevalier & Febvre, 1984 and *sensu* Mikrjukov 1994)" suggesting that this new name corresponded to the species described by Febvre-Chevalier and Febvre in 1984 and himself. However, in the present paper, he writes "Febvre-Chevalier and Febvre (1984) and Mikrjukov (1994) studied a stalked marine centrohelid, *Heterophrys minutus*". My questions therefore are: is the species name *Heterophrys Febvre-Chevalierae* synonymous with *H. minutus?* If so, why has the name been changed? (The drawing illustrating the species *H. minutus* in the present paper does not resemble that found in Villefranche sur Mer by Febvre-Chevalier and Febvre). I am not in agreement with K. Mikrjukov when he creates new combinations for genera and/or species insufficiently described. Is it useful, for instance to substitute the name *Heterophrys minutus* comb. nov. to *Actinolophus minutus* Walton 1905 if this change cannot be justified by an accurate comparison? Walton's diagnosis was based on a very succinct description of a single specimen of a tiny heliozoan bearing very thin, short radiating pseudopods. The cell body was borne at the tip of a stalk (70μm long) and covered with a gelatinous coat. Taking the general behaviour of heliozoa into account, especially those forming a mucous stalk, none of these characters is stable enough to be regarded as a valuable criterion. The size of the cell depends on both food and life cycle; the stalk being secreted, its length increases progressively and the heliozoan can periodically leave its mucous envelope and reconstitute a new one; the axopods being extremely versatile features, their length varies according to the retraction or extension states. My opinion is therefore that changing the generic or specific names without giving sufficient arguments is unhelpful.

In the paper entitled "Taxonomy and phylogeny of heliozoa. II. The order Dimorphida Siemensma, 1991 (Cercomonadea classis n.): diversity and relatedness with cercomonads" K. Mikrjukov proposes to change the status of the genus *Dimorpha* Gruber, 1882 on his own interpretation of a drawing (Fig. 1A) of Blockmann (1894) (erroneously referred to in the first version of the MS as Gruber, 1882). K. Mikrjukov writes, "A clearly visible nucleus is shown in his (Blockmann's) figure near the posterior periphery of the cell but it is surely wrongly labelled as a food vacuole". I am not in agreement with this opinion and think that Blockman's drawing shows something resembling more a prey organism in a food vacuole than a nucleus. On many occasions, K. Mikrjukov uses a peremptory tone "surely wrongly labelled...", "...This (Shouteden) mistake was followed later by Belar, Penard... ", "...which was wrongly considered by further investigations... ". Considering in particular the remarkable quality of the ultrastructural description of *Dimorpha mutans* by Brugerolle and Mignot, in comparison with Blockmann's sketch drawing dating from 1894, I persist in finding that this part of the paper is based on intuitive judgment. K. Mikrjukov also reconsiders the taxonomic position of the species *Dimorpha floridanis* whose behavior was described by Bovee in the 60s. As noted by K. Mikrjukov himself, "... no morphological basis is presented in the description of this organism". Therefore, what are the arguments for such a change?

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Letter to the Editor

A new species of the genus *Rhizoplasma* Verworn (1896)

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In the littoral zone of the coast from Santa Cruz (La Palma, Canary Islands), a marine plasmodial rhizopod was found whose network looked very similar to that of the freshwater rhizopod *Reticulomyxa filosa*. In March 1999 the specimen was collected and brought to Jena in a plastic flask. The central cytoplasm (400-600 μm across) of this marine rhizopod was orange coloured (Figs. 1-2). When picked out of the dish and put into a new one, half an hour later the cytoplasm formed a new network of about 6 to 20 mm (Fig. 3). The cytoplasmic transport of mitochondria and vesicles within the reticulopods was bi-directional with mean velocity of about 15-20 μm s^{-1}. The ectoplasm was thin and clear with attached debris, the endoplasm was granular containing numerous vesicles filled with prey and debris. The rhizopod feeded on bacteria, flagellates and mostly diatoms, whose empty shells marked the tracks of the former network (Figs. 1 and 4). Large prey was transported on the surface of the reticulopods. The six found multinucleated specimen lived for five to seven month in Petri dishes at room temperature (22-24 °C) on the window-sill (west side) of the laboratory. The seawater was replenished once a week without supplementing additional food. The diatoms growing in the seawater were prey enough. Some weeks later, two other reticulates of the same species were found in sand-water samples from Puerto de la Cruz (Tenerife, Canary Islands), indicating a broader distribution of plasmodial rhizopods in this region.

Reticulate amoebae are important grazers of bacteria in marine and freshwater sediments and play an important role in cycling of benthic carbon and nutrients (Grell 1994, Rogerson *et al.* 1995, Hausmann and Hülsmann 1996). They seem to be cosmopolitan organisms, which are not yet well investigated. Only two marine genera of order *Athalamida* are known: *Rhizoplasma* and *Pontomyxa*. *Pontomyxa flava*, described by Topsent in 1892 (see Lee *et al.* 1985), is larger (contracted 2-3 cm long) than our species from La Palma and yellow coloured. The species *Rhizoplasma kaiseri*, found by Verworn in 1896 (see Lee *et al.* 1985), has also an orange central body but its network revealed less connections between the pseudopodia. Following the principle of nomenclature applied by Grell (1994) for *Synamoeba arenaria* the new isolate is proposed to be named *Rhizoplasma arenaria* because it also lives between sand grains.

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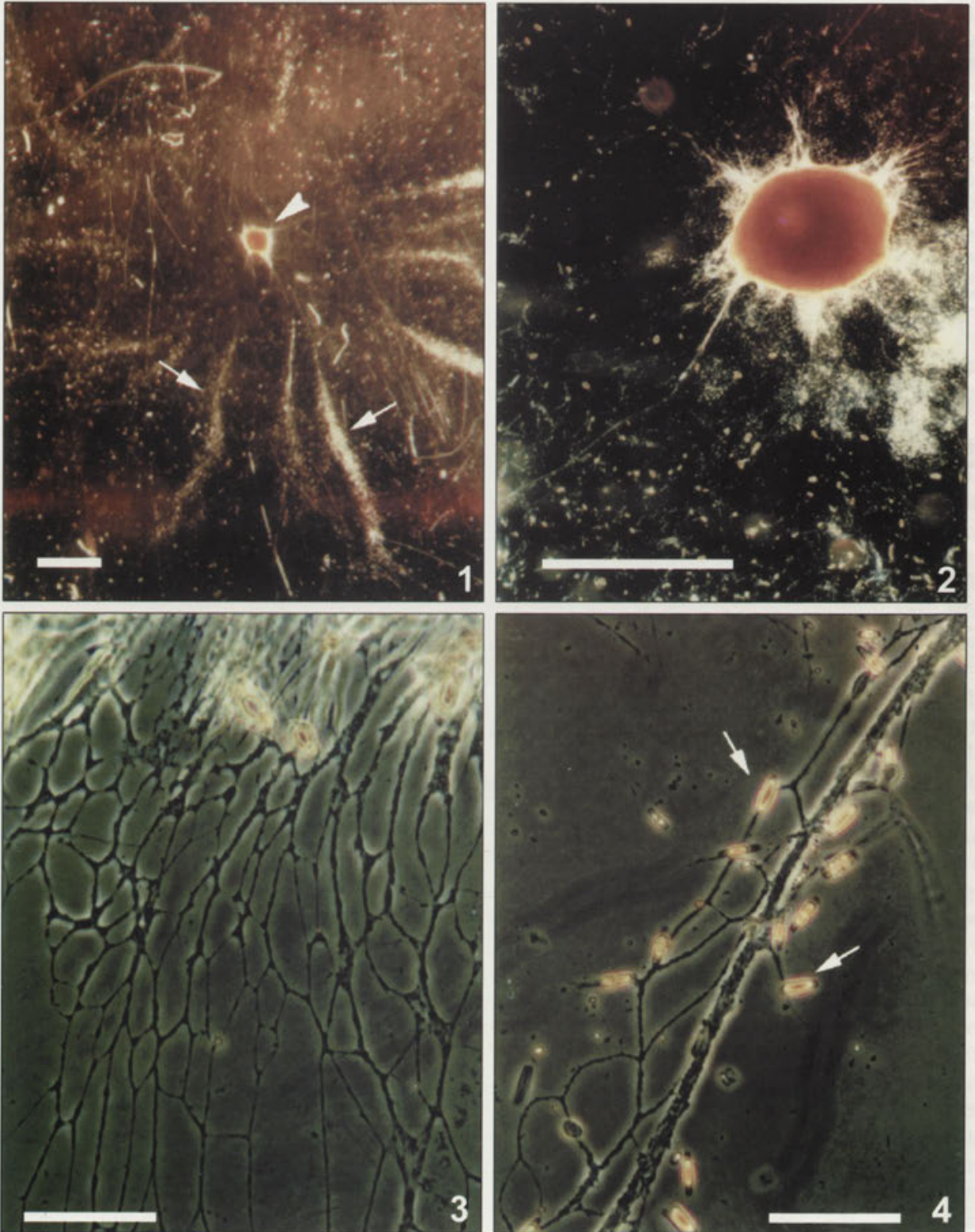
Fig. 1. *Rhizomyxa* sp. Overview. The arrowhead and the arrows indicate the central body and tracks of the network marked by empty diatom shells, respectively. Scale bar - 1000 μm
Fig. 2. Larger image of the central body with outgrowing new network. Scale bar - 1000 μm
Fig. 3. Larger image of the network. Scale bar - 50 μm
Fig. 4. Diatoms (arrows) transported along the rhizopds. Scale bar - 50 μm

Unfortunately the organisms did not divide or produce cysts or any resting stages and died after some months. So there are only photos and videos, which the authors would like to show to protozoologists who are interested in or have seen a similar marine plasmodial rhizopod.

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Book Review

Identification and Ecology of Limnetic Plankton Ciliates by W. Foissner, H. Berger and J. Schaumburg. Bayerisches Landesamt für Wasserwirtschaft (Herausgeber und Verlag), München 1999. 793 pp., with 2783 figures, 59 tables

The book by W. Foissner, H. Berger and J. Schaumburg entitled „Identification and Ecology of Limnetic Plankton Ciliates" was published at the end of 1999. This extensive monograph is the effect of 3 years hard working, with success! It consists of 793 pages with 2783 photographs and line drawings, and 59 tables. It gives for the first time a wide view on the limnetic plankton ciliates in all aspects. The book guides the reader through the subject trying to render all information in an attractive and easy to understand way. The authors are hoping that this book „not only shows how taxonomy and revisions should be performed, but also will contribute to make identifications more usual and reliable in protozooplankton science". Furthermore, as authors pointed out, „pelagic ciliates and protozoa in general have been ignored for a long time by plankton ecologist", in spite that „they form an integral part of the planktonic food web".

The book consists of 7 chapters, and 3 of them seem to be most essential. In „Investigation of Plankton Ciliates and Species Concept" the reader might found the basic information how to work with living organisms and a list of methods useful for ciliates observation and measurement. Detailed descriptions of staining, fixation and preparation for scanning electron microscopy are included. One can also learn the most useful methods for the best visualization of different intracellular structures, thereby effective observation.

The chapter „General Ecology" contains a lot of information about i.e. abundance, biomass, productivity, diversity, and gives a list of 725 ciliate species from freshwater and saline inland pelagials, while the subchapter „Ecological Characterization of the Species De-scribed in this Book" contains the list of chosen 118 species and subspecies.

The most important chapter is „Identification, Description and Ecology of Species". „Key to the Main Groups and Species" deserves particular attention. It is clear, legible, and easy to use and it allows very quick identification of the examined organism. It allows to identify a given specimen alive, or after cell silver and protargol impregnation. After that the reader can focus on its precise recognition. In „Description and Ecology of Species", the main part of the book, one can found all universal, up to date information about 118 Ciliate species. Line drawings and photographs either of protargol impregnated or visualized by means of scanning microscope, are best ever seen. Each cilium, each spine or bristle are perfectly visualized as well as all details of the cell surface. Description of the species includes complete information about it's taxonomy, internal and external composition, morphologically similar species, and precise ecological data.

The chapter „Glossary to Ciliate Morphology" is full of general terms, useful and indispensable, for understanding of structural details. At the end - „Literature" gives the enormous list of references, those older and up to date.

I am sure that this book should be at the shelves of all libraries of protozoology, ecology, hydrobiology, biotechnology, zoology etc. departments, and at all places where it could serve as a model monograph.

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Häder D-P., Reinecke E. (1991) Phototactic and polarotactic responses of the photosynthetic flagellate, *Euglena gracilis*. *Acta Protozool.* **30:** 13-18

Books:

Wichterman R. (1986) The Biology of *Paramecium*. 2 ed. Plenum Press, New York

Articles from books:

Allen R. D. (1988) Cytology. In: *Paramecium*, (Ed. H.-D. Görtz). Springer-Verlag, Berlin, Heidelberg, 4-40

Zeuthen E., Rasmussen L. (1972) Synchronized cell division in protozoa. In: Research in Protozoology, (Ed. T. T. Chen). Pergamon Press, Oxford, **4:** 9-145

Illustrations

All line drawings and photographs should be labelled, with the first author's name written on the back. The figures should be numbered in the text as Arabic numerals (e.g. Fig. 1). Illustrations must fit within either one column (86 x 231 mm) or the full width and length of the page (177 x 231 mm). Figures and legends should fit on the same page. Lettering will be inserted by the printers and should be indicated on a tracing-paper overlay or a duplicate copy.

Line drawings (three copies + one copy without lettering)

Line drawings should preferably be drawn about twice in size, suitable for reproduction in the form of well-defined line drawings and should have a white background. Avoid fine stippling or shading. Computer printouts of laser printer quality may be accepted, however *.TIF, *.PCX, *.BMP graphic formats (**300 DPI at least**) on disk are preferred.

Photographs (three copies + one copy without lettering)

Photographs at final size should be sharp, with a glossy finish, bromide prints. Photographs grouped as plates (in size not exceeding 177 x 231 including legend) must be trimmed at right angles accurately mounted and with edges touching and mounted on firm board. The engraver will then cut a fine line of separation between figures. Magnification should be indicated. Colour illustration on transparent positive media (slides 60 x 45 mm, 60 x 60 mm or transparency) are preferred.

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Authors will receive one set of page proofs for correction and are asked to return these to the Editor within 48-hours. Fifty reprints will be furnished free of charge. Orders for additional reprints have to be submitted with the proofs.

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