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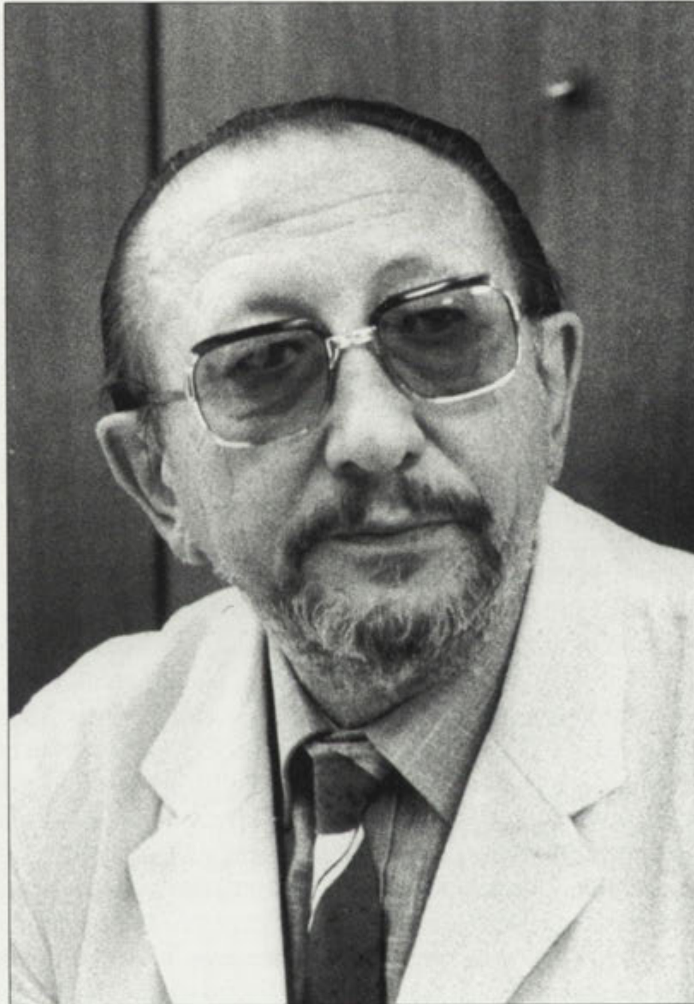

Karl-Ernst Wohlfarth-Bottermann (1923-1997)

Karl-Ernst Wohlfarth-Bottermann, professor emeritus of the University of Bonn died on September 29, 1997, at the age of 74. His death profoundly shocked and distressed many his friends all over the world. He counted among most outstanding authorities in the knowledge of the structural background and physiology of cellular movements. Protista, in particular the plasmodia of acellular slime moulds and free-living amoebae, were the preferred subjects of his studies. The death of Professor Wohlfarth-Bottermann is therefore a great loss for the general cell biology, as well as for the experimental protistology.

He was born on May 23, 1923. At the age of 19, immediately from the high school, he was enrolled into the army and sent to the East Front; soon wounded, he remained in hospitals till the end of the war. Then, for two years he was a farm worker in Low Saxony. The hard experience of these 5 years of the war and post-war period deeply

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influenced his personality, the sensitivity to human problems, openness to other people and other ideas, sense of reality and a hate of any prejudice or dogmatism as well in the life as in science.

Wohlfarth-Bottermann became student of biology in 1947 at the University of Köln, he obtained the doctor rer. nat. degree at the University of Münster in 1951 and began there his scientific career in the Department of Electron Microscopy. After a brief period spent in Braunschweig, he worked in Stockholm with F.S. Sjøstrand. Here his scientific interest has crystallised and concentrated on the ultrastructure of the ground cytoplasm. After habilitation he was appointed as the first Director of the Zentrallaboratorium für Angewandte Übermikroskopie at the University of Bonn. Due to his efforts a new research unit of the Bonn University, the Institut für Cytologie und Mikromorphologie, has been founded in 1965. Wohlfarth-Bottermann became its first Director and the first professor of cell biology in Germany. He retired in 1988, at the age of 65.

When Wohlfarth-Bottermann started in the late fifties and early sixties his study of the mechanism of cellular movements a much was presumed but virtually nothing was positively known about the nature and localisation of the conjectural contractile apparatus in the slime mould plasmodia and amoeboid cells. Since 1956 he started the systematic research on the ultrastructure of the groundplasm (cytoplasmic matrix) and the cytoskeleton in the large amoebae and slime moulds, mostly in the plasmodia of *Physarum polycephalum* a perfect subject to study the mechanism of cytoplasmic contractility and protoplasmic streaming. Wohlfarth-Bottermann used simultaneously the electron microscope methods and phase contrast light microscope, and he applied the technique of semi-thin sections, for following the changes in the ultrastructure of the cytoskeleton. This made it possible to correlate the formation and dissociation of the bundles of actomyosin filaments with the contraction and relaxation stages in the cytoplasm. He demonstrated the ATP-ase activity and contractility of these fibers and stressed the importance of an isometric contraction state for their formation.

These studies of Wohlfarth-Bottermann became well known after presentation at the first international symposium on the cell motility which was held in 1963, in Princeton. Nevertheless, for more than 10 years some scientists considered them as an investigation of artefacts. Only in the seventies the work of Wohlfarth-Bottermann became generally accepted, after being confirmed with other methods, including immunofluorescence, and in other material, the tissue culture cells. His publications were later considered in Current Contents, as the citation classics.

The research of Wohlfarth-Bottermann on the slime mould plasmodia was parallel to more biochemically oriented studies of Japanese workers, and it was also run in cooperation with Polish and Russian groups. After 1970, Wohlfarth-Bottermann developed the methods of correlating the study of dynamic organisation of the cytoskeleton with quantitative tensiometric measurements of forces produced during endogenous, oscillatory contractions of plasmodial strands. Together with co-workers he confirmed his earlier suggestions that in slime moulds the isometric contraction promotes the stabilisation of actin bundles, whereas the isotonic contraction results in their dispersion. Similar conclusions concerning stress fibers in the tissue culture cells have been advanced much later, in the nineties.

Wohfarth-Bottermann and his school made also great contributions to elucidating the nature and the generation site of the motor force of amoeboid movement. In his laboratory, over 30 years ago, it was for the first time demonstrated that the peripheral layer of the cytoplasm of amoebae, which appeared in the light microscope as optically empty and was called hyaloplasm, is in reality a submembrane network of actin microfilaments which produce the force needed for cell locomotion. In the course of following years Wohlfarth-Bottermann and his students, as well as other groups in U.S.A., Japan, U.K. and Poland, have confirmed the discovery of the cortical cytoskeleton, identified its components by immunocytochemical techniques, described their dynamics and functions in many cells. This should be remembered by younger generations, which accept now the presence and the crucial role of the cell cortex and cytoskeleton in cell machinery as something which is self-evident, as one of paradigms of the modern cell biology.

Professor Wohfarth-Bottermann was a talented teacher and many of his Ph.D. students and post-doctoral foreign associates became on their turn professors at different universities.

In recognition of his scientific merits and competence professor Wohlfarth-Bottermann was, i. a., elected a member of the Scientific Council of the Max-Planck-Geselschaft and a fellow of the Deutsche Akademie der Natürforscher "Leopoldina". He was one of the founders of the German Society for Cell Biology and its vice-president, and a member of the Board of Governors of ECBO, the European Cell Biology Organisation.

Also his editorial activity has significantly contributed to the progress of international cooperation in the fields of cell biology and experimental protistology. He was for several years one of the editors of old and prestigious journal,

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Protoplasma. In 1968 he founded a new journal, Cytobiologie, which quickly became well internationally known and 10 years later has been transformed into the European Journal of Cell Biology. Wohlfarth-Bottermann was its editor-in-chief until his retirement in 1988.

Karl-Ernst Wohlfarth-Bottermann belonged to those who have built the bases for the modern cell biology and he remarkably stimulated the international cooperation in this field for at least three decades. His involvement in the research, his friendly relations with foreign colleagues and fatherly attitude to young co-workers will be always remembered by those who had the privilege of meeting him.

Andrzej Grębecki and Włodzimierz Korohoda

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Acta Protozoologica (1998) 37: 5-15

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Review Article

Antarctic Tintinnids: Their Ecology, Morphology, Ultrastructure and Polymorphism

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Summary. Ciliates from the suborder Tintinnina are the cosmopolitan organisms living mainly in the upper parts of oceans and seas. In consideration of their morphology and role in food web they are an interesting material for researches. Besides the attributes typical for all Ciliata they have some internal and external features typical only for this group. I started my examination of Antarctic tintinnids from the analysis of species composition and seasonal succession in waters of Admiralty Bay and the Weddell Sea (Wasik and Mikołajczyk 1990, 1994a). *Cymatocylis convallaria*, with a short lorica horn dominated during the austral summer, while *C. affinis* with the long aboral part of the lorica dominated during the winter. In spring and fall the transition in loricae shape from one form to the other was observed, a finding which may indicate that some previously separate species are in fact only polymorphs of one species. To furnish evidence for this suggestion, protoplast morphology and ultrastructure were compared (Wasik and Mikołajczyk 1991, 1992) with special emphasis on the pattern of somatic and oral ciliature (Wasik and Mikołajczyk 1994); the results unequivocally showed that both *C. affinis* and *C. convallaria* are indeed polymorphic forms of one species. Tintinnids are known as ciliates with a protoplast protected by different forms of loricae. Depending on the species, the lorica might be hyaline and partially or totally agglutinated by mineral and/or biological particles. The possibility that tintinnids select particles during lorica formation is still being debated, but I tried to address this issue. The results indicated that particles are selected by them. The investigations were not limited only to an examination of the material agglutinating the loricae, but included analyses of the morphology and microarchitecture of the lorica wall (Wasik *et al.* 1996; 1997a,b). On the base of electron scanning and transmission microscopies it appeared that depending on species lorica surface morphology revealed differences, while wall microarchitecture is very much the same in a form of honeycomb.

Key words: infraciliature, lorica, microarchitecture, polymorphism, seasonal succession, ultrastructure of protoplast.

INTRODUCTION

Ciliates belonging to the suborder Tintinnina are cosmopolitan organisms, though most are found in marine waters (Pierce and Turner 1993). It must be stressed, however, that they are to be found in different environments and climatic zones, although particular genera may typically be found only in certain areas. Despite their geographic distribution, as much as 90 % of tintinnids are found in the upper water column, which may be about the top 100 m in warmer waters (Middlebrook *et al.* 1987) and only 50 m in the Southern Ocean (Buck *et al.* 1992).

Tintinnids have a number of special attributes which distinguish them from other Ciliata, including (1) the lorica

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covering protoplast, intracellular structures such as (2) morulas, with lorica-building material and (3) capsules responsible, probably, for balance maintenance during fast swimming and (4) the reduction in the somatic ciliature (Corliss 1979). The latter, however, no longer participates in locomotion, but is involved instead in lorica formation and thus in protoplast protection, in the removal of waste products from the lorica, and in the positioning of the ciliate itself within the lorica. Cell movement is effected by an oral ciliature that extends beyond the lorica in the form of crown of membranelles (Laval-Peuto and Brownlee 1986).

Loricae have been of use in palaeontological studies. By 1885 Rüst had found tintinnid fossils in Jurassic coprolites in Europe, while Lagerheim (1901) identified the modern species *Codonella cratera* in Pleistocene deposits. Fifty years after tintinnid fossils were found for the first time, loricae were noticed in Jurassic and Cretaceous rocks also in Australia, Cuba, Mexico, Venezuela, and the United States (see Tappan and Loeblich 1968). The oldest fossils of tintinnid loricae originate from the Ordovician, i.e., 500 million years (Chennaux 1968).

Scientific interest in ciliates belonging to the suborder Tintinnina began towards the end of the nineteenth century (Entz 1884, Daday 1887), when they were identified in plankton samples. From this point they have been described systematically. Many years later Zeitzschel (1967) considered that these organisms comprise an important element of the food web, linking producers and higher level consumers (Middlebrook *et al.* 1987). Some algae and bacteria are too small to be consumed by larger plankton feeders (e.g. the microzooplankton) (Fenchel 1987), but food particles consumed by tintinnids covers a broad spectrum in terms of size, ranging from 1-2 μm in diameter to *ca* 40-45 % of the lorica's opening diameter. Thus, small tintinnids such as *Lohmaniella spiralis*, feed mainly on bacteria (<1 - 2 μm), while the largest consume dinoflagellates, diatoms and small ciliates, whose diameter does not exceed 40 μm (Heinbokel 1978).

Sherr *et al.* (1986) noted that tintinnids have a high grazing capacity, and pointed out that they can consume up to 60% of an ecosystems primary production. This is possible since they have high metabolic and reproduction rates (Taniguhi and Kawakami 1985; Verity 1985, 1986). Other authors (Heinbokel and Beers 1979, Capriulo and Carpenter 1980), however, report considerably lower tintinnid grazing rates (20-40% of primary production), but did not question their role in marine ecosystems. Tintinnids are not only important as consumers but also as a food source for mesozooplankton, which, in turn, provide food for birds, and marine mammals (Rakusa Suszczewski and Nemoto 1989, Alder and Boltovsky 1991).

LORICA - A PROTOPLAST PROTECTING STRUCTURE

Tintinnids are ciliates with protoplast protected by a lorica. The loricae, depending on the genera and species, differ morphologically, being hyaline or agglutinated by mineral and/or biogenic particles. Foreign material may be adhered to the whole lorica or only to its lower part which is called the bowl. In the latter case the upper part (collar) always remains hyaline. Many years ago they played a significant role in tintinnid identification, a system then based solely on lorica morphology (Kofoid and Campbell 1929, Marshall 1969). Even small changes in lorica shape and size would lead to the description of a new species. More recently it has transpired that tintinnids show considerable polymorphism, and their classification has thus had to be revised.

Polymorphism is induced by variations in the environment (Laval-Peuto and Brownlee 1986) which influence the secretion of lorica building material. It was believed initially that the "old" lorica divided together with the protoplast or it might originated as a result of peeling of the cell's outer surface (see Tappan and Loeblich 1968). Later, Campbell (1927) found in *Favella* that the place at which the lorica-building material is secreted, is a region near the oral apparatus, and that the secretion occurs before cell fission is complete. This process was described in most detail by Biernacka (1952), who agreed with Campbell (1927) on the site, but suggested that secretion occurred soon after binary fission (Fig. 1). She found in *Tintinnopsis* that the posterior fissiont (opist) remained in the "old" lorica, while the necked anterior fissiont (proter) constructed the "new" one. Lorica building material flowed back along the rotating cell and formed the lorica from the aboral to the adoral part. During this time foreign particles can be agglutinated to the forming lorica surface.

Tappan and Loeblich (1968) pointed on existing in the literature divergences in data concerning lorica formation process. It was proposed that lorica-building material might be secreted by the whole cell surface and lorica might be constructed from the adoral to aboral part. It was also suggested that various parts of the loricae may be constructed differently. Gold and Morales (1976) maintained that the bowl of *Tintinnopsis parva* lorica is formed by material secreted through the whole surface of the

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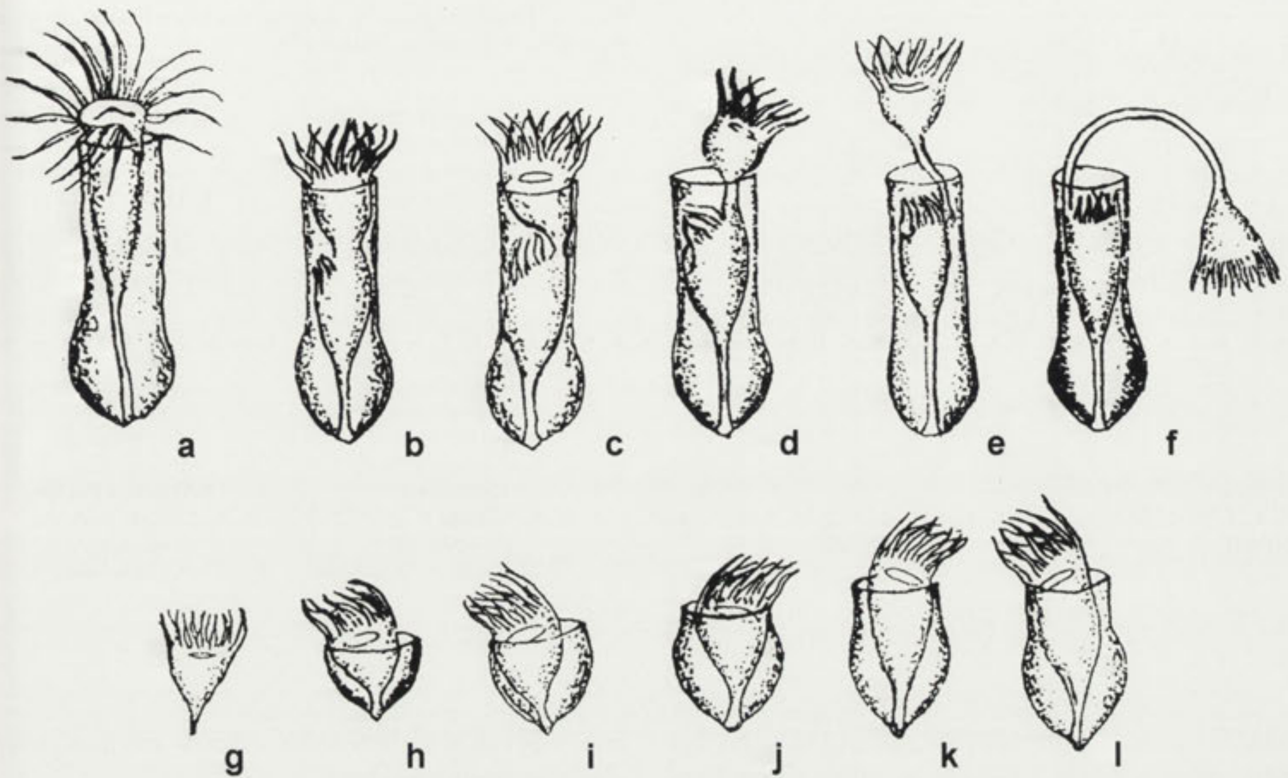

Fig. 1. Binary fission and lorica formation process (according to Biernacka 1952)

proter, while the upper lorica part - by material secreted near the cytostome. Clearly, the process of lorica formation requires much further study.

RESEARCH AREA

Polish studies of tintinnids to date have concerned only those from Baltic Sea, and focusing on species composition and distribution in coastal waters (Kirchner 1937; Biernacka 1948, 1952; Witek 1993). My work is the first to consider Antarctic tintinnid ecology, together with protoplast and loricae morphology and ultrastructure.

Through cooperation with the Department of Antarctic Biology of the Polish Academy of Sciences, I had the opportunity to examine material from the Southern Ocean around the South Shetland Islands, principally Admiralty Bay (Fig. 2). This bay is the largest on King George Island, and covers some 122 km^2 and attains a depth of > 600m. The Polish Antarctic station Henryk Arctowski is located here, and this provided the chance to study material collected over a year. Due to its location and hydrological conditions, the bay is an interesting site for marine research. It opens widely to the Bransfield Strait from where waters originating largely in the Bellingshausen Sea flow in (Tokarczyk *et al.* 1991). The annual salinity of the surface layer varies through the presence of melting icebergs and sea-ice in the summer, and it occasionally freezes in winter. On the other hand, the water temperature varies little, with a range of about -1.9°C to 3.1°C (Rakusa-Suszczewski 1993).

SPECIES COMPOSITION AND SEASONAL SUCCESSION

Ciliates comprise a significant part of the protozooplankton biomass (Garrison and Buck 1989). Though naked ciliates are the most numerous, tintinnids are also found in large numbers (Garrison 1991, Pierce and Turner 1992).

In Admiralty Bay and in the region between the South Orkneys and Elephant Island, 19 tintinnid species were represented in planktonic samples collected during the

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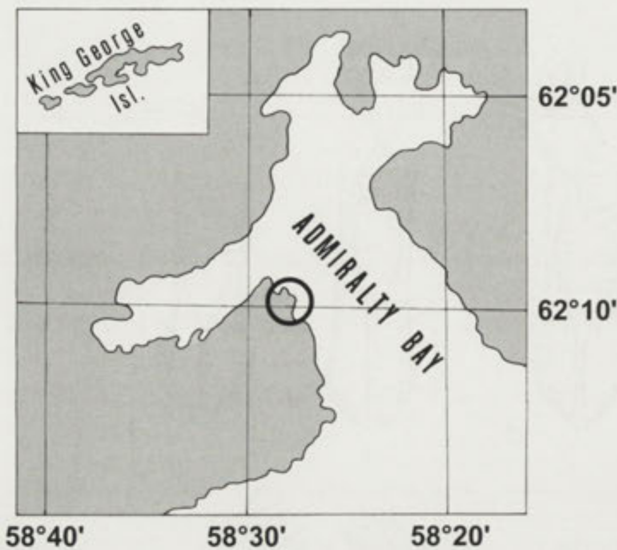

Fig. 2. Research area and location of Polish Antarctic Station (circle)

Table 1. Biogeographical localization of chosen Tintinnina genera (according to Pierce and Turner 1993)

1. Cosmopolitan	**4. Austral**
Amphorellopsis	*Cymatocylis*
Codonella	*Laackmanniella*
Codonellopsis	**5. Warm water**
Dictyocysta	*Amplectella*
Epiplocylis	*Brandtiella*
Eutintinnus	*Cyttarocylis*
Salpingella	*Dadayiella*
2. Neritic	*Epiorella*
Favella	*Petalotricha*
Helicostomella	*Rhabdonella*
Leprotintinnus	*Stelidiella*
Metacylis	*Undellopsis*
Stenosemella	*Xystonella*
Tintinnopsis	**6.Tropical Pacific**
3. Boreal	*Amplectellopsis*
Parafevella	*Codonopsis*
Ptychocylis	*Cricundella*

austral summer of 1988/1989 (Wasik and Mikołajczyk 1990); these included *Cymatocylis affinis/convallaria* (Fig. 8) the numerically dominant species which belongs to a genus endemic (Table 1) to the Southern Ocean. The cosmopolitan species *Codonellopsis balechi* (Fig. 3) and an endemic species, *Cd. gaussi* (Fig. 4) were also abundant but only at some sampling stations. Similar local dominations were reported by Boltovskoy and Alder (1992) in the central and south Weddell Sea for *Cd. gaussi* and *Laackmanniella naviculaefera* (Fig. 5). It was found (Wasik and Mikołajczyk 1990) that the total number of tintinnids in Admiralty Bay in December 1988 numbered up to 4000 cells/m^3, and was higher near Elephant Island where waters originating in the Bellingshausen Sea were encountered (Tokarczyk *et al.* 1991). At the other sampling stations, total cell numbers were considerably lower. The proximity of land might partially explain local increases in tintinnid density; such a relationship was described by Burkowsky (1976) for the White Sea. Conversely, such a correlation was not found to apply to two interchangeable Baltic dominants, *Helicostomella subulata* (Fig. 6) and *Tintinnopsis lobiancoi* (Fig. 7) (Wasik and Mikołajczyk 1996). At two closely situated stations, the number of tintinnid cells was occasionally found to vary considerably. According to Taniguchi and Kawakami (1985) this variation may be attributable to local differences in the distribution of phytoplankton. Since when tintinnids encounter dense prey concentrations they can being to feed immediately and thus grow at a maximum potential rate.

Tintinnid numbers are not only regulated by food availability, but also by temperature. Verity (1986) found this to be an important factor, and suggested that in Narragansett Bay tintinnids started multiplying only when the water temperature exceeded 6°C. In Admiralty Bay (Wasik and Mikołajczyk 1994b) despite stable and low surface waters temperature, number of tintinnids changes depending on the season, what is closely correlated with blooms in the phytoplankton. Seasonal changes in tintinnid numbers can also be found in the Indian Ocean, where water temperature varies little around 28 °C (Gilron and Lynn 1989). It should be stressed that temperature is an important factor in controlling the phytoplankton population, and thus it control tintinnid density. Light is another factor that cannot be overlooked. Such factors explain the occurrence of tintinnids in the upper water column only, since ciliates there find the temperature, light, and food regimes most conducive to growth.

POLYMORPHISM

Polymorphism in tintinnids requires that the systematics should be revised, and not based simply on loricae size and shape, but should also consider protoplast morphology and ultrastructure. Such new approaches can be

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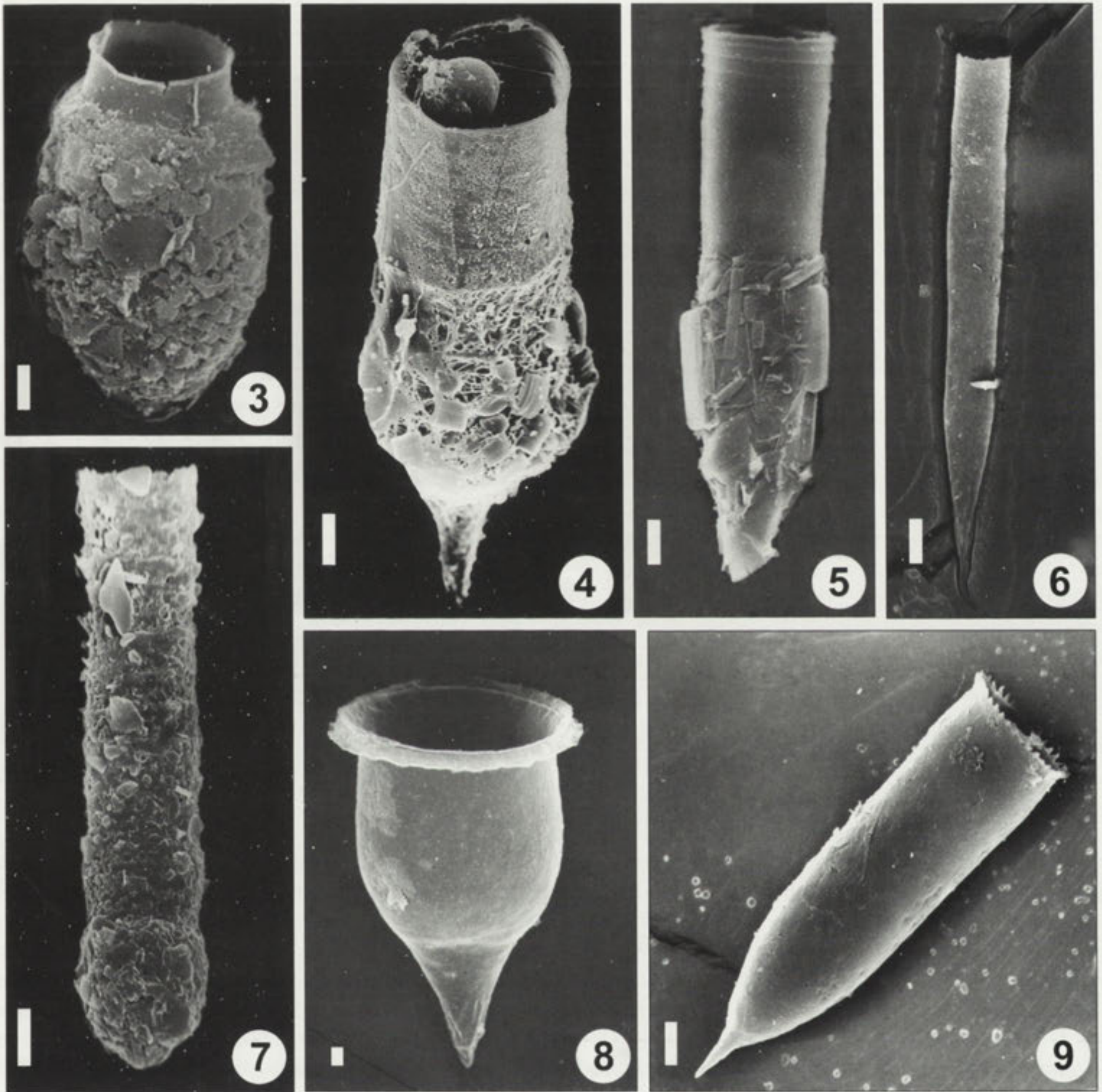

Figs. 3 - 9. Scanning electron micrographs of tintinnid species. 3 - *Codonellopsis balechi*, 4 - *Cd. gaussi*, 5 - *Laackmanniella naviculaefera*, 6 - *Helicostomella subulata*, 7 - *Tintinnopsis lobiancoi*, 8 - *Cymatocylis affinis/convallaria*, 9 - *Parafavella denticulata*. Scale bars - 10 μm

found in classifications prepared by Small and Lynn (1985) or Corliss (1994).

On the basis of morphometric analyses, Boltovskoy *et al*. (1990) suggested that previously separated Antarctic endemics, *C. affinis* and *C.convallaria*, are only polymorphic forms of one species and proposed that it be named *C. affinis/convallaria* (Fig. 8). Analysis of material collected in over almost one year (early 1990 to early 1991) from Admiralty Bay, provided us (Wasik and Mikołajczyk 1994b) with evidence that this suggestion is in fact correct. It appeared that forma *convallaria*, which is characterized by a shortening of the aboral horn of the lorica, was dominant in summer, while forma *affinis* with an elongated lorica dominated during winter. Spring and fall were periods during which transitional forms occurred, and transformation from one form to the other was

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evident through changes in lorica length. On the basis of differences in the size of the lorica's horn, it was possible to determine during which season the cells were caught.

During investigations of seasonal succession in the tintinnid population, it appeared that their high total numbers in Admiralty Bay during the austral summer were characterized by low species diversity, and dominance of the population by *C. affinis/convallaria* forma *convallaria* (90% by number). During the austral winter, low tintinnid numbers accompanied diverse species composition, with predominantly *C. affinis/convallaria* forma *affinis* (60% of total cell numbers), and also by *C. vanhoffeni*, *C. drygalskii* and *Codonellopsis balechi* (Wasik and Mikołajczyk 1994b).

During examination of plankton in Canadian coastal waters, Middlebrook *et al.* (1987) found that Tintinnina species with a protoplast protected by a hyaline loricae always dominated in summer, whilst those with an agglutinated one were more frequent in winter. Only the first part of this suggestion seems to apply to Admiralty Bay waters, since during winter the number of species with loricae agglutinated with particles increased, but they never dominated.

MORPHOLOGY AND ULTRASTRUCTURE OF *C. affinis/convallaria*

To date there have been no morphological or ultrastructural studies of the protoplasts in Antarctic tintinnids. On the other hand, those from other regions have been considered by a number of authors (Laval 1971,1972; Hedin 1975, 1976a,b; Brownlee 1977; Foissner and Wilbert 1979; Snieżek *et al.* 1991; Choi *et al.* 1992).

As a detailed analysis of the protoplast is a prerequisite to identification of the *C. affinis* and *C. convallaria* as polymorphs, we examined them both microscopically (Wasik and Mikołajczyk 1992, 1994a). To all intents and purposes, they appeared identical. Both were covered by two membranes, an internal one called the plasmalemma and an external one, the perylemma (Fig. 10). The perylemma is typical for the Choreotrichida (tintinnids) but has also been recorded in members of Oligotrichida and Stichotrichida (Bardele 1981); only in the tintinnids, however, it cover the cell body and spread onto the oral and somatic cilia.

Beyond the lorica extended a crown of 19 adoral membranelles. On the tips of *C. affinis/convallaria* cilia distinct swellings were located (Wasik and Mikołajczyk 1991). They were called by Tamarin *et al.* (1974) discocilia or paddle cilia and until now have only been observed in some marine invertebrates such as sponges (Bergquist *et al.* 1977) or molluscs (Verni 1985) and in one ciliate, *Euplotes* sp. (Verni 1985).

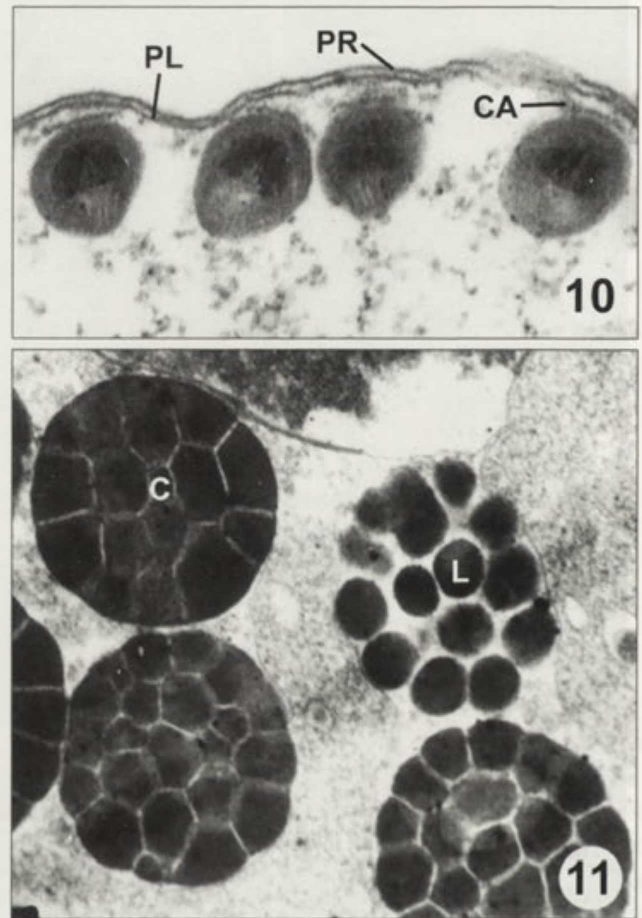

Fig. 10. TEM micrograph of capsules in *C. affinis/convallaria*. CA - cap, PL - plasmalemma, PR - perylemma. x 40000. Fig. 11. TEM micrograph of morulas in *C. affinis/convallaria*. C - closely packed, L - loosely packed. x 20000

Some features may be typical to a number of ciliates, but one of the intracellular structures that is found only in some tintinnids is the striae, located on the adoral membranelles. Their number differs between species; only two were found in *Tintinnopsis parva* (Laval-Peuto *et al.* 1979), three in *C. affinis/convallaria*, while more than 10 have been reported in members of the genera *Petalotricha* and *Cyttarocylis* (Laval 1971, 1972).

In striae, and in the cytoplasm at the posterior end of *C. affinis/convallaria* were found electron dense capsules (Fig. 10) (Wasik and Mikołajczyk 1992). Variations in their structure and size, according to Laval (1971), may be a feature that can be used in species identification,

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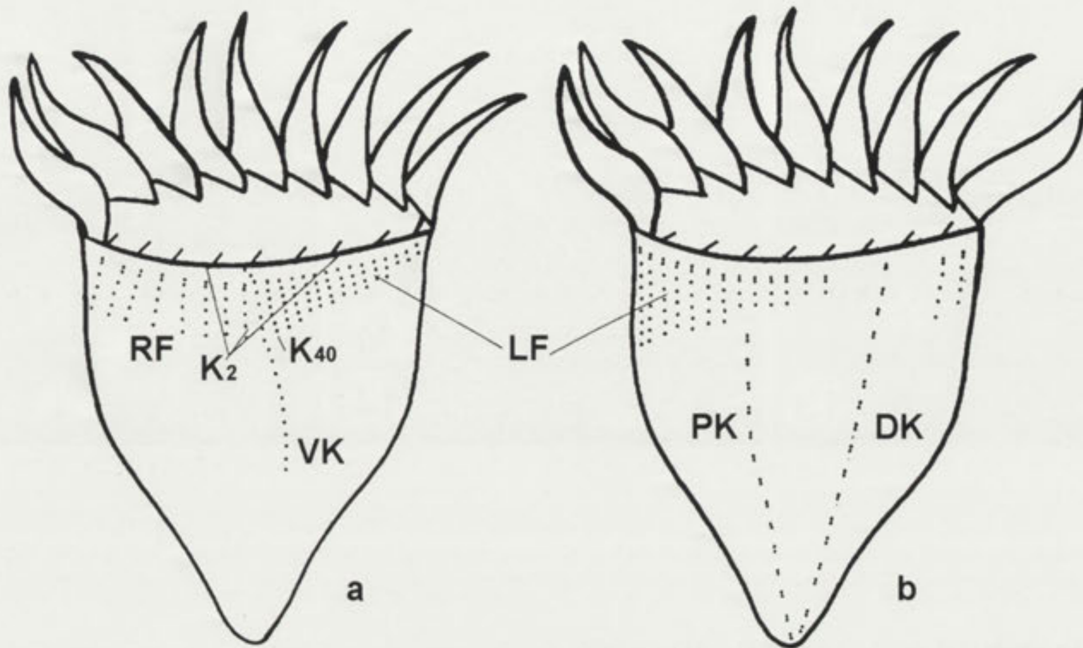

Fig. 12. Diagram of somatic ciliature of *Cymatocylis affinis/convallaria*. a - ventral surface, b - dorsal surface. DK - dorsal kinety, K_2 - second somatic kinety, K_{40} - last somatic kinety, LF - left ciliated field, PK - posterior kinety, RF - right ciliated field, VK - ventral kinety

and they thus became the basis of three groupings. Using transmission electron microscopy (TEM) I classified *C. affinis/convallaria* capsules as "grossly spherical, with a straight conical anterior pole". The pole is directed towards the plasmalemma and in this region (over the capsule) the electron dense structure called the cap (Fig. 10) is located. I found that the capsule and the cap, as well as the cap and plasmalemma, are connected by fibrils, which stabilize the whole structure with respect to the membrane (Wasik and Mikołajczyk 1992). The role of the capsules is not known, but it has been suggested that they might be a form of microtoxocysts, or may function as statocysts that help maintain balance during fast swimming (Laval 1971).

The most interesting structure that is typical only for Tintinnina are electron dense, osmiophilic granules containing material for new lorica formation (Biernacka 1952, Laval 1972, Hedin 1975). Depending on the species they can exist in form of sparse corpuscles (*Parafavella gigantea, Tintinnopsis parva*) or covered by unit membrane morulas (*Petalotricha ampula, Cyttarocylis brandii*) (Fig. 11). Morulas found to date in examined species were composed of either loosely or closely packed elements (Laval 1972). In *C. affinis/convallaria* cytoplasm we found both types existing simultaneously (Wasik and Mikołajczyk 1992). Their location changes during the cell's life cycle. In interphase they have been found in the posterior region of the cell. Before cell division they are observed directly under the crown of adoral membranelles such that after fission are found in the proter only.

A definitive answer on the existence of a polymorphic form is provided only by comparative analysis of oral and somatic ciliature; examination of protoplast ultrastructure alone is insufficient (Laval-Peuto and Brownlee 1986). In this respect, we considered *C. affinis* and *C. convallaria* in whom the pattern appears to be identical (Fig.12). The somatic ciliature was formed by 1 ventral (VK), 1 dorsal (DK), and 1 posterior kineties (PK), and two fields, the left built of 28 kineties (LF) and the right of 10 (RF) (Wasik and Mikołajczyk 1994a).

Comparative examinations of the *C. affinis* and *C. convallaria* (Wasik and Mikołajczyk 1992, 1994 a,b) have confirmed that these previously separate species have to be treated as polymorphic forms. These results were included by Petz *et al.* (1995) in a guide to Antarctic Ciliata.

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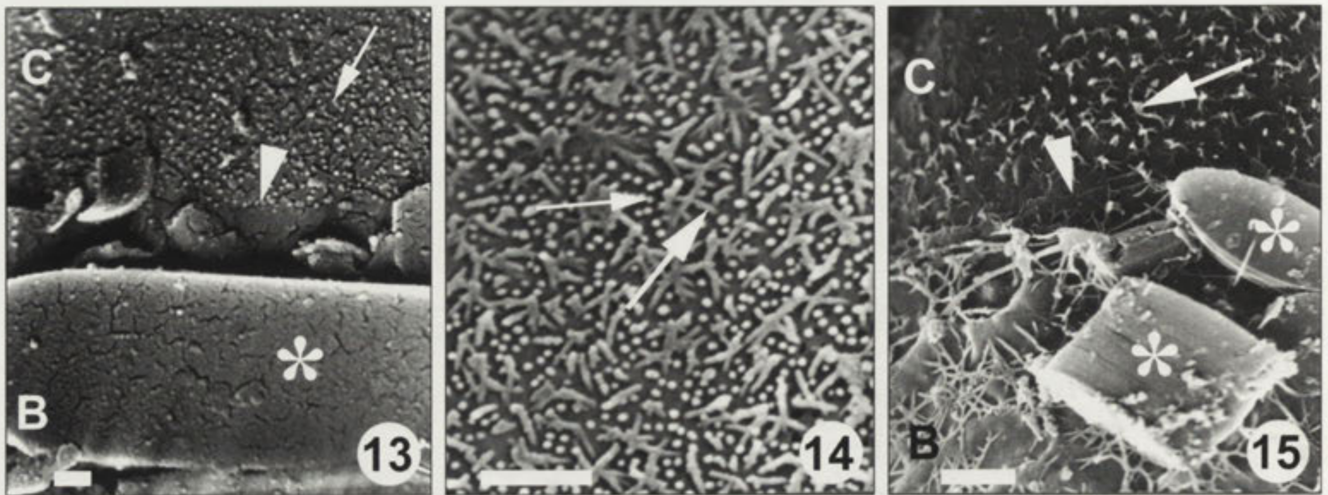

Figs. 13 - 15. Lorica surface morphology. 13 - *Laackmanniella naviculaefera*, 14 - *Parafevella denticulata*, 15 - *Codonellopsis gaussi*. B - bowl, C - collar, arrowhead - border between collar and bowl, small arrow -granules, big arrow - bristles, asterisk - diatoms. Scale bars - 1 μm

LORICA MORPHOLOGY AND MICROARCHITECTURE

Loricae are interesting but as yet not fully described structures. It might be suspected that different methods of lorica construction (Biernacka 1952, Tappan and Loeblich 1968, Gold and Morales 1976) give rise to the high variety of shapes and different properties of the collar and bowl, and thus variations in its surface morphology and wall microarchitecture. To address this issue I conducted a comparative examination of the surfaces and internal structure of three morphologically different loricae (Table 2) belonging to species from different areas (Pierce and Turner 1993), since environmental conditions may substantially affect lorica construction (Laval-Peuto and Brownlee 1986).

The microarchitecture of tintinnid loricae has also been a poorly addressed subject, even though Kofoid and Campbell (1929), and later Laval-Peuto and Brownlee (1986) pointed out that their structure might be vacuolar, tubular or compact; it has in fact only been described precisely for three species. In *Parafavella gigantea* it appeared to be trilaminar, comprising hexagonal prisms (Burkovsky 1973, Hedin 1975) whilst in *Favella ehrenbergii* (Hedin 1975) it had an irregular network of ridges and alveoli. In *C. affinis/convallaria* it is heterogeneous, being trilaminar in the adoral region, with distinct internal layering which becomes thin and finally decline (Wasik and Mikołajczyk 1992).

Comparison of the loricae from seven species (Table 2) revealed major differences between these in terms of their surface morphologies (Wasik *et al.* 1997a). In the case of the totally hyaline *H. subulata*, *C. drygalskii* and *C. vanhoffeni*, as well as in the hyaline collar of *L. naviculaefera* (Fig.13), the surface appeared to be granular, while in the hyaline lorica of *P. denticulata* (Figs. 9, 14) it was covered by both granules and bristles. On the other hand, on the lorica surface of the partially agglutinated *Cd. gaussi* (Fig. 15) only bristles of various lengths were observed. The surfaces of loricae that were totally or partially agglutinated by particles could be examined only in places free of foreign material, and always appeared smooth, as did the internal surfaces of all examined loricae.

Table 2. Examined tintinnid species

Species	Lorica	Occurrence
Helicostomella subulata	hyaline	Baltic
Cymatocylis drygalskii	"	Admiralty Bay
Cymatocylis vanhoffeni	"	"
Parafevella denticulata	"	White See
Tintinnopsis lobiancoi	totally agglutinated	Baltic
Laackmanniella naviculaefera	partially agglutinated	Admiralty Bay
Codonellopsis gaussi	"	"

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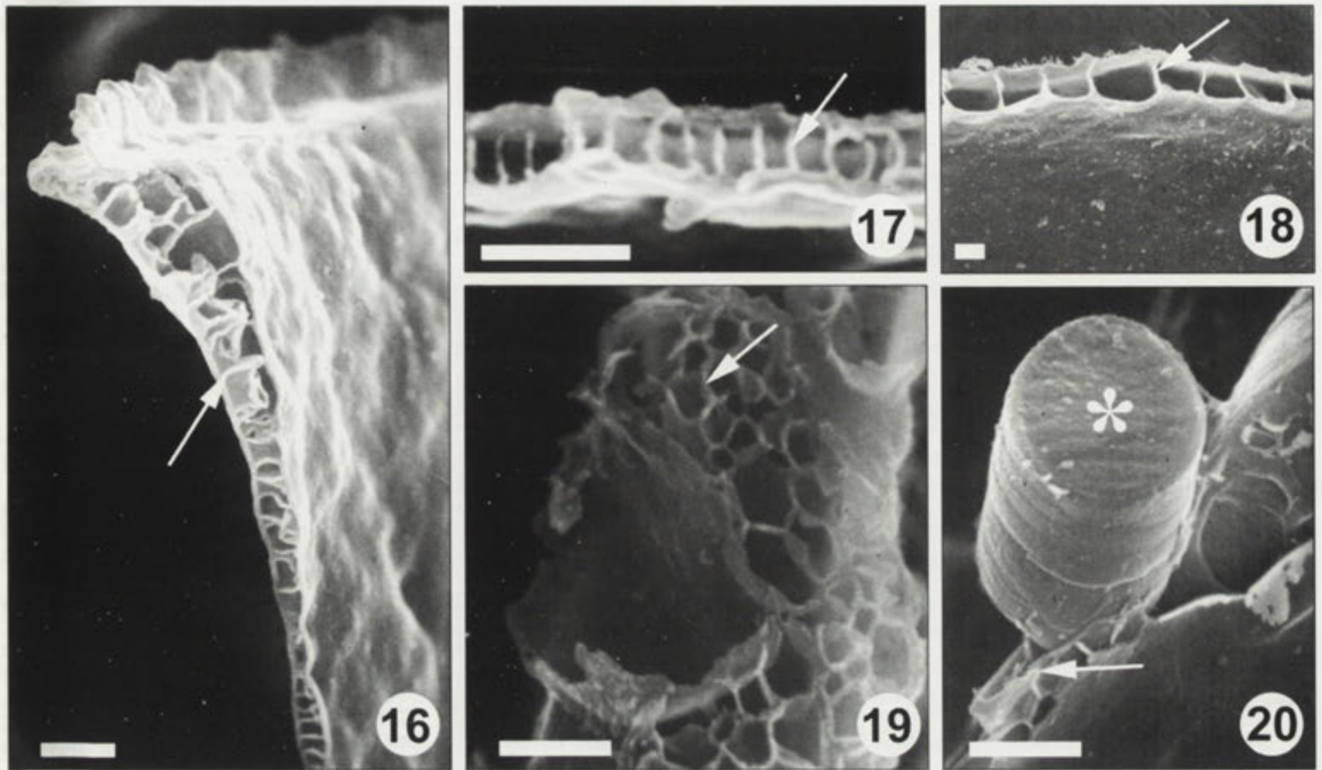

Figs. 16 - 20. Microarchitecture of loricae wall. 16 - *Cymatocylis vanhoffeni*, 17 - *Helicostomella subulata*, 18 - *Parafevella denticulata*, 19 - *Tintinnopsis lobiancoi*, 20 - *Laackmanniella naviculaefera*. Arrow - trilaminar construction of lorica wall, asterisk - diatom. Scale bars - 1 μm

In spite of the diverse loricae surface morphologies, their microarchitecture appeared similar (Wasik *et al.* 1997a). In all species it was trilaminar with a honeycomb-like structure (Figs. 16-20). Variations comprised only the size of the wall elements, as well as multiplication of the alveoli in the internal layer, and laminae construction.

Loricae are composed of chitin (Kofoid and Campbell 1929) or tektin (Dogiel *et al.* 1962) a complex of proteins and carbohydrates. X-ray microanalysis confirmed the organic nature of building material (Gold and Morales 1975, Wasik *et al.* 1997b), while staining with bromophenol blue showed the major constituents to be proteins (Gold and Morales 1975, Wasik *et al.* 1997b). By using alcian blue, we found that the partially agglutinated loricae of *Laackmanniella* and *Codonellopsis* contain acidic mucopolysaccharides (Wasik *et al.* 1997b).

LORICAE AGGLUTINATION

Though the active role of tintinnids in "catching" particles for lorica formation from the water column is not questioned, discussion centers on how and if they select them. In the case of partially or totally agglutinated loricae (Figs. 3-5,7), the proportions of particles of mineral or biological origin differs depending on the species. Those agglutinated solely by mineral particles are known as "arenaceous", whilst those with both mineral and biological material are described as "agglomerated" (Gold and Morales 1976).

The existence of arenaceous loricae confirms that tintinnids can select particles since they choose only mineral particles even in the presence of those of biotic origin (Gold and Morales 1975, Gowing and Garrison 1992). On the basis of my examinations it appeared that only empty diatom frustules comprised the bulk of the agglutinated biotic particles (Wasik *et al.* 1996).

Mechanisms of particle selection and agglutination have not been elucidated. Suggestions by Gold and Morales (1976) that arenaceous loricae might be formed in sediments and agglomerated ones in the water column seems unlikely. Gowing and Garrison (1992) considered it as impracticable because cells with arenaceous loricae would have to travel after division from deep waters

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(i.e. 2000m) to the surface. Such continuous migrations are difficult to accept considering that tintinnids divide at the rate of about 0.5 to 3 times per day (Verity 1986, Gilron and Lynn 1989).

The classification of loricae according to the nature of the agglutinating material proposed by Gold and Morales (1976) is incomplete since it did take into account loricae agglutinated by biological material exclusively. I found such phenomenon in *Laackmanniella naviculaefera* (Fig.20)(Wasik *et al.* 1996).

Additionally, on the surface of *Tintinnopsis lobiancoi* described as arenaceous, apart from mineral particles I found diatoms, particularly from the genus *Thalassiosira*. I also saw differences in diatoms composition on loricae of species belonging to one genus. On the surface of the Antarctic *Codonellopsis gaussi* empty *Fragillariopsis pseudonana* frustules dominated, while loricae of *Codonellopsis balechi* appeared to be more typically "arenaceous". In detail, both the nature and number of particles agglutinated to the loricae of *L. naviculaefera* were examined (Wasik *et al.* 1996). Those collected in 1988 and 1990 from the central part of Admiralty Bay were compared with those from various regions of the Weddell Sea collected during the austral summer of 1988/1989. It appeared that depending on the sampling time and place, the dominant diatom on the loricae changes between *Fragilariopsis cylindrus* and *F. pseudonana*. Comparing this observation with those of Ligowski and Kopczyńska (1991) and Kopczyńska (1993) for diatom species composition in the environment showed that dominants on tintinnid loricae were present in the water only in small number, what confirmed that tintinnids might select particles.

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ACTA
PROTOZOOLOGICA

Abundance and Diversity of Gymnamoebae at Varying Soil Sites in Northeastern U. S. A.

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Summary. Population densities, morphotypes, sizes and diversity of soil gymnamoebae were assessed at six different soil sites located at Torrey Cliff Nature Preserve and on the campus of the Lamont-Doherty Earth Observatory in Palisades, N.Y. Gymnamoebae were categorized into four morphotypes based on a previously published method. Only the pine grove and swampy soil contained populations of all four types of gymnamoebae. The mean densities (cells/g dry soil) were 1,182 (pine grove soil), and 1,399 (swamp soil). Only type 4 gymnamoebae (flattened, discoidal) such as *Platyamoeba* and *Vannella* sp. were detected in the remaining four sites. These, however, were in large quantities, with mean densities ranging from 3,218 for the soil under the rock to 5,055 for soil containing gravel. Diversity indices varied from a high value of 3.7 in the swampy soil to 1.8 to 2.6 in other locations.

Key words: biodiversity, ecology, soil gymnamoebae.

INTRODUCTION

Protozoa as members of all major ecosystems have a crucial role in the flow of energy and nutrients by promoting microbial activity and mineralization of essential nutrients at the base of the food chain (Band 1959; Coleman *et al.* 1980; Stout 1980; Rogerson and Berger 1981; Foissner 1987; Clarholm 1981, 1989; Casida 1989; Zwart *et al.* 1994; Anderson 1996). In addition, protozoa are useful as model systems in elucidating principles and theories of ecology and cellular physiology.

Gymnamoebae have been recognized as major components of soil biota, comprising 50 to 90% of the protozoa in soils and litters (Bamforth 1985). Nonetheless, they are relatively neglected in protozoan ecology, and additional studies comparing different niches are needed to assess abundances, diversity of morphotypes and variations in size that may correlate with predatory activity (Clarholm 1981, Schönborn 1986, Meisterfeld 1989, Cowling 1994). The present study uses a newer method of enumeration by laboratory culture methods that has been shown to be particularly useful in accounting for more gymnamoebae than some previously used methods (Anderson and Rogerson 1995, Darbyshire *et al.* 1996, Anderson 1997). Data are provided on abundances of morphotypes, sizes, and diversity of soil gymnamoebae inhabiting six different terrestrial locations during the summer months of one year at sites not altered by human activity. This investigation provides information on the numerical significance of soil-dwelling gymnamoebae in

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natural habitats within a relatively circumscribed geographical locale during the same summer season. These data provide additional information on the significance of gymnamoebae in terrestrial sites other than agriculturally worked soil where substantial data have been accumulated (Darbyshire and Greaves 1967, 1973; Clarholm 1981).

MATERIALS AND METHODS

Study Sites and Sampling Procedures

Six separate soil sampling sites were identified at the Torrey Cliff Nature Preserve and on the campus of the Lamont-Doherty Earth Observatory at a location approximately 1 km^2 (41° 00' 28" N, 73° 54' 39" W) near Palisades N.Y., U. S. A. All sampling took place during the months of July, August and September of 1995. These sites were chosen because they differed in ecological setting and physical structure and were considered likely to be characterized by diverse populations of gymnamoebae. The sites were: (1) - soil in a pine stand on a ridge near a paved lane, (2) - soil from the edge of a fresh water swamp, (3) - soil under a large but moveable, granite rock, (4) - soil under a dead and rotting log in the later stages of decomposition, (5) - soil under a bed of moss, and (6) - soil containing gravel. The gravel-containing soil was found near a road that had been abandoned for many years.

Two cylindrical soil core samples were taken from each site using cork borers, one for enumeration of amoebae and the other for moisture and organic content. All soil samples were placed in sealed plastic bags to prevent evaporation of moisture from the soil sample, and immediately taken to the laboratory at Lamont-Doherty Earth Observatory for analysis. The temperature of each soil site was measured *in situ*, and the time of day was recorded. Each site was sampled on two separate occasions during the same day. Data from replicate samples were compared to determine patchiness and variability in gymnamoeba abundances. Soil moisture was determined gravimetrically based on differences in weight before and after drying. Organic content was determined by differences in weight before and after combustion at 350°C.

Enumeration of Gymnamoebae

The number of amoebae per unit weight of soil was determined by laboratory culture methods (Anderson and Rogerson 1995, Anderson 1997) using multi-well plastic culture dishes containing nutrient agar as food source to support bacterial prey for the amoebae (Page 1983). A weighed portion of soil was suspended in a measured volume of micropore-filtered pond water and 10 µl aliquots were inoculated into each well of the culture dishes. Based on the proportion of wells containing amoeba growth out of the total number of wells, the number of amoebae per unit weight of soil was determined according to our standard method. The length and width of the amoebae in µm were also recorded using an ocular reticle in a phase contrast light microscope. Since there was a high diversity of species, and identification to species level by light microscopy is not always possible, the gymnamoebae were categorized into one of four broad morphotypic groups (Anderson and Rogerson 1995, Anderson 1997). Type 1 gymnamoebae are those with extended lobose or fine sub-pseudopodia during locomotion. Genera of this type include *Chaos*, *Echinamoeba*, *Acanthamoeba* and the familiar *Amoeba proteus*. Type 2 gymnamoebae are non-eruptive limax gymnamoebae, including *Hartmannella* and *Glaeseria*. Type 3 gymnamoebae are eruptive limax gymnamoebae, and type 4 gymnamoebae are characterized by a broad or fan shaped pseudopodia. An example of the former is *Vahlkampfia* and the latter are *Platyamoeba* and *Vannella*. In some samples, large reticulate or elongate gymnamoebae were found (e.g. *Arachnula*). These were categorized as "other". Diversity of amoebae in samples from each site was determined by the H equation (Anderson 1987).

RESULTS

Abundance and Diversity

The number and diversities of each gymnamoeba morphotype in the six soil sites (tabulated in relation to organic content, moisture, and pH of the soil) are presented in Tables 1 and 2.

The two samples from the pine grove are generally similar. All four types of gymnamoebae were found in both samples. The most abundant, however, was type 4. The second sample contained twice as many type 4 gymnamoebae as the first. The cause of this difference is not known. The moisture content is higher in the second sample (Table 1) due to a substantial rainfall that occurred between the morning and afternoon samplings. This is not a probable cause for the difference since doubling times for gymnamoebae are generally longer than several hours. The differences may reflect patchiness as may be expected in a site with considerable heterogeneity such as this pine stand location. Soils contain many microsites, where bacterial growth alternates with predation and death within millimeters (Clarholm 1981). The existence of microniches in a visually homogeneous sample may be an explanation for population variations. Moreover, type 4 gymnamoebae (typically *Vannella* and *Platyamoeba*) are usually small, fan-shaped or discoidal amoebae. They may respond more rapidly to opportunistic variables associated with microsite variability (Anderson 1997). The pine grove sample also had significant numbers of types 1, 2 and 3 gymnamoebae with total populations of 1,044 cells/g and 1,311 cells/g for samples 1 and 2 respectively. The diversity indices (2.2 and 2.6) are not the highest among the six sites sampled largely due to fewer numbers of type 1 gymnamoebae (less than 100) compared to densities of over 100 for the other three morphotypes. Moreover, the relatively large density of type 4 gymnamoebae tends to reduce diversity at this site. The major genera found in the pine soil samples were

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Table 1. Densities (cells/g dry soil) of gymnamoeba morphotypes in the pine soil and swamp soil sites in relation to amount of organics, moisture content, pH and diversity

Date	Site	Organics (%)	Water (%)	pH	Type 1	Type 2	Type 3	Type 3	Other	Total	Diversity
7/26	Pine Grove 1	5.1	11.9	6.2	73	254	145	536	36	1,044	2.6
7/26	Pine Grove 2	5.0	20.8	6.2	49	146	49	1067	0	1,311	2.2
										$\bar{x}$ = 1,182	
7/27	Swampy Soil 1	9.1	40.0	6.4	298	447	347	198	0	1,290	3.7
7/27	Swampy Soil 2	10.3	37.3	6.3	151	377	226	754	0	1,508	3.1
										$\bar{x}$ = 1,399	

Table 2. Densities[1] (cells/ g dry soil) of gymnamoeba morphotypes in the moss, rock, log, and gravel sites in relation to amount of organics, moisture content, pH and diversity

Date	Site	Organics (%)	Water (%)	pH	Type 3	Type 4	Other	Total	Diversity
8/14	Under Rock 1	7.8	18.0	5.9	0	3,118	54	3,172	2.1
8/14	Under Rock 2	6.8	16.5	6.4	0	3,255	0	3,255	2.0
								$\bar{x}$ = 3,218	
8/15	Under Log 1	34.0	33.5	6.1	0	4,398	0	4,398	1.9
8/15	Under Log 2	56.0	5.4	6.3	0	5,660	0	5,660	2.2
								$\bar{x}$ = 5,029	
8/17	Gravel Soil 1	6.6	4.7	6.3	0	4,398	0	4,398	2.5
8/17	Gravel Soil 2	5.6	5.4	6.3	0	5,660	53	5,713	2.5
								$\bar{x}$ = 5,055	
9/16	Mossy Soil 3	10.7	34.0	5.7	0	4,146	0	4,146	1.8
9/16	Mossy Soil 4	10.4	32.0	5.6	65	3,106	0	3,171	2.0
								$\bar{x}$ = 3,658	

[1]Type 1 and 2 gymnamoebae were not present in detectable numbers at these sites in late summer

Cashia (*limacoides*?), *Echinamoeba*, *Glaeseria*, *Vexillifera*, *Vahlkampfia*, *Hartmannella*, *Platyamoeba* and *Vannella*.

The soil sampled from the edge of the swamp was relatively high in diversity compared to the other five sites, though the abundances were not among the highest. Sample 1 with a total of 1,290 gymnamoebae/g had high diversity (3.7) with more equivalent balance in numbers of all four types of gymnamoebae. The abundances of each morphotype were found to be generally similar for the two samples except for type 4 in the second sample, where 754 cells/g of soil were found compared to 198 cells/g of soil in the first sample, again possibly related to differences in microporosity and available food supply.

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The greater density of type 4 gymnamoebae in the second sample reduced the diversity relative to the first sample. The major genera in the swamp samples were *Echinamoeba* (*exudans*?), *Glaeseria, Mayorella, Vahlkampfia, Hartmannella*, and *Vannella.*

Samples taken at all other sites contained largely type 4 gymnamoebae. The moss site contained respectively 4,146 and 3,106 gymnamoebae /g (Table 2). Only *Vannella* and perhaps *Platyamoeba* species were observed in these samples as indicated by the high numbers of type 4 gymnamoebae at this site. The soil sample from under the rock, under the log, and the gravel soil site were completely dominated by type 4 gymnamoebae (largely *Vannella*) and had the highest abundances of all the six sites. These soil samples had a range of properties as shown in Table 1. This may indicate the capacity of type 4 gymnamoebae to exploit diverse soil habitats.

Sizes of Gymnamoebae

Type 1 gymnamoebae were generally low in abundance throughout the six sites at our sampling locations and varied widely in size. For example, the range was 13 to 40 μm in the pine stand and swamp soil samples where they occurred most consistently in detectable numbers. The mean lengths (± standard error) for the two pine soil sites sampled were 14 μm (±1.0) and 25 μm (±6.7). For the two swamp samples, the mean lengths were 28 μm (± 4.2) and 44 μm (±1.0). The remainder of the samples did not contain any detectable type 1 amoebae. However, a large reticulate species, possibly *Arachnula* sp. (60 μm), was found in the second sample of the pine soil site. Type 2 gymnamoebae generally ranged in length from 10 to 38 μm at these two sites. However, the mean size varied. The mean lengths were 15 μm (±3.9) and 17 μm (±3.5) in the pine soils and 19 μm (±2.9) to 27 μm (±2.4) in the swamp soil sites. The eruptive limax amoebae including *Vahlkampfia* spp. (type 3) were also smaller in the pine soil site (15 ±2.3 to 18 μm ±1.5) compared to those in the swamp site (20 ±4.3 to 30 μm ±10.3).

Larger species tended to dominate the other sites with mean size ranges of 13 ±0.9 to 17 μm ±0.8 (soil under rock), 14 ±0.7 to 15 μm ±0.7 (gravel containing soil with maximum sizes of 30-38 μm), and 14 ±0.7 to 18 μm ±0.5 (moss covered soil site). Some larger species of type 4 gymnamoebae also occurred in the moss site ranging in size up to 25 or 30 μm. Type 4 gymnamoebae (*Vannella* and *Platyamoeba*) were generally small in all of the six sites with large numbers of species less than 10 μm. Since these are likely to be largely bactivorous and contribute to remineralization by their microbial trophic activity, we determined the percentage of type 4 gymnamoebae < 10 μm at each soil site: pine stand, 31%; swamp, 7%; under rock, 35%; under moss bed, 18%; under log, 73%; and mixed with gravel, 39%.

DISCUSSION

Abundance, morphotypes, and size of gymnamoebae varied substantially in soils of different composition across six sites (sampled July to September 1995) within a relatively small geographic area in northeastern U. S. A. The summer of 1995 was unusually dry with mean monthly precipitation in inches of 3.13 (July), 0.53 (August), and 3.22 (September, late in the month). However, most of the sites were quite moist in the range of 12 to 40 % (Tables 1 and 2), with the exception of the gravel soil site (*ca* 5% moisture) dominated by type 4 gymnamoebae. Although our enumeration method does not distinguish between encysted and active stages, the data present a conservative estimate of viable gymnamoebae. It is interesting how successfully the type 4 gymnamoebae have survived at this relatively unprotected site exposed to wind and insolation.

Of the six soil sites sampled, only the swampy soil and the pine soil contained all four types of gymnamoebae. Of these two, the swampy soil contained the highest abundances of all morphotypes (Table 1). Both of these soil sites were characterized by a relatively less acidic pH (6.2 to 6.4) compared to other sites where abundances were lower. Moreover, the swampy soil had by far the greatest moisture content (approximately 40%) as well as moderate organic content (*ca* 10 %). The swamp soil may contain microsites with sufficient moisture to support growth and motility of gymnamoebae, and sustain high bacterial populations as prey. The large proportion of gymnamoebae less than 10 μm in size is consistent with the presence of small microsites where these microamoebae may invade and engulf prey more successfully. The results suggest that among the six sites we examined, a swampy soil environment, characterized by a slightly acidic pH and a relatively high moisture content may be the most suitable to support a diverse range of gymnamoebae. We note that the abundances of gymnamoebae at our pine stand site are less than those reported by Clarholm (1981) for a podsolized pine forest site (10^5 to 2 x 10^6 amoebae/g of dry soil). These abundances, however, were reported from the humus layer at a site four days after a rain event. Our

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location is much more exposed and at an elevated site where there is likely to be more susceptibility to drying and insolation.

A more detailed analysis of the data in Tables 1 and 2 suggests that the single most important factor affecting gymnamoeba populations is the amount of soil moisture. However, not all morphotypes appear to be equally dependent on high moisture content to support relatively large populations of viable amoebae. Type four gymnamoebae were found in large numbers in August and September during drier periods and do not seem to be restricted to areas with large moisture content. The relative small size of the type 4 gymnamoebae <10 μm (in some locations up to 70 % of the total sample) may enable them to invade smaller pore spaces where water is retained and thus survive in areas with minimal moisture. They also may form cysts more readily and survive unfavorable conditions for longer times compared to other genera. This requires further examination.

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A Revision of Choanoflagellate Genera *Kentrosiga* Schiller, 1953 and *Desmarella* Kent, 1880

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Summary. A revision of the choanoflagellate genera *Desmarella* Kent, 1880 and *Kentrosiga* Schiller, 1953 is presented following a light and electron microscopical investigation of the type species of both genera: *Desmarella moniliformis* and *Kentrosiga thienemanni*. It is argued that differences between the type species of the two genera represents differences at the species level. The main generic character of *Kentrosiga* Schiller, 1953, namely, the presence of tentacle-like threads at the posterior end of the cell, is not considered to be a character even at the species level, since most choanoflagellate species illustrate this feature. On the basis of the data presented we consider the generic name *Kentrosiga* Schiller, 1953 is invalid as a younger synonym of *Desmarella* Kent, 1880. A revised list of species attributable to *Desmarella* Kent, 1880 is also given.

Key words: choanoflagellates, *Desmarella*, *Kentrosiga*, taxonomy.

INTRODUCTION

The two choanoflagellate genera *Desmarella* Kent, 1880 and *Kentrosiga* Schiller, 1953 belong to the family Codonosigidae (= Monosigidae) Kent, 1880, which is represented by naked forms either solitary or colonial, marine or freshwater. Species of both genera have a planktonic colonial stage in their life cycle, which is represented by a ribbon-like row of cells numbering 2 to 30 or more (Zhukov and Karpov 1985). All cells of a colony are usually located in mucilage which can be visualised by staining with methylene blue. Shape in colonial cells varies from ovoid to pyriform (Zhukov and Karpov 1985).

Colonies of both genera are similar in most respects. In fact, Schiller (1953) created the genus *Kentrosiga* using only one character, namely the presence of threads at the posterior end of the cells (Leadbeater 1985). The validity of the genus *Kentrosiga* has already created some doubts (Leadbeater 1985, Thomsen and Buck 1991), but the formal unequivocal justification of its invalidity has not been provided. To decide this matter it is necessary to compare the type species of each genus by isolating them as clonal strains and maintaining them in laboratory conditions.

The strain of one type species, *Kentrosiga thienemanni*, has already been investigated by light and electron microscopical methods (Karpov 1982, 1985). In the spring of

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1996 we received an unidentified strain of *Kentrosiga/Desmarella* sp. from the culture collection of the Institute of Inland Waters RAS (Borok, Russia) which we identify here as *Desmarella moniliformis*. This now makes it possible to revise the two genera.

MATERIALS AND METHODS

A strain of *Kentrosiga/Desmarella* sp. was received from the culture collection of the Institute of Inland Waters RAS (Borok, Russia). This strain was originally isolated from a freshwater sample collected under the ice of Rybinsk reservoir in April 1996. To obtain a culture with a high cell density, the strain was adapted to a medium comprising Pratt's solution (KNO_3 - 0.1 g/l, $MgSO_4 \cdot 8H_2O$ - 0.01 g/l, $K_2HPO_4 \cdot 8H_2O$ - 0.01 g/l, $FeCl_3 \cdot 8H_2O$ - 0.001 g/l; pH 7.0) and cereal leaf extract in a ratio of 5:1 respectively.

For light microscopy, cells were concentrated by centrifugation for 5 min., fixed with 0.7% glutaraldehyde, and then transferred to a slide for investigation. Observation of living and fixed cells was made using a Leitz Ortholux II light microscope fitted with Nomarski optics at magnification 1500x with oil immersion.

Cell measurements have been carried out on 25 cells. For species identification the key descriptions by Kent (1880), Schiller (1953) and Zhukov and Karpov (1985) have been used.

For electron microscopy, cells were fixed with 0.5% of osmium tetroxide in 0.05 M phosphate buffer at pH 7.2 for 10 min on ice, then fixed by 1.3% glutaraldehyde in 0.07 M phosphate buffer for 2 h at 4°C in the dark. Then, after postfixation with 1% osmium tetroxide in the same buffer for 1h at 4°C, a pellet was dehydrated in an alcohol series and embedded in Epon resin. Cells were sectioned using a diamond knife on the ultramicrotome "Ultracut" (Reichert). After staining with uranyl acetate and lead citrate, serial sections were investigated with an electron microscope JEM-1200 (Jeol).

RESULTS AND DISCUSSION

Identification of *Kentrosiga/Desmarella* sp. strain as *Desmarella moniliformis*

A general view of a colony is shown in Fig. 1. The number of cells within colonies in culture varies from 2 to 20, but the average number is 8. Each colony has a ribbon-like form, which may be slightly curved because of the length of the colony. Cells are ovoid in form and are attached to each other by short lateral bridges which are not visible with light microscopy. Posterior tentacles were not observed on the majority of cells; they have been found on solitary cells but rarely on cells of colonies.

The measurements of individual cells in a colony show the following dimensions: average length of cell body is 7.3 µm (range 4.3-8.7 µm), width of cell body - 5.7 µm (range 2.9-7.25 µm), length of collar - 10.7 µm (range 8.7-14.5 µm), length of flagellum - 15.8 µm (range 11.6-20.3 µm). So, the flagellum in our strain is approximately 2-3 times longer than the cell body, and the collar is 1.5 times longer than the body.

Based on the shape and dimensions of cells and the average number of cells in a colony we have compared this clone of *Kentrosiga/Desmarella* sp. with all other previously described species of these two genera (Table 1). Our present strain has the same characters as those presented in the original description of *Desmarella moniliformis* Kent, 1880 and *D. phalanx* Kent, 1880 (Kent 1880) (see Table 1). The length of the cell body in our strain is slightly less than in *D. moniliformis*, but the latter has the smallest cells among other species of this genus. Both species have the same ratio of flagellar length to cell length; bridges between cells in colonies are not visible with light microscopy, and the average number of cells in a colony is similar.

Desmarella phalanx has the same main characters as *D. moniliformis* (Table 1), but has only been given a different name because of its freshwater habitat (Kent 1880). Saedeleer (1927) compared *D. phalanx* with *D. moniliformis*, and found differences in the following: cell length, dimensions of the gap between cells in a colony, nuclear diameter, and the different habitats. To discuss this view we have to say that cell length variability in *D. moniliformis* covers the range found in *D. phalanx* (Saedeleer, 1927); differences in habitats are not essential, as some species of choanoflagellates occur in both marine and freshwater habitats (Zhukov and Karpov 1985). The only character of significance is a gap between cells. There is no gap between cells in *D. moniliformis*. So, concerning this character our strain belongs to *D. moniliformis*.

From this short discussion we can conclude that *D. phalanx* should be considered as a synonym of *D. moniliformis*, and the latter species occurs in both marine and freshwater habitats. Thus, we are identifying our *Kentrosiga/Desmarella* sp. strain as *Desmarella moniliformis* Kent, 1880.

Electron microscopical characters of *D. moniliformis*

The main ultrastructural characters of *D. moniliformis* are shown in Figs. 2-5. There is a lateral cytoplasmic bridge between the cells of a colony, which has a transverse electron dense amorphous septa with a hole in the centre (Figs. 2,3). The general distribution of organelles within the cell is the same as in other choanoflagellates. The number of collar tentacles is about 25. The proximal part of the flagellar basal body is surrounded by a ring of electron dense material, from which the rootlet microtu-

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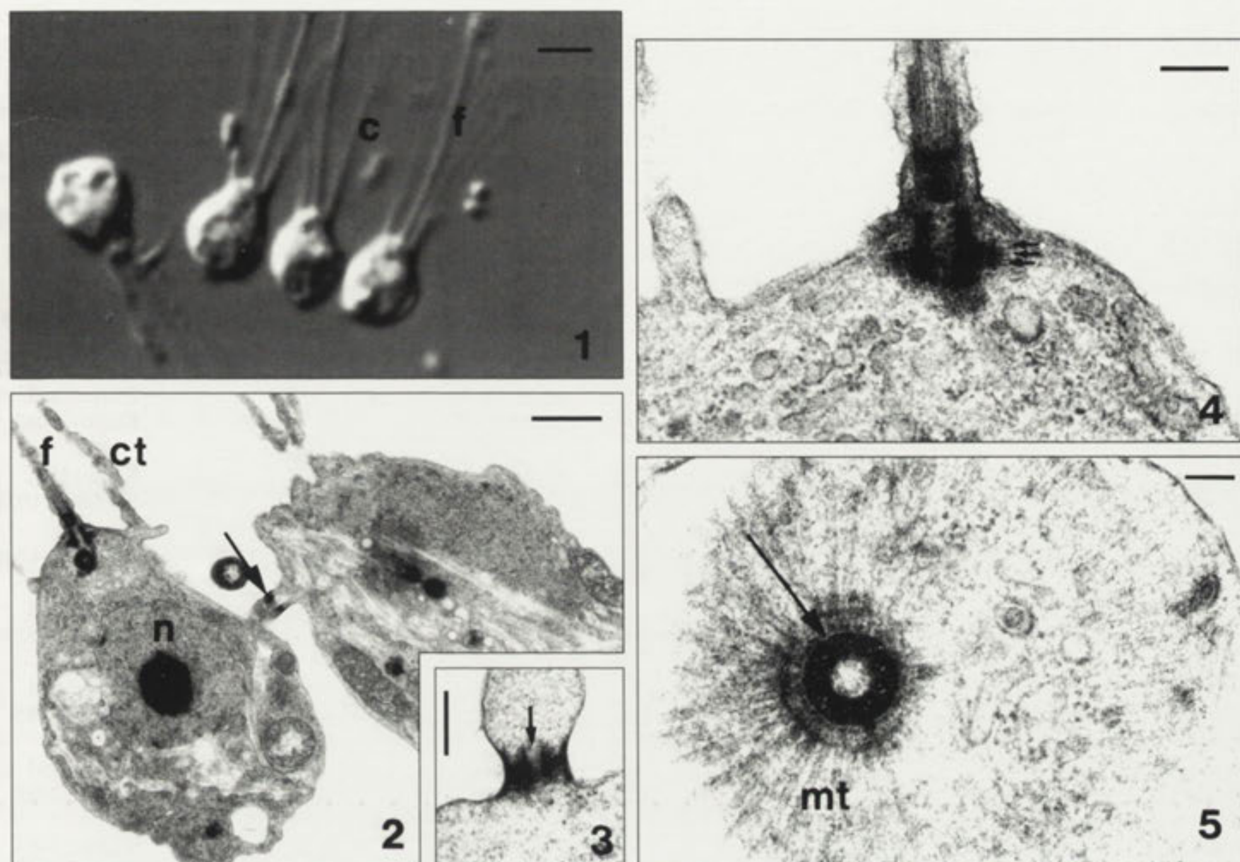

Figs. 1-5. Main characters of *Desmarella moniliformis* at light (1) and electron microscopical (2-5) levels. 1 - light microscopical view of fixed colony, c - collar, f - flagellum. Scale bar - 4 μm. 2 - ultrathin section through the 2 cells of colony showing a lateral bridge (arrow); ct - collar tentacles, f - flagellum, n - nucleus. Scale bar - 1 μm. 3 - electron dense septa in cytoplasmic bridge under higher magnification; arrow shows a hole in the centre of septa. Scale bar - 200 nm. 4 - longitudinal section of the flagellar basal body with microtubular rootlets, arrows show three levels of microtubules. Scale bar - 250 nm. 5 - cross section of the flagellar microtubular rootlet system; arrow shows a dense fibrillar ring, from which microtubules (mt) originate. Scale bar - 100 nm

bules pass radially (Fig. 5). The rootlet microtubules are arranged in two or three layers, as seen in longitudinal sections (Fig. 4), and their complete number is between 60-90.

Comparison of *D. moniliformis* Kent, 1880 with *K. thienemanni* Schiller, 1953

The type species of *Kentrosiga*, *K. thienemanni*, has been studied by light and electron microscopy (Karpov 1982, 1985; Zhukov and Karpov 1985) and has the following main characters. Colonies are ribbon-like, forming an arch when they have a large number of cells, which may reach 30-40. An average cell number is about 8. Lateral bridges between cells are hardly visible when viewed with light microscopy. Cells are inserted in mucilage and have at their posterior end the tentacle-like projections. The length of the cell body is 9-12 μm, and the flagellum is 3-4 times longer than the body.

Electron microscopical observations reveal the existence of cytoplasmic bridges between cells in colonies of *K. thienemanni* (Karpov 1982, 1985; Zhukov and Karpov 1985). Each bridge contains a transverse electron dense septum which is perforated by several pores. The flagellar rootlet system of *K. thienemanni* differs from that of *D. moniliformis* in several respects. It is arranged with 4-5 focuses, instead of one ring in *D. moniliformis*, and the microtubules are organised in 5-7 layers. The complete number of microtubules in the rootlet system of *K. thienemanni* is estimated at between 150-200 compared with 60-90 in *D. moniliformis*.

Thus the ultrastructural differences between *Desmarella moniliformis* are significant. Together with light micros-

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Table 1. Main characteristics of choanoflagellates species of genera *Desmarella* and *Kentrosiga*

Spp. names	Length of cell body (in µm)	Flagellum/cell ratio	Lateral bridges	Posterior projections	Number of cells in colony	References
Kentrosiga/ *Desmarella* sp.	4.3-8.7 (mean 7.3)	2-3	-	+	2-12	present paper
D. moniliformis	6-11	2	-	+	4-12	Kent, 1880; Boucaud-Camou, 1967; Throndsen, 1974; Zhukov and Karpov, 1985
D. phalanx	6-11	2	-	+	4-12	Kent, 1880; Saedeleer 1927
D. irregularis	8-11	no data	-	+	up to 50	Boucaud-Camou, 1967; Zhukov and Karpov, 1985
D. pyriformis	10-13	3	+	-	4-8	Zhukov and Karpov, 1985
D. sphaeroidea	10-11	2	+	-	4	Zhukov and Karpov, 1985
K. thienemanni	9-12	3-4	+	1-3	4-8	Schiller, 1953; Zhukov and Karpov, 1985
K. cylindrica	18-21	no data	+	-	4	Schiller, 1953
K. setifera	21-23	1	-	3-5	1	Schiller, 1953
K. skujae	15	2-3	+	2	2-4	Schiller, 1953

copy they give us the possibility of distinguishing these two species very easily. The question that is posed is whether these differences are of generic or specific significance?

Estimation of *Kentrosiga* and *Desmarella* characters

Earlier Hibberd (1975), after investigating the choanoflagellate *Codonosiga botrytis*, proposed that the development of the flagellar rootlet system in choanoflagellates could be related to the dimensions of the cell: the larger the cell the more extensive the rootlet system. Then Karpov (1981) showed that the development of rootlet system in choanoflagellates correlates with the ratio of flagellar length to body length. This can be reasonably justified since the main function of the rootlet system is to anchor the flagellum to the remainder of the cell. Differences in the ratio of flagellar length to cell length do not reflect characters at the generic level in choanoflagellates. For example, solitary cells of *Monosiga ovata* have almost the same ratio of flagellar length to the body length (2:1) (Karpov, 1982) as do cells of the colonial species *D. moniliformis* (present paper). In spite of the differences in body dimensions (cells of *M. ovata* are almost half the size of *D. moniliformis*), both species have the same structure of rootlet system including the number of microtubules (about 60 in *M. ovata*). Two other species from different genera (*K. thenemanni* and *C. botrytis*) have the same structure of flagellum (Karpov 1981): flagellum/cell ratio is 3-4, number of rootlet microtubules is 120-200, and they arranged in 4-5 focuses around the flagellar base. Moreover, these characters are essentially different in frame of one genus *Codonosiga* (*C. botrytis* differs from *C. gracilis*, which has the ratio

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2-3, and 80-120 rootlet microtubules, which are arranged in a circle as in *M. ovata* (Karpov 1981). Therefore we can conclude that the correlation between the ratio of flagellar length to body length on one hand and the development of the rootlet system on the other are only characters of specific importance (see also Table 1). Thus, in spite of certain ultrastructural differences between the two type species, *D. moniliformis* and *K. thienemanni*, they reveal only differences at species level.

The posterior tentacles in *Kentrosiga* species, described by Schiller (1953) as a character of generic significance, have been found in species of both genera (Table 1). Our previous investigations have also showed that many choanoflagellates can produce tentacle-like projections from the posteriorly and laterally from the cell (Karpov 1980, 1981, 1982, 1985; Zhukov and Karpov 1985). These projections never form a collar-like structure, but serve for attachment to a substratum. Thus, the ability to produce tentacles is a common character of all choanoflagellates independent of species or genus.

Based on these data presented here we consider it justified to replace the generic name *Kentrosiga* Schiller, 1953 (as a younger synonym) by the generic name *Desmarella* Kent, 1880.

A list of *Desmarella* species

Four species have been ascribed to the genus *Kentrosiga* (Schiller, 1953), and these are now transferred to the genus *Desmarella*. Unfortunately, only one species, the type species *Kentrosiga thienemanni*, has been investigated in culture, so we cannot be sure that all of them are valid. This matter must be left for the future. Here we present a list of all species of the genus *Desmarella*.

D. *moniliformis* Kent, 1880 (= *D. phalanx* Kent, 1880, = *D. brachycalix* Skuja, 1939) - type species.

D. irregularis Stokes, 1888

D. sphaeroidea (Schiller) Bourrelly, 1957

D. pyriformis (Schiller) Bourrelly, 1957

D. thienemanni (Schiller) Karpov and Coupe

D. setifera (Schiller) Karpov and Coupe

D. skujae (Schiller) Karpov and Coupe

D. cylindrica (Schiller) Karpov and Coupe

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D. gyrinopsis (Schiller) Bourrelly, 1957

D. lithoporum (Schiller) Karpov and C

D. aculeata (Schiller) Karpov and Coupe

D. plana (Schiller) Karpov and Coupe

D. caloferitis (Schiller) Karpov and Coupe

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Redescription of *Ascobius lentus*, a Rare Freshwater Folliculinid (Ciliophora: Heterotrichida) from a Pond in Germany

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Summary. A rare freshwater species of folliculinid ciliates, *Ascobius lentus* Henneguy 1884, is redescribed. The redescription includes data on the habitat, abundance, morphology, infraciliature, and ultrastructure. *Ascobius lentus* was rediscovered in high numbers on artificial substrate in the eutrophic lake "Alte Kiesgrube" near Grietherbusch, Germany, during the summer months. The genus is characterized by a simple, lying, flat lorica without flaps or chambers, short, broad and flexible peristomial wings, a paroral kinety closely apposed to the adoral zone of membranelles (AZM), a compact macronucleus, and a spatulate or broadened holdfast organelle. *Ascobius lentus* is the type species and the only so far described freshwater species of the genus. It differs from marine species especially by its highly vacuolated anterior part; the network of vacuoles is probably an osmoregulatory organelle. The macronucleus of *A. lentus* is surrounded by a filamentous network that has not yet been described in other folliculinid species. In summary, *Ascobius* has a unique combination of morphological and ultrastructural features which justifies, at the present state of knowledge, its classification in a separate genus.

Key words: ciliates, ecology, Heterotrichida, morphology, taxonomy, ultrastructure.

INTRODUCTION

Folliculinids are sessile, filter-feeding heterotrichs. The trophic cells (trophonts) are characterized by a wing-shaped peristome. Each individual is fixed by a holdfast organelle inside a chitinous lorica. The trophont divides asymmetrically: the proter becomes a motile swarmer, the opisthe develops into a trophont.

Until now, most of the about 70 folliculinid species so far described (for most recent review see Hadzi 1951) were collected from marine habitats. We redescribe one of the four rare freshwater species, *Ascobius lentus*. *A. lentus* was first reported from Montpellier, France, by Henneguy (1884) who recognized it as a *Bursaria*-like ciliate. Hadzi (1951) re-interpreted the drawings of Henneguy and placed *A. lentus* as the type species of the genus *Ascobius* to the folliculinidae. The original description of *A. lentus* is quite poor. We add here information

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about the swarmer stage, about the morphology and ultrastructure of the trophont, and we include also data about the habitat where the organism was found.

MATERIAL AND METHODS

Collecting the organisms

The study was conducted originally to monitor for macrozoobenthos organisms which settle during the year on an artificial substrate. Artificial substrate samplers (ASS) were placed at three sites: the river Rhine, a gravel-pit lake permanently connected with the Rhine, and the "Alte Kiesgrube" (AKG). *Ascobius* was only found in samples from the AKG (for description of the habitat see Result section).

The ASS consisted of a rectangular, perforated 10 l garbage can in which a frame was fixed. 5 PVC plates (size: 0.5 x 16 x 16 cm) were vertically inserted in the frame with a distance of about 3 cm from each other.

Between March and October 1995, one series of PVC plates was exposed for one month only. Each plate was then removed and replaced by the next one (monthly plates). Another series of plates was exposed from the beginning of the experiment in March 1995; one plate of this series was removed each month (cumulative plates). The exposure time of the cumulative plates thus varied from 1 to 8 months. After exposure, the plates were examined under a dissecting microscope and the number of loricae per plate was determined.

For light microscopical observations of the ciliates, we inserted glass slides in the ASS for certain periods during the autumn 1995 and during the summer 1996. In parallel, Thermanox plastic cover slips (Lux Scientific Cooperation, California) were exposed, in order to obtain cells for transmission electron microscopical analyses.

Light microscopy

Morphology of living cells and of formol-fixed and protargol-stained cells was documented by video records and photography using a Zeiss IM-35 microscope equipped with a flash system and an Olympus OM-4 camera. Living cells were measured on a video screen; the nuclear dimensions were determined on fixed and stained material directly under the microscope.

Protargol staining was performed on about 50 specimen by the method of Wilbert (1975) adapted to the organism. After fixation with Stieve's fluid (after Foissner, 1991) for at least 30 min, the cells were bleached for 1-5 min in 0.5% (v/v) sodium hypochloride. The cells were stained after washing at 60°C in 1% (w/v) protargol for 1 h. The material was developed until the kineties become visible, poststained in 0.2 % (w/v) gold chloride (30 s) and fixed in sodium thiosulfate.

Transmission electron microscopy

We analyzed 3 individuals collected in September 1995 and 6 individuals collected in June 1996 by transmission electron microscopy (TEM).

The Thermanox cover slips with the attached folliculinids were washed with millipore-filtered, natural pond water and transferred in ice cold 2.5 % (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) with 5 mM $MgCl_2$, supplemented with 1 % (w/v) OsO_4 after 5 min (Mulisch and Hausmann 1983). Fixation lasted for 30 min on ice. Washing was carried out with 0.1 M sodium cacodylate at room temperature. The cells were dehydrated in increasing concentrations of ethanol, transferred through propylene oxide and flat embedded in Araldite. *Ascobius lentus* was identified under a dissecting microscope, cut out and glued onto a resin block. Sectioning was performed with a Reichert Ultracut. The sections were double-stained with uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963) and observed in a ZEISS TEM 109.

RESULTS

Improved diagnosis

Length of extended trophont 250-310 μm, swarmer about 150 x 60 μm, lorica 158-220 x 130-160 μm. Trophic cell with spatulate or axe-shaped holdfast organelle attached inside a simple, laterally attached lorica with a short neck. On average 47 longitudinal somatic kineties. Peristomial wings short, broad, flat and flexible; not visible when contracted. AZM with closely apposed paroral kinety. Buccal cavity surrounded by 2 turns of the AZM. Trophont transparent and colorless except for some light blue pigment around the macronucleus; swarmer with light blue pigment stripes and dark blue spots at anterior and posterior ends. Trophont and swarmer contain usually one ellipsoid or spherical macronucleus and two to three micronuclei in the posterior third. Contractile vacuole consists of anterior network of vacuoles.

Neotype material

No type material from *A. lentus* has been mentioned in the literature. Thus we deposit five slides with fixed, protargol-stained and embedded cells of *A. lentus* at the Oberösterreichisches Landesmuseum (LI), Linz, Austria.

Habitat

The AKG is a gravel-pit lake where gravel was mined until the end of the sixties. It is used now as a bath lake. The AKG is situated in the floodplain of the Lower Rhine between the cities Rees and Emmerich (geographical coordinates of AKG: 51°48' N, 6°20'E). Because of this location, the AKG is flooded at high waters of the river Rhine. Flooding occurred 8 times during the last 25 years (last high waters in December 1993 and February 1995). Water fluctuations up to 1.5 m are also visible in years without a high water due to fluctuations of the groundwater level.

The AKG has no surface flow. The physico-chemical analysis characterizes the AKG as an eutrophic lake with

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a stable summer stratification (Graef 1985). The temperature at the place of exposure of the ASS (between 10°C and 25°C), and the secchi disk transparency from April to November 1995 are shown in Fig. 1.

The ASS were exposed near the bank in a depth of about 2 m. Here, the bank wood plant consists of willows that shaded the exposure place. The bank substrate consists mainly of gravel with a more or less thick layer of mud, depending on the steepness of the bank. Typical macrozoobenthos organisms found in the ASS were the sponge *Ephydatia fluviatilis*, the gastropods *Bythinia tentaculata* and *Potamopyrgus antipodarum*, the bivalve *Dreissena polymorpha* and the bryozoan *Plumatella emarginata*.

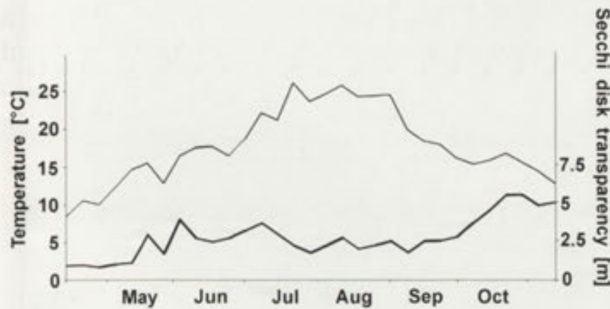

Fig. 1. Profiles of temperature (thin line) and secchi disk transparency (thick line) in the AKG during summer 1995

A. lentus was detected on the PVC plates in July 1995. At the monthly plate, the abundance was about 1.5 individuals/mm^2, whereas it was more than 15 individuals/mm^2 on the cumulative plate. In August, about 1.6 individuals/mm^2 on the monthly plate and nearly 17 individuals/mm^2 on the cumulative plate were counted. The abundance on the monthly plates decreased to 0.05 individuals/mm^2 in September, and to 0.015 individuals/mm^2 in October. On the cumulative plates, more than 20 individuals/mm^2 were found in September, but none in October. In conclusion, most individuals were counted during July and August, when the temperature in the AKG was at its maximum (20-25°C).

A. lentus was also observed (but not counted) in high numbers on artificial substrates during the summer 1996. No specimens were detected on stones or water plants collected from the vicinity of the place of exposure of the AAS. We also could not detect any swarmer in plankton samples from this place.

Morphology and ultrastructure of the trophic stage

Lorica (Fig. 2)

The shape and dimensions of the loricae of *A. lentus* are little variable (Table 1). The ampulla is extremely flat. It is laterally attached to the substrate by a thin but clearly visible basal plate. As viewed from above, the ampulla is broad and ellipsoidal; it lacks flaps or chambers. The neck is upright, narrow and very short; its wall is not sculptured.

Table 1. Dimensions of *Ascobius lentus* in μm

	Character	Range	Arithmetic mean	No. of individuals	Standard deviation	Coefficient of variation
Lorica	total length	158-173	169	12	12	7.1
	total width	133-161	148	12	11.4	7.7
	L/W-index	1.1-1.2	1.16	12	0	4
	brim diameter	34-39 x 18-28	39 x 23	9	1.6	4
Trophont	total length (extended)	250-310	280	5	23	8.1
	width (extended)	55-101	78	9	15	18
	PW length (extended)	75/100		2		
	PW width (extended)	70/75		2		
	holdfast width (extended)	50-130	92.5	8	20	21.6
	Ma diameter	15x20 - 20x35	20 x 30	12	3.9	18
	no. of micronuclei	2-4	3	7	0.6	8.7
	Mi diameter	3.0-4.2	3.7	10	0.3	3.1
	distance Ma from posterior	20-50	34	13	7.6	22.1
	number of kineties	41-51	47	7	3.1	6.7

L/W - length/width, Ma - macronucleus, Mi - micronucleus; PW - peristomial wings

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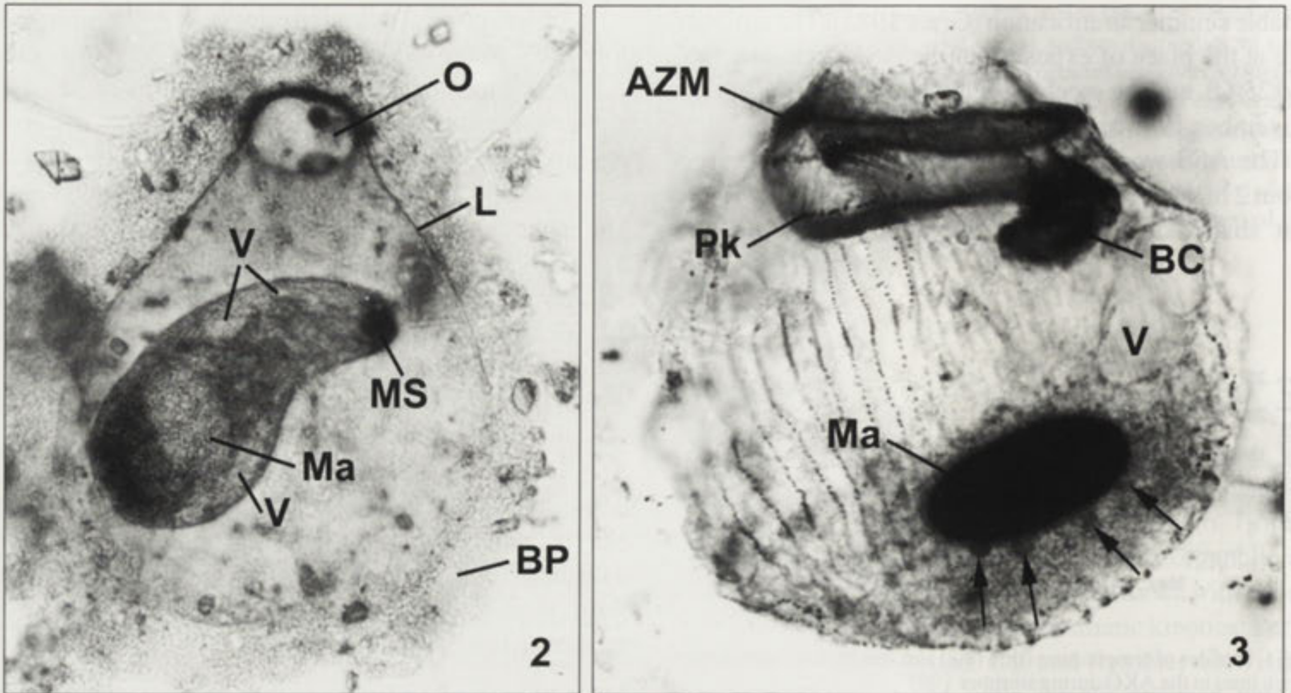

Figs. 2, 3. Light micrographs of *A. lentus*. 2 - living swarmer inside a lorica (L). The lorica is attached by a prominent basal plate to a glass slide that was exposed in the AKG for several months. Note the characteristic, ellipsoidal opening of the lorica. The swarmer has an anterior membranellar spiral and includes the ellipsoid macronucleus and large vacuoles. x 400. 3 - dorsal view of a protargol-impregnated, contracted trophont. The adoral zone of membranelles and the paroral kinety surround the anterior area. The ellipsoid macronucleus and four micronuclei (arrows) can also be recognized. x 1000. AZM - adoral zone of membranelles, BC - buccal cavity, BP - basal plate, L - lorica, Ma - macronucleus, MS - membranellar spiral, O - opening, PK - paroral kinety, V - vacuoles

The ellipsoidal opening may be surrounded by a small brim. The wall of the lorica is colourless in newly formed loricae and consists of electron-transparent, loosely arranged fibrillar material. Older loricae may have a bluish color; their basal plates may be blue or brownish.

Trophic cell (see Table 1 for dimensions)

The trophont is transparent and almost colorless. Some blue pigment is concentrated around the macronucleus; no cortical pigmentation has been detected. The cell can be divided into four distinct zones (Fig. 4): the wing-shaped anterior oral area, the highly vacuolated postoral area, the dense nuclear region and the posterior holdfast structure.

The anterior half of the cell is shaped as two broad, flat and flexible peristomial wings surrounded by an adoral zone of membranelles (AZM). The paroral kinety is situated close to the AZM (Figs. 3, 4). At the first view, the extended wings appear to be of unequal length, the right one being larger. In contrast, analyses of video images of several individuals revealed similar dimensions for the right and the left peristomial wing. The apparent asymmetry is probably due to the fact that the left wing is usually more twisted and more contracted than the right one. Both wings may or may not have pointed tips. The anterior of contracted cells looses its wing-shaped appearance (Fig. 3). The peristomial field is large and has a wide opening to the buccal cavity at the base of the right wing. The buccal cavity is flat and surrounded by 2 turns of the adoral zone of membranelles (Figs. 3, 4).

The anterior two thirds of the cell are highly vacuolated (Figs. 4, 6-8). The vacuoles surround the buccal cavity leaving only a narrow channel for food vacuoles pinched off from the cytostome. Small, transparent vacuoles from the posterior continually fuse with a network of large anterior vacuoles. The network might represent an osmoregulatory organelle as its shape and dimensions eventually (but not dramatically) change. This is supported by the fact, that the ultrastructure of the anterior cell part was poorly preserved in all our preparations. The large vacuoles tend to swell, to fuse or to burst during fixation (Fig. 3).

Most cytoplasm, food vacuoles, pigment and the macronucleus are situated in the posterior third of the cell (Figs. 3-8). Only this part of the cell is visible at low

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magnification in the light microscope using bright field illumination. The macronucleus is compact and relatively small compared to the cell size (Figs. 3-7). It may be ellipsoid or spherical. Some cells with two closely apposed, spherical macronuclei have been observed. Macronuclear shape and diameter are similar in swarmers and trophonts. As seen in the TEM, the macronucleus is filled with a spongy mass of chromatin. Small, distinct chromatin bodies are aligned along the nuclear envelope (Fig. 9). Many nucleoli are scattered throughout the nucleus. The nuclear envelope is perforated by many pores and surrounded by a filamentous layer which is perforated at the

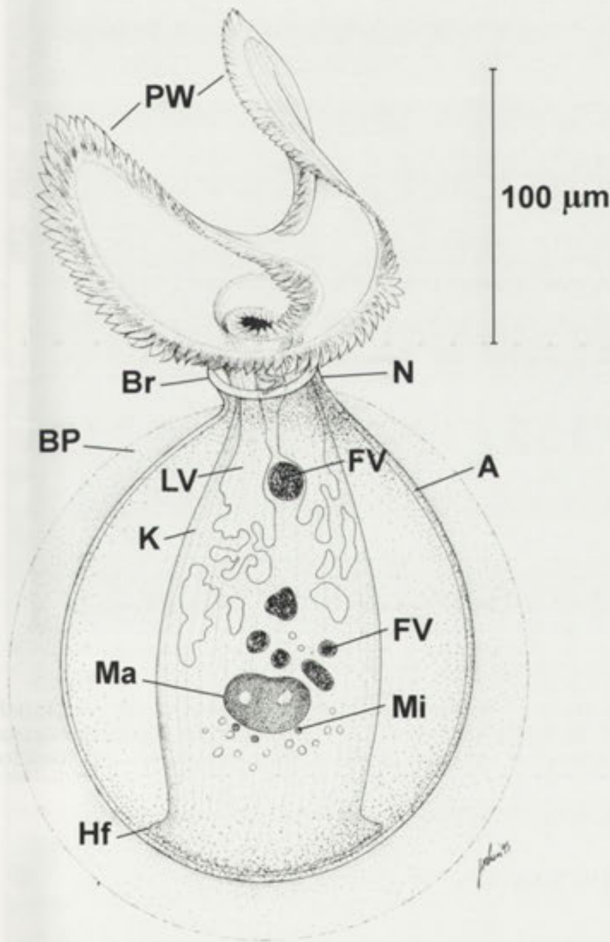

Fig. 4. Drawing (summary of a number of copies from a video screen) of an extended, living trophont of *A. lentus* as seen from above (= dorsal view). Note the typical appearance of the peristomial wings and the axe-shaped holdfast. A - ampulla, AZM - adoral zone of membranelles, BC - buccal cavity, BP - basal plate, Br - brim, FV - food vacuole, Hf - holdfast, K - kinety, LV - large vacuole, Ma - macronucleus, Mi - micronucleus, N - neck, PW - peristomial wings

sites of the nuclear pores (Figs. 9, 10), and thus appears as a regular meshed network in oblique sections (Fig. 10). In places, additional bundles of filaments can be detected at the periphery of the macronucleus (Fig. 9). Up to 4 (usually 2-3) micronuclei per cell have been counted. The micronuclei are situated close to the macronucleus (Figs. 3, 4). They contain dense chromatin and have a diameter of about 3.5 µm (Fig. 11).

The cytoplasm around the nuclei is filled with small cisternae of the rER and many dictyosomes, that are in close association with the rER (Fig. 9). Small vesicles, some of them resembling those in the cortical ribs, are scattered throughout the cytoplasm. All cells analyzed by electron microscopy were heavily infected by bacteria (Fig. 15).

The cell is fixed in the posterior part of the ampulla by a broad holdfast organelle the shape of which appears to be highly variable among otherwise similar-looking individuals. No matter how much the cell is contracted, the holdfast may be spatulate, or it may extend in a pseudopod-like manner towards the lateral walls of the lorica, thus looking like an axe as seen from above (Fig. 4-8). The spatulated holdfast organelle (Fig. 7) is fixed to the lorica wall by regularly spaced attachment points that each appears to be connected to a short, longitudinal, cytoplasmic fibril. The axe-shaped holdfast organelle looks smooth and transparent in the light microscope (Fig. 6). Its cytoplasm has a fine granular/fibrillar appearance in the TEM; it includes almost no organelles and only few bacteria. The contact zone between the cell and the lorica shows no special attachment devices: in most areas, the plasma membrane appears to adhere directly to the wall of the lorica.

The somatic cortex is characterized by longitudinal ribs interspaced with in average 47 kineties (Table 1). A rib contains the overlapping ribbons of postciliary microtubules (km fiber), a thick bundle of myonemes surrounded by a large cisterna, and some extrusive (?) vesicles with an electron-lucent content (Fig. 12). Each kinety consists of dikinetids: an anterior and a posterior kinetosome with associated microtubular and microfibrillar structures (see Figs. 14-16 for details). The posterior kinetosome bears the cilium in most parts of the cell, except for the dorsal side of the peristomial wings, where the anterior kinetosome is ciliated and its transverse microtubules are elongated (Fig. 13).

The swarmer

Swarmers have been observed only inside loricae (Fig. 2), most of them directly after division of the trophic cell. The cells have a size of about 150 x 60 µm and are transparent with some dark-blue pigment concentrated at

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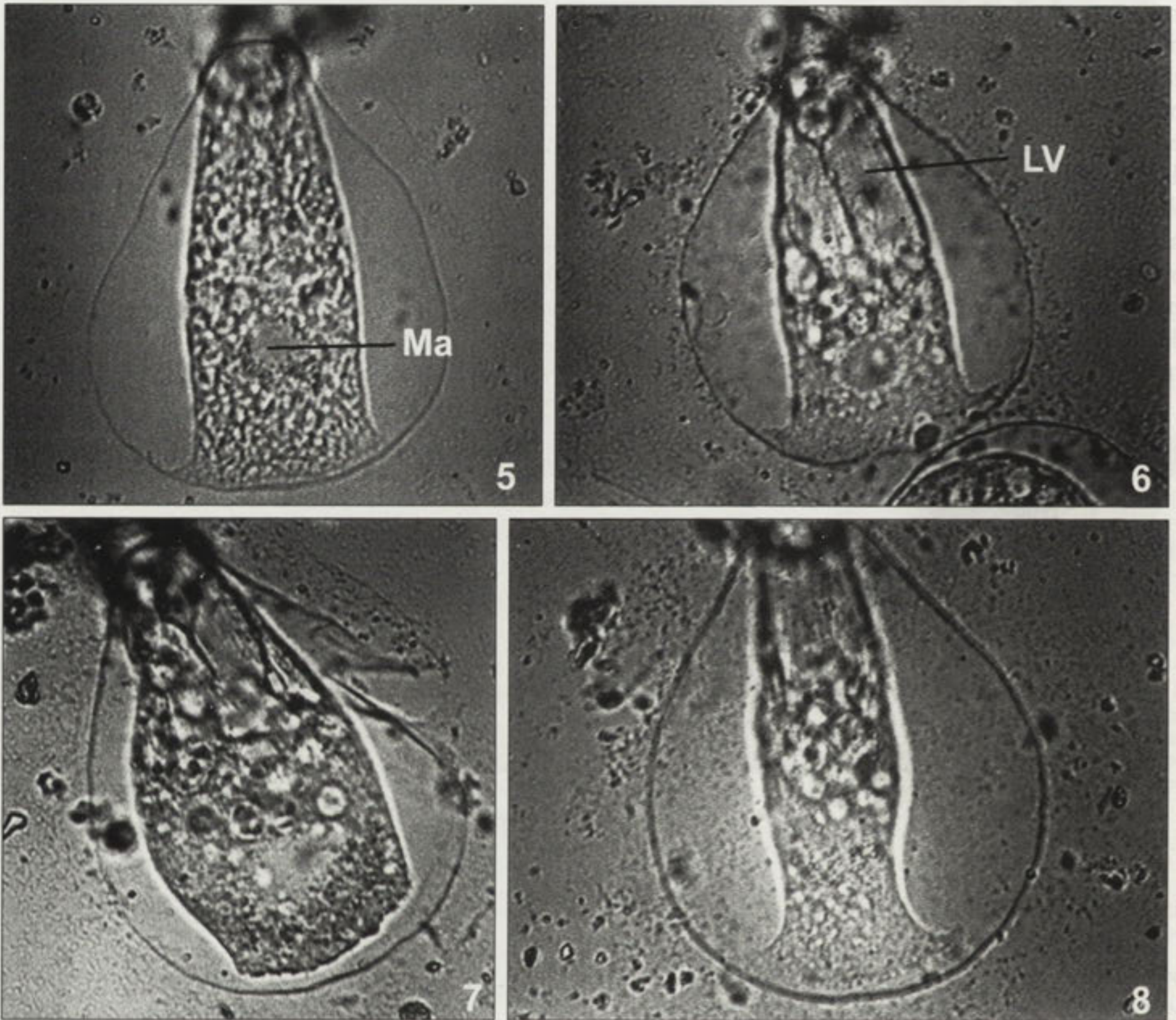

Figs. 5-8. Light micrographs (video prints) of living, extended trophonts of *A. lentus* grown on a monthly plate collected in October 1995. In order to illustrate the variability in shape of holdfast organelles, only cell bodies inside the loricae are shown. 5, 6, 8 - the attachment sites are broader than the cells; the holdfasts look more or less axe-shaped. 7 - this cell has a spatulate holdfast organelle. x 400. LV - large vacuole, Ma - macronucleus

the anterior and posterior end. The anterior cytoplasm contains large vacuoles. There are light blue pigment stripes between the longitudinal kineties. The rounded macronucleus is situated in the posterior third of the cell. It is surrounded by 2-3 micronuclei.

We observed one proter of a late divider (stage 7 after Mulisch and Patterson 1987) by TEM (results not shown). The future swarmer resembles the trophont in the network of anterior vacuoles. It differs from the trophont by its more dense cytoplasm and by its high content of electron-dense vesicles which may represent pigment granules.

DISCUSSION

Identification and comparison of *Ascobius lentus* with related species

Freshwater folliculinids are extremely rare and usually badly documented (Dioni 1972, Ruttner-Kolisko 1953, see also Hadzi 1951).The family Folliculinidae contains about 70 nominal species; only four of them were collected from freshwater habitats: *Folliculina boltoni* Kent 1881, *Diafolliculina thomseni* Hadzi 1951 (= *F. boltoni*

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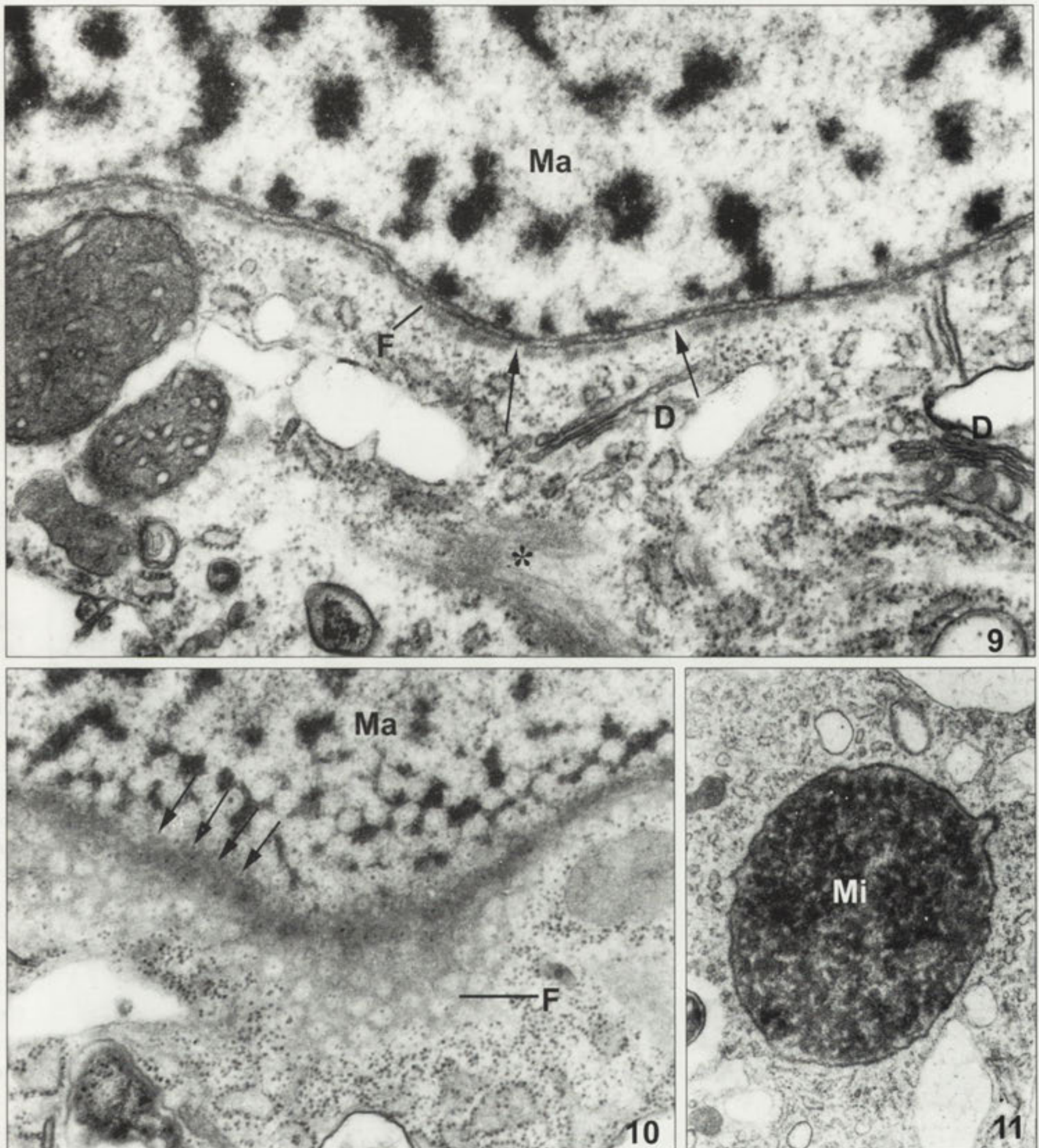

Figs. 9-11. Transmission electron micrographs of nuclei of *A. lentus*. 9 - cross-section through the macronuclear periphery. The nuclear envelope is at its cytoplasmic face covered by a filamentous layer that is perforated at the sites of the nuclear pores (arrows). Dictyosomes and filament bundles (*) occur in the vicinity of the macronucleus. x 52 000. 10 - oblique section through the macronuclear periphery. Note the filamentous network around the nuclear envelope. The mesh size roughly corresponds to the size of the nuclear pores (arrows). x 45 000. 11 - the micronucleus contains dense chromatin. x 17 000. D - dictyosomes, F - filamentous bundle, Ma - macronucleus, Mi - micronucleus

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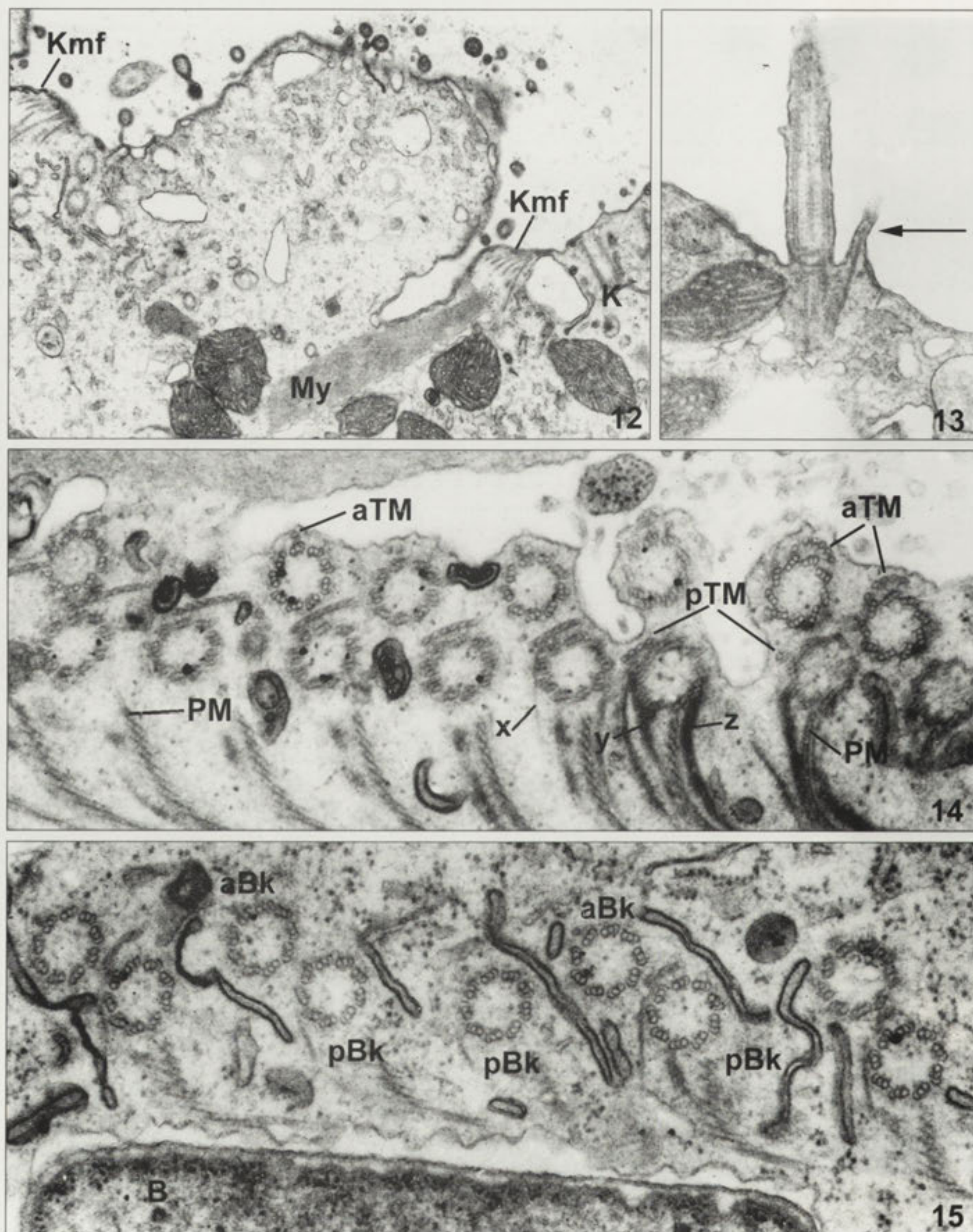

Figs. 12-15. Transmission electron micrographs of somatic cortex and infraciliature of *A. lentus*. 12 - cross-section through a cortical rib between two kineties. The cortex contains only few, electron lucent vesicles. x 12 000. K - kinety, Kmf - km fiber, My - myoneme. 13 - (anterior) ciliated kinetosome at a peristomial wing. Note the elongated transverse microtubules that give rise to a small protuberance (arrow) beside the cilium. x 35 000. 14, 15 - cross-sections at different levels through dikinetids of somatic kineties of the cell body. The anterior basal body is associated with transverse microtubules. These are prominent at the dorsal side of the peristomial wings, but reduced in posterior cell areas. The posterior basal body is associated with transverse and postciliary microtubules. Three fibers (x, y, z) accompany the postciliary microtubules. x 50 000. aBk - anterior basal body, aTM - anterior transverse microtubules, pBk - posterior basal body, PM - postciliary microtubules, pTM - posterior transverse microtubules

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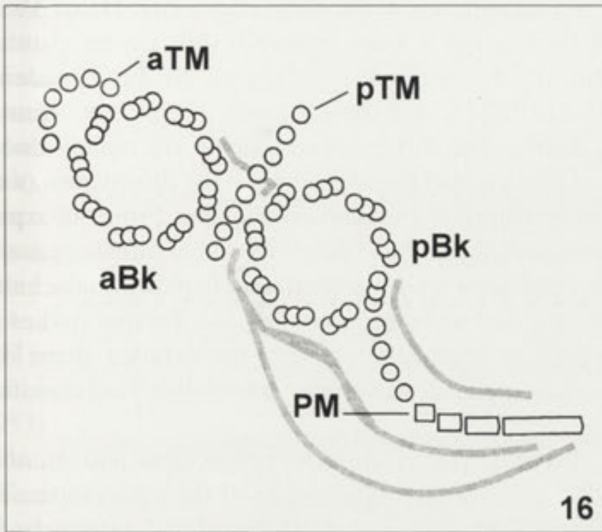

Fig. 16. Scheme of the somatic dikinetid of *A. lentus*. The shaded areas represent fibrous structures. See Figs. 14 and 15 for legend and abbreviations

Kent *sensu* Thompson, 1921), *Botticula ringueletti* Dioni 1972, and *Ascobius lentus* Henneguy 1884.

Folliculina boltoni, *Diafolliculina thomseni* and *Ascobius lentus* have been regarded as being identical, until Hadzi (1951) placed them into different genera. The genus *Folliculina* Lamarck 1816 is characterized by simple, broad and laterally flattened loricae without flaps or chambers. The cells are attached to the posterior inner wall by a narrow, stalk-like holdfast organelle (Hadzi 1951). *Ascobius* Henneguy 1884 differs from *Folliculina* by having a broad holdfast organelle. The genus *Diafolliculina* was created by Hadzi (1951) for folliculinids with a broad holdfast organelle and a lorica with a valve-like structure between ampulla and neck. *Botticula* Dioni 1972 differs from the three genera described in the shape of the lorica (double-walled ampulla and long neck) and by its beaded macronucleus.

Although recent data on ultrastructure and morphogenesis of some species (Mulisch *et al.* 1981, Mulisch and Patterson 1987, Mulisch *et al.* 1993) indicate that the current classification of the folliculinids should be revised (Mulisch *et al.* 1993), we accept Hadzi's (1951) concept until more detailed data become available. From the present state of knowledge, the shape of the holdfast organelle as well as valve-like structures in the lorica are valuable systematic criteria. Since the folliculinid detected by us has a broad holdfast organelle and its lorica lacks flaps or chambers, we regard it as a member of the genus *Ascobius*. Until now, this genus includes 6 species (Hadzi 1951): five are marine, only *A. lentus* Henneguy was isolated from a freshwater habitat (Henneguy 1884).

A. lentus has been very poorly described. Henneguy (1884) neither observed extended trophonts nor swarmers or other stages of the life cycle. Henneguy compared the organism with *Bursaria* and placed it only in the vicinity of the family Folliculinidae. Neither the genus *Ascobius* nor the (type-)species *A. lentus* were accepted widely, until Hadzi (1951) characterized the species from the drawings of Henneguy.

A. lentus resembles our organism in the transparency and light blue color of the cell body, but differs from it by the much more pronounced asymmetry of the peristomial wings (as interpreted by Hadzi 1951, from the drawing of the contracted cell), by its size, and by some features of the lorica. The lorica of Henneguy's population measured about 220 x 145 μm; thus it is longer and more slender than the lorica of the organism described in this paper. The basal plate of Henneguy's population is mentioned to be sticky and thus covered with foreign material, whereas in our specimen it appears transparent and quite clean, even at artificial substrates that were exposed for longer periods. Despite these differences we regard our population and *A. lentus* as identical species.

Habitat

Ecological data on folliculinids, especially on freshwater folliculinids are rare (for reviews see Hadzi 1951, Ruttner-Kolisko 1953), since the species were found only sporadically. Some detailed data are available from the "Lunzer Untersee" (Austria), where a freshwater species (named *Folliculina boltoni*) was reported to occur (only) during the winter 1951/52 in high densities (Ruttner-Kolisko 1953). The folliculinid observed had a simple lorica, a broad holdfast, blue-green pigment stripes, unequal wings and a deep buccal cavity (Ruttner-Kolisko 1953); it thus resembles neither *F. boltoni* Kent, 1881 nor *Diafolliculina thomseni* Hadzi 1951 nor *A. lentus*. The species settled on *Fontinalis* at a depth of 11-15 m. Ruttner-Kolisko (1953) characterized the preferred habitat of her *F. boltoni* as cold (4-12 °C), oligotrophic and light-protected. Reck (1987) recorded *Botticula ringueletti* Dioni 1972 and *Folliculina boltoni* Kent 1881 in the pelagial of the Plußsee, a small, shaded pond in the north of Germany. Both species were found only during the second half of May at depths between four and twenty meters. The water temperature was 4.3 - 11.0°C (oxygen: 1.1-10.4 mg/l; pH: 7.3-8.89; NH_4: 89-218 μg/l). Other reports on freshwater folliculinids point to similar prefer-

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ences (for literature see Ruttner-Kolisko 1953). In contrast, our observations on *A. lentus* suggest that this species prefers warm and eutrophic habitats, since we found it only during the summer. Henneguy (1884), however, detected its population during the winter, in an artificial pond in the botanical garden (Jardin des Plantes, Montpellier). Vucetich (1972) recorded *A. lentus* in Argentina. The few data indicate that there may exist more than the described freshwater species.

At the first view, the differences in abundance of *A. lentus* on the monthly and the cumulative plates in July are not comprehensible. Two explanations may be conceivable: (1) *A. lentus* settled on the plates before it was recognized for the first time (this is possible because we focused on macrozoobenthos species). The cumulative sum therefore could be higher than the abundance counted on the monthly plate. (2) *A. lentus* prefers a substrate for settlement which is covered with a thin layer of periphyton. Consequently, the new monthly plates would be a less preferred substrate than the older cumulative plates. This explanation is less probable, since from our experience with marine species folliculinids appear to be early colonizers of virgin resources (Mulisch *et al.* 1986).

Morphology and comparison with other folliculinid genera

Morphological details and ultrastructural data are only available from three further genera, namely *Lagotia* (Mulisch *et al.* 1993), *Eufolliculina* (Mulisch *et al.* 1981; Mulisch and Hausmann 1983, 1984, 1988; Mulisch and Patterson 1983), and *Metafolliculina* (Uhlig 1965), the two latter being very similar (Mulisch, unpublished observations) and likely synonymous. In contrast to *Ascobius*, these genera are attached to the lorica wall by a bundle of short cilia that arise at a pointed or stalked end (see literature above, and unpublished observations of M. Mulisch). Their holdfast includes a fibrillar bundle (Mulisch and Hausmann 1983). In *A. lentus*, no such structures have been detected. The longitudinal fibrils in the spatulate holdfast organelle observed by light microscopy are described also from the marine species *Ascobius faure-fremieti* (= *Folliculina ampulla* Fauré-Fremiet, 1932; see Hadzi 1951). They probably represent myonemes or km fibers, since no additional fibrillar bundles could be detected by TEM. The holdfast organelle of *Ascobius* is more simply organized than the stalked one, and thus it is probably a primitive character of the genus.

The variability of the shape of the holdfast organelle in *A. lentus* is remarkable. Claparède and Lachmann (1858) draw an axe-shaped holdfast organelle for their *Freia ampulla* (now *Ascobius claparedei* Hadzi 1951). The holdfast of *A faure-fremieti* is shown to be spatulate, that of *A. simplex* is reported to be broadened (Hadzi 1951). All these shapes appear to occur in *A. lentus*. The differences in shape are mainly due to variations in cell breadth whereas the dimensions of the attachment sites are relatively constant. From our experience on *Eufolliculina uhligi*, stalked holdfast organelles may also show some variability in shape at the attachment site, but they never become spatulate. Further studies are needed to clarify the value of the detailed shape of a holdfast organelle as a character for folliculinid classification.

Lagotia, *Eufolliculina/Metafolliculina* and *Ascobius* differ also by the organization of their peristome. The wings of *Ascobius* are short, broad and extremely flat compared to *Eufolliculina/Metafolliculina*. The wings of *Lagotia* are relatively stiff and held in a typical manner (like the ears of a hare), even in the contracted state. This is probably due to horizontally arranged microtubular ribbons in the peristomial wings which may prevent extensive twisting or bending (see also discussion in Mulisch *et al.* 1993). These ribbons are absent in *Eufolliculina/Metafolliculina* (Mulisch and Hausmann 1984, and unpublished observations of M. Mulisch) and apparently also in *A. lentus*, species with very flexible wings. In contrast to the other genera, the wings of *A. lentus* disappear totally during contraction. The apparent lack of peristomial wings in contracted trophonts has been already mentioned by Henneguy (1884). *Eufolliculina/Metafolliculina* and *Ascobius* have a flat buccal cavity, compared to the *Lagotia*-species. On the other hand, *Ascobius* resembles *Lagotia* in the position of the paroral kinety that is closely apposed to the AZM.

In summary, *Ascobius* has a unique combination of morphological and ultrastructural features which justifies, at the present state of knowledge, its classification in a separate genus.

The filamenous network at the outside of the nuclear envelope of *A. lentus* has not been described yet in other heterotrichs, including folliculinids. It does not resemble the fenestrated cisterna around the nuclei of *Stentor coeruleus* (Mulisch 1988), and it is certainly absent in *E. uhligi* (Mulisch *et al.* 1981, and unpublished observations of M. Mulisch) which has a beaded macronucleus. Ultrastructural data on the nuclear organisation of folliculinids with a compact macronucleus have been published only from *Lagotia minor* (Mulisch *et al.* 1993).

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The macronuclear envelope in *L. minor* was not well preserved. However, the thickness of the envelope suggests that it is covered by an additional layer. A similar layer as in *A. lentus* can be clearly identified around the ellipsoidal macronucleus of *Parafolliculina violacea* (Mulisch and Smock, unpublished observations). It may have a skeletal function supporting the compact macronuclear shape. Studies on more genera may show if this feature is characteristic for all folliculinids with a compact macronucleus.

As reported from other freshwater folliculinids (Hadzi 1951), *A. lentus* lacks a typical "pulsating" contractile vacuole found in other heterotrich ciliates. Ruttner-Kolisko (1953) suggested osmoregulation to occur by a defecation vacuole located near the tip of the right peristomial wing. Penard (1919) identified lacunae growing from vesicles in the ectoplasm near the nucleus and also observed "busting" of the lacunae in *Folliculina boltoni*. Both authors interpreted the lack of pulsating vacuoles as an argument for a primary marine origin of the freshwater folliculinids. It is even argued (e.g., Ruttner-Kolisko 1953) that marine species (or species from brackish water) eventually invade freshwater habitats thus explaining the sporadic occurrence of the freshwater species. Folliculinids (always identified as *F. boltoni*) have been sporadically found in the river Rhine (Schmitz, personal communication). Thus it could be argued that *A. lentus* is an originally marine species (it looks almost identical to *A. faure-fremieti*), which from the North-Sea via the river Rhine and its high waters was transported into the small lake near Grietherbusch. However, more autecological data are needed to prove this idea. Our studies on *E. uhligi* indicate that marine folliculinids may be extremely resistant to a slow, step-wise decease in the osmolarity of the medium. However, even after staying for weeks in sea-water diluted to an osmolarity of 20 milliosmol, *E. uhligi* did not develop any structure resembling an osmoregulatory organelle, and the trophonts did not reproduce but swelled and finally bursted (Mulisch, unpublished observations).

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Ultrastructural Study and Description of *Flabelliforma magnivora* sp. n. (Microspora: Duboscqiidae), a Microsporidian Parasite of *Daphnia magna* (Crustacea: Cladocera: Daphniidae)

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Summary. The new microsporidium *Flabelliforma magnivora* sp. n. is described based primarily on ultrastructural characteristics. All life cycle stages have isolated nuclei. Merogonial and sporogonial reproductions are by rosette- or finger-like budding. The sporogony yields 4-16, most frequently 12, sporoblasts. A fragile sporophorous vesicle is formed by the sporont. Spores are lightly pyriform, with both poles blunt, often with one surface slightly convex. Unfixed spores measure 2.34-3.03 x 4.07-4.93 µm. The exospore, which is five-layered with an internal double-layer, measures 35-40 nm thick. The polar filament is isofilar with 14-17, 77-94 nm wide coils arranged in two irregular layers of coils in the posterior half of the spore. The polaroplast, which ends close to the anterior polar filament coils, is composed of two regions of regularly arranged lamellae: narrow lamellae anteriorly, wider lamellae posteriorly. The host is the cladoceran *Daphnia magna*. The primary site of infection is the fat body, but infection was also observed in the hypodermic cells and the ovaries. In the laboratory the parasites are transmitted with nearly 100% fidelity from mother to offspring. Transversely sectioned polyribosomes appeared as characteristic circular configurations of 9 ribosomes. Teratological development, including incomplete separation of sporoblasts, macrospores with supernumerary polar filament coils, and disturbed coiling, was observed. The identification of the species and the genus position are briefly discussed.

Key words: *Daphnia magna*, *Flabelliforma magnivora* sp. n., polyribosomes, taxonomy, teratological development, ultrastructure.

Abbreviations: A - anchoring disc, E - exospore, EN - endospore, F - polar filament, N - nucleus, P - plasma membrane, PA - anterior polaroplast, PP - posterior polaroplast, PS - polar sac, S - sporophorous vesicle, T - tubules, V - posterior vacuole.

INTRODUCTION

Daphnia species, like other Cladocera, host numerous microsporidia, and mixed infections of two or more species are frequently observed. Our first observations of the microsporidium of *Daphnia magna* treated herein were in mixed infections of field collected specimens, and it was impossible to identify and characterize the species until a laboratory reared clone was established. Comparison with microsporidia of Cladocera in the collection of Dr. Otto Jírovec, Prague, revealed what is probably the same microsporidium, together with another species recorded under the name *Thelohania cladocera*. The species, which is new to science, is briefly described herein

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with emphasis on the ultrastructural characteristics. The identification and taxonomic position are discussed. The new species is placed in the genus *Flabelliforma* Canning, Killick-Kendrick and Killick-Kendrick, 1991. The ecology of this microsporidium has partly been described under the name *Tuzetia* sp. by Mangin and *et al.* collaborators (1995).

MATERIALS AND METHODS

Infected specimens of the species *Daphnia magna* Strauss, 1820 were collected from ponds in southern Oxfordshire, UK (populations 8, 11 and 24), and near Moscow, Russia, and studied directly in the field collected samples and/or in clones established from them. The sources of material were:

population 8 (Latitude 51°37,25', Longitude 1°22,70'), sampling November 18, 1992: series No. 921218-(K-M);

clones established from population 11 (Latitude 51°37,25', Longitude 1°22,30'): series No. 940812-(A-C), 950727-(A -E) (A = 11:2, the numbers denote population and clone; A3 type series; B = 11:15, C = 11:4, D = 11:19, E = 11:18);

population 24 (Latitude 51°52,80', Longitude 1°7,20') sampling October 29, 1992: series No. 921218 (A-F). Clones established from population 24: series No. 950727-(L-N) (L =24:1, M = 24:3, N = 24:2);

clone C1 isolated from a mud sample from a pond 50 km north of Moscow, Russia (description in Mangin *et al.* 1995): series No. 930914-(Å-Ö) and 950727-H.

Fresh squash preparations were made by the agar method of Hostounský and Žižka (1979) and studied using phase contrast microscopy. Permanent squash preparations were lightly air-dried and fixed either in methanol for at least 15 min for Giemsa staining, or in Bouin-Duboscq-Brasil (BDB) solution for at least 1 h prior to staining with Heidenhain´s iron haematoxylin. For paraffin sectioning whole animals were fixed in BDB solution overnight or longer. After washing and dehydration in a graded series of ethanols specimens were cleared in butanol and embedded in Paraplast (Lancer St. Louis, MO, USA). Sections were cut more or less longitudinally at 10 µm and stained with haematoxylin or with a modification of the polychromatic staining according to Vetterling and Thompson (1972) where the nuclear staining was substituted by Heidenhain´s haematoxylin. For details on the histological techniques used see the manual by Romeis (1968). All permanent preparations were mounted in D.P.X. (BDH Chemicals Ltd., Poole, England). Measurements were made with an eye-piece micrometer at x 1,000 or using an image analysis program (Micro Macro AB, Gothenburg, Sweden).

For transmission electron microscopy infected hosts were fixed in 2.5 % (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at room temperature (18-23°C) for 24 h. After washing in cacodylate buffer and post fixing in 2 % (w/v) osmium tetroxide in cacodylate buffer for 90 min at 4°C, the specimens were washed and dehydrated in an ascending series of buffer-acetone solutions to absolute acetone and embedded in epon. Sections were stained using uranyl acetate and lead citrate (Reynolds 1963).

Cultures were established from field samples (populations 11 and 24) or hatched from ephippia (resting egg, Russian population). Isofemale lines were established in the laboratory and kept in boiled, filtered and aerated water under standard laboratory conditions at room temperature (18-23°C) with *Chlamydomonas rheinhardii* as food.

The species has been identified in two slides (smears, Giemsa staining) belonging to the collection of Dr. Otto Jírovec, Prague. One slide is labelled "*Thelohania cladocera*", the other one "*Thelohania cladocera*, D. m., Lohovec". On both slides are added in ink by Jírovec´s hand "*Dafnia pulex*, *Thelohania cladocera*, Stary kveten 1937".

RESULTS

Pathogenicity and transmission

Infected cladocerans had the whitish tone common for many microsporidioses. Infection was primarily restricted to the adipose tissue (Fig. 1), but small foci were also found in the hypodermis and in the ovaries. Every cell of an infected fat body lobe was invaded by the parasite. Infected cells became distinctly hypertrophic when packed with spores. Lysis was seen in central regions of the lobes, but real syncytia were not formed.

Infected hosts suffered about 40-60 % reduction in fecundity and died after 30-40 days (controls survived longer than 50 days). All host offspring was infected with the parasite and parasites were transmitted to the resting eggs of the host. No evidence of horizontal transmission was found in the laboratory, but it is possible that laboratory conditions were not appropriate for horizontal transmission. In large mass cultures occasionally uninfected hosts were discovered. These outcompeted very rapidly the infected hosts. Under these conditions vertical transmission was not perfect, although very close to 100%. A detailed description of the biology of the microsporidium was published by Mangin and collaborators (1995) under the preliminary name *Tuzetia* sp.

Mixed infections

In field collected specimens of the series No. 921218 the species was mixed with a microsporidium of the genus *Tuzetia* in the adipose tissue , and simultaneously another microsporidium, *Agglomerata cladocera* (see Larsson *et al.* 1996), occurred in the hypodermis.

Presporal stages and life cycle

All life cycle stages observed displayed isolated nuclei, and there were no indications of reductional division in the initial phase of the sporogony. The light microscopic preparations revealed the sporogony, but stages belonging to the merogonic reproduction were rare (Fig. 3). In ultrathin sections both the merogonial and sporogonial

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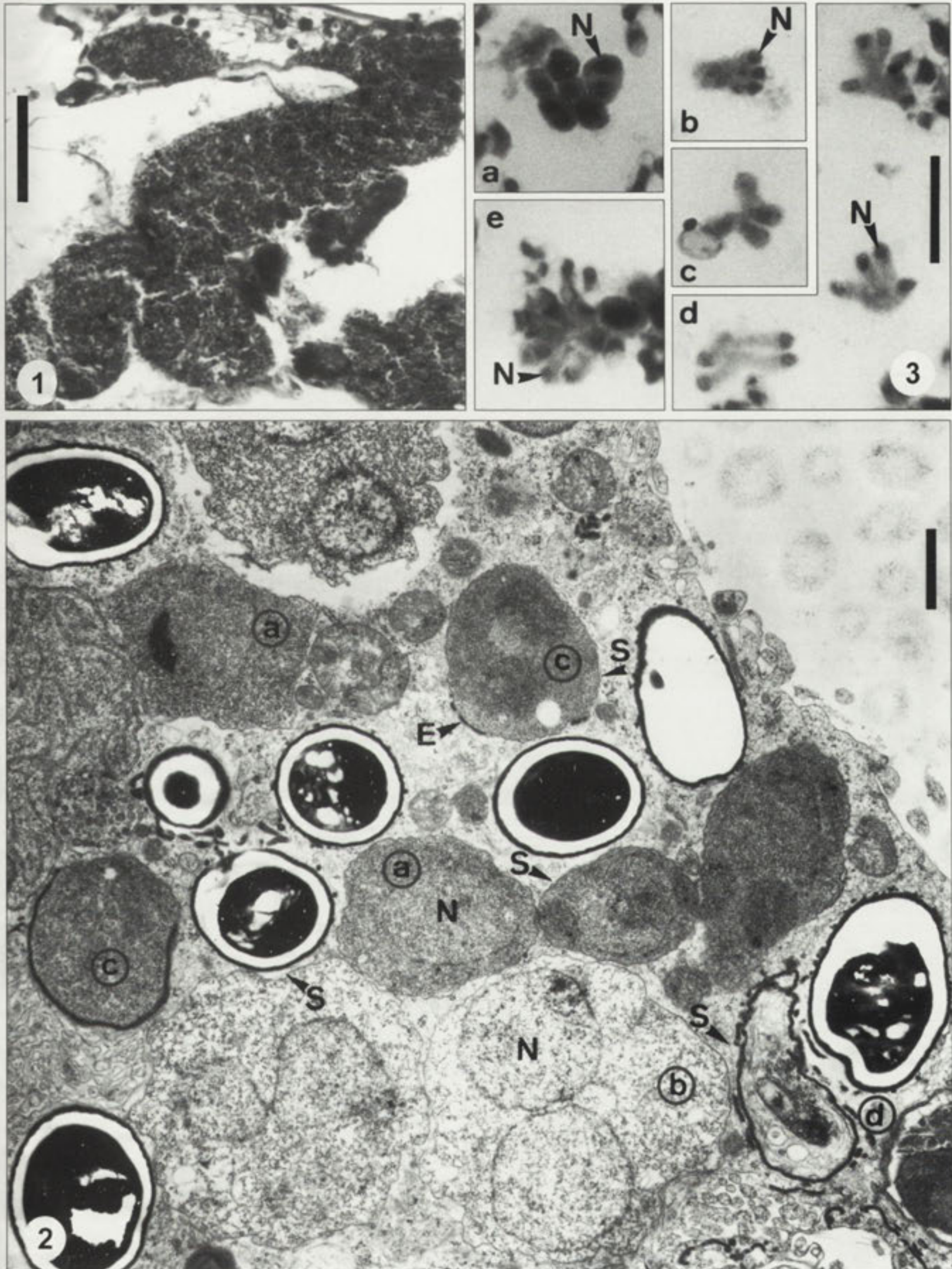

Figs. 1-3. The early development and effect on the host of *Flabelliforma magnivora* sp. n. 1 - fat body lobes of *Daphnia magna* completely filled with microsporidia (haematoxylin). 2 - ultrathin section of fat tissue with developing microsporidia: young sporonts with developing sporophorous vesicle (a); older sporonts with complete sporophorous vesicle (b); still older sporonts with complete sporophorous vesicle and developing exospore layer (c); and sporophorous vesicles with mature and anomalous spores (d). 3 - end of merogony and sporogony (Giemsa stain): a- a group of merozoites maturing to sporonts (two are binucleate sporonts); b- sporogonial plasmodium prior to cytoplasmic fission; c- four-lobed sporogonial plasmodium; d- 4- and 5-nucleate finger-like dividing sporonts; e- at least 8-nucleate lobed sporont. Scale bars: 1 - 50 µm, 2 - 1 µm, 3 - 10 µm

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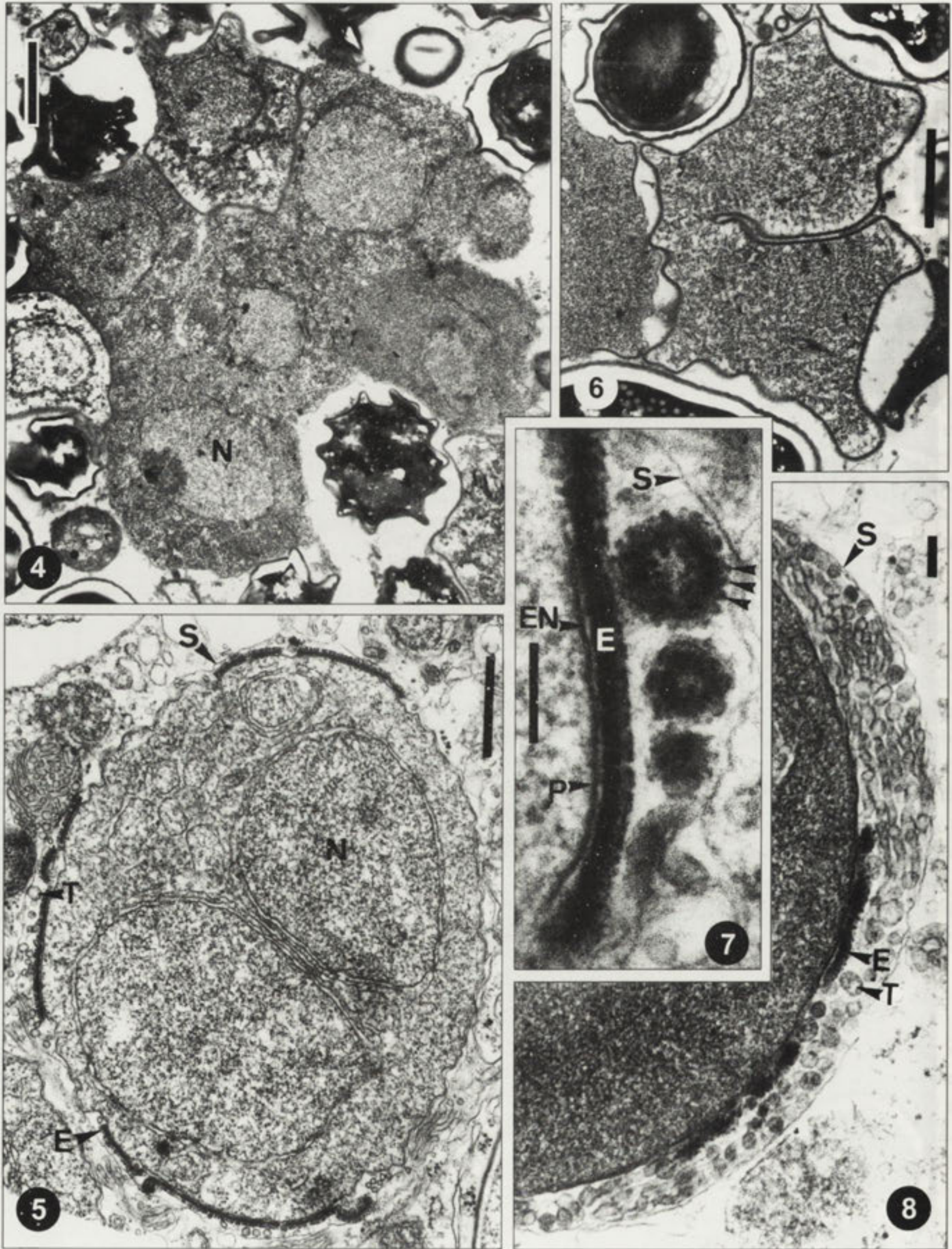

Figs. 4-8. Late merogony and early sporogony. 4 - rosette-like merogonial plasmodium, no traces of a sporophorous vesicle. 5 - young sporont with two nuclei visible; complete sporophorous vesicle, with thin walled tubules in the episporontal space and with developing exospore layer. 6 - finger-like lobed sporogonial plasmodium with complete exospore layer. 7 - sporont with developing exospore layer; thick-walled tubular structures of exospore material, with lobed periphery (arrowheads), protrude into the episporontal space. 8 - detail of a sporophorous vesicle filled with thin-walled tubular material. Scale bars: 4, 6 - 1 µm, 5 - 0.5 µm, 7, 8 - 100 nm

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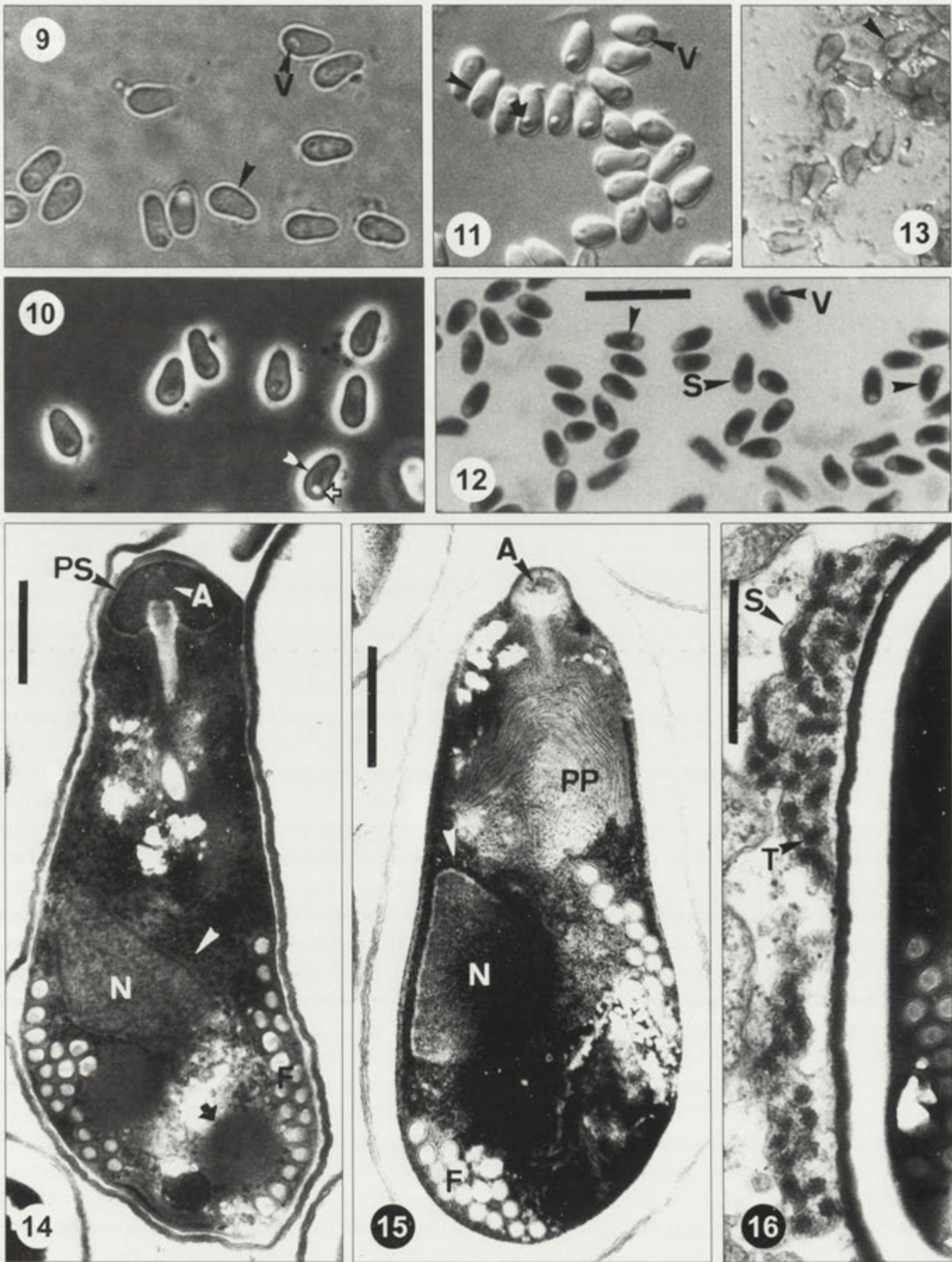

Figs. 9-16. Different aspects of mature spores. 9-11 living spores viewed in bright field (9), phase contrast (10), and interference phase contrast (11); arrows point at globular inclusions, arrowheads at the convex surface of the spore 12 - spores stained with haematoxylin. 13 - one of the two "*Thelohania cladocera*" in Coll. Jírovec, Prague, obviously the same species. 14, 15 - longitudinally sectioned immature and mature spores; arrow points at globular inclusion, white arrowheads at polyribosomes. 16 - sporophorous vesicle around mature spores, with exospore-derived tubules. Scale bars: 9-13 (with common bar on 12) - 10 μm, 14-16 - 0.5 μm

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reproduction were distinct (Figs. 2, 4). Merogonial plasmodia and merozoites had an about 8 nm thick plasma membrane without external reinforcements. The cytoplasm contained numerous free ribosomes, but there were few or none traces of endoplasmic reticulum (Fig. 4). Nuclei were of the normal eukaryotic type with a nuclear envelope of double unit membranes, separated by a perinuclear space, and with pores. In fixed and stained smears merogonial nuclei measured up to 2.6 µm in diameter. Merozoites were released by rosette-like budding (Fig. 4). It is unknown if there is more than one sequence of merogonial reproduction. The number of merozoites is unknown, but at least exceeds 4.

The last generation of merozoites matured to sporonts. Merozoites and young sporonts had a fairly dense cytoplasm with numerous free ribosomes, while the cytoplasmic density of older sporonts was variable (Fig. 2). The endoplasmic reticulum was weakly developed also in sporogonial stages, but a few cisternae were usually seen around the nuclei. Dividing nuclei exhibited up to 142 nm wide electron dense spindle plaques in shallow depressions of the nuclear envelope, and the radiating intranuclear mitotic spindle tubules measured approximately 18 nm in diameter. At the beginning of the sporogony the thin envelope of the sporophorous vesicle, approximately half as thick as a unit membrane, was detached from the surface (Figs. 2, 5). This was followed by secretion of electron-dense material on the surface of the sporont, building the future exospore layer (Figs. 2, 5, 7). The exospore was initiated over wide areas, not spot-wise, and it grew to a continuous coat before sporoblasts were budded off (Fig. 6). Approximately simultaneously with the initiation of the exospore, the lucent primordium of the endospore appeared (Figs. 5, 7). The exospore primordium, which measured about 31 nm thick, had a wide electron-dense, continuous internal layer and a surface layer which was moderately electron dense in the internal zone, prominently dense externally (Fig. 7). The surface layer was not continuous and transversely sectioned protrusions of exospore material exhibited a more or less folded periphery (Fig. 7). Simultaneously with the initiation of the thick sporont wall, exospore material produced in excess formed 20-70 nm wide thin-walled vesicles or tubules (Figs. 5, 7, 8), protruding into the sporophorous vesicle (Figs. 5, 8). Sporonts with a small number of nuclei, and not completely enclosed in a sporophorous vesicle, had up to 2.3 µm wide nuclei (in sections). Nuclei of sporogonial plasmodia with complete sporophorous vesicle, and with partially developed thick exospore-layer, were smaller, up to 1.8 µm wide (in sections and smears).

The cytoplasmic cleavage of the sporogonial plasmodia began already when the plasmodia were four-nucleate. Sporoblasts were formed by rosette- or finger-like budding (Figs. 3, 6). Sporoblasts were enveloped in folds of the sporophorous vesicle, and vesicle folds persisted to some extent also around peripheral spores. As the sporophorous vesicles were fragile, distinct groups of sporoblasts were not common in squash preparations. However, groups with 4 to 16 spores were seen, but 12 appeared to be the most common number. Plasmodia visible in light microscopic preparations had 4 or 8 fingers. The initiation and differentiation of the sporal organelles conformed with the normal for microsporidia, including the late initiation of the polaroplast and the maturing of the polar sac-anchoring disc complex (Fig. 14). In the immature spore, which has the complete number of polar filament coils but still not the definite shape and completely developed endospore layer, the polar sac is swollen like the cup of a mushroom and the anchoring disc appears as a dark globule on the top of the polar filament (Fig. 14).

The mature spore

Mature spores were pyriform, with both poles blunt. Typically one side was lightly convex (Figs. 9-12). Unfixed spores measured 2.34-3.03 x 4.07-4.93 µm (n = 50), fixed and stained spores 1.55-2.47 x 3.55-4.36 µm (n = 50). Obliquely and close to the posterior pole was a vacuole, measuring about one third of the spore length (Figs. 9-12). In haematoxylin stained preparations the vacuole of most spores was pale, while the nucleus was seen as a dark area in the centre of the spore (Fig. 12). A spherical refractive granule was frequently seen in the vacuole region of living spores (Figs. 10,11). The posterior vacuole was never well fixed in preparations for electron microscopy, but a distinct membrane lining was revealed. In ultrathin sections of immature, and sometimes also mature, spores the granule was visible as a homogeneous moderately or prominently dense substance (Fig. 14).

The spore wall measured 157-199 nm, except most anteriorly where sizes down to 55 nm were recorded (Fig. 15). It had the normal three subdivisions: a 35-40 nm thick exospore, a wide structure-less endospore, which was more narrow anteriorly, and an approximately 8 nm wide plasma membrane. The exospore had five layers which in direction outwards were (Fig. 17: 1-5): an about 8 nm thick double-layer (1), an 8-10 nm wide fairly lucent layer (2), a narrow dense zone of about 5 nm (3), an 8-10 nm thick moderately dense material (4), and a dense surface layer (about 5 nm) (5). The tubular projections formed in connection with the development of the sporont

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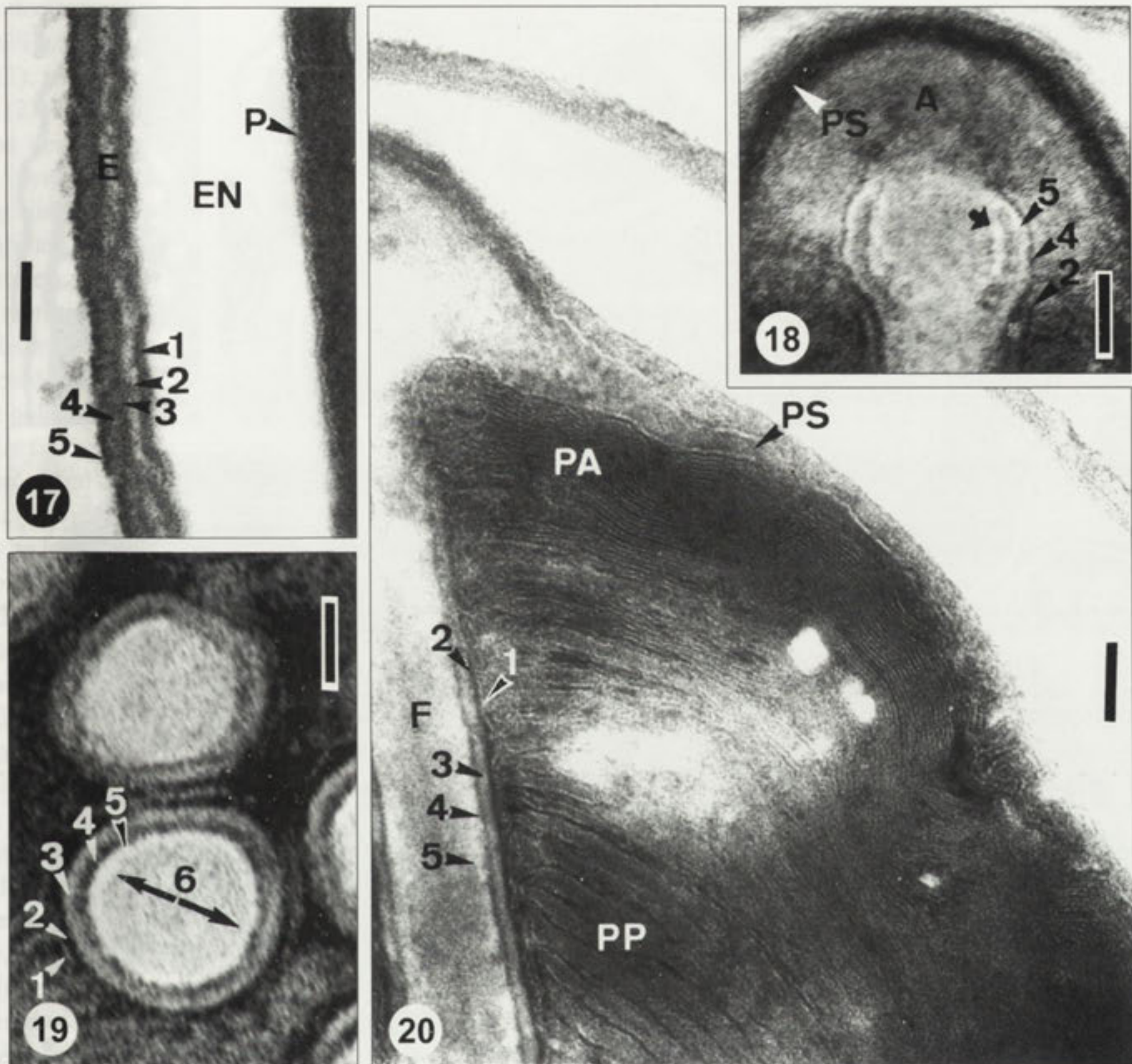

Figs. 17-20. Ultrastructural details of the mature spore. 17 - longitudinally sectioned spore wall, 1-5 indicate the layers of the exospore. 18 - connection between the polar filament and the anchoring disc; the layers of the filament are broken at the attachment, arrow points at a filament layer not present outside the polar sac. 19 - transversely sectioned polar filament coils; layers indicated 1-6. 20 - anterior part of a mature spore exhibiting the construction of the polaroplast. Scale bars - 50 nm

wall remained with unchanged structure also in vesicles with mature spores (Figs. 2, 16).

The polar filament was attached to a moderately electron-dense, convex anchoring disc at the anterior pole of the spore (Fig. 15). The widest sectioned disc measured 285 nm in diameter. Close to the disc the filament widened to form a globular body, about 1/4 wider than the filament proper (Fig. 18). The filament was anteriorly up to 117 nm wide. It proceeded straight backwards along the central axis of the spore for about 1/3 of the spore length, then turned sidewards and touched the spore wall about 1/3 from the posterior pole of the spore (Fig. 15). The final part was coiled in two irregular rows of anteriorly directed coils close to the spore wall. In the longitudinally sectioned spore the group of coils to one side was localized to the posterior 1/3 of the spore, while the group to the other side was in the equator plane (Fig. 15). The angle of tilt of the most anterior coil to the long axis of the spore was about 30°. The rows of coils extended for about 1/4 of the spore length. There were totally 14-17 isofilar, 77-94 nm wide coils. The transversely sectioned filament exhibited a series of layers of varying thickness and electron density.

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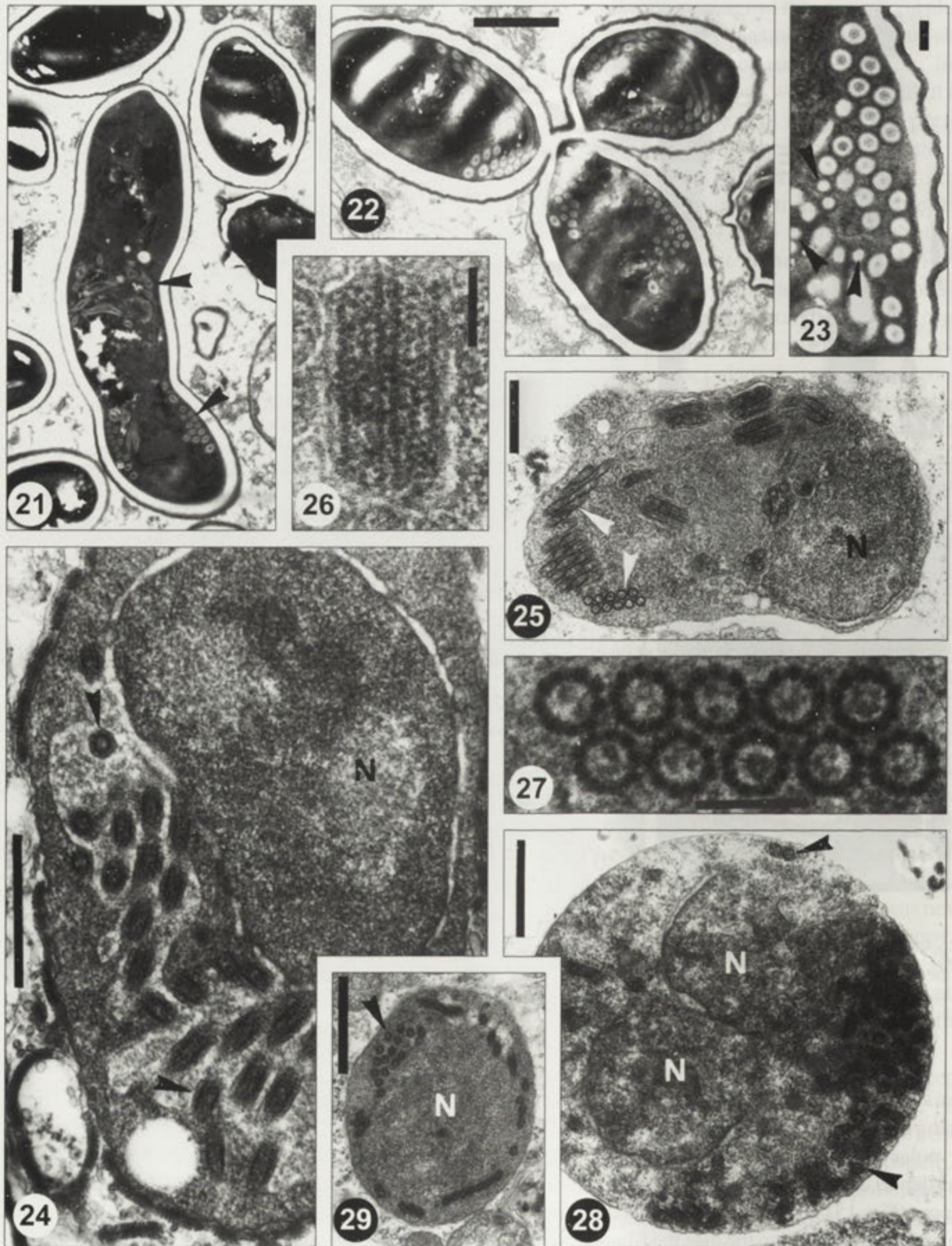

Figs. 21-29. Anomalies (21-23) and unusual cytology of the polyribosomes (24-29). 21 - anomalous macrospore with duplicated coiling (arrowheads). 22 - triskelion-like configuration caused by incomplete fission. 23 - irregular coiling with supernumerary immature coils (arrowheads). 24, 25 - sporont lobes with tubulus-like polyribosomes (arrowheads). 26 - longitudinally sectioned polyribosomes. 27 - transversely sectioned polyribosomes; each circle composed of 9 ribosomes. 28 - young sporont with two nuclei visible exhibiting polyribosomes (arrowheads). 29 - sporont lobe with circumnuclear arrangement of the polyribosomes (arrowheads). Scale bars: 21, 22, 29 - 1 μm, 23, 26, 27 - 100 nm, 24, 25, 28 - 0.5 μm

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In direction inwards the following layers could be discriminated (Figs. 18-20: 1-6): a 5 nm thick unit membrane cover (1); two approximately 6 nm thick layers, electron dense (2) and moderately dense (3); a more narrow electron dense zone (4); an approximately 6 nm wide lucent layer of fibrillar nature (5); and the centre (6) lacking distinct stratification. The two external layers (1, 2) joined with the polar sac. They were absent from the swollen part of the filament, where a new layer of lucent material appeared in zone 6 (Fig. 18). The layering was absent from the area where the polar filament connected with the anchoring disc (Fig. 18).

The polaroplast, which surrounded the uncoiled part of the polar filament and terminated slightly anteriorly of the most proximal coil (Fig. 15), was of the generalized type for microsporidia. The anterior short section, approximately as long as the diameter of the polar filament, was composed of densely packed 7-13 nm wide lamellae (Fig. 20). The posterior part had regularly arranged lamellae, wider and of fairly uniform thickness, 17-20 nm. The unit membrane-lined polar sac enclosed the anchoring disc, and its posteriorly directed umbrella-like part covered approximately 2/3 of the polaroplast (Fig. 20). It was filled with uniform electron-dense material. The approximately 5 nm thick unit membrane components of the polar filament, the polaroplast lamellae and the polar sac belonged to the same membrane system.

The irregularly shaped nucleus, when seen in sectioned spores, was localized slightly below the equator of the spore (Figs. 14, 15). The widest section through a nucleus measured 0.8 µm in diameter. The cytoplasm was electron-dense with prominent strands of polyribosomes in the proximity of the nucleus, filament coils and polaroplast (Figs. 14,15).

The sporophorous vesicle remained also enclosing mature spores. The envelope was uniform and thin, about 6 nm. Most of the tubular projections formed in the initial phase of the sporogony disappeared when the sporoblasts matured, but occasionally even fairly great numbers of tubules remained together with the mature spores (Fig. 16).

Anomalies and unusual cytology

Two clear anomalies were observed in sporogonial stages. In series No. 940812-A2 elongated macrospores of a size up to three times the normal length were observed (Fig. 21). The internal organization was disturbed with the polar filament coils arranged at two levels. In series No. 930914-Å duplicated or triskelion-like arrangement of matures spores was seen (Fig. 22). This was a result of a disturbed sporogonial fission where the sporoblast have been unable to separate.

In series No. 940812-A otherwise normal spores exhibited anomalous morphogenesis of the polar filament. The filament was longer than normal, and the coiling was disorganized in that immature and hence more narrow coils with less complex structure were mingled with the completely developed and wide ones (Fig. 23).

In one of the series (No. 940812-A) ribosomes were arranged in such way that they gave the impression of forming tubular structures (Figs. 24-29). Transverse sections of the aggregations were completely circular (Fig. 27). Each circle, which measured 70-100 nm in diameter, consisted of 9 ribosomes. The ribosomes were not associated with visible membraneous structures (Fig. 27). The configurations, which must be characterized as polyribosomes, were often arranged concentrically around the nucleus (Fig. 29). They were present from the beginning of the sporogony (Fig. 28).

DISCUSSION

Cytology

Anomalous sporogony is not unusual among microsporidia. Triskelion-like configurations of spores (Fig. 22), the result of incomplete separation of sporoblasts, appears to be especially common among microsporidia with rod-shaped spores, for example observed in *Bacillidium strictum* (see Larsson 1992). Anomalies in the coiling of the polar filament, including the production of supernumerary coils (Fig. 21), is also previously known from microsporidia, for example observed in *Cystosporogenes deliaradicae* (see Larsson *et al.* 1995).

It is a normal feature of an immature spore that the posterior part of the growing polar filament is more narrow and still lacking some structural components. However, normally only the last 1-2 coils are immature. In the spores seen here (a detail reproduced as Fig. 23) the immature part consists of several coils and the coiling is irregular.

The "ribosome-tubules" is probably not an anomaly. We have seen the same arrangement in one more species (undescribed observation), and similar structures are visible in spores of *Heterosporis finki* (in Fig. 9, Schubert 1979). The structures are present already in young sporonts (Fig. 28), and when viewed at low magnification they might be taken for early initiation of the polar filament coils like the process characteristic for the *Enterocytozoon* species (Desportes *et al.* 1985, Cali and Owen 1990),

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which is not the typical way of filament initiation (Vávra 1976, Larsson 1986). Transverse sections of the aggregations, viewed at great magnification (Fig. 27), revealed that each circle was composed of 9 ribosomes. Superficially the configurations resembled the way the doublet microtubules are arranged in cilia and flagellae. Membraneous structures were not observed together with the circles. Polyribosomes are characteristic components of the microsporidian spore, but irrespective of the plane of sectioning they are normally seen as lines of paired structures sandwiched between membranes (in Figs. 14, 16, 17, Larsson 1984). The arrangement of polyribosomes in the spore has been described as "Spiralstrukturen" (Schubert 1979), which must denote the arrangement around the nucleus. Spiralized polyribosomes, like the arrangement visible in Fig. 3:6 in Cormack (1984), has not been observed in microsporidia.

Taxonomy

Although if the microsporidium has been seen by previous investigators of the microsporidia of microcrustaceans, we cannot find that it has been named. The species is present in the collection of Otto Jírovec, Prague, where it is one of two microsporidia listed under the name "*Thelohania cladocera*". One of these, with a spore size corresponding with Pfeiffer´s species *Glugea cladocera*, has recently been redescribed and transferred to the genus *Agglomerata* (Larsson *et al.* 1996). The host of this microsporidium was clearly stated to be *Daphnia magna*. The host of the second microsporidium (Fig. 13) is uncertain. On one of the two labels the host is indicated as *Daphnia magna* ("D. m."), which must denote the primary identification. However, on both slides Jírovec has later added "*Dafnia pulex*". It is unclear if this addition is a mistake or if the primary identification was made tentatively.

A century ago Pfeiffer described the new species *Glugea leydigii* from the fat body of German specimens of *Daphnia pulex* (see Pfeiffer 1895). The pathogenic action described is commonly encountered in microsporidioses of Cladocera: infected animals were chalky white and their movements were slowed down. Infection spread from the fat body to the hypodermis. The description is very brief and as no data about dimensions is provided *G. leydigii* must actually be considered to be a *nomen dubium*. The sporogony and spores are illustrated. Sporogonial plasmodia are rounded and multinucleate, and the sporogony yields 8, 12 or 24 pyriform spores in a round or ellipsoid "Sporocyste", which is drawn with a thick, distinct wall (Fig. 37 B: 6-7 in Pfeiffer 1895). Even if there are few characters that can be compared, the obviously more acute spore shape, the prominent and obviously persistent sporophorous vesicles, and the different host species tell the difference to the microsporidium studied by us.

Pleistophora ellipsoidea, another microsporidium invading the fat body of *Daphnia magna*, produces oval to lightly pyriform spores (Vidtmann 1990). Living spores measure 2,6-3,1 x 4.2-5.1 µm, fixed and stained spores 2.4-3.1 x 3.3-4.4 µm. The sporogonial plasmodium is elongated oval, the sporogony is polysporoblastic yielding up to 16 sporoblasts by rosette-like budding. The sporophorous vesicle is fragile and destroyed when making preparations. *P. ellipsoidea* and the microsporidium treated herein share the host, the tissue affinity, probably the number of spores produced, and the size of the spores. Ultrastructural characters cannot be compared. However, as two differences appear fairly clear, the sporogony of *P. ellipsoidea* involves multinucleate rounded sporogonial plasmodia, and the spores appear more stout and blunt, we feel convinced that the species treated herein is different from *P. ellipsoidea*.

Isolated nuclei in all stages of the life cycle, polysporoblastic sporogony (yielding at least 4 spores) in sporophorous vesicles, and spores exhibiting normal microsporidian cytology is characteristic for nearly 30 microsporidian genera. Restricting the comparison to genera considered to be monomorphic, having isofilar polar filament, producing four or more sporoblasts by rosette- or finger-like budding in sporontogenetic sporophorous vesicles uniting all daughter cells of the sporont, the number is reduced to 4: *Flabelliforma*, *Glugea*, *Larssonia*, and *Tardivesicula*. Two of them can be disregarded. The *Glugea* species are parasites of fish (Canning and Lom 1986) producing oval spores. *Tardivesicula*, so far with the only species *T. duplicata*, has elongated, almost rod-shaped spores (Larsson and Bylén 1992).

The two remaining genera cannot be compared in all details, but there are a few distinct differences, most obvious in the spore shape. The spores of *Larssonia obtusa*, so far the only species of the genus, are distinctly pyriform (Vidtmann and Sokolova 1994), while the spores of *Flabelliforma montana*, the type species of *Flabelliforma*, are flattened on one side, convex on the other (Canning *et al.* 1991), resembling the spores of the species treated herein (Figs. 9-12). It is generally accepted that the spore shape is constant for a microsporidian genus. A closer comparison with *F. montana* reveals further similarities: finger-like divisions in the sporogony

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(Figs. 15, 16 in Canning *et al.* 1991), initiation of the sporophorous vesicle without formation of blisters, and tubular inclusions of the episporontal space (visible in Figs. 32 and 34 of the description in Canning *et al.* 1991). Like in *F. montana* folds of the sporophorous vesicle persist around sporoblasts and, at least to some extent, also around peripheral mature spores. The mature spores of *F. montana* were not well preserved and detailed comparison of the sporal cytology is hardly possible. The exospore appears fairly thick and some kind of layering is visible (Fig. 34 of the description in Canning *et al.* 1991). With clear similarities in taxonomically important characters (e. g. spore shape and sporogonial division), and without indications of distinct differences in the ultrastructural cytology, the species described herein fits well in genus *Flabelliforma*.

Two more species have been assigned to the genus *Flabelliforma*, both of them are parasites of microcrustaceans: *F. ostracodae*, a parasite of Ostracoda (Bronvall and Larsson 1994), and *F. diaptomi*, a parasite of Copepoda (Voronin 1996). They are clearly different from the species treated herein. Spores of both species are smaller than the spores of this species, and in addition the hosts are not closely related with the Cladocera. *F. ostracodae* was tentatively identified to belong in *Flabelliforma*, which makes detailed comparison superflous. *F. diaptomi* has the spore shape typical for the genus, and a distinctly stratified exospore, with a layering resembling the layering of *F. magnivora*.

DESCRIPTION

Flabelliforma magnivora sp. n.

Merogony: merogonial stages with isolated nuclei. Reproduction by rosette-like budding. The number of merozoites unknown but exceeds 4. It is unknown if there is more than one merogonial cycle.

Sporogony: sporogonial plasmodia with isolated nuclei yield 4-16, usually 12, uninucleate sporoblasts by finger-like budding.

Spore: pyriform, with both ends blunt, often with one side curved. Distinct posterior vacuole in oblique position in the posterior third of the spore. Refractive globular granule visible in vacuoles of living spores. Unfixed spores measure 2.34-3.03 x 4.07-4.93 µm, fixed and stained spores 1.55-2.47 x 3.55-4.36 µm. The spore wall measures 157-199 nm, with a 35-40 nm wide five-layered exospore. The polar filament is isofilar, arranged in 14-17, 77-94 nm wide coils, in two irregular layers of coils below the equator of the spore. The angle of tilt is about 30°. Sectioned anchoring discs are up to 285 nm wide. Polaroplast with a short anterior, and a long posterior section of regularly layered lamellae. Anterior lamellae measures 7-13 nm, posterior ones 17-20 nm. Nucleus (sectioned) up to 0.8 µm in diameter, in the posterior half of the spore.

Sporophorous vesicle: a thin, fragile envelope is formed at the beginning of sporogony, simultaneously all over the sporont; vesicle folds enclose sporoblasts. The episporontal space is traversed by thick-walled and thin-walled tubules of exospore-derived material.

Site of infection: adipose tissue, hypodermis and ovary.

Type host: *Daphnia magna* Strauss, 1820 (Crustacea: Cladocera: Daphniidae).

Type locality: UK, a pond near Oxford (Latitude 51°37,25', Longitude 1°22,30').

Types: syntypes on slides No. 970727-A3-(2-5).

Deposition of types: in the International Protozoan Type Slide Collection at Smithsonian Institution, Washington DC, and in the collections of Dr. J. Weiser, Charles University, Prague, Czech Republic, and of, R. Larsson, Department of Zoology, University of Lund, Sweden.

Etymology: derived from the name of the host and the Latin verb *voro* = devour.

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Eimeria gnui sp. n. (Apicomplexa: Eimeriidae) from White-tailed Gnu, *Connochaetes gnu*

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Summary. Oocysts of *Eimeria gnui* sp. n. are described from the faeces of the white-tailed gnu, *Connochaetes gnu* from the Riyadh Zoo, Saudi Arabia. Sporulated oocysts are elongate-ellipsoid 43.5 x 26.3 (40-49 x 24-29) µm, and posses finely pitted wall and bilayered, 2 (1.5-2.5) µm; micropyle is present but a polar granule and oocyst residuum are absent. Sporocysts are elongate-ovoid, 18.7 x 9 (16-20 x 7.6-10) µm, with a sporocyst residuum and Stieda body. Sporozoites are elongate, club shaped, and contain two prominent refractile bodies.

Key words: *Connochaetes gnu*, description, *Eimeria gnui*, white-tailed gnu.

INTRODUCTION

Coccidial infection is common in oryx and gazelle of Riyadh public Zoo (Kasim and Al-Shawa 1988, Kasim *et al.* 1991). Although three species of *Eimeria* have been reported from members of the subfamily Alcelaphinae (Pellerdy 1974), only one *Eimeria connochaetei*, has been described from *Connochaetes gnu* (Levine and Ivens 1970).

During a survey of the parasitic fauna of Riyadh Zoo, three white-tailed gnu were examined for *Eimeria* infection, two of which were found positive for an undescribed species. The present paper describes the morphological characteristics of this new species of *Eimeria*.

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MATERIALS AND METHODS

Freshly passed faecal samples from three adult white-tailed gnus, *Connochaetes gnu*, were collected separately during December, 1996. Each sample was put in a labelled vial and kept cool during the journey from Riyadh Zoo to the parasitology Laboratory at the Zoology Department, College of Science, King Saud University. Routine faecal examinations using Sheather's sugar flotation (Levine 1973) was performed on each sample. Positive samples were incubated in a thin layer of 2.5% (w/v) aqueous potassium dichromate solution at 28 ± 2°C and examined periodically to determine the sporulation time. Fifty sporulated oocysts and fifty sporocysts from each sample were measured with a microscope fitted with a 100x apochromatic oil immersion objective and a 10x ocular micrometer. The number of layers of the oocysts wall, its thickness, and the detailed structure of the sporocysts were examined

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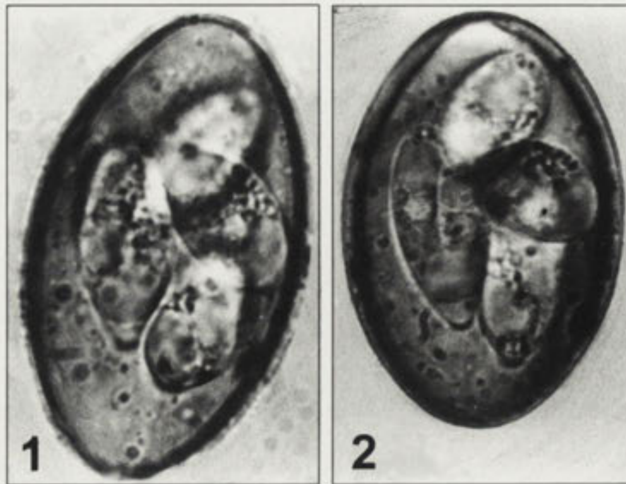

Fig. 1,2. Photomicrographs of living sproulated oocysts of *Eimeria gnui* sp. n. x1000

Fig. 3. Camera lucida drawing of sporulated oocysts of *E. gnui* sp. n. Scale bar - 15 µm

after crushing the oocysts by pressure on the coverslip. Drawing was made with the aid of camera lucida. All measurements are in micrometers (µm) with the mean followed by the range in parentheses.

RESULTS

Eimeria gnui (Figs. 1-3)

Description

Sporulated oocysts elongate-ellipsoid, flattened at both ends, 43.5 x 26.3 (40-49 x 24-29) µm (n = 50). Shape index (length/width ratio) 1.65 (1.58-1.79). Oocyst wall bilayered (confirmed by crushing oocysts with gentle coverslip pressure), 2 (1.5-2.5) µm thick. Outer wall layer yellowish-brown, finely pitted, about two-thirds of total thickness; inner layer dark brown. Micropyle present

Table 1. Comparative of species of *Eimeria* reported from the subfamily Alcelaphinae

Original structure	*E. gnui*	*E. connochaetei*	*E. gorgonis*	*E. talboti*
Oocyst				
shape	slightly ellipsoid	roughly ellipsoid	ellipsoid	ovoid
size in µm	40-49 x 24-29 (43.5 x 26.3)	20 - 27 x 13-15 (22.1 x 14)	20.5-26 x 15-18 (22.7 x 16.5)	35-38 x 22.27 (36.2 x 24.7)
shape index	1.65	1.58	1.38	1.47
micropyle	present	absent	absent	absent
polar granule	absent	absent	present	absent
Sporocyst				
shape	elongate ovoid	ovoid	lemon	-
size in µm	16-20 x 7.6-10 (18.7 x 9.0)	12-13 x 6-7	12-15 x 4.5-6	12.5-15 x 8.7-10 (14 x 9.6)
Host	*Connochaetes gnu*	*Connochaetes gnu*	*Gorgon taurinus*	*Alcelaphus cokei*

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5 (4-6) µm wide; oocyst residuum and polar granule absent. Sporocysts elongate-ovoid, 18.7 x 9 µm (16-20 x 7.6-10) (n = 50); L/W ratio 2.08 (1.78-2.38); Stieda body present; substiedal body absent. Sporocyst residuum present, composed of many scattered granules. Sporozoites elongate-club shaped, lying head to tail in the sporocysts, each with a small anterior 3.5 (3-4) and large posterior 5.5 (4-6) refractile bodies.

Taxonomic summary

Type host: white-tailed gnu, *Connochaetes gnu* (Alcelaphinae).

Type locality: Riyadh Zoo.

Prevalence: found in two of three (67%) *C. gnu*.

Site of infection: unknown, oocysts recovered from faeces.

Sporulation time: five days at 28 ± 2°C.

Etymology: the specific name *gnui*, is derived from the species name of the host.

Type specimens: oocysts in 10% formalin and a phototype are deposited in the parasitological collection, Zoology Department, College of Science, King Saud University, Riyadh both as KSUC-97.

DISCUSSION

To our knowledge, there are only three species of *Eimeria* have been described previously from members of the subfamily Alcelaphinae (Pellerdy 1974). These are *E. chonnochaetei* (Levine and Ivens 1970) from *Connochaetes gnu*, *E. gorgonis* (Prasad 1960) from *Gorgon taurinus*, and *Eimeria talboti* (Prasad and Narayan 1963) from *Alcelaphus cokei*. *Eimeria gnui* can be easily distinguished from all these three *Eimeria* species in having much larger oocysts and sporocysts, possessing a micropyle and in having a finely pitted outer layer of the oocyst wall. Our new species differs from *E. gorgonis* by lacking a polar granule (Table 1). These structural and host differences lead us to suggest that *E. gnui* is a distinct species.

Acknowledgements. We wish to thank the Riyadh Zoo directors for their co-operation.

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ACTA
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Light and Scanning Electron Microscopy of *Chloromyxum vanasi* sp. n. (Myxozoa: Myxosporea) Infecting Gallbladder of the Nile Catfish *Bagrus bayad* (Forskal, 1775) (Teleosti: Bagridae)

Mohamad A. ALI

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Summary. Light and SEM description of a new myxosporean species *Chloromyxum vanasi* which infected the gallbladder of the Nile catfish *Bagrus bayad* (Forskal, 1775) are presented. The host fishes were examined from two localities in Egypt, Nile River and Wadi El-Raiyan Lake. Prevalence of infection was very low but the infection was severe when found. Pseudoplasmodia are polysporic, rarely disporic, round to ellipsoid with holdfast projections and pseudopodia. Spores are subspherical with 2 supracapsular loops, 2-3 extrasutural ridges. Some spores contained a transverse furrow at the surface of the posterior third of the shell. Polar filament pores are very conspicuous and arranged in zig-zag position. Dissimilar arrangement of shell ridges was evident and asymmetrical shell valves were observed.

Key words: *Chloromyxum*, Egypt, fish parasites, Myxosporea.

INTRODUCTION

About 53 myxosporean species belonging to 9 genera have been described so far from freshwater fishes in Africa. Out of these myxosporeans, only three members of the genus *Chloromyxum* Mingazzini, 1890 are reported; two from marine fishes of coastal areas (Kudo 1920) and one from a freshwater fish host (Fomena and Bouix 1991). The three species were presented in light microscopy which seems to be of little taxonomic value in describing this genus. Lom and Dykova (1993) stressed on the use of scanning electron microscopy (SEM) as a prerequisite for any well based description of *Chloromyxum* species.

In this paper, light and SEM description is presented for *Chloromyxum vanasi* sp. n. from the freshwater Nile catfish *Bagrus bayad*.

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MATERIALS AND METHODS

Fresh or live fish were collected from two localities: (i) - fish markets of Cairo representing the Nile catch and, (ii) - samples from Wadi El-Raiyan Lake in the western desert of Egypt. Nile samples (90 fishes) were collected from November 1996 to June 1997; the fish ranged from 37-71 cm in total length. The Wadi El-Raiyan Lake samples (47 fishes) ranged from 24-73 cm in total length and were collected during September 1996 to April 1997.

The contents of the gallbladder were checked microscopically for myxosporean parasites. Detected spores and pseudoplasmodia were

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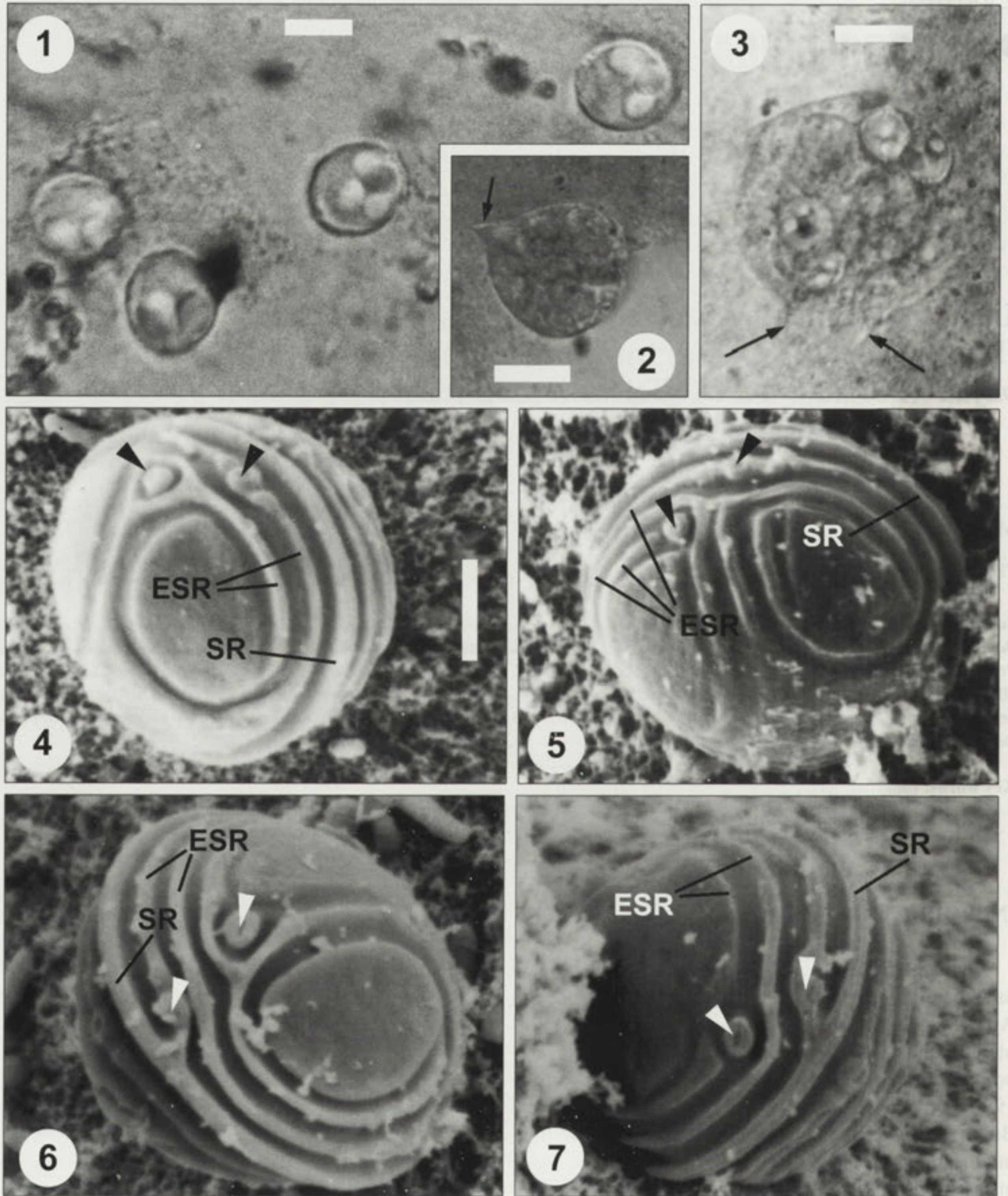

Figs. 1-7. *Chloromyxum vanasi* sp. n. from *Bagrus bayad*. 1 - light micrographs of fresh spores; 2,3 - polysporic pseudoplasmodia with stalk-like holdfast projections (arrows), 4-7 - SEM micrographs; 4,5 - typical mature spores with supracapsular loops, second polar filament pore lie in a triangular pit; 6,7 - mature spores with two asymmetrical spore shells and the first extrasutural ridges merging into the main sutural ridge. ESR - extrasutural ridge, SR - sutural ridge, arrowhead - polar filament pore, Scale bars: 1 - 4.5 µm; 2,3 - 13 µm; 4-7 - 2 µm

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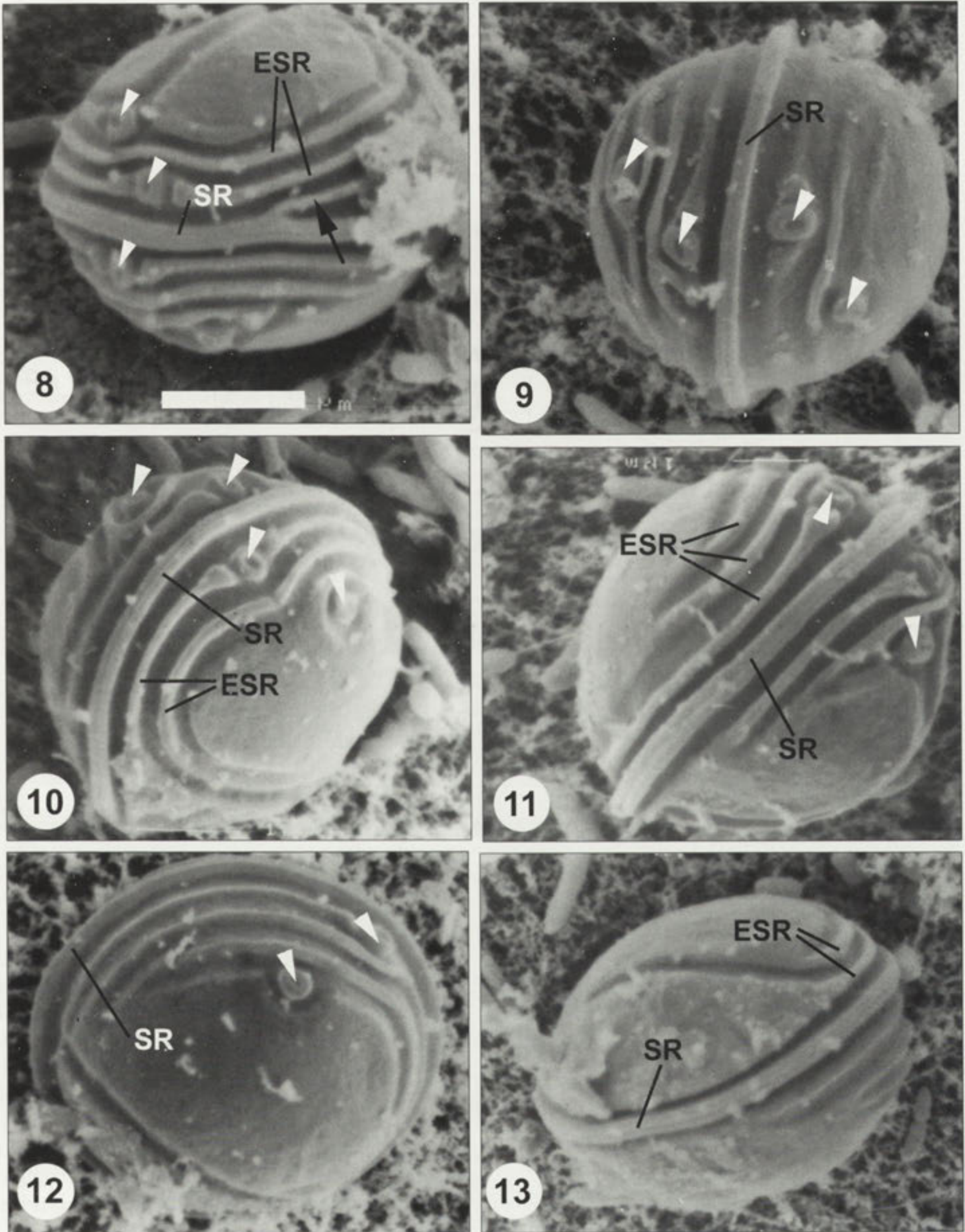

Figs. 8-13. *Chloromyxum vanasi* sp. n. from *Bagrus bayad*. 8 - mature spore with incontinuous ridge merging into the main sutural ridge (arrow); 9 - immature spore showing the different pattern of the shell ridges at both sides; 10,11 - spores at premature stage 12,13 - spores with a posterior transverse furrow. ESR - extrasutural ridge, SR - sutural ridge, arrowhead - polar filament pore. Scale bar: 8-13 - 2 μm

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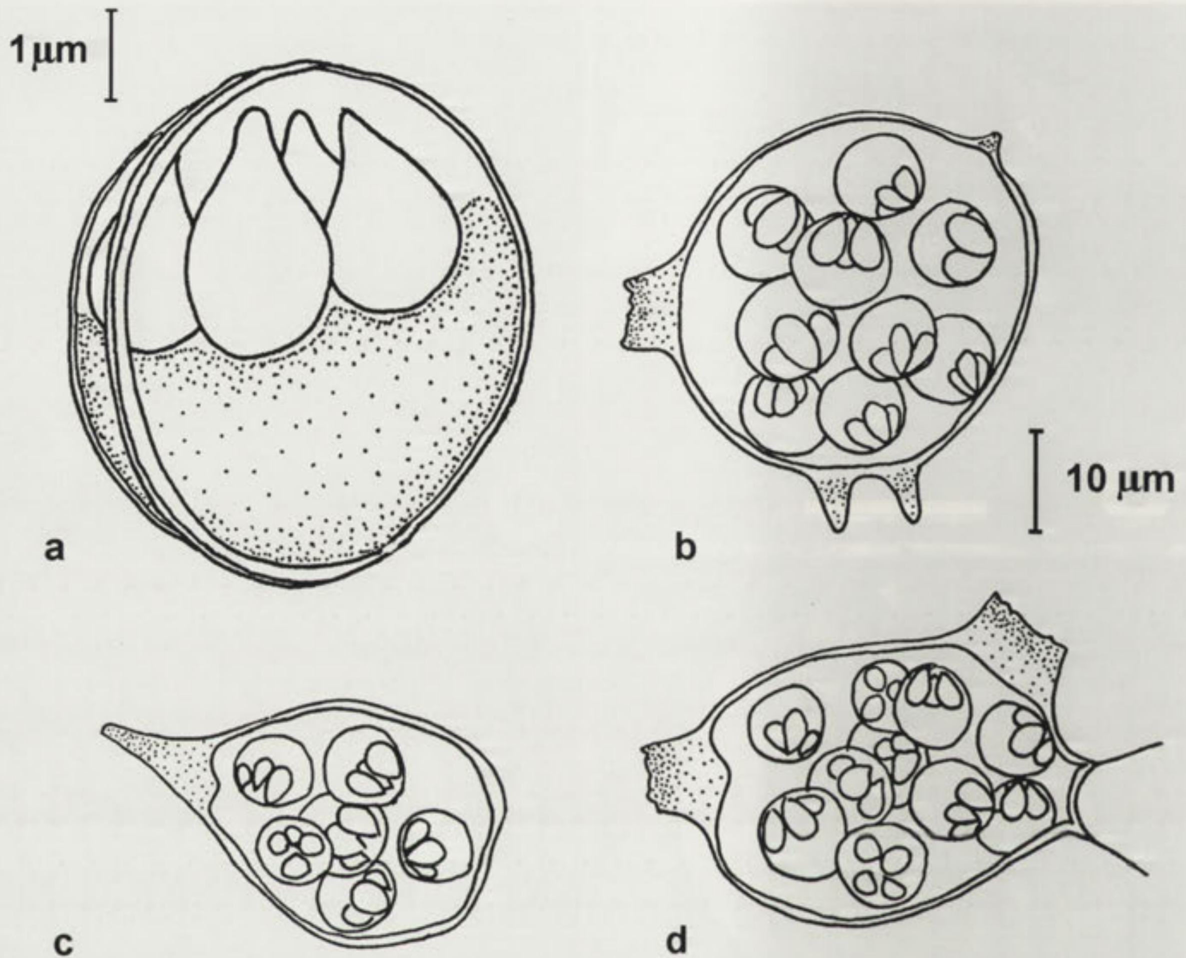

Figs. 14 a-d. Schematic drawings of *Chloromyxum vanasi* sp. n.; a - spore; b-d - different shapes of pseudoplasmodia showing the pseudopodia and the holdfast projections. Scale bars: a - 1 µm, b-d - 10 µm

measured and photomicrographed while fresh. The positive gallbladders were fixed in 5% neutral buffered formalin and used for SEM.

Bile fluid and squashed bile contents were washed and then dehydrated in ethanol gradient. The material was then critical point dried, sputtered with gold and studied in a Jeol Winsen JSM 6400 at 5 kV.

Terminology of the spore description followed Listebarger and Mitchell (1980) and Lom and Dykova (1993).

RESULTS

Fish samples collected from the Nile and Wadi El-Raiyan Lake showed a very low prevalence of infection, 1.1 % (1/90) and 4.3% (2/47), respectively. In the single infected Nile specimen, the infection was severe and the gallbladder attained a turbid deep bluish green color. Microscopic examination revealed that bile turbidity was due to enormous amounts of fine fragments and adhered aggregates of pseudoplasmodia. Masses of these plasmodia were also attached to the epithelia of the gallbladder.

Pseudoplasmodia are polysporic (up to 12 spores were observed) and rarely disporic. The plasmodia have irregular shapes but are mostly rounded to ellipsoid, reaching 34x42 µm. They have granular and refractile endoplasm with a clear hyaline zone of ectoplasm. Most of the plasmodia showed pseudopodia and holdfast outgrowths ranging from hair-like to stalk-like projections (Figs. 2, 3, 14 b-d).

Spores are subspherical (Figs. 1,14a) measuring 6.5 ± 0.4 (5.9-6.9) µm in length and 6.0 ± 0.3 (5.4-6.8) µm in thickness (n=25). The four polar capsules are oval and nearly equal measuring 3.3 ± 0.5 (3.1-3.9)

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x 2.5 ± 0.5 (1.8-2.9) μm. The number and pattern of polar filaments could not be discerned.

The surface pattern of the spores as revealed by the SEM showed some variability. The most salient feature of the mature spore was the two supracapsular smooth areas or "loops" which occupy more than half of the anterior part of the spore valve (Figs. 4, 5). Fully formed spore usually posses one prominent sutural ridge (SR) and 2 -3 extrasutural ridges (ESRs). The first ESR is continuous through the whole circumference of the spore and interrupted anteriorly by the first polar filament pore (PFP). In some spores, the first ESR is merged into the main SR (Figs. 6, 7). The second ESR runs parallel to the SR at the anterior half of the spore then wound centrally to form loops which delimit two supracapsular clear areas. These loops are usually separated by a small vertical ridge. Sometimes the second ESR also is incontinuous and merge into the first one (Fig. 12). A transverse furrow may exist in some spores at the posterior third and it is delimited by the first and second ESRs (Figs. 12,13). The PFPs are very conspicuous and arranged in zig-zag position. The second PFP is located in different positions. In some spores, it is found inside a triangular pit (Figs. 4,10) while in others it may be next to the third ESR (Figs. 5-8). The surface ridges of the two shell valves were dissimilar (Figs. 9, 11) and many mature spores showed different shell sculpture on both sides (Figs. 4, 7, 10).

DISCUSSION

Kudo (1920) illustrated two *Chloromyxum* species from fishes at coastal areas of Africa, *C. magnum* Awerinzew, 1913 and *C. quadratum* Thelohan, 1985. Both species differ from the present material; *C. magnum* is characterized by extraordinary large size, while, *C. quadratum* has quadranular spores. The third *Chloromyxum* species in Africa, *C. birgii* Fomena and Bouix, 1991 varies in having smooth shells. *C. fluviatile* Thelohan, 1892 (type A) as reported by Lom and Dykova (1993) is the only closely resembling species to our material. The similarity lies in the presence of two supracapsular clear areas. However, *C. vanasi* differ in the host, plasmodia, spore dimensions, and, especially, the sculpture of the shell valves. Therefore, the present material is believed to be a new myxosporean and the specific name is dedicated to Professor Van As, Bloemfontein, South Africa. The type material of the present spores is in the possession of the author.

During the preparation of *C. vanasi* sp. n. for SEM, the adhering fragments of pseudoplasmodia were squashed to release the spores. This technique should consider the likelihood of presence of many immature spores in the examined material and consequently a careful choice of the fully formed spores in describing the variability of the (mature) spores is necessary.

Many spores of *C. vanasi* sp. n. showed variable pattern of ridges on both shells of the same spore. This probability of asymmetry of shell valves is not included in the characters of the genus mentioned by Lom and Noble (1984).

Acknowledgment. The author is greatly indebted to Prof. Van As for his invitation to visit the University of Orange Free State (South Africa) in which the present SEM work was done. Thanks are due to Dr. Linda Basson for preparing the materials in SEM.

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ACTA PROTOZOOLOGICA

Karl-Ernst Wohlfarth-Bottermann - obituary notice. 1

REVIEW ARTICLE

A. Wasik: Antarctic tintinnids: their ecology, morphology, ultrastructure and polymorphism 5

ORIGINAL ARTICLES

P. J. Bischoff and O. R. Anderson: Abundance and diversity of Gymnamoebae at varying soil sites in Northeastern U. S. A. 17

S. A. Karpov and S. J. Coupe: A revision of Choanoflagellate genera *Kentrosiga* Schiller, 1953 and *Desmarella* Kent, 1880 23

M. Mulisch, T.Heep, W. Sturm and J. Borcherding: Redescription of *Ascobius lentus*, a rare freshwater folliculinid (Ciliophora: Heterotrichida) from a pond in Germany 29

J. I R. Larsson, D. Ebert, K. L. Mangin and J. Vávra: Ultrastructural study and description of *Flabelliforma magnivora* sp. n. (Microspora: Duboscqiidae), a microsporidian parasite of *Daphnia magna* (Crustacea: Cladocera: Daphniidae) 41

M.S. Alyousif and Y. R. Al-Shawa: *Eimeria gnui* sp. n. (Apicomplexa: Eimeriidae) from white-tailed gnu, *Connochaetes gnu* 53

M. A. Ali: Light and scanning electron microscopy of *Chloromyxum vanasi* sp. n. (Myxozoa: Myxosporea) infecting gallbladder of the Nile catfish *Bagrus bayad* (Forskal, 1775) (Teleosti: Bagridae) 57

1998 FEBRUARY

VOLUME 37 NUMBER 1

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