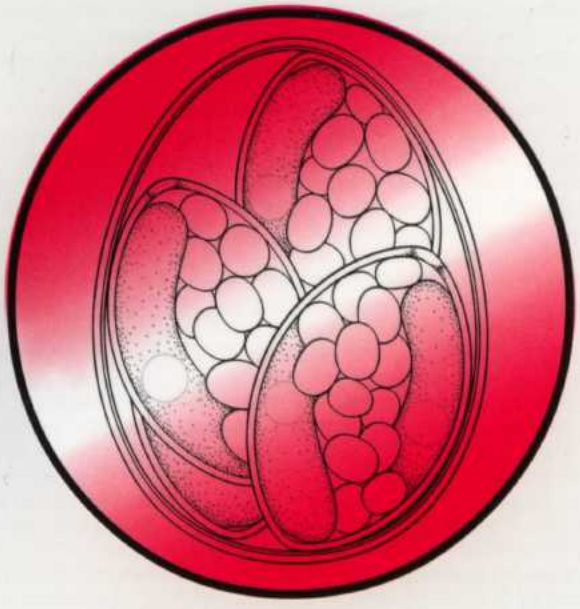
ACTA PROTOZOOLOGICA



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Review Article

Biogeography and Dispersal of Micro-organisms: A Review Emphasizing Protists

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Summary. This review summarizes data on the biogeography and dispersal of bacteria, microfungi and selected protists, such as dinoflagellates, chrysophytes, testate amoebae, and ciliates. Furthermore, it introduces the restricted distribution and dispersal of mosses, ferns and macrofungi as arguments into the discussion on the postulated cosmopolitism and ubiquity of protists. Estimation of diversity and distribution of micro-organisms is greatly disturbed by undersampling, the scarcity of taxonomists, and the frequency of misidentifications. Thus, probably more than 50% of the actual diversity has not yet been described in many protist groups. Notwithstanding, it has been shown that a restricted geographic distribution of micro-organisms occurs in limnetic, marine, terrestrial, and fossil ecosystems. Similar as, in cryptogams and macrofungi about, 30% of the extant suprageneric taxa, described and undescribed, might be morphological and/or genetic and/or molecular endemics. At the present state of knowledge, micro-organism endemicity can be proved/disproved mainly by flagship species, excluding sites (e.g., university ponds) prone to be contaminated by invaders. In future, genetic and molecular data will be increasingly helpful. The wide distribution of many micro-organisms has been attributed to their small size and their astronomical numbers. However, this interpretation is flawed by data from macrofungi, mosses and ferns, many of which occupy distinct areas, in spite of their minute and abundant means of dispersal (spores). Thus, I suggest historic events (split of Pangaea etc.), limited cyst viability and, especially, time as major factors for dispersal and provinciality of micro-organisms. Furthermore, the true number of species and their distribution can hardly be estimated by theories and statistics but require reliable investigations on the number of morphospecies in representative ecosystems. Generally, the doubts on Beijerinck´s famous metaphor "in micro-organisms everything is everywhere" can be focussed on a simple question: If the world is teeming with cosmopolitan unicells, where is everybody?

Key words: cryptogam spores, cyst viability, flagship species, Gondwana, human dispersal, Laurasia, local *vs*. global diversity, protozoa, undersampling.

INTRODUCTION

A comprehensive review on biogeography and dispersal of micro-organisms does not exist so far. Consequently, the relevant literature is highly scattered, often vague, and burdened with numerous misidentifications due to methodological shortcomings and flaws, patchy information, and, lastly, the widespread practice to use Holarctic identification literature to determine species from other regions (Foissner 1987, 1998; Lhotský 1998; Hoffmann 1999; Alongi *et al*. 2002). The view was developed that prokaryotes, unicellular eukaryotes and small multicellular organisms have a cosmopolitan distribution because of their minute sizes and their ability to form dormant stages (cysts, eggs, spores), which facilitate dispersal by air, dust, and migrating animals. As early as in 1913, this peaked in Beijerinck's famous

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metaphor "in micro-organisms, everything is everywhere, the environment selects", which soon became a paradigm in microbial ecology. Part of this view is likely to have been caused by the intuitive feeling that such minute creatures must have simple ecologies. This assumption, however, could not be supported in many recent studies (Laybourn-Parry 1992, Weisse *et al.* 2001, Azovsky 2002, Hausmann *et al.* 2003, Weisse 2004).

In recent times, Beijerinck's metaphor has been emphasized in particular by Fenchel *et al.* (1997), Finlay (2002), and Azovsky (2000, 2002), who applied ecological methods and concepts on both original studies and literature data of heterotrophic protists, mainly ciliates. This approach tends to be flawed by the fact that more than half of the protist world is still undescribed, especially the rare species (World Conservation Monitoring Centre 1992, Foissner *et al.* 2002). Accordingly, concepts which work well with higher plants and animals must fail if applied to micro-organisms where the global diversity is largely unknown. Furthermore, it is well established that even intensive studies of a certain habitat provide only a small fraction of global protist diversity (Finlay and Maberly 2000, Foissner *et al.* 2002). Thus, the postulate of Finlay and Esteban (1998) that "all species of freshwater protozoa could eventually be discovered in one small pond" is unlikely to be ever confirmed experimentally or by classical ecological and morphological studies. It has been shown repeatedly that the carrying capacity of habitats for species is limited not only for multicellular organisms but also for protists (Foissner 1999b). Accordingly, local distribution patterns must exist. This is supported by the meta-analysis of Hillebrand *et al.* (2001), who showed a decreasing similarity of species composition with increasing geographic distance both in diatoms and ciliates.

Data which fail to support the "everything is everywhere" paradigm have largely been ignored in the past, although they convincingly demonstrate a rather restricted distribution of many "flagship" genera and species belonging to various groups of protists (Bonnet 1983, Dragesco and Dragesco-Kernéis 1986, Tyler 1996, Vyverman 1996, Foissner 1999b, Foissner *et al.* 2002). Refined morphological methods and more sophisticated techniques, such as electron microscopy and molecular analyses, increasingly support the taxonomists' view on the existence of provincial micro-organisms, including small metazoans (Ferris and Ferris 1985; Bayly 1995; Reid 1998; Lee 2000; Gómez *et al.* 2002; Segers 2001, 2003; Trontelj 2004), protists (Foissner *et al.* 2001, Kristiansen 2001, Pawlowski and Holzmann 2002, Schlegel and Meisterfeld 2003, Fokin *et al.* 2004, Pochon *et al.* 2004) and bacteria (Cho and Tiedje 2000, Papke and Ward 2004). On the other hand, it is obvious that small organisms *tend* to have broader geographic ranges than large ones. This can be attributed, however, also to their high phylogenetic age that gave them more time to disperse. Thus, I basically agree with the statement of Fenchel (1993): "… smaller organisms tend to have wider or even cosmopolitan distribution, a higher efficiency of dispersal, a lower rate of allopatric speciation and lower rates of local and global extinction than do larger organisms". The more strict view that all microorganisms are distributed globally, however, receives increasing criticism not only from protistologists, like me and Coleman (2002), but also from other disciplines, especially molecular ecologists (Zettler Amaral *et al.* 2002, López-García *et al.* 2003, Stoeck and Epstein 2003, Lachance 2004, Šlapeta *et al.* 2005).

Related to the distribution problem is that of species diversity. The cosmopolitan school argues that microorganisms have low species richness because of the obvious lack of opportunities for allopatric speciation (Fenchel 1993, Wilkinson 2001, Finlay 2002). On the other hand, many micro-organisms are much older than the extant multicellular organisms giving them sufficient time for accumulating a high diversity, even if speciation rates are low! This interpretation is supported by bacteriologists (Cohan 2002) and mycologists (Hawksworth 2001), who envisage millions of species, nearly approaching May's (1986) famous correlation between body size and species numbers. The question of species richness has been addressed in my previous reviews, where I have provided evidence that, at least in ciliates, more than half of the free-living species have not been described yet (Foissner 1999b, Foissner *et al.* 2002). Notably, Aptroot (2001) has identified 200 species of ascomycetes associated with a single *Elaeocarpus* tree in Papua New Guinea, a figure which is similar to the numbers of species of soil nematodes per sampling site (Ettema 1998). Since some 160 ciliate species were found by Foissner (1999b) under 100 m^2 of beech forest soil, soil ciliates might be as diverse as nematodes and microfungi (World Conservation Monitoring Centre 1992, Hawksworth 2001, Foissner *et al.* 2002).This and the figures mentioned by the World Conservation Monitoring Centre (1992) should be kept in mind if one tries estimating the number of endemic taxa. Obviously, we

know mainly the common, euryoecious species, which usually have wider geographic ranges than the more demanding species.

The highly divergent views on microbial diversity and dispersal illustrate the need for a comprehensive review. The present paper is a first attempt at compiling the literature dealing with the geographic distribution of representative groups of unicellular organisms, from bacteria to ciliates. This review highlights an urgent need for more detailed research and emphasizes provinciality in limnetic, marine, terrestrial, and fossil microbial ecosystems. Furthermore, the distribution patterns of mosses, ferns and macrofungi are compared with the distribution of micro-organisms. The main dispersal forms, i.e. the spores, of these organisms are similar in size and abundance to large bacteria and small protists. Notwithstanding, many of these mosses, ferns and fungi exhibit pronounced endemism. This reinforces the assumption that also micro-organisms do have biogeographies, which remain to be unravelled.

Terminology

The terms endemic, endemism, endemicity, provinciality and restricted geographic distribution are used throughout the paper in the same, very general sense (see Anderson 1994 for a detailed discussion of this matter). The same applies to the term "ubiquity", where I adopt the usual meaning, that is, the occurrence in a wide variety of habitats. Names of higher taxa, such as orders and classes, are usually vernacular, as outlined by Margulis *et al.* (1990). The authorities of the genera and species are omitted because they can be found in the literature cited.

Some basic considerations and major problems in estimating diversity and distribution of micro-organisms

"Everything is everywhere" is not falsifiable and thus a metaphor

Beijerinck's statement cited above is widely considered as a scientific hypothesis. However, scientific hypotheses must be falsifiable (Popper 1962). This is not the case with Beijerinck's statement testing of which would require the existence of at least two identical habitats in different biogeographical regions. Further, these habitats should have a representative size and age to allow the establishment of a micro-organism community such as found, for instance, in old ponds. Such conditions do not exist in the real world, and it is unlikely that they can be created experimentally. It might be important to note that "similar" or "almost identical" would be insufficient because this could imply that differences in species composition are due to genuine differences in the habitats. Similar considerations were made also by Finlay *et al.* (2004).

The local *vs*. global diversity measure is futile unless the global diversity is known

The ratio of local:global diversity is an important measure in conservation biology because it indicates "hot spots" of biodiversity. It is derived from Mac Arthur's and Wilson's theory of island biogeography and based on the fact that the number of species increases with the size of the area under investigation. In protists, this measure was first used by Fenchel *et al.* (1997). They found that about 10% of the estimated global diversity of free-living ciliates (3000 species) could be detected in local samples. From this, they conclude that "everything is (almost) everywhere" (Fenchel *et al*. 1997) and that "all species of fresh-water protozoa could eventually be discovered in one small pond" (Finlay and Esteban 1998). At first glance, this conclusion appears reasonable because the ratios of local:global diversity in protozoa is much higher than those found in metazoan and plant communities (usually < 1%). However, it is obvious that this method must be seriously flawed if the global diversity is largely unknown, as is the case in protists. The following example may illustrate the problem for the about 800 described species of soil ciliates (Foissner 1998, 2000; Foissner *et al*. 2002). At least 270 of them have been reliably recorded from Germany (Foissner 2000). In 1987, when just 250 species of soil ciliates were known globally (Foissner 1987), nearly 100% of the global diversity occurred in Germany. Today, with some 800 described species of soil ciliates (Foissner *et al*. 2002), the percentage has dropped to 34%, and if the 500 hitherto undescribed species, which I have in my records (Foissner 1998), are added, the percentage would drop to about 23%. Finally, when the very conservative estimate of 2000 species of soil ciliates globally is used in our calculations (Foissner 1997b, Chao *et al*. 2006), the ratio of local: global diversity drops to 14%. This is, compared to higher organisms, still a high value. However, the assumed global diversity of 2000 species is likely to be an underestimate.

These simple calculations show that the local: global diversity measure is flawed, as long as the actual global

diversity of the group under consideration is unknown. Unfortunately, Fenchel and Finlay (2004) refuse such evidences and stick to their view that global diversity of free-living ciliates is sufficiently known to use the local:global diversity measure. However, global ciliate diversity is not known, but likely more near to 30,000 species (Foissner *et al.* 2002) than to the 3,000 species proposed by Finlay (2001). Potentially, the species-area curves used by Azovsky (2002) are also influenced by this phenomenon. He compares the total world macrofauna with just a small fraction of the protists, i.e. the free-living ciliates.

Taken together, both Beijerinck's original metaphor and its interpretation by Finlay and Esteban (1998) are flawed not only by ecological theory but also by the common knowledge that nobody has ever found all species of a certain taxonomic group (e.g., ciliates) in a single pond, lake, or area. For instance, only 256 ciliate taxa have been reported from Priest Pot, Finley's well-studied model pond (Finlay and Maberly 2000). Likewise, an estimated maximum of only 200 ciliate species occurs in a 100 m² area of beech forest (Foissner 1999b), that is, about 10% of the estimated global soil ciliate diversity (Foissner *et al*. 2002).

Undersampling: the key to understand diversity and distribution of micro-organisms

Micro-organisms are difficult to recognize because they are small and dormant for extended periods of time. Thus, various culture methods are required to make them "visible". Then, however, a very selective community may develop and many of the "rare" species may escape detection among the few highly abundant and ubiquitous species. Only if a variety of different culture methods has been applied and the samples were carefully inspected have these rare species a chance to be identified. It is well known that such "rare" species comprise more than 80% of the total species pool in most communities studied so far (Schwerdtfeger 1975, Foissner *et al*. 2002), and that far less than 10% of the environmental bacteria can be grown on the commonly used culture media (Cohan 2002). Accordingly, it is likely that we know the majority of the more common, euryoecious species of protists quite well, but that we are rather ignorant about the more than 90% uncultured species. The effect of undersampling becomes especially evident if samples are manipulated to create new niches (Fenchel *et al*. 1997) or if the same site is investigated repeatedly (Foissner *et al*. 2002): 13 sampling campaigns, distributed over a period of 17 months, were required to find 80% of the 160 ciliate species identified until now in just 100 m² of an Austrian beech forest soil.

These problems in recognizing protist diversity concern also the recognition of undescribed species which are notoriously undersampled and whose large proportion becomes evident only if many samples from a large area are analysed (Foissner 1997b, Foissner *et al*. 2002). While most individual samples provide only 1 to 3 (4-7%) new species, the samples collectively show that up to 50% of the species found are undescribed.

Undersampling is not only caused by the methodological problems discussed above, but also by misidentifications, which are quite common (Foissner 1987, 1998; Alongi *et al*. 2002); by neglecting the rare and very rare species, a quite usual practice; by classifying distinct, but undescribed species as malformed individuals of known species; and the strongly decreasing number of well educated alpha-taxonomists (Cotterill 1995). All these problems mean that the diversity of microbial communities is usually greatly underestimated and distribution data become skewed to the common, euryoecious and/or easy-to-identify species. Undersampling is even a serious problem in higher organisms, such as fish and mammals (Kodric-Brown and Brown 1993).

Flagship taxa: an "ultimate" proof of endemism

Species with conspicuous size, morphology and/or colour are called "flagship taxa". They are the elephants of the microscopic world. Tyler (1996) has summarized the reasons why such taxa have the greatest probability of real endemism: "Because they are so showy, or so novel, it is unlikely that such species would be overlooked if indeed they were widely distributed. If the Australian endemics occurred in Europe or North America then they would have been seen there, long ago".

Many more flagships are known from algae than from heterotrophic protists. In my opinion, this is simply because the latter found less attention in the past. Thus, it is relatively easy to discover new flagship species in ciliates, especially in Africa and South America and the marine environments (see below).

A brief review on restricted geographic distribution of bacteria and microfungi

Using data from recent molecular studies, Dykhuizen (1998) estimated that thirty grams of forest soil contain over half a million bacterial species, that there are 2,000 different bacterial communities, and possibly a trillion (!)

of bacterial species globally. More recent analyses tend to surpass these estimations (Gans *et al.* 2005)! Although such numbers depend highly on the species concept used, it is clear that the number of bacterial species is enormous. Modern bacterial species concepts even suggest that the named molecular species are more like genera than species (Cohan 2002).

Molecular techniques also provided evidence for a restricted geographical distribution of soil bacteria, such as *Pseudomonas* and *Rhizobium* on the basis of autofluorescence and multilocus enzyme electrophoresis (Cho and Tiedje 2000, Souza *et al*. 1992). Indeed, the most recent studies, using refined molecular methods, reveal biogeographies in various bacteria and endemicity of genotypes at continental and local level (Borneman and Triplett 1997, Hagström *et al*. 2000, Madrid *et al.* 2001, Whitaker *et al*. 2003, Bavykin *et al*. 2004, Lawley *et al*. 2004, Papke and Ward 2004, Silva *et al*. 2005).

Lhotský (1998), who believes that there are only a few really cosmopolitan algal species, re-evaluated Komárek's studies on cyanobacteria: of 20 exclusively planktonic freshwater cyanophytes only 25% were cosmopolitan, 40% were tropical species, 5% were restricted to the neotropic region, and 15% were species known only from Cuba. Likewise, 50% of the chlorococcal algae have a restricted distribution. Potentially, temperature is a major factor controlling the distribution of the marine species of cyanobacteria (Hoffmann 1999).

About 120,000 fungal species have been described, but their global diversity has been estimated to be well above one million species (Hawksworth 2001). While it is textbook knowledge that most macrofungi occupy rather distinct areas, notwithstanding their dispersal by microscopical spores (see chapter on dispersal), the distribution of microfungi is much less clear because most are associated with particular host plants and animals as parasites or mutualists; therefore they have a distribution similar to those of their hosts (Hawksworth 2001). However, even "free-living" microfungi appear to occupy restricted areas, for instance, the soil and litter species belonging to the genus *Aspergillus* (Klich 2002). This is emphasized by data of Green and Bohannan (2006) for ascomycete fungi: using intergenic spacer analysis (ARISA), geographic distance was a more useful predictor for ascomycete fungi community than habitat across scales of 1 m to ~ 100 km. *Geosiphon pyriforme*, a coenocytic soil fungus living in endocytobiotic association with a cyanobacterium, *Nostoc punctiforme*, represents a remarkable case of local endemism. At the hyphal tips, the fungus forms unicellular, multinucleated 1-2 mm bladders, which host the symbiotic cyanobacteria. Although this fungus is of almost macroscopic size and known since 1915, a region in the Spessart Mountains (Germany) is the only known natural habitat so far (Schuessler and Kluge 2000).

A brief review on restricted geographic distribution of autotrophic and heterotrophic protists

According to Taylor and Pollingher (1987), Tyler (1996), Vyverman (1996), and Coleman (1996, 2001), there is little doubt that both freshwater and marine algae do have biogeographies, although it is sometimes difficult to distinguish between patchy search results, restricted distributions, and genuine invasions into a certain region (see dispersal chapter). In addition to a large cluster of pantropical and temperate-montane species with representatives in all major taxonomic groups, there are large numbers of taxa endemic to each of the different tropical regions. Among the heterotrophic protists, which have been much less intensively studied so far, we can at least distinguish genera and species with a restricted Laurasian/Gondwanan distribution. However, I am convinced that increased research will reveal distribution patterns of heterotrophic protists that are basically similar to those found in algae.

Dinoflagellates (Figs 1; 3e, f)

Older data were extracted from the valuable reviews of Taylor and Pollingher (1987) and Pollingher (1987). Both freshwater and marine dinoflagellates show endemism in the strict sense of the word, i.e. are restricted to only one particular region, despite the potential continuity of all oceans and genuine man-made introductions into a given region.

It might be expected that the Polar Regions would host endemic species because of the great distance between north- and south polar regions. This is indeed the case, with roughly 80% endemicity in each polar region. As with other groups, the Indo-West Pacific region possesses a variety of endemic species, most of which are neritic (Fig. 1). One such species is *Dinophysis miles*, a distinctively shaped, neritic dinophysoid "flagship" which occurs from the Western Indian Ocean and Red Sea to the Gulf of Tonkin. *Ceratium dens* (Fig. 3e) is another species with such a restricted distribution, which is difficult to understand in a region in which a strong inter-ocean flow exists that reverses seasonally

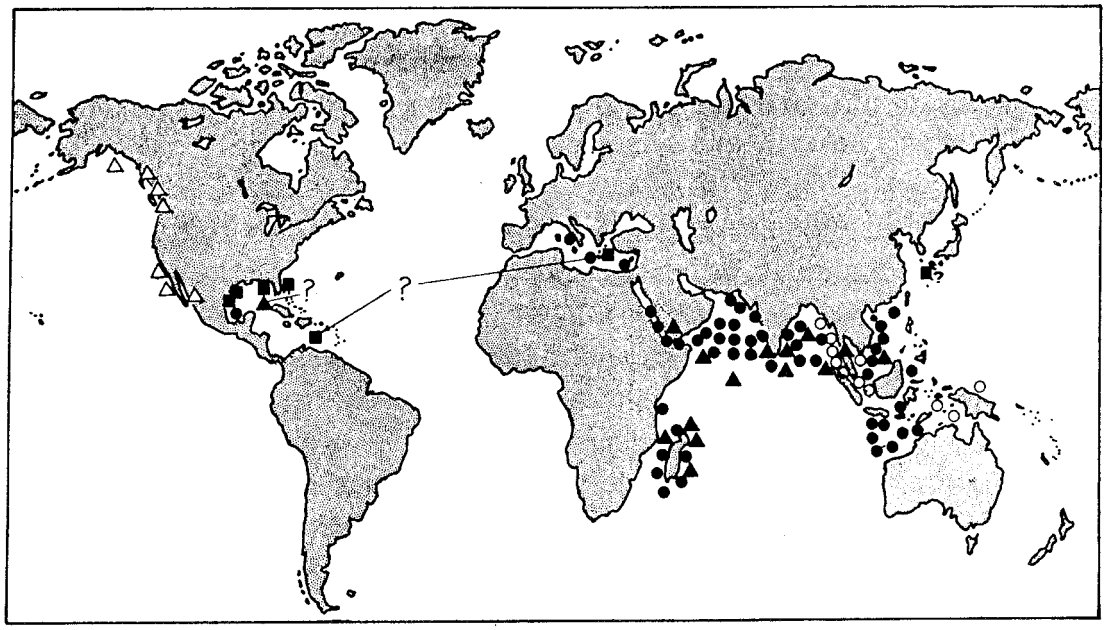


Fig. 1. Distribution of some endemic dinoflagellates (from Taylor and Pollingher 1987). *Dinophysis miles* (closed circle), *Dinophysis miles* var. *schroeteri* (open circle) and *Ceratium dens* (closed triangle) are Indo-west Pacific taxa; *Oxyphysis oxytoxoides* (open triangle) is a Pacific endemic, and *Ptychodiscus brevis* (square) is probably a Caribbean endemic.

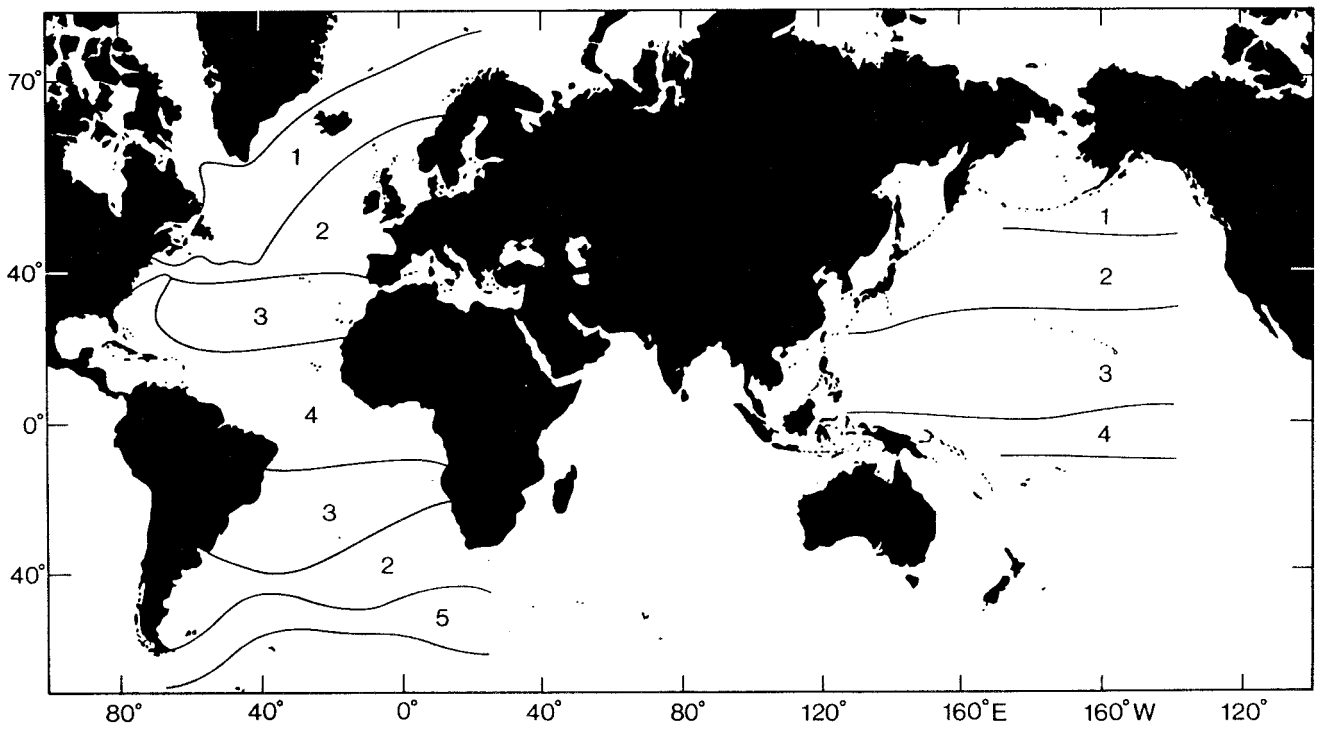
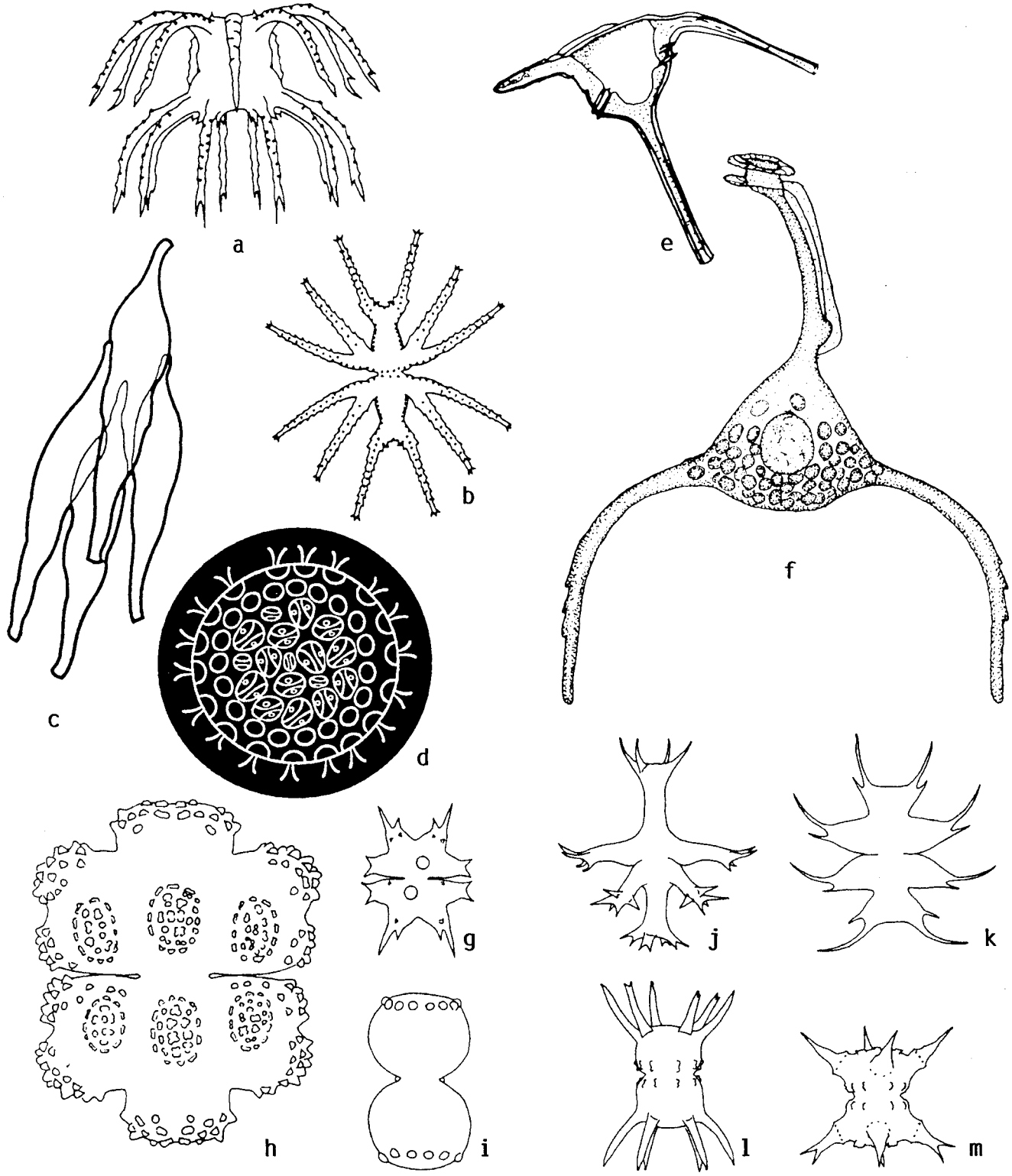


Fig. 2. Biogeographic coccolithophore zones from the Atlantic and Pacific Oceans (from Winter *et al.* 1994). 1 - Subarctic, 2 - Temperate (Transitional), 3 - Subtropical (Central), 4 - Tropical (Equatorial), 5 - Subantarctic. Similar distribution patterns are found in foraminifera.



Figs 3a-m. Some endemic algae, viz., desmids (a, b, g-m), dinoflagellates (e, f), and chrysophytes (c, d). **a-d** - Australian flagship endemites (from Tyler 1996): *Amscottia gulungulana, Micrasterias hardyi, Dinobryon unguentariforme,* and *Tessellaria volvocina;* **e, f** - *Ceratium dens,* an Indo-west Pacific species, see also figure 1 (from Taylor 1987) and *Tripsolenia intermedia,* a tropical marine species (from Dodge and Lee 1985); **g-i** - Neotropic desmids (from Coesel 1996): *Euastrum grandiornatum, Euastrum foersteri,* and *Cosmarium redimitum*; **j-m** - Desmids from equatorial Africa (from Coesel 1996): *Allorgeia incredibilis, Micrasterias sudanensis, Staurastrum rhoskae,* and *Staurastrum fuellebornii* var. *evolutum.*

with the monsoons. Another species, *Ceratium egyptiacum* occurs only in the Suez Canal, the Red Sea and the south-eastern Mediterranean. Other species are restricted to the tropical Atlantic Ocean (e.g., *Gessnerium monilatum*, *Ptychodiscus brevis*) and the North Pacific, for instance, *Oxyphysis oxytoxoides* (Fig. 1).

Also, there is molecular evidence for provinciality and cryptic speciation in dinoflagellates. Montresor *et al.* (2003b) performed morphological and genetic investigations on several populations of the cosmopolitan marine species *Scrippsiella trochoidea.* They found that very similar morphs of *Scrippsiella trochoidea* exhibit genetic differences in the order of different species. Recently, Pochon *et al.* (2004) showed biogeographic partitioning and host specialization of the foraminiferan dinoflagellate symbiont *Symbiodinium*. They discovered a distinctive biogeographic break between the kinds of symbionts found in *Sorites* from the East Pacific and Caribbean. Garcia-Cuetos *et al.* (2005) suggest that the strong host-symbiont specificity observed in soritid foraminifera is a combined effect of a selective recognition mechanism, vertical transmission of symbionts, and biogeographical isolation.

In freshwater environments, the most striking examples for local endemism come from studies in the large, old lakes. Lake Baikal harbours four dinoflagellate species, three of which are endemic forms: *Gymnodinium baicalense, G. coeruleum* and *Peridinium baicalense*. In Japan, Lake Biwa contains endemic algae from various groups, and Lake Ohrid in the former Yugoslavia contains, *inter alia*, the endemic dinoflagellate *Cystodinium dominii*. A lot of endemic dinoflagellates occur also in Australia (Tyler 1996, Hallegraeff *et al.* 2004), and the very common "cosmopolitan" *Ceratium hirundinella* seems to be absent from Indonesia (Vyverman 1996), Venezuela, the Amazonian basin and the southern part of South America (Pollingher 1987).

Diatoms

The siliceous, highly structured frustules of the diatoms provide excellent possibilities for biogeographical research, especially when scanning electron microscopy is used. Diatoms show distinct global distribution patterns and even local endemism. For instance, 8% (30 taxa) of the diatom flora of Lake Tanganyika can be considered as regional endemics (Vyverman 1996). Kienel (2003) described a restricted distribution of a common planktonic species, viz., *Aulacoseira subarctica*. Probably, this provinciality is caused by a preference for low temperatures, low light intensities and high concentrations of silicate and mineral nutrients. And recently, Shayler and Siver (2004) discovered several endemic diatoms and *Mallomonas* in a pond area of Florida, U.S.A. They suggest that such specific flora is related to the history of the ponds which suffered a transition from alkaline to very acidic conditions over the years. Thus, strong environmental changes could have promoted the evolution of locally adapted lines that eventually speciated. Another excellent example is represented by the genus *Actinella* which presently comprises 29 species, only two of which (*A. brasiliensis*, *A. punctata*) have a worldwide distribution (Sabbe *et al.* 2001). Nine *Actinella* species are endemic to Australasia, and there are even marked differences in the geographic distribution at smaller spatial scales: some species are only known from a few lakes (Sabbe *et al.* 2001).

Coccolithophores (Fig. 2)

Coccolithophores are marine, planktonic algae with highly structured, calcified scales. It is a relatively small group presently comprising about 200 species. Probably, their diversity is much higher because a number of novel species have been described recently. Biogeography of species is still rather incomplete because reliable identification requires an electron microscopical analysis. However, the gross distribution patterns are well established and described here, using the excellent reviews of Brand (1994), Winter *et al.* (1994) and Roth (1994).

Coccolithophores, like most phytoplankton and microzooplankton, can be classified according their occurrence in five major latitudinal zones: Subarctic, Temperate (or Transitional), Subtropical (or Central), Tropical (or Equatorial) and Subantarctic (Fig. 2). Distinct species assemblages occur in each of these zones, which are, in most cases, similar to those in the corresponding zones of the other hemisphere. Exceptions are found in the Subarctic-Subantarctic zones, which host similar genera but few "bipolar" species.

The fossil records reveal a distinct provinciality of many coccolithophore species in the Cretaceous and Tertiary periods (Green *et al.* 1990). Moreover, the biogeographic distribution of individual coccolithopore species changes over geological times, obviously in response to changing environmental conditions and the global distribution of the water masses. For instance, the subpolar *Coccolithus pelagicus* expanded its range towards the equator during the last glacial peak, while several tropical species (e.g., *Calcidiscus leptoporus*, *Syracosphaera pulchra*) exhibited markedly reduced biogeographic ranges closer to the equator during this

period (Brand 1994). Similar changes were observed in the various fossil freshwater and marine algal communities (Coleman 1996, Adey and Steneck 2001).

Chrysophytes (Figs 3c, d)

The biogeographic data dealing with the silica-scaled chrysophytes were excellently summarized by Kristiansen (2001) and Kristiansen and Funch Lind (2005): "It is now evident from electron-microscopical examinations of samples from lakes and ponds all over the world that the silica-scaled chrysophytes show distinct distribution patterns. Differences in ecological requirements determine local occurrence, and on a global basis several distribution types can be established. Best known in this respect is the genus *Mallomonas* with 172 described taxa. Of these, 31 taxa are cosmopolitan or widely distributed, 59 taxa have a northern temperate-subarctic-arctic distribution, of these 22 are endemic. A total of 17 taxa have a bipolar distribution. Eighteen taxa are restricted to the tropics, seven of which are endemics. Seventeen taxa have scattered distributions with scant occurrence. Including the already mentioned species, altogether 69 taxa must be deemed endemic, although several of them may lose this status after further investigations. The genus *Synura* apparently has a similar biogeography. The very small species of *Paraphysomonas* and *Spiniferomonas* have been argued to be cosmopolitan, although there are so far no satisfactory investigations (see Foissner *et al*. 2002 for detailed discussion).

Desmids (Figs 3a, b, g-m)

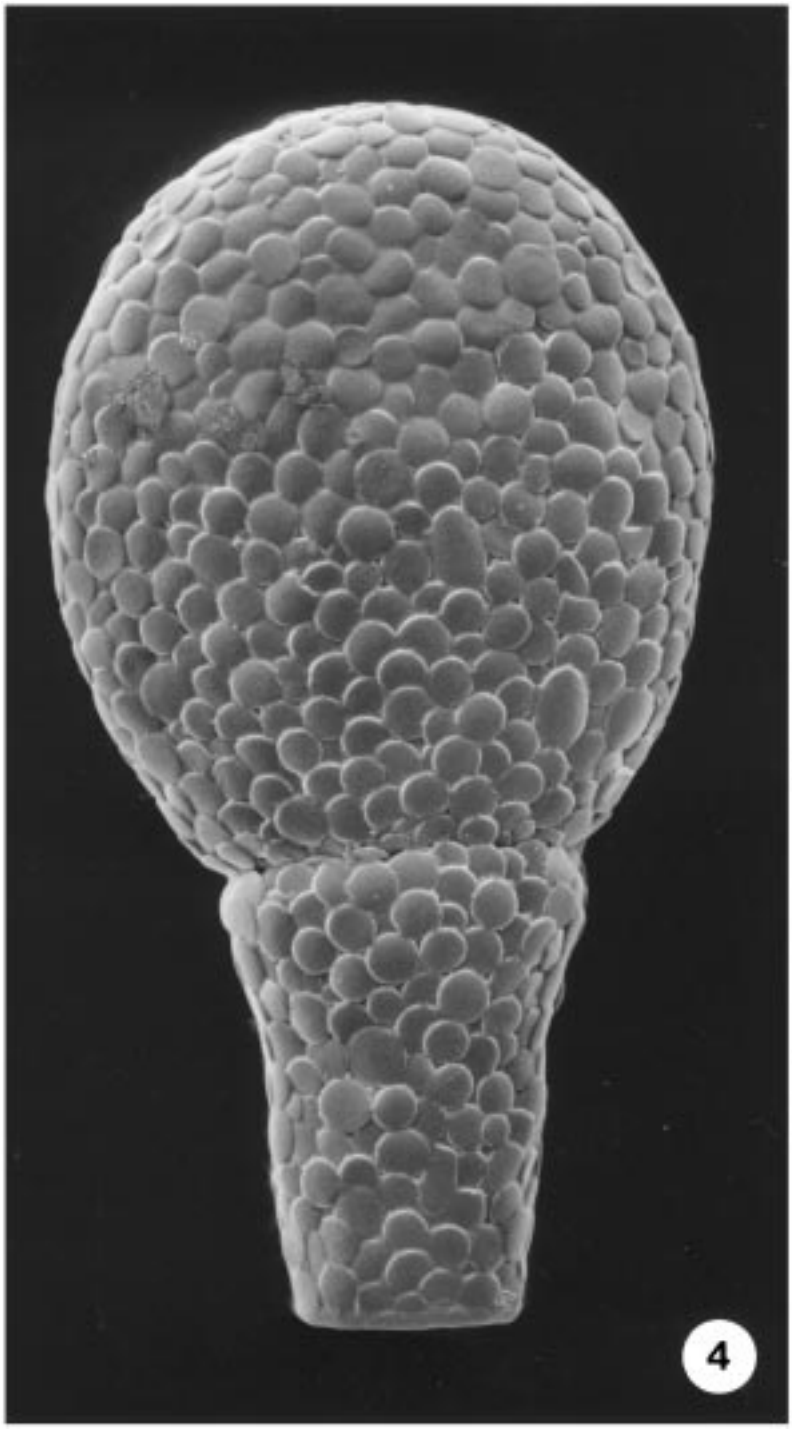
These fancy organisms have attracted many professionals and amateurs. Many desmids are obviously cosmopolitan, while others have a restricted Laurasian/Gondwanan distribution, for instance, the "northern" *Micrasteria crenata* and the "southern" *M. alata* from Amazonia (Bremer 2002). Today, 10 regions with well known desmid floras can be distinguished, with a most pronounced endemism in the Indo-Malaysian/Northern Australian region, in tropical America, and equatorial Africa (Coesel 1996; Fig. 3a, b, g-m). It is likely that endemism is much more common in this group than previously assumed. In an extensive literature search, Vyverman (1996) found that among the more than 2680 desmids recorded from the Indo-Malaysian/North Australian region, about 800 (31%) have never been found elsewhere, and 4% of the desmids of Papua New Guinea can be considered as paleotropic taxa. Sometimes, related species show distinct or partially overlapping geographic distributions. For instance, *M. cruxmelitensis* has its main occurrence in temperate regions, while *M. radians* has a pantropical distribution. Similar distribution patterns are known, *inter alia*, also from diatoms and dinoflagellates (Vyverman 1996): in some tropical lakes, the genus *Surirella* is abundant in the plankton, while most temperate species of this genus are benthic; and the dinoflagellate genus *Peridinium* seems to replace the genus *Ceratium* forwards the equator (see above).

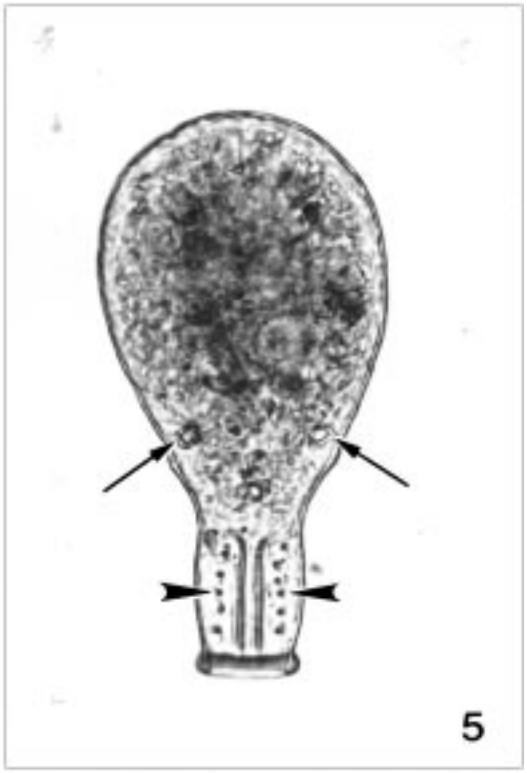
Naked amoebae, heliozoa, and heterotrophic flagellates (Fig. 6)

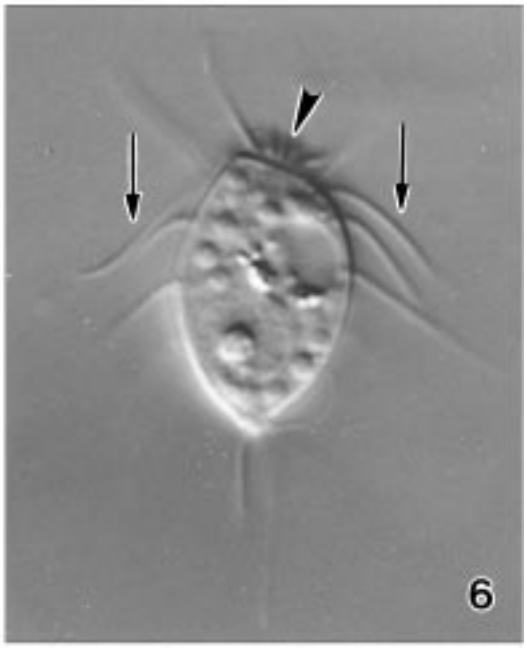
Naked amoebae are difficult to identify. Thus, reliable faunistic data are scarce. Nonetheless, there is some evidence for a restricted distribution of several species (Page 1976, 1991) and molecular varieties (Smirnov *et al*. 2002). *Naegleria italica*, for instance, could not be found elsewhere than in Italy during a worldwide search (Jonckheere 2002). Likewise, a study of amoebae from lens cases in Korea found that ~ 88% of these were potential keratitis pathogens, with distribution of isolates reflecting geographic regions of the country (Schuster and Visvesvara 2004). Cavender *et al*. (2002) reported evidences that the assemblage of dictyostelid slime moulds from New Zealand is quite distinctive and reflects the isolation of this land mass; five of the thirteen species found were undescribed.

The available data on Heliozoa were reviewed by Siemensma (1991). About 100 species are known globally, but many novel species have been described in the eighties suggesting that many more might be discovered in future research. A reliable identification often requires scanning electron microscopy (SEM); the lack of SEM analysis might have flawed many of the older data. The few studies which used SEM in the past discovered not only many new species but also suggest that some might have a restricted Laurasian/Gondwanan distribution.

For the free-living, heterotrophic flagellates, Lee and Patterson (1998) favour a model in which there are relatively few species most of which have cosmopolitan distribution. On the other hand, the structure of their data highly resembles those reported by Foissner (1997b) for soil ciliates: there are few new species in the individual samples, but many if one adds together data from several samples from larger areas. Thus, Foissner (1999b) concluded that free-living flagellate diversity is probably also larger than presently assumed. This appears to be confirmed by recent electron microscopical and molecu-







Figs 4-6. Gondwanan testate amoebae (4, 5) in the scanning electron and bright field microscope, and a Gondwanan heterotrophic flagellate (6) in the interference contrast microscope (originals). **4** - *Apodera vas* is an eye-catching species with a length of about 170 µm and a characteristic shell shape; **5** - An even more distinct "flagship" is *Certesella certesi*, which is about 130 µm long and has highly characteristic teeth (arrowheads) at both sides of the neck and two pores on each side (arrows); **6** - Although *Hemimastix amphikineta* is only about 20 µm long, it is a "flagship" flagellate because it has two highly characteristic flagella rows (arrows) making it looking like a small ciliate. Arrowhead marks mouth entrance.

lar work that discovered a great variety of undescribed taxa (Zoological Record, Moon-van der Staay *et al*. 2001, Scheckenbach *et al*. 2005).

There is at least one soil flagellate, *Hemimastix amphikineta*, with a distinct biogeography. This highly characteristic organism (Fig. 6) has been found in about 50 out of 300 soil samples from Gondwanaland (South America, Africa, Australia) and the transition zone of Gondwana and Laurasia (Central America, Malaysia), but never in Laurasia (mainly Europe and about 60 samples from North America), even not in a recent study of soils from twelve natural forest stands in Austria, where I specifically looked for this organism (Foissner *et al*. 2005).

Foraminifera

Extant and fossil foraminifera have distinct biogeographies at large scale (Fig. 2), controlled mainly by temperature, water currents, and food availability (Bé 1977, Kemle-von Mücke and Hemleben 1999, Kucera and Darling 2002, Groves *et al*. 2003). A considerable number of species appears to have a restricted distribution (Bé 1977). However this endemism might be less pronounced in the deep sea species (Gooday 1999).

Recently, molecular investigations revealed not only a considerable cryptic diversity in foraminifera, but also highlighted the endemic character of the allogromiid foraminifera from Explorers Cove, Antarctica (Kucera and Darling 2002, Pawlowski and Holzmann 2002). Notably, none of the sequences derived from non-Antarctic species clusters with the Antarctic ribotypes. This and other data support the hypothesis that the morphological resemblance between foraminiferal taxa from the northern and southern hemispheres is due to convergence rather than evidence for a common origin (Pawlowski *et al*. 2002). However, very recent data suggest another mechanism for the "bipolar cosmopolitan" *Neogloboquadrina pachyderma* (Darling *et al.* 2004). Fossil and molecular data demonstrate a stepwise progression of diversification starting with the allopatric isolation of Atlantic Arctic and Antarctic populations after the onset of the Northern Hemisphere's glaciation. Similar genetic divergence has also been reported between a bipolar planktonic dinoflagellate from the high polar water mass and Antarctic Sea ice (Montresor *et al*. 2003a).

Many foraminifera occupy wide geographic ranges (Bé 1977). However, these potentially cosmopolitan morphospecies exhibit a high genetic diversity. For instance, the highly variable and cosmopolitan *Globorotalia truncatulinoides* consists of four different rRNA lineages, which should be considered as distinct species: lineages 1 and 2 occur mainly in subtropical waters, lineage 3 is abundant only in the Subantarctic Convergence, and lineage 4 inhabits subantarctic waters (Vargas *et al*. 2001).

Testate amoebae (Figs 4, 5, 7; 8a-v)

Among the heterotrophic protists, testate amoebae provide the most convincing evidence for a restricted geographic distribution of many genera and species. Most of these endemics are "flagships" with a large size (> 100 µm) and distinct morphology, for instance, *Apodera vas* (Fig. 4), which is quite common in Gondwanan areas but absent from the northern hemisphere. Meisterfeld (2002b) summarizes this quite concisely: "Testate amoebae are found world-wide, but they are neither cosmopolitan nor ubiquitous. Several species, mainly from the Nebelidae, Distomatopyxidae, and Lamtopyxidae have a restricted geographical distribution, which is certainly not a result of uneven sampling effort". Briefly, there are distinct differences in the Laurasian and Gondwanan testacean faunas and, in addition, between the temperate and tropical regions (Hoogenraad and de Groot 1979, Bonnet 1983, Bobrov 2001). Furthermore, molecular investigations showed that some of the common, cosmopolitan "species" are genetically highly diverse; they consist of several ribospecies which, subsequently, could be distinguished also morphologically (Vargas *et al*. 2001, Wylezich *et al*. 2002).

The following compilation, based on the authoritative reviews of Meisterfeld (2002a, b), shows that a restricted distribution is not an exceptional phenomenon in testate amoebae. Certainly, the knowledge is still incomplete for many genera and species because reliable biogeographical data are rare. It seems possible that some of the interstitial marine testacean genera and species also have a restricted distribution. The genera *Heteroglypha* (Fig. 8q), *Lamtopyxis* (Fig. 8m), *Lamtoquadrula* (Figs 8b, c), *Moghrebia* (Figs 8f, g), *Paracentropyxis* (Fig. 8n), *Pentagonia* (Figs 8h, i), and *Pseudonebela* (Fig. 8d) occur only in (mostly tropical) Africa. *Alocodera*, *Ampullataria* (Fig. 8v), *Certesella* (Fig. 5), *Cornuapyxis* (Fig. 8o), *Oopyxis* (Fig. 8l), *Pileolus* (Fig. 8r), and *Suidifflugia* occur only in Central and South America. *Wailesella*, *Deharvengia* (Fig. 8u), and *Playfairina* occur only in Indonesia and Australia. *Microquadrula* (Fig. 8a) appears to be a

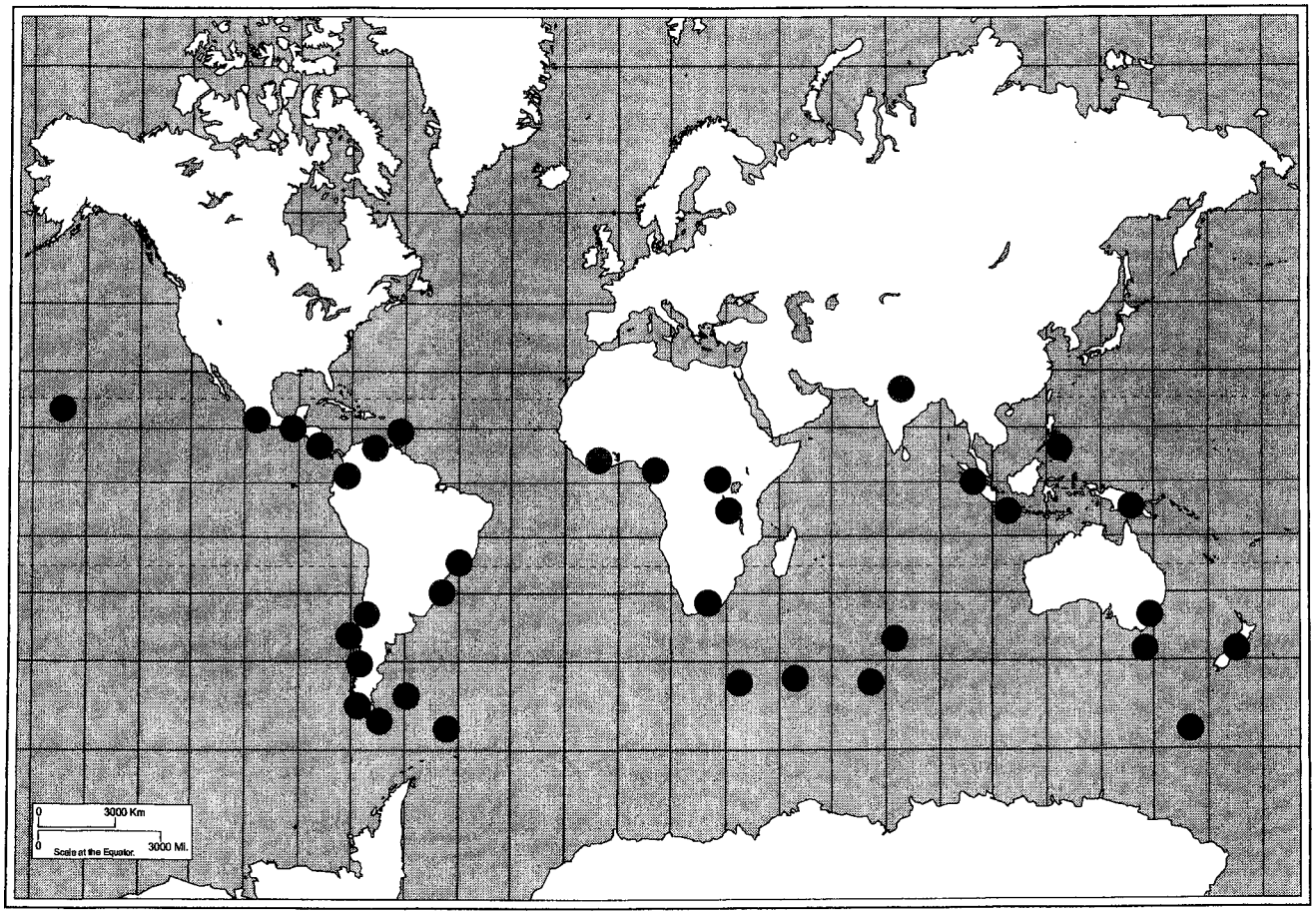


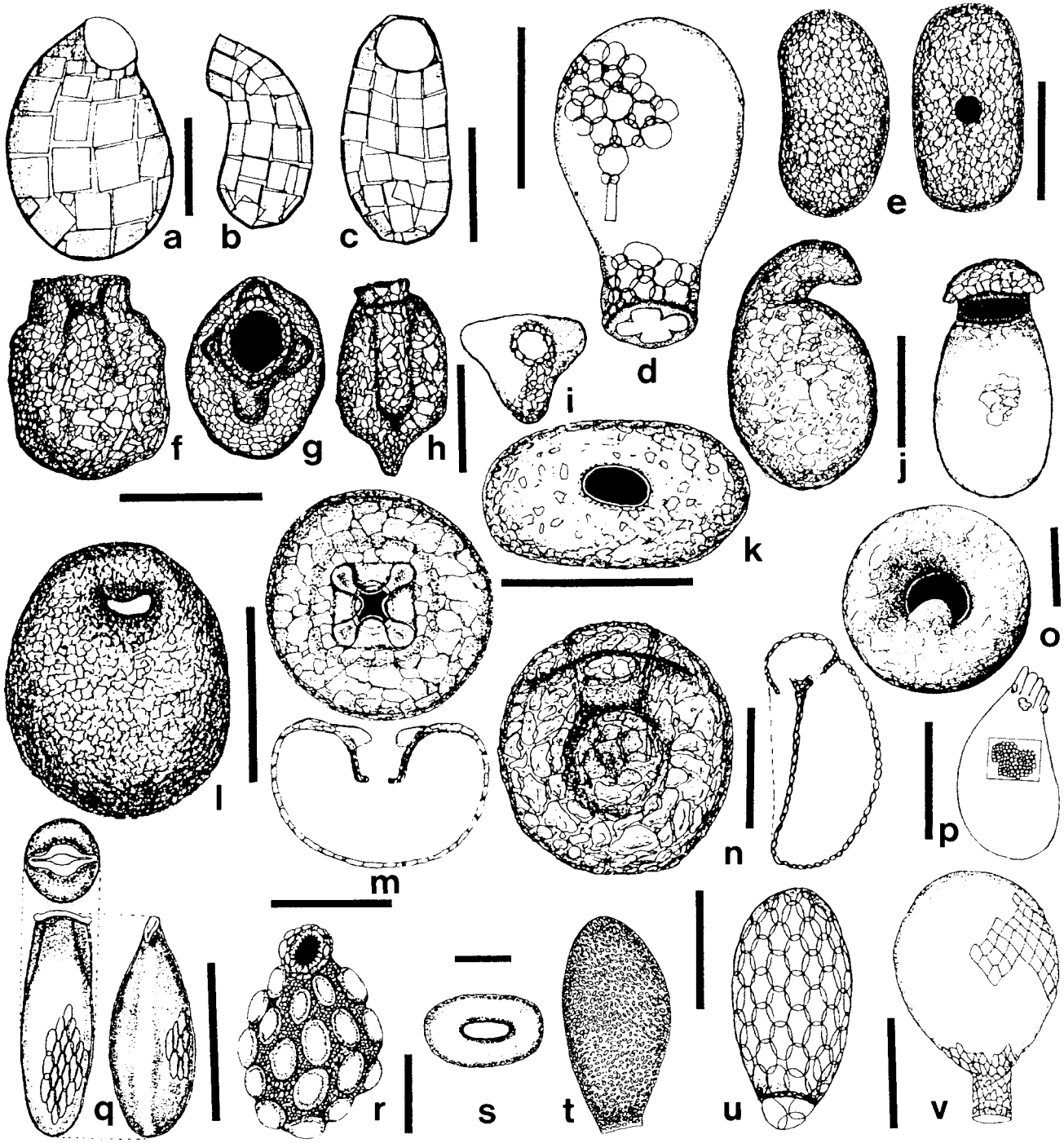
Fig. 7. Recorded occurrences of *Nebela (Apodera) vas*, a Gondwanan testate amoebae (cp. Figure 4). From Smith and Wilkinson (2005). This compilation disproves the statement of Finlay *et al*. (2004) that *N. vas* occurs in the Holarctic.

Cuban endemic, and *Feuerbornia* (Fig. 8p) has been reported only from South America and New Zealand. *Jungia* and *Ellipsopyxis* (Fig. 8k) are restricted to Indonesia, Africa and South America. *Hoogenraadia* (Fig. 8j) occurs in Africa and East Asia. *Ellipsopyxella* (Fig. 8e) appears to be restricted to Africa and South America. *Distomatopyxis* occurs in North and Central America, East Asia and Spain. *Matsakision* (Figs 8s, t) is probably a Laurasian endemic.

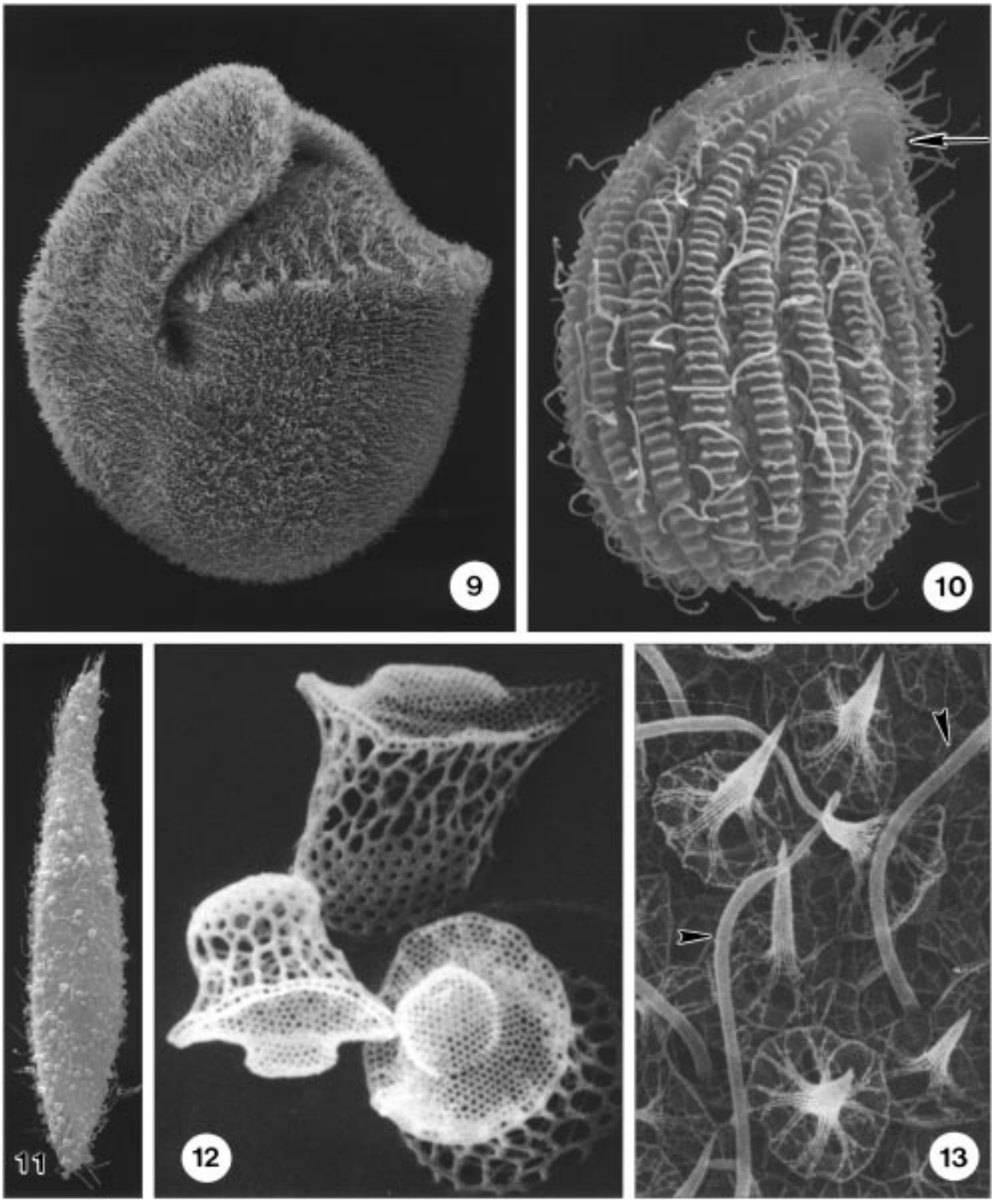
Recently, Finlay *et al.* (2004) claimed that *Apodera* (formerly *Nebela*) *vas*, a Gondwanan flagship species (Fig. 4), might be a misidentified *Pontigulasia* and thus occurs in the Holarctic. This claim is based on very old literature from around 1900, which contains some misidentifications, and, of course, cannot take into account the more recent contributions of acknowledged taxonomists, such as Bonnet, Grospietsch, Meisterfeld and Golemansky. Smith and Wilkinson (2005) carefully reviewed the literature on the occurrence of *A. vas* (Fig. 7). According to this compilation, *A. vas* has never been found in the Holarctic despite the presence of many suitable habitats. Thus, Smith and Wilkinson (2005) suggest that the patterns of atmospheric circulation do not enable cysts to be carried from Nepal across high montane Tibet, or from Mexico across the desert states of USA, to reach suitable habitats further north. Lastly, also the distinct distribution of other Gondwanan flagship nebeliids, such as *Certesella certesi* (Fig. 5) and *Alocodera cockayni* is beyond any doubt.

Ciliates (Figs 9-21a-h)

Ciliates exhibit a broad spectrum of distinct morphologies (Corliss 1979), but rather complicated methods (various silver impregnation techniques, scanning elec-



Figs 8a-v. Some endemic testate amoebae genera (compiled from Meisterfeld 2002a, b). Further genera, see figures 4 and 5. African endemics: *Lamtoquadrula* (b, c), *Pseudonebela* (d), *Moghrebia* (f, g), *Pentagonia* (h, i), *Lamtopyxis* (m), *Paracentropyxis* (n), *Heteroglypha* (q). Central and South American endemics: *Oopyxis* (l), *Cornuapyxis* (o), *Pileolus* (r), *Ampullataria* (v). Indonesian endemic: *Deharvengia* (u). Cuban endemic: *Microquadrula* (a). Laurasian endemic: *Matsakision* (s, t). *Ellipsopyxella* (e) occurs in Africa and South America; *Hoogenraadia* (j) occurs in Africa and East Asia; *Feuerbornia* (p) occurs in South America and New Zealand; *Ellipsopyxis* (k) occurs in Asia, Africa and South America. Scale bars: 10 µm (q-u), 20 µm (b, c, n), 50 µm (d-q, v), 100 µm (f, g, m).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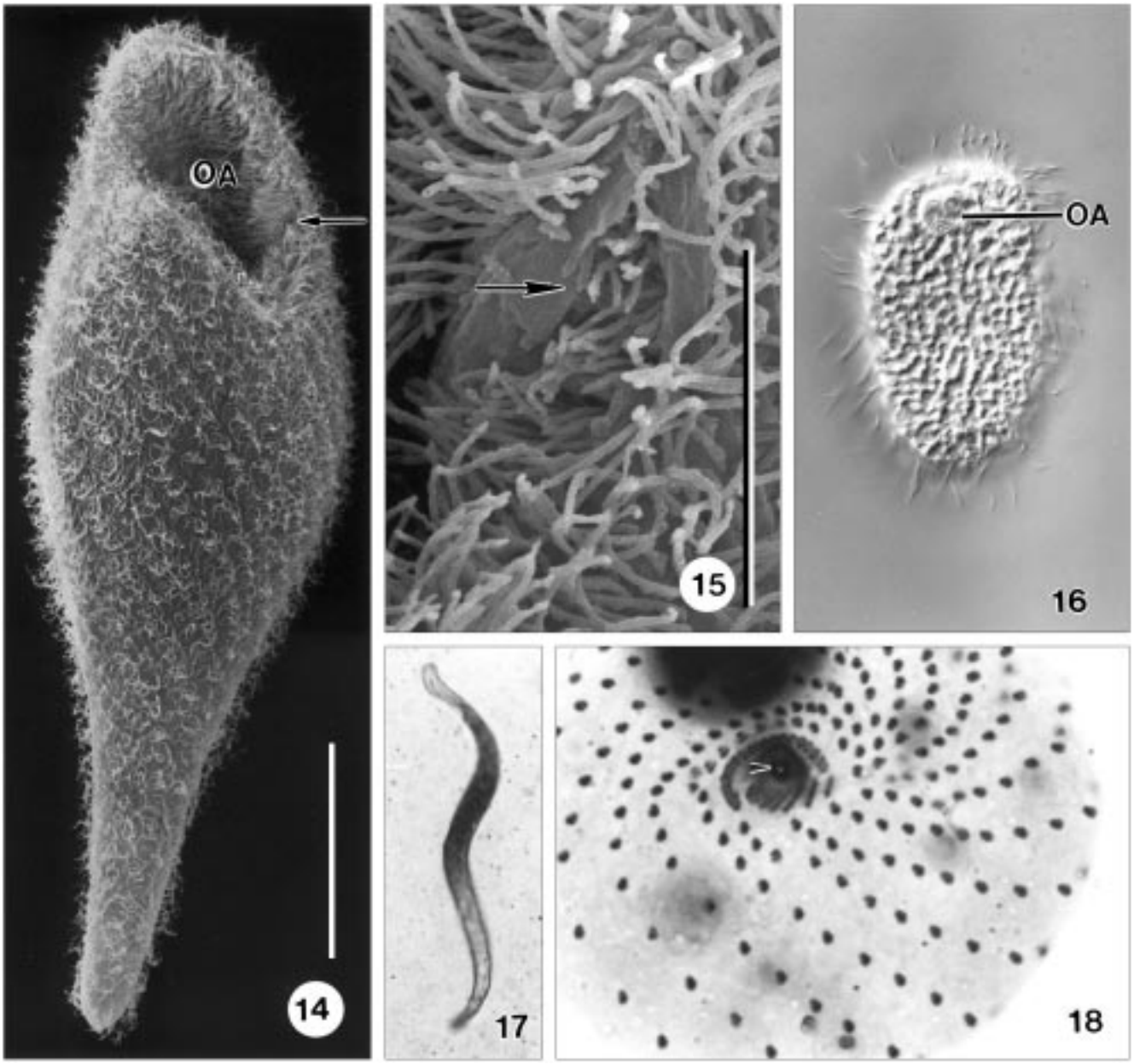


Figs 14-18. Endemic ciliates in the light (16-18) and scanning electron (14, 15) microscope. **14, 15** - a still undescribed tetrahymenid ciliate from bromelian tanks in the Dominican Republic (from Foissner *et al.* 2003). This species reaches a length of 800 µm and is thus a size "flagship" which would have been recognized in Europe, if it were there. The bromelian ciliate represents a new genus and family because it has many peculiarities, for instance, possesses a large, functional oral apparatus (OA) and a minute, likely functionless original mouth (arrows) with partially reduced adoral membranelles. Scale bars: 50 µm and 10 µm; **16, 18** - *Fungiphrya strobli* has been found only on the Table Mountain of the Republic of South Africa (from Foissner 1999a). This small (about 50 µm) ciliate is highly characteristic because the oral apparatus (OA) possesses a minute feeding tube used to penetrate fungal hyphae and spores; **17** - *Circinella arenicola* is a worm-like hypotrichous ciliate which reaches a length of 600 µm (from Foissner 1994). Among 1000 soil samples investigated, it occurred only in an inland sand dune of Utah, USA, although many similar habitats were investigated in Namibia (Southwest Africa) and Australia.

Figs 9-13. Endemic soil ciliates in the scanning electron microscope (from Foissner 1993, 2005). **9** - *Bresslauides discoideus* is a massive, up to 600 µm-sized "flagship" occurring only in Laurasia and the transition zone to Gondwana (Fig. 19). With its large, table-like mouth, it can feed even on rotifers; **10** - *Cosmocolpoda naschbergeri*, although being only up to 70 µm long, is also eye-catching because it has highly characteristic ridges. The arrow marks the small mouth entrance. Among 1000 soil samples investigated, this species occurred only in a single sample from the Caribbean coast of Costa Rica, Central America; thus it is possibly a regional endemic; **11-13** - *Sleighophrys pustulata* (11, 12) and *Luporinophrys micelae* (13) are rod-shaped, about 200 µm long Venezuelan flagships with highly characteristic epicortical scales (12, 13), which are 5-12 µm high and thus easily recognizable also in the light microscope.

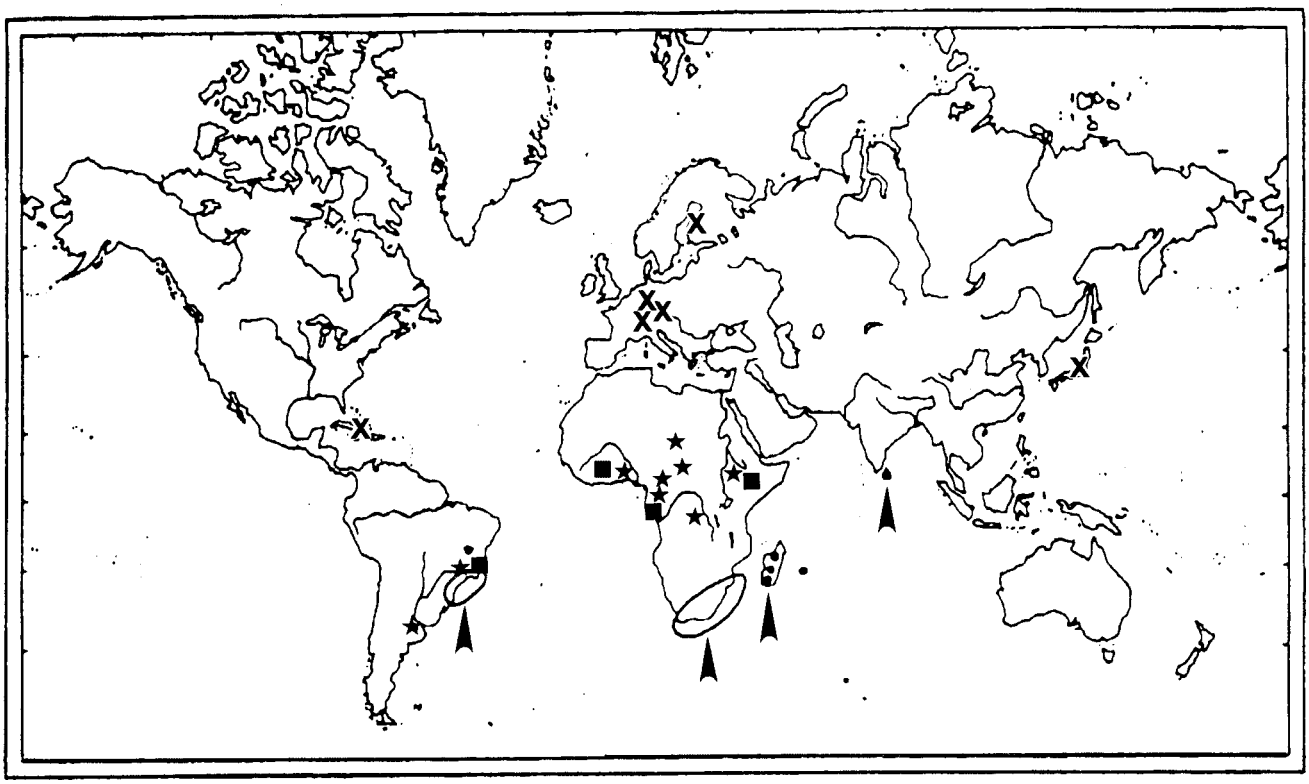


Fig. 19. Gondwanan areas of the moss *Campylopus twaitesii* (arrowed dots and ellipses; from Frahm 2001) and the ciliates *Neobursaridium gigas* (asterisk) and *Frontonia vesiculosa* (square); after data from Dragesco and Dragesco-Kernéis (1986). *Bresslauides discoideus* (**x**), in contrast, is a Laurasian ciliate so far found only in Europe, Japan, and in mud from tank bromeliads of the Dominican Republic (based on Foissner 1993 and more recent, unpublished data).

tron microscopy) are required to distinguish the huge amount of genera and species (Foissner *et al*. 2002). Therefore, parts of the old faunistic literature, mainly based on live observation, might be inconclusive or even misleading.

Genetic and molecular biogeographic studies are still sparse in ciliates, but those available show that ciliate diversity is larger by at least one order of magnitude than anticipated by morphologists and ecologists (Dini and Nyberg 1993, Hackstein 1997, Nanney *et al*. 1998, Foissner *et al*. 2001, López-García *et al*. 2003, Katz *et al*. 2005, Šlapeta *et al*. 2005). Obviously, most ciliate morphospecies consist of multiple, microscopically indistinguishable species whose distinct molecular distances indicate continuous speciation over long periods of time. The fact that several sibling species are often found in the same sample indicates that they might occupy different ecological (micro)-niches (Nanney 2004). Significant evidence for these conclusions comes from studies on *Paramecium*, *Tetrahymena* and *Euplotes*, the "pets" of the ciliatologists.

The *Tetrahymena pyriformis* complex presently consists of 25 species. Some are cosmopolitan or near cosmopolitan (e.g., *T. cosmopolitanis*), while others are highly restricted in their distribution. For instance, *T. thermophila*, which has been collected hundreds of times in eastern North America, has never been found outside this region (Nanney *et al*. 1998, Nanney 2004). Likewise, *Paramecium novaurelia*, a member of the *P. aurelia* complex, has never been collected outside of Europe, where it is the most widespread species of the complex (Przyboś and Fokin 2000, Fokin *et al*. 2004). Another example is *P. schewiakoffi* which is confined to Asia, as shown by Fokin *et al*. (2004) in a combined morphological and molecular approach. Basically, the same pattern is recognizable in *Euplotes*. For instance, *Euplotes octocarinatus*, which is quite common in ponds of North America, has never been found in central

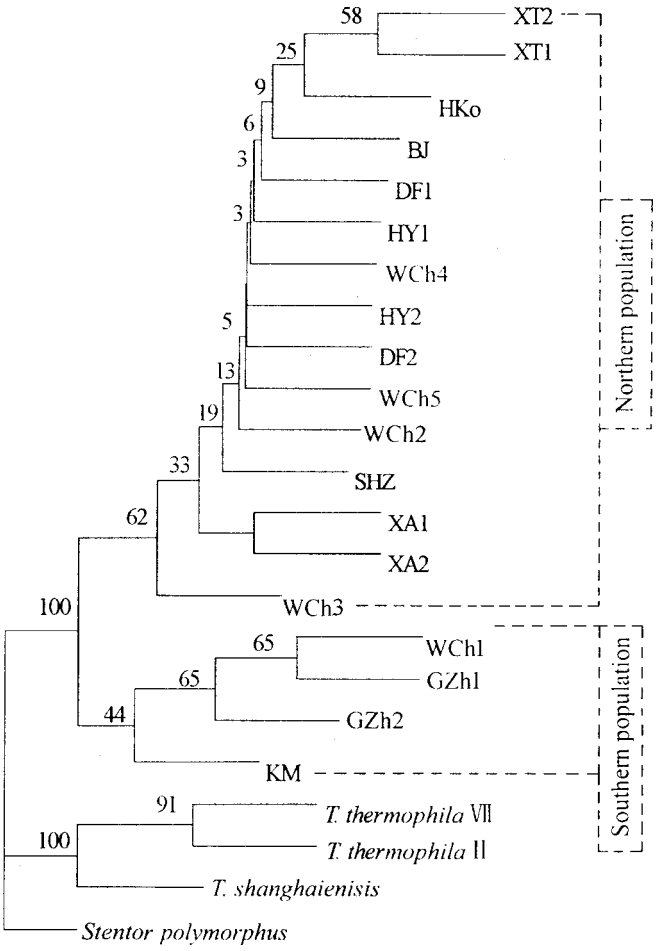


Fig. 20. Neighbour-joining tree of 19 populations of *Carchesium polypinum* (from Miao *et al*. 2004). The tree is based on 18S-ITS1-5.8S ribosomal DNA and shows a well-supported separation of the *Carchesium* populations found in northern and southern China.

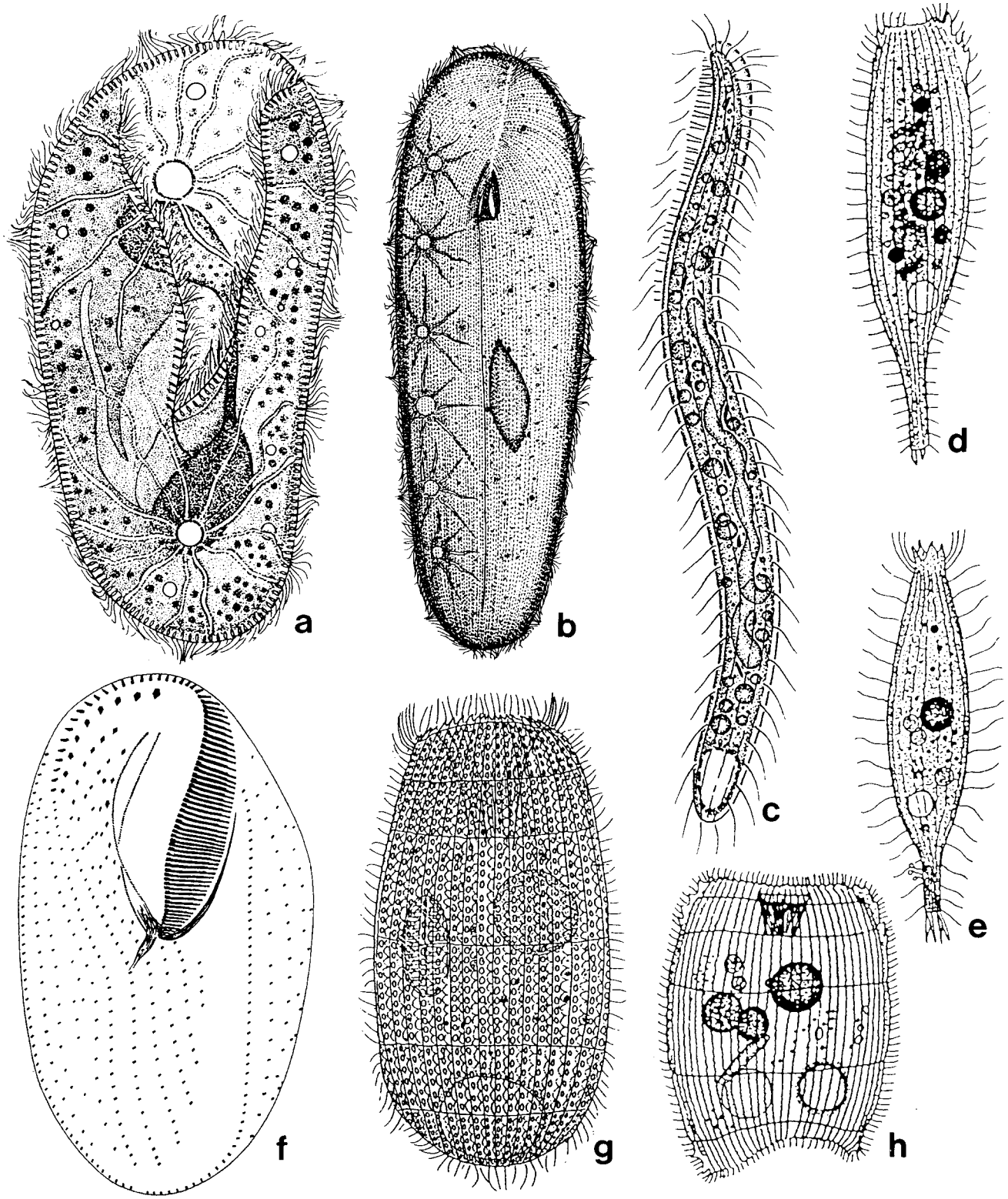
Europe, as mentioned by Heckmann (pers. inform.), a recognized specialist for this species. Provinciality is also known for the common "cosmopolite" *Stylonychia mytilus*, which now consists of four reproductively isolated species three of which can be separated also morphologically, albeit by sophisticated features such as the dorsal bristle pattern and micronucleus size (Gupta *et al*. 2001, Shi and Ammermann 2004). While *S. mytilus* and *S. lemnae* are probably cosmopolites, *S. ammermanni* and *S. harbinensis* are restricted to India and China.

Speciation as a consequence of geographical barriers has been highlighted in an excellent study by Miao *et al.* (2004). They investigated the abundant cosmopolitan ciliate *Carchesium polypinum* in China (Fig. 20). The 18S-ITS1 rDNA sequences separated the 19 populations analysed in this study into a northern and a southern phylogroup, basically matching those found in fish. Within the large northern territory, some fine-scaled differences were found.

This brief overview shows that the genetic and molecular data argue consistently for a geographically restricted distribution of ciliate species, both at a global scale (Gondwanan/Laurasian distribution, Nanney *et al*. 1998, Pucciarelli *et al*. 2003) and at the continental level (Nanney *et al*. 1998, Foissner *et al*. 2001, Droste *et al*. 2003, Fokin *et al*. 2004, Katz *et al*. 2005) and local level (Nanney *et al*. 1998, Przyboś and Fokin 2000, Snoeyenbos-West *et al*. 2002). This is strongly supported by field data discussed in the following paragraphs.

There is solid morphological evidence that a number of "flagship" families, genera and species have a restricted global distribution. These eye-catching species, which approach a size of about 1 mm would have been found in Europe, if they were there. Well-known examples are *Neobursaridium gigas* (Fig. 21a) and *Frontonia vesiculosa* (Fig. 21b), which occur only in South America and Africa (Fig. 19); and *Loxodes rex*, an endemic of tropical Africa (Dragesco and Dragesco-Kernéis 1986; a recent record from Bangkok is doubtful, see Foissner *et al*. 2002). *Neobursaridium gigas* is a stenothermic ciliate well adapted to the tropics, which has its highest growth rates at temperatures between 71.6°F and 78.8°F (Dragesco 1968). Other excellent examples are the Colepidae from Lake Baikal (Figs 21d, e, h). These ciliates have distinct body shapes and highly structured calcareous scales which make a misidentification unlikely. Interestingly, a potentially endemic colepid genus was discovered in Lake Tanganyika (Fig. 21g).

Impressive examples for recently described flagship species are three heterotrichs from very different habitats and two holotrichs (haptorids) from Venezuela. The first is *Stentor araucanus*, an up to 300 µm long, blue-green planktonic ciliate, which is frequent in South American lakes (Foissner and Wölfl 1994). The second is *Maristentor dinoferus*, an up to 1 mm long, purple, *Stentor*-like ciliate, which is common on the coral reefs of Guam, Mariana Islands, in the Pacific Ocean (Lobban *et al*. 2002). Finally, *Heterostentor coeruleus*, a 200-300 µm long, bluish ciliate was discovered in the littoral of Potter Cove, King George Island, Antarctica (Song and Wilbert 2002). The two haptorids from astatic grassland ponds at the north coast of Venezuela are each about 200 µm in size and have complex, up to 12 µm high



Figs 21a-h. Eye-catching ciliates with restricted biogeographic distribution. **a, b** - *Neobursaridium gigas* and *Frontonia vesiculosa* are up to 1 mm long freshwater ciliates occurring only in South America and tropical Africa (from Dragesco and Dragesco-Kernéis 1986; see also figure 19); **c** - *Apobryophyllum vermiforme*, a 200 µm long species from ephemeral habitats in Namibia (from Foissner *et al*. 2002); **d, e, g, h** - endemic Colepidae from Lake Baikal (d, e, h; from Obolkina 1995) and Lake Tanganyika (g; from Dragesco and Dragesco-Kernéis 1991). These species, each representing a distinct genus, are real "flagships", although being only 100-150 µm long, because they have highly characteristic body shapes and/or calcareous plates in the cortex; **f** - *Gigantothrix herzogi*, an up to 400 µm long, stichotrichine soil ciliate from Kenya (from Foissner 1999c).

scales easily recognizable with the light microscope (Figs 11-13, Foissner 2005). Each of these species is a flagship with distinct morphology, considerable size and/or conspicuous colour. If they would be ubiquitous they could not have been overlooked, at least in Europe. These five flagship ciliates demonstrate that even common - in their region - and abundant species were unknown until recently, demonstrating our ignorance of free-living ciliate diversity.

Recently, Foissner (2003) and Foissner *et al.* (2003) opened the door to another large ecosystem, viz., the endemic ciliate fauna in the tanks of bromeliads, with species reaching a length of up to 800 µm (Figs 14, 15). Bromeliads occur mainly in Central and South America and their tanks, which are formed by the coalescing leaf axils, form minute ponds. Altogether the tanks form a specific ecosystem whose extensive compartmentalization obviously fosters speciation. Thus, an impressive mixture of cosmopolitic (e.g., *Leptopharynx costatus*) and endemic (e.g., *Bromeliophrya brasiliensis*) ciliates occur in the tanks, often representing new genera and families. One of the new families, which are represented by three new genera and four novel species, is an excellent example for the evolution of ciliate genera and species due to a long-lasting spatial separation from the main gene pool, since these species are closely related to *Glaucoma scintillans*, a widely distributed species in "ordinary" limnetic habitats. The differences between the endemic and the ubiquitous species became obvious only through a combined morphological, molecular, and ecological approach (Foissner *et al.* 2003). A similar situation is found in certain metazoans. Packer and Taylor (1997), for instance, suggest that the presence of morphologically unrecognized species may be more common among widespread, easily identified "species" than it is generally assumed.

Detailed faunistic data are available from soil ciliates (Foissner 1998). Of 643 species identified in 1000 samples collected worldwide, 44% have been found so far only in one biogeographic region. Potentially, further investigations will show that one or the other (or even many) of these species occur also in other areas of the world (Foissner *et al.* 2002). On the other hand, such studies will invariably reveal a large number of novel species, which trivially, have not been described previously in other environments (Foissner *et al.* 2002, 2005). Thus, it is unlikely that the percentage of species with restricted distribution will decrease significantly even after intensified sampling. As these figures are based on 1000 samples, it is very likely that there exist many endemic soil ciliates, which await their discovery, similar to the situation in soil testate amoebae (Bonnet 1983). Some of the supposed endemics are shown in Figures 9, 10, 16-18, 21c, f. All of them have large size and/or a very distinct morphology, for instance, *Circinella arenicola*, which was discovered in an inland sand dune of Utah, USA (Fig. 17). This species, which is highly conspicuous due to its size (up to 600 µm), did not occur in the plethora of sand dune samples from the Namib Desert (Foissner *et al.* 2002) and Australia (Foissner, unpubl.), although all these habitats look quite similar. It is likely that *Fungiphrya strobli* is another example of local endemism: although it is a small species (~ 50 µm), it is very distinct because it is an obligate mycophage with a

Table 1. Number of ciliate species in rainforest litter and soil from Borneo and Malaysia.

Sample no.	Time elapsed since collection	Species number
Fresh samples		
1	< 10h	32
2	< 10h	29
3	~ 12 h	32
4	~ 12 h	28
5	< 10h	28
6	< 10h	12
7	< 10h	19
8	< 10h	22
		$\overline{X}$ 25
Air dried and stored up to 9 months[a]		
9	~ 6 m	36
10	~ 6 m	34
11	~ 5 m	29
12	~ 5 m	50
13	~ 5 m	35
14	~ 5 m	12
15	~ 9 m	15
		$\overline{X}$ 30
Air-dried and stored 9-65 months[a]		
16	~ 59 m	12
17	~ 65 m	7
18	~ 65 m	4
19	~ 25 m	7
20	~ 41 m	2
		$\overline{X}$ 6.4
Air-dried and stored 82 months[a]		
21	82 m	4
22	82 m	3
23	82 m	1
24	82 m	1
25	82 m	0
		$\overline{X}$ 1.8

[a] Investigated with the non-flooded Petri dish method for four weeks (Foissner *et al.* 2002).

very special oral apparatus (Figs 16, 18). Among the 1000 soil samples investigated so far, *F. strobli* occurred only in one sample from the Table Mountain in the Republic of South Africa. This area is well known for its numerous endemic plants, which characterize it as a distinct phytogeographical region (Sitte *et al*. 1991).

The high number of endemites in soil is not surprising because soil is a highly compartmentalized ecosystem with unfathomed niches for minute protists. Each plant species may provide specific niches by providing specific root exudates and particular microenvironments. Thus, it is difficult to believe that the fractal structure of soil limits ecological niches, and, consequently, protistan species diversity, as postulated by Finlay and Fenchel (2001). Furthermore, it has been shown that self-similarity leads to a distribution of species-abundance which deviates considerably from the commonly assumed log-normal distribution and predicts considerably more rare species than the latter (Harte *et al*. 1999). Exactly this has been observed in the above-mentioned 1000 soil samples: a very limited number (~50) of common (frequent) species is opposed to a very high number (> 700) of rare species (Foissner *et al*. 2002).

Very recently, Chao *et al*. (2006) used improved statistical tools to estimate global soil ciliate diversity and distribution based on 359 samples from five continents. The "abundance-based coverage estimation" model provided a global soil ciliate diversity of at least 1900 species which is consistent with the findings of Foissner (1997b), who used a probability-based method. Further, Chao *et al*. (2006) provided evidences for a distinct separation of the Laurasian and the Gondwanan soil ciliate biota, and thus for the influence of historical events on the distribution of micro-organisms.

Dispersal

Cysts and passive dispersal

Many micro-organisms, especially those living in terrestrial and limnetic habitats, can produce dormant stages named spores, stomatocysts, resting cysts, or cysts (my preferred general term), depending on the group of organisms investigated and personal taste. Cysts are generally assumed to be the major dispersal vehicle of unicellular organisms, since they remain viable under various harsh influences (e.g., drought and cold) for a long period of time (for reviews, see Corliss and Esser 1974 and Foissner 1987).

However, the biogeographical discussion ignored that cysts have very different properties, depending on the region and habitat in which they have been produced. While ciliate and flagellate cysts formed in extreme habitats withstand desiccation, drought and frost for years (Foissner 1996, Foissner *et al*. 2002) or even decennia (Foissner 1987), most of those produced in rainforest soil are viable for only a few months (Foissner 1997a and Table 1). Likewise, cysts from cultivated cells are often much "weaker" than those from environmental specimens, and frequently species lose the ability to produce cysts at all on prolonged laboratory cultivation (Corliss and Esser 1974; Foissner 1987, 1997a). This makes sense. Soil (and, of course, laboratory cultures) from evergreen rainforests is slightly wet most of the time due to the frequent rainfalls and the high air humidity. Thus, most protists are probably permanently active and hardly forced to produce desiccation-resistant dormant stages (Foissner 1997a). Accordingly, these species have little chance to disperse via cysts over large areas. This and the lack of glaciation might be the main reasons that endemics are more frequent in tropical than temperate regions.

It is widely assumed that trophic cells and cysts of micro-organisms are dispersed by wind and animals, especially migrating birds. There are many investigations supporting this view (some cited in Corliss and Esser 1974 and Foissner 1987). Unfortunately, cysts often could be reactivated only partially and most organisms were not identified to species level. But it appears that mainly common ubiquists are found, emphasizing the restricted cyst viability reported above.

However, not all cosmopolitan protists can produce cysts. Well known examples are the ciliate complexes *Paramecium aurelia* and *Tetrahymena pyriformis*, some of whose sibling species appear to have a restricted distribution (Elliott 1973, Nanney and McCoy 1976, Sonneborn 1975). This indicates that time is a crucial factor for becoming a cosmopolite, as discussed below.

Dispersal by human activities

Biogeographic changes due to human activities have been largely ignored in the discussion of distribution of protists and small metazoans, although a number of examples are well known for a long time. For example, several tropical and indopacific species of foraminifera entered the Mediterranean Sea via the Suez Canal (Lesseps' immigrants) and tropical aquaria. Moreover, it is likely that certain toxic dinoflagellates spread by human activities (Hallegraeff and Bolch 1992). In roti-

fers, many of which have a similar size as ciliates, *Brachionus havanaensis* and *Keratella americana* have been introduced to southeast Asia by human activities (Segers 2001).

Shipping (ballast water), the transport of goods, and the construction of canals are three major reasons for the artificial dispersal of protists. Millions of tons of water and many thousands of tons of soil are transported across the world each year. Hallegraeff and Bolch (1992) and Hülsmann and Galil (2002) suppose that since the introduction of water as ballast in the middle of the 19th century, many protists may have spread globally, unheeded by protozoologists. The diatoms *Odontella sinensis* and *Coscinodiscus wailesii* entered the North Sea and the Baltic Sea rather recently, together with their parasites (Kühn 1997, Hülsmann and Galil 2002). Likewise, *Lagenophrys cochinensis*, an ectosymbiotic ciliate of wood-boring, marine isopods, has probably been transported from New Zealand to California in wooden ship hulls rather recently (Clamp 2003), while the coccolithophore *Emiliania huxleyi* invaded the Black Sea about 1500 years ago (Winter *et al*. 1994). Elliott (1973) proposed that a species of the *Tetrahymena pyriformis* complex entered the Pacific Islands when man migrated westward from South and perhaps Central America. The same might have happened more recently with *Paramecium quadecaurelia*, a member of the *P. aurelia* sibling species complex. This species, which was known only from Australia, was recently reported from a pond of the city of Windhoek, the capital of Namibia, Africa (Przyboś *et al.* 2003). Dispersal by ship's ballast water might also be responsible for the occurrence of four euryhaline psammobiontic (obligate sand-dwelling) testate amoeba species in the Great Lakes, Canada (Nicholls and MacIsaac 2004).

Another impressive example is the appearance of *Hydrodictyon* in New Zealand where this very distinctive alga had never been seen before. It was found in a pond belonging to a hatchery supplying fish and aquatic plants to aquarists. Obviously, *Hydrodictyon* had been imported together with fish or aquatic plants from East Asia (Kristiansen 1996).

Spores of macrofungi, mosses and ferns show that small size and high numbers do not necessarily cause cosmopolitan distribution

It is a matter of fact that, at the morphospecies level, higher plants and animals usually have a more restricted distribution than micro-organisms. The reasons are unclear and difficult to exploit experimentally, but small size and high numbers are assumed to play a major role (Fenchel 1993, Finlay *et al*. 1996, Finlay 2002). A more differentiated view is held by Kristiansen (2000), who concisely summarized the hypotheses on micro-organism dispersal and biogeography: "Round remarked that the occurrence of so many freshwater algae throughout the world is a reflection of ease of transport - yet for the majority there is no information on transport mechanisms. Dispersal may thus be successful only rarely and under especially favourable circumstances; still the dispersal mechanisms will work if only there is time enough. This has been expressed by Gislen 'Wherever a biotope suitable for a certain organism exists, that organism will appear there as soon as sufficient time has elapsed to allow it to be transported to and to settle in that locality'. This adds a time dimension to Beijerinck's statement that, in micro-organisms, everything is everywhere, the environment only selects. Thus, time is a crucial factor. This means that we can consider the distribution of a species as a momentary picture based on: Age of the species - Available dispersal vectors -Adaptations for dispersal -Suitable available localities - and Sufficient time. In a cosmopolitan species, all these parameters are in optimum".

Basically, I agree with all these statements and hypotheses, but they are incomplete because they do not explain why mosses, ferns and macrofungi have indisputable biogeographies, although their main dispersal means (spores) are in the size of large bacteria or small protists and are produced in gigantic numbers (Tryon and Tryon 1982, Webster 1983, Carlile and Watkinson 1994, Kramer *et al*. 1995, Schwantes 1996, Frahm 2001). Even some groups of flowering plants have seeds in the size of middle-sized *Paramecia* (100-300 µm), for instance, many species of the families Pyrolaceae, Orobanchaceae and Orchidaceae (Sitte *et al.* 1991; Foissner, unpubl.), and the Orchidaceae comprise more than 20,000 species! Further, many seeds of higher plants have highly effective morphological adaptations for air dispersal, but are not cosmopolites, although many of these "exotics" grow well in our home gardens. Morphological adaptations for air dispersal are unknown in cysts and spores of micro-organisms, suggesting that this kind of distribution never played a major role.

Mosses, ferns and macrofungi, altogether well above 40,000 species, have distinct biogeographies, although their spores are in the size of those of small protists, that is, have a usual range of 10-50 µm and are produced in astronomical numbers, for instance, a single *Agaricus campestris* releases 1.6×10^{10} spores in six days

(Webster 1983), which exceeds the abundance of ciliates in a m² of forest soil by several orders of magnitude (Meyer *et al*. 1989). This fact has been completely ignored by protist and general ecologists (Fenchel 1993; Azovsky 2000, 2002; Wilkinson 2001; Finlay 2002; Hillebrand 2002). In my opinion, it is impossible to explain their restricted distribution by more complex ecologies and/or biologies (e.g., sex) because these are found also in micro-organisms (Weisse *et al*. 2001, Hausmann *et al*. 2003). Even the mycotrophic orchids are no exception, if one assumes global distribution of micro-organisms (Finlay 2002). The same has been observed in freshwater invertebrates, where potential and actual dispersal rates differ greatly (Bohonak and Jenkins 2003). Although nobody will deny that micro-organisms, macrofungi, mosses, and ferns usually have larger areals than flowering plants and higher animals, the minute size of the dispersal stages does not cause global distribution. This casts strong shadow on the hypothesis of Wilkinson (2001) that organisms with a size of less than 100-150 µm are generally cosmopolitan because their low mass facilitates air distribution. As a consequence, the hypotheses that micro-organisms are distributed globally and have low species richness because of lack of opportunities for allopatric speciation (Fenchel 1993, Finlay 2002, Wilkinson 2001) are also doubtful. Thus, I favour the more differentiated view of Kristiansen (2000), cited above.

Conclusions: Not everything is everywhere

A rapidly increasing number of studies, ranging from bacteria to small metazoans, are beginning to challenge Beijerinck's "everything is everywhere" metaphor and its contemporary variants. The data available suggest that micro-organisms have distribution patterns similar to those known from higher plants and animals, and that these patterns reflect historical (Gondwanan/Laurasian), ecological (tropical/temperate), and continental/local conditions. However, the biogeographical patterns of protists are usually less distinct than those of multicellular organisms because (i) they appear to occupy wider ranges, (ii) reliable distribution data are rare for most groups, and (iii) at least half of their diversity is still undescribed, especially that of the rare species which tend to have more restricted distributions than the common euryoecious species. Notwithstanding these limitations by a lack of data, it is realistic to assume that at least 30% of the protists are endemic (in the broadest sense).

Further research is necessary to assess to what degree historic, biological, climatic and habitat factors have contributed in creating the distribution patterns of micro-organisms. However, most specialists would agree on the following reasons:

(i) Historic (separation of Pangaea into Gondwana and Laurasia) and/or more recent (continental) geographic isolation were pivotal in limnetic and terrestrial ecosystems (Elliott, 1973; Bonnet 1983; Ferris and Ferris 1985; Najt and Weiner 1996; Reid 1998; Foissner 1999b; Kristiansen 2001; Foissner *et al*. 2002; Meisterfeld 2002a, b; Pawlowski and Holzmann 2002; Pucciarelli *et al*. 2003), while water current systems, temperature, and food played a major role in marine environments (Taylor and Pollingher 1987, Brand 1994, Roth 1994, Winter *et al*. 1994, Kemle-von Mücke and Hemleben 1999). More recently, artificial dispersal of micro-organisms by human activities increasingly masks the genuine distribution patterns.

(ii) Age of the species, that is, sufficient time to disperse. As many micro-organisms are much older than higher plants and animals (Cohan 2002, Arber 2004), this explains their usually wider distribution.

(iii) Limited cyst viability could be responsible for the restricted distribution of certain micro-organisms at large scale. At smaller scales, protected dispersal stages (cysts) explain the fast local distribution of euryoecious species, experimentally shown by Wanner and Dunger (2002) and others.

Lastly, we must take into account the 300 years and numerous taxonomists that were required to describe the world's mammals and higher plants, and even these lists are not yet complete (Cotterill 2005). Scientific protistology is just 150 years old and has attracted few taxonomists. Thus, it is not surprising that the majority of the protists is still undescribed, especially if their cryptic diversity due to developmental homeostasis is taken into account (Dini and Nyberg 1993, Nanney *et al*. 1998, López-García *et al*. 2003, Šlapeta *et al*. 2005). Thus, I agree with Nanney (2004): "We are all blind men trying to describe a monstrous elephant of ecological and evolutionary diversity".

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ACTA
PROTOZOOLOGICA

Redescription of *Tintinnopsis cylindrica* Daday, 1887 (Ciliophora: Spirotricha) and Unification of Tintinnid Terminology

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Summary. Although *Tintinnopsis cylindrica* Daday, 1887 is apparently widely distributed in the plankton of marine and brackish coastal waters, its ciliary pattern remained unknown. Without detailed knowledge of the cell morphology, however, the proposed synonymies cannot be proved. Hence, the cell and lorica features of *T. cylindrica* are redescribed from live and protargol-impregnated specimens collected in mixo-polyhaline basins at the German North Sea coast. An improved species diagnosis and a comprehensive unified terminology are provided. The somatic ciliary pattern of *T. cylindrica* is complex, comprising a ventral, dorsal, and posterior kinety as well as a right, left, and lateral ciliary field. Accordingly, the species differs from its congener *T. cylindrata* that has merely a right and left ciliary field and ventral organelles. On the other hand, the genera *Codonella*, *Codonellopsis*, *Cymatocylis*, *Helicostomella*, *Leprotintinnus*, and *Stenosemella* share this pattern. The oral primordium of *T. cylindrica* develops hypoapokinetally posterior to the lateral ciliary field as in *Codonella cratera* and *Cymatocylis convallaria*.

Key words: biogeography, ciliary pattern, ecology, morphology, ontogenesis, taxonomy, Tintinnina.

INTRODUCTION

Entz (1884, 1909b), Bütschli (1887-1889), Daday (1887), Brandt (1907), Schweyer (1909), and Hofker (1931) emphasized the significance of cytological features for a natural tintinnid taxonomy. Nevertheless, the majority of the ~ 1,200 tintinnid species was described in the following years, using merely lorica features (e.g., Kofoid and Campbell 1929, 1939). It was only in the eighties and nineties of the last century, that the investigation of the cell morphology experienced a renaissance by the redescription of 16 tintinnid species (Foissner and Wilbert 1979, Song and Wilbert 1989, Blatterer and Foissner 1990, Foissner and O'Donoghue 1990, Sniezek *et al.* 1991, Snyder and Brownlee 1991, Choi *et al.* 1992, Song 1993, Wasik and Mikołajczyk 1994, Petz *et al.* 1995). The number of reinvestigated species is, however, still too low for a revision of the classification and a comparison with the gene trees (Snoeyenbos-West *et al.* 2002, Strüder-Kypke and Lynn 2003). Hence, a further tintinnid species, *viz.*, *Tintinnopsis cylindrica*, is

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redescribed in the present paper, including both, features of the lorica and the cell.

MATERIALS AND METHODS

Collection. The samples were collected between 1991 and 1993 in the basins of two polders, *viz.*, the Beltringharder Koog and the Speicherkoog Dithmarschen, at the North Sea coast of Schleswig-Holstein, northern Germany. These shallow (up to 15 m deep) basins contain brackish water of changing salinities as they are temporarily connected with the Wadden Sea by sluice gates and have freshwater inflow by rainwater, ground water, and streams. Likewise, they are characterized by reduced tidal currents, high turbidity, and eutrophication due to nutrient loads drained from agricultural areas. The abiotic factors that prevailed during the investigation period were described in detail by Agatha *et al.* (1994) and Riedel-Lorjé *et al.* (1998). Samples were taken monthly by bucket from December to February, fortnightly in March and November, and weekly during all other months at the bank of the basins. One subsample was immediately preserved with 1% Lugol's iodine solution and analyzed latest six month after sampling for abundances (Agatha *et al.* 1994), while an unpreserved subsample was used for taxonomical investigations.

Taxonomic studies. All observations are from field material as culture trials failed, using a temperature of ~ 12°C, a 12 h light to 12 h dark cycle with an irradiance of ~ 15 µE m^{-2} s^{-1}, and a mixture of flagellates from the sampling sites as prey. Cell movement was studied in a Petri dish (~ 6 cm across; water depth ~ 2.5 cm) under a dissecting microscope at ~ 20°C. Cell morphology was investigated under a compound microscope equipped with a high-power oil immersion objective as well as bright-field and interference contrast optics. Protargol impregnation followed the protocol of Song and Wilbert (1995). For scanning electron microscopy, cells were fixed for 30 min in a modified Parducz' solution made of 6 parts of 2% OsO_4 (w/v) in sea water and one part of saturated aqueous $HgCl_2$ (Valbonesi and Luporini 1990); further steps followed Foissner (1991).

Counts and measurements on protargol-impregnated cells were performed at ×1,000; *in vivo* measurements were made at ×40-1,200.

The kinetal density index is the ratio of kinety number to cell circumference posterior to the membranellar zone [kineties/µm] in protargol-impregnated cells (Snyder and Brownlee 1991). Usually, it was impossible to count all somatic kineties in a specimen as the curved and densely spaced ciliary rows could not be discerned in the laterally orientated fields; hence, the kinetal index was not calculated.

Illustrations. Drawings of live specimens summarize information and are based on mean measurements, while those of protargol-impregnated specimens were made with a camera lucida. The kinetal map depicts the morphostatic ciliary pattern of a protargol-impregnated specimen in two dimensions (Foissner and Wilbert 1979, Choi *et al.* 1992), that is, the cortex is drawn as cut longitudinally along the dorsal kinety; it is also based on mean measurements. Horizontal bars symbolize the collar membranelles, diagonal bars those membranelles that are partially or entirely in the buccal cavity, namely, the elongated collar membranelles and the buccal membranelles. The ratio of cell circumference to length of kineties is 1:1. Kinetids are equidistantly arranged in the ciliary rows and the kinety curvature is neglected, except for the ventral and last kinety whose course might be of taxonomic significance. The somatic cilia are symbolized by oblique lines, differences in their length are not considered.

Neotype material. A slide with protargol-impregnated neotype specimens is deposited with the relevant cells marked in the Biology Centre of the Museum of Upper Austria (LI) in A-4040 Linz (Austria). The reasons for and the problems with neotypification are discussed by Foissner (2002), Foissner *et al.* (2002), and Corliss (2003).

RESULTS

Terminology

Since more than thirty years, the orders Halteriida and Oligotrichida have been separated from the order Choreotrichida by the shape of the adoral zone of membranelles (C-shaped *vs.* ring-shaped; Fauré-Fremiet 1970). However, Kim *et al.* (2005) discovered a new member of the family Strombidinopsidae (order Choreotrichida) with a slightly open membranellar zone, representing a transitional stage. Hence, a different terminology for the large and small membranelles/polykinetids in the Halteriida and Oligotrichida (anterior and ventral membranelles) on the one hand and the Choreotrichida (external and internal membranelles) on the other hand seems not any longer justified. Accordingly, a unifying and neutral terminology concerning the oral ciliature is introduced here. Additionally, the confusing terminology of the somatic ciliary components and some further features in the suborder Tintinnina is unscrambled here.

Adoral zone of membranelles. The adoral zone of membranelles is an orderly arrangement of membranelles around the peristomial field, terminating in the buccal cavity. The term was probably introduced by Bütschli (1887-1889) and is favoured, as the younger term "oral polykinetids" (Sniezek *et al.* 1991, Snyder and Brownlee 1991, Choi *et al.* 1992, Wasik and Mikołajczyk 1994) is restricted to the basal bodies of the membranelles and their associated fibres; both are only recognizable after silver impregnation. Furthermore, the expression "adoral zone of membranelles" is also used in the related class Hypotrichea (hypotrichs and stichotrichs).

Collar membranelles. The collar membranelles form a closed zone on the peristomial rim and constitute the anterior portion of the oral primordium in early dividers. They have larger polykinetids and longer cilia than the buccal membranelles. Some collar membranelles are elongated into the eccentric buccal cavity (praeorale

Membranellen, Foissner and Wilbert 1979; Buccalmembranellen, Blatterer and Foissner 1990; somatic adoral membranelles, Foissner and O'Donoghue 1990). Although there are several other names for these membranelles (adorale Pectinellen, Entz 1909b; Membranulae, Entz 1929; Peristomal-Pektinellen, Entz 1937; adoral membranelles, Foissner and Wilbert 1979, Foissner and O'Donoghue 1990, Petz and Foissner 1993; external membranelles, Petz *et al.* 1995), the term "collar membranelles" is chosen, as it clearly explains the position of the membranelles and can also be used for the large membranelles in the Halteriida and Oligotrichida.

Buccal membranelles. The buccal membranelles are entirely situated in the buccal cavity and constitute the posterior portion of the oral primordium in early dividers. Note that some authors do not differentiate between elongated collar membranelles and buccal membranelles, but lump them to infundibular membranelles/polykinetids (e.g., Wasik and Mikołajczyk 1994). The term "buccal membranelles" is preferred instead of "internal membranelles" (Petz *et al.* 1995), as it clearly describes the position of the membranelles in the buccal cavity and is also applicable for the small membranelles in the Halteriida and Oligotrichida.

Endoral membrane. The endoral membrane extends across the peristomial field into the buccal cavity. It is usually named paroral membrane; however, due to its monostichomonad structure and probable homology to the endoral membrane of the stichotrichs, halteriids, oligotrichids, and most hypotrichs, this undulating membrane should likewise be called endoral membrane (Agatha 2004a, b).

Numbering of somatic kineties. The numbering commences with the ventral kinety or, when this ciliary row is absent, with the leftmost kinety of the right ciliary field and continues in clockwise direction when the cell is viewed from anterior (Chatton *et al.* 1931). Despite the fact that this numbering opposes that in the closely related suborder Strobilidiina (order Choreotrichida; Deroux 1974), it is maintained here to avoid confusion.

Ventral kinety. The ventral kinety is on the left side of the right ciliary field and on the right side of the oral primordium. It is the longest entirely monokinetidal ciliary row on the ventral side (Figs 1b, f). Although older expressions existed (frange bordante, frange ondulante, Fauré-Fremiet 1924; ciliary membrane, Campbell 1926, Kofoid and Campbell 1939, Tappan and Loeblich 1968), the term "ventral kinety" was introduced by Snyder and Brownlee (1991) to indicate the position of the ciliary row.

Right ciliary field. The right ciliary field is on the right side of the ventral kinety, the ventral organelles, or a blank stripe. The name for this ciliary field on the right cell side was introduced by Snyder and Brownlee (1991).

Dorsal kinety. The dorsal kinety is separate from the right and left ciliary field between which it is situated dorsally. It is the longest kinety, usually extending from the membranellar zone to the base of the stalk. The term was introduced by Choi *et al.* (1992). Foissner and Wilbert (1979), Foissner and O'Donoghue (1990), and Petz *et al.* (1995) named it "ventral kinety", although this ciliary row is on the cell side almost opposite to the eccentric buccal cavity.

Left ciliary field. The left ciliary field is on the left side of the dorsal kinety. Its ciliary rows are more closely spaced than those of the right ciliary field. The name for this ciliary field on the left cell side was introduced by Snyder and Brownlee (1991).

Lateral ciliary field. The lateral ciliary field is between the ventral kinety and the left ciliary field, with which it is occasionally lumped (Laval-Peuto 1994, Wasik and Mikołajczyk 1994). The term was introduced by Fauré-Fremiet (1924), and there are two similar expressions: Lateralfeld (Foissner and Wilbert 1979) and lateral field of kineties (Petz *et al.* 1995).

Posterior kinety. The posterior kinety is posterior to the lateral ciliary field. The term was introduced by Choi *et al.* (1992) and is favoured, as the names "Ventro-Lateralkinete" (Foissner and Wilbert 1979, Foissner and O'Donoghue 1990, Petz and Foissner 1993) and "dorsolateral kinety" (Petz *et al.* 1995) do not emphasize its unique position in the posterior cell portion.

Ventral organelles. The ventral organelles comprise a transverse (V1) and an oblique (V2) organelle, i.e., two short dikinetidal kineties posterior to the ventral collar membranelles. The term was introduced by Foissner and Wilbert (1979).

Capsules. Capsules are probably extrusive organelles that are attached to the cell membrane of cytoplasmic extensions, such as, accessory combs, striae, and tentaculoids. They are subspherical, 200-600 nm in size, and often form clusters; three morphotypes are known (Laval-Peuto and Barria de Cao 1987). Laval (1971 cited in Laval 1972) introduced the term "capsules torquées", but in the English literature only "capsules" is used (Hedin 1975, Gold 1979, Laval-Peuto *et al.* 1979, Capriulo *et al.* 1986, Wasik and Mikołajczyk 1992); the

older expressions “Bacterioidkörperchen” (Entz 1909b) and “trichocysts” (Campbell 1926) are less specific and are thus rejected.

Accessory combs. Accessory combs are conspicuous intermembranellar ridges. The term was introduced by Campbell (1926); the alternative names “Begleitkämme” (Entz 1909b) and “crêtes adorales” (Laval-Peuto 1994) were rarely used in the literature.

Striae. Striae are beaded, longitudinal cytoplasmic strands that are enclosed together with an collar membranelle by the perilemma (Laval 1972, Laval-Peuto 1994). The term was introduced by Entz (1929) and more often used than the expressions “lames de revêtement” (Laval-Peuto *et al*. 1979) and “Deckplättchen” (Entz 1909b).

Tentaculoids. Tentaculoids are small, finger-like, and possibly contractile cytoplasmic extensions between the collar membranelles (Corliss 1979, Laval-Peuto and Brownlee 1986). The term was introduced by Haeckel (1873).

Lorica. A lorica is a house, fitting the cell loosely, with an anterior (oral) and occasionally posterior (aboral) opening. It is carried about by free-swimming species or fixed to the substratum by sessile ones (Corliss 1979). A lorica should not be confused with the distended and often reticulate posterior cell surface of the related Oligotrichida.

Protolorica. A protolorica is built by the proter just after cell division (Laval-Peuto and Brownlee 1986).

Paralorica. A paralorica is a replacement lorica formed by a morphostatic cell (Laval-Peuto and Brownlee 1986).

Epilorica. An epilorica is a spiralled or annulated portion frequently added to the anterior end of a proto- or paralorica (Laval-Peuto and Brownlee 1986).

Tintinnopsis cylindrica Daday, 1887 (Figs 1-3, Table 1)

1887 *Tintinnopsis Davidoffii* var. *cylindrica* - Daday, Mitt. zool. Stn Neapel 7: 553.

1907 *Tintinnopsis cylindrica* - Wright, Ann. Rep. Dept. of Marine and Fisheries, Fisheries Branch, Ottawa 39: 11 (raise to species rank).

1913 *Tintinnopsis radix* forma *cylindrica* - Laackmann, Akad. Wiss. Wien, Math. nat. Kl. 122: 145.

1929 *Tintinnopsis davidoffi* var. *cylindrica* Daday, 1887 - Kofoid and Campbell, Univ. Calif. Publs Zool. 34: 33 (first revisers).

1929 *Tintinnopsis cylindrica* Daday - Kofoid and Campbell, Univ. Calif. Publs Zool. 34: 33 (first revisers).

1932 *Tintinnopsis kofoidi* sp. nov. - Hada, Zool. Inst., Fac. Sci. Hokkaido Imp. Univ., Sapporo 30: 210 (new subjective synonym).

1981 *Tintinnopsis kofoidii* - Hargraves, J. Plankton Res. 3: 85.

1983 *Tintinnopsis kofoidi* - Stoecker *et al.*, Mar. Biol. 75: 293 (growth experiments).

1986 *Tintinnopsis kofoidi* - Verity, Mar. Ecol. Prog. Ser. 29: 117 (growth rates).

1990 *Tintinnopsis kofoidi* - Kamiyama and Aizawa, Bull. Plankton Soc. Jap. 36: 137 (excystment).

1997 *Tintinnopsis kofoidi* - Kamiyama, J. Oceanogr. 53: 299 (excystment).

2005 *Tintinnopsis cylindrica* - Kamiyama and Matsuyama, J. Plankton Res. 27: 307 (ingestion rate).

Non *Tintinnopsis cylindrica* n. sp. - Daday, 1892, Természetr. Füz. 15: 201 (junior homonym; now *Tintinnopsis cylindrata* Kofoid and Campbell, 1929).

Non *Tintinnopsis cylindrica* Daday - Entz, 1905, Áll. Közl. 4: 204 (now *Tintinnopsis cylindrata* Kofoid and Campbell, 1929).

Non *Tintinnopsis cylindrica* (Daday) - Entz, 1909a, Math. naturw. Ber. Ung. 25: 204 (now *Tintinnopsis cylindrata* Kofoid and Campbell, 1929).

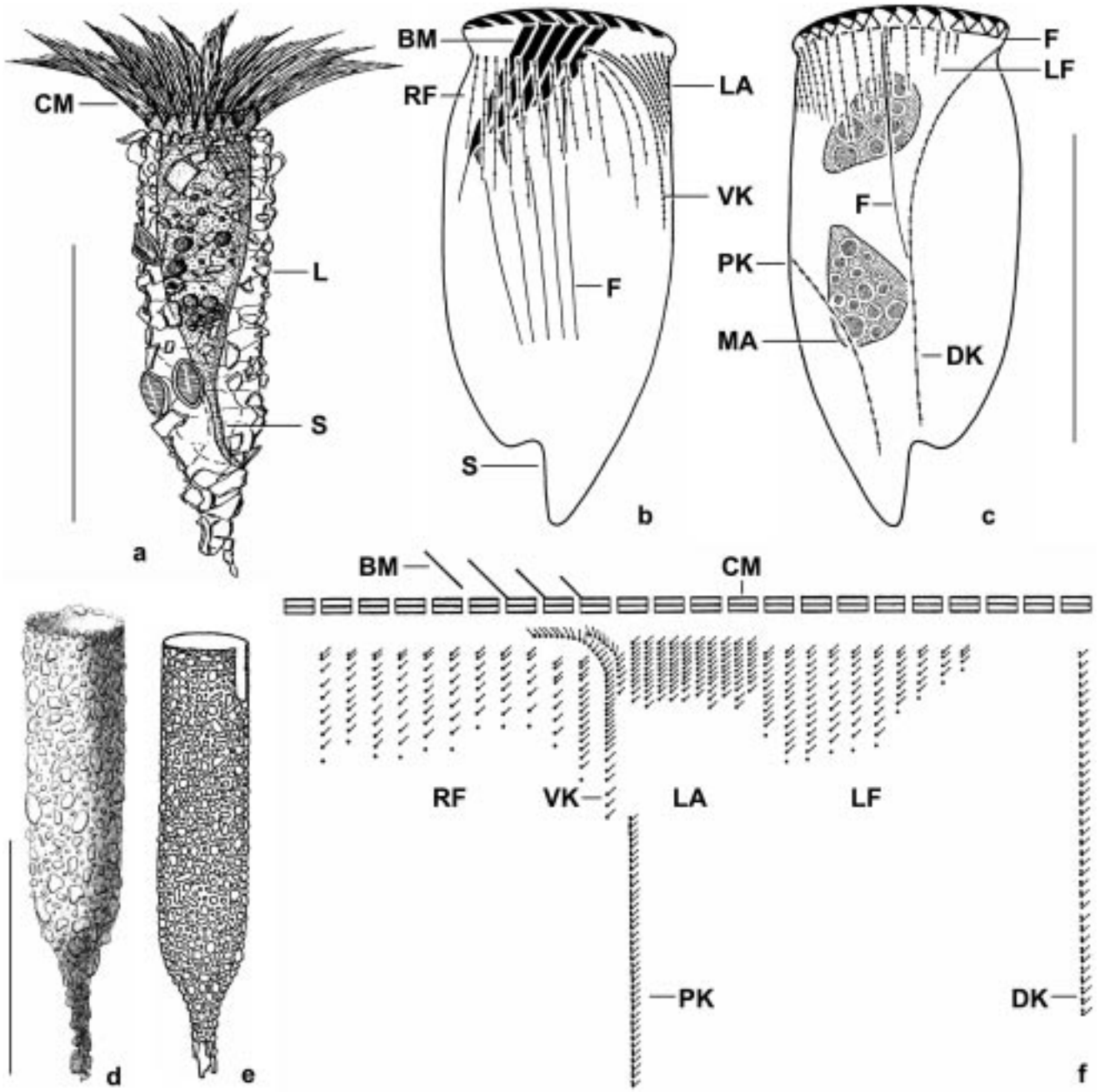
Non *Tintinnopsis cylindrica* Daday - Entz, 1909b, Arch. Protistenk. 15: 118 (now *Tintinnopsis cylindrata* Kofoid and Campbell, 1929).

Non *Tintinnopsis cylindrica* Daday - Jaczó, 1940, Fragm. faun. hung. 3: 59 (now *Tintinnopsis cylindrata* Kofoid and Campbell, 1929).

Non *Tintinnopsis cylindrica* sp. n. - Meunier, 1910, Campagne Arctique de 1907: 140 (junior homonym; now *Tintinnopsis spiralis* Kofoid and Campbell, 1929).

Neotype material. Neotypified from plankton of the mixo-polyhaline basin (54°32'58" N, 08°52'59" E) in the Beltringharder Koog, as (i) no type material is available, (ii) the original description lacks many morphologic features, and (iii) the species has several proposed subjective synonyms.

Improved diagnosis (based on data from the type and neotype population). Lorica on average 150-240 µm long and 45-50 µm wide orally, with agglutinated particles; Pasteur pipette-shaped, *viz.*, cylindroidal for on average 65-75% of total length, posteriorly tapered, merging into straight cylindroidal process ~ 20 µm



Figs 1a-f. *Tintinnopsis cylindrica* (a-d, f) and a supposed synonym (e) from life (a, e), after protargol impregnation (b, c, f), and preserved with mercuric chloride (d). **a -** a representative specimen from the neotype population; **b, c -** ciliary pattern of ventral and dorsal side. Note the fibres that are associated with the oral and somatic ciliature; **d -** a lorica from the type population (from Daday 1887); **e -** *Tintinnopsis kofoidi* (from Hada 1932a); **f -** kinetal map of a morphostatic specimen. BM - buccal membranelle, CM - collar membranelles, DK - dorsal kinety, F - probably fibrillar structures, L - lorica, LA - lateral ciliary field, LF - left ciliary field, MA - macronuclear nodules, PK - posterior kinety, RF - right ciliary field, S - stalk, VK - ventral kinety. Scale bars: 100 µm (a, d, e); 50 µm (b, c).

Table 1. *Tintinnopsis cylindrica* morphometric data. Measurements in µm. CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, n - number of individuals investigated, SD - standard deviation, SE - standard error of arithmetic mean, $\bar{x}$ - arithmetic mean.

Characteristics[a]	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Lorica, total length[b]	151.3	148.0	25.1	5.2	16.6	75.0	220.0	23
Lorica, oral diameter[b]	48.4	49.0	5.2	1.1	10.8	34.0	56.0	23
Lorica length:oral diameter, ratio[b]	3.2	3.0	0.6	0.1	19.4	1.6	5.2	23
Lorica, length of cylindroidal portion[b]	98.1	95.0	18.1	4.7	18.4	69.0	144.0	15
Lorica, length of tapered portion[b]	26.2	28.0	8.5	2.2	32.6	6.0	38.0	15
Lorica, process length[b]	22.1	20.0	9.7	2.4	43.9	0.0	38.0	15
Lorica, process diameter[b]	10.1	10.0	0.7	0.2	7.3	8.0	11.0	15
Cell, length	71.0	68.5	12.8	2.6	18.0	52.0	89.0	24
Cell, width	31.7	32.5	6.2	1.3	19.5	22.0	48.0	24
Macronuclei, length	15.6	16.0	4.5	1.0	29.2	9.0	23.0	19
Macronuclei, width	8.1	8.0	2.3	0.5	28.2	3.0	12.0	19
Macronuclei, number	2.0	2.0	0.0	0.0	0.0	2.0	2.0	19
Micronuclei, diameter	2.0	2.0	0.7	0.2	34.0	1.0	3.0	14
Micronuclei, number	2.0	2.0	0.0	0.0	0.0	2.0	2.0	14
Ventral kinety, length[c]	22.1	23.0	3.7	1.4	16.8	17.0	28.0	7
Ventral kinety, number of kinetids	35.8	33.0	12.3	5.5	34.4	25.0	55.0	5
Dorsal kinety, length[c]	46.8	43.5	9.4	4.7	20.2	40.0	60.0	4
Dorsal kinety, number of kinetids	36.1	36.0	9.6	3.6	26.5	25.0	55.0	7
Posterior kinety, length[c]	33.4	31.0	14.6	6.5	43.7	19.0	50.0	5
Posterior kinety, number of kinetids	26.1	21.0	10.7	3.6	41.0	15.0	45.0	9
Left ciliary field, number of kineties	10.4	11.0	1.5	0.5	14.5	8.0	12.0	8
1. kinety in left field, length	2.4	2.0	0.7	0.3	31.3	2.0	4.0	8
1. kinety in left field, number of kinetids	2.9	3.0	0.6	0.2	22.3	2.0	4.0	8
2. kinety in left field, length	3.9	4.0	1.6	0.6	42.4	2.0	7.0	8
2. kinety in left field, number of kinetids	3.9	3.5	2.0	0.7	50.6	2.0	8.0	8
3. kinety in left field, length	5.8	5.0	1.2	0.4	20.3	5.0	8.0	8
3. kinety in left field, number of kinetids	5.5	5.0	1.1	0.4	19.4	4.0	7.0	8
4. kinety in left field, length	8.3	8.5	2.9	1.0	35.3	3.0	12.0	8
4. kinety in left field, number of kinetids	7.9	8.0	2.6	0.9	33.6	3.0	11.0	8
5. kinety in left field, length	9.8	10.0	2.5	0.9	26.1	6.0	15.0	8
5. kinety in left field, number of kinetids	9.8	9.5	2.0	0.7	20.3	7.0	13.0	8
6. kinety in left field, length	10.6	9.5	2.8	1.0	26.6	8.0	16.0	8
6. kinety in left field, number of kinetids	10.1	10.0	1.6	0.6	16.2	8.0	13.0	8
7. kinety in left field, length	11.1	9.0	4.2	1.5	37.7	8.0	20.0	8
7. kinety in left field, number of kinetids	10.1	9.5	2.7	1.0	27.1	7.0	16.0	8
8. kinety in left field, length	11.5	9.5	4.8	1.7	41.3	8.0	19.0	8
8. kinety in left field, number of kinetids	11.5	10.5	4.0	1.4	35.1	7.0	18.0	8
9. kinety in left field, length	11.7	11.0	4.5	1.7	38.7	6.0	18.0	7
9. kinety in left field, number of kinetids	12.0	12.0	4.4	1.7	36.6	7.0	18.0	7
10. kinety in left field, length	11.4	8.0	5.6	2.5	49.5	6.0	18.0	5
10. kinety in left field, number of kinetids	11.0	10.0	3.4	1.5	30.8	7.0	15.0	5
11. kinety in left field, length	10.6	10.0	4.8	2.2	45.5	5.0	16.0	5
11. kinety in left field, number of kinetids	10.6	9.0	3.6	1.6	34.4	7.0	15.0	5
12. kinety in left field, length	11.5	-	-	-	-	9.0	14.0	2
12. kinety in left field, number of kinetids	11.0	-	-	-	-	8.0	14.0	2
Lateral ciliary field, number of kineties	11.1	10.0	2.9	1.1	25.6	8.0	16.0	7
Right ciliary field, number of kineties	10.6	11.0	1.3	0.5	12.0	9.0	12.0	7
1. kinety of right field, length	16.3	15.0	3.9	1.6	24.1	13.0	23.0	6
1. kinety of right field, number of kinetids	14.3	14.0	4.1	1.4	28.5	9.0	19.0	8
2. kinety of right field, length	11.0	10.5	2.8	1.1	25.1	7.0	15.0	6
2. kinety of right field, number of kinetids	7.3	7.0	1.8	0.7	25.3	5.0	11.0	8
3. kinety of right field, length	9.6	9.0	2.3	0.9	24.0	7.0	14.0	7
3. kinety of right field, number of kinetids	6.4	6.0	1.7	0.6	26.4	5.0	9.0	8
4. kinety of right field, length	9.5	9.5	2.4	1.0	25.6	6.0	13.0	6
4. kinety of right field, number of kinetids	6.8	6.0	2.1	0.8	31.4	5.0	10.0	8
5. kinety of right field, length	10.3	9.5	3.8	1.5	36.6	5.0	16.0	6
5. kinety of right field, number of kinetids	6.6	6.0	2.4	0.8	36.0	4.0	11.0	8

Table 1. (contd)

6. kinety of right field, length	13.2	14.0	3.2	1.3	24.2	8.0	17.0	6
6. kinety of right field, number of kinetids	8.4	8.0	2.8	1.0	33.1	5.0	13.0	8
7. kinety of right field, length	13.4	14.0	3.8	1.7	28.2	9.0	17.0	5
7. kinety of right field, number of kinetids	8.6	8.0	3.2	1.2	37.4	6.0	14.0	7
8. kinety of right field, length	14.4	17.0	4.5	2.0	31.3	9.0	18.0	5
8. kinety of right field, number of kinetids	9.1	7.0	3.8	1.4	41.6	6.0	15.0	7
9. kinety of right field, length	13.5	12.5	3.3	1.7	24.6	11.0	18.0	4
9. kinety of right field, number of kinetids	10.3	9.5	3.6	1.8	35.1	7.0	15.0	4
10. kinety of right field, length	12.0	11.0	2.6	1.5	22.0	10.0	15.0	3
10. kinety of right field, number of kinetids	9.0	8.0	2.6	1.5	29.4	7.0	12.0	3
11. kinety of right field, length	15.0	-	-	-	-	-	-	1
11. kinety of right field, number of kinetids	8.0	-	-	-	-	-	-	1
12. kinety of right field, length	10.5	-	-	-	-	9.0	12.0	2
12. kinety of right field, number of kinetids	8.0	-	-	-	-	7.0	9.0	2
Collar membranelles, number[d]	21.7	22.5	1.8	0.7	8.1	19.0	23.0	6
Buccal membranelle, number	1.0	1.0	0.0	0.0	0.0	1.0	1.0	18

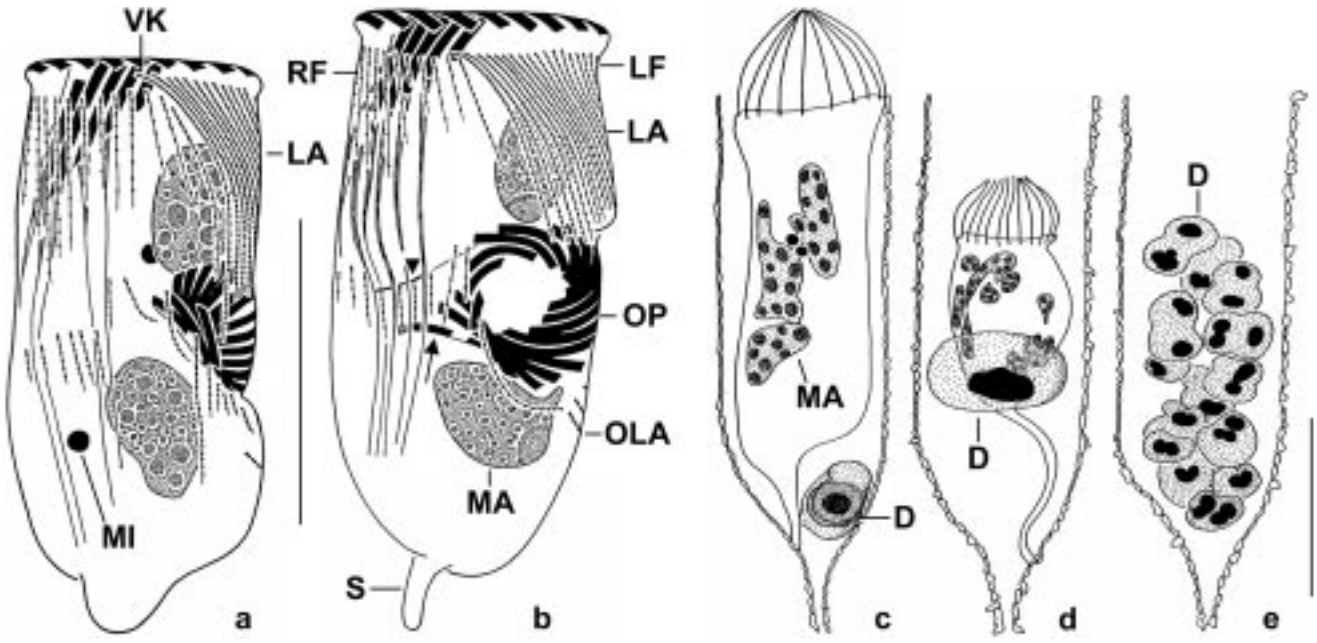
[a]Data are based, if not stated otherwise, on protargol-impregnated and mounted specimens from field material; [b]material preserved with Lugol's iodine solution; [c]measured as cord of organelle; [d]counted in properly orientated morphostatic specimens or oral primordia of middle dividers.

long and 10-15 µm wide. Cell on average 125-210 × 40-45 µm and elongate obconical, highly contractile. 2 macronuclear nodules and 2 micronuclei. Ventral kinety commences anterior to right ciliary field, consists of ~ 36 monokinetids. On average 11 kineties in right and 10 in left ciliary field, all composed of monokinetids and one anterior dikinetid, except for second and third ciliary row with two anterior dikinetids. About 36 dikinetids in dorsal kinety and 26 in posterior kinety, with a cilium only at each posterior basal body. Lateral ciliary field composed of ~ 11 monokinetidal kineties. On average 22 collar membranelles of which 4 extend into buccal cavity; single buccal membranelle. Marine and brackish waters.

Description of polder specimens. Loricae 75-220 µm long and 34-56 µm wide orally after preservation with Lugol's iodine solution; Pasteur pipette-shaped, *viz.*, cylindroidal for about two thirds of total length, anterior end transversely truncate, posteriorly tapered, merging into cylindroidal process. Process aborally open and usually transversely truncate, straight, 8-11 µm in diameter but highly variable in length possibly because it easily breaks off (Table 1). Matrix hyaline, incrustrated to various degrees by particles of non-biogenic origin (probably silt particles), diatom frustules and their fragments, and green globular organisms; no distinct spiralled or annulated structures recognizable (Figs 1a, d, e; 3a-e).

Fully extended cells 75-185 × 30-55 µm *in vivo*, elongate obconical, body proper gradually merges into slender, wrinkled stalk, attached to tapered portion of lorica (Figs 1a; 2c, d; 3a); disturbed or preserved cells contracted by ~ 40% and almost ellipsoidal (Figs 1b, c; 2a, b; 3b, d; Table 1). One ellipsoidal macronuclear nodule each in anterior and posterior cell half, with some large (~ 4 µm across) and several small (1-2 µm across) dark inclusions (probably nucleoli). Micronuclei adjacent to macronuclear nodules, globular, faintly impregnated with protargol. No contractile vacuole recognized. Cytopyge near mid-body. Myonemes not impregnated. Accessory combs rarely recognized *in vivo* (Fig. 3a), while striae, tentaculoids, and capsules not recognizable. Cytoplasm colourless, finely granulated, contains food vacuoles with coccal organisms (4-9 µm across) as well as centric (5-8 µm across) and pennate (10-16 × 2-3 µm) diatoms. Swims slowly (~ 0.1 mm s^{-1}) forward, twitches back on obstacles. Disturbed specimens retract quickly (< 1 sec) into posterior portion of lorica, with motionless collar membranelles bent to centre of peristomial field (Fig. 3b); lorica abandonment never observed. When inconvenience stops, specimens slowly (> 1 min) extend and spread out the collar membranelles almost perpendicularly (Fig. 1a).

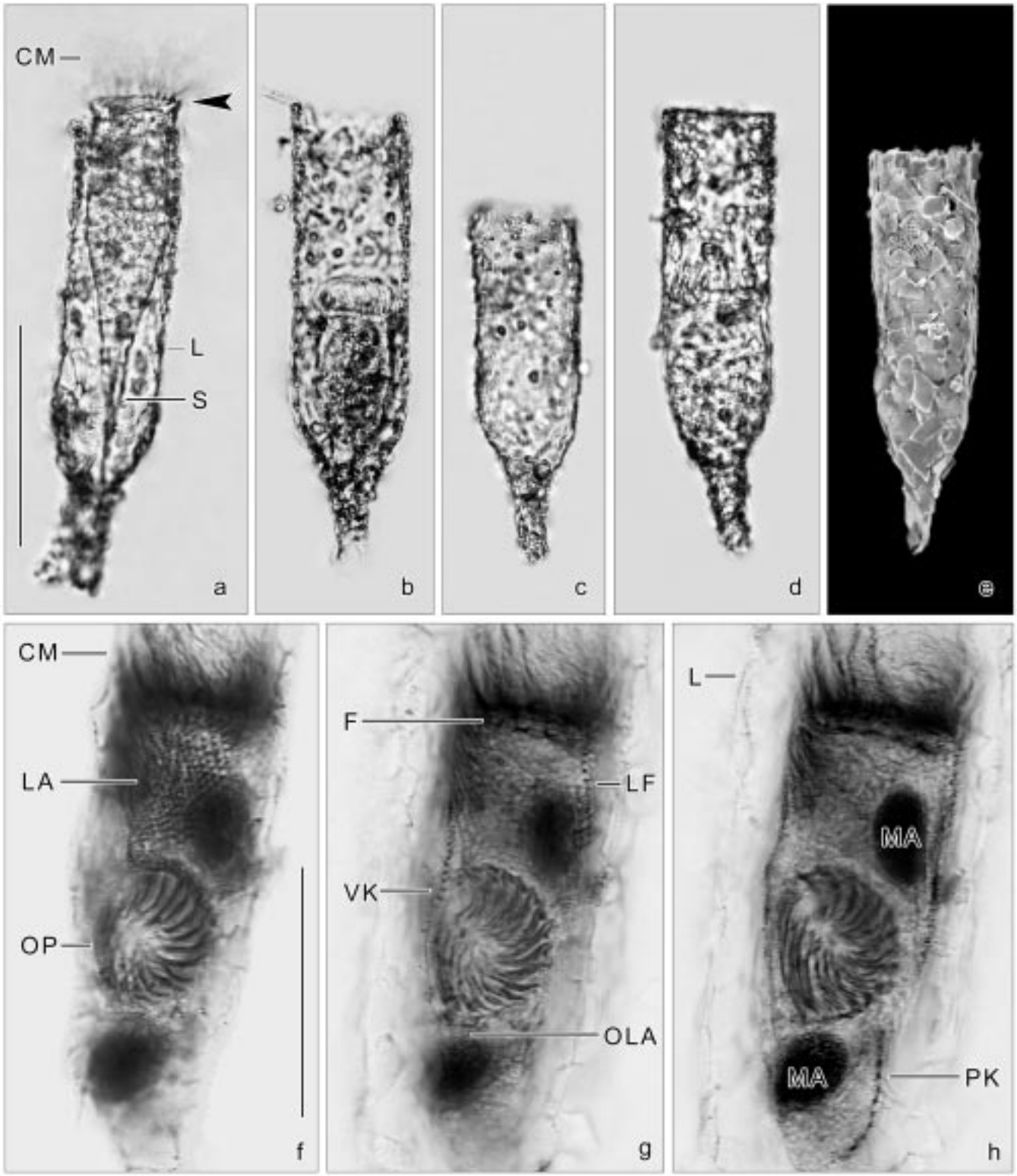
General pattern of somatic ciliature as described in 'Terminology' (Figs 1b, c, f). Length of kineties and number of kinetids usually highly variable possibly due to basal body proliferation or resorption in postdividers (see below; Table 1). Ventral kinety commences anterior to second or third kinety of right ciliary field, performs



Figs 2a-e. *Tintinnopsis cylindrica* after protargol impregnation. **a, b -** ventral views of middle dividers showing the oral primordium in a subsurface pouch posterior to the proter's lateral ciliary field whose kineties are distinctly longer than in morphostatic specimens (cp. Figs 1b, c, f). Note the replication bands in the macronuclear nodules (b). Arrow marks the single buccal membranelle of the opisthe. Arrowhead denotes the short endoral membrane of the opisthe; **c-e -** lateral views of specimens in successive stages of dinoflagellate infection. The dinoflagellate completely sucks out the tintinnid cell (c, d), before it obtains the sporogenetic stage (e). Note the morphologic changes of the macronuclear nodules due to the infection (c, d). D - parasitic dinoflagellate (probably *Duboscquella* sp.), LA - proter's lateral ciliary field, LF - left ciliary field, MA - macronuclear nodules, MI - micronuclei, OLA - opisthe's lateral ciliary field, OP - oral primordium, RF - right ciliary field, S - contracted stalk, VK - ventral kinety. Scale bars: 50 µm.

distinct leftward curvature, terminating near anterior end of posterior kinety, composed of densely spaced monokinetids; cilia decrease in length from ~ 11 µm in anterior portion to 3-4 µm in posterior. Kineties of right ciliary field composed of monokinetids and one anterior dikinetid, except for its first and second kinety that probably have two dikinetids anteriorly; cilia 2-4 µm long, except for the 9-11 µm long anteriormost dikinetidal cilia (soies, Fauré-Fremiet 1924), often apparently absent in posteriormost kinetids. Dorsal kinety commences at same level as right and left ciliary field and curves leftwards to base of stalk; cilia ~ 10 µm long and only at each posterior dikinetidal basal body. Posterior kinety commences in second quarter of cell posterior to last or penultimate kinety of lateral ciliary field and curves rightwards to base of stalk; cilia 6-7 µm long and only at each posterior dikinetidal basal body. Kineties of left ciliary field composed of monokinetids and one anterior dikinetid, almost gradually elongated from left to right; cilia 2-4 µm long, except for the 9-11 µm long anteriormost dikinetidal cilia (soies, Fauré-Fremiet 1924), often apparently absent in posteriormost kinetids. Kineties of lateral ciliary field commence ~ 1 µm anterior to those of left and right ciliary field, densely spaced and slightly curved, except for the rightmost kinety that extends parallel to the distinctly curved ventral kinety, 8-10 µm long (n = 6), composed of closely spaced monokinetids with cilia 2-4 µm long. Longitudinal argyrophilic fibres connect kinetids in ciliary rows.

Oral apparatus occupies anterior cell portion, perpendicular to main cell axis. Collar membranelles form closed spiral on peristomial rim, 1-2 µm apart, composed of three rows of basal bodies with cilia up to 25-30 µm long (Figs 1a-c; 3a). Two argyrophilic fibre bundles extend from each collar membranelle rightwards and leftwards, merging into a horizontally orientated circular fibre underneath the membranellar zone. Another argyrophilic fibre bundle commences at the left half of the circular fibre and extends posteriorly, terminating between the posterior and dorsal kinety (Fig. 1c). Eccen-



Figs 3a-h. *Tintinnopsis cylindrica* from life (a-d), in the scanning electron microscope (e), and after protargol impregnation (f-h). **a-d -** lateral views showing lorica variability, concerning the degree of incrustrated particles and length of the cylindroidal portion. Accessory combs (arrowhead; a) are rarely recognizable; **e -** the lorica wall has many silt particles and fragments of diatom frustules incrustrated; **f-h -** same middle divider at three focal planes. The new oral apparatus develops in a subsurface pouch posterior to the proter's lateral ciliary field. CM - collar membranelles, F - probably fibrillar structures, L - lorica, LA - proter's lateral ciliary field, LF - left ciliary field, MA - macronuclear nodules, OLA - opisthe's lateral ciliary field, OP - oral primordium, PK - posterior kinety, S - stalk, VK - ventral kinety. Scale bars: 100 µm (a-e); 50 µm (f-h).

tric buccal cavity contains one buccal membranelle and the proximal portions of four elongated collar membranelles, each associated with a longitudinal argyrophilic fibre bundle extending to the posterior quarter of cell proper (Fig. 1b). Endoral membrane inconspicuous, as apparently short and restricted to the buccal cavity.

Ontogenesis. Only few sufficiently impregnated division stages were found in the preparations (Figs 2a, b; 3f-h). Stomatogenesis commences with the apokinetal development of a small, cuneate field of basal bodies posterior to the lateral ciliary field. The oral primordium sinks into a subsurface pouch and membranelles differentiate. The posterior portion of the oral primordium performs a distinct rightwards curvature until the opisthe's right side faces the proter's ventral side. The endoral membrane is apparently very short and entirely located in the buccal cavity. In middle dividers, the proter's ciliary fields are elongated compared to morphostatic specimens: the left field by ~ 80%, the right field by ~ 50%, and the lateral field by ~ 240%. In the opisthe, the right field is about one third shorter than in morphostatic specimens, whereas the lateral field is almost of same length; length of the left ciliary field is not recognizable. The ventral kinety curves along the lower right margin of the oral primordium. One replication band each traverses the macronuclear nodules. Only when the new oral apparatus evaginates, the two nodules fuse. Loricae embracing a resting cyst were not found.

DISCUSSION

Comparison of populations. The loricae from the type population are longer than those of the polder specimens (243 µm *vs.* 75-220 µm), while the oral diameter is almost identical (45 µm *vs.* 48 µm; Daday 1887). According to Laval-Peuto and Brownlee (1986), the oral diameter of a lorica is the least variable dimension and a taxonomically reliable character, whereas its length increases during lorica formation. Hence, the identification of the polder specimens with *Tintinnopsis cylindrica* is beyond reasonable doubt, especially, as Daday (1887) observed a similar number of collar membranelles and a similar position of the two macronuclear nodules. Schweyer (1909) recognized tentaculoids which were not found in the polder specimens; however, these organelles are probably contractile (Laval-Peuto 1994) and their absence should thus not be overestimated.

The literature data concerning the lorica dimensions of *T. cylindrica* and its synonyms fall into a size range of 105-300 × 33-60 µm (Brandt 1907; Okamura 1907; Laackmann 1913; Rossolimo 1922; Wailes 1925, 1943; Hada 1932a, c, 1937; Marshall 1934; Orsi 1936; Balech 1948, 1951; Biernacka 1948; Silva 1952; Cosper 1972; Gold and Morales 1975; Bakker and Phaff 1976; Kršinić 1980; Rampi and Zattera 1982; Stoecker *et al.* 1983; Yoo *et al.* 1988; Lipej 1992).

Comparison with similar species. There are several species and subspecies from marine and brackish waters which might be synonyms of *Tintinnopsis cylindrica*: *T. davidoffii* with two globular macronuclear nodules in posterior cell half; *T. davidoffii* var. *longicauda* and *T. curvicauda* with a curved lorica process (Daday 1887); *T. pseudocylindrica* with irregular aboral opening (Hada 1964); *T. fracta* with an obliquely truncate lorica process (Brandt 1906, 1907); *T. coronata* with an irregularly expanded oral lorica rim (Kofoid and Campbell 1929); *T. levigata* with a lorica 50-70 × 20-30 µm in size (Wailes 1925); *T. platensis* without agglutinated particles at the lorica process (Cunha and Fonseca 1917); *T. aperta* (Brandt 1906, 1907), *T. lindeni* (Daday 1887), *T. panamensis*, and *T. tocantinensis* (Kofoid and Campbell 1929) with a bulbous zone between the cylindroidal portion and the lorica process; *Codonella annulata* with an annulated lorica structure (Daday 1886); *C. radix* with a lorica up to 480 µm long (Imhof 1886); and *Tintinnus annulatus*, *T. helix* (Claparède and Lachmann 1859), and *T. fistularis* (Möbius 1887) with a spiralled lorica structure. The often very short descriptions of these taxa consider only or mainly lorica features, using small discrepancies in structure and shape for the establishment of a new species or variation. Although the formation of spiralled or annulated lorica structures is part of the life cycle in several tintinnids (paralorica and epilorica; Laval-Peuto 1981, 1994) and the lorica process might vary in shape and size due to environmental conditions, investigations of the cell morphology are required to justify a synonymization of the species mentioned above.

In only 16 tintinnid species, the main cytological features are known. The general ciliary pattern of *T. cylindrica* matches that of *Codonella cratera* (Foissner and Wilbert 1979), *Stenosemella lacustris* (Foissner and O'Donoghue 1990), *Codonellopsis glacialis*, *Cymatocylis calyciformis* (Petz *et al.* 1995), and the *Cymatocylis affinis/convallaria*-group (Wasik and Mikołajczyk 1994, Petz *et al.* 1995). Small and Lynn

(1985), Laval-Peuto and Brownlee (1986), and Laval-Peuto (1994) provided some original or modified illustrations from Brownlee's unpublished Master and Doctoral Theses, showing protargol-impregnated tintinnids with an apparently similar ciliary pattern: *Tintinnopsis baltica*, *T. subacuta*, *Stenosemella steini*, *Favella* sp., *Climacocylis scalaroides*, and *Protorhabdonella simplex*. Furthermore, the somatic ciliature of *Tintinnopsis rapa*, *T. fimbriata*, *T. tubulosoides*, *T. campanula*, *Helicostomella subulata*, and *Leprotintinnus pellucidus* are very much alike (own observ.). Further studies are required to elucidate whether the observed subtle differences in the structure, position, and curvature of the kineties are species- or genus-specific. On the other hand, the congener *Tintinnopsis cylindrata* resembles *Tintinnidium fluviatile* and *Tintinnidium pusillum* in the presence of ventral organelles and the absence of a dorsal kinety, a posterior kinety, and a lateral ciliary field (Foissner and Wilbert 1979). Nevertheless, its generic affiliation is not changed as the ciliary pattern of the type species of the genus *Tintinnopsis*, *T. beroidea*, is unknown.

Ontogenetic comparison. In only seven tintinnid species, ontogenesis was investigated after protargol impregnation: *Tintinnopsis* sp., *Favella* sp. (Brownlee 1983, Laval-Peuto 1994), *Tintinnopsis cylindrata*, *Tintinnidium pusillum*, *T. semiciliatum*, *Codonella cratera* (Petz and Foissner 1993), and *Cymatocylis convallaria* (Petz *et al.* 1995). *Tintinnopsis cylindrata* and the *Tintinnidium* species differ from *Tintinnopsis cylindrica* in the ciliary pattern (see above); hence, they are excluded from the following comparison.

The division stages of *T. cylindrica* match the observations on *Codonella cratera* (Petz and Foissner 1993), *Cymatocylis convallaria* (Petz *et al.* 1995), and apparently *Favella* sp. (Laval-Peuto 1994) very well in the position of the oral primordium. Likewise, the kineties of the proter are elongated and those of the opisthe are shortened compared to morphostatic specimens, indicating a resorption of basal bodies by the proter and a second round of basal body proliferation by the opisthe in late dividers or postdividers (this study, Brownlee 1983, Petz and Foissner 1993). Petz and Foissner (1993) assumed that an anteriorly elongated ventral kinety occurs only in dividers or postdividers, while we agree with Petz *et al.* (1995) in regarding it as the morphostatic state of the ciliary row.

Occurrence and ecology. The following compilation comprises merely records of *Tintinnopsis cylindrica* and the synonyms mentioned above. Note that only few of them were substantiated by morphometric data and/or illustrations (see 'Comparison of populations') and that the morphologic variability of the species is unknown; thus, misidentifications cannot be excluded.

Daday (1887) discovered *T. cylindrica* in the Gulf of Naples. In the Mediterranean Sea and adjacent brackish water lagoons, the species was also found by several other authors (Brandt 1906, 1907; Schweyer 1909; Laackmann 1913; Orsi 1936; Rampi 1939, 1948, 1950; Margalef and Morales 1960; Kršinić 1979, 1980, 1987; Rassoulzadegan 1979; Rampi and Zattera 1982; Lakkis and Novel-Lakkis 1985; Lipej 1992; Lam-Hoai *et al.* 1997; Rougier and Lam Hoai 1997; Ounissi and Frehi 1999; Lam-Hoai and Rougier 2001; Sabancý and Koray 2001; Modigh and Castaldo 2002; Moscatello *et al.* 2004; Balk's and Wasik 2005). Likewise, it was recorded in the North Atlantic (Wright 1907; Silva 1952; lorica of *T. cylindrica* less distinctly tapered, Cosper 1972; Gold and Morales 1975; Hargraves 1981; Stoecker *et al.* 1983; Verity 1986, 1987; Middlebrook *et al.* 1987; Sanders 1987; Gilron and Lynn 1989; Pilling *et al.* 1992; Leakey *et al.* 1993; Pierce and Turner 1994; Paulmier 1995; Tempelman and Agatha 1997; Urrutxurtu *et al.* 2003; Urrutxurtu 2004), South Atlantic (Balech 1948, 1951; Akselman and Santinelli 1989), Red Sea (Aboul-Ezz *et al.* 1995), Indian Ocean (Krishnamurthy and Santhanam 1978; Krishnamurthy *et al.* 1979, 1987; Damodara Naidu 1983; Damodara Naidu and Krishnamurthy 1985), North Pacific (Okamura 1907; Wailes 1925, 1943; Hada 1932a, b, c, 1937; Wang and Nie 1934; Konovalova and Rogachenko 1974; Yoo *et al.* 1988; Yoo and Kim 1990; Kamiyama and Aizawa 1990, 1992; Kamiyama and Tsujino 1996; Kamiyama 1997; Uye *et al.* 2000; Kamiyama *et al.* 2001; Kamiyama and Matsuyama 2005), and South Pacific (Brandt 1906, 1907; Marshall 1934; Burns 1983). *Tintinnopsis cylindrica* also occurred in the brackish waters of the Black (Rossolimo 1922) and Baltic Sea (Brandt 1906, 1907; *T. cylindrica* with exceptionally long lorica process, Biernacka 1948, 1968) and lagoons at the coast of the North Atlantic (this study, Bakker and Pauw 1975, Bakker and Phaff 1976, Bakker 1978, Agatha and Riedel-Lorjé 1997, Riedel-Lorjé *et al.* 1998), North Pacific (Hada 1937, Godhantaraman and Uye 2003), and Indian Ocean (Godhantaraman 2001, 2002). Accordingly, records substantiated by morphometric data are available from marine and brackish waters of subarctic, temperate, subtropical, and equatorial areas, while the species was apparently not found in polar regions. The spatial distribution of *T. cylindrica* might, however,

change when further species or subspecies will definitely be synonymized and the morphologic variability of the species is better known.

In the polder basins, *Tintinnopsis cylindrica* was mainly recorded at salinities higher than 10‰ and temperatures above 8°C, that is, mostly during summer. It occasionally dominated the tintinnid community and had maximum abundances of ~ 4,400 individuals per litre in September 1991 and July 1992 in the mixo-polyhaline basins of the Beltringharder Koog and Speicherkoog Dithmarschen. The seasonal dynamics observed in the polder basins match the findings from other regions (Hada 1937; Bakker and Pauw 1975; Bakker and Phaff 1976; Bakker 1978; Hargraves 1981; Sanders 1987; Verity 1987; Leakey *et al.* 1993; Pierce and Turner 1994; Kamiyama and Tsujino 1996; Godhantaraman 2001, 2002).

Specimens infested by a parasitic dinoflagellate, probably *Duboscquella* sp., were occasionally found (Figs 2c-e). Hada (1932a) as well as Akselman and Santinelli (1989) described similar infection stages in *Tintinnopsis kofoidi*, whereas the infection was interpreted as sexual reproduction in the possibly synonymous species *Tintinnus helix* (Laackmann 1907). While the infection rate was apparently low in the polder basins, *Duboscquella* sp. significantly decimated ciliate stocks at the northeast coast of the USA (Coats and Heisler 1989).

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ACTA
PROTOZOOLOGICA

Redescriptions of Three Cyrtophorid Ciliates from Marine Biofilm, with Establishment of a New Genus, *Wilbertella* nov. gen. (Ciliophora: Cyrtophorida: Lynchellidae)

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Summary. The morphology of live cells and the infraciliature of three marine cyrtophorid ciliates, *Chlamydonellopsis calkinsi* (Kahl, 1928) Blatterer *et* Foissner, 1990, *Chlamydonella pseudochilodon* (Deroux, 1970) Petz, Song *et* Wilbert, 1995 and *Chlamydonella derouxi* Song, 2003, collected from the coastal water off Qingdao, China, were investigated. An improved diagnosis of the little-known *C. calkinsi* is suggested: marine *Chlamydonellopsis* with asymmetrical oval body shape, 35-70 × 20-40 µm *in vivo*, two rows of club-shaped protuberances on ventral side; 15-18 somatic kineties; two contractile vacuoles diagonally positioned; 12-15 nematodesmal rods, two micronuclei. Based on the Qingdao populations and previous descriptions, redefined diagnoses of *C. pseudochilodon* and *C. derouxi* are also supplied. A new genus *Wilbertella* nov. gen. is suggested: Lynchellidae with a distinct blank zone in left posterior of cell; perioral kineties in flattened Y-shaped; left kineties distinctly shortened, right kineties extending to posterior end; no distinct gap between left and right ciliary field. Two nominal species are transferred into this new genus: *W. distyla* (Wilbert, 1971) nov. comb. [basionym: *Parachilodonella distyla* Wilbert, 1971] and *W. stricta* (Deroux, 1976) nov. comb. [basionym: *Chlamydonella stricta* Deroux, 1976].

Key words: *Chlamydonellopsis*, *Chlamydonella*, morphology and infraciliature, Lynchellidae, *Wilbertella* nov. gen.

INTRODUCTION

Cyrtophorid ciliates are commonly found in aquatic biofilms or periphytons on a variety of immersed surfaces such as on stones (Cairns and Yongue 1968, Foissner *et al*. 1992), macrophytes (Baldock *et al*. 1983, Gismervik 2004) and artificial substrates (Deroux 1970, Gong *et al*. 2005a). In the 1970s, using glass slides as artificial substrate and silver staining methods, Deroux investigated extensively more than 50 cyrtophorid species from French coast off the Atlantic, contributing greatly to the taxonomy and biogeography of these taxa (Deroux 1970, 1976).

More recently, during surveys of ciliate fauna of the Yellow Sea, north China, we isolated diverse cyrtophorids from marine biofilms. Many of these ciliates were found to be new or little known (Gong and Song 2003, 2004 a, b, c, 2006; Gong *et al*. 2002, 2003, 2005b). This paper describes three lynchellid species from the Jiaozhou Bay off Qingdao, and suggests a new genus, *Wilbertella* nov. gen.

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MATERIALS AND METHODS

Glass slides as artificial substrate were submerged in the marine water at a depth of 1 m allowing microbial colonization. After 15 days' exposure, glass slides with developed biofilm were transported to a jar with seawater from the sample sites. Specimens were transferred with a micropipette and then maintained in the laboratory for about 4 days in Petri dishes with adding several rice grains to support microbial growth.

The populations of *Chlamydonellopsis calkinsi* (Kahl, 1928) Blatterer *et* Foissner, 1990 and *Chlamydonella derouxi* Song, 2003 were collected in August 2002, from an open abalone culturing pond near Qingdao. Isolation of *Chlamydonella pseudochilodon* (Deroux, 1970) Petz, Song *et* Wilbert, 1995 was made from a scallop farming water in the Jiaozhou Bay, Qingdao.

Live cells were observed with differential interference contrast microscopy and their infraciliature was revealed using the protargol impregnation method according to Wilbert (1975). The Chatton-Lwoff silver nitrate method was used to reveal the silverline system (Song and Wilbert 1995). Live individuals were examined and measured at 1,000× magnification; counts, measurements and drawings of stained specimens were performed at 1,250× with the aid of a camera lucida.

Terminology and taxonomic scheme are according to Deroux (1976), Gong *et al.* (2002) and Corliss (1979), respectively.

Protargol impregnated voucher slides of three species are deposited in the Laboratory of Protozoology, OUC, China, with the following registration numbers: *Chlamydonellopsis calkinsi*, G020810011; *Chlamydonella pseudochilodon*, G001122012; *Chlamydonella derouxi*, G03081031.

RESULTS AND DISCUSSION

Redescription of *Chlamydonellopsis calkinsi* (Kahl, 1928) Blatterer *et* Foissner, 1990 (Figs 1, 2; Tables 1, 2)

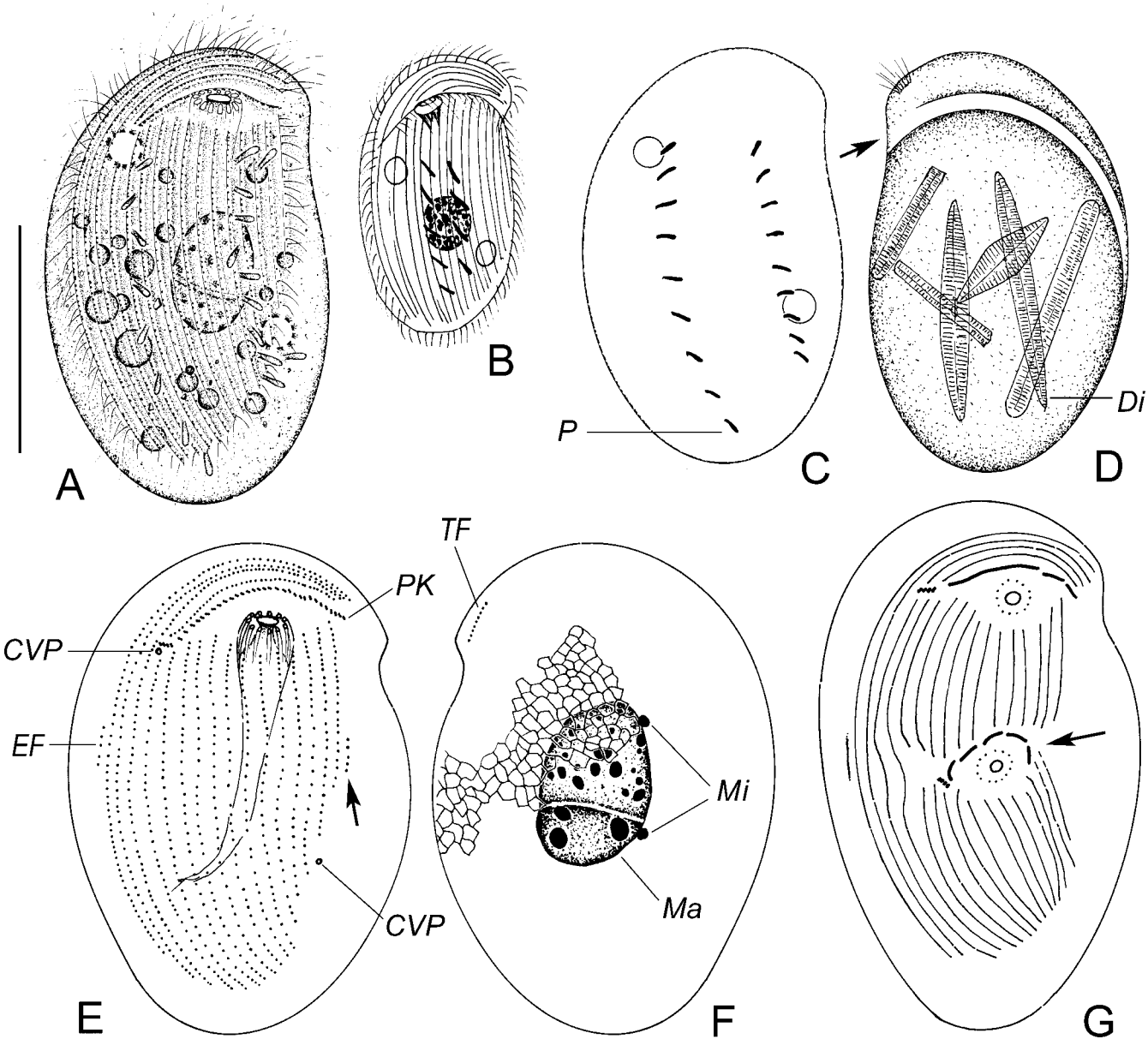
Improved diagnosis. Marine *Chlamydonellopsis* with asymmetrical oval body outline, size 35-70 × 20-40 µm *in vivo*; 15-18 somatic kineties, 12-15 nematodesmal rods; two contractile vacuoles diagonally positioned; two rows of club-shaped protuberances on ventral surface; two micronuclei.

Description. Size 35-70 × 20-40 µm *in vivo*, usually 60 × 35 µm (Table 1). Body reniform to oval in outline, with anterior left end inconspicuously prominent; both ends broadly rounded (Figs 1A, C-F; 2A). Dorsoventrally flattened, ratio of width to thickness ~ 2:1. Ventral side flat, dorsal side hunched, with an arched depression at anterior 1/6 of body length (arrows, Figs 1D, 2D). Cytostome prominent, ~5 µm in diameter, sub-apically located. Cyrtos extending into endoplasm slightly left-posteriorly. Endoplasm colorless, containing several large globular granules (2-5 µm in diameter) and numerous tiny particles (across < 1 µm); many rod- and shuttle-like ingested diatoms (Di) frequently observed (Fig. 1D). Macronucleus ovoid, positioned near body center. About 18 immobile club-shaped protuberances (P) on ventral surface, each 3 µm long and 0.8 µm in diameter, arranged in two longitudinal rows (Figs 1A, C; arrows in Fig. 2C). Two contractile vacuoles (arrows in Fig. 2B), each ~ 4 µm in diameter, diagonally located, pulsing interval 2 mins on average. Cilia 8 µm long *in vivo*. Movement slow, gliding on substrates, sometimes swimming with rotation.

A total of 15-18 somatic kineties on ventral side (see Table 1). The rightmost four kineties almost extending over whole body length, with anterior portion transversely arched and anterior of oral field; usually the inner one or two rows interrupted by perioral kinety (PK). Other kineties terminating anteriorly below cytostome level; the leftmost 5-6 rows slightly progressively shortened from right to left. One terminal fragment (TF) consisting of about 10 basal bodies, dorso-anteriorly positioned (Figs 1F, 2G). Usually two short equatorial fragments (EF), one on right and one on left margin (arrow in Fig. 1E) of ciliary field, composed of 2-14 and 0-12 unciliated kinetosomes, respectively (Figs 1E; 2E, I). Macronucleus (Ma) oval, positioned in body center; usually two micronuclei (Mi) adjacent to macronucleus, each ~ 2 ìm in diameter. Mostly two contractile vacuole pores (CVP) recognizable in protargol impregnated specimens, anterior one between kinety 3 and 4 from right, posterior one near the end of kinety 2 or 3 from left (Figs 1E, 2F).

Oral structure typical of genus: one perioral kinety basically transversely arched and continuous, composed of ~ 30 dikinetids, with the rightmost 3-4 basal body pairs slightly detached (Figs 1E, 2E). Perioral kinety in opisthe are formed by ~ 6 short segments of kineties, which can be detected at late stages of morphogenetic process (Figs 1G, 2J). Cyrtos composed of 12-15 nematodesmal rods, extending ~ 60% of cell length. Silverline system irregularly reticulate (Figs 1F; 2K, H).

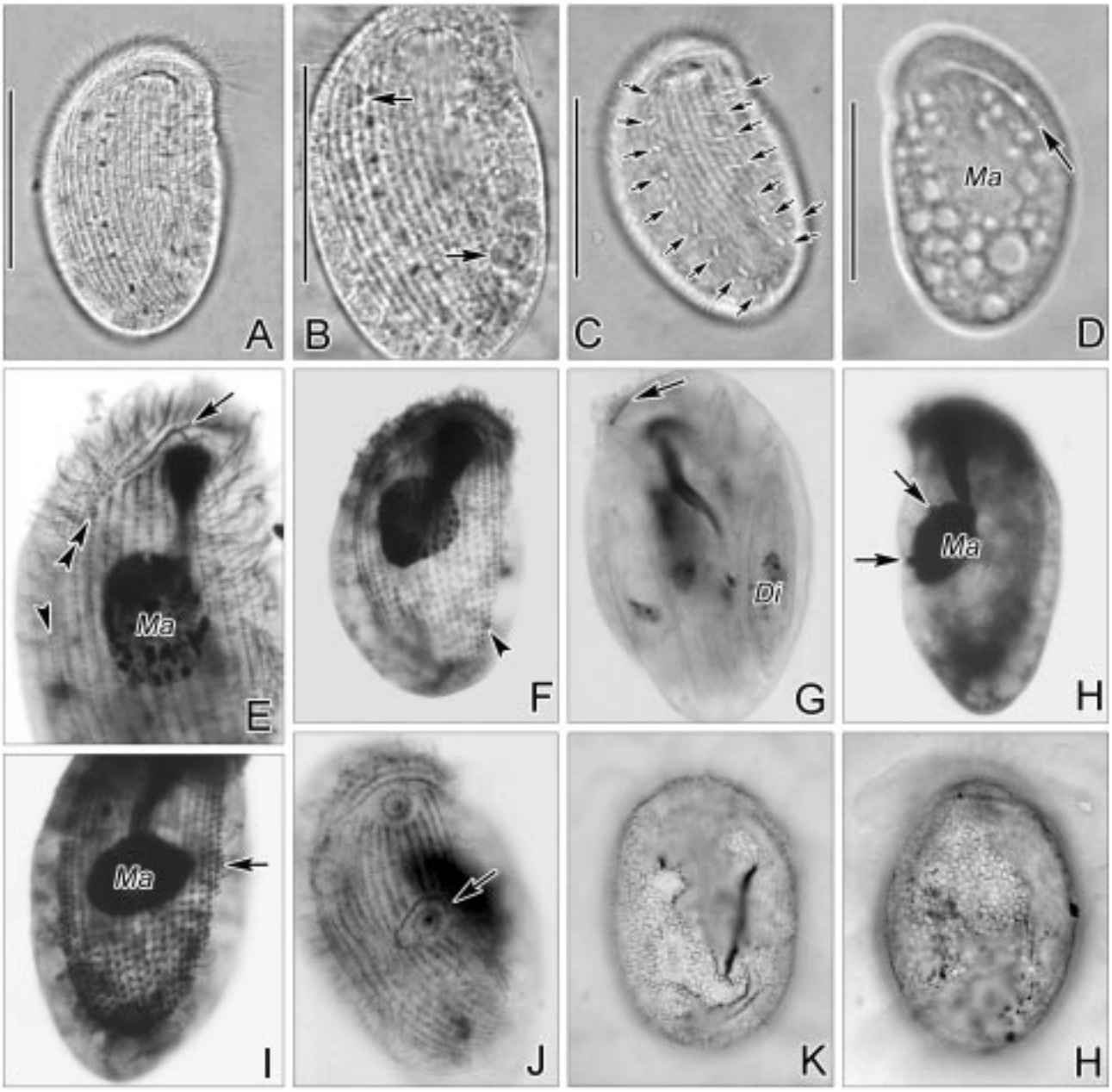
Remarks. Kahl described two new *Chilodon* species in 1928: *C. calkinsi* and *C. pediculatus*, which both were emended as members of the genus *Chilodonella* Strand, 1926 because the generic name *Chilodon* Ehrenberg, 1834 had been synonymized with the latter (Kahl 1931, Aescht 2001). Meanwhile, Kahl (1931) regarded *Chilodonella pediculatus* as a synonym of *Chilodonella calkinsi*. This revision was obviously accepted by Blatterer and Foissner (1990), who trans-



Figs 1A-G. Morphology and infraciliature of *Chlamydonellopsis calkinsi* from life (A, C, D, original; B, after Kahl 1931), after protargol (E-G) and Chatton-Lwoff impregnation (F). **A, B** - ventral views; **C, D** - showing the ventral club-shaped protuberances (C) and dorsal view (D), arrow indicates the arched depression; **E, F** - ventral (E) and dorsal (F) views of infraciliature and a part of silverline system; arrow marks the left equatorial fragment; **G** - an individual in morphogenesis, to note the fragmented perioral kinety (arrow) in opisthe. CVP - contractile vacuole pore; Di - diatom; EF - equatorial fragment; Ma - macronucleus; Mi - micronuclei; P - club-shaped protuberances; PK - perioral kinety; TF - terminal fragment. Scale bar: 30 µm.

ferred *Chilodonella calkinsi* Kahl, 1928 into their newly erected genus *Chlamydonellopsis* Blatterer *et* Foissner, 1990.

According to the original descriptions by Kahl (1928), *Chilodon pediculatus* is very similar to *Chilodon calkinsi* in terms of the body shape, size, position of terminal fragment and marine habitat. As for ventral protuberances, Kahl (1928) gave no information for the latter, but stated that there were 14-15 ventral protuberances in the former. Despite this difference, we believe



Figs 2A-H. Photomicrographs of *Chlamydonellopsis calkinsi* from life (A-D), after protargol (E-J) and Chatton-Lwoff impregnation (K, H). **A-C** - ventral views, focusing on cytostome and ciliary rows (A), contractile vacuoles (B, arrows) and two rows of club-shaped protuberances (C, arrows); **D** - dorsal view, arrow marks the arched depression on dorsal surface; **E** - ventral view of the anterior portion, to note the perioral kinety (arrow), anterior contractile vacuole pore (double-arrowheads) and left equatorial fragment (arrowhead); **F** - to note the posterior contractile vacuole pore (arrowhead); **G** - arrow indicates the terminal fragment; **H** - showing the two micronuclei (arrows) adjacent to macronucleus. **I** - arrow refers to the left equatorial fragment; **J** - infraciliature of an individual in morphogenesis, arrow indicates the segmented perioral kinety in opisthe; **K, L** - dorsal views of silverline system. Di - diatom; Ma - macronucleus. Scale bars: 30 µm.

that the synonymization of these two taxa should be reasonable because, as Kahl (1931) mentioned, the protuberances can be easily overlooked during living observation. The redescription of *C. calkinsi* by Kahl (1931) is thus virtually authoritative for species identification: oval body shape and size (40-60 µm), having ~ 15 ciliary rows, two rows of club-shaped protuberances on ventral side, two diagonally positioned

Table 1. Morphometric characteristics of *Chlamydonellopsis calkinsi* (upper line), *Chlamydonella pseudochilodon* (middle line) and *Chlamydonella derouxi* (lower line). Data from protargol impregnated specimens; measurements in µm. CV - coefficient of variation in %, Max - maximum, Mean - arithmetic mean, Min - minimum, n - number of individuals examined, SD - standard deviation.

Characters	Min	Max	Mean	SD	CV	n
Body length	38	70	54.7	7.2	13.2	34
	49	67	59.1	5.7	9.7	21
	17	29	22	3.3	15.0	20
Body width	24	40	32.1	4.3	13.4	34
	38	51	45.1	4.8	10.7	21
	12	19	15.1	2.7	17.9	20
Number of somatic kineties	15	18	16.5	0.8	4.9	34
	18	19	18.8	0.4	2.1	21
	12	13	12.1	0.3	2.5	20
Number of preoral kineties	4	4	4	0	0	34
	4	4	4	0	0	21
	4	4	4	0	0	20
Number of nematodesmal rods	12	15	13.4	0.8	6.0	12
	16	21	17.8	1.4	7.7	21
	11	13	12.3	0.8	6.5	20
Number of basal bodies in terminal fragment	6	12	9.3	1.9	20.4	18
	7	10	8.6	0.8	9.5	21
	4	6	4.4	0.6	13.6	20
Number of basal bodies in right equatorial fragment	2	14	6.8	3.7	54.4	34
	0	13	6.8	4.8	69.8	21
	0	4	1.4	1.0	71.4	16
Number of basal bodies in left equatorial fragment	0	12	5.8	3.8	65.5	34
	0	14	7.4	4.0	54.1	21
	0	3	2.1	1.1	52.4	9
Length of macronucleus	13	24	17.9	2.5	14.0	18
	15	34	25.9	4.9	18.9	21
	6	12	8.2	1.6	19.5	18
Width of macronucleus	6	16	10.7	2.6	24.3	18
	6	10	7.8	1.3	16.2	21
	4	9	5.8	1.1	19.0	20
Number of micronuclei	1	2	1.8	0.5	27.8	12
	1	6	4.0	2.5	62.5	21
	1	1	1	0	0	14
Number of contractile vacuole pores	2	3	2.1	0.3	14.3	18
	2	2	2	0	0	21
	2	2	2	0	0	5

contractile vacuoles and marine habitat (Kahl 1931; Fig. 1B). Our organisms match well the authoritative description by Kahl (1931) and the infraciliature-based diagnosis for the genus *Chlamydonellopsis* by Blatterer and Foissner (1990), so that the identification is beyond doubt.

Comparison with related species. *Chlamydonellopsis calkinsi* (Kahl, 1928) has two congeners: *C. plurivacuolata* Blatterer *et* Foissner, 1990 and *C. polonica* (Foissner, Czapik *et* Wiackowski, 1981) Blatterer *et* Foissner, 1990, which are both freshwater species (Blatterer and Foissner 1990, Foissner *et al.* 1991). Morphologically, *Chlamydonellopsis calkinsi* differs from the type species *C. plurivacuolata* in the numbers of somatic kineties (15-17 *vs.* 19-23), preoral kineties (4 *vs.* 5-8), nematodesmal rods (12-15 *vs.* 17-20), contractile vacuoles (2 *vs.* 4), ventral protuberances (*ca* 18 *vs. ca* 10) and macronuclear size (18 × 11 *vs.* 27 × 16 µm) (Foissner *et al.* 1991; Table 2).

Chlamydonellopsis calkinsi is similar to *C. polonica* with respect to the body shape and size, macronuclear size, number and position of contractile vacuoles (Foissner *et al.* 1981; Table 2). However, the former can be recognized by having fewer somatic kineties (15-17 *vs.*

18-20), preoral kineties (4 *vs*. 6), nematodesmal rods (12-15 *vs*. 16-18) and the biotopes (marine *vs*. freshwater).

Redescription of *Chlamydonella pseudochilodon* (Deroux, 1970) Petz Song *et* Wilbert, 1995 (Figs 3, 4; Table 1)

The previously invalid genus *Chlamydonella*, originally established by Deroux (1970), has been reactivated according to ICZN (1999) with the designation of *Chlamydonella pseudochilodon* as the type (Petz *et al*. 1995, Aescht 2001). To authors' knowledge, however, the type species has never been clearly defined.

Improved diagnosis: Marine *Chlamydonella* 30-75 × 20-50 μm *in vivo*, asymmetrically oval to reniform in outline; ~ 14-20 somatic kineties, of which 4 rows extend apically; 11-21 nematodesmal rods; two contractile vacuoles diagonally positioned; one to several micronuclei.

Description of the Qingdao population: Size 50-70 × 35-50 μm *in vivo*, usually 60 × 45 μm. Body oval in outline, with inconspicuous indentation on anterior left; both ends rounded, left margin roughly straight, right convex (Figs 3A, B). Conspicuously dorsoventrally flattened, ratio of width to thickness ~ 4:1. Ventral side flat, dorsal slightly vaulted. Pellicle robust and flexible. Cytoplasm colourless and hyaline with posterior portion slightly grayish, usually containing several ingested diatoms and numerous tiny granules (~ 1 μm across). Cytostome (Cs) prominent, located slightly left of median and at anterior 1/5 of body length, diagonally oriented, ~12 μm long *in vivo*. Cyrtos (Cy) composed of 16-21 nematodesmal rods (Figs 3C, 4B). Macronucleus longitudinally positioned, slightly left of midline; size about 26 × 8 μm after protargol impregnation, containing differently-sized, globular to elongated nucleoli, usually with central portion more heavily impregnated than others (Figs 3D, 4D). One to six (on average 4) globular micronuclei, each 1.5-3 μm in diameter, mostly arranged left of macronucleus (Figs 3D; 4E, F). Two contractile vacuoles (CV), typically diagonally positioned (Figs 3A, B); two contractile vacuole pores (CVP) symmetrically positioned between kinety 3 and 4, from outer to inner (Figs 3C; 4G, H). Club-shaped protuberances (P) mostly arranged near perimeter of ventral surface, each 2-3 μm long (Fig. 3B). Cilia ~5 μm long *in vivo*. Movement slow, gliding on substrate; highly thigmotactic, attaching to substrate firmly when stimulated.

Somatic kineties ~17 rows (Figs 3C, 4A; Table 1). Four frontoventral kineties, of which the outermost one is loosely ciliated, especially in its anterior portion (Fig. 3D; arrowheads in Fig. 4B), and the innermost one is bisected by perioral kinety near the anterior contractile vacuole pore (Figs 3C, 4B). Other kineties slightly C-shaped, terminating anteriorly at about cytostome level and posteriorly at body margin. Basal bodies generally densely spaced; no distinct sparsely-ciliated zone (= zone de raréfaction cinétosomienne, Deroux 1970) recognizable. Terminal fragment (TF) consisting of *ca* 9 basal bodies, dorsally positioned on left-anterior end of cell (Fig. 3D; arrow in Fig. 4C); right (REF) and left equatorial fragment (LEF, arrow in Fig. 3C) composed of ~7 and 4 basal bodies, respectively. Several leftmost somatic kineties join to form the oral primordium during stomatogenesis (Fig. 4I).

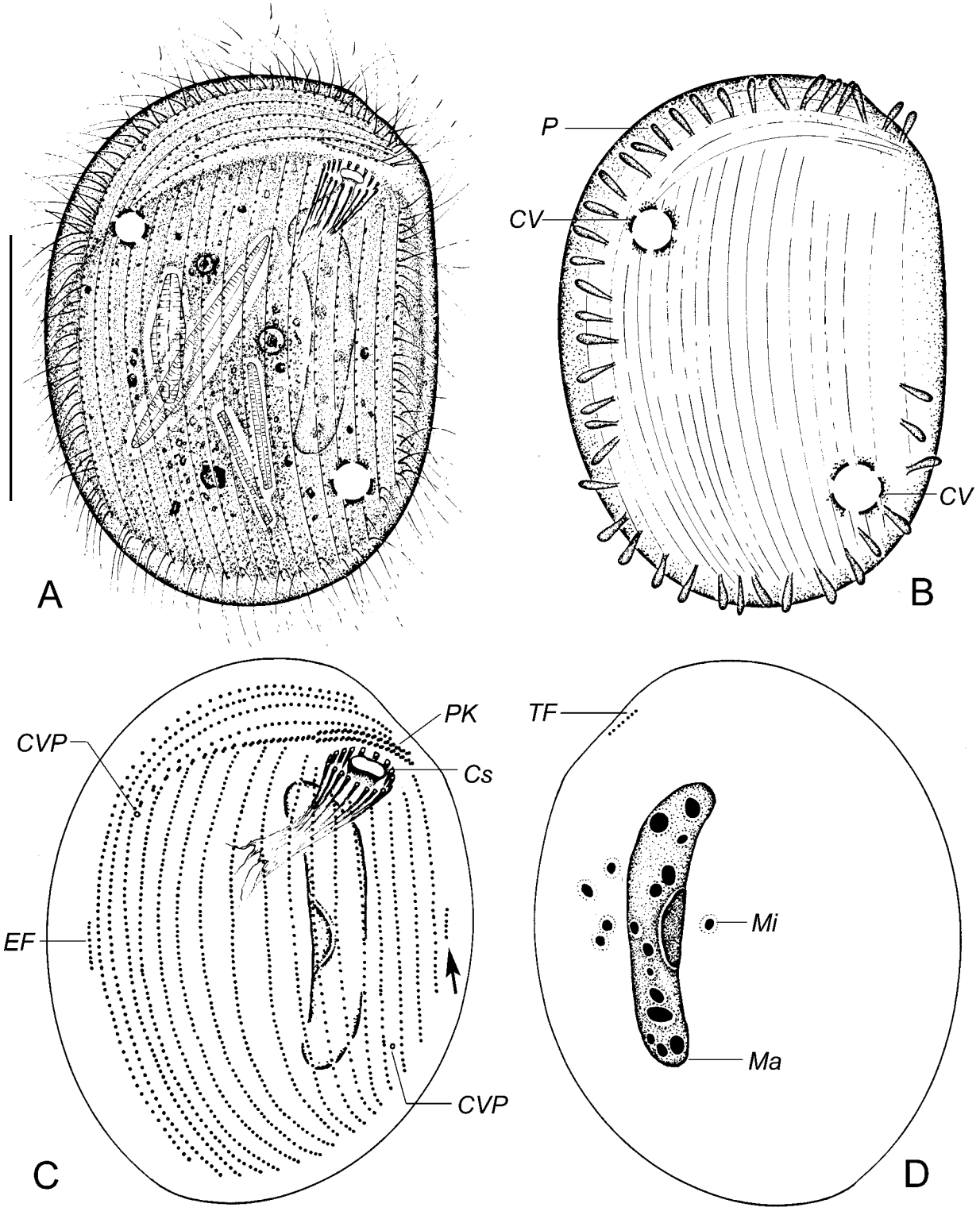
Oral structure typical of genus: Y-shaped perioral kineties (2 short arms on left and 1 long on right), completely composed of dikinetids (Fig. 3C; arrow in Fig. 4B).

Remarks: Considering the body shape and size, the infraciliature and the number/position of contractile vacuoles, the Qingdao population basically corresponds with the original description by Deroux (1970) and the redescription by Petz *et al.* (1995). The differences are the shape (elongate *vs.* oval or ellipsoidal) and the size (see Table 3) of the macronucleus, which is basically considered to be an intra-species feature as to authors' knowledge. In addition, there are moderately large coefficients of variations for macronuclear length (18.9%) and width (16.2%) in the Qingdao population (Table 1), indicating the unreliability of merely using macronuclear shape/size for taxa separation at species levels.

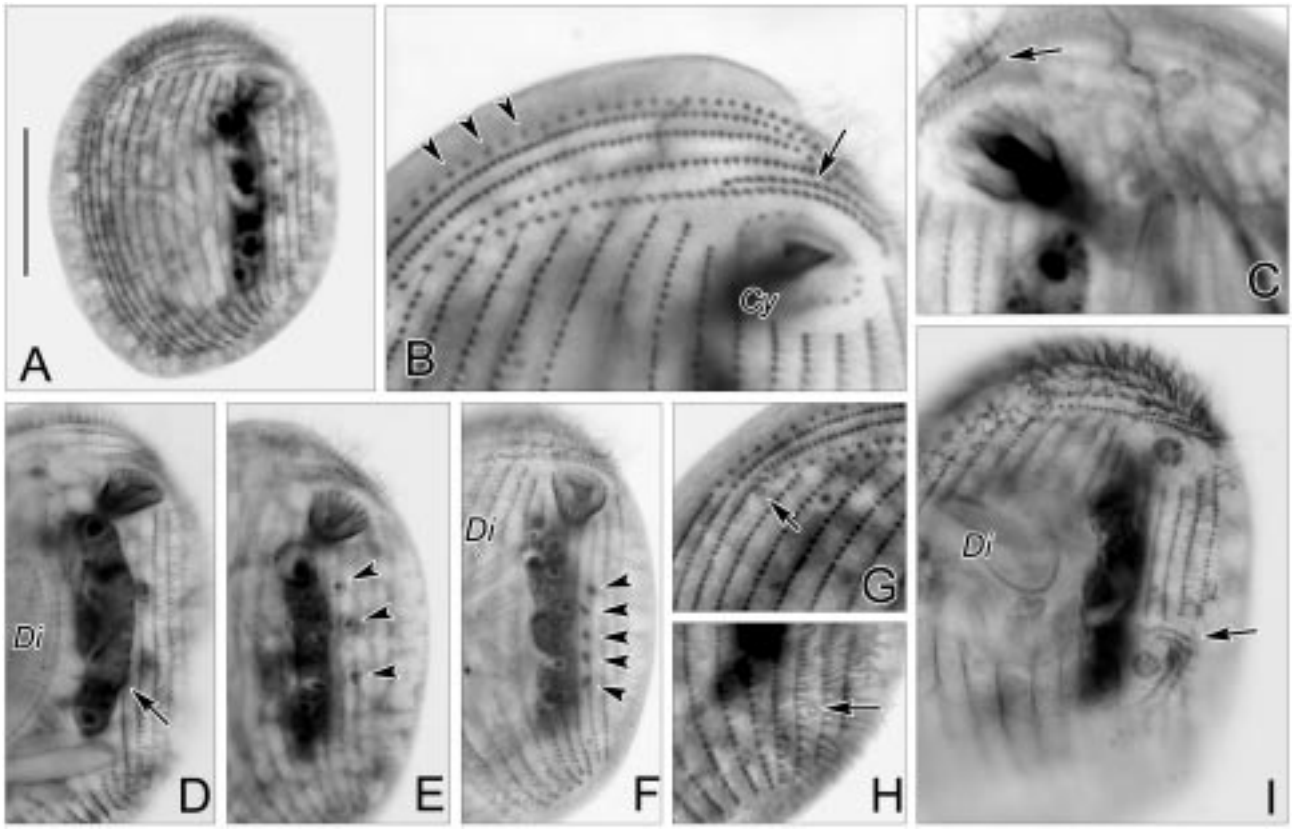
As for the feature of ventral protuberances, our observation is basically in accordance with Deroux's (1970). While Petz *et al.* (1995) did not mention these organelles, which could be overlooked during observations of live cells. Nevertheless, we identify our population mainly based on the infraciliature.

Redescription of *Chlamydonella derouxi* Song, 2003 (Figs 5A-C, 6; Table 1)

This population corresponds perfectly with the original description in living morphology (body size and shape, number of nematodesmal rods, and position of contractile vacuoles) and infraciliature (numbers of somatic kineties and preoral kineties) (Song 2003; Figs 5A-C, 6A-F; Table 1), by contrast, the numbers of (i) club-shaped protuberances on ventral side (1 *vs*. 3); (ii) basal bodies in equatorial fragments (1 *vs*. 1-4); and (iii) micronuclei (2 *vs*. 1) are different. Thus, we provide



Figs 3A-D. Morphology and infraciliature of the Qingdao population of *Chlamydonella pseudochilodon* from life (A-B) and after protargol impregnation (C, D). **A** - ventral view. **B** - showing the club-shaped protuberances; **C, D** - ventral (C) and dorsal (D) infraciliature. Cs - cytostome; CV - contractile vacuole; CVP - contractile vacuole pore; EF - equatorial fragment; Ma - macronucleus; Mi - micronuclei; P - club-shaped protuberances; PK - perioral kinety; TF - terminal fragment. Scale bar 30 µm.



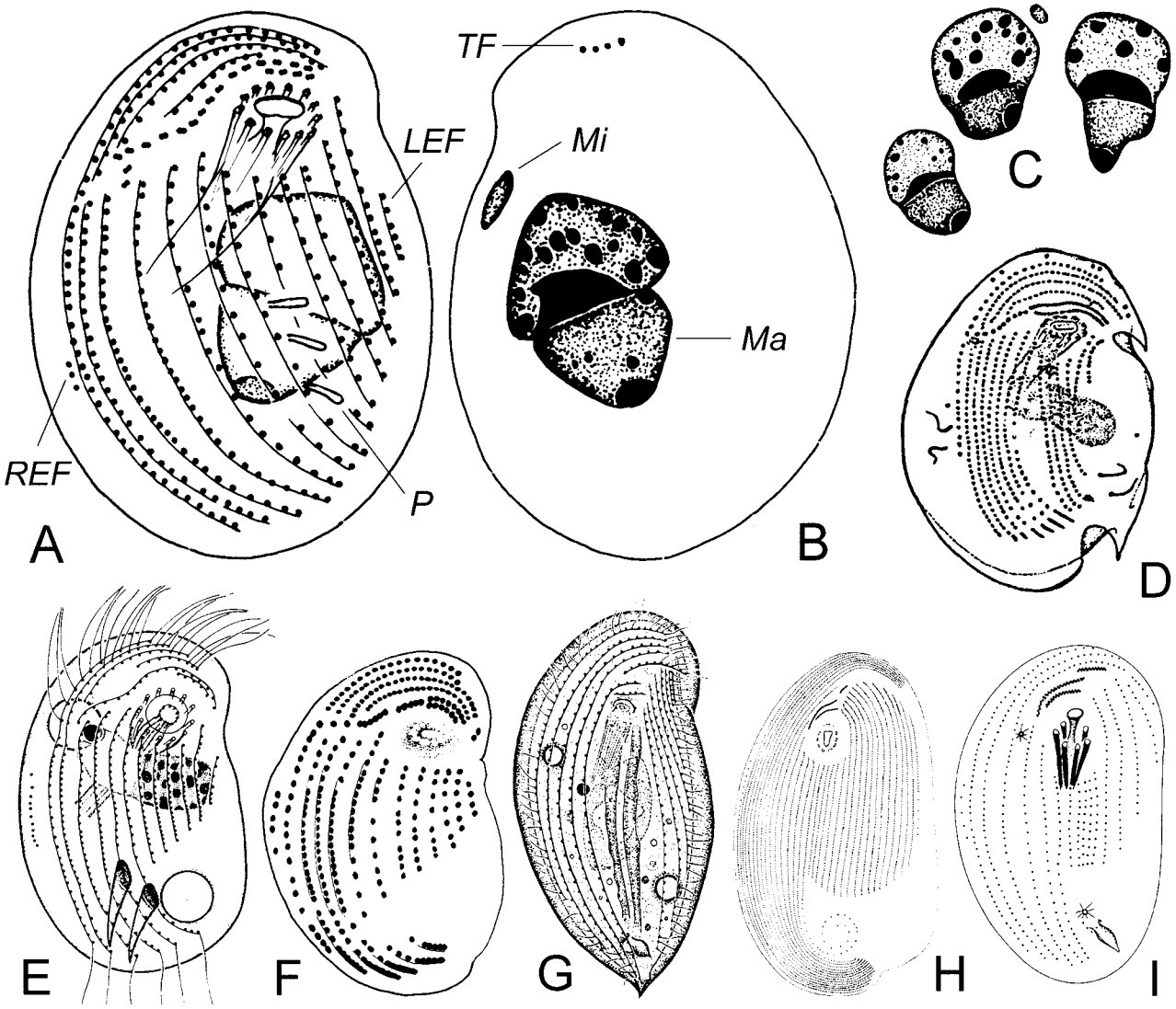
Figs 4A-I. Photomicrographs of *Chlamydonella pseudochilodon* after protargol impregnation. **A** - ventral view; **B** - oral field, arrow marks the Y-shaped perioral kineties, arrowheads indicate the loosely spaced basal bodies in the rightmost kinety; **C** - showing the terminal fragment (arrow); **D-F** - to note the macronucleus (arrow) and micronuclei (arrowheads); **G, H** - the right anterior (G) and left posterior (H) contractile vacuole pores; **I** - a stage of morphogenesis, arrow indicates the oral primordium for the opisthe. Cy - cyrtos; Di - diatom. Scale bar 30 µm.

photomicrographs and suggest an improved definition for this species.

Improved diagnosis: Small marine *Chlamydonella* about 20-30 × 12-20 µm *in vivo*, body shape asymmetrical oval in outline; with one to several club-shaped protuberances on ventral surface, located slightly left of median and posterior portion of cell; ~12 somatic kineties and 12 nematodesmal rods; two contractile vacuoles diagonally positioned.

Remarks: Two nominal species *Parachilodonella distyla* Wilbert, 1971 and *Chlamydonella stricta* Deroux, 1976 resemble *C. derouxi* Song, 2003 in terms of the body size (*ca* 20-30 µm in length) and the basic ciliary pattern (~12 somatic and 4 preoral kineties; Wilbert 1971, Deroux 1976), whereas the distinctness of these three taxa has not been explicitly discussed. We will review this matter briefly.

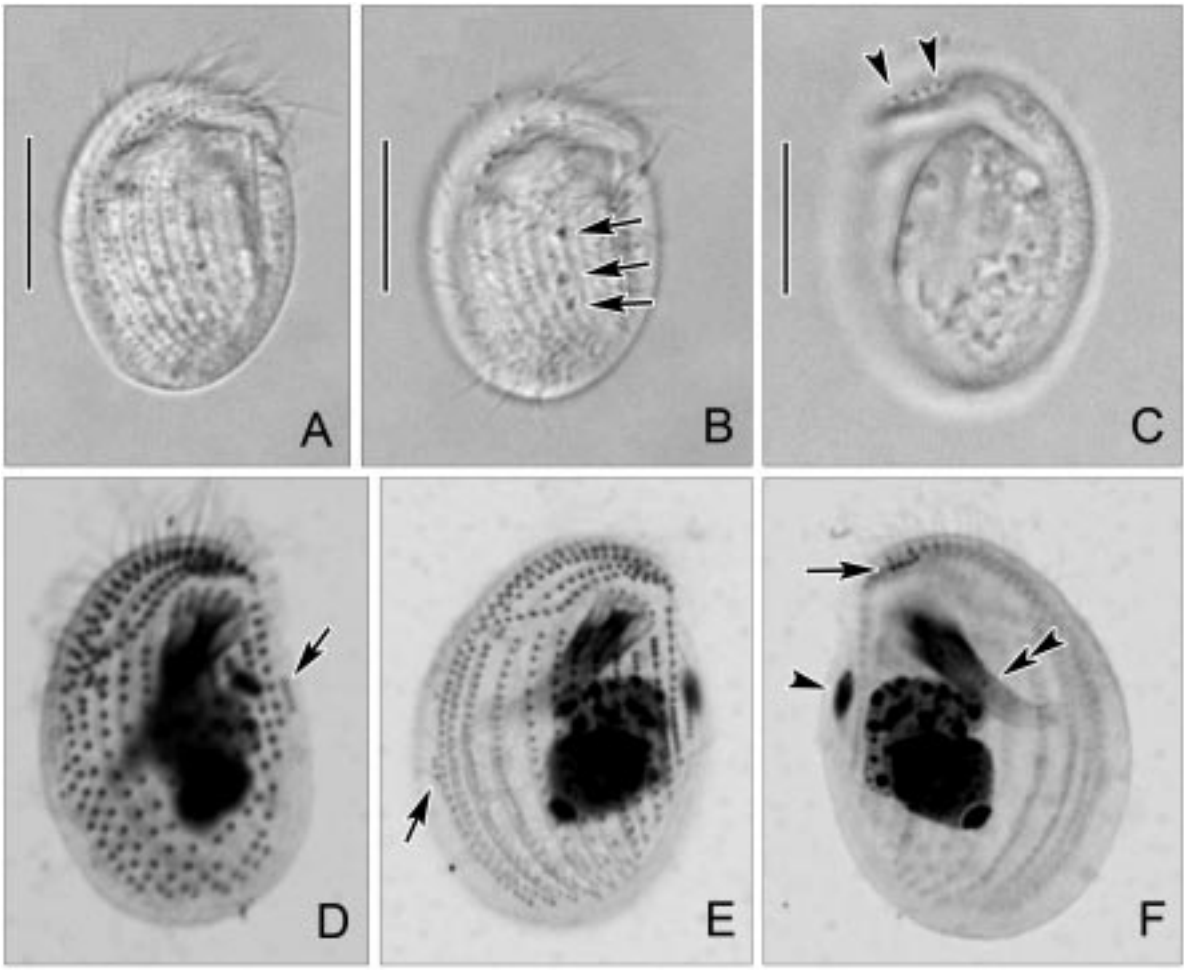
It should be noted that two specialized plasmatic organelles in cyrtophorids, the podite (or glandular adhesive organelle, Griffel etc.) and the ventral protuberances (so called tentacle-like structures, finger-like tentacles, feet etc.) might not be clearly outlined in previous studies, which may hence lead to misunderstanding or misidentifications. Based on previous and the present work, we herein summarize the distinguishable aspects of the podite and the protuberances as follows: (1) shape (blade-like or foliform *vs.* club, finger-like); (2) size (usually 8-10 *vs.* 3-5 µm in length); (3) number and position (consistently one, at posterior ventral surface *vs.* usually several to many, regularly or irregularly distributed between ventral kineties); (4) movement (actively mobile, moving around the base effectively *vs.* immobile); (5) with *vs.* without a furrow for discharging adhesive substance; and (6) usually several kinetosome-like dots present (*vs.* absent) beneath the base of organelles after protargol impregnation (this paper; Gong and Song 2003, 2004a, b, c, 2006; Gong *et al*. 2002, 2003). Furthermore, the podites are presumably used for



Figs 5A-I. Infraciliature of *Chlamydonella derouxi* (A-C, original) and related taxa (D-I). **A, B** - ventral (A) and dorsal (B) views; **C** - shape of macronucleus from three different individuals; **D** - *Atopochilodon distichum* (from Deroux 1976); **E** - *Wilbertella distyla* (from Wilbert 1971); **F** - *W. stricta* (from Deroux 1976); **G** - *Parachilodonella lygiae* (from Dragesco 1966); **H** - *Brooklynella hostilis* (from Lom and Nigrelli 1970); **I** - *Brooklynella sinensis* (from Gong and Song 2006). LEF - left equatorial fragment; Ma - macronucleus; Mi - micronucleus; P - club-shaped protuberances; REF - right equatorial fragment; TF - terminal fragment.

attaching the ciliate to the substrate (Fauré-Fremiet *et al.* 1968, Lom and Corliss 1971), whereas the ventral protuberances, though the function remains unclear, are apparently not used for clinging to substrate (Foissner *et al.* 1991, Lynn and Foissner 1994). Taxonomically, the presence of podites is exclusive for ciliates in two cyrtophorid families namely Hartmannulidae and Dysteriidae, whereas ventral protuberances have been only observed so far in lynchellid taxa, e.g. *Coeloperix sleighi* Gong *et* Song, 2004, *Chlamydonellopsis* Blatterer *et* Foissner, 1990, *Lynchella gradata* Kahl, 1933 and *Chlamydonella derouxi* Song, 2003 (Kahl 1933, Foissner *et al.* 1991, Gong and Song 2004c, this paper).

The genus *Parachilodonella* was erected by Dragesco (1966) to include a single marine species, *P. lygiae* Dragesco, 1966. Main generic characteristics



Figs 6A-F. Morphology and infraciliature of the Qingdao population of *Chlamydonella derouxi* from life (A-C) and after protargol impregnation (D-F). **A, B** - ventral views, arrows indicate the three club-shaped protuberances on ventral side; **C** - dorsal view, arrowheads mark the terminal fragment; **D, E** - ventral infraciliature, showing the left (arrow in D) and right (arrow in E) equatorial fragment; **F** - dorsal infraciliature, to note the terminal fragment (arrow), the micronucleus (arrowhead), and the cyrtos (double-arrowheads). Scale bars 10 µm.

of this taxon are: (1) *Chilodonella*-like infraciliature and (2) possessing a typical podite on ventral side (Dragesco 1966, Fig. 5G). According to these two points, the nominal species *P. distyla*, a freshwater form, is likely misplaced because it has a different ciliature-pattern and two ventral cytoplasmic protuberances (Wilbert 1971). Nevertheless, although similar to the well-known *Chlamydonella* regarding the general ciliature, *P. distyla* can be clearly distinguished from *Chlamydonella* (including *C. derouxi* described above) in the presence (*vs*. absence) of a distinct non-ciliated zone in left posterior of ventral surface (Fig. 5E), which should be considered as a feature at the generic level. Likewise, another nominal species, *Chlamydonella stricta*, which was originally isolated from marine biofilms (Deroux 1976), should be a congener with *P. distyla* (Fig. 5F). Based on these understanding, a new genus, *Wilbertella* is suggested:

***Wilbertella* nov. gen.**

Diagnosis: Lynchellidae with a distinct blank zone in left posterior portion of cell because left kineties dis-

Table 2. Comparison between *Chlamydonellopsis calkinsi* and its congeners.

Characters	*C. calkinsi*	*C. plurivacuolata*	*C. polonica*
Body size (µm)	50-70 × 30-45	50-110 × 30-55	55-80 × 38-52
Number of protuberances	*ca* 18	*ca* 10	?
Number of preoral kineties	4	5-8 (mean 6)	6
Number of somatic kineties	15-17 (mean 16)	19-23 (mean 21)	18-20 (mean 19)
Number of nematodesmal rods	12-15 (mean 13)	17-20 (mean 18)	16-18
Number of basal bodies in terminal fragment	6-12	10-20	12
Macronuclear size (µm)	18 × 11	27 × 16	22 × 12
Number and position of contractile vacuoles	2, diagonally positioned	4, two on left, another two on right	2, diagonally positioned
Habitat	marine	freshwater	freshwater
Data resource	present work	Blatterer and Foissner 1990	Foissner *et al*. 1981

? Data not available.

Table 3. Comparison between populations of *Chlamydonella pseudochilodon.*

Character	*C. pseudochilodon*	*C. pseudochilodon*	*C. pseudochilodon*
Body length (in µm) *in vivo*	50-70	30-45	43-73
Body width (in µm) *in vivo*	35-50	20-25	24-38
Number of somatic kineties	18-19	14-20	14
Number of preoral kineties	4	4	4
Number of nematodesmal rods	16-21	11-16	12-18
Club-shaped protuberances	present	present	?
Macronuclear length	15-34	7-15	29
Macronuclear width	6-10	8-10	10
Macronuclear shape	elongate	oval	ellipsoidal
Number of micronuclei	1-6	1	1
Number of contractile vacuoles	2	2	2
Habitat	Marine	Marine	Marine
Sample location	Yellow Sea, China	Atlantic, France	Antarctica
Data source	Original	Deroux 1970	Petz *et al*. 1995

? Data not available.

tinctly shortened, right kineties extending to posterior end; perioral kineties in flattened Y-shaped; no distinct gap between left and right ciliary fields.

Type species: *Parachilodonella distyla* Wilbert, 1971.

Dedication: This new genus is dedicated to Prof. Norbert Wilbert, Institut für Zoologie, Universität Bonn, Germany, in recognition of his eminent contribution to ciliatology. Feminine gender.

Species assignable: *Wilbertella distyla* (Wilbert, 1971) nov. comb. [basionym: *Parachilodonella distyla* Wilbert, 1971]; *Wilbertella stricta* (Deroux, 1976) nov. comb. [basionym: *Chlamydonella stricta* Deroux, 1976].

Comparison with related genera: Both the genera *Chlamydonella* Deroux in Petz Song *et* Wilbert, 1995 and *Atopochilodon* Deroux, 1976 resemble *Wilbertella* nov. gen. with respect to the flattened Y-shaped perioral kineties (Deroux 1970, 1976; Petz *et al*. 1995; this paper; Fig. 5D). However, the new genus can be distinguished from the former two in the structure of the left kineties which are distinctly shortened posteriorly (*vs*. extending to cell end in *Chlamydonella* and *Atopochilodon*). In

addition, the left and right ciliary fields are distinctly separated in *Atopochilodon* (*vs*. continuous in *Wilbertella*).

Compared with *Wilbertella*, another similar taxon, *Parachilodonella* Dragesco, 1966 can be recognized by having a podite (*vs*. ventral club-shaped protuberances or absent in *Wilbertella*) and *Chilodonella*-like infraciliature (Dragesco 1966, Fig. 5F), that is, left and right ciliary fields are separated as parenthesis marks (*vs*. continuous in *Wilbertella*).

The posterior ends of left kineties in *Brooklynella* Lom *et* Nigrelli, 1970 are also greatly shortened relative to right ones, leaving a blank area in posterior left of ventral surface (Lom and Nigrelli 1970, Gong and Song 2006; Figs 5H, I). Nevertheless, *Brooklynella* differs from *Wilbertella* in: (1) arrangement of oral structure (one preoral and two circumoral kineties *vs*. flattened Y-shaped perioral kinety); (2) absence (*vs*. presence) of the interruption between frontoventral and oral kineties; and (3) presence (*vs*. absence) of a typical podite.

Thigmogaster Deroux, 1976 may also resemble *Wilbertella* in terms of the shortened left kineties (Deroux 1976). However, *Thigmogaster* can be easily recognized by the following features: (1) macronucleus centric (*vs*. juxtaposed) heteromerous; (2) perioral kineties *Chilodonella*-like (one preoral and two circumoral kineties *vs*. flattened Y-shaped); (3) somatic kineties in right field continuous (*vs*. interrupted by perioral kineties); and (4) left and right ciliary fields slightly detached (*vs*. continuous).

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A Redescription of *Amphizonella violacea* (Amoebozoa: Arcellinida)

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Summary. We redescribe the lobose testate amoeba *Amphizonella violacea* Greeff, 1866. We have collected this conspicuous but often overlooked species in xerophilic mosses from six localities in Germany. The overall shape is much more variable than hitherto known. Non moving cells are usually roundish or oval but can emit pseudopods, moving amoebae adopt a more longish tongue or fan-like shape sometimes non moving amoebae take a stretched form with pseudopods at opposite ends. In contrast to previously published images the outer gelatine-like mucus layer is not always present. After excystation this layer is secreted *de novo*. Its thickness is variable and can reach up to 23 µm. Normally it contains numerous rod-shaped bacterial exobionts of unknown function. In SEM the outer surface is smooth. The untextured organic shell-wall is flexible and opens in a large not well defined aperture.

Key words: *Amphizonella violacea*, Arcellinida, ecology, Microcoryciidae morphology, SEM, testate amoebae, *Zonomyxa violacea*.

Abbreviations used: DIC - differential interference contrast, PBS - phosphate buffered saline, SEM - scanning electron microscope.

INTRODUCTION

The genus *Amphizonella* was erected by Greeff (1866) with *Amphizonella violacea* as type species. In 1888 the same author corrected and amended his original description. Penard (1902, 1906) provided a detailed redescription. Beside some secondary entries in keys and textbooks (Deflandre 1953, Harnisch 1958, Schönborn 1966, Meisterfeld 2002) only Thomas (1957) has published a short note dealing essentially with the nature of the conspicuous colour of the organism.

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This species has not been studied in further detail for about one hundred years until today. The aim of our study is to correct and supplement the previous descriptions and to document different life stages of this largely overlooked species with modern methods.

MATERIALS AND METHODS

A total of about fifty samples of xerophilic mosses (epiphytic or epilitic) from roofs and brick or concrete walls in different areas of Germany were studied. *Amphizonella violacea* could be discovered in six of these locations (Table l). Positive samples came exclusively from roofs. Usually only single or few cells were found, only two of these 50 samples had high abundances x = 14.8 (7-24) specimens per cover slip 18 × 18 mm.

Prior to extraction the samples were wetted for up to twenty four hours with none carbonated mineral water Volvic until saturated. Then the samples were squeezed out or washed out. To remove coarse particles the suspension was sieved through a small meshed screen (0.5-l mm). Mainly living specimens of *Amphizonella violacea* were studied.

If the suspension contains not too much detritus, the amoebae stay alive for days or up to two weeks. Pseudopodial activity can be observed best if the slides are left undisturbed in a moist chamber for several hours or with an inverted microscope. If the evaporated water is replaced daily such preparations can be studied for several weeks.

Cells for SEM preparation were first fixed in saturated sublimate solution ($HgCl_2$) for 10 min and after washing in tap water postfixed in glutaraldehyde (6.25% in PBS) for 20 min, washed in PBS and dehydrated in an alcohol series. From absolute alcohol the cells were transferred into HMDS (Hexamethyldisilazan, Merck) (Oshel 1997). The nuclei were stained in fixed material with bromophenolblue.

REDESCRIPTION

The outer shape of *Amphizonella violacea* is largely mutable (Figs 1-4). The envelope is flexible, organic, transparent, 1 to 5 µm thick, with undulations and a wrinkled surface (Fig. 13). The envelope is usually attached to the cell but sometimes small parts are not attached.

The colour depends on the stage and can vary from almost colourless over violet to brownish yellow (during cyst formation).

The pseudostome is large but its shape is not very well defined and changing continuously with the movement of the cytoplasm. Generally it looks like an indentured and ramified slit (Figs 4, 5, 11, 12) but it can sometimes be more or less circular.

The envelope is normally covered by a hyaline gelatine-like layer which can be up to 25 µm thick (Fig. 13). In many specimens this layer contains rod like bacteria (Figs 6-8, 14). The ovular nucleus is ovoid or spherical, the nuclear membrane is easily visible, the numerous (usually more than thirty) nucleoli are mainly concentrated below the nuclear membrane (Fig. 6)

Numerous (usually more than 30) contractile vacuoles are evenly distributed and discharge in the thin space between envelope and cell surface (Figs 5, 6). In most stages the cytoplasm contains countless small (< 1 µm) violet granu1es which give the whole cell a light to dark violet tint. We have also observed specimens lacking the violet pigment. The colour of these cells appeared yellowish to light brown. Prior to cyst formation the cytoplasm becomes dark brown.

Dimensions

Greeff (1866) in his original description specifies a length of 150 µm. Later Penard (1906) in his redescription expanded the range to 125-250 µm. Our own measurements (rounded forms only, long axis exc1uding the galantine like layer) differ slight1y between different populations. The population from Magdeburg ranges from 120-239 µm with a mean length of 170 µm (n=15) while one population from Aachen is smaller: length of 95-170 µm, mean 125 µm (n=20).

Occasionally extremely large or small amoebae were observed. Stretched forms are often much longer than the average (Fig. 3). One stretched individual of 558 µm was observed as well as several small individuals (75-86 µm). The small individuals from Aachen lack the violet colour.

Motility and locomotion

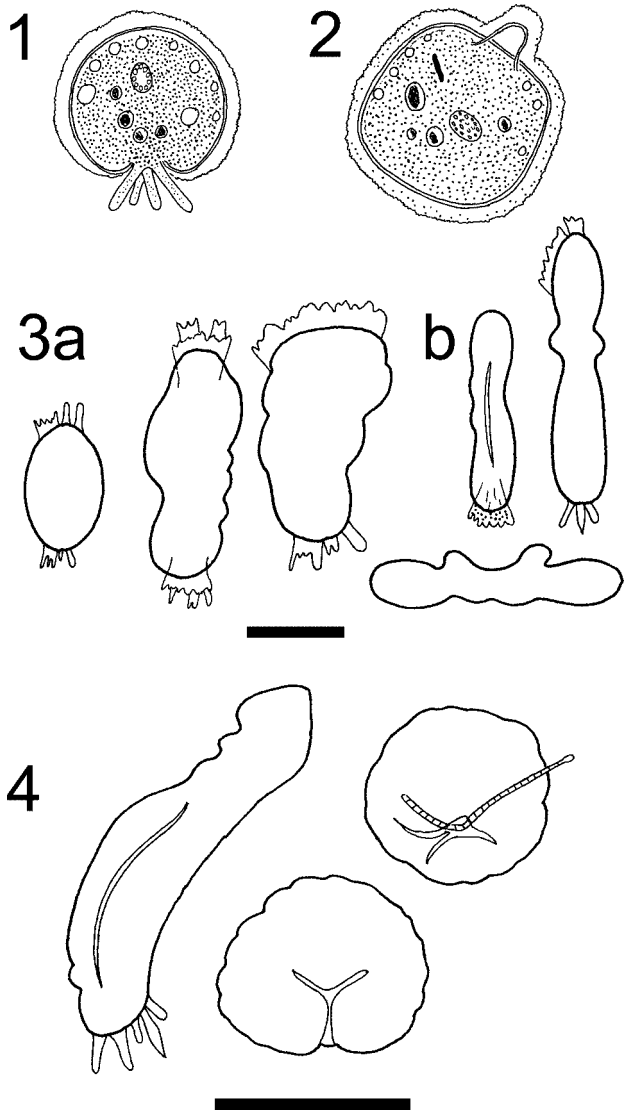
Amphizonella is able to change its shape to a large extent. Three distinct active forms are common: (i) Stationary non moving form (this is not a cyst), the cell is more or less spherical with flattened ventral sides; nevertheless this form can emit pseudopods (Figs 1, 2, 5). This is the most common form immediately after extraction from the moss. (ii) Moving form (with pseudopods). The cell is fiat, outstretched, ovoid, tongue or fanlike (Fig. 3a). (iii) Stretched form, irregularly longish, pseudopods often emitted simultaneously at both poles, without locomotion (Fig. 3b).

It takes several hours after rewetting of the moss until the moving and stretched forms can be observed.

Due to the pigment granules the movement and streaming of the cytoplasm can be easily observed. The amoebae move by lobose fan, finger or sword like pseudopods (Figs 1, 3, 4, 9) which are always emitted through a slit-like aperture. These pseudopods have a broad ectoplasmic seam. Short finger like pseudopodia can be completely ectoplasmic. The motion of *Amphizonella violacea* is extremely slow (0.65 µm s^{-1} at 20°C). Often the pseudopods of crawling amoebae are emitted under the cell and therefore difficult to observe.

Feeding

Active amoebae contain several food vacuoles. The content is often already digested but diatoms, testate amoebae e.g. *Arcella arenaria, Difflugia lucida* as well as members of the moss microflora like



Figs l-4. *Amphizonella violacea.* **1 -** lateral view (after Penard 1902); **2** - dorsal view (after Penard 1906); **3a** - stretched forms, often the pseudopodia that are emitted through the aperture are only visible at both poles of the cell; **3b** - aperture of the shell is sometimes only a long narrow slit; **4** - variability of apertural shapes. The individual on the right is ingesting a filamentous cyanobacterium. Scale bars: 200 µm.

cyanobacteria or green algae can be distinguished. The aperture of *Amphizonella violacea* is very large and flexible (Figs 4, 10-12) enabling the amoebae to ingest large food items like the lobose testate amoeba *Microcorycia flava* (diameter 125 µm). Faeces are excreted through the aperture by flat pseudopodia (Fig. 10).

Habitat

The preferred habitat of *Amphizonella violacea* are xerophilic mosses with extremely fluctuating water contents which can typically be found on roofs and with 1ower frequencies on walls, concrete surfaces or rocks. These biotopes are characterised beside the rapidly changing humidity by large temperature changes (daily and seasonally). In contrast to Bartoš (1940) and our results, Penard (1906) gives moist or even submersed mosses as preferred habitat. We do not believe that he had mistaken *Amphizonella violacea* with *Zonomyxa violacea* but it cannot be ruled out that he had taken xerophilic mosses that were only wet when the samples were taken.

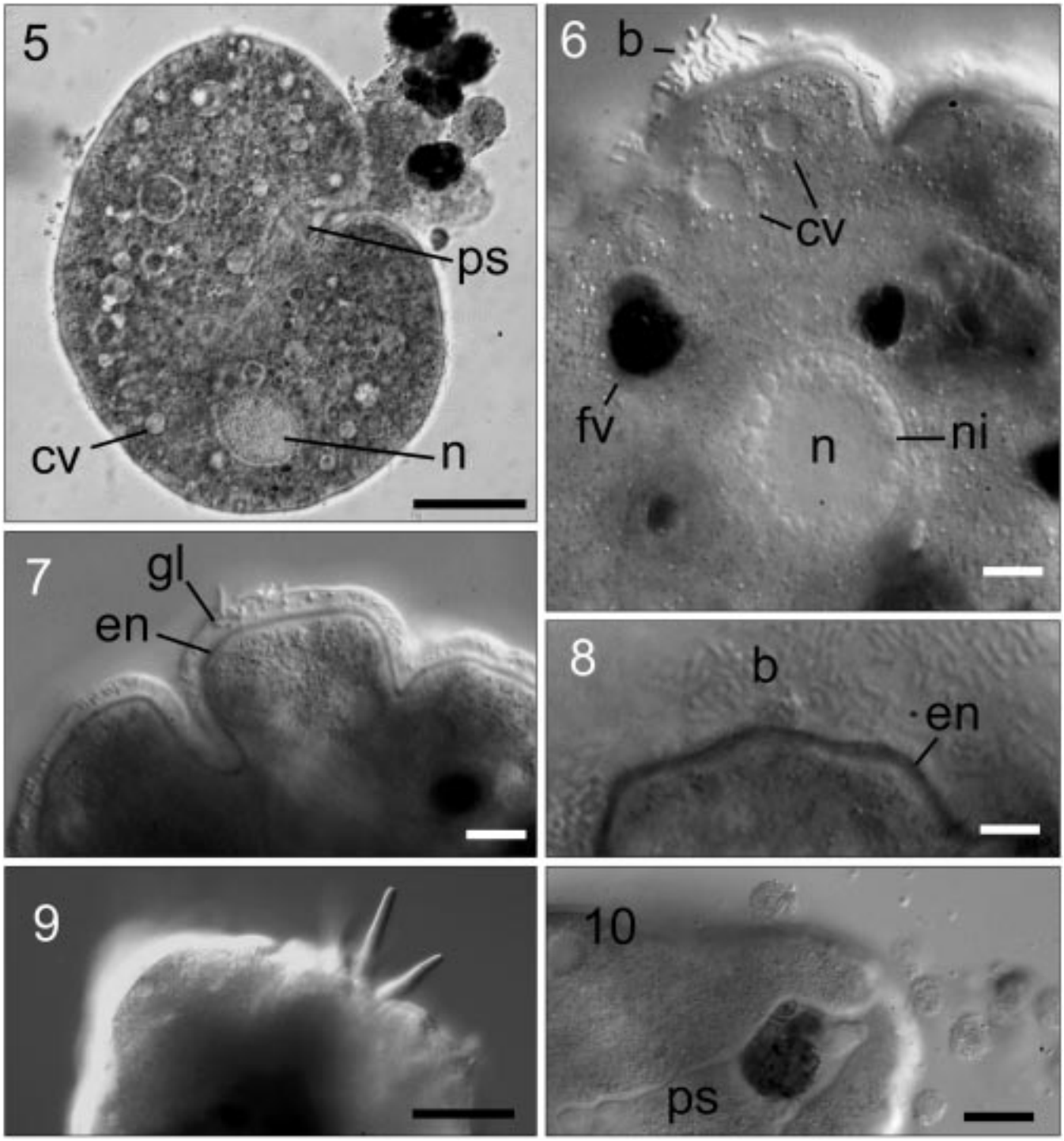
Cysts

Amphizonella violacea can survive under unfavourable environmental conditions as precysts or cysts. Precysts are formed in response to a rapid desiccation of the moss. In this case *Amphizonella* takes an irregular roundish shape, during dehydration the cell becomes dark brown and almost opaque.

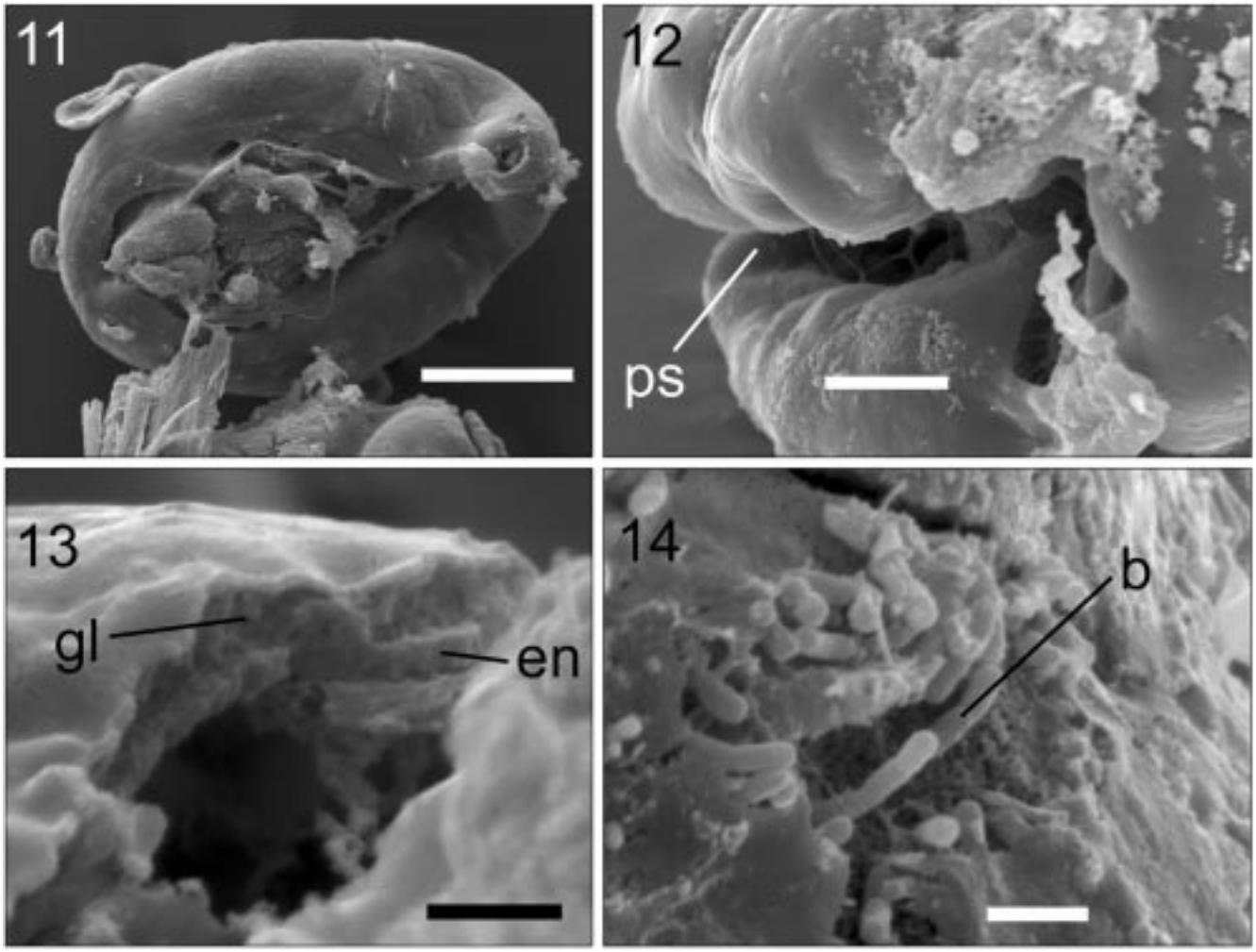
Prior to cyst formation the colour of the cytoplasm also changes from violet to brown. The cell becomes more or less spherical. In this early stage the surface of the envelope is still relatively smooth. The cytoplasm is now almost completely opaque. Details of the cell structure are hardly visible. Only peripheral vacuoles appear as pale spots. At the same time the outer hyaline layer, which has become thicker during encystment, spreads out over the surface of the substrate (slide). With further dehydration the interior of the cyst brightens partially and the spherical shape wrinkles more and more and becomes an irregular often relative flat cyst with a hyaline seam. In contrast to precysts the cysts are formed in moist or only slowly drying mosses.

Differential diagnosis

Amphizonella violacea can easily be confused with *Zonomyxa violacea* Nüsslin, 1884. *Zonomyxa violacea* can be distinguished from *Amphizonella violacea* by the following characters: A thinner test, no external mucus layer, and most significant1y numerous nuclei. According to our observations (Meisterfeld unpublished) *Zonomyxa* normally has 4 but Penard 1906 reports up to 32. These are smaller (~20 µm) than the single large nucleus of *Amphizonella violacea.* Nüsslin 1884 and Penard 1906 describe the locomotive form of *Zonomyxa violacea* as pointed at the front but broadly rounded at



Figs 5-10. *Amphizonella violacea.* **5 -** lateral view of a compressed individual during food uptake (algae). Numerous contractile vacuoles at the periphery of the cell and an ovular nucleus. The pseudostome is in this view a narrow cleft; **6** - ovular nucleus with numerous peripheral nucleoli, contractile vacuoles, food vacuoles, numerous dark pigment-granules and bright crystals; **7** - envelope of the cell with the transparent gelatine-like layer. Note the large embedded and attached bacteria; **8 -** detail of the shell surface with envelope and rod shaped bacteria in the gelatine layer. **9** - fingerlike pseudopodia (ectolobopodia); **10 -** apertural view (inverted microscope) of an excreting cell. A large pseudopodium is emitted from the apertural cleft. b - bacteria, cv - contractile vacuoles, en - envelope of the cell; fv - food vacuoles, gl - gelatine-like layer, n - nucleus, ni - peripheral nucleoli, ps - pseudostome. Brightfield (5), DIC (6-10). Scale bars: 40 µm (5); 10 µm (6, 7); 5 µm(8); 40 µm (9); 20 µm (10).



Figs 11-14. *Amphizonella violacea* SEM. **11 -** apertural view, the large pseudostome extends across the whole length of the specimen; **12 -** detail of the cleft-like shell aperture (ps). Smooth areas of the surface alternate with patches that show attached and embedded bacteria; **13 -** cut open shell surface showing the homogeneous envelope (en) and the gelatine-like layer (gl); **14 -** detail of the cell surface with embedded and attached bacteria (b). Scale bars: 50 µm (11); 10 µm (12); 2 µm (13, 14).

Table 1. New locations in Germany where *Amphizonella violacea* has been found during this study.

Location	District	State
Klaber	Güstrow	Mecklenburg- Vorpommem
Warnkenhagen OT Tellow	Güstrow	Mecklenburg-Vorpommem
Gardelegen	Altmark-Kreis Salzwedel	Sachsen-Anhalt
Loburg	Anhalt-Zerbst	Sachsen-Anhalt
Magdeburg		Sachsen-Anhalt
Aachen OT Seffent		Nordrhein-Westfalen

the posterior end. But this character is of little value because active cells can emit several finger-like pseudopodia giving the cells an outline similar to *Amphizonella violacea* (Meisterfeld unpublished). The average dimension is 150 µm but large individuals can reach up to 200 µm. Stretched, moving cells can be up

to 250 µm long (Nüsslin 1884). The habitat requirements for *Zonomyxa* are completely different: so far it has only been reported from Sphagnum or aquatic vegetation (e.g. Nüsslin 1884, Penard 1906).

DISCUSSION

Greeff (1866) describes the shape of *Amphizonella violacea* as more or less spherical. Even Penard (1902/1906) depicts *Amphizonella violacea* in his drawings as a rather spheroid rhizopod. These figures later served other authors as templates for their own illustrations (Deflandre 1953, Harnisch 1958, Schönbron 1966, Meisterfeld 2002). For this reason the published picture of *Amphizonella violacea* dos not reflect the actual range of existing life stages. Based on our observations of a large number of individuals, *Amphizonella violacea* is only spherical in the non locomotive form. Moving amoebae display a considerable variability of shapes. A so called peripheral gelatine or mucus layer was reported in several descriptions of the species although it was not mentioned in the original description (Greeff 1866). Not until his description of 1888 did Greeff explicitly describe the existence of the double envelope, a peripheral hyaline usually broad layer and underneath the actual shell. According to Greeff (1888) the outer layer is only formed by larger seemingly full-grown individuals but he gives no further information about the nature of this layer. In his redescription Penard (1902/1906) interprets it as mucus layer and illustrates *Amphizonella violacea* constantly with this layer. This led to the impression that *Amphizonella violacea* is always covered by a peripheral mucus layer which is definitely not the case. After excystation the amoeba initially do not have it, only during the course of the active phase a mucus layer is eventually formed. This layer is usually colonised by large rod like bacteria. Occasionally we have found single individuals with a thick mucus layer of 13 to 23 µm (sampling site 4, Table 1). Such a thick mucus layer is obviously only rarely formed. The conditions that induce this process are unclear.

Little is known about the nature of the violet pigment. Already Penard (1906) had shown that the pigment granules bleach and eventually become rose if the cells are kept in an acid medium. Thomas (1957) hypothesized that the colour is a result of the feeding of *Amphizonella* on *Gloeocapsa compacta* and related Cyanobacteria which were common in the samples he had studied and were frequently found in the food vacuoles. *Gloeocapsa compacta* contains gloeocapsin, a pigment that together with scytonemin is believed to be a powerful protective agent against UV-radiation (Garcia-Pichel and Castenholz 1993). These pigments are widely distributed among cyanobacteria of extreme habitats like rock surfaces. Whether the pigment of *Amphizonella* is Gloeocapsin and *Gloeocapsa* is really the source of the pigment has not been proven. In our samples these or related species were not present but filamentous cyanobacteria could be observed.

Classification

According to recent classifications (Meisterfeld 2002, Cavalier-Smith *et al*. 2004, Adl *et al*. 2005) the position of *Amphizonella* in the system is as follows:

Phylum: Amoebozoa Lühe, 1913
Class: Lobosea Carpenter, 1861
Order: Arcellinida Kent, 1880
Suborder: Arcellinina Haeckel, 1884
Family: Microcoryciidae

Below Arcellinida this classification is based on morphological characters alone. The relation to typical lobose testate amoebae like *Arcella, Difflugia* or *Nebela* remains uncertain until molecular data are available.

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Description of a New Freshwater Heterotrophic Flagellate *Sulcomonas lacustris* Affiliated to the Collodictyonids

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Summary. This new heterotrophic flagellate has two flagella inserted in the subapical region and a conspicuous ventral groove where bacteria are phagocytosed. Several microtubular fibres are connected to the two basal bodies: a dorsal root that plays the role of a MTOC for cortical microtubules, a left and a right ventral root that border the ventral groove, a root on the left side inserted between the Golgi body and the nucleus, and two fibrils that outline the bottom of the groove. Several features suggest a phylogenetic relationship with collodictyonids, i. e. a similar constriction at the base of the flagella, a similar desmose between the basal bodies that includes the origin of the left root, a similar arrangement of flagellar roots, and tubular mitochondrial cristae. Several features are more specific, such as a fibrillar rhizostyle adhering to a mitochondrial lobe and the single, small dictyosome.

Key words: collodictyonid affiliation, freshwater flagellate, *Sulcomonas lacustris* n. sp., ultrastructure.

INTRODUCTION

The widespread application of molecular methods such as the SSU rRNA (small subunit ribosomal nucleic acid) gene sequences to the identification of uncultivated environmental organisms (Dawson and Pace 2002) has led to the discovery of new phylotypes, that are presumably new species and subgroups of protists (Moon-van der Staay *et al.* 2001; Lopez-Garcia *et al.* 2001, 2003; Diez *et al.* 2001; Moreira and Lopez-Garcia 2002; Massana *et al.* 2002, 2004; Berney *et al.* 2004; Scheckenbach *et al.* 2005; Slapeta *et al.* 2005). These methods are highly efficient in estimating the diversity of the organisms in environmental samples but are not free of artefacts such as the occurrence of chimeric sequences (Berney *et al.* 2004). These methods therefore require isolation of the organisms corresponding to the phylotypes for morphological and physiological studies in order to characterize the species and to understand their role in aquatic ecosystems (Fenchel 1987, 2002; Finlay 1990; Finlay and Fenchel 2004). In traditional morphology, light and electron microscopy have proved important and necessary tools for the identification of species and their taxonomic affiliation and for providing insights into their biology (Ragan and

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Chapman 1978, Margulis *et al.* 1990, Taylor 1999, Lee *et al.* 2001, Hausmann *et al.* 2003, Adl *et al.* 2005). Several free-living flagellates in aquatic environments have yet to be identified (Patterson and Zölffel 1991, Tong *et al.* 1998, Patterson 1999, Bernard *et al.* 2000, Patterson *et al.* 2001, Schroeckh *et al.* 2003, Lee *et al.* 2005). This study describes a protist flagellate not yet reported in the protistology or ecology literature, and which is probably rare in aquatic environments. Several features revealed by electron microscopy suggest that it is affiliated to *Diphylleia (= Aulacomonas*) and the collodictyonids, which are a small group of flagellates with tubular mitochondrial cristae phylogenetically distant from other flagellate groups (Brugerolle and Patterson 1990, Brugerolle *et al.* 2002).

MATERIALS AND METHODS

Origin. This flagellate was isolated from the intestinal content of a tadpole of *Bufo bufo* from Lake Chauvet in the Clermont-Ferrand region (Central France). The gut content was mixed with a few ml of a Ringer's solution in a Petri dish and left for 48 h at room temperature. Several protists developed, among which *Sulcomonas lacustris* was the most abundant and was collected, using a micropipette for light microscopy observations and preparation for electron microscopy. The flagellate population then decreased as ciliates invaded the dish and fed on both the flagellates and bacteria.

Microscopy. Living cells were observed under a phase contrast microscope (Leitz), transformed into drawings, and then photographed after fixation. Electron microscopy was performed by collecting the cells using a micropipette under a stereo-microscope followed by fixation in 1:1 mixture of 1% glutaraldehyde and 2% osmium tetroxide (Polysciences) in 0.1 M phosphate buffer at pH 7 for 1 h. Cells were washed twice in the buffer by centrifugation and then post-fixed in 1% osmium tetroxide in 0.1M phosphate buffer for 1 h. After washing in water, the cells were pre-embedded in 1% agar (Difco) and stained 'en bloc' in saturated uranyl acetate in 70% ethanol for 15 h. After complete dehydration in an alcohol series to 100% alcohol and 1,2-propylene oxide, the cell pellet was embedded in Epon 812 resin (Merck). Ultrathin sections were obtained using a Reichert Ultracut S microtome (Leica), and stained for 15 min with lead citrate before examination under a JEOL 1200EX electron microscope at 80 KV.

RESULTS

Light microscopy

The cells have an ovoid shape with a conspicuous ventral groove, and an average length of 9.8 µm (8.4-11.2 µm), for an average breath of 5.6 µm, from 50 measured cells (Figs 1, 3). The two flagella are inserted in the sub-apical region. The anterior flagellum is shorter, about two times the cell length, and oriented forward, while the posterior flagellum is longer, about three times the cell length, and oriented backwards (Fig. 3). In the living cell, the posterior flagellum generally lies in the ventral groove and sometimes adheres to the substratum, but in the fixed cells both flagella are often deflected forwards. Swimming is not very efficient since the two opposite beating flagella do not propel the cell quickly. The nucleus is situated in the anterior part, the cell cortex appears rigid, and the cytoplasm contains food vacuoles.

Electron microscopy

Flagella. The two basal bodies (1, 2) are arranged in parallel, one above the other in the longitudinal axis of the cell and both oriented toward the ventral side (Figs 2, 4). Basal bodies are of a quite normal length of 0.5 µm, and are interlinked by a dense fibre which caps their proximal section (Figs 2, 4, 8) and is connected to the 'rhizostyle' (see below). The basal body cylinder does not show an evident cartwheel structure and the transitional zone is relatively long at 0.1 µm (Figs 5, 8). Remarkably, the proximal part of the flagellum is occupied by an electron-dense constriction zone about 0.05 µm long where the flagellar membrane is in contact with the doublets of the axoneme (Fig. 5). A transverse section across this constriction shows that the central pair of microtubules is surrounded by a very dense sleeve (Fig. 5c). The flagella do not bear any mastigonemes on their surface.

Flagellar roots. The two flagella arise from an aperture whose dorsal border is supported by a microfibrillar zone (Figs 4, 13). The anterior basal body (1) is connected to a dorsal microtubular root comprising about five microtubules that is oriented toward the dorsal left side (Figs 2, 6, 7, 10). Distantly spaced microtubules arise along this fibre and run under the plasma membrane (Figs 6, 11). They cover the anterior part of the cell cortex, and also accumulate along the two ridges of the ventral groove (Figs 2, 15). These cortical microtubules arise from microfibrillar material present along the dorsal root and close to the anterior basal body that acts as a MTOC (Figs 10, 11). At the right side of the basal body (1) is attached a second root (rvR) of five interlinked microtubules that runs along the right border of the ventral groove under the plasma membrane (Figs 2, 7, 9, 14). The posterior basal body (2) is connected to the anterior basal body by a desmose that includes the left ventral root (lvR) comprising five to six interlinked

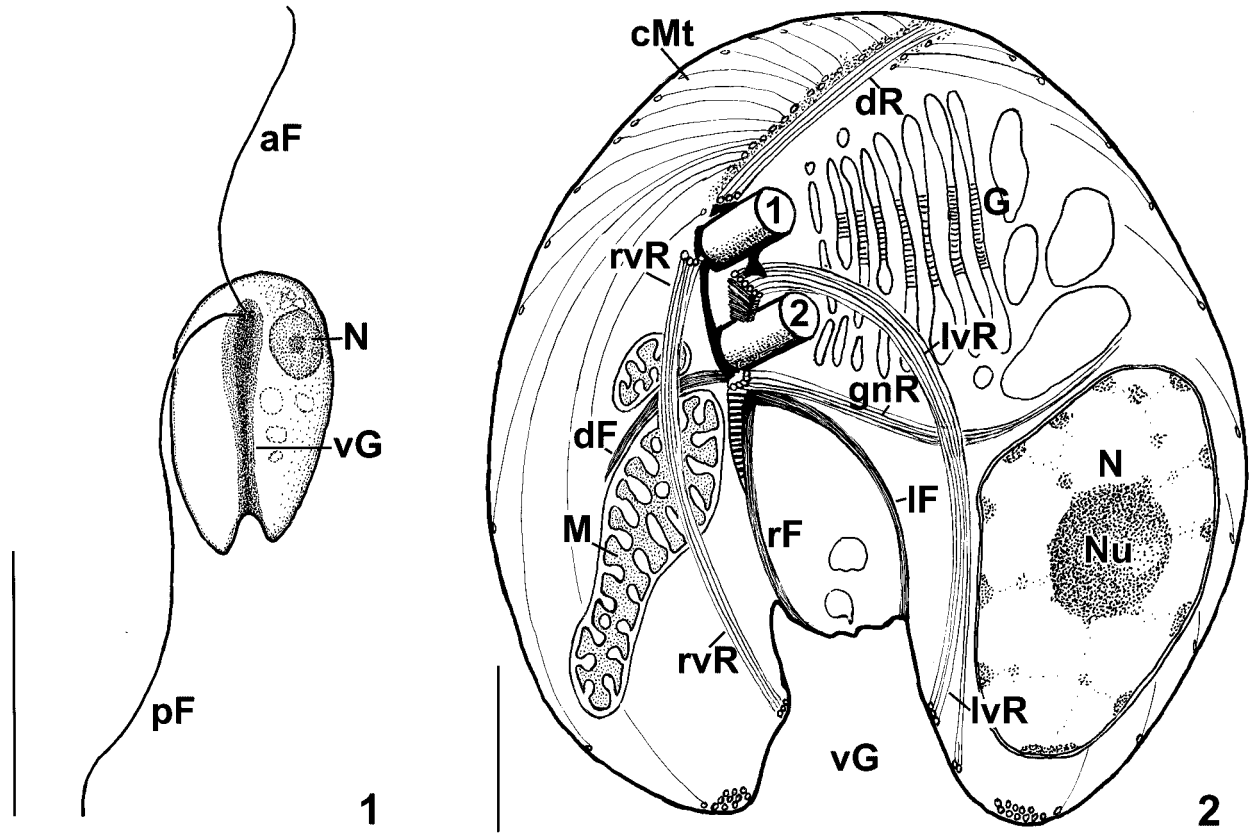
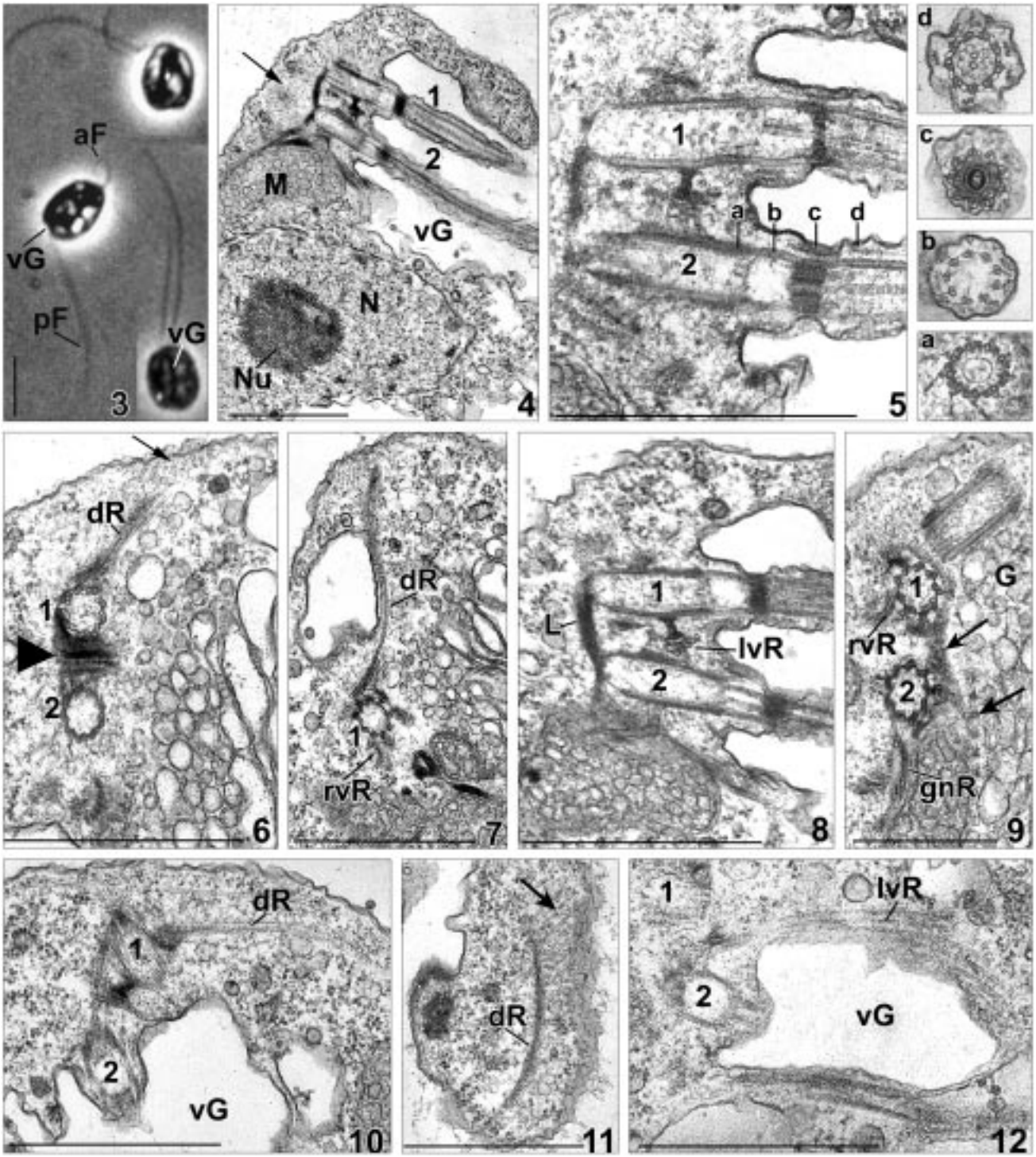


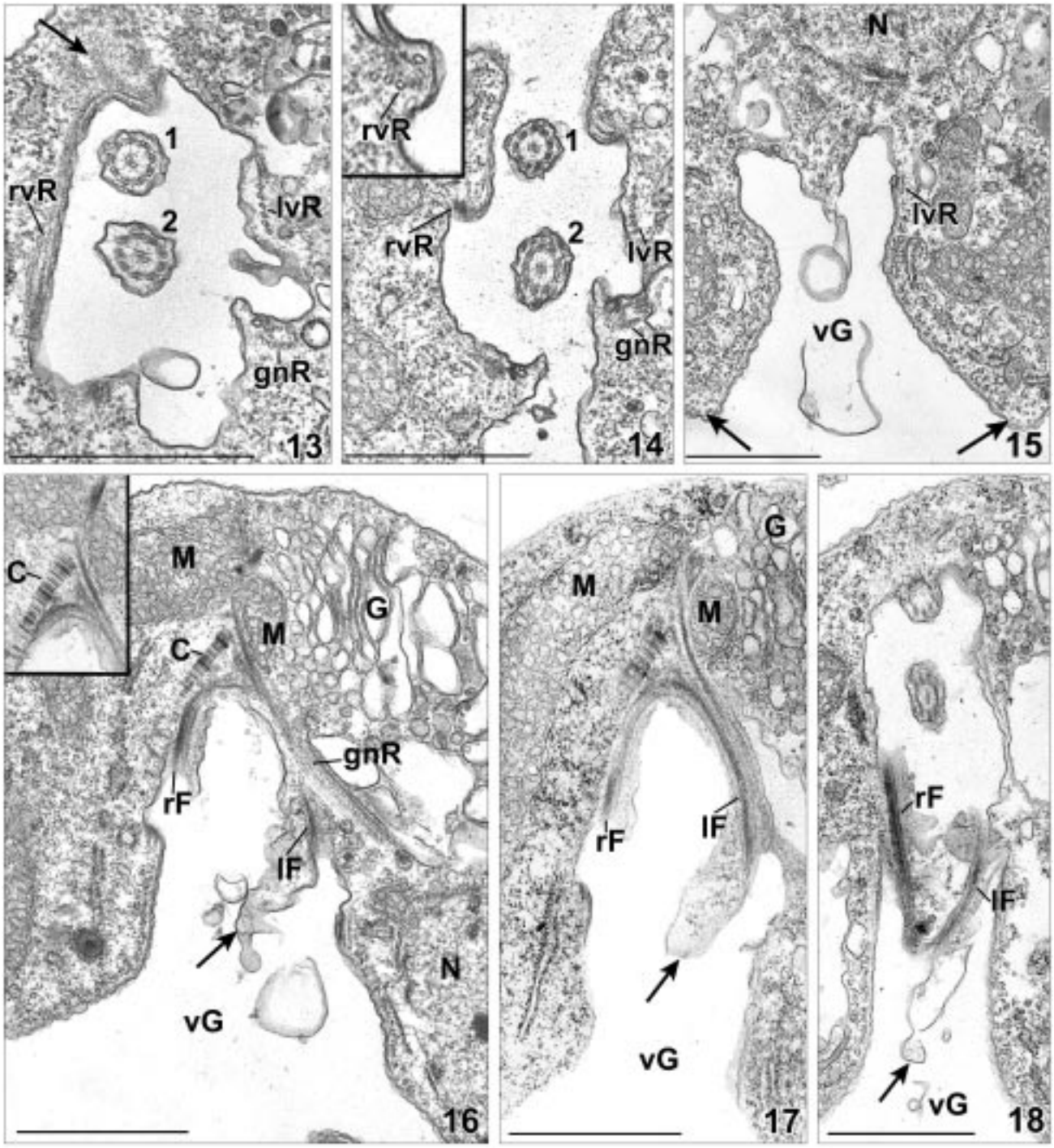
Fig. 1. Light microscopy representation of *Sulcomonas lacustris* showing the two flagella (aF - anterior flagellum, pF - posterior flagellum) apically-inserted, the longitudinal ventral groove (vG) and the nucleus (N). Scale bar 10 μm.
Fig. 2. Reconstruction scheme of the flagellar system of *Sulcomonas lacustris*, view onto the anterior pole. The two basal bodies (1, 2) are connected to the dorsal root (dR) from which arise cortical microtubules (cMt) covering the anterior part, the right ventral root (rvR) oriented to the right side of the groove (vG), the left ventral root (lvR) oriented to the left side of the groove, the Golgi nucleus root (gnR) inserted between the Golgi body (G) and the nucleus (N) with a nucleolus (Nu), the two ventral fibrils (rF, lF) that develop on the upper bottom of the groove and the rizostyle-like dorsal fibril (dF) linked to a mitochondrial lobe (M). Scale bars: 1 μm.

microtubules (Figs 2, 6, 8). This desmose comprises a dense connector attached to the anterior basal body (1) on one side and a microfibrillar striated structure attached to the posterior basal body (2) on the other side (Fig. 8). Distally, this left microtubular root is oriented to the anterior left face of the ventral groove and merges with cortical microtubules of the left border of the groove (Figs 12-15). At the posterior side of the basal body (2) is attached a microtubular root of five microtubules (gnR) oriented to the left side of the groove and distally inserted between the Golgi body and the nucleus (Figs 2, 9, 13, 16). This root shares a common origin with several electron-dense fibrils. The ventral fibril divides into two branches (rF) and (lF) that outline the upper bottom of the groove (Figs 2, 16-18). The base of the right side branch is doubled by a striated cord (Fig. 16). The dorsal fibril (dF) is oriented dorsally and similarly to a small rhizostyle it is attached to a large mitochondrial lobe indenting its surface (Fig. 19). In addition, on the left side of the basal body pair there is a striated structure that links the two basal bodies and continues along the Golgi stack of cisternae (Fig. 9, arrows).

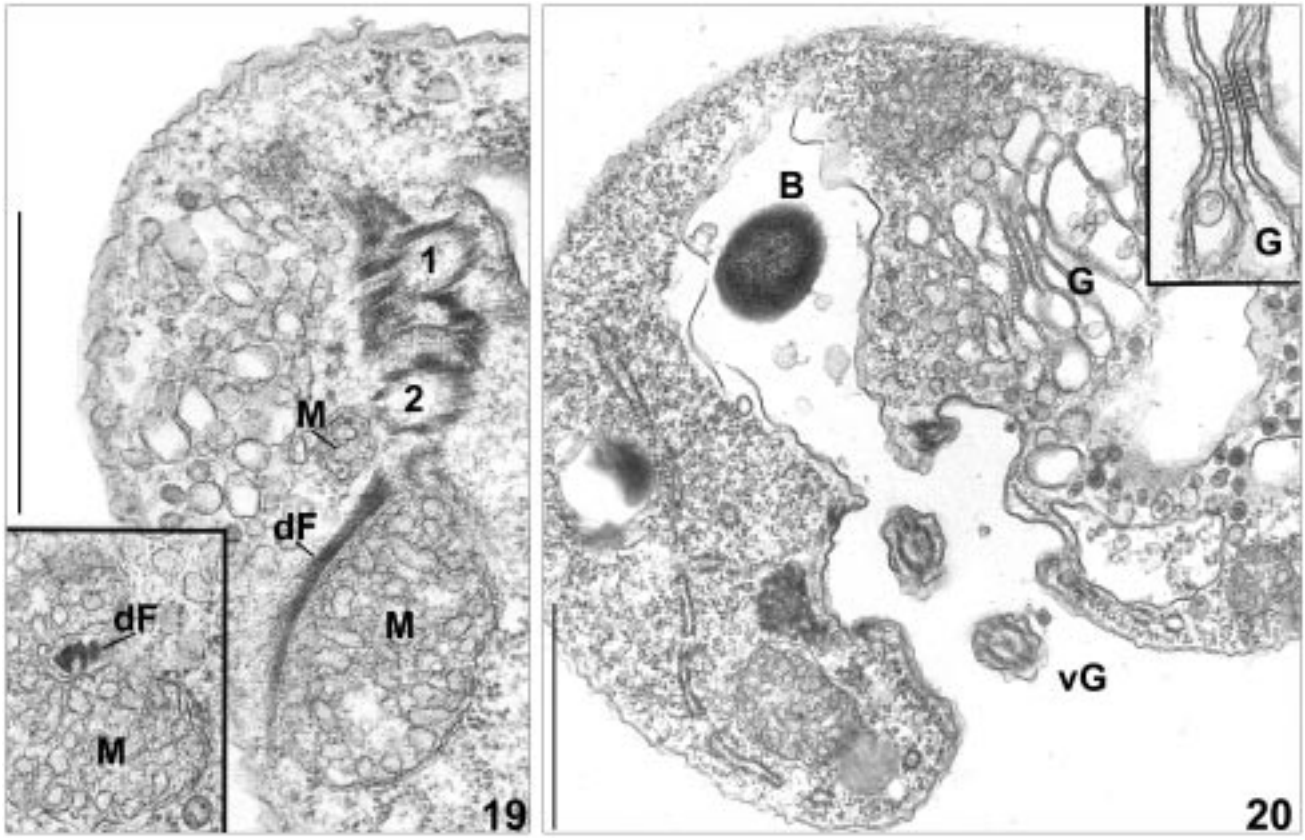
The Golgi body is situated on the left side of the basal bodies with its “trans” face close to the basal bodies and its “cis” face oriented toward the cell surface (Fig. 20). The “cis” face of the Golgi body comprises large vesicles that widen to form vacuoles close to the cell surface in the zone in front of the nucleus (Figs 2, 20). Inside the staggered Golgi cisternae there is a peculiar structure composed of bridges that link the two closely apposed membranes inside a cisterna. Examination of the sections through the cell suggests that the mitochon-



Figs 3-12. Light microscopy micrographs (3) and electron micrographs (4-12) of *S. lacustris*; **3** - phase contrast micrographs showing the two unequal flagella (aF, pF) inserted at the top of the ventral groove (vG); **4** - view of the two basal bodies / flagella (1, 2) inserted subapically in a dorsal microfibrillar zone (arrow) and arising in the ventral groove (vG), the mitochondrion (M), and the nucleus (N) with a nucleolus (Nu); **5** - ultrastructure of the basal bodies/flagella (1, 2) and transverse sections at different levels (a, b, c, d); notice the constriction zone and the special ultrastructure at the # c level; **6**, **7** - sections showing the dorsal microtubular root (dR) attached to basal body (1) from which arise cortical microtubules (arrow), the desmose structure between the basal bodies (arrowhead) and the origin of the right ventral root (rvR); **8** - origin of the left ventral root (lvR) between the basal bodies (1, 2); basal body connecting link (L); **9** - section showing the right ventral root (rvR) attached to the anterior basal body (1), the Golgi nucleus root (gnR) attached to the posterior basal body (2) and a striated structure (arrows) connecting the basal bodies and the Golgi zone (G); **10**, **11** - dorsal root (dR) connected to basal body (1) and the origin of the cortical microtubules close to this root (arrow); **12** - origin of the left ventral root (lvR) between the two basal bodies (1, 2). Scale bars: 10 µm (3); 1 µm (4-12).



Figs 13-18. Electron micrographs of *S. lacustris*. **13**, **14** - transverse sections of the upper part of the groove where the two flagella (1, 2) arise, dorsal microfibrillar border of the groove (arrow), right ventral root (rvR), left ventral root (lvR), Golgi nucleus root (gnR); **15** - transverse section at the nucleus level (N) showing the microtubules (arrows) accumulated in the two borders of the ventral groove (vG) and microtubules of the left ventral root (lvR); **16-18** - oblique section showing the Golgi nucleus root (gnR) inserted between the Golgi body (G) and the nucleus (N), as well as the two fibrils (rF, lF) that outline the bottom of the groove, the striated connecting cord (C) and pseudopodial processes (arrow) at the bottom of the groove (vG). Scale bars: 1 µm.



Figs 19-20. Electron micrographs of *S. lacustris.* **19** - the dorsal fibril (dF) connected to basal body (2) that adheres to a mitochondrial lobe (M) indenting its surface (inset); **20** - transverse section to show the endocytosis of a bacterium (B) at the bottom of the groove (vG) and the peculiar bridges between the membranes within the Golgi (G) (inset). Scale bars: 1 µm.

drion is a mitochondrial network with tubular or ampullar cristae and an electron-dense matrix. The nucleus is situated on the left dorsal part of the cell and contains a central nucleolus (Figs 2, 4). Digestive vacuoles containing bacteria are scattered within the cytoplasm. These digestive vacuoles are formed at the upper bottom of the groove (Fig. 20), a place where small pseudopods develop (Figs 15-18), and this area is a privileged zone of endocytosis of food particles.

DISCUSSION

This flagellate shows common features with *Diphylleia* and *Collodictyon*, two heterotrophic free-living flagellates with an undetermined phylogenetic position among flagellate groups (Brugerolle and Patterson 1990, Brugerolle *et al.* 2002). The basal part of the flagella has a similar constriction and ultrastructure, and the typical desmose between basal bodies including the origin of the left microtubular root is also similar. The other roots that compose the cytoskeleton are also very similar: the dorsal root is associated with an MTOC for cortical microtubules and the ventral roots outline the ventral groove. Comparatively to *Diphylleia* and *Collodictyon* other features are more specific, such as the kind of rhizostyle that links the mitochondrion to the basal bodies and the limited extension of the Golgi body.

The features of the flagellar apparatus did not allow to suggest a close phylogenetic relationship with any other group of flagellates, despite the mitochondrion with tubular cristae (Taylor 1999). From light microscopy observation, the presence of a deep groove could suggest a phylogenetic relationship with a member of the Excavata super-group (Adl *et al.* 2005), but a precise examination of the flagellar apparatus organization by electron microscopy did not support a close relationship with any of the diverse groups composing the Excavata. The use of SSU rRNA sequences of *Diphylleia* to locate this group in the tree of life has not fulfilled its

promise (Brugerolle *et al*. 2002). Unfortunately this flagellate could not be collected again in the nature in order to identify it by SSU rRNA sequencing and use it for further phylogenetic analysis.

The history of the origin of this species did not indicate that it is a free-living species. However, since free-living protozoa such as a ciliate, a euglenid of the genus *Scytomonas* and a volvocid of the genus *Polytoma* developed within the culture dish, this suggests that they were ingested with food by the young tadpoles. To the best of my knowledge, no such flagellate has been thus far been described in the literature. The only flagellate to date that has a similar morphology as revealed by light microscopy is *Protaspis simplex* Vørs, 1992, but this species has a shallow ventral groove and a marine habitat (Tong *et al.* 1998). Unfortunately there is no electron microscopy image of a *Protaspis* species available to compare with. I propose to create a new species name to identify this flagellate.

Diagnosis

Sulcomonas lacustris n. g., n. sp. from the Latin *sulco* that means groove or furrow and *lacustris* from lake.

Cell of about 10 µm length with two sub-apical flagella and a ventral groove extending across the whole cell length. The anterior flagellum is two times the cell length and the posterior flagellum is three times the cell length. The constriction at the base of the flagella and the desmose between the two basal bodies including a microtubular fibre are typical. The cortical surface is sustained by microtubules that originate from a dorsal MTOC and the ventral groove is outlined by three microtubular roots together with dense fibrils. There is a kind of rhizostyle-like fibril that attaches the posterior basal body to a lobe of the mitochondrion that has ampullar cristae. The Golgi body is situated on the anterior left part above the nucleus. This flagellate lives in freshwater and feeds on bacteria.

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ACTA
PROTOZOOLOGICA

Two Eimerian Coccidia (Apicomplexa: Eimeriidae) from the Critically Endangered Arakan Forest Turtle *Heosemys depressa* (Testudines: Geoemydidae), with Description of *Eimeria arakanensis* n. sp.

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Summary. Coprological examination of nine Arakan forest turtles *Heosemys depressa* freshly imported from Myanmar revealed the presence of two species of *Eimeria*, one of which is described as a new species. Oocysts of *Eimeria arakanensis* n. sp. are broadly oval to subspherical, 28.0 (24-30) × 23.6 (22-25) µm, with a smooth, colourless, uni-layered wall ~ 0.6 µm thick, possessing a large globular oocyst residuum. Sporocysts are ellipsoidal to oval, 12.8 (12-15) × 7 (6-8) µm, with a smooth and colourless sporocyst wall; Stieda body present, knob-like, ~ 1 × 1-2 µm, substieda body homogenous, sub-globular, 1-1.5 × 1.5-2 µm. Additionally, oocysts of *Eimeria* cf. *mitraria* (Laveran *et* Mesnil, 1902) were found in all nine examined turtles. *H. depressa* represents a new host for this coccidium. Host specificity and taxonomy of *E. mitraria*, yet recorded by previous authors from turtles of four families, is discussed.

Key words: Apicomplexa, Coccidia, *Eimeria arakanensis* n. sp., *Eimeria* cf. *mitraria*, Geoemydidae, *Heosemys depressa*, Myanmar.

INTRODUCTION

Although the South and Southeast Asia represent an area of the highest diversity of turtle species (Van Dijk *et al*. 2000), only nine species of *Eimeria* are described from turtles of this region. Six of them parasitize softshell turtles (family Trionychidae) of India (Simond 1901, Das Gupta 1938, Chakravarty and Kar 1943, Kar 1944) and three are eimeriids from turtles of the speciose family Geoemydidae (= Bataguridae). Those in the latter family are *E. mitraria* (Laveran *et* Mesnil,1902) from *Chinemys reevesii* (Gray,1831), *E. kachua* Široký *et* Modrý, 2005 from *Kachuga tentoria circumdata* Mertens, 1969, and *E. patta* Široký *et* Modrý, 2005, parasitizing *Melanochelys trijuga edeniana* Theobald, 1876 (Laveran and Mesnil 1902, Široký and Modrý 2005). Among these species, *E. mitraria* is exceptional in its wide host spectrum and geographical range of distribution, as previous authors have reported it from 12 turtle species of four families.

The Arakan forest turtle *Heosemys depressa* (Anderson, 1875) [for the latest taxonomy see Diesmos *et al*.

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(2005)], is endemic to Myanmar (formerly Burma) (Win Maung and Win Ko Ko 2002), and belongs to the rarest turtles of the world (Iverson and McCord 1997), and than being listed as critically endangered (IUCN 2004). Recently, we had a chance to examine nine specimens of *H. depressa*, freshly imported from western Myanmar. Two species of *Eimeria* were found to parasitize this turtle, one of which is described in this paper as new.

MATERIALS AND METHODS

Nine Arakan forest turtles, *H. depressa*, were imported into the Czech Republic from China and are currently kept alive in a private collection. Originally, these animals were exported by a Chinese pet animal dealer from western Myanmar to China in August 2003. Based on the dealer's information all specimens were freshly caught in the wild, which corresponds with observed ticks' infestation and perfect state of health of the animals. Despite this fact, exact locality of their origin was not given.

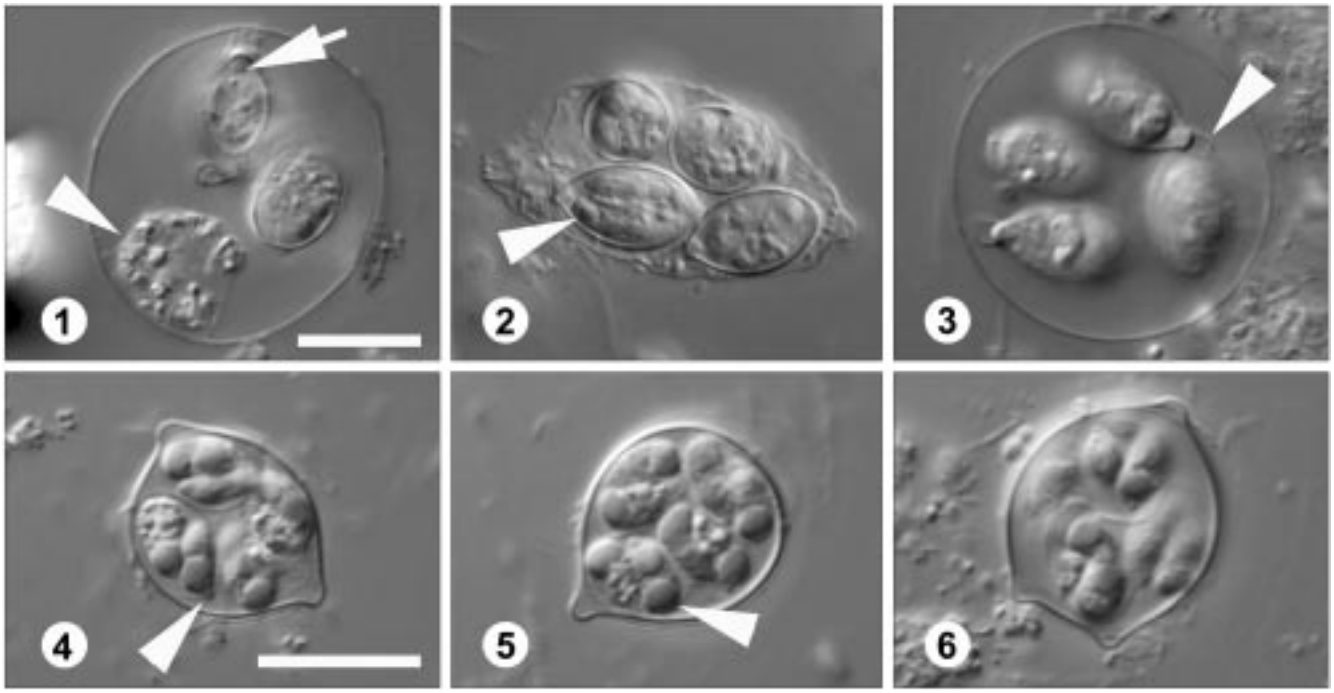
During quarantine, turtles were housed separately in plastic boxes. Individually collected fresh faeces were placed in 2.5% aqueous (w/v) potassium dichromate ($K_2Cr_2O_7$), mixed thoroughly, and then allowed to sporulate in open shallow plastic containers at 20-23°C. Samples were examined after concentration by flotation using a modified Sheather's sugar solution (specific gravity 1.3). Oocysts were measured and photographed using an Olympus Provis AX 70 microscope equipped with Nomarski interference contrast optics (NIC). Morphological features were described according to Duszynski and Wilber (1997), and measurements were made on 30 oocysts using a calibrated ocular micrometer and are reported in micrometers (µm) as means, followed by ranges in parentheses.

RESULTS

Coprological examination revealed eggs of undetermined nematodes and the presence of oocysts of two eimerians. Three *H. depressa* were infected with a thus far undescribed *Eimeria* species; all nine harboured the oocysts of *Eimeria* cf. *mitraria* (Laveran *et* Mesnil, 1902). Moreover, two ticks of the genus *Amblyomma* were found on a single turtle during the initial inspection.

Eimeria arakanensis n. sp. (Figs 1-3, 7)

Description of oocysts: Oocysts are broadly oval to sub-spherical, 28.0 (24-30) × 23.6 (22-25), oocyst shape index (SI, length/width) 1.19 (1.08-1.36), oocyst wall



Figs 1-3. Nomarski interference contrast photographs of oocysts of *Eimeria arakanensis* n. sp., all in the same scale. **1 -** sporulated oocyst, note large oocyst residuum (arrowhead) and subglobular substieda body (arrow). Scale bar: 10 µm; **2 -** sporocysts in collapsed oocyst, note refractile body (arrowhead); **3 -** sporulated oocyst from a polar view, note membranous cupola-like structure covering the Stieda body (arrowhead).
Figs 4-6. Nomarski interference contrast photographs of oocysts of *Eimeria* cf. *mitraria* (Laveran *et* Mesnil, 1902), all in the same scale. **4 -** sporulated oocyst, note the Stieda body (arrowhead). Scale bar: 10 µm; **5 -** sporocysts tightly packed in sporulated oocyst, prominent refractile body is marked by arrowhead; **6 -** overall shape of sporulated oocyst with distinct projections.

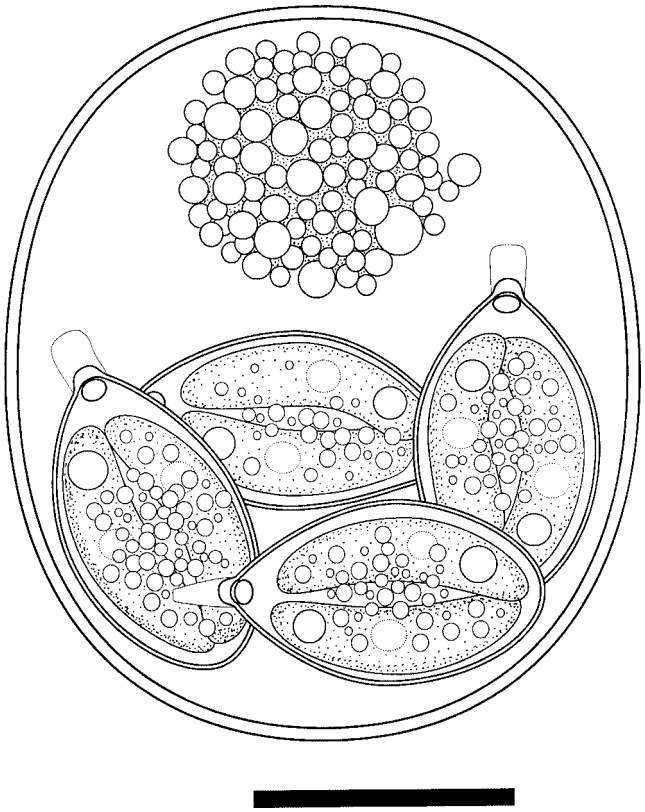


Fig. 7. Composite line drawing of sporulated oocyst of *Eimeria arakanensis* n. sp. Scale bar: 10 μm.

single-layered, colourless, smooth and ~ 0.6 thick. Micropyle and polar granule absent. Globular oocyst residuum composed of fine granules present, measuring approximately 10 - 15 in diameter. Sporocysts ellipsoidal to oval, 12.8 (12-15) × 7 (6-8), sporocyst SI 1.84 (1.63-2.17), with smooth, colourless and less than 0.5 thick sporocyst wall. Stieda body present, knob-like, ~ 1 high and 1-2 wide (n = 12). Substieda body homogenous, subglobular, 1-1.5 high and 1.5-2 wide (n = 12). Very thin, membranous, cap-like structure over-layering Stieda body. Sporocyst residuum present as small granules of irregular sizes usually scattered in high number among sporozoites. Sporozoites elongate, cucumber-shaped, lying head to tail within sporocyst. Each sporozoite bears one sub-spherical to spherical refractile body (~1-2 × 1.5-2) at one end, smaller nucleus (~1-2 in diameter) located sub-centrally.

Type host: Arakan forest turtle, *Heosemys depressa* (Anderson, 1875) (Reptilia: Testudines: Geoemydidae).

Type material: Photosyntypes are deposited at the Department of Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, under collection number R 100/05.

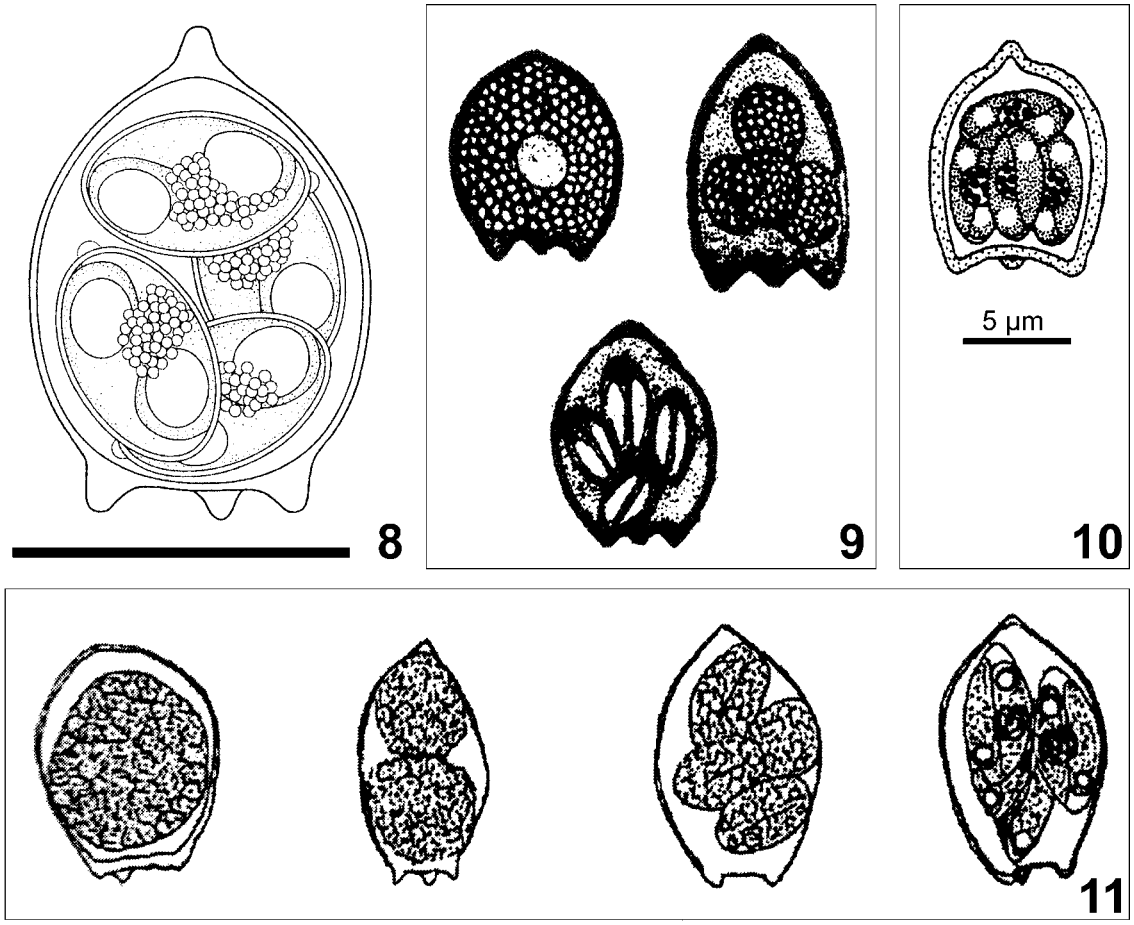
Type locality: Western Myanmar (Burma), detailed locality unknown.

Prevalence: Three of nine examined specimens of *H. depressa* were infected.

Sporulation: Exogenous. Oocyst became fully sporulated within 3-5 days at 20-23°C.

Table 1. Review of turtle species reported to date as hosts of *Eimeria mitraria* (Laveran *et* Mesnil, 1902), asterisk marks the type host.

Host	Locality	Size of oocysts	References
Geoemydidae Theobald, 1868			
Chinemys reevesii (Gray, 1831)*	Asia	15 × 10	Laveran and Mesnil (1902)
Heosemys depressa (Anderson, 1875)	Myanmar	14.9 × 10.1	This study
Emydidae Rafinesque, 1815			
Chrysemys picta bellii (Gray, 1831)	Iowa (USA)	10-15 × 5-11	Deeds and Jahn (1939)
	Iowa (USA)	10 × 7.6	Wacha and Christiansen (1976)
Emydoidea blandingii (Holbrook, 1838)	Iowa (USA)	Not given	Wacha and Christiansen (1980)
Emys orbicularis (Linnaeus, 1758)	Galicia (Spain)	12.1 × 9.5 (10-14 × 7-11)	Segade *et al.* (2004)
Graptemys geographica (Le Seur, 1817)	Iowa (USA)	11 × 8.3	Wacha and Christiansen (1976)
Graptemys pseudogeographica (Gray, 1831)	Iowa (USA)	9.2 × 8.1	Wacha and Christiansen (1976)
Graptemys versa Stejneger, 1925	Texas (USA)	Not given	McAllister *et al.* (1991)
Pseudemys texana Baur, 1893		10 × 8 (8-12 × 6-9)	McAllister and Upton (1989)
Trachemys scripta elegans (Wied-Neuwied, 1839)	Texas (USA)	Not given	McAllister and Upton (1988)
		10 × 8 (8-12 × 6-9)	McAllister and Upton (1989)
Chelydridae Agassiz, 1857			
Chelydra serpentina serpentina (Linnaeus, 1758)	Iowa (USA)	Not given	Wacha and Christiansen (1980)
Kinosternidae Agassiz, 1857			
Kinosternon flavescens flavescens (Agassiz, 1857)	Texas (USA)	10 × 8 (8-12 × 6-9)	McAllister and Upton (1989)
Kinosternon flavescens spooneri Smith, 1951	Iowa (USA)	11.5 × 8.6	Wacha and Christiansen (1976)



Figs 8-11. Comparison of *E. mitraria* from *H. depressa* with drawings from previous studies. **8 -** composite line drawing of our isolates of *E. mitraria*. Scale bar: 10 µm. **9 -** *E. mitraria* from original description (Laveran and Mesnil 1902). **10 -** *E. mitraria* from nearctic turtle species (modified after Wacha and Christiansen 1976). **11 -** process of sporulation of *E. mitraria* from *Chrysemys picta bellii* (after Deeds and Jahn 1939).

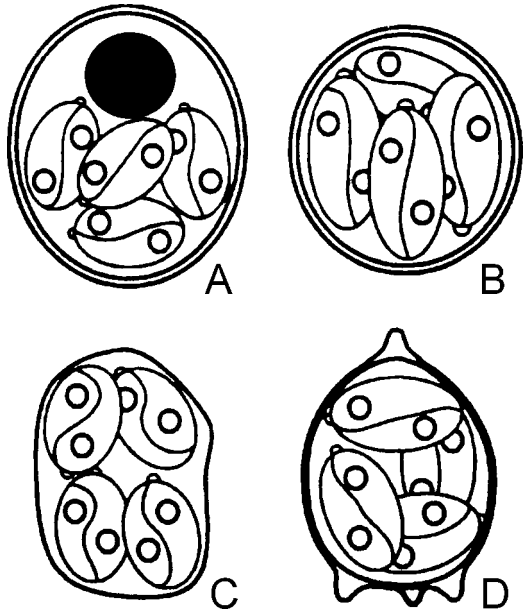
Site of infection: Unknown, collected from faeces.

Etymology: The specific epithet *arakanensis* is derived from the name of Arakan hills, the "terra typica" of the type host.

Remarks: *Eimeria brodeni* Cerruti, 1930 described from *Testudo graeca* Linnaeus, 1758 from Cagliari, Sardinia, Italy is the only *Eimeria* from turtles of the Testudinoidea that resembles *Eimeria arakanensis* n. sp. morphologically. However, oocysts of *E. brodeni* are narrower and lack an oocyst residuum (Cerruti 1930). *E. chrysemydis* Deeds *et* Jahn, 1939 from *Chrysemys picta bellii* (Gray, 1831) from Iowa, USA possesses oocysts of similar size, but are pear-shaped (Deeds and Jahn 1939) compared to broadly oval of *E. arakanensis* n. sp. All other coccidian species from testudinoid hosts are significantly smaller.

***Eimeria* cf. *mitraria* (Laveran *et* Mesnil, 1902) (Figs 4-6, 8-11)**

Description of oocysts: Oocysts ovoid, 14.9 (13-16) × 10.1 (9-12); SI is 1.48 (1.27-1.78); micropyle, polar granule and oocyst residuum absent. Oocyst wall smooth, colourless, under light microscopy single-layered, ~ 0.5 thick. Each pole of oocyst bears blunt conical projections, ~ 1.2 (1-1.5) long. Most oocysts possess three projections on one ("flat") pole, and one on the opposite ("conical") end, giving the oocyst projectile-like appearance. Nevertheless, specimens with two and three projections were rarely seen. Sporocysts oval to ellipsoidal, 6.8 (6-8) × 4.2 (3.5-5), with smooth and colourless sporocyst wall and sporocyst SI 1.64 (1.3-2). Stieda body present as tiny knob-like projection. Substieda



Figs 12A-D. Schematic drawings of morphological types of oocysts of *Eimeria* spp. described from turtles. **A** - oocysts of various shape possessing distinct oocyst residuum, **B** - elliptical, sub-spherical to spherical oocysts lacking oocyst residuum; **C** - thin-walled oocysts with shape influenced by position of sporocysts inside, and lacking oocyst residuum; **D** - mitra-shaped (*Eimeria mitraria* - like) oocysts without oocyst residuum.

body not discernible. Sporozoites elongate, arranged head to tail within sporocyst. Each sporozoite bears a prominent spherical to sub-spherical refractile body (~ 2-3 × 2) at one end, nucleus not discernible. Sporocyst residuum present, usually as compact granular sphere, ~ 2-3 in diameter.

DISCUSSION

Eimerian parasites are traditionally considered to be highly host specific (Duszynski 1986) and host systematics and geographic origin are common tools in their taxonomy. However, strict host specificity of *Eimeria* spp. parasitizing reptiles was repeatedly questioned (i.e. Wacha and Christiansen 1974, 1976; McAllister and Upton 1989). Thus, *Eimeria* spp. described and named from turtles of families Emydidae, Geoemydidae, and Testudinidae [superfamily Testudinoidea sensu Gaffney and Meylan (1988)] are considered in the differential diagnosis of *E. arakanensis* n. sp. given above.

Genus *Eimeria* represents the most specious genus within both protozoan and metazoan organisms. Until recently, more than 1700 named *Eimeria* species have been described on the base of the qualitative and quantitative traits of their sporulated oocyst and their host specificity (Duszynski and Upton 2001). Despite the great oocyst variability, there is an evident trend of repetition of typical morphological features in *Eimeria* species, originating from different hosts from different zoogeographic regions. Thorough evaluation of oocyst morphology reveals many such "morphotypes". *Eimeria leukarti* Reichenow, 1940 from horses and *E. cameli* Reichenow, 1952 from dromedaries can be used as a most prominent example of such similarity. Recent studies on rodent and bat *Eimeria* species revealed that some morphological features (namely the presence of the oocysts residuum) show a clear correlation to the phylogenetic relationships as determined by the molecular genetic methods (Zhao and Duszynski 2001a,b; Zhao *et al*. 2001). It is probable, that further studies reveal phylogenies, showing even more such "subgroups" within evidently polyphyletic genus *Eimeria*.

Also among chelonian *Eimeria*, the oocyst shape and absence vs. presence of the oocyst residuum can be used to group species together (Fig. 12). Howsoever arbitrary such a grouping looks like, above-mentioned case of rodent coccidia suggests the evolutionarily significance of some aspects of the oocyst morphology.

Then, the following morphological types can be delimited within chelonian *Eimeria*: (**a**) species with oocysts of various shape possessing an oocyst residuum, i.e. *E. chrysemydis* Deeds *et* Jahn, 1939; *E. cooteri* McAllister *et* Upton, 1989; *E. delagei* (Labbé, 1893); *E. kachua* Široký *et* Modrý, 2005; *E. marginata* Deeds *et* Jahn, 1939; (**b**) species with elliptical, sub-spherical to spherical oocyst without the oocyst residuum (*E. geochelona* Couch, Stone, Duszynski, Snell *et* Snell, 1996; *E. koormae* Das Gupta, 1938; *E. mascoutini* Wacha *et* Christiansen, 1976; *E. paynei* Ernst, Fincher *et* Stewart, 1971; *E. serpentina* McAllister, Upton *et* Trauth, 1990); (**c**) *Eimeria* spp. with peculiar thin walled oocysts of polymorphic shape without oocyst residuum (*E. motelo* Hůrková, Modrý, Koudela *et* Šlapeta, 2000; *E. patta* Široký *et* Modrý, 2005); and, (**d**) species with *Eimeria mitraria*-like oocysts, which also lack oocyst residuum and typically possess peculiar projections of the oocyst wall [*E. mitraria* (Laveran *et* Mesnil, 1902); *E. stylosa*, McAllister *et* Upton, 1989]. At least the latter case is probably a case of synapomorphy rather than convergence and it is possible, that actually each of these morphologically defined groups represent a separate evolutionary lineage, emergence of which predates the separation of continents.

Chelonians of Eurasian and American continents have been geographically separated since the middle Miocene (Danilov 2005). Originally, *E. mitraria* was described from *Chinemys reevesii* from SE Asia. Later authors considered further eimerian isolates from 10 Nearctic turtle taxa of other three families (including phylogeneticaly distant families Chelydridae and Kinosternidae) to be conspecific with *E. mitraria* (Table 1, Figs 8-11). Moreover, we currently found morphologically very similar oocysts in Neotropical chelid turtle *Batrachemys heliostemma* McCord, Joseph-Ouni *et* Lamar, 2001 from Peru (data not shown).

It is hard to imagine that protozoan with a short generation intervals can survive for 15 millions years without further speciation. Thus, wide occurrence of *E. mitraria* in hosts of two suborders in two distant continents makes the conspecificity of isolates rather improbable. Moreover, all *E. mitraria*-like coccidia reported to date from Nearctic turtles have smaller oocysts (Table 1) comparing to the size given for *E. mitraria* by Laveran and Mesnil (1902). We believe that mitra-shaped oocysts reported to date from 13 turtle hosts (McAllister and Upton 1989, Lainson and Naiff 1998, Segade *et al*. 2004) is a common, synapomorphic trait of *Eimeria* from aquatic turtles. Thus, *E. mitraria*, as presently used, possibly represents a "morphotype" rather than a species. Similar cases can be found in coccidia of other hosts; i.e. *Eimeria callospermophyli* Henry, 1932 is reported from both Palaearctic and Nearctic ground squirrels (Wilber *et al*. 1998).

So far, a universal species concept is not even discussed in the area of coccidian taxonomy and variable combination of morphological, biological and evolutionary species concepts is used throughout the taxonomical studies by various authors. Such a discouraging situation calls for intense research and broad, interdisciplinary discussion aimed to provide a species concept adequate to the current state of knowledge.

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The Variation of Testacean Assemblages (Rhizopoda) Along the Complete Base-Richness Gradient in Fens: A Case Study from the Western Carpathians

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Summary. Most recent ecological studies of testate amoebae were conducted in acid *Sphagnum*-dominated mires. Little data exists about the variation in testacean assemblages along the complete gradient from extremely poor acid fens to strongly calcareous spring fens. This so called poor-rich gradient is the most important gradient for the majority of mire biota and can display a time dimension as well. In order to fill this gap we studied modern testacean assemblages in spring fens in the Western Carpathians (Czech Republic and Slovakia). The DCA of all testacean assemblages revealed two major factors controlling species variation, the poor-rich gradient and the microhabitat-type: waterlogged bare sediments versus bryophytes. Sampled sediments of poor acidic fens have species composition similar to that of bryophyte tufts from mineral-richer fens. The ordination of the subset of samples taken from bryophyte tufts shows the clear poor-rich gradient on the first axis and moisture gradient causing variation of "acid" samples along the second axis. Based of DCA results, testacean assemblages were divided into six major types for which the diagnostic species were determined using *phi*-coefficient. Nearly all types are dominated by *Euglyphidae*, *Centropyxidae*, *Cyclopyxidae* and *Difflugiidae* were characteristic for calcareous habitats. Species richness did not correlate with the poor-rich gradient. The species variation in testate amoebae assemblages is better predicted by both the species composition of the vegetation as a whole and by the composition of bryophyte tuft than by long-term averages of water chemistry variables. The autecology of many species living in acid *Sphagnum* fens corresponds well with published data with several exceptions. On the other hand, the study of the complete poor-rich gradient revealed different species optima reported from higher pH *Sphagnum* fens, and thus it changed our view on the indicative value of some species.

Keywords: bog, calcium, fen, mire, peatlands, pH, *Sphagnum*, spring, testaceans, testate amoebae, vegetation, wetland.

INTRODUCTION

Testate amoebae (Rhizopoda) are often studied in both active *Sphagnum* bogs and Holocene peat deposits. They are used as biondicators of past and present environmental changes because of (i) their high abundances in wetland habitats (e.g. Warner 1987, Charman and Warner 1992, Booth 2001), (ii) well-defined ecological preferences (e.g. Tolonen *et al.* 1992, Bobrov *et al.* 1999, Lamentowicz and Mitchell 2005), (iii) rapid response to environmental change (e.g. Jauhiainen 2002, Mitchell 2004) and (iv) possibility of species-level identification even in fossil peat sections (Tolonen 1986). Numerous ecological studies dealing with recent species responses to the water level gradient in *Sphagnum* mires provide a good basis for the usage of testate amoebae as paleoindicators of past hydrological changes

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(Hendon *et al.* 2001, Booth 2002, Schnitchen *et al.* 2003, Davis and Wilkinson 2004, Caseldine and Gearey 2005) and also as recent indicators of hydrological changes during restoration or ongoing climate change (Jauhiainen 2002, Kishaba and Mitchell 2005, Schoning *et al.* 2005). However, the responses of testate amoebae species to other environmental factors such as pH and mineral richness are less known in mires. The pH and calcium concentration are the major factors controlling species distribution of mire plants especially at a landscape scale (variation between individual mires) (Waughmann 1980, Vitt 2000, Hájková *et al.* 2004, Nekola 2004, Tahvanainen 2004). The botanical composition, vegetation structure and species richness all change along this base-richness gradient from strongly acid bogs and poor fens to the extremely-rich fens. The high concentrations of calcium and hydrogencarbonates in extremely-rich fens make the occurrence of even calcitolerant *Sphagna* impossible and the so called "brown mosses" (mostly *Amblystegiaceae*) are therefore dominant (Gorham and Janssens 1992, Vitt 2000). The extremely high mineral concentration leads to calcium carbonate (tufa) precipitation (Boyer and Wheeler 1989, Hájek *et al.* 2002) and the occurrence of (sub)halophytic conditions (Cooper 1995, Horsák 2006). This conspicuous species turnover was named poor-rich fen gradient (e.g. Malmer 1986, Økland *et al.* 2001, Hájek *et al.* 2002, Tahvanainen 2004). Changes in species composition along the poor-rich gradient have also been observed in molluscan (Horsák and Hájek 2003), algal (Poulíčková *et al.* 2003) and fungal (Vašutová 2005) communities. Because of this, there is a strong presumption that the same pH/calcium gradient should also influence the variation in testacean assemblages, but current ecological knowledge does not provide sufficient evidence. Most ecological studies of testate amoebae have been conducted in acid, ombrotrophic or only slightly minerotrophic mires (Charman and Warner 1992, Bobrov *et al.* 1999, Mitchell *et al.* 2000). Whenever less acid habitats were included they were either strongly underrepresented (Mitchell *et al.* 1999, Lamentowicz and Mitchell 2005) or represented by the habitats still populated by *Sphagnum* species (Heal 1961, Warner 1987, Charman and Warner 1992, Booth and Zygmunt 2005). Tolonen *et al.* (1992, 1994) included several "brown-moss" rich fens in their study, but these were slightly acid or subneutral and non-calcareous, with the calcium concentration not exceeding 9 mgl^{-1}. Some studies present data on testate amoebae in truly calcareous wetlands, but only this habitat type was studied as opposed to the entire poor-rich fen gradient (Casper and Schönborn 1985, Bonny and Jones 2003, Mattheeussen *et al.* 2005). We therefore hypothetise that the often reported fact, that moisture is the primary and pH only the secondary factor controlling variation in testate amoebae assemblages (Warner 1987, Charman and Warner 1992, Tolonen *et al.* 1994, Charman 1997, Bobrov *et al.* 1999, Mitchell *et al.* 2000), is primarily due to the rather short base-richness gradient studied. The importance of a length of the gradient for appreciating of environmental control of species-data variation is evident from the results of Booth (2001), who found water-table depth to be the most important factor for variation in testacean assemblages from *Sphagnum* substrate, whereas pH turned to be the most important factor when the entire data set including non-*Sphagnum* samples was analysed. Nevertheless, even Booth did not include the complete base-richness gradient in his study as the most "basicole" species had pH optima between pH 5.5 and 6.0. In another study, Booth and Zygmunt (2005) described the species composition of some mineral-rich *Sphagnum*-fens in the Rocky Mountains, which were also rather acid. The lack of recent information about testate amoebae preferences along the poor-rich gradient has important consequences in ecology, i.e. an unprecise interpretation of Holocene environmental change during succession from minerotrophic cyperaceous-rich fens to ombrotrophic bogs and an insufficient partitioning of the effects of pH and water regime in the habitats where these two variables coincide. In order to fill this apparent gap in ecological knowledge, we studied the recent testacean assemblages in spring fens in the Western Carpathian flysh zone (Czech Republic and Slovakia). The study region represents a suitable model area where particular, isolated spring fens vary from calcium-poor acid fens tending to ombrotrophy (about 2 mgl^{-1} of calcium) to extremely calcium-rich petrifying fens (to 300 $mg.l^{-1}$ of calcium). The poor-rich gradient is developed here at the landscape level, independent of the moisture gradient which is displayed only at the within-site scale (Hájková *et al.* 2004). Additionally, the complete poor-rich gradient covered in this study area represents a good opportunity to study the relationships among the botanical composition of the fen, of the moss sample, and the composition of testacean assemblages. The only attempt to test such a relationship was done by Mitchell *et al.* (2000), who found only marginally significant correlations between plant- and testacean species compositions in acidic mires. In the Western Carpathian spring fens, the existing studies showed that the botanical composi-

tion of a fen site or of a moss sample, as expressed by DCA site scores, is a better determinant of variation in the species composition of molluscan, algal and fungal assemblages than the directly measured environmental data including water chemistry and moss water content (Horsák and Hájek 2003, Poulíčková *et al.* 2004, Vašutová 2005). By analogy, vascular plant species composition contributes significantly to water pH when explaining variation in bryophyte assemblages (Hájková and Hájek 2004). In this study, we therefore aimed not only to reveal the species distribution pattern along the poor-rich gradient, but also to test the relative importance of plant species composition and directly measured water-chemistry data for the explanation of variation in testacean assemblages in fens.

MATERIALS AND METHODS

Study area

The Western Carpathians have been chosen due to the variable chemistry of aquifers and, on the other hand, due to similarities in hydrological characteristics and in the origin of spring fens within this area (Rybníèková *et al.* 2005). The study area is located on the western margin of the Western Carpathians and forms part of the flysch belt, in which sandstone and claystone of variable calcium content alternate. Marls, lime-rich claystone, calcareous sandstone and limestone prevail in the south-western part of the study area. Groundwaters are carbonatogenic there and their dominant mineralization process is carbonate dissolution which leads to the calcium-(magnesium)-bicarbonate type of chemistry (Rapant *et al.* 1996). This chemistry type supports cold water travertine (tufa) formation. Not only the extreme values of calcium concentration, but also the high magnesium concentration characterises the springwaters of this geological unit (Hájek *et al.* 2002). Towards the north-east the groundwater is characterised by constantly high concentrations of calcium, but lower contents of magnesium and higher contents of sodium, potassium and iron (Rapant *et al.* 1996, Hájek *et al.* 2002). The northern part of the study area is formed mostly by decalcified, often iron-cemented sandstone which causes that the hydrolythic dissolution of silicates at the interface between water and psammitic-psephitic rocks dominate in a mineralisation process. In this part the calcium concentration is the lowest within the entire study area. Altitude, humidity, temperature, and geographical position correlate with the main chemical gradient, but water chemistry seems to have the most important influence on spring biota (Hájek *et al*. 2002, Horsák and Hájek 2003). Calcium-rich rocks are situated in the south-western part, which is warmer (annual mean temperature is about 8°C) and drier (annual mean precipitation is about 700 mm). The altitudes of the south-western fens are lower though always exceeding 340 m and springs often develops on rather steep slopes. On the contrary, north-eastern fens are located at higher altitudes (max. 911 m) but at more gentle slopes. In addition, the climate of the northeastern part is more humid and cooler. The mean annual precipitation often reaches more than 1400 mm; the mean annual temperature can decrease to *ca* 5°C (Vesecký 1966)

Fourteen sites distributed along the complete water-chemistry and geographical gradients were selected for the research (Fig. 1). In all these sites, monitoring of water chemistry was conducted three times a year during 1999-2003. In order to obtain a balanced set of habitat types, seven localities were selected within calcareous spring fens with different degree of calcium carbonate precipitiation (Hrnčiarky, Hrubý Mechnáč, Valašské Kloubouky, Semetín, Jasenka, Hrubé Brodské and Kelčov) and seven within *Sphagnum*-fens with different share of calcitolerant and calcifobe *Sphagnum* species (Zajacovci 1, Zajacovci 2, Polková, Jančíkovci, Biely Kríž, Obidová 1, and Obidová 2). For the details about vegetation and geographical coordinates see Appendix 1, for further details and information about site selection see Hájek and Hekera (2004) and Poulíčková *et al.* (2005).

Vegetation sampling and analysing

Vegetation was recorded in 16 m^2 plots (Chytrý and Otýpková 2003) and using the nine-degree Braun-Blanquet cover scale modified by van der Maarel (1979). All vegetation plots used in this paper are the same as those utilized in a more detailed study of vegetation-water chemistry relationships in the study area (Hájek *et al.* 2002). In addition, the bryophyte species composition of the sample for testacean research was recorded using simple four-degree scale (3 - dominance; 2 - sub-dominance or co-dominance; 1 - presence; 0 - absence). Nomenclature follows Kubát *et al*. (2002) for vascular plants and Kučera and Váňa (2003) for bryophytes.

The vegetation plots were subjected to detrended correspondence analysis (DCA) using CANOCO software. The first axis was interpreted clearly as the poor-rich vegetation gradient (Fig. 2). The site scores on the first DCA axis were used as an environmental variable in further analyses. By analogy, the bryophyte composition of 29 samples for testate amoebae research was also subjected to DCA, nine samples from non-bryophyte substrate (sediments) were omitted. The site scores on the first axis, interpreted as a combined gradient of base-richness and moisture, see Fig. 3, were also used as an environmental variable in further analyses to involve moisture variation. The moisture characteristics were not measured directly in this study.

Water chemistry sampling

Water samples were collected from permanent plots (shallow pits), which were located in the surroundings of the major stream or headspring at each locality. The shallow pits were always re-exposed and water was pumped-out of them and allowed to refill before sampling. The contact of sampled springwater with air was short. The groundwater was several centimeters below the surface in many cases. Water conductivity, pH and redox-potential were measured in situ using portable instruments (CM 101 and PH 119, Snail Instruments). The readings were standardized to 20°C (pH, conductivity) and Ag/AgCl reference electrode (redox-potential). Conductivity caused by hydrogen ions was subtracted (Sjörs 1952). Afterwards, water was placed in plastic bottles using a syringe. Preservatives were added to divided samples: for metallic elements, 0.5 ml of 65% HNO_3 per 100 ml of sample; for anions, 3 ml of chloroform per 1000 ml.

Since most water samples were turbid due to colloidal suspensions, filtration or centrifugation (4000 turns per minute, 10 minutes)

was necessary. The concentrations of sulphates, phosphates, nitrates, ammonium ions, and chlorides were determined by DR 2000 spectrophotometry following colour reactions with certificated HACH-reagents Metallic and semimetallic cation (Ca^{2+}, Mg^{2+}, Si^{4-}, K^{+}, Na^{+} and Fe^{3+}) concentrations were determined using a GBC AVANTA atomic absorption spectrometer. All plots were sampled from 1999 to 2003, three times a year (April, July and October). The values of all seasonal measurements were averaged in this study. The calcium concentrations were transformed logarithmically to approximate normal distribution.

Testacean data collecting

Samples for testacean research were taken on the 11th and 12th July 2002. Several samples of moss tufts were taken at each locality. In addition, bottom sediments from small depressions were also sampled. The moss samples were transported in live state to the laboratory and particular bryophyte species were identified (det.: M. Hájek); bottom sediments were preserved in 4% formaldehyde. Mosses were moistened before handling. After extracting the water, 0.1 ml of extract was analysed. Testate amoebae were identified (det.: V. Opravilová) and the total number of individuals was counted. Nomenclature of testate amoebae follows Aescht and Foissner (1989). The complete list of all recorded species is published in Opravilová (2005).

Testacean data processing

The number of testate amoebae individuals was transformed to the scale appropriate for ordination analyses. The highest number of individuals found in the whole species-by-sample matrix was considered as 100%; all other numbers were transformed into the percentage scale. This method was applied in order to avoid over-weighting of species in the samples with low total number of individuals. The resulting percentage scale was logarithmically transformed in ordination analyses. This transformation allowed us to focus primarily on species composition of the testacean assemblage and its changes along environmental gradients. The changes in species abundances were suppressed in this way. Transformed abundance values were subjected to indirect ordination analysis, the detrended correspondence analysis (DCA) with downweighting of rare species, which revealed the main directions of variation in testacean assemblages. The analysis showed, among others, that the substratum type influenced substantially the species composition of the assemblage and impedes an axes interpretation. Therefore, one more ordination was done using only testacean data from bryophytes samples. Both ordination analyses showed that six clear types of assemblages, nearly discontinuously delimited from each other, can be distinguished. The diagnostic species for these six clear types of assemblages were calculated using the *phi*-coefficient. This coefficient shows the concentration of the species in the group of samples - a higher frequency of the species in the group as compared to the species frequency outside the group means a higher *phi*-coefficient (Chytrý *et al.* 2002). In order to calculate *phi*-coefficient properly, all six groups were standardised to equal size whereas the size of target group is always one sixth of the total data set. Zero fidelity was given to the species with the significance of non-random occurrence in target group yielded by Fisher´s exact test lower than 0.01. JUICE software (Tichý 2002) was used for this computation. In addition, the percentage representation of particular testacean families in samples of six major types was calculated. The total number of individuals found in each sample group was 100% in this case.

All ion concentrations, altitude, slope degrees, DCA site scores of the vegetation and of the bryophyte sample composition and categorical variable indicating character of substratum (bryophytes versus bare sediments) were used as explanatory variables in a canonical correspondence analysis with forward selection of environmental variables. The Monte-Carlo permutation test was used to test the usefulness of each variable to extend the set of explanatory variables used in the ordination model (Lepš and Šmilauer 2003). The goal of this analysis was to find the best set of predictors of species composition of testate amoebae assemblages. The analysis was conducted separately for (i) entire data set and (ii) assemblages from bryophyte samples only.

The correlation between species richness (the number of species found in an assemblage) and environmental factors was assessed using the Pearson´s correlation coefficient.

RESULTS

The DCA of all testacean assemblages revealed two major factors controlling species variation in testacean assemblages. The first axis represented the poor-rich gradient from calcareous spring fens to poor acid spring fens tending to ombrotrophy. The second axis separated samples taken in bare waterlogged sediments, not populated by bryophytes, from the bryophyte samples. The DCA scatter (Fig. 4a) showed an interesting ecological pattern, the shift in species composition of the community from poor-fen sediments towards that of circumneutral *Sphagnum*-fens and, in one case, even towards base-rich “brown-moss” fens. The basicole species forming the left part of the scatter (Fig. 4b, *Difflugia rubescens* Penard, *Difflugia bryophila* Penard, *Difflugia pyriformis* Perty and *Quadrulella symmetrica* (Wallich)) had their optima in calcareous waterlogged bare sediments. *Difflugia bryophila* and *Cyphoderia ampulla* (Ehrenberg) were the best indicators of bare sediments in our study reaching 78% frequency and *phi*-coefficient > 0.5 in samples from sediments. The right part of the scatter plot was composed of acidophilous species that were distributed along the second axis representing a moisture gradient. The ordination of the subset of samples taken from bryophyte tufts showed the clear base-richness gradient from calcareous fens to mineral-poor acid ones. Poor fen samples were divided into drier hummock microhabitats and wetter microhabitats along the second axis. Four separate and ecologically defined sample groups appeared in the scatter plot (Fig. 5a). The position of only one from the 38 samples was unexpected: one assemblage from high-productive calcare-

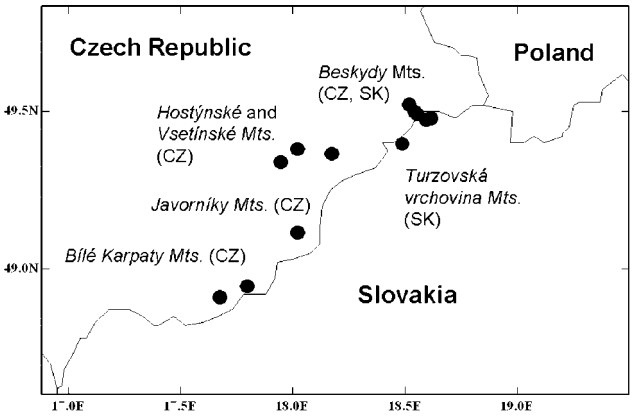

Fig. 1. Distribution of study sites in the Western Carpathians. Coordinates are in WGS 84 system.

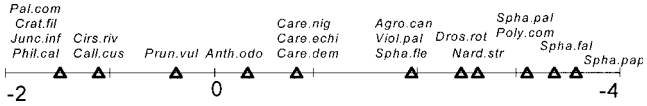

Fig. 2. Position of the most important plant species along the first DCA axis. Species with a fit above 50% and weight above 10% were selected. For the full names of the species see Appendix 2.

Fig. 3. Position of the most important species along the first CA axis of the bryophyte sample composition. Species with a fit above 10% were selected into the graph. For the full names of the species see Appendix 2.

ous fen was placed among moderately-rich *Sphagnum* fens.

The species distribution pattern (Fig. 5b) also showed a higher variation in acid habitats, from wet assemblages with *Sphenoderia fissirostris* Penard to *Assulina seminulum* (Ehrenberg) assemblages from drier microhabitats. The species *Arcella catinus* Penard and *Cyclopyxis eurystoma* Deflandre occupied drier microhabitats on the transition between poor- and moderately-rich fens. The species *Difflugia bryophila* Penard, *Difflugia pyriformis* Perty, *Centropyxis constricta* (Ehrenberg), *Centropyxis cassis* Deflandre had their optima in bryophyte tufts of calcareous fens.

The species *Pseudodifflugia gracilis* Schlumberger were the best indicators of calcareous fen sediments (Table 1). Four other species displayed notable fidelity to this microhabitat type. *Cyphoderia ampulla* (Ehrenberg) reached the lowest fidelity as it overlapped into bryophyte tufts of moderately-rich *Sphagnum*-fens. *Arcella hemisphaerica* Perty characterised poor- and moderately-rich fen sediments. Seven species were characteristic for bryophyte tufts of calcareous fens, but only *Paraquadrula irregularis* (Archer) and *Centropyxis discoides* Penard were restricted to bryophyte tufts practically not living in bare sediments. Bryophyte tufts of moderately-rich *Sphagnum*-fens were characterised by *Arcella discoides* Ehrenberg. The poor acid fens had a large group of diagnostic species. The moisture gradient in poor fen assemblages was evident. The species diagnostic of poor fen lawns overlapped to poor-fen sediments and moderately-rich fen bryophytes and displayed therefore lower fidelity values, e.g. *Nebela collaris* (Ehrenberg) *Phryganella acropodia* (Hertwig and Lesser), *Sphenoderia fissirostris* Penard. The species diagnostic of *Sphagnum* hummocks seemed, on the other hand, not to tolerate mineral-richer habitats and reach high fidelity to this microhabitat (*Nebela militaris* Penard, *Nebela tincta* (Leidy), *Assulina seminulum* (Ehrenberg), *Assulina muscorum* Greeff, *Heleopera petricola* Leidy), with the exception of *Corythion dubium* Taranek and *Nebela bohemica* Taranek that had a wider ecological amplitude.

The analysis of the family level in the six major types of assemblages (Table 2) showed that all types were dominated by *Euglyphidae* with the exception of calcareous bare sediments dominated by *Centropyxidae, Cyclopyxidae* and *Difflugiidae*. *Euglyphidae* prevailed nearly absolutely in bryophyte tufts of moderately-rich fens. Acid, calcium-poor sediments were also characterised by *Pseudodifflugiidae* and *Arcellidae*. *Hyalospheniidae* characterised all extremely acid habitats, especially *Sphagnum* hummocks. On the other hand, a high share of *Centropyxidae* was a characteristic of both calcareous habitats.

The species variation of testate amoebae assemblages was best predicted by the species composition of the vegetation as a whole (Table 3). The site score of the vegetation plot on the first DCA axis accounted for more variation in testacean assemblages than did the long-term (i.e. more precise) averages (1999-2003) of water chemistry variables. The composition of bryophyte tuft was the second most important factor and the type of the substratum (bare sediment versus mosses) was the third one.

Table 1. List of species that differentiate six principal types of testacean assemblages presented in Figs 4, 5. The numbers represent the values of standardised *phi*-coefficient of the species concentration in a particular cluster. Zero fidelity is given to the species with significance of non-random occurrence in target group yielded by Fisher´s exact test lower than 0.01.

Group No.	1	2	3	4	5	6
Number of samples	5	4	9	5	6	9
Species differentiating sediments of calcareous fens						
Pseudodifflugia gracilis	80.8	.	.	.	.	.
Difflugia glans	74.9	.	.	.	.	.
Difflugia gramen	74.5	.	.	.	.	.
Cyclopyxis kahli	62.2	.	.	.	.	.
Cyphoderia ampulla	50.6	.	.	.	.	.
Species differentiating sediments of poor and moderately-rich fens						
Arcella hemisphaerica	.	72.5	.	.	.	.
Species differentiating bryophyte tufts of calcareous fens						
Paraquadrula irregularis	.	.	81.1	.	.	.
Centropyxis discoides	.	.	63.2	.	.	.
Centropyxis constricta	.	.	52.3	.	.	.
Centropyxis aculeata oblonga	.	.	51.3	.	.	.
Difflugia pyriformis s.l.	.	.	45.5	.	.	.
Trinema enchelys	.	.	33.2	.	.	.
Centropyris cassis	.	.	32.0	.	.	.
Species differentiating bryophyte tufts of moderately-rich fens						
Arcella discoides	.	.	.	54.4	.	.
Species differentiating bryophyte lawns of poor acid fens						
Trinema complanatum	.	.	.	.	56.9	.
Euglypha cristata	.	.	.	.	53.5	.
Sphenoderia fissirostris	.	.	.	.	47.9	.
Nebela collaris	.	.	.	.	39.9	.
Phryganella acropodia	.	.	.	.	38.5	.
Species differentiating bryophyte hummocks of poor acid fens						
Nebela militaris	.	.	.	.	.	69.7
Nebela tincta	.	.	.	.	.	67.4
Assulina seminulum	.	.	.	.	.	59.5
Heleopera petricola	.	.	.	.	.	61.7
Corythion dubium	.	.	.	.	.	41.9
Nebela bohemica	.	.	.	.	.	34.4
Species differentiating both poor-fen lawn and poor-fen hummocks						
Assulina muscorum	.	.	.	.	48.4	48.4

No environmental variable correlated with the species richness of testate amoebae assemblages, except for a marginally significant and difficult-to-interpret iron concentration. We observed no trends even using non-linear techniques. For these reasons, the correlation coefficients are not shown in this paper.

DISCUSSION

The poor-rich gradient

Our data set from the poor-rich fen gradient was governed by a dominant primary gradient of pH and

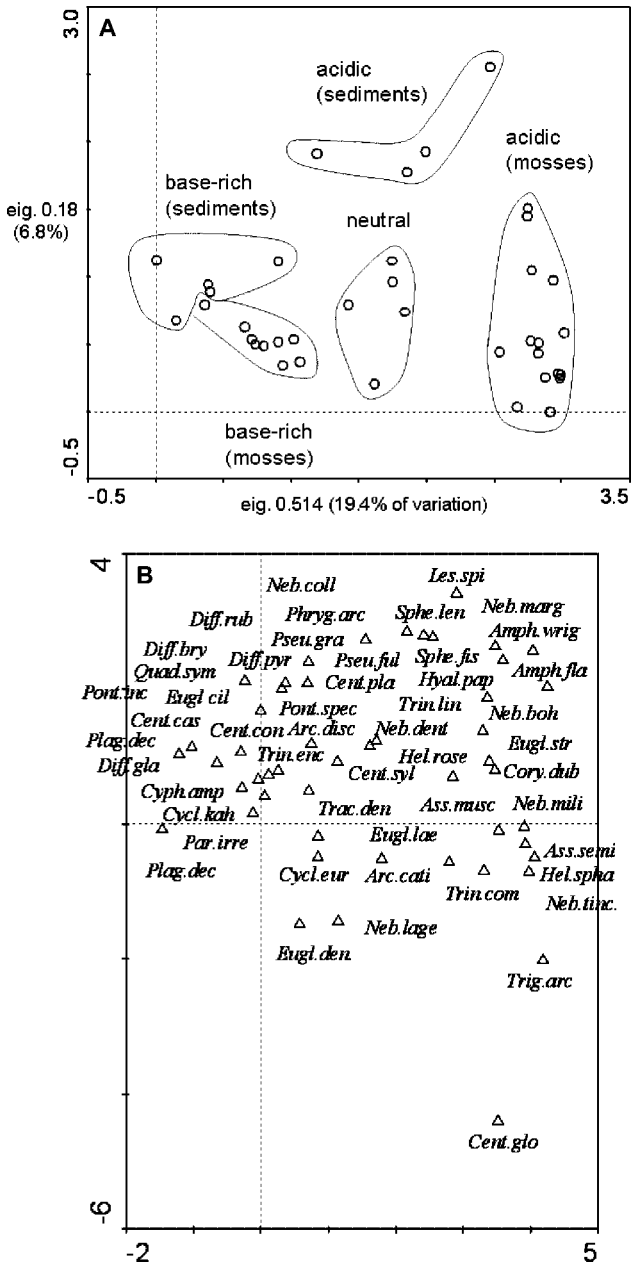

Fig. 4. Detrended correspondence analysis of all testatean assemblages (A) and their species (B). Ecologically interpretable groups of assemblages are indicated, eigenvalues and percentage of explained variance are presented at the axes. For the full names of the species see Appendix 2.

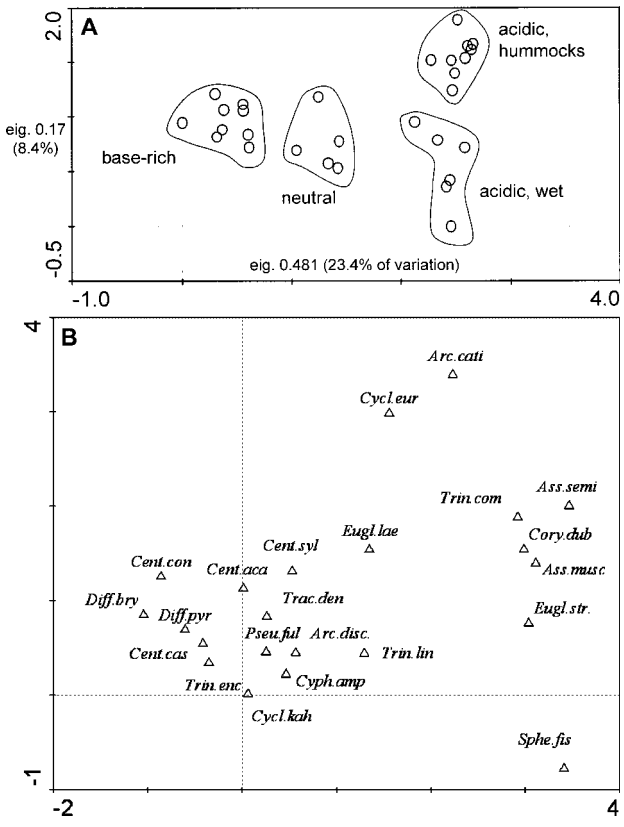

Fig. 5. Detrended correspondence analysis of testatean assemblages from bryophyte tufts (A) and their species (B). Ecologically interpretable groups of assemblages are indicated, eigenvalues and percentage of explained variance are presented at the axes. For the full names of the species see Appendix 2.

calcium, whose influence, according to comparison of DCA eigenvalues, is nearly three times stronger than that of the secondary gradient which has been interpreted as the moisture gradient. Base-richness is only scarcely reported as the most important environmental control for species variation in testacean assemblages. Mattheeussen *et al.* (2005) found such a result using data from arctic wetlands and Beyens *et al.* (1986), by analogy, from arctic mosses sampled across a wide range of wetland and terrestrial habitats. Booth (2001) found a similar pattern in non-*Sphagnum* wetland microhabitats. Booth and Zygmunt (2005) also suggested mineral richness to be a possible explanation for differences in assemblage composition between two regions of North America. The results of Mitchell *et al.* (1999) suggest that pH may be a major determinant even if the incomplete poor-rich gradient is analysed. On the other hand, Tolonen *et al.* (1994) analysed a longer poor-rich gradient than Mitchell *et al.* (1999) stretching beyond *Sphagnum* fens towards rich brown-moss fens, but the most important variable was still the water regime. The data from the Jura mountains (Mitchell *et al.* 1999) and

Table 2. Percentage representation of particular families in samples of six major types. The highest values for each column and each row are in bold.

Substrata Reaction	sediment base-rich	mosses base-rich	mosses neutral	sediments acid	mosses acid, wet	mosses acid, hummocks
LOBOSEA						
Arcellidae	< 1%	< 1%	< 1%	**10.43%**	< 1%	< 1%
Microchlamyidae	**< 1%**	< 1%	-	-	-	-
Centropyxidae	**41.32%**	11.44%	3.85%	4.26%	< 1%	1.64%
Cyclopyxidae	**13.70%**	4.18%	< 1%	-	< 1%	7.73%
Difflugiidae	**17.81%**	1.56%	< 1%	12.34%	< 1%	< 1%
Heleoperidae	1.83%	1.69%	1.67%	1.06%	1.27%	**2.49%**
Hyalospheniidae	5.71%	7.74%	1.91%	11.28%	23.73%	**36.83%**
Lesquereusiidae	1.37%	< 1%	< 1%	**4.26%**	< 1%	1.09%
Plagiopyxidae	4.11%	< 1%	-	-	-	-
Cryptodifflugiidae	-	-	-	-	< 1%	< 1%
Phryganellidae	1.14%	< 1%	< 1%	**4.26%**	1.10%	< 1%
FILOSEA						
Amphitrematidae	-	-	-	-	< 1%	< 1%
Cyphoderiidae	**2.28%**	< 1%	< 1%	1.70%	-	< 1%
Euglyphidae	10.05%	**71.60%**	**90.47%**	**38.51%**	**73.02%**	**48.07%**
Pseudodifflugiidae	-	< 1%	< 1%	**11.91%**	< 1%	< 1%

Table 3. Results of forward selection in CCA. Only variables significant at the P level 0.01 (Monte-Carlo test) were included to the model.

Entire data set			
Step	Variable	Lambda	Cumulative explained variance
1.	1st DCA axis of vegetation	0.434	16%
2.	1st DCA axis of sample composition	0.226	25%
3.	sediment / mosses	0.159	31%
4.	Ca (log-transformed)	0.085	36%
5.	SO_4^{2-}	0.082	39%
Moss samples only			
Step	Variable	Lambda	Cumulative explained variance
1.	1st DCA axis of vegetation	0.417	20%
2.	1st DCA axis of sample composition	0.160	28%
3.	Si	0.089	33%

from the Western Carpathians (this study) showed one more analogy: in both cases the second ordination axis, interpreted as the axis of moisture, was markedly clearer at the poor, more acid end of the gradient.

Booth (2001) reported a poor-rich gradient very similar to that described in this study. He primarily studied the within-wetland variation and observed a correlation between pH and moisture. Waterlogged depressions in acid wetlands are often not only wetter, but also less acid due to contact with mineral-richer groundwater or due to water aeration caused by water flow (Tahvanainen and Tuomaala 2003). The competition for mineral nutrients between peat mosses and invertebrates also plays a significant role. Our assemblages from waterlogged microhabitats of poor acidic fens had species composition corresponding to that of the bryophyte tufts from mineral-richer fens (Fig. 5a). A similar pattern was found in the case of molluscan assemblages (Horsák and Hájek 2003). Booth (2001) found that the testacean species characterising wetter sites also had higher pH optima. This holds not only for testate amoebae, but probably also for other biota in poor fens, diatoms for example (Poulíčková *et al.* 2004). Our study covered a wider range of pH and calcium concentration and the species turnover extended further, to more alkaline habitats characterised by a high representation

of *Difflugiidae* and *Centropyxidae*. The differentiation among major habitat types distinguished along the poor-rich gradient is therefore less influenced by covariation with moisture.

Our study showed that both the species composition of the vegetation, involving vascular plants and bryophytes, and the species composition of the moss sample characterise testacean assemblages better than even long-term measured water-chemistry data. Mitchell *et al.* (2000) reported rather different results. In their study testate amoebae reflected the chemistry of the groundwater and to a lesser extent the botanical composition of the moss carpet in acid *Sphagnum* mires. In such habitats direct measurements of water chemistry are necessary. On the other hand, our data suggest that the composition of the mire vegetation can substitute for directly measured water chemistry in the research of testate amoebae responses to the poor-rich gradient. The vegetation reflects not only a long-term development of environmental conditions but also possible antagonistic or synergistic influences of environmental factors on the fen biota.

When the species composition of our assemblages is compared with that of bogs and extremely poor fens across Europe (Mitchell *et al.* 2000), the assemblages from the Carpathian poor acid fens correspond rather to subcontinental Fennoscandian bogs than to oceanic bogs of Great Britain and the Netherlands. It is partly inconsistent with the species composition of plants. The studied Carpathian poor fens harbour several suboceanic, but very few boreal higher-plant species (Poulíčková *et al.* 2005). This comparison supports the results of Mitchell *et al.* (2000) that testate amoebae respond to different large-scale environmental factors in acidic mires. On the other hand, the mineral-richer mires studied by Booth and Zygmunt (2005) in North America have some species and groups in common with our rich fens (e.g. *Centropyxis casis*, *Quadrulella symmetrica*, *Pseudodifflugiidae*).

The species richness of vascular plants, bryophytes and some invertebrates is extremely low in the most acid poor fens and increases towards circumneutral habitats or further towards calcareous fens (Hájková and Hájek 2003, Chytrý *et al.* 2003, Horsák and Hájek 2003, Poulíčková *et al.* 2003, Tahvanainen 2004). This pattern has been explained by a large calcicole species pool that has resulted from historical and evolutionary processes (for details see Pärtel 2002 and Chytrý *et al.* 2003). The lack of correlation between testacean species richness and pH (this study) as well as between fungal species richness and pH (Vašutová 2005) suggests that the historical processes considered cannot provide a satisfactory explanation of mire biodiversity patterns or that they are not relevant for some taxonomic groups. Indeed, for both testate amoebae and fungi a set of specialised species populating acidophilous *Sphagnum* plants exists.

Autecology

The autecology of many species living in *Sphagnum* fens corresponds well to published data. *Amphitrema wrightianum* Archer, *A. flavum* (Archer) and *Hyalosphenia papilio* (Leidy) have their optima in wet microhabitats of poor acid fens; the latter species tolerate enhanced mineral supply (Meisterfeld 1979, Charman and Warner 1992, Tolonen *et al.* 1992, Booth 2001, Schnitchen *et al.* 2003, Booth and Zygmunt 2005, Lamentowitz and Mitchell 2005). Nonetheless, these species have a rather wide ecological amplitude and are not significantly a characteristic of any microhabitat type (Table 1). *Assulina muscorum, A. seminulum, Arcella catinus, Nebela militaris, N. bohemica, Trigonopyxis arcula* (Leidy) and *Corythion dubium* characterise rather drier poor-fen microhabitats, the latter species overlap to moderately-rich fens (latter references plus Beyens *et al.* 1986, Tolonen *et al.* 1994, Bobrov *et al.* 1999, Mitchell *et al.* 2000, Opravilová and Zahrádková 2003, Vincke *et al.* 2004). The species *Trinema enchelys* (Ehrenberg), *Tracheleuglypha dentata* (Penard), *Centropyxis cassis* Deflandre, *Cyclopyxis kahli* Deflandre, *Cyphoderia ampulla* (Ehrenberg), *Difflugia glans* Penard and *Quadrulella symmetrica* (Wallich) were often reported either as characteristic for the "higher-pH habitats", i.e. moderately calcium-rich *Sphagnum* fens, or generally as inhabitants of all acid habitats (Heal 1961, Beyens *et al.* 1986, Tolonen *et al.* 1992, Mitchell *et al.* 1999, Mitchell 2004, Vincke *et al.* 2004, Lamentowitz and Mitchell 2005). Having now studied the complete poor-rich gradient, we have found that the majority of these species, namely *Cyclopyxis kahli, Quadrulella symmetrica, Centropyxis cassis, Trinema enchelys, Difflugia glans* and *Cyphoderia ampulla* have their optimum in "brown-moss" calcareous fens (see also Mattheeussen *et al.* 2005).

A disagreement was found in the case of *Pseudodifflugia gracilis* which behaves as an indicator of calcareous fens in our study area, whereas Bobrov *et al.* (1999) reported its occurrence from dry and acid bog hummocks. By analogy, the *Centropyxis aculeata* group reported from acid hummocks by Bobrov *et al.* (1999)

or from moderately-rich *Sphagnum* fens (Tolonen *et al.* 1992, Lamentowitz and Mitchell 2005) is characteristic of calcareous fens in our study. The taxonomical differences or relatively low number of samples used in presented study could be the reason of the differences described above.

The next indicator of calcareous fens, *Paraquadrula irregularis*, is generally not an obligate inhabitant of this habitat type. Nguyen-Viet *et al.* (2004) report its common occurrence in *Tortula muralis* tufts, but exclusively in unpolluted areas.

Lamentowitz and Mitchell (2005) found that assemblages from laggs in north-western Poland, i.e. from moderately-rich and poor fens, are species-poor and strongly dominated by *Arcella discoides*. This species is the best indicator of moderately-rich fens also in our study area.

An interesting analogy between testacean- and plant ecology is drawn by the comparison of the distribution pattern of some testate amoebae with the results of a fertilisation experiment made by Mitchell (2004). The abundance of *Tracheuglypha dentata* and *Phryganella acropodia* increased during fertilisation of wet arctic tundra. In our study area these species are typical for the middle part of the poor-rich gradient. The numerous studies in mire ecology suggest that under extreme pH conditions the availability of major nutrients (N, P, K) is low. Many nutrient-demanding meadow species grow in mires of circumneutral pH in our study area (Hájek and Hekera 2004, Hájková and Hájek 2004). The tendency of some species like *Tracheuglypha dentata* to prefer N- and P-rich habitats is probably the reason why one assemblage from a highly calcareous albeit high-productive fen had a species composition more similar to moderately-rich *Sphagnum* fens than to calcareous fens. Nonetheless, the low number of replications is a certain limitation of this conclusion.

The study of the complete poor-rich gradient improves our view on the indicative value of some species. Species which are often regarded to be ubiquitous such are *Assulina muscorum, Nebella militaris, N. tincta, Heleopera petricola,* and *Corythion dubium* (e.g. Mitchell *et al.* 1999) are among the best indicators of acid environment in our study area.

Knowledge about the responses of testate amoebae species to the poor-rich gradient is still limited as compared to their responses to the moisture gradient. The improvement of understanding the species response to the poor-rich gradient has an important implication for palaeoecology. During the Holocene, not only hydrological regime, but also base-richness can change, as confirmed by macrofossil analyses from central Europe (e.g. Rybníček and Rybníčková 1968), Scandinavia (e.g. Mörnsjö 1969) and northern America (e.g. Kuhry *et al.* 1993). Warner and Charman (1994) compared modern testate amoebae assemblages with fossil ones and found a combined poor-rich and moisture gradient very similar to that presented in this study. Their rich fens were characterised by the genera *Cyclopyxis* and *Centropyxis*, poor fens by the species *Amphitrema flavum*, *Assulina muscorum* and *Heleopera sphagni* and drier poor-fen hummocks by the species *Nebela militaris* and *Trigonopyxis arcula*.

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Appendix 1. List of localities, their geographical coordinates, altitudes, dominants and long-term averages of physical-chemical characteristics. Cation concentrations are in mgl^{-1}, cond - electrical conductivity of water.

Site name	coordinates	commune	altitude	Ca	Mg	Fe	water pH	cond	Dominants
Biely Kríž	N:49°29'54", E:18°32'47"	Biely Kríž	910	6.72	0.95	4.3	4.63	45	*Sphagnum palustre, S. papillosum, S. fallax, Carex echinata*
Hrnčiarky	N:48°54'30", E:17°40'34"	Strání	440	173.35	16	8.59	7.49	672	*Cratoneuron commutatum, Campylium stellatum, Eriophorum angustifolium, Equisetum telmateia*
Hrubé Brodské	N:49°21'59", E:18°10'30"	Nový Hrozenkov	530	86	11.2	20.67	7.13	431	*Aulacomnium palustre, Homalothecium nitens, Eriophorum latifolium, E. angustifolium*
Hrubý Mechnáč	N:48°56'33", E:17°47'54"	Lopeník	640	79.91	14.68	3.73	7.55	430	*Campylium stellatum, Cratoneuron commutatum, Eriophorum angustifolium, E. latifolium*
Jančíkovci	N:49°29'26", E:18°33'22"	Klokočov	750	3.24	0.74	6.12	4.21	27	*Sphagnum palustre, S. papillosum, S. fallax, Drosera rotundifolia*
Jasenka	N:49°22'41", E:18°01'24"	Vsetín	565	107.48	8.77	81.02	7.19	491	*Cratoneuron commutatum, Fissidens adianthoides, Eriophorum latifolium*
Kelčov	N:49°23'03", E:18°28'47"	Nižný Kelčov	660	63.08	5.29	71.76	7.08	292	*Scorpidium cossonii, Campylium stellatum, Bryum pseudotriquetrum, Eriophorum latifolium*
Obidová 1	N:49°31'03", E:18°31'23"	Visalaje	735	7.34	1.7	11.35	5.43	46	*Sphagnum contortum, S. warnstorfii, Eriophorum angustifolium*
Obidová 2	N:49°31'03", E:18°31'25"	Visalaje	730	10.8	2.53	29.28	5.97	77	*Sphagnum flexuosum, Carex echinata, Viola palustris*
Polková	N:49°28'24", E:18°35'53"	Klokočov - Hlavice	610	6.9	2.36	55.97	5.59	57	*Sphagnum flexuosum, S. subsecundum, Carex nigra*
Semetín	N:49°20'18", E:17°56'51"	Vsetín-Semetín	400	174.42	19.11	11.76	7.23	494	*Tomenthypnum nitens, Bryum pseudotriquetrum, Eriophorum angustifolium, Succisa pratensis*
Valašské Klobouky	N:49°06'47", E:18°01'24"	Valašské Klobouky	370	193.42	5.74	7.84	7.51	463	*Cratoneuron commutatum, Calliergonella cuspidata, Equisetum telmateia, Valeriana simplicifolia*
Zajacovci 1	N:49°28'46", E:18°37'03"	Zajacovci	660	6.64	1.84	2.81	5.23	43	*Sphagnum capillifolium, S. fallax, Carex nigra, Drosera rotundifolia*
Zajacovci 2	N:49°28'40", E:18°37'01"	Zajacovci	640	10.77	2.21	30.57	5.59	71	*Sphagnum subsecundum, S. contortum, Carex nigra, C. lasiocarpa*

Appendix 2. Abbreviations of the names used in ordination diagrams.

Testate amoebae (Figs 4-5)			
Arcella catinus	Arc.cati	Pseudodifflugia fulva	Pseu.ful
Arcella discoides	Arc.disc	Pseudodifflugia gracilis	Pseu.gra
Assulina muscorum	Ass.musc	Quadrulella symmetrica	Quad.sym
Assulina seminulum	Ass.semi	Sphenoderia fissirostris	Sphe.fis
Amphitrema flavum	Amph.fla	Sphenoderia lenta	Sphe.len
Amphitrema wrightianum	Amph.wrig	Trigonopyxis arcula	Trig.arc
Centropyxis aculeata	Cent.aca	Tracheleuglypha dentata	Trac.den
Centropyxis cassis	Cent.cas	Trinema complanatum	Trin.com
Centropyxis constricta	Cent.con	Trinema enchelys	Trin.enc
Centropyxis globulosa	Cent.glo	Trinema lineare	Trin.lin
Centropyxis sylvatica	Cent.syl		
Corythion dubium	Cory.dub	**Plants (Figs. 2-3)**	
Cyclopyxis eurystoma	Cycl.eur	Agrostis canina	Agro.can
Cyclopyxis kahli	Cycl.kahl	Anthoxanthum odoratum	Anth.odo
Cyphoderia ampulla	Cyph.amp	Calliergonella cuspidata	Call.cus
Difflugia bryophila	Diff. bry	Campylium stellatum	Camp.ste
Difflugia glans	Diff. gla	Carex demissa	Care.dem
Difflugia pyriformis	Diff. pyr	Carex echinata	Care.echi
Difflugia rubescens	Diff.rub	Carex nigra	Care.nig
Euglypha ciliata	Eugl.cil	Cirsium rivulare	Cirs.riv
Euglypha denticulata	Eugl.den	Cratoneuron filicinum	Crat.fil
Euglypha laevis	Eugl.lae	Drosera rotundifolia	Dros.rot
Euglypha strigosa	Eugl.str	Juncus inflexus	Junc.inf
Heleopera rosea	Hel.rose	Nardus stricta	Nard.str
Heleopera sphagni	Hel.spha	Palustriella commutata	Pal.com
Hyalosphenia papilio	Hyal.pap	Philonotis calcarea	Phil.cal
Lesquereusia spiralis	Les.spi	Plagiomnium elatum	Plag.ela
Nebela bohemica	Neb.boh	Polytrichum commune	Poly.com
Nebela collaris	Neb.coll	Prunella vulgaris	Prun.vul
Nebela dentistoma	Neb.dent	Sphagnum denticulatum	Spha.den
Nebela lageniformis	Neb. lage	Sphagnum fallax	Spha.fal
Nebela marginata	Neb.marg	Sphagnum flexuosum	Spha.fle
Nebela militaris	Neb. mili	Sphagnum palustre	Spha.pal
Nebela tincta	Neb. tinc	Sphagnum papillosum	Spha.pap
Paraquadrula irregularis	Par.irre	Sphagnum rubellum	Spha.rub
Phryganella acropodia	Phryg.acr	Sphagnum subsecundum	Spha.sub
Plagiopyxis declivis	Plag.dec	Straminergon stramineum	Stra.str.
Pontigulasia incisa	Pont.inc.	Tomenthypnum nites	Tome.nit
Pontigulasia spectabilis	Pont.spec	Viola palustris	Viol.pal

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ACTA
PROTOZOOLOGICA

Short Communication

Tubulin is not Posttranslationally Modified (Acetylated or Polyglutamylated) in *Tetrahymena* Macronucleus

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Summary. In the interphase of the cell cycle of *Tetrahymena pyriformis* GL tubulin was not demonstrated in the macronucleus by confocal microscopy using antibodies to α-tubulin, acetylated α-tubulin and polyglutamylated tubulin antibodies or Flutax-1. During cell division, from the formation of the new oral apparatus to the beginning of cytokinesis, tubulin was diffusely present in the nucleus, however (i) it did not form visible microtubules in confocal microscope- and (ii) it was not acetylated or polyglutamylated. As acetylation stabilizes microtubules, the results point to the dynamism of nuclear tubulin which could have a role in the transport of the numerous macronuclear chromosomes.

INTRODUCTION

Ciliates are relatively large and highly polarized cells, whose locomotion, feeding, cortical architecture and development are heavily dependent on diverse microtubules (Frankel 1999). These diverse microtubules have distinct morphologies and stabilities. *Tetrahymena* expresses only α- and two β-tubulin genes (and also γ-tubulin gen), thus it is unlikely that the diversity of microtubules are derived from diverse tubulin genes (Gaertig *et al*. 1993). Considering this diversity of microtubules it may derive mainly from (a) association with different microtubule-associated proteins (MAPs), with structural MAPs or molecular motors; or from (b) different posttranslational modifications (PTMs), as acetylation (α-tubulin), detyrosination (α-tubulin), glutamylation (α-and β-tubulins), glycylation (α-and β-tubulins), or phosphorylation (α-and β-tubulins); the different properties of microtubule species and their stability emerge from the regulation of post-translational modifications (Gaertig 2000).

α-Tubulin acetylation appears to be the most widespread PTM as it was detected in ciliary, cortical and intramicronuclear microtubules of *Tetrahymena* (MacRae 1997). This PTM affects the stability of microtubules, and this is due to the binding of specific MAPs to the walls of acetylated microtubules (Piperno and Fuller 1985). It was found that microtubule bundles formed in MAP_{2c}- or tau-transfected cells were stabilized against microtubule depolymerizing reagents and were enriched in acetylated alpha tubulin (Marute *et al*. 1986). Thus,

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probably acetylation (and possibly also the detyrosination or polyglutamylation) may play a role in the maintenance of stable populations of microtubules. It was found that stable microtubules defined as those that had not undergone polymerization within 1 h after injection of biotin-tubulin and they were posttranslationally modified; furthermore dynamic microtubules were all unmodified. Posttranslational modification, however, is not a prerequisite for microtubule stability and vice versa. *Potorous tridactylis* kidney cells have no detectable acetylated microtubules but do have a sizable subset of stable ones, and chick embryo fibroblast cells are extensively modified however, hardly have stable microtubules (Schulze *et al*. 1987).

Tubulin have been reported within the dividing macronucleus of ciliated protozoa (Williams and Williams 1976), but little is known about their precise role in the division process itself, or about their posttranslational modification(s).

In our previous experiments connection of microtubular and signaling systems was observed in *Tetrahymena* (Kovács *et al*. 2000, Kovács and Pintér 2001), and data were obtained on the effect of drugs affecting microtubular assembly (Kovács and Csaba 2005). The structure of microtubular system (longitudinal and transverse microtubule bands) in cells exposed to 0.1 mM indomethacin became frequently irregular, a phenomenon which was not seen in control cells. In a considerable amount of cells stomatogenesis were seen, whereas in these cells the macronucleus did not elongate. Considering these facts we concluded that indomethacin impaired the signaling system which was able to connect the events of divisional morphogenesis; it ceased the junction between the cytoskeletal and nuclear processes (Kovács and Pállinger 2003). The aim of the present study is to obtain additional data on the posttranslational modification (eg. acetylation) of tubular stuctures in the nuclei of *Tetrahymena*, and on the possible role of microtubular stability/dynamics in the mitotic events of this unicellular organism.

MATERIALS AND METHODS

Chemicals. Mouse monoclonal anti-acetylated tubulin, anti-polyglutamylated-tubulin and anti-α-tubulin antibodies, FITC-labeled anti-mouse goat IgG, Flutax-1 and tryptone were obtained from Sigma (St Louis, MO, USA). Yeast extract was purchased from Oxoid (Unipath, Basingstoke, Hampshire, UK). All other chemicals used were of analytical grade available from commercial sources.

Cultures. In the experiments, *Tetrahymena pyriformis* GL strain was tested in the logarithmic phase of growth. The cells were cultivated at 28°C in 0.1 % yeast extract containing 1 % tryptone medium. Before the experiments the cells were washed with fresh culture medium and were resuspended at a concentration of 5×10^4 cells ml^{-1}.

Confocal scanning laser microscopy. To localize tubulin-containing structures, cells were fixed in 4% paraformaldehyde dissolved in PBS, pH 7.2. After washing with wash buffer [WB] (0.1% BSA in 20 mM Tris-HCl; 0.9% NaCl; 0.05% Tween 20, pH 8.2) the cells were incubated with monoclonal anti-acetylated tubulin antibody or monoclonal anti-a-tubulin antibody diluted 1:500 with antibody [AB] buffer (1% BSA in 20 mM Tris-HCl; 0.9% NaCl; 0.05% Tween 20, pH 8.2) for 45 min at room temperature. After three washings with WB the anti-tubulin antibody treated cells were incubated with FITC-labelled anti-mouse goat IgG (diluted to 1:500 with AB buffer) for 45 min at room temperature.

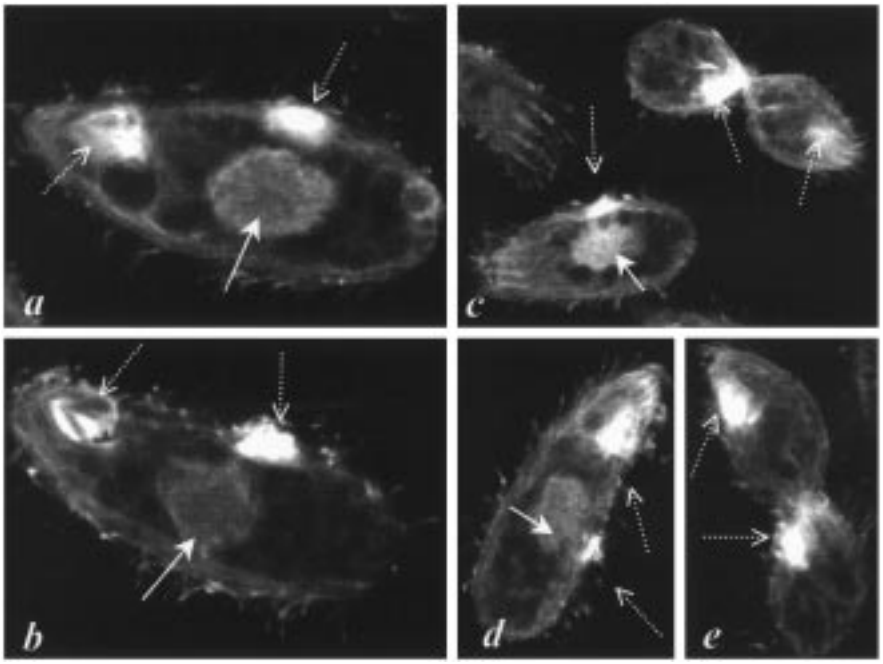
Beyond that some cells were labeled with fluorescent taxoid derivative 7-O-[N-(4'-fluoresceincarbonyl)-L-alanyl]taxol (Flutax-1). As Flutax-1 reversibly interacts with the taxol binding sites of microtubules with high affinity, it serves to image the microtubule cytoskeleton (Arregui *et al*. 2002). The binding of Flutax-1 was analysed on fixed cells (in 4% paraformaldehyde dissolved in PBS, pH 7.2). The stock solution of Flutax-1 was 10^{-4} M dissolved in DMSO, and diluted with AB buffer to 10^{-6}M. The cell suspensions were incubated with 10^{-6}M Flutax-1 (v/v) for 20 min. After these incubations the cells were washed four times with WB, and were mounted onto microscopic slides.

The mounted cells were analyzed in a Bio-Rad MRC 1024 confocal scanning laser microscope (CSLM) equipped a krypton/argon mixed gas laser as a light source. Excitation carried out with the 480 nm line from the laser.

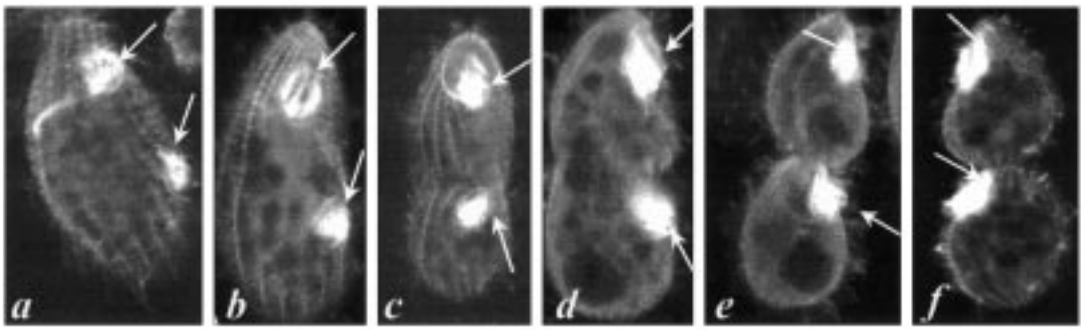
RESULTS AND DISCUSSION

According to Fujiu and Numata (1999) the macronuclear division of *Tetrahymena* is classified into six stages, considering the morphology of the macronucleus. They found that tubulin is substantially excluded from interphase nuclei, but it is present in dividing (amitotic?) ones - from the appearance of „anarchic field", the formation of new oral apparatus (about stage 2) to the beginning of cytokinesis, when the division furrow appears (about stage 5). In their observations tubulin forms microtubules throughout the macronuclear division however, these microtubules are biochemically different from the cytoskeletal (cytoplasmic) ones. Investigating electron-microspically, Williams and Williams (1976) demonstrated the presence of these nuclear microtubules mainly in connection with the nuclear envelope.

In our present experiments in the interphase nucleus tubulin was not demonstrable (Figs 4a-c), it was labeled



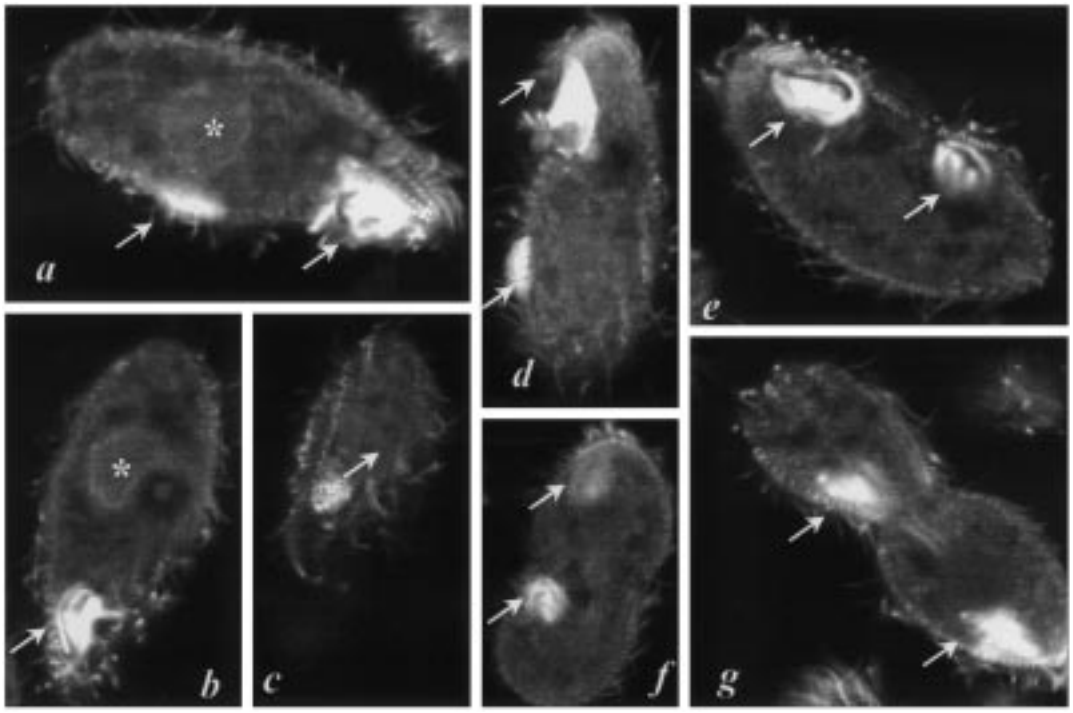
Figs 1a-e. Binding of FITC-labeled anti α-tubulin antibody to the tubulin containing systems of *Tetrahymena*. The nuceli are labeled from the formation of new oral apparatus to the beginning of appearance of division furrow. Arrows - nuclei; dotted arrows - oral apparatus. Confocal scanning laser microscopic pictures. Magnification: 1400× (1a, b); 900× (1c-e).



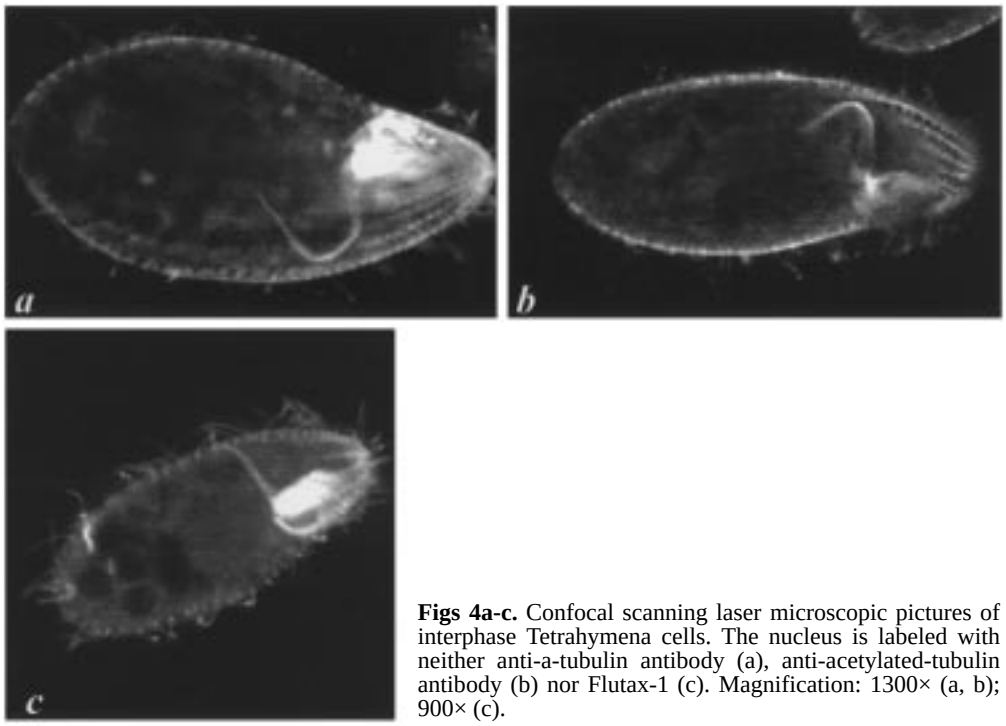
Figs 2a-f. Binding of FITC-labeled anti acetylated-tubulin antibody to the microtubular system of *Tetrahymena*. Confocal scanning laser microscopic pictures. Arrows - oral apparatus. The labeling is absent in all stages of cell division. Magnification 1100×.

neither with antibodies against a-tubulin and acetylated tubulin nor Flutax-1. During cell division of *Tetrahymena* the nuclear tubulin appears, however, it did not form confocal microscopically demonstrable microtubules. It was at all times labeled with anti-a-tubulin antibodies (Figs 1a-d) and Flutax-1 (Figs 3a,b), however this tubulin is neither acetylated (Figs 2a-f) nor polyglutamylated (data not shown). In late cytokinesis some microtubular structures were visible between the isolated two macronuclei - largely in the Flutax-labeled cells (Fig. 5c).

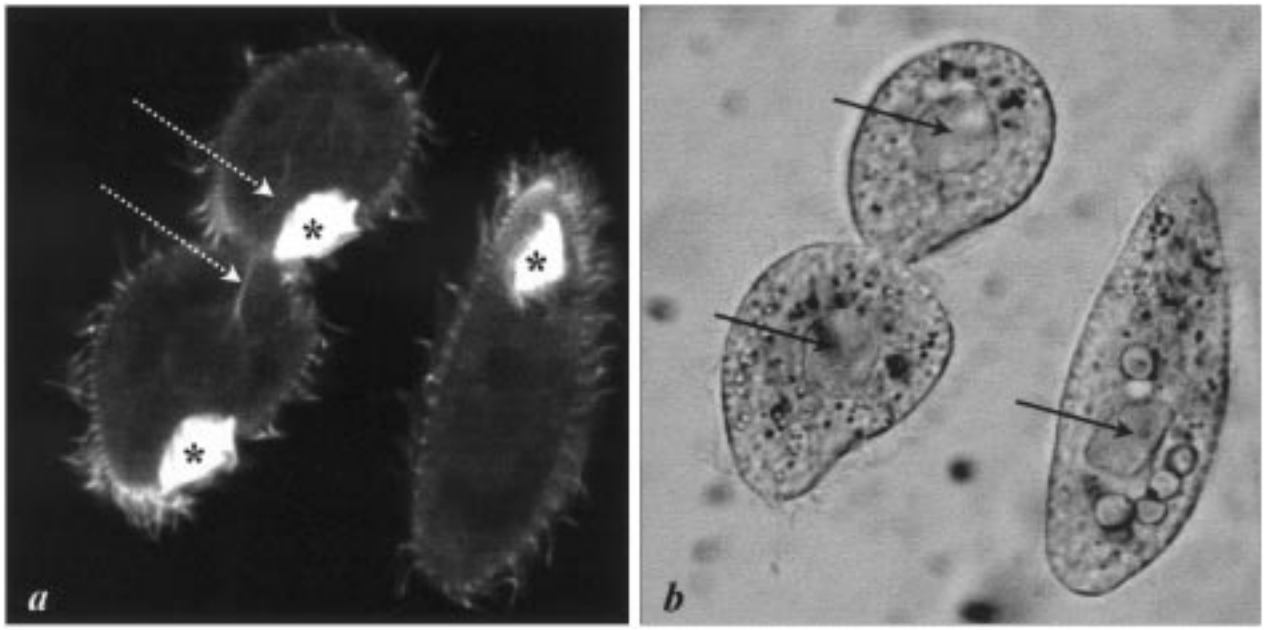
In tissue-culture of mammalian cells tubulin has been implicated as one of the nuclear proteins. It was found distributed throughout the nucleus and particularly in association with the chromatin. Tubulin comprised about 6.5% of the nonhistone chromosomal proteins. Nuclear tubulin appears in a nonmicrotubular form (as in our present experiments). Fluorescence microscopical examinations on metaphase chromosomes revealed that tubulin was present on the chromosomes. These data suggest a structural role for chromatin-associated tubulin



Figs 3a-g. a - binding of Flutax-1 to the microtubular system of *Tetrahymena*; **b, c** - same cell; optical sections at middle of the cell (b) and the surface of cell (c). Nuclar-labeling is visible at the formation of new oral apparatus, later this labeling disappears. Arrows - oral apparatus; asterisk - nuclei. Confocal scanning laser microscopic pictures Magnification: 1200× (a, e, g); 900× (b-d); 800× (f).



Figs 4a-c. Confocal scanning laser microscopic pictures of interphase Tetrahymena cells. The nucleus is labeled with neither anti-a-tubulin antibody (a), anti-acetylated-tubulin antibody (b) nor Flutax-1 (c). Magnification: 1300× (a, b); 900× (c).



Figs 5a, b. Interphase and dividing *Tetrahymena* cells. **a** - labeled with Flutax; **b** - same cells in brightfield. Arrows - nuclei; dotted arrows - microtubule bundles between the nuclei of daughter cells. Asterisk - oral apparatus. Confocal scanning laser microscopy Magnification 1200×.

(Menko and Tan 1980). The ciliate macronucleus (the transcriptionally active „somatic" nucleus) typically does not show recognizable chromosomes, and conventional mitotic spindles are not seen. Cell division (amitosis?) in this case involves an elongation of the nucleus within the persisting nuclear envelope, and an eventual pulling apart into the two daughter nuclei (Williams and Williams 1976). It was shown that colchicine applied to *Tetrahymena* late in the cell cycle had no blocking effect on either cell or macronuclear division. However, nuclear cleavage was unequal; many daughter cells were produced with nuclei which were either smaller or larger than normal, and some had no nuclei at all (Tamura *et al.* 1969). It has been shown that significant elongation of the macronucleus can occur in the absence of tubulin, and that microtubules are not required to maintain an elongation and separation once it has occured normally. However, autonomous nuclear constriction and separation do not occur without nuclear tubulin; the macronucleus is cut in two by the advancing cytoplasmic fission furrow.

In ciliated protoza the macronucleus contains the somatic genome, which is fragmented, polyploid and actively expressed. In the *Tetrahymena thermophila* the macronucleus consists of 200-300 identifiable autonomously replicating pieces („MAC chromosomes"), derived from the five pairs of germline-derived chromosomes (Conover and Brunk 1986). Macronuclear chromosomes lack centromeres and kinetochores (Davidson and LaFountain Jr. 1975), sister chromatids segregate randomly and spindle pole bodies do not form (Doerder 1979); accordingly the macronuclear microtubules operate in no way like the „real" kinetochor-microtubules. The huge number of MAC chromosomes to which tubulin can be sticked could explain the diffuse appearance of anti-tubulin fluorescence during cell division. This tubulin which does not form microtubules could have a role in the transport of „MAC chromosomes". The nuclear envelope associated microtubules which were not demonstrated immunocytochemically, but were shown in electron microscopic pictures have a role in the terminal stages of nuclear elongation and separation (Williams and Williams 1976).

It is likely that the macronuclear microtubules are very dynamic structures: the enhanced stability of microtubules alters normal microtubule dynamics, leading to the nuclear aberrant phenotypes. In a b-tubulin mutant *Tetrahymena thermophila* (btu1-1), where microtubules are strongly stabilized polymers, and increasing amount of amacronucleate cell formation occurs. Also

the microtubule-stabilizing drugs (taxol and DMSO) promote amacronucleate cell formation (Smith *et al*. 2004). This helps to understand why acetylated (stabilized) tubulin in the macronucleus of dividing *Tetrahymena* was not found and can explain the biochemical difference between nuclear and cytoplasmic tubulin, mentioned by Fujiu and Numata.

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PROTOZOOLOGICA

BOOK REVIEW

ANTARCTIC MARINE PROTISTS, edited by Fiona J. Scott & Harvey J. Marchant. Published by Australian Biological Resources Study (ABRS), Canberra & Australian Antarctic Division, Hobart, 563 pp. ISBN: 0 642 56835 9 (hardcover) Price AU$ 95.00 (price includes surface postage for overseas orders, and GST and postage within Australia). Order from ABRS, GPO Box 787, Canberra, A.C.T. 2601, Australia.

The best discoveries happen by chance. A flyer of a book entitled ANTARCTIC MARINE PROTISTS arrived in my "pigeon-hole" right in the middle of my fruitless quest for records of marine scale-bearing protists from the Southern Hemisphere. I ordered the book without delay, and when it landed in my hands it was just what I had been hoping for - a compilation of more than 560 Antarctic marine protists, more than 1300 pictures (light, and electron micrographs, and drawings), description of little-known protistan groups, and plentiful records. My next aim was, indisputably, the bibliography. And *voi-la*…there they were: the much-longed-for "Southern Hemisphere" references, and many more on related subjects! ANTARCTIC MARINE PROTISTS is a very useful book. It is beautifully presented, with inviting deep-blue hard covers featuring dimly-lit underwater images of pelagic 'armoured' protists (diatoms, parmales, coccolithophores, dinoflagellates). The book has 15 chapters, 12 contributing authors and it has been edited by Fiona Scott and Harvey Marchant. The chapters are (1) introduction, (2) diatoms, (3) dinoflagellates, (4) silicoflagellates, (5) haptophytes (Order Prymnesiales), (6) haptophytes (Order Coccolithophorales), (7) chrysophytes, (8) prasinophytes, (9) chlorophytes, (10) cryptophytes, (11) euglenoids, (12) cyanophytes, (13) choanoflagellates, (14) ciliates, and (15) Protista *incertae sedis*. A glossary of terms, an extensive bibliography, copyright permission, and a well-organised Index complete the opus. The initiative of presenting one single list of references at the end of the book is very welcome and it makes the search for bibliographic references effortless.

Chapter 1 is the Introduction. It gives a useful overview of the biological zones in the Polar Front of the Southern Ocean, a brief description of the biology and diversity of Antarctic marine protists (cell size range, shape, presence of silica and other mineralised walls and scales, feeding strategies, etc), an historical account (although too brief - see below) of the origins of protistological research in Antarctica, and the importance of Antarctic marine protists. The section "Sample collection and processing" offers details of the different methods and techniques followed to study the diverse protist groups treated in the book. However, the section is perhaps a little misleading for the organisms shown in the book are not the outcome of a planned research programme whose results are published in the form of a single volume ("For the purpose of this volume, organisms were collected in a variety of ways from the different depths of the water column…"), but a compilation of essentially previously-published work - a task that should not be underestimated but which in my view should have been made clearer in the introduction. The chapter finishes with a list of the systematic arrangement of the 560 taxa covered by the book.

Chapter 2 is the longest - a 188-page chronicle of Antarctic diatoms. As with the majority of the remaining taxa dealt with in the book each species description includes basionym and synonyms, additional references, distribution, and in most cases a brief comparison with similar species (including Tables in some instances). This chapter is illustrated with 575 light and electron (SEM) micrographs, and 19 drawings. Chapter 3 is a 47-page detailed account of the Antarctic dinoflagellates that includes almost one hundred (mostly SEM) micrographs of these protists.

A total of 22 species (mostly of the genus *Protosperidinium*) recorded in Antarctic marine environments are not treated in the chapter, and, unfortunately, no explanation is given for their exclusion. However, to compensate such an omission, the full list and further reading is provided. The silicoflagellates form Chapter 4. These protists are well recorded in the fossil record and (as explained in the chapter) their 'skeletons' have been used as tools to study Tertiary Antarctic climates. The three extant recognised species *Dictyocha fibula, D. octonaria*, and *D. speculum*, are all present in Antarctic waters and are described and illustrated in the chapter.

Chapters 5 and 6 deal with the haptophyte orders Prymnesiales and Coccolithophorales (heterococcolithophores and holococcolithophores), respectively. These protists are small, they have the cell covered with scales (siliceous, organic or in the case of coccolithophores, calcite), and bear two flagella and a haptonema. Two genera of the order prymnesiales are treated in chapter 5, *Phaeocystis* (one species) and *Chrysochromulina* (21 species and morphotypes). Although the chapter claims that *Chrysochromulina* species are rarely reported from Antarctic waters it describes and illustrates 21 (8 species and 13 "types")! Bearing in mind that there are currently only 55 named *Chrysochromulina* species but probably in excess of 100 including undescribed and open-nomenclature species, a total of 21 do not seem to suggest they are species poor. Coccoliths from coccolithophores are very well known in paleoceanography and paleoclimatic research. Six of the thirteen families recognised within the Order Coccolithophorales are present in the polar region of the Southern Ocean, with eleven genera and 20 species positively identified in the region, most of which are described and illustrated (using high-quality TEM and SEM micrographs) in this book.

Chapter 7, on the chrysophytes, includes the little-known parmales - all profusely illustrated. With the exception of the latter, only two genera of chrysophytes are covered in this chapter, i.e. *Paraphysomonas* and *Meringosphaera*. At this point, a (probably unintentional) incomplete list of geographical records of some of the species needs to be brought to attention, for *Paraphysomonas antarctica* has also been reported from the Baltic, Danish Wadden Sea, and tropical waters [see Vørs N. (1992) Heterotrophic amoebae, flagellates and heliozoa from the Tvärminne area, Gulf of Finland, in 1988-1990. *Ophelia* **36:** 1-109].

The Prasinophytes are described in Chapter 8. These protists are solitary or colonial forms that have the cell and the flagella covered with organic scales - characters that are impressively illustrated in the EM figures of the chapter. Chapter 9 is one page long and it deals with only one chlorophyte species, *Polytoma papillata*. The author does not specify whether this is the only species of chlorophyte ever found in Antarctic sea water. The next Chapter is devoted to the autotrophic cryptophytes. As in the previous chapter, it is brief and deals with one known species (*Geminigera cryophila*) and with another species that, although not given a binomial name, is probably a new genus, the main characters of which are superbly illustrated in the SEM pictures.

Euglenoids (excluding kinetoplastids, which are treated as *incertae sedis* protists in the last chapter of the book, Chapter 15) and cyanophytes make up Chapters 11 and 12, respectively. There are not many records of marine euglenoids in Antarctica, and only three known species and a *Eutreptiella* sp. are described and illustrated in the chapter. The cyanophytes in Chapter 12 are represented by *Synechococcus* sp. Chapter 13 focuses on the choanoflagellates, with 32 marine species described and illustrated (SEM and/or TEM micrographs, many of which are the author's originals).

The ciliate chapter (Chapter 14) is a compilation of the author's observations in addition to other records of ciliates from Antarctic marine habitats, both pelagic and non-pelagic. The general introduction to the biology, morphology, and ecology of ciliates is very good and informative. The chapter describes and illustrates an impressive 161 species. However, perhaps a little criticism can be tolerated insofar as there is a distinct lack of micrographs of live forms and silver-impregnated specimens. In contrast, the number of drawings (many of them the author's originals) exceed 350.

The last chapter of the book is dedicated to Protista *incertae sedis*. Some protists included in this section currently enjoy a better taxonomic status than the one given by the title of the chapter but the authors chose to deal with them as of uncertain status as the aim of the book is to present a compilation of Antarctic protists rather than judge the validity of taxonomic schemes or recent results of molecular tools applied to such organisms. Protists included in this section are (in order of appearance) naked and testate amoeba spp., foraminifera, heliozoa, kinetoplastids, bisocoecids and other heterokonts, and heterotrophic flagellates. Amongst all these groups the heliozoa and the heterotrophic flagellates are the ones given most attention. As the book deals mainly (although not

exclusively) with pelagic protists, only one foraminiferan is described, i.e. *Neogloboquadrina pachyderma*, apparently the only foraminiferal morphospecies recorded so far in Antarctic plankton. As the book is on Antarctic marine protists this section should undoubtedly have included some account of "other" foraminifera. There is a big literature on Antarctic benthic foraminifera going back to the 1840's with Ehrenberg's (1844) examination of samples returned from the *Terror* and *Erebus* expeditions of James Clarke Ross (both expeditions are alluded to by the authors in Chapter 1) and further subsequent expeditions and geological research as well as the introduction of box and multiple coring have greatly expanded knowledge of Antarctic benthic foraminifera [see Cornelius N. and Gooday A. J. (2004) "Live" (stained) deep-sea benthic foraminifera in the western Weddell Sea: trends in abundance, diversity and taxonomic composition in relation to water depth. *Deep-Sea Research* II **51:** 1571-1602]. In my view, a brief reference to the rich diversity of Antarctic benthic forams with a few key bibliographic citations should have been incorporated in this chapter.

But minor shortcomings do not cast a shadow on the book. ANTARCTIC MARINE PROTISTS is a "must have" - well presented, packed with very useful information, species descriptions, bibliographic references and breath-taking micrographs. These are Antarctic protists as you have probably not seen before.

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Articles from books: Neto E. D., Steindel M., Passos L. K. F. (1993) The use of RAPD's for the study of the genetic diversity of *Schistosoma mansoni* and *Trypanosoma cruzi*. In: DNA Fingerprinting: State of Science, (Eds. S. D. J. Pena, R. Chakraborty, J. T. Epplen, A. J. Jeffreys). Birkhäuser-Verlag, Basel, 339-345

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