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Comparative Ultrastructure and Elemental Composition of Envelopes of *Trachelomonas* and *Strombomonas* (Euglenophyta)

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Summary. The ultrastructure and composition of envelopes of *Trachelomonas* and *Strombomonas* from natural freshwater bodies in Argentina and Brazil were analyzed. Examination of the surfaces of different species of *Trachelomonas* by scanning electron microscopy showed several types of ornamentation and a uniform internal surface, whereas numerous particles adhered to the surfaces of most *Strombomonas* envelopes. Transmission electron-microscopic images showed the microarchitecture of both genera to consist largely of electron-opaque granules, but some envelopes also contained fibrillar or needle-like components interwoven with the granules. Energy-dispersive X-ray analysis indicated that iron and/or silicon were present in most of the organisms from Brazil in higher levels than manganese; the latter was absent in all the samples from Argentina.

Key words. Electron-microscopic energy-dispersive X-ray spectroscopy, elemental composition; envelope, Euglenophyta, *Strombomonas*, *Trachelomonas*.

INTRODUCTION

The euglenoid flagellates represent a diverse group of organisms found in natural collections from around the world. All euglenoids produce and secrete copious amounts of mucopolysaccharides; in some organisms they are retained by the cell and ultimately form an ornate, mineralized structure, generally termed envelope, lorica or test. In those genera that produce such a structure, namely *Trachelomonas* (Ehr.) and *Strombomonas* (Defl.), the morphology of the envelope is a key taxonomic criterion at both the generic and species levels. Deflandre (1930) based the separation of the two genera on rather poorly defined morphological characters of the envelope. Pringsheim (1953) showed that environmental conditions affect these features and proposed a new taxonomic scheme for *Trachelomonas* based on cytological characteristics, even though he adopted Deflandre's classification.

Subsequently, many studies of the envelopes using scanning and transmission electron microscopy have been carried out (e.g., Dodge 1975, Leedale 1975, Hager 1979, West and Walne 1980, Tell and Conforti 1984, Couté and Thérézien 1985, Conforti and Tell 1986, Dunlap and Walne 1987, Rino and Pereira 1990, Rosowski 1993). Dunlap et al. (1986) reported that envelope development, microarchitecture, elemental

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composition and cytochemical staining properties of *Strombomonas conspersa* (Pascher) Defl. are identical to those of several species of *Trachelomonas*. However, on the basis of comparative light and scanning electron microscopy of 20 taxa of *Strombomonas*, Tell and Conforti (1988) proposed some new combinations and reassigned several species of *Trachelomonas* to the genus *Strombomonas*.

In an attempt to more fully understand this longstanding problem, we present new observations on the comparative ultrastructure and elemental composition of the envelope in several species of *Strombomonas* and *Trachelomonas* from field samples.

MATERIALS AND METHODS

Collections from numerous freshwater sites in the province of Chaco, Argentina, and from Camaleão Lake, Manaus, Brazil, were provided by Dr. H. Tell (University of Buenos Aires), Lic. Y. Zalocar (Cecoal, Corrientes, Argentina) and Maria Rodrigues (Universided de São Luis, Maranhão, Brazil). The organisms were collected with a 20-µm mesh net. The Argentine samples were subsequently fixed in 4.0% formaldehyde, and the Brazilian samples in Transeau solution. The fixed samples were deposited in the collection of the Limnology Laboratory, Biology Department, University of Buenos Aires.

For scanning electron microscopy (SEM), cells were isolated under a dissecting microscope, then dehydrated in an ethanol series (50-100%), air dried on a cover glass and coated with gold-palladium. Envelopes were photographed and their elemental compositions were determined with a Philips 505 scanning electron microscope, operating at 20 kV and equipped with an EDAX X-ray detector and 9100 Multichannel Analyzer.

For transmission electron microscopy (TEM) and X-ray microanalysis (EDS), samples from the SEM fixation were dehydrated in an acetone series (25-100%) and embedded and cured in Spurr's resin, according to the procedures described by Reymond and Pickett-Heaps (1983). After examination by light microscopy, individual cells were selected for TEM analysis, mounted on resin stubs, thin sectioned on a Reichert OMU-3 ultramicrotome, and examined in an Hitachi H-800 TEM, outfitted with a Link Pentafet X-ray detector and QX 2000 processor.

RESULTS

Species of *Trachelomonas* and *Strombomonas* collected from freshwater bodies in Chaco, Argentina, and from Camaleão Lake, Manaus, Brazil, (Fig. 1) were examined and analyzed by SEM, TEM and energy-dispersive spectroscopy (EDS).

Fig. 1. Map of the study areas

Trachelomonas. The surface morphologies of envelopes of *T. volvocina* Ehr. from Argentina, and *T. similis* (var. *similis* fo. *similis*) Stokes from Brazil are shown in Figs. 2 and 3, respectively, with correlative SEM-EDS analyses of the envelopes (Figs. 4,5). The spectra are similar in having iron (Fe) as the major mineralizing element and lesser amounts of silicon (Si). Manganese (Mn) is also present in *T. similis* (var. *similis* fo. *similis*) but absent in *T. volvocina*. Small amounts of Mn are present, however, in samples of *T. volvocina* from Brazil. The relative amounts of these elements in several species of *Trachelomonas* and *Strombomonas* are shown in Table 1.

Table 1.

Relative amounts of elements in envelopes of *Trachelomonas* and *Strombomonas* from Chaco, Argentina, and Manaus, Brazil

	Argentina	Brazil			
% element	*T. volvocina*	*T. volvocina*	*T. similis*	*S. planctonica*	*S. deflandrei*
Si	7.10	9.30	9.37	25.73	46.87
Fe	46.30	51.01	31.75	9.08	8.57
Mn	—	1.16	5.70	0.77	0.58

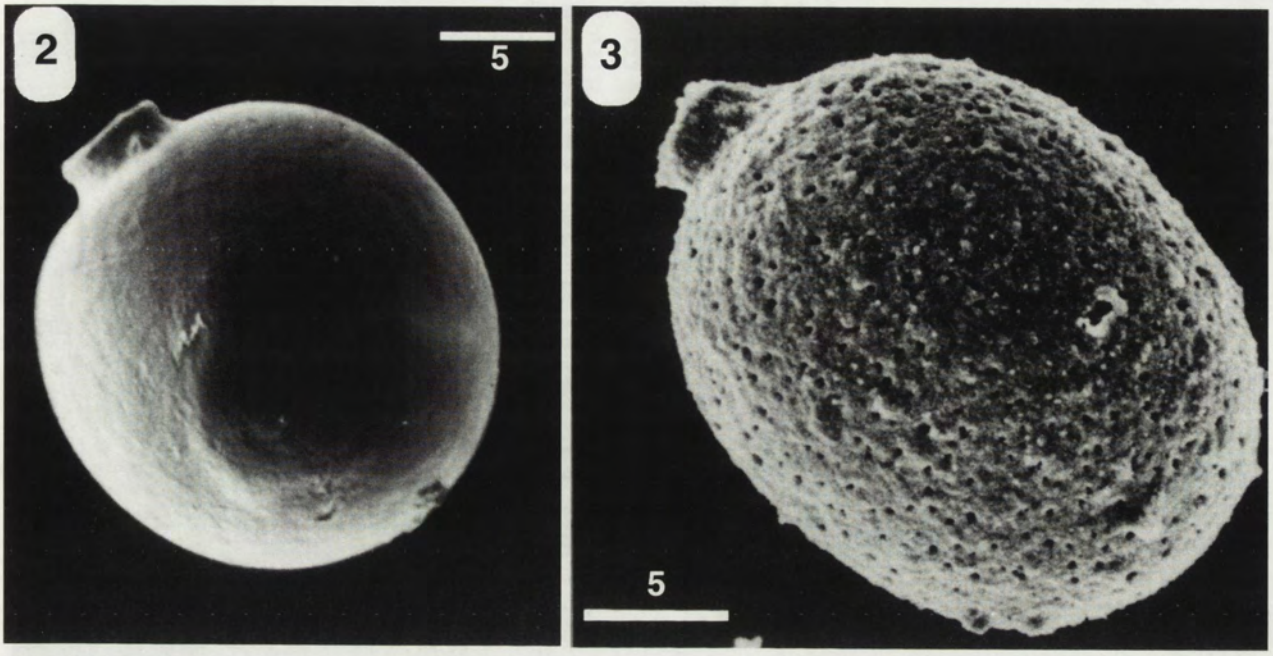


Fig. 2. SEM of *Trachelomonas volvocina* envelope shows the neck at the anterior end and the smooth surface. Bar - 5 µm
Fig. 3. SEM of envelope of *T. similis* (var. *similis* fo. *similis*) shows the punctate and rather rough surface. Bar - 5 µm

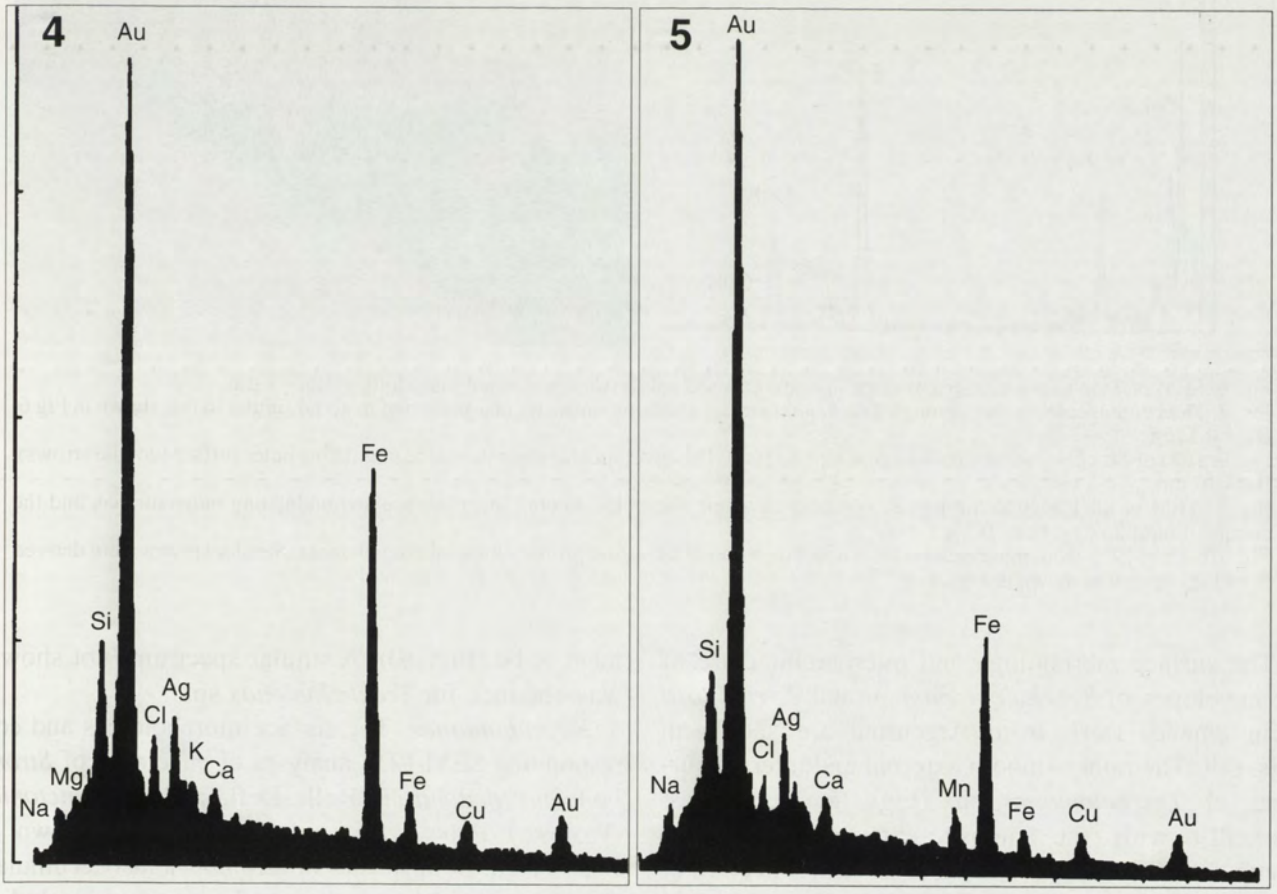


Fig. 4. SEM-EDS of *T. volvocina* envelope shows Fe as the major mineralizing element. The Au peaks are artifacts of the Au coating during specimen preparation
Fig. 5. SEM-EDS of envelope of *T. similis* (var. *similis* fo. *similis*) shows Fe as the major mineralizing element

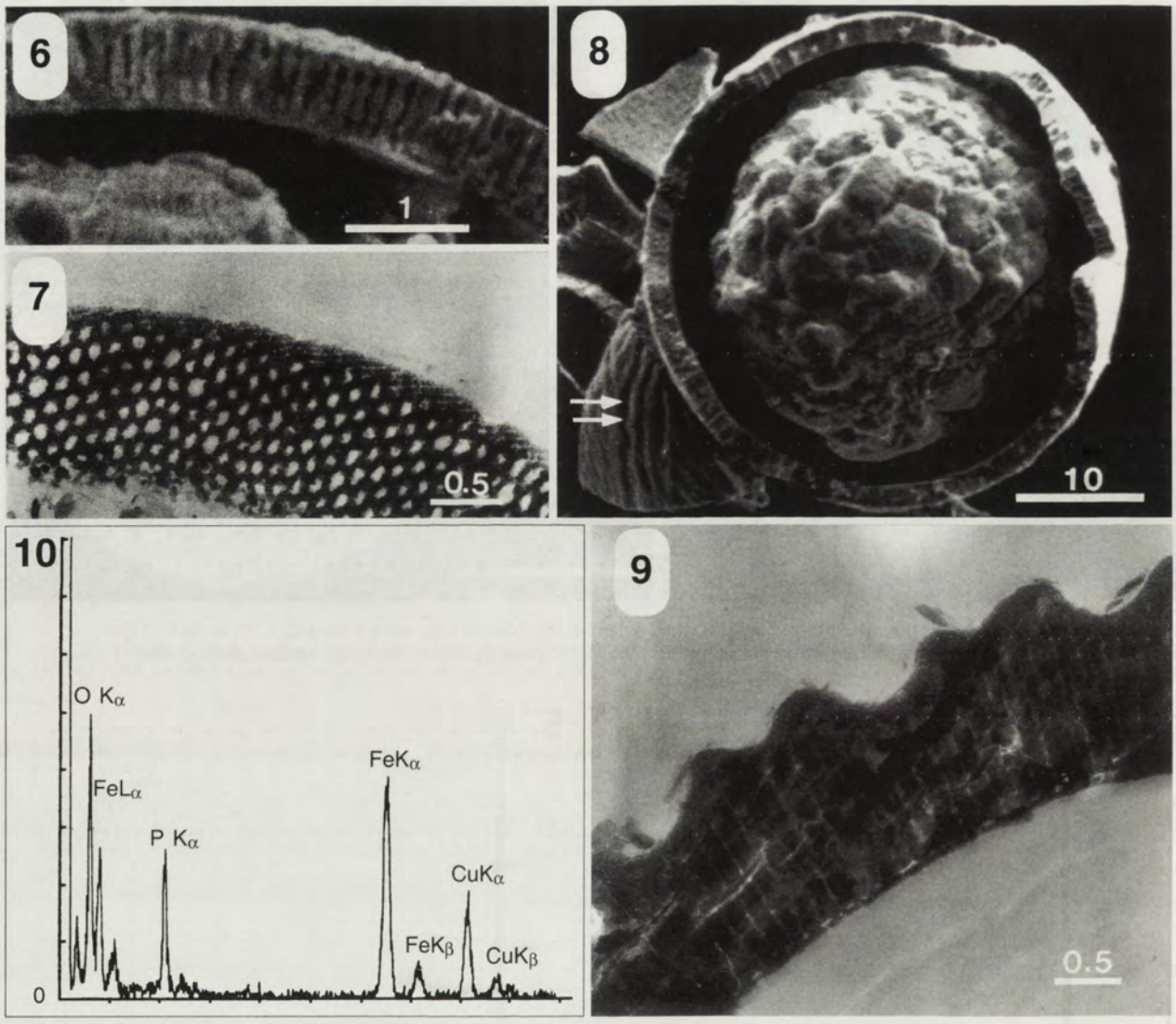
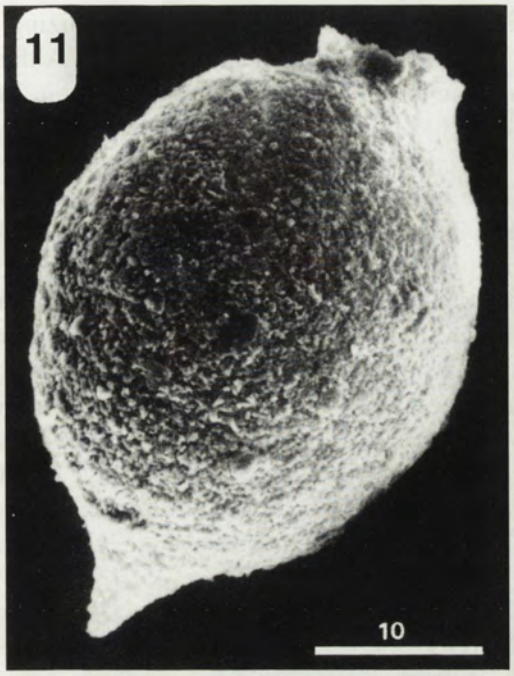
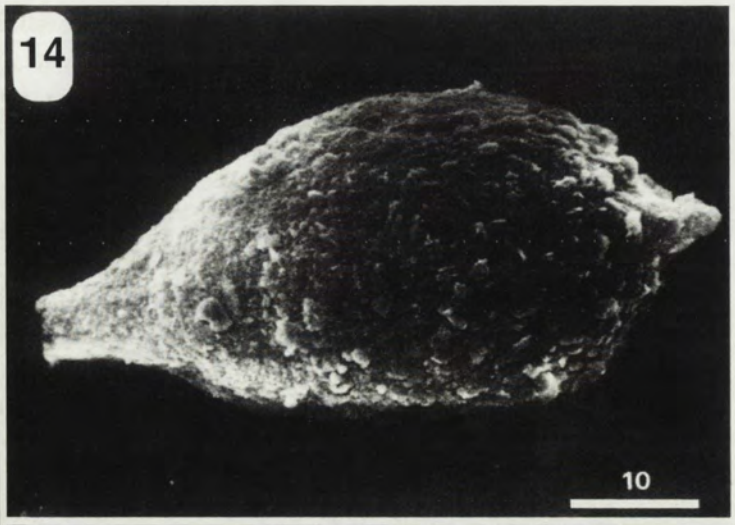


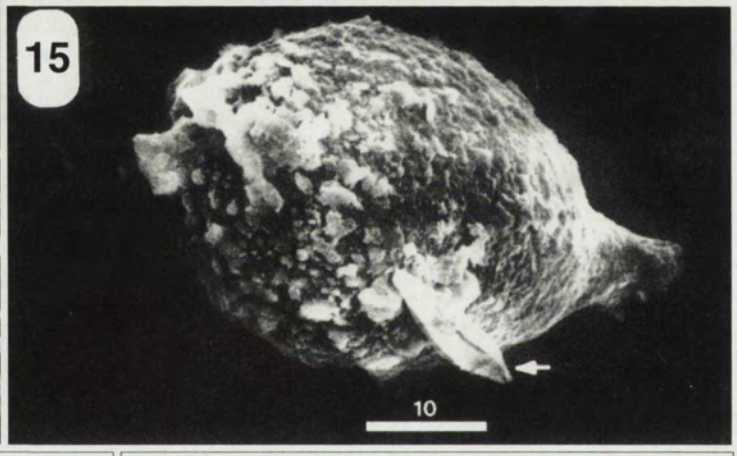
Fig. 6. SEM of *Trachelomonas* sp. envelope in transverse section shows a perforated morphology. Bar - 1 µm
Fig. 7. TEM of tangenial section through *Trachelomonas* sp. envelope shows regular pattern of material similar to that shown in Fig 6. Bar - 0.5 µm
Fig. 8. SEM of *Trachelomonas rugulosa* envelope and cell. The envelope fragment shows the undulating outer surface (double arrows). Bar - 10 µm
Fig. 9. TEM of thick section through *T. rugulosa* envelope shows the smooth inner surface and undulating outer surface, and the columnar morphology. Bar - 0.5 µm
Fig. 10. TEM-EDS from the envelope shown in Fig. 9 shows Fe as the primary mineralizing element. Similar spectra were derived from the organisms shown in Figs. 6-8

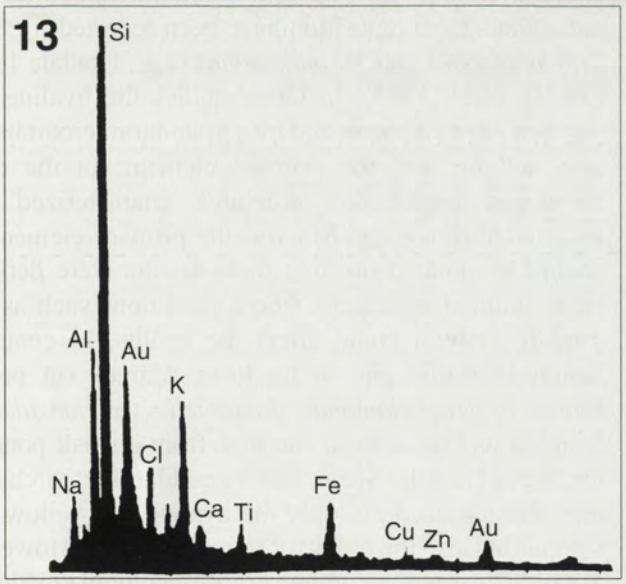
The surface morphology and microarchitecture of the envelopes of *Trachelomonas* sp. and *T. rugulosa* Stein emend. Defl. from Argentina are shown in Figs. 6-9. The rather smooth external and internal surfaces of *Trachelomonas* sp. (Figs. 6, 7) contrast markedly with the rugose exterior surface of *T. rugulosa* (Figs. 8, 9). The basic microarchitecture of both species is granular (Figs. 7, 9), and as shown in a representative spectrum obtained from TEM-EDS analyses of *T. rugulosa*, the major mineralizing element is Fe (Fig. 10). A similar spectrum (not shown) was obtained for *Trachelomonas* sp.

Strombomonas. The surface morphologies and corresponding SEM-EDS analyses of envelopes of *Strombomonas deflandrei* (Roll) Defl. and *S. planctonica* (Wolosz.) Popova (var *planctonica*) are shown in Figs. 11-16. Both species of *Strombomonas* accumulate extraneous particles on the envelope surface, including sand grains (Fig. 12) and even occasional diatom frustules (Fig. 15, arrow). The EDS spectra for both species









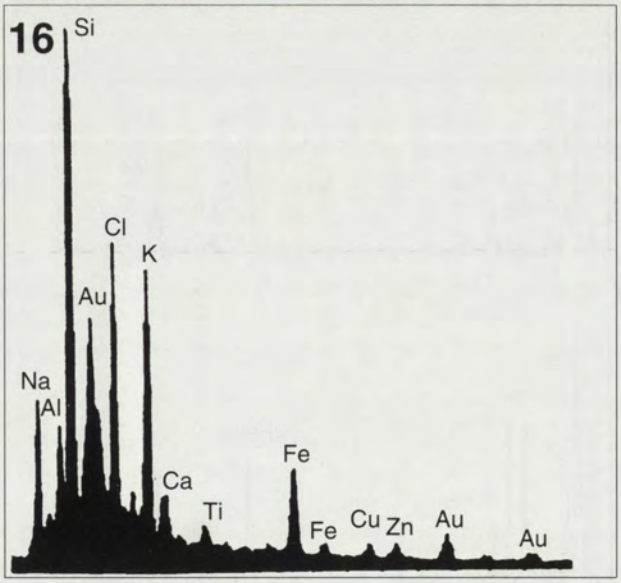


Fig. 11. SEM of *Strombomonas deflandrei* envelope shows general morphology. Bar - 10 µm
Fig. 12. Higher magnification of portion of envelope shown in Fig. 11 shows accumulation of extraneous material on the surface. Bar - 1 µm
Fig. 13. SEM-EDS of *S. deflandrei* envelope shows Si as the major element; some Fe is also present
Fig. 14. SEM of *S. planctonica* var. *planctonica* envelope shows general morphology. Bar - 10 µm
Fig. 15. SEM of envelope of *S. planctonica* var. *planctonica* with accumulated material, including a diatom frustule (arrow), on the envelope surface. Bar - 10 µm
Fig. 16. SEM-EDS spectrum of envelope of *S. planctonica* var. *planctonica* shows Si as the primary element; some Fe is also present

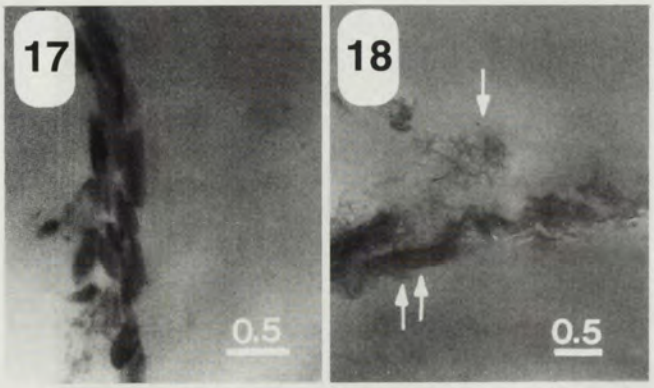


Fig. 17. TEM thick section through envelope of *Strombomonas* sp. shows an array of large, regularly shaped electron-opaque structures. Bar - 0.5 μm

Fig. 18. TEM thick section through envelope of *Strombomonas* sp. shows clusters of granular material (arrow) and large electron-opaque structures (double arrows, cf. Fig. 17). Bar - 0.5 μm

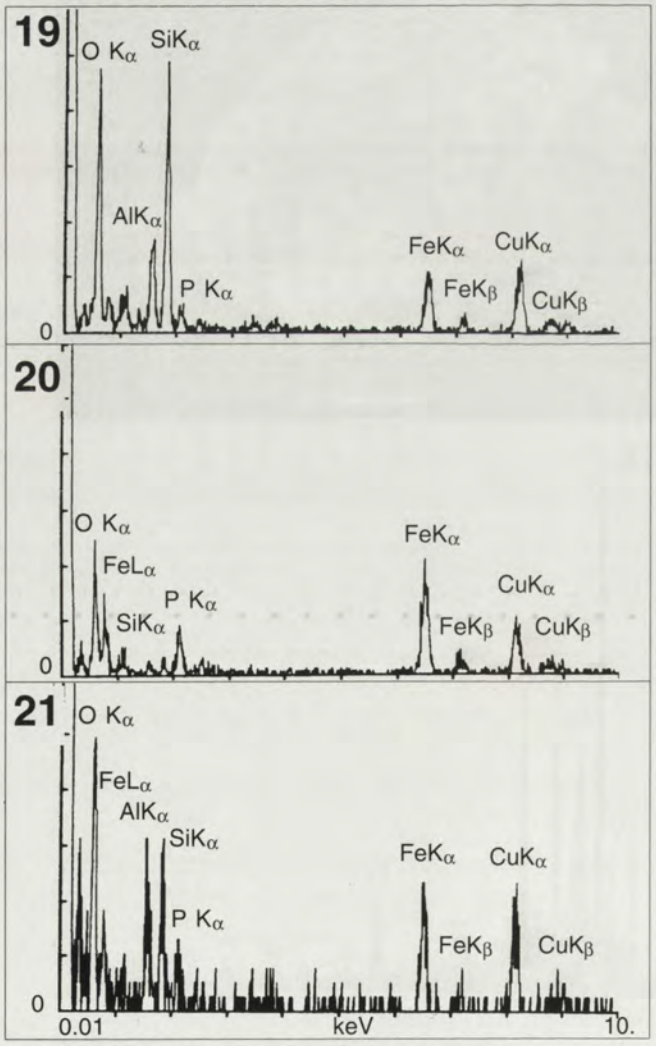


Fig. 19. TEM-EDS spectrum from envelope shown in Fig. 17. Si is the primary element; Fe is also present

Fig. 20. TEM-EDS spectrum from envelope shown in Fig. 18 (arrow). Fe is the primary element, and some Si is present

Fig. 21. TEM-EDS spectrum of envelope region (double arrows) shown in Fig. 18. Fe and Si are present in nearly equal amounts

are similar, showing Si as the major mineralizing element, with Fe in lesser amounts (Figs. 13,16). Although very small amounts of Mn are indicated in Table 1, Mn peaks in the spectra are not clearly discernible.

The TEM microarchitecture of *Strombomonas* sp. shows large, electron-opaque components, often at or near the envelope surface (Figs. 17,18), as well as accumulations of smaller granular deposits (Fig. 18). TEM-EDS analysis of regions containing the large structures shown in Fig. 17 indicates Si as the predominant element, with some Fe present (Fig. 19). EDS analysis of the granular regions shown in Fig. 18 indicates Fe as the major element, with a smaller peak for Si (Fig. 20, cf., Tab. 1), whereas EDS analysis of the large electron-opaque regions (Fig. 18, double arrows, cf. Fig. 17) indicates the presence of Si and Fe (Fig. 21).

DISCUSSION

Several studies have shown that Fe and Mn are the primary mineralizing elements in all species of *Trachelomonas* analyzed to date (e.g., Leedale 1975, Dunlap et al. 1983, Dunlap and Walne 1985, Donnelly Barnes et al. 1986). Similar results have been reported for *Strombomonas*, although only one species, *S. conspersa*, has been investigated (Dunlap et al. 1986). Positive correlations of envelope color, microarchitecture and elemental composition have been reported for both *Trachelomonas* and *Strombomonas* (e.g., Leedale 1975, Dunlap et al. 1983). In those studies the hyaline envelopes were characterized by a granular microarchitecture and Fe was the primary element. In the dark envelopes needle-like structures characterized the microarchitecture and Mn was the primary element. It should be pointed out that these results were derived from cultured organisms where conditions such as the growth medium could affect the results. In contrast, Sanchez-Castillo and de la Rosa Alamos (in press) found, in *Trachelomonas pseudofelix* var. *nevadensis* Sanchez and de la Rosa var. nov. from a small pond in the Sierra Nevada, Spain, that a granular microarchitecture characterized not only the hyaline and yellow envelopes but also the reddish-brown envelopes. However, X-ray microanalyses of the variously colored envelopes were not provided. In our analyses of organisms from several bodies of freshwater in Argentina and Brazil, the color of the envelope does not necessarily correlate with its elemental composition in the way suggested by Dunlap et al. (1983). Why the dark-colored envelopes do not

contain Mn, as would have been predicted from previous studies that did correlate envelope color, microarchitecture and elemental composition (Dunlap et al., 1983) is not fully understood. Our present data do, however, indicate a correlation between envelope microarchitecture and elemental composition. Additionally, differences in fixation protocols cannot be ruled out as a reason for the absence of Mn in the samples from Argentina. However, our experience from other studies of mineralization in euglenoids and other flagellates suggests that fixation protocols do not affect either the basic microarchitecture or the minerals present in the envelopes.

In the *Sphaerotilus-Leptothrix* group of bacteria it was shown that Fe^{2+} is readily oxidized to Fe^{3+} between pH 6 and 8, and it was also demonstrated that Mn oxidation and deposition in the sheath of the *Sphaerotilus-Leptothrix* bacteria are inhibited by phosphates in the culture medium (van Veen et al. 1973). Thus, in our present study the presence or accumulation and oxidation of Fe may not only add to the predominance of Fe in the analyzed envelope, it may also suggest why the envelopes appear dark, since oxidized Fe is rust colored. If so, then environmental conditions may be involved, since the growth habitat must be conducive to the oxidation of Fe. Moreover, as shown for studies on *Trachelomonas* envelopes from oxbow lakes in Alabama (Joo et al. 1991) and from Lake Eibsee in Germany (Steinberg and Klee 1984), information on habitat water chemistry will also be crucial to our understanding of qualitative and quantitative mineralization events in future investigations.

Genetic differences between species or even within the same species could affect the type(s) of mucopolysaccharides destined for the envelope. In the present study, the mucopolysaccharides may influence the local environment by inducing the oxidation of Fe. As discussed previously (van Veen et al. 1978, Dunlap and Walne 1985), Fe deposition onto the organic component of the envelope may be a passive process not requiring biological oxidation, whereas Mn deposition may be an active process requiring cellular activity, possibly involving some type of Mn oxidizing enzyme.

The low levels or total absence of Mn in all analyzed envelopes may suggest that Mn is absent or has low concentrations in the areas sampled. Alternatively, Mn may be present but the organisms are unable to use it, a suggestion that agrees with the observations of Singh (1956) and Moss and Gibbs (1979). Singh showed that two (of six) species of *Trachelomonas* never produced dark envelopes, even when grown in media with Fe and Mn supplements. Moss and Gibbs (1979) also reported that the levels of Fe and Mn in two English rivers did not affect the presence or absence of either element in the trachelomonad envelope.

The effects of environmental conditions were also reported by Dunlap and Walne (1985). In those studies (and other unpublished studies by JRD and PLW) on *Trachelomonas* species which normally do not accumulate Mn in their envelopes, the addition of high levels of Mn to the culture medium did not enhance the uptake of Mn into the envelope. Similar results were found when Fe was added to the culture medium of a species which under standard culture conditions, would not accumulate Fe. Conversely, when the nitrogen level (as NH_4OH) of the growth medium was increased, Fe was accumulated in the envelope, even in species which do not accumulate Fe under standard culture conditions. The accumulation of Fe in the envelope was associated with an appearance of electron-opaque granular material in the envelope (see also West and Walne 1980). The effect of the increased nitrogen was not determined, i.e., (1) whether it resulted in a change in the pH of the culture medium thus favoring Fe oxidation, or an alteration of the envelope organic component(s) already in place, or (2) whether it altered the mucopolysaccharides that would eventually become a part of the envelope, or (3) some combination of these phenomena. Clearly, however, nitrogen did affect both envelope microarchitecture and elemental composition. Thus, in both natural and cultured materials environmental factors have been shown to play a role in envelope color, microarchitecture and elemental composition. Moreover, in the species examined thus far, Fe deposition may be environmentally induced, whereas Mn deposition may not.

With respect to basic microarchitecture, the envelopes of the *Trachelomonas* and *Strombomonas* species examined in the present study are similar, but the *Strombomonas* species all accumulated exogenous particles on or within the envelope (see also Tell and Conforti 1988). Based on their morphology and elemental composition, these exogenous particles are probably sand grains. That the *Strombomonas* species accumulated this material and the *Trachelomonas* species did not may reflect differences in the type of extracellular mucopolysaccharides produced and secreted by the cell, independent of the envelope mucopolysaccharides themselves. Dunlap and Walne (1985) suggested that cells may produce several types of mucilage, some of

which become mineralized and some do not. Similar results have also been reported for the green alga *Phacotus* (Pocratsky and Walne 1981), in which the presence of exogenous particles was attributed to the non-mineralizing mucilage; since it was extruded through the pores in the envelope, the exogenous particles stuck to it, causing an accumulation on the surface of the envelope. A recent finding of bacterial chains perpendicular to the envelope surface in laboratory cultures of *Trachelomonas grandis* Singh (Rosowski 1993) raises interesting questions about the role of such bacteria not only in surface ornamentation but also in the secretion of different mucilages that might be involved in particle accumulation. Thus, the accumulation of exogenous particles may reflect differences in the type(s) of mucilage produced by different genera. Additional information on the mucopolysaccharides produced by *Trachelomonas* and *Strombomonas* may help to resolve the differences in envelope morphology and hence genetic taxonomy.

The results presented here further illustrate the need to understand the effects of environmental conditions, especially water chemistry data, on envelope characteristics since these are used as the primary taxonomic criteria in this diverse group of organisms.

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Infraciliature of *Cymatocylis affinis/convallaria* (Tintinnina)

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Summary. No differences in oral ciliature and kinetom of two polymorphic forms of *Cymatocylis affinis/convallaria* were noted. The oral ciliature consists of 15 peristomial and 4 infundibular polykinetids (OPk). The kinetom built of 39-42 kineties, is usually composed of 28 kineties of the left field (LF), 10 of the right field (RF), 1 ventral (VK), 1 dorsal (DK) and 1 unnumbered posterior kinety (PK). The scheme of somatic ciliature seems to qualify this species for the second group (according to Laval-Peuto and Brownlee 1986) wherein tintinnids with moderate reduction of the kinetom are included.

Key words. *Cymatocylis affinis/convallaria*, kinetom, oral ciliature, protargol-silver staining, somatic ciliature.

INTRODUCTION

Tintinnina, free swimming ciliated protozoa, are protected by the lorica so only adoral membranelles and the anterior part of the cell body can extend beyond the shell. The oral ciliature is responsible for both locomotion and food capture, together with transference of food particles to the cytostome. Somatic ciliature is reduced and no longer functions in swimming. According to Laval-Peuto and Brownlee (1986) it participates in formation of the lorica, removal of waste products, and in the positioning of the protoplast within the lorica.

The commonly used classification of the Tintinnina was based mainly upon characteristics of the lorica, a structure which can vary in shape, structure and ornamentation (Laval-Peuto and Brownlee 1986, Choi et al. 1992). Many authors (Bakker and Phaff 1976; Laval-Peuto 1981, 1983; Laval-Peuto and Brownlee 1986) have reported that ciliates of this suborder demonstrate high natural polymorphism, such that even loricea of the same species can be significantly different. This is why lorica morphology cannot be the only descriptive feature taken into consideration. The most important factor which should be investigated is the cell's ultrastructure, with special emphasis on the organization of the ciliature and infraciliature (Brownlee 1977, Foissner and Wilbert 1979, Laval-Peuto and Brownlee 1986, Snieżek et al. 1991). Choi et al. (1992) emphasized that much larger numbers of species must be examined at generic and suprageneric levels using TEM, SEM and protargol impregnation methods, to clarify an old Tintinnina classification.

Only few Tintinnina species have been carefully examined using the silver impregnation staining method (Brownlee 1977, Foisner and Wilbert 1979, Laval-Peuto and Brownlee 1986, Snieżek et al. 1991, Snyder and

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Brownlee 1991, Choi et al. 1992). The analysis of protargol treated tintinnids led to the distinction of 4 groups according to the degree of somatic ciliature reduction. Specifically, the division was based on kinetal density index, the length of the kineties, and the position of the oral apparatus. In the first group one can find cells with long kineties, little specialization of somatic cilia, and the most anteriorly located oral apparatus. In the second group are species with specialized ciliature and ventralized oral apparatus. To the third group belong species with short kineties and infundibulum with slight ventralization, whereas to the fourth - species with few short kineties and a ventral infundibulum (Laval-Peuto and Brownlee 1986).

Boltovskoy et al. (1990) suggested that the ciliate endemic to Antarctic waters, *Cymatocylis affinis* Laackmann,1909, and *C. convallaria* Laackmann, 1909, in spite of showing differences in lorica morphology, are polymorphic forms (Fig. 1) and ought to be treated as one species named *C. affinis/convallaria*. The morphology and ultrastructure of *C. affinis/convallaria*, forma *convallaria* was described in detail in our previous papers (Wasik and Mikołajczyk 1992, 1994), in which the problem of infraciliature was only slightly addressed. Investigation of each polymorphic form's occurrence demonstrates that they are interchangeable during the year. The *C. affinis/convallaria*, forma *convallaria* is dominant in the austral summer, while forma *affinis* is restricted to the winter. The aim of this paper was to recognize the species specific ciliary pattern of both polymorphic forms, which will provide final confirmation of whether they can be combined, and if so, to which group according to Laval-Peuto and Brownlee's (1986) clustering, this species belongs.

MATERIALS AND METHODS

Cells used for examination were collected in Admiralty Bay, King George Island, between February 1990 and January 1991 during the XIVth Antarctic expedition organized by the Department of Antarctic Biology Polish Academy of Sciences. Ciliates were fixed in precooled 4% formalin buffered with calcium carbonate at 0-3°C immediately after collection. Samples were then ported to the Nencki Institute of Experimental Biology for further work.

The protargol impregnation procedure was carried out according to Hedin (1976) with minor modifications as described previously (Wasik and Mikołajczyk 1992). Protargol stained material was examined and photographed under a light microscope (Leitz and Jenalumar, Carl Zeiss, Jena). Film negatives were analyzed for description of the ciliature pattern, using a viewer (Carl Zeiss, Jena). For SEM, cells were prepared as described previously (Wasik and Mikołajczyk 1991).

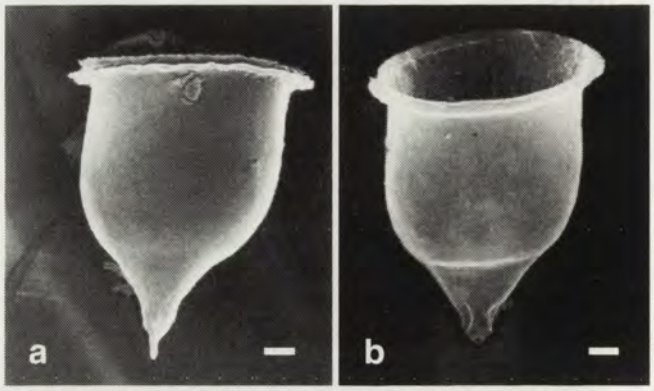


Fig. 1. Scanning electron micrograph of *C. affinis/convallaria* loricae. a - forma *affinis*, b - forma *convallaria*. Bar - 10 µm

RESULTS AND DISCUSSION

Examination of the protargol-silver stained oral and somatic ciliature of polymorphic forms of *Cymatocylis affinis/convallaria* revealed no differences. Therefore, it is reasonable to consider them as one species as proposed by Boltovskoy et al. (1990).

Oral ciliature

Oral infraciliature (Fig. 2) of *C. affinis/convallaria* consists of 18 (17-19) adoral membranelles, composed of three kinetosomal rows (Wasik and Mikołajczyk 1992). The same arrangement was also reported by Choi et al. (1992) in other species, such as *Eutintinnus spp.* Oral polykinetids built with two rows of kinetosomes (*Nolaclusilis bicornis*) can also be found (Snyder and Brownlee 1991). In *C. affinis/convallaria* there are usually 19 oral polykinetids (OPk): 15 peristomial and 4 infundibular (Table 1). The number of infundibular polykinetids may vary with species, from 1 as in *Tintinnidium mucicola*, or up to 6 or 7 as in *Stenosemella steini* (Brownlee 1977). The last two infundibular polykinetids in *C. affinis/convallaria*, OPk_{19} and OPk_{18}, originate deeply, and are in fact very near to the cytostome (Fig. 3). A distal tip of OPk_{19} does not emerge from the infundibulum; OPk_{18} is longer and curves onto the peristomial rim. OPk_{17} and OPk_{16} begin at successively shallower depths and extend out of the infundibulum. The peristomial polykinetid OPk_1 curves down into the infundibulum.

The paroral membrane (PM) in *C. affinis/convallaria* begins near the cytostome and extends along the right wall of the peristome, terminating below OPk_6 (Fig. 3), and consisting of a single row of kinetosomes (Wasik

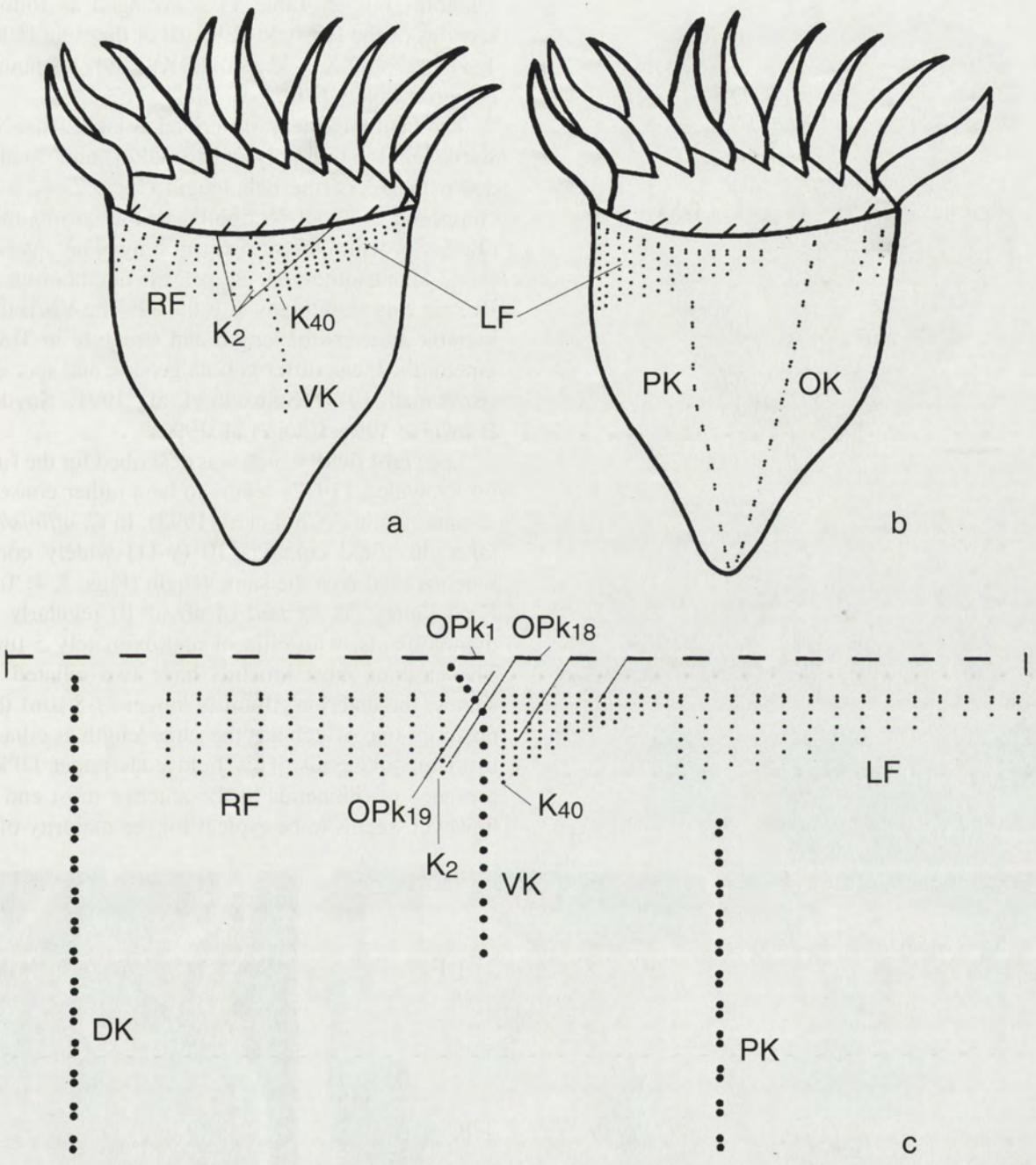
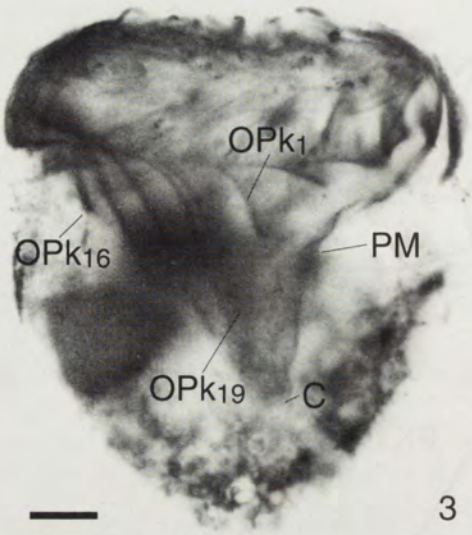


Fig. 2. Semi-diagrammatic illustrations of a - ventral surface, b - dorsal surface, c - kinetal map of *C. affinis/convallaria*. Abbreviations: DK - dorsal kinety, K_2 - second somatic kinety, K_{40} -last somatic kinety, LF - left ciliated field, OPk_1 to OPk_{19} - oral polykinetids, PK - posterior kinety, RF - right ciliated field, VK - ventral kinety

and Mikołajczyk 1992). This arrangement seems to be common in tintinnids, although species with more than one row can also be found (Brownlee 1977). In *Eutintinnus angustatus*, extension of the paroral membrane with a form of lightly staining cilia-like structures is also evident.

Somatic ciliature

We numbered somatic kineties as proposed by Choi et al. (1992) who followed the convention of Brownlee (1977). The total number of somatic kineties of *C. affinis/convallaria* varies from 39 to 42. Usually the



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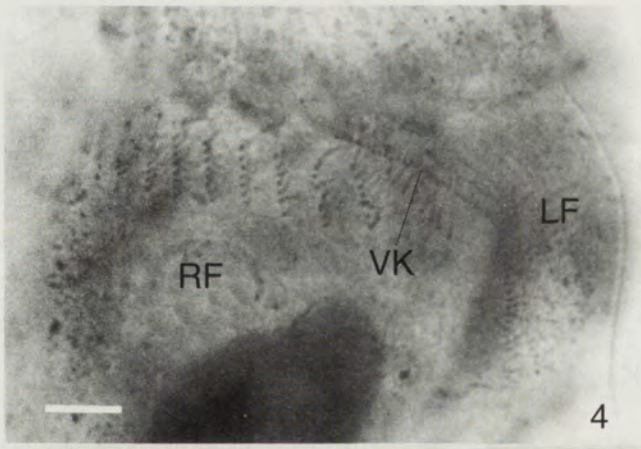

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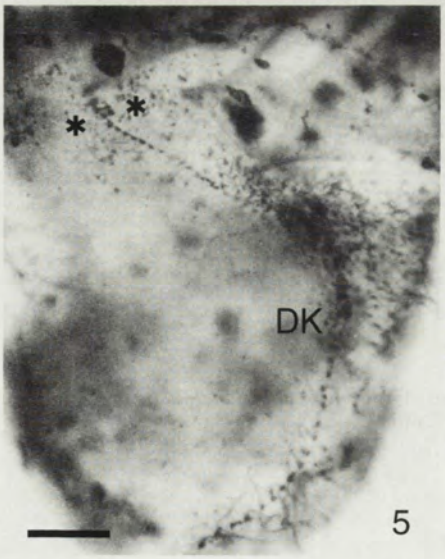
kinetom (Fig. 2, Table 1) is arranged as follows: 28 kineties of the left field (LF), 10 of the right field (RF), 1 ventral (VK), 1 dorsal (DK), and 1 unnumbered posterior kinety (PK).

The **ventral kinety**, described as kinety number one, starts near the oral polykinetid (OPk_1) and usually runs down to 2/3 of the cell length (Figs. 2, 4, 10). It is composed of about 60 tightly composed monokinetids (Table 1), with cilia of 5-6 μm long. The anteriormost 6 to 7 kinetosomes extend over the neighboring LF and RF, curving slightly towards the RF. The VK is the most variable in terms of length and structure in Tintinnina kinetom's. It can differ at both generic and species level (Brownlee 1977, Snieżek et al. 1991, Snyder and Brownlee 1991, Choi et al. 1992).

The **right field**, which was described for the first time by Brownlee, (1977) seems to be a rather conservative somatic feature (Choi et al. 1992). In *C. affinis/convallaria* this field contains 10 (9-11) widely composed kineties of almost the same length (Figs. 2, 4; Table 1). Each kinety is formed of about 10 regularly spaced monokinetids, with cilia of approximately 5 μm long. The anterior most kinetids have two ciliated kinetosomes; the anterior cilium is longer (7-8 μm) than the posterior one, which has the same length as cilia arising from monokinetids. This field ends under OPk_6. The presence of dikinetids at the anterior most end of RF, however, seems to be typical for the majority of tintin-



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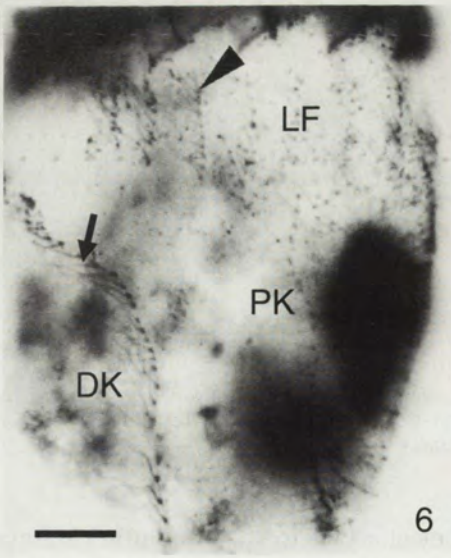

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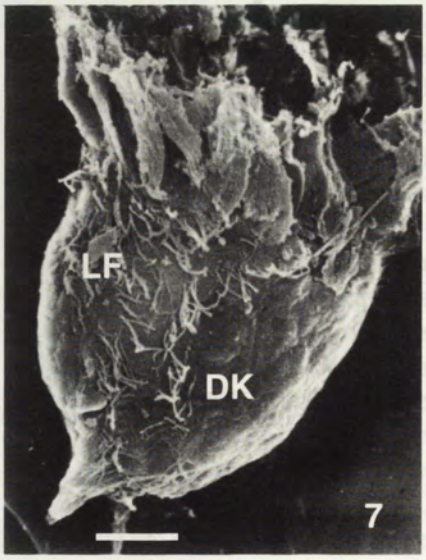

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Figs. 3 – 5. Fig. 3. Protargol impregnated oral area. OPk_{16} to OPk_{19} - infundibular polykinetids, OPk_1 - peristomial polykinetid, c - cytostome, PM - paroral membrane. Bar - 10 μm Fig. 4. Ventral view showing position of the ventral kinety (VK) relative to the right (RF) and left (LF) ciliated fields. Protargol impregnated cell. Bar - 10 μm Fig. 5. Dorsal view showing position of the dorsal kinety (DK). Unciliated area on both sides of DK (stars). Fig. 6. Widely spaced kineties of LF. Arrow-head shows cilia of unequal length emerging from the anterior most kinetosomes. Arrow points to long cilium arising from the posterior kinetosome of the dorsal kinety (DK) dikinetid. PK - posterior kinety. Figs. 4, 5 - protargol stained cells. Fig. 7. Scanning electron micrograph of the dorsal kinety (DK) and the widely spaced kineties of LF. Bars - 10 μm

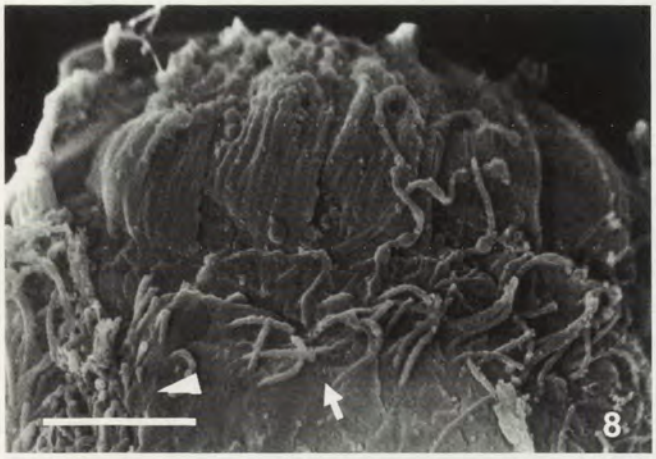

Fig. 8. Closely (arrow head) and widely (arrow) spaced kinetids of LF. SEM. Bar - 10 μm

nids; in some species (*Eutintinnus augustatus*, *E. tenuis*, *Salpingacantha spp.*) it can be absent (Brownlee 1977, Choi et al. 1992).

The **dorsal kinety** (Figs. 2, 5-7) starts at OPk_8 and runs down as far as the end of the tail, curving in the central part of the cell body. This kinety is composed of 64-66 dikinetids (Table 1), which are closely arranged anteriorly and become progressively more distant from each other posteriorly. Cilia arise only from posterior kinetosomes and are longer than the other somatic cilia, and are approximately 10 μm long. Both sides of DK toward RF and LF, usually of one OPk width are unciliated (Figs. 2, 5). In some species, such as *Eutintinnus spp.* , more than one DK is present (Choi et al. 1992), whilst in some species (*Nolaclusilis spp.*, *Tintinnidium spp.*) this kinety is absent (Foissner and Wilbert 1979, Song and Wilbert 1989, Snieżek et al. 1991, Snyder and Brownlee 1991). Although in most of the examined species the DK is composed of dikinetids, in some (*Eutintinnus angustatus*, *Codonella cratera*, *Stenosemella steini*) it is formed of monokinetids (Brownlee 1977, Choi et al. 1992).

The **left field** in *C. affinis/convallaria* is divided into two sectors of differently composed kineties (Fig. 2, Table 1). The first, which begins at OPk_{10}, is formed by 13-14 widely spaced kineties (Figs. 2, 6-10) with the second starting near OPk_{15} being built with 14-15 closely spaced kineties (Figs. 2, 4, 8, 10). The division of LF into two subfields seems to be quite frequent in tintinnids (*Tintinnopsis spp.*, *Stenosemella spp.*, *Codonella spp.*), although this phenomenon is not observed (Faure-Fremiet 1924, Brownlee 1977, Choi et al. 1992) in some species (*Amphorellopsis acuta*, *Eutintinnus spp.* except *E. tenuis*). In each sector, the kinetal length gradually

Table 1

Infraciliature of *C. affinis/convallaria*

	mean	SD	range	number of cells
Oral polykinetids number				
infundibular	4	± 0	-	20
peristomial	15	± 0.2	14-16	30
Kinety number				
right field	10	± 0.8	9-10	40
left field				
closely spaced sec.	14	± 0.3	14-15	24
widely spaced sec.	14	± 0.4	13-14	20
ventral kinety	1	± 0	-	20
dorsal kinety	1	± 0	-	20
posterior kinety	1	± 0	-	20
Kinetosome number				
right field	10	± 0.7	7-11	30
left field				
closely spaced sec.				
longest kinety	25	± 0.5	23-26	20
shortest kinety	4	± 0.2	3-4	20
widely spaced sec.				
longest kinety	12	± 0.3	11-12	15
shortest kinety	2	± 0.2	2-3	15
ventral kinety	60	± 1.6	54-63	17
dorsal kinety	66	± 0.5	64-66	15
posterior kinety	50	± 0.4	50-52	20

increases from 2-3 single ciliferous kinetosomes in K_{13}, to about 12 in K_{26} (widely spaced sector), and from 3-4 ciliated monokinetids in K_{27}, to 23-26 in K_{40} (closely spaced sector). The anterior most kinetids of widely spaced kineties have two ciliferous kinetosomes. The anterior cilium is longer than the posterior one (Fig. 6), as in RF. Cilia length differences in terms of sector are also noticed. Those from closely spaced kineties are much shorter (ca. 2 μm) than those from widely spaced ones (5-6 μm).

Under the LF, between K_{17} and K_{18}, the **posterior kinety** (Figs. 2, 6, 9, 11) originates and runs down to the end of the cell's tail. It is composed of ca. 50 dikinetids (Table 1), with increasing distance between each of them towards the posterior end of the cell. From the posterior kinetosomes of each dikinetid, 10 m long cilia arise. The PK is a rather variable structure of the kinetom. In a number of species for example (*Tintinnopsis spp.*, *Stenosemella steini*) it is formed of dikinetids, whilst in others (*Codonella cratera*, *C. scalaroides*) by monokinetids (Brownlee 1977). In *Eutintinnus spp.* the PK is absent (Choi et al. 1992).

We found that in *C. affinis/convallaria*, irregularly scattered groups of two dikinetosomes (Fig. 11) are located, and if present are always found on each side of

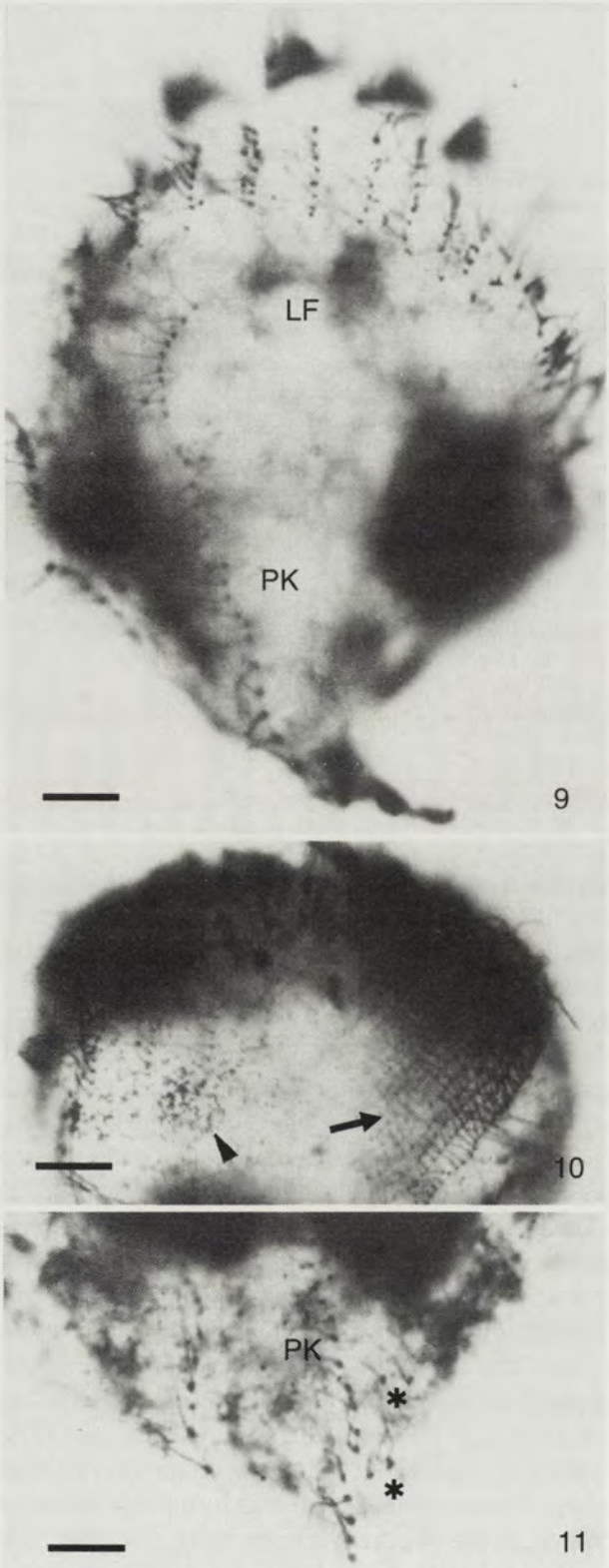

Fig. 9. View of widely spaced kineties of the left field (LF) with the posterior kinety (PK) located below. Fig. 10. Closely spaced kineties (arrow) of LF. Slightly marked widely spaced kineties (arrow head) of LF. Fig. 11. Irregularly scattered groups of dikinetosomes (stars) located near the posterior kinety (PK). Figs. 9-11 protargol stained cells. Bar - 10 μm

PK. It is probable that they are the remnants of the long kineties. Kinetal fragments, but located near DK, were found in *Eutintinnus pectinis* (Brownlee 1977, Choi et al. 1992). Brownlee (1977) suggested that they may be transformed PK.

Laval-Peuto and Brownlee (1986) proposed that Tintinnina species be arranged into 4 groups according to the degree of infraciliature reduction (see INTRODUCTION). The analysis of the ciliature and infraciliature of *C. affinis/convallaria* seems to qualify this species to the second group, characterized by moderate reduction of the somatic ciliature.

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Cytoskeletal Organization of *Disematostoma colpidioides* (Ciliophora, Frontoniidae).

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Summary. The cytoskeletal arrangement of *Disematostoma colpidioides* has been studied by silver impregnation and permeabilization methods. The somatic cortex is supported by the epiplasm, kinetosomes and their fibrillar derivates, and by the outer and infraciliary lattices. The buccal cytoskeleton presents a more complicated structure. The right side is mainly maintained by nematodesmal bundles arising from the vestibular and paroral kineties, and microfibrillar tracts contribute to the maintenance of this side. The left side is supported by the microfibrillar nets and the postciliary microtubules coming from the kinetosomes of the membranelles. The bottom of the buccal cavity is surrounded by the deep net and postciliary microtubules of the peniculi, and by the filamentous reticulum and nematodesmata belonging to the paroral infraciliature.

Key words. Cytoskeleton, *Disematostoma*, permeabilized cells.

INTRODUCTION

Morphology and morphogenesis of the peniculid ciliate *Disematostoma* have been studied in detail at light microscopy (Martín-González et al. 1990, Serrano et al. 1990), but ultrastructural data about this genus have only been reported by Didier (1970).

Cytoskeletal structures of peniculid ciliates have been studied by several authors (Cohen el al. 1982; Cohen and Beisson 1988; Didier 1970; Garreau de Loubresse et al. 1988; Guinea et al. 1987, 1990; Iftode et al. 1989; Martín-González et al. 1986). According these studies, the general organization of the cortical elements within this group can be summarized as follows: (1) a granular subalveolar epiplasm described in all the species studied, (2) an outer lattice, (3) an infraciliary lattice defined by Cohen and Beisson (1988) as "a network of bundles of microfilaments running under the cortex", and (4) a number of somatic kineties composed by dikinetids or monokinetids, or by both, double and single kinetosomes.

The aim of this work is to contribute to the knowledge of the cellular architecture of *D. colpidioides*, mainly those related with the oral area and both sutures, studying control and permeabilized cells.

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MATERIALS AND METHODS

Specimens of *Disematostoma colpidioides* were collected in a natural pond of "Dehesa de la Villa" (Madrid, Spain). The ciliates were isolated and maintained at room temperature (20°C) in soil medium inoculated with *Chlorogonium* sp.

Light microscopy studies of the cytoskeletal structures were performed using Fernandez-Galiano's silver impregnation method (1976).

Permeabilization of cells was achieved by transferring them into 1% Triton X-100 in PHEM buffer + 3% BSA + 0.1% Tween 20 for 5 min (PHEM buffer contains 60mM PIPES, 25mM HEPES, 10mM EGTA and 2mM $MgCl_2$) (Schliwa and van Blerkom 1981, Jerka-Dziadosz, personal communication). After washing in PHEM buffer, treated and control cells were fixed 60 minutes in 2% glutaraldehyde in 50 mM sodium cacodylate, pH 7.2. Post-fixation was done with 1% OsO_4 in PHEM buffer during 45 min. The specimens were dehydrated, embedded in Epon 812, and sectioned with a Reichert-Jung Ultracut ultramicrotome. Staining was made with uranyl acetate followed by lead citrate. A Philips 300 transmission electron microscope (60-80 kV) was used.

RESULTS

General Morphology

Disematostoma colpidioides is a kidney-shaped ciliate with 90-95 longitudinal somatic kineties and the dorsal polar band characteristic of this genus on the dorsal side (Figs. 1 and 2). On the ventral side, the oral infraciliature is constituted by one paroral kinety, three peniculi, and 3-5 vestibular kineties (Fig. 3). Two sutures: preoral and postoral ones, can also be observed on this side (Figs. 1, 3, 4).

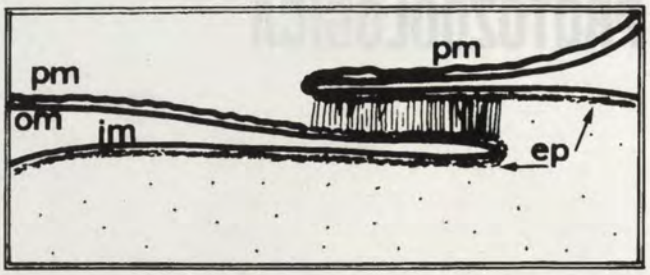
Somatic Cortex

At the somatic cortex, cytoskeletal structures as the outer and infraciliary lattices, epiplasmic layer and the classical fibrillar elements, can be observed (Fig. 5).

Preoral Suture. The preoral suture, located at the anterior zone of the ciliate body, is constituted by the convergence of the anterior extremes of the somatic kineties except for the left ventral ones. This zone is reinforced by very long kinetodesmal fibers crossing one another, which arise from kinetosomes placed on the both sides (Figs. 3, 6).

Postoral Suture and Dorsal Polar Band. The postoral suture departs from the posterior zone of the buccal cavity going backward to the posterior pole (Fig. 1). The argentophilic line observed in silver impregnated

Scheme 1



specimens, the cytoproct (Fig. 4), is constituted by two epiplasmic layers linked by microfibrils (Scheme 1, Figs. 7, 8).

Below to the dikinetids placed on both sides of the suture there are nematodesmal plates originating long nematodesmata (Figs. 4, 8, 9) that penetrate deeply in the endoplasm reaching the vicinity of the macronucleus (Fig. 10).

The postoral suture finishes at the posterior pole of the cell being in contact with the "dorsal polar band", in which the dorsal kineties converge. In this zone, we observe the existence of cilia with membranous folding that might correspond with the infraciliature of the dorsal polar band (Figs. 2, 11).

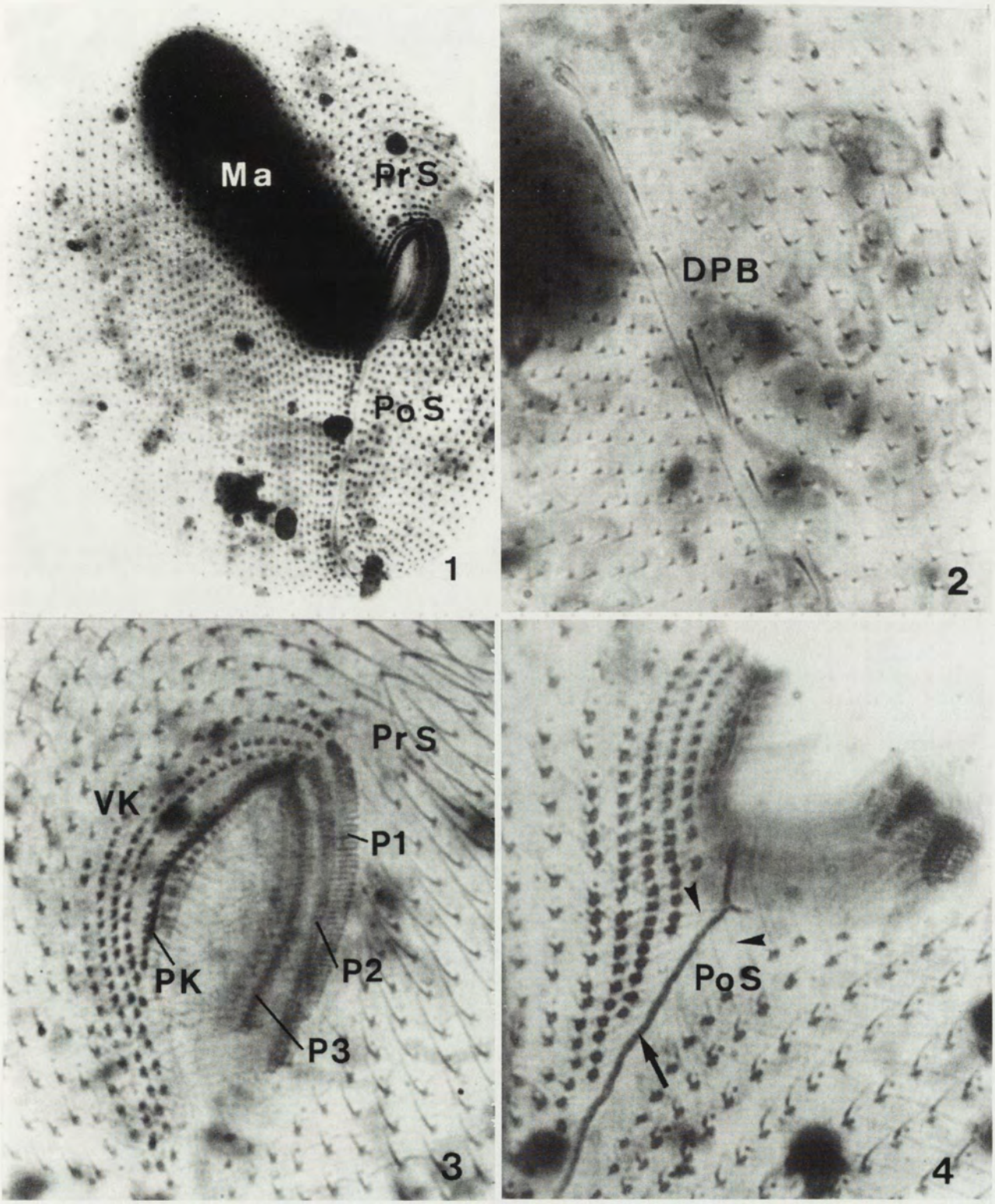
Oral region

The skeleton of the oral area is formed by the kinetosomes, fibrillar and microfibrillar derivates of the peniculi, and the paroral and vestibular kineties.

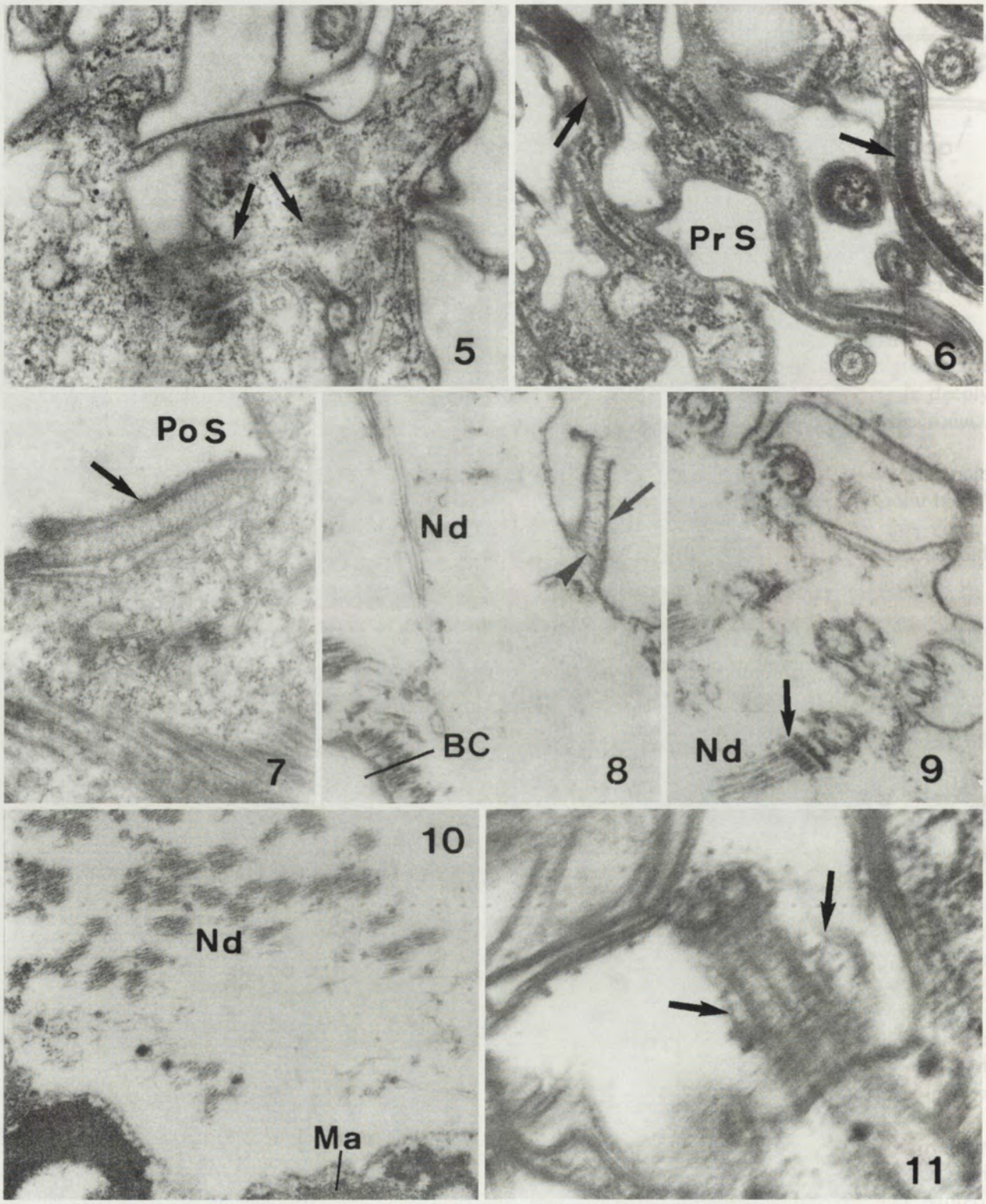
At the right side of the buccal cavity, the cytoskeleton is reinforced by long nematodesmata originating from the paroral and vestibular kineties (Fig. 12). Nematodesmal plates connect with the kinetosomes in this zone through microfibrillar bundles (Figs. 12, 13). Moreover, all the nematodesmal plates of each kinety are also connected one to another through microfibrillar tracts (Fig. 13).

From the nematodesmal plates situated under the paroral kinetosomes, microfibrillar bundles depart connecting with the ribbed wall (Fig. 14). The paroral kinetosomes are also in contact at a deeper level with the filamentous reticulum (Figs. 12, 15).

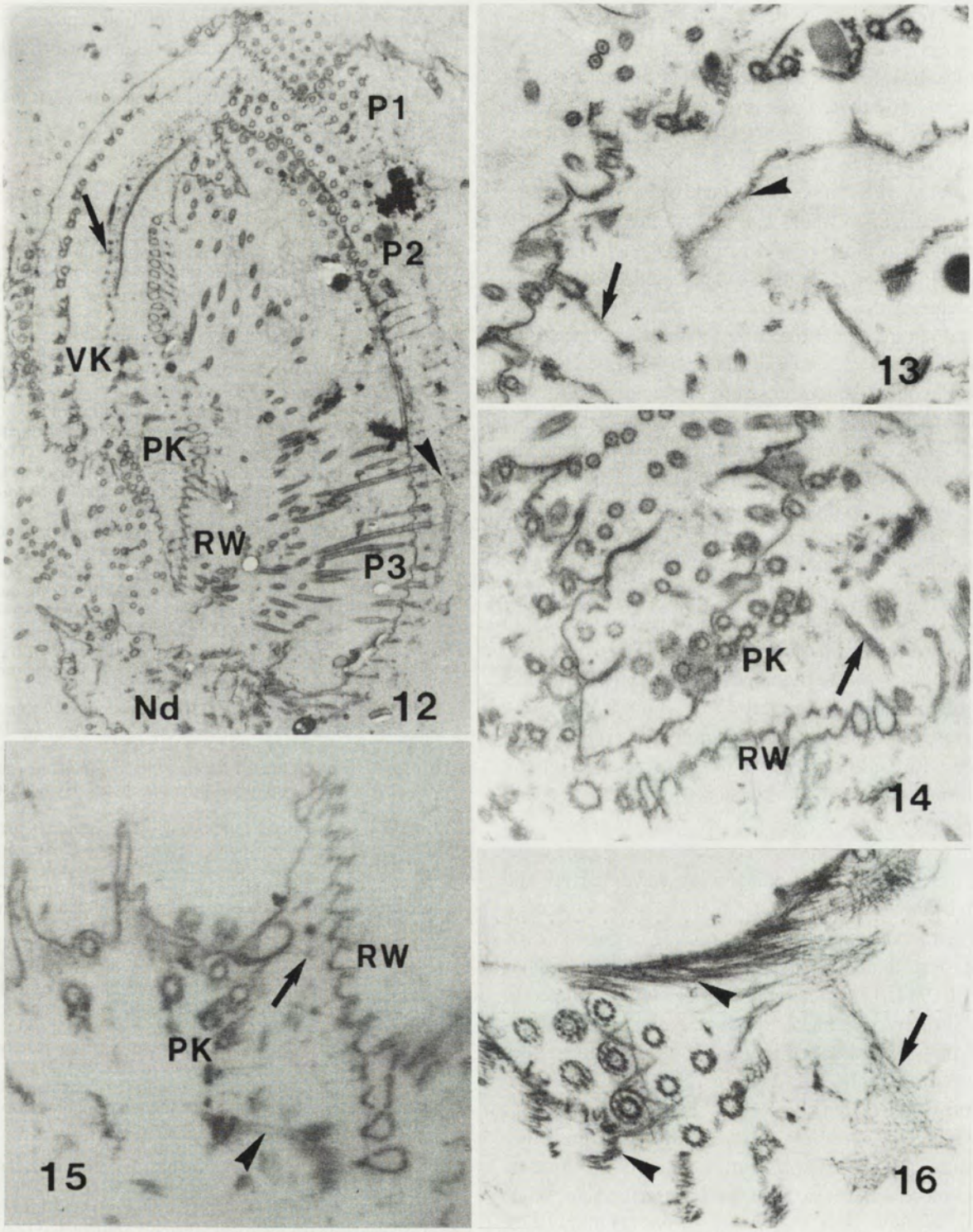
On the left side, the oral cytoskeleton is reinforced by the postciliary microtubules and the microfibrillar nets originated from the peniculi (Fig. 12). Moreover, from the kinetosomes of each peniculus depart bundles of postciliary microtubules that reach the right side of the buccal cavity (Fig. 12). These microtubular ribbons form thick bundles which surround the deeper zone of the buccal cavity (Fig. 16).



Figs. 1-4. *Disematostoma colpidioides* impregnated with silver carbonate. Fig. 1 - general view of a specimen by its ventral side, PrS - preoral suture, PoS - postoral suture, Ma - macronucleus (x 600); Fig. 2 - detail of the dorsal polar band (DPB) (x 1500); Fig. 3 - buccal cavity of the vegetative cell showing the arrangement of the oral infraciliature and the preoral suture. P1, P2 and P3 -peniculi, PK - paroral kinety, VK - vestibular kineties, PrS - preoral suture (x 1500); Fig. 4 - postoral suture (PoS). Note the cytoproct (arrow) and the slight striations (arrow heads) of this zone (x 1600)



Figs. 5-11. Somatic cortex of *D. colpidioides*. Figs. 5, 6, 7 and 11 are control cells, and Figs. 8, 9 and 10 are sections of permeabilized cells. Fig. 5 - somatic cortex. The infraciliary lattice (arrows) can be observed (x 35500); Fig. 6 - grazing section through the preoral suture (PrS). Long kinetodesmal fibers are crossing in the middle of this zone (arrows)(x 37000); Fig. 7 - transversal section through the postoral suture (PoS) showing the pellicular fold in the middle part of this zone (arrow) (x 28700); Fig. 8 - oblique section through the postoral suture showing the epiplasmic fold (arrow) and the microfibrils linking the epiplasmic layers (arrow head). BC - buccal cavity, Nd - nematodesmata (x 21000); Fig. 9 - nematodesmal plates (arrow) and nematodesmata (Nd) of the postoral suture (x 33300); Fig. 10 - bundles of nematodesmata (Nd) coming from the postoral kineties reaching the proximity of the macronucleus (Ma) (x 22850); Fig. 11 - cilium of the dorsal polar band with membranous folding (arrow) (x 68100)



Figs. 12-16. Buccal cavity of *D. colpidioides*. Permeabilized cells; Fig. 12 - planar view of the buccal cavity showing the oral cytoskeleton. P1, P2 and P3 - peniculi, PK - paroral kinety, VK - vestibular kineties, Nd - nematodesmata, RW - ribbed wall. Arrow indicates the filamentous reticulum, and arrow head points the deep net of the peniculi (x 7500); Fig. 13 - detail of the cytoskeleton at the right side of the buccal cavity. Note the fibrillar connections between the kinetosomes of the vestibular kineties and the kinetodesmal plates (arrow), and the microfibrillar tracts that relate the nematodesmal plates beneath each kinety (arrow head) (x 18850); Fig. 14 - parallel section to Fig. 12. Detail of the right side showing the microfibrillar bundles (arrow) connecting the paroral kinety (PK) with the ribbed wall (RW) (x 16900); Fig. 15 - oblique section through the paroral kinety (PK). The filamentous reticulum (arrow), the ribbed wall (RW), and the microfibrillar bundles (arrow head) can be observed (x 18700); Fig. 16 - cytoskeleton of the left side of the buccal cavity. Microfibrillar nets (arrow) and microtubular bundles (arrow heads) originated from the peniculi can be observed (x 20000)

DISCUSSION

Results about the cytoskeleton of the somatic cortex of *Disematostoma colpidioides* agree with those obtained in other ciliates (Cohen et al. 1982; Cohen and Beisson 1988; Didier 1970; Fleury 1991a, b; Gil 1986, 1988; Jerka-Dziadosz et al. 1987) it is constituted by the infraciliature: basal bodies, related fibers and several networks. These structures are in close relationship with each other and with the epiplasm, constituting a superficial support of the ciliary shape.

Preoral suture, constituted by the convergence of the anterior extremes of somatic kineties, has not yet been described in other peniculines at ultrastructural level. Our observations indicate that this structure does not present specific cytoskeletal components, but in this zone the kinetodesmal fibers arising from the kinetosomes of both sides are longer than the other somatic ones. These fibers intersect one another reinforcing this area as in *Paramecium* (Fernández-Galiano 1978).

Our observations about the postoral suture in *D. colpidioides* coincide with those obtained in *Frontonia* (Gil 1984, 1988) and *Paramecium* (Cohen and Beisson 1988). Epiplasmic folds of the cytoproct could represent a local differentiation related to excretory function.

With respect to the oral cytoskeleton of *D. colpidioides*, we agree with Didier (1970) in the general arrangement and the fibrillar systems associated to the penicular and paroral kinetosomes. Moreover, we have observed that there are longitudinal and transversal connections at the level of the nematodesmal plates related with paroral and vestibular kineties.

The nematodesmal composition of *D. colpidioides* is less complex than that observed in *Frontonia* (Didier 1970; Gil 1984, 1988): nematodesmal bundles related to a microfibrillar layer placed at the right side of the vestibular kineties, and series of nematodesmata located at both extremes of the left side of the buccal cavity of *Frontonia* (Didier 1970; Gil 1984, 1988) have not been observed in *Disematostoma colpidioides*. On the other hand, the structure of the oral cytoskeleton of *Disematostoma* is more complex than that described in other peniculid ciliates as *Urocentrum turbo* (Didier 1970, Guinea et al. 1987), in which oral nematodesmata are not present.

Finally we want to point out that the cytoskeletal elements at the oral area of *D. colpidioides* are connected. Postciliary microtubules departing from the peniculi reinforce the dorsal side of the buccal cavity, reaching the right side under the filamentous reticulum. Moreover, at the bottom of the buccal cavity, the filamentous reticulum is related with the deep microfibrillar net of the peniculi.

Acknowledgement. This work was supported by grant PB 87/0220 from the CICYT-CSIC.

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Ca^{2+} Ions Mediate the Photophobic Response in *Blepharisma* and *Stentor*

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Summary. The effects of changes in external free Ca^{2+} ions and presence in external solution of Ca^{2+} channel blockers or ionophore on light-dependent motile responses in *Blepharisma* and *Stentor* protozoans, were studied using microscope video recording. Cells entering an illuminated field show a step-up photophobic response, these usually occur with some delay. A decrease in the concentration of external free Ca^{2+} or the addition of the Ca^{2+} channel blockers, pimozide or diltiazem, to the medium significantly lowers the photosensitivity and prolongs the delay of the step-up photophobic responses in *Stentor* and *Blepharisma*. The Ca^{2+} ionophore A23187 added to the medium inhibits photophobic response and markedly increase the response time at lower extracellular Ca^{2+}. The results suggest that Ca^{2+} ions are involved in the light transduction chains in *Blepharisma* and *Stentor* ciliates.

Key words. Photophobic response, Ca^{2+}-ions, *Blepharisma* and *Stentor*.

INTRODUCTION

The pigmented ciliates *Blepharisma japonicum* and the related *Stentor coeruleus* respond to a changes in light intensity with a light avoiding motile response (Jennings 1904, Mast 1906, Giese 1981). A step-up increase in light intensity elicits in both ciliates a step-up photophobic response consisting of a delayed cessation of swimming (stop response), period of backward swimming (ciliary reversal) and finally resumption of forward movement (Wood 1976, Song et al. 1980a, Matsuoka 1983a, Kraml and Marwan 1983). In lateral light, *Stentor* cells swim away from light source without displaying distinct ciliary reversal (negative phototaxis) (Song et al. 1980b). In both ciliates a step-up increase in light intensity induces depolarizing photoreceptor potential which may trigger an action potential associated with the stop/ciliary reversal response (Wood 1976, Fabczak et al. 1993a,b).

Beside the limited literature reports on the effects of several uncouplers and ionophores (TPMP, FCCP, ionomycin) on the phototactic response in *Stentor* (Chang et al. 1981, Prusti and Song 1986, Walker at al. 1981, Passarelli et al. 1984), the role of Ca^{2+} ions in the photophobic response in the ciliates has not received a detailed investigation. In order to elucidate the role of Ca^{2+} ions in the photophobic response in *Stentor* and *Blepharisma,* we analyzed the effects of Ca^{2+}, Ca^{2+} ionophore A23187 and the Ca^{2+} channel blockers, pimozide and diltiazem, on the photosensitivity of these ciliates.

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MATERIALS AND METHODS

Cells

Stock cultures of heterotrichous ciliates, *Stentor coeruleus* and *Blepharisma japonicum*, were maintained in the culture medium of the following composition: 1 mM $CaCl_2$, 1 mM $MgSO_4$, 1 mM $NaNO_3$, 0.1 mM KH_2PO_4, 0.3 mM Na_2HPO_4 at pH 7.2 for *Stentor* and 6.9 for *Blepharisma* (22°C). Food for the cells was an axenic culture of *Tetrahymena pyriformis*. For the experiments described, samples of the *Blepharisma* or *Stentor* cells were washed in fresh culture medium without nutritional components (control solution) and then suspended in a test solution in an experimental chamber. Before the photoresponse assays, the cells were adapted to darkness in fresh medium for 30-40 min. The temperature of the cell samples in the photosensitivity analysis chamber was maintained automatically at 22°C (Fig. 1).

Solutions

Ca^{2+} channel blockers diltiazem (Sigma, U.S.A.), pimozide (Sigma, U.S.A.) and Ca^{2+} ionophore A23187 (Sigma, U.S.A.) were initially dissolved in DMSO (Sigma, U.S.A.) to obtain stock solutions. The test solutions containing fresh culture medium and an appropriate Ca^{2+} channel blocker, were prepared shortly before each experiment to minimize the decomposition of these substances in aqueous solution (Davis et al. 1986). Low concentrations of free Ca^{2+} ions in test solutions were obtained by adding appropriate amounts of $CaCl_2$ to the fixed 2 mM concentration of EGTA (correct $CaCl_2$/EGTA proportion) in Ca^{2+}-free culture medium (Portzehl et al. 1964). The EGTA based Ca buffer was chosen to avoid depletion of cellular Mg^{2+} ions because low stability constant of EGTA for Mg^{2+}.

Photomotility recordings

The cell swimming behaviour was observed with a microscope (Ergaval Zeiss, Germany) with a video system equipped with an infrared sensitive CCD camera (SCAR OS-458, Taiwan), a video monitor and recorder (SLV-426EE Sony, Japan) (Fig. 1). The camera was mounted on the head of a microscope through a video relay lens adapter (F37.820 Edmund Sci., U.S.A.). The light-induced response of an individual cell in the chamber placed on the microscope stage was recorded and then studied in detail by frame-by-frame analysis of the recorded tape. The positions of an image of a particular cell were analyzed directly on the monitor screen at 40 ms time intervals. The light source for the photic stimulation was provided by a microscope illuminator lamp with a variable power supply, in conjunction with a 700 nm long-pass plastic filter (FRF-700 Westlake Plastics Co., U.S.A.) which was placed between the microscope stage and its illuminating system. At 700 nm and above, there is no induction of movement by light in both *Blepharisma* and *Stentor* (Fabczak et al. 1992a,b). The plastic filter had a hole of about 1 mm in diameter to provide a light trap, i.e. field of white or monochromatic light (Song et al. 1980a, Kraml and Marwan 1983). Interference and neutral density filters were used to provide monochromatic light of different intensities. The intensity of light applied to the light trap was monitored with a calibrated silicon photodiode (VTA 9313 EG & Vactec, U.S.A) linked to a digital voltmeter (V 628 Meratronic, Poland). Temperature in the experimental chamber was set by a temperature controller based on a Peltier semiconductor element (Peltron PKE 72, Germany) (Fig. 1).

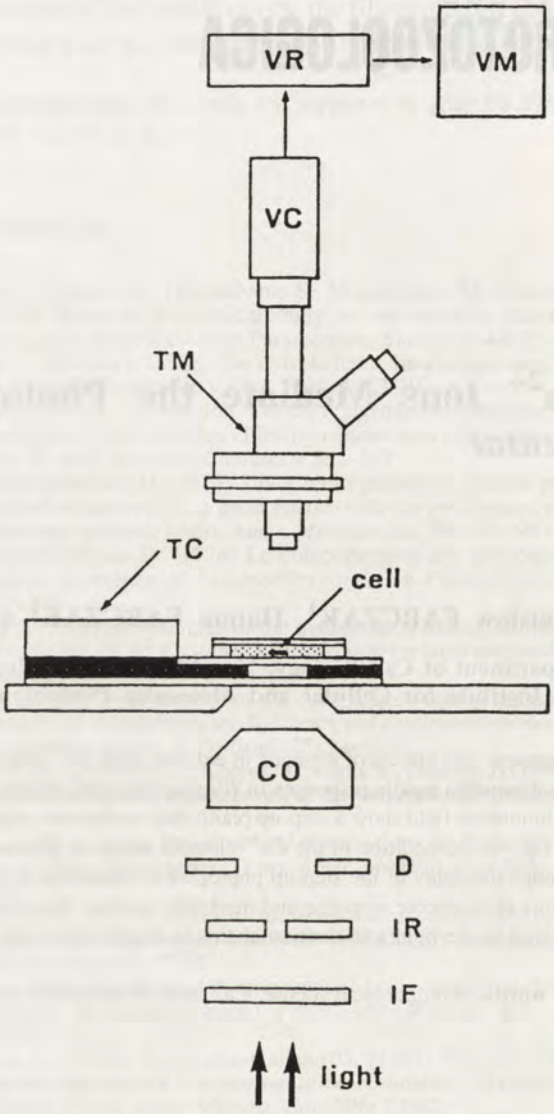

Fig. 1. Schematic diagram of infrared video recording set-up: TM-trinocular microscope, VC-infrared sensitive video camera with relay lens adapter, VR-video recorder, VM-video monitor, TC-temperature controller, CO-condenser objective, D-diaphragm, IR-infrared filter with central hole, IF-interference filter

To illustrate the degree of reorientation in swimming direction during photophobic responses by *Blepharisma* and *Stentor*, the cells were also photographed (Fig. 2). The pictures of cell swimming in thin sheet of culture medium were taken from above and against a black background (Dryl 1958).

RESULTS

The pear-shaped cells of *Stentor* swim rather slowly (200 to 300 $\mu m\ s^{-1}$) and in a straight line under infrared light (Fig. 2a), as a result of positive photokinesis (Iwat-

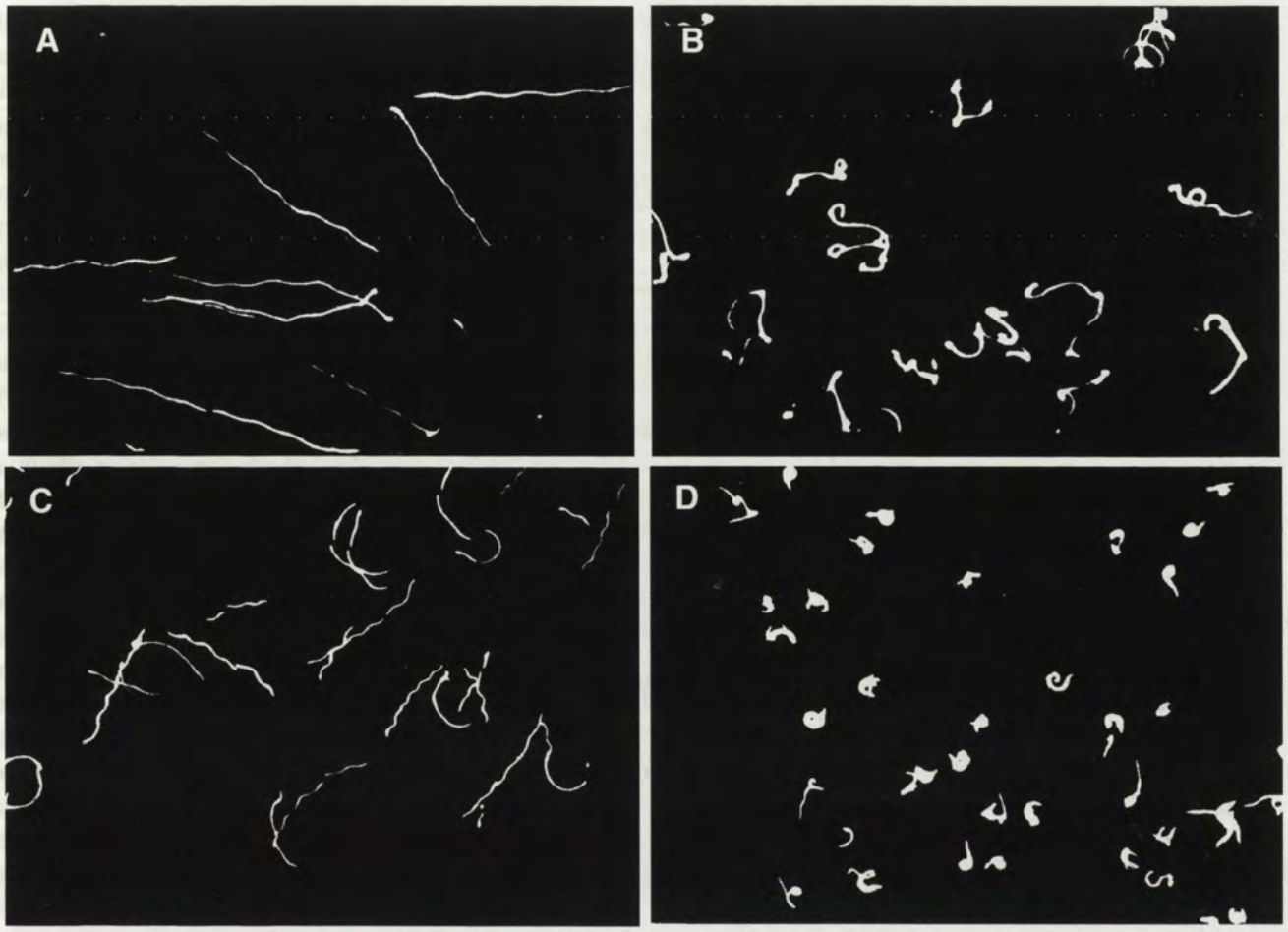

Fig. 2. Schematic illustration of step-up photophobic responses by (A) *Blepharisma* and (B) *Stentor*. When cells enter a white or monochromatic light trap they stop (with some delay), swim transiently backwards for some period, and finally continue swimming forward swimming in new direction (away from the light trap)

suki 1992). Unlike *Stentor,* most cells of *Blepharisma* under infrared light swim in a curved path, with a lower velocity (80 to 120 $\mu m\, s^{-1}$) resulting from a more pronounced in *Blepharisma* positive photokinesis (Fig. 2c) (Matsuoka 1983a, Kraml and Marwan 1983). Some cells remain motionless in such "dark" light conditions. Both ciliates occasionally display ciliary reversals during swimming. "Spontaneous" ciliary reversal in darkness are supposedly induced by some local mechanical or chemical stimulation which may exist in solution (Ghetti 1991).

When dark/infrared light adapted *Blepharisma* or *Stentor* cells accidentally enter a bright light field (light trap), they momentarily modify the ciliary beating pattern and hence mode of swimming. A typical light-dependent response exhibited by *Blepharisma* and *Stentor*, known as step-up photophobic response, is illustrated in Fig. 2b and 2d and schematically reproduced in Fig. 3. Both ciliate organisms after entering the illuminated area, continue to swim forward with the same speed for a short time and then they arrest the ciliary motion. This results in transient cessation of forward swimming (stop response) followed, at lower light intensity, by recovery of ciliary beating and forward movement in the same direction. The higher light intensity applied to the light trap elicits in both cells stop response followed by the reverse of the direction of ciliary stroke (ciliary reversal) and a period of backward swimming. *Stentor* moves backwards in straight line and this movement is terminated when the cell pivots, usually through an acute angle with respect to its former path of swimming (Figs. 2b, 3b). In contrast, *Blepharisma* mostly moves backwards in more or less circular path (rotatory movement) (Figs. 2d, 3a). Both *Blepharisma* and *Stentor* after period of backward swimming resume forward movement in a new direction (away from the light trap). The delay in stop response depends on light intensity within the light trap, the cell enters the light trap of higher

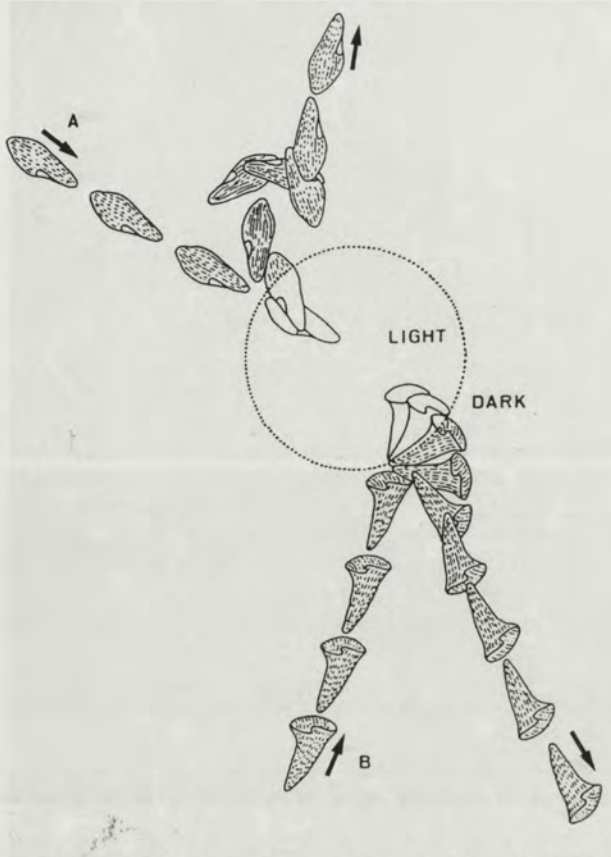

Fig. 3. Free swimming in dark (in infrared light), and phobically reacting *Blepharisma* (A) and *Stentor* (B). Pictures are taken under dark field and 10 s exposure. Cells are suspended in culture (control) solution of pH 6.9 (*Blepharisma*) and 7.2 (*Stentor*)

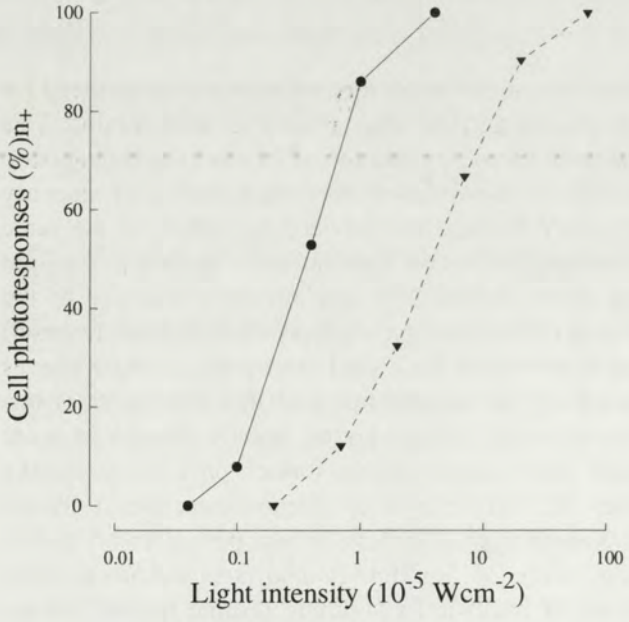

Fig. 4. Intensity-response curves for photophobic responses in *Stentor* (solid line) and *Blepharisma* cells (dashed line) in culture (control) solutions, 22°C, pH 7.2 (*Stentor*) and 6.9 (*Blepharisma*)

intensity with the very tip of its body only. In both protozoa forward swimming speed after ciliary beat reversal is always higher than the previous speed (positive photokinesis) (Matsuoka 1983a, Fabczak and Fabczak 1993, unpublished data). The present observations on photomovement in *Blepharisma* and *Stentor* are consistent with previous data when photoresponses in these ciliates were elicited by short overall illumination (light pulses) (Fabczak et al. 1993a,b).

To analyze the photosensitivity of both *Blepharisma* and *Stentor* cells in various experimental conditions the fluence rate-response curves were determined (Fig. 4). From these curves, which show a sigmoidal dependence with the fluence rate, the ED_{50} values of the effective light stimulus (Table 1), i.e. the light intensity which induces phobic responses in 50% of the cells, were estimated (Venulet and Wójcik 1960). The reciprocal of the ED_{50}, i.e. $1/ED_{50}$ value was then used to characterize the cell light sensitivity. The statistical method used minimizes dispersion of the data which stems from differences in photosensitivity of individual cells and their positions during swimming in relation to light source (Kraml and Marwan 1983, Matsuoka 1983b, Ghetti 1991, Wood 1991). The delay of the phobic response, defined as the time elapsed between the cell's entry into the light trap and its response was measured for each photoresponding cell directly from video recordings (Fabczak et al. 1993a,b). Under normal conditions (i.e. dark adapted cell in culture medium, 22°C), the light of 580 nm at fluence rate of 4.7×10^{-5} W cm^{-2} in the trap induces the step-up photophobic responses in 50% of *Blepharisma* cells Fig. 4), and the delay of stop response is about 0.6 s, while in *Stentor* the similar effect is attained at $0.32.5 \times 10^{-5}$ W cm^{-2} of 610 nm (Fig. 4) and the cells respond with an average delay of 0.2 s. The delay in the photophobic response observed in both ciliates in control solution corresponds to data reported for *Blepharisma* and *Stentor* elsewhere (Ghetti 1991, Iwatsuki and Kobayashi 1991, Matsuoka 1983a, Kraml and Marwan, 1983).

Changes in external free $[Ca^{2+}]_o$ concentration significantly affect the photoresponses of *Blepharisma* and *Stentor* (Fig. 5). A decrease in $[Ca^{2+}]_o$ concentration between 10^{-4} M and 10^{-7} M results in a substantial decrease of the photosensitivity. Solutions containing 10^{-7} M of free Ca^{2+} or less, fully inhibit the cell response. For both ciliates the shortest delay in swimming stop response was noted in the control solution (culture medium of 10^{-3} M free Ca^{2+}), a decrease in $[Ca^{2+}]_o$ results in its prolongation (Fig. 6). The inhibitory effect

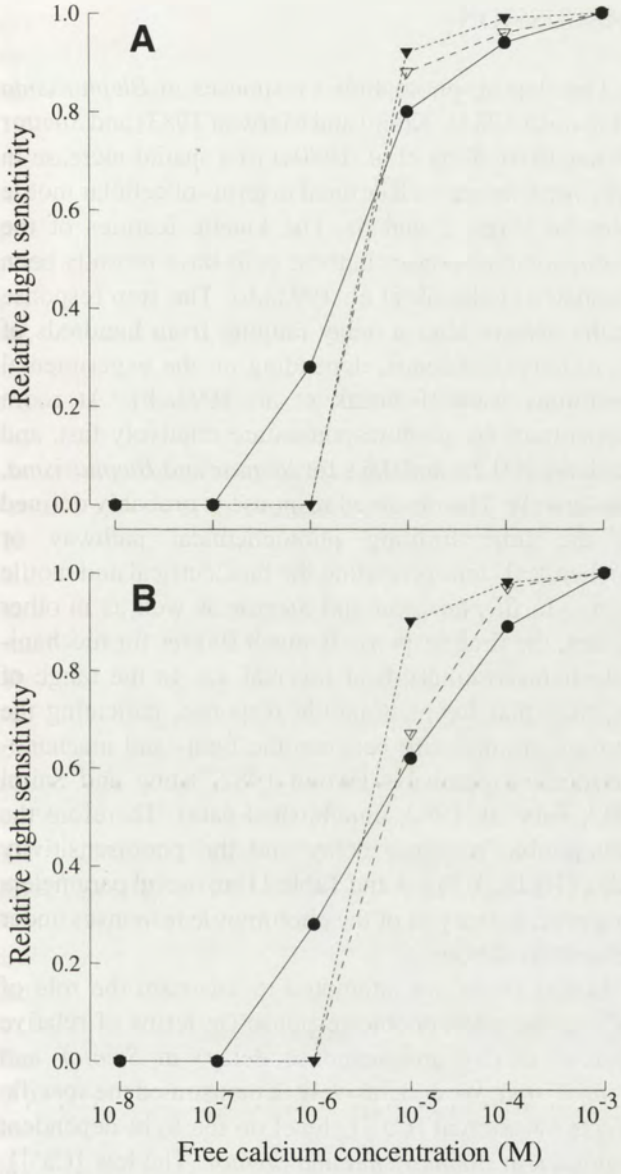

Fig. 5. Rate of inhibition of relative light sensitivity in (A) *Stentor* and (B) *Blepharisma* by decreasing external free Ca^{2+} concentration without the ionophore A23187 (solid line), and at 1.25×10^{-6} M (dotted line) or 2.5×10^{-6} M (dashed line) added

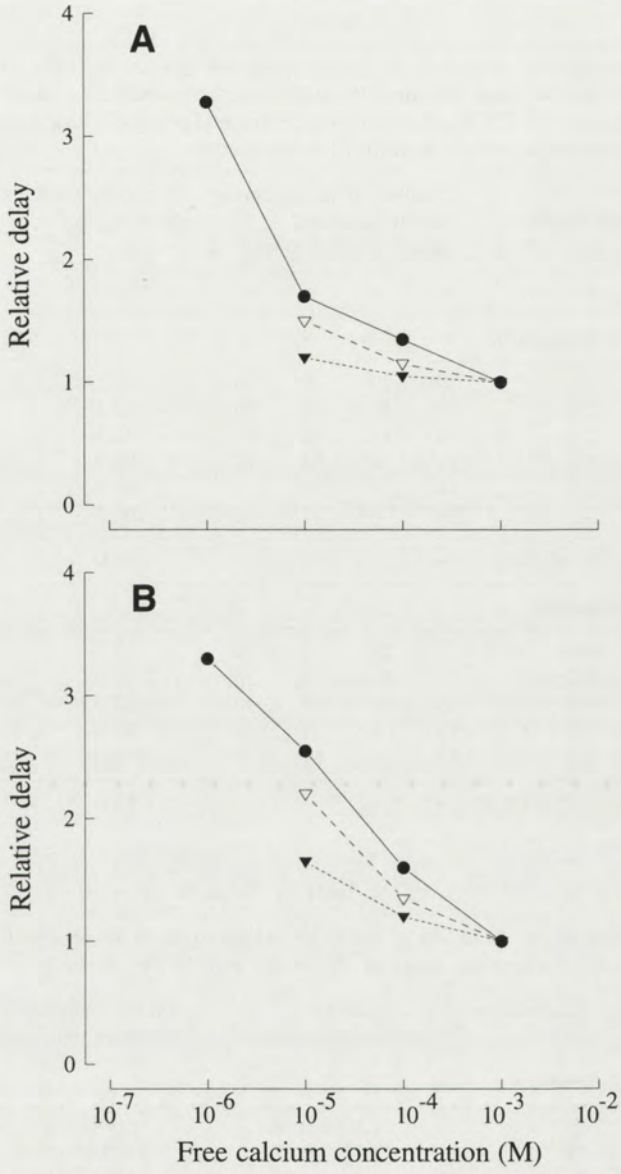

Fig. 6. Relative response delay in (A) *Stentor* and (B) *Blepharisma* in solutions with various concentrations of free Ca^{2+} without the ionophore A23187 (solid line), and containing 1.25×10^{-6} M (dashed line) or 2.5×10^{-6} M of A23187 (dotted line)

of low $[Ca^{2+}]_o$ on the photophobic response was fully eliminated by cell incubation in control solution for about 30-40 min. The solutions containing higher levels of free Ca^{2+} ions than in culture medium, enhance the photosensitivity of *Blepharisma* cells (data not shown), as has been shown previously for *Stentor* (Colombetti et al. 1982, Iwatsuki and Song 1989).

The lipophilic ionophore A23187 added to the culture medium did not affect the photoresponse of the cells (Fig. 5). A decrease in $[Ca^{2+}]_o$ levels to 10^{-5} M in the presence of micromolar concentrations of A23187 (1.25×10^{-6} M or 2.5×10^{-6} M), reduces the cell photosensitivity and increases the response delay only slightly in comparison with that in absence of the ionophore. The greatest reduction in photosensitivity or delay elongation in *Stentor* (Fig. 5a) and *Blepharisma* (Fig. 5b) are observed at concentrations below 10^{-5} M $[Ca^{2+}]_o$. The medium with A23187 at 10^{-6} M free Ca^{2+} or less completely inhibited light responses in both *Stentor* and *Blepharisma*.

Diltiazem and pimozide, reagents known to inhibit L-type Ca^{2+} channels in a variety of excitable tissues, also alter photophobic responses in *Blepharisma* and *Stentor*. As shown in Table 2 both blockers (10^{-6} M

Table 1

Photophobic responses of *Blepharisma* and *Stentor* to light of 580 nm (A), and 610 nm (B) under standard condition (culture solutions, 22 °C). Examples of an experimental protocol illustrating the statistical method used for ED_{50} calculation

light intensity (I_L) and (ED_{50})	Number of cells showing positive (n_+) and negative (n_-) responses		% of cells showing positive (n_+) responses	
I_L (10^{-5} Wcm^{-2})	n_+	n_-	n_+	n_-	(%)n_+
(A) *Blepharisma*					
0.2	0	20	0	56	0
0.7	5	13	5	36	12.1
2.0	6	11	11	23	32.3
7.0	13	8	24	12	66.7
20.0	14	4	38	4	90.4
70.0	29	0	67	0	100.0
4.7 (ED_{50})	–	–	–	–	50.0
(B) *Stentor*					
0.04	0	24	0	59	0
0.1	3	19	3	35	7.9
0.4	15	10	18	16	52.9
1.0	19	6	37	6	86.0
4.0	25	0	62	0	100.0
0.32 (ED_{50})	–	–	–	–	50.0

Table 2

Effects of 10^{-5} M diltiazem and $5x10^{-6}$ M pimozide on the photosensitivity and response delay in (A) *Stentor* and (B) *Blepharisma*

Inhibitor (M)	Latency (relative units)	Phobic response (relative units)
(A) *Stentor*		
0	1.00+/-0.08	1.00+/-0.07
10^{-5} diltiazem	1.39+/-0.13	0.53+/-0.08
$5x10^{-6}$ pimozide	1.36+/-0.09	0.65+/-0.04
(B) *Blepharisma*		
0	1.00+/-0.05	1.00+/-0.17
10^{-5} diltiazem	2.29+/-0.23	0.60+/-0.05
$5x10^{-6}$ pimozide	1.30+/-0.18	0.85+/-0.11

diltiazem or $5x10^{-6}$ M pimozide) markedly reduce to differing degrees, cell photosensitivity. Simultaneously, the delay in the photophobic response increase in the presence of these inhibitors. DMSO, used as a solvent for the stock solutions of A23187 and Ca^{2+} channel blockers, somewhat depress the cell photosensitivity of both ciliates, thus the photomotile response data with these drugs are compared with these in the control solution containing only the solvent in appropriate levels.

DISCUSSION

The step-up photophobic responses in *Blepharisma* (Matsuoka 1983a, Kraml and Marwan 1983) and *Stentor* (Wood 1976, Song et al. 1980a) to a spatial increase in light intensity are well defined in terms of cellular motile behavior (Figs. 2 and 3). The kinetic features of the photophobic responses in these cells have recently been elucidated (Fabczak et al. 1993a,b). The stop response occurs always after a delay ranging from hundreds of ms to tens of seconds, depending on the experimental conditions used (Fabczak et al. 1993a,b). At room temperature the photoresponses are relatively fast, and the delay is 0.2 s and 0.6 s for *Stentor* and *Blepharisma*, respectively. The observed response is probably defined by the time limiting photochemical pathway or biochemical steps preceding the bioelectrical and motile events. In *Blepharisma* and *Stentor* as well as in other ciliates, the time response is much shorter for mechanically induced ciliary beat reversal, i.e. in the range of ms, than that for photomotile response, indicating the primary dissimilarity between the light- and mechanotransduction pathways (Wood 1982, Kung and Saimi 1982, Fabczak 1992, unpublished data). Therefore the photophobic response delay and the photosensitivity index ($1/ED_{50}$) (Fig. 4 and Table 1) are useful parameters for a precise analysis of the photomotile responses under defined conditions.

In this study, we attempted to ascertain the role of Ca^{2+} in the photophobic response (in terms of relative photosensitivity and response delay) in *Stentor* and *Blepharisma*. We conclusively demonstrated the specific effects of external $[Ca^{2+}]_o$ level on the light-dependent responses in *Blepharisma* and *Stentor*. The low $[Ca^{2+}]_o$ and Ca^{2+} channel blockers such as diltiazem and pimozide, retard the photosensitivity of these cells (Figs. 5 and 7). The inhibitory effect of Ca^{2+} ionophore A23187 on the photoresponse at low $[Ca^{2+}]_o$ (Fig. 5) is consistent with the assumed role of Ca^{2+} in the photo-signal transduction (Song 1981) owing to its ability to equilibrate the free Ca^{2+} concentrations at both sides of the cell membrane (Reed and Lardy 1972, Pohl et al. 1980, Lee and Vidaver 1984). Normally, cytoplasmic or axonemal Ca^{2+} levels in ciliated protozoa is maintained at low level (Eckert and Naitoh 1972, Naitoh 1973, Wood 1982). The lack of photoresponse in *Stentor* and *Blepharisma* at 10^{-6} M $[Ca^{2+}]_o$ in the presence of A23187 corresponds to the intracellular or intraciliary free Ca^{2+} concentration of around 10^{-6} M (Fig. 5). In the control solution Ca^{2+} moving down its electrochemical

gradient will enhance the intracellular Ca^{2+} level and depolarize membrane potential (receptor potential), as observed for *Blepharisma* and *Stentor* (Fabczak et al. 1993 a, b). In analogy to the ionic mechanism of mechanosensory transduction in *Paramecium* and *Stentor* as well (Machemer 1976, Wood 1982), the receptor potential elicits voltage-dependent Ca^{2+} fluxes (action potential) and leads to the ciliary reversal. Accordingly, present results show that the low $[Ca^{2+}]_o$ or the presence of Ca^{2+} channel inhibitors, which decrease the membrane permeability to Ca^{2+} ions, inhibit the Ca^{2+} fluxes across the cell membrane of *Blepharisma* or *Stentor* and hence the photophobic response.

Various protonophores specifically inhibit the light-dependent responses in *Stentor* and *Blepharisma* (Song 1981, Walker et al. 1981, Fabczak et al. 1993c, Passarelli et al. 1984)). Furthermore, it has been demonstrated that in *Stentor,* the cell photoreceptor stentorin, in an excited state, releases H^+ ions to the cell cytoplasm (Walker et al. 1981, Song 1981). Similar results have been reported recently by Matsuoka et al.(1992) with blepharismin, a photoreceptor in the related *Blepharisma*. Song et al. (1980a, 1981), Walker et al. (1981) and Fabczak et al. (1993c) hypothesized that the release of H^+ may act as the initial signal following light absorption by the photoreceptor (stentorin or blepharismin) (Song 1981, Passarelli et al. 1984). These transient increases in intracellular free H^+ in *Blepharisma* and *Stentor* may activate an electrogenic pump which provides a H^+/Ca^{2+} exchange (Song 1981, Wood 1991). The net Ca^{2+} inward flux will then cause a receptor potential as indicated (Fabczak et al. 1993 a,b). The photoreceptor potential eventually triggers an action potential (typical for other ciliates) and subsequent photophobic orientation (stop/ciliary reversal response).

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Isospora, *Caryospora* and *Eimeria* (Apicomplexa: Eimeriidae) in Passeriform Birds from Czech Republic

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Summary. Passeriform birds were studied as hosts of monoxenous coccidia (Eimeriidae). 571 faecal samples from 46 species were examined. Coccidia were found in 210 samples (36.8%) from 34 host species (73.9% of examined species). 36 oocyst types mostly belonging to the genus *Isospora* were found (33 types); oocysts of the genera *Caryospora* and *Eimeria* were also noted. *Caryospora* is reported for the first time from the genus *Acrocephalus*. Infections were mostly single (91.4%), but multiple infections were also found. Problems of species determination in eimeriid coccidia from birds are discussed, as well as the possibility that every species of passerine bird in Central Europe can act as a host for at least one species of monoxenous coccidia.

Key words. Coccidia, Passeriformes, Eimeriidae, *Isospora*, *Caryospora*, *Eimeria*.

INTRODUCTION

Passerine birds have been known as hosts of monoxenous coccidia for one hundred years: in 1893 Labbé found oocysts in *Carduelis carduelis* (ex Levine 1982). However, unlike species from domestic fowl, the coccidia of free-living birds have not been intensively studied. The aim of this study was to ascertain which genera or species of monoxenous coccidia (Eimeriidae) occur in passerine birds from the Czech Republic and their level of infection.

MATERIALS AND METHODS

Passerine birds were net caught between 1988 and 1992 in different areas of the Czech Republic. They were kept in tissue bags for max. 1 hour and released. Faeces were removed, maintained in a 2% aqueous solution of potassium bichromate ($K_2Cr_2O_7$) at room temperature for 3 days to allow sporulation, and then stored at 4°C.

Each sample was examined directly without any flotation method. Oocysts were measured (n=10) using a calibrated ocular micrometer, drawn and photographed. The data were statistically processed and average, maximal and minimal lengths and widths of oocysts, as well as the standard error were determined.

Supposing that eimeriid coccidia are genus-specific parasites, the data obtained were compared with all descriptions and findings pertaining to a particular genus only. To compare data from different individuals of one genus, the multiple range test was used.

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RESULTS

A total of 571 individuals of 46 passeriform bird species belonging to 28 genera were examined. Oocysts of monoxenous coccidia (Eimeriidae) were found in 210 individuals (36.8%) of 34 species (73.9% of species infected) and 22 genera (78.6% genera infected). Thirty

seven oocyst types were found. Seven types were identified as previously described species, and for 5 types the identification was uncertain. A further 25 oocyst types found are probably new species (see Tables 1 and 2, Figs. 1-37). Most of the hosts released oocysts of the genus *Isospora* (two sporocysts in an oocyst; 99.0% of infected individuals); the genera *Caryospora* (one sporocysts in an oocyst) and *Eimeria* (four sporocysts in an oocyst) were also found (see Table 3). The majority of infections were single with only one type of oocyst (91.4% of infections), in a lesser extent double and triple infections with two types of *Isospora* or *Caryospora* and *Isospora* (see Table 4).

DISCUSSION

Despite the many extant descriptions of monoxenous coccidia from passeriform birds, species determination using oocysts is problematic. With the older descriptions being deficient, practically all findings of the genus *Isospora* were classified as *I. lacazei* (Labbé, 1893). Pellérdy (1974) stated that in addition to the house sparrow (*Passer domesticus*) 40 to 50 species of passeriform bird can host this *Isospora*. Actually, monoxenous coccidia are thought to be genus-specific (Levine 1982), although exceptions exist: *I. xerophila* infects 4 genera of the family Ploceidae (Barré and Troncy 1974), *Eimeria dispersa* from turkey (*Meleagris gallopavo*) can develop in the pheasant (*Phasianus colchicus*) and other members of the order Galliformes (Doran 1978). However, we cannot generalize this information, and without positive tests of infectivity for other genera we should consider monoxenous coccidia to be genus-specific (Long and Joyner 1984). Attempts to infect the canary (*Serinus canaria*, Fringillidae) with the oocysts of *Isospora* from house-sparrow (Passeridae) failed (Box 1970, Černá 1972), in the same way in that attempts to infect the canary with the oocysts from *Hesperiphona vespertina* (Khan and Desser 1971).

In identifying oocysts from passerine birds, the morphology is still of utmost importance, despite the fact that their length, width and shape may change during infection and as a function of inoculum (Cheissin 1947, 1957). The oocyst and sporocyst size varies amongst host individuals of one species, or among different species of one genus (Gardner and Duszynski 1990). Precise information on size (average length and width, maximum and minimum size, number of measurements, standard error) in combination with morphological characteristics could facilitate species identification. However, in much of the older descriptions this information is missing, so species-identification is difficult. Another problem is represented by descriptions which mention several genera of birds as hosts of one species of *Isospora*. More detailed data, e. g. experiments with infections by one oocyst or biochemical data are difficult to obtain due to problems with accessibility and rearing of hosts as free-living birds.

One of the most complicated examples are the *Isospora* in the house-sparrow. The above mentioned species *I. lacazei*, as which earlier findings from sparrows were considered, is believed to be a parasite of the goldfinch (*Carduelis carduelis*) (Levine 1982). As a sparrow parasite however, *I. passeris* was established (Levine 1982), its description being given previously by Levine and Mohan (1960). Scholtyseck (1954) stated two length frequency maxima of oocysts from sparrows (24 μm and 34 μm). Milde (1979) supposed that the sparrow is the host of two species of *Isospora* on the basis of such maxima (24 μm and 28 μm) and of the ultrastructure of intestinal and extraintestinal stages. Grulet et al. (1982) described 12 species of *Isospora* from sparrows on the basis of oocyst morphology. According to Landau (1989, pers. com.) this speciation took place in isolated populations of sparrows which later fused. Experiments with infections by one oocyst to exclude the influence of intraspecific variability were not carried out. The numbers of oocysts measured are very low (10 or less), and was unable to detect the morphological differences presented by the authors. I found two length frequency maxima of oocysts (21 μm and 30 μm) and two morphological forms. These results lead me to conclude that there are only two species of *Isospora* in the house-sparrow from the Czech Republic.

The observation of the genus *Caryospora* is of special interest. Some species of this genus have a direct life-cycle but are also able to survive in an intermediate host (*Caryospora bubonis*) (Stockdale and Cawthorn 1981). Species from snakes can complete a large part of their life cycle in rodents: *C. bigenetica* and *C. simplex* (Wacha and Christiansen 1982, Upton et al. 1984); but not all *Caryospora* from snakes are able to do this (Upton et al. 1983, Upton and Sundermann 1990). *Caryospora* from passeriform birds are probably monoxenous because of the feeding habit of their hosts; unfortunately, information about their life cycles are not available. There are only three descriptions of the genus *Caryospora* from Passeriformes: in *Dives atrogularis* (Icteridae) (Pellérdy 1967), in the robin *Erithacus*

Table 1

Monoxenous coccidia found in passeriform birds (Nomenclature by Hudec 1983)

Host species	n	+	Parasite species (notes)
Hirundo rustica	2	0	(none)
Delichon urbica	4	4	*Isospora* sp. type 1 (probably new species)
Anthus trivialis	2	0	(none)
Anthus pratensis	1	0	(none)
Motacilla cinerea	10	10	*Isospora* sp. type 2 (probably new species)
Motacilla alba	2	1	*Isospora* sp. type 2 (probably new species)
Troglodytes troglodytes	9	5	*Isospora* sp. type 3 *(probably new species)*
Prunella modularis	11	2	*Isospora* sp. type 4 (probably new species)
		1	*Isospora* sp. type 5 (probably new species)
Erithacus rubecula	37	15	(5 double infections)
		14	*I. erithaci* Anwar, 1972 (type 6)
		6	*C. jiroveci* Černá, 1976 (type 7)
Phoenicurus ochruros	6	1	*Isospora* sp. type 8 (probably new species)
Turdus merula	19	7	*I. turdi* Schwalbach, 1959 (type 9)
Turdus philomelos	5	1	*I. turdi* Schwalbach, 1959 (type 9)
		2	?*I. robini* McQuistion et Holmes, 1988 (type 10)
Locustella naevia	1	1	*Isospora* sp. type 12 (probably new species)
Acrocephalus schoenobaenus	8	3	*Isospora* sp. type 12 (probably new species)
Acrocephalus palustris	6	3	(1 triple, 1 double infection)
		2	*Isospora* sp. type 12 (probably new species)
		2	*Isospora* sp. type 13 (probably nes species)
		2	*Caryospora* sp. type 14 (probably new species)
Acrocephalus scirpaceus	9	4	(2 double infections)
		4	*Isospora* sp. type 15 (probably new species)
		2	*Isospora* sp. type 12 (probably new species)
Acrocephalus arundinaceus	2	0	(none)
Hippolais icterina	81	64	*Isospora* sp. type 16 (probably new species)
Sylvia curruca	4	1	?*I. sylviae* Schwalbach, 1959 (type 17)
Sylvia communis	6	3	(1 double infection)
		2	?*I. sylviae* Schwalbach, 1959 (type 17)
		2	?*I. sylvianthina* Schwalbach, 1959 (type 18)
Sylvia borin	10	4	(2 double infections)
		2	?*I. sylviae* Schwalbach, 1959 (type 17)
		3	?*I. sylvianthina* Schwalbach, 1959 (type 18)
		1	*E. depuytoraci* Černá, 1976 (type 19)
Sylvia atricapilla	19	10	(2 double infections)
		7	?*I. sylviae* Schwalbach, 1959 (type 17)
		5	?*I. sylvianthina* Schwalbach, 1959 (type 18)
Phylloscopus collybita	9	5	(2 double infections)
		5	*Isospora* sp. type 20 (probably new species)
		2	*Isospora* sp. type 21 (probably new species)
Phyloscopus trochilus	6	1	(1 double infection)
		1	*Isospora* sp. type 20 (probably new species)
		1	*Isospora* sp. type 21 (probably new species)
Regulus regulus	1	0	(none)
Regulus ignicapillus	1	0	(none)
Muscicapa striata	2	1	*Isospora* sp. type 22 (probably new species)
Ficedula hypoleuca	1	1	*Isospora* sp. type 23 (probably new species)
Aegithalos caudatus	5	1	*Isospora* sp. type 24 (probably new species)
Parus palustris	6	0	(none)
Parus montanus	6	0	(none)
Parus ater	1	0	(none)
Parus caeruleus	66	3	*Isospora* sp. type 25 (probably new species)
Parus major	106	2	*Isospora* sp. type 25 (probably new species)
		3	*Isospora* sp. type 26 (probably new species)
Sitta europaea	7	3	?*I. sittae* Golemanski, 1977 (type 27)
Certhia familiaris	2	1	*Isospora* sp. type 28 (probably new species)
Lanius collurio	6	1	*Isospora* sp. type 29 (probably new species)
Passer domesticus	52	24	*Isospora* sp. type 30 (undescribed species)
			Isospora sp. type 31 (undescribed species)
Fringilla coelebs	13	5	*I. fringillae* Yakimoff et Gousseff, 1938 (type 32)
		1	*Isospora* sp. type 33 (probably new species)
Fringilla montifringilla	1	1	(1 double infection)
		1	*Isospora* sp. type 33 (probably new species)
		1	*Isospora* sp. type 34 (probably new species)
Serinus serinus	2	0	(none)
Carduelis	6	0	(none)
Pyrrhula	9	6	*I. perroncitoi* Carpano, 1937 (type 35)
Coccothraustes coccothraustes	1	0	(none)
Emberiza citrinella	5	4	*Isospora* sp. type 36 (probably new species)
Emberiza schoeniclus	3	1	?*Isospora* sp. Mačulskij, 1941 (type 37)

n - number of examined individuals;
+ - number of infected individuals

Table 2

Description of oocysts found in passerine birds

Oocysts							Sporocysts			
Type	x	min/max	SEl	SEw	shape	polar body	shape	Stieda body	substieda body	residuum
1	27.0x23.7	24.0-31.3x19.5-26.3	0.26	0.25	ellipsoidal	big	ovoid	prominent	small	compact
2	20.8x18.7	16.5-26.5x16.5x21.0	0.24	0.16	ellipsoidal	medium	ellipsoidal	prominent	small	compact
3	24.0x21.5	19.0-29.0x17.0-25.2	0.32	0.26	subspherical	big	ovoid	prominent	medium	diffuse
4	20.1x19.3	18.5-22.0x18.0-21.0	0.26	0.27	subspherical	present	ovoid	flat	medium	diffuse
5	26.1x20.7	21.0-29.4x19.0-21.0	0.95	0.21	ellipsoidal	present	ellipsoidal	medium	small	transient
6	19.0x18.3	16.0-22.0x16.0-22.0	0.18	0.18	subspherical	present	pyriform	prominent	big	diffuse
7	18.2x17.0	16.5-21.0x15.0-18.9	0.14	0.17	subspherical	present	ovoid	small	medium	diffuse
8	19.9x19.1	18.0-22.0x18.0-21.0	0.71	0.56	subspherical	present	ovoid	small	medium	transient
9	18.8x17.2	16.5-21.4x15.0-19.7	0.17	0.20	subspherical	present	ellipsoidal	small	medium	diffuse
10	27.8x20.9	23.1-32.0x19.0-21.7	1.06	0.23	ovoid	present	ovoid	prominent	small	compact
11	23.4	21.5-25.5	0.48		spherical	small	ovoid	wide	medium	diffuse
12	26.0x23.0	22.0-30.0x16.0x27.3	0.19	0.33	subspherical	present	wide	wide	medium	diffuse
13	20.1x17.7	18.0-21.0x16.5-21.0	0.32	0.37	subspherical	present	wide	prominent	big	compact
14	31.8x24.3	28.0-35.7x20.0-27.3	0.40	0.33	ellipsoidal	present	ovoid	prominent	small	compact
15	21.3x20.1	16.8-25.2x14.7-25.1	0.55	0.72	subspherical	present	ovoid	prominent	small	diffuse
16	26.8x24.8	19.5-33.6x18.0-31.5	0.13	0.13	subspherical	present	ovoid	prominent	medium	transient
17	30.1x26.1	25.5-33.6x21.0-30.0	0.26	0.25	ellipsoidal	present	ellipsoidal	prominent	small	compact
18	26.1x25.0	21.0-29.4x21.0-27.3	0.24	0.21	subspherical	present	ovoid	wide	medium	diffuse
19	15.3x13.5	15.0-15.5x13.0-14.0	0.20	0.12	subspherical	present	ellipsoidal	small	absent	diffuse
20	27.6x26.3	22.8-35.7x21.0-31.5	0.42	0.36	subspherical	present	ellipsoidal	prominent	big	diffuse
21	29.3x26.3	25.2-33.6x22.0-30.0	0.60	0.72	ovoid	present	ovoid	prominent	small	compact
22	20.4x19.4	19.0-21.5x18.0-21.0	0.26	0.30	subspherical	small	ovoid	wide	small	diffuse
23	21.2	19.5-22.0	0.38		spherical	present	ovoid	small	medium	diffuse
24	28.2x25.7	25.6-29.9x21.4-29.2	0.45	0.64	subspherical	present	ovoid	prominent	small	compact
25	26.8x24.1	23.8-30.0x21.5-26.6	0.32	0.26	subspherical	present	ovoid	prominent	medium	compact
26	29.7x26.1	25.5-33.6x23.0-29.6	0.35	0.40	subspherical	present	ellipsoidal	wide	small	transient
27	21.4x21.2	19.5-25.0x19.5-23.5	0.35	0.30	subspherical	small	ovoid	prominent	medium	diffuse
28	26.0x21.8	24.0-27.0x20.0-24.0	0.38	0.40	subspherical	big	ovoid	prominent	small	compact
29	24.3x19.3	22.5-27.0x18.0-21.0	0.40	0.39	ovoid	big	ovoid	prominent	small	compact
30	22.3x21.2	15.0-27.0x15.0-26.5	0.28	0.25	subspherical	present	ovoid	prominent	small	compact
31	30.1x29.4	29.4-32.0x27.0-31.5	0.34	0.46	subspherical	present	ellipsoidal	wide	medium	transient
32	21.8x20.8	18.1-25.2x18.0-25.2	0.28	0.29	subspherical	present	ovoid	small	medium	diffuse
33	26.2x23.0	24.0-29.6x19.7-26.3	0.45	0.38	ellipsoidal	1 - 2	ovoid	prominent	small	compact
34	18.0x15.2	16.5-19.7x13.3-16.5	0.33	0.32	ellipsoidal	present	long	small	small	diffuse
35	23.5x22.5	19.5-27.0x18.0-27.0	0.27	0.29	subspherical	small	ovoid	flat	small	diffuse
36	29.6x27.3	23.1-35.7x22.0-33.6	0.56	0.42	subspherical	small	ellipsoidal	flat	big	diffuse
37	25.9x23.5	21.0-28.5x19.5-27.0	0.72	0.70	ellipsoidal	big	ovoid	prominent	small	compact

Type - number of oocyst type (see table 1); x - average length and width; min/max -minimal and maximal length and width; SEl - length standard error; SEw - width standard error

Table 3

Level of coccidia infection found in passeriform birds

Taxon	n	%	+
Eimeriidae	210	36.8	100
Isospora	208	36.4	99.0
Caryospora	8	1.4	3.8
Eimeria	1	0.2	0.5

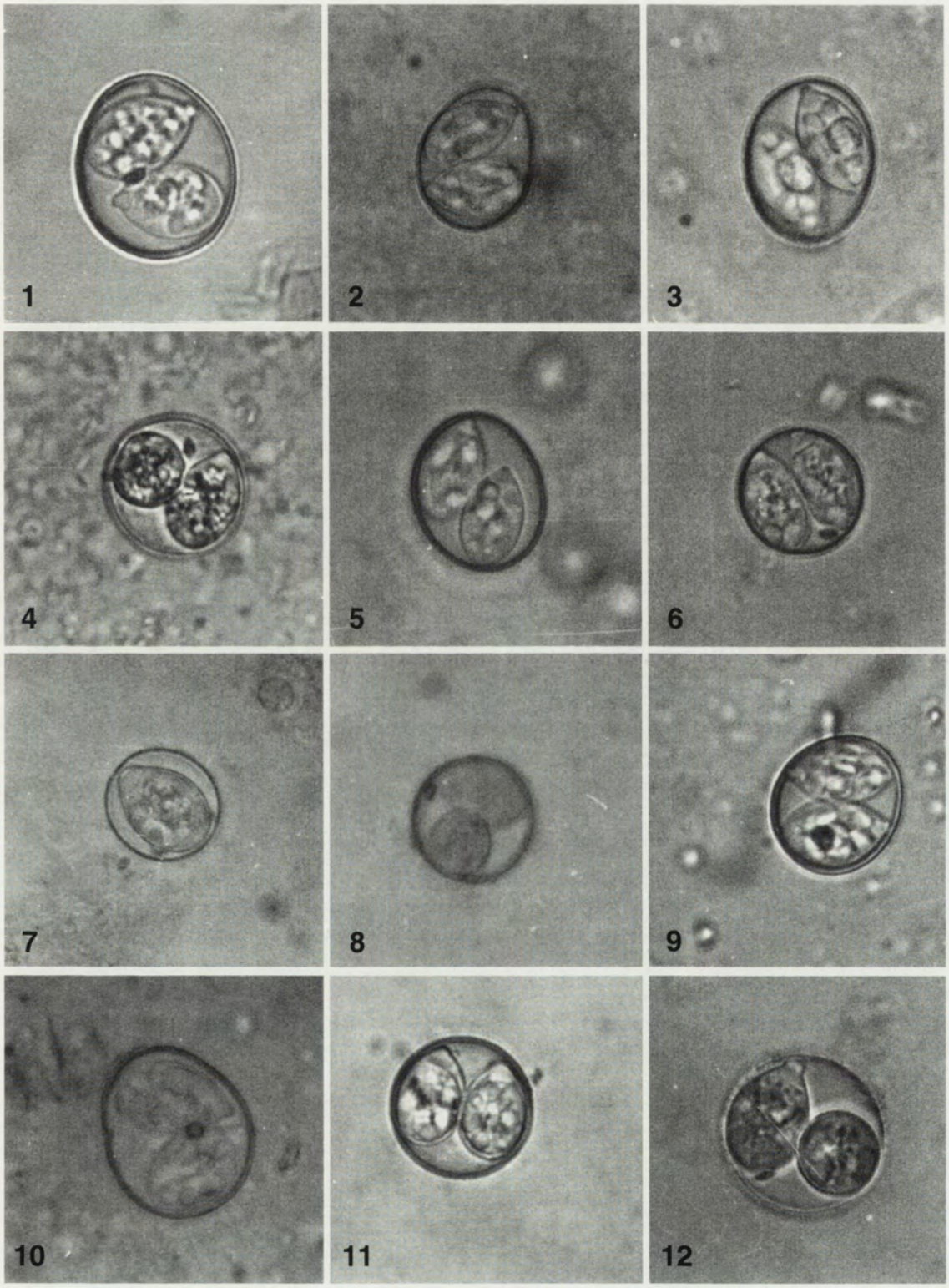
n - number of infected individuals; % - percent of examined individuals; + - percent of infected individuals

Table 4

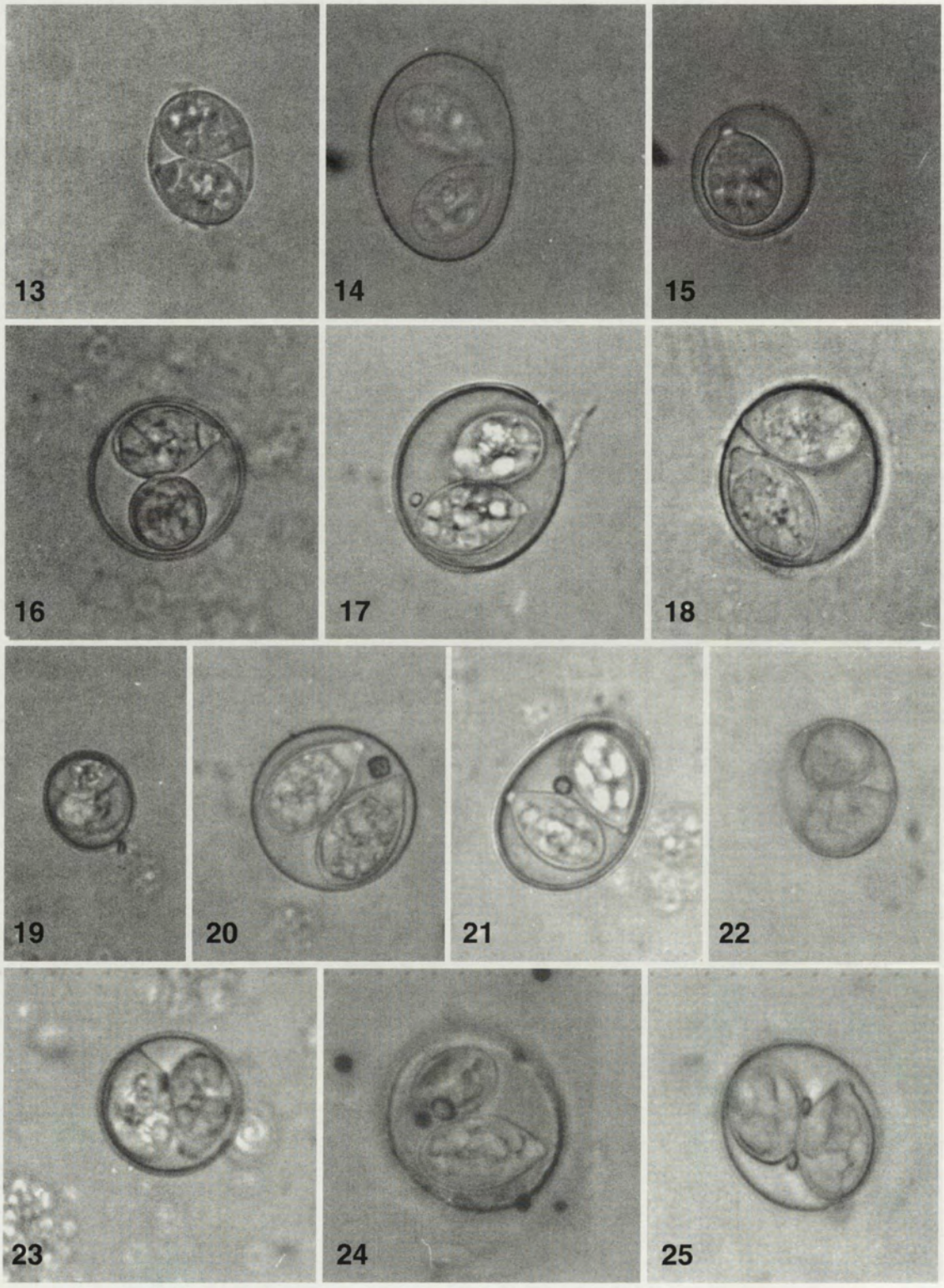
Frequency of different infection type

Infection type	n	%	+
Single	192	33.6	91.4
Double	17	3.0	8.1
Triple	1	0.2	0.5

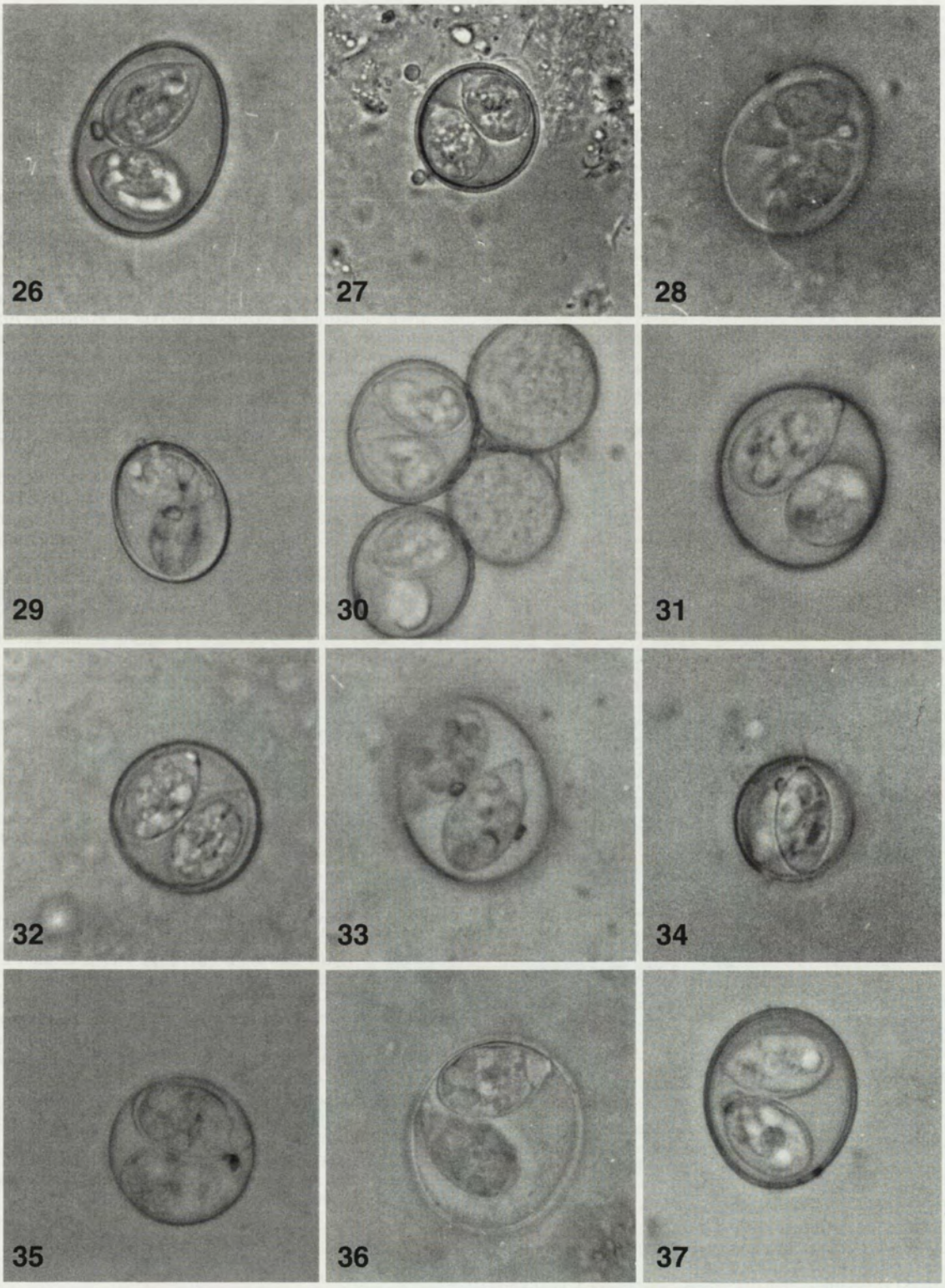
n - number of infected individuals; % - percent of examined individuals; + - percent of infected individuals



Figs. 1-12. Oocyst type - host species. 1. *Isospora* sp.- *Delichon urbica*, 2. *Isospora* sp.- *Motacilla cinerea*, 3. *Isospora* sp.- *Troglodytes troglodytes*, 4,5. *Isospora* sp.- *Prunella modularis*, 6. *Isospora erithaci* - *Erithacus rubecula*, 7. *Caryospora jiroveci* - *E. rubecula*, 8. *Isospora* sp. - *Phoenicurus ochruros*, 9. *Isospora turdi* - *Turdus merula*, 10. *Isospora* sp. - *T. philomelos*, 11. *Isospora* sp. - *Locustella naevia*, 12. *Isospora* sp. - *A. schoenobaenus*. Magnification 1000 x



Figs. 13-25. Oocyst type - host species. 13. *Isospora* sp. - *Acrocephalus palustris,* 14. *Isospora* sp. - *A. scirpaceus*, 15. *Caryospora* sp. - *A. palustris*, 16. *Isospora* sp. - *Hippolais icterina*, 17. *Isospora* sp. - *Sylvia curruca*, 18. *Isospora* sp. - *S. borin*, 19. *Eimeria depuytoraci* - *S. borin*, 20. *Isospora* sp. - *Phyloscopus collybita*, 21. *Isospora* sp. - *P. collybita*, 22. *Isospora* sp. - *Muscicapa striata*, 23. *Isospora* sp. - *Ficedula hypoleuca*, 24. *Isospora* sp. - *Aegithalos caudatus*, 25. *Isospora* sp. - *Parus caeruleus*. Magnification 1000 x



Figs. 26-37. Oocyst type - host species. 26. *Isospora* sp. - *Parus major*, 27. *Isospora* sp. - *Sitta europaea*, 28. *Isospora* sp. - *Certhia familiaris*, 29. *Isospora* sp. - *Lanius collurio*, 30,31. *Isospora* sp. - *Passer domesticus*, 32. *Isospora fringillae* - *Fringilla coelebs*, 33,34. *Isospora* sp. - *F. montifringilla*, 35. *Isospora perroncitoi* - *Pyrrhula pyrrhula*, 36. *Isospora* sp. - *Emberiza citrinella*, 37. *Isospora* sp. - *E. schoeniclus*. Magnification 1000 x

rubecula (Černá 1976), and in *Diphyllodes magnificus* (Paradiseidae) (Varghese and Yayabu 1981, Upton and Sundermann 1990). I found oocysts of the *Caryospora*-type in 6 robins and 2 reed-warblers, *Acrocephalus palustris*; in all but one case there were mixed infections of both *Caryospora* and *Isospora* oocysts. Nevertheless, anomalous sporulation exists in *Isospora*, during which *Caryospora*-like oocysts and transient forms develop, e.g. in sparrows (Černá 1974) or in crested larks *Galerida cristata* (Golemanski 1977). Transient forms, however, were not present in my material; in addition, the case described by Černá (1974) was also of a pure *Caryospora* infection (Černá 1992, pers. com.). So *Caryospora* from my material are probably true species. Another finding of *Caryospora* in two *Hippolais* sp. from Ongudai, Russian Altai (Svobodová 1990, unpub.) indicate that *Caryospora* of passeriform birds are not so rare, although they are not as common as *Isospora*.

The number of infected birds (36.8%) from those of examined (571) corresponds to data given by Scholtyseck and Przygodda (1956). They found that 40% of 632 examined passeriform individuals were infected. I found oocysts in 74% of 46 examined species, and in 86% of 28 examined genera. Nevertheless, descriptions of oocysts from the majority of genera, which were in my case found not to release oocysts, do exist in *Hirundo rustica* and *Anthus pratensis* (Schwalbach 1959), *Serinus canarius* (Box 1975), and *Carduelis chloris* (Anwar 1966). The only two genera without description are *Regulus* and *Coccothraustes*; but oocysts of *Isospora* were found in *C. coccothraustes* by Černá (1973, unpub.) and in *R. ignicapillus* by Svobodová (1993, unpub.). These facts support my conclusion that probably all passeriform birds from Central Europe are potential hosts for at least one species of coccidia.

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Freshwater Gymnamoebae With a New Type of Surface Structure *Paradermamoeba valamo* and *P. levis* sp. n. (Thecamoebidae), and Notes on the Diagnosis of the Family

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Summary. Two species of freshwater gymnamoebae *Paradermamoeba valamo* and *P. levis* sp. n. are included in this recently established genus, which is classified in the family Thecamoebidae. These species are undoubtedly thecamoebians on the basis of their general morphology, but are distinguished from the other members of this family by the fine structural organization of the cell surface. In *Paradermamoeba* spp. the surface consists of a layer of regularly arranged, helical glycostyles embedded in an electron-transparent matrix. The results of this light and electron-microscopical study of *P. valamo* and *P. levis* are compared briefly with previous descriptions of other species of Thecamoebidae, with the purpose of reconsidering the taxonomic accuracy of the modern diagnosis of the family proposed by Page (1987).

Key words. *Paradermamoeba valamo*, *Paradermamoeba levis* sp. n., Gymnamoebia, Thecamoebidae, taxonomy, ultrastructure, cell surface.

INTRODUCTION

The family Thecamoebidae was established to combine amoebae that "... do not form pseudopodia during locomotion. The shape in locomotion is ovoid to circular in outline and subject to slight change only ..." (Schaeffer 1926). Initially Thecamoebidae included only two genera: *Thecamoeba* Fromentel, 1874 and *Rugipes* Schaeffer, 1926. Subsequently, the generic and species composition of this family were changed repeatedly by many authors (see: Page 1991). However, as noted by T.K.Sawyer, "Taxonomic problems remain unresolved in spite of efforts... to establish a united system of classification at the familial level" (Sawyer 1975a). This statement is still generally correct, although some progress has been made in resolving the problems, due largely to the work of F. C. Page (Page 1977, 1983, 1987, 1988; Page and Blakey 1979; Page 1991).

The results of the light-microscopical study and electron-microscopical investigation of surface structure of two naked lobose amoebae that are included now into the recently established genus *Paradermamoeba*(Smirnov and Goodkov 1993), are presented here. Genus *Paradermamoeba* was classified within the family Thecamoebidae according to the reasons initially proposed by A.A.Schaeffer (1926) for this taxon. The morphological peculiarities of *Paradermamoeba* spp. present some additional information for the correct taxonomic definition of the family (see: Discussion).

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MATERIALS AND METHODS

All the samples were collected during June-August 1991 from Lake Leshevoe (Valamo Island, Lake Ladoga) at depths of 0.5-6m. Samples were inoculated in Petri dishes on 1.5% Bacto-agar made with sterilized water from the same lake. Suspension of *Aerobacter* in sterilized water was added to each dish and incubated at room temperature. Four clones of *Paradermamoeba valamo* and one clone of *P. levis* were established.

Light-microscopic investigations were carried out using the phase--contrast and differential-interference-contrast optics to observe the clonal cultures and amoebae collected in field material. Not less than 100 amoebae from each clone were measured in order to calculate the average and max/min length, breadth and length/breadth ratio (L/B). The nuclei of living specimens were also measured (20 amoebae from each clone) and then examined in preparations fixed with Bouin's solution and stained with iron hematoxylin.

For the elecron-microscopical investigations amoebae were fixed in 4% glutaraldehyde (in 0,1M phosphate buffer pH 7.4) for 1h, then in 2% osmium tetroxide in the same buffer for 1h. The fixation was made at the room temperature. After dehydration in the grade series of ethanols the amoebae were embedded in Epon-Araldite. Sections were stained with saturated solution of uranyl acetate in 50% ethanol and with Reinolds' lead citrate and examined in a "Tesla BS-500" electron microscope.

RESULTS

Paradermamoeba valamo Smirnov et Goodkov, 1993

Light-microscopical observations. Locomotive form of the amoeba is flattened, oblong, usually lancet-shaped (Figs. 1-6). Amoeba moves flowingly; neither pseudopodia, nor subpseudopodia are formed. Lateral parts of the body are considerably more flattened than the axial part. The frontal zone of hyaloplasm has long lateral extensions toward the posterior end of the body. As a rule these extensions are not longer than 3/4 of the body length, but sometimes they extend along the whole amoeba. Lateral edge of the body commonly is wavy, uneven and the anterior end of the body is rounded. The dorsal surface of the locomotive form is smooth, without any folds or wrinkles. The wide rounded uroidal region sometimes is of a not clearly differentiated morular type; but the uroidal structures are often lacking in locomotion. The length of the locomotive form is 51-107μm (the average length is 62-76μm, depending on the clone); the breadth in the widest part of the body is 16-30μm (the average breadth is 19-25μm). Length/breadth ratio (L/B) is 2.8-3.5.

To change the direction of locomotion, the amoeba can form a wide, triangular lobe with rounded end (Fig.7). Then this new pseudopodia quickly becomes a leading one, and the normal locomotive form of the amoeba is thus restored. Two, three or more pseudopodia can be formed when the amoeba is changing the direction of locomotion, but only one of them will then be the leading one. The amoeba can also change the direction of locomotion by bending the body, and in this case it does not lose the characteristic locomotive form.

The amoeba can also move slowly, often changing the direction of movement ("indirect" movement). In this case, it does not take a typical locomotive form, but forms wide, triangular extensions with rounded ends (Figs. 9-12).

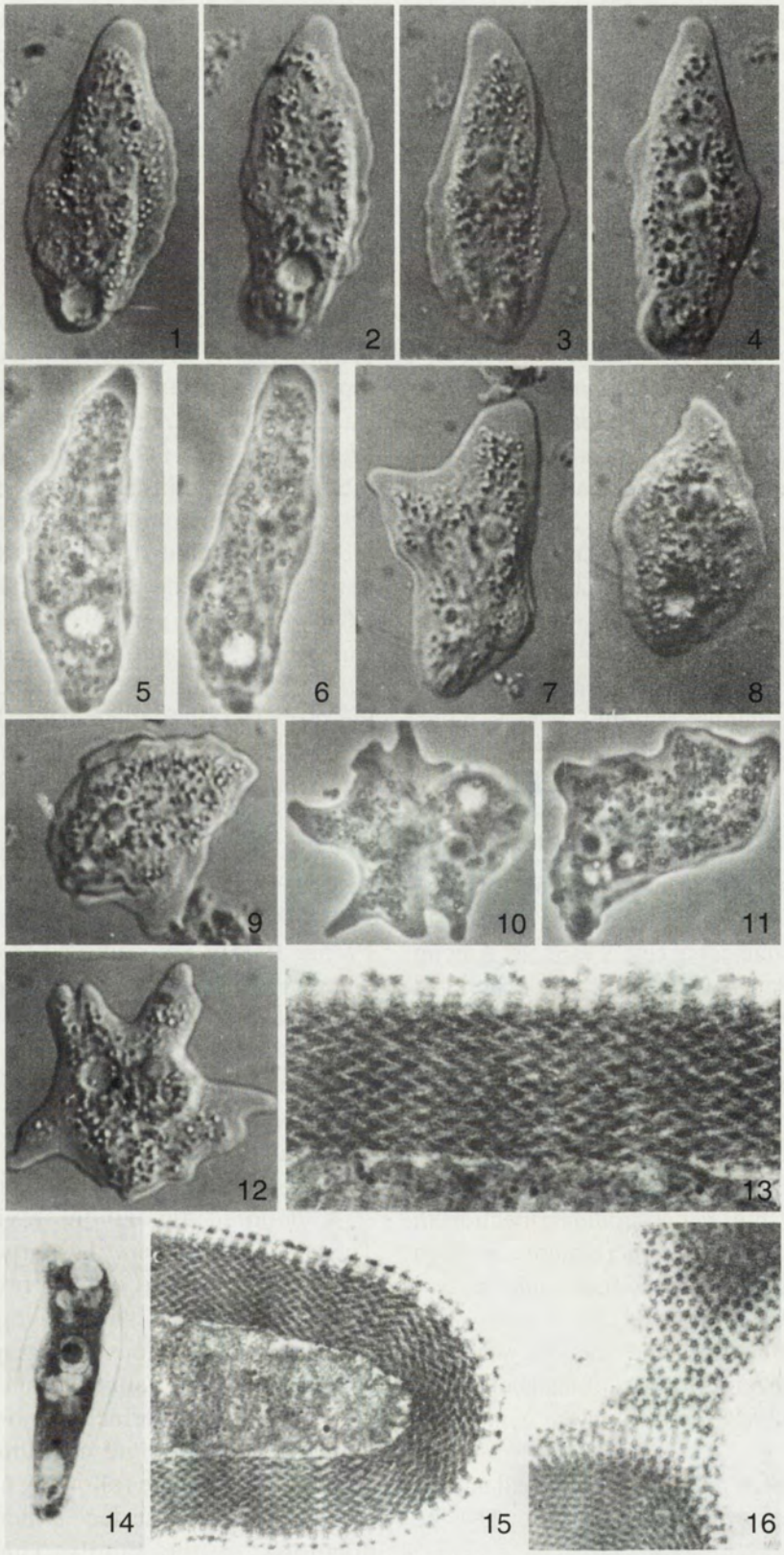
When not moving ("resting"), the amoeba is flattened; its surface is always smooth (Fig.8). Generally, there is a hyaline border around the whole amoeba body. The amoeba produce short, triangular hyaline lobes, and sometimes a number of short pseudopodia of different forms.

A typical floating form is irregular, with 3-4 short, broad, conical pseudopodia with rounded ends.

The cell has one spherical nucleus with a single central endosome (Fig. 14). The nucleus is about 8μm in diameter, the endosome is about 5μm in diameter (in living specimens). There is one contractile vacuole, which is usually situated in the posterior part of the amoeba body (Figs. 1, 2, 5, 6). The most common inclusions in the cytoplasm are small, dark, optically opaque granules, small food vacuoles. We never observed crystals in the cytoplasm of amoebae. Cysts were not found.

P. valamo commonly feeds on bacteria, small unicellular algae, cysts of other protists.

Cell surface structure. Ultrastructural examination shows a distinct surface structure, about 520 nm of total thickness, which consists of a layer of regularly arranged tightly packed helical-shaped glycostyles (Figs. 13, 15, 16). Coils of neighboring helices overlap. Each glycostyle terminates with a hollow straight funnel-shaped structure, which sometimes seems to be pentagonal in cross-section (Fig. 16). The length of the spiral part of the glycostyles is about 400 nm and the diameter is about 100 nm; length of the straight terminal part of glycostyles is about 120 nm. Helices form 7-7.5 turns. They are embedded in an unstructured electron-transparent matrix which does not seem to prevent the neighboring glycostyles from moving in relation to each other (Fig. 15). We never observed considerable deformation of the glycostyles at the site of their contact with the substrate or in any other place, so it is possible to suggest that they are rigid.



Figs. 1-16 - *Paradermamoeba valamo*. 1-6 - locomotive form; 7 - amoeba during changing the direction of locomotion; 8 - "resting" amoeba; 9-12 - amoeba during "undirected" movement; 13 - part of cell surface; note plasma membrane, glycostyles, hollow terminal structures; 14 - stained preparation, showing nucleus; 15 - cell surface, total view; 16 - section of glycostyles, showing pentagonal cross-section of terminal structures. Figs. 1-4, 7-9 - differential interference contrast, x 670; 5, 6, 10, 11 - phase contrast, x 670; 13 - x 45800, 14 - x 540; 15 - x 25400, 16 - x 35000

***Paradermamoeba levis* sp. n.**

Light-microscopical observations. The locomotive form of this amoeba (Figs. 17-23) is similar in general to the locomotive form of the *P. valamo* but the lateral flattened extensions of the body are less pronounced and sometimes they disappear. The length of locomotive amoeba ranges from 23 to 53μm (average length 35-46μm). The breadth is about 6-18μm. Average length/breadth ratio (L/B) is 3.5-3.9.

The amoeba changes the direction of movement commonly by bending the body without losing its typical locomotive form; pseudopodia formation rarely occurs during this process and it seems to be not typical for this species. Broad extensions with rounded ends are formed during "undirected" movement (Fig. 21). The body shape of the "resting" amoeba is irregular, often with a hyaline border around the whole amoeba body. Short pseudopodia with rounded ends as well as hyaline lobes can be formed (Fig. 25).

The floating form is of the radial type (Fig. 24). There is a central mass of granuloplasm with 5-7 thick, blunt, hyaline pseudopodia often of unequal length; the longest ones are 4-5 times longer than the diameter of the central granular mass.

The nucleus is of the vesicular type. In living specimens it measures 2.3-5.7μm in diameter (average diameter is 4μm); the central endosome is about 1.5-2.9μm. The number of nuclei per cell is variable. Among 46 simultaneously inspected amoebae there were 32 with one nucleus, 12 with two nuclei and 2 with three nuclei. When an amoeba contains two nuclei, they are unequal in size and often closely associated with each other. In living specimens such associations often look like one nucleus with two endosomes (Figs. 22, 23), but the presence of two separate nuclei may be confirmed in stained preparations (Fig. 27). It should be mentioned, that sometimes nearly all amoebae in a culture contained two nuclei, but during other periods only a few binucleate specimens were observed.

The amoeba has one contractile vacuole, which is generally situated at the posterior part of the body (Figs. 20, 22). We never observed crystals in the cytoplasm. Cysts were not found.

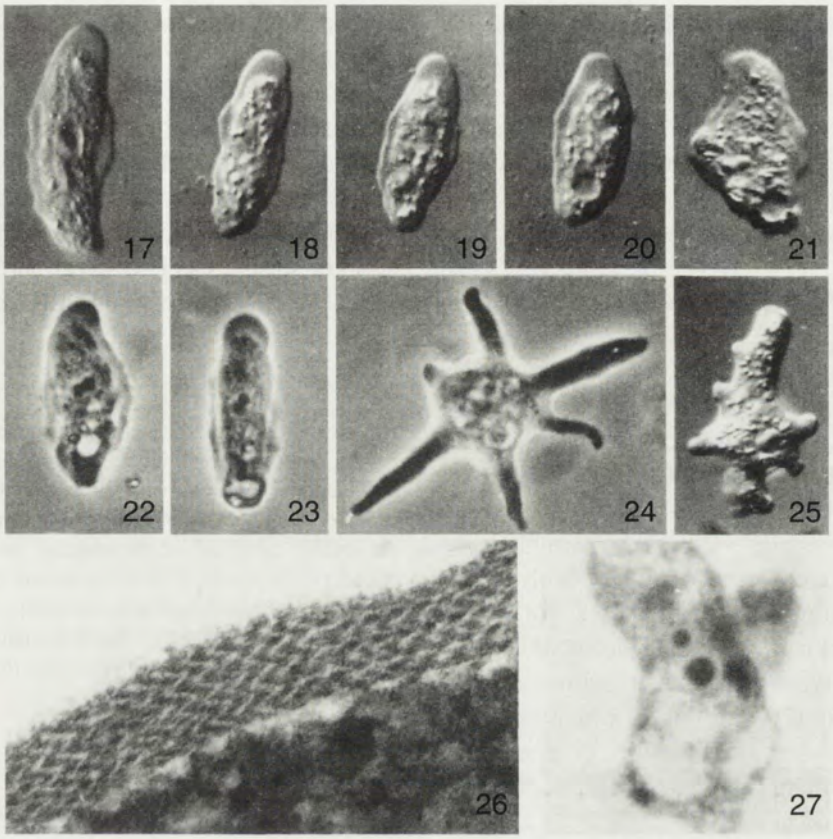
P. levis commonly feeds on bacteria and small unicellular algae; small cysts of other protists can also be engulfed.

Cell surface structure. As observed with electron microscopy, the structural organization of the cell surface of *P. levis* (Fig. 26) is similar to that of *P. valamo* (see above), but *P. levis* lacks the distinct straight terminal structures on the distal end of glycostyles. The total thickness of the cell surface is about 220 nm; the diameter of the helices is about 90 nm, and they form 4 turns.

DISCUSSION

As we mentioned above, the generic composition of the family Thecamoebidae has been changed repeatedly by many authors since 1926, when it was established by A.A. Schaeffer to include *Thecamoeba* Fromentel, 1874 and *Rugipes* Schaeffer, 1926. Thus, the following additional genera have been recognized in the Thecamoebidae in different years (some of them were excluded from this family or declared as non-valid by other authors) (listed alphabetically): *Clydonella*, Sawyer 1975; *Dermamoeba*, Page et Blakey 1979; *Lingulamoeba*, Sawyer 1975; *Paradermamoeba*, Smirnov et Goodkov 1993; *Pessonella*, Pussard 1973; *Platyamoeba*, Page 1969; *Pseudothecamoeba*, Page 1988; *Sappinia*, Dangeard 1896; *Striamoeba*, Jahn, Bovee et Griffith 1974; *Thecochaos*, Page 1981; *Vannella*, Bovee 1965 (see: Bovee 1970, 1985; Bovee and Sawyer 1979; Jahn et al. 1974; Page 1976a, 1976b, 1977, 1978, 1981, 1983, 1987, 1988; Page and Blakey 1979; Page 1991; Sawyer 1975a, 1975b; Smirnov and Goodkov 1993). On the contrary, Singh and co-workers proposed to subdivide *Thecamoeba* into eight independent genera on the basis of nuclear structure and mitotic pattern, and classified them and other traditional thecamoebian genera into different families (Singh and Hamemaiah 1979, Singh et al. 1982). Thus, they did not recognize Thecamoebidae as a valid taxon.

A detailed taxonomic revision of the family Thecamoebidae is not the purpose of present report, however a great part of such revision was carried out by F.C. Page in his successive publications (see references in the previous paragraph). His modern classification of the family (Page 1987, 1988, 1991) conforms to the principles basically proposed by Schaeffer (1926) and is quite acceptable in our opinion, but with the following notes. Such a character as organization of surface structure is included in the diagnosis of the family Thecamoebidae. Representatives of five genera, whose ultrastructure has been studied, possess quite different types of cell surface structure: thick, amorphous glycocalyx



Figs. 17-27 - *Paradermamoeba levis* sp. n. 17-23 - locomotive form; 21 - amoeba during "undirected" movement; 24 - floating form; 25 - "resting" amoeba; 26 - cell surface, note plasma membrane, glycostyles; 27 - stained preparation, showing binucleate cell. Figs. 17-20, 21, 24, 25 - differential interference contrast, x 670; 22, 23 - phase contrast, x 670; 26 - x 54000; 27 - x 1360

(*Thecamoeba*, *Sappinia*), distinct filaments radiating outward from plasmamembrane (*Pseudothecamoeba*), thick, structured, non-mucoid cover, termed a "cuticle" (*Dermamoeba*), a layer of regularly arranged, tightly packed helical glycostyles (*Paradermamoeba*); the fine structure of *Thecochaos* is still unknown (see: Goodfellow et al. 1974; Page 1978, 1983, 1988, 1991; Page and Blakey 1979; Page 1991; Smirnov and Goodkov 1993). As seen from this list, the surface structure is a considerable taxonomic character for thecamoebians on the generic, but not on the familial level. Thus there are no reasons to include this feature in the diagnosis of the family. This is also true for the nucleus of thecamoebians, whose organization is variable even within some genera.

We propose to emend the diagnosis of the family Thecamoebidae, based on the reasons discussed above. Investigation of *P. levis* forced us to make some changes in the diagnosis of the genus *Paradermamoeba* and of *P. valamo*, so these diagnosis are also presented.

SYSTEMATIC ACCOUNT

Family Thecamoebidae (Schaeffer, 1926) Page, 1987; emend.

Amoebae flattened, with more or less regular outline, commonly oblong, monopodial with rare exceptions; at light-microscopical level surface often appears as pellicle-like layer, which may be distinctly wrinkled; hyaloplasm usually a deep, anterolateral crescent, sometimes with long lateral extensions toward the posterior end, but continuous anterior hyaloplasm never occupies up to half of body length; a variety of nuclear structures; no cytoplasmic crystals.

Dermamoeba, Paradermamoeba, Pseudothecamoeba, Thecamoeba, Thecochaos, Sappinia.

Genus *Paradermamoeba* (Smirnov et Goodkov, 1993)

Amoebae flattened, oblong, slightly narrower anteriorly, with length commonly more than 2.5 times width; crescent-shaped anterior hyaloplasm with long

extensions along lateral sides of the body; surface in locomotion always smooth; commonly uninucleate, but may temporarily have supplementary nuclei in some species; cell coat consists of a layer of regularly arranged tightly packed helical glycostyles embedded in electron-transparent matrix.

Type species: *P. valamo*, freshwater.

Paradermamoeba valamo Smirnov et Goodkov, 1993

Locomotive form flattened, oblong, commonly lancet-shaped; usually without differentiated uroidal structures, but sometimes with wide uroid of morular type. Length in locomotion 51 - 107μm (average length 62 - 76μm), breadth 16 - 30μm (average breadth 19 - 25μm), length/breadth ratio (L/B) 2.8 - 3.5. Uninucleate, vesicular nucleus about 8μm diameter with single central endosome about 5μm diameter (in living specimens). Total thickness of cell surface coat is about 500 nm, each glycostyle is terminated with a straight hollow structure about 120 nm length, which seems to be pentagonal in cross-section. Floating form irregular, often with a few blunt conical pseudopodia with rounded ends. Cysts unknown.

Known habitat: Freshwater, North-Western Russia.

Paradermamoeba levis sp. n.

Locomotive form flattened, oblong; uroid of bulbous type sometimes present. Length in locomotion 23 - 53μm (average length 35 - 46μm), breadth 6-18μm (average breadth 9 - 14μm), length/breadth ratio (L/B) 3.5 - 3.9. Uninucleate, with tendency to have one or, rarely, two supplementary nuclei. Nucleus of vesicular type 2.3 - 5.7μm in diameter (average diameter4 m) with single central endosome 1.5 - 2.9μm diameter (average diameter 2.3μm) (in living specimens). When binucleate, nuclei are closely associated with one another. Total thickness of cell surface coat is about 220 nm. No differentiated terminal structures on each glycostyle. Typical floating form is of radial type with long, blunt hyaline pseudopodia with rounded ends. Cysts unknown.

Known habitat: Freshwater, North-Western Russia.

Type slides are deposited in the museum of preparations of the Laboratory of Invertebrate Zoology, Biological Research Institute, St.Petersburg State University: Holotype, 1992: 648; paratypes 651, 652. Bottom sediments of Lake Leshevoe (Valamo Island, Lake Ladoga), from depths of 0.5 - 5m, July 1991.

Differential diagnosis

Paradermamoeba levis and *P. valamo* differ in their dimensions, uroidal structures and organization of the nucleus. The total thickness of the cell surface coat of *P. levis* is 220 nm whereas in *P. valamo* it reaches up to 500 nm. Glycostyles of *P. levis* lack terminal hollow straight structures. The spiral part of the glycostyles in *P. levis* forms 4 turns, and in *P. valamo* it forms 7 - 7.5 turns.

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A New Species of *Eimeria* (Apicomplexa) from the Orange-Fronted Conure, *Aratinga canicularis* (Psittaciformes), in Costa Rica

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Summary. A new species of coccidian (Apicomplexa, Eimeriidae) is described from the faeces of the Orange-Fronted Conure, *Aratinga canicularis* (Psittaciformes, Psittacidae), from Costa Rica. Oocysts of *Eimeria aratinga* sp. n. were found in 3/21 (14%) birds and are ellipsoidal, 35.0 x 25.9 (32.4-37.6 x 23.8-28.0) µm, with a smooth, bilayered wall and a shape index (length/width) of 1.35 (1.22-1.50). A micropyle and oocyst residuum are absent but a fragmented polar granule is present. Sporocysts are ovoidal, 19.2 x 9.8 (17.6-20.8 x 9.0-10.4) µm, and possess Stieda and substieda bodies; shape index 1.97 (1.76-2.22). Each sporozoite has spherical anterior and subspherical to ellipsoidal posterior refractile bodies.

Key words. *Eimeria aratinga* sp. n., *Aratinga canicularis* host

INTRODUCTION

Although over 340 species of psittaciform birds have been described to date (Clements 1981, Forshaw 1989), little is known of their coccidia. As part of a larger study involving parrot behavior in Costa Rica, we were recently able to examine fecal samples from several psittaciform species for coccidia. Several birds, all Orange-fronted Conures (*Aratinga canicularis*), were passing coccidian oocysts unlike any reported thus far from other members of the Psittaciformes. This bird is one of four species of parrots commonly found in the dry forest habitat of northwestern Costa Rica (Forshaw 1989). It ranges widely in flocks of 2-200 as it forages for seeds, fruits, and flowers. Like most parrots, it nests in cavities, although it is unusual in that it constructs its own nest hollows in arboreal termite mounds rather than using existing tree hollows (Hardy 1963). Below we present a description of this new species of coccidian.

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MATERIALS AND METHODS

All birds were trapped live during June to August, 1992 and February to July, 1993 using mist nets and placed temporarily in cloth bags. Fecal samples were collected from within the bags, which were then placed in screw cap vials in a thin layer of 2.5% (w/v) aqueous solution of potassium dichromate and shipped to Kansas State University. Oocysts were then concentrated by flotation in an aqueous sucrose solution (specific gravity 1.30). Measurements and photomicrographs were taken using Nomarski interference-contrast optics and a calibrated ocular micrometer and are reported in micrometers (µm) as means, followed by the ranges in parentheses.

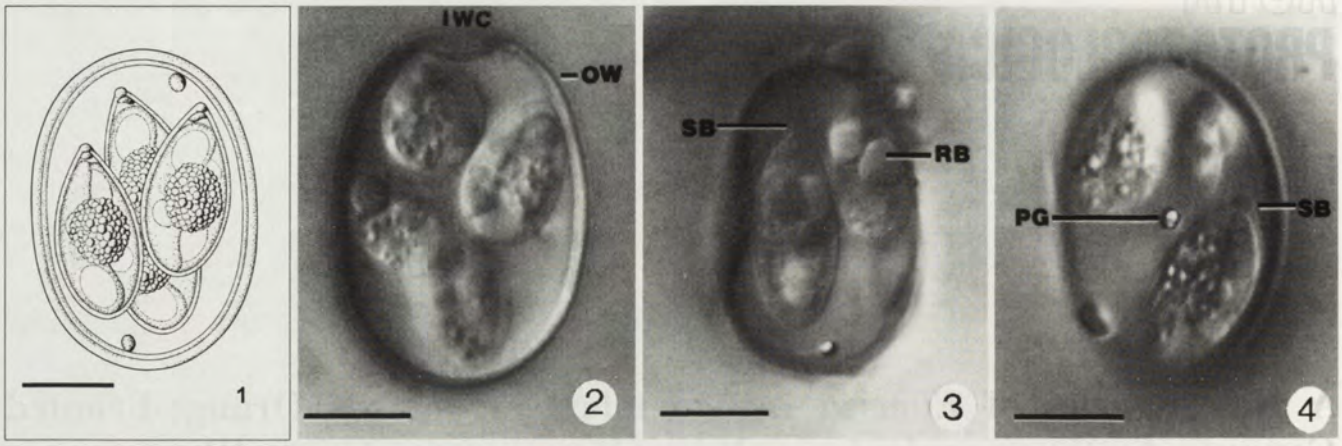

Fig. 1. Composite line drawing of sporulated oocyst of *Eimeria aratinga* sp. n. Bar - 10 µm
Figs. 2-4. Nomarski interference-contrast photomicrographs of sporulated oocysts of *Eimeria aratinga* sp. n. Bars - 10 µm Abbreviations: IWC - inner wall collapse at pole of oocyst after exposure to sucrose solution, OW - oocyst wall, PG - polar granule; RB, refractile body, SB - Stieda body

RESULTS

Fecal samples were collected from a total of 75 parrots: *Amazonia albifrons* (26 adults/juveniles and 6 nestlings), *A. auropalliata* (4 adults/juveniles and 7 nestlings), *Aratinga canicularis* (21 adults), and *Brotogeris jugularis* (11 adults). Coccidian oocysts were recovered only from the feces of 3/21 (14%) *Aratinga canicularis*. Upon sporulation, these oocysts were found to represent a previously unreported species, which is described below.

Eimeria aratinga sp. n. (Figs. 1-4)

Description of oocysts: Oocysts ellipsoidal, 35.0 x 25.9 (32.4-37.6 x 23.8-28.0), with a smooth, bilayered wall ca 1.5-1.8 thick; inner layer 0.4-0.5; outer layer 1.0-1.4; shape index (length/width) of 1.35 (1.22-1.50). Micropyle and oocyst residuum absent, fragmented polar granule present, consisting as 2-5 granules. Sporocysts ovoidal, 19.2 x 9.8 (17.6-20.8 x 9.0-10.4), with smooth, thin wall ca 0.5 thick; shape index 1.97 (1.76-2.22); Stieda body present, consisting as small, button-like structure; small substieda body present. Sporocyst residuum present, 7.5 x 6.5 (6.4-8.0 x 4.8-7.2), consisting as many granules in compact mass. Sporozoites elongate, arranged head-to-tail in sporocyst, 15.6 x 4.2 (13.6-17.6 x 3.8-5.0). Each sporozoite with spherical anterior refractile body, 3.3 (2.6-4.0), and subspherical to ellipsoidal posterior refractile body, 5.2 x 4.0 (4.0-6.4 x 3.6-4.8). Nucleus located between refractile bodies.

Type-host: *Aratinga canicularis* (Linné) "Orange-Fronted Conure" (Psittaciformes, Psittacidae)

Type locality: Area de Conservación Guanacaste, Costa Rica; 10°45'N, 85°35'W.

Site of Infection: unknown. Oocysts recovered from feces.

Sporulation: unknown. Oocysts arrived at Kansas State University fully sporulated and may have sporulated en route. Other known coccidia from Psittaciform birds are known to sporulate exogenously.

Prevalence: 3/21 (14%) *Aratinga canicularis* were passing oocysts.

Etymology: the specific epithet reflects the host genus.

Type specimens: phototypes of *Eimeria aratinga* from *Aratinga canicularis* have been deposited in the U.S. National Parasite Museum in Beltsville, Maryland as USNM No. 83814.

DISCUSSION

Prolonged exposure to sucrose solution often resulted in the partial collapse of the inner oocyst wall at one pole (Fig. 2). Thus, even though a true micropyle was not noted, modification of the wall at one pole may occur.

To date, only 5 members of the apicomplexan family Eimeriidae (3 eimerians and 2 isosporans) have been reported from Psittaciform birds (Table 1). Oocysts and sporocysts of *Eimeria psittacina* Gottschalk, 1972 are considerably smaller and cannot be confused with the species reported herein (Gottschalk 1972). Oocysts of

Table 1

Named and some unnamed species of coccidia (Eimeriidae) reported from Psittaciform birds.

Species	Host	Oocysts (μm)	Locality	Reference(s)
"Coccidia"				
sp.	*Amazona aestiva*	No data	Argentina	Tsai et al. (1992)
sp.	*Melopsittacus undulatus*	No data	No data	Morelli (1956); Panigrahy et al. 1981a 1981b)
sp.	*Melopsittacus undulatus*	No data	Philippines	Tsai et al. (1992)
sp.	*Trichoglossus haematodus*	No data	Indonesia	Tsai et al. (1992)
Eimeria spp.				
aratinga sp. n.	*Aratinga canicularis*	35.0 x 25.9 (32.4-37.6 x 23.8-28.0)	Costa Rica	This study
dunsingi	*Melopsittacus undulatus*	33.7 x 22.8 (27.8-35 x 20-23)	Cosmopolitan	Brada (1966); Farr (1960); Keymer (1958); Todd et al. (1977)
haematodi	*Trichoglossus haematodus*	32.3 x 27.6 (24.7-40.0 x 20.8-35.0)	New Guinea	Varghese (1977)
psittacina	*Melopsittacus undulatus*	22.0 x 17.3 (19.4-26.3 x 15.0-19.7)	Bulgaria	Gottschalk (1972)
Isospora spp.				
melopsittaci	*Melopsittacus undulatus*	24.0 x 18.5 (20-28 x 17.3-21.3)	India	Bhatia et al. (1973)
psittaculae	*Psittacula eupatria*	28.6-33.0 x 24.2-28.6	India	Chakravarty & Kar (1946)

E. dunsingi Farr, 1960 are only slightly smaller but are pear-shaped rather than ellipsoidal (Farr 1960, Todd et al. 1977). In addition, sporocysts of *E. dunsingi* are less elongate. Oocysts of *E. haematodi* Varghese, 1977 are less elongate, the sporocysts are shorter, and an oocyst residuum is present (Varghese 1977).

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Ceratomyxa daysciaenae sp. n. (Myxozoa: Ceratomyxidae) a Myxosporean Parasite in the Gallbladder of an Teleost from the Hooghly Estuary, West Bengal, India

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Summary. A new myxosporean, *Ceratomyxa daysciaenae* sp.n. (Myxozoa: Ceratomyxidae), has been described from the gallbladder of an estuarine teleost *Daysciaena albida* (Cuv.) (Sciaenidae). The parasite possesses a disporous trophozoite, a large strongly arcuate spore with a central ellipsoidal region and laterally drawn-out, downwardly directed, thin-walled and symmetrical shell valves with sharply pointed, often recurred tips. The suture is thin but distinct and slightly sinuous. It possesses a pair of small, spherical polar capsules. A binucleate sporoplasm fills the spore cavity. Large granules makes the central region more denser. The dimensions of spore and polar capsule are 5.0-7.0 (6.04) µm x 55.0-75.0 (65.13) µm and 1.8-3.0 (2.14) µm respectively.

Key words. *Ceratomyxa daysciaenae* sp. n., Myxozoa, parasitic, gallbladder, *Daysciaena albida* (Cuv.), estuarine.

INTRODUCTION

During an investigation of the parasitic Protozoa of the fishes of West Bengal, India, a myxozoan species was found infecting the gallbladder of an estuarine teleost (Sciaenidae) from Hooghly River-Bay of Bengal estuary near Kakdwip of West Bengal. This parasite has been described as a new species of *Ceratomyxa* Thelohan 1892.

MATERIALS AND METHODS

All necropsies were conducted on frozen fish under the microscope. The myxozoan parasite obtained from the gallbladder was studied in detail from wet smears treated with Lugol's iodine and from dry smears stained with Giemsa after fixation in absolute methanol, under the oil immersion lens of a research microscope. All the measurements were given in micrometers (µm). The width of the spore represents the distance between the two tips of the spore passing through the center of the central spore body and the sutural diameter denotes the height of the spore at the suture. The illustrations were made with the aid of a camera lucida from the type materials.

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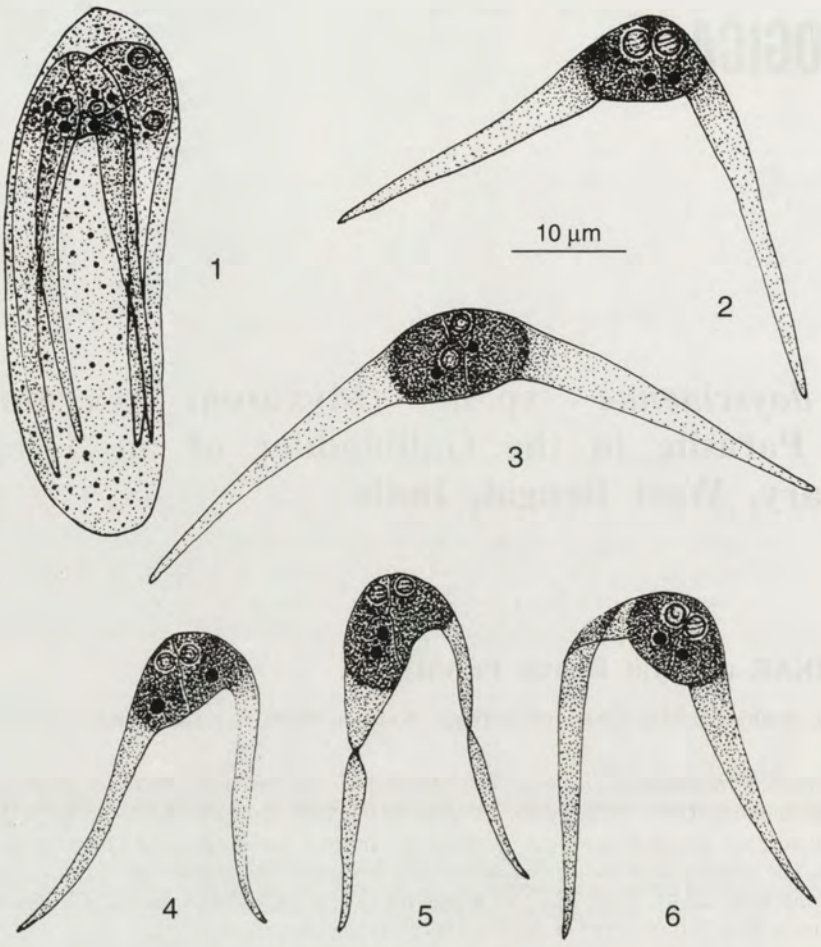
OBSERVATIONS

Ceratomyxa daysciaenae sp.n.

The early trophic forms were small and ovoidal measuring 15.0 x 8.0 µm. The fully grown trophozoites were large and ellipsoidal with one end more pointed than the other (Fig. 1). The average dimension of the spore producing trophozoite was 80.2 x 25.65 µm. The trophozoites were disporous and floated in the bile. The



Figs. 1-6. *Ceratomyxa daysciaenae* sp.n. 1 - a fresh disporous trophozoite - Lugol's iodine, 2 - a fresh spore in side view - Lugol's iodine, 3 - a fresh spore in capsular view - Lugol's iodine, 4 and 6 - strongly arcuate spores in side view - Lugol's iodine, 5 - a fresh spore with a pair of unequal polar capsules and twisted extensions of shell valves - Lugol's iodine

free-floating mature spores were strongly arcuate to crescent-like with a broad ellipsoidal center and two very long laterally drawn-out, downwardly directed shell valves (Figs. 2-6). The central part of each spore was arched or convex anteriorly while its posterior edge was slightly or weakly convex. The two laterally drawn-out parts were the continuous extensions of the two shell valves which were symmetrical, smooth, equal, thin-walled, greatly compressed - parallel to the sutural axis and gradually tapered into sharply pointed (often recurred) tips. Sometimes, the lateral extensions of the shell valves became twisted near their midpoint (Fig. 5). There was no septum between the central part and the lateral extension of each shell valve. The suture was very thin, intercapsular, slightly sinuous but never straight. The two polar capsules were small, spherical and usually subterminal (Figs. 2, 4 and 6) but rarely terminal (Fig. 5), usually equal but rarely unequal (Fig. 5). The polar filament in each polar capsule was insufficiently distinct to count coils. Often a dot-like capsulogenous nucleus was found near the base of each capsule (Fig. 5). The sporoplasm filled the central cavity of spore with a mass of coarser granules and a pair of large, spherical nuclei. Any vacuole of iodinophilous or non-iodinophilous type was not seen in the sporoplasm. No triad spore forms were found.

Measurements (based on thirty fresh spores, treated with Lugol's iodine, from a single frozen host) were: spore sutural diameter - 5.5-7.0 (6.04) µm; width - 55.0-75.0 (65.13) µm; polar capsule diameter - 1.8-3.0 (2.14) µm.

Infection locus: gallbladder.

Incidence: 3/38 (7.9%).

Pathogenicity: not apparent.

Host: *Daysciaena albida* (Cuv.), (Sciaenidae)

Habitat: Hooghly River estuary near Kakdwip of West Bengal, India.

Period of study: 1990-1992.

Infection period: November-January, 1991-1992.

Material: syntypes on slide No. Mx. - 11 are deposited to the Department of Zoology, R. B. C. College, Naihati 743165, West Bengal, India.

DISCUSSION

Thelohan (1892) erected the genus *Ceratomyxa* while describing its type species *Ceratomyxa arcuata* from the gallbladder of *Pagellus centrodontus* (cited from Kudo 1919). The diagnostic features were recently revised by Lom and Noble (1984) to include elongated, crescent-shaped or arcuate spores with shell valves often conical or flattened processes, with rounded or pointed tips, exceeding in length the axial diameter of the spore. Spherical polar capsules, sometimes unequal in size, have capsular foramina near the sutural line at the anterior pole of the spore. Exceptionally the capsules open at the opposite side of the central sutural line. Binucleate sporoplasm does not completely fill the spore cavity; two uninucleate sporoplasms were also reported. Trophozoite monosporous to polysporous, mostly disporous; coelozoic in marine fishes, rarely histozoic. The present myxozoan species well satisfies the diagnostic characters mentioned above, warranting its placement in *Ceratomyxa* Thelohan, 1892.

There are altogether nine *Ceratomyxa* spp. recorded from Indian fishes (Chakravarty 1939, 1943; Choudhury and Nandi 1973; Sarkar 1986; Das et al. 1988; Narasimhamurti et al. 1990; Rajendran and Janardanan 1992) as well as a brief preliminary reports of *Ceratomyxa* spp. in the gallbladder of some fishes by Ray (1933) and Setna (1942). The spore shape of the present *Ceratomyxa* sp. closely resembles only *Ceratomyxa sagarica* Choudhury and Nandi (1973) reported from the gallbladder of an estuarine teleost, *Boleopthalmus boddaerti* Palbs. However, the spores of the former species are larger (both in sutural diameter and in width) than the later species (spore dimension of *C. sagarica* - 3.3-4.1 μm x 26.5-36.3 μm). Moreover, the spores of *C. sagarica* possess a pair of distinct noniodinophilous vacuoles in the sporoplasm which are not found in the present myxozoan species. Thus, the present *Ceratomyxa* sp. is distinct and different from *C. sagarica.*

The present *Ceratomyxa* sp. is also similar either in size or in shape with a few members of this genus recorded from countries other than India viz., *Ceratomyxa sphairophora* Davis 1917, *C. atenuatta* Davis 1917, *C. flagellifera* Davis 1917, *C. aggregata* Davis 1917, *C. spinosa* Davis 1917, *C. porrecta* Dogiel 1948 (cited from Shulman 1966), *C. moenei* Meglitsch 1960, *C. orientalis* (Dogiel) Shulman 1966 and *C. acuta* Shulman 1966. Of these, the spores of *C. sphairophora, C. attenuata, C. flagellifera* are larger in sutural diameter, in width and also in the diameter of polar capsules than of the present species (12.0 x 115.0-140 μm and 6.0 μm, 9.0 x 115.0 μm and 4.5 μm, 12.0 x 118.0 μm and 6.0 μm are the dimensions of spores and the polar capsules of *C. sphairophora, C. attenuata* and *C. flagellifera* respectively).

Among the rest of similar species, *C. aggregata, C. moenei, C. porrecta* and *C. acuta* have spores with smaller dimensions particularly in width than the present myxozoan (spore dimensions are 6.0-7.0 x 50.0 μm, 4.5-7.1 x 25.3-35.5 μm, 4.0-5.0 x 50.0-64.0 μm and 5.0-6.0 μm x 39.0-52.0 μm of four above mentioned spp. respectively). Moreover, except *C. acuta*, the spores of the first three *Ceratomyxa* spp. have a straight posterior edge of the central part of the spore while this is convex to weakly convex in the present myxozoan. The central part of the spore of *C. acuta* is also similar to the present species but its monosporous as well as disporous trophozoite and smaller spore width are dissimilar. Of the other *Ceratomyxa* spp., *C. spinosa* has been transferred to the genus *Davisia* by Laird (1953) on the ground that the laterally drawn-out extensions of the shell valves are the separate entities from the central part of its spore and thus is quite different from the present species. Lastly, although the width of spore and the diameter of polar capsules of *C. orientalis* are nearly similar to the present species, both symmetrical and asymmetrical spores, much wider sutural diameter (7.0-11.0 μm), straight posterior edge of the central part of spore and the thick, straight suture of *C. orientalis* are different from the present myxozoan. Considering all the differences with the most similar *Ceratomyxa* spp. the present myxozoan is regarded as a new species and designated as *Ceratomyxa daysciaenae* sp.n. after the generic name of its host.

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Book review

Foissner, W. (1993) COLPODEA (Ciliophora)

Protozoenfauna Vol. 4/1 (ed. D. Matthes), 798 pp., 2900 figs., 38 tabs., 17 x 24 cm, hard cover. ISBN (Stuttgart) 3-437-30677-4, ISBN (New York) 1-56081-351-2. Gustav Fischer, Stuttgart-Jena-New York, price 296.- DM

It is no exaggeration to say that no other class of ciliates has ever been treated in a more comprehensive way than the Colpodea by W. Foissner in vol. 4/1 of the "Protozoenfauna". The Colpodea are a relatively small group of ciliates with about 170 valid species, almost ubiquously found in any terrestrial environment, but rather difficult to identify correctly as far as it concerns the genera *Colpoda, Maryna, Platyphrya* and *Bryometopus.*

The book begins with a concise General Section which covers the following topics: Light microscopic and ultrastructural morphology - Ecology, occurrence, geographic distribution - Parasitism - Phylogeny and evolution. It also gives a general introduction on the historic development of current classification of the Colpodea and their identification as a separate class, admittedly influenced by the highly conservative colpodean kinetid pattern first recognized by Denis Lynn. Oral structures of colpodid ciliates at the first glance look very diverse which led to a misplacement of several true colpodids among heterotrichs (e.g. *Bursaria*), haptorids (e.g. *Sorogena*), gymnostomes (e.g. *Platyophrya*). In the General Section all earlier classifications of colpodid ciliates beginning with Kahl are listed. These lists, at least to experts, give a vivid view of the earlier turmoil at times of constant reshuffling of colpodean classification through contributions by de Puytorac, Corliss, Jankowski, Lynn and Foissner. Technical notes on collecting, culturing, observing, and staining of the colpodid ciliates finish off the General Section.

The major part of the book is devoted to the Systematic Section. On some 700 pages the orders Colpodida, Grossglockneriida, Bursariomorphida, Sorogenida, Bryophryida, Cyrtolophosidida and Bryometopida are treated according to the following scheme: Diagnosis of the order, remarks to the diagnosis, key to the families, diagnosis and remarks to the families, detailed description of the type genus, its somatic and oral ciliature, cytological data, life cycle, asexual reproduction (with notes on morphogenesis where known), conjugation and cyst formation. This scheme is then recapitulated for all valid species of a genus, this time starting with a complete list of synonyms, their authors with references. The detailed description of every listed species is copiously illustrated by accurate drawings of the living specimen, high-quality light micrographs of silver-stained

specimen and beautiful scanning micrographs, long tables of morphometric data, probably helpful to -taxonomists, comprehensive data on occurrence and ecology of every single species are present in an overall pleasing style. In many cases drawings from the literature are reproduced which greatly help to understand the description of earlier workers, and the most complete body of literature on colpodid ciliates ever published will be of unsurpassed value for generations of ciliatologists to come. Nonetheless, the users need to be patient if they want to take profit of this huge collection of data, a better known species e. g. *Bursaria truncatella,* is treated on some 40 pages of text and figures. Almost every detail reported on this ciliate seems to be compiled in this monograph. Thirty pages of literature references, with an estimate of 900 entries, and a systematic index with all valid species names, junior homonyms, synonyms and outdated combinations complete this monograph. This book certainly sets a new standard in species revision of ciliated protozoa.

Nothing to criticize ? Isn't every reviewer forced to find something he doesn't like? Why not me, too ? I don't buy the author's ideas on the origin of colpodid ciliates from haptorids. I think in this point Foissner is too much biased by the importance he puts to the silverline pattern. The phylogenetic relationships among the

different orders of the Colpodea is yet another unsolved problem. Though Foissner's most recent revision, as published in this book, can be regarded as a temporary status of consolidation, it will be interesting to see to what extent this classification will be corroborated by molecular studies. But no doubt, the morphological and ecological data, the neat drawings, morphometric tables will be of permanent actuality.

This outstanding piece of work would have been the appropriate starter to the very ambitious series "Protozoenfauna", published by the Gustav Fischer company: For sure, this series will be no bargain for the publisher (nor for the author, who instead has put a considerable amount of private money into his "labour of love"). Nonetheless, the publisher, standing in a great tradition of protozoological publications (Kahl, Doflein-Reichenov, Archiv für Protistenkunde) is encouraged to continue with this project. Wilhelm Foissner, who will apologize if I call him an "illegitimate, late-born scientific son" of Alfred Kahl, is involved in the volumes on ciliates to come. Thanks to his unflinching diligence and endurance he is a good guarantor for a successful continuation and final completion of the "Protozoenfauna".

Sisyphus was an unhappy hero, while Wilhelm Foissner can be proud of his work.

Christian F. Bardele, Tübingen

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