ACTA PROTOZOO- LOGICA

VOLUME 26

Number 3

ACTA PROTOZOOŁOGICA

International Journal of Protozoology

Morphology and Infraciliature in *Urotricha nais* sp. n. and *Urotricha castalia* sp. n. (*Ciliophora, Prorodontida*)

A. MUÑOZ, C. TÉLLEZ and D. FERNÁNDEZ-GALIANO

Departamento de Microbiología, Facultad de Biología
28040 Madrid, España

Synopsis. The morphology and the infraciliature of the ciliates *Urotricha nais* sp. n. and *U. castalia* sp. n., are described. *U. nais* is small in size (16–34 μm × 12–32 μm) and posesses 18–21 somatic kineties, a circumoral corona of 9–10 pairs of kinetosomes, a "brosse" formed by two membranelle-like structures and one caudal cilium. *U. castalia* is 44–67 μm × 39–65 μm in size, and posesses 47–50 somatic kineties, a circumoral corona of 20–25 pairs of kinetosomes, a "brosse" formed by three membranelle-like structures and 5–7 caudal cilia.

The genus *Urotricha*, established in 1859 by Claparède and Lachmann (Corliss 1979) includes small rounded or ovoid ciliates, with an apical oral aperture surrounded by a circumoral infraciliature of pairs of kinetosomes, and uniform somatic ciliature in longitudinal kineties that extend only part way down so that the posterior pole is free of cilia with the exception of one or more caudal cilia. One of the most significant morphological features of the genus is the presence of a "brosse" constituted by membranelle-like structures of two rows of kinetosomes.

The genus includes 20 species, 12 of which are described in Kahl's (1930–35) book of ciliates. Five of them have been redescribed using silver impregnation methods (Gelei 1954, Dragesco et al. 1974, Golière 1977, Foissner 1979 and 1984). Since then, eight new species have been described (Dragesco et al. 1974, Czapik and Jordan 1976, Grolière 1977, Foissner 1983, Alekperov 1984 and Martín-González et al. 1985).

Traditionally, the species of the genus *Urotricha* are placed within the order *Prostomatida*, characterized by the possession of an unspecialized circumoral infraciliature (Corliss 1979), but the study with transmission electron microscopy of de Puytorac and Grain (1972) demonstrates that the circumoral infraciliature differs from the somatic. Also, the study of the

morphogenesis in *U. puytoraci* by Dragesco et al. (1974) and our own observations on the morphogenesis in other species of this genus (unpublished), show that the morphogenesis of *Urotricha* spp. varies with respect to that seen in other prostomatid ciliates in some aspects.

Earlier, this genus was placed in the families *Holophryidae* (Kahl 1935), in the *Prorodontidae* (Corliss 1979) and lately in the family *Plagiocampidae* (Foissner 1983). However, Corliss (1979) considers that it deserves a family of its own, and Small and Lynn (1985) created the new family *Urotrichidae* within the order *Prorodontida*.

Different species of *Urotricha* appear rather frequently in water samples taken from lakes, ponds and streams collected near Madrid, and we have made detailed studies of the infraciliature of the various populations found. As a result, we describe here two new species for this genus: *U. nais* and *U. castalia*.

Materials and Methods

The species of *Urotricha* here described were collected in an artificial pond in "Parque de Berlín" located in Madrid, Spain, during the months of October, 1983 through May, 1984. Large populations of *U. castalia* first appeared, later decreased and then were replaced by abundant populations of *U. nais*.

The samples were enriched in the laboratory with wheat grains or semolina that provide food for the small flagellates that serve as prey for the ciliates.

The morphological features and especially the infraciliature were observed in silver impregnated specimens following the method of Fernández-Galiano (1976).

Results

Urotricha nais[1] sp. n.

This ciliate, oval in shape, varies in size from 16–34 μm long by 12–32 μm wide (measured from fixed specimens). The nuclear apparatus consists of large oval macronucleus of 8–11 μm in length and a spherical micronucleus (Fig. 1).

Silver impregnation demonstrates that the somatic kinetosomes are distributed in 18 to 21 meridional kineties (Pl. I 3) that run from the anterior pole, close to the oral cavity and extend only part way down the cell and leave the posterior third of the cell barren of cilia with the exception of a single long caudal cilium (Pl. I 4). The number of kinetosomes in each kinety is very variable, even in the same specimen, and ranges from 5 to 11 kinetosomes per kinety. The somatic kinetosomes bear a kinetodesmal fiber at its right side that

---

[1] *Nais*: from the greek *naides*, the ones that swim, nymphs of rivers.
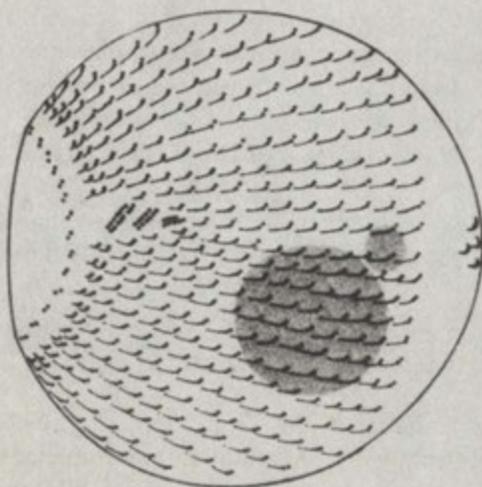
Fig. 2. *Urotricha castalia* sp. n. Diagram showing the somatic and circumoral infraciliature
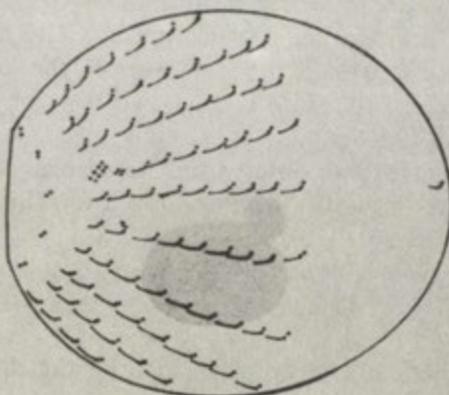

Fig. 1. *Urotricha nais* sp. n. Diagram showing the somatic and circumoral infraciliature

runs towards the anterior kinetosome. Associated with each somatic kineto-some is a single parasomal sac (Pl. I 4).

One of the somatic kineties is shorter than the rest and leaves an anterior space that is occupied by the kinetosomes that form the "brosse". The "brosse" is constituted by two organelles similar in appearance to membranelles formed by obliquely oriented rows of kinetosomes. The upper or first membranelle is formed by two rows of four kinetosomes each, and the second one by two rows of two kinetosomes only. The kinetosomes of the membranelles do not bear kinetodesmal fibers (Pl. I 4).

The oral infraciliature is represented by a circumoral corona of 9–10 pairs of kinetosomes surrounding the anterior orly cavity (Pl. I 4). This species has somatic toxicysts (Pl. I 3).

Urotricha castalia[2] sp. n.

This species, spherical in shape, is 44–67 µm long by 39–65 µm wide. Nuclear apparatus is constituted by a large rounded macronucleus that varies in size from 11–19 µm and an spherical micronucleus of 3.5–4.5 µm in diameter (Fig. 2).

The somatic infraciliature is distributed in 47–50 meridional kineties that leave an aboral zone free of cilia with the exception of a tuft of 5–7 long caudal cilia (Pl. I 6, 7 and 8).

5–7 of this kineties are shorter than the rest and contain 12–15 kinetosomes each, whereas the longer ones have in general from 16–18 kinetosomes each (Pl. I 6 and 7). The somatic kinetosomes bear a kinetodesmal fiber and an associated parasomal sac.

The "brosse" is represented by three membranelles, rectangular in shape, obliquely disposed, that decrease in size posteriorly. The first, or anterior, membranelle is formed by two rows of 5–9 kinetosomes; the second one by two rows of 4–6 kinetosomes, and the third one by two rows of 3–5 kinetosomes (Pl. I 6 and 7).

Surrounding the oral cavity, we observe the circumoral corona formed by 20–25 pairs of kinetosomes obliquely disposed (Pl. I 6). This species presents abundant somatic toxicysts (Pl. I 9).

Discussion

According to our experience, in order to identify the different species of *Urotricha*, it is necessary to consider the various features of the infraciliature as a whole. The attempts to utilize only a single character, like the infraciliature

---

[2] *Castalia*: from the greek *Kastalia*, greek nymph loved by Apollo.

of the membranelles of the "brosse", which G r o l i è r e (1977) considered an specific feature, may lead to erroneus identification.

The characters of the infraciliature that we have taken into account to distinguisch species in the genus *Urotricha* are: (1) number of somatic kineties; (2) number of kinetosomal pairs in the circumoral corona; (3) number, size, position and infraciliature of the membranelles that constitute the "brosse"; (4) number of short kineties behind the "brosse"; (5) number of caudal cilia and (6) number of kinetosomes per kinety. In general we do not weigh any one character more than others, but in some species a few characters can be sufficiently significant or diagnostic by themselves to permit us differentiate one species from others. Other features that should be considered are the morphology of the macronucleus and the presence or absence of toxicysts. In our studies of silver impregnated specimens, only the presence of toxicysts can be taken into account; their absence can not be definitively interpreted as it can depend on the fixation.

Morphological characters as size are also used as diagnostic features but should not either be considered significant by themselves. Size in species of the genus *Urotricha* is generally small and may show important variations (up to 20 μm in organisms of a maximum size of 35 μm).

The species we name here *Urotricha nais* can be easily distinguished from all other *Urotricha* species previously described, mainly because the infraciliature is much reduced with respect to other species of this genus. The number of meridian kineties, the number of kinetosomes per kinety, the presence of a

Table 1

Characterization of the different species of *Urotricha*

	Size μm	Number kineties	Short kineties	Brosse membranelles	Circumoral corona	Nuclear apparatus	Caudal cilia	Toxicysts
U. castalia	44–67 × 39–65	47–50	5–7	3	23–25 pairs of kinetosomes	Mn: spherical 11–19 μm	5–7	present
U. puytoraci Dragesco 1974	50–60	48–51	3	3	26–27 pairs of kinetosomes	Mn: sausage shaped	several	absent
U. sphaerica Grolière 1977	48–55 × 46–51	59–61	2	3	26 pairs of kinetosomes	Mn: spherical	1	—
U. apsheronica Alekperov 1984	75 × 55–60	60	5	3	30–35 pairs of kinetosomes	Mn: spherical 18 μm	18	—

single short kinety and the number of kinetosomal pairs that form the perioral corona are smaller, in general, than in other *Urotricha* species and, more significatively, the "brosse" is also reduced as it is constituted only by two membranelle-like structures. The presence of this reduced "brosse" makes necessary to modify the characterizations of the genus *Urotricha*, which is considered as having a "brosse anterior as three short oblique rows" (Small and Lynn 1985), in order to include *U. nais.*

Urotricha castalia can be distinguished from *U. puytoraci*, described by Dragesco et al. (1974), by its number of short kineties and the number of kinetosomes per kinety (according to Dragesco's images and figures) and also by the shape of the macronucleus and the presence of toxicysts (Pl. I 9). It differs from *U. sphaerica* (Grolière 1977) in the number of somatic kineties, the number of short kineties and the number of caudal cilia. It is also different from *U. apsheronica* (Alekperov 1984) in size, number of somatic kineties, number of kinetosomes of the circumoral corona and in the number of caudal cilia. (See Table 1).

REFERENCES

Alekperov J. X. 1984: New species of ciliates (*Gymnostomata*) from bodies of Azerbaijan (in russian). Zool. Zh., 63, 1417–1418.

Corliss J. O. 1979: The ciliated *Protozoa*. Characterization, Clasification and Guide to the Literature. Second Edition. Pergamon Press, London and New York.

Czapik A. et Jordan A. 1976: Les ciliés psammophiles de la mer Baltique aux envirous de Gdańsk. Acta Protozool., 15, 423–445.

Dingfelder J. H. 1962: Die ciliaten vorübergehender Gewasser. Arch. Protistenk., 105. 509–650.

Dragesco J., Iftode F. et Fryd-Versavel G. 1974: Contribution à la connaisance de quelques ciliés Holotriches Rabdophores; I Protomiens. Protistologica, 10, 59–75.

Fernández-Galiano D. 1976: Silver impregnation of the ciliated protozoa: procedure yielding good results with the silver carbonate method. Trans. Am. Microsc. Soc., 95, 557–560.

Foissner W. 1979: Ökologische und systematische Studien über das Neuston alpiner Klein-gewasser, mit besonderer Berücksichtigung der Ciliaten. Int. Rev. ges. Hydrobiol., 64, 99–140.

Foissner W. 1983: Taxonomische studien über die Ciliaten des Grobglocknergebietes (Hohe Tauver, Österreich). I Familien *Holophryidae, Prorodontidae, Plagiocampidae, Colepidae, Enchelyidae* und *Lacrimariidae* nov. fam. Ann. Naturhist. Mus. Wien, 84/B, 49–85.

Foissner W. 1984: Morphologie und Infraciliatur einiger limnischer Ciliaten (*Protozoa: Ciliophora*). Schweiz. Z. Hydrol., 46/2, 211–223.

Gelei Von J. 1954: Über die Lebensgemeinschaff einiger Temporärer Tümpel auf einer Bergwiese im Borzsönygebirge (Oberungarn). III Ciliaten. Acta Biol., 5, 259–343.

Grolière C. A. 1977: Contribution à l'étude des ciliés des sphaignes et des étendues d'eau acides. I. Description de quelques espèces de Gymnostomés, Hypostomes, Hymenostomes et Heterotriches. Ann. Stn. Biol. Besse-en-Chandesse, 10, 265–297.

Kahl A. 1930-35: Wimpertiere oder Ciliata (Infusoria). In: Die Tierwelt Deutschlands, Dahl F. ed: Jena, G. Fischer.

Martin-González A., Serrano S. and Fernández-Galiano D. 1985: *Urotricha vitrea* n. sp. (*Ciliophora, Prorodontidae*). General morphology and cytological events during the conjugation process. Can. J. Zool., 63, 1885–1891.

Puytorac de P. et Grain J. 1972: Bactéries intramitochondriales et particularités de l'ultra-structure cytostomo-pharyngienne chez le cilié *Urotricha ovata*, Kahl. C. R. Soc. Biol., 166, 604–611.

Small E. B. and Lynn D. H. 1985: Phylum *Ciliophora*. In: An Illustrated Guide to the *Protozoa*. (Lee J. J., Hunter S. H. and Bovee E. C. eds.) Society of Protozoologist, Kansas.

EXPLANATION OF PLATE I

3: Apical view of *Urotricha nais* showing the somatic kineties and oral cavity
4: Side view of *U. nais* showing the somatic and circumoral infraciliature. The kinetodesmal fiber (Kd) can be observed as well as the pairs of kinetosomes of the circumoral corona (CC) and the "brosse" (arrow)
5: Specimen of *U. nais* showing extruded toxicysts (T)
6: *U. castalia*. Apical view showing the somatic kineties, the infraciliature of the circumoral corona (CC) and the three membranelles of the "brosse" (arrow)
7: *U. castalia*. Lateral view showing the somatic kineties covering the body only part way down. Mn — macronucleus, mn — micronucleus and B – "brosse"
8: *U. castalia*. View of naked posterior pole of the cell where the kinetosomes of the caudal cilia can be observed (arrow)
9: *U. castalia* showing the body covered with toxicysts
All images are of silver impregnated specimens

PLATE I
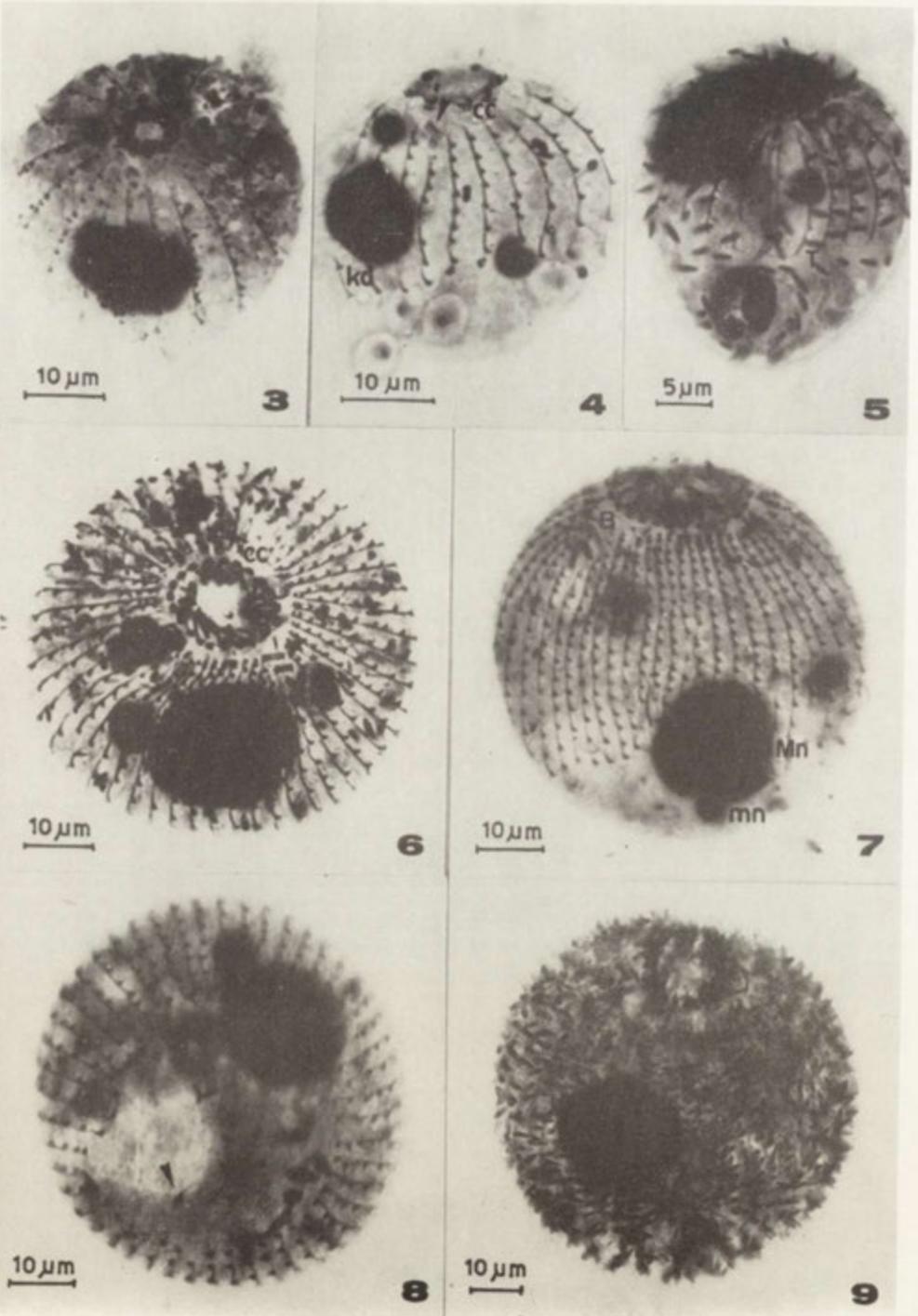
A. Muñoz et al.

auctores phot.

Formation of the Oral Apparatus in the Absence of Macronuclear Anlage Differentiation during Sexual Reproduction in *Paramecium tetraurelia*

Stephen F. NG and Ann NEWMAN

Zoology Department, University of Hong Kong, Hong Kong

Synopsis. By preventing macronuclear anlage differentiation through centrifugation of conjugating *Paramecium tetraurelia*, we have obtained exconjugants lacking macronuclear anlage but possessing functional oral apparatuses of normal appearance. We conclude that the anlage is not essential for the final stomatogenetic stages, which usually take place when macronuclear anlagen begin to differentiate. Exconjugants lacking macronuclear anlagen nevertheless possessed fewer food vacuoles than usual. The stomatogenic implications of these findings are discussed.

During sexual reproduction of *Paramecium*, the pre-existing oral apparatus is resorbed and a new one is produced. The stomatogenic events proceed closely in parallel with the micronuclear division cycle which culminates in the production of new micronuclei and macronuclear anlagen (detailed in Ng and Newman 1984 a). It is now clear that the micronucleus is indispensable for stomatogenesis during sexual reproduction (Ng and Mikami 1981, Ng and Newman 1984 b, reviewed in Ng and Newman 1984 a and Ng 1986). Specifically, the postmeiotic nuclei and zygotic nucleus are strongly implicated in controlling an early step of oral membranelle assembly (Ng and Newman 1984 a, b, Tam and Ng 1986).

The development of the oral apparatus during sexual reproduction starts with the proliferation of an oral field of basal bodies, and thereafter their gradual organization into oral membranelles in the new buccal cavity (stages 1–6, Ng and Newman 1984 a). The elaboration of the mature pattern of the oral membranelles at stage 6, together with the final step involving the formation of the postoral fibres (a group of microtubules intimately associated with the formation of food vacuoles) (Cohen et al. 1984) (stage 7), leading to the production of a functional oral apparatus engaged in feeding (stage 8), take place during the differentiation of macronuclear anlagen after the second postzygotic division (Ng and Newman 1984 a). Using colchicine to interfere

with macronuclear anlage differentiation, we have shown that the anlage is dispensable in the completion of a functional oral apparatus (Ng and Newman 1985). *Paramecium tetraurelia* is thus different from *Euplotes aediculatus,* in which the anlage was shown to be essential for the second round of oral reorganization during conjugation (Kloetzel 1981).

Our previous study employed colchicine to disrupt the mitotic spindles of the second postzygotic division (Ng and Newman 1985). Normally, the second postzygotic spindles extend from the anterior to posterior end of the cell. The two anterior nuclei form micronuclei, while the other two reaching the posterior cytoplasm differentiate into macronuclear anlagen, which develop under the influence of posterior cytoplasmic factors (Mikami 1980, Grandchamp and Beisson 1981). Colchicine effectively disrupted the second postzygotic spindles and produced cells lacking macronuclear anlage, but it also resulted in the formation of abnormal oral structures (in particular, abnormal postoral fibres) and affected feeding (Ng and Newman 1985, Ng unpublished). Centrifugation of conjugants during postzygotic stages has been shown to result in the deviation of the number of new micronuclei and macronuclear anlagen from the normal ratio of 2 : 2, particularly in the reduction of the latter (Sonneborn 1954). In the present study we have employed centrifugation as a means to disturb the position of the second postzygotic nuclei, in order to prevent macronuclear anlage differentiation. This allows us to assess, at the cytological level, whether the oral apparatus developed in the absence of the anlage and also its functioning is normal.

Materials and Methods

Cell and Culture

Paramecium tetraurelia, stock 51, mating types VII and VIII were used. The cells were cultured in cerophyl medium (2.5 g/l; phosphate-buffered at pH around 7) supplemented with stigmasterol (5 mg/l), and bacterized with *Enterobacter aerogenes*. Culture methods and handling followed that of Sonneborn (1950, 1970).

Harvesting Synchronous Conjugants for Centrifugation

The mating cultures were initiated by growing postautogamous cells of opposite mating types in test tubes for 3 days at 27°C. Upon mixing in petri dishes, cells of opposite mating types agglutinated. The mating cultures were fed 1.25 h later with medium to terminate conjugation of loose-pairs and also to prevent further formation of new pairs. Tight-pairs were then collected at the 2nd h after agglutination. About 6 h later, when the mating partners of 50% of the pairs had separated, at the stage corresponding to the second postzygotic metaphase (Ng and Newman

1984 a), they were subjected to centrifugation in a clinical centrifuge for 1 h. The period of centrifugation thus covered the postzygotic stages for most of the cells. Eight centrifugation experiments were performed, at speeds 100 g, 700 g, 1200 g, 1400 g (three), 1500 g and 1600 g, in that order. Only two at 1400 g (designated A and B) yielded cells without macronuclear anlage, whereas the remaining generated various degrees of abnormal distribution of the number of micronuclei and macronuclear anlagen.

Cytology

At about 11 h after agglutination, the centrifuged cells were collected for staining with basic fuchsin and indigo carmine (Butzel 1953, Delamater 1948, Sonneborn 1950), and also for silver impregnation (Chatton and Lwoff 1936, Corliss 1953) with modifications. Basic fuchsin-stained and favorable silver-impregnated samples allowed an assessment of the presence of micronuclei, macronuclear anlagen and food vacuoles. In addition, silver impregnation revealed structural details of the oral apparatus. The cells were studied under × 1000 phase contrast optics.

Statistics

Comparison of two sample means by Student's t-test and the Wilcoxon test, and comparison of a single observation with the mean of a sample were according to Sokal and Rohlf (1969).

Results and Discussion

Formation of the Oral Apparatus in the Absence of Macronuclear Anlage

Of the eight centrifugation experiments, two (A and B) yielded a total of 25 exconjugants lacking macronuclear anlagen (Table 1 a and footnote c).

All 25 cells possessed food vacuoles, and hence functional oral apparatuses. Of the 21 possessing micronuclei but lacking macronuclear anlage from experiment A, 12 were from the silver-impregnated sample. The latter allowed us to determine that their oral apparatuses, especially the oral membranelles and postoral fibres, looked normal. Hence, in the absence of the macronuclear anlage, the exconjugants proceeded to complete the final steps of stomatogenesis, producing normal-looking and functional oral apparatuses.

We believe that the absence of the macronuclear anlage in the exconjugats was a consequence of a disturbance in the distribution of the second postzygotic mitotic nuclei. The two nuclei normally found in the posterior cytoplasm, and hence destined to form the macronuclear anlagen, were instead prevented from doing so because of their abnormal positioning resulting from centrifugation. We rule out the idea of prior development of the macronuclear anlagen and their subsequent extrusion from the cell during centrifugation, for the following reasons: (1). The exconjugant normally possesses two micronuclei

Table 1

Distribution of postautogamous cells in terms of the presence and number of macronuclear anlagen (ma) and micronuclei (mic)

Experiment	(a) Presence (+)/absence (−) of ma and mic in a cell				(b) No. of nuclei (ma+mic) per cell					(c) Total
	ma− mic+	ma− mic−	ma+ mic−	ma+ mic+	4			> 4	< 4	
					2 : 2	x : y	0 : 4			
A	21	1	1	227	170	33	21	16	11	251
B	1	1	0	41	28	4	1	8	2	43

(b) 2 : 2, each cell has 2 mic and 2 ma; x : y, unequal numbers of mic and ma but together making up 4 nuclei in a cell; 0 : 4, each cell has no ma but 4 mic > 4, up to 8 nuclei in one case. < 4, either 2 or 3 nuclei in a cell. (c) In experiment A, 178 cells tallied were fuchsin-stained and 73 were silver-impregnated. In experiment B, 43 cells talls tallied and included in the Table were fuchsin-stained. As to the silver-impregnated sample of B, 52 cells were observed: one possessed 4 micronuclei, food vacuoles but no macronuclear anlage; the remaining 51 cells all possessed macronuclear anlagen but the micronucleus cannot be clearly defined in these. The silver sample of experiment B was thus not included in the Table. Together, experiments A and B yielded 25 cells lacking macronuclear anlage.

and two macronuclear anlagen derived after second postzygotic mitosis. Centrifugation was effective in producing deviations from this typical distribution of nuclei, while maintaining four nuclei per cell (Table 1 b, "x : y"). (2). Of the 23 cells from experiments A and B possessing micronuclei but lacking macronuclear anlage (Table 1 b and footnote c), all of them in fact possessed four micronuclei, corresponding to the normal complement of nuclei after the second postzygotic mitosis. In addition, most of the exconjugants after centrifugation possessed four nuclei each $(170 + 33 + 21/251 = 90\%$ in A; $28 + 4 + 1/43 = 77\%$ in B). It is thus highly unlikely that all of the 23 cells have initially possessed macronuclear anlagen, hence > 4 second postzygotic nuclei, only later to have the anlagen removed from them upon centrifugation. Rather, they must have possessed 4 second postzygotic nuclei all along, but none of these has developed into macronuclear anlage because of the positional disturbance.

Does the Macronuclear Anlage Play any Role at All in Stomatogenesis?

Our present results show that the macronuclear anlage is dispensable for the formation of a functional oral apparatus of normal appearance. This conclusion is consistent with, and extends the previous work based on interference of anlage development by colchicine (Ng and Newman 1985). In this current work, all oral apparatuses presently produced in the absence of macronuclear anlage appeared normal, in contrast to some abnormal ones

formed in the presence of colchicine. Even so, it remains difficult to exclude the possibility that the macronuclear anlage plays some stomatogenic role. Not even ultrastructural investigations would be decisive in this matter. An indirect approach would be to test the function of the oral apparatus, by comparing the numbers of food-vacuoles of exconjugants with and without macronuclear anlage (1). If there were no differences, then (a) obviously the anlage is not playing any role in the construction of a fully functional oral apparatus, but (b) it may still be involved in the development of other oral components having little to do with food vacuole formation. (2). Conversely, if cells lacking macronuclear anlage accumulate fewer food vacuoles, then (a) the anlage may participate in the assembly of oral components involved in feeding, but (b) it is equally possible that the cell's metabolism is slowed down in the absence of anlage nuclear RNA synthesis.

Our scoring of food vacuoles in the two groups of exconjugants in experiment A indicated that a slightly, but significantly smaller number of food vacuoles were present in cells lacking macronuclear anlage but possessing micronuclei, than in centrifuged exconjugants with a normal complement of nuclei, $(32.7 \pm 5.29$ [18, 23–42] vs. 36.6 ± 8.60 [39, 19–55], mean No. of food vacuoles per cell $\pm$ S.D. [sample size, range]; $0.025 > p > 0.01$, one-tailed Student's test; $p = 0.025$, one-tailed Wilcoxon test). This observation therefore does not offer support for the view that the anlage is not playing any role in the construction of a fully functional oral apparatus, but exactly how the absence of the anlage may affect feeding is yet to be investigated.

Stomatogenic Role of the Zygotic and Postzygotic Nuclei

Two exconjugant cells from experiments A and B possessing neither macronuclear anlage nor micronucleus, both from fuchsin-stained samples, are of interest in this regard (Table 1 a). These rare cases were also observed in the previous study with colchicine treatment of conjugants (Ng and Newman 1985, Table 1, footnote c). It can be inferred that these two cells have at some stage lost the zygotic nucleus or the postzygotic mitotic derivatives, for two reasons. Firstly, for 50% of the cells centrifugation began at the 2nd postzygotic metaphase (see Materials and Methods). For a few cells this could have started even earlier, at the late zygotic stage, judging from the degree of asynchrony of conjugation with the present protocol (Ng unpublished, Chau M. F. unpublished). The development of these nuclei might thus be affected, leading to their degeneration.

Secondly, we have reported observations strongly implicating a stomatogenic role of the prezygotic nuclei and/or the zygotic nucleus, particularly in the early steps of oral membranelle assembly (stage 2) (Ng and Mikami 1981, Ng and Newman 1984 a, b, Tam and Ng 1986). In the present study, the

two exconjugants possessing neither macronuclear anlage nor micronucleus could nevertheless possess food vcuoles. Thus it can be inferred that they must have gone through stage 2, but have lost their postzygotic nuclei, or have even earlier lost their late zygotic nuclei. The disapperance of the zygotic nucleus or postzygotic nuclei thus offers an opportunity to assess their involvement in the later stages (3–8) of stomatogenesis.

The two exconjugants possessed substantially fewer food vacuoles (6 and 18) than those with micronuclei and/or macronuclear anlagen $[(36.6 \pm 8.6)$ $(n = 39)]$. These rare cases provide the first indication that the zygotic and postzygotic nuclei may be involved in the later stomatogenic steps. We are currently designing experiments to test this notion.

REFERENCES

Butzel H. M. Jr. 1953: A counterstain for macronuclear anlagen in *Paramecium aurelia*. Microb. Genet. Bull., 7, 33.

Chatton E. and Lwoff A. 1936: Techniques pour l'etude des Protozoaires, specialement de leurs structures superficielles (cinetome et argyrome). Bull. Soc. Fr. Micr., 5, 25–39.

Cohen J., Garreau de Loubresse N. and Beisson J. 1984: Actin mobilization during phagocytosis in *Paramecium*. J. Submicrosc. Cytol., 16, 103–104.

Corliss J. O. 1953: Silver impregnation of ciliated protozoa by the Chatton-Lwoff technic. Stain Technol., 28, 97–100.

Delamater E. D. 1948: Basic fuchsin as a nuclear stain for fungi. Mycologia, 40, 423–429.

Grandchamp S. and Beisson J. 1981: Positional control of nuclear differentiation in *Paramecium*. Dev. Biol., 81, 336–341.

Kloetzel J. A. 1981: Nuclear roles in the postconjugant development of the ciliated protozoa *Euplotes aediculatus*. I. Evidence for sequential roles of the differentiating macronucleus in exconjugant development. Dev. Biol., 83, 20–32.

Mikami K. 1980: Differentiation of somatic and germinal nucleus correlated to intracellular localization in *Paramecium caudatum*. Dev. Biol., 80, 46–55.

Ng S. F. 1986: The somatic function of the micronucleus of ciliated protozoa. Progress in Protistology, 1, 215–286.

Ng S. F. and Mikami K. 1981: Morphogenetic role of the germ nucleus in *Paramecium tetraurelia*. Protistologica, 17, 497–509.

Ng S. F. and Newman A. 1984 a: The role of the micronucleus in stomatogenesis in sexual reproduction of *Paramecium tetraurelia*: micronuclear and stomatogenic events. Protistologica, 20, 43–64.

Ng S. F. and Newman A. 1984 b: The role of the micronucleus in stomatogenesis in sexual reproduction of *Paramecium tetraurelia*: conjugation of amicronucleates. Protistologica, 20, 517–523.

Ng S. F. and Newman A. 1985: The macronuclear anlage does not play an essential role in stomatogenesis in conjugation in *Paramecium tetraurelia*. Protistologica, 21, 391–398.

Sokal R. R. and Rohlf F. J. 1969: Biometry. W. H. Freeman and Co., San Francisco, 776 pp.

Sonneborn T. M. 1950: Methods in the general biology and genetics of *Paramecium aurelia.* J. Exp. Zool. 113, 87–148.

Sonneborn T. M. 1954: Patterns of nucleocytoplasmic integration in *Paramecium.* Caryologia, 6 (Suppl.), 307–325.

Sonneborn T. M. 1970: Methods in *Paramecium* research. Methods in Cell Physiology, 4, 241–339.

Tam L. W. and Ng S. F. 1986: The role of the micronucleus in stomatogenesis in sexual reproduction of *Paramecium tetraurelia*: laser microbeam irradiation of the micronucleus. J. Cell Sci., 86, 287–303.

On Division in a Fresh-Water *Mesodinium acarus*

Henry TAMAR

Department of Life Sciences, Indiana State University, Terre Haute,
Indiana 47809, USA

Synopsis. The later gross cytoplasmic aspects of the homothetogenic fission of two
specimens of a fresh-water form of *Mesodinium acarus* are described. One of these
cells suddenly split into two filial organisms before the constriction of cell division
became apparent. Immediately following fission, all daughter organisms had reduced
anterior portions (cones). A brief description of the observed form of *M. acarus* is
included. An addendum reports *Mesodinium velox* from the East Coast of North
America.

Although species of the genus *Mesodinium* (*Ciliophora*, class *Litostomatea*,
subclass *Haptoria*, order *Haptorida*, family *Mesodiniidae*) have been observed
and studied by many research workers, the life cycles of these organisms
remain poorly known. Penard (1922) saw several specimens of a fresh-water
Mesodinium pulex dividing, and drew one individual in an early stage of cell
division (Fig. 62, right drawing, p. 59). However, the other researchers who
investigated mesodiniums did not find an individual undergoing fission, and
this led baffled protozoologists to hypothesize that perhaps species of *Mesodi-
nium* reproduce only at night (K. Gold, personal communication). Lindholm
(1981) reported observing cell division in a few small and intermediate-sized
cells (forma minor *sensu* Leegaard?) of *Myrionecta* (formerly *Mesodinium*)
rubra, and offered a photograph. However, in a 1985 review Lindholm
indicated that these cells had not been dividing, but had only formed mirror
images (doublets). In the same review, Lindholm (1985) described the earlier
stages of binary fission from new specimens of *Myrionecta* (*Mesodinium*) *rubra*,
and supplied photographs and drawings. Yet, in spite of strenuous efforts, it
was not possible to observe the final stages of fission.

In this paper observations are presented on the later stages of binary fission
in a fresh-water form of *Mesodinium acarus*.

Materials and Methods

On 23 May 1986 water samples were collected in and around Everglades National Park, at the southern tip of the state of Florida, U.S.A.

One of these samples was taken from the fresh-water Tamiami Canal, which flows adjacent to, and immediately north of, the Tamiami Trail (Highway 41). The point of collection lay directly north of the central, Shark Valley portion of Everglades National Park, at 25°45.7′N, 80°44.2′W. At the time and depth of collection the water temperature was 31°C, and Florida elodea (*Hydrilla verticillata* Royle) and algae were included in the sample.

Another sample was drawn inside Everglades National Park from the fresh-water Paurotis Pond, which lies close to Highway 27 northeast of Flamingo, at 25°16.8′N, 80°48.1′W. At the time of collection the water temperature was 29.5°C, and cone-spur bladderwort (*Utricularia gibba* L.), algae and detritus were included in this sample.

Between 23 and 27 May the Paurotis Pond sample jar was kept in a cooler at predominantly 24–27°C, whereas the Tamiami Canal jar had higher temperatures. On 27 May both jars were brought to the author's laboratory and placed in an incubator which provided continuous fluorescent illumination and normally was maintained at 27–29°C. However, the incubator malfunctioned during the night of 30–31 May, and the temperature in the jars reached 32°C. The jars were occasionally aerated. Small quantities of skimmed milk powder were added to the Tamiami Canal jar on 31 May, 3 June and 16 June, and to the Paurotis Pond jar on 31 May and 13 June, to stimulate increases in the numbers of any *Halteria* species present.

On 17 June 1986, 0.15–0.3 ml water from the Tamiami Canal jar were placed on a slide and examined with a stereomicroscope equipped with a 2X auxiliary lens. Several mesodiniums were found, captured with a micropipette, and transferred in a drop of water to a second slide. A few pieces of thin thread now were placed around the drop, and a coverglass was applied. When this preparation was examined by dark phase-contrast microscopy at 500X, a dividing specimen of the to-be-described fresh-water form of *Mesodinium acarus* was seen, and could be studied.

On 18 June another dividing individual of the same form was found in water from the Paurotis Pond jar. It was studied by similar means.

Both dividing specimens were photographed with a microflash device, using the technique given in Tamar (1986 a).

Results

Attributes of the Organism

The form in which cell division was observed, like all mesodiniums, was divided into an anterior portion, the cone, and a posterior portion, the flask, by a constriction at which the cirri and membranelles originated (Pl. I 4). The broad cone usually widened a bit from the constriction to create proximal bulges, and then tapered to a typically wide and flat anterior end. The cirri were bifurcated distally for about half of their lengths, the posterior branch of the bifurcation being the shorter one (Pl. I 4), and had no barbs. Some specimens yielded counts of as many as 21 cirri. In individuals at rest the cirri formed 2 circlets. The membranelles clearly extended beyond the posterior end of the cell body in some specimens (Pl. I 4), but did not seem to extend that far

in most individuals. There appeared to be 8 tentacular processes. Two contractile vacuoles, one on each side, were located in the basal portion, or near the middle of the anterior-posterior axis, of the cone. Almost all of the specimens were active continuously. They most often crawled or darted forward and then hesitated (temporarily stopped moving) before jumping or darting backward. Both longer and shorter rapid forward and backward excursions were seen.

Preliminary measurements of the *Mesodinium* form were made by the Negative — Magnifier Method (Tamar 1986 a). The body length of 14 specimens varied between about 12 and 20 μm. The greatest body width of 16 specimens ranged from about 8 to, at the most, 15 μm. The lengths of the cirri of 16 specimens were at least 9 μm, and reached up to about 12 μm.

The organism in question was identified as a fresh-water form of *Mesodinium acarus* on the following three main bases: (1). The fact that the cone tapered toward its anterior end without having a distal cylindrical portion or a distal widening (the last 2 features are characteristics of *Mesodinium pulex*). (2). That the specimens showed continuous activity (this activity also resembled in its nature that of the marine *M. acarus*) and (3). The relatively small size of the organism. The obtained measurements and most of the observed characteristics of this fresh-water form of *M. acarus* indicate that it is quite similar to the marine *M. acarus* (see Tamar 1977).

Fission

Tamiami Canal Sample

The cell division of the specimen of a fresh-water *Mesodinium acarus* obtained from the Tamiami Canal jar on 17 June was observed shortly after midday, between 12:15 and 12:30 P.M. When first examined, the specimen was elongated, but the constriction of cell division was not seen. There were both an anterior group of cirri, those of the proter, and a posterior group of cirri, those of the opisthe, the last cirri apparently having lengths equal to those of the first. Anterior membranelles, immediately behind the anterior cirri, were also discerned. The cone was flattened (abnormally short and lacking visible distal structure). A transverse constriction now appeared at approximately the middle of the individual, and after this the division of the body into two cells by transverse (homothetogenic) binary fission proceeded at a very rapid rate. The room temperature at this time was 29–30°C.

The individual was photographed during cell division (Pl. I 6, 7), and in the pictures the posterior membranelles are visible.

Immediately after cell division had been completed (Pl. I 8), each of the two filial specimens had a flattened cone.

During the process of cell division the above specimen was able to move about at speeds comparable to those of other, interphase specimens. However, before the constriction of cell division appeared, it had been inactive for much of the time while presenting polar views.
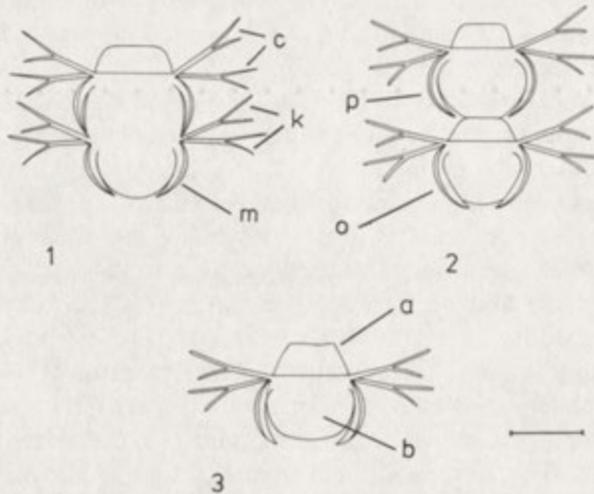

Fig. 1–3. Drawings of a fresh–water form of *Mesodinium acarus* during and after cell division. 1 — 18 June specimen shortly before it split into two filial cells. 2 — 17 June specimen during cell fission. 3 — Proter descended from the 17 June specimen, immediately after its fission from the opisthe. a — cone, b — flask, c — cirri of proter, k — cirri of opisthe, m — membranelles of opisthe, o — opisthe, p — proter. Scale bar represents 10 μm

Paurotis Pond Sample

The cell division of the specimen obtained from the Paurotis Pond jar on 18 June was observed in the afternoon, at about 4:25 P.M. This individual was moving about at normal rates when first seen, and was immediately photo-graphed (Pl. I 5) while in motion. As in the case of the 17 June specimen, the body was elongated and the cone was flattened, being abnormally short and lacking visible distal differentiation of structure and form.

The specimen now remained in place under the coverglass in a sideways position. As in the 17 June specimen, an anterior group of cirri, anterior membranelles, and a posterior group of cirri could be identified (also, as in the 17 June cell, the lengths of the posterior cirri appeared to approximate those of the anterior cirri). However, the posterior membranelles visible in Pl. I 5 were not seen directly. While the specimen remained in place as described, both anterior cirri and posterior cirri were individually moved anteriorly.

The constriction of cell division had not yet become visible when the immobile specimen, with a sudden motion, split or jerked apart into two new cells! This occurred within a minute or two after the organism had ceased swimming about. During this time the room temperature was 24–25°C.

Immediately after the sudden splitting of the specimen, the two resulting daughter cells each had a flattened cone. Soon the cones of both new specimens increased in length, and then tentacular processes appeared at the anterior ends of both specimens (see Pl. I 4). The two filial organisms thus were developing the normal shape of the adult specimens.

Discussion

The related findings indicate that, during reproduction of the observed fresh-water form of *Mesodinium acarus*, fission (*sensu stricto*) of the cell body takes place within a very short time span, and that a sudden splitting of the cell body can occur. The quick separation of the 18 June specimen into two filial products seems to be unique among ciliates, and the genus *Mesodinium* may offer an opportunity to investigate the basis for such extremely rapid cytokinesis.

The rapid cell fission met in the investigated *Mesodinium* form provides a possible partial explanation for the dearth of observations of cell division in this genus. Lindholm (1985) hypothesized that, in the presumably closely-related *Myrionecta* (*Mesodinium*) *rubra*, cell fission might occur by a very rapid splitting or "cytosplitting" of the cell body. The present observations lend support to this hypothesis. Lindholm (1985) also noted that the strong vacuolization in the central portion of *Myrionecta rubra* might be an adaptation for rapid fission.

The reduced state of the cones of the filial organisms immediately following cell fission, and their subsequent increase in length to assume the shape of the adult cone, suggest that stomatogenesis (oral replacement?) may occur after fission.

Cell division in the genus *Mesodinium* patently requires further study. More observations are needed of the fission of the cell body, both within a fluid volume and on substrates. Further, nothing is known of stomatogenesis, or of the nuclear changes during binary fission, in mesodiniums. The nuclear processes could be interesting, since normally one cannot see nuclear material in living mesodiniums with dark phase-contrast microscopy, and the nuclear matter of *Mesodinium pulex* differs from that of other types of Protozoa in not staining well with the DNA-specific fluorochrome DAPI (4', 6-diamidino--2-phenylindole) (E. Sherr, personal communication).

ADDENDUM

In the course of the collecting trip during which the samples described previously in this paper were gathered, at least one specimen of *Mesodinium velox* Tamar, 1986 (Tamar 1986 b) was also obtained. This specimen was found in a sample of sea water containing sea grass, algae, detritus

and sand which had been collected from a sand bar off the harbor of Flamingo (25°08.2'N, 80°56.7'W) in Everglades National Park on 23 May 1986. At the time of collection, in the morning near low tide, the water at the collection site had a depth of under 15 cm and a temperature of about 28°C.

The specimen of *M. velox*, which was photographed, had 17 cirri, typical for this species at Santa Catalina Island near Los Angeles. Its cell body and cirri did not exceed the previously determined size range. The presence of this specimen in the above-described sample shows that *M. velox* exists not only on the Pacific Coast, but also, at least in shallow water, on the East Coast, of North America.

ACKNOWLEDGEMENTS

REFERENCES

Lindholm T. 1981: On the ecology of *Mesodinium rubrum* (Lohmann) (Ciliata) in a stagnant brackish basin on Åland, SW Finland. Kiel. Meeresforsch., Sonderh. 5, 117–123.

Lindholm T. 1985: *Mesodinium rubrum* — a unique photosynthetic ciliate. In: Advances in Aquatic Microbiology (eds. Jannasch H. W. and LE B. Williams P. J.), Academic Press, London, 3, 1–48.

Penard E. 1922: Études sur les Infusoires d'Eau Douce. Georg. et Cie, Genève.

Tamar H. 1977: Three marine *Mesodinium* species (*Didiniidae*). In: Abstracts, Vth Int. Congr. Protozool. (ed. Hutner S. H.), New York, p. 336.

Tamar H. 1986 a: Several photomicrographic methods for measuring ciliates. Acta Protozool., 25, 123–129.

Tamar H. 1986 b: Four marine species of *Mesodinium* (*Ciliophora: Didiniidae*). I. *Mesodinium velox* n. sp. Trans. Am. Microsc. Soc., 105, 130–140.

EXPLANATION OF PLATE I

4–8: A fresh-water form of *Mesodinium acarus*
4: Typical adult, interphase specimen
5: 18 June specimen before it split apart, and while still in movement
6–8: 17 June specimen
6: Shortly after the appearance of the constriction of cell division
7: A few moments later. The constriction has progressed
8: Immediately after separation of the filial products. a — cone, b — flask, c — cirri of proter, k — cirri of opisthe, m — membranelles of opisthe, o — opisthe, p — proter. Scale bars represent 10 μm
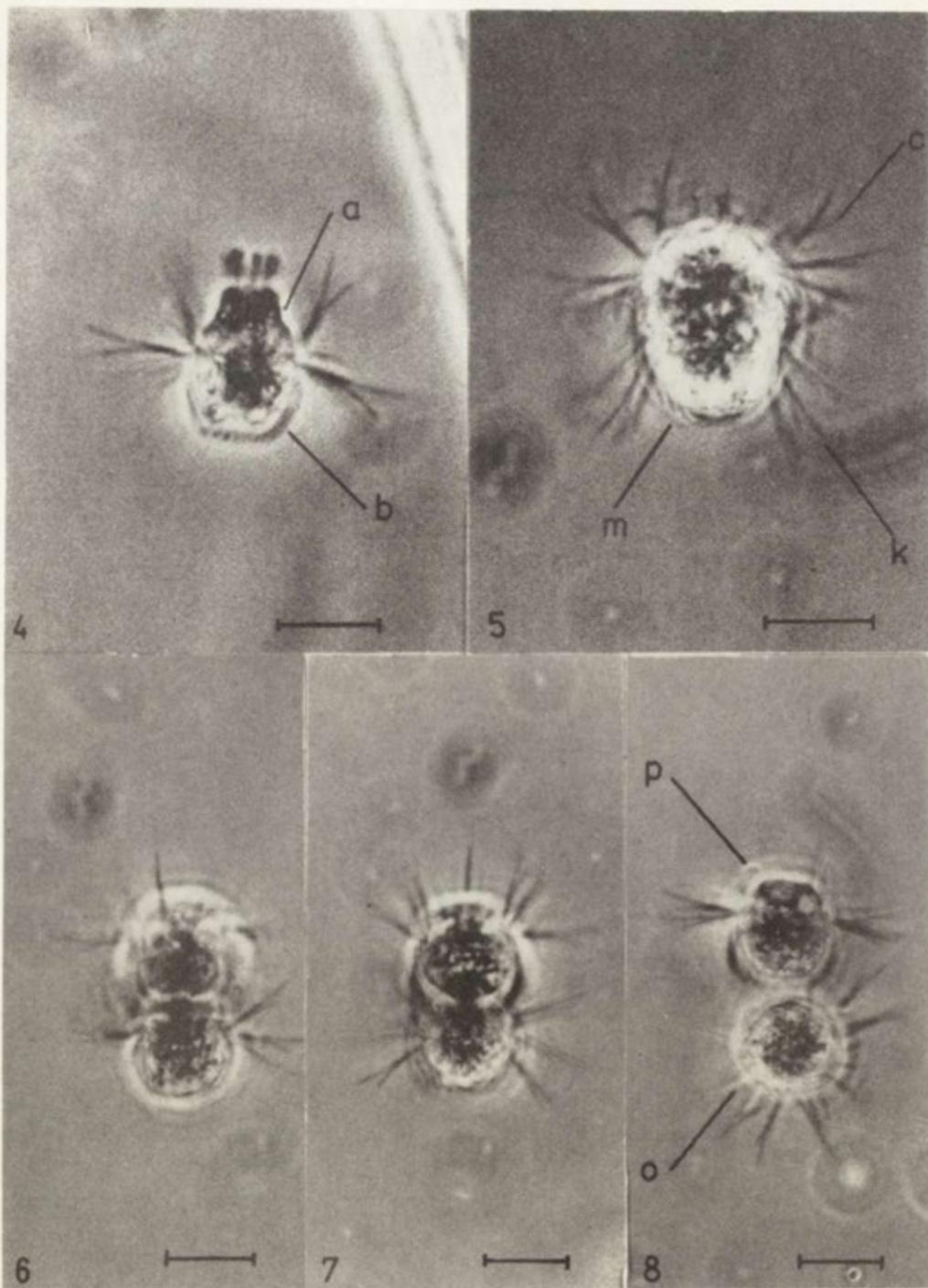
Magnetic Orientation in *Paramecium caudatum*

Irwin R. ISQUITH and Janice SWENSON

Department of Biological Sciences, Fairleigh Dickinson University,
Teaneck, NJ 07666 and Department of Biology, King's College, Wilksboro, PA 18711, USA

Synopsis. Paramecium caudatum was shown to demostrate altered orientational behavior when subjected to high intensity magnetic fields. Although the organisms continue to swim back and forth between the north and south poles of the experimental apparatus, they do spend a greater amount of time at the south.

Numerous organisms, including such diverse forms as bacteria and vertebrates, have been shown to have the direction of their locomotion affected by magnetic fields (i. e., display magnetotaxis). Protists shown to be included in this group are *Paramecium caudatum* (Brown 1962, Kogan and Tikhonova 1965) and Volvox (Palmer 1963). The discovery of magnetite particles in magnetotactic bacteria by Blakemore (1975) helps explain the phenomenon and has stimulated interest in it (Frankel et al. 1979). The book, Magnetite Biomineralization and Magnetoreception in Organisms, (Kirschvink et al. 1985) contains an excellent series of articles describing the current research in the area. The purpose of this study was to confirm and further elucidate magnetically altered bahavior in *Paramecium caudatum*.

Materials and Methods

The movement of *Paramecium caudatum* populations was observed in a channel, 27 mm × 3.5 mm × 1 mm, placed on the stage of a Wild stereoscope. The channel was delineated into three sections each 9 mm long. Permanent magnets were then oriented to the channel producing nine different magnetic orientations in the channel, as shown in Fig. 1; the configurations are defined in Table 1. Magnetic measurements were made with a gaussmeter (Model 505, RFL Industries, Boonton, NJ).

The protozoa used were all laboratory cultures initially received from Connecticut Valley Biological Supply Co. They were maintained in 0.1% Cerophyl at 18–21°C, in the dark. At the

beginning of an experiment, cells were individually picked and placed into a sterile Cerophyl solution; after 10 min 10–25 of these organisms were again individually picked and placed into the channel for experimentation. The medium in the channel was also 0.1% Cerophyl.

The data consist of the number of organisms in each third of the channel at one minute intervals. Three different conditions were studied: (1) before a magnet was in place ("pre-magnetic"),
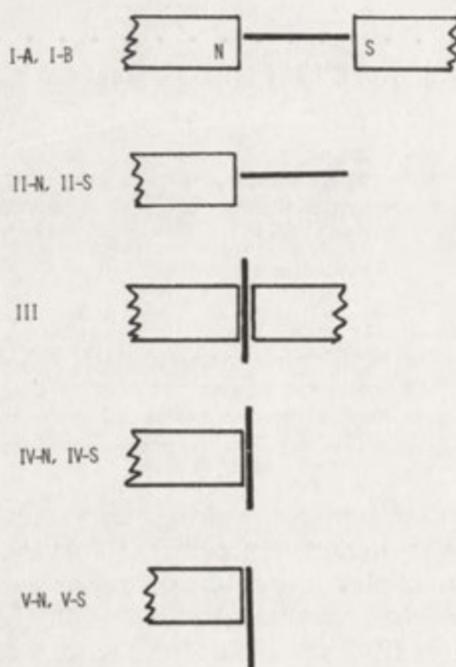

Table 1

Magnetic strengths in the experimental apparatus.
Figure 1 illustrates the orientations

Orientation	Poles	Field strength (Gauss) in Channel		
		Polar End (S)	Middle	Non-polar End (S)
I-A	N and S	2400	1600	—
I-B	N and S	340	130	—
II-N	N	275	70	30
II-S	S	260	65	25
III	N and S	—	185	100
IV-N	N	—	100	75
IV-S	S	—	100	75
V-N	N	100	80	40
V-S	S	100	76	40

(2) while a magnet was in place ("magnetic") and (3) after a magnet was removed ("post-magnetic"). Each group of organisms that was placed in the channel was used to gather 4–9 "pre-magnetic" readings, 9–12 "magnetic", and 4–9 "post-magnetic". All the readings for each condition under a given magnetic orientation were pooled.

Results

Table 2 contains the data representing the total number of organisms in each third of the channel under each of the nine experimental orientations for "pre-magnetic" and "magnetic" conditions. Table 3 contains analogous data for "pre-magnetic" and "post-magnetic" conditions. In all the cases, organisms

Table 2

Comparison of Premagnetic and Magnetic Distributions

Orien-tation	Condition								
	Magnetic				Premagnetic				
	Total	"N" (or left)	"M"	"S" (or right)	Total	"N" (or left)	"M"	"S" (or right)	P
Ia	8928	2774 31.1%	2717 30.4%	3437 38.5%	4065	1410 34.7%	1211 29.8%	1444 35.5%	> 0.001
Ib	4322	1247 28.8%	1300 30.1%	1775 41.1%	2111	753 35.7%	640 30.3%	718 34.0%	> 0.001
II-N	2585	829 32.1%	870 33.6%	886 34.3%	1740	615 35.3%	515 29.6%	610 35.0%	> 0.001
II-S	2998	914 30.5%	897 29.9%	1187 39.6%	1893	661 34.9%	567 30.0%	665 35.1%	> 0.001
III	2203	777 35.3%	649 29.4%	777 35.3%	1220	425 34.8%	370 30.3%	425 34.8%	0.5–0.7
IV-N	3335	1223 36.7%	906 27.2%	1206 36.2%	1872	644 34.4%	589 31.5%	639 34.1%	> 0.001
IV-S	2312	735 31.8%	782 33.8%	795 34.4%	1442	506 35.1%	459 31.8%	477 33.1%	> 0.001
V-N	5513	1881 34.1%	1633 29.6%	1999 36.2%	2617	929 35.5%	803 30.7%	885 33.8%	> 0.005
V-S	4016	1416 35.2%	1119 27.9%	1481 36.9%	2200	788 34.3%	733 31.9%	779 33.9%	> 0.001

Each number represents the number of organisms found under the stated condition, cummulatively in all series of experiments. "N" — third of the channel closest to the magnet's north pole; "S" is equivalent for the south pole, and "M" is the middle third of the channel. For conditions III, IV-N, and IV-S, when the magnet (s) were perpendicular to the middle of the channel, "N" — left third of the channel; "S" — right third of the channel, and "M" = the middle third. Eight orientations show a significant distributional difference between the magnetic and pre-magnetic conditions. The one condition that did not produce a significant difference was III

I. R. ISQUITH AND J. SWENSON

Table 3

Comparison of Premagnetic and Postmagnetic Distributions

Orientation	Total	Condition						
		Premagnetic			Postmagnetic			
		"N" (or left)	"M"	"S" (or right)	Total	"N" (or left)	"M"	"S" (or right)
Ia	4065	1410 34.7%	1211 29.8%	1444 35.5%	4151	1430 34.5%	1256 30.2%	1465 35.3%
Ib	2111	753 35.7%	640 30.3%	718 34.0%	2089	737 35.3%	647 31.0%	705 33.7%
II-N	1740	615 35.3%	515 29.6%	610 35.0%	1670	545 32.6%	517 31.0%	608 36.4%
II-S	1893	661 34.9%	567 30.0%	665 35.1%	1830	633 34.6%	570 31.1%	627 34.3%
III	1220	425 34.8%	370 30.3%	425 34.8%	1058	352 33.3%	308 29.1%	398 37.6%
IV-N	1872	644 34.4%	589 31.5%	639 34.1%	1657	561 33.8%	523 31.6%	573 34.6%
IV-S	1442	506 35.1%	459 31.8%	477 33.1%	1310	437 33.4%	406 31.0%	467 35.6%
V-N	2617	929 35.5%	803 30.7%	885 33.8%	2515	883 35.1%	796 31.6%	836 33.2%
V-S	2200	788 35.8%	633 28.8%	779 35.4%	1985	707 35.6%	562 28.3%	716 36.1%

Each number represents the number of organisms found under the stated condition, cummulatively in all series of experiments. The letter designations are based upon magnetic condition designations. "N" — third of the channel closest to the magnet's north pole; "S" is equivalent for the south pole, and "M" is the middle third of the channel. For conditions III, IV-N, and IV-S, when the magnet(s) were perpendicular to the middle of the channel, "N" — left third of the channel; "S" — right third of the channel, and "M" — the middle third. No significant differences in distribution were found between the premagnetic and postmagnetic conditions for any field type.

continue to swim back and forth along the length of the channel. The data from the "magnetic condition" and the data from the "post-magnetic" condition are compared to the "pre-magnetic" data using Chi square analysis. The data clearly indicate that in eight of the nine experimental orientations, the organisms distribute themselves differently during the "magnetic" condition than during the "pre-magnetic" condition. However, in none of the nine orientations is there a significant difference between the "pre-" and "post-magnetic" conditions. The case where there is no significant difference among any of the three conditions is when both the north and south poles are adjacent to the center third of the channel (Orientation III).

Discussion

It is obvious from these data that the movement *Paramecium caudatum* is influenced by magnetic fields. In orientations Ia, Ib, IIN and IIS, where the magnetic field is parallel to the channel, the organisms clearly spend more time in the third of the channel closer to the south the pole of the magnet and less in the third near the north. In the conditions where the magnets are perpendicular to the channel, the results are not as clear, but there are definite patterns: when the magnet's N pole is perpendicular to the middle of the channel (IV-N), cells tend to collect at both ends of the channel compared to "pre-magnetic" condition; when the N pole is perpendicular to an end of the channel (V-N), again there are fewer cells near the magnet, with the cell number increasing at the end of the channel furthest from the magnet. The results for these two orientations when the magnets are reversed (i.e., the south pole is used) i.e., in condition IV-S, cells show a slight tendency to collect at the middle of the channel and in V-S there is a slight increase at the end near the south pole. In all eight of these conditions (four parallel and four perpendicular) the generalization can be made that these organisms "prefer" the south pole over the north pole.

The one orientation (III) in which no statistical difference between the "magnetic" and "pre-magnetic" condition is found does not offer the organism a choice between a north and south pole. The field is perpendicular to the middle of the channel; the paramecia are swimming up and down a magnetic gradient, but are not presented with a choice of north versus south. The results for this condition therefore do not contradict the conclusions that paramecia "prefer" the south. This is the same conclusion that K o g a n and T i k h o - n o v a (1965) reached.

The comparison of the pre-magnetic and post-magnetic conditions clearly indicate that there is no after-effect. This would tend to indicate that the magnetotatic response is not due to an alteration in the chemical environment, which would most likely last for some time after the magnets have been removed. This observation is in disagreement with the work of K o g a n and T i k h o n o v a (1965). It is more reasonable to assume that the organisms are responding directly to the magnetic field, perhaps in a manner similar to magnetotatic bacteria.

The magnetic response of these organisms is a very weak one, in that the organisms do not collect and remain in any region of the channel, but continue to swim back and forth. This could possibly indicate that the observed data are being produced by altered swimming velocities. If the organisms swim slower in the region near the south pole and more rapidly in the region near the north pole, a larger number of the organisms would be found in the region near the south. If this were the case, the phenomenon observed here would be a case of

magnetokinesis and not magnetotaxis. Using a videotape analysis, however, it was not possible to detect any significant difference in swimming velocities.

The subtlety of this response would cause one to doubt that it is a major orienting force for these organisms in nature. Response to environmental factors such as water currents, chemical gradients and temperature certainly would innundate any magnetotactic response.

REFERENCES

Blakemore R. P. 1975: Magnetotatic Bacteria. Science, 190, 377–379.
Brown F. A., Jr. 1962: Responses of the planarian, *Dugesia* and the protozoan, *Paramecium* to very weak horizontal magnetic fields. Biol. Bull., 123, 264–281.
Frankel R. B., Blakemore R. P. and Wolfe R. S. 1979: Magnetite in Freshwater Magnetotatic Bacteria. Science, 203, 1355–1356.
Kirschvink J. L., Jones D. S. and MacFadden B. J. (eds.) 1985: Magnetite Biomineralization and Magnetoreception in Organisms. Plenum Press, New York.
Kogan A. B. and Tikhonova N. A. 1965: Effect of a constant magnetic field on the movement of *Paramecia*. Biofizika, 10, 292–296.
Palmer J. D. 1963: Organismic spatial orientation in very weak magnetic fields. Nature, 198, 1061–1062.

Effect of External Agents on Cytoplasmic Streaming in *Paramecium*. IV. Starvation in Mineral Medium [1]

Jerzy SIKORA and Anna WASIK

Department of Cell Biology, M. Nencki Institute of Experimental Biology,
3 Pasteur Str., 02-093 Warszawa, Poland

Synopsis. Starvation of aposymbiotic *Paramecium bursaria* leads to decline of cytoplasmic streaming velocity and of food vacuoles formation rate. The gradual decrease of endocytosis rate during starvation is supposed to be dependent on the cell's diminished reception ability. Since the velocity of cytoplasmic streaming is also lowered during starvation it makes us more confident about the occurrence of receptors in the *Paramecium bursaria* cell surface which are involved in triggering two physiologically related processes: endocytosis and intracellular transportation system.

It is known that *Paramecium* cells are extremely susceptible to the variation of their surrounding medium. Since the dependence of the velocity of cytoplasmic streaming within the *Paramecium* on temperature (Kuźnicki and Sikora 1973), life cycle phase (Sikora and Kuźnicki 1975, 1976) and the presence of particulate suspension in the medium (Wasik 1983, Wasik and Sikora 1981, 1984 a, b, Wasik et al. 1984) have been described, these ciliates became a useful subject for the investigation of the motile phenomena within the protozoan cells, especially those which seem to be not involved in locomotion (Sikora 1981). So far the only approach which enables the evaluation of intracellular motile activity *in vivo* is the measuring of the velocity of cytoplasmic streaming (Sikora et al. 1979, Sikora 1981).

In the previous papers (Wasik 1983, Wasik and Sikora 1984 a) we presented data showing a distinct effect of particulate material on the rate of food vacuoles formation and on the cytoplasmic streaming velocity in *Paramecium bursaria*. It was found that both endocytosis and the motility of cytoplasm are in some limits dose-dependent on solid particles within the

surrounding medium. The response of paramecia is not simply proportional to the concentration of particulate material but depends also on its nature (Wasik et al. 1987). Aposymbiotic *Paramecium bursaria* deprived of the capacity to form food vacuoles, remains susceptible to mechanical stimuli. Upon an increase of concentration of carmine particles in the medium, a substantial enhancement of cytoplasmic streaming velocity has been noticed (Wasik and Sikora 1984 b). This finding leads to the conclusion that the mechanism responsible for the propulsion of cytoplasm in *Paramecium bursaria* might be regulated by external stimuli. Although distinct changes in the velocity of cytoplasmic streaming evoked by solid particles, like carmine or latex spheres in the medium, have been stated, the records are often dispersed and inconsistent. Searching for the source of data variability, we ask in the present paper whether the duration of starvation of paramecia in a mineral maintenance solution prior the experiment would affect the cytoplasmic streaming velocity, as it affects endocytosis (Wasik et al. 1987).

Materials and Methods

Paramecium bursaria devoid of symbiotic *Chlorellae* were obtained from pure culture maintained in the laboratory. Cells were grown in darkness in the Scottish grass infusion inoculated with *Klebsiella aerogenes*, at room temperature $19 \pm 2°C$, as previously described by Sonneborn (1970). Owing to these conditions it was possible to reduce to a great extent the number of symbiotic *Chlorellae* and eventually eliminate them, in order to avoid disturbances in measuring the motility of cytoplasm within the host cell. When the culture reached an early stationary phase, cells were pelleted from growth medium at $300 \times g$ for 1.5 min and washed twice in the maintenance solution (Wasik et al. 1987). Paramecia resuspended in the maintenance solution at the initial concentration of 1000 cells ml^{-1} were starved for 1, 2, 3, 12 and 24 h. After the appropriate time of starvation, samples of cells were fed with 1.04 μm in diameter dyed-latex-microspheres (Polyscience) used in final concentration 10^8 particles ml^{-1}, for 3 min and then treated with the solution of $NiCl_2$ to immobilize *Paramecium* cells (Sikora and Wasik 1978, Sikora 1981). After 10 min incubation, the cells were gently pelleted at the bottom of depression slide, washed with the maintenance solution and transferred in a small drop on a cover glass and mounted by means of pure vaseline on the microscope slide to prevent evaporation and squeezing of ciliates since that impairs cytoplasmic streaming. The velocity of cytoplasmic streaming was estimated under bright field microscope (Sikora et al. 1979, Sikora 1981). In order to determine the endocytosis rate, paramecia, after being fed for 3 min with latex spheres, were fixed in 1.5% glutaraldehyde and the number of food vacuoles counted under phase interference-contrast microscope. In addition to the protocols mentioned above, we considered the phagocytosis rate as a good control of the physiological state of the *Paramecium bursaria* cultures.

Results

Although *Paramecium bursaria* easily survived the 24 h incubation in the maintenance solution, there was no clear evidence whether the cytoplasmic

streaming velocity is affected after a prolonged starvation. During 30 min routine observation time for estimation of the velocity of cytoplasm any distinguishable changes have been noticed (Sikora unpublished). As it is shown in Fig. 1 the routinely used concentration of the latex spheres has evoked only small changes of the velocity of cytoplasmic streaming in paramecia starved during the first 3 h. The shift towards significant (Student's t-test at $P > 0.95$) retardation of velocity is seen after 12 h of starvation, while after 24 h the mean velocity decreased to 72.8% of its initial value (Table 1). The number of food vacuoles formed in paramecia starved during analogous duration showed a
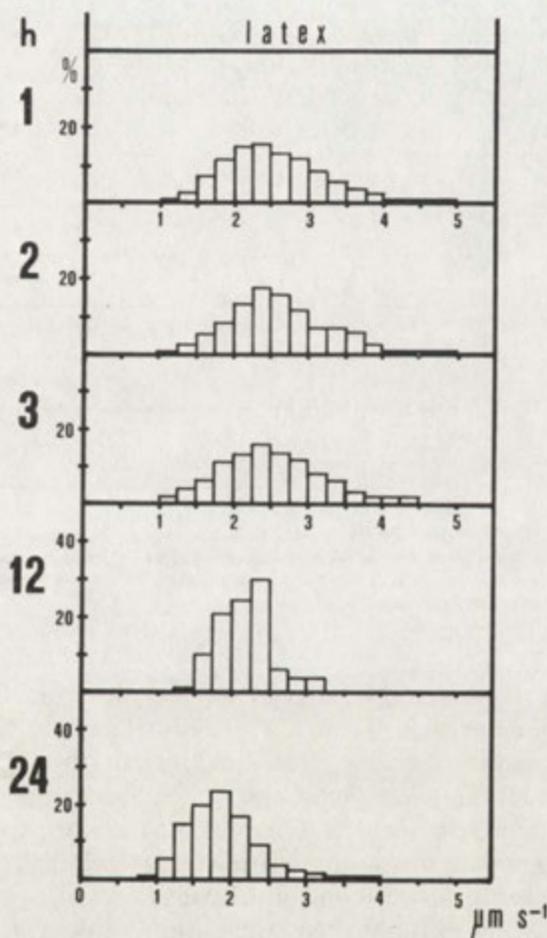
Fig. 1. Dependence of cytoplasmic streaming velocity in aposymbiotic *Paramecium bursaria* on duration of starvation in mineral maintenance solution. Histograms are expressed in percentage of cells showing matched velocities of stated times after 3 min feeding with dyed-latex-microspheres and immobilized by means of $NiCl_2$ solution

more distinct decrease (Table 1, Fig. 2). After 24 h over 60% of the ciliates did not form food vacuoles during the first 3 min when exposed to the latex spheres. An average number of food vacuoles formed was lowered almost 8 times in comparison with records obtained after starvation during the first 3 h. Linear regression coefficient between the time of starvation and cytoplasmic

Table 1

Endocytosis and cytoplasmic streaming rate in *Paramecium bursaria* cells according to the stated duration of starvation

Duration of starvation time in hours	Mean number of food vacuoles* formed	Mean value of cytoplasmic streaming velocity*** in $\mu m\,s^{-1}$
1	9.32 ± 3.69	2.57 ± 0.52
2	11.01 ± 3.90	2.70 ± 0.59
3	11.72 ± 3.94	2.47 ± 0.59
12	6.31 ± 3.47	2.18 ± 0.38
24	$2.10**$	1.87 ± 0.32
Regression coefficient against time	-0.95	-0.97

* Cells exposed to dyed-latex-microspheres for 3 min and fixed in glutaraldehyde
** Approximated mean, since in this case frequency distribution has expotential character
*** Measured after 3 min feeding with dyed-latex-microspheres and followed by immobilization by means of NiCl₂ solution

streaming velocity as well as the rate of food vacuoles formation were of the same high order of magnitude (Table 1, Fig. 2). Interestingly, the paramecia in the course of starvation became more uniform in their response to the stimulation of cytoplasmic streaming and endocytosis (Fig. 1, Table 1).

It should be pointed out that the data presented in this paper are expressed in terms of the number of vacuoles per average cell and the velocity of randomly chosen small (up to 3 μm in diameter) crystals — the markers of cytoplasmic motility, and that there were quite wide variations between individual cells. At one extreme there were cells which have not formed any food vacuoles during 3 min feeding affected by experimental conditions or influenced by internal factors. On the other hand, cells which formed unusually large number of food vacuoles also may appear in the same conditions.
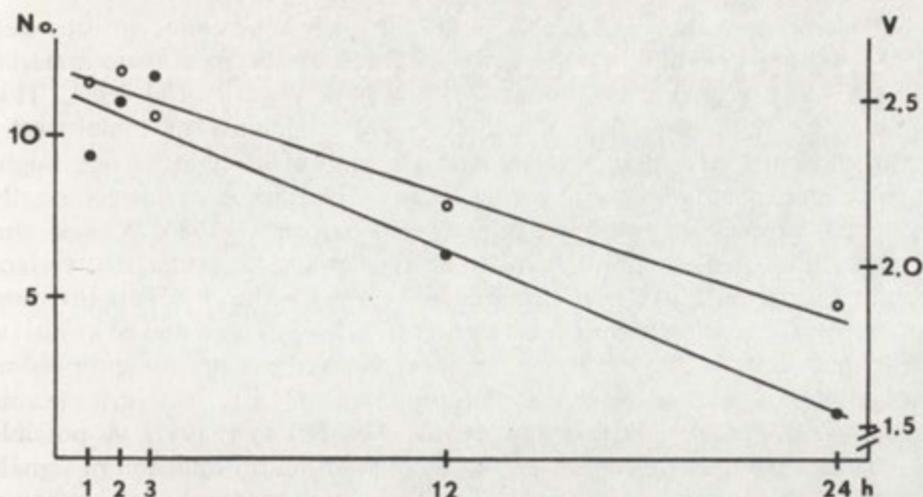
Fig. 2. Comparison of linear regression of food vacuoles formation rate (closed circles) and cytoplasmic streaming velocity (open circles) against time of starvation of aposymbiotic *Paramecium bursaria*

Variations concern the values of cytoplasmic streaming velocity as well. Thus the presented results have to be considered as an expression of values to which the majority of cells conform.

Discussion

Starvation might be regarded as a normal physiological condition to which most cells may be exposed during shorter or longer periods of their life. Free living ciliates, frequently used in studies on food uptake, seem to be suitable subjects to follow the effect of starvation on the phagocytotic activity and related processes.

The particulate matter is usually present in the surrounding medium of paramecia and has a pronounced influence on the rate of food vacuoles formation as well as on cytoplasmic streaming velocity (Wasik 1983, Wasik et al. 1984 a, b, Wasik et al. 1984). Thus the purpose of the present paper was to reveal to what extent the cytoplasmic motile activity in *Paramecium bursaria* correlates with the ability of the ciliate to form food vacuoles after different period of starvation. Additionaly that would be a methodically valuable information since, in order to standardize the response of the ciliates before experimental feeding, different periods of starvation in mineral solutions or in non-bacterized medium are routinely applied. As we found, the starvation affects the cytoplasmic streaming velocity, but in lesser degree if compared with

food vacoules formation rate (Table 1). On the other hand endocytosis as well as cytoplasmic streaming are distinctly enhanced by the particulate material (Wasik 1983, Wasik and Sikora 1981, 1984 a, Wasik et al. 1984). This implies that both physiological processes are stimulated or inhibited by common factors. However, this associations are not as tight as one might suppose, since the inhibition of food vacuoles formation does not necessarily lower the cytoplasmic streaming velocity (Sikora et al. 1984, Wasik and Sikora 1984 b). Therefore it seems to be appropriate to assume that surface receptors or receptor like structures (see for review Csaba 1985) are involved not only in the sensing of nutrients (Lenhoff 1974, Sattler and Staehelin 1974), but also in the transmission of signals triggering the propulsion mechanism of intracellular transportation system i.e., the cytoplasmic streaming in *Paramecium* (Sikora et al. 1979, Sikora 1981). A possible involvement of surface receptors of *Paramecium* in the transmission of signals evoked by the particulate matter in the surrounding medium and showing a dose-dependent influence on the motility of cytoplasm and endocytosis will have to be verified in much more detail. In conclusion, the present investigation of cytoplasmic streaming in starved *Paramecium bursaria* has revealed further relations between phagocytosis and intracellular transportation system. A short-lasting starvation do not impair the cytoplasmic motility, while a prolonged incubation in mineral maintenance solution leads to a decrease of streaming velocity.

REFERENCES

Csaba G. 1985: The unicellular *Tetrahymena* as a model cell for receptor research. Int. Rev. Cytol., 95, 327–377.

Kuźnicki L. and Sikora J. 1973: Cytoplasmic streaming within *Paramecium aurelia*. III. The effect of temperature on flow velocity. Acta Protozool., 12, 59–66.

Lenhoff H. N. 1974: On the mechanism of action and evolution of receptors associated with feeding and digestion. In: Coelenterate Biology (eds. L. Muscatine and H. N. Lenhoff), Academic Press, New York, pp. 238–239.

Sattler C. A. and Staehelin L. A. 1974: Ciliary membrane differentiation in *Tetrahymena pyriformis*. J. Cell Biol., 62, 473–490.

Sikora J. 1981: Cytoplasmic streaming in *Paramecium*. Protoplasma, 109, 57–77.

Sikora J. and Kuźnicki L. 1975: Cytoplasmic streaming during binary fission and conjugation. J. Protozool., 22, 74–75.

Sikora J. and Kuźnicki L. 1976: Cytoplasmic streaming within *Paramecium aurelia*. IV. Cyclosis during binary fission and conjugation. Acta Protozool., 15, 173–178.

Sikora J. and Wasik A. 1978: Cytoplasmic streaming within Ni^{2+} immobilized *Paramecium aurelia*. Acta Protozool., 17, 389–397.

Sikora J., Wasik A. and Baranowski Z. 1979: The estimation of velocity distribution profile of *Paramecium* cytoplasmic streaming. Eur. J. Cell Biol., 19, 184–188.

Sikora J., Kubalski A., Wasik A. and Kuźnicki L. 1984: The role of mechanical stimuli in the control of the cytoplasmic streaming in *Paramecium bursaria*. Postępy Biol. Kom., 11, 543–546.

Sonneborn T. M. 1970: Methods in *Paramecium* research. In: Methods in Cell Physiology, 4 (ed. D. M. Prescott), Academic Press Inc., New York, pp. 241–339.
Wasik A. 1983: Effect of external agents on cytoplasmic streaming in *Paramecium*. I. Influence of carmine suspension. Acta Protozool., 22, 183–189.
Wasik A. and Sikora J. 1981: Acceleration of cytoplasmic streaming velocity by external stimuli in *Paramecium bursaria*. In: Progress in Protozoology, VI Int. Congr. Protozool., Warszawa, 387.
Wasik A. and Sikora J. 1984 a: Effect of external agents on cytoplasmic streaming in *Paramecium*. II. Influence of media free of suspension. Acta Protozool., 23, 107–113.
Wasik A. and Sikora J. 1984 b: Effect of external agents on cytoplasmic streaming in *Paramecium*. III. Influence of endocytosis cessation. Acta Protozool., 23, 115–121.
Wasik A., Sikora J. and Kuźnicki L. 1984: Effect of different suspensions on phagocytosis and cytoplasmic streaming in *Paramecium bursaria*. Postepy Biol. Kom., 2, 529–532.
Wasik A., Sikora J. and Krucińska M. 1987: Endocytotic ability of starved *Paramecium bursaria*. Acta Protozool., 26, 133–141.

Steroid Hormone (Prednisolone) Influence on the Unicellular *Tetrahymena* (an Electron Microscopic Study)

György CSABA and András Kristóf FÜLÖP

Department of Biology, Semmelweis University of Medicine,
H-1445, Budapest, Hungary

Synopsis. Treatment of the unicellular *Tetrahymena* with 10^{-8} M prednisolone gave rise to both early and late ultrastructural changes, of which nuclear euchromatinization, increased cellular synthetic activity, nucleolar association and distension of the perinuclear gap were most characteristic. These changes have indicated a distinct response of the unicellular to steroids, which involves changes partly similar to, partly different from those elicited in the target cells of higher organisms.

The unicellular *Tetrahymena* either possesses, or is able to form, receptors for hormones of multicellular organisms in presence of these (Csaba 1980, 1981, 1985). Its interaction with the hormones of higher organisms is indicated not only by the binding of those signal molecules, but also by a (not infrequently specific) response to them. For example, the *Tetrahymena* responds to insulin (Csaba and Lantos 1975) or epinephrine (Csaba and Lantos 1976) by altering its glucose metabolism, to histamine or serotonine (Csaba and Lantos 1973) by increasing its phagocytic activity, and to thyroxine or its precursors by changing its mitotic rate (Csaba and Németh 1980). Moreover, the *Tetrahymena* contains itself many hormones, among others several polypeptide hormones (Berelowitz et al. 1982, Le Roith et al. 1982, 1983, Schwabe et al. 1983).

We demonstrated earlier (Csaba et al. 1985) presence of steroid hormones in the *Tetrahymena*, and formation of adequate receptors in its cytosol under the influence of massive steroid exposure. In the present study we investigated the response of the *Tetrahymena* to a steroid hormone. For lack of a "target organ" the electron microscopic detection of ultrastructular changes had been done.

Materials and Methods

Tetrahymena pyriformis GL cells, in the logarithmic phase of growth (3-day cultures), were maintained in 0.1 per cent yeast extract containing 1 per cent Bacto-tryptone medium (Difco, Michigan, USA) at 28°C. under continuous shaking. Part of the cultures was used as control, part was exposed to 10^{-8} M prednisolone (di-aresone-F-aquosum, Organon-Oss, Holland). The treated cultures were either fixed after exposure for 15 or 45 min, or were returned to plain medium after 1 h, and fixed at 4, 24 or 48 h after the beginning of treatment.

All cultures were fixed in a 2 per cent glutaraldehyde solution (in 0.1 M, pH 7.4 phosphate buffer), post-fixed in 1 per cent OsO_4 (in Millonig buffer) for 1 h, and embedded in araldite. The sections were counter-stained with uranyl acetate and lead citrate, and were examined in a EMV 100B electron microscope.

Results

The ultrastructural changes elicited by prednisolone can be seen in the electron micrographs presented in Pl. I–IV. In normal conditions, the nucleoli form an orderly row beneath the nuclear membrane. After 15-min treatment with prednisolone, nucleous-like bodies, which could not be distinguished with certainity from condensated clumps of chromatine (heterochromatine), made appearence in the central region of the nucleus (Pl. I). After 45 min there were indications of an increased euchromatinization and, in places, of nucleolar rarefaction (Pl. I b). The perinuclear gap was distended, and large electron lucent areas could be seen (Pl. I b, c). Four hours after the beginning of treatment (i.e., 3 h after its conclusion), the local gap distension was of similar degree, but nuclear euchromatinization was much more pronounced (Pl. II). After 24 h, the gap distensions were still conspicuous (Pl. III), euchromatinization was distinct, and confluence of the nucleoli was observed in places (Pl. III b). After 48 h, a further progression of euchromatinization, and appearance of enormous masses of endoplasmic reticulum, which is not normally characteristic of the *Tetrahymena*, could be seen (Pl. IV a). Many mitochondria appeared among the canalicules of the rough-surfaced endoplasmic reticulum. No nucleoli could be seen at the nuclear periphery (beneath the nuclear membrane), but enormous nucleolar associations made appearance in the central nuclear region (Pl. IV b). The different nuclear regions exhibited varying degrees of euchromatinization.

Discussion

The ultrastructural observations strongly suggest that the steroid receptors of the *Tetrahymena* are able to mediate the information carried by the hormone to the cytoplasmic structures, and even to the nucleus. Increased euchromat-

inization and proliferation of the rough-surfaced endoplasmic reticulum were equally indicative of the stimulation of cellular synthetic activity by prednisolone.

The hormone treatment lasted 1 h, and although its effects (euchromatinization and nucleolar association) became obvious very soon, the most distinct changes appeared by 48 h. This indicated that after entering the cell, the steroid stimulated the cellular response durably, also in the progeny generations. Thus the prolonged action, which characterizes the steroid hormones in higher organisms (Bentley 1980) seems to have taken effect also in the Tetrahymena. While, however, in higher organisms the prolonged action is being developed in the same target cell, in the unicellular it was developed over several progeny generations, for, taking into consideration about 10 generation changes within 48 h, the intracellular hormone concentration finally present was only a residue of that which had originally entered the unicellular organisms. Earlier studies based on the analysis of receptor kinetics (Csaba et al. 1985) indicated that the formation of steroid receptors in the *Tetrahymena* presupposes a lasting exposure. The fact that in the present study, steroid action was demonstrable relatively soon, seems to indicate that the development of action preceded the establishment of binding. This does not, of course, imply that steroid action was developed without binding at receptor level; it only suggests that the morphological (ultrastructural) changes of the cells are more sensitive indicators of interaction with the hormone than the methods available for the demonstration of its binding to the receptor.

The influence of steroid hormones on the *Tetrahymena* can be well characterized as dramatic if it is taken into consideration that part of the prednisolone-induced changes were more pronounced, and part even not usual (nucleolar migration towards the centre, distension of the perinuclear gaps and euchromatinization) compared to those elicited by the steroids in eukaryotic cells.
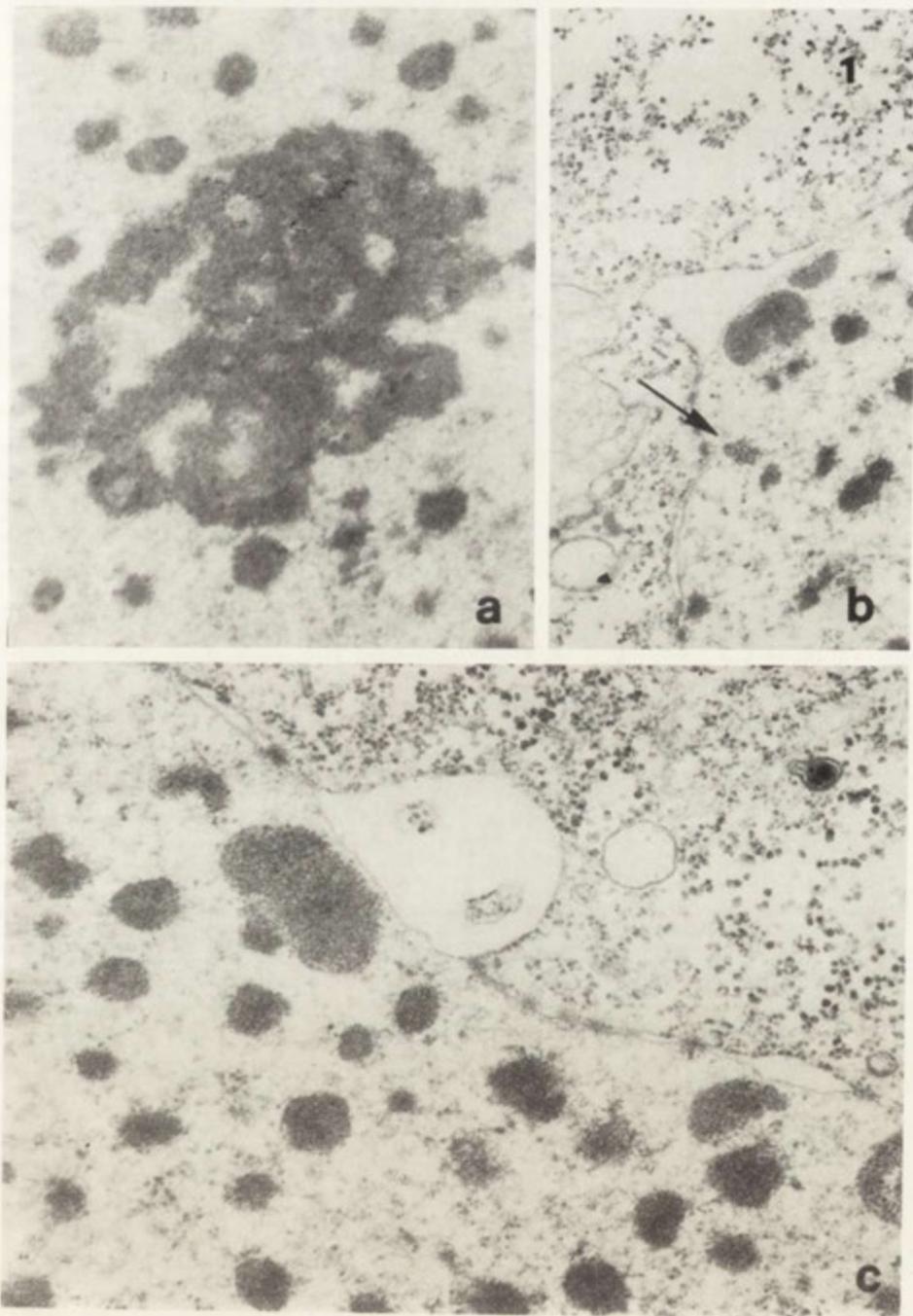
REFERENCES

Bentley P. J. 1980: Endocrine Pharmacology. Cambridge Univ. Press, London–New York.
Berelowitz M., Le Roith D., van Schenk H., Newgaard C., Szabo M., Frohman L. A. Shiloach J. and Roth J. 1982: Somatostatin-like immunoreactivity and bioactivity is native to *Tetrahymena pyriformis*, a unicellular eukaryote. Endocrinology, 110, 1939–1944.
Csaba G. 1980: Phylogeny and ontogeny of hormone receptors: the selection theory of receptor formation and hormonal imprinting. Biol. Rev., 55. 47–63.
Csaba G. 1981: Ontogeny and Phylogeny of Hormone Receptors. Karger, Basel–New York.
Csaba G. 1985: The unicellular *Tetrahymena* as a model cell for receptor research. Int. Rev. Cytol., 95, 327–377.
Csaba G. and Lantos T. 1973: Effect of hormones on *Protozoa*. Studies on the phagocytotic effect of histamine, 5-hydroxytryptamine and indoleacetic acid in *Tetrahymena pyriformis*. Cytobiologie, 7, 361–365.

Csaba G. and Lantos T. 1975: Effect of insulin on glucose uptake of *Tetrahymena*. Experientia, 31, 1097–1098.
Csaba G. and Lantos T. 1976: Effect of epinephrine on glucose metabolism in *Tetrahymena*. Endokrinologie, 68, 239–240.
Csaba G. and Németh G. 1980: Effect of hormones and their precursors on protozoa — the selective responsiveness of *Tetrahymena*. Comp. Biochem. Physiol., 65B, 387–390.
Csaba G., Inczefi-Gonda Á. and Fehér T. 1985: Induction of steroid binding sites (receptors) and presence of steroid hormones in the unicellular *Tetrahymena pyriformis*. Comp. Biochem. Physiol., 82A 567–570.
Le Roith D., Liotta A. S. Roth, J. Shiloach J. Lewis M. E., Pert C. B. and Krieger D. T. 1982: ACTH an β-endorphin-like activities are native to unicellular organisms. Proc. Natl. Acad. Sci. USA, 79, 2086–2090.
Le Roith D., Shiloach J., Berelowitz M., Frohman L., Liotta A. S., Krieger D. T. and Roth J. 1983: Are messenger molecules in microbes the ancestors of the vertebrate hormones and tissue factors? Fed. Proc., 42, 2602–2607.
Schwabe C., Le Roith D., Thompson R. P., Shiloach J. and Roth J. 1983: Relaxin extracted from protozoa (*Tetrahymena pyriformis*): molecular and immunological properties. J. Biol. Chem., 258, 2778–2781.

EXPLANATION OF PLATES I–IV

Pl. I. a — Appearance of the nucleolus in the nuclear centre of *Tetrahymena* after 15-min prednisolone treatment. × 60 000
b — Detachment of the nucleolus (arrow) after 45-min treatment with prednisolone. × 49 000
c — Local distension of the perinuclear gap after 45-min treatment with prednisolone. × 90 200
Pl. II. a — *Tetrahymena* treated with prednisolone for 4 h. The arrows point to "immigrant" or "absent" nucleoli. Note the distinct euchromatinisation. × 8000
b — Untreated (control) *Tetrahymena* at 4 h. × 8500
Pl. III. a — Prednisolone-exposed tetrahymenas after 24 h. Note local distension of the perinuclear gap. × 90 200
b — Prednisolone-exposed *Tetrahymena* after 24 h. Note unusual nucleolar configuration in the nuclear centre. × 50 000
Pl. IV. a — Prednisolone-exposed *Tetrahymena* after 48 h. Note enormus proliferation of the endoplasmic reticulum and appearance of mitochrondria between its canalicules, migration of the nucleoli to the central region, and distinct indications of euchromatinisation. × 10 700.
b — Prednisolone-exposed *Tetrahymena* after 48 h. Note local euchromatinisation at bottom left. The nucleoli have migrated to the centre and assumed queer shapes reminding of the nucleoli of higher organisms rather than of those of the *Tetrahymena*. × 22 700
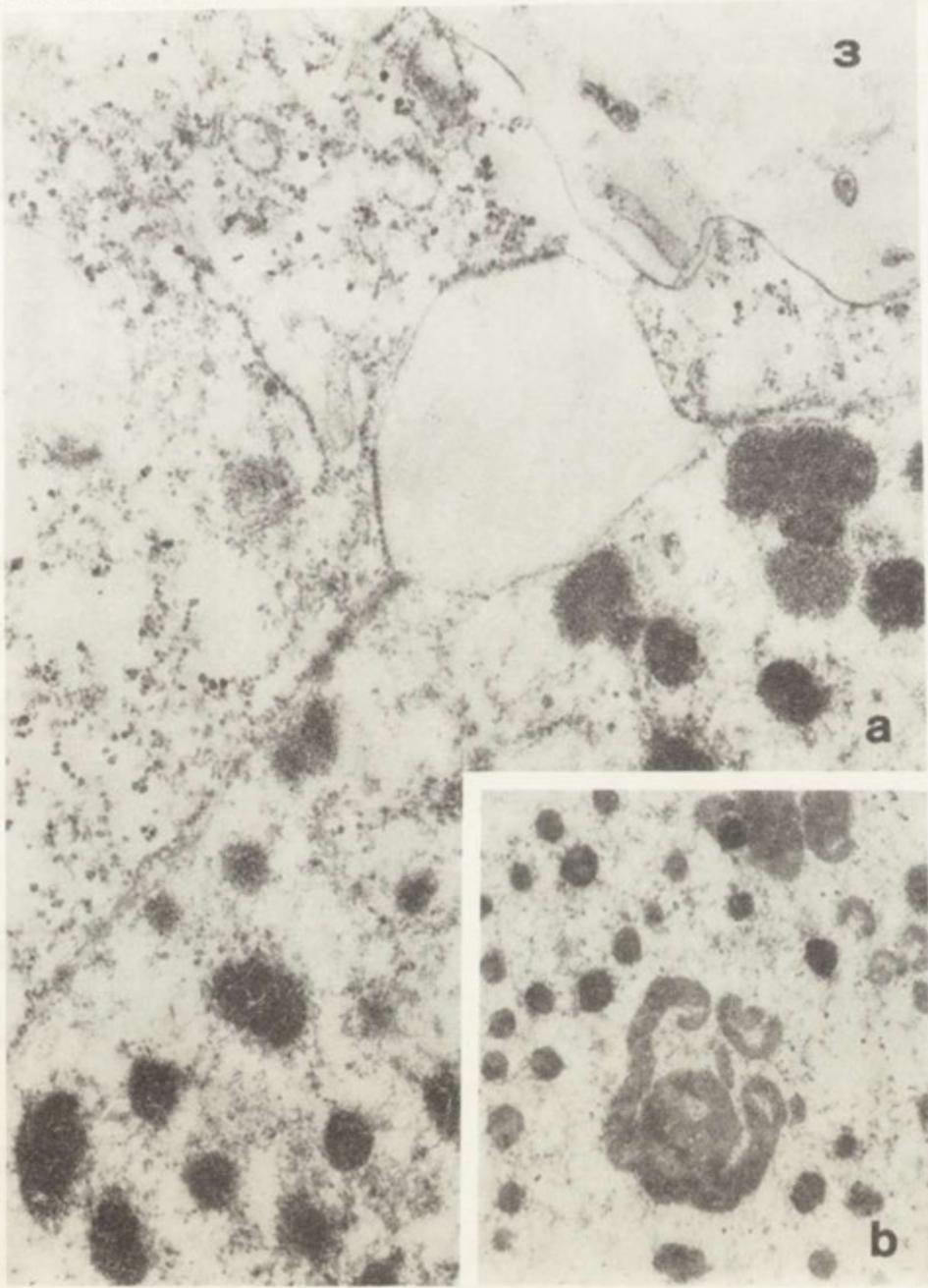
G. Csaba et A. K. Fülöp

auctores phot.

PLATE II
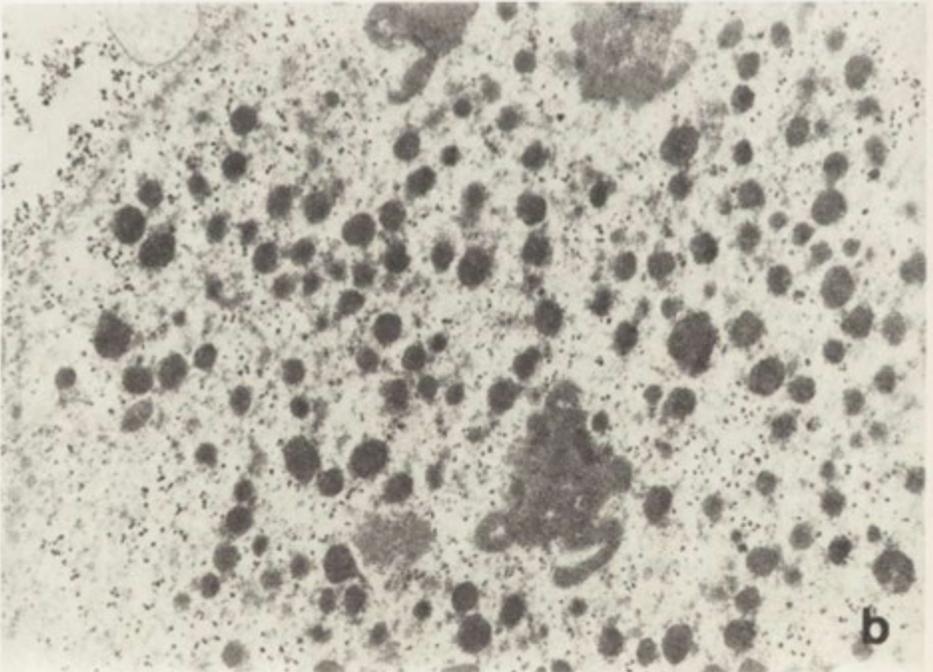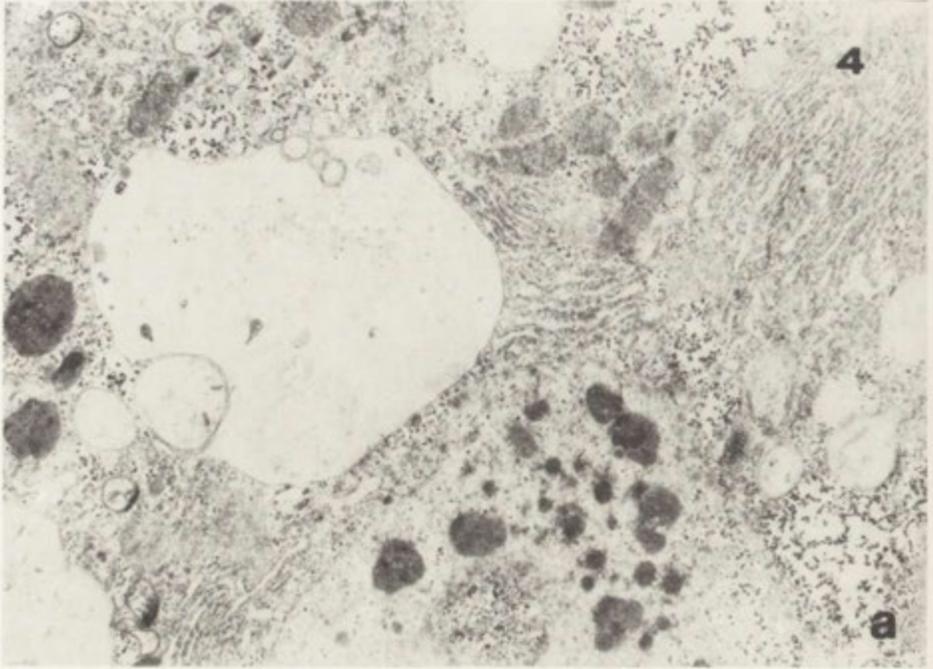

G. Csaba et A. K. Fülöp   auctores phot.

PLATE III
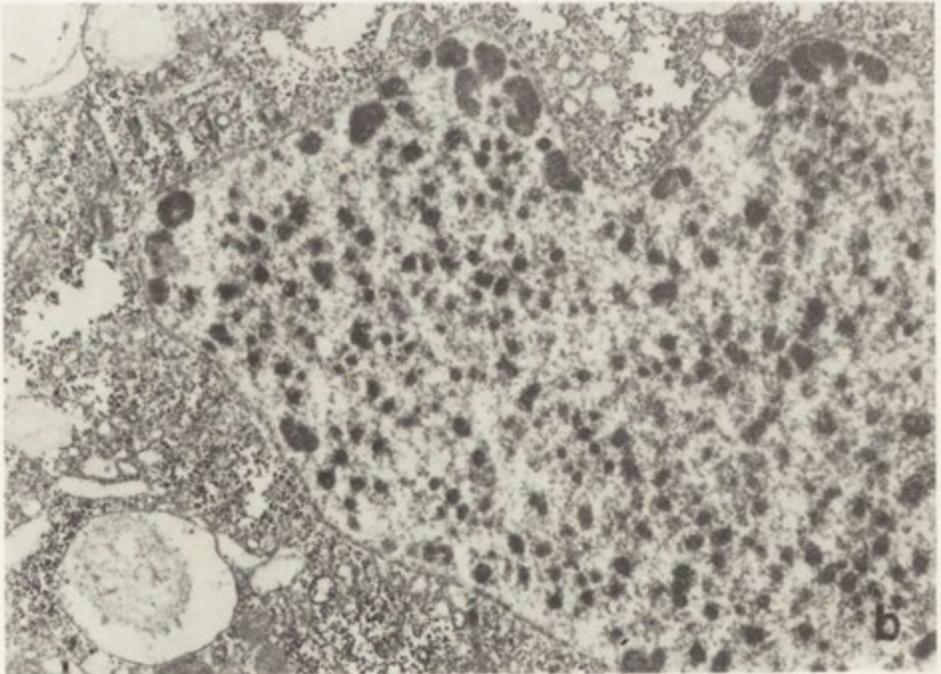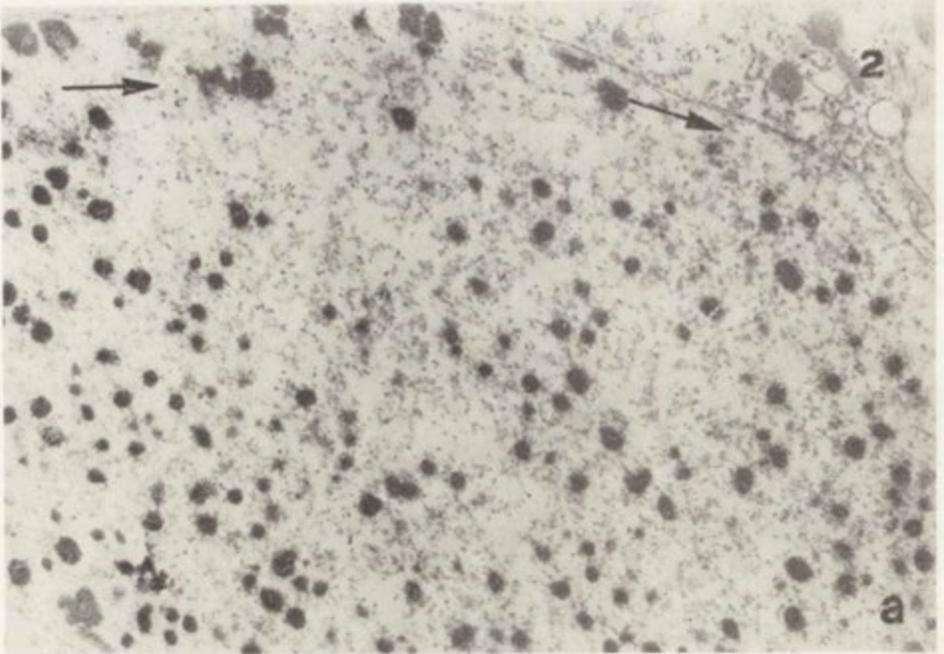
G. Csaba et A. K. Fülöp

auctores phot.

G. Csaba et A. K. Fülöp   auctores phot.

Grebneckiella ramachandrani sp. n. (*Apicomplexa: Cephalina*) from the Centipede, *Scolopendra morsitans* Linné

S. PREMA and K. P. JANARDANAN

Parasitology Laboratory, Department of Zoology, Calicut University 673 635 Kerala, India

Synopsis. Grebneckiella ramachandrani sp. n. from the intestine of the centipede *Scolopendra morsitans* Linné collected from the Calicut University Campus, is described in detail and its systematic position discussed. The sporonts reach 2400 by 480 μm. Sporont has the ratios here: PL:TL = 1:14.9; PW:DW = 1:1.86. Gametocyst spherical, dehiscence by pseudocyst formation. Spores cylindrical, 13 by 6 μm, with round ends and a convex thickening at one end.

The existing information on the gregarines of Indian centipedes is limited to the reports on four species belonging to the three genera, *Stenophora* Labbe, 1899, *Grebneckiella* Bhatia, 1938 and *Mecistophora* Ganapati and Narasimhamurti, 1966. The contributions came from Mitra and Chakravarty (1937), Chakravarty (1938, 1939), Misra (1942) and Ganapati and Narasimhamurti (1966).

The present authors recovered a species of cephaline gregarine of the genus *Grebneckiella* from the intestine of the centipede, *Scolopendra morsitans*, collected from the campus of the Calicut University. Morphological and biological details of the gregarine proved that the present form is distinct from the known species of *Grebneckiella* and is hence reported as *Grebneckiella ramachandrani* sp. n. Our reasons are discussed elsewhere.

Materials and Methods

The centipedes, *Scolopendra morsitans* Linné, collected from the Calicut University Campus, were at once examined for their gregarines or maintained in the laboratory and studied for the trophozoite, sporont and gametocyst stages at convenience. The trophozoites and sporonts were

studied from smears of infected midguts, fixed in Schaudinn's and stained with Heidenhain's hematoxylin. Pieces of the midgut, fixed in Bouin's fluid and sectioned at 5 μm were stained as above for studying the intracellular stages of development. Gametocysts were collected from the hindgut or faecal pellets of the infected hosts and maintained in moist chamber for further development. Sporozoites were released by exposing mature spores to the centipede's midgut fluid and observed under a phase-contrast microscope. Various development stages of live gregarines were studied by staining them supravitally with neutral red or methylene blue.

Sketches were made with the aid of a camera lucida; descriptions are based on the measurements of a minimum of 15 specimens.

Abbreviations used in this paper are: DL = deutomerite length; DW = deutomerite width; PL = protomerite length; PW = protomerite width; TL = total length. The ratios used are, the protomerite length to total length (PL:TL), and protomerite width to deutomerite width (PW:DW).

Results

Grebneckiella ramachandrani sp. n.

Description

Sporonts (Fig. 1 *1*) solitary, milky-white, elongate, anterior third broadest, gradually tapering to a round caudal end. Protomerite hemispherical; apical papilla and apical pore absent; epicyte covering over protomerite uniformly thick, longitudinally striated; the striations continuous with those on deutomerite; protomerite endocyte granular, translucent. Septum circular, flat; constriction at septum conspicuous.

Deutomerite elongate, cylindrical, widest a little behind septum and tapering to a round caudal end; epicyte hyaline, uniformly thick, longitudinally striated; endocyte granular, opaque.

Nucleus spherical to ovoid, not visible in fresh sporonts, variable in position. Endosome round or irregular, varying from 1 to 9 in number, deep-staining with hematoxylin. Nucleus in a sporont of 2400 μm long measured 75.9 μm in diameter.

Measurements (in micrometers): Measurements of sporonts (with mean in parentheses) are noted below.

TL = 1024–2400 (1516.85); DW = 144–480 (303.7)

PL = 64.8–256 (107.1); PW = 112–320 (165.1)

Ratios: PL:TL = 1:14.9; PW:DW = 1:1.86

Gametocysts (Fig. 1 *2*). Gametocysts spherical, opaque, milky-white. Cyst wall with epicyst and endocyst; epicyst uneven, 77 to 320 μm thick, of hyaline material impregnated with debris particles; endocyst hyaline, 7.7 to
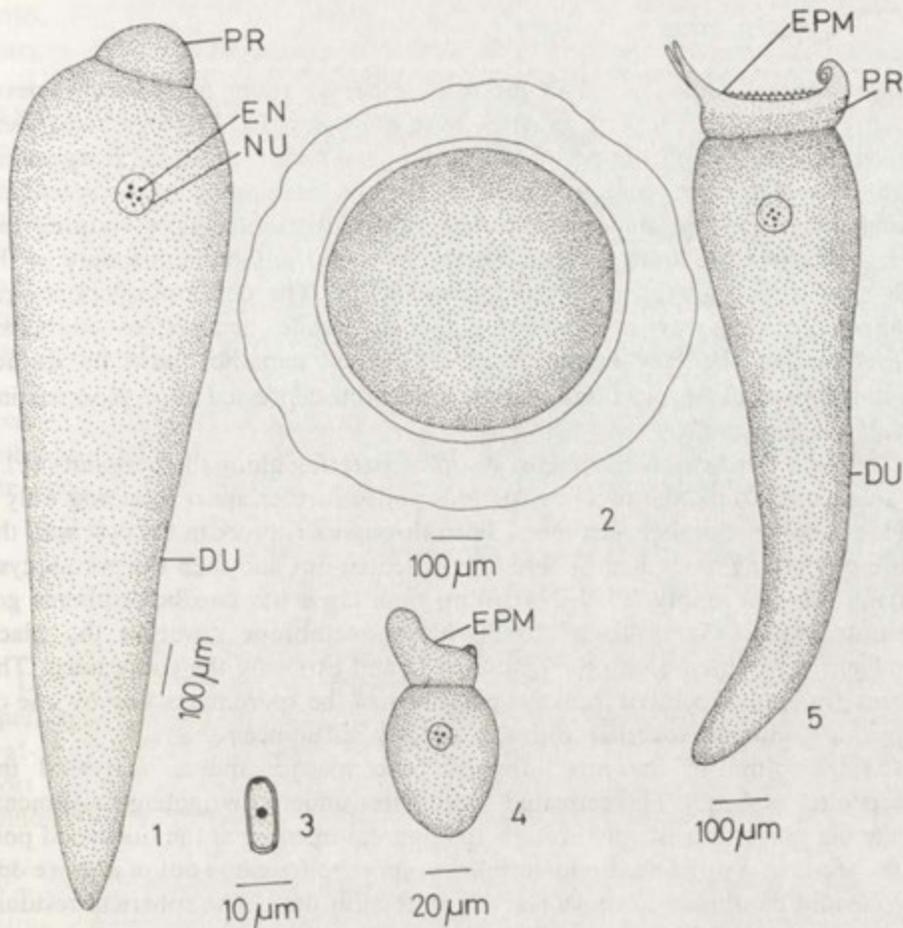
Fig. 1. *1–5. Grebneckiella ramachandrani* sp. n. *1* — Sporont, *2* — Gametocyst, *3* — Spore,
4 5 — Trophozoites. DU — deutomerite, EN — endosome, EPM — epimerite, NU — nucleus,
PR — protomerite

15.4 µm thick; line of association clearly visible in newly formed gametocysts.
Fresh gametocysts measured 352 to 592 µm (mean, 400.8 µm).

Gametes. Gametes anisogamous; microgametes motile, filamentous, 4.5
to 5 µm long, with elongated rod-like head and a drawnout tail; macrogametes
non-motile, spherical, 3 µm in diameter, with vacuolated cytoplasm containing
basophilic irregularly distributed granules.

Spores (Fig. 1 *3*). Spores cylindrical, with round ends and a convex
thickening at one end; spore wall thick, dark-brown; sporoplasm with a central
spherical residual body. Fresh spores measured 13 by 6 µm.

Sporozoites. Sporozoites vermiform, 12 by 1.5 µm; 8 per spore; anterior
end pointed, posterior end blunt.

Life-cycle Stages

Gametocysts, maintained in moist chamber at room temperature, developed gametes in 20 h. The gametes were anisogamous. Microgametes were actively moving along the periphery of the gametocyst. In 20–22 h, spherical zygotes were formed, and in the next 2 h the developing zygotes became cylindrical, its cytoplasm contained irregularly distributed deep-staining bodies. The immature spores were observed in 24–30 h. One hemisphere of the cyst now turned grey, the other remained white. The developing spores got confined to the grey hemisphere while the white hemisphere contained a pseudocyst. The grey hemisphere then turned metallic black; its surface exhibited papillary projections and an equatorial depressed zone representing a dehiscence band.

In 48–50 h the two hemispheres started separating along the periphery (Pl. I 1). In another fours, the two hemispheres pulled further apart retaining only a bridge of connection between them. Then through a rupture in the cyst wall the spore-containing black hemisphere first squeezed out and then the pseudocyst, leaving the cyst empty (Pl. I 2–4). With time lapse the two hemispheres got separated (Pl. I 5, 6). In 60 to 72 h the membrane covering the black hemisphere ruptured along the dehiscence band exposing the spore mass. The spores gradually liberated from the periphery of the spore mass, one by one or in groups adhered together through an oily substance.

Midgut fluid of the host, applied onto mature spores, activated the sporozoites in 4 min. The activated sporozoites undergo wriggling movements inside the spore, and escape through an apparent opening at the thickened pole of the spore in 5 to 7 min. Eight vermiform sporozoites came out of a spore one by one and continued to move actively in the gut fluid. The spherical residual body remained inside the spore wall.

The centipedes seem to pick up infection by feeding on food materials containing viable spores of the gregarine. The sporozoites are released in midgut, obviously under the influence of the midgut fluid, get attached to the midgut epithelium and develop into trophozoites. Intracellular development stages were not observed in serial sections of the midgut.

The smallest trophozoite observed measured 19.8 by 13.2 μm, with 3.3 μm long protomerite and 16.5 μm long deutomerite. Its epimerite was boat-shaped, with a single 6.6 μm long arm on one side; nucleus was spherical, measuring 6.6 μm. The trophozoite (Fig. 1 4) measuring 85.8 μm long developed on the epimerite two arm-buds, each 5.1 μm long, opposed to the long arm, now measuring 18.3 μm long; protomerite measured 16.5 by 33 μm and deutomerite 69.3 by 49.5 μm. The trophozoite measuring 356.4 μm long had a boat-shaped epimerite; its long arm measured 125 by 18.3 μm, and the paired

arms, 16.5 by 9.9 µm. Widely spaced denticles were also present on the apical margin of the epimerite.

The largest observed trophozoite was 1500 µm long, with a boat-shaped, 18.8 µm long epimerite, a rectangular, 75 by 277.5 µm protomerite and an elongated, 1406.2 by 326.2 µm deutomerite; its nucleus was spherical, 64 µm in diameter, with 1–9 round or irregular endosomes. The paired arms on the epimerite measured 150 µm long and the long arm 282.5 µm. The trophozoite remained attached to the epithelium with its expanded epimerite and appeared T-shaped as its asymmetrical arms extended at right angles to the protomerite. In a trophozoite forcibly detached from the epithelium the long arm remained curved inward (Fig. 1 5).

Fully grown trophozoites got detached from the midgut epithelium and became sporonts. The sporont epimerite was invaginated into the protomerite with the tip of the long arm alone showing through the protomerite apex. In late sporonts the epimerites disappeared.

Taxonomic Summary

Diagnosis: Sporonts solitary, elongate, anterior third broadest, gradually tapering to a round caudal end; protomerite hemispherical. Trophozoites with boat-shaped epimerites carrying a long curved arm on one side, and paired short arms against it. Gametocysts spherical; dehiscence by pseudocyst; spores cylindrical, with round ends and a convex thickening at one end.

Measurements: TL = 1024–2400 (1516.85); DW = 144–480 (303.7); PL = 64.8–256 (107.1); PW = 112–320 (165.1).
Ratios: PL:TL = 1:14.9; PW:DW = 1:1.86.
Host: *Scolopendra morsitans* Linné.
Location in host: Intestine.
Type locality: Calicut University Campus, Kerala, India.
Date of collection: July to November 1982 and 1984.
Holotype: Deposited in the parasitological collection in the Department of Zoology, University of Calicut, Kerala, India.

Discussion

The present gregarine from *Scolopendra morsitans* Linné resembles *Grebneckiella pixellae* Misra, 1942 infecting *Scolopendra morsitans* and *G. japonica* (Hoshide 1952) Levine, 1976 infecting *Scolopendra subspinipes mutilans*. Comparative characters in Table 1 clearly show that the present gregarine is

Table 1

Comparitive characters

Comparative characters	*Grebneckiella pixellae* Misra, 1942	*G. japonica* (Hoshide, 1952) Levine, 1976	*G. ramachandrani* sp. n.
Sporonts	Solitary, 1050-4050 µm long	Solitary, 4000 µm long, 390 µm wide	Solitary, 1024-2400 µm long, 144-480 µm wide
PL : TL	1 : 15-25	1 : 18-24	1 : 14.9
PW : DW	1.2-2.5 : 1	1.1-2.1 : 1	1 : 1.86
Trophozoites	Epimerite present; passes through an intracellular phase of development	Epimerite present; without intracellular development stages	Epimerite present; without intracellular development stages
Gametocysts	Spherical, 208-672 µm	Spherical, 800-1000 µm	Spherical, 352,592 µm
Epimerite	Caducous; digitiform protuberances with thread-like filaments; the long arm with a vesicular nucleus at its distal end; shorter arm with bifid distal extremity	Boat-shaped; short spines with thread-like filaments; long arm without nucleus; short arms paired	Boat-shaped; widely spaced denticles without thread-like filaments; long arm without nucleus; short arms paired
Sporocysts	Cylindrical or long ovoidal; 10-13 µm by 4-5 µm; operculum absent; united in oblique chains	Ellipsoidal; 13 by 6 µm; united in chains diagonally	Cylindrical; a convex thickening at one end; 13 by 6 µm; not united in chains
Host	*Scolopendra morsitans* Linné	*Scolopendra subspinipes mutilans* L. Koch	*Scolopendra morsitans* Linné
Locality	Lucknow, U. P. (India)	Murozumi, Naruto, Iwakuni, Tabuse Yamaguti Pref. (Japan)	Calicut University Campus, Kerala (India)

significantly different from *G. pixellae* and *G. japonica*. The gregarine differs from *G. pixellae* in its measurements and ratios and in having (1) epimerite without filaments, (2) no nuclear body in the long arm, (3) extracellular development, and (4) no epispore for the spores, which are not united in oblique chains. The gregarine differs from *G. japonica* in measurements and ratios, in having epimerites without filaments, and cylindrical spores which are not united in chains diagonally. The gregarine is, therefore, considered as a new taxon and named *Grebneckiella ramachandrani* sp. n. The species is named after Dr. P. Ramachandran, teacher of one of the present authors (K. P. J.), in recognition of his contributions to the study of parasitic protozoans. This is the first report of a cephaline gregarine from a centipede in Kerala.

ACKNOWLEDGEMENTS

REFERENCES

Chakravarty M. 1938: Observations on the life-history of *Nina navillae* Mitra and Chakravarty, from the intestine of the *Scolopendra* sp. Arch Protistenkd. 40, 502–506.

Chakravarty M. 1939: On the morphology and life-history of a new cephaline gregarine, *Stenophora shyamaprasadi* n. sp., from the intestine of chilopod *Cormocephalus dentipes* Poc. Arch. Protistenkd., 92, 67–72.

Ganapati P. M. and Narasimhamurti C. C. 1966: On a new cephaline gregarine *Mecistophora legeri* n. gen., n. sp., from a centipede *Mecistocephalus punctifrons* Newp. Arch. Protistenkd., 104, 554–558.

Hoshide H. 1952: *Chilophoda* gregarinides. Zool. Mag., 61, 196–200.

Levine N. D. 1976: Some nomenclatural corrections among septate gregarines (*Protozoa, Apicomplexa*). Proc. Zool. Soc., Calcuta, 29, 21–28.

Misra P. L. 1942: On the life history of a new gregarine, *Grebneckiella pixellae* sp. nov., from the centipede, *Scolopendra morsitans* Linné, with a note on the family *Dactylophoridae* Leger, 1892. Rec. Ind. Mus., 44, 323–337.

Mitra A. N. and Chakravarty M. 1937: Observation on *Nina navillae* n. sp. from *Scolopendra* sp. Proc. Indian Sci. Congr., XXIV, 291.

EXPLANATION OF PLATE I

1–6: Gametocyst of *Grebneckiella ramachandrani* sp. n. under various stages of dehiscence
1: Gametocyst with spore-containing hemisphere and pseudocyst under separation, note the epicyst with impregnated debris particles
2: Spore — containing hemisphere came out of the gametocyst
3: Pseudocyst coming out of the gametocyst
4: Spore-containing hemisphere and pseudocyst free from the gametocyst wall
5: Spore-containing hemisphere
6: Pseudocyst. ENC — endocyst, EPC — epicyst, PC – pseudocyst, SH — spore-containing hemisphere

PLATE I
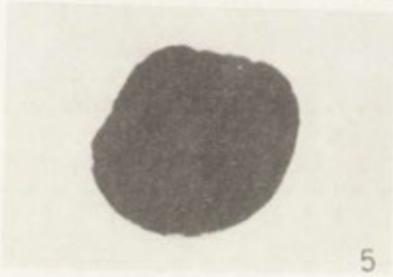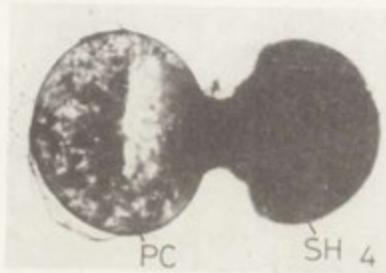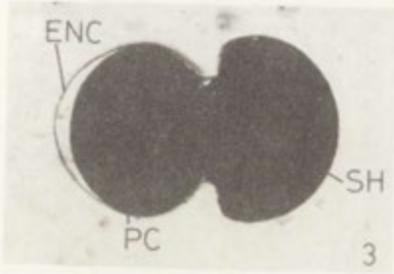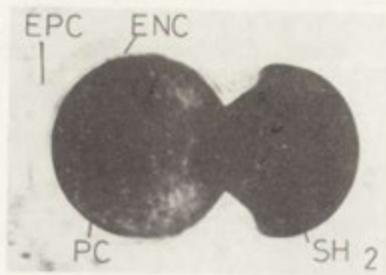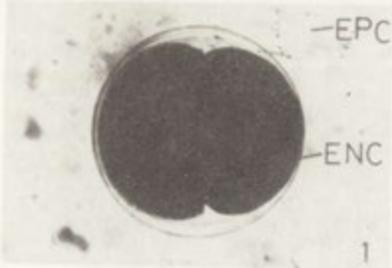
S. Prema et K. P. Janardanan

auctores phot.

Three New Septate Gregarines in *Oryzaephilus mercator* (F.), Infesting Different Stored Food Items

S. GHOSE, S. K. GUPTA and D. P. HALDAR

Protozoology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India

Synopsis. Three distinctly different species of septate gregarines belonging to the genus *Didymophyes* Stein from the common pest. *Oryzaephilus mercator* F. have been described in this communication. These are *D. oryzaephilae* from ground nuts, *D. lipai* from cashew nuts and *D. rigidus* from rice grain. These variations have been attributed to the change of food items consumed by the hosts.

Oryzaephilus mercator (F.) (*Insecta: Coleoptera*) is a common pest occurring in rice grains, ground nuts and cashew nuts in the Gangetic West Bengal. Occasionally, the damage caused by this tenebrionid is alarmingly high. As such, a programme was undertaken to study whether these had any internal animal parasites in their gut, and if so, whether these helped the hosts in the digestion of food taken by these pests. Interestingly it has been revealed that the hosts collected from all the three sources are parasitized by septate gregarines (*Apicomplexa: Sporozoea*), infection reaching about 50% at times. Upon preliminary examination the gregarines were identified as belonging to the genus *Didymophyes* Stein. However, detailed study on their morphology and life history has shown that these are altogether different species depending upon the stored food item infested by their hosts. The gregarines are described as three separate species in this communication.

Materials and Methods

The host insects *Oryzaephilus mercator* (Favrel) were collected from different grocer's shops at Chinsurah, Kanchrapara and Calcutta in West Bengal and brought alive to the laboratory for investigation. The insects were decapitated, their guts carefully dissected out under a binocular and gently pressed for the parasites to come out from the gut lumen. For permanent preparation

smears were fixed in Schaudinn's fluid and subsequently stained with Heidenhain's haematoxylin. For studying the intracellular stages of development of the parasites, portions of midgut of infected hosts were fixed in Bouin's fluid and 5 μm thick sections were stained as above. Cysts were collected from the midgut of the infected hosts and cultured in moist chamber for sporulation (Sprague 1941). The following abbreviations have been used: TL = Total length; LE = Length of epimerite; LP = Length of protomerite; LD = Length of deutomerite; LN = Length of nucleus; WE = Width of epimerite; WP = Width of protomerite; WD = Width of deutomerite; WN = Width of nucleus.

Observations

Didymophyes oryzaephilae sp. n.

Host: *Oryzaephilus mercator* (F.) from *Juglans regia* L. (Ground nuts)
Incidence: 234 out of 506 (46.2%) hosts examined are infected with this gregarine.

Development. Intracellular; as revealed in sections; the earliest stage is an ovoidal body with peripherally placed spherical nucleus. The infected cell shows a clear area around the parasite.

Trophozoite. Small, vase-like body measuring 24.3 μm to 32.4 μm in total length. The epimerite is small, cashew nuts like (Fig. 1 *1*); when viewed from the top it appears disc like. The protomerite is rectangular while deutomerite is vase-like in shape. Nucleus is spherical to rounded and located centrally in the deutomerite. The pellicle is thin and the epicyteal striations cannot be observed. The amount of cytoplasm is less in epimerite but high in proto- and deutomerite.

Sporadin and association. Solitary as well as biassociative. A young sporadin (Fig. 1 *2*) is cylindrical in shape with spade-shaped protomerite and elongated deutomerite. Nucleus is rectangular and situated medially. The mature sporadin (Fig. 1 *3* and *4*) is cylindrical in shape with globular protomerite and long, cylindrical deutomerite. The nucleus is elliptical in shape with prominent nuclear membrane. Cytoplasm is uniformly granulated throughout the proto- and deutomerite.

The sporadins in syzygy are obtained in large numbers. The association is always caudo-frontal in nature. The associated partners, viz., the primite and the satellite are morphologically different. The protomerite of the primite is hemispherical whereas in the satellite the protomerite is absent, a distinguishing generic character (Fig. 1 *5*). The associating partners exhibit bending manoeuvres and ultimately become enclosed in cyst wall (Fig. 1 *6*). Occasionally the primite is observed to retain the papilla-like epimerite during early association (Fig. 1 *7*).

Gametocyst and Spore. Freshly collected gametocysts are blackish white in colour, double walled, spherical bodies measuring 47.2 μm × 42.6 μm in dimension (Fig. 1 *8*). After 48 h of development in the moist chamber the
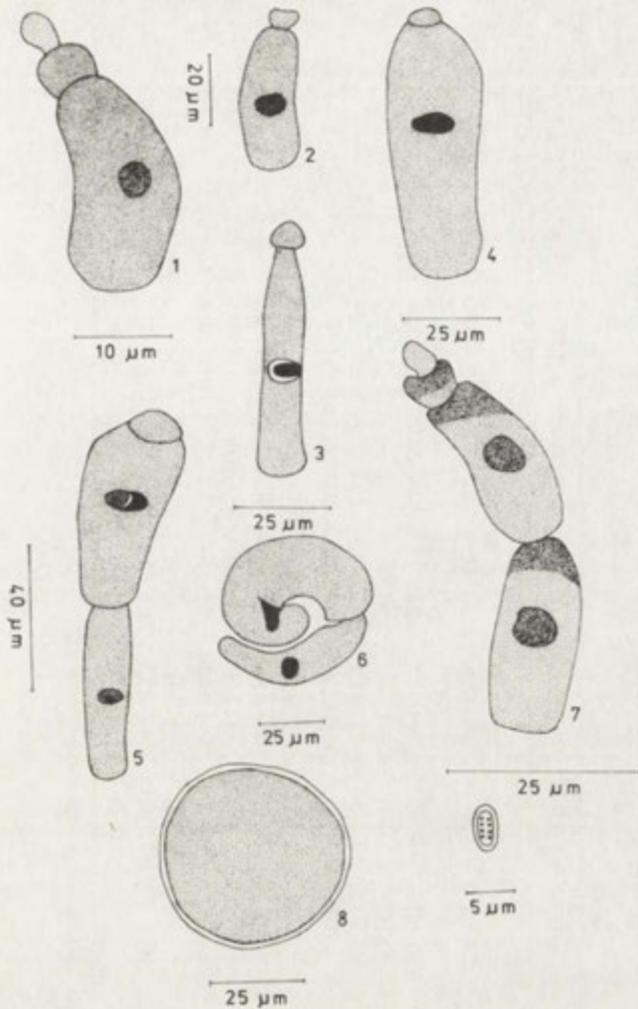
Fig. 1. *1–9*. Camera lucida drawings of *Didymophyes oryzaephilae* sp. n. *1* — Mature trophozoite, *2* — Young sporadin, *3–4* — Mature sporadins, *5* — Syzygy, *6* — Syzygy showing bending manoeuvres, *7* — Syzygy showing epimerite in the primite, *8* — Gametocyst, *9* — Spore

cysts dehisce by simple rupture liberating spores in short chains. Spores are double walled, smooth ellipsoidal (Fig. 1 9) measuring 7.5 μm × 4.1 μm. After 40 h, eight rounded sporozoites are developed in each spore arranged in two rows.

Measurements (in microns). The summary of measurements of 20 specimens of trophozoites and sporadins with the mean within parenthesis is given in Table 1. Details of measurements of individual specimens are given in Table 2.

Table 1

Showing the summary of measurements of trophozoites and sporadins of three species of *Didymophyes* Stein

	TL	LE	WE	LP	WP	LD	WD	LN	WN	LP:TL	WP:WD
Trophozoite											
D. oryzaephilae	24.3–32.4	5.4–8.1	5.4–8.1	5.4	8.1–8.6	12.1–18.9	11–16.2	5.4–6.7	5.4–6.7		
	(28.5)	(6.5)	(6.2)		(8.2)	(16.53)	(13.56)	(5.8)	(6.4)		
D. lipai	22.95–35.1	5.4–6.75	4.86–6.75	4.05–6.75	5.4–10.8	12.15–21.6	8.64–20.25	4.05–6.75	4.05–9.45		
	(29.7)	(6.3)	(5.7)	(5.5)	(7.5)	(17.9)	(13.4)	(5.7)	(6.5)		
D. rigidus	23.8–51.3	4.0–5.4	3.2–5.4	2.7–6.8	5.4–9.5	16.2–40.5	10.8–17.6	2.7–5.4	5.4–6.8		
	(42.5)	(4.9)	(4.7)	(5.1)	(8.1)	(32.4)	(15.0)	(4.8)	(5.7)		
Sporadin											
D. oryzaephilae	24.3–108.0			5.4–10.8	8.1–18.9	18.9–98.0	10.8–43.2	5.4–9.4	5.4–16.2	1:4.5–12.3	1:1.3–2.9
	(74.2)			(8.6)	(13.3)	(65.5)	(26.0)	(7.0)	(9.2)	(7.8)	(1.9)
D. lipai	20.25–121.5			5.4–10.8	5.94–14.04	13.5–110.7	12.7–27.54	4.05–10.8	4.05–12.15	1:3.0–14.3	1:1.3–2.3
	(64.3)			(8.3)	(10.3)	(56.0)	(18.2)	(7.0)	(9.0)	(7.1)	(1.7)
D. rigidus	10.8–94.5			2.7–8.1	4.0–13.5	8.1–86.4	5.4–37.1	2.2–8.1	2.4–13.5	1:8.02	1:1.96
	(44.0)			(5.2)	(7.7)	(38.8)	(15.8)	(5.0)	(6.2)		

Figures within parentheses are average of 20 specimens (except in case of trophozoites)

Table 2

Showing details of measurements (in microns) of different parts of 20 specimens
of *Didymophyes oryzaephilae*

Sl. No.	TL	LE	LP	LD	LN	WE	WP	WD	WN	LP : TL	WP : WD
(1)	30.2	5.9	5.4	18.9	5.7	8.1	8.1	16.2	6.7	1 : 5.6	1 : 2.0
(2)	27.0	5.4	5.4	16.2	5.4	5.4	8.1	13.5	6.7	1 : 5.0	1 : 1.6
(3)	24.3	6.7	5.4	12.1	5.4	5.4	8.6	11.1	5.4	1 : 4.5	1 : 1.2
(4)	32.4	8.1	5.4	18.9	6.7	5.9	8.1	13.5	6.7	1 : 6.0	1 : 1.6
(5)	64.8	—	8.1	56.7	8.1	—	12.6	22.9	10.5	1 : 8.0	1 : 1.8
(6)	68.8	—	9.4	59.4	7.5	—	10.8	24.3	9.4	1 : 7.2	1 : 2.2
(7)	67.5	—	8.1	59.4	6.7	—	13.5	24.3	10.8	1 : 8.3	1 : 1.8
(8)	89.1	—	8.1	81.0	7.5	—	14.8	27.0	13.5	1 : 11.0	1 : 1.8
(9)	89.1	—	10.8	78.3	5.4	—	18.9	29.7	8.1	1 : 8.2	1 : 1.5
(10)	99.9	—	8.1	91.8	6.7	—	14.8	43.2	13.5	1 : 12.3	1 : 2.9
(11)	67.5	—	8.1	59.4	5.9	—	12.1	22.9	9.4	1 : 8.3	1 : 1.8
(12)	81.0	—	10.8	70.2	6.7	—	13.5	24.3	9.4	1 : 7.5	1 : 1.8
(13)	91.8	—	9.4	82.3	5.4	—	14.8	35.1	14.8	1 : 9.7	1 : 2.3
(14)	41.8	—	6.7	35.1	8.1	—	11.3	17.5	9.4	1 : 6.2	1 : 1.5
(15)	62.1	—	8.1	54.0	5.4	—	10.8	24.3	13.5	1 : 7.6	1 : 2.2
(16)	54.0	—	8.1	45.9	8.1	—	13.5	21.6	12.7	1 : 6.6	1 : 1.6
(17)	70.2	—	9.4	60.7	9.4	—	11.3	20.2	10.8	1 : 7.4	1 : 1.7
(18)	106.6	—	9.4	97.2	8.1	—	16.2	32.4	16.2	1 : 11.3	1 : 2.0
(19)	24.3	—	5.4	18.9	5.4	—	8.1	10.8	5.4	1 : 4.5	1 : 1.3
(20)	108.0	—	9.9	98.0	8.1	—	16.2	35.1	16.2	1 : 10.9	1 : 2.1

Material. Holotype; trophozoite on slide No. $AP_{2/11}$ prepared from the contents of the midgut of *Oryzaephilus mercator* (Favrel), from *Juglans regia* L. at Chinsurah, Hooghly, West Bengal, India by S. Ghose on July 7, 1983. Paratype; many on the above-numbered slide and on other slides; other particulars are the same as for the holotype material.

Affinities. The gregarine under report is found in association of two, its spherical cyst dehisces by simple rupture, liberating ellipsoidal spores and during association the satellite is without any septum. As such its placement in the genus *Didymophyes* Stein, 1848 is beyond any doubt. The gregarine possesses close resemblances with *D. minuta* (Ishii) 1914 in the WP : WD values. Its elongated, cylindrical sporadins and larger primite during association brings it closer to *D. longissima* (Von Siebold) Frantzius, 1848, otherwise it differs from it in all other characters. It appears, therefore, that *Oryzaephilus mercator* infesting ground nuts is parasitized by a new species of *Didymophyes*, *D. oryzaephilae* sp. n., after the generic name of the host.

Didymophyes lipai sp. n.

Host: *Oryzaephilus mercator* (larvae and adult) from *Anacardium occidentalis* L. (cashew nuts). Incidence: 85 out of 255 (33.3%) hosts examined are infected with this gregarine. Development: Intracellular.

Trophozoite. Ovoidal, 22.95 µm to 35.1 µm in total length; epimerite egg-shaped or conical with pointed anterior end (Fig. 2 *1* and *2*). However, the protomerite is variously shaped, i.e., hemispherical, ovoidal or hat-shaped with
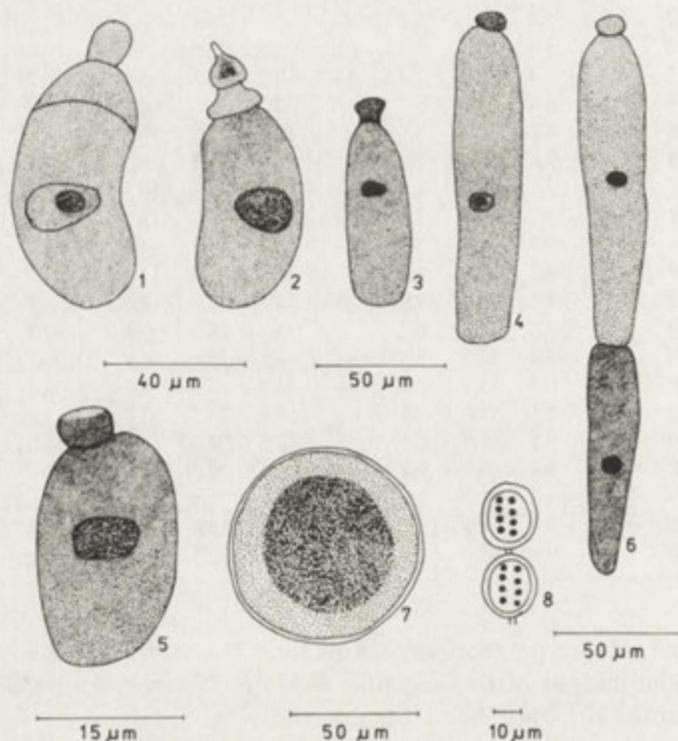

Fig. 2. *1–8*. Camera lucida drawings of *Didymophyes lipai* sp. n. *1–2* — Mature trophozoites, *3* — Young sporadin, *4–5* — Mature sporadins, *6* — Syzygy, *7* — Gametocyst, *8* — Spores

constriction at the epimerite-protomerite junction. Deutomerite is cashew nut like, obese or flat. Cytoplasm is uniformly granulated throughout epimerite, protomerite and deutomerite.

Sporadin and association. Solitary as well as biassociative. A young sporadin is elongated to ovoidal in shape (Fig. 2 *3*) with hemispherical or egg-shaped protomerite, broader than long. Deutomerite is elongated to obese in shape. The mature sporadins (Fig. 2 *4* and *5*), however, are cylindrical to vase-like with ellipsoidal to rhomboidal protomerite and cylindrical deutomerite.

During association the primite is larger than the satellite (Fig. 2 6), the protomerite of the primite is ellipsoidal. The satellite, as usual, is without any septum.

Gametocyst and Spore. Gametocysts are blackish white in colour, rounded in shape (Fig. 2 7) measuring 67.2 μm in dimension. The cyst wall is thin with condensed central zone and thinner peripheral zone. After 120 h of development inside the moist chamber the cyst bursts by simple rupture liberating spore in chains. Spores are spherical, double walled (Fig 2 8) measuring 8.25 μm × 6.6 μm in the average. Thirty hours after release, the spores develop sporozoites which are arranged linearly in two rows, four in each.

Measurements. The summary of measurements of 20 specimens of trophozoites and sporadins with the mean within parenthesis is given in Table 1. Details of measurements of individual specimens are given in Table 3.

Material. Holotype; trophozoite on slide No. KA/6 prepared from the contents of the midgut of the insect *Oryzaephilus mercator* (F.), from cashew nuts collected at Calcutta, West Bengal, India by S. Ghose on January

Table 3

Showing details of measurements (in microns) of the different parts of 20 specimens of *Didymophyes lipai* from the larvae and adults of *Oryzaephilus mercator* (F.)

Sl. No.	TL	LE	LP	LD	LN	WE	WP	WD	WN	LP : TL	WP : WD
(1)	35.1	6.75	6.75	21.6	6.75	6.75	9.45	20.25	9.45	1 : 5.2	1 : 2.1
(2)	22.95	6.75	4.05	12.15	5.4	5.4	5.4	8.64	5.4	1 : 5.6	1 : 1.6
(3)	27.0	5.4	5.4	16.2	4.05	5.4	6.75	10.8	4.05	1 : 5.0	1 : 1.6
(4)	31.05	6.75	5.4	18.9	6.75	4.86	6.75	13.5	8.1	1 : 5.75	1 : 2.0
(5)	32.4	5.94	5.94	20.52	5.4	5.94	10.8	14.04	5.4	1 : 5.5	1 : 1.3
(6)	25.65	—	8.1	17.55	4.05	—	9.45	13.5	4.05	1 : 3.1	1 : 1.4
(7)	25.65	–	5.4	20.25	5.4	—	5.94	14.04	8.1	1 : 4.75	1 : 2.3
(8)	20.25	—	6.75	13.5	5.4	—	8.1	13.5	8.1	1 : 3.0	1 : 1.6
(9)	35.1	—	10.0	25.1	5.4	—	8.1	12.7	5.4	1 : 3.5	1 : 1.5
(10)	83.7	—	8.1	75.6	8.1	—	12.15	18.9	10.8	1 : 10.3	1 : 1.5
(11)	68.85	—	6.75	62.1	6.75	—	9.45	14.85	10.8	1 : 10.2	1 : 1.6
(12)	36.45	—	5.4	31.05	5.4	—	8.1	13.5	5.4	1 : 6.75	1 : 1.6
(13)	56.7	—	8.1	48.6	5.4	—	9.45	13.5	5.4	1 : 7.0	1 : 1.4
(14)	116.1	—	8.1	108.0	8.1	—	12.15	24.3	9.45	1 : 14.3	1 : 2.0
(15)	121.5	—	10.8	110.7	9.45	—	13.5	26.65	11.34	1 : 11.25	1 : 1.9
(16)	56.7	—	10.8	45.9	8.64	—	10.8	16.2	6.75	1 : 5.25	1 : 1.5
(17)	94.5	—	8.1	86.4	10.8	—	14.04	27.54	13.5	1 : 11.6	1 : 1.9
(18)	85.05	—	8.1	76.95	8.1	—	12.15	24.3	12.15	1 : 10.5	1 : 2.0
(19)	66.15	—	9.45	56.7	8.1	—	9.45	18.9	12.15	1:7.0	1 : 2.0
(20)	72.9	—	10.8	62.1	5.4	—	12.15	22.95	11.34	1 : 6.75	1 : 1.8

2nd, 1984. Paratype; many, on the above-numbered slide and on other slides; other particulars are the same as for the holotype material.

Affinities. The gregarine possesses satellite without septum and spherical or rounded cyst dehiscing by simple rupture. As such, it can at once be placed in the genus *Didymophyes* Stein, 1848 under the family *Didymophyidae*. Its LP:TL and WP:WD values closely resemble those of *D. paradoxa* Stein, 1848 and *D. minuta* (Ishii) Watson, 1916 respectively. Its primite is larger than the satellite, a feature which is very similar to that of *D. longissima* (Von Siebold) Frantzius, 1848. However, the present form differs these three species in all other characters. Its egg shaped or conical epimerite and spherical spores distinguishes it from all other described species under the genus. The parasite is thus, given a separate species status and is named *D. lipai* sp. n., after the eminent Polish gregarinologist, J. J. Lipa.

Didymophyes rigidus sp. n.

Host: *Oryzaephilus mercator* (F.) from rice grain.
Incidence: 33% of the hosts are infected with this gregarine.

Development: Intracellular.

Trophozoite. Vase-like, measuring 23.8 μm × 10.8 μm; epimerite papilla-like, it becomes disc-like with prominent ridges radiating from central area when viewed from the top (Fig. 3 *1–3*). Protomerite is rectangular or hemispherical and is broader than long. Deutomerite is rectangular or cylindrical in shape. Cytoplasm is uniformly granulated throughout protomerite and deutomerite.

Sporadin and association. Solitary, biassociative. Young sporadin is ovoidal in shape (Fig. 3 *4*) with hemispherical protomerite and obese deutomerite. The mature sporadin is cylindrical (Fig. 3 *5* and *6*), consists of dome-shaped protomerite and cylindrical deutomerite.

During association the posterior end of the primite firmly fits in the cup-like depression at the anterior end of the satellite (Fig. 3 *9–11*). The protomerite of the primite is hemispherical while it is absent in satellite. The posterior end of the deutomerite of the primite is rounded while that of the satellite is gradually narrower (Fig. 3 *7*, *8*).

Gametocyst and spore. Gametocysts are opaque white in colour, spherical (Fig. 3 *12*) measuring 49.3 μm × 46.6 μm. As the cyst develops inside the moist chamber, the inner mass separates off from the outer wall so that a clear area is seen around the latter. After 48 h spores are liberated by simple rupture of the cyst. Spores are smooth, ellipsoidal (Fig. 3 *13*) measuring 3.0 μm × 2.2 μm. Eight sporozoites are arranged in two rows inside each spore.

Measurements. The summary of measurements of 20 specimens of trophozoites and sporadins with the mean within parenthesis is given in Table 1. Details of measurements of individual specimens are given in Table 4.

Material. Holotype; Trophozoite on slide No. KP/1, prepared from the contents of the midgut of the coleopteran insect, *Oryzaephilus mercator* (F.) from rice collected at Kanchrapara, West Bengal, India by S. Gupta on 13th April, 1983. Paratype; many, on the above numbered slide and on other slides; other particulars are the same as for the holotype material.

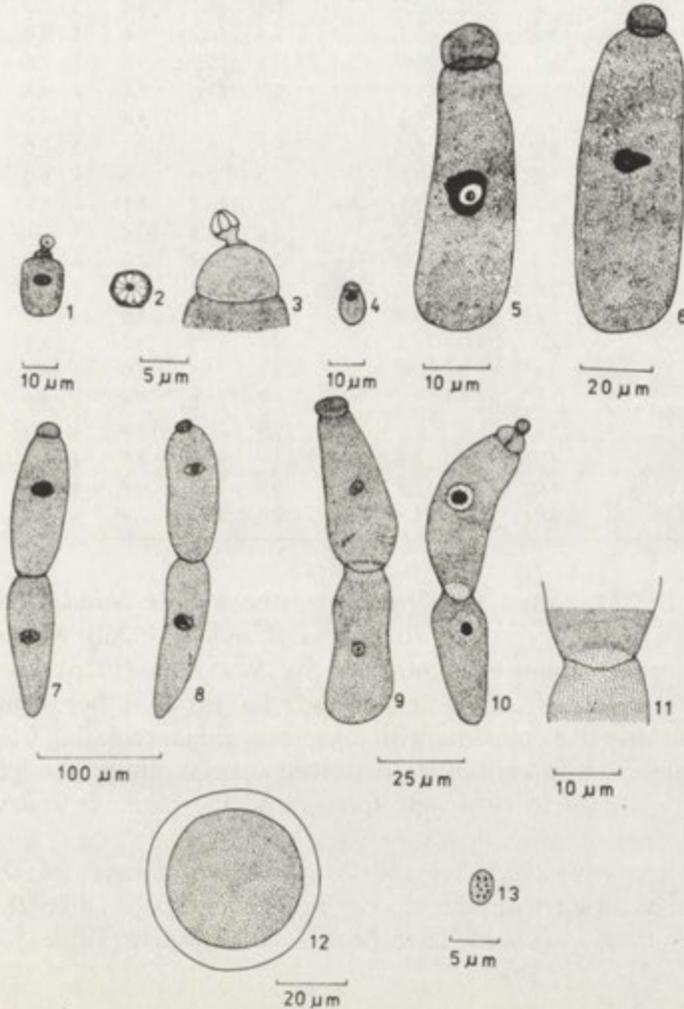Affinities. There can be no doubt about the placement of the gregarine under the genus *Didymophyes* Stein, 1848 as its satellite lacks a septum during

Fig. 3. *1–13*. Camera lucida drawings of *Didymophyes rigidus* sp. n. *1* — Trophozoite, *2* — Enlarged polar view of the epimerite, *3* — Anterior part of trophozoite, *4* — Young sporadin, *5–6* — Mature sporadins, *7–9* — Sporadins in association, *10* — Sporadins in association, primite still bearing an epimerite, *11* — Portion of caudo-frontal association, *12* — Gametocyst, *13* — A spore with eight sporozoites

Table 4

Showing details of measurements (in microns) of the different parts of 20 specimens of *Didymophyes rigidus* from the adults of *Oryzaephilus mercator* (F.)

Sl. No.	TL	LE	LP	LD	LN	WE	WP	WD	WN	LP : TL	WP : WD
(1)	23.8	4.9	2.7	16.2	2.7	5.4	5.4	10.8	5.4	1 : 8.8	1 : 2.0
(2)	37.8	5.4	5.4	27.0	5.4	4.3	6.8	16.2	6.8	1 : 7.0	1 : 2.4
(3)	48.6	4.0	5.4	39.2	5.1	3.2	9.4	14.2	5.4	1 : 9.0	1 : 1.5
(4)	51.3	5.4	6.8	39.1	5.4	5.4	9.5	16.2	5.4	1 : 7.5	1 : 1.7
(5)	50.8	4.9	5.4	40.5	5.4	5.4	9.5	17.6	5.4	1 : 9.4	1 : 1.8
(6)	10.8	—	2.7	8.1	2.2	—	5.4	8.1	3.4	1 : 4.0	1 : 1.5
(7)	47.2	—	6.7	40.5	8.1	—	8.1	15.7	7.4	1 : 7.0	1 : 1.9
(8)	32.4	—	5.4	27.0	4.8	—	7.5	13.5	5.9	1 : 6.0	1 : 1.8
(9)	72.9	—	5.4	67.5	8.1	—	8.1	16.2	8.1	1 : 13.5	1 : 2.0
(10)	86.4	—	8.1	78.2	5.4	—	·10.8	32.4	10.8	1 : 10.6	1 : 3.0
(11)	13.5	—	2.7	10.8	2.4	—	5.4	5.4	2.4	1 : 5.0	1 : 1.0
(12)	28.4	—	5.4	23.0	3.2	—	4.0	10.8	4.0	1 : 5.3	1 : 2.7
(13)	31.0	—	5.4	25.6	4.6	—	8.1	15.7	4.9	1 : 5.7	1 : 1.9
(14)	33.0	—	4.0	29.0	5.4	—	8.1	12.2	5.4	1 : 8.3	1 : 1.5
(15)	35.1	—	5.4	29.7	4.0	—	8.1	15.5	4.0	1 : 6.5	1 : 1.5
(16)	51.3	—	5.4	45.9	5.4	—	6.8	10.8	5.4	1 : 9.5	1 : 1.6
(17)	94.5	—	8.1	86.4	8.1	—	13.5	37.1	13.5	1 : 11.7	1 : 2.8
(18)	22.9	—	4.0	18.9	5.4	—	5.4	12.2	5.4	1 : 5.7	1 : 2.3
(19)	21.6	—	4.0	17.6	2.7	—	6.8	12.2	6.8	1 : 5.4	1 : 1.8
(20)	78.3	—	5.4	72.9.	4.8	—	9.4	18.9	5.4	1 : 14.5	1 : 2.0

syzygy. Its LP : TL values closely resemble those of *D. paradoxa* Stein, 1848. The association is very similar to that of *D. minuta* (Ishii) Watson, 1916 as evidenced from the illustrations given by Watson (1916). However, the present form differs from these two species in all other characters. Its characteristic disc-like epimerite with prominent ridges radiating from a central area separates it from all other described species under the genus. It is, therefore, considered to be a new species and the name *D. rigidus* sp. n. is proposed for it.

The comparative characters of the three new species of *Didymophyes* described here along with the closely resembling species like *D. paradoxa*, *D. minuta* and *D. longissima* have been summarized in Table 5.

Diagnosis

Didymophyes oryzaephilae sp. n.

Trophozoite vase-like; epimerite small, cashew nut like; sporadins in syzygy;

gametocysts spherical; dehiscence by simple rupture; spores double walled, smooth, ellipsoidal; early development intracellular.

$$LP:TL = 1:4.5–12.3 \ (7.8);$$

$$WP:WD = 1:1.3–2.9 \ (1.9).$$

Didymophyes lipai sp. n.

Trophozoite ovoidal; epimerite egg-shaped or conical with pointed anterior end; sporadins in caudo-frontal association; gametocysts rounded; dehiscence by simple rupture; spores double walled, spherical; early development intracellular.

$$LP:TL = 1:3.0–14.3 \ (7.1);$$

$$WP:WD = 1:1.3–2.3 \ (1.7).$$

Didymophyes rigidus sp. n.

Trophozoite vase-like; epimerite small, papilla-like; sporadins in association; gametocysts spherical; simple rupture; spores smooth ellipsoidal; early development intracellular.

$$LP:TL = 1:4.0–14.5 \ (8.02);$$

$$WP:WD = 1:1.0–3.0 \ (1.96).$$

Discussion

The role of food of arthropods in gregarine infections was studied by various authors earlier and has been summarized by Lipa (1967) in his classical monograph. The author is of the opinion that high density of insects and individuals of various generations is the reason for frequent infection by a variety of protozoans, and the kind of food, phylogeny, pH of gut, etc. play a definite role in the incidence of gregarines in their insects. In our recent studies we have observed that the larva of *Tribolium castaneum* is infected with a gregarine, *Eliptocystis triboli* (Sengupta et al. 1986) while the adult of the same host with another gregarine, *Hirmocystis oxeata* (Ghose et al. 1986). This may be due to the fact that insects after metamorphosis change the environment they inhabit as larvae, since with the change of environment a change of food and a reaction of gut contents is frequently connected, as suggested by Lipa (1967). Sengupta and Haldar (1986) also came to a similar conclusion from their studies on gregarine parasite from insects.

It will be revealed from this communication that the three species differ

Table 5

Showing the comparative characters of *D. paradoxa, D. minuta, D. longissima, D. oryzaephilus, D. lipai* and *D. rigidus*

Characters	*Didymophyes paradoxa* Stein, 1848	*D. longissima* (Von Siebold) Frantzius, 1848	*D. minuta* (Ishii) Watson, 1916	*Didymophyes oryzaephilae* sp. n.	*Didymophyes lipai* sp. n.	*Didymophyes rigidus* sp. n.
Total length	—	—	188 µm in length	121.5 µm in length	108 µm in length	94.5 µm in length
Epimerite	—	—	—	The epimerite is egg-shaped or conical with pointed anterior end	Small, cashew-nut like; when viewed from the top it appears as a disc	Papilla-like, it becomes disc-like with prominent ridges radiating from central area when viewed from the top
Protomerite	Dome-shaped, considerably flattened; twice as wide as high; a little wider than deutomerite	—	Somewhat flattened; twice as wide as high	Hemispherical, ovoidal or hat-shaped	Rectangular	Rectangular or hemispherical and is broader than long
Deutomerite	Primite: Cylindrical; same or 1.5 times longer than satellite; satellite: tapering to a blunt point	Primite longer than satellite	Cylindrical in both primite and satellite; equal in length; posterior end broadly rounded in satellite	Primite larger than satellite. Cylindrical to ovoidal in both primite and satellite	Primite larger than satellite. Deutomerite of the primite ends blindly whereas in satellite it gradually narrows, posteriorly	Posterior end of the primite firmly fits in the cup like depression at the anterior end of the satellite. Deutomerite of the primite is rounded while that of the satellite is gradually narrower

Nucleus	Spherical; large	Spherical with one karyosome	Spherical with one large karyosome	Egg-shaped or rhomboidal with nuclear membrane and an endosome	Elliptical or spherical in shape with prominent nuclear membrane	Ovoidal to elliptical
Sporadin	Biassociative; short	Elongate cylindrical	Biassociated; elongated	Solitary as well as biassociative; elongated, cylindrical to obese in shape	Solitary as well as bi-associative; cylindrical	Solitary, biassociative; Ovoidal to cylindrical
Gametocyst	—	—	—	Rounded	Spherical	Spherical
Spore	—	—	—	Spherical	Ellipsoidal	Ellipsoidal
LP : TL	1 : 7 to 1 : 9	1 : 30	1 : 23	1 : 30–14.3 (7.1)	1 : 4.5–12.3 (7.8)	1 : 8.02
WP : WD	1 : 1 to 1 : 1.1	1 : 1	1 : 1.5	1 : 1.3–2.3 (1.7)	1 : 1.3–2.9 (1.9)	1 : 1.96
Host	Geotrupes sp. and G. stercorarius (L.)	Gamarus pulex	Tribolium ferrugineum (F)	Oryzaephilus mercator (F.)	Oryzaephilus mercator (F.)	Oryzaephilus mercator
Locality	Berlin and Poitiers	Germany	Japan	India	India	India

conspicuously in their morphological and morphometrical characters, and as such, leave no doubt in erecting new species status for them. The variations may be attributed to the change of their food habits. The role of the gregarines in damaging the stored products is now under investigation in this laboratory.

ACKNOWLEDGEMENTS

REFERENCES

Ghose S., Sengupta T. and Haldar D. P. 1986: Two new Septate Gregarines (*Apicomplexa: Sporozoea*), *Gregarina basiconstrictonea* sp. n. and *Hirmocystis oxeata* sp. n. from *Tribolium castaneum* (Herbst). Acta Protozool., 25, 93–108.

Lipa J. J. 1967: Studies on Gregarines (*Gregarinomorpha*) of arthropods in Poland. Acta Protozool., 5, 97–179.

Sengupta T. and Haldar D. P. 1986: Does food habit of an host play any role in determining its protozoan parasitic fauna? Proc. Fifth Nat. Zool. Conf., Bangladesh.

Sengupta T., Ghose S. and Haldar D. P. 1986: The biology of *Eliptocystis triboli* n. gen., n. sp. infecting the larvae of *Tribolium castaneum* (Herbst) in walnuts. Arch. Protistend., (in press).

Sprague V. 1941: Studies on *Gregarina blattarum* with particular reference to the chromosome cycle. Ill. Biol. Monogr., 18, 5–57.

On the Occurrence of *Isospora sibporensis* sp. n. (*Protozoa: Apicomplexa: Eimeriidae*) from a Common House Rat, *Rattus rattus arboreus* (Horsefield) from West Bengal, India

S. BANDYOPADHYAY, R. NANDI and A. K. DAS

Zoological Survey of India, 8, Lindsay Street, Calcutta-700087, India

Synopsis. A new species, *Isospora sibporensis* has been described from a common house rat, *Rattus rattus arboreus* (Horsefield) in West Bengal, India and compared with all the previously recorded isosporan species of rats.

Only three species of *Isospora* have been reported so far from rats. Levine and Ivens (1965) described *I. ratti* from *Rattus norvegicus* in the United States of America. Later Kshirsagar (1980) reported *I. aurangabadensis* and *I. krishnamurthyi* from *Rattus rattus rattus*[1] in India. During the course of our investigation of protozoan parasites of small mammals, another species was recorded from the common house rat, *Rattus rattus arboreus* in West Bengal, whose taxonomic account is presented.

Materials and Methods

Specimens were collected from various parts of India by using field traps and baits; those belonging to suborder *Myomorpha* (includes rats and allied rodents) were collected from the field and human settlements. Fresh faecal samples were suspended in physiological saline and subjected to direct examination. In the positive cases, the faecal material was strained to eliminate debris and placed in 2.5% potassium dichromate solution for subsequent sporulation of the oocysts. Sporulation time was recorded. Both the unsporulated and sporulated oocysts were studied under low power and under oil immersion lens. Camera lucida drawings were made. Measurements were taken with an ocular micrometer.

---

[1] Identity of this subspecies of rat is doubtful as it does not occur in India in natural state

Description

Isospora sibporensis sp. nov.
(Fig. 1)

Type-host: *Rattus rattus arboreus* (Horsefield)
Type-locality: Sibpore, Dist. Howrah, West Bengal, India
Site of infection: Oocytes obtained in the faeces
Life cycle: Unknown

The oocysts are subspherical to spherical in shape and measure 21–24.75 μm by 19.5–22.5 μm (average 22.05 μm × 20.7 μm). The oocyst wall is yellowish in colour and 1 μm (average) in thickness. It consists of two layers of equal thickness. Oocysts possess distinct micropyle with micropylar cap and

10 μm

Fig. 1. Camera lucida drawing of *Isospora sibporensis* sp. n. (Sporulated oocyst)

polar body. No oocyst residuum is noticed. Sporocysts are ovoid bearing a conspicuous steida body and measure 12–15.75 μm by 9–11.25 μm (average 14.1 μm × 10.5 μm). Sporocysts are two in number, each with four sporozoites. Sporozoites are somewhat broad and elongate in shape measuring 4.5–6 μm by 2.45–3 μm (average 5.4 μm × 2.85 μm). They have neither refractile area nor definite pattern of orientation. Sporocyst residuum is prominent, its course granules are scattered irregularly. Sporulation time varies from 36 to 48 h at room temperature (35–37°C).

Diagnosis

Oocysts subspherical to spherical measuring 21–24.75 μm × 19.5–22.5 μm (average 22.05 μm × 20.75 μm). Oocyst wall yellowish, bilayered and 1 μm thick. Polar body present but oocyst residuum absent. Sporocysts ovoid with steida body, ranging from 12–15.75 μm × 9–11.25 μm (average 14.1 μm × 10.5 μm). Sporozoites broad and elongate and measure 4.5–6 μm × 2.45–3 μm (average 5.4 μm × 2.85 μm). Sporocyst residuum conspicuous and in the form of course granules.

Table 1

Comparison of *Isospora sibporensis* sp. n. with related species

Isospora species	Oocyst characters					Sporocyst characters			
	Shape and size	Wall	Micropyle	Polar granule	Residuum	Shape and size	Stieda body	Sporozoites	Residuum
I. ratti Levine and Ivens (1965)	Subspherical, 22-24 μm × 20-21 μm	Monolayered, smooth, 1 μm in thickness, pale tan to tan in colour	−	+ (two in number)	−	Asymmetrical, broadly ovoid, 16 μm × 11 μm	+	Exhibited no particular orientation	+ (Compact)
I. aurangabadensis Kshirsagar (1980)	Spherical or subspherical, 32-44 μm × 32-40 μm (mean 35.46 × 33.96 μm)	Monolayered, 1.5-2 μm in thickness, yellowish brown in colour	−	+ (one in number)	−	Oval, globose or elongate, 18-24 μm × 12-15.2 μm (mean 19.86 × 12.96 μm)	+	Exhibited a refractile body at the broader end	+ (As ovoid, granular, central mass)
I. krishnamurthyi Kshirsagar (1980)	Spherical or slightly ovoid, 36-48 μm × 35.2-40 μm (mean 42.40 × 37.10 μm)	Monolayered, 1.8 μm in thickness, pale yellowish in colour	−	+ (one in number)	−	Elongated or ovoid, 20.8-26 μm × 17.6-21.5 μm (mean 23.64 × 19.25 μm)	+	Sporozoites showed no refractile body	+ (In the form of few coarse granules)
I. sibporensis sp. n.	Subspherical to spherical, 21-24.75 μm × 19.5-22.5 μm (mean 22.5 × 20.7 μm)	Bilayered, total thickness 1 μm, yellowish in colour	+ (with micropylar cap)	+ (one in number)	−	Ovoids, 12-15.75 μm × 9-11.25 μm (mean 14.1 × 10.05 μm)	+	Sporozoites without any refractile body, exhibited no definite pattern of orientation	+ (In the form of scattered coarse granules)

http://rcin.org.pl

Remarks

In Table 1, the present species, *Isospora sibporensis* sp. n., has been compared in detail with all the three earlier recorded species of *Isospora* from rats.

The Table itself is explanatory. It clearly reveals that *I. sibporensis* sp. n. sufficiently differs from all the other isosporan species of rats described so far, particularly, in the presence of micropyle with micropylar cap and bilayered oocyst wall. During the present investigation 208 specimens of rats belonging to the genus *Rattus* were examined for coccidian parasites, of which only one was found infected with the present parasite.

REFERENCES

Kshirsagar H. S. 1980: Two new species of *Isospora* Schneider, 1881 from rats in India. Arch. Protistenkd., 123, 267–271.
Levine N. D. and Ivens V. 1965: The coccidian parasites (*Protozoa : Sporozoa*) of rodents. Illinois-Biol. Monogr., 33, 1–365.

Oocysts of Six New *Coccidiomorpha* Species from Pinnipeds of King George Island (South Shetlands, Antarctic)

Jan DRÓŻDŻ

W. Stefański Institute of Parasitology, Polish Academy of Sciences,
3 Pasteur Str., P. O. Box 153, 00-973 Warszawa, Poland

Synopsis. A coproscopical examination of samples of faeces of 5 species of pinnipeds inhabiting King George Island (South Shetlands) revealed the occurrence of oocysts of six new *Coccidiomorpha* species. Specific names are given to three more frequent and numerous species for which a process of sporulation has been observed. The following species were found: *Isospora mirungae* sp. n. in young sea elephants (*Mirunga leonina*), *Eimeria* sp. 1 in crab eater (*Lobodon carcinophagus*) and *Eimeria weddelli* sp. n., *E. arctowskii* sp. n., *Eimeria* sp. 2 and *Eimeria* sp. 3 in Weddell seal (*Leptonychotes weddelli*). In sea-leopard (*Hydrurga leonina*) and eared seal (*Arctocephalus gazella*) no coccidian parasites have been found.

Eimeria phocae Hsu et al., 1974 is the only one *Coccidiomorpha* species hitherto described from Antarctic. It was found in two young individuals of *Phoca vitulina concolor* caught in 1972 in Portland (Hsu et al. 1974, Howard et al. 1981). This situation has inclined the author to undertake investigation on the occurrence of coccidian oocysts in faeces of marine pinnipeds from King George Island during the whole year.

Materials and Methods

A segment of the sea shore of King George Island, from Point Thomas to Patelnia was chosen for an investigation area. On this territory observations on seals and eared seals were made and samples of faeces were collected to be examined in the laboratory. They were collected in monthly intervals from April 1981 to January 1982. In the same time observations of seals and collection of samples of their faeces were made every week on the territory from Point Thomas to Ecology Glacier. Besides, several surveys were made along the shores of Keller Penninsula and Point Henneguin in order to collect samples.

Collected samples of fresh faeces were subjected to flotation with saturated NaCl and centrifuged. The oocysts were measured and photographed. Cultures of the oocysts were maintained by an addition of 1–2% potassium bichromate to small amount of faeces. They were kept at room temperature (5–10°C).

A total of 474 samples were examined from all pinniped species inhabiting King George Island. The number of samples from particular host species was as follows:

(1) *Mirunga leonina* — 320 samples from all months of the investigation period,

(2) *Leptonychotes weddelli* — 65 samples from all months,

(3) *Lobodon carcinophagus* — 43 samples from August and September 1981,

(4) *Hydrurga leptonyx* — 4 samples from December 1981 and January 1982,

(5) *Arctocephalus gazella* — 42 samples from April and June 1981 and January 1982.

Results

Six species of oocysts were found. As all the oocysts clearly differed from *Eimeria phocae*, the only one known species of marine pinnipeds, they were regarded as new species. The names *Isospora mirungae* sp. n., *Eimeria weddelli* sp. n. and *Eimeria arctowskii* sp. n. are given to more frequently occurring and numerous species. The remaining three species occurred rarely in examined samples of faeces and their sporulation process was not observed. They are described as *Eimeria* sp. 1, *Eimeria* sp. 2 and *Eimeria* sp. 3.

Isospora mirungae sp. n. (Pl. I 1)

Oocysts ellipsoidal or egg-shaped, greyish transparent in colour. Oocyst wall smooth, bilayered, thin. Zygote spherical. Oocyst dimensions 18–22 × 12–14 μm, mean 20.2 × 12.2 μm. Width to length ratio 1.65. Sporulation time 6–10 days. Sporocysts spherical, 8–10 μm in diameter.
Host: *Mirunga leonina*

Eimeria weddelli sp. n. (Pl. I 3)

Oocysts egg-shaped or ellipsoidal yellowish brown. Oocyst wall smooth, bilayered, about 2 μm thick. Micropyle well marked, about 3 μm in diameter. Zygote spherical. Oocyst dimensions 45–52 × 28–30 μm, mean 48.1 × 29.6 μm. Width to length ratio 1.62. Sporulation time 9–10 days. Sporocysts 12–16 μm long.

Host: *Leptonychotes weddelli*

Eimeria arctowski sp. n. (Pl. I 4)

Oocyst ellipsoidal or barrel-shaped, yellowish brown. Cyst wall bilayered, 2 μm thick, covered with numerous small protuberances on the whole surface. A funnel shaped invagination of the cyst wall occurs around the micropyle. Micropyle well marked, 5 μm in diameter. Zygote elliosoidal. Oocyst dim-

ensions 44–48 × 24–30 μm, mean 46 × 28.1 μm. Width to length ratio 1.64. Sporocyst length 14–18 μm. Sporulation time 7–10 days.

Host: *Leptonychotes weddelli*

Eimeria sp. 1 (Pl. I 2)

Oocysts egg-shaped, yellowish grey. Oocyst wall smooth, bilayered, thin. Micropyle not visible. Oocyst dimensions 38–40 × 24–25 μm, mean 30 × 24.3 μm. Width to length ratio 1.60.

Host: *Lobodon carcinophagus*

Eimeria sp. 2 (Pl. I 5)

Oocyst ellipsoidal cherry-orange in colour. Oocyst wall bilayered, thin, wrinkled. Micropyle absent. Zygote fills almost the whole space inside the cyst wall. Oocyst dimensions 22–31 × 14–22 μm, mean 25.7 × 18.8 μm. Width to length ratio 1.37.

Host: *Leptonychotes weddelli*

Eimeria sp. 3 (Pl. I 6)

Oocysts spherical in shape, grey. Oocyst wall smooth, thin. Micropyle absent. Zygote fills almost the whole space inside the cyst. Oocyst diameter 25–33 μm.

Host: *Leptonychotes weddelli*

Oocysts of *I. mirungae* sp. n. were found in 4 out of 8 examined young sea elephants, 3 months old, born on King George Island. In all cases mass infection occurred. In adult sea elephants no oocysts were found despite a great number of samples examined. Single oocysts of *Eimeria* sp. 1 were found in 6 crab-eaters. The remaining 4 species were found in Weddell seal: *E. weddelli* sp. n. in 14 samples, *E. arctowskii* sp. n. in 10 samples, *Eimeria* sp. 2 in 5 samples and *Eimeria* sp. 3 in 3 samples. Oocysts of the two first mentioned species were usually numerous or very numerous, while those of *Eimeria* sp. 2 and *Eimeria* sp. 3 occurred always singly and in seals harbouring Keller Peninsula and Point Henneguin. Season of the year had no bearing on the prevalence and intensity of infestation of Weddell seal.

In samples of faeces from *Hydrurga leptonyx* and *Arctocephalus gazella* no oocysts were found.

REFERENCES

Howard E. B., Britt J. O. I. and Matsumaoto J. 1981: Parasitic disease. In: Pathobiology of Marine Mammal Diseases. Vol. 1, (ed. Howard E. B.), Boca Raton, Florida, USA, CRS Press Inc., 119–232.

Hau C. K., Melby E. C. and Altman N. H. 1974: *Eimeria phocae* sp. n. from the harbor seal (*Phoca vitulina concolor*). J. Parasitol., 60, 399–402.

J. Dróżdż

auctor phot.

BOOKS RECEIVED

Ciliés libres de l'Afrique intertropicale. Introduction à la connaissance et à l'étude des Ciliés. Jean DRAGESCO et Armelle DRAGESCO-KERNÉIS, avec le concours de Ghislaine FRYD-VERSAVEL. Éditions de l'ORSTOM, Institut Francais de Recherche Scientifique pour le Dèveloppement en Coopération, Collection Faune Tropicale No XXVI, Paris, 1986, pp. 559, 154 pl. avec 1136 dessins.

Table des matières: Préface de J. O. Corliss, Résumé, Summary. Introduction.

Généralités. Dèfinition des Infusoires ciliés, Structure des Ciliés, Écologie des Ciliés, Biologie des Ciliés, Systématique des Ciliés, Définitions succinctes des principaux termes utilisés en ciliatologie, Récoltes, cultures, techniques de préparation et d'observation des Ciliés, Étude des préparations extemporanées ou définitives.

Étude systématique des Ciliés d'Afrique intertropicale. Carte des lieux de récolte des Ciliés, Liste des abréviations utilisées dans l'illustration, Classe des *Kinetophragminophora*, Classe des *Oligohymenophora*, Classe des *Polyhymenophora*, Bibliographie, Table alphabétique des genres et espèces décrits, Table des matières

Systematics of Protozoa and their Phylogenetic Links with Lower Eukaryotes. Editor M. V. KRYLOV. Proceedings of the Zoological Institute. Vol. 144, Leningrad, 1986, pp. 116, 14 pl.

Contents. Introduction. Starobogatov Ya. I. — On the Number of Kingdom of Eucaryotic Organisms, Raikov I. B. — Pathways of Evolution of the Mitotic Apparatus in Lower Eukaryotes, Belova L. M. — Polymorphism of Malat Dehydrogenase (E.C.1.1.1.37) at *Blastocrithida gerricola* and its Significance for Taxonomy, Podlipaev S. A. — *Phytomonas elmassiani* (*Mastigophora* : *Trypanosomamonida*) from the Plants *Cynachum sibiricum* (*Asclepiadaceae*) in the Middle Asia and Kazakhstan, Mikhalevich V. I., Rodionova M. K. and Siniakova G. N. — The Wall Ultrastructure of the Two Agglutinated Foraminiferal Genera with the Quinqueloculine Type of the Shell, Jankowski A. W. — New and Little Known Genera of Ciliated Protozoa (phylum *Ciliophora*), Lukashenko N. P. and Rybakova Z. I. — To Genetics of Populations and Evolution of Infusoria *Tetrahymena* and *Paramecium*, Seravin L. N. — The Nature and Origin of Spongia, Abstracts

NOTICE TO AUTHORS

ACTA PROTOZOOLOGICA is intended as a journal serving for the publication of original papers embodying the results of experimental or theoretical research in all fields of protozoology with the exception of faunistic notices of the local character and purely clinical reports. The papers should be concise as will not be accepted if they have been previously published elsewhere. After acceptance by the Editors papers will be printed in the possibly shortest time.

Papers are accepted in English, French, German and Russian. Every paper should begin with the name and postal address of the laboratory, name and the surname of the author and title in the language of the text. The titles in German and French should be translated into English according to the demands of Current Contents. The paper should be accompanied by synopsis in the language of the text not exceding 100 words and by short summary in one of 4 languages accepted in the Journal. In the Russian text also the name and the postal address of the laboratory, legends of tables, plates and text illustrations must be translated, the translation of the summary may be somewhat more extensive, and the name of the authors should be given additionally also in the Latin characters.

Manuscripts should be doublespaced typescript (30 lines one side of a sheet) with a normal margin. No elements of the text should be fully typed in capitals nor in spaced set (only underlining with pencil is admittable). In decimal fractions points not commas should be used. The generally accepted abbreviations and symbols are recommended. Nomenclature must agree with the International Code of Zoological Nomenclature, London 1961. The original and one carbon copy of the whole text material should be supplied.

References must be cited in the text indicating only the authors and year thus:

"Kinosita (1954) found that, etc."

Only all references cited in the text should be listed. The list must be arranged as follows:

Ehret C. F. and Powers, E. L. 1959: The cell surface of *Paramecium*. Int. Rev. Cytol., 8, 97–133.

Gelei J. von 1939: Das aussere Stutzgerustsystem des Parameciumkörpers. Arch. Protistenk., 92, 245–272.

Titles of references are given in their original language (not translated). In papers written in English, French or German, the Cyrillic type of the Russian is transliterated according to the international system (ISO Recommendation R 9 September 1954). This regulation is not applied to names if there exists in their traditional spelling. Also author may freely choose in transliteration of his own name. In Russian papers, the Russian references are cited in Cyrillic, the others in the Latin characters, but they must be listed all together in the Latin alphabetical order.

The following material should be supplied on separate sheets: (1) the running title for the page headlines, (2) tables, (3) legends for text figures, (4) legends for plates. Line-drawings will be published in the text, photographs and raster figures on separate plates. No coloured photographs can be published presently. Lettering on photographs and drawings should be marked in pencil. With no regard to the language of the text, only the Latin lettering, arabic numerals or generally accepted symbols are admittable for marking on illustrations. Numbering of text-figures, plates and tables must be also marked in pencil, as well in the legends as in the text.

Galley proofs are sent to the authors. Authors receive 75 reprints without covers.

In preparation

L. N. S e r a v i n and A. V. G o o d k o v: *Euhyperamoeba fallax* Seravin et Goodkov, 1982 (*Lobosea, Gymnamoebia*) — Multinucleate Marine Limax Amoeba — Morphology, Biological Peculiarities and Systematic Position — Z. Ž i ž k a: Ultrastructure of Trophozoites and Cysts of the Amoeba *Malamoeba locustae* King et Taylor, 1936, Parasitizing the Locust *Locusta migratoria* R. et F. — A. C z a p i k et J. F r y d a: L'etude sur la morphologie et stomatogenése de *Spathidium muscicola* Kahl, 1930 — N. R i c c i, C. M i c e l i and R. G i a n n e t t i: The Ethogram of *Euplotes crassus* (*Ciliata, Hypotrichida*) II. The Paralyzed Mutant — W. K a s p r z a k, J. W i n i e c k a and A. C. M a j e w s k a: Antigenic Differences among *Giardia intestinalis* Isolated from One Geographic Area — D. V. R. K. P r a s a d and C. K a l a v a t i: Morphology and Life-cycle of Two New Species of *Leptomonas, L. indica* sp. n. and *L. bakeri* sp. n. from Lygaeid Insects — R. S. T a n d o n, R u p L a l and V. V. S. N a r a y a n a R a o: Effects of Malathion and Endosulfan on the Growth of *Paramecium aurelia* — T. M i c h a ł o w s k i, I. L a n d a, P. M u s z y ń s k i and P. S z c z e p k o w s k i: The Influence of Non-Protein-Nitrogen on the Growth of Rumen Ciliate *Entodinium caudatum in vitro* — T. M i c h a ł o w s k i: The Volatile Fatty Acids Production by Ciliate Protozoa in the Rumen of Sheep — Books Received

CONTENTS