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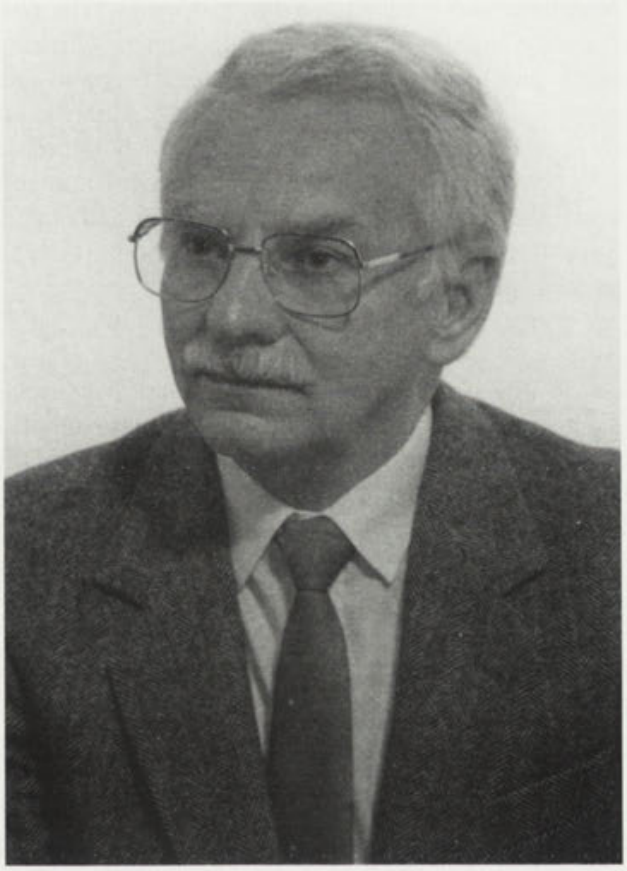

Witold Kasprzak
(1927-1996)

Professor Witold Kasprzak suddenly died on 13th November 1996. He was a world known protozoologist and parasitologist, an outstanding expert in protozoan parasites of man, especially amoebae and giardia.

Born on 29 July 1927 in Leszno Wielkopolskie, he studied biology at Mathematics and Natural Sciences Faculty of Poznań University in 1947-1951. As a student of the IVth course he undertook work in the Laboratory of Biology and Parasitology of the Medical Academy in Poznań under the guidance of Professor Czesław Gerwel, a well known Polish parasitologist. After the first research on epidemiology, carried out with the staff of the Laboratory, he concentrated his interest in parasitic protozoans of man and the diseases caused by them. The subject of his doctor's thesis (1960) - *Entamoeba histolitica* and amoebiosis caused by it, was later continued in the thesis for doctor of sciences degree (1967). Simultaneously, dr Kasprzak was studying medicine being graduated from the Academy of Medicine in Poznań in 1965. In 1974, after the death of Professor Gerwel, he became the director of the Laboratory of Biology and Medical Parasitology in the Institute of Biostructure of the Medical Academy in Poznań. In 1977 he became the Associate Professor and in 1986 the Full Professor.

The first object of dr Kasprzak's study was *Entamoeba histolitica*. The study began from epidemiology of amoebosis caused by this protozoan, and led through experimental research on biology to the recognition of the character of particular strain and its infection ability. Soon around dr Kasprzak his future collaborators gathered and more and more ambitious research with many aspects was carried by this team. One of such aspects were free living amoebae of the genus *Naegleria* and their pathogenic properties. The research aimed at recognition of pathogenic properties in already known isolates of this amoeba as well as searching for new pathogenic strains in natural environment. The research has been expanded to other pathogenic amoebas, among others to the representatives of the genus *Acanthamoeba*. These investigations have attracted attention to the Poznań research center and yielded many cooperations contacts with various research laboratories in the world.

During the succeeding years, Professor Kasprzak and his collaborators commenced the studies on flagellates of the genus *Giardia*. They ascertained the zoonotic character of giardiasis and obtained much valuable information about this parasite. During the recent years Professor Kasprzak undertook research on cryptosporidian parasites, however only primary results have been obtained before his death.

In general, Professor Kasprzak published 61 original papers, 67 abstracts and 55 articles and surveys. Several further works are still in press.

Summing up scientific activity of Professor Kasprzak and his team one is stricken up by many aspects touched in these investigations and their actuality. The biochemical and molecular methods were used as well as methods for genetic differentiation of parasites. Professor Kasprzak was a good chief of large and well differentiated team so many broad projects might have been realized. He was a good teacher and Master for his students and collaborators. He promoted 5 doctoral and 5 post-doctoral dissertations as well as a dozen of master's theses. Professor Kasprzak was found of his collaborators being precise in work but not hard. His great laboriousness and perfectionism were connected with great friendliness and readiness to help. He joined wisdom with kindness and humor with great optimism.

Professor Kasprzak carried out broad international cooperation - the list of institutions engaged is long; among others it embraces Center for Disease Control, Atlanta, USA; Institute of Hygiene and Epidemiology, Belgium; Rijkinstitut voor Volksgezondeheid en Milieuhygiene, Bilthoven, Holland etc. For many of them the death of Professor Kasprzak will be a great loss.

Professor Kasprzak was also a excellent teacher; in lectures on general and medical parasitology he transmitted modern and reliable knowledge to his students. He was a good preceptor of young men.

He fulfilled also many academical duties acting as pro-dean of the Faculty of Medicine and the member of Senate of K. Marcinkowski Medical Academy in Poznań. He was acting also as a member of many commissions for parasitic and tropical diseases and commission for teaching parasitology in the country. He was also an active member of WHO Expert Advisory Panel on Parasitic Diseases.

Professor Kasprzak was an active member of scientific societies, especially of the Polish Parasitological Society in which he was the president in 1987-1991. He was also the member of the Committee for Parasitology of the Polish Academy of Sciences and member of scientific councils of a number of institutions. He was also the member of editorial boards of some journals such as Wiadomości Parazytologiczne (chairman since 1992), Acta Protozoologica, Folia Parasitologica etc. He was also an active participant of many conferences and congresses, native and international ones, concerning protozoology, parasitology and medicine (especially those concerning parasitic diseases of man) as well as an organizer of some of them (Ist Research Coordination Meeting of International Atomic Energy Agency, Poznań 1987, vice-president of VIth International Congress of Protozoology, Warszawa 1981 etc.).

Sudden death of Professor Kasprzak is a great loss for Polish and world protozoology and parasitology, for his mother Academy and, above all, for his students and friends.

Stanisław L. Kazubski

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Review article

Peculiarities of the Symbiotic Systems of Protists with Diverse Patterns of Cellular Organisation

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Summary. The analysis of symbiotic systems' occurrence in the representatives of several protists' macrotaxa with various patterns of cellular organisation (amoebae, flagellates and ciliates) has been performed. The frequency of symbionts' occurrence in different cell compartments has been evaluated on the basis of data obtained from literature and original electron-microscopical investigations. Cell coat, cytoplasm, perinuclear space, karyoplasm and various cell organelles has been studied. No reliable cases of symbionts' maintenance in protists' mitochondria and plastids were established. The occurrence of symbionts' associations in different taxa varies essentially. Symbiotic systems of three protists' groups analysed are shown to possess qualitative differences. In amoebae (Lobosea, Gymnamoebia) in 24 species out of 61 species studied symbionts are found in cytoplasm only; they are maintained neither on the cell coat nor in the nucleus and other organelles. Symbiotic systems of flagellates and ciliates in general have much in common. For each of these groups more than a hundred species of symbionts are described. Symbionts occupy the cell surface, cytoplasm, karyoplasm and perinuclear space. For different ecological groups of flagellates high frequency of occurrence of various symbionts on the cell surface is noted (in more than 30 species), reports of ectobionts in ciliates being less numerous and mostly restricted to metanobacteria. The results of analysis performed show that the formation of symbiotic relationship in lower eukaryotes' evolution is not a partial result of accidental ancestral partners' combinations. It is a natural phenomenon, widely distributed of protists. It is pronounced in diverse forms and with various frequency in different evolutionary branches. We suppose that the regularities of appearance of stable symbiotic associations are determined by the pattern of cellular organisation of the host to a great extent and depend upon the morphophysiological preadaptations of prokaryotic microorganisms trying to use the protists' cells as their environment in the course of their co-evolutionary process.

Key words: amoebae, cellular symbiosis, ciliates, co-evolution of partners, flagellates, lower eukaryotes, protists, pattern of cellular organisation, symbiotic systems.

INTRODUCTION

The study of symbiotic interactions between protozoa and prokaryotes attracts the attention of cell biologists for more than 20 years since L. Margulis revived on a new level the idea of symbiotic origin of eukaryotes (Margulis 1970, 1981, 1993). Nowadays the interest for this spectre of problems did not subside but increased greatly. The studies of the symbiotic systems of protists and prokaryotes are actively held in two directions: detailed research on the limited number of widely used model objects from flagellates, ciliates, actinopods and amoebae with the use of all the modern methods (Preer and Preer 1984; Lee et al. 1985b, 1995; Ossipov et al. 1986; Görtz 1986, 1988; Fokin and Görtz 1993; Rautian et al. 1993; Jeon 1995a,b) and comparative evolutionary analysis of symbionts in various groups of protists (Ball 1969; Gromov 1978;

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Podlipaev and Ossipov 1983; Cavalier-Smith and Lee 1985; Lee and Corliss 1985; Lee et al. 1985a,b; Ossipov et al. 1986; Corliss et al. 1990; Margulis et al. 1990, Heckmann and Görtz 1991; Lee and Anderson 1991; Soldo and Fokin 1994).

In the present review the comparative evolutionary analysis of the origin of cellular symbiosis is used. In the course of the evolutionary process the protists' cell have been used as environment by various microorganisms which occupied different cellular compartments: pellicle, cytoplasm, nucleus, the system of cisterns of endoplasmic reticulum (Ball 1969, Podlipaev and Ossipov 1983, Ossipov et al. 1986, Margulis et al. 1990). The representatives of the most diverse groups of organisms turned to symbiotic existence in protists: viruses, bacteria, fungi, as well as other protists. Various combinations of partners, arising in the course of co-evolution, on some stage become stable systems, which probably provides for their greater selective value in some conditions (Podlipaev and Ossipov 1983; Soldo 1983; Lee and Corliss 1985; Lee et al. 1985a, b; Rautian and Ossipov 1985; Ossipov et al. 1986; Corliss et al. 1990; Fenchel and Finlay 1991; Soldo et al. 1992; Fenchel and Bernard 1993).

To understand the regularities of origin and the trends of evolution of the symbiotic relationships in lower eukaryotes it is important to estimate comparatively the frequency of the originating and the peculiarities of mutual adaptation of the partners of the symbiotic systems among protists' groups differing by their pattern of cellular organisation, by their physiology and the peculiarities of cell and life cycles. Reviews of the kind, concerning particular macrotaxa, have been already undertaken. Quite full is the generalised information on the symbiotic systems of ciliates (Preer and Preer 1984, Ossipov et al. 1986, Heckmann and Görtz 1991, Fokin 1993). Recently we have performed comparative ultrastructural analysis of the occurrence of cytobionts in the representatives of two orders of naked amoebae within the class Lobosea (Smirnov et al. 1995) and the representatives of 17 phyla of flagellates (Ossipov et al. 1996).

The notion of "the pattern of cellular organisation" usually includes fundamental morphophysiological, ultrastructural and ontogenetic characteristics of the cell. They are essentially different in various macrotaxa of lower eukaryotes (Taylor 1978, Raikov 1982, Karpov S. 1990, Margulis et al. 1990, Harrison 1991, Kuźnicki and Walne 1993, Patterson D. 1994, Sleigh 1995). For interphase cells of vegetative stage such characteristics are: the presence of characteristic set of cellular organelles and structures; or on the contrary, the absence of some organelles; the presence of cytostome and its specialised ultrastructures; cell coat and the degree of cell shape stability; organelles of cell motility and cytoskeleton. For reproducing protists' cells characteristic are the forms of nuclear division, various types of sexual and asexual reproduction. All these parameters of the pattern of cellular organisation are subject to most essential changes on different stages of the life cycle and depend upon the presence (absence) of sexual process in the cycle and upon its form (copulation, conjugation or other forms). Adaptive ecologo-physiological characteristics undoubtedly influence the pattern of cellular organisation (life form) of the protists (freshwater, marine, parasitic, microanaerobic, obligatory anaerobic and other forms).

Taking into account major differences of the laws of the evolutionary process on cellular level in protists (marked for Protozoa, in the classification previously accepted), the pattern of cellular organisation has always been assigned substantial role in lower eukaryotes' evolution (Polyansky and Raikov 1976; Margulis 1981; Polyansky 1981; Patterson D. 1988, 1994; Karpov S. 1990; Margulis et al. 1990; Seravin 1992; Kuźnicki and Walne 1993; Hülsmann and Hausmann 1994; Cavalier-Smith 1995; Sleigh 1995). In this regard, a certain conservatism of the patterns of cellular organisation is usually marked for macroevolutionary process on multicellular level in Planta and Animalia.

We deliberately leave outside the scope of the present review the considerations on a vast and very interesting problem of the sustaining of viruses and virus-like bodies in the protists' cells. To those interested in the problem a number of reviews and original articles may be recommended, containing quite a full account of the matter (Lemke 1976; Teras 1986, 1990; Patterson J. 1990; Wang and Wang 1991; Lipscomb and Riordan 1995). Now we only mark that the viruses and virus-like bodies have been found in the representatives of the flagellates Chrysophyta, Cryptophyta, Dinophyta, Kinetoplastida and a number of taxa within amoebae and ciliates. At the same time the viruses have not been recorded in Choanoflagellida, Euglenophyceae, Opalinata, Parabasalia, Proteromonadida and other groups. When discussing the major issue of the present review it is necessary to take into account the fact that a considerable part of populations of all bacteria groups in natural ecosystems contains various bacteriophages (Proctor and Fuhrman 1990). Viruses circulating in microorganisms (as lower links of ecosystems' trophic chains) are in the course of microevolutionary process constantly involved as substantial additional component (genetic vector) in representatives of many protists'

macrotaxa (Lipscomb and Riordan 1995). Viruses are also characteristic for some symbiotic systems, for example, for symbiotic bacteria of ciliates of the genus *Paramecium* (Preer and Preer 1984, Quackenbush 1988).

In the present review we attempted a comparative evolutionary estimation of the frequency of mainly prokaryotic cytobionts' occurrence in various cell compartments of the representatives of protists' groups differing by the pattern of cellular organisation. As in our previous reviews (Smirnov et al. 1995, Ossipov et al. 1996) we did not aim at the collection of all the references of symbionts in protists' groups analysed, but based the research first of all upon the data of through ultrastructural investigations.

Results of the analysis of original and literature data on the occurrence of ecto- and endosymbionts in various compartments of the protists' cell are represented in consolidated tables. Unfortunately, not in all the investigations used protists and their symbionts are identified on the species level. In such cases only generic names are presented in the table, abbreviated as "sp.", or "spp." if the symbionts occurred in several species of the same genus.

Symbiotic systems of gymnamoebae

Endocellular symbiosis seems to be rather widely distributed among gymnamoebae, though this problem never was a subject of detailed consideration. There are some literary data devoted to the symbiotic systems of particular species, of which strains of *Amoeba proteus* containing X-bacteria, are by far the most thoroughly studied (Jeon and Lorch 1967; Jeon 1983, 1995a,b; Soldo and Fokin 1994). For the representatives of the order Acanthopodida - acanthamoebae - various forms of relationship with bacteria have been described (Holden et al. 1984, Byers 1986, Hagnere and Harf 1993, Gautom and Fritsche 1995). Some amoebae species are known to contain in the cytoplasm unicellular algae - "zoochlorellae" (Cann 1981; Goodkov et al. 1993; Jeon 1995a,b). In the same time, in the comparative cytological aspect intracellular symbiosis in gymnamoebae has never been an object of special study, excluding our recent review (Smirnov et al. 1995). However, the results of the ultrastructural investigations of the representatives of different amoebae families (Daniels 1973; Page 1983, 1987, 1988, 1991) indicate that many species of gymanmoebae contain bacteria in their cytoplasm.

In the present paper we tried to estimate the extent of the presence of cytobionts in the members of the orders Euamoebida and Leptomyxida (Rhizopoda, Lobosea) accepting them as proposed by Page (Page 1987). This analysis was mostly performed basing on literary ultrastructural data, and also some light-microscopical studies of the representatives of these orders were taken into account. As a part of this analysis we considered our original electron-microscopical data on the symbiotic bacteria in the cytoplasm of different amoebae species. They were obtained in the course of the investigation of the naked amoebae from the bottom sediments of the freshwater lakes of the Valamo Archipelago (Lake Ladoga). The basic results of our analysis are demonstrated in the Table 1. The species marked as "sp." are new to science, they are not yet described and therefore have no specific names.

In most cases the discovery of bacteria inside the amoebae has been a side result of the study and the authors did not pay special attention to it. Direct references to the absence of bacteria in the cytoplasm of the studied amoebae were found only in several articles. Therefore in the majority of cases the absence of the cytobionts cannot be considered an established fact. The results of light-microscopical observations are analysed only if endocytobionts were observed with certainty. We did not take into consideration rather numerous references of the beginning of this century, the authors of which reported the presence in amoebae of various symbionts (parasites) on light microscopical level. A suppressing majority of these references deal with the results of observations of solitary amoebae taken directly from nature and it is difficult now to estimate the reliability of these data.

Data represented in Table 1 demonstrate that the symbionts have been found in the cytoplasm of 21 out of 49 of the studied representatives of the order Euamoebida and of 3 out of 12 studied representatives of the order Leptomyxida. We note once more, though, that the absence of references of the cytobionts' presence in a certain studied species does not mean ultimate absence of symbionts in this species.

Out of 61 studied species of Gymnamoebae 24 species are shown to possess symbionts in the cytoplasm, and no cases of symbionts' sustaining on the cell surface and in the organelles are demonstrated. In *Paramoeba eilhardi* bacteria do penetrate into the nucleus, but it results in rapid death of the infected cell (Grell and Benwitz 1970). Not a single case of the symbionts' sustaining in perinuclear space and in karyoplasm of amoebae has been shown. The presented material allows to postulate with certainty the presence of bacteria in the cytoplasm of the representatives of the order Euamoebida as a widely distributed phenomenon.

Table 1. Distribution of cytoplasmic endobionts among members of the orders Euamoebida and Leptomyxida (Lobosea, Gymnamoebia) - a summary table (after: Smirnov et al. 1995)

Systematic position of the host	Species known	Species studied with TEM	Species containing endobionts	Location of endobionts in the cytoplasm
Order Leptomyxida				
Suborder Leptoramosina				
Family Gephryamoebidae				
Genus *Gephryamoeba*	1	0	0	
Family Stereomyxidae				
Genus *Stereomyxa*	2	2	1	in the vacuoles
Genus *Corallomyxa*	1	1	0	
Suborder Rhizoflabellina				
Family Flabellulidae				
Genus *Flabellula*	4	4	0	
Genus *Paraflabellula*	1	1	1	free
Family Leptomyxidae				
Genus *Balamuthia*	1	1	0	
Genus *Leptomyxa*	2	1	0	
Genus *Rhizamoeba*	6	2	1	free
Order Euamoebida				
Family Paramoebidae				
Genus *Janickina*	2	1	1	free
Genus *Korotnevella*	2	3*	3	free or in vacuoles
Genus *Mayorella*	9	6	3	free or in vacuoles
Genus *Paramoeba*	2	2	2	in vacuoles
Family Thecamoebidae				
Genus *Dermamoeba*	2	0	0	
Genus *Paradermamoeba*	2	0	0	
Genus *Parvamoeba*	1	1	0	
Genus *Pseudothecamoeba*	1	1	0	
Genus *Sappinia*	2	1	1	free
Genus *Thecamoeba*	8	2	1	free
Genus *Thecochaos*	2	0	0	
Family Vannellidae				
Genus *Vannella*	11	4	0**	
Genus *Platyamoeba*	9	2	0	
Family Vexilliferidae				
Genus *Neoparamoeba*	3	3	0	
Genus *Pseudoparamoeba*	1	1	0	
Genus *Vexillifera*	8	3	0	
Family Amoebidae				
Genus *Amoeba*	4	4	3	free or in vacuoles
Genus *Chaos*	3	2	2*	free or in vacuoles
Genus *Deuteramoeba*	2	2	1	free
Genus *Hydramoeba*	1	1	0	
Genus *Parachaos*	1	1	1	unknown
Genus *Polychaos*	3	2	1	free or in vacuoles
Genus *Trichamoeba*	4	1	0	
Family Hartmannellidae				
Genus *Cashia*	1	1	0	
Genus *Glaeseria*	1	1	0	
Genus *Hartmannella*	4	2	0	
Genus *Saccamoeba*	6	2	2	free

"*" - including our unpublished data
"**" - in one species bacteria-like cytoplasmic inclusions were observed with light microscopy (Hayes et al. 1991)

Bacteria are situated in symbiontophoric vacuoles or freely in the amoebae cytoplasm, not surrounded by the symbiontophoric vacuole's membrane, with approximately equal frequency. Both free-lying and enclosed into vacuoles bacteria can be sustained within the same cell (for example, in *Polychaos dubium*). Bacteria enclosed in vacuoles are most likely to get into the cell in the course of phagocytosis; the ways of free-lying bacteria's appearance in the cytoplasm has not been ever traced.

In three amoebae species of the orders in question chlorellae were found in the cytoplasm, symbiotic association being artificially obtained in one of them. In the cytoplasm of *Parachaos zoochlorellae* the chlorellae are not constantly present and there is evidence to suggest here a case of "non-obligatory" symbiosis (Karpov A. et al. 1991). In general symbiosis with unicellular algae is less widely distributed in naked amoebae than, for example, in ciliates (Ossipov 1981, Preer and Preer 1984, Görtz 1986, Ossipov et al. 1986).

The character of relationship of amoebae and bacteria is poorly studied and in majority of cases remains obscure. Recently Michel and co-authors (1995) considered in details *Saccamoeba* spp. containing bacteria. They indicated that in this case bacteria are obligatory symbionts of the amoebae. Bacterial infections have been described, leading to rapid death of amoebae. In some cases after infection parasite-host system might stabilise somehow and start self-sustaining, later giving rise to the dependence of the host upon the parasite as well (Jeon 1983, 1987, 1995a,b). In infected amoebae of one of the *Rhizamoeba saxonica* clones the number of bacteria in the cell is large enough; electron-microscopical photographs show that they occupy a substantial part of the cell volume (Page 1980). At the same time in the majority of other amoebae species symbiotic algae do not fill a large part of the cell volume.

The relationships of partners are much better studied in representatives of the order Acanthopodida than in representatives of the two orders studied in our review (Smirnov et al. 1995). For *Acanthamoeba* peculiar trophic relations with *Legionella pneumophilla* are described (Tyndall and Domingue 1982, Anand et al. 1983, Harf and Monteil 1989). It has been demonstrated experimentally that amoebae excrete into the culture medium some soluble factor, necessary for bacteria reproduction. Bacteria are actively phagocyted by amoebae and remain viable inside the food vacuoles for a long time (Holden et al. 1984, Kilvington and Price 1990). Acanthamoebae that phagocyted clones of *Aeromonas* sp. bacteria resistible to mercury ions were noted to become more resistible to these ions themselves (Hagnere and Harf 1993). The transmissibility of bacterial endosymbionts between different isolates of *Acanthamoeba* was specially studied by Gautom and Fritsche (1995). It is likely that acanthamoebians are well enough preadapted to sustaining bacteria in the cytoplasm and that bacterial species do exist that are potentially capable of becoming amoebae's endocytobionts. Symbiotic systems like these are probably originating all the time in natural microbiocenoses. In the course of wider ecological populational research similar evolutionary "young" symbiotic associations are quite likely to be found in representatives of other gymnamoebae orders as well.

Symbiotic systems of flagellates

According to modern data the degree of flagellates' biodiversity is estimated by not less than eleven thousand species described (Andersen 1992, Corliss 1994, Scarlato et al. 1994), whereas ultrastructurally studied are less than several hundreds. Like for the representatives of other taxa we considered the lack of references in literature on the revealing of cytobionts in a certain studied species to be insufficient to postulate their true absence. In our review (Ossipov et al. 1996) we have estimated for the first time the frequency of cytobionts' occurrence in representatives of the largest possible number of macrotaxa (phyla) of flagellates on the basis of original electron-microscopical data and those obtained from literature.

E c t o b i o n t s. Ectobiotic bacteria are situated on the cell surface and are associated with it during the larger part of flagellates' life cycle. They have been revealed in more than 30 species of flagellates from different systematic groups: Chrysophyceae, Dinophyceae, Hypermastigida, Kinetoplastida, Oxymonadida, Pelobiontida, Trichomonadida (Table 2). Unfortunately, ectobionts' identification was usually restricted to the description of external appearance, for example: "rod-like bacteria", "bacilli", "long bacteria" etc. In the majority of cases the ectosymbionts have not been identified at all and only in several cases, for example, in oxymonad *Polymastix melolonthae* (Brugerolle 1981) and dinoflagellate *Ornithocercus thumii* (Taylor 1990) exact species names of ectobionts are known (Table 2).

The character of ectobionts' interaction with superficial structures differs essentially in various flagellate species. Ectobiotic bacteria in pelobiontids *Pelomyxa palustris* and *Mastigamoeba aspera* fit closely the plasmalemma of the host lying below the glycocalix layer (Griffin 1988). In oxymonad *Pyrsonympa* the numerous spirochetes are rigidly attached to the superficial structures of the cell (Bloodgood and Fitzharris 1976).

Table 2. Prokaryotic ectocytobionts in flagellates (after: Ossipov et al. 1996)

Systematic position of the host	Ectocytobionts
Parabasalia: (Trichomonadida)	
Kirbynia spp.	Long bacteria
Foania spp.	Spirochets
F.decipiens	Idem
Pseudodevescovina uniflagellata	»
Hyperdevescovina torquata	»
Macrotrichomonas hirsuta	Long bacteria
Devescovina	Idem
Mixotricha paradoxa (Hypermastigida)	Spirochets and unidentified bacteria
Holomastigotoides spp.	Idem
Joenia spp.	»
Joenia annectens	»
Projoenia	»
Trichonympha	»
Barbulanympha	Spirochets and rod-like bacteria
Urinympha	Rod-like bacteria
Oxymonada: (Oxymonadida)	
Pyrsonympha	Spirochets and rod-like bacteria
Polymastix melolonthae	*Fusiformis melolonthae*
Polymastix spp.	Long bacteria
Oxymonas spp.	Spirochets and other bacteria
Microrhopalodina	Idem
Pelobiontida:	
Pelomyxa palustris	Unidentified bacteria
Mastigamoeba aspera	Idem
Kinetoplastida:	
Cryptobia spp.	»
Dinophyceae:	
Ornithocercus thumii	*Synechococcus carcerius*
O. magnificus	*Synechococcus* and *Synechocystis*
Citharistes apsteinii	Unidentified bacteria
Histioneis panda	Idem
Parahistioneis sp.	»
Chrysophyta:	
Spumella elongata	»

Some ectosymbiotic bacteria are undoubtedly participating in the protozoons' cell motility, though in majority of oxymonads and hypermastigids the largest amount of work during movement is certainly performed by the flagella. Among ectobiotic bacteria the most effective movers are spirochetes. Representatives of the genus *Devescovina* move by means of bacilli that stick all over the cell with their flagella rotating actively (Tamm 1982).

Thus a conclusion can be made that the flagellates' superficial structures are used by diverse forms of prokaryotic microorganisms, spirochetes occupying a noticeable place among them. It should be noted that the latter are absent among the flagellates' endobionts.

E n d o b i o n t s. Prokaryotic endobionts are found in more than 70 flagellate species. Bacterial species, with rare exceptions, have not been identified. All of them are Gram-negative, i.e. lack of mureine in the cell wall. Generalised data, presented in Table 3, permit to evaluate the frequency of endobionts' occurrence in various compartments of the host cell.

Symbiotic bacteria are most often sustained in the cytoplasm of flagellates (51 species). They can either be situated in symbiontophoric vacuole or lie freely in cytoplasm. Methanogenic bacteria *Methanobacterium formicicum* are associated with hydrogenosomes in the cytoplasm of *Psalteriomonas* (Fenchel and Finlay 1991). Karyoplasm of flagellates is inhabited by symbionts much rarely. Nuclear endobionts are found in 8 host species, and they are never surrounded by membrane structures of the host cell. The only case of membrane being found around bacterium in the nucleus of trichomonad *Calonympha grassi* (Joyon et al. 1969) is quite doubtful, for endobionts

Table 3. Data on the presence and localization of endobionts in cell compartments of the representatives of flagellates' phyla (after: Ossipov et al. 1996, upgrated)

Systematic position of the host	Cell compartments				
	cytoplasm	karyoplasm	perinuclear space	chloroplasts	mitochondria
1	2	3	4	5	6
Glaucocystophyta:					
Cyanophora paradoxa	+	-	-	-	-
Gloeochaete wittrockiana	+	-	-	-	-
Paulinella chromatophora	+	-	-	-	-
Glaucocystis nostochinearum	+	-	-	-	-
Glaucosphaera vacuolata	+	-	-	-	-
Chlorophyta:					
Volvox carteri	+	-	-	-	-
Bryopsis hypnoides (see text)	+	-	-	-	-
Caulerpa prolifera (see text)	+	-	-	-	-
Syphonales (see text)	+	-	-	-	-
Euglenophyceae:					
Strombomonas conspersa	-	+	-	-	-
Trachelomonas oblonga var. *punctata*	-	+	-	-	-
Euglena spirogira	-	+	-	-	-
E. tristella	+	-	-	-	-
E. mutabilis	+	-	-	-	-
Eutreptiella eupharingea	+	-	-	-	-
Colacium vesiculosum	+	-	-	-	-
Lepocinclis ovum	+	-	-	-	-
Kinetoplastida:					
Rhynchomonas nasuta	+	-	-	-	-
Pleuromonas jaculans	-	-	+	-	-
Cryptobia sp.	-	-	+	-	-
Bodo designis	+	-	-	-	-
B saltans	+	-	-	-	-
B. curvifilus	+	-	-	-	-
Rhynchobodo armata	+	-	-	-	-
Phyllomitus apiculatus	+	-	-	-	-
P. amylophagus	+	-	-	-	-
Cryptobia vaginalis	+	-	-	-	-
Blastocrithidia culicis	+ DS	-	-	-	-
Crithidia oncopelti	+ DS	-	-	-	-
C. dianei	+ DS	-	-	-	-
C. desouzai	+ DS	-	-	-	-
Trypanosoma corbitis	+ DS	-	-	-	-
Herpetomonas roitmani	+ DS	-	-	-	-
H. muscarum	+ DS	-	-	-	-
Cercomonadida:					
C. activus	+				
C.crassicauda	+	-	-	-	-
Spongomonadida:					
Rhipidodendron splendidum	+	-	-	-	-
Thaumatomonadida:					
Thaumatomonas seravini	-	-	+EPR	-	-
Choanomonada:					
Choanoeca perplexa	+	-	-	-	-
Pelobiontida:					
Pelomyxa palustris	+	-	-	-	-
Mastigella	+	-	-	-	-
Mastigamoeba	+	-	-	-	-
Mastigina hylae	+	-	-	-	-
Dinamoeba mirabilis	+	-	-	-	-

Table 3 (continued)

1	2	3	4	5	6
Raphidophyceae:					
Gonyostomum semen	+	-	-	-	-
Chattonella marina	-	+	-	-	-
Xanthophyceae:					
Vaucheria sessilis	+	-	-	-	-
Chrysophyta:					
Mallomonas caudata	+	-	-	-	-
M. papillosa	+	-	-	-	-
M. calceolus	+	-	-	-	-
Chrysosphaerella brevispina	-	-	+	-	-
Chrysamoeba radians	+	-	-	-	-
Paraphysomonas vestita	-	-	+EPR	-	-
P. diademifera	-	-	+	-	-
P. undulata	-	-	+	-	-
Dictyocha speculum	+	-	-	-	-
Dinophyceae:					
Noctiluca scintillans	+	-	-	-	-
Gymnodinium splendens	-	+	-	-	-
Glenodinium foliaceum	-	+	-	-	-
Gyrodinium instriatum	-	+	-	-	-
Polykrikos herdmannii	+	-	-	-	-
Woloszynskia pascheri	-	-	-	+?	+?
Cryptophyceae:					
Chroomonas sp.	-	-	+EPR	-	-
Heterolobosea:					
Psalteriomonas vulgaris	+	-	-	-	-
P. lanterna	+	-	-	-	-
Parabasalia:					
Joenia annectens	+	+	-	-	-
Trichonympha	+	-	-	-	-
Barbulanympha	+	-	-	-	-
Urinympha	+	-	-	-	-
Calonympha grassi	-	-	+?	-	-
Oxymonadida:					
Pyrsonympha	-	-	-	-	-

"+" - the presence of symbionts in the host's compartment
"-" - the absence of symbionts in the hosts's compartment
"DS" - diplosomes
"EPR" - symbionts present also in the rough endoplasmic reticulum cisternae of the host
"?" - questionable situation, commentaries in the text

are situated at the periphery of the nucleus and lie, in our opinion, in perinuclear space.

In 9 flagellate species symbionts occupy perinuclear space. Despite the fact that perinuclear space and rough reticulum (EPR) are the same compartment of the cell, in some flagellates the endobionts are noted in EPR, and in others - in perinuclear space only (Fokin and Karpov 1995). Bacteria have been found in both sections of this compartment only in colourless chrysomonad *Paraphysomonas vestita* (Tanichev and Karpov 1992).

Discovery of symbionts in chloroplasts and mitochondria in dinoflagellate *Woloszynskia pascheri* (Wilcox 1985) is a unique case among protists. Unfortunately, after the description of the very fact of prokaryotes' presence in these cell organelles, further investigation has not been performed. We suppose other interpretations of electronmicrographs presented by the author to be possible as well. For example, in protists' mitochondria electron-dense inclusions of various kind of non-cellular nature may be encountered. Let us mark that the structures enclosed in chloroplasts and mitochondria on

electron photographs presented by Wilcox possess well pronounced coating and rather homogenous contents.

In representatives of Glaucophyceae the cytoplasm of all investigated species contains cyanellae, that actually are unidentified cyanobacteria. Previously they were erroneously regarded as independent flagellates' organelles. However, by the pigment composition, by the presence of cell wall and the features of its organisation, by molecular biological properties and the degree of their dependence upon the host genome cyanellae cannot be considered organelles - chloroplasts (Kies and Kremer 1990). The cell wall of symbiotic cyanobacteria in different species of Glaucophyceae is present on various stages of reduction. Symbiosis of this kind is probably very ancient and is quite widely distributed among other protists, and also in the kingdoms of animals and plants (Reisser et al. 1985, Schenk et al. 1987).

In regard to the discussion of the origin of red algae chloroplasts by means of symbiosis of the ancestral form of eukaryotes with cyanobacteria Glaucophyceae are a happy relic discovery. Their cyanellae can be discussed as a kind of transitional link between the free-living cyanobacteria and red algae chloroplasts. Cyanellae differ from the latter by the presence of the cell wall, peculiarities of pigment composition and the degree of dependence upon the nuclear genome (Kies and Kremer 1990).

For understanding of the mechanisms of the symbiotic systems' origin in Protista molecular biological data on Kinetoplastida are of great interest. Free-living kinetoplastids (bodonids) quite often contain Gram-negative bacteria in their cytoplasm. We managed to discover that bacteria can also occupy perinuclear space both in free-living bodonid *Pleuromonas jaculans* (Karpov and Zhukov 1983) and in parasitic cryptobiid *Cryptobia* sp. (Fokin and Karpov 1995). Among other parasitic kinetoplastids - trypanosomatids - endobionts are also widely distributed, but occupy the cytoplasm only and are always represented by a single large dividing cell surrounded by membrane. Symbionts like these were named diplosomes by analogy with true cell organelles (Chang 1974).

It is established that the presence of symbionts (diplosomes) in trypanosomatids is somehow connected with molecular characteristics of the host genome. The study of nucleotide sequences of the gene of 18S rRNA in symbiont-containing kinetoplastids *Crithidia deanei, C. oncopelti* and *C. desouzai* (Du and Chang 1993) revealed that their homology with the same gene of *C. fasciculata* is more than 90%.

When comparing rRNA genes sequences of *Blastocrithidia* and *Crithidia* symbionts is has been established that they share many peculiarities: G+C content equals 53% and coding region identity reaches 97,3% (Du et al. 1994). It has been demonstrated that symbionts of *Crithidia* and *Blastocrithidia* belong to β-subgroup of purple proteobacteria. According to the analysis of nucleotide sequences these symbionts are most close to a free-living bacteria *Bordetella bronchiseptica*. This permitted Du and co-authors (Du et al. 1994) to suggest to name the symbionts of blastocrithidia and crithidia, respectively, *Kinetoplastibacterium blastocrithidii* and *K. crithidii*, in according to generally accepted rules of binomial nomenclature for prokaryotes. Comparison of gene nucleotide sequences also showed that symbionts of *Blastocrithidia culicis* are most close to the symbionts from the hosts of *Crithidia* genus. This fact gives evidence to monophyletic origin of symbionts from two kinetoplastid genera. Trypanosomatid symbionts defy cultivation outside the host (Du et al. 1994) hence the possibility to suppose them to be very ancient obligatory symbionts of this flagellate group.

Free-living kinetoplastids being phagotrophic, i.e. being capable of engulfing bacteria by means of cytostome and cytopharynx, bacteria transition to the cytoplasm is quite possible. Therefore the facts of wide distribution of cytoplasmic symbionts in bodonids (Vickerman 1991) may be easily explained. Parasitic kinetoplastids not being phagotrophic, it is reasonable to suggest that diplosomes have been inherited by trypanosomatids from bodonids and are not a new acquisition. This idea has been formulated by Frolov (Frolov 1993) who also suggested that all diplosome containing trypanosomatids form one group of monophyletic species (Frolov and Karpov 1995). These statements have been further confirmed by the works of Du and co-authors (Du and Chang 1993, Du et al. 1994).

The presence of symbionts substantially tells on morphology and physiology of the host (Freymuller and Camargo 1981). In this respect the flagellate species containing endosymbionts (*Crithidia deanei, C. oncopelti, Blastocrithidia culicis*) were compared with those without symbionts (*Leptomonas seymouri, L. collosoma, L. samueli, Crithidia fasciculata, C. luciliae, C. acanthocephali, Herpetomonas megaseliae, H. mariadeanei, H. samuelpessoai, H. muscarum muscarum, Trypanosoma cruzi*). Paraxial strands in flagella were found only in the latter group of species (without endobionts). Besides, it is in these flagellate species that kinetoplastic DNA forms on the ultrastructural level fibrils that are larger and situated more loosely than in symbiont-containing species. Species with symbionts are also noted by mitochondria periphery branching accompanied by the

lack of submembrane microtubules in the sites where mitochondria are adjacent to the cell membrane.

The analysis of trophic requirements of host clones deprived of symbionts by means of antibiotics treatment has shown that their need in certain growth factors increases (Chang 1980). For normal functioning of such aposymbiotic flagellates a supplementary set of metabolites is required: haem, purines, a number of aminoacids and vitamins (Du et al. 1994).

The presence of symbionts may also influence physiological characteristics of the host's cell surface. For instance, lectines agglutination from the cell surface of trypanosome *Crithidia dianei* is known to take place more actively if the cells do not contain endosymbionts (Esteves et al. 1982). The presence of a symbiont in *Herpetomonas roitmani* changes the composition of plasmalemma polysaccharides (for example, xylose and inositol are absent) and superficial anion groups, and also the pathways of haem synthesis and urea cycle, elicits supplementary requirements in trophic factors as compared to clones that lost the symbionts (Faria e Silva et al. 1994). The process of cell differentiation also changes substantially: in the cell population of the "wild type" clone of flagellates (with symbionts) the share of opisthomastigots amounts to 37% whereas in the cell population deprived of symbionts it amounts to 72%.

Thus, at close consideration, interinfluence of partners in "*Kinetoplastibacterium - Trypanosoma*" symbiotic system happens to be very essential and the system itself is undoubtedly one of the most archaic symbiotic associations among protists (Frolov 1993, Du et al. 1994, Frolov and Karpov 1995).

As shown by analysis of data presented in Tables 2 and 3, frequency of occurrence of symbiotic associations differs essentially in various flagellate macrotaxa. Cytobionts are recorded in various cell compartments in more than a hundred species from 17 phyla of flagellates; most often - in the representatives of Euglenophyceae, Kinetoplastida, Chrysophyceae, Dinophyceae and Parabasalia. Two species with symbionts have been found among Cercomonadida (10 species studied), and one species in Choanoflagellida (about 20 species studied) and Thaumatomonadida (6 species studied). On the contrary, within Pelobiontida endobionts have been found in all the 5 species investigated.

Material analysed permits us to state with confidence that symbiosis of prokaryotic microorganisms and flagellates is a widely distributed phenomenon among these protists. Consequently a remarkable fact is the lack of references of symbiotic organisms in all representatives of Opalinata, Proteromonadida, Haptophyceae, Eustigmatophyceae, rather thoroughly studied flagellate taxa (Karpov S. 1990, Margulis et al. 1990).

Thus, data considered allow to postulate that prokaryotic microorganisms have occupied a variety of flagellate cell compartments. In the evolution of symbiotic systems of flagellates, undoubtedly, both previously stated strategies of relationship formation (on ultrastructural level) between the symbiont and the protist cell have been used (Ossipov et al. 1986). The first strategy being utilised, the invaded microorganism stops being recognised by the protist's cell as an alien creature. In such evolutionary advanced system the symbionts turn, as a matter of fact, into supplementary organelles of the host. The second strategy being used, the symbionts are localised in such host cell compartments and have such membrane surroundings that the possibility of hydrolytic attack of the host lysosomic enzymes is excluded topologically. In the course of evolution of such symbiotic systems the endobionts are sustained with stability either in the perinuclear space or in the karyoplasm of the host.

Symbiotic systems of ciliates

The problem of ciliates' symbiosis with microorganisms was a subject of a number of reviews devoted to the systematics, to the morphological and functional relationships of the partners (Kirby 1941; Ball 1969; Preer et al. 1974; Ossipov et al. 1976, 1986; Gromov 1978; Ossipov 1981; Ossipov and Podlipaev 1981; Podlipaev and Ossipov 1983; Preer and Preer 1984; Görtz 1986, 1988; Corliss et al. 1990; Fenchel and Finlay 1991; Heckmann and Görtz 1991; Fokin 1993; Embley and Finlay 1994; Rosati 1994; Soldo and Fokin 1994; Fokin and Karpov 1995). Different species of symbionts are as a rule characterised by a strict localisation in the host's cell or on its surface.

External conditions varying widely, symbiotic microorganisms of the ciliates may provide the host with certain selective advantage by means of utilising supplementary trophic and energy sources (Margulis 1970, 1981; Ossipov and Podlipaev 1981; Podlipaev and Ossipov 1983; Soldo 1983; Lee and Corliss 1985; Görtz 1986; Ossipov et al. 1986; Fenchel and Bernard 1993; Embley and Finlay 1994). For many species of ciliates' symbiotic microorganisms the symbiosis is obligatory, whereas it is non-obligatory for the host in majority of cases.

According to modern data the biodiversity of ciliates in the world fauna is estimated approximately as 7500 species (Margulis et al. 1990, Scarlato et al. 1994). Only several hundred ciliate species have been studied on electron-microscopical level. In these investigations many

ciliate species were represented by random samples from natural populations or single clones. Number of ciliate species for which stable symbiotic associations have been described exceeds a hundred by much. Representatives of various systematic groups of Ciliophora may form symbiotic relationships with bacteria, lower fungi, flagellates and other microorganisms. According to electron-microscopical and molecular-biological data symbionts occur in various systematic groups of Ciliophora (Table 4). This undoubtedly demonstrates high frequency of the formation of symbiotic associations within this evolutionary advanced Protista macrotaxon. The level of knowledge of various ciliates' groups differs essentially, not permitting to compare with certainty the frequency of occurrence of symbiotic system within the phylum.

It should be taken into consideration that the majority of investigations on the symbiontology of ciliates has been performed on the representatives of a certain number of groups (genera, species). The representatives of the following genera are undoubtedly such favourite model object of symbiotic relationships study: *Paramecium* (Wichterman 1953, 1986; Sonneborn 1959; Ossipov 1981; Preer and Preer 1984; Görtz 1986, 1988; Fokin 1993), *Euplotes* (Heckmann and Görtz 1991), *Parauronema* (Soldo 1983).

Axenic cultures of *Tetrahymena pyriformis* are rather widely used in recent times in medical biological investigations as model objects for estimation the capacity of free-living ciliates to sustain in natural ecological systems bacteria, pathogenic to men (Fields et. al. 1984, Pushkareva et al. 1990). Tetrahymens has been shown to be capable of sustaining in the cytoplasm some bacterial species which prove to be resistable to digestion and reproduce within the host. The degree of stability in such originated symbiotic associations is still obscure.

Table 4. Data on the presence and the character of localization of endobionts in the cells of the representatives of the phylum Ciliophora (after: Fokin 1993, upgrated).

Systematic position of the host	Endobiont species	Cell compartment			
		Cytoplasm	Macronucleus	Micronucleus	Perinuclear space
1	2	3	4	5	6
Class Kinetofragminophorea					
Chilodonella cucullulus	*	+	-	-	-
Cyclopostium sp.	*	+	+	-	-
Discophrya buckei	*	+	-	-	-
Discophrya erlangensis	*	+	-	-	-
Discophrya helophori	*	+	-	-	-
Discophrya hochi	*	+	-	-	-
Discophrya ochthebii	*	+	-	-	-
Entodinium sp.	*	+	-	-	-
Helicoprorodon multinucleatum	*	-	+	-	-
Isotricha intestinalis	*	-	+	-	-
Isotricha prostoma	*	-	+	-	-
Lacrimaria cucumis	*	+	-	-	-
Loxophyllum meleagris	*	-	+	-	-
Placus striatus	*	+	-	-	-
Pseudoprorodon arenicola	*	-	+	-	-
Trimyema compressum	*	+	-	-	-
Trithigmostoma cucullulus	*	-	+	-	-
Class Oligohymenophorea					
Ancistrum mytili	*	+	+	-	+
Conchophtirius curtus	*	-	+	-	-
Cyclidium porcatum					
Mesnilella trispiculita	*	-	+	-	-
Metaradiophrya gigas	*	-	+	-	-
Neobursaridium gigas	*	-	+	-	
Parauronema acutum	"Xenosome"	+	-	-	-
Plagiopyla frontata	*	+	-	-	-
Plagiopyla nasuta	*Methanobacterium formicicum*	+	-	-	

Table 4 (continued)

1	2	3	4	5	6
Proboveria rangiae	*	-	+	-	-
Protophrya ovicola	*	+	-	-	-
Trichodina pediculus	*	+	-	-	-
Trichodina sulcatus	*	+	-	-	-
Vorticella similis	*	-	+	-	-
Zoothamnion pelagium	*Holospora* sp.	-	+	-	-
Class Polyhymenophorea					
Blepharisma japonicum	*	+	-	-	-
Brachonella caduca	*	+	-	-	-
Caenomorpha lata	*	+	-	-	-
Climacostomum virens	"Theta"	+	-	-	-
Cyttarocylis brandti	*	+EPR	-	-	-
Discomorphella pectinata	*	+	-	-	-
Euplotes aediculatus	*Polynucleobacter necessarius*	+	-	-	-
Euplotes crassus	*	-	+	-	-
Euplotes daidaleos	*Polynucleobacter* sp.	+	-	-	-
Euplotes eurystomus	>>	+	-	-	-
Euplotes minuta	>>	+	-	-	-
Euplotes octocarinatus	>>	+	-	-	-
Euplotes patella	>>	+	-	-	-
Euplotes plumipes	>>	+	-	-	-
Euplotes woodruffi	>>	+	-	-	-
Euplotidium itoi	*	+	-	-	-
Favella ehrenbergii	*	+	-	-	-
Frontonia sp.	*	+	-	-	-
Halteria bifurcata	*	-	+	-	-
Halteria geleiana	*	+MT	-	-	-
Metopus contortus	*Methanoplanus endosymbiosus*	+	-	-	-
Metopus palaeformis	*	+	-	-	-
Metopus striatus	*Methanobacterium formicicum*	+	-	-	-
Metopus vestitus	*	+	-	-	-
Parablepharisma sp.	*	+	-	-	-
Paramecium aurelia	*	+	+	-	-
Paramecium dubosqui	*Caedibacter macronucleorum*	-	+	-	-
Paramecium dubosqui	*Perinucleobacter macronucleatus*	-	-	-	+
Paramecium woodruffi	*Pseudolyticum minutum*	+	-	-	-
Paramecium woodruffi	*Perinucleobacter heteronucleophilus*	-	-	-	+
Paramecium woodruffi	*	+	-	-	-
Paramecium woodruffi	*Holospora bacillata*	-	+	-	-
Paramecium aurelia	*Tectibacter vulgaris*	+	-	-	-
Paramecium aurelia	*Pseudocaedibacter conjugatus*	+	-	-	-
Paramecium aurelia	*Ps. minutus*	+	-	-	-
Paramecium aurelia	*Ps. glomeratus*	+	-	-	-
Paramecium aurelia	*Ps. falsus*	+	-	-	-
Paramecium aurelia	*Lyticum flagellatum*	+	-	-	-
Paramecium aurelia	*L. sinuosum*	+	-	-	-
Paramecium aurelia	*Holospora caryophila*	-	+	-	-
Paramecium aurelia	*Caedibacter taeniospiralis*	+	-	-	-
Paramecium aurelia	*C. varicaendens*	+	-	-	-
Paramecium aurelia	*C. pseudomutans*	+	-	-	-
Paramecium aurelia	*C. paraconjugatus*	+	-	-	-

Table 4 (continued)

1	2	3	4	5	6
Paramecium bursaria	*Pseudocaedibacter chlorellopellens*	+	-	-	-
Paramecium bursaria	*Holospora acuminata*	-	-	+	-
Paramecium bursaria	*H. curviuscula*	-	+	+	-
Paramecium bursaria	*	-	-	+	-
Paramecium bursaria	*	+	-	-	-
Paramecium calkinsi	*Holospora bacillata*	-	+	-	-
Paramecium calkinsi	*H. curvata*	-	+	-	-
Paramecium calkinsi	*	-	+	-	-
Paramecium calkinsi	*	+	-	-	-
Paramecium caudatum	*Pseudolyticum multiflagellatum*	+EPR	-	-	-
Paramecium caudatum	*Nonospora macronucleata*	-	+	-	-
Paramecium caudatum	*Holospora obtusa*	-	+	-	-
Paramecium caudatum	*H. elegans*	-	-	+	-
Paramecium caudatum	*H. undulata*	-	-	+	-
Paramecium caudatum	*H. recta*	-	-	+	-
Paramecium caudatum	*H. caryophila*	-	+	-	-
Paramecium caudatum	*Cytobacter aggregatum*	+	-	-	-
Paramecium caudatum	*Caedibacter caryophila*	-	+	-	-
Paramecium multimicronucleatum	*Perinucleobacter pseudoheteronucleophilus*	-	+	-	
Petalotricha ampulla	*	+	-	-	-
Plagiotoma lumbrici	*	-	+	-	-
Saprodinium dentatum	*	+	-	-	-
Sicuophora xenopi	*	+MT	-	-	-
Spirostomum ambiguum	*	-	+	-	-
Stentor multipormis	*	-	+	-	-
Stentor multipormis	*	+	-	-	-
Stentor polymorphus	*Holospora* sp.	-	+	-	-
Stylonychia mytilus	*	-	+	-	-
Urostyla grandis	*	+	-	-	-

"+" - the presence of symbionts in the host's compartment
"-" - the absence of symbionts in the host's compartment
"*" - endobiont not identified
"MT" - mitochondria
"EPR" - endoplasmic reticulum

Frequency of symbiotic associations' occurrence to a large extent depends upon a number of ecological peculiarities of the habitats of particular ciliates' groups. In the cytoplasm of some ciliate species symbiotic bacteria (mostly methanogenic or sulphur bacteria) can be found out under certain conditions, like the lowering of oxygen content or in anaerobic conditions, the increased hydrogen sulphide concentration and the increased saprobic level (Fenchel and Finlay 1991, Embley and Finlay 1994). Such qualities are peculiar to characteristic ciliate fauna of sapropel and psammon, both freshwater and marine (Schrenk and Bardele 1991), in the rumen of ruminants (Vogels et. al. 1980).

Ectobionts. Surficial structures of ciliates (cortex) is not the favourite cell compartment for symbiotic microorganisms' sustaining. Not many reliable cases of ectobionts' sustaining are known for ciliates. Ectosymbiosis in ciliates, as a rule, is the specific character.

In astomatous psammophyte ciliate *Kentrophoros fistulosum* many a hundred of sulphur bacteria are sustained on the dorsal surface of the flattened cell. Some of these sulphur bacteria are constantly included into food vacuoles by means of the invaginations of the cell surface and are digested (Raikov 1971). On the cell surface of the hypotrich marine ciliate *Euplotidium itoi* hundreds of bacteria (epixenosomes) are sustained. These ectobionts penetrate into the cytoplasm of the host both by means of cell membrane invaginations and through the superficial cortical structures, this constituting a unique peculiarity of this symbiotic system. There they are surrounded by

endoplasmic reticulum and turn into endobionts (Rosati 1994). It is interesting to note that, well-studied cytoplasmic symbiotic bacteria (xenosomes) of the marine ciliate *Parauronema acutum* during experimental infection through the culture media penetrate into the host cell also through by means of cortical structures invaginations (Soldo et al. 1992, 1993).

It is established that the ciliates living in anaerobic conditions are incapable of using oxidative phosphorylation in energy metabolism and are very sensitive to even insignificant quantities of oxygen. These ciliates usually contain in their cytoplasm mitochondrial analogies - hydrogenosomes and numerous symbiotic bacteria, ectobionts included, specific and systematic status of which is not known with certainty (Fenchel and Finlay 1991). For the symbiotic ciliates of the rumen, living in anaerobic conditions, contacts of the cell surface with metanobacteria are also characteristic (Vogels et al. 1980).

Ectobionts are found in several families of ciliates from anaerobic condition: Trichostomatida (*Trimyema, Plagiopyla, Sonderia*), Scuticociliatida (*Cristigera*), Heterotrichida (*Parablepharisma, Metopus, Caenomorpha*), Odontostomatida (*Myelostoma*) and in Entodiniomorphida (Fenchel and Finlay 1991).

Endobionts. Data on the symbiotic microorganisms of ciliates' cell compartments are quite numerous. They are presented in Table 4. Certain species of endobionts may be sustained in the cytoplasm without symbiontophoric vacuole, whereas other species are always surrounded by the host's membrane. Some endosymbionts are surrounded by the flattened endoplasmic reticulum cisternae (Ossipov et al. 1986, Fokin 1993). It should be noted that there are no reliable data on endobionts' sustaining in ciliates mitochondria except for one reference (Pyutorac and Grain 1972). In general, the ciliates are characterised by a great variety of internuclear symbiotic bacteria (Ossipov et al. 1976; 1986; Ossipov 1981; Görtz 1986, 1988; Heckmann and Görtz 1991; Fokin 1993). Endosymbionts are sustained both in micronucleus and macronucleus. As the symbiotic systems of ciliates were the subjects of quite full reviews, in this part of our review we won't consider them in detail as we have done above for two other protistan groups - amoebae and flagellates.

For stable symbiotic systems of Ciliophora a high degree of specificity of the partners on the species level is characteristic. On the one hand, a rather limited number of symbiotic microorganisms is known, which are sustained in the cells of a particular species of the host or even in its certain intraspecific groups (subspecies, syngenes, clones, etc.). Potential number of symbionts may differ significantly even for closely related host species, as seen observed within the limits of *Paramecium* genus (Table 4). In a number of cases the presence of this or that symbiont is a generally accepted specific character of the host. For example, the presence of symbiotic algae is a specific character for the ciliate *Paramecium bursaria*.

On the other hand, a certain species of symbiotic microorganisms is sustained, as a rule, only in a definite ciliate species, or even in a certain intraspecific groups: syngenes or clones of certain genotypes (Sonneborn 1959, Preer and Preer 1984). Symbiotic systems of intranuclear bacteria and ciliates are shown to posses functional complementation of symbiont-host relationships, the possibility of stable associations formation being determined by genotype peculiarities of both the host and the symbiont (Skoblo and Lebedeva 1993; Rautian et al. 1990, 1993).

Frequency of occurrence of certain species of symbionts may differ essentially even in spatially and geographically close natural populations of ciliates (Ossipov et al. 1989). The degree of dependence of the partners of symbiotic associations varies greatly: from temporal relations, sporadically met with, to strict obligatory relations for both symbionts and ciliates. In some cases the partners' relationships reach such a high degree of physiological and genetical integration that the symbionts may be, actually, considered as a special extraorganelle of the ciliate cell. It is the case for *Euplotes* (Heckmann and Görtz 1991) and *Parauronema* (Soldo 1983). Spontaneous or induced loss of obligatory endobionts leads to lethal consequences for the ciliates or lowers abruptly their viability.

DISCUSSION

The analysis of peculiarities of the symbiotic systems of amoebae, flagellates and ciliates, presented in the previous publications (Podlipaev and Ossipov 1983; Cavalier-Smith and Lee 1985; Ossipov et al. 1986, 1996; Corliss et al. 1990; Fokin 1993; Smirnov et al. 1995) permits us to compare quite accurately the relative frequency of symbionts' occurrence in the hosts' cells in various protists' macrotaxa (Table 5), differing by their pattern of cellular organisation.

Evident qualitative differences can be noticed for symbiotic systems of amoebae and those of flagellates and ciliates. In amoebae of all the cell compartments only the cytoplasm is inhabited by prokaryotic microorganisms, whereas the cell coat, perinuclear space and karyoplasm

Table 5. Frequency of prokaryotes' occurrence on and in various cell compartments of protists with diverse patterns of cellular organisation

Hosts	cell surface	cytoplasm	perinuclear space	karyoplasm	References
Amoebae	-	+++	-	-	Smirnov et al. 1995
Flagellates	+++	+++	++	++	Ossipov et al. 1996
Ciliates	++	+++	++	+++	Ossipov et al. 1986, Fenchel and Finlay 1991, Heckmann and Görtz 1991, Fokin 1993, Rosati 1994, Fokin and Karpov 1995

"-" - absence of reliable data on the presence of cytobionts;
"++" - several records known;
"+++" - phenomenon widely distributed.

remain inaccessible for the symbionts (Smirnov et al. 1995). The noted limitations in symbiotic associations formation are probably connected with such characteristics of the pattern of amoebae cell organisation as dynamic body shape, amoeboid movement and peculiarities of EPR membrane conversion (Sleigh 1989, Bovee 1991).

On the contrary, symbiotic systems of flagellates and ciliates in general have much in common, though there are evident differences as well. References of ectobionts in ciliates (Raikov 1971, Fenchel and Finlay 1991, Rosati 1994) are not so numerous as compared to a noted high frequency of symbionts' occurrence on the surface of flagellates (Table 2). This might be also connected with essential differences in cortical structures organisation in flagellates and ciliates (Margulis et al. 1990, Karpov S. 1990, Lynn and Corliss 1991, Vickerman et al. 1991). We suppose that the wide distribution of symbionts in karyoplasm of ciliates nuclear apparatus is undoubtedly connected with two major tendencies in the evolution of this macrotaxon. The first is the appearance of unique form of the nuclear apparatus of ciliates on the basis of nuclear dualism and polygenomic nature of macronucleus, and also the closed type of division of somatic and generative nuclei (Ossipov 1981; Raikov 1982, 1989, 1994, 1995). Secondly, it is conjugation - a form of sexual process with no formation of typical gametes, followed by abrupt reconstruction of cellular organisation and removing of numerous inclusions takes place. The conjugants, retaining the continuity of cytoplasmic structures, exchange only by sexual nuclei - pronuclei, which promotes the preservation of endobionts' population (Ossipov 1979, 1981). In our opinion, these two trends in the evolution of ciliates as of a progressive phylum of protists have substantially increased the stability of cell compartments as the endobionts' environment. Exceptionally high phagocytic activity of these protists and large size of the cell (up to several hundred microns and more) should be considered as essential factors, causing the high frequency of the origin of endosymbiotic associations of ciliates and bacteria.

Nowadays a postulate is widely accepted that the flagellate pattern of cellular organisation is primary towards all other patterns, amoeboid one included (Taylor 1978; Cavalier-Smith 1992, 1993, 1995; Corliss 1994; Sleigh 1995). At the root of the most recent phylogenetical trees of lower protists flagellate forms stand, among which the most close to ancestral forms are Pelobiontida and Diplomonadida.

It is generally accepted that on the earlier stages of evolution the prokaryotic microorganisms, due to the peculiarities of their ultrastructural organisation and physiology of cell membranes, were utterly incapable of symbiotic systems' formation among themselves (Margulis 1970, 1981, 1993). After transition to the eukaryotic level of organisation the ancestral protists acquired, on the basis of phagocytic activity, the ability to form symbiotic associations with prokaryotes. Even in the lowest flagellates (amitochondrial, non-photosynthetic, feeding heterotrophically, by means of phagocytosis as well) the ability arises to form a great diversity of symbiotic systems with the bacteria. Symbiogenesis of some cell organelles of primitive eukaryotes is supposed by many authors to take place on this basis (Margulis 1970, 1981; Sitte 1993; Cavalier-Smith 1995; Sleigh 1995).

We believe that the study of basic stages of the origin of symbiotic relationships of prokaryotes and flagellates will make it possible to formulate the major principles of the origin of symbiotic systems of protists.

Intracellular parasitism is characteristic for all representatives of the phylum Microsporidia, one of the most ancient phyla among eukaryotes. Microsporidia are considered to turn to parasitc way of life before the appear-

ance of flagellar organisation of their free-living ancestors (Karpov S. 1990, Brugerolle 1991, Patterson D. 1994). According to our opinion, the particularities of patterns of Microsporidia cell organisation, but not only their parasitism, should be considered as the primary cause of the symbiont absence in this phylum. This explains, in our opinion, an absence of symbionts in microsporidia. Symbiotic systems are widely represented among some protistan macrotaxa, originated on the basis of flagellate ancestor and possessing rather complicated life cycles with alternation of the patterns of cellular organisation. Such is the case, for example, for numerous representatives of the following taxa: Granuloreticu-losa (class Foraminiferea), Heliozoa, Radiolaria (Cavalier-Smith and Lee 1985; Lee et al. 1985a,b; Lee and Anderson 1991).

It is interesting to compare the data on the evolutionary close relationship of the groups, differing greatly by their patterns of cellular organisation, such as Dinophyceae, Apicomplexa and Ciliophora (Schlegel 1991, Cavalier-Smith 1995), with the peculiarities of symbiotic systems of these groups. As shown in our review (Tables 3 and 4), symbiotic associations of Dinophyceae are diverse though not as numerous as those of Ciliophora, whereas data on the symbionts of Apicomplexa are absent. These comparative evolutionary data, obtained with the use of molecular biological methods, demonstrate with certainty that the peculiarities of cellular organisations of protists macrotaxa influence essentially the ability to use the host's cell as a habitat for associated microorganisms.

During the evolutionary prime of the protists (Kuźnicki and Walne 1993, Cavalier-Smith 1995), in certain taxonomic groups, for example, in amoebae, and in connection with the transition to intracellular parasitism (Apicomplexa, Plasmodiophora), the primary ability of eukaryotic cell to the formation of symbiotic systems, in our opinion, was subject to partial or complete reduction. This process is especially noticeable in the point of transition to multicellular level of organisation, for example, in animals (Gromov 1978).

We suppose that the formation of the symbiotic relations in the lower prokaryotes' evolution is not a partial result of accidental ancestral partners' combinations. It is a common phenomenon, distributed in protistan according to certain principles. It is pronounced in various degrees and diverse forms in different evolutionary branches. Symbiotic associations are of different geochronological age - from evolutionary ancient to the young ones, constantly appearing nowadays as well in many species of protists. All the transitional forms are present: from accidental, temporary associations of partners to obligatory stable symbiotic relationships. The presented analysis demonstrates that the appearance of stable symbiotic associations is to a great extent determined by the pattern of cellular organisation of the host and depends upon the morphophysiological preadaptations of microorganisms trying to use the protists' cells as their habitat in the course of neoevolutionary process.

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REFERENCES

Anand C.M., Skinner A.R., Malic A., Kurtz J.B. (1983) Interaction of *L. pneumophila* and a free-living amoeba (*Acanthamoeba palestinensis*). *J.Hyg.* **91:** 167-178

Andersen R.A. (1992) Diversity of eukaryotic algae. *Biodiversity and Conservation.* **1:** 267-292

Ball G.H. (1969) Organisms living on and in Protozoa. In: Research in Protozoology, (Ed. T.T. Chen). Pergamon Press, New York, **3:** 565-718

Bloodgood R.A., Fitzharris T.P. (1976) Specific associated of prokaryotes with symbiotic flagellate *Protozoa* from the hindgut of the termite *Reticulitermes* and the wood-eating roach *Cryptocercus*. *Cytobios.* **17:** 103-122

Bovee E.C. (1991) Sarcodina. In: Microscopic Anatomy of invertebrates, (Eds. F.W. Harrison and J.O. Corliss). Wiley-Liss, New York, 161-259

Brugerolle G. (1981) Etude ultrastructurale du flagelle parasite *Polymastix melolonthae* (Oxymonadida). *Protistologica.* **17:** 139-145

Brugerolle G. (1991) Flagellar and cytoskeletal systems in amitochondrial flagellates: Archamoeba, Metamonada, and Parabasalia. *Protoplasma* **164:** 70-90

Byers T.J. (1986) Molecular biology of DNA in *Acanthamoeba, Amoeba, Entamoeba, and Naegleria*. *Int.Rev.Cytol.* **99:** 311-341

Cann J.P. (1981) An ultrastructural study of *Mayorella viridis* (Leidy) (Amoebida: Paramoebida) - a rhizopod, containing zoochlorellae. *Arch. Protistenk.* **124:** 353- 360

Cavalier-Smith T. (1992) Origin of the cytoskeleton. In: The Origin and Evolution of the Cell, (Eds. H. Hartman, and K. Matsuno). World Sci., Singapore, 79-106

Cavalier-Smith T. (1993) Kingdom Protozoa and its 18 phyla. *Microbiol. Rev.* **57:** 953-994

Cavalier-Smith T. (1995) Zooflagllate phylogeny and classification. *Cytology.* **37**: 1010-1029

Cavalier-Smith T., Lee J.J. (1985) Protozoa as hosts for endosymbiosis and the conversion of symbionts into organelles. *J. Protozool.* **32**: 376-379

Chang K.-P. (1974) Ultrastucture of symbiotic bacteria in normal and antibiotic treated *Blastocrithidia culicis* and *Crithidia oncopelti*. *J. Protozool.* **21:** 699-707

Chang K.-P. (1980) Modulation of polyamine level and biosynthetic enzymes by bacterial endosymbionts in Trypanosomatid protozoa. In: Endocytobiology. Endosymbiosis and Cell Biology. (Eds. W. Schwemmler , and H. Shenk) Gruyter Comp., Berlin, New York, 348-359

Corliss J.O. (1994) An interim utilitarian (user-friendly) hierarchical classification and characterization of the protists. *Acta Protozool.* **33:** 1-51

Corliss J.O., Fujisima M., Lee J.J., Lee R.E., Soldo A.T., Jeon K.W. (1990) Endosymbionts of Protozoa. *Zool. Sci.* Suppl. **7:** 167-177

Daniels E. (1973) Ultrastructure. In: The Biology of Amoeba, (Ed. K.W. Jeon) New York, London, 125-169

Du Y., Chang K.-P. (1993) PCR amplification and sequence analysis of pre-ribosomal RNA genes from *Crithidia* endosymbionts. In: Endocytobiology, (Eds. S. Sato, M. Ishida, and H. Ishikawa). Tubingen Univ. Press, Tubingen, 499-504

Du Y., Maslov D.A., Chang K.-P. (1994) Monophyletic origin of β-division proteobacterial endosymbionts and their coevolution with insect trypanosomatid protozoa *Blastocrithidia culicis* and *Crithidia* spp. *Proc. Nat. Acad. Sci. USA*. **91:** 8437-8441

Embley M., Finlay B., (1994) The use of small subunit rRNA sequences to unravel the relationships between anaerobic ciliates and their methanogen endosymbionts. *Microbiology*. **140:** 225-235

Esteves M.J.G., Andurade A.F.B., Angluster J., De Souza W., Mundim M.H., Roitman J., Perreira M.E.A. (1982) Cell surface carbohydrates in *Crithidia dianei*: influence of the endosymbionte. *Eur. J. Cell Biol*. **26:** 244-248

Faria e Silva P.M., Fiorini J.E., Soares M.J., Alviano C.S., De Souza W., Angluster J. (1994) Membrane-associated polysaccharides composition, nutritional requirements and cell differentiation in *Herpetomonas roitmani*: influence of endosymbiont. *J. Euk. Microbiol*. **41:** 55-59

Fenchel T., Bernard C. (1993) Endosymbiotic purple nonsulfur bacteria in anaerobiotic ciliated protozoon. *FEMS Microbil. Lett*. **110:** 21-25

Fenchel T., Finlay B.J. (1991) The biology of free-living anaerobic ciliates. *Europ. J. Protistol*. **26:** 201-215

Fields B.S., Shotts E.B., Feeley J.C. (1984) Proliferation of *Legionella pneumopfila* as an intracellular parasite of the ciliated protozoan *Tetrahymena pyriformis*. *Appl. Environm. Microbiol*. **47:** 467-471

Fokin S. (1993) Bacterial endobionts of ciliates and their employment in experimental protozoology. *Tsitologia*. **35:** 59-91 (In Russian with English summary)

Fokin S., Görtz H.-D. (1993) *Caedibacter macronucleorum* sp. nov., a bacterium inhabiting the macronucleus of *Paramecium duboscqui*. *Arch. Protistenkd*. **143:** 319-324

Fokin S., Karpov S. (1995) Bacterial endocytobionts inhabiting the perinuclear space of protista. *Endocytobiosis and Cell Res*. **11:** 81-94

Freymuller E., Camargo E.P. (1981) Ultrastructural differences between species of Trypanosomatids with and without endosymbionts. *J. Protozool*. **28:** 175-182

Frolov A.O. (1993). The origin of the trypanosomatides. *Parasitologiya* **27:** 97-107 (In Russian with English summary)

Frolov A.O., Karpov S.A. (1995) Comparative morphology of kinetoplastids. *Tsitologia* **37:** 1072-1094 (in Russian with English summary)

Gautom R.K., Fritsche T.R. (1995) Transmissibility of bacterial endosymbionts between isolates of *Acanthamoeba* spp. *J. Euk. Microbiol*. **42:** 452-456

Goodkov A.V., Karpov A.S., Malysheva L.V. (1993) Electron-microscopical investigation of artificially obtained (newly formed) symbiotic association of amoebae with chlorophyte algae. *Tsitologia* **35:** 127-133 (in Russian with English summary)

Görtz H.-D. (1986) Endonucleobiosis in ciliates. *Int. Rev. Cytol*. **102:** 169-213

Görtz H.-D. (1988) Endocytobiosis. In: Paramecium, (Ed. H.-D. Görtz). Springer- Verlag, Berlin, Heidelberg, New York, 393-405

Grell K., Benwitz G. (1970) Ultrastruktur mariner Amoben. I. *.Paramoeba eilhardi* Schaudinn. *Arch. Protistenk*. **112:** 119-137

Griffin J.L. (1988) Fine structure and taxonomic position of the giant amoeboid flagellate *Pelomyxa palustris*. *J.Protozool*. **35:** 300-315

Gromov B. V. (1978) Bacteria - intercellular symbionts of animals. *Uspechi Microbiologii*. **18:** 50-72 (in Russian with English summary)

Hagnere C., Harf C. (1993) Symbiotic interactions between free-living amoeba and harboured mercury-resistant bacteria. *Europ. J. Protistol*. **29:** 155-159

Harf C., Monteil H. (1989) Interaction between free-living amoebae and *Legionella* in the environment. *Wat. Sci. Technol*. **20:** 35-239

Harrison F.W. (1991) Micoroscopic Anatomy of Invertebrates. V.1. Protozoa. Wiley-Liss, New York

Hayes S.J., Hayes K.P., Robinson B.S. (1991) Geosmin as an odorous metabolite in cultures of a free-living amoeba, *Vannella* sp. (Gymnamoebia, Vannellidae). *J. Protozool*. **38:** 44-47

Heckmann K., Görtz H.-D. (1991) Prokaryotic symbionts of ciliates. In: The Prokaryotes, (Eds. A. Balows, H.G. Truper M. Dworkin, W. Harden, and K.H. Schleifer). Springer-Verlag, New York, Berlin, Heidelberg, 3865-3890

Holden D.P., Winkler H.H., Wood D.O., Leinbach E.D. (1984) Intracellular growth of *Legionella pneumophila* within *Acanthamoeba castellanii*. *Infect. Immunol*. **45:** 18- 24

Hülsmann N., Hausmann K. (1994) Towards a new perspective in protozoan evolution. *Europ. J. Protistol*. **30:** 365-371

Jeon K.W. (1983) Integration of bacterial endosymbiont in amoebae. *Int. Rew. Cytol*. **14:** 29-47

Jeon K.W. (1987) Change of the cellular pathogens into required cell components. *Ann. N. Y. Acad. Sci*. **503:** 359-371

Jeon K.W. (1995a) The large, free-living amoebae: wonderful cells for biological studies. *J. Euk. Microbiol*. **42:** 1-7

Jeon K.W. (1995b) Bacterial endosymbiosis in Amebas. *Trends in Cell Biol*. **5:** 137-140

Jeon K.W., Lorch I.J. (1967) Unusual intracellular bacterial infection in large, free- living amoebae. *Exp. Cell Res*. **48:** 236-240

Joyon L., Mignot J.-P., Kattar M.-R., Brugerolle G. (1969) Complements a l'etude des *Trichomonadida* et plus particulierment de leur cinetide. *Protistologica*. **5:** 309-326

Karpov S.A. (1990) The system of protists. Mezhvuzovskaya tip., OmPI, Omsk (in Russian)

Karpov S.A., Zhukov B.F. (1983) The ultrastructure of *Pleuromonas jaculans* Perti (Kinetoplastida, Zoomastigophorea, Sarcomastigophorea). In: Protozoa of activated sludge. Ser. Protozoology, Nauka, Leningrad. **8:** 153-156 (in Russian)

Karpov A.S., Goodkov A.V., Afonkin S.Yu. (1991) Survival of chlorellae - the symbionts of *Paramecium bursaria* - in the cytoplasm of large free-living amoebae of different species and clones differing by the pattern of the first stages of feeding. *Tsitologia*. **33:** 104-115 (in Russian with English summary)

Kies L., Kremer B.P. (1990) Phylum Glaucocystophyta. In: Handbook of Protoctista, Jones and Bartlett Publ., Boston, 152-166

Kilvington S., Price J. (1990) Survival of *Legionella pneumophila* within cysts of *Acanthamoeba polyphaga* following chlorine exposure. *J. Appl. Bacteriol*. **68:** 519- 525

Kirby H. (1941) Organisms living on and in Protozoa. In: Protozoa in biological research, (Eds. G.N. Calkins, and F. M. Summers). Columbia University Press, New York, 1009-1113

KuŸnicki L., Walne P. L. (1993) Protistan evolution and phylogeny: current controversies. *Acta Protozool*. **32:** 135-140

Lee J.J., Anderson O.R. (1991) Symbiosis in foraminifera. In: Biology of Foraminifera (Eds. J.J. Lee, and O.R. Anderson) Academic Press, London

Lee J.J., Corliss J.O. (1985) Symposium on Symbiosis in Protozoa : Introductory remarks. *J. Protozool*. **32:** 371-372

Lee J.J., Lee M.J., Weis D.S. (1985a) Possible adaptive value of endosymbionts to their protozoan hosts. J. *Protozool*. **32:** 380-382

Lee J.J., Soldo A.N., Reisser W., Lee M.J., Jeon K.W., Görtz H.-D. (1985b) The extent of algal and bacterial endosymbioses in Protozoa. *J. Protozool*. **32:** 391-403

Lee J.J., Wray C. G, Lawrence C. (1995) Could foraminiferal zooxantellae be derived from environmental pools contributed by different coelenterate hosts? *Acta Protozool*. **34:** 75-85

Lemke P.A. (1976) Viruses of eukaryotic microorganisms. *Ann. Rev. Microbiol*. **30:** 105-145

Lipscomb D.L., Riordan G.P. (1995) Ultrastructural examination of virus-like particles in a marine rhizopod (Sarcodina, Protista). *Acta Protozool*. **34:** 35-44

Lynn D.H., Corliss J.O. (1991) Ciliophora. In: Microscopic Anatomy of Invertebrates, (Eds. F.W. Harrison, and J.O. Corliss). Wiley-Liss, New York **1:** 333-467

Margulis L. (1970) Origin of Eukaryotic Cells. Yale Univ. Press, New Haven, London

Margulis L. (1981) Symbiosis in Cell Evolution. W.H. Freeman and Co, San Francisco

Margulis L. (1993) Symbiosis in Cell Evolution. 2 ed., W.H. Freeman and Co, New York

Margulis L., Corliss J.O., Melkonian M., Chapman D.J. (Eds) (1990) Handbook of Protoctista. Jones and Bartlett Publ., Boston

Michel R., Muller K.D., Schmid E.N. (1995) *Ehrlichia* like organisms (KL-1) observed as obligate intracellular parasites of *Saccamoeba* species. *Endocytobiosis and cell res.* **11:** 69-80

Ossipov D.V. (1979) On possible reasons of the absence of free gamets in the sexual process of Ciliophora . *Tsitologia.* **21:** 257-270 (In Russian with English summary)

Ossipov D.V. (1981). Problems of nuclear heteromorphism of the unicellular organisms. Nauka, Leningrad (In Russian)

Ossipov D. V., Karpov S.A., Rautian M.S. (1996) Occurrence of prokaryotic ecto- and endocytobionts in flagellated protists. *Tsitologia* **38:** 411-426 (In Russian with English summary)

Ossipov D. V., Podlipaev S.A. (1981) Theoretical and practical aspects of studies on the relationships between protozoa and microorganisms. In: The Interaction between Protozoa and Viruses, (Ed. J.H. Teras). Nauka, Leningrad, **6:** 5-30 (in Russian with English summary)

Ossipov D.V., Fokin S.I., Skoblo I.I. , Borchsenius O.N., Rautian M. S. (1986) Ecologo-morphological principles of evolution of endosymbiotic systems in Protozoa (ultrastructural aspect). *Tsitologia* **28:** 1155-1164 (In Russian with English summary)

Ossipov D.V., Fokin S.I., Borchsenius O.N., Lebedeva N.A., Rautian M. S., Skoblo I.I. (1989) Diversity and distribution of the intranuclear symbionts of ciliates of the Soviet Union. In: Ecology of Marine and Fresh-water Protozoans (Proceedings of the II Symposium, 12-15 Sept., 1989). (Ed. Y.I. Polyanski), Yaroslavl, 53

Ossipov D.V., Gromov B.V., Mamkaeva K. A., Rautian M.S., Skoblo I.I., Borchsenius O.N. (1976) The use of symbiotic bacteria for analysis of the structure and function of nuclear apparatus of ciliates. In: Karyology and Genetics of Protozoa, (Ed. I.B. Raikov). Nauka, Leningrad, **1:** 101-139 (In Russian with English summary)

Page F. C. (1980) A light and electrone microscopical comparison of limax and flagellate marine amoebae belonging to four genera. *Protistologica.* **16:** 7-78

Page F.C. (1983) Marine Gymnamoebae. Inst. Terr. Ecol., Cambrige.

Page F. C. (1987) The classification of 'naked' amoebae (Phylum *Rhizopoda*). *Arch. Protistenk.* **133:** 199-217

Page F.C. (1988) A new key to freshwater and soil gymnamoebae (with instructions for culture). Freshwater Biol. Ass., Ambleside

Page F.C. (1991) Nackte Rhizopoda. Protozoenfauna, Stuttgart, New York, **2:** 1-170

Patterson D.J. (1988) The evolution of Protozoa. Mem. Inst. Oswaldo Cruz, Suppl. 1, **83:** 580-600

Patterson D.J. (1994) Protozoa: evolution and systematics. In: Progress in Protozoology, (Eds. K. Hausmann, and N. Hülsmann). Gustav Fischer Verlag, Stuttgart, Jena, New York, 1-14

Patterson J.L. (1990) Viruses of protozoan parasites. *Exp. Parasitol.* **70:** 111-113

Podlipaev S.A., Ossipov D.V. (1983) The cell of Protozoa as the environment of symbionts. In Biological Research Institute Transactions, (Ed. J.I. Polyansky). Leningrad University, Leningrad, **34:** 73-112 (in Russian with English summary)

Polyansky Y. I. (1981) About specificity of evolutionary process in large taxa (on example from the protozoan evolution). In: The Problems of the Modern History of Evolutionary Sstudies, (Ed. Y. M. Gall). Nauka, Leningrad (in Russian)

Polyansky Y. I., Raikov I.B. (1976) Polymerisation and oligomerisation phenomenes in protozoans evolution. *Trans. Amer. Microsc. Soc.* **95:** 314-326

Preer J.R., Preer L.B. (1984) Endosymbionts in Protozoa. In: Bergey's Manual of Systematic Bacteriology, (Eds. N.R. Krieg, and J.G. Holt). Williams and Wilkins, Baltimore, London, **1:** 795-811

Preer J.R. Jr., Preer L.B., Jurand A. (1974.) Kappa and other endosymbionts in *Paramecium aurelia. Bacteriol. Rev.* **38:** 113-163

Proctor L.M., Fuhrman J.A. (1990) Viral mortality of marine bacteria and cyanobacteria. *Nature* **43:** 60-62

Pushkareva V.I., Litvin V.J., Konstantinova N.D., Popov, V.L. (1990) Analysis of the mechanisms of interpopulational interactions of yersins with infusoria *Tetrahymena pyriformis* on cellular and subcellular levels. *Zh. Medi. Epid.* **23:** 3-8 (in Russian)

Puytorac P. de, Grain J. (1972) Bacteries intermitochondriales et particularites de l'ultrastructure cytostomo-pharryngienne chez le cilie *Urotricha ovata* Kahl. *C.R. Soc. Biol.* **166:** 604-607

Quackenbush R.L. (1988) Endosymbionts of killer paramecia. In: Paramecium, (Ed. H.-D. Görtz). Springer Verlag, Heidelberg, New York, 406-418

Raikov I.B. (1971) Bacteries epizoiques et mode de nutrition du cilie psammophile *Kentrophoros latum* Raikov, cilie holotriche mesopsammique. *Cah. Biol. mar.* **15:** 379-393

Raikov I.B. (1982) The Protozoan nucleus. Morphology and evolution. In: Cell Biol. Monogr., (Eds. M.A. Berkeley, W. Beermann, L. Goldstein, K.R. Porter, and P. Sitte). Springer-Verlag, Wien, New York, **9:** 1-174

Raikov I.B. (1989) Nuclear genome of the Protozoa. In: Progress in Protistology, (Eds. Corliss J.O., and Patterson D.J). Biopress Ltd, Bristol **3:** 21-46

Raikov I.B. (1994) The diversity of forms of mitosis in Protozoa: a comparative review. *Europ. J. Protistol.* **30:** 253-269

Raikov I.B. (1995) Structure and genetic organisation of the polyploid macronucleus of Ciliates: a comparative review. *Acta Protozool.* **34:** 151-171

Rautian M.S., Ossipov D.V. (1985) Genetic effects of the intracellular symbiosis. *Tsitologia.* **27:** 124-135 (In Russian with English summary)

Rautian M.S., Skoblo I.I., Lebedeva N.A., Ossipov D.V. (1990) Complementation in the symbiont-host system formation between the clones of the ciliate *Paramecium caudatum* and the stoks of the bacterium *Holospora obtusa. Tsitologia* **32:** 584-591 (in Russian with English summary)

Rautian M.S., Skoblo I.I., Lebedeva N.A., Ossipov D.V. (1993) Genetics of symbiotic interactions between *Paramecium bursaria* and the intranuclear bacterium *Holospora acuminata*, natural genetic variability by infectivity and susceptibility. *Acta Protozool.* **32:** 165-173

Reisser W., Meier R., Görtz H.-D., Jeon K.W. (1985) Establishment, maintenance, and integration mechanisms of endosymbionts in Protozoa. *J. Protozool.* **32:** 383-390

Rosati G. (1994) Entry of foreign bacteria and of its own epibionts (Epixenosomes) into an hypotrichous ciliate, *Euplotidium itoi*, by cortical invaginations. *Arch. Protistenkd.* **144:** 283-288

Scarlato O.A., Starobogatov Ya.I., Lobanov A.L., Smirnov I.S. (1994) The data bases in zoologycal systematics and the information concerning higher taxa of animals. *Zool. Zh.* **73:** 100-116 (In Russian with English summary)

Schenk H.E., Bayer M.G., Zook D. (1987) Cyanelles. From symbionts to organelle. *Ann. N.Y. Acad. Sci.* **503:** 151-167

Schlegel M. (1991) Protist evolution and phylogeny as discerned from small subunit ribosomal RNA sequence comparison. *Europ. J. Protistol.* **27:** 207-219

Schrenk H.-G., Bardele C. F. (1991) The fine structure of *Saprodinium dentatum* Lauterborn 1908 as a representative of the odontostomatida (Ciliophora). *J. Protozool.* **109:** 57-77

Seravin L.N. (1992) Eukaryotes lacking the most important cellular organelles (flagella, Golgi-complex, mitochondria), and the main purpose of organellogy. *Tsitologia.* **34:** 3-33 (in Russian)

Skoblo I.I, Lebedeva N.A. (1993) The infectiousness of *Holospora acuminata* - an intranuclear bacterial symbiont of *Paramecium bursaria* (Ciliata). *Tsitologia* **35:** 92-99 (in Russian with English summary)

Sitte P. (1993) Simbiogenetic evolution of complex cells and complex plastids. *Europ. J. Protistol.* **29:** 131-143

Sleigh M.A. (1989) Protozoa and other protists. Arnold, London

Sleigh M.A. (1995) Progress in understanding the phylogeny of flagellates. *Cytology.* **37:** 985-1009

Smirnov A.V., Ossipov D.V., Rautian M.S. (1995) Endocytobionts in representatives of two amoebian orders, *Euamoebida* and *Leptomyxida* (Lobosea, Gymnamoebia). *Tsitologia.* **37:** 403-414 (in Russian with English summary)

Soldo A.T. (1983) The biology of the xenosome, an intercellular symbiont. *Int. Rev. Cytol.* **14:** 79- 109

Soldo A.T., Fokin S. (1994) Interactions in Symbiosis. In Progress in Protozoology. (Eds. K. Hausmann, and N. Hülsmann). Gustav Fisher Verlag, Stuttgart, Jena, New York, 113-120

Soldo A.T., Brickson S.A., Vazquez D. (1992) The molecular biology of a bacterial endosymbiont. *J. Protozool.* **39:** 196-198

Soldo A.T. , Musil G., Brickson S.A. (1993) The invasive nature of an infectious bacterial symbiont. *J. Euk. Microbiol.* **40:** 33-36

Sonneborn T.M. (1959) Kappa and related particles in *Paramecium. Advan. Virus Res.* **6:** 229-356

Tamm S.L. (1982) Flagellated ectosymbiotic bacteria propel a eukaryotic cell. *J. Cell Biol.* **94:** 497-509

Tanichev A.I., Karpov S.A., (1992) Ultrathin structure of the colourless chrysomonade *Paraphysomonas vestita* from the Baikal. *Tsitologia* **34:** 101-105 (in Russian with English summary)

Taylor F.J.R. (1978) Problems in the development of an explicit hypothetical phylogeny of the lower eukaryotes. *BioSystems* **10:** 67-89

Taylor F.J.R. (1990) Symbioses in marine Protozoa. In: Ecology of Marine Protozoa. Oxford Univ. Press, New York, Oxford, 323-340

Teras J.H., (1986) Protozoal viruses and the interaction of protozoa with mammalian viruses. *Insect. Sci. Applic.* **7:** 355-361

Teras J.H., (1990) The relationship between protozoa and viruses. 4. Protozoa as hosts of mammalian viruses. *Proc. Estonian. Acad. Sci. Biol.* **39:** 237-241

Tyndall R.L., Domingue E. L. (1982) Cocultivation of *Legionella pneumophila* and free- living amoeba. *Appl. Env. Microbiol.* **44:** 954-959

Vickerman K. (1991) Organization on the bodonid flagellates. In: The Biology of Free-living Heterotrophic Flagellates. (Eds. D.J. Patterson, and J. Larsen) Clarendon Press, Oxford, 159-176

Vogels G.D., Hoppe W.F., Stumm C.K. (1980) Association of methanogenic bacteria with rumen ciliates. *Appl. Environ. Microbiol.* **40:** 608-612

Wang A.L., Wang C.C. (1991) Viruses of Protozoa. *Ann. Rev. Microbiol.* **45:** 251-263

Wichterman R. (1953) The biology of *Paramecium.* Blakiston and McGraw-Hill, New York

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

Wilcox L.W. (1985) Prokaryotic endosymbionts in the chloroplast stroma of the dinoflagellate *Woloszynskia pascheri. Protoplasma* **135:** 71-79

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Different Resting Stages of the Plasmodial Rhizopod *Reticulomyxa filosa*

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Summary. It has been shown that the freshwater plasmodial rhizopod *Reticulomyxa filosa* produces uncovered resting stages which are formed throughout the year in response to a deterioration of general environmental conditions. In summer, cysts with a thick bilayered wall (covered cysts) additionally develop. These cysts are able to endure drying and low temperatures. Because of their relatively high resistance, covered cysts seem to be relevant for dispersion not only by water as the uncovered resting stages but also by air.

Key words: covered cysts, dispersion, plasmodial rhizopod, *Reticulomyxa filosa*, uncovered resting stages.

INTRODUCTION

Reticulomyxa filosa is a plasmodial rhizopod that was first isolated in 1937 during a field collection in woods in the metropolitan area of New York City. At this location, *Reticulomyxa* was found together with slime moulds on decaying leaves at the edge of a tidal swamp. Nauss (1949) placed this multinucleated organism within a new genus and named it *Reticulomyxa filosa* because of its anastomosing filose pseudopodia.

Hülsmann (1984) found *Reticulomyxa* species in ponds of the Botanical Garden of the University of Bochum (Germany) and in Pays-de-Calais (France). Schliwa and Koonce (Schliwa et al. 1984, Koonce et al. 1986) discovered *Reticulomyxa* in a freshwater fish tank in the Department of Zoology at the University of California at Berkeley, USA. Since that time the plasmodial rhizopod has been used as a model system of intracellular transport. The knowledge on its physiological behaviour is based mainly on laboratory observations (Nauss 1949; Hülsmann 1984, 1987; Ostwald 1988; Gothe 1994).

Nauss (1949) mentioned the apparent encystment of *R. filosa* when cells were maintained at lowered temperatures or when prolonged in culture along with algae. Hülsmann (1987) demonstrated in a film on cell motility, food capture, and reproduction that *R. filosa* forms cysts in response to unfavourable environmental conditions. Also Ostwald (1988) found cysts with a thick cyst wall.

The present study describes for the first time uncovered resting stages besides the formation of thick covered cysts from *R. filosa* and discusses their importance in relation to dispersion events.

MATERIALS AND METHODS

A strain of *Reticulomyxa filosa* supplied by Schliwa (Munich) was used. The cells were cultured in plastic Petri dishes containing natural spring water, boiled and filtered before use. This freshwater medium was supplemented with wheat germ flakes which stimulated the growth

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of bacterial and protistan prey essential for the development of *R. filosa*. The medium was replenished every 4-5 days. Cultures were maintained under natural conditions at 22-24°C. Some studies were performed in a day/night simulator realizing 12 h of light /12 h of dark and a temperature of 24°C.

The light microscopic observations were made using a Zeiss-Axioplan microscope equipped with a water immersion 40x phase contrast objective. This lens permitted long-term examination of cells without the stress imposed by observing cells under a coverslip. Observations at higher magnifications were made with an oil immersion 100x objective. In this case the coverslips were sealed with Lanolin to form a closed chamber, containing settled *R. filosa*.

The encystment and excystment events were documented by video tapes.

For electron microscopy, the specimen were fixed for 1 h at room temperature in a solution of 1% formaldehyde and 0.5% glutaraldehyde in 10 mM Pipes (pH 7.2), 1 mM EGTA and 1 mM $MgSO_4$, postfixed in 0.2% osmium tetroxide for 1h, 0.05% tannin for 45 min and stained with 0.5 uranyl acetate for 12 h, dehydrated in acetone, and embedded in Vestopal. Observations were made using a Tesla BS 540 or a Philips 400 T transmission electron microscope.

RESULTS

Reticulomyxa filosa was found to produce two types of dispersion forms: uncovered resting stages and durable covered cysts. Throughout the year uncovered resting stages (Figs. 1a-c) with a smooth surface and without an outer envelope were formed in response to a deterioration of environmental conditions. They were of different sizes, 10-200 µm diameter, and different morphological forms: most were spherical, but some were oviform or irregular. Additionally, cysts with a thick, mucous envelope (covered cysts, Figs. 4-6) were formed. Unlike the temporary resting stages these cysts appeared in our cultures only from May to September.

The development of uncovered resting stages was promoted by an excessive proliferation of bacteria, algae, or other protists within the cultures. Electron microscopic images show numerous vacuoles filled with bacteria

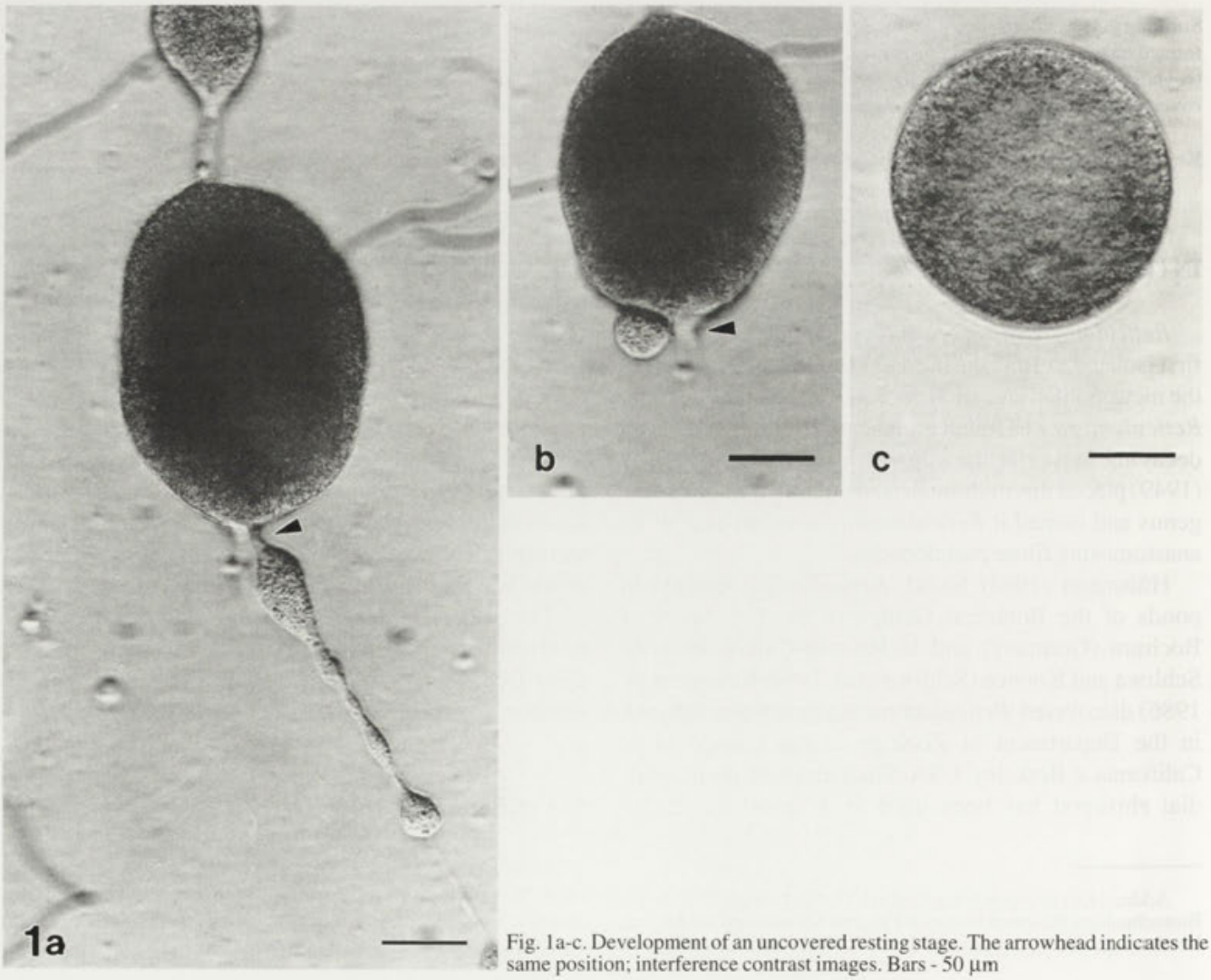
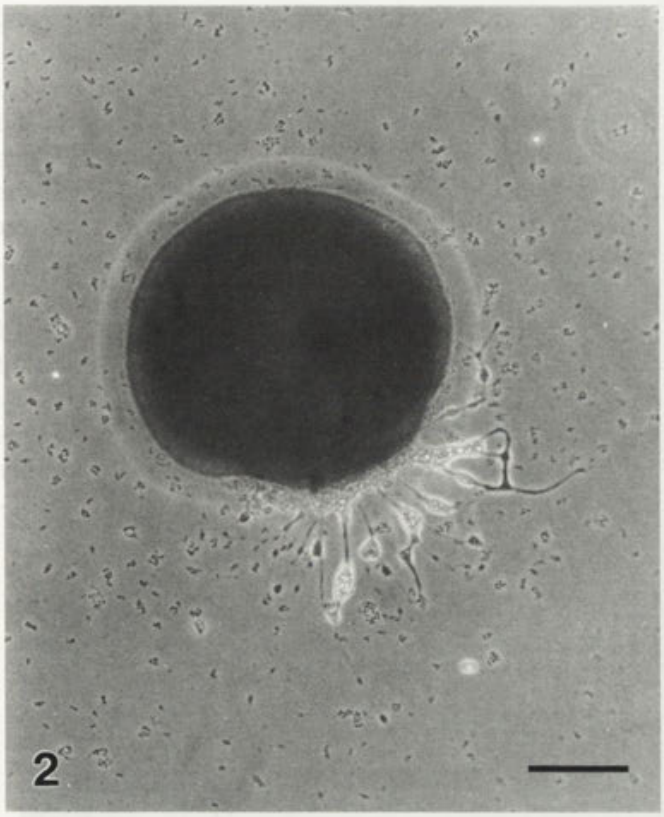
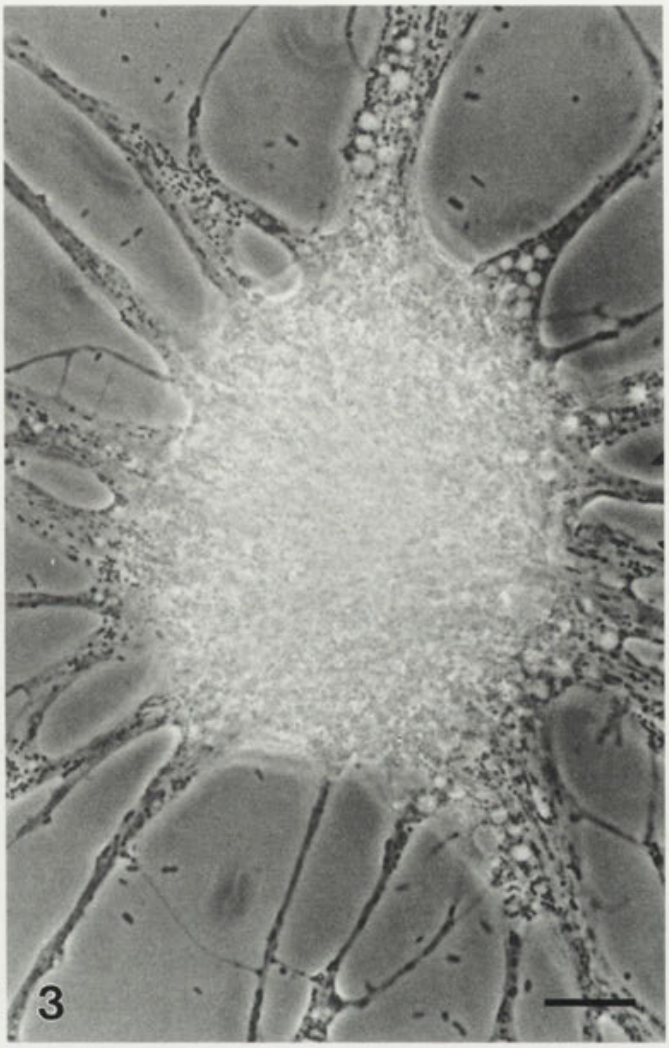


Fig. 1a-c. Development of an uncovered resting stage. The arrowhead indicates the same position; interference contrast images. Bars - 50 µm





Figs. 2 ,3. Development of a network from an uncovered resting stage. 1-h interval between (2) and (3), phase contrast images. Bars - 50 µm (2), 10 µm (3)

(Fig. 9). In cultures which were left without replenishing the medium, uncovered resting stages developed after 12-14 days and were produced from ***all*** plasmodia of the population in a culture dish. Such resting stages developed faster in cultures maintained at reduced temperatures. For example, after only two days at 6°C in a dark refrigerator the protoplasm contracted, the reticulate network disappeared, and several differently sized uncovered resting stages were formed from every plasmodial rhizopod. Darkness alone did not cause these processes. The uncovered resting stages did not tolerate extreme conditions: They did not survive storage at -16°C, desiccation, or contact with air. When they were moved to the air/water interface their cytoplasm expanded some 10 fold followed by disruption of the cell membrane.

On the other hand, uncovered resting stages survived for two to three weeks at the unfavourable environment and could be transported easily by floating in water. Upon improvement of living conditions, e.g., by translocation into fresh spring water at room temperature, they rapidly responded by radiating thin reticulopodial strands within just a few minutes (Fig. 2) followed by formation of complete reticulate networks within 1 h (Fig. 3). A much longer stay at adverse conditions causes the resting stages to die. In this case they were digested by bacteria and other protists.

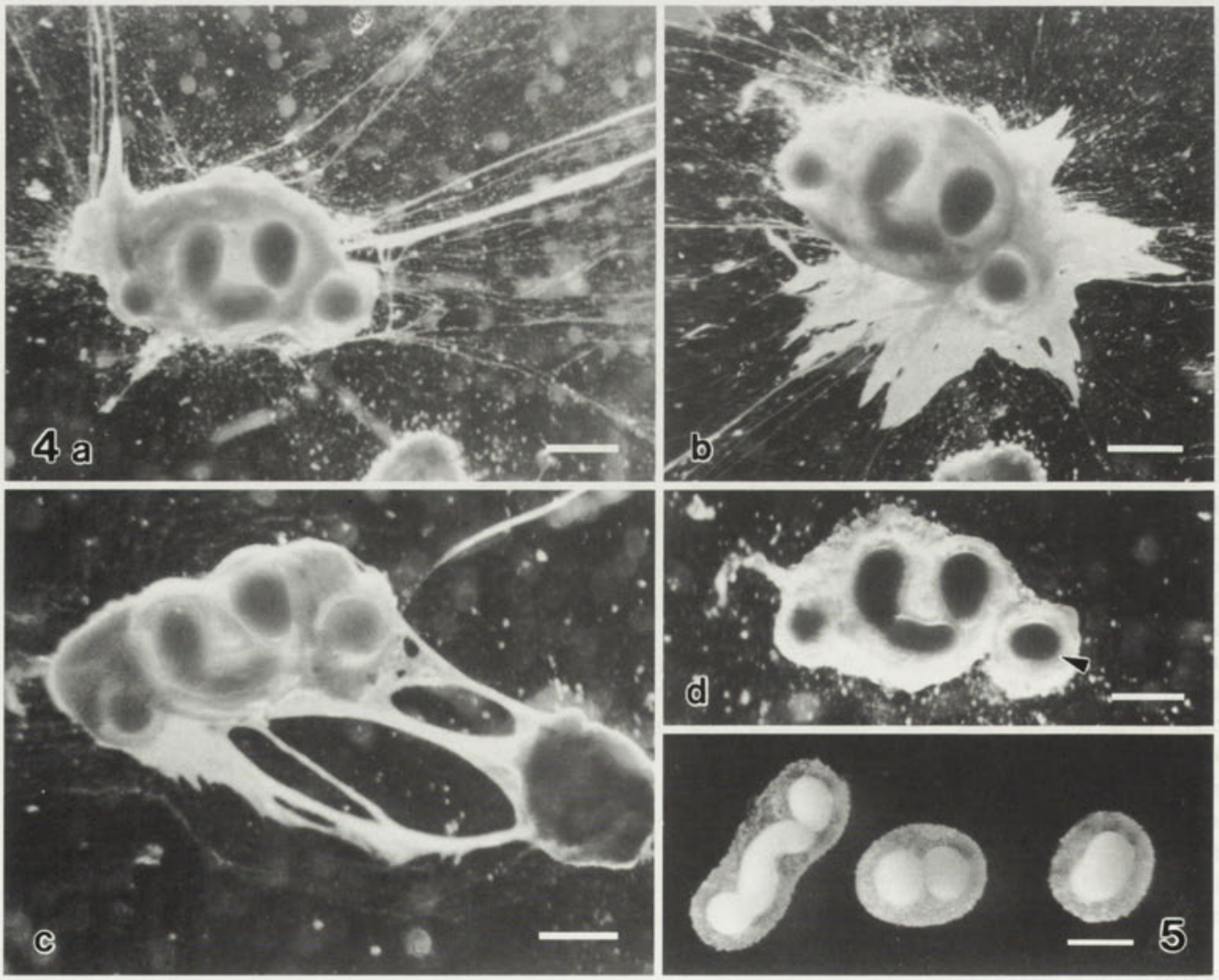
Covered cysts were formed by restreaming of the cytoplasm to the centre of the pseudopodial network (Figs. 4a-d, 5). A cover consisting of two layers, a thin (1-2 µm) elastic inner layer and a thicker (20-30 mm) outer one (Figs. 6, 7) appeared. The outer layer seems to be developed from mucous metabolic end products like the „false body or replica" (Nauss 1949) which the moving plasmodium left behind after a cleansing process. Beneath the cyst covers numerous reserve granules appea-red (Fig. 10b). The number of nuclei per volume unit in both uncovered resting stages and in covered cysts is found to be twofold higher than in the spread plasmodial network (Figs. 9, 10).

In contrast to formation of uncovered resting stages, covered cysts developed in summer cultures independently on replenishing of medium. It has to be mentioned that **not all** plasmodia of a population formed covered cysts. Some networks remained spread until responding to adverse conditions with developing uncovered resting stages.

Covered cysts had a high ability to withstand adverse environmental conditions. They were able to endure complete desiccation in the surrounding biotop. Addition of fresh medium to cysts kept up to 23 days without water caused them to swell markedly. Two to three days later, the cysts revealed a disruption of about 15-25 µm in their cover. At this site the new plasmodium is released (Figs. 7a, b) leaving the cyst coat behind.

Some dried covered cysts also survived freezing and storage at -16°C (at least up to 5 days). In addition, covered cysts withstand pressure. When compressed under a coverslip they showed a certain elasticity and did not lyse like the uncovered resting stages. However, when the pressure was gradually increased by pressing the coverslip e.g., with a preparation needle, the cyst cover burst and the cytoplasm streamed out forming a new reticulate network (Fig. 8).

Over three years we obtained about 50 covered cysts in summertime. Uncovered resting stages could be induced at every time of the year. Under laboratory conditions the development of uncovered resting stages into plasmodial networks was nearly 100%, but only 20% of covered cysts were creeping out and develop-



Figs. 4a-d. Development stages of covered cysts from a network. 12-h interval between the images in (4a) and (4d), the arrowhead in (4d) indicates the cyst shown in (6). (4a-d), phase contrast images. Bars - 100 µm Fig. 5. Covered cysts, darkfield image. Bar - 100 µm

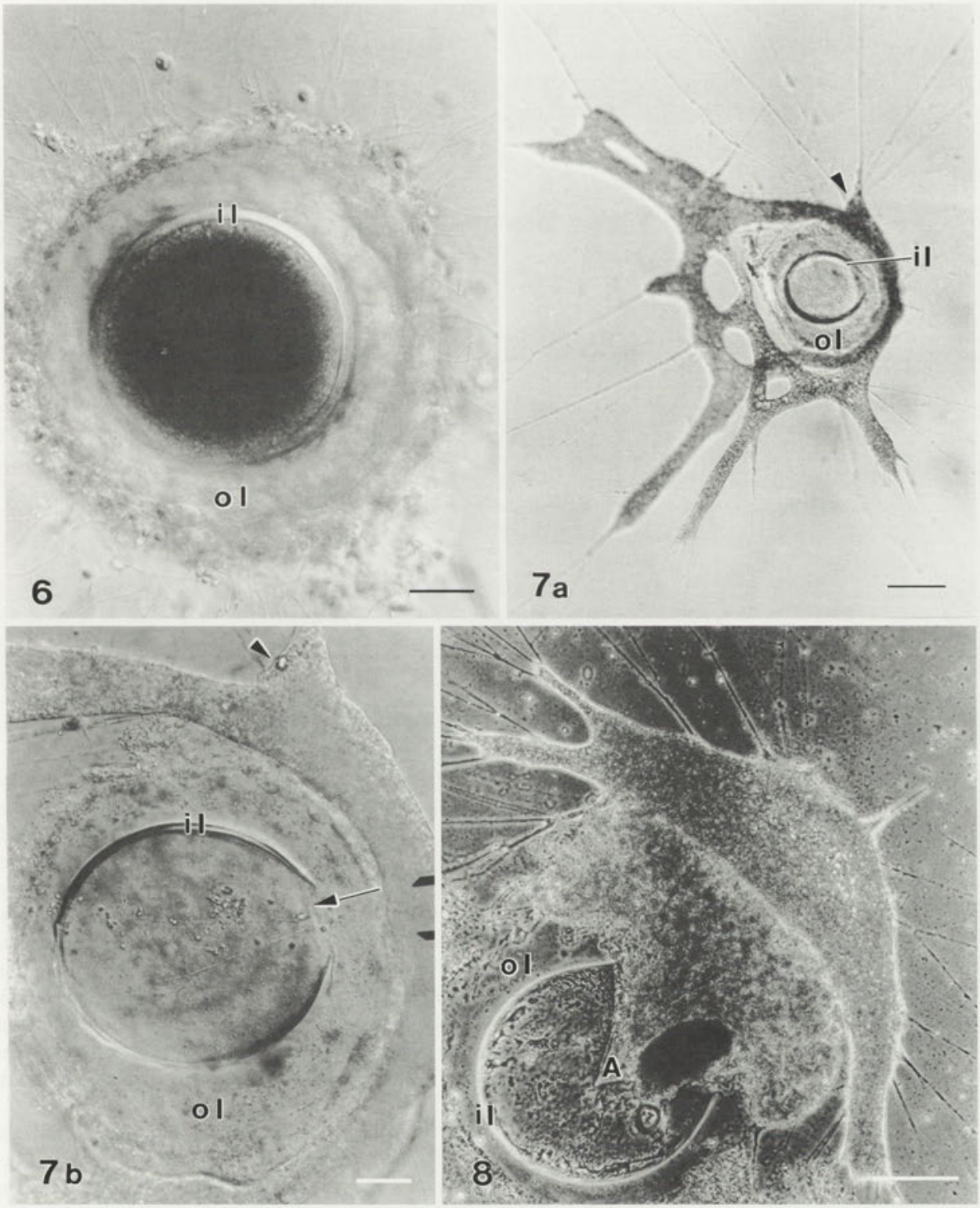
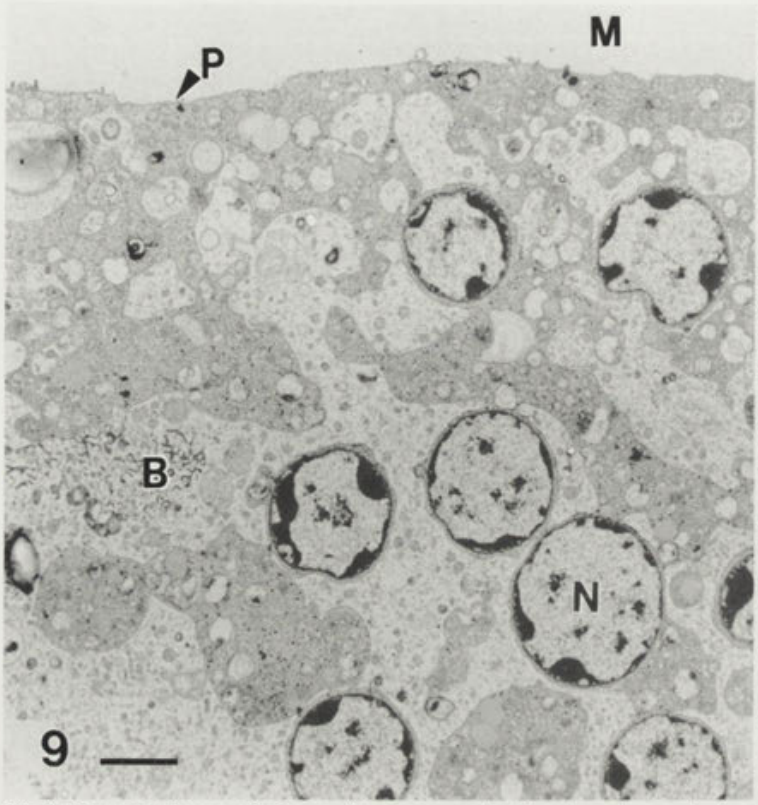
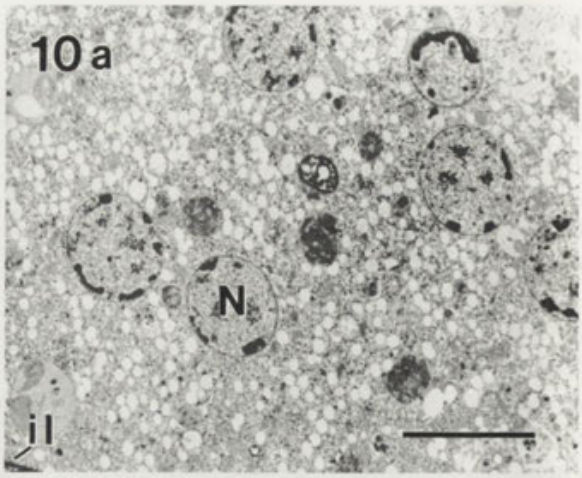


Fig. 6. Covered cyst, isolated from the cyst pack demonstrated in Fig. 4d (arrowhead). The thin filaments around the cyst cover are cyanobacteria. Bar - 25 µm

Fig. 7a, b. Excystment of a covered cyst. 7a- plasmodium creeped out of the cyst presented in Fig. 6. Bar - 100 µm. 7b- higher magnification of Fig 7a. The arrowheads mark the same position in both images. The arrow shows the gap where the plasmodium creeped out of the cyst. Note that the empty cyst cover did not collapse after outgrowing of the plasmodium indicating a mechanical stability of the cover. Bar - 25µm. ol - outer layer, il - inner layer of cyst cover, interference contrast images

Fig. 8. Plasmodium released by mechanical pressing the covered cyst between a coverslip and the bottom of the culture dish. (A) indicates the artificial wedge aperture. Bar - 50 µm





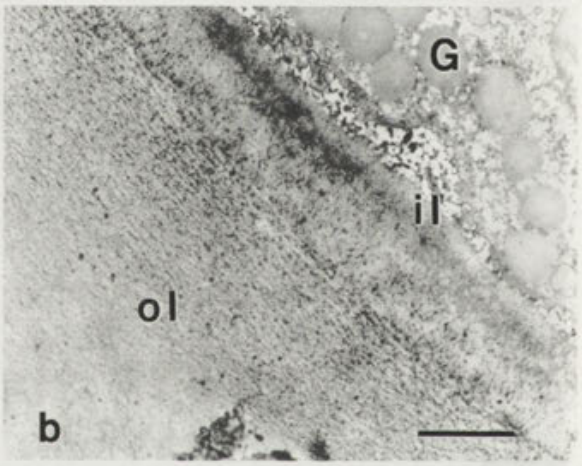


Fig. 9. Electron microscopic image of an uncovered resting stage demonstrating that it has no wall or cover. B - bacteria, P - plasmamembrane, M - culture medium. Bar - 2 µm

Fig. 10a, b. Electron microscopic images of a covered cyst showing the thick outer layer (ol) and the elastic inner layer (il), nuclei (N) and reserve granula (G). Bars - 5 µm (10a); 1 µm (10b)

ing new plasmodial networks. The reasons for that are unknown yet.

DISCUSSION

Many protozoa develop cysts to withstand adverse environmental conditions, e.g., periods of desiccation, high or low temperatures, or food deficiency. Cyst formation is also important in the dispersal of species (Corliss and Esser 1974). The cyst forming behaviour and morphology are different in rhizopoda (see Schönborn 1966, Laminger and Sturn 1984, Chardez 1989, Bovee 1991, Page and Siemensma 1991, Calvo-Mendez et al. 1993, Hülsmann 1993). In *Reticulomyxa filosa* there are two strategies to resist adverse conditions and to disperse:

First, the cultures respond rapidly to deterioration of environmental conditions (low temperature, deterioration of water following overgrowth of other organisms) by developing uncovered resting stages. Although they do not have a cover, they can be regarded as simple cysts, because they are produced to cope with bad conditions. In these cases, **all** individuals within a culture showed a synchronous response, e.g., they began to develop uncovered resting stages immediately after conditions began to deteriorate, regardless of the time of the year. Some testate amoebae form preencystment stages without producing a cyst wall (Schönborn 1966) for coping periods of biotop desiccation. Also in colpodea „thin-walled cysts" are seen (Foissner 1993) which are pluripotent. At acceptable living conditions they develop reproductive cysts, at unfavourable conditions they develop resting cysts. In *R. filosa* we have never seen a development of covered cysts from uncovered resting stages.

Second, the plasmodia produce durable covered cysts. This happened only in summer when the possibility of biotop desiccation may become maximum. It was not possible to initiate the formation of covered cysts in cultures at other times of the year. It seems that light is important in controlling this event since covered cysts have never been found in cultures maintained in total darkness. However, in studies over 15 weeks in winter controlling light levels in a day/night simulator with 12 h

of light / 12 h of dark and a temperature of 24°C, no covered cysts were produced suggesting that the control mechanisms governing encystment are more complicated. Moreover, it has been mentioned that **not all** individuals of a population formed covered cysts. This is surprising since an external triggering factor would act on the entire population of rhizopods. The fact that encystment was not induced in all cells suggests remarkable physiological differences between the cells within a culture.

The occurrence of the distinctive freshwater plasmodial rhizopod at geographical locations distant thousands of kilometres from each other suggests that *R. filosa* is a cosmopolitan organism. The question of how this species can be transported such distances is not completely resolved. The present study, which has shown that *R. filosa* can form both uncovered resting stages and covered cysts, does address part of this problem. Dispersal of uncovered resting stages is only possible in running water. However, covered cysts, which can tolerate desiccation, could be additionally carried in air over long distances.

But why do the rhizopods develop uncovered resting stages and not only covered cysts for survival of unfavourable environmental conditions and dispersion? One of natural habitats of *R. filosa* was found to be the edge of tidal swamps (Nauss 1949). If the water quality deteriorates, which can happen all over the year, uncovered resting stages rapidly form and are sufficient to disperse or to overcome conditions of minor environmental stress. But, to overcome seasonal extreme conditions (desiccation in summer followed by low temperature in winter) the organism could use a prophylactic mechanism for long-term protection. Development of covered cysts in summer seems to fulfil such a requirement. At times when no desiccation is expected no covered cysts are necessary, in particular because of their longer excystment time and lower excystment rate. Hence, it seems to be ecologically advantageous that one part of the population develops covered cysts for the case of complete desiccation, the other part only uncovered resting stages which are able to grow to a new network within a short period if no complete desiccation occurs, for instance in a rainy summer and autumn.

Over a period of three years we could observe about 50 covered cysts. Further studies are necessary to extent the knowledge on the mechanisms of the dimorphic trend in development of resting stages in *R. filosa*. Because there was no possibility to induce the development of covered cysts up to now, the experimental research on the mechanisms of encystment and excystment of covered cysts is more difficult than in the case of the uncovered resting stages. As a basis for such research, we originally intended to enlarge the number of culture dishes to get more covered cysts for larger experimental series. Unfortunately our strain of *R. filosa* died due to a laboratory accident and we have not been able to get a new culture up to now.

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REFERENCES

Bovee E.C. (1991) Cysts and spores. In: Microscopic Anatomy of Invertebrates (Eds. F.W. Harrison and J. O. Corliss). John Wiley and Sons, New York, 191-194

Calvo-Mendez C., Villagomez-Castro J.C., Lopez-Romero E. (1993) Rapid loss of ornithine decarboxylase activity during encystation of *Entamoeba invadens*. *FEMS Microbiol. Letters* **112:** 131-134

Chardez D. (1989) On the multiplication of *Centropyxis discoides* and the medium influence on the morphology of the test rhizopoda Testacea. *Acta Protozool.* **28:** 15-18

Corliss J.O., Esser S.C. (1974) Comments on the role of the cyst in the cycle and survival of free-living protozoa. *Trans. Amer. Micros. Soc.* **93:** 578-593

Foissner W. (1993) Class Colpodea (Ciliophora). In: Protozoenfauna, (Ed. D. Matthes). Gustav Fischer Verlag, Stuttgart

Gothe G. (1994) *Reticulomyxa filosa*, ein interessanter plasmodialer Rhizopode des Süßwassers. *Mikrokosmos* **83:** 301-306

Hülsmann N. (1984) Biology of the genus *Reticulomyxa filosa* (Rhizopoda). *J. Protozool.* **34:** 55a

Hülsmann N. (1987) Bewegung, Nahrungsaufnahme und Fortpflanzung bei *Reticulomyxa filosa* (Rhizopoda). Film Nr. C 1639, IWF, Göttingen

Hülsmann N. (1993) *Lateromyxa gallica* n. g., n. sp. (Vampyrellidae): a filopodial amoeboid protist with a novel life cycle and ultrastructural characters. *J. Euk. Microbiol.* **40:** 41- 49

Koonce M.P., Euteneuer U., Schliwa M. (1986) *Reticulomyxa*: a new model system of intracellular transport. *J. Cell Sci.* Suppl. **5:** 145-159

Laminger H., Sturn R. (1984) Influence of nutrition on encystment and excystment of testacea protozoa, rhizopoda. *Pedobiologia* **27:** 241-244

Nauss R.N. (1949) *Reticulomyxa filosa* gen. et spec. nov., a new primitive plasmodium. *Bull. Torrey Bot. Club* **76:** 161-173

Ostwald H. (1988) Ein Riese unter den Wurzelfüßern: *Reticulomyxa filosa*. *Mikrokosmos* **77:** 123-125

Page F.C., Siemensma F.J. (1991) Nackte Rhizopoda und Heliozoa. In: Protozoenfauna (Ed. D. Matthes). Gustav-Fischer-Verlag, Stuttgart, 150-157

Schliwa M., McDonald K.L., Koounce M., Euteneuer U. (1984) *Reticulomyxa*, a new model system for the study of intracellular organelle transport. *J. Cell Biol.* **99:** 239a

Schönborn W. (1966) Beschalte Amöben (Testaceae), Ziemsen Verlag Wittenberg

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Morphology and Microstructure of Selected Tintinnina Loricae

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Summary. The morphology and microstructure of three types of tintinnid loricae, hyaline, partially and totally agglutinated by foreign particles, from different geographic areas, are described. Their external surface morphology differs; on the hyaline surfaces there are granules or/and bristles, while the surfaces of agglutinated regions, when particle free are always smooth. Internally, each is similarly trilaminar, giving a honeycomb-like structure. Modifications are restricted to element size, number, and construction of laminae.

Key words: *Codonellopsis gaussi*, *Cymatocylis drygalskii*, *C. vanhoffeni*, *Helicostomella subulata*, honeycomb-like structure, *Laackmanniella naviculaefera*, *Parafavella denticulata*, tintinnid loricae, *Tintinnopsis lobiancoi*.

INTRODUCTION

Ciliates belonging to the suborder Tintinnina are characterized by having a lorica protecting the protoplasts. Loricae may be hyaline or partially or totally agglutinated by foreign particles (Gold and Morales 1976a, b; Gowing and Garrison 1992; Wasik et al. 1996), with particle adhesion facilitated through the adhesive of the lorica.

The structure of the lorica has long been of interest since its morphology formed the basis of tintinnid taxonomy (Kofoid and Campbell 1929, 1939; Hada 1970; Sassi and Melo 1986). Its internal structure, described mainly during protoplast examination (Laval 1972, Burkovsky 1973, Hedin 1975, Laval-Peuto 1980, 1981), has been described as vacuolar, tubular or compact, among others (Laval-Peuto and Brownlee 1986).

Tappan and Loeblich (1968) suspected that loricae with different structure, thickness and composition, might be built in different way. Gold and Morales (1976b) found that various mechanisms are involved in the formation of different parts of a lorica. Formation, however, is always directed from the posterior to the anterior end (Biernacka 1952, Laval-Peuto 1981, Laval-Peuto and Brownlee 1986). Using *Tintinnopsis parva*, Gold and Morales (1976b) showed that the basal region of the bowl is built from material secreted over the surface of the newly divided cell, while the collar comprises material secreted from a specialized region located anteriorly.

We suspected that differences in the way the lorica are formed, their morphologies, and adhesive properties, may be reflected in their microstructure. We chose *Helicostomella subulata*, *Cymatocylis vanhoffeni* and *C. drygalskii*, *Parafavella denticulata*, *Laackmanniella naviculaefera*, *Codonellopsis gaussi* and *Tintinnopsis lobiancoi*, since their loricae represent three morphologically different types (hyaline, partially, totally agglutinated). Moreover, these species are found in different geographic areas (Pierce and Turner 1993); many aspects of tintinnid structure and morphology are influenced by environmental factors during lorica formation (Laval-Peuto and Brownlee 1986).

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MATERIALS and METHODS

Loricae of ciliates from six Tintinnina genera were examined: *T. lobiancoi, H. subulata* from the Baltic Sea, and *P. denticulata* from the White Sea collected by the Center of Marine Biology in Gdynia. *C. drygalskii, C. vanhoffeni, Cd. gaussi* and *L. naviculaefera* collected in Admiralty Bay, King George Island, South Shetland Islands, by the Department of Antarctic Biology in Warsaw.

Lorica morphology, architecture and ultrastructure, were determined using a JEOL 1200 EX transmission electron microscope, and the same but equipped with ASID 19 scanning attachment operating at 40 Kv. Loricae were prepared as described by Wasik and Mikołajczyk (1991). To show lorica microstructure, individual loricae previously prepared for SEM were broken mechanically by micromanipulation and recoated with gold.

RESULTS

The tintinnid loricae examined represent morphologically different types; the hyaline *H. subulata* (Fig. 1A), *C. drygalskii* (Fig. 2A), *C. vanhoffeni* and *P. denticulata* (Fig. 3A); the partially agglutinated *L. naviculaefera* (Fig. 4A) and *Cd. gaussi* (Fig. 5A); the fully agglutinated *T. lobiancoi* (Fig. 6A).

Non-agglutinated loricae

In terms of structure, the simplest hyaline lorica we observed was that of the neritic *H. subulata* (Fig. 1A). It is divided to collar with spiral turns (Fig. 1B) and an elongated, narrow bowl. The spiral turns are visible on the TEM micrographs as electron dense strips (Fig. 1C). The collar ends with an entire rim (Fig. 1B). Externally, the lorica surface is slightly granular, while internally it is smooth (Fig. 1D). The internal structure comprises *ca* 0.3 μm thick trilaminar wall (Fig. 1D). External and internal laminae (*ca* 0.07 μm) are solid and separated by perpendicular septa *ca* 0.2 μm high, which occasionally branch (Fig. 1D). Septa form *ca* 0.1 μm wide alveoli (Fig. 1D) giving a honeycomb-like structure (Fig. 1E).

The hyaline loricae of the austral *C. drygalskii* (Fig. 2A) and *C. vanhoffeni* are more elaborated. The oral rim of *C. drygalskii* is formed of three short denticulated crests (Fig. 2B), while that of *C. vanhoffeni* has two (Fig. 2C). The external surface of each lorica is granular, but internally smooth (Fig. 2D). Distinct wrinkles (Fig. 2C) in the anterior part of the *C. vanhoffeni* lorica

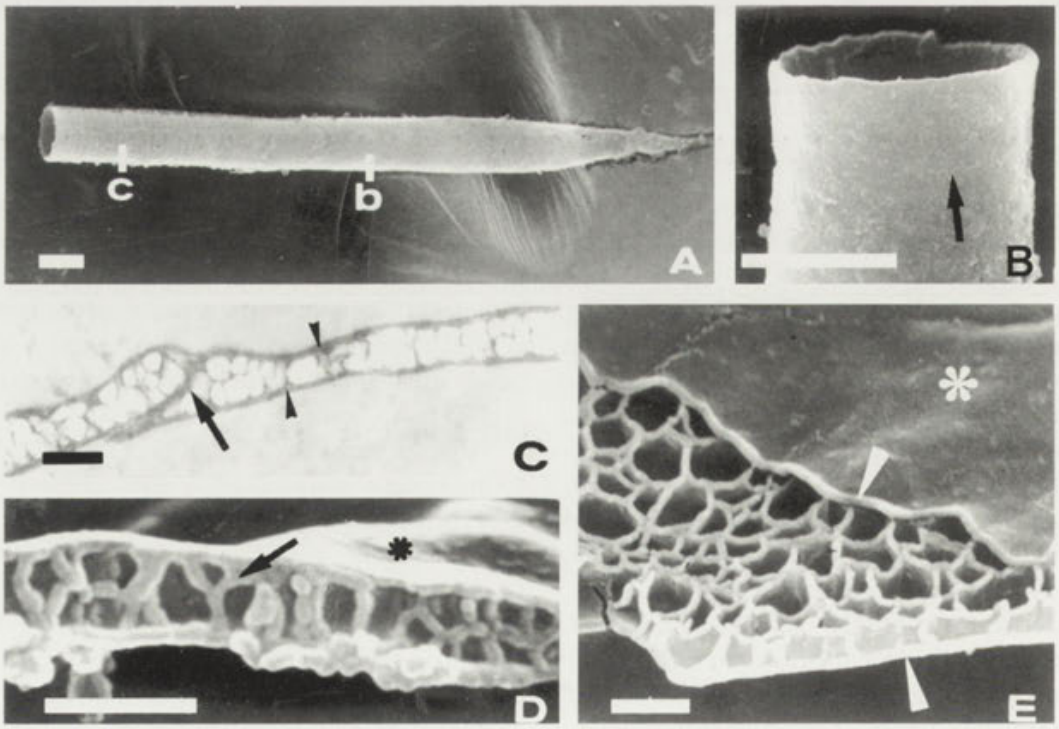

Fig. 1A-E. Scanning and transmission electron micrographs of *Helicostomella subulata* lorica. A - general view, b - elongated bowl, c - helicoidal collar, bar - 10 μm; B - collar with slightly marked turn (arrow), bar - 10 μm; C - TEM of collar, laminae (arrowheads), turn (arrow), bar - 1 μm; D - wall microstructure, branching septum (arrow), smooth inner surface (asterisk), bar - 1 μm; E - honeycomb-like structure, laminae (arrowheads), smooth inner surface (asterisk), bar - 1 μm

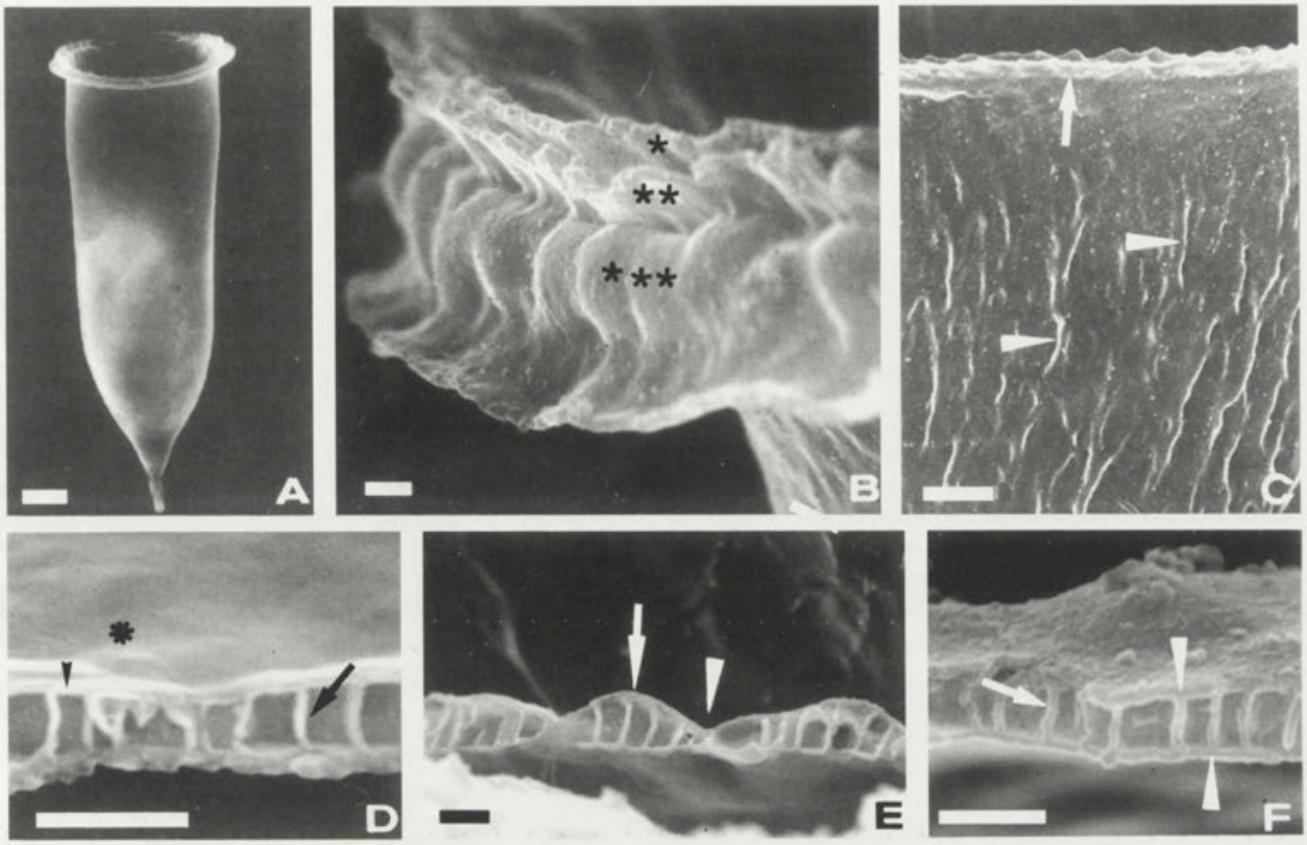

Fig. 2A-F. Scanning electron micrographs of *Cymatocylis drygalskii* and *C. vanhoffeni* loricae. A - general view of *C. drygalskii*, bar - 10 µm; B - three denticulated crests (stars) of *C. drygalskii* collar, bar - 1 µm; C - anterior part of *C. vanhoffeni*, with two denticulated crests (arrow), wrinkles (arrowheads), bar - 10µm; D - trilaminar wall of *C. drygalskii*, inner lamina (arrowhead), perpendicular septum (arrow), smooth inner surface (asterisk), bar - 1µm; E - microstructure of *C. vanhoffeni* wrinkled collar with short (arrowhead) and long (arrow) septa, bar - 1µm; F - microstructure of *C. drygalskii* bowl, ramifying laminae (arrowheads), septum (arrow), bar - 1 µm

are formed by periodic shortening and elongation of the perpendicular septa (Fig. 2E).

In the *ca* 0.8 µm thick *C. vanhoffeni* lorica wall there are 0.15 µm internal and external laminae separated by perpendicular septa 0,5 µm in length. These septa can branch to form additional alveoli. Laminae bordering the middle honeycomb-like layer, ramify and form the wall of septa (Fig. 2F).

The general scheme of the internal wall in the *C. drygalskii* lorica is very like that described above. Differences amounted only to the size and thickness of the internal elements, which are generally smaller in this species (Fig. 2D).

The lorica of the boreal *P. denticulata* (Fig. 3A) is non-agglutinated but has the most complex structure. Its solid, oral rim has smooth surface and is constructed with a single denticulated crest (Fig. 3B). The external surface of the bowl is coated by ca 0.5 µm bristles and small granules (Figs. 3B-D), while the internal surface is smooth (Fig. 3E). Pores of various sizes sometimes occur in the wall. The structure of the broken loricae shows a 2.5 µm thick wall, with *ca* 0.2 µm internal and external laminae, separated by *ca* 2 µm perpendicular septa (Fig. 3E) forming honeycomb-like patterns. The sides of each septum diverge to form an equilateral triangle with circular openings (Figs. 3E, F); the height of this triangle is 0.3 µm. The diameter of the openings is *ca* 0.04 µm. We were surprised to find that both external and internal laminae of the loricae exhibit their own honeycomb-like structure, with small perpendicular septa *ca* 0.07 µm long (Fig. 3G).

Partially agglutinated loricae

The tubular lorica of the austral *L. naviculaefera* (Fig. 4A) has a well defined hyaline collar with spiral turns (Fig. 4B). Its outer surface is lumpy. Granules are largest

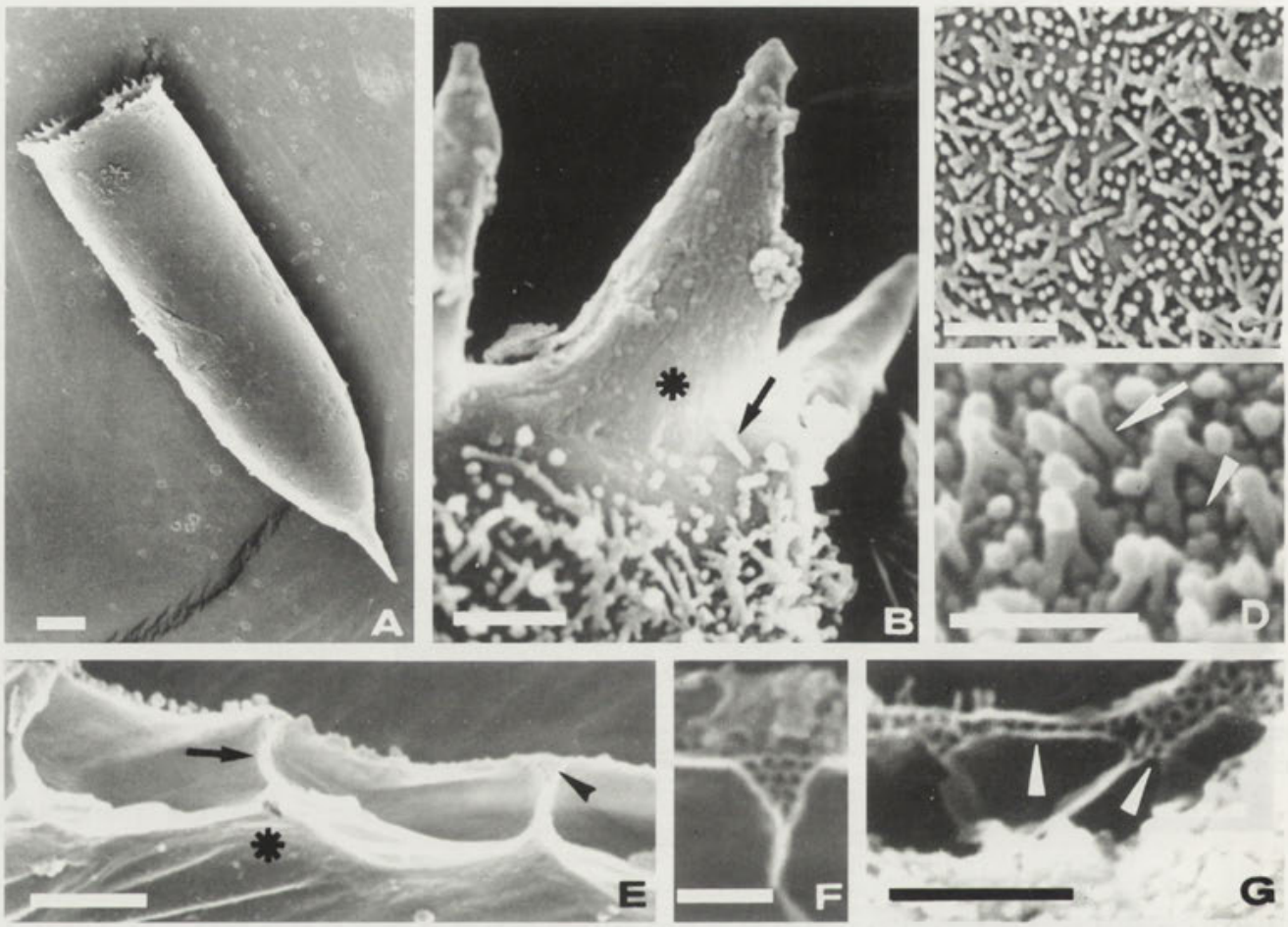

Fig. 3A-G. Scanning electron micrographs of *Parafavella denticulata* lorica. A - general view, bar - 10 µm; B - denticulated crest, bristle (arrow), smooth surface (asterisk), bar - 1 µm; C - bristles and granules, bar - 1 µm; D - high magnification of bristle (arrow) and granules (arrowhead), bar - 1 µm; E - microstructure of wall, septum (arrow), smooth inner surface (asterisk), triangle with openings (arrowhead), bar - 1 µm; F - high magnification of triangle, bar - 0,5 µm; G - microstructure of external lamina (arrowheads), bar - 1 µm

and most closely packed at its lower part (Fig. 4C), and become smaller and less densely packed toward the oral rim (Fig. 4B). The collar's inner surface is smooth (Fig. 4D). The longitudinal view of the collar shows 0.5 µm wall with a typical, trilaminate structure. Both the inner and outer 0.1 µm laminae are solid and limit a single alveolar layer (Fig. 4D).

The border between the collar and bowl is sharp and distinct (Fig. 4C). The bowl is usually not fully covered by particles; where naked it's surface is smooth (Fig. 4C). The bowl's internal structure is similar to that of the collar, except in places where foreign particles are agglutinated. Particles can be adhered to the bowl's external surface or embedded in the middle of the honeycomb-like layer (Fig. 4E). In the latter case, proliferation of the alveoli around the particle occurs. The internal surface of the bowl is smooth with tiny pores (Fig. 4E).

The lorica collar of the cosmopolitan *C. gaussi* is covered, except at the oral rim, by bristles (Fig. 5B). Bristle length changes from very short at the upper part of the collar (Figs. 5B, C) to long (1 µm) at the lower part (Fig. 5D), and bristle coverage terminates rapidly where the collar turns to the bowl (Fig. 5D). The bowl is wound by threads of different lengths, between which foreign particles are visible (Fig. 5E). When not covered by particles, the bowl's surface is smooth with tiny pores (Fig. 5E). Internally, the structure of the whole lorica represents a typical trilaminar texture with solid laminae and 0.4 µm long septa.

Totally agglutinated loricae

The neritic tintinnid, *T. lobiancoi* has a tube-shaped lorica, often with a broadened aboral end (Fig. 6A). The different sized pores are irregularly distributed all over the

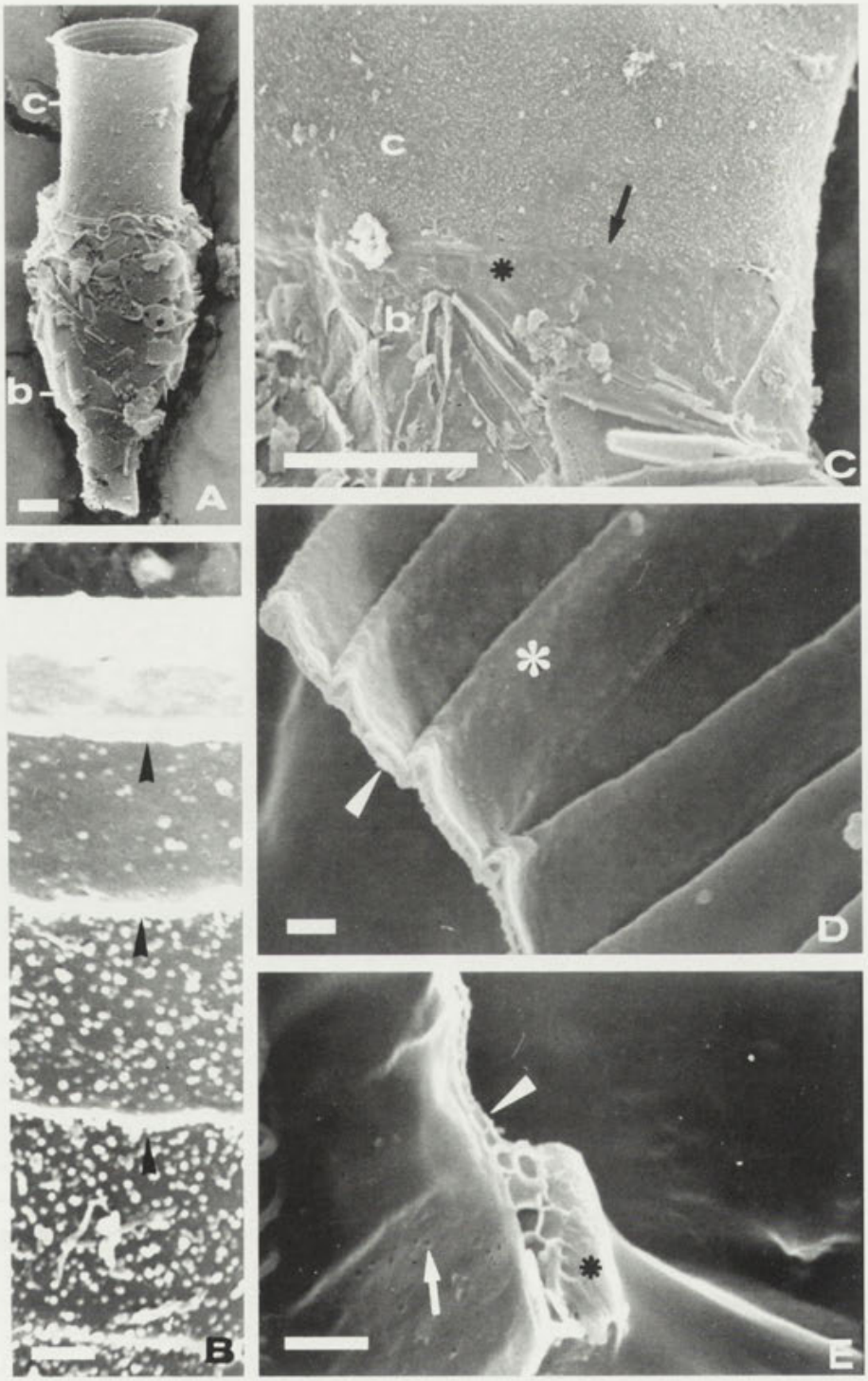

Fig. 4A-E. Scanning electron micrographs of *Laackmanniella naviculaefera* lorica. A - general view, b - bowl, c - collar, bar - 10µm; B - anterior part of collar, turns (arrowheads), bar - 1µm; C - border (arrow) between collar (c) and bowl (b); surface free of particles (asterisk); bar - 10µm; D - broken trilaminar wall of collar (arrowhead); smooth inner surface (asterisk), bar - 1µm; E - wall of broken bowl (arrowhead) with embedded foreign particle (asterisk), perforation of wall (arrow), bar - 1µm

lorica (Fig. 6B). The internal surface of the lorica wall and the external surface where particle free, are smooth (Fig. 6B).

Although its internal architecture is also trilaminar, it differs from those described above. External and internal laminae are thick (*ca* 0.3 µm), solid, and separated by

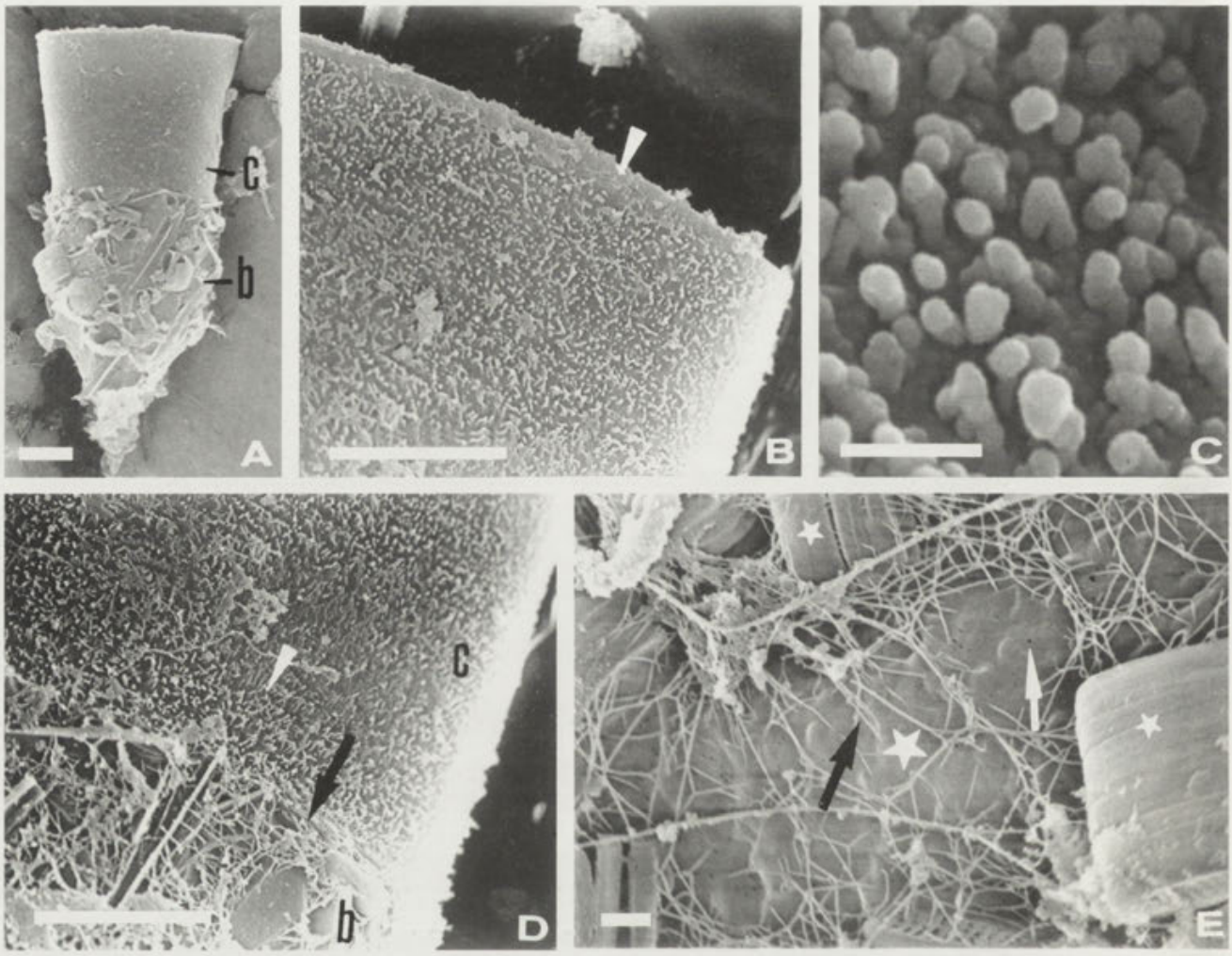

Fig. 5A-E. Scanning electron micrographs of *Codonellopsis gaussi* lorica. A - general view, b- bowl, c- collar, bar - 10μm; B - collar with bristles; smooth oral rim (arrowhead), bar - 10μm; C - high magnification of the short bristles, bar - 1μm; D - border (arrow) between collar (c) and bowl (b), long bristles (arrowhead), bar - 10μm; E - fragment of bowl, foreign particles (small asterisks), pores (white arrow), smooth surface (big asterisk), threads (black arrow), bar - 1 μm

alveoli forming an extended honeycomb-like structure over 2 μm thick (Figs. 6C-E).

The entire external surface of the lorica is able to adhere foreign particles. These can be adhered to, or even set into the external lamina (Fig. 6D), as well as be partly or fully embedded in the honeycomb-like layer (Fig. 6E).

DISCUSSION

We have described a range of tintinnid loricae selected according to three criteria, origin, morphology and adhesive properties. They belong to four biogeographic categories (Pierce and Turner 1993), cosmopolitan *Codonellopsis gaussi*, neritic *Helicostomella subulata* and *Tintinnopsis lobiancoi*, boreal *Parafavella denticulata*, and austral *Cymatocylis drygalskii*, *C. vanhoffeni* and *Laackmanniella naviculaefera*. The loricae also represent three morphologically different types, hyaline, partially and totally agglutinated by foreign particles thus having different adhesive properties.

We assumed that such differences may be reflected in the microstructure, which until now has not been well described. Kofoid and Campbell (1929), and later Laval-Peuto and Brownlee (1986), found that loricae texture may be alveolar, vacuolar, tubular, lacunar or compact and considered the microstructure to be taxonomically important. The well studied lorica wall of

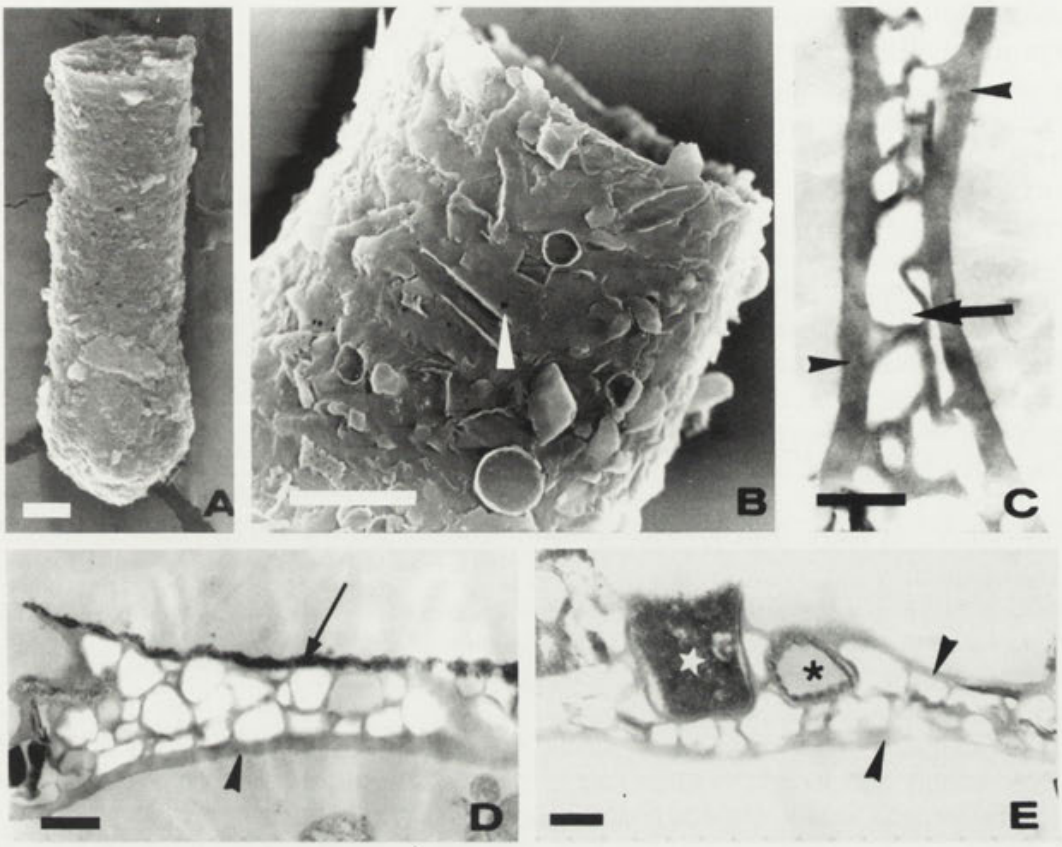

Fig. 6A-E. Scanning and transmission electron micrographs of *Tintinnopsis lobiancoi* lorica. A - general view, bar - 10µm; B - anterior end; pore (arrowhead), bar - 10µm; C-E - TEM of wall microstructure, alveoli (thick arrow), laminae (arrowheads), particle set into outer lamina (thin arrow), particles (asterisks) embedded in alveolar layer, bar - 1 µm

Paraftavella is composed of a single layer of hexagonal prisms (Burkovsky 1973, Hedin 1975, Sokolova and Gerassimova 1984) and that of *Favella* (Hedin 1975) of an irregular network of ridges and alveoli. Also well described is the lorica ultrastructure of a species endemic to Antarctica, *Cymatocylis affinis/convallaria* (Wasik and Mikołajczyk 1992); it is generally trilaminar, but its netted layer changes depending on the lorica's region.

Detailed morphological studies of hyaline tintinnid loricae revealed differences in their external surface structure, with those of *H. subulata, C. drygalskii* and *C. vanhoffeni* being granular, while the surface of *P. denticulata* is coated by both compact granules and bristles. The hyaline collars of the partially agglutinated *L. naviculaefera* and *Cd. gaussi* also differ in terms of their surface structure; in the former there are granules of different size and arrangement, while in the latter there are bristles whose length varies, depending on their localization on the collar. Generally the outer surface of their bowls, as well as the surface of the totally agglutinated *T. lobiancoi*, is smooth in areas that are particle free. The inner surfaces of the lorica in all species considered in this work appeared to be smooth.

It was surprising that in spite of differences in surface morphology and sample origin, the internal structure of each lorica was trilaminar, giving a honeycomb-like structure. Modifications arose mainly in the size of the elements forming the trilaminar wall, in multiplication of alveoli, and laminae construction.

The hyaline loricae we studied showed differing degrees of microarchitecture complexity. The lorica of *H. subulata* seemed to be the simplest, not only morphologically but also in terms of microstructure. Other hyaline species, *C. drygalskii* and *C. vanhoffeni*, have more elaborate loricae, while the most complex belonging to *P. denticulata*. TEM by Gold and Morales (1975) and Hedin (1975) of *P. gigantea*, and by Burkovsky (1973)

of *P. denticulata* appeared to show simple trilaminar loricae with prismatic elements at the ends of septa. Our SEM observations of *P. denticulata* confirmed their observations, showing that both laminae have their own honeycomb-like structure.

The entire agglutinated lorica of *T. lobiancoi* exhibits sticky properties that allow foreign particles to cement to, or set into its wall. Its internal texture also appeared to be a thick and multilayered honeycomb.

Clearly both anterior and posterior parts of totally hyaline and totally agglutinated loricae have the same microstructure, but it is interesting that partially agglutinated species, *Laackmanniella* and *Codonellopsis* which have morphologically different collars and bowls share the same internal structure. Differences in sticky properties of the respective collars and bowls does not change the general internal scheme.

To conclude, there are no major differences in loricae microstructure despite the fact that they vary in shape, surface morphology, adhesive properties and develop under different environmental conditions. The common appearance of the honeycomb-like structures in tintinnid loricae is understandable since it provides a lightweight, elastic, yet strong structure ideally suited to protecting protoplasts.

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REFERENCES

Biernacka I. (1952) Studies on the reproduction of some species of the genus *Tintinnopsis* Stain. *Ann. Univ. Mariae Curie-Skłodowska* **6:** 211-247 (in Polish with English summary)

Burkovsky I. V. (1973) Variability of *Parafavella denticulata* in the White Sea. *Zool. J.* **52:** 1277-1285

Gold K., Morales E. A. (1975) Tintinnida of the New York Bight: loricae of *Parafavella gigantea, P. parundentata*, and *Ptychocylis obtusa*. *Trans. Amer. Micros. Soc.* **94:** 142-145

Gold K., Morales E. A. (1976a) Observations on the nature and significance of the particles used by Tintinnida during lorica-building. *Trans. Amer. Micros. Soc.* **95:** 69-72

Gold K., Morales E. A. (1976b) On Tintinnida using scanning electron microscopy. *Trans. Amer. Micros. Soc.* **95:** 707-711

Gowing M. M., Garrison D. L. (1992) Abundance and feeding ecology of larger protozooplankton in the ice edge zone of the Weddell and Scotia Seas during the austral winter. *Deep-Sea Res.* **39:** 893-919

Hada Y. (1970) The protozoan plankton of the Antarctic and Subantarctic seas. *Jare Scien. Rep., Ser. E* **31:** 1-51

Hedin H. (1975) On the ultrastructure of *Favella ehrenbergii* (Claparede and Laackmann) and *Parafavella gigantea* (Brandt), Protozoa, Ciliata, Tintinnida. *Zoon* **3:** 11-18

Kofoid C. A., Campbell A. S. (1929) A conspectus of the marine and fresh water Ciliata belonging to the suborder Tintinnoinea with descriptions of new species principally from the Aggasiz expedition to the eastern tropical Pacific, 1904-1905. *Univ. Calf. Publ. Zool.* **34:** 1-403

Kofoid C. A., Campbell A. S. (1939) The Ciliata: the Tintinnoinea. *Bull. Nus. Comp. Zool. Harv.* **84:** 1-403

Laval M. (1972) Ultrastructure de *Petalotricha ampulla* (Fol) comparison avec d'autres tintinnides et avec les autres de Ciliés. *Protozoologica* **8:** 369-386

Laval-Peuto M. (1980) Ultrastructure of the lorica-wall of Tintinnina. (Ciliata, Spirotricha). *J. Protozool.* **27:** 83A

Laval-Peuto M. (1981) Construction of the lorica in ciliate Tintinnina. *In vivo* study of *Favella ehrenbergii*: variability of the phenotypes during the cycle, biology, statistics, biometry. *Protistologica* **17:** 249-272

Laval-Peuto M., Brownlee D. C. (1986) Identification and systematics of the Tintinnina (Ciliophora): evaluation and suggestions for improvement. *Ann. Inst. Oceanogr. Paris* **62:** 69-84

Pierce R. W., Turner J. T. (1993) Global biogeography of marine tintinnids. *Mer. Ecol. Prog. Ser.* **94:** 11-26

Sassi R., Melo G. N. (1986) Tintinnina (Protozoa-Ciliophora-Oligotrichida) from the First Brazilian Expedition to the Antarctic. *Ann. Acad. Bras. Cienc. (Suppl.)* **58:** 63-83

Sokolova Y. Y., Gerassimova Z. P. (1984) The ultrastructure of the ciliates *Parafavella denticulata* Ehrenberg, 1840. *Citologia* **26:** 1237-1245 (in Russian with English summary)

Tappan H., Loeblich, A. R. Jr. (1968) Lorica composition of modern and fossil Tintinnida (Ciliate Protozoa), systematics, geologic distribution, and some new tertiary taxa. *J. Paleontol.* **42:** 1378-1394

Wasik A., Mikołajczyk E. (1991) Discocilia (paddle cilia) in the marine ciliate *Cymatocylis convallaria* (Tintinnina). *Cell Biol. Inter. Rep.* **15:** 485-491

Wasik A., Mikołajczyk E. (1992) The morphology and ultrastructure of the Antarctic ciliate, *Cymatocylis convallaria* (Tintinnina). *Acta Protozool.* **31:** 233-239

Wasik A., Mikołajczyk E., Ligowski R. (1996) Agglutinated loricae of some Baltic and Antarctic Tintinnina species (Ciliophora). *J. Plank. Res.* **18:** 1931-1940

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ACTA
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Ultrastructural Characteristics of a *Lankesterella*-like Coccidian Causing Pneumonia in a Northern Cardinal (*Cardinalis cardinalis*)

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Summary. An unusual coccidian was identified in lung tissue from a northern cardinal *(Cardinalis cardinalis)* that died in respiratory distress. Ultrastructural observations revealed both asexual and sexual stages in lung tissue, including schizonts, gamonts, and sporulated oocysts. The parasite displayed characteristics that have been observed in a variety of Apicomplexa, and most closely resembled coccidia of the genus *Lankesterella*.

Key words: *Cardinalis cardinalis, Lankesterella,* parasite, passeriformes, protozoan, pulmonary.

INTRODUCTION

Little is known of the protozoan parasites of cardinals. Recently, Baker et al. (1996) reported an unidentified coccidian causing pneumonia in a northern cardinal (*Cardinalis cardinalis*) from the U.S.A. The purpose of this report is to describe the ultrastructural characteristics of the coccidian parasite reported by those authors.

MATERIALS AND METHODS

In June 1993, a nestling cardinal (*Cardinalis cardinalis*) was collected near Tucson, Arizona for a songbird research project (Baker et al. 1996). At 46 days of age the bird developed anorexia, lethargy, and upper respiratory congestion with occasional sneezing and was found dead in its cage 2 days later. A necropsy was performed within 30 min of death. The lungs were perfused in situ with 10% neutral buffered formalin. For the present study, lung tissue was post-fixed in osmium tetroxide and was processed for transmission electron microscopy.

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RESULTS

Pathologic findings

Histologically, there was generalized interstitial pneumonia characterized by large numbers of parasites predominantly within air capillaries and the interstitium. Tertiary bronchi were relatively spared. The interstitium contained small to moderate numbers of mononuclear inflammatory cells. Larger blood vessels

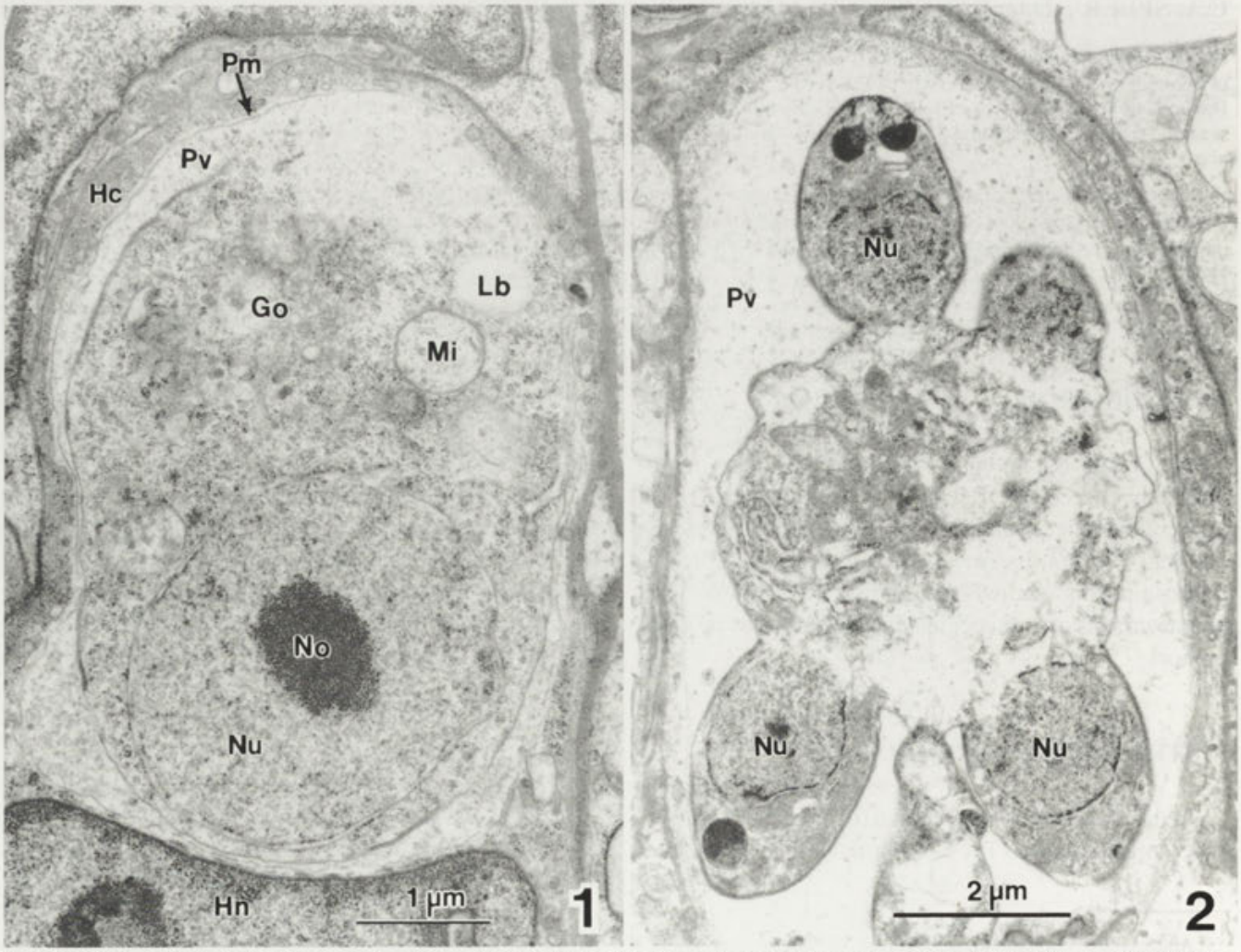
were congested and had a marked increase in circulating leukocytes. Both asexual and sexual stages of the parasite were seen. Neither lesions nor parasites were identified in other tissues examined, including liver, lung, heart, spleen, kidney, pancreas, small intestine, eyes, and brain (Baker et al. 1996).

Host cells

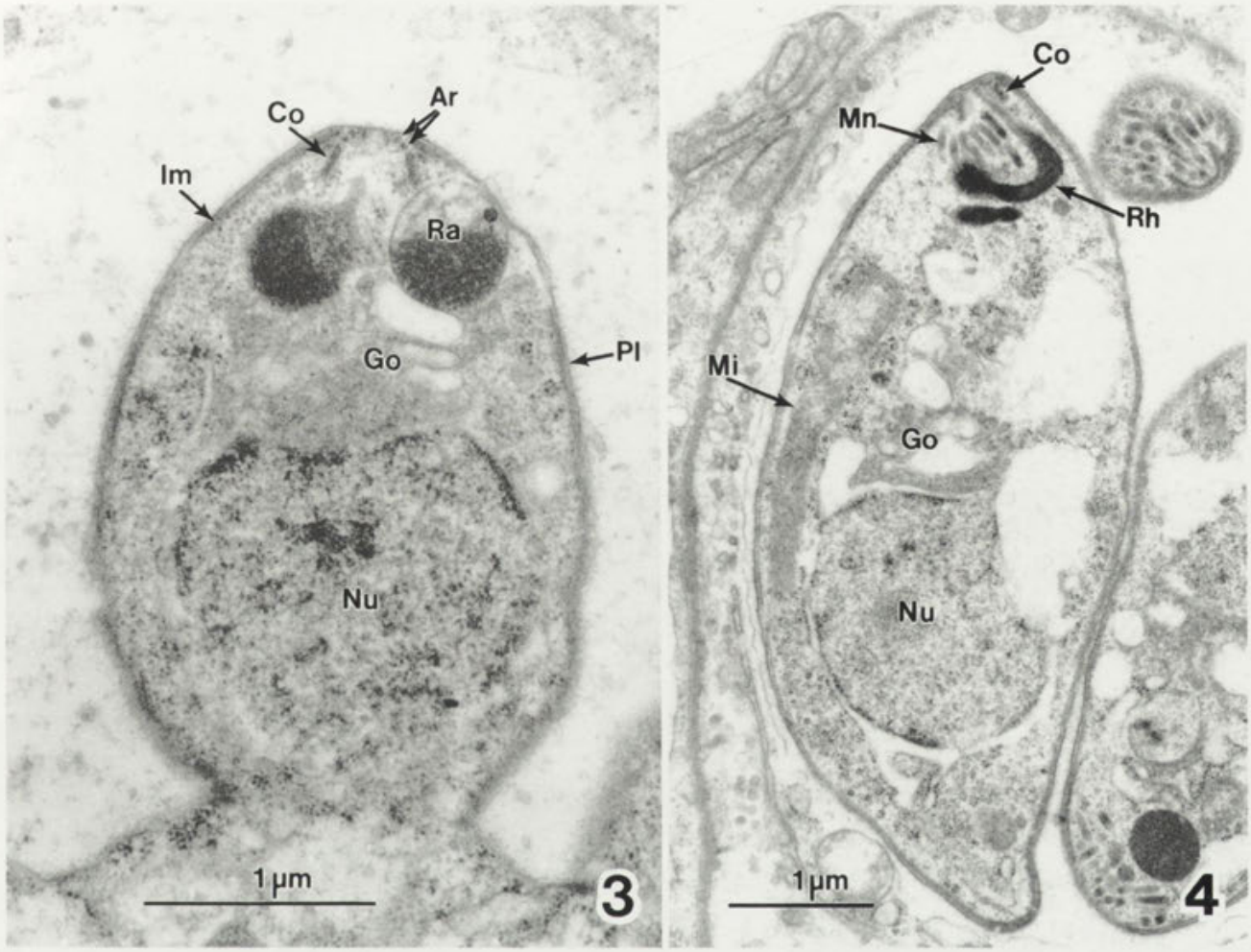
Parasites were located intracellularly primarily within endothelial cells of the pulmonary capillaries (Fig. 1) and rarely in the alveolar epithelium. They were spheroidal to cylindrical in shape, usually conforming to the shape of the pulmonary capillary, and were situated in a parasitophorous vacuole. The parasitophorous vacuolar membrane was in close proximity to the parasite plasmalemma except in areas where the parasitophorous vacuole contained numerous tubular and spheroidal vesicles and finely granular material.

Trophozoites

Trophozoites were spheroidal to ellipsoidal and measured 6.4 x 4.6 µm (5.2-8.2 x 3.8-5.5 µm, n = 8). They contained a nucleus with a prominent nucleolus, a few micronemes, several mitochondria and multiple membrane bound vesicles, 1 to 3 large vacuoles containing a finely granular material and several vesicles, a Golgi complex, smooth and rough endoplasmic reticulum, polysomes, ribosomes, centrioles, cytoplasmic microtubules, many small vesicles, a few micropores and a few lipid droplets (Fig. 1). The trophozoite pellicle consisted of a plasmalemma with a few segments of inner membrane complex located immedi-



Figs. 1-2. Transmission electron micrographs of a coccidian parasite in the lung tissues of a cardinal. 1 - trophozoite in capillary endothelial cell; Golgi complex (Go); host cell cytoplasm (Hc); host cell nucleus (Hn); lipid body (Lb); mitochondrion (Mi); nucleus of parasite (Nu); nucleolus (No); parasitophorous vacuolar membrane (Pm); parasitophorous vacuole (Pv); x 19,100. 2 - schizont with merozoites budding radially from a central residual body; nucleus of budding merozoite (Nu); parasitophorous vacuole (Pv); x 12,500



Figs. 3-4. Transmission electron micrographs of a coccidian parasite in the lung tissues of a cardinal. 3 - higher magnification of budding merozoite in figure 2 showing two apical rings (Ar); conoid (Co); Golgi complex (Go); inner membrane complex (Im); nucleus (Nu); plasmalemma (Pl); rhoptry anlagen (Ra); x 32,000. 4 - merozoite showing conoid (Co); Golgi complex (Go); micronemes (Mn); mitochondrion (Mi); nucleus (Nu); two rhoptries (Rh); x 20,000

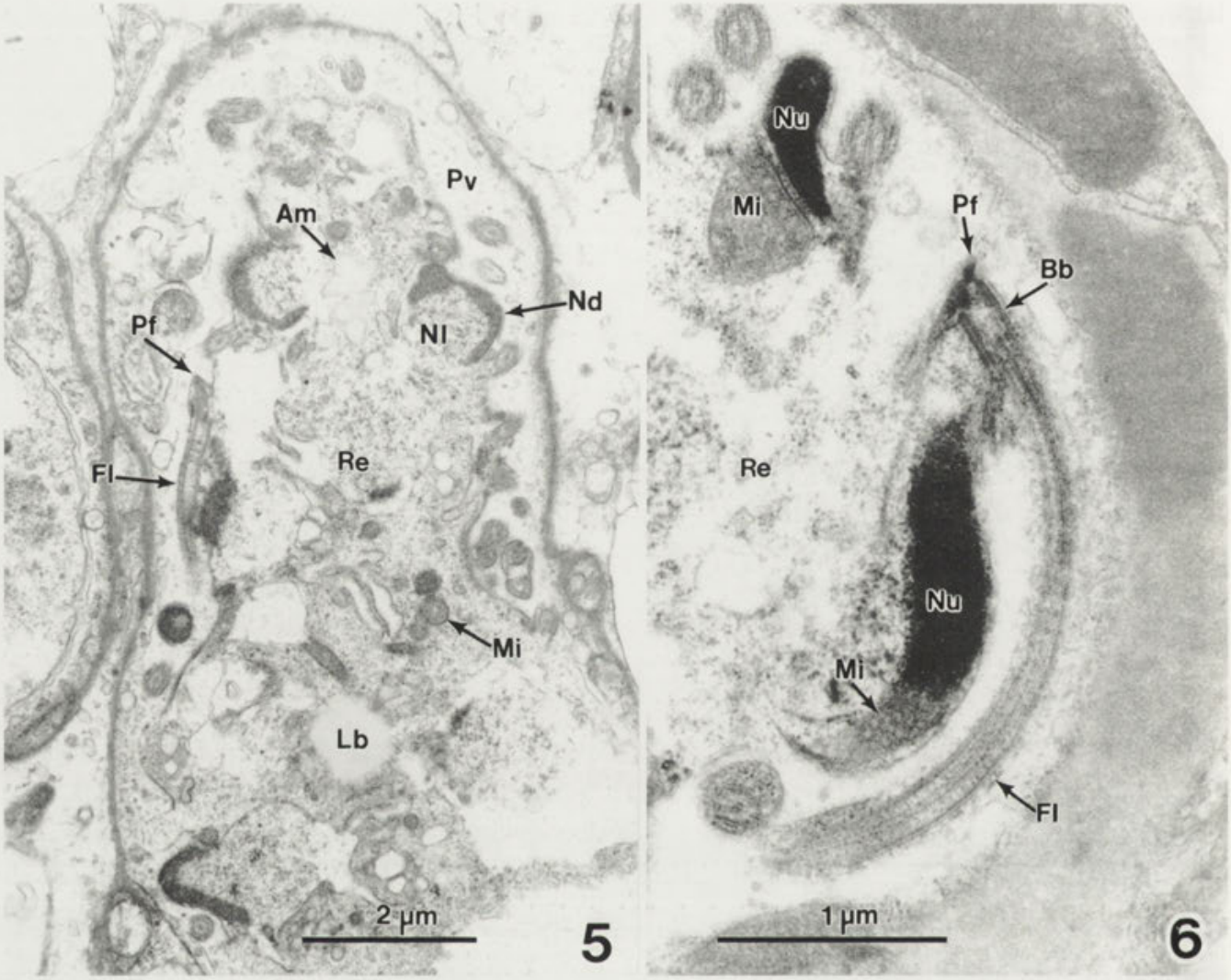
ately below. No amylopectin granules or micronemes were seen.

Schizonts

Immature schizonts were larger than trophozoites and measured 8.9 x 5.2 µm (5.0-15.4 x 4.3-6.7 µm, n=10) (Fig. 2). They contained 2 or more nuclei, all organelles and inclusion bodies found in trophozoites, and several large vacuoles filled with granular material. Schizont nuclei in various stages of division contained intranuclear spindles, one at each pole of the nucleus, a nucleolus that became rod-shaped and eventually pinched in two, and a nuclear envelope that remained intact during division. Two centrioles were located immediately above and slightly lateral to each centrocone. Merozoite formation began at the schizont surface (Fig. 2) where 2 merozoites developed at each pole of each nucleus. The nuclei became situated just beneath the parasite plasmalemma and a Golgi complex and 2 membrane-bound vesicles (rhoptry anlagen) were located in the cytoplasm between each centrocone and the schizont surface. Apical rings, conoid, polar rings, inner membrane complex, and subpellicular microtubules appeared immediately above the 2 rhoptry anlagen (Fig. 3). As each merozoite elongated, the inner membrane complex and its associated subpellicular microtubules extended posteriorly, incorporating half of the nucleus, which pinched off from the other half (Fig. 3). The rhoptry anlagen elongated and became electron-dense to form 2 rhoptries (Figs. 3, 4). Eventually, the merozoites separated from the small residual body. Mature schizonts contained a maximum of 12 merozoites.

Merozoites

Merozoites measured 6.0 x 2.2 µm (5.0-7.0 x 1.9-2.5µm, n=11), and each contained a nucleus with a prominent nucleolus, relatively few micronemes, 2 rhoptries, several mitochondria, smooth and rough endoplasmic reticulum,



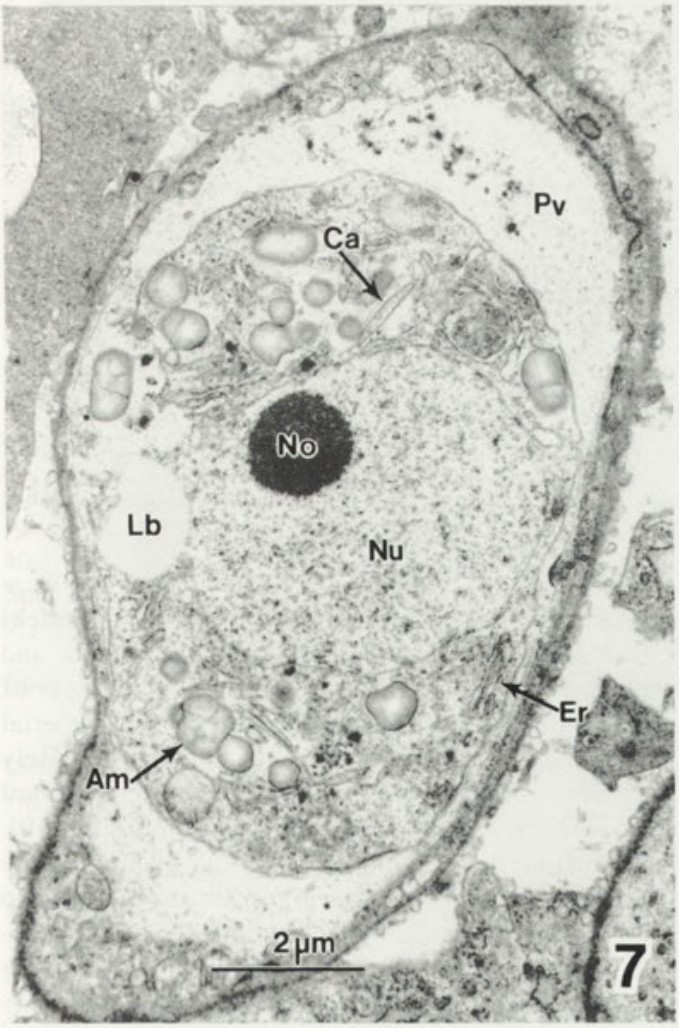
Figs. 5-6. Transmission electron micrographs of a coccidian parasite in the lung tissues of a cardinal. 5- microgamont in early stage of microgamete formation; amylopectin granule (Am); flagellum (Fl); lipid body (Lb); mitochondrion (Mi); electron-lucent portion of nucleus (Nl); electron-dense portion of nucleus (Nd); perforatorium (Pf); parasitophorous vacuole (Pv); residual body (Re); x 12,500. 6 - microgamete; basal body (Bb); flagellum (Fl); mitochondrion (Mi); nucleus (Nu); perforatorium (Pf); residual body (Re); x 10,000

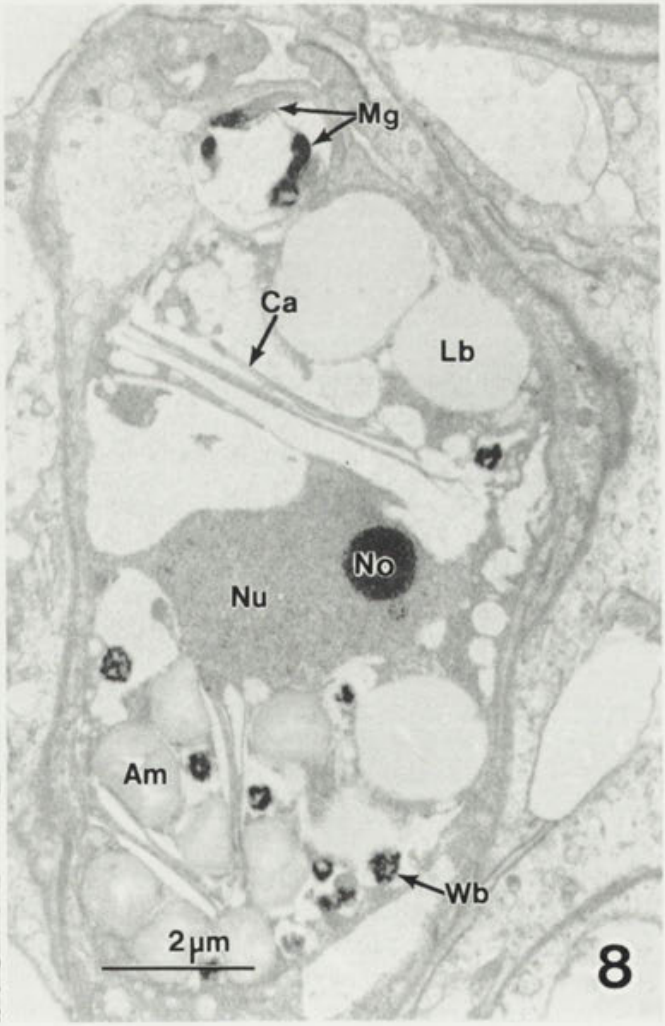
Golgi complex, micropores, posterior pore, polysomes and ribosomes, several vesicles, conoid, 22 subpellicular microtubules, inner membrane complex, and a few lipid droplets, but no amylopectin granules (Fig. 4). The apical complex was turned slightly to one side so that it was not in line with the longitudinal axis of the merozoite (Fig. 4).

Microgamonts

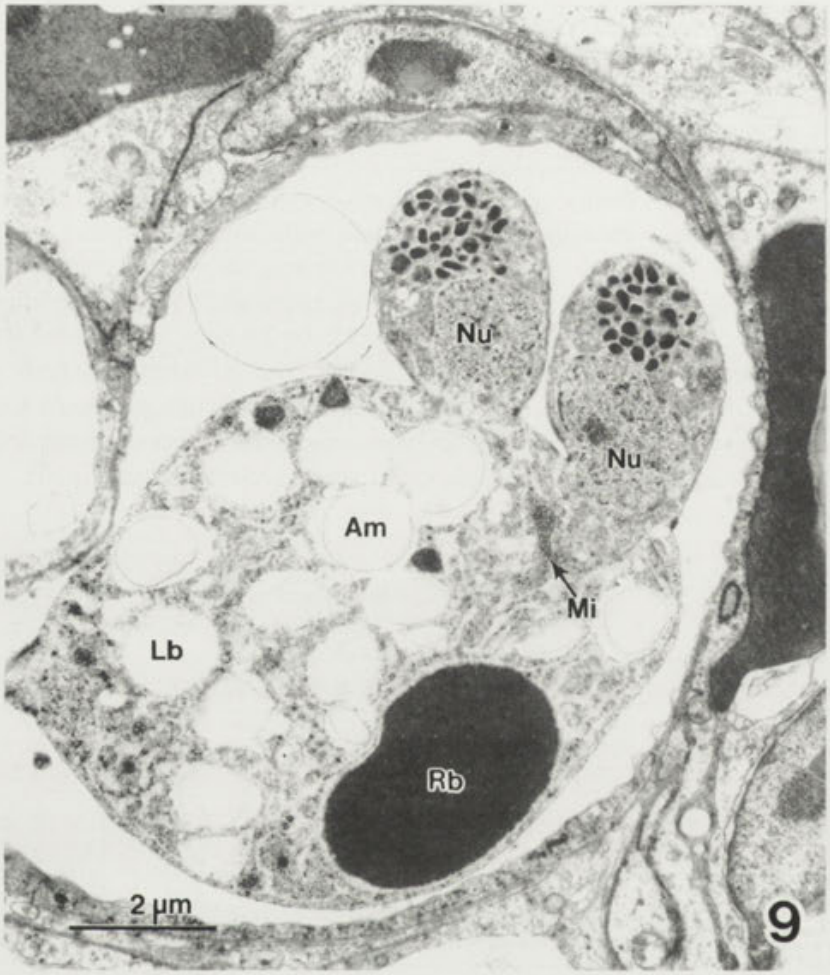
Microgamonts were usually spheroidal and differed from schizonts by possessing more and smaller nuclei, rod-shaped mitochondria and numerous amylopectin granules (Fig. 5). Nucleoli were present in the nuclei of early microgamonts but were absent in more advanced stages. Dividing nuclei had a centrocone, a spindle apparatus and 2 centrioles located at each pole of the nucleus. Nuclear division was completed by indentation of the nuclear envelope in the mid-region of the nucleus. As nuclear divisions proceeded, the nuclei decreased in size and the chromatin became more electron-dense near the outer margin of the nucleus (Fig. 5). Some mitochondria were located in the interior of the gamont, but many were close to the plasmalemma and closely associated with the nuclei. In early microgamonts, a single plasmalemma surrounded the parasite, but in later stages an inner membrane complex of 2 closely applied membranes was situated just beneath the plasmalemma and immediately peripheral to each nucleus. In slightly more advanced stages, a flagellum developed from each of 2 basal bodies

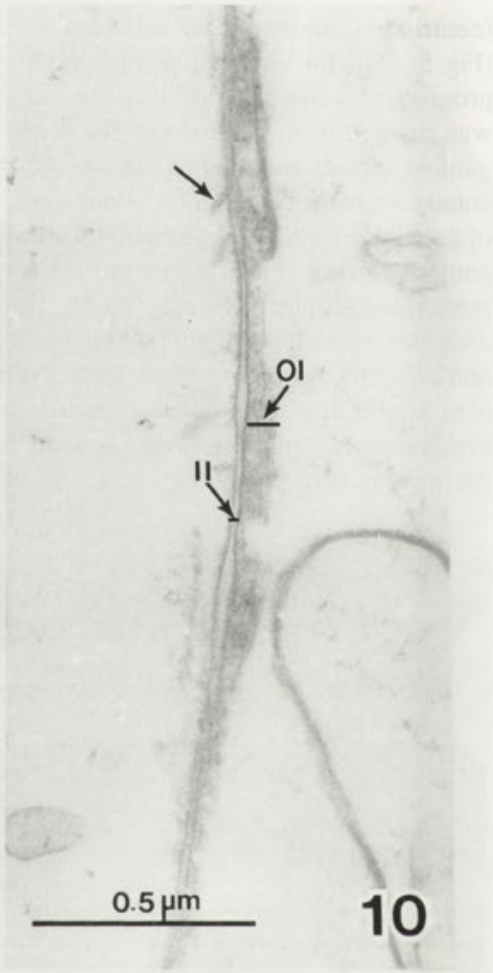
(centrioles), immediately adjacent to each nucleus (Fig. 5). With further development, the chromatin became progressively more compact and dense, and eventually was situated in that portion of the nucleus nearest the gamont surface and the less electron-dense portion oriented toward the center of the gamont (Fig. 5). The body of each microgamete was formed by protruding from the gamont surface. Each protrusion contained the dense portion of a peripherally located nucleus, a mitochondrion, a segment of the inner membrane complex, 2 basal bodies and 2 flagella. As microgamete formation progressed, the area connecting the immature microgamete with the residual portion of the gamont gradually decreased with the microgamete eventually pinching off the surface. Only the electron-dense portion of the nucleus was incorporated into the microgamete, the lucent portion remained with the gamont residual body. Mature microgamonts were spheroidal and measured 11.5 x 8.0 mm (8.6-15.3 x 5.2-12.2 mm, n=6). After microgamete formation was completed, the residual body of the gamont contained electron-lucent parts of residual nuclei, amylopectin granules, mitochondria, polysomes, ribosomes, vesicles, endoplasmic reticulum and a few lipid bodies. Microgametes measured 2.7 x 0.5 mm (2.0-3.0 x 0.4-0.6 mm, n=5) and consisted of 2 basal bodies, 2 flagella, a nucleus, a mitochondrion, a segment of inner membrane complex, a pointed perforatorium at the apex immediately above the basal bodies, and a plasmalemma (Fig. 6).





Figs. 7-8. Transmission electron micrographs of a coccidian parasite in the lung tissues of a cardinal. 7- early macrogamont; amylopectin granule (Am); canaliculi (Ca); endoplasmic reticulum (Er); lipid body (Lb); nucleolus (No); nucleus (Nu); parasitophorous vacuole (Pv); x 10,000. 8- mature macrogamont with microgametes (Mg) in its cytoplasm; amylopectin granules (Am); canaliculi (Ca); lipid bodies (Lb); nucleolus (No); nucleus (Nu); type II wall forming bodies (Wb); x 9,900





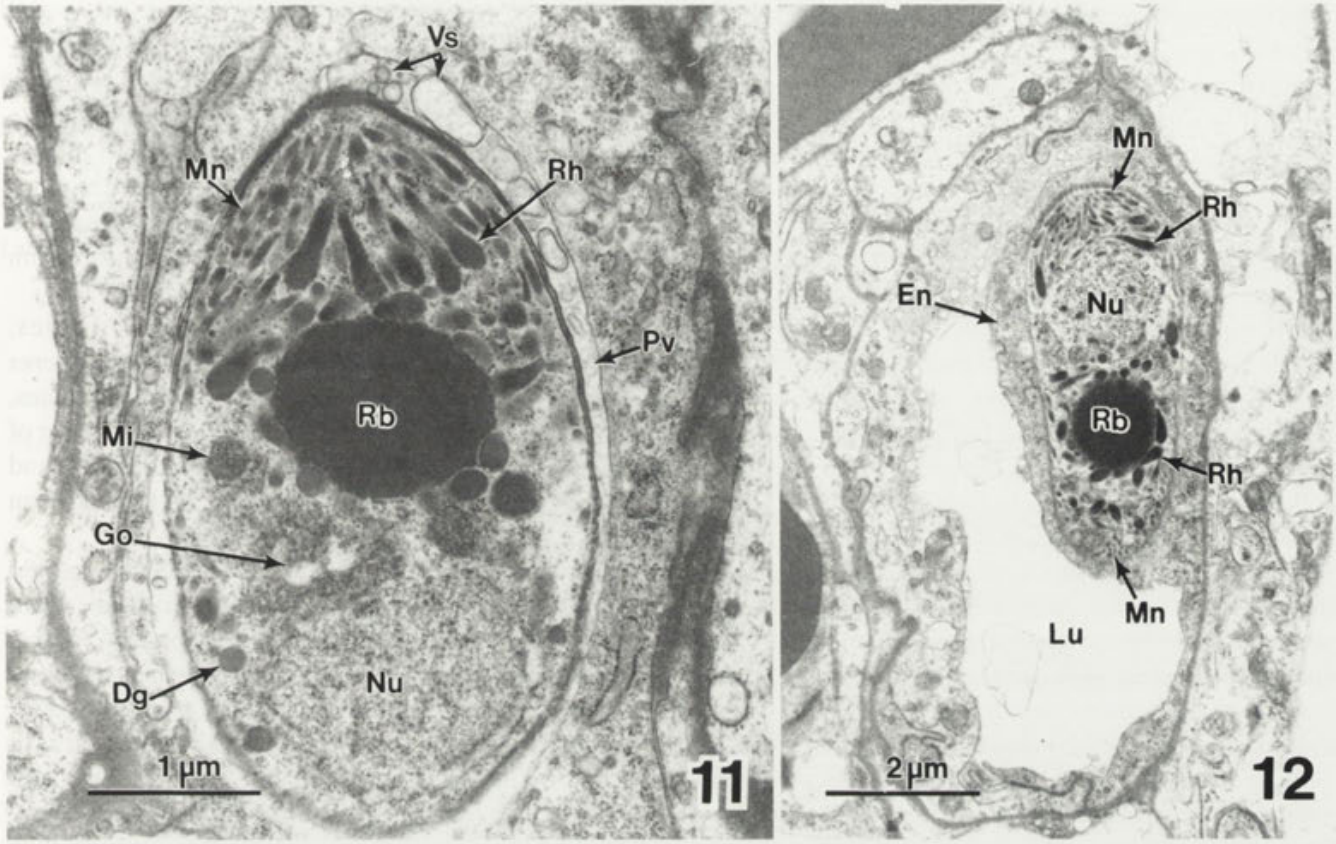
Figs. 9-10. Transmission electron micrographs of a coccidian parasite in the lung tissues of a cardinal. 9- oocyst in intermediate stage of sporulation with sporozoites budding radially from the sporont; amylopectin granule (Am); lipid body (Lb); mitochondrion (Mi); nucleus (Nu); refractile body (Rb); x 10,000. 10 -oocyst wall consisting of inner (Il) and outer (Ol) layers; note rod-shaped structures (arrow) projecting inward from the inner layer; x 99,000

Macrogamonts

Early macrogamonts characteristically contained a large nucleus and nucleolus, canaliculi, unusually large amylopectin granules and lipid droplets, in addition to other organelles and inclusion bodies (Fig. 7). Mature macrogamonts measured 14.8 x 7.7 µm (7.6-23.4 x 4.5-11.5 µm, n=8), contained type II wall forming bodies, large lipid droplets and large amylopectin granules. Type II wall forming bodies were granular, labrynthine and electron-dense and situated within the cisternae of the rough endoplasmic reticulum (Fig. 8). Type I wall forming bodies were absent. Occasionally, microgametes were observed in the host cell cytoplasm in close proximity to macrogamonts or in the cytoplasm of macrogamonts (Fig. 8).

Oocysts

Unsporulated oocysts contained a centrally located nucleus, canaliculi, lipid droplets, large amylopectin granules and 1 or 2 large refractile bodies. They were surrounded by a thin oocyst wall (48-132 nm thick) consisting of an inner layer, 16-32 nm thick and comprised of two membranes, and an outer layer of moderately electron-lucent finely granular material 16-100 nm thick (Fig. 9). In some sections, moderately electron-dense rods (83.5 x 16.7 nm) projected inward from the inner layer of the oocyst wall (Fig. 10). Sporozoite development occurred by budding at the surface of the sporont (Fig. 10) in a manner similar to that described for merozoite formation. During sporulation, a conoid, apical rings, polar rings, an inner membrane complex, subpellicular microtubules, several rhoptries, and numerous micronemes were formed within the sporozoite bud. As budding proceeded, 1 or 2 refractile bodies, a nucleus and several mitochondria moved into the bud from the sporont protoplasm (Fig. 10). Completely sporulated oocysts contained



Figs. 11-12. Transmission electron micrographs of a coccidian parasite in the lung tissues of a cardinal. 11- intracellular sporozoite; dense granules (Dg); Golgi complex (Go); micronemes (Mn); mitochondrion (Mi); nucleus (Nu); parasitophorous vacuole (Pv); refractile body (Rb); rhoptry (Rh); vesicles in parasitophorus vacuole (Vs); x 12,000. 12 - sporozoite in endothelial cell of pulmonary capillary; note rhoptries (Rh) in close proximity to refractile body (Rb) and micronemes (Mn) at both poles of the sporozoite; endothelial cell (En); lumen of pulmonary capillary (Lu); nucleus (Nu); x 10,000

8 sporozoites and a centrally located residuum with lipid droplets, canaliculi, vesicles, amylopectin granules and various cytoplasmic organelles and inclusion bodies. Sporulated oocysts measured 12.4 x 9.0 µm (11.0-14.0 x 7.3-10.0 µm, n=5).

Sporozoites

Sporozoites (Figs. 11, 12) measured 6.4 x 2.4 µm (5.2-7.5 x 1.9-3.1 µm, n=6) and contained 2 apical rings, 2 polar rings, conoid, an inner membrane complex, 22 subpellicular microtubuless, 1 or 2 refractile bodies, dense granules, a nucleus, Golgi complex, several rhoptries, numerous micronemes, several mitochondria and a few micropores. Rhoptries and micronemes were located at both ends of the sporozoite, but were more numerous at the apical end and some were clustered around the surface of refractile bodies (Fig. 12). Sporozoites were free in the lumen or within endothelial cells of pulmonary capillaries (Fig. 12), and a few were seen within lymphocytes within blood vessels. Sporozoites were never seen within alveoli.

DISCUSSION

Considerable confusion exists concerning the identity of coccidian parasites in visceral tissues of passerine birds. Species of the genera *Toxoplasma*, *Atoxoplasma*, *Sarcocystis*, *Isospora*, and *Lankesterella* have been reported from passerine birds. In our study, the cardinal coccidian was not *Toxoplasma* because it replicated by schizogony and gametogony, whereas *Toxoplasma* in birds divides into 2 by endodyogeny. It was also not *Sarcocystis* because merozoites in schizonts lack rhoptries (Dubey et al. 1989). The parasite was not *Isospora* because gamonts and sporulated oocysts were found in the lung tissue.

The cardinal parasite was not *Atoxoplasma* because sexual stages were present in the lung tissue. There is much confusion concerning the identity of *Atoxoplasma* and *Lankesterella* in birds. Garnham (1950) reviewed earlier reports of *Toxoplasma*-like parasites in mononuclear cells of passerine birds and proposed the genus *Atoxoplasma* for single or paired organisms found in monocytes and lymphocytes of visceral tissues. Lainson

(1959) synonymized *Atoxoplasma* with *Lankesterella* because he found schizogony and gametogony in visceral tissues of sparrows and sporozoite-like organisms in mites removed from the infected birds. Box (1970, 1977) proposed the name *Isospora serini* for *Atoxoplasma*-like parasites in canaries. She found sexual stages in the intestinal epithelium and asexual stages in mononuclear cells of visceral tissues; she did not report nor explain the existence of the sexual stages reported by Lainson (1959) and Dissanaike (1967) in visceral tissues of sparrows and mynah birds, respectively. Levine (1982) reviewed in detail *Atoxoplasma*-like parasites and recommended retaining the genus *Atoxoplasma*, but he also did not confirm the existence of sexual stages in visceral tissues of birds.

Previous reports clearly show that birds have 2 types of coccidian parasites in their visceral tissues and blood (Baker et al. 1972, Levine 1982). One group consists of *Atoxoplasma* (*Isospora serini*-type) with organisms in mononuclear cells; the second group contains parasites which resemble haemogregarines. Haemogregarines have typically heteroxenous coccidian life cycles that can be divided into obligatory 2-host cycles. One group includes gametogony in the vertebrate host and sporogony in the invertebrate host; examples of this group are *Hepatozoon*, *Caryospora*, and *Haemogregarina*. Members in the second group occur in the family Lankesterellidae Nöller, 1902 and contain *Lankesterella* Labbè, 1899, *Lainsonia* Landau, 1973 and *Schellackia* Reichenow, 1919. Coccidia of the genus *Schellackia* contain 8 naked sporozoites in oocysts located in the intestinal lamina propria and epithelial cells of lizards and toads. Members of the genus *Lankesterella* are typically parasites of amphibia and occasionally birds, with schizogony, gametogony and sporogony occurring in reticuloendothelial cells and oocysts containing many naked sporozoites. In the genus *Lainsonia*, schizogony, gametogony and sporogony occur in visceral reticuloendothelial cells and oocysts contain 8 naked sporozoites. Species of this parasite have been reported only from New World lizards (Landau 1973, Landau et al. 1974).

In the cardinal coccidian, schizogony, gametogony, and sporogony were only observed in the lung tissue. Therefore, from the evidence in our study we can only state that the parasite in our cardinal was different from the one studied by Box because she did not find sexual stages in the lungs (Box 1970, 1977). We did not see single nor double zoites in mononuclear cells that are typical of *Atoxoplasma*; the zoites of this parasite are PAS-negative and clearly distinguishable from the sporozoites we observed in the capillary endothelium in the cardinal because sporozoites are PAS-positive and contain large refractile bodies. In the present study, the oocysts contained 8 sporozoites and relatively few (< 15) microgametes were seen. In the *Lankesterella garnhami* infection in sparrows reported by Lainson (1959) there were 60-100 microgametes and the oocysts contained a large number of sporozoites. The cardinal parasite is therefore a species different from *L. garnhami*.

Dissanaike (1967) described a new species, *Lankesterella lainsoni*, from a mynah bird (*Acridotheres tristis melanosternus*) from Ceylon, in which sporozoites, schizonts, gamonts and oocysts were seen in a number of visceral tissues. There were numerous microgametes and oocysts contained 30 or more sporozoites. The parasite in the cardinal is, therefore, not *L. lainsoni*.

Khan and Desser (1971) and Desser (1980) reported on a *Lankesterella*-like parasite from grosbeaks from Canada which Levine (1982) considered to be *Atoxoplasma* and proposed a new name, *A. desseri*. Khan and Desser (1971) saw usually single and occasionally up to 26 merozoites in peripheral blood mononuclear cells as well as in visceral tissues, where developing schizonts were also seen. Electron microscopy confirmed that at least some parasites divided by schizogony to produce 2 or more than a dozen merozoites (Desser 1980). Endodyogeny and endopolygeny were not observed and gamonts were not seen. Ultrastructurally, trophozoites and schizonts of the *Lankesterella*-like coccidian described by Desser (1980) appear to be similar to the parasite in the cardinal.

In the present study, blood smears were not available for microscopic examination. However, single sporozoites were occasionally found free or in lymphocytic cells within various pulmonary vessels. No other parasite stages were seen in blood cells and a definitive genus and species assignment could not be made.

REFERENCES

Baker D.G., Speer C.A., Yamaguchi A., Griffey S.M., Dubey, J.P. (1996) An unusual coccidian parasite causing pneumonia in a northern cardinal (*Cardinalis cardinalis*). *J. Wildl. Dis.* **32:** 130-132

Baker J.R., Bennett G.F., Clark G.W., Laird M. (1972) Avian blood coccidians. *In:* Advances in Parasitology. Academic Press, London, **10:** 1-30

Box E.D. (1970) *Atoxoplasma* associated with an Isosporan oocyst in canaries. *J. Protozool.* **17:** 391-396

Box E.D. (1977) Life cycles of two *Isospora* species in the canary, *Serinus canarius* Linnaeus. *J. Protozool.* **24:** 57-67

Desser S.S. (1980) An ultrastructural study of the asexual development of a presumed *Isospora* sp. in mononuclear, phagocytic cells of the evening grosbeak (*Hesperiphona vespertina*). *J. Parasitol.* **66:** 601-612

Dissanaike A.S. (1967) *Lankesterella lainsoni* sp.nov. from the Ceylon mynah bird *Acridotheres tristis melanosternus*. *Ceylon J. Sci. (Bio. Sci.)* **6:** 225-229

Dubey J.P., Speer C.A., Fayer R. (1989) Sarcocystosis of animals and man. CRC Press, Boca Raton, Florida

Garnham P.C.C. (1950) Blood parasites of East African vertebrates, with a brief description of exo-ervthrocytic schizogony in *Plasmodium pitmani*. *Parasitol.* **40:** 328-337

Khan R.A., Desser S.S. (1971) Avian *Lankesterella* infections in Algonquin Park, Ontario. *Can. J. Zool.* **49:** 1105-1110

Lainson R. 1959 *Atoxoplasma* Garnham, 1950, as a synonym for *Lankesterella* Labbé, 1899. Its life cycle in the English sparrow (*Passer domesticus domesticus*, Linn.). *J. Protozool.* **6:** 360-371

Landau I. (1973) Diversité des mécanismes assurant la pérennité de l'infection chez les sporozoaines coccidiomorphes. *Mém. Mus. Nat. Hist. Nat., Série A, Zoologie* **77:** 1-62

Landau I., Lainson R., Boulard Y., Shaw J.J. (1974) Transmission au laboratoire et description de l'Hémogrégarine *Lainsonia legeri* n.sp. (Lankesterellidae) parasite de lézards brésiliens. *Ann. Parasitol. Hum. Comp.* **49:** 253-263

Levine N. (1982) The genus *Atoxoplasma* (Protozoa, Apicomplexa). *J. Parasitol.* **68:** 719-723

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Ultraviolet Radiation Effects on Pigmentation in the Cyanobacterium *Phormidium uncinatum*

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Summary. The Baikal strain of the cyanobacterium *Phormidium uncinatum* was found to possess the photosynthetic pigments chlorophyll *a*, carotenoids, phycocyanin and allophycocyanin, while the Tübingen strain of *Phormidium* contained, in addition to these, the biliprotein phycoerythrin. Sucrose gradient centrifugation of the pigment extracts resulted in a separation of the phycobiliproteins into several bands, which according to their absorption and fluorescence properties, were identified as monomers, trimers and hexamers. With increasing UV-B irradiation the heavier aggregates were broken down into smaller components. Photobleaching of these accessory pigments also occurred. FPLC gel filtration analyses of the pigments also showed loss of heavier aggregates of the phycobilins and bleaching of the pigments. SDS-polyacrylamide gel electrophoresis of the sucrose gradient and FPLC fractions indicated loss of the biliproteins with increasing UV-B irradiation. The loss of the β- were more rapid than that of the α-subunits. Increasing levels of ultraviolet irradiation is therefore deleterious to these organisms.

Key words: allophycocyanin, cyanobacteria, *Phormidium uncinatum*, phycocyanin, phycoerythrin, UV-B radiation.

INTRODUCTION

The photosynthetic apparatus of cyanobacteria is closely similar in structure and function to that located in chloroplasts of photosynthetic eukaryotes. The two reaction centers, photosystem II (P680) and photosystem I (P700), possess light harvesting antennae made up of several protein pigment complexes. Chlorophyll *a* is the universal pigment of the reaction centers in cyanobacteria, red algae and cryptophyceae; in addition, these algae contain phycobiliproteins which are accessory pigments for the operation of photosystem II (O'Carra and O'Eocha 1976, Glazer 1981). The accessory light-harvesting antenna of cyanobacteria is a water-soluble multicomponent complex. The light energy harvested is transferred predominantly to photosystem II with about 90 % efficiency (Glazer 1984).

The phycobilisomes of cyanobacteria are arranged in short or long rows, evenly spaced out on the stromal surface of the thylakoid membranes, mostly in a discoidal or hemidiscoidal shape or in an ovoid, globular or bundle shape (Gantt 1980, Rippka et al. 1974, Guglielmi et al. 1981). They consist mainly of biliproteins. Phycocyanin and allophycocyanin are the two universal constituents of biliproteins in red algae and cyanobacteria. Phycoerythrin is present in most red algae and in many cyanobacteria, while phycoerythrocyanin has only a narrow taxonomic distribution, primarily occurring in heterocyst-forming cyanobacteria which do not contain phycoerythrin (Bryant et al. 1976, Rippka et al. 1979). The biliproteins absorb

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light in a broad spectral region between 500 and 650 nm, complementary to the absorption bands of the chlorophyll *a*-protein complexes (Cohen-Bazire and Bryant 1982, Mullineaux 1992). Phycobiliproteins represent a considerable fraction (up to 50 %) of the total cellular proteins of these organisms (Myers and Kratz 1955; Giraud 1963; Bennett and Bogorad 1973; Gantt and Lipschultz 1973, 1974; Tandeau De Marsac 1977). It is therefore not surprising that some cyanobacterial cultures (such as *Spirulina*) are used as food for humans as well as cattle and others for many other commercial purposes. The cyanobacteria and other phytoplankton play an important role as primary producers in the food chain in marine and freshwater ecosystems. They are very important both as oxygen producers and carbon dioxide consumers. Any factor affecting their growth and survival would therefore bring about an ecological imbalance in the biosphere. In addition, most cyanobacteria are capable of fixing atmospheric nitrogen into useable forms for higher plants. They therefore constitute a natural source of fertilizer applied mostly in rice fields.

Recently, the increased emission of anthropogenic gases (such as chloro-fluorocarbons) into the atmosphere has led to the gradual depletion of the ozone layer in the stratosphere. Since the ozone layer is the protective shield against hazardous short wavelength ultraviolet radiation from the sun, all organisms on earth would therefore be prone to the deleterious effects of short wavelength ultraviolet stress. These deleterious effects include DNA damage, decline in plant growth and yield affecting agricultural and timber crops. The stressful effects of increased UV-B radiation may be more pronounced in phytoplankton and other microorganisms since they have no protective epidermal layer. In this paper, the effects of UV-B radiation on pigmentation in two strains of the cyanobacterium *Phormidium uncinatum* were investigated.

MATERIALS AND METHODS

Organisms and culture conditions

Two strains of *Phormidium uncinatum* (Baikal and Tübingen strains) obtained from the Göttingen algal culture collection (Schlösser 1982) were used in this work. These strains have been selected because of their sensitivity to UV-B and the previous knowledge of the effects of enhanced solar and artificial UV-B radiation on absorption, fluorescence, motility and orientation (Häder 1984, Häder et al. 1986, Häder and Häder 1990, Donkor and Häder 1991). The organisms were grown on agar (0.35%) in C 6 mineral medium in 100-mm Petri dishes (Nultsch and Häder 1974) covered with 200-µm pore membrane filters (SM 11307, Sartorius, Göttingen, FRG) and moistened with 5 ml C 6 mineral solution. The cultures were kept under constant white light from mixed fluorescence lamps at 6 W m^{-2} in a temperature-controlled room (20° C).

UV irradiation

Organisms were exposed in liquid medium in Petri dishes (90 mm in diameter) at room temperature to artificial UV radiation from a transilluminator (11-350-M, Bachofen, Reutlingen, Germany) with a peak wavelength of 312 nm. Half of the irradiance (23 W m^{-2}) was in the UV-B range (280 - 315 nm) and half in the UV-A range (315 - 400 nm). The emission spectrum of this radiation source has been published recently (Gerber and Häder, 1995). Alternatively, the cells were exposed to solar radiation at noon time in summer at Erlangen (50° N).

Separation of phycobilins with sucrose gradient centrifugation

Organisms from two fully grown plates were harvested and cooled on ice chips. A buffer solution of pH 7.5, made up of 50 mM K_2HPO_4 and KH_2PO_4, was used in washing the organisms by centrifugation (Beckman, J2-21 M/E, UK) twice at 3000 rpm for 5 min at 5°C using rotor JA 20. Three ml of the buffer solution was added to 3.4 g of the organisms and 500µl of 1 mM PMSF solution (in isopropanol) added in order to prevent protein breakdown by proteases. The organisms were homogenized (15000 rpm, 60 s) and cooled in ice. The cells were then broken by passing the homogenized organisms twice through a French press cell (Aminco, Urbana, Illinois, USA) at 1000 bar, and the sample collected at a flow rate of 1 - 2 drops per second. Seventy µl of Triton X-100 was added, thoroughly mixed and the suspension incubated on ice for about 1 h. The suspension was then centrifuged at 15000 rpm for 5 min at 5°C using the rotor JA 20. The supernatant was removed, the absorption spectrum measured and the crude extract subjected to continuous sucrose gradient centrifugation, in a sucrose gradient of 5 - 50% in 150 mM Tris/HCl buffer at pH 7.5, using an ultracentrifuge (Beckman L8 - 70M, USA) at 40000 rpm and 10°C for 16 h (rotor type SW 40). The various phycobilin bands were carefully removed with a syringe, absorption and fluorescence measurements were taken and the samples stored in a deep freezer for FPLC or SDS-polyacrylamid gel electrophoresis analyses.

Separation of biliproteins with FPLC

A gel filtration column, Superdex 200 prep grade, was used to separate the biliproteins on the FPLC (Fast Protein Liquid Chromatography). A buffer solution containing 0.15 M Tris/HCl (pH 7.5) was used as eluent. Biliprotein samples were filtered through a 0.2 µm sterile filter, 1.8 ml of which were loaded onto the column and allowed to run at a flow rate of 0.5 ml/min for 3 h. The samples were collected and their absorption spectra measured with a single beam spectrophotometer (DU-70, Beckman, Palo Alto, USA). Measurements of fluorescence spectra were also made using a spectrofluorimeter (RF 5000, Shimadzu, Japan). Some of the eluted samples were also used for running SDS-polyacrylamide gel electrophoresis.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Vertical SDS gradient gel electrophoresis was prepared according to Lämmli (1970), using a stacking gel and a resolving gel. The thickness of the gel was 1.5 mm, with a resolving gel surface area of

12.5 x 15.5 cm², composed of 5 -15 % T. Ten µg protein were applied to each well. The electrophoresis was run initially at 300 V and 30 mA for about 1 h. When the samples had run into the resolving gel, the power was increased to 600 V and 60 mA. The gel was stained using the procedure developed by Merril (1990). The gels were finally dried in a gel drier (BIO-RAD), Richmond, California, USA) or stored in polythene bags in glycerol.

RESULTS

Effects of UV on phycobiliproteins

Sucrose gradient centrifugation of the pigment extracts showed that the control samples separated into seven bands. Absorption spectra of the seven bands are recorded in Fig. 1. Some of the bands were missing in the UV-B irradiated samples. Loss of the heavier phycobilin aggregates (fractions 5 and 7 of the control) occurred in the irradiated samples. A comparison of the absorption spectra of fraction 1 of the control and irradiated samples showed increases in amplitude of the peaks of the irradiated samples, especially at 412 nm, Fig. 2), whereas a drastic decline in amplitude of peaks corresponding to the phycobilins occurred with increasing UV irradiation (Fig. 3).

Fluorescence emission and excitation spectra of the sucrose gradient fractions from the Baikal and Tübingen stains of *Phormidium* also indicated a more rapid decline in the phycocyanin peaks than the phycoerythrin and allophycocyanin peaks (data not shown). The emission spectra of the first fraction from the Baikal strain monitored at an excitation wavelength of 436 nm showed an

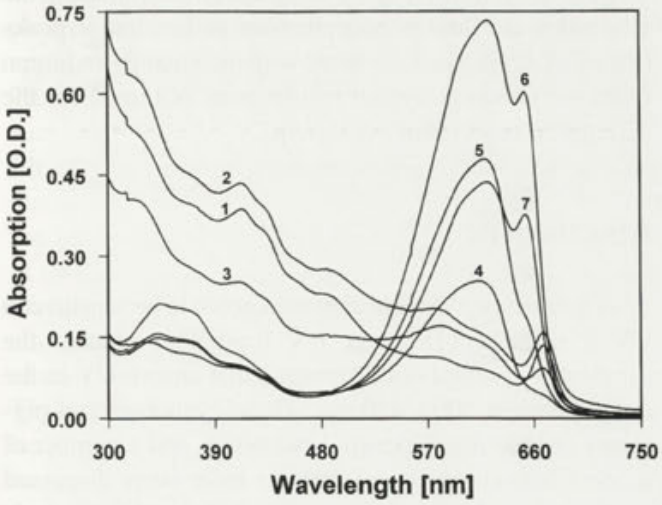

Fig. 1. Absorption spectra of sucrose gradient fractions (1 - 7) of the unirradiated control sample of *Phormidium uncinatum* (Baikal strain)

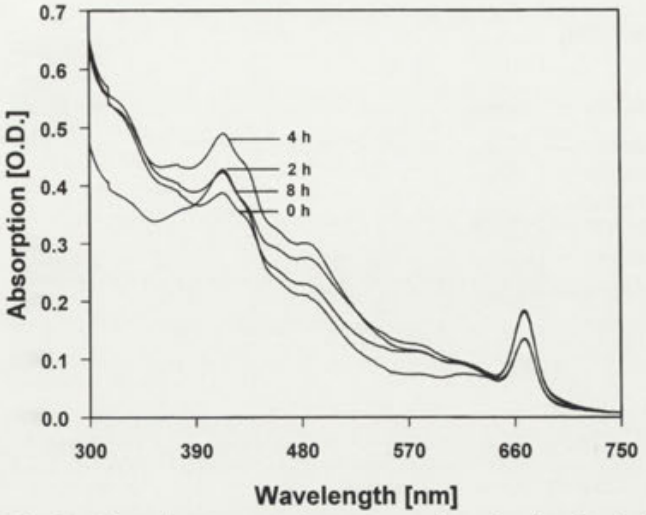

Fig. 2. Absorption spectra of sucrose gradient fraction 1 of *P. uncinatum* (Baikal strain) after exposure to artificial UV radiation for 0, 2, 4 and 8 h

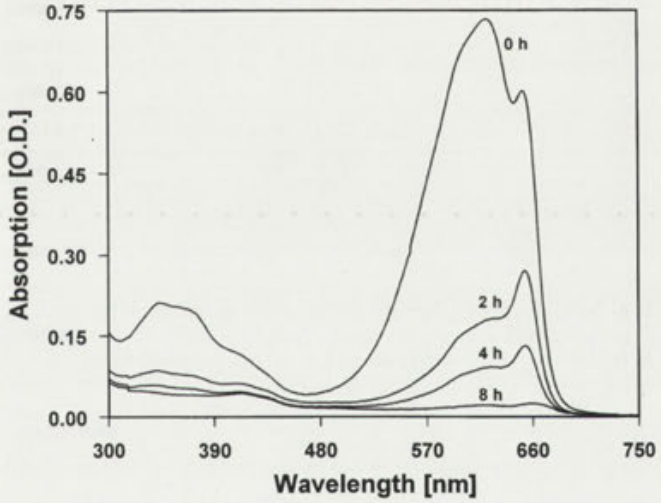

Fig. 3. Absorption spectra of sucrose gradient fraction 6 of *P. uncinatum* (Baikal strain) after exposure to UV radiation for 0, 2, 4 and 8 h

increase in amplitude at the peaks in the 2-h irradiated sample. However, further irradiation caused a decline in these peaks (data not shown).

SDS-polyacrylamide gel electrophoresis of the various sucrose gradient fractions of both the Tübingen and Baikal strains showed that fractions of the unirradiated control sample contained phycobiliprotein bands of about 17.2, 18.2, 18.3, 20, 22 and 24 kDa bands. The 22 - 24 kDa bands disappeared completely in the 2-h and 4-h irradiated samples. A gradual decline occurred in the 20 kDa band with increasing irradiation. The 18 kDa bands were also diminished with irradiation and almost disappeared in the 4-h irradiated sample (Fig. 4). In fraction 4, gradual reduction occurred in the 20 - 24 kDa bands with increasing irradiation, whereas the 18 kDa bands in-

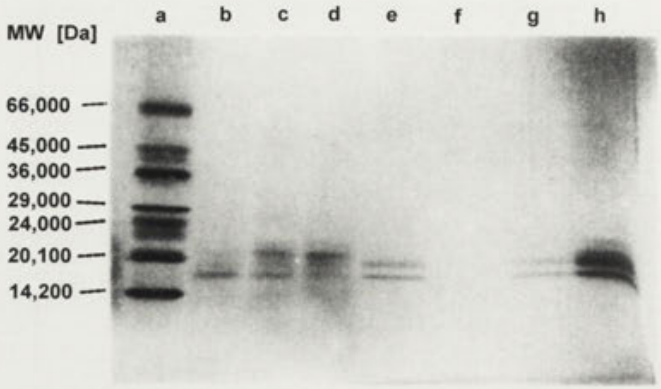

Fig. 4. SDS-polyacrylamide gel of sucrose gradient fractions of *P. uncinatum* (Tübingen strain): (a) marker 6H, (b) fraction 6 of 4 h, (c) 2 h and, (d) control, (e) fraction 7 of control, (f) fraction 4 of 4 h, (g) 2h and (h) control

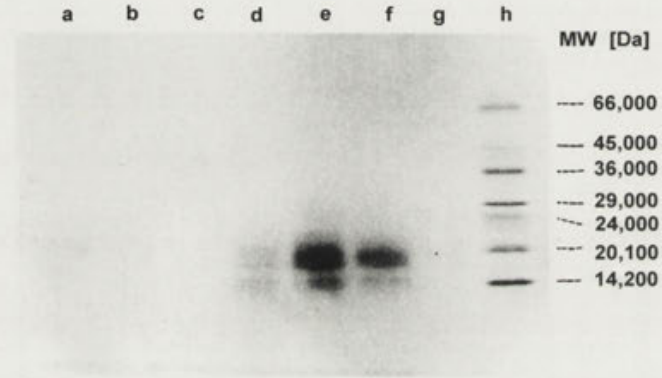

Fig. 5. SDS-polyacrylamide gel of sucrose gradient fractions of *P. uncinatum* (Tübingen strain): fraction 1 of (a) 2 h, (b) 4 h and (c) 8 h, fraction 2 of (d) control, (e) 4 h and (f) 8 h, (g) marker 6H

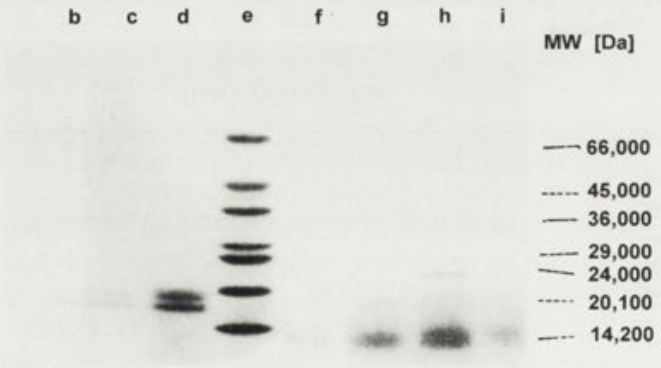

Fig. 6. SDS-polyacrylamide gel of sucrose gradient fractions of *P. uncinatum* (Baikal strain): fraction 6 of (b) 4 h, (c) 2 h, (d) control, (e) marker 6H, fraction 1 of (f) 8 h, (g) 4 h, (h) and (i) control

creased. In fractions 1 - 3, the 2 h- and 4 h-irradiated samples showed more prominent bands in the 24, 18 and smaller kDa bands as compared to the control. All these bands, however, were diminished in the 8 h-irradiated sample (Figs. 5 and 6).

The FPLC gel filtration of the Baikal strain showed three major and three minor peaks on the elution diagram

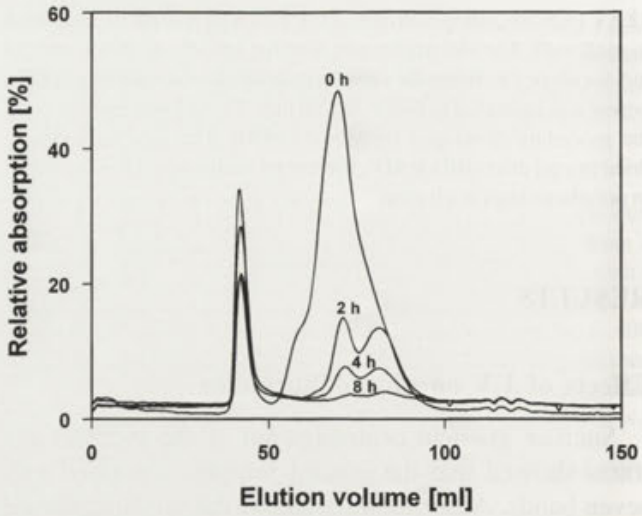

Fig. 7. FPLC gel fraction diagram of *P. uncinatum* (Baikal strain) comparing the elution at 280 nm of the control sample and samples exposed to UV radiation for 0, 2, 4 and 8 h

(data not shown). The first major peak contained fractions which showed chlorophyll absorption peaks. The next was a minor elution peak, from which a blue-colored fraction was obtained, which showed a phycocyanin absorption maximum. From the second major elution peak a blue-colored fraction was obtained, which showed phycocyanin and allophycocyanin absorption maxima. The next two minor elution peaks contained blue-colored phycocyanin-containing fractions. From the last major peak colorless fractions were obtained, which did not show any absorption maxima.

In the 2, 4 and 8-h irradiated samples the FPLC elution diagram showed a shift of the peaks corresponding to the phycocyanin and allophycocyanin containing fractions to longer elution times (Fig. 7). Decreases in amplitude in the absorption and fluorescence emission and excitation peaks of the FPLC fractions occurred with increasing irradiation (data not shown). Similar results were obtained for the Tübingen strain (data not shown).

DISCUSSION

A number of cyanobacteria are known to be sensitive to UV-B radiation. However UV irradiation induces the synthesis of a number of pigments that absorb UV in the range between 300 - 450 nm. These cyanobacterial pigments include mycosporins, scytonemin and a number of unidentified chromophores which have been discussed extensively in the literature (Garcia-Pichel and Castenholz 1991; Garcia-Pichel et al. 1992, 1993; Scherer et al. 1988). Also the strains studied in this work produce UV screening

pigments as indicated by the increase in the broad peak centered around 412 nm. Also in this case the chemical identity of the chromophore has not yet been revealed.

In the fractions obtained from the sucrose gradient centrifugation of the pigment extracts, the loss of fractions, which had longer phycocyanin and allophycocyanin absorption wavelengths (including phycoerythrin peaks in the Tübingen strain of *Phormidium*) in the UV-B irradiated samples indicate breakdown of heavier aggregates of phycocyanin and phycoerythrin by UV-B radiation into smaller subunits, which confirms earlier observations made during *in vivo* absorption and fluorescence measurements (Donkor and Häder 1991).

The fact that the absorption peaks of the first sucrose gradient fractions of the irradiated samples were higher than the control in the Baikal and Tübingen strains of *Phormidium* suggests an increase of some degradation products caused by UV irradiation. Also, increases in the 481 nm peak in the UV irradiated samples could be due to an increment in the carotenoid to chlorophyll ratio with UV irradiation in order to afford some photoprotection. Prolonged irradiation, however, caused bleaching of the pigments.

In vivo, the phycobiliproteins are arranged in high molecular mass aggregates and are structurally composed of phycobilisomes (Gant and Conti 1966), which in electron microscopic studies have been found to be attached to Photosystem II particles and arranged in rows on the surface of thylakoids of cyanobacteria and red algae (Mörschel and Rhiel 1987). The subunits are connected by linker proteins (Lundell et al. 1981, Glazer 1982). Drastic decline in absorption peaks of the phycobilin-containing fractions vividly shows drastic loss of the phycobiliproteins with increasing UV-B irradiation, which appeared to be faster in phycocyanin than allophycocyanin. The fluorescence emission and excitation spectra of the sucrose gradient fractions and fractions of the FPLC gel filtration confirmed this observation. The shifts of the maxima to shorter wavelengths with increasing irradiation, the loss of the second fraction of the FPLC gel filtration of the Baikal strain of *Phormidium*, and the shift of elution of the phycobilins of the irradiated samples to longer elution times also confirm breakdown of higher aggregates of these pigments to smaller subunits by UV-B radiation. This is clearly illustrated in the Tübingen strain, where the heavier aggregated phycobilin fractions, which were hexamers and trimers drastically decreased while the smaller aggregated phycobilin fraction, which was eluted later (basically monomers) increased in the irradiated samples.

The degradation kinetics of the phycobilins indicates that during UV irradiation the phycobilisomes disintegrate from the outside toward the core (Fischer and Häder 1992a). It could be speculated that the disassembly of the phycobilisomes by UV treatment is caused by damage of linker proteins. In addition, it needs to be stressed that exposure to UV radiation induces a general decrease in protein concentration which has also been found in other algal systems (Zündorf and Häder 1991, Häder and Brodhun 1991, Fischer and Häder 1992b).

The decrease in protein content with UV irradiation is clearly illustrated by the results of the SDS-polyacrylamide gel electrophoresis of the sucrose gradient and FPLC fractions, in which protein bands of the fractions containing the phycobilins declined in intensity with increasing irradiation. The rapid loss of the 20.1 and 22 kDa protein bands with irradiation as compared to the 17.3 and 18.2 kDa bands indicates that the loss of the β-subunits was greater than that of the α-subunits. It has also been demonstrated that solar radiation had a much more drastic effect on the phycobiliprotein degradation and protein loss than UV radiation alone. This is illustrated by the complete loss of all pigments in the 8 h-irradiated samples of the Baikal and Tübingen strains of *Phormidium* exposed to unfiltered solar radiation (data not shown). These samples were bleached completely, and the sucrose gradients showed no bands at all. Obviously, the strong visible component of sunlight also plays a decisive additional role in the destruction of the biliproteins (Nultsch and Agel 1986).

From this study it can be concluded that with the aid of sucrose gradient centrifugation, FPLC gel filtration and SDS-PAGE, as well as the absorption and fluorescence properties of these samples it has been clarified that with increasing UV-B irradiation the heavier phycobilin aggregates (hexamers and trimers) were broken down into smaller components (monomers). Also, photobleaching of these accessory pigments occurred. The loss of the β-subunits was more rapid than that of the α-subunits.

Photodestruction of these accessory pigments would result in a serious decline in energy transfer for photosynthetic activity and on primary productivity at large. These observations indicate that the persistence of the gradual deletion of the ozone layer in the stratosphere, resulting in increasing amounts of UV-B radiation reaching the Earth's surface is bound to augment the stress factor of this radiation on phytoplankton organisms. Recently, solar UV-B radiation has been found to penetrate far deeper into the euphotic zone of the oceans than considered before, indicating a substantial hazard

(Gieskes and Kraay 1990, Smith et al. 1992). Investigations on marine phytoplankton have indicated that the photosynthetic productivity may decrease by a factor of 6 - 12 % due to increased solar UV-B radiation under the condition of the Antarctic ozone hole (Smith et al. 1992). These clearly indicate that persistence of this problem of the ozone layer depletion may lead to a number of ecological consequences including reduced biomass production, which will consequently affect the biological food web up to man; reduced sink capacity for atmospheric carbon dioxide - which would contribute to the greenhouse effect. This would lead to global warming - which might also lead to partial melting of the polar ice caps and worldwide sea level rise; decreased oxygen production; impaired nitrogen fixation, which may mean that more money may have to be sunk into buying artificial fertilizer, especially in the tropics, where there is heavy dependence on cyanobacteria for nitrogen supply in rice fields (Kumar and Kumar 1988).

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REFERENCES

Bennett D. A., Bogorad L. (1973) Complementary chromatic adaptation in a filamentous blue-green alga. *J. Cell Biol.* **58:** 419-435

Bryant D. A., Glazer A. N., Eiserling F. A. (1976) Characterization and structural properties of the major biliproteins of *Anabaena* sp. *Arch. Microbiol.* **110:** 61-75

Cohen-Bazire G., Bryant D. A. (1982) Phycobilisome composition and structure. In: The Biology of Cyanobacteria (Eds. N. G. Carr and B. A. Whitton), Blackwell Scientific Publications, Oxford, London, 143 -190

Donkor V., Häder D.-P. (1991) Effects of solar and ultraviolet radiation on motility, photomovement and pigmentation in filamentous gliding cyanobacteria. *FEMS Microbiol. Ecol.* **86:** 159-168

Fischer M., Häder D.-P. (1992a) UV effects of photosynthesis and phycobiliprotein composition in the flagellate *Cyanophora pardoxa*. *FEMS Microbiol. Ecol.* **101:** 121-131

Fischer M., Häder D.-P. (1992b) UV effects on the pigmentation of the flagellate *Cyanophora paradoxa* - Biochemical and spectroscopic analysis. *Europ. J. Protistol.* **28:** 163-169

Gantt E. (1980) Structure and function of phycobilisomes: light harvesting pigment complexes in red and blue-green algae. *Int. Rev. Cytol.* **66:** 45-80

Gantt E., Conti S. F. (1966) Phycobiliprotein localization at algae. *Brookhaven Symp. Biol.* **19:** 393-405

Gantt E., Lipschultz C. A. (1973) Energy transfer in phycobilisomes from phycoerythrin to allophycocyanin. *Biochim. Biophys. Acta* **292:** 858-861

Gantt E., Lipschultz C. A. (1974) Phycobilisomes of *Porphyridium cruentum*: Pigment analysis. *Biochem.* **13:** 2960-2966

Garcia-Pichel F., Castenholz R.W. (1991) Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. *J. Phycol.* **27:** 395-409.

Garcia-Pichel F., Sherry N.D., Castenholz R.W. (1992) Evidence for a UV sunscreen role of the extracellular pigment scytonemin in the terrestrial cyanobacterium *Chlorogloeopsis* sp. *Photochem. Photobiol.* **56:** 17-23

Garcia-Pichel F., Wingard C.E., Castenholz R.W. (1993) Evidence regarding the UV sunscreen role of a mycosporin-like compounds in the cyanobacterium *Gloeocapsa* sp. *Appl Environ Microbiol* **59:** 170-176

Gerber S., Häder D.-P. (1995) Effects of artificial and simulated solar radiation on the flagellate *Euglena gracilis*: physiological, spectroscopical and biochemical investigations. *Acta Protozool.* **34:** 13-20

Gieskes W. C., Kraay G. W. (1990) Transmission of ultraviolet light in the Weddell sea: report on the first measurements made in Antarctic. *Biomass Newsletter* **12:** 12-14

Giraud G. (1963) Les pigments et les caracterisitques fonctionnelles de l'appareil photosynthetique de diverses algues. *Physiol. Veg.* **1:** 203-255

Glazer A. N. (1981) Photosynthetic accessory proteins with bilin prosthetic groups. In: Biochemistry of Plants (Eds. M. D. Hatch and N. K. Boardman), Vol. VIII, Photosynthesis. Academic Press, New York, 51-96

Glazer A. N. (1982) Phycobilisomes: structure and dynamics. *Ann. Rev. Microbiol.* **36:** 173-198

Glazer A. N. (1984) Phycobilisome. A macromolecular complex optimized for light energy transfer. *Biochim. Biophys. Acta* **768:** 29-51

Guglielmi G., Cohen-Bazire G., Bryant D. A. (1981) The structure of *Gloeobacter violaceus* and its phycobilisomes. *Arch. Microbiol.* **129:** 181-189

Häder D.-P. (1984) Effects of UV-B on motility and photoorientation in the cyanobacterium, *Phormidium uncinatum*. *Arch. Microbiol.* **140:** 34-39

Häder D.-P., Brodun B. (1991) Effects of ultraviolet radiation on the photoreceptor proteins and pigments in the paraflagella body of the flagellate, *Euglena gracilis*. *J. Plant Physiol.* **137:** 641-646

Häder D.-P., Häder M. (1990) Effects of solar radiation on motility, photomovement and pigmentation in two strains of the cyanobacterium, *Phormidium uncinatum*. *Acta Protozool.* **29:** 291-303

Häder D.-P., Watanabe M., Furuya M. (1986) Inhibition of motility in the cyanobacterium, *Phormidium uncinatum*, by solar and monochromatic UV irradiation. *Plant Cell Physiol.* **27:** 887-894

Kumar A., Kumar H. D. (1988) Nitrogen fixation by blue-green algae. In: Plant Physiology Research (Ed. S. P. Sen), New Delhi: Society for Plant Physiology and Biochemistry, 1st International Congress of Plant Physiology, 15-22

Lämmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680-685

Lundell D. L., Williams R. C., Glazer A. (1981) Molecular architecture of a light harvesting antenna. *J. Biol. Chem.* **256:** 3580-3592

Merril C.R. (1990) Original gel-staining techniques. In: Methods in Enzymology (Ed. M. P. Deutscher), Vol. 182, Guide to Protein Purification. Academic Press, Inc., San Diego, 477-488

Mörschel E., Riehl E. (1987) Phycobilisomes and thylakoids: the light-harvesting system of cyanobacteria and red algae. In: Electron Microscopy of Proteins (Eds. J. K. Harris and R. W. Horne), Membranous Structure. Academic Press, London **6:** 209-254

Mullineaux C. W. (1992) Excitation energy transfer from phycobilisomes to photosystem I in a cyanobacterium. *Biochim. Biophys. Acta* **1100:** 285-292

Myers J., Kratz W. A. (1955) Relations between pigment content and photosynthetic characteristics in a blue-green alga. *J. Gen. Physiol.* **39:** 11-22

Nultsch W., Agel G. (1986) Fluence rate and wavelength dependence of photobleaching in the cyanobacterium *Anabaena variabilis*. *Arch. Microbiol.* **144:** 268-271

Nultsch W., Häder D.-P. (1974) Über die Rolle der beiden Photosysteme in der Photophobotaxis von *Phormidium uncinatum*. *Ber. Dtsch. Bot. Ges.* **87:** 83-92

O'Cara P., O'Eocha C. (1976) Algal biliproteins and phycobilins. In: Chemistry and Biochemistry of Plant Pigments. (Ed. T. W. Goodwin), Vol. 1, 2nd ed. Academic Press, New York 328-376

Rippka R. Waterbury J. B., Gohen-Bazire G. (1974) A cyanobacterium which lacks thylakoids. *Arch Microbiol.* **100:** 419-436

Rippka R. Deruelles J., Waterbury J. B., Herdman M., Stainer R. Y. (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* **111:** 1-61

Scherer S., Chen T.W., Boger P. (1988) A new UV-A/B protecting pigment in the terrestrial cyanobacterium *Nostoc commune*. *Plant Physiol.* **88:** 1055-1057

Schlösser U. G. (1982) Sammlung von Agenkulturen. *Ber. Dtsch. Bot. Ges.* **9:** 181-276

Smith R. C., Prezelin B. B., Baker K. S., Bidigare R. R., Boucher N. P., Coley T., Karentz D., Macintyre S., Matlick H. A., Menzies D., Ondrusek M., Wan Z., Waters K. J. (1992) Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. *Science* **255:** 952-959

Tandeau De Marsac N. T. (1977) Occurrence and nature of chromatic adaptation in cyanobacteria. *J. Bact.* **130:** 82-91

Zündorf I., Häder D.-P. (1991) Biochemical and spectroscopic analysis of UV effects in the marine flagellate *Cryptomonas maculata*. *Arch Microbiol.* **156:** 405 -411

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Induced Defence in the Ciliate *Euplotes octocarinatus* is Reduced when Alternative Prey are Available to the Predator

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Summary. Some species of the genus *Euplotes* (Protista, Ciliophora) are known to change shape in the presence of predators, making them more difficult to ingest. The extent of this induced defence has been shown to be proportional to predator density, but the exact nature of the stimulus and the mechanism of its reception by the prey remain largely unknown. This experiment addressed the effect of the availability of alternative prey on the extent of the induced defence. *Euplotes octocarinatus* was confronted with the omnivorous ciliate predator *Stylonychia mytilus* in the presence or absence of an alternative prey, the green flagellate *Chlorogonium elongatum*. Predation of *E. octocarinatus* by *S. mytilus* was greatly reduced in the presence of an abundant flagellate population. Lower predation risk was accompanied by weaker anti-predator cell transformation response in *Euplotes*, indicating that the prey reacts rather to the actual level of predation and not simply to the presence of a predator.

Key words: alternative prey, morphological defence, phenotypic plasticity, predator-prey interaction, Protista.

INTRODUCTION

All predators have restricted size ranges of prey they can feed on. These ranges are mostly constrained by morphology. However, within the predator's diet, items of food differ in terms of their profitability, so we should expect predators to exhibit prey preference. If given a choice, an animal should feed on prey that maximize its net energy gain per unit of foraging time. In response to the predator's diet range and food selectivity, natural selection should act on the prey species either to remove it from the predator's diet range or to make it less profitable. Consequently we observe various kinds of prey defences.

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Prey defence may be permanent or inducible. Induced defence is mostly expressed on a behavioural level, but in some groups an induced morphological defence has also evolved. It is economical, as its costs are only incurred when the prey is actually endangered. Induced morphological defence has been recorded in several aquatic invertebrates (see Harvell 1984, Havel 1987 for reviews), protozoans (Kuhlmann and Heckmann 1985, Wicklow 1988), one fish species (Brönmark and Miner 1992) and some terrestrial plants (e.g. Young 1987, Milewski et al. 1991).

The freshwater ciliate *Euplotes octocarinatus* Carter, 1972 is able to modify its morphology in the presence of some predators. The ciliate responds by developing lateral "wings" and dorsal projections which make it more difficult to ingest (Kuhlmann and Heckmann 1985). The change in cell shape is the result of a cytoskeletal reorganization. Transformed cells have more microtubular triads

Table 1. Experimental design. Each treatment contained a different combination of organisms, in initial quantities as indicated. The initial volume of the medium in each experimental dish was 4 ml; five independent replicates per treatment were made.

Initial number of organisms in each treatment (cells/dish)			
Treatment	*Euplotes* 2000	*Stylonychia* 140	*Chlorogonium* 1.7×10^6
E (*Euplotes* alone)	+	-	-
EC (*Euplotes* + *Chlorogonium*)	+	-	+
ES (*Euplotes* + *Stylonychia*)	+	+	-
ESC (*Euplotes* + *Stylonychia* + *Chlorogonium*)	+	+	+

and additional single microtubules in the cell cortex (Jerka-Dziadosz et al. 1987). This structural modification does not require cell division for its completion and is perpetuated during subsequent divisions, as long as the inducing signal is present in the environment (Kuhlmann and Heckmann 1985). For some of *Euplotes*' predators the inducing signal molecules have been isolated. These predator-released substances have been demonstrated to induce defensive cell reorganization in *Euplotes* without the predator present (Kusch and Heckmann 1992, Kusch 1993a).

Cell transformation in *Euplotes* is a graded response. As shown in laboratory experiments, the lower the prey/predator ratio the higher the magnitude of the defence (Kusch 1993b, 1995). Thus the extent of cell modification seems to be adjusted to the actual risk of predation, and the cost of defence minimized. In natural systems, other factors such as the presence of alternative prey may influence the risk of predation.

The aim of this study was to examine whether induced defence in *Euplotes octocarinatus* is affected by the presence of alternative prey to the omnivorous predator *Stylonychia mytilus*, that is, whether *Euplotes* reacts to predator density or to the actual predation risk.

MATERIALS AND METHODS

The predatory species *Stylonychia mytilus* used in the experiments was originally collected from a small, organically polluted stream in the north part of Cracow, Poland. The prey species *Euplotes octocarinatus* was isolated from a freshwater aquarium in the Department of Hydrobiology, Jagiellonian University, Cracow. The ciliates were grown and cultivated as clonal cultures. Only one clone of *E. octocarinatus* and one clone of *S. mytilus* were used in the experiments. The stock cultures were kept in Petri dishes (5 cm diameter) at 20-23°C. Volvic mineral water (Société des Eaux de Volvic, Puy-de-Dôme, France) was used as the medium. Every other day both species were fed with the green flagellate *Chlorogonium elongatum*. *Stylonychia mytilus* was transferred to fresh medium every three days, and *E. octocarinatus* once a week.

For the experiment the cultures of ciliates were prepared in the following way. Two days before the experiment, populations from several dishes were mixed together and relocated to new vessels with fresh medium in order to provide similar culture conditions. On the day preceding the experiment all protozoans were well fed with *Chlorogonium*. After twelve hours, *Euplotes* cells from different dishes were concentrated and transferred into a common beaker from which small samples were taken to estimate population density. Then fresh medium was added to the beaker in order to arrive at a concentration of 1000 cells/ml. The same procedure was repeated with *Stylonychia* and its density was brought to 140 cells/ml. The density of *Ch. elongatum* used as alternative prey was 1.7×10^6 cells/ml. These initial cultures were used to set up the experiment.

The experiment was carried out in Petri dishes (5 cm diameter). There were four treatments, each with five independent replicates, that is, five separate vessels per treatment (see Table 1 for the experimental design). All dishes in each treatment were supplied with 2 ml of *Euplotes* culture (1000 cells/ml) taken from the initial culture after stirring. Depending on the treatment, different combinations of medium, *Stylonychia* or *Chlorogonium* cultures were added to the experimental vessels so as to end up with a volume of 4 ml in each dish. The "*Euplotes*" treatment (E) contained only *Euplotes* cells, and had 2 ml of medium added. For the "*Euplotes-Chlorogonium*" treatment (EC), 1 ml of *Chlorogonium* culture and 1 ml of medium were added. The two remaining treatments were supplied with 1 ml of *Stylonychia* culture (140 cells/ml). One of these latter (ES) was provided with 1 ml of medium and the other one (ESC) with 1 ml of *Chlorogonium* culture as the alternative prey for *Stylonychia*. Thus the initial densities of the prey and the predator in the experimental dishes were 500 and 35 cells/ml, respectively. The ciliates were incubated for 9 h at 23°C. The duration of the experiment and the initial densities were chosen according to earlier experiments which had shown that at these concentrations very strong cell transformation occurred after 9 h. Two treatments containing the predator (ESC and ES) were used to measure the extent of predator-induced morphological transformation in *Euplotes* with and without *Chlorogonium* present, and also to calculate the final number of both ciliates. The treatments without predator (E and EC) were only used to estimate the effect of *Chlorogonium* on the final *Euplotes* number in the absence of the predator.

After 1.5, 5 and 7.5 h, 0.5 ml of *Chlorogonium* culture was added to the treatments that had originally contained these algae (EC and ESC) to maintain a continuous surplus of the flagellate through the whole

experiment. The same amount of medium was simultaneously added to the vessels of the two other treatments (E and ES).

To measure *Euplotes* cell length and width, the ciliates of the "*Stylonychia*" treatments (ES and ESC) were video-recorded at the beginning and end of the experiment. The recording was done directly through the bottom of the experimental dishes with an inverted microscope (Olympus IMT-2), in such a way as to preclude the possibility of encountering the same individual more than once. The video equipment included a Bischke CCD-500 camera, Aver 2000 frame grabber (ADDA Technologies), Panasonic NV-J35EE video recorder, and an IBM-compatible computer with an SVGA monitor. The recording and measuring were done with a 10x lens, 2.5x ocular, and an additional 1.5x magnification factor. With this magnification a distance of 50 µm corresponded to about 18 mm on the screen. From each tape sequence representing a single replicate, the cell length and width of the first 30 *Euplotes* individuals detected with sharp images were measured using a MultiScan image analysis programme (Computer Scanning Systems Ltd).

After 9 h of incubation the content of each dish was fixed with Lugol solution (final concentration 1%) to estimate the number of both ciliates. The estimation was performed in plankton chambers. Each fixed sample was left in a chamber for a few days to allow cell sedimentation, and then the cells were counted in 25 randomly chosen visual fields at 60x and the final ciliate number for each replicate was calculated (the scanned area covered 1/10 of a chamber bottom surface).

The mean values of 30 *Euplotes* cell dimensions were calculated for each replicate of the ES and ESC treatments (Hurlbert and White 1993). The means from the ES treatment were compared with those of the ESC treatment. One-way ANOVA was used for comparison of cell length. Due to the heterogeneity of variances, cell width was compared by Kruskal-Wallis ANOVA by ranks. The final numbers of ciliates were compared by one-way ANOVA and Tukey's test for *post-hoc* comparison of means.

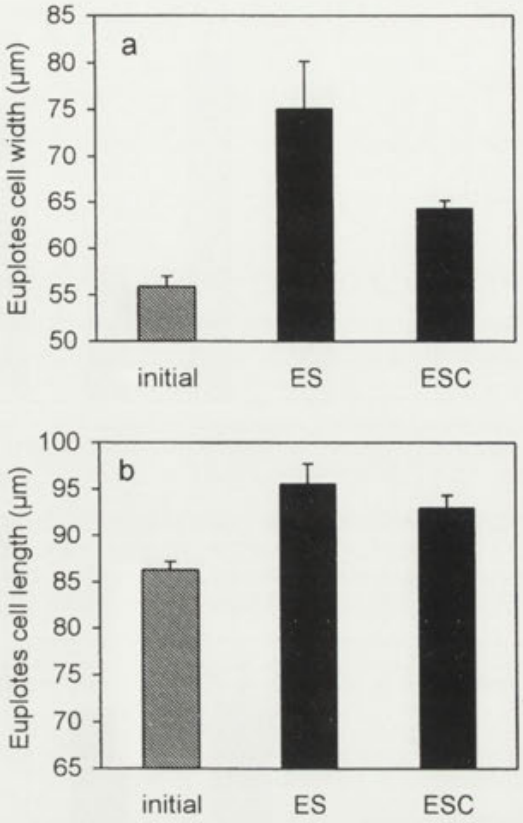

Fig. 1. Morphological transformation in *Euplotes octocarinatus* after 9 h exposure to *Stylonychia mytilus* in the absence (ES) and presence (ESC) of *Chlorogonium elongatum*. Each bar represents the mean and SD of 5 independent replicates (experimental units). 30 individuals (evaluation units) were measured per replicate

RESULTS

At the beginning of the experiment, the *Euplotes octocarinatus* cells were 55.9 ± 0.5µm wide and 86.3 ± 0.4 µm long (mean ± SE). After 9 h of exposure to *Stylonychia mytilus* they enlarged in both treatments (Fig. 1). However, the change in *Euplotes* cell width was significantly less pronounced in the presence of alternative prey (ESC) than in the treatment without *Chlorogonium* (ES) (Kruskal-Wallis: $H = 6.82$, $df = 1$, $N = 10$, $P = 0.009$). No significant difference between treatments was found in cell length (ANOVA: $F_{[1,8]} = 5.03$, $P = 0.06$, Fig. 1).

The number of *Stylonychia* increased in both treatments from an initial 140 up to approximately 220 cells/dish. No significant difference was found between treatments (ANOVA: $F_{[1,7]} = 0.006$, $P = 0.94$). The final cell numbers of *Euplotes* differed remarkably among the treatments ($F_{[3,15]} = 29.97$, $P < 0.0001$). *Post-hoc* analysis revealed that this result was entirely due to the ES treatment, as the number of *Euplotes* was significantly reduced by *Stylonychia* predation only in the absence of the alternative prey. The means of the three remaining treatments (E, EC and ESC) did not differ significantly from each other and were slightly higher than the initial values (Fig. 2). *Chlorogonium elongatum*, used here as alternative food for *Stylonychia*, is food for *Euplotes* too. However, comparison of the final number in the treatments without predator (E and EC in Fig. 2) shows that the effect of *Chlorogonium* on *Euplotes* growth rate was not statistically significant ($F_{[1,8]} = 4.54$, $P = 0.066$). Thus the difference in final *Euplotes* numbers between treatments containing *Stylonychia* (ES and ESC) resulted from intense predation in ES.

The predator/prey ratio, initially the same, doubled in ES compared to ESC during the experiment. In order to check whether this change could have affected the observed transformation in *Euplotes*, an additional experiment was carried out using the same basic methodology. This time the effect of two different initial densities of *Stylonychia* (70 and 140 cells/dish) on the response of *Euplotes* (2000 cells/dish) was compared. After 9 h the mean numbers of *Stylonychia* increased up to 177 and 94

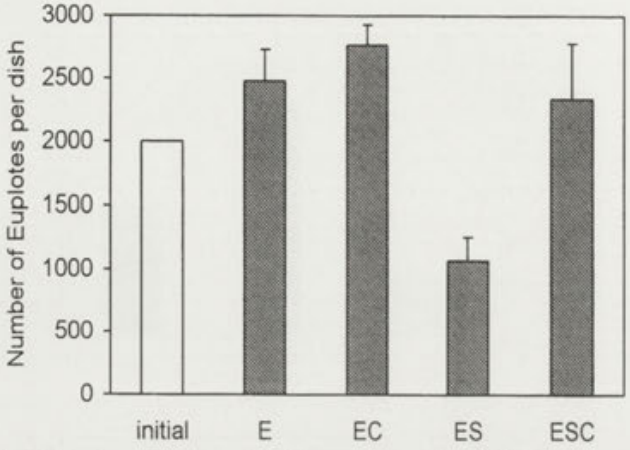

Fig. 2. Number of *Euplotes* after 9 h experiment. Symbols: E - *Euplotes* alone; EC - *Euplotes* + *Chlorogonium*; ES - *Euplotes* + *Stylonychia*; ESC - *Euplotes* + *Stylonychia* + *Chlorogonium*. The number of *Euplotes* in ES is significantly lower than that in all other treatments. The three remaining treatments do not differ significantly from each other

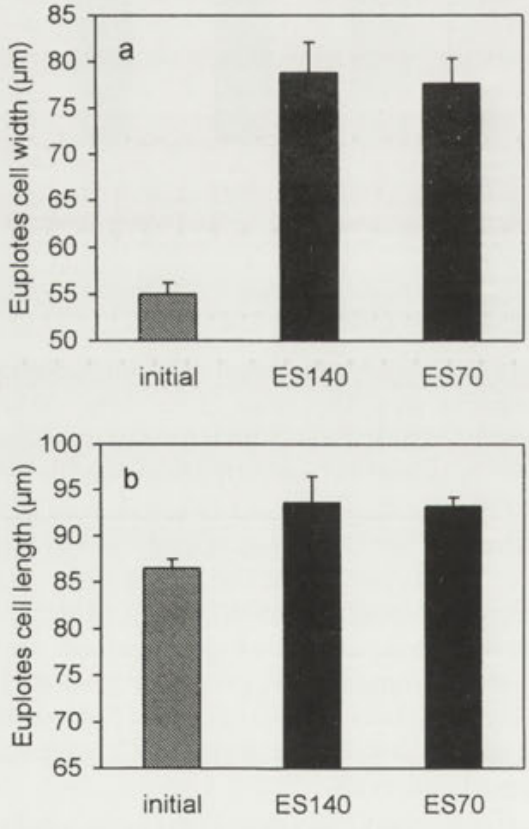

Fig. 3. The effect of two different initial densities of *Stylonychia* (70 and 140 cells/dish) on *Euplotes* transformation. No significant difference in cell width and length. Each bar represents the mean and SD of 5 independent replicates (experimental units). 30 individuals (evaluation units) were measured per replicate

per dish, while those of *Euplotes* dropped to 1311 and 1662 in the ES140 and ES70 treatments respectively. Thus the initial two-fold difference in predator/prey ratio between treatments had increased during the experiment. No significant difference in *Euplotes* cell width between the ES140 and ES70 treatments was observed ($F_{[1,8]} = 0.379$, $P = 0.55$) (Fig. 3).

DISCUSSION

The results reported in Fig. 3 suggest that both predator/prey ratios were high enough to induce maximum response in the prey, so the difference in the transformation observed in the main experiment (Fig. 1) should not be attributed to the change in the predator/prey ratio during the experiment but rather to the presence of *Chlorogonium* in the ESC treatment. The extent of *Stylonychia*-induced defence in *Euplotes* was affected by the availability of alternative prey. As predator-caused mortality was much lower in ESC (Fig. 2), the prey must have adjusted its defensive response to predation pressure, that is, the predator's actual feeding behaviour and not simply its presence.

Such a result is consistent with evolutionary predictions on inducible defences. Their evolution is favoured when the defence is beneficial in the presence of the predator but involves some fitness costs that are not compensated when the predator is absent (Dodson 1989; Adler and Harvell 1990; Harvell 1990a,b; Riessen 1992). Transformed *Euplotes* cells benefit from decreased vulnerability to their predators compared to untransformed conspecifics (Kuhlmann 1992, Kuhlmann and Heckmann 1994, Kusch 1995), but the defence must be costly, as the cells tend to return to their normal ovoid shape as soon as the inducing factor disappears. Several apparent fitness costs of this defence are in the literature: "winged" morphs have longer generation time than ovoid ones (Kuhlmann 1992); protein synthesis is necessary for the induced transformation (Kusch and Kuhlmann 1994); both the extent and maintenance of the cell modification are adversely affected by poor nutrition (Wiąckowski and Szkarłat 1996).

Since inducible defence has costs and benefits, *Euplotes* should accommodate the degree of defence to actual predation pressure and minimize costs. Earlier studies showed that the response was proportional to predator density (Kusch 1993b, 1995). Our experiment shows that when *Stylonychia* predation pressure decreases because of an abundance of alternative food (*Chlorogonium*) (Fig. 2), the transformation response is diminished (Fig. 1a).

Euplotes is able to develop stronger defensive structures in better nutritional conditions (Kuhlmann and

Heckmann 1994, Wiąckowski and Szkarłat 1996). In ESC *Euplotes* had better conditions to produce a stronger defence: a permanent surplus of food. Even so, the degree of defence was significantly lower in this treatment (Fig. 1a), which reinforces our result.

The risk of predation seems to be the ultimate factor behind the degree of predator-induced cell transformation. We are planning experiments to specify the proximate factors directly responsible for the defence reaction in our system.

Acknowledgements. We thank Jan Kozłowski for comments on the manuscript and Michael Jacobs for helping to edit it.

REFERENCES

Adler F. R., Harvell C. D. (1990) Inducible defenses, phenotypic variability and biotic environments. *Trends Ecol. Evol.* **5:** 407-410

Brönmark C., Miner J. (1992) Predator-induced phenotypical change in body morphology in crucian carp. *Science* **258:** 1348-1350

Dodson S. (1989) Predator-induced reaction norms. *BioScience* **39:** 447-452

Harvell C. D. (1984) Predator-induced defense in a marine bryozoan. *Science* **224:** 1357-1359

Harvell C. D. (1990a) The ecology and evolution of inducible defences. *Quart. Rev. Biol.* **65:** 323-340

Harvell C. D. (1990b) The evolution of inducible defence. *Parasitology* **100:** 53-61

Havel J. E. (1987) Predator-induced defenses: a review. In: Predation. Direct and Indirect Impacts on Aquatic Communities, University Press of New England, Hanover, London, 263-278

Hurlbert S. H., White M. D. (1993) Experiments with freshwater invertebrate zooplanktivores - quality of statistical analyses. *Bull. Mar. Sci.* **53:** 128-153

Jerka-Dziadosz M., Dosche C., Kuhlmann H.-W., Heckmann K. (1987) Signal-induced reorganization of the microtubular cytoskeleton in the ciliated protozoon *Euplotes octocarinatus*. *J. Cell. Sci.* **87:** 555-564

Kuhlmann H.-W. (1992) Benefits and costs of predator-induced defenses in *Euplotes*. *J. Protozool.* **39:** 49A

Kuhlmann H.-W., Heckmann K. (1985) Interspecific morphogens regulating prey-predator relationships in protozoa. *Science* **227:** 1347-1349

Kuhlmann H.-W., Heckmann K. (1994) Predation risk of typical ovoid and winged morphs of *Euplotes* (Protozoa, Ciliophora). *Hydrobiologia* **284:** 219-227

Kusch J. (1993a) Predator-induced morphological changes in *Euplotes* (Ciliata) - Isolation of the inducing substance released from *Stenostomum sphagnetorum* (Turbellaria). *J. Exp. Zool.* **265:** 613-618

Kusch J. (1993b) Induction of defensive morphological changes in ciliates. *Oecologia* **94:** 571-575

Kusch J. (1995) Adaptation of inducible defence in *Euplotes daidaleos* (Ciliophora) to predation risk by various predators. *Microb. Ecol.* **30:** 79-88

Kusch J., Heckmann K. (1992) Isolation of the *Lembadion*-factor, a morphogenetically active signal that induces *Euplotes* cells to change from their ovoid form into a larger lateral winged morph. *Dev. Genet.* **13:** 241-246

Kusch J., Kuhlmann H.-W. (1994) Cost of *Stenostomum*-induced morphological defence in the ciliate *Euplotes octocarinatus*. *Arch. Hydrobiol.* **130:** 257-267

Milewski A. V., Young T. P., Madden D. (1991) Thorns as induced defenses - experimental evidence. *Oecologia* **86:** 70-75

Riessen H. P. (1992) Cost-benefit model for the induction of an antipredator defense. *Am. Nat.* **140:** 349-362

Wiąckowski K., Szkarłat M. (1996) Effects of food availability on predator-induced morphological defence in the ciliate *Euplotes octocarinatus* (Protista). *Hydrobiologia* **321:** 47-52

Wicklow B. J. (1988) Developmental polymorphism induced by intraspecific predation in the ciliated protozoan *Onychodromus quadricornutus*. *J. Protozool.* **35:** 137-141

Young T. P. (1987) Increased thorn length in *Acacia drepanolobium* - an induced response to browsing. *Oecologia* **71:** 436-438

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ACTA
PROTOZOOLOGICA

Updating the Trachelocercids (Ciliophora, Karyorelictea). IV. Transfer of *Trachelocerca entzi* Kahl, 1927 to the Gymnostomatea as a New Genus, *Trachelotractus* gen. n. (Helicoprorodontidae)

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Summary. The morphology and infraciliature of *Trachelocerca entzi* Kahl, 1927, a highly contractile mesopsammal ciliate found on the French Atlantic coast at Roscoff, were studied in live and protargol impregnated specimens. The somatic infraciliature consists of ciliated monokinetids, except for two specialized kineties which have ciliated dikinetids forming a brush near the anterior end and spiral around the cytopharyngeal opening. The head and neck kinetids bear distinct nematodesmata, i.e. are oralized somatic kinetids. The head has a crown-like peribuccal ridge containing long extrusomes (toxicysts). The oral apparatus is simple, i.e. composed of a conical cytopharyngeal basket whose anterior end is surrounded by a few dikinetids possibly belonging to the anterior end of the spiral (brush)kineties. These characters largely match those known from *Helicoprorodon*. A glabrous stripe framed by a specialized bristle kinety, as found in trachelocercid and loxodid karyorelictids, is lacking. Thus, *T. entzi* is transferred from the karyorelictids to the gymnostomatids, viz. the family Helicoprorodontidae as a new genus, *Trachelotractus* gen. n., differing from *Helicoprorodon* by the possession of a typical gymnostomatous brush and the arrangement of the head extrusomes, which form a single circumpharyngeal bundle in *Trachelotractus* and a distinct spiral in *Helicoprorodon*.

Key words: infraciliature, *Helicoprorodon*, mesopsammon, Trachelocercidae, *Trachelotractus entzi* (Kahl, 1927) gen. n., comb. n.

INTRODUCTION

Little substantial alpha-taxonomic work has been done on karyorelictid ciliates since the pioneering study by Dragesco (1960), probably because they are rather fragile and thus difficult to preserve and stain. Notable exceptions are the papers by Raikov (1962), Dragesco and Dragesco-Kernéis (1986) and Wilbert (1986). Only recently, Foissner (1995, 1996a-c) and Foissner and Dragesco (1996a, b) updated the matter and provided detailed descriptions of the infraciliature of some time-honoured and some new genera. During these studies, we found also *Trachelocerca entzi* Kahl, 1927, a conspicuous, highly contractile species, which has never been investigated in detail since the original description. Only Dragesco (1960) provided a brief redescription confirming Kahl's observations.

The present paper describes in detail the morphology and infraciliature of *T. entzi* and shows that it does not belong to the karyorelictid trachelocercids but to an entirely different group, the gymnostomatid helicoprorodontids. The same previously happened to *Trachelocerca fusca* Kahl, 1928, which became type of the (gymnostomatid or trichostomatid) genus *Paraspathidium* Noland, 1937, and *Trachelocerca trepida* Kahl, 1928, which became type of another gymnostomatid genus, *Pseudotrachelocerca*

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Song, 1990. Very likely, several other trachelocercids are also misplaced, e.g. *Trachelocerca conifera* Kahl, 1930 and *T. minor* Gruber, 1888.

MATERIALS, METHODS AND TERMINOLOGY

Trachelotractus entzi and *Helicoprorodon* sp. were found on 14. 9. 1994 in the mesopsammon, i.e. in the upper 0 - 4 cm sand layer of the French Atlantic coast at Roscoff (W 4°, N 48°50'), France. Samples were collected and treated as described by Fauré-Fremiet (1951). The upper 0 - 4 cm sand layer of shallow pools was taken with a small shovel during the tide, put into a 1 litre jar, and was allowed to settle for at least 24 hours. During this time many trachelocercids and other ciliates move upwards and enrich in the upper 1 cm of sand. About 20 ml sand and sea water from this layer were collected with a large-bore (5 mm) pipette and mixed with about 5 ml of a 12% $MgCl_2$ solution to detach the ciliates. The mixture was then gently rotated in a petri dish so that the sand collected in the centre and the detached ciliates could be picked up individually with a capillary pipette from the clear supernatant.

Cells were studied *in vivo* using a high-power oil immersion objective (Foissner 1991). The infraciliature was revealed by protargol impregnation [Foissner 1991; protocol B (Wilbert's method)], using the fixative described by Foissner and Dragesco (1996a): 5 ml glutaraldehyde (25%), 5 ml saturated, aqueous mercuric chloride, 3 ml aqueous osmium tetroxide (2%), and 1 ml glacial acetic acid are mixed just before use. This fixative preserves most mesopsammal ciliates very well, but does not prevent contraction in contractile species. Specimens were fixed for 10 - 15 min and washed three times in distilled water. The nuclear apparatus and the cortical granules were also studied in transient preparations stained with methyl green-pyronine (Foissner 1991).

Counts and measurements on silvered specimens were performed at a magnification of x 1,000. *In vivo* measurements were conducted at a magnification of x 40 - 1,000. Although these provide only rough estimates, it is worth giving such data as specimens usually shrink in preparations and contract during fixation. Illustrations of live specimens were based on free-hand sketches, those of impregnated cells were made with a camera lucida. All figures are oriented with the anterior end of the organism directed to the top of the page. Terminology is according to Corliss (1979) and Foissner and Foissner (1988).

RESULTS

Trachelotractus gen. n.

Diagnosis: Helicoprorodontidae with few (two) parallel brush kineties spiralling around cytopharyngeal opening in single turn. Brush subapical, composed of ciliated dikinetids. Head extrusomes in single circumpharyngeal bundle attached to peribuccal ridge.

Type species: *Trachelocerca entzi* Kahl, 1927.

Etymology: Composite of the Greek noun *trachelos* (neck) and the Latin noun *tractus* (extension), referring to the similarity with *Trachelocerca* in general appearance and contractility. Masculine gender.

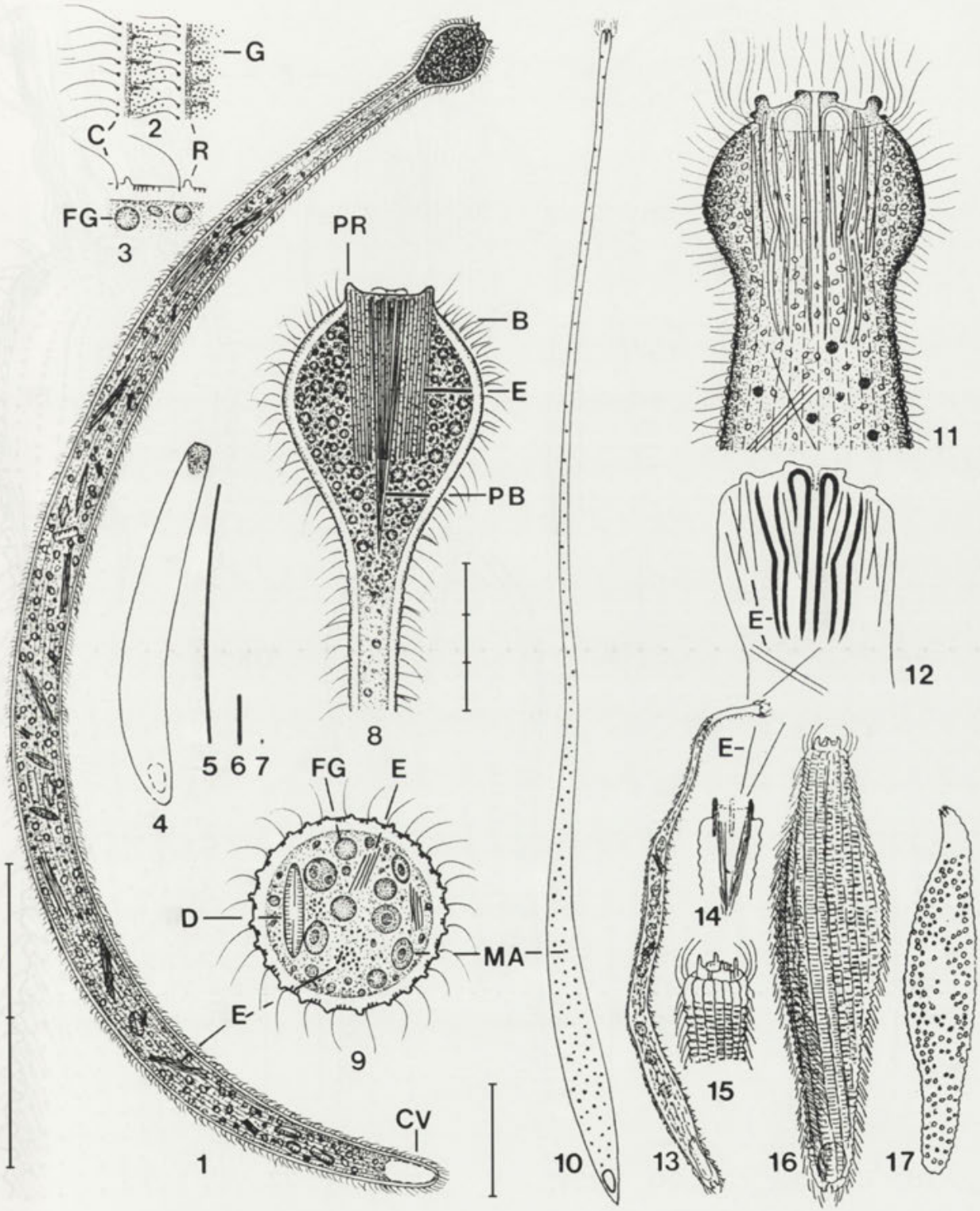
Redescription of *Trachelotractus entzi* (Kahl, 1927) comb. n. (Figs. 1-9, 18-27, 29-41, Table 1)

Improved diagnosis: Extended about 1000 x 35 µm, contracted about 300 x 50 µm. Vermiform, head globular with crown-like peribuccal ridge, distinctly set off from cylindroid neck which gradually widens to trunk. About 150 - 600 macronuclei. One contractile vacuole in posterior end. Three types of rod-shaped extrusomes, i.e. long (35 µm) and short (7 µm) toxicysts in head and trunk, and minute (< 1 µm) mucocysts in cortex. An average of 28 ciliary rows on trunk and 20 on head. Brush consisting of two rows indistinctly separate from respective somatic kineties.

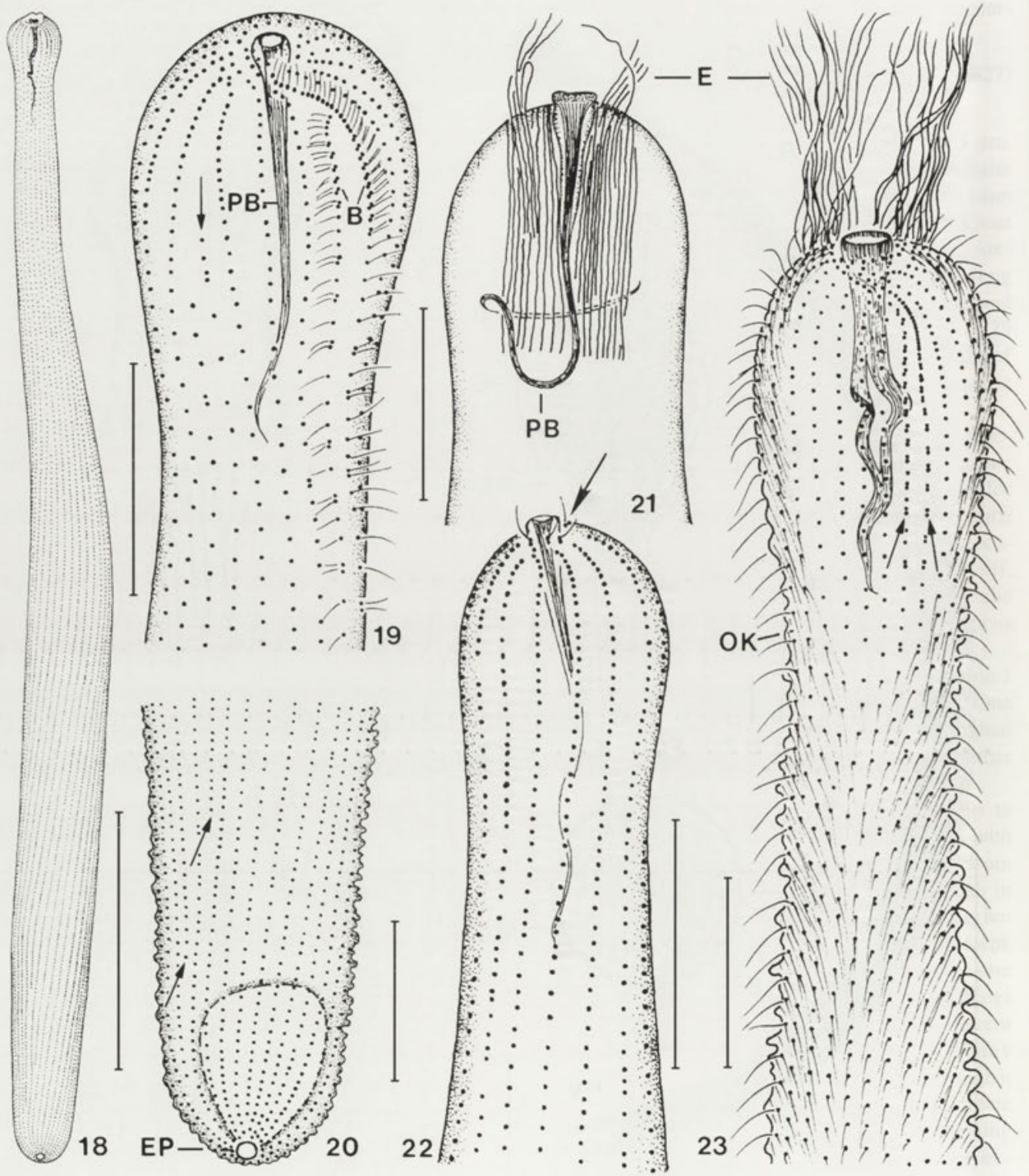
Specimens investigated and type material: The redescription is based on 15 well-impregnated specimens; some others were of useable quality and served for completing morphometry. No type material from *T. entzi* has been mentioned in the literature. Thus, I have deposited two neotype slides with specimens from Roscoff, prepared as described, in the Oberösterreichische Landesmuseum in Linz (LI), Austria. Relevant specimens are marked by a black ink circle on the cover glass.

Redescription: Morphometric data shown in Table 1 are repeated in this section only as needed for clarity. One specimen was excluded from the analysis because it had about 55 kineties and thus possibly belonged to another species.

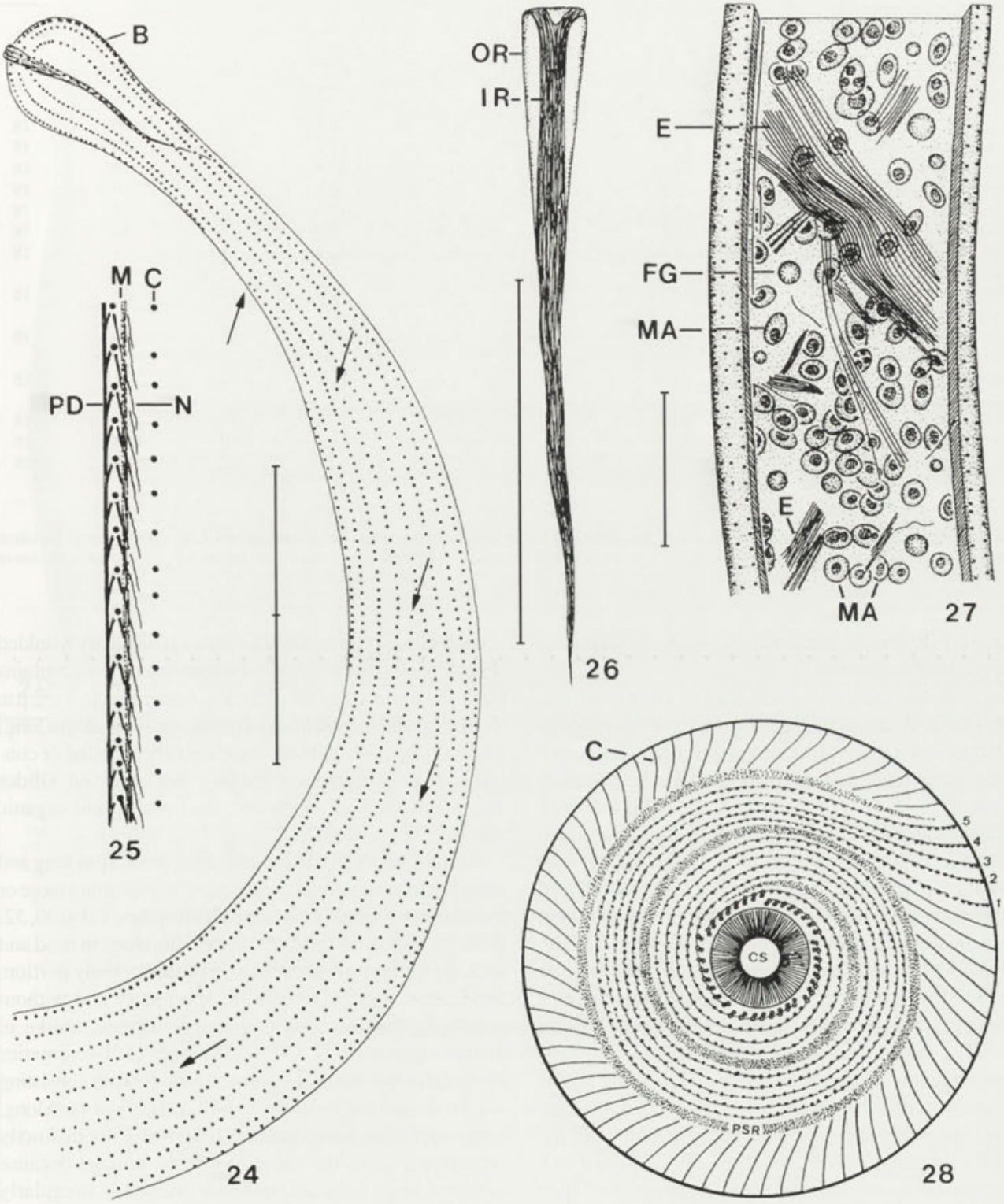
Largest, possibly fully extended specimens up to 1300 µm long and about 35 µm wide. Vermiform with conspicuous, globular dark head distinctly set off from narrowed, greyish neck; trunk cylindroid, brownish in dissecting microscope, gradually narrowed posteriorly but not tail-like (Figs. 1, 9, 18, 29). Head globular to pyriform, about as wide as trunk, black at low magnification because packed with highly refractile, 1-3 µm sized fat globules and 1-2 µm sized irregularly shaped granules different from the ellipsoidal inclusions found in many trachelocercids (Foissner and Dragesco 1996a,b); peribuccal ridge circular, rather flat and with some minute processes, contains tightly packed extrusomes forming conspicuous core around cytopharyngeal basket, respectively, in central portion of head (Figs. 8, 11, 15, 21, 31). Very flexible and contractile, fully contracted specimens cylindroid and about 300 µm long (Fig. 4); contraction very likely due to fibre (myoneme), rarely impregnated with protargol (Fig. 25), located in small ridge left of each ciliary row (Figs. 2, 3, 9), rather slow, length and



Figs. 1 - 17. *Trachelotractus entzi* from life (1 - 11, 13 - 16) and after hematoxylin staining (12, 17). Figures 1 - 9 originals, 10 - 12 from Dragesco (1960), 13 - 17 from Kahl (1927). 1, 10, 13 - extended specimens; 2, 3 - surface view and optical section of cortex; 4, 16 - contracted specimens, length about 300 µm; 5, 6, 7 - long (35 µm) and short (7 µm) toxicyst and cortical granule (mucocyst ? 0.3 µm), drawn to scale; 8, 11, 12, 14, 15 - fine structure of head. Note curved thick "fibres" in Figs. 11, 12, very likely bundles of exploding toxicysts; 17 - nuclear apparatus. B - (dorsal)brush, C - ciliary rows, CV - contractile vacuole, D - diatom, E - extrusomes, FG - fat globules, G - cortical granules (mucocysts ?), MA - macronuclei, PB - pharyngeal basket, PR - peribuccal ridge, R - cortical ridge containing myoneme. Scale bar division 100µm (Figs. 1, 10) and 10µm (Fig. 8)



Figs. 18 - 23. *Trachelotractus entzi*, oral and somatic ciliary pattern (infraciliature) after protargol impregnation. 18 - total view of typical specimen; 19, 20 - anterior and posterior body portion at higher magnification. Arrows mark shortened kineties; 21 - head with circumpharyngeal extrusome bundle partially extruded (cp. Fig. 31); 22 - oral infraciliature. Arrow marks dikinetids around pharyngeal opening; 23 - head and neck infraciliature. Note brush kineties (arrows) spiralling around pharyngeal opening and nematodesmata of oralized somatic kinetids (OK). B - (dorsal)brush, E - extrusomes, EP - excretory pore of contractile vacuole, OK - oralized somatic kinetids, PB - pharyngeal basket. Scale bars 100 µm (Fig. 18) and 20 µm (Figs. 19 - 23)



Figs. 24 - 28. *Trachelotractus entzi* (24 - 27) and *Helicoprorodon gigas* (28; from Raikov and Kovaleva 1980), infraciliature after protargol impregnation (24 - 27) and transmission electron microscopy (28). 24 - reduction of ciliary rows (arrows) in head and neck region; 25 - somatic fibrillar system in head and neck region, where kinetids have an associated nematodesma; 26 - pharyngeal basket; 27 - cytoplasmic inclusions in trunk region; 28 - schematic apical view showing five (numbered 1 - 5) somatic kineties spiralling around cytostome (CS). Spiral kineties have dikinetids at anterior end and are separated by the peribuccal ridge (PSR) which contains long extrusomes (cp. Figs. 42 - 44). B - brush, C - ciliary rows, E - extrusomes, FG - fat globules, IR - inner region of oral basket, M - myoneme, MA - macronuclei, N - nematodesma, OR - outer region of oral basket, PD - postciliodesma. Scale bar division 20μm

Table 1. Morphometric data from *Trachelotractus entzi* *

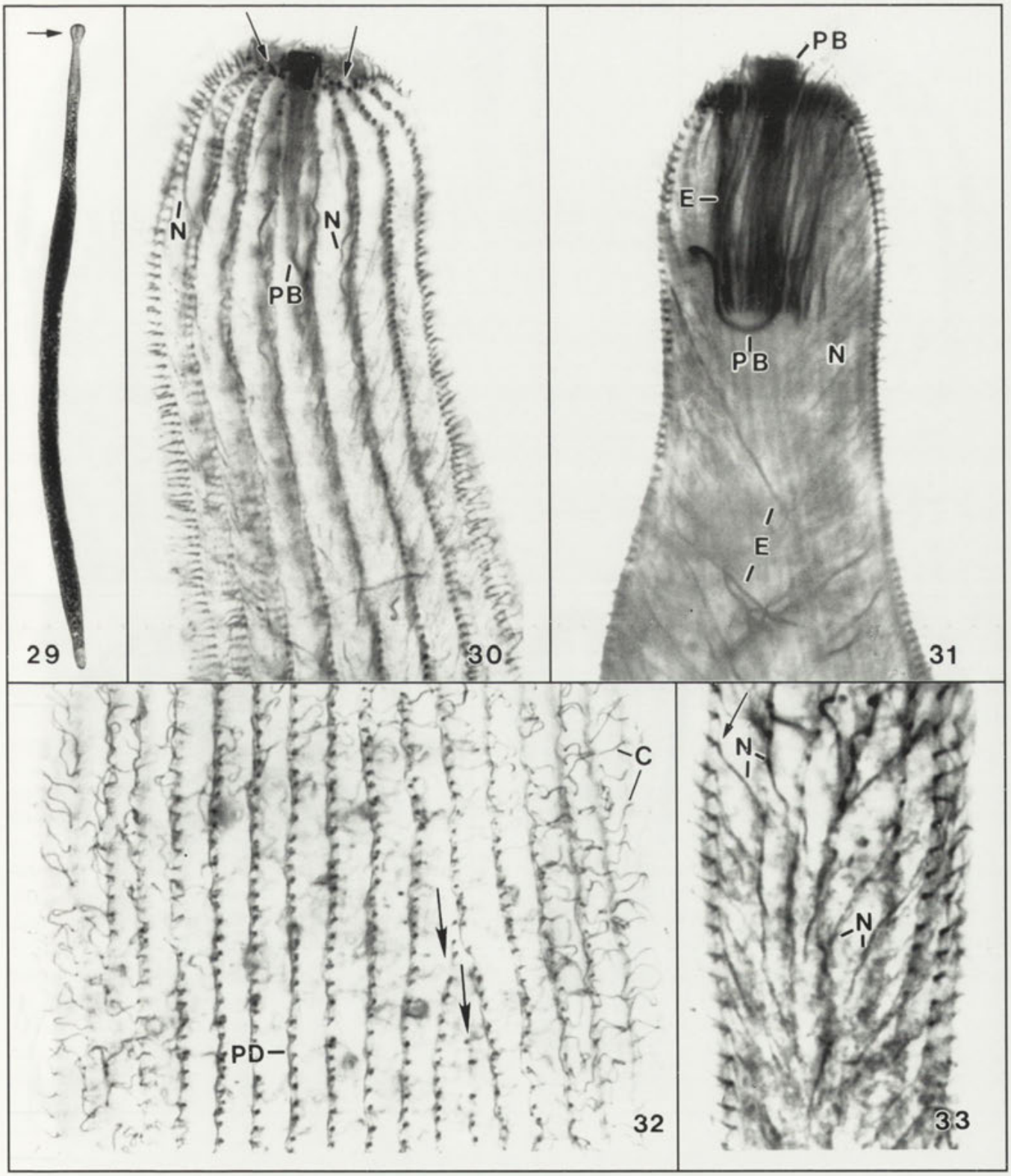
Character	$\bar{X}$	M	SD	$SD_{\bar{x}}$	CV	Min	Max	n
Body, length	753.3	775.0	220.3	51.9	29.2	350	1150	18
Body, maximum width	38.6	37.5	8.4	2.0	21.7	26	54	18
Body, width at head	22.8	24.0	3.6	0.8	15.6	16	29	18
Macronucleus, length	4.1	4.0	0.9	0.2	20.9	3	6	18
Macronucleus, width	3.6	3.5	0.7	0.2	18.6	3	5	18
Oral basket, length	34.0	35.0	6.7	1.6	19.6	18	44	18
Oral basket, width	4.0	4.0	0.9	0.2	22.9	3	6	18
Distance anterior end to end of right brosse kinety	17.4	16.5	5.0	1.2	28.9	10	30	18
Distance anterior end to end of left brosse kinety	21.8	20.0	6.8	1.5	31.3	15	40	18
Somatic kineties, maximum number on trunk	29.4	28.0	4.5	1.1	15.2	25	40	18
Somatic kineties, number on head	19.1	20.0	3.0	0.7	15.6	13	24	18
Cilia, number in a row	400.3	420.0	108.6	25.6	27.1	220	650	18
Brosse kineties, number	2.0	2	0.0	0.0	0.0	2	2	18

* Data based on protargol-impregnated and mounted specimens from field. Measurements in µm. Abbreviations: CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, n - number of individuals investigated, SD - standard deviation, $SD_{\bar{x}}$ - standard deviation of mean, $\bar{X}$ - arithmetic mean

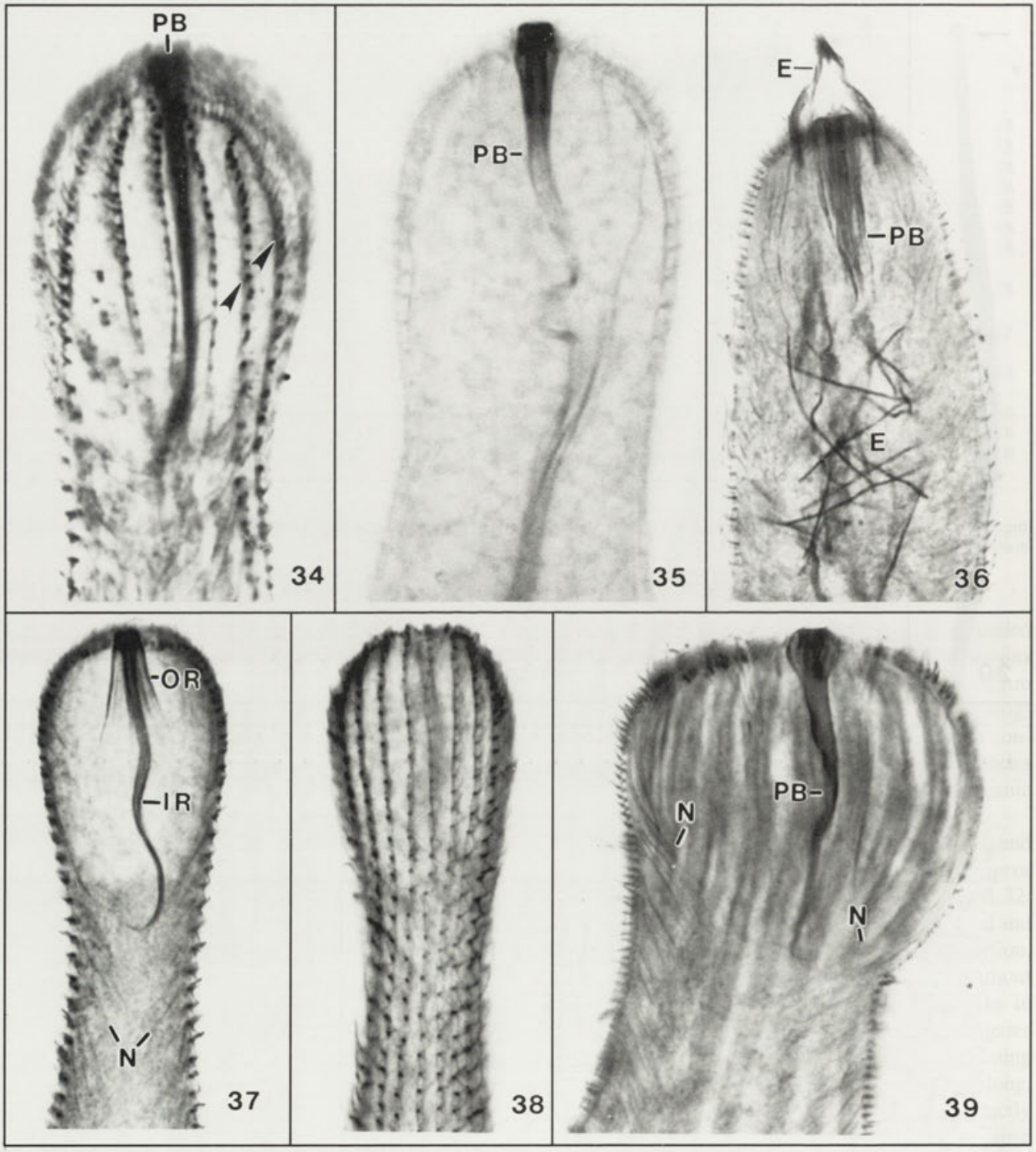
shape of cell thus comparatively well preserved in protargol slides (Table 1).

Macronuclei small, globular to slightly ellipsoidal, distributed throughout body, exact number and micronuclei difficult to ascertain because of many similar sized cytoplasmic inclusions, 300 - 600 nuclei seem to be common, with possibly up to 1000 in largest cells (Figs. 9, 27). Contractile vacuole distinct, in rear end with single excretory pore in centre of posterior pole (Figs. 1, 20). Two size-types, 25 µm and 7 µm long, of thin, rod-shaped extrusomes in peribuccal ridge and scattered throughout cytoplasm singly and in large bundles (Figs. 1, 27, 36), never attached to somatic cortex, unlike in *Helicoprorodon* (Figs. 42, 44). Long extrusomes usually curved and/or wrinkled in protargol slides, those attached to peribuccal ridge often completely or partially extruded providing cells with conspicuous apical beard (Figs. 21, 23, 31, 36); if completely extruded, head centre appears more pale than head margin and neck, where the nematodesmata of the oralized somatic kinetids extend (Figs. 23, 37, 38). Cortex conspicuous because ornamented and forming about 2µm thick, vitreous layer sharply separated from granular cytoplasm, contains many minute (about 0.3 x 0.15 µm) granules (mucocysts ?) irregularly arranged in broad stripe left of ciliary rows (Figs. 2, 3, 7); ornamented by small ridges extending left of each ciliary row and laterally between ciliary rows, lateral ridges especially pronounced in contracted specimens whose cortex is distinctly wrinkled (Figs. 2, 3, 9, 16, 20, 23). Cytoplasm colourless, contains many 1 - 5 µm sized fat globules, innumerable 3 x 2 µm sized ellipsoid inclusions, and many small (7 - 20µm long) diatoms; whether diatoms were actively ingested or contained in prey organisms has been not observed. Glides and winds elegantly between sand grains and organic debris.

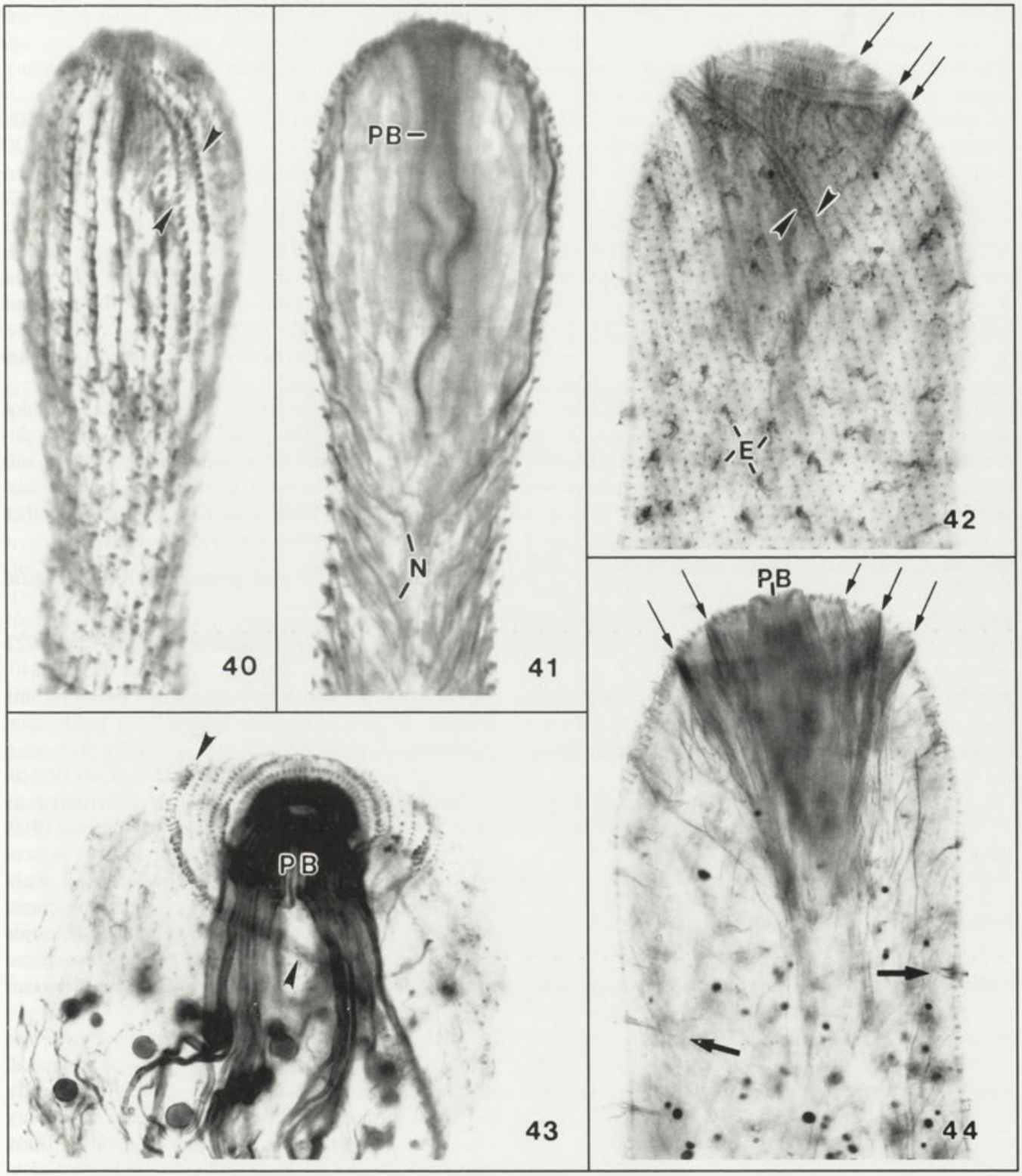
Surface very densely ciliated, cilia about 8µm long and arranged in longitudinal rows (kineties) becoming more or less distinctly spiral in contracted cells (Figs. 1, 18, 30, 32; Table 1); about one third of kineties shortened in head and neck region and subterminally in posterior body portion, abridgement occurs over whole perimeter and without regularity, thus no secant system is formed, unlike in trachelocercids (Figs. 19, 20, 24; Table 1). Two kineties subapically specialized to distinct (dorsal) brush consisting of paired basal bodies (dikinetids) having about 4µm long, rather stiff cilia; brush kineties posteriorly not distinctly separated from their respective somatic kineties because of rather large zone where brush dikinetids irregularly alternate with somatic monokinetids; at least one brush kinety continues apically and curves around cytopharyngeal opening, thus most ciliary rows abut to brush kineties (Figs. 8, 19, 23, 34, 40). Two kinds of somatic monokinetids, viz. normal ones having a distinct postciliary microtubule ribbon in trunk, and oralized somatic kinetids



Figs. 29 - 33. *Trachelotractus entzi*, infraciliature after protargol impregnation. 29 - total view of typical specimen with globular head (arrow); 30 - head and anterior neck region. Arrows mark dikinetids surrounding pharyngeal opening; 31 - head and anterior neck region of specimen with intact circumpharyngeal extrusome bundle (cp. Fig. 21); 32 - surface view of posterior neck region showing shortened kineties (arrows); 33 - neck region showing oralized somatic kinetids which have a distinct nematodesma associated with the basal body (arrow). C - cilia, E - extrusomes, N - nematodesmata, PB - pharyngeal basket, PD - postciliodesma



Figs. 34 - 39. *Trachelotractus entzi*, infraciliature of head and anterior neck region after protargol impregnation. 34 - arrows mark brush kineties composed of dikinetids (cp. Fig. 19); 35 - overbleached specimen with wrinkled pharyngeal basket; 36 - specimen just extruding circumpharyngeal extrusome bundle; 37, 38 - same specimen focused to centre and surface. The head appears pale because the circumpharyngeal extrusome bundle has been extruded (cp. Fig. 31); 39 - the head and neck kinetids have associated nematodesmata obliquely extending to neck midline. E - extrusomes, IR - inner region of oral basket, N - nematodesmata, OR - outer region of oral basket, spread fan-like in this specimen, PB - pharyngeal basket



Figs. 40 - 44. *Trachelotractus entzi* (40, 41) and *Helicoprorodon* sp. (42 - 44), oral and somatic infraciliature after protargol impregnation. 40, 41 - same specimen focused to surface and centre. Arrows mark brush kineties spiralling around pharyngeal opening; 42, 43, 44 - like *Trachelotractus*, *Helicoprorodon* has two specialized kineties (arrowheads) spiralling around pharyngeal opening; however, the kineties are not composed of dikinetids but associated with distinct nematodesmata. The peribuccal ridge contains long extrusomes which, together with the nematodesmata of the neighbouring spiral kineties, form a conspicuous, spiral core (thin arrows; cp. Fig. 28). Thick arrows mark extrusome bundles attached to somatic cortex. E - extrusomes, N - nematodesmata of oralized somatic kinetids, PB - pharyngeal basket

having not only a postciliary microtubule ribbon but also a distinct nematodesma in head and neck region; nematodesmata about 30 µm long, those of head kinetids almost parallel to cell surface, those of neck obliquely extending to neck midline; postciliary microtubule ribbons of neighbouring kinetids overlap and thus form conspicuous postciliodesma close to right of each kinety (Figs. 23, 25, 30-33, 36-39, 41).

Oral apparatus and infraciliature simple, in centre of apical end. Pharyngeal basket conical with bulbous anterior portion, about as long as head (Table 1), composed of two distinct zones, viz. an outer lightly and an inner heavily impregnated region, sometimes curved loop-like or bifurcate (Figs. 8, 19, 21-24, 26, 31, 34-36, 39, 41); outer region very rarely spread fan-like (Fig. 37). Pharyngeal opening surrounded by few dikinetids possibly belonging to anterior ends of brush kineties (Figs. 22, 30); details, however, difficult to recognize because minute and well-oriented apical views were not obtained due to the vermiform shape of the organism.

Occurrence and ecology: *T. entzi* has been reported from littoral sands in Italy (Entz 1884), Germany (Bock 1952, Kahl 1927, 1928), France (Dragesco 1960, present study), England (Hartwig and Parker 1977), the Black Sea (Petran 1967) and the Caspian Sea (Agamaliev 1983). It colonizes fine and coarse sands (Bock 1952) and even saprobic sites (Dragesco 1960). Biernacka's (1963) report from the Polish sea coast is very likely based on a misidentification because the specimens lacked a contractile vacuole.

DISCUSSION

Identification and synonymy

Trachelotractus entzi was first described by Entz (1884) as *Trachelocerca phoenicopterus* Cohn, 1866. However, Entz (1884) obviously mixed at least three species, viz. *T. phoenicopterus* Cohn, 1866, *T. entzi* Kahl, 1927 and *Lagynus sulcatus* Gruber, 1888. Kahl (1927), who recognized Entz's mistake, provided a very detailed description of *T. entzi* (Figs. 13 - 17), which largely agrees with my observations. Thus, there can be no doubt as to the identification. There is only one significant difference, viz. the number of kineties, about 20 according to Kahl (1927) and 25-40 (29, Table 1) in my specimens, which matches the value ("about 30") observed by Dragesco (1960). Thus, it is reasonable to assume that Kahl (1927), not having the advantage of silver impregnation, underestimated kinety number. Interestingly, I also underestimated the number of kineties in live specimens, i.e. recorded, very much like Kahl (1927), "about 20 ciliary rows" in my notebook.

Great variation has been reported in body length [300 - 1000 µm (Kahl 1927), 200 - 900 µm (Dragesco 1960), up to 1300 µm (this study)], body shape [club-shaped (Dragesco 1960; Fig. 10), cylindroid-fusiform (Kahl 1927, this study; Figs. 1, 13)] and number of macronuclei [several hundreds (Kahl 1927), 150-200 (Dragesco (1960), about 300 - 600 (this study)], indicating that *T. entzi* could be a complex of different species. However, size and shape are very difficult to ascertain in this species because of its high contractility; more systematic measurements, using video microscopy, are required.

Trachelocerca minor Gruber, 1888 is possibly a senior synonym of *T. entzi* Kahl, 1927. However, Gruber's description is too incomplete for a reliable identification, and thus *T. minor* should be considered as species indeterminata. Alternatively, this name could be used for another *Trachelotractus* species, if one should be discovered.

Trachelotractus as a new genus and its systematic position

Dragesco (1960) fixed *Trachelocerca entzi* Kahl, 1927 as type of the genus *Trachelocerca* Ehrenberg, 1840, which would prohibit establishing a new genus for this species. However, Dragesco's subsequent typification proved to be invalid because the type of *Trachelocerca* was fixed by monotypy (Foissner and Dragesco 1996b).

Traditionally, *T. entzi* has been considered as karyorelictid trachelocercid (Kahl 1930, Dragesco 1960, Carey 1992). This is not supported by the present investigations. *Trachelotractus entzi* lacks all main infraciliary characteristics of the karyorelictids in general and the trachelocercids in particular, viz. somatic dikinetids and a glabrous stripe surrounded by a highly specialized bristle kinety (Dragesco and Foissner 1996b, Foissner 1996c).

A proper classification of *T. entzi* must take into account its four main characteristics, viz. the peribuccal ridge with its typical extrusomes, the somatic monokinetids, the oralized somatic kinetids, and the specialized ciliary rows curving around the pharyngeal opening. These characters are exactly those found in *Helicoprorodon*, which has a gymnostomatid (haptorid) ultrastructure (Dragesco 1960, Lipscomb and Riordan 1991, Raikov and Kovaleva 1980, Puytorac and Kattar 1969; Figs. 28, 42 - 44). Thus, *Trachelotractus* is assigned to the family Helicoprorodontidae Small and Lynn, 1985, suborder

Helicoprorodontina Foissner and Foissner, 1988, class Gymnostomatea Bütschli, 1889.

Trachelotractus entzi possesses, like most gymnostomatids, a typical brush consisting of two rows of paired basal bodies with shortened cilia (Figs. 19, 23, 34, 40). This is markedly different from *Helicoprorodon*, which very likely lacks a brush (Puytorac and Kattar 1969, Raikov and Kovaleva 1980, Lipscomb and Riordan 1991), although two of the somatic kineties which spiral around the anterior end, are specialized, i.e. associated with distinct nematodesmata making the subapical kinetids more distinct than the neighbouring normal somatic ones (Fig. 42). Thus, these kineties might be homologous to the brush kineties of *Trachelotractus* and other gymnostomatids. Another main difference concerns the spiral kineties, which perform about 1 turn in *Trachelotractus* (Figs. 19, 23, 34, 40) and 4 - 6 turns in *Helicoprorodon* (Dragesco 1960, Puytorac and Kattar 1969, Raikov and Kovaleva 1980, Lipscomb and Riordan 1991; Figs. 28, 43). This is associated with a marked difference in the arrangement of the extrusomes, which form a simple, circumpharyngeal bundle in *Trachelotractus* and a distinct spiral, which parallels the apical spiral kineties, in *Helicoprorodon* (Raikov and Kovaleva 1980; Figs. 28, 43,44).

In sum, the differences between *T. entzi* and *Helicoprorodon* spp. do not appear to be excessively large, but are nevertheless distinct enough to warrant generic separation. In fact, *T. entzi* looks like a simplified *Helicoprorodon*, indicating that it might be close to the stem species from which both *Trachelotractus* and *Helicoprorodon* evolved.

Spiroprorodon (a junior synonym of *Gymnozoum*; Petz et al. 1995a) is another genus resembling *Trachelotractus* and *Helicoprorodon*, especially in having some kineties curving around the cytopharyngeal apparatus. However, *Gymnozoum* is very likely closely related to the cyrtophorids, as indicated by morphological and nuclear characteristics (Petz et al. 1995a) as well as morphogenetic (Petz et al. 1995b) and ultrastructural (Sniezek and Small 1993) evidence. *Gymnozoum* is broadly ellipsoidal, acontractile, and lacks extrusomes and is thus easily distinguished from *Trachelotractus* and *Helicoprorodon*.

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REFERENCES

Agamaliev F. G. (1983) Ciliates of the Caspian Sea. Taxonomy, ecology, zoogeography. Nauka, Leningrad (in Russian)

Biernacka I. (1963) Die Protozoenfauna in Danzinger Bucht II. Die Charakteristik der Protozoen in Untersuchten Biotopen der Seekuste. *Polskie Archwnm Hydrobiol.* **11:** 17-75

Bock K. J. (1952) Zur Ökologie der Ciliaten des marinen Sandgrundes der Kieler Bucht I. *Kieler Meeresforsch.* **9:** 77-89

Bütschli O. (1887-1889) Protozoa. Abt. III. Infusoria und System der Radiolaria. In: Klassen und Ordnung des Thier-Reichs (Ed. H. G. Bronn). C. F. Winter, Leipzig, **1:** 1098-2035

Carey P. G. (1992) Marine interstitial ciliates. Chapman and Hall, London, New York, Tokyo, Melbourne, Madras

Cohn F. (1866) Neue Infusorien im Seeaquarium. *Z. wiss. Zool.* **16:** 253-302

Corliss J. O. (1979) The ciliated protozoa. Characterization, classification and guide to the literature. 2nd ed. Pergamon Press, Oxford, New York, Toronto, Sydney, Paris, Frankfurt

Dragesco J. (1960) Ciliés mésopsammiques littoraux. Systématique, morphologie, écologie. *Trav. Stn biol. Roscoff* (N.S.) **12:** 1-356.

Dragesco J., Dragesco-Kernéis A. (1986) Ciliés libres de l'Afrique intertropicale. *Faune tropicale* **26:** 1-559

Ehrenberg C. G. (1840) Das grössere Infusorienwerk (Diagnosen von 274 neuen Infusorien). *Ber. Verh. K. Preuss. Akad. Wiss. Berl.*, year **1840:** 197-219

Entz G. (1884) Über Infusorien des Golfes von Neapel. *Mitt. zool. Stn Neapel* **5:** 289-444

Fauré-Fremiet E. (1951) The marine sand-dwelling ciliates of Cape Cod. *Biol. Bull. mar. biol. Lab., Woods Hole* **100:** 59-70

Foissner W. (1991) Basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa. *Europ. J. Protistol.* **27:** 313-330

Foissner W. (1995) *Kentrophoros* (Ciliophora, Karyorelictea) has oral vestiges: a reinvestigation of *K. fistulosus* (Fauré-Fremiet, 1950) using protargol impregnation. *Arch. Protistenk.* **146:** 165-179

Foissner W. (1996a) Updating the trachelocercids (Ciliophora, Karyorelictea). II. *Prototrachelocerca* nov. gen. (Prototrachelocercidae nov. fam.), with a redescription of *P. fasciolata* (Sauerbrey, 1928) nov. comb. and *P. caudata* (Raikov & Dragesco, 1966) nov. comb. *Europ. J. Protistol.* **32:** 336-355

Foissner W. (1996b) A redescription of *Remanella multinucleata* (Kahl, 1933) nov. gen., nov. comb. (Ciliophora, Karyorelictea), emphasizing the infraciliature and extrusomes. *Europ. J. Protistol.* **32:** 234-250

Foissner W. (1996c) The infraciliature of *Cryptopharynx setigerus* Kahl, 1928 and *Apocryptopharynx hippocampoides* nov. gen., nov. spec. (Ciliophora, Karyorelictea), with an account on evolution in loxodid ciliates. *Arch. Protistenk.* **146:** (years 1995/96): 309-327

Foissner W., Dragesco J. (1996a) Updating the trachelocercids (Ciliophora, Karyorelictea). I. A detailed description of the infraciliature of *Trachelolophos gigas* n. g., n. sp. and *T. filum* (Dragesco & Dragesco-Kernéis, 1986) n. comb. *J. Euk. Microbiol.* **43:** 12-25

Foissner W., Dragesco J. (1996b) Updating the trachelocercids (Ciliophora, Karyorelictea). III. Redefinition of the genera *Trachelocerca* Ehrenberg and *Tracheloraphis* Dragesco, and evolution in trachelocercid ciliates. *Arch. Protistenk.* **147:** 43-91

Foissner W., Foissner I. (1988) The fine structure of *Fuscheria terricola* Berger et al., 1983 and a proposed new classification of the subclass Haptoria Corliss, 1974 (Ciliophora, Litostomatea). *Arch. Protistenk.* **135:** 213-235

Gruber A. (1888) Weitere Beobachtungen an vielkernigen Infusorien. *Ber. naturf. Ges. Freiburg i. B.* **3:** 57-70

Hartwig E., Parker J. G. (1977) On the systematics and ecology of interstitial ciliates of sandy beaches in North Yorkshire. *J. mar. biol. Ass. U.K.* **57:** 735-760

Kahl A. (1927) Neue und ergänzende Beobachtungen holotricher Ciliaten. I. *Arch. Protistenk.* **60:** 34-129

Kahl A. (1928) Die Infusorien (Ciliata) der Oldesloer Salzwasserstellen. *Arch. Hydrobiol.* **19:** 50-123

Kahl A. (1930) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 1. Allgemeiner Teil und Prostomata. *Tierwelt Dtl.* **18:** 1-180

Lipscomb D. L., Riordan G. P. (1991) Ultrastructure of *Helicoprorodon multinucleatum* (Ciliophora). *Trans. Am. micr. Soc.* **110:** 93-117

Noland L. E. (1937) Observations on marine ciliates of the Gulf coast of Florida. *Trans. Am. micr. Soc.* **56:** 160-171

Petran A. (1967) Cercetări asupra faunei de ciliate psamobionte la plajele din sudul litoralului românesc al mării negre. *Ecol. mar.* **2:** 169-191

Petz W., Song W., Wilbert N. (1995a) Taxonomy and ecology of the ciliate fauna (Protozoa, Ciliophora) in the endopagial and pelagial of the Weddell Sea, Antarctica. *Stapfia (Linz)* **40:** 1-223

Petz W., Song W., Wilbert N. (1995b) Morphogenesis of *Gymnozoum viviparum* Meunier, 1910 and *G. sympagicum* Petz et al. 1995, cyrtophorid ciliates from Antarctic sea ice. *Europ. J. Protistol.* **31:** 454 (Abstract 183)

Puytorac P. de, Kattar M. R. (1969) Observations sur l'ultrastructure du cilié *Helicoprorodon multinucleatum* Dragesco 1960. *Protistologica* **5:** 549-560

Raikov I. B. (1962) Les ciliés mésopsammiques du littoral de la Mer Blanche (U.R.S.S.) avec une description de quelques espèces nouvelles ou peu connues. *Cah. Biol. mar.* **3:** 325-361

Raikov I. B., Kovaleva V. G. (1980) Fine structure of the circumoral ciliature and pharyngeal apparatus of the marine ciliate *Helicoprorodon gigas* (Gymnostomata). *Protistologica* **16:** 177-198

Small E. B., Lynn D. H. (1985) Phylum Ciliophora. In: Illustrated Guide to the Protozoa (Eds. J. J. Lee, S. J. Hutner and E. C. Bovee). Allen Press, Lawrence, Kansas, 393-575

Sniezek J. H., Small E. B. (1993) Ultrastructural analysis of *Spiroprorodon* sp.: phylogenetic implications. *J. Euk. Microbiol.* **40:** 41A (Abstract 242)

Song W. (1990) Infraciliature and silverline system of the ciliate *Pseudotrachelocerca trepida* (Kahl, 1928), nov. gen., nov. comb., and establishment of a new family, the Pseudotrachelocercidae nov. fam. (Ciliophora, Haptoria). *Europ. J. Protistol.* **26:** 160-166

Wilbert N. (1986) Die orale Infraciliatur von *Tracheloraphis dogieli* Raikov, 1957 (Ciliophora, Gymnostomata, Karyorelictida). *Arch. Protistenk.* **132:** 191-195

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Isospora ubique: A New Coccidian Parasite from the Wedge-billed Woodcreeper (*Glyphorynchus spirurus*) from South America

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Summary. A new species of *Isospora* is described from the faeces of the wedge-billed woodcreeper, *Glyphorynchus spirurus* from eastern Ecuador and Guyana. Oocysts of *Isospora ubique* sp. n. were found in 18/41 wedge-billed woodcreepers. Sporulated oocysts are subspherical to ovoidal, 23.4 x 21.8 (21-27 x 19-24) µm, with a smooth, colorless bilayered wall; the inner wall is thinner and darker. The average shape index is 1.07. No micropyle or oocyst residuum are present but the oocyst contains one ovoid polar granule. Sporocysts are ovoidal, 14.8 x 10.1 (14-16 x 9-11) µm, average shape index is 1.46 with a smooth single layered wall, and composed of a nipple-like Stieda body but no substieda body. The sporocyst residuum is usually composed of coarse granules scattered among the sporozoites or sometimes loosely clustered with a thin membrane surrounding the sporozoites and residuum granules within the sporocyst. Sporozoites are vermiform with an ovoid, posterior refractile body and an ovoid centrally located nucleus and lying randomly in the sporocyst.

Key words: *Glyphorynchus spirurus, Isospora ubique* sp. n.

INTRODUCTION

The wedge-billed woodcreeper *(Glyphorynchus spirurus),* the only species in its genus, is a geographically widespread, common and numerous member of lowland, tropical, humid forest. This, the smallest woodcreeper (Order: Passeriformes, Suborder: Tyranni, Family: Dendrocolaptidae), eats very small arthropod prey encountered as it pecks on large treetrunks (Pierpont 1986). The species is divided into 4 subspecies according to location, plumage and song.

Glyphorynchus spirurus is one of the most numerous birds in its large range from the entire Amazon River basin, the Maracaibo basin of northwestern Venezuela, north to southern Mexico, eastward from the Paria Peninsula and the Guayas, to along the Brazilian coast south to Bahia (Ridgely and Tudor 1994). Yet no prior studies have reported coccidian parasites using *G. spirurus* as its host. This paper describes a new coccidian species found in the wedge-billed woodcreeper, *Glyphorynchus spirurus.*

MATERIALS AND METHODS

During bird collecting expeditions to Ecuador in August, 1990 and March, 1991 and Guyana in August, 1994, samples were taken from the contents of the small and large intestines of 41 wedge-billed woodcreepers and sent to the first author's laboratory for examination. Procedures for preserving faecal material and for measuring and photographing oocysts were as described by McQuistion and Wilson (1989). All measurements are given in µm with size ranges in parentheses following the means. Oocysts were 1-2 months old when examined, measured and photographed.

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RESULTS

Isospora ubique sp. n. (Figs. 1-3)

Description of oocysts: oocysts subspherical, 23.4 x 21.8, (21-27 x 19-24) (N=113) with a smooth, bilayered wall *ca* 2.0 thick; the inner wall is darker. The shape index (length/width) is 1.07 (1.00-1.23). Micropyle and oocyst residuum absent but one ovoid polar granule is present. Sporocysts ovoidal, 14.8 x 10.1 (14-16 x 9-11) (N=98); shape index 1.46 (1.36-1.67). The Stieda body is nipple-like with no substieda body. Sporocyst residuum usually composed of coarse granules scattered among the sporozoites or sometimes loosely clustered. A thin membrane appears to surround the sporozoites and residuum granules within the sporocyst. Sporozoites vermiform shaped with an ovoid, posterior refractile body and a ovoid, centrally located nucleus. Sporozoites are arranged randomly in sporocyst.

Type host: *Glyphorynchus spirurus rufigularis* Zimmer, 1934, (Passeriformes: Dendrocolaptidae). An adult male collected in August 1993 by A. P. Capparella (APC #4024) has been deposited at The Academy of Natural Sciences of Philadelphia Museum as ANSP 186826.

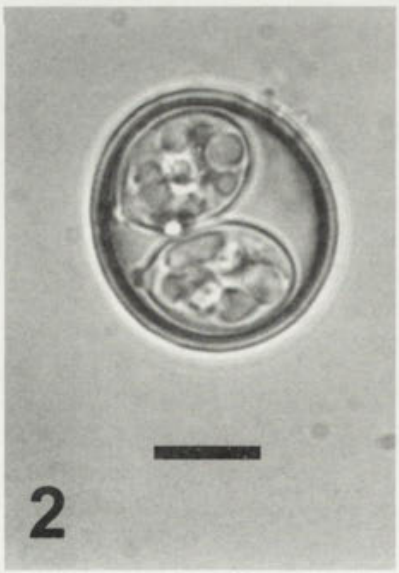
Type specimens: a phototype series and formalin-preserved sporulated oocysts are deposited in the U.S. National Parasite Collection, Beltsville, Maryland 20705, accession no. USNPC No. 86671 (phototypes) and USNPC No. 86672 (syntypes).

Type location: Ecuador, Provincia de Morona-Santiago, *ca* 5 km SW of Taisha, 2° 22' S, 77° 30', 425 m elevation.

Prevalence: 18/41 total; 0/3 of *G. s. sublestes* (Peters, 1929) were infected from Ecuador, Provincia de Esmeraldas, *ca* 20 road km N-NW of Alto Tambo, 0° 57' N, 78° W, 275 m elevation, July 1990: 2/7 of *G. s. rufigularis* were infected from Ecuador, Provincia de Morona-Santiago *ca* 5 km SW of Taisha, 2° 22' S, 77° 30' W, 425 m elevation, July 1990: 0/2 *G. s. rufigularis* were examined from Ecuador, Provincia de Sucumbios near Imuya Cocha, 0° 34' S, 75° 17' W, 200 m elevation, March 1991: 7/11 of *G. s. rufigularis* were infected from Ecuador, Provincia de Sucumbios, *ca* 14 km N of Tigre Playa, 220 m elevation, July 1993: 3/5 of *G. s. rufigularis* were infected from Ecuador, Provincia de Sucumbios, *ca* 20 km NE of Lumbaqui, 600 m elevation, July 1993: 5/9 of *G. s. spirurus* (Vieillot, 1819) were infected from Guyana, *ca* 5 km N of Rockstone on east bank of the Essequi River, 25 m elevation, August 1993: 1/4 of *G. s. spirurus* were infected from Guyana, *ca* 5 km NW of Mabura Hill between the Essequi and Demerara Rivers, elevation; 100 m, August 1993.

Sporulation time: unknown; oocysts were partially sporulated when received at the laboratory and became fully sporulated after exposed to air for several days prior to examination.

Site of infection: the specific site in the intestine is not known, oocysts found in faeces.



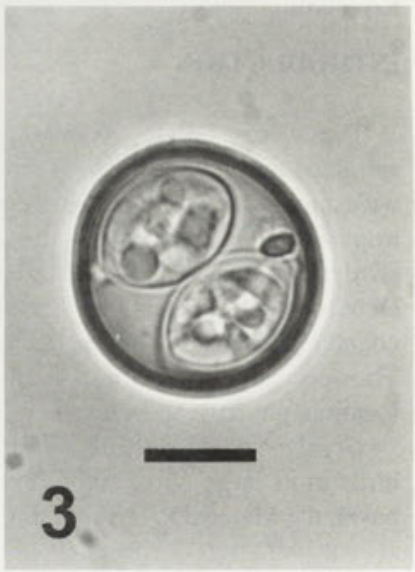


Fig. 1. Composite line drawing of sporulated oocyst of *Isospora ubique* sp. n. from *Glyphorynchus spirurus*. Bar - 10µm Figs. 2-3. Photomicrographs of sporulated oocysts of *Isospora ubique* sp. n. Bars - 10µm. 2- full lateral view of oocyst showing double layered oocyst wall and general subspherical shape; 3- note thin membrane enclosing sporozoites and sporocyst residuum in lower sporocyst and the single, ovoid polar body

Table 1. Comparison of Coccidian Parasites in the Avian Family Dendrocolaptes

Species	*I. magna*	*I. concentrica*	*I. ocellati*	*I. striata*	*I. ubique*
Host	*Dendrocolaptes certhia*	*Dendrocolaptes certhia*	*Xiphorhynchus ocellatus*	*Xiphorhynchus ocellatus*	*Glyphorynchus spirurus*
Oocyst size (µm)	29.7 x 24.9	26.6 x 22.7	20 x 17	18 x 16	23.4 x 21.8
Shape Index	1.20	1.20	1.20	1.20	1.07
Polar granules	1	1-2	1	1	1
Sporocyst size (µm)	15.8 x 12.6	17.2 x 11	12 x 8	11 x 8	14.8 x 10.1
Shape Index	1.3	1.6	1.5	1.4	1.5
Stieda body	nipple-like	square	dome-like	nipple-like	nipple-like
Substieda body	wavy border	bubble-like	ellipsoidal	rectangular	none
Sporocyst residuum	large, lobate	ovoid	scattered	small, spherical	scattered
Sporozoite shape	sausage-shape	vermiform	sausage-shape	vermiform	vermiform
No. of refractile bodies	2	1	1	1	1
Size nucleus	medium	small	large	small	small
Unique features	none	concentric lines	none	concentric lines	none

Etymology: the specific epithet, *ubique,* is the Latin word for everywhere in reference to the widely scattered locations where the parasite was found.

Remarks: the fecal samples collected represented three subspecies of *Glyphorynchus spirurus.* Three samples were collected from *G. s. subletus* which is located on the western slope of the Andes Mountains in the Provincia de Esmeraldas of Ecuador. Unfortunately, all of these samples were negative for coccidia.

The precise host subspecies assignment of the samples taken in the lowlands of eastern Ecuador (Provincia de Sucumbios and Provincia de Morona-Santiago) is difficult because two subspecies meet there; *G. s. rufigularis* Zimmer 1934 and *G. s. casteinaudii* Des Murs 1855 (Peters 1951). Judging from the plumage, behavior and song of the hosts captured in this region, we are confident that the samples collected were from the subspecies *G. s. rufigularis.* Therefore, all of the samples that were positive for coccidia were from hosts located east of the Andes Mountains with the Guyanan subspecies, *G. s. spirurus,* representing the second subspecies of *Glyphorynchus spirurus* to host *I. ubique.*

DISCUSSION

Four other isosporan parasites have been described from sympatric avian species with *Glyphorynchus spirurus* in the family Dendrocolaptes (Table 1). *Isospora magna* and *I. concentrica* were described from the barred woodcreeper, *Dendrocolaptes certhia* (McQuistion and Capparella 1995) and *I. ocellati* and *I. striata* were described from the ocellated woodcreeper, *Xiphorhynchus ocellatus* (McQuistion and Capparella In Press). All four isosporan parasites are quite different in oocyst structure compared to *I. ubique.* The average oocyst size of *I. ubique* is smaller than *I. magna* and *I. concentrica* but larger than *I. ocellati* and *I. striata* and the shape index of *I. ubique* is subspherical (1.07) compared to the more ovoidal shape (1.20) for the other four coccidians.

When comparing the morphological characteristics of the sporocysts or sporozoites, the difference between *I. ubique* and the other isosporan species is even more striking. *I. ubique* has no substieda body and an apparent membrane enclosing the sporocysts residuum and the sporozoites. The other isosporan species has various shaped substieda bodies and no membrane enclosing the sporozoites and sporocyst residuum. The sporozoites of *I. concentrica* and *I. striata* have concentric bands or lines around the anterior end of the sporozoites while *I. ubique* lacks concentric bands. *I. magna* and *I. ocellati* have sausage -shaped sporozoites compared to the vermiform sporozoites of *I. ubique* and the sporozoites of *I. magna* have two refractile bodies.

Glyphorynchus spirurus shows substantial river-delimited genetic variation on opposite banks of two rivers in Amazonia (Napo, upper Amazon), implying that rivers act as partial or complete barriers to gene flow (Capparella 1987, 1988). Nevertheless, there were no major differences in morphological characteristics be-

tween *I. ubique* from eastern Ecuador and Guyana despite the interposition of several rivers and a geographic distance of *ca* 1200 km.

Acknowledgments. We are indebted to Mark Robbins and David Agro and the Academy of Natural Scieces of Philadelphia for their help in collecting the faecal samples, identifying the avian hosts, and furnishing the museum accession numbers of the hosts. Thanks are due also to Jay Helton for the composite drawing and Ms. Mary Ellen Martin for assistance in naming the parasite.

REFERENCES

Capparella A. P. (1987) Effects of riverine barriers on genetic differentiation of Amazonian forest undergrowth birds. Ph. D. dissertation. Louisiana State University, Baton Rouge, Louisiana

Capparella A. P. (1991) Neotropical avian diversity and riverine barriers. *Acta Congressus Internationalis Ornithologici.* **20:** 307-316

Peters, J. L. (1951) Check-list of Birds of the World. Volume VII. Museum of Comparative Zoology, Cambridge, Massachusetts

Pierpont N. (1986) Interspecific aggression and the ecology of woodcreepers (Aves: Dendrocolaptidae). Ph. D. dissertation. Princeton University, Princeton, New Jersey

McQuistion T. E., Wilson M. (1989) *Isospora geospizae,* a new coccidian parasite (Apicomplexa: Eimeriidae) from the small ground finch (*Geospiza fuliginosa)* and the medium ground finch (*Geospiza fortis)* from the Galapagos Islands. *Syst. Parasitol.* **14:** 141-144

McQuistion T. E., Capparella A. P. (1995) Two new isosporan parasites (Apicomplexa: Eimeriidae) from the South American woodcreeper *Dendrocolaptes certhia. Acta Protozool.* **34:** 299-302

McQuistion T. E., Walden K. K. O., Capparella A. P. (1977) Two new species of *Isospora* (Apicomplexa: Eimeriidae) from the ocellated woodcreeper, *Xiphorhynchus ocellatus* (Passeriformes: Dendrocolaptidae). *Arch. Protistenkd.* (in press)

Ridgely R. S., Tudor G. (1994) The Birds of South America, Volume II. The Suboscine Passerines. University of Texas Press, Austin, Texas

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Book Review

Ciliates: Cells as Organisms. Edited by Klaus Hausmann and Phyllis C. Bradbury. Gustav Fischer Verlag, Stuttgart, Jena, Lübeck, Ulm, 1996. IX + 485 pp., 323 micrographs, 154 line drawings, 67 diagrams, 20 tables, hard cover; DM 248

Ciliates represent a group of organisms fascinating in many respects. These "perfect complete animalcules" as the editors call them following the example of Ehrenberg's famous treatise (1838), have a cellular complexity unmatched by any other eukaryotic protistan cell. This alone would suffice to draw attention to their research; until now, more than 8000 species have been described, some of them being well known even to wide public from school textbooks. Ciliates are easy to find in all kinds of water environment, including salt brine in arctic ice or warm springs. Very many live as commensals or parasites in other animals, presenting examples of extreme intricacies in host-parasite relationships. They have served as excellent tools in studies in cell biology, biochemistry, genetics and recently molecular biology. However, until now no comprehensive monograph has been available to present in an authoritative way a complete natural history of ciliates. There only exist purely taxonomical treatises such as the classical work of Kahl, a more recent book of Corliss (1979), some monographs restricted to much studied genera, e.g., *Tetrahymena* (Elliot 1973) or *Paramecium* (Wichtermann 1985) or books paying attention to some aspects of the study of ciliates (e.g., Nanney 1980). The gap has now been successfully filled by a large format, lavishly illustrated book, edited by two authorities in ciliate research, Professor Klaus Hausmann from the Free University of Berlin and Professor Phyllis C. Bradbury from North Carolina State University. They engaged 23 contributors from various countries to author 17 chapters of the book.

The first chapter on "Morphology and cytology of ciliates" by the late Jerome J. Paulin is a well balanced overview of the cell cycle and structures of ciliates, supported by a selection of outstanding electron microscope micrographs. The part on "Cytoskeleton of ciliates" has been expanded, to include the most recent data, in an additional short chapter by A. Adoutte and A. Fleury, with a very extensive list of references.

The chapter on "Systematics of ciliates" by D. H. Lynn introduces a classification based on, in addition to morphology and morphogenesis, the evidence of biochemical and genetical methods. I feel that it has been redundant to discuss in detail the now outdated system of Jankowski (1980), completed before later discoveries on *Stephanopogon* and on the ultrastructure of many ciliates. The fast development of views on ciliate classification is witnessed by the fact, that a paper co-authored by Dr. Lynn (J. Euk. Miocrobiol. 1996, p. 225) presents some new modifications (exclusion of Protocruziids from Karyorelictea etc). The next chapter on the "Evolution of ciliates" (M. Schlegel and K. Eisler) links up with the preceding one. It depicts in an instructive way the present idea on the evolution of ciliates as a sister group of dinoflagellates and the origin of somatic and buccal

ciliature. Contrary to earlier assumptions, ciliates with elaborate buccal ciliary organelles are taken as basic branches in the phylogenesis while the "gymnostomial" types represent a derived state. Similarly, equally upsetting older ideas, the paroral membrane is supposed to had given rise to somatic ciliature and not *vice versa*. These conclusions are supported by both small and large subunit rRNA sequence comparisons.

The chapter by W. Foissner on "Ontogenesis in ciliated protozoa, with emphasis on stomatogenesis" is an impressive piece of work. For a comprehensive discussion of all types of division and stomatogenesis, it reviews publications on ciliate ontogenetic processes in the period of 1870 to 1993, with the emphasis on stomatogenesis; the list of references contains 852 items! Published data on 600 ciliate species are extracted and surveyed in a list giving their types of fission and stomatogenesis. An ensuing cladistic system of ciliates concurs with the phylogenetic views expressed in the previous chapters.

A short chapter on "Ciliate metabolism" has been written by J.J. Blum, who was earlier engaged in these studies but later converted to the metabolism of kinetoplastids. In "Phagotrophy of ciliates", R. Radek and K. Hausmann review, mainly from the structural point of view, various processes of endo- and exocytosis, pino- and phagocytosis, of filter feeding, herbivory and carnivory.

I.B. Raikov in the chapter "Nuclei of ciliates" gives a succinct synthesis of main facts on the structure and behaviour of micro- and macronucleus (and their components) during the cell cycle. It appears that there are still many mysteries to be unraveled in the macronucleus.

Two chapters, "Fertilization and sexuality in ciliates" by A. Miyake and "Ciliate genetics" by L. K. Bleyman deal with related research areas that have been traditionally paid a wide attention. The reader will find a rather detailed insight into sexual cycles, mating types, gamones that determine them, gamotypes, into analysis of sexual processes, methods of ciliate genetic analysis and topics like cortical, organellar and behavioral genetics, supported by a respectable total of 20 pages of references.

In "Cilia: structure and molecular biology", P. Satir and K.L. Barkalow provide a state of art of the research on these, as they say, *sine qua non* organelles of the group. It includes intricate details of the ciliary structure, mechanisms of motility and molecular biology of axonemal proteins.

Selected major topics of "Molecular biology of ciliates" form the body of this chapter by H.J. Schmidt. They are mostly related to the study of ribosomal RNA genes, ribosomal proteins, surface antigens and mating type pheromones. A discussion is also included on the significance of deviations from the universal genetic code used by eukaryotes to translate mRNA into polypeptides, found first in ciliate surface antigens.

Two chapters, relating mainly to developments of the last two decades, may be read with special interest since their topic has not been largely represented in protozoological literature. They give an account of studies, prevailing with higher metazoans but performed at the ciliate cell level. Both are written in a clear, readable style. The first, "Sensory-motor coupling and motor responses" by H. Machemer and P.F.M. Teunis deals with principles controlling ciliate behavior; e.g., with responses to stimuli and their classes, adaptation, habituation and related topics. They conclude that data from sensory perception to motility suggest that the ciliate cell generates behavior at a level comparable to that of small invertebrates. In "Ethology of ciliates", N. Ricci analyses more complex behaviors by means of adapted ethograms (=behavioral pattern of a certain species). This is aimed at understanding the problems of behavior and daily life, of behavior and sex and provides a deeper insight into ciliate adaptive biology.

Of the many aspects of ciliate ecology, B.J. Finlay and T. Fenchel in "Ecology: role of ciliates in the natural environment" focus their attention on the participation of ciliates in the flow of energy and material - and factors involved - in various ecosystems. The present shift from e.g., the one-time recording of species in various habitats or monitoring of organic pollution becomes very obvious.

Symbiotic relationships of ciliates are dealt with by H.-D. Görtz, "Symbiosis in ciliates" and by P.C. Bradbury, "Pathogenic ciliates". It seems that both chapters, concluding the volume, are slightly "undernourished"; although ciliates as disease agents are no match to those causing e.g., malaria or coccidiosis, the plethora of intriguingly various ways of life on or within their hosts calls for more space. In the first chapter, bacterial and algal symbioses are adequately represented; however, the phenomena of rumen, colon or epibiotic ciliates are mentioned too briefly. The same applies to trichodinids or epibiontic ciliates; there is no mention of astome ciliates.

A work of this volume and size necessarily contains some errors; e.g., *Ophyridium* (sic!) is erroneously named in reference to a picture of *Scyphidia*; Fig. 6c, p. 63, should be courtesy of J. Lom instead of E.B. Small; in chapter 2, Hausmann and Mulisch is missing in the references.

The book has a pronounced aesthetic appeal; it has a nice look, is finely printed and almost all of its well selected

line drawings and photographs are of top quality. Quite impressive - to name two examples to represent many - is the visualisation of separate elements of *Paramecium* cytoskeleton reminiscent of a fine, fragile lace (p.43) or the awfully opened enormous mouth of the giant specimen of *Onychodromus* engulfing a smaller kin. "Ciliates: Cells as Organisms" is a valuable source of useful, up-to-date information (there are many references to publications that have appeared in 1995) which can be recommended both to professional protozoologists and to other interested readers. It is also a book which is a pleasure to read and have on the book shelve. It is certainly welcome by ciliatologists and will find its readership also among zoologists, biologists and those engaged in teaching biology from high school level upward.

Jiří Lom, Českie Budějovice, Czech Repuplic

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