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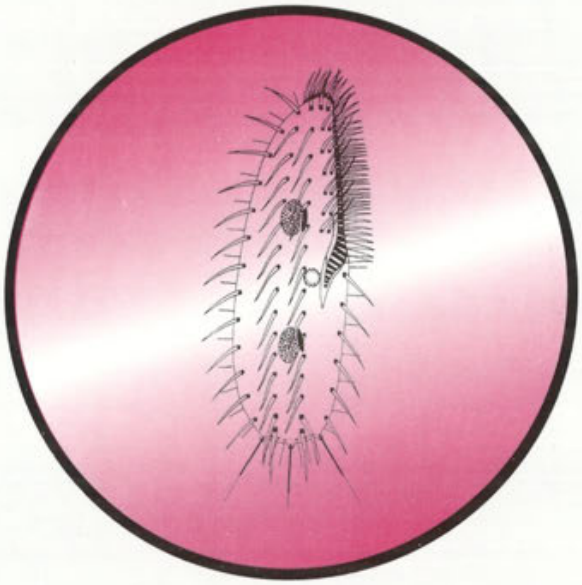

NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY
WARSAW, POLAND
1995
VOLUME 34 NUMBER 3
ISSN 0065-1583

Polish Academy of Sciences
Nencki Institute of Experimental Biology

ACTA PROTOZOOLOGICA

International Journal on Protistology

ACTA PROTOZOOLOGICA appears quarterly.

The price (including Air Mail postage) of subscription to ACTA PROTOZOOLOGICA at 1996 is: US $ 180.- by institutions and US $ 120. - by individual subscribers. Limited number of back volumes at reduced rate are available. For matters regarding ACTA PROOTOZOLOGICA, contact Managing Editor, Nencki Institute of Experimental Biology, ul. Pasteura 3, 02-093 Warszawa, Poland; Fax: (4822) 225342; E-mail: jurek@ameba.nencki.gov.pl

Front cover: *Wallackia schiffmanni.* In: W. Foissner (1976) *Wallackia schiffmanni* nov. gen., nov. spec. (Ciliophora, Hypotrichida) ein alpiner hypotricher Ciliat. *Acta Prootozol.* **15:** 387-392

Printed at the MARBIS, ul. Kombatantów 60, 05-070 Sulejówek, Poland

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1995) 34: 151 - 171

Review Article

Structure and Genetic Organization of the Polyploid Macronucleus of Ciliates: a Comparative Review

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Key words. Macronucleus, polyploidy, nuclear division, DNA molecules, minichromosomes, subchromosomes.

CONTENTS

1. INTRODUCTION

Fourteen years have passed since the conclusion of the author's latest review on morphology and evolution of the polyploid macronucleus (in Raikov 1982, chapter 8). Since that time, much research has dealt with the macronucleus, above all biochemical and molecular research. Although reviews on this kind of somatic nuclei did appear during these years, they dealt mainly with the genetic organization of the macronucleus and with alterations of the ciliate genome during macronuclear development (Ammermann 1985; Blackburn and Karrer 1986; Steinbrück 1986, 1990; Raikov 1989 and especially Prescott 1994). Some reviews were devoted to the molecular biology of the macronucleus in only certain groups of ciliates or even a single ciliate genus (Prescott 1984, 1992; Hufschmid 1985; Brunk 1986; Karrer 1986; Klobutcher and Prescott 1986; Kraut et al. 1986; Preer 1989; Ammermann 1990; Jahn 1991), others to only some special kind of molecules in that nucleus, such as the rDNA (Blackburn 1982, Yao 1982, Engberg 1985) or to only the telomeric sequences of the molecules (Blackburn 1986a, b, 1991; Blackburn and Szostak 1984). A broad review of recent advances in the field of research of somatic nuclei is clearly needed. The aim of the present review is to update and supplement the chapter 8, dealing with the polyploid macronucleus in various ciliates, of the author's book (Raikov 1982). Excluded are the macronuclei of karyorelictid ciliates

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which are not polyploid and which have been considered elsewhere (Raikov 1985, 1994).

The macrosystem of ciliates adopted here is the "utilitarian" system of J. O. Corliss (1994).

2. STRUCTURE OF THE MACRONUCLEUS

2.1. Chromatin

Recent investigations have confirmed the fact already known in 1982 that the macronuclear chromatin is largely compact and consists either of many discrete chromatin lumps, the so-called "small bodies", which are however interconnected by filaments (apparently DNP), or of larger discrete chromatin bodies which are not visibly interconnected, or else of a continuous reticulum of rather thick trabeculae of condensed chromatin. The "small bodies" type occurs, e.g., in *Tetrahymena* (Fig. 1) or *Paramecium*; more recently, it has been observed also in the colpodean *Pseudoplatyophrya* (De Puytorac et al. 1983), the heterotrich *Stentor* (Kawakami 1984) and the hypotrichs *Histriculus similis* (Calvo et al. 1986) and *Euplotes eurystomus* (Lin and Prescott 1986). Larger discrete chromatin bodies occur in the entodiniomorph *Epidinium* (Furness and Butler 1983), in the hypotrichs *Holosticha* sp. and *Euplotes crassus* (Lin and Prescott 1986) and, as was already known, in trophonts of Suctoria, e.g. *Ephelota* (Grell and Benwitz 1984) and *Trichophrya* (Mogenson and Butler 1984). A continuous network of chromatin strands (Fig. 2) was shown to occur in the litostomatean *Homalozoon* (Leipe and Hausmann 1992), the apostomatean *Conidophrys* (Bradbury and Tyson 1982), the heterotrichs *Climacostomum* (Hufschmid 1983) and *Transitella* (Iftode et al. 1983), the tintinnid *Parafavella* (Sokolova and Gerassimova 1984), and the hypotrichs

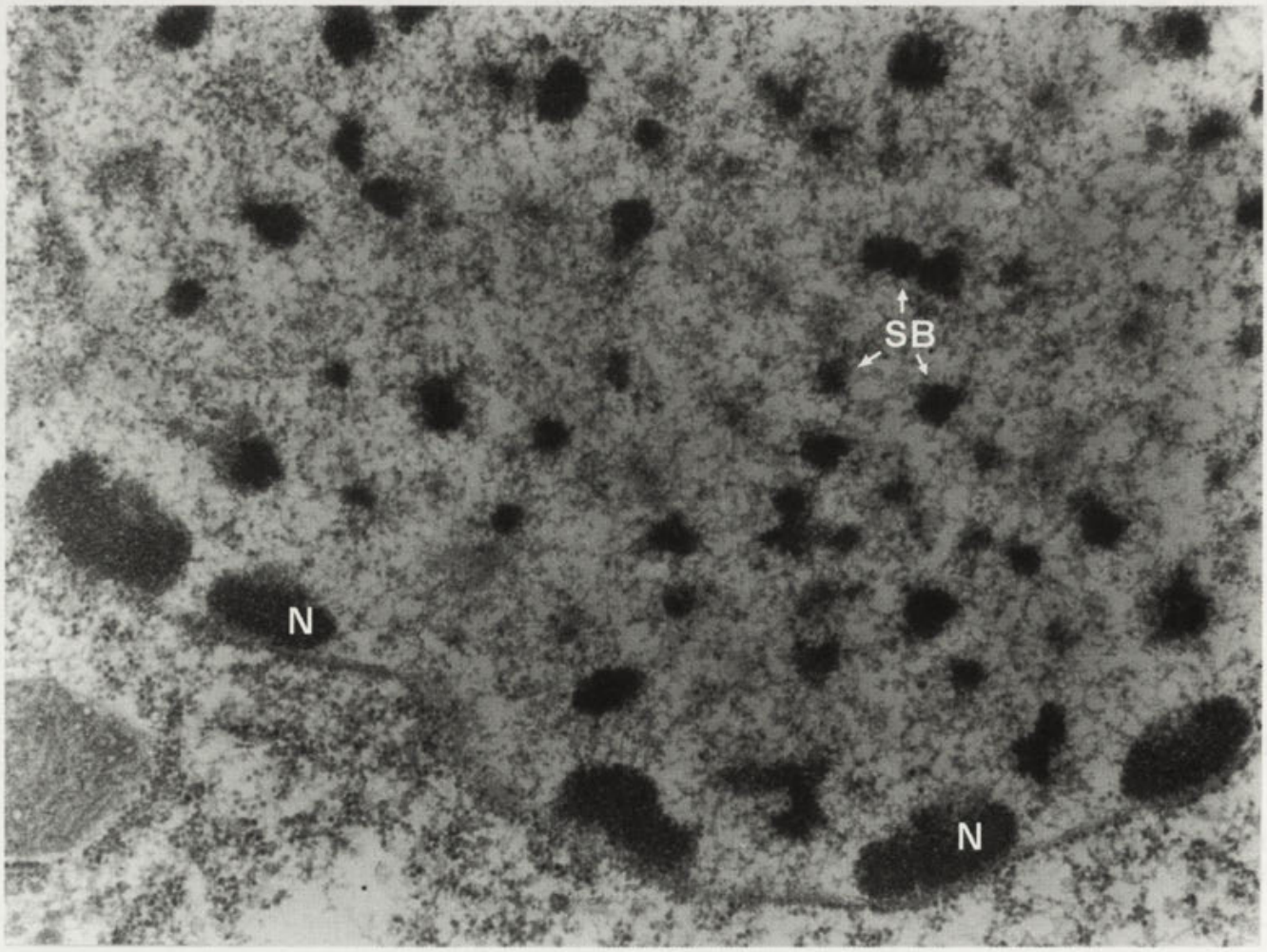

Fig. 1. Part of the macronucleus of *Tetrahymena pyriformis* GL showing discrete small bodies of chromatin (SB), and peripheral cup-shaped nucleoli (N). Glutaraldehyde and osmium tetroxide fixation, uranyl acetate and lead citrate staining (x 37 400). Original

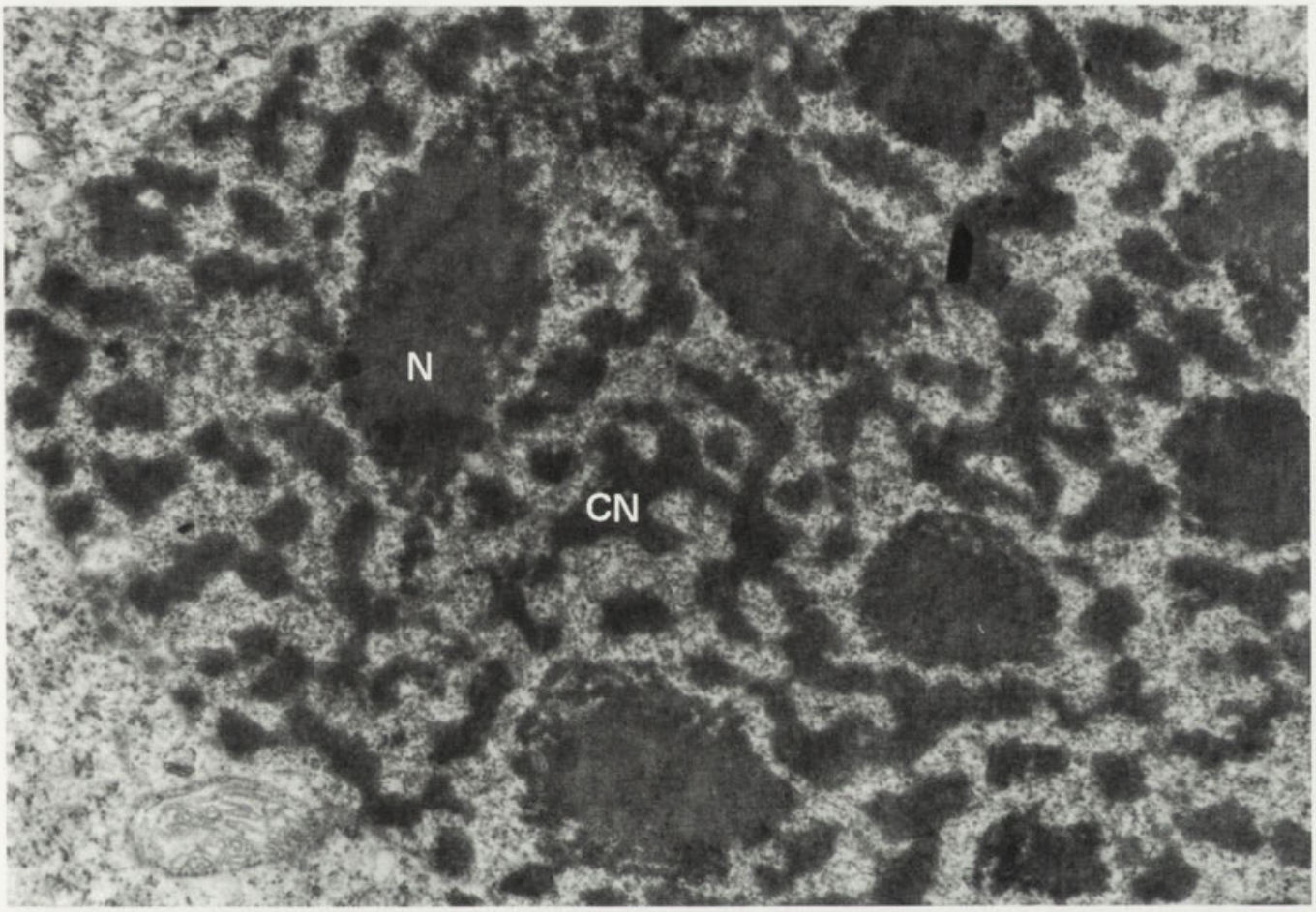

Fig. 2. Part of a node of the moniliform macronucleus of *Homalozoon vermiculare* showing continuous chromatin net (CN) and several nucleoli (N), in its meshes. Osmium tetroxide fixation, conventional staining (x 26 000). Original

Euplotes rariseta (Dallai et al. 1985), Urostyla cristata, *Styx* sp. (Lin and Prescott 1986) and *Aspidisca* sp. (Rosati et al. 1987). The chromatin structure (and the gross morphology of the macronucleus) shows considerable variation depending on the life cycle stage in forms with a complicated cycle, e.g. in Suctoria (Fox et al. 1992).

Spreading of the macronuclear chromatin has shown that the condensed chromatin always consists of regularly arranged nucleosomes appearing as beads on nucleosomal fibrils. The nucleosomes aggregate into supranucleosomes, and both the supranucleosomal and nucleosomal fibrils form radial loops upon artificial decondensation of the chromatin ("small") bodies, as shown in the colpodean *Bursaria truncatella* (Martinkina et al. 1983). At a definite stage in the cell cycle of *Bursaria*, natural decondensation of the chromatin occurs and the nucleosome arrangement becomes less regular (Vengerov et al. 1983). The macronuclear chromatin of *Bursaria* is especially compact in cysts, where chromatin crystalloids of the liquid crystal type are formed; the nucleosome structure is concomitantly lost (Sergejeva et al. 1987, Sergejeva and Bobyleva 1988). Crystal-like structures (a form of chromatin packing?) occur also in the compact chromatin of precystic forms of *Euplotes rariseta* (Dallai et al. 1985). During excystment, the chromatin in *Bursaria* is activated and naturally decondensed; transcription complexes occur on the nucleosomal threads (Tikhonenko et al. 1984). During early division, the macronuclear chromatin of *Bursaria* is condensed but begins decondensation (formation of radial loops) at late stages of division (Popenko et al. 1988). Similar results have been obtained while studying the macronuclear chromatin structure in the cell cycle of the heterotrich *Spirostomum* with the spreading technique (O. Borchsenius et al. 1988).

A highly condensed state of the macronuclear chromatin in cysts (where the four macronuclei of the vegetative cell fuse together) has been observed also in the hypotrich *Gastrostyla*, the total volume of the mac-

ronuclei decreasing to one-fifth, probably as a result of dehydration (Gutierrez 1985). However, the DNA/histone ratio here remains about the same. Four macronuclei fuse into a single one also in resting cysts of two other hypotrichs, *Laurentiella acuminata* (Gutierrez and Pérez-Silva 1983) and *Onychodromus acuminatus* (Jareño 1985). Fusion of chromatin bodies into larger masses occurs in the macronucleus of resting cysts of both *Laurentiella* and *Onychodromus*. During excystment, the cyst macronucleus of *Onychodromus* stretches and at least one end of it comes into contact with the cortex of the cell (Jareño 1984).

The DNA content of the macronuclei of many ciliates has been determined and expressed in absolute units (picograms). These data, together with some earlier ones, are presented in Table 1.

The macronuclear chromatin appears to contain a more or less typical set of histone fractions, while the micronucleus has an aberrant set (Caplan 1977). Most of the variation is due to the linker histone H1 or similar. In *Tetrahymena*, the macronucleus has a more or less typical H1, and the micronucleus has instead three proteins only partly homologous to H1 (Chicoine et al. 1985). In *Stylonychia*, the macronucleus has a full set of histones but the micronucleus lacks H1 and H3 (Schlegel et al. 1990). The lacking fractions gradually appear during macronuclear development. The same occurs in *Tetrahymena* where the micronucleus-specific histones are eliminated from macronuclear primordia and macronucleus-specific fractions are then accumulated (Allis and Wiggins 1984, Wenkert and Allis 1984).

Table 1

DNA content of the macronuclei (Ma) of some ciliates in the G1 phase (in picograms)

Species	DNA ofMa	Reference
Uronema nigricans	1.4	Soldo et al. 1981a
Parauronema acutum	1.9	Soldo et al. 1981a
Parauronema virginianum	2.0	Soldo et al. 1981a
Miamiensis avidus	2.3	Soldo et al. 1981a
Tetrahymena thermophila	9-11	Gibson and Martin 1971, Soldo et al. 1981a
Tetrahymena pyriformis	9-11	Gibson and Martin 1971, Soldo et al. 1981a
Paramecium octoaurelia	46	Soldo et al. 1981a
*Oxytricha similis**	51	Steinbrück et al. 1981
Blepharisma americanum	69	Santangelo 1990
Paramecium primaurelia	70	Cummings 1975, McTavish and Sommerville 1980
Paramecium tetraurelia	70	Cummings 1975, McTavish and Sommerville 1980
*Uroleptus caudatus**	96	Ammermann and Münz 1982
*Oxytricha nova**	116	Lauth et al. 1976
Paramecium caudatum	170	Soldo et al. 1981a
*Stylonychia putrina**	174	Ammermann and Münz 1982
Paramecium bursaria	277	Steinbrück et al. 1981
Blepharisma japonicum	288	Santangelo 1990
*Paraurostyla weissei***	348	Ammermann and Münz 1982
Euplotes aediculatus	380	Ammermann and Münz 1982
*Stylonychia lemnae**	788	Ammermann and Münz 1982
*Stylonychia mytilus**	1004	Ammermann & Schlegel 1983
Stentor coeruleus	3000	Ammermann 1985
Bursaria truncatella	38000	Winkler et al. 1982

* Data for the sum of 2 Ma; ** Data for the sum of all Ma

The DNA content of the macronucleus is variable even within a clone (Seyfert and Cleffman 1982). This is known to occur due to incomplete or excessive DNA replication, inegalities of DNA distribution during macronuclear division, and eventual extrusion of some chromatin from the macronucleus (for earlier references see Raikov 1982). Since then, strong variations of the macronuclear DNA content have been reported, related to culture age (Takagi and Kanazawa 1982), feeding conditions (Dupy-Blanc 1982), clonal age (Delmonte-Corrado et al. 1986), starvation (Brunk and Bohman 1986), a complicated life cycle including hypertrophic growth in *Ophryoglena* (Morat 1982), etc. Incomplete or excessive DNA replication in the macronucleus has been reported in many ciliates, e.g. in *Chilodonella* (Golembiewska-Skoczylas and Radzikowski 1992). The problem of regulation of the macronuclear DNA content has been reviewed by Berger (1988). Unequal division of the macronucleus (mean difference of DNA distribution between the two sister nuclei, 10%) occurs in the hypotrich *Laurentiella* (Torres et al. 1982). It occurs also in the cyrtophorid *Chilodonella* (Radzikowski 1983, 1985). Chromatin extrusion was shown to occur at almost every cell division in *Tetrahymena*, the size of extrusion bodies being larger in macronuclei with higher than average DNA content. The DNA of the extrusion bodies is capable of replication; thus, it does not appear to be defective (Bodenberger et al. 1992).

2.2. Nucleoli

The macronuclear nucleoli are known to be highly labile structures, responding to many external factors and depending on the stage of the life and cell cycles (Raikov 1982). The typical fine structural components of the nucleoli are a fibrillar core and a granular cortex. The fibrillar core displays a nucleolus-organizing region which contains ribosomal DNA (rRNA genes). The nucleoli vary from small and compact to large reticulate when the granular parts of several nucleoli fuse (Fig. 3). Segregation of nucleoli into the fibrillar and the granular components occurs under the action of certain drugs.

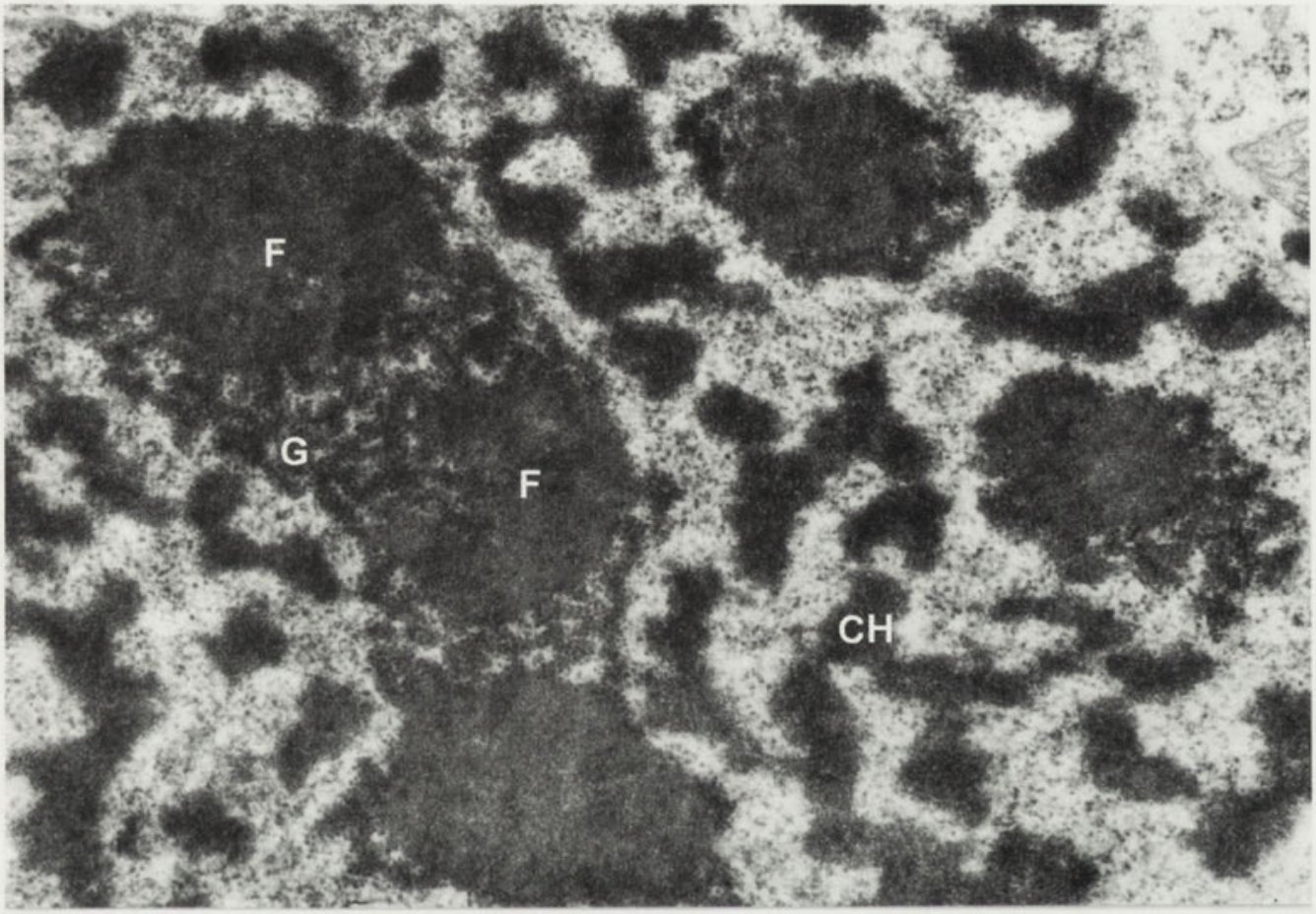

Fig. 3. Nucleoli with fused granular parts (G), and separate fibrillar cores (F), in the macronucleus of *Homalozoon vermiculare*; chromatin trabeculae (CH). Fixation and staining as in Fig. 2 (x 42 500). Original

Fusion, degranulation and/or segregation of nucleoli occurs in *Tetrahymena* under the action of RNA synthesis inhibitor Actinomycin D (Caratero et al. 1983, Nilsson 1985), cadmium ions (Pyne et al. 1983), ultraviolet irradiation (Caratero et al. 1984). The total volume occupied by the nucleoli decreases in *Paramecium tetraurelia* with aging of the clones (Heifetz and Smith-Sonneborn 1981). In the stationary phase of culture growth, the nucleoli of *P. caudatum* lose their granular cortex which peels off in form of granular lamellae (Fok and Allen 1981). During excystment of the hypotrich *Onychodromus acuminatus*, the macronuclear nucleoli become largely degranulated (Jareño 1988).

The nucleoli have recently been mass isolated from macronuclei of *Tetrahymena* and have been shown to be able to synthesize RNA in vitro (Matsuura and Higashinakagawa 1992).

Active NORs in *Tetrahymena* macronuclear nucleoli can be revealed, as NORs in nucleoli of other cells, by silver nitrate staining (Sabaneyeva et al. 1984).

2.3. Filaments, microtubules, and the macronuclear envelope

Microfilaments were known to occur but rarely in polyploid macronuclei, e.g. in *Lacrymaria* or *Stentor* where they form polymorphous nuclear inclusions (references in Raikov 1982). Their chemical nature remained obscure, though they seemed to be proteinaceous.

During the last years, actin has been revealed in macronuclei of *Tetrahymena* in form of bundles of microfilaments which can be induced by DMSO treatment (Katsumaru and Fukui 1982) and decorated with heavy meromyosin (Méténier 1984). Similar bundles occur in the macronucleus of *Stylonychia* treated with thioacetamid, a known F-actin inductor (Raminder Chadha et al. 1983). Microfilaments of unknown nature occur also in the isthmus between the two macronuclei of the hypotrich *Kahliella* (Fleury et al. 1985). Fibrils of the type of intermediate filaments (about 10 nm thick)

occur in the macronuclear chromatin, both condensed and preparing for replication (A. Olins and D. Olins 1990).

Bundles of microtubules (perhaps, a stock of tubulin) have been known to occur in the non-dividing macronucleus of trophonts of many Suctoria (references in Raikov 1982). This has been confirmed with respect to *Trichophrya* (Mogenson and Butler 1984). The same occurs in some other ciliates, e.g. the hypotrich *Histriculus* (Calvo et al. 1986). Bundles of microtubules have been seen at early stages of excystment in outgrowths of the fused macronucleus of resting cysts of *Onychodromus* (Jareño 1985).

The nuclear envelope of macronuclei belongs usually to the classical type and lack conspicuous intranuclear or cytoplasmic specializations (Raikov 1982). However, a fenestrated cytoplasmic cisterna has been revealed in *Stentor* enveloping macronuclear nodes (and micronuclei), so that the perinuclear ground cytoplasm is reduced to a thin rim between the nuclear envelope and the cytoplasmic cisterna (Mulisch 1988). Nuclear pores have been shown to be unequally distributed in the macronuclear envelope of *Euplotes crassus*: hexagonally packed in some regions of the envelope and totally absent in other regions, notably those where the condensed chromatin adheres from the inside to the envelope (Dallai and Luporini 1982).

2.4. Heteromeric macronuclei

It has long been known that macronuclei of the Phyllopharyngea (Cyrtophortida and Chonotrichia) have a special, so-called heteromeric structure (see Raikov 1982). They consist of a DNA-rich orthomere and a DNA-poor paramere, the latter often containing however a small DNA-containing body, the endosome. The orthomere and the paramere may lie side-by-side (juxtaposed type) or the orthomere may encircle the paramere (concentric type).

In the last years, only the concentrically-shaped heteromeric macronucleus of *Chilodonella steini* has been investigated (Fig. 4). It has been shown that the DNA content of macronuclei was variable due to both unequal DNA distribution during division and differential DNA replication in response to culture conditions (Radzikowski 1983). The replication of the macronuclear DNA starts and ends in the paramere earlier than in the orthomere. The DNA of the endosome does not replicate at all. In the next cell generation, the DNA synthesized in the paramere partly remains there, partly migrates to the orthomere where it is probably amplified (Radzikowski 1985). Anyway, virtually all fluctuations of the macronuclear DNA content depend on the orthomere which can also divide unequally. Some DNA of the paramere, probably unable to further replication, goes to the endosome. The DNA synthesized in the orthomere remains there and some of it goes to the endosome, where it does not replicate any more. There is no migration of DNA from the orthomere to the paramere (Radzikowski 1985). So the paramere seems to be the part of the macronucleus where the DNA is prepared for amplification and the orthomere, where it is actually amplified.

3. REPLICATION AND DIVISION OF THE MACRONUCLEUS

3.1. DNA replication

DNA replication is known to occur in the macronucleus in the interphase. Its timing in respect to the generation time is variable and not correlated or only partly correlated with DNA replication in the micronucleus. Generally, the S period lasts much longer in the macronucleus than in the micronucleus, suggesting a certain asynchrony of DNA replication in the somatic nucleus (for references see Raikov 1982). More recently it has been shown, for example, that in *Didinium* the S phase occupies the larger part of the interphase and that the ribosomal DNA is replicated in early S (Karadzhan 1987).

In *Paramecium tetraurelia,* the time of beginning of the macronuclear S period is determined in the preceding cell cycle and does not depend on the actual nutritional conditions (Ching and Berger 1986a). But the rate of DNA synthesis already started depends on the nutrient level: it is lower in starving cells (Ching and Berger 1986b). With less DNA in the macronucleus (due to unequal division) the S period starts earlier, but in the following cell cycle only (Rasmussen et al. 1986). Macronuclear and cell division may occur when the DNA of this nucleus is not yet fully replicated; the transition point when the cell becomes division-competent corresponds to about 75% of the full duplication of DNA and coincides with the start of micronuclear mitosis (Rasmussen et al. 1985).

A special case of macronuclear DNA replication when it is spatially restricted and occurs as a wave of replication passing through the macronucleus is that of the replication bands typical of the hypotrichs and

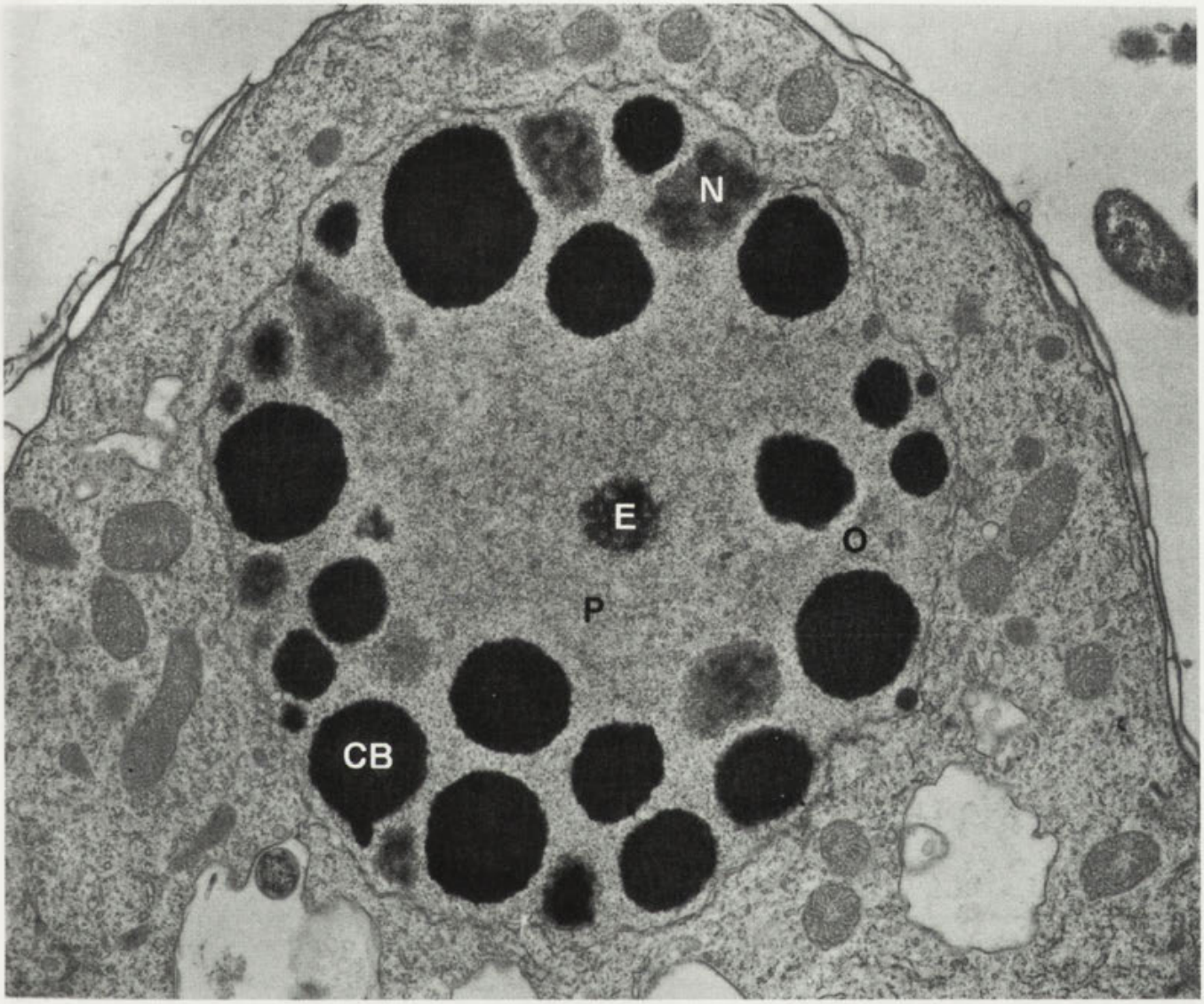

Fig. 4. Heteromeric macronucleus of the concentric type in *Chilodonella uncinata* containing large discrete chromatin bodies (CB) and nucleoli (N) in the peripheral orthomere (O) and the chromatin endosome (E) in the central paramere (P) (x 16700). After Pyne (1978)

some other ciliates (for more detail, see Raikov 1982). Recently, much research has been done with the hypotrich replication bands as unique models of DNA replication. The morphology of replication bands in various hypotrichs (*Holosticha, Styx, Urostyla,* and two species of *Euplotes*) has been described by Lin and Prescott (1986). As usual, a forward zone consisting of a plexus of uniform 40-70 nm thick fibres, and a rear zone consisting of much thinner filaments, possibly individual DNA molecules, can be distinguished in the replication band. Stereo electron microscopy of thick sections of *Oxytricha, Styx* and *Euplotes* has revealed 40-50 nm thick fibrils containing tightly packed nucleosomes in the forward zone and 10 nm chromatin fibrils in the rear zone (A. Olins et al. 1981). DNA synthesis has been shown by high resolution autoradiography to occur only near the leading edge of the rear zone (Lin and Prescott 1985). Cytochemically, the replication bands of *Euplotes* proved to contain much acid-soluble argentophilic protein and many thiol groups in other protein(s) (Allen and D. Olins 1984). These proteins have been found by electron microscope cytochemistry mainly in the forward zone, in addition to much DNA stained with osmium amine; the DNA synthesis (rear) zone appeared highly hydrated (A. Olins et al. 1988).

Isolated macronuclei of *Euplotes* have been found to synthesize DNA *in vitro* in their replication bands (D. Olins and A. Olins 1987). Replication bands have been successfully mass isolated from macronuclei, and many replication forks of DNA found on spreads of them (Allen et al. 1985). Monoclonal antibodies against their proteins have been raised, including one specific for the rear zone (Allen et al. 1986). The rear zone also contains the proliferating cell nuclear antigen / cyclin peculiar of various proliferating cells (D. Olins et al. 1989). Replication bands react with antibodies against a 14-nm-filament forming protein (49 kD) found, e.g., in *Tetrahymena* (Numata et al. 1991). Also, sugar-binding substances (lectins) occur in the replication bands of *Euplotes* (D. Olins et al. 1988).

Initiation of DNA synthesis in replication bands requires the presence of a micronucleus, as shown in *Euplotes* by Mikami et al. (1985).

3.2. Condensation and division

The macronuclear division is generally preceded by contraction of complexely shaped macronuclei (moniliform, ribbon-shaped, etc.) into a compact body, the so-called division macronucleus. When there are several macronuclei in a vegetative cell, they generally fuse (see Raikov 1982).

The process of contraction of the moniliform macronucleus of the litostomatean *Homalozoon* has been observed by Leipe and Hausmann (1992). The contraction is accompanied by a change in chromatin morphology: from coarsely reticular it becomes finely fibrillar, and the nucleoli are degranulated and decrease in size. Having divided in two, the daughter macronuclei renodulate, while bundles of microtubules occur in the connecting bridges between the nodes.

The macronuclei are known to be able to divide not only in two but also in several parts at once, or to bud off smaller bodies which thereafter regenerate to the adult size (Raikov 1982). All this indicates that the macronuclear genome must be repeated many times in this nucleus. Separate chromatin aggregates supposed to represent genomes occur during division in various colpodids, e.g. *Tillina* (Frenkel 1982). They have been supposed to be diploid (Fig. 5). However, in *Colpoda cucullus* such chromatin aggregates have been shown to be not always diploid (Morat et al. 1981).

The macronuclear division itself seems to be under the control of a region of the cell cortex which seems to assure proper positioning and direction of elongation of the nucleus (Tucker et al. 1980, De Terra 1983). In

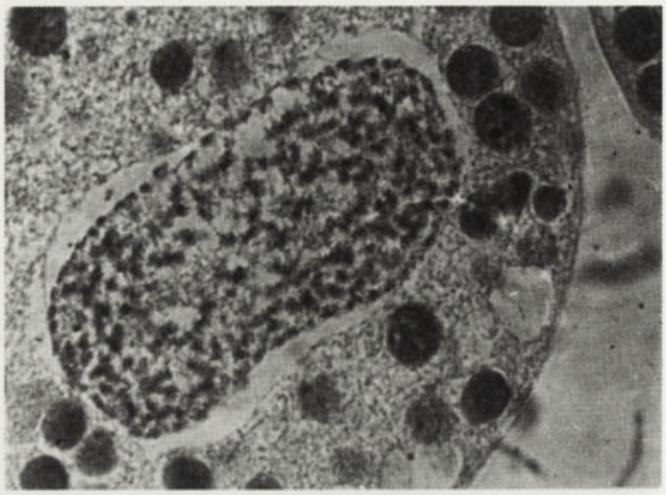

Fig. 5. Chromatin aggregates in the dividing macronucleus of *Tillina magna* (first division in a reproduction cyst). Light micrograph of a section (Zenker fixation, Feulgen staining) (x 940). After Frenkel (1982)

Paramecium, there are at least two populations of microtubules in the dividing macronucleus, one of which assures flattening of the nucleus against the dorsal cortex (a ring of microtubules spanning the nucleus), the other assures stretching of the macronucleus, is assembled later, and consists of longitudinal bundles of microtubules (Tucker et al. 1980).

4. MACRONUCLEAR DNA MOLECULES

A detailed review of the structure and development of the macronucleus in terms of molecular genetics and of the organization of macronuclear genes is beyond the scope of the present article; for that, the reader is referred to the excellent recent review of D. M. Prescott (1994). However, a brief overview of the molecular biology of the macronucleus cannot be avoided here.

At least two fundamental types of macronuclear organization can presently be distinguished. It has already been known in 1982 that the chromosomes in the macronucleus of hypotrichs are fragmented in short molecules of DNA containing one gene each, and such fragments are strongly amplified. However, the molecular organization of macronuclei containing longer molecules of DNA (such as those of *Tetrahymena, Paramecium,* and the heterotrichs) was not clear (Raikov 1982). It became known only later due to the method of pulsed field gel electrophoresis (molecular karyotyping), which has shown that these

molecules are subchromosome-sized, i.e. correspond to chromosome parts.

4.1. Gene-sized DNA molecules

The size of the genome fragments varies in the hypotrichs between 0.4 and 20 kilobase pairs (kbp) or, on spreads, 0.1-6 μm (mean 0.8 μm), which corresponds to one transcribed gene with some spacer DNA probably including regulatory regions (Herrick 1992, reviews: Klobutcher and Prescott 1986, Raikov 1989, Ammermann 1990, Steinbrück 1990, Prescott 1994). The gene-sized DNA molecules have the characters of mini-chromosomes: they are capable of autonomous replication and transcription, and have typical telomeric inverted repeats of an octonucleotide C4A4/G4T4. However, they do not have centromeres and do not attach microtubules, i.e. they have no mechanism of regular segregation of replicated copies into daughter nuclei.

The telomeres consist of regions of double-stranded DNA (C4A4 in one strand and G4T4 in the complementary one) which are 20 bp long in *Oxytricha* and *Stylonychia* and 28 bp in *Euplotes,* and of 16 base single-stranded "tails" of the G4T4 strand (Klobutcher et al. 1981, Pluta et al. 1982, Henderson and Blackburn 1989, and others). The two telomeres are inverted in respect to each other. Such a structure is probably necessary for the correct replication of the gene-sized DNA molecules according to the model suggested by Zahler and Prescott (1988) and reviewed by Prescott (1994).

There is probably a higher order structure in the macronucleus, or a scaffold to which mini-chromosomes are attached, possibly due to interactions of telomeres with a protein core or with some filament-forming protein (Lipps et al. 1982, Klobutcher and Prescott 1986, A. Olins and D. Olins 1990).

It has been demonstrated that some mini-chromosomes are amplified in the macronucleus stronger than others, i.e. there is over-amplification of some genes in that nucleus (Steinbrück 1983).

Several genes of hypotrichs have been shown to exist in mini-chromosomes of definite length, sometimes in two mini-chromosomes differing in the amount of spacer DNA. For example, α-tubulin genes of *Stylonychia lemnae* occur in molecules 1730 and 1850 bp in length (Helftenbein 1985), whereas α-tubulin genes of *Euplotes octocarinatus* occur only in molecules 1587 bp long, and β-tubulin genes, in molecules of 1524 bp (Liang et al. 1994). The α-tubulin gene of *Euplotes focardii* occurs in molecules 1800 bp long, whereas there are three different β-tubulin genes in this species, occurring in molecules of 2150, 1900 and 1600 bp (Miceli et al. 1994). Comparable data have been obtained for the histone and tubulin genes of *Stylonychia mytilus* (Elsevier et al. 1978, Dollak 1982), the actin genes of *Oxytricha fallax* (Kaine and Spear 1980, 1982) and *O. nova* (Greslin et al. 1989, Prescott and Greslin 1992), the phospholipase gene of *Euplotes crassus* (Klobutcher et al. 1991), the mating pheromone genes of *Euplotes* species (Miceli et al. 1989, F. Meyer et al. 1992), the telomeric protein gene of *Oxytricha nova* (Mitcham et al. 1992), and some others. In all, at least 48 macronuclear genes of hypotrichs have been isolated and sequenced (reviewed by Prescott 1994, see also Miceli et al. 1994).

The occurrence of two or more forms of mini-chromosomes carrying the same gene apparently depends on the phenomenon of alternative processing, when the sequences flanking the gene are cut in a different but definite way, leaving different lengths of spacer beside the gene. This has been observed in *Oxytricha fallax* (Cartinhour and Herrick 1984; Herrick et al. 1987a, b) and *O. nova* (Klobutcher et al. 1988).

Replication forms of the mini-chromosomes of *Euplotes* and *Styx* have been studied on spreads with the electron microscope and shown to have one or two sites of initiation of replication (Murti and Prescott 1983). Each mini-chromosome replicates autonomously when getting into the replication band passing through the macronucleus.

It has also been shown that the ends of the telomeric sequences of DNA (especially the single-stranded G4T4 tails) are protected by a special dimeric protein different from histones and thus form a telomere complex (Gottschling and Cech 1984; Gottschling and Zakian 1986; Price and Cech 1987, 1989; Price et al. 1992; Fang and Cech 1993).

Among ciliates other than the hypotrichs, fragmentation of the macronuclear genome into gene-sized DNA molecules has been revealed in at least two taxa, the Oligotrichida (*Halteria*) and the Cyrtophorida (*Chilodonella*). In *Halteria grandinella,* the macronuclear DNA is 2-20 kbp long (Méténier and Hufschmid 1988), and in *Chilodonella uncinata* and *Ch.* (*Trithigmostoma*) *cucullulus,* it is 0.35-30 kbp long (Lahlafi and Méténier 1991).

4.2. Subchromosome-sized DNA molecules

The second model of macronuclear structure occurs in many ciliates, especially the Hymenostomatia, e.g. in

Tetrahymena, and the Peniculinia, e.g. in *Paramecium* (reviews: Brunk 1986, Karrer 1986, Preer 1989, Raikov 1989, Steinbrück 1990). Here, the chromosomes are fragmented but not so strongly as in the case of gene-sized DNA. On electron micrographs of the spread DNA the molecules are generally too long to be measured. The same occurs following chromatin spreading: the nucleosomal fibres are also long and usually loop-like, being issued by bodies of condensed chromatin (see above). The exact nature of the macronuclear DNA of *Tetrahymena* failed to be determined for a long time: sedimentation methods tended to underestimate the size of the DNA due to shearing, usual electrophoresis did not resolve molecules longer than 50-100 kbp, whereas viscoelastic measurements tended to overestimate the DNA size and led to a conclusion that the macronuclear DNA was chromosome-sized (see Raikov 1982).

The incertitude ended when the method of pulsed-field gel electrophoresis (PFGE), capable to separate DNA molecules up to 2000 kbp in length, was introduced. It has been shown that the size of the macronuclear DNA of *Tetrahymena, Paramecium* and some other ciliates falls in this range. Altschuler and Yao (1985) first demonstrated that the macronuclear genome of *Tetrahymena thermophila* consists of 70 or more molecules up to 1100 kbp, whereas the micronuclear genome consists of only 5 chromosomes. Conover and Brunk (1986) found ~270 different DNA molecules in the macronucleus of the same species, ranging from 100 to 1500 kbp (mean: 800 kbp). Then, each micronuclear chromosome of this ciliate must at average be fragmented into ~50 subchromosomes. Palacios et al. (1992a) confirmed this but gave a higher average length value for the subchromosomes (1000-1100 kbp) of four species of *Tetrahymena.* Subchromosomes ranging in size from 100 to 800 kbp (average: 450 kbp) have been found in the macronucleus of *Paramecium tetraurelia* (Phan et al. 1989).

At the ends of the subchromosomes of *Tetrahymena* there are typical inverted telomeric repeats of 50-70 copies of a hexanucleotide C4A2/G4T2 which form a double-stranded sequence with single-stranded G4T2 "tails" (Yao and Yao 1981, Yokoyama and Yao 1986, Larson et al. 1987, Henderson and Blackburn 1989). In *Paramecium primaurelia* and *P. tetraurelia,* the telomeric repeat unit may be not only C4A2 but also C3A3 (Baroin et al. 1987, Forney and Blackburn 1988). Nonetheless, the subchromosomes seem to have no centromere and no means to attach spindle microtubules.

Other ciliates also seem to have DNA molecules of subchromosomal length in the macronucleus. In the heterotrich *Blepharisma,* usual electrophoresis has shown that the macronuclear DNA is high molecular and not resolved by the method (Salvini et al. 1983). The use of PFGE, however, demonstrated that these DNA molecules have the size of 100-500 kbp, and up to 2000 kbp (Piras et al. 1989). In another heterotrich, *Climacostomum,* chromatin spreading has revealed that the macronucleus contains long nucleosome fibrils (20 μm), too long to be formed by gene-sized DNA molecules (Hufschmid 1983). The same has been observed in the macronucleus of the colpodean *Bursaria* (Martinkina et al. 1983 and others - see above). In four *Colpoda* species, the average size of macronuclear DNA, as determined by PFGE, lies between 1000 and 1200 kbp (Palacios et al. 1992b).

As in mini-chromosomes, the genes of ciliates with long DNA are localized in definite subchromosomes. This shows that the fragmentation into subchromosomes is an ordered and not a stochastic process. For example, the variant surface antigen A (VSA-A) gene is found in *Paramecium tetraurelia* only in macronuclear subchromosomes 320 kbp long (Phan et al. 1989).

The number of macronuclear genes sequenced steadily grows. In *Tetrahymena,* this number includes histone H4, H1 and H2B genes (Bannon et al. 1984, Min Wu et al. 1986, Nomoto et al. 1987), the myosin gene (Mitchell and Martin 1989), the α-tubulin gene (Callahan et al. 1984, Barahona et al. 1988), the β-tubulin genes (Barahona et al. 1988), the actin genes (Cupples and Pearlman 1986, Hirono et al. 1987), the ribosomal protein gene (Nielsen et al. 1986), the surface protein gene SerH3 (Kile et al. 1988), the ubiquitine genes (Neves et al. 1988), etc. In *Paramecium* (*aurelia* group), the β-tubulin gene (Dupuis 1992) and the immobilization surface antigen genes (E. Meyer et al. 1985, Preer et al. 1987) have been investigated.

5. MACRONUCLEAR DEVELOPMENT AND GENOME TRANSFORMATIONS

The development of a new macronucleus occurs in ciliates after conjugation or autogamy. This phenomenon leads to formation of a DNA-rich somatic nucleus from a synkarion derivative which is generally a diploid non-differentiated nucleus. The macronuclear differentiation is coupled with processes of deep reorganization of the genome of the micronucleus including deletion of some

DNA sequences, transposition of other sequences, fragmentation of the chromosomes, and amplification of the conserved DNA molecules. Reviews concerning these phenomena have been published by Ammermann (1985), Blackburn and Karrer (1986), Steinbrück (1986), Prescott (1992, 1994), and others.

5.1. The hypotrichs

In the hypotrichs, macronuclear development is known to include polytenization of the chromosomes, producing a banded pattern, which is followed by transverse fragmentation of the polytene chromosomes through the interbands and excessive DNA diminution leading to the reduction of the genome size (see Raikov 1982, 1989). Reviews dealing with polytene chromosomes in ciliates are available (Alonso 1978, Kraut and Lipps 1984, Ammermann 1987a).

In the last 15 years, it was shown that in some cases (*Stylonychia lemnae*) only some of the micronuclear chromosomes polytenize and play a role in macronuclear development, whereas other chromosomes (the so-called germline-restricted ones), present in a variable number, degenerate (Ammermann 1987b). In other hypotrichs, there are no germline-restricted chromosomes, e.g. in *Euplotes octocarinatus* where all the chromosomes of the synkarion polytenize (Kuhlmann and Heckmann 1991). Some evidence has been obtained that polytene chromosomes undergo somatic pairing of the homologues which is sometimes incomplete, and that polytene chromosomes can form puffs at definite stages of their development (Jareño 1990). The number of polytene chromosomes in *Euplotes* tends to decrease with the progress of polytenization, probably as a result of end-to-end chromosome fusion (Sato 1989). Spreading of the polytene chromosomes has shown that, unlike classical polytene chromosomes from salivary gland cells of *Diptera*, polytene chromosomes of *Stylonychia* consist of two kinds of DNP filaments: thinner axial ones and thicker ones which form the DNA bands and have the form of lateral loops (G. Meyer and Lipps 1984). The latter are preferentially destroyed (after closing up into free circles) when the DNA diminution occurs.

The size of the macronuclear genome is always reduced in hypotrichs in comparison with the micronuclear genome and brought to a more or less uniform value between 20 and 55 million base pairs (Mbp), as shown in Table 2, upper half. At the same time, the size of the micronuclear genome is one or even two orders of magnitude larger: about 500 Mbp in *Euplotes aediculatus* (Ammermann and Münz 1982), 620 Mbp in *Oxytricha nova* (Lauth et al. 1976), some 3800 Mbp in *Paraurostyla weissei* (Ammermann and Münz 1982), and 8300 Mbp in *Stylonychia lemnae* (Ammermann and Schlegel 1983). In other words, the larger the micronuclear genome, the stronger it is reduced during macronuclear development.

Of interest are the processes leading to the formation of many mini-chromosomes constituting together the macronuclear unit genome from the initial much larger micronuclear genome. The process of deletion of many parts of the genome may at first involve deletion of whole (germline-restricted) chromosomes, as outlined above (e.g., in *Stylonychia).* However, in most other hypotrichs this step is missing. Second, mass DNA deletion occurs after polytene chromosomes in the macronuclear primordia are fragmented through the interbands and when the material of the bands is isolated into vesicles. At this stage, much DNA including most repetitive sequences is destroyed (reviewed by Raikov 1982, Dawson et al. 1984), and some micronucleus-specific genes are deleted as well (Ammermann 1990).

Table 2

Size of the macronuclear unit genome and its average copy number in the macronucleus (DNA content : genome size)

Species	Genome size (Mbp)	Copy number	References
Ciliates with gene-sized DNA molecules:			
Paraurostyla weissei	21.5	~15500**	Steinbrück et al. 1981
Oxytricha similis	26	~2000*	Steinbrück et al. 1981
Euplotes aediculatus	43	8100	Steinbrück et al. 1981
Stylonychia lemnae	48	~15000*	Steinbrück et al. 1981
Oxytricha nova	55	2000*	Lauth et al. 1976
Other ciliates:			
Tetrahymena thermophila	123	~73	Gorovsky 1980
Tetrahymena pyriformis	~190	~45	Yao and Gorovsky 1974
Tetrahymena pyriformis	56	186	Soldo et al. 1981a
Paramecium primaurelia	46	1400	Cummings 1975
Paramecium primaurelia	80	840	McTavish and Sommerville 1980
Paramecium octoaurelia	58	~750	Soldo et al. 1981a
Paramecium caudatum	23	~6500	Soldo et al. 1981a
Paramecium bursaria	17	15400	Steinbrück et al. 1981
Uronema nigricans	40	33	Soldo et al. 1981a
Parauronema acutum	43	42	Soldo et al. 1981a
Parauronema virginianum	42	46	Soldo et al. 1981a
Miamiensis avidus	49	45	Soldo et al. 1981a
Stentor coeruleus	92	30000	Pelvat and De Haller 1976

* Data for the sum of two macronuclei; ** Data for the sum of all macronuclei in a cell.

The processing of the conserved DNA fragments has been studied in *Euplotes crassus* (Roth and Prescott 1985). At first, the terminal spacers are deleted, which corresponds to the major part of the eliminated DNA. Then, telomere repeats are added in excess to the ends of the gene. And finally, the telomeres are trimmed to the species-specific size (review: Prescott 1994).

It became clear during the last years, that the processing of the macronuclear chromosomes is even more complicated. Inside most genes, there are the so-called internal eliminated sequences (IES), which have to be deleted during the development of active macronuclear genes from silent micronuclear genes (Klobutcher et al. 1984, Klobutcher 1987). These deletions occur earlier than chromosome fragmentation, still at the polytene chromosome stage (Tausta and Klobutcher 1990). Additionally, it has recently been shown that, in some genes, the conserved portions of the gene (the macronucleus-destined sequences or MDS) must be reordered to produce a functional gene. This has been demonstrated with the actin I gene (Greslin et al. 1989, Prescott and Greslin 1992) and the telomeric protein encoding gene of *Oxytricha nova* (Mitcham et al. 1992). The same occurs in the actin I gene of *O. trifallax* (Prescott and Greslin 1992). This "scrambled gene" phenomenon is reviewed by Prescott (1992, 1994).

Even earlier than the deletions of the IES, another type of deletion of DNA sequences has been recently discovered in *Euplotes crassus* (Jahn et al. 1989, Krikau and Jahn 1991, Jaraczewski and Jahn 1993). These are transposon-like elements designated as Tec1 and Tec2, which are excised during chromosome polytenization (reviewed by Prescott 1994). Each of these elements, about 5.3 kbp long, is dispersed in about 30 000 copies in the micronuclear genome (Baird et al. 1989) but is absent from the macronucleus.

5.2. *Tetrahymena* and other Ciliates

The development of the macronuclear primordia in *Tetrahymena, Paramecium,* the heterotrichs such as *Blepharisma* and *Stentor,* and the litostomateans such as *Didinium* and *Dileptus,* are known to include no stage of formation of giant polytene chromosomes (review: Raikov 1982). Instead, the primordia contain inconspicuous flocculent material, sometimes some transient chromatin accumulations, e.g. in *Paramecium caudatum* (Mikami 1987, review: Mikami 1988). Then, the first nucleoli are formed and the chromatin "small" bodies appear throughout the macronuclear primordium.

However, a transitory drop of the DNA content or a slowing down of the rate of the DNA synthesis indicating deletion of some DNA does occur in these ciliates. Thus, in *Tetrahymena thermophila* disappearance of some DNA sequences occurs early in development of the macronuclear primordia, when they have only 4 c to 8 c DNA (Austerberry et al. 1984, Brunk and Conover 1985). In the same species, the DNA replication in the macronuclear primordia has been followed by photometry from 2 c to 32 c, and at the transition from 8 c to 16 c an incomplete round of DNA duplication (or a complete one coupled with DNA deletion) has been detected (J. Roth and Cleffmann 1986). Macronuclear primordia of *Tetrahymena* have been separated by flow cytofluorimetry into 4 c, 8 c, 16 c and 32 c fractions, which demonstrates that DNA replication normally proceeds there stepwise (Allis and Dennison 1982, Brunk and Bohman 1986). However, the DNA replication becomes asynchronous starting with the 16 c stage, which may indicate DNA deletions (Brunk and Bohman 1986). Anyway, deletions occur by site-specific cuts at many places in the genome, and then by elimination of the region between two cuts, with rejoining of the free ends produced (Austerberry and Yao 1987). The number of such sites in the genome of *Tetrahymena* has been estimated at 5000 (Yao et al. 1984). Separate from the sequence deletion is the process of fragmentation of the chromosomes into subchromosomes, which occurs later in primordium development at sites marked by a special 15 bp signal sequence (Yao et al. 1987, 1990; Yao 1989).

A transient reduction of the DNA content has been also observed during macronuclear development in *Dileptus* where the four macronuclear primordia divide into numerous macronuclei; the DNA reduction amounts to 1.3-1.4 fold and occurs during division of the primordia (Karadzhan 1985). In *Blepharisma,* this stage of DNA reduction (if any) has not been detected photometrically, and the DNA accumulation in the primordia seems to be continuous (Dass et al. 1982).

Most recent morphological studies on macronuclear development in species lacking polytene chromosomes deal with late stages of development, especially nucleologenesis. In *Tetrahymena,* ribosomal RNA transcription starts only with appearance of the nucleoli and only in fed animals (Bro et al. 1981). The first nucleoli form as a large aggregate in the centre of the primordium (Caratero et al. 1983). The newly formed nucleoli intensely incorporate 3H-uridine, whereas earlier stages show only weak incorporation (Weiske-Benner and Eckert 1985). The same occurs in

macronuclear primordia of *Blepharisma* (Dass et al. 1982).

The development of the macronucleus is accompanied with severe changes in the histone composition. The micronucleus-specific histones disappear still during meiosis, so that the young macronuclear primordia of *Tetrahymena* contain none of them. Then, macronucleus-specific histones are synthesized in the cell and accumulate in the growing macronuclear primordia (Allis and Wiggins 1984).

The genome size in ciliates with subchromosomal DNA is reduced during macronuclear development again to a more or less uniform level usually comprised between 17 and 92 Mbp (Table 2, lower half). Only in the macronucleus of *Tetrahymena* the unit genome appears to be substantially larger, up to 123 Mbp and even to 180-200 Mbp (Table 2). However, Soldo et al. (1981a) gives for *Tetrahymena* the kinetic complexity of the macronuclear DNA of 56 Mbp, which is well within the above range.

The degree of reduction of the genome size during macronuclear development depends, as in hypotrichs, mainly on the genome size of the micronucleus. The larger the initial micronuclear genome, the stronger is it reduced. In *Tetrahymena*, the degree of reduction of the genome size is the smallest: the macronuclear unit genome is only about 15% smaller than the micronuclear genome (Yao and Gorovsky 1974, Yao and Gall 1979; reviews: Gorovsky 1980, Raikov 1989). In *Paramecium* (*aurelia* group), the micronuclear genome of 270-320 Mbp (Ammermann 1985) is reduced in the macronucleus 3-6 fold, to 46-80 Mbp (Table 2). But in *Paramecium bursaria* the genome size of the micronucleus, which is 3500 Mbp (Ammermann 1985), is reduced to 17 Mbp, i.e. more than 200 times.

Consequently, there is no drastic difference between the degrees of genome reduction between ciliates with gene-sized DNA (mini-chromosomes) and subchromosome-sized DNA. Both groups include species with weak (*Euplotes minuta,* Ammermann 1985; *Tetrahymena*), medium (*Oxytricha nova, Paramecium aurelia*-group) and strong genome reduction (*Stylonychia, Paramecium bursaria*).

The chromosomes of ciliates with the subchromosomal type of macronuclear organization are cut at many sites, as already mentioned, well before the actual fragmentation of the chromosomes into subchromosomes. During the former process, the micronucleus-specific sequences of DNA are deleted with rejoining of the ends. In some cases, sequences which are normally deleted (micronucleus-specific ones) can be conserved in the macronucleus, which is apparently the molecular basis of exceptional viability of some amicronucleate clones of *Tetrahymena* (Karrer et al. 1984). Such facultatively deleted sequences have been cloned and found to occur only in some cell lines (White and Allen 1986). In a broader sense, this is one of the manifestations of the phenomenon of alternative processing, when the same chromosome can be cut in two or more alternative ways (Austerberry and Yao 1988). This phenomenon occurs also in *Paramecium* (Caron 1992).

6. RIBOSOMAL RNA GENES

The family of ribosomal RNA genes, the so-called ribosomal DNA (rDNA), has in the early eighties already been known to consist in all ciliates of short free molecules, and that not only in ciliates with gene-sized DNA in the macronucleus, but also in ciliates with larger DNA which is now known to be subchromosomal (reviews: Blackburn 1982; Raikov 1982, 1989). This family of genes, which is usually transcribed as a single molecule of preribosomal RNA, contains genes for 16-18 S rRNA, 5.8 S rRNA, and 26-28 S rRNA.

The rDNA of *Tetrahymena* is known to be represented by free molecules about 21 kbp in size, i.e. which are smaller than any other subchromosomes (Altschuler and Yao 1985). These molecules are palindromic dimers, i.e. have two sets of ribosomal genes in mutually inverted position. The genes for 17 S rRNA are near the centre of the molecule, the genes for 26 S rRNA are distal, and the genes for 5.8 S rRNA are between them (Karrer and Gall 1976, reviewed by Din and Engberg 1979, Blackburn 1982, Raikov 1982, Engberg 1985, Hufschmid 1985, Raikov 1989). Both replication and transcription start near the centre of the molecule and proceed towards the free ends (Truett and Gall 1977, Borkhardt and Nielsen 1981, Cech and Brehm 1981, Cech et al. 1982). The rDNA molecules are assembled inside the nucleoli; they combine with histones to form nucleosomes, and represent the so-called r-chromatin (review: Vavra et al. 1982).

A complete sequence of the palindromic rDNA molecule of *Tetrahymena thermophila* has been obtained recently (Engberg and Nielsen 1990). Intervening sequences (introns) have been found inside the 26 S rRNA genes of some species of *Tetrahymena*; they are transcribed with the gene but later excised from the RNA by autocatalytic

self-splicing (Din et al. 1979, Wild and Gall 1979, Kan and Gall 1982, Kruger et al. 1982, Cech 1985, Sharp 1985). Both ends of the palindromic rDNA molecules of *Tetrahymena* have telomeric repeats of 20-70 copies of the hexanucleotide C4A2 (G4T2 with its overhanging single-chain terminus in the complementary chain) (Blackburn and Gall 1978).

The palindromic rDNA molecules of *Tetrahymena* form by excision of the ribosomal gene from a micronuclear chromosome. This gene in the micronuclear genome is, exceptionally, single-copy and monomeric (not palindromic) (Yao and Gall 1977, Yao 1981, Pan et al. 1982). Then, the excised gene is amplified, but at first in a monomeric form, only 11 kbp long and with a telomeric repeat (C4A2)n at only one end (Pan and Blackburn 1981). These non-palindromic molecules exist in developing macronuclei and are gradually replaced with maturation of the macronuclei by the inverted dimeric (palindromic) form (Yao et al. 1985).

The copy number of palindromic rDNA molecules in *Tetrahymena,* resulting from the process of amplification, is about 170-200 per unit genome (Engberg 1985). If this genome is repeated about 45 times in the adult macronucleus, the total number of rDNA molecules in it can reach 7500-9000. However, the copy number of rDNA strongly depends on feeding and other external conditions.

Yet in another tetrahymenine ciliate, *Glaucoma chattoni,* the rDNA molecules are non-palindromic free monomers 9.3 kbp long (Katzen et al. 1981). Unlike the intermediate monomeric form of rDNA in *Tetrahymena,* they have telomeric repeats of the hexanucleotide C4A2 at both ends (review: Blackburn 1982).

The rDNA of *Paramecium tetraurelia* proved to consist mainly of tandemic repeats of a 8 kbp large monomer, the number of repeats in one molecule varying from 2 to 13 (Findly and Gall 1978, 1980). The monomer consists of the usual small subunit, 5.8 S, and large subunit ribosomal RNA genes (one copy of each). The copy number of rDNA (the number of monomers) is in *Paramecium tetraurelia* 32-100 per unit genome or 62 to 170 thousand per macronucleus (Raikov 1989).

In the hypotrichs, the ribosomal gene family exists as free monomeric molecules. It includes as usual one gene for the small subunit rRNA, one gene for 5.8 S rRNA, and one gene for the large subunit rRNA (Lipps and Steinbrück 1978, Spear 1980, Swanton et al. 1982). The mini-chromosomes containing rDNA are not very conspicuous among the other mini-chromosomes; they measure about 7.5 kbp (Lipps and Steinbrück 1978, Swanton et al. 1982). As other mini-chromosomes, the rDNA molecules have telomeric repeats of the octonucleotide C4A4/G4T4 at both ends (Klobutcher et al. 1981). The copy number of the rDNA mini-chromosome is about 50 per unit genome or about 750 thousand for the entire macronucleus in *Stylonychia* (Lipps and Steinbrück 1978). The small subunit rRNA gene of *Oxytricha* and *Stylonychia* has been sequenced (Elwood et al. 1986). The rDNA of the hypotrichs forms nucleosomes (Butler et al. 1984).

So far little is known about the ribosomal DNA in other ciliates. In the scuticociliate *Uronema*, the rapidly renaturating DNA component (kinetic complexity about 15 kbp) seems to be rDNA (Soldo et al. 1981b). The same occurs in the macronucleus of the colpodean *Bursaria* (S. Borchsenius and Sergejeva 1979). In both cases, the ribosomal DNA seems to exist as free molecules. In the phyllopharyngean *Chilodonella,* which has a heteromeric macronucleus (see above), the rDNA is localized in the orthomere and the endosome but not in the DNA-poor paramere (Radzikowski and Steinbrück 1989).

The 5 S rRNA genes were already known to occur in the macronucleus separately from the main rRNA gene family (Raikov 1982). In *Tetrahymena*, they are united in tandemic clusters in some subchromosomes, with 5-20 genes in each (Pederson et al. 1984b). They also display an altered nucleosomal structure which is possibly related to rapid transcription (Pederson et al. 1984a). In *Euplotes eurystomus* and probably in other hypotrichs, each 5 S gene forms a separate mini-chromosome which is present in about one million copies in the macronucleus. This molecule has been entirely sequenced and found 930 bp long, with the only coding region of 120 bp. This mini-chromosome has typical telomeres and forms 4 nucleosomes (Robertson et al. 1989).

7. COPY NUMBER CONTROL AND MACRONUCLEAR DIVISION

Interesting is the question as to how the genetic balance of the macronucleus is maintained, i.e. how the increase of aneuploidy in daughter macronuclei is avoided during asexual reproduction. Prolonged amitotic division would lead to a loss of some genes and, as a consequence, to increased mortality. As a matter of

fact, however, the ciliates are capable of a long and sometimes indefinite vegetative reproduction. During reproduction, one allele of a gene is more or less rapidly lost, but somehow this does not happen to both alleles; in other words, all the loci are conserved though they finally become homozygous as a result of the outnumbering of one allele by the other (review: Larson et al. 1991).

In former years, there existed various hypotheses explaining the maintenance of genetic completeness during amitotic divisions of the macronucleus. Most hypotheses assumed that macronuclear genomes somehow maintained their physical integrity inside the polyploid macronucleus, in form of separate "subnuclei". Therefore, even random segregation of such complete genomes would not lead to aneuploidy, but only to variations in the ploidy degree (the so-called subnuclear hypotheses, see Raikov 1982). However, we know now that the macronucleus contains not only no subnuclei but even no complete chromosomes. The chromosomes are always fragmented in hundreds or thousands of pieces containing either a single gene (mini-chromosomes) or several genes (subchromosomes). But in both cases the chromosome fragments have no mechanism for precise segregation of replicated molecules between daughter macronuclei, since both lack centromeres. Consequently, the chromosome fragments can be segregated only at random.

For ciliates with a very small macronuclear unit genome repeated thousands of times (e.g., in *Stylonychia lemnae* or *Paramecium bursaria*, see Table 2), it can still be supposed that the segregation of their macronuclear DNA molecules (be it mini-chromosomes or subchromosomes) is in fact only random. In this case, the maintenance of genic completeness can for a long time (hundreds of generations) be assured by purely statistical laws. The "stock" of copies of each gene would last for 400-500 generations before lethal loss of genes begins (Ammermann 1971, Preer and Preer 1979). Such is in fact the observed longevity of clones of most ciliates. But this hypothesis contradicts the facts when we consider the ciliates with a relatively low average copy number of subchromosomes in the macronucleus, such as *Tetrahymena* where this number is about 45. Calculations show that mortality should begin here within dozens of generations; though, amicronucleate *Tetrahymena* clones can live for decades. Consequently, mechanisms for regulating the copy number of each subchromosome or mini-chromosome should exist (Larson et al. 1991, Prescott 1994). The mechanism should somehow distinguish, which DNA molecules are underrepresented and which are overrepresented (probably beyond certain limits) in result of random distribution at division. In this case the former would replicate two or more times and the latter would not replicate at all in the next cell cycle.

In the recent years, such a mechanism of copy number control has actually been observed in both *Tetrahymena* and the hypotrichs. This has been demonstrated especially with respect to the rDNA sequence of *Tetrahymena*, the copy number of which is about 200 times higher than that of other subchromosomes and is controlled independently (Larson et al. 1991). It also proved to be strain-specific (Kunze and Cleffmann 1991). The copy number of another gene (probably, the copy number of the respective subchromosome) is normally reduced when the cells enter the stationary phase, unlike several other genes which do not significantly change their copy numbers (Kunze and Cleffmann 1991). The copy number of molecules carrying various H4 histone gene versions in *Tetrahymena* consistently differs as much as tenfold; the copy number control mechanism distinguishes loci but not alleles of one locus (Brunk and Havas 1992).

In the hypotrichs, the various gene-sized macronuclear DNA molecules are amplified to an unequal degree. In *Stylonychia lemnae,* the degree of amplification of different mini-chromosomes increases differentially with aging of the clones and the copy number of each molecule is genetically controlled (Steinbrück 1983). The multiple (6 in all) size classes of gene-sized molecules belonging to the 81-MAC family in *Oxytricha fallax* all display independent control of their copy number (Herrick et al. 1987a, b). Different copy numbers are maintained independently for each mini-chromosome also in *Euplotes crassus* (Baird and Klobutcher 1991) and some of them increase differentially during clonal aging in *Oxytricha nova* (Harper et al. 1991).

So it is almost certain that the subchromosomal or gene-sized molecules in the macronucleus are segregated at random but then there is a mechanism recognizing their copy number and correcting it thereafter.

Acknowledgements. This work was carried out with the financial support of the Russian Basic Research Foundation under the project No. 93-04-21803 and the Russian State Scientific and Technical Program "Frontiers in Genetics", Section III-1. This publication was made possible also in part by Grant No. R4X000 from the International Science Foundation.

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Received on 24th January, 1995

Acta Protozoologica (1995) 34: 173 - 180

ACTA
PROTOZOOLOGICA

A Zoological Classification System of Cryptomonads

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Summary. Some zoological classification systems of cryptomonads, a group of ambiregnal protists, are reviewed. In addition, a system recently erected under the International Code of Botanical Nomenclature is provided with a zoological equivalent, that is, a system where taxa are named according to the International Code of Zoological Nomenclature. A "dual" (ICZN - ICBN) classification system of cryptomonads is needed to avoid a potentially destabilizing removal of the group from one or the other Code under which it has traditionally been treated. The system allows for the existence of 3 orders based on the presence and position of the nucleomorph. In the order Goniomonadida (single family: Goniomonadidae), nucleomorphs and plastids are absent. In the order Pyrenomonadida (single family: Pyrenomonadidae), the nucleomorph is positioned in an invagination of the pyrenoid. In the order Cryptomonadida, which is subdivided into 2 families (Cryptomonadidae and Hemiselmidae) based on the position of the flagella, the nucleomorph is positioned outside the pyrenoid. The system is consistent with available data on small subunit ribosomal RNA sequences.

Key words. Ambiregnal protists, Cryptomonadea, Cryptomonadida, Cryptomonadidae, cryptomonads, Goniomonadida, Goniomonadidae, Hemiselmidae, nomenclature, Pyrenomonadida, Pyrenomonadidae, systematics.

INTRODUCTION

Cryptomonad flagellates (cryptomonads) are nutritionally heterogeneous. Many are photosynthetic, but 2 genera (*Chilomonas* and *Goniomonas)* are entirely heterotrophic. In addition, several photosynthetic forms are not obligately so, and can feed heterotrophically depending on environmental or culture conditions. Owing to their nutritional heterogeneity, the cryptomonads fall within the concept of "ambiregnal" protists (Patterson 1986; Patterson and Larsen 1991, 1992) - protists whose nomenclature is regulated both by the International Code of Zoological Nomenclature (ICZN) and the International Code of Botanical Nomenclature (ICBN). The nomenclatural problems raised by the ambiregnal status have been summarised and discussed by Patterson (1986), Corliss (1992), Patterson and Larsen (1991, 1992), Hawksworth (1994), and Hawksworth et al. (1994). Some authors have also proposed formal revisions of the ICBN and ICZN to deal specifically with ambiregnal protists (e.g. Taylor et al. 1987).

Perhaps the single most important nomenclatural consequence of the ambiregnal status is that a given taxon may be known under two different names, each one correct under its respective Code. As a result, in recent

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taxonomic works on ambiregnal protists (e.g. Larsen and Patterson 1990) it is becoming customary to specify the correct name of taxa under each Code.

In a recent paper (Novarino and Lucas 1993b), some botanical classification systems of cryptomonads were briefly reviewed. A new system was also proposed in which suprageneric taxa were named according to the ICBN. In the present paper, following a brief review of some zoological classification systems, the botanical system of Novarino and Lucas (1993b) is provided with a zoological equivalent, that is, a system in which taxa are named according to the ICZN.

SOME ZOOLOGICAL CLASSIFICATION SYSTEMS BASED ON LIGHT MICROSCOPY

Amongst zoologists, interest in the classification of the cryptomonads has dealt mainly with their position within the general systematic framework of the unicellular eukaryotes and their kin, with relatively little attention paid to suprageneric classification within the cryptomonads themselves. In the case of traditional, light microscopy-based classification, this can be easily appreciated from Hall's (1953) comprehensive review of classification systems available at his time of writing. Many authors of our century have considered the cryptomonads as an order of flagellate or phytoflagellate protozoans (e.g. Doflein and Reichenow 1928, Borradaile and Potts 1935, Hyman 1940, Hall 1953, Mackinnon and Hawes 1961, Honigberg et al. 1964, Lepsi 1965, Kudo 1966, Grell 1973, Levine et al. 1980, Lee et al. 1985, Puytorac et al. 1987, Mehlhorn and Ruthmann 1992). Amongst those authors, only Hall (1953), Lepsi (1965), Kudo (1966) and Puytorac et al. (1987) addressed the question of suprageneric classification below the level of order. Hall (1953) recognised 3 families: the Cryptochrysidae, where an open "furrow" is present on the ventral cell surface; the Cryptomonadidae, without a "furrow" but with a closed gullet; and the Nephroselmidae, with laterally attached flagella. Lepsi (1965) followed Hall (1953) except for the fact that he did not recognise the Cryptochrysidae. Kudo (1966) recognised 2 suborders: the Phaeocapsina (not subdivided into families), where non-motile stages are dominant, and the Eucryptomonadina, where the motile flagellate stage is dominant. In the Eucryptomonadina he recognised 2 families: the Cryptomonadidae, with anterior flagella, and the Nephroselmidae, whose definition corresponded to that of Hall (1953). Puytorac et al. (1987) recognised 3 families: the Cryptomonadidae (no definition given); the Planonephridae, with laterally attached flagella; and the Cyathomonadidae, based on *Cyathomonas truncata* sensu auctorum, a phagotrophic cryptomonad correctly referred to as *Goniomonas truncata* (Fresenius) Stein, 1878.

Alternative views on cryptomonad classification, though few in number, have been available since the early 1900's. Calkins (1909) considered the genera of cryptomonads known to him (*Cryptomonas* and *Chilomonas)* as members of the phytoflagellate order Chrysoflagellida. This order, which was not subdivided further, included all of the photosynthetic flagellates "with yellow chromatophores". Doflein (1909) considered the cryptomonads as a suborder (Cryptomonadina, not subdivided further) of the flagellate order Chromomonadina, which - analogous to the Chrysoflagellida of Calkins (1909) - included the photosynthetic flagellates with yellow plastids. A similar view was taken by Claus et al. (1932), except that those authors considered the cryptomonads as forming a single family (Cryptomonadidae) within the order Chromomonadina. By contrast, in the textbook by Grassé (1952), the cryptomonads were considered as forming a class of flagellates in their own right. This class was named "Cryptomonadines" and was not subdivided further.

All of the classification systems cited above appear inadequate for two reasons. First, they include suprageneric taxa based on genera no longer recognised as belonging to the cryptomonads, or of doubtful status. (For a selected list of those genera, see Novarino and Lucas 1993b). Second, because they are based on light microscopy only they do not provide a framework where taxa described using electron microscopy (Santore 1984; Hill 1991a, b; Hill and Wetherbee 1986, 1988, 1989, 1990; Novarino 1991a, b; Novarino and Lucas 1993a; Novarino et al. 1994) can be easily accommodated.

RECENT CLASSIFICATION SYSTEMS BASED ON ELECTRON MICROSCOPY

Very few classification systems of cryptomonads have been proposed in recent years. Based on ultrastructural rather than light-microscopical features, they still pay little attention to suprageneric classification within the cryptomonads themselves. Cavalier-Smith (1989, 1993) considered the cryptomonads as forming 2 classes within the

kingdom Chromista, phylum Cryptista. These classes were named Cryptomonadea and Goniomonadea (synonym: Cyathomonadea) and were not subdivided further. The Cryptomonadea were defined as cryptomonads with plastids, periplastidial membranes, periplastidial ribosomes, and nucleomorphs, whereas the Goniomonadea lacked plastids and nucleomorphs, and possessed a different type of flagellar ornamentation. Corliss (1994) maintained this system, except that he used the phylum name Cryptomonada instead of Cryptista.

To the authors' knowledge, there are only 2 more classification systems based on electron microscopy. Both systems address the question of suprageneric classification within the cryptomonads themselves, but have no zoological equivalents because they have been erected under the ICBN. One of these systems has been discussed briefly by Novarino and Lucas (1993b), who have also proposed the other one. The system which follows provides a zoological equivalent of the system of Novarino and Lucas (1993b).

CLASS CRYPTOMONADEA *emend.* Cavalier-Smith, 1989 *emend.* Novarino and Lucas

Equivalent to Cryptophyceae *emend.* Cavalier-Smith (1989) *emend.* Novarino and Lucas (1993b) (ICBN).

Plastids and nucleomorphs either present or absent. Otherwise as in the class Cryptomonadea *emend.* Cavalier-Smith, 1989. Three orders:

I. Order Goniomonadida (Cavalier-Smith) Novarino and Lucas

Equivalent to Goniomonadales (Cavalier-Smith) Novarino and Lucas (1993b) (ICBN).

Basionym: Goniomonadea Cavalier-Smith, 1993 (Synonym: Cyathomonadea Cavalier-Smith, 1989).

The order Goniomonadida was first introduced by Novarino and Lucas (1993b) as Goniomonadales (ICBN), a new name and status for a class originally described by Cavalier-Smith (1989) under the name Cyathomonadea Cavalier-Smith, 1989. However, Novarino and Lucas (1993b) overlooked the fact that Cavalier-Smith (1993) had changed the name Cyathomonadea Cavalier-Smith, 1989 to Goniomonadea Cavalier-Smith, 1993. This name should now be considered as the basionym of Goniomonadida. Plastids and nucleomorphs absent. One family:

Family Goniomonadidae Hill, 1991 (ICZN)

Equivalent to the family Goniomonadaceae Hill (1991c) (ICBN) [Synonym: family Cyathomonadaceae Pringsheim (1944), sub Cyathomonadidae in Puytorac et al.(1987)].

With the characters of the order. One genus:

Goniomonas Stein, 1878 (ICZN, ICBN).

II. Order Pyrenononadida Novarino and Lucas

Emended spelling of Pyrenomonadales Novarino and Lucas (1993b) (ICBN), and thereto equivalent.

Nucleomorph positioned in an invagination of the pyrenoid. One family:

Family Pyrenomonadidae Novarino and Lucas (ICZN)

Corrected spelling (ICZN, Articles 32c iii and 32d) of Pyrenomonadaceae Novarino and Lucas (1993b) (ICBN), and thereto equivalent.

With the characters of the order. Two genera:

Pyrenomonas Santore, 1984 (Santore 1984) (ICZN, ICBN). Synonym: *Rhodomonas* Karsten, 1898 (Karsten 1898) *emend.* Hill and Wetherbee, 1989 (Hill and Wetherbee 1989) [See Novarino 1991a]

Rhinomonas Hill and Wetherbee, 1988 (Hill and Wetherbee 1988) (ICZN, ICBN)

III. Order Cryptomonadida Novarino and Lucas

Non Cryptomonadida *sensu auctorum.*

Emended spelling of Cryptomonadales Novarino and Lucas (1993b) (ICBN) *(non* Cryptomonadales *sensu auctorum),* and thereto equivalent.

Nucleomorph positioned outside the pyrenoid. Two families:

Family Cryptomonadidae Stein, 1878 (ICZN)

Corrected spelling (ICZN, Articles 32c iii and 32d) of Cryptomonadina Stein, 1878. Equivalent to the family Cryptomonadaceae *sensu* Butcher (1967) (ICBN).

Flagella inserted apically or subapically. Five genera:

Chilomonas Ehrenberg, 1831 (Ehrenberg 1831) (ICZN, ICBN)

Chroomonas Hansgirg, 1885 (Hansgirg 1885) (ICZN, ICBN)

Cryptomonas Ehrenberg, 1831 (Ehrenberg 1831) (ICZN, ICBN)

Plagioselmis Butcher, 1967 (Butcher 1967) *emend.* Novarino, Lucas and Morrall, 1994 (Novarino et al. 1994) (ICZN, ICBN)

Proteomonas Hill and Wetherbee, 1986 (Hill and Wetherbee 1986) (ICZN, ICBN).

Family Hemiselmidae Butcher, 1967 (ICZN)

Corrected spelling (ICZN, Articles 32c iii and 32d) of Hemiselmidaceae Butcher, 1967 (Butcher 1967).

Equivalent to the family Hemiselmidaceae Butcher ex Silva (1980) (ICBN) [Synonym: Planonephraceae Christensen (1967), sub Planonephridae in Puytorac et al. (1987)].

Flagella inserted laterally. One genus:

Hemiselmis Parke, 1949 (Parke 1949) (ICZN, ICBN). Synonym: *Planonephros* Christensen, 1967 (Christensen 1967).

Subgenera *Hemiselmis* Parke, 1949 (Parke 1949) (ICZN), equivalent to *Hemiselmis* Butcher, 1967 (Butcher 1967) (ICBN); *Plagiomonas* Butcher, 1967 (Butcher 1967) (ICZN, ICBN).

DISCUSSION

Systematics

In our classification system, most suprageneric taxa are defined on the basis of one character only. In future, this could help to reduce the proliferation of unnecessary descriptions of suprageneric taxa. It might be asked if suprageneric taxa based on few characters are meaningful from a phylogenetic point of view. This is difficult to establish in the case of the families included in our system, owing to the lack of relevant molecular data. However, the existence of 3 separate orders based on a single character is consistent with molecular data (see below).

Previous classification systems based on light microscopy made use of such features as the presence or absence of a ventral "furrow" in the vestibular region of the cell from which the flagella arise, and the importance of non-motile stages throughout the life-cycle (Hall 1953, Lepsi 1965, Kudo 1966). Those features appear to be inadequate for the purpose of suprageneric classification for the following reasons: (1) the term "furrow" may encompass artefactual and non-artefactual structures alike; non-artefactual "furrows" appear to be taxonomically significant at most at the species level (Novarino 1991b, Novarino et al. 1994); (2) it has long been recognised that many cryptomonads may assume a non-motile state depending on culture or environmental conditions (see Novarino and Lucas 1993b).

The present system considers that the major subdivisions (orders) of the cryptomonads can be defined on the basis of nucleomorph features:

(1) nucleomorphs and plastids absent (order Goniomonadida);

(2) nucleomorph placed inside an invagination of the pyrenoid (order Pyrenomonadida); and

(3) nucleomorph placed outside the pyrenoid (order Cryptomonadida).

This view is supported by molecular evidence. A tree of eukaryotes based on small subunit ribosomal RNA sequences (McFadden et al. 1994) suggests that *Cryptomonas* strain Φ (a member of the Cryptomonadida because the nucleomorph is placed outside the pyrenoid: see the ultrastructural study by Gillot and Gibbs 1980) and *Pyrenomonas salina* (a member of the Pyrenomonadida because the nucleomorph is placed inside the pyrenoid: see Santore, 1984) are phylogenetically distinct from one another, and both are distinct from *Goniomonas truncata* (Goniomonadida). Thus, the existence of the 3 orders Goniomonadida, Pyrenomonadida and Cryptomonadida is consistent with the molecular data available to date.

Some genera of cryptomonads described using electron microscopy are not included in the present classification system because it is felt that further information on those genera is desirable (Novarino and Lucas 1993b). This is also the case with some recently described genera for which no ultrastructural information is available, i.e. *Platychilomonas* Larsen and Patterson, 1990 (Larsen and Patterson 1990), and *Pseudocryptomonas* Bicudo and Tell, 1988 (Bicudo and Tell 1988).

Nomenclature

The class Cryptomonadea *emend.* Cavalier-Smith (1989) only includes cryptomonads with plastids and nucleomorphs. This definition excludes *Goniomonas truncata* (Fresenius) Stein, 1878 from that class. *Goniomonas truncata* (under the incorrect designation of *Cyathomonas truncata*) was placed by Cavalier-Smith (1989) in a class of its own, Cyathomonadea Cavalier-Smith, 1989, whose name was changed to Goniomonadea Cavalier-Smith, 1993 on a later occasion (Cavalier-Smith 1993). Novarino and Lucas (1993b) included *Goniomonas truncata* in the class Cryptophyceae (ICBN) by emending the diagnosis of that class to include forms lacking plastids and nucleomorphs, whilst at the same time lowering the rank of Cavalier-Smith's (1989) class based on *Goniomonas ('Cyathomonas') truncata* to that of an order. Molecular evidence that *Goniomonas truncata* is related to the host component of cryptomonads (McFadden et al. 1994) now lends further support to the idea that *Goniomonas truncata* should be placed in the same class as all the other cryptomonads, albeit in an order of its own to reflect its distinct ultrastructural features (Novarino and

Lucas 1993b). This change in classification with respect to the system of Cavalier-Smith (1989, 1993) is in agreement with the view of Patterson and Larsen (1992) that "the only legitimate changes in [protistan] classification are those which seek to better reveal insights into [phylogenetic] relationships". This change also suggests that taxonomic ranks are not superfluous concepts from a phylogenetic point of view, in agreement with Corliss (1994) but in contrast with the views of Patterson and Larsen (1992). In our opinion, the case of *Goniomonas* and the order based on it suggests that classification systems can be made to better reflect phylogenetic affinities by choosing the most appropriate rank for each suprageneric taxon.

The ICZN seems to give little weight to the concept of "rank", as shown, for instance, by the fact that the name of the original author of a taxon is maintained after a change in rank of that taxon (ICZN, Art. 50c). However, this is only mandatory for taxa in the family, genus, or species group, since the ICZN makes no provisions for taxa above the rank of superfamily. In the past, there has been a recommendation that, when elevating the rank of a suprageneric taxon of protists/protozoa, "... the responsibility for the name of the newly created, distinct, high-level group should be that of the person who established the concept (definition, boundaries, etc.) of that group" (Honigberg et al. 1964, Levine et al. 1980). This suggestion was very likely put forward to reduce the loss of information deriving from the strict application of Art. 50c of the ICZN to such systematically and nomenclaturally unstable assemblages of organisms as the protists/protozoa. However, in a recent protistan/protozoological classification scheme (Corliss 1994), Art. 50c of the ICZN has been effectively extended to higher-level taxa, resulting for instance in the recognition of a phylum "Cryptomonada Ehrenberg, 1838", when Ehrenberg (1838) did not erect a phylum for the cryptomonads but merely a family. In our classification system, the recommendations of Honigberg et al. (1964) and Levine et al. (1980) concerning the authorship of suprageneric taxa after a change in rank are extended to such cases where the rank of a taxon is lowered. Following the recommendation of Patterson and Larsen (1992), it also retains the botanical practice of the citation of basionyms (ICBN, Art. 33.2). This is more informative than non-citation, and therefore more suited for the needs of the protists/protozoa with their systematic and nomenclatural instability.

In the present system, the spelling of class and order names (not covered by the ICZN) follows the recommendations of Honigberg et al. (1964) and Levine et al. (1980). As far as the nomenclature of families is concerned, the following notes apply:

(1) the concept of the family Goniomonadidae corresponds to that of the family Cyathomonadidae as defined by Puytorac et al. (1987). The name Cyathomonadidae found in Puytorac et al. (1987), which can be considered as a corrected spelling (ICZN, Articles 32c iii and 32d) of the name Cyathomonadaceae Pringhseim (1944), is not used here because it is based on a misidentified type-genus (ICZN, Art. 65b);

(2) the definition of the family Cryptomonadidae corresponds in substance to that given by Kudo (1966). The most senior (earliest available) name for a family based on the genus *Cryptomonas* Ehrenberg appears to be Cryptomonadina [sic] Ehrenberg, 1838. (Earlier appearances of this name in the literature are disregarded here owing to the lack of verbal descriptions). This name is not adopted here because Ehrenberg (1838) assigned the genus *Chilomonas* to the family Monadina [sic] rather than Cryptomonadina, thus failing to recognise that genus as a genus of true cryptomonads. The next most senior names appear to be Cryptomonadina [sic] Perty, 1852, a name applied by Perty (1852) to a family which only included the type-genus, and Cryptomonadina [sic] Stein, 1878, a name applied by Stein (1878) to a family including both the genera *Cryptomonas* and *Chilomonas*. The family Cryptomonadaceae *sensu* Butcher (1967) (ICBN) also includes both the genera *Cryptomonas* and *Chilomonas* (Novarino and Lucas 1993b). Therefore, Stein's family (under the corrected spelling of Cryptomonadidae), is adopted here as the zoological equivalent of the family Cryptomonadaceae *sensu* Butcher (1967) (ICBN);

(3) the definition of the family Hemiselmidae corresponds to that of the family Nephroselmidae given by Hall (1953). The name Nephroselmidae found in Hall (1953), which can be considered as a corrected spelling (ICZN, Articles 32c iii and 32d) of the name Nephroselmidaceae Pascher (1913), is not used here because the genus *Nephroselmis* is not a genus of cryptomonads. (There is here a complex nomenclatural and taxonomic case for which the reader is referred to Silva (1980) for further details). The definition of the family Hemiselmidae also corresponds to that of family Planonephridae given by Puytorac et al. (1987), whose name can be considered as a corrected spelling (ICZN, Articles 32c iii and 32d) of the name Planonephraceae Christensen (1967). The name Planonephridae (Planonephraceae) is based on the generic name *Planonephros* Christensen, 1967 (Christensen

1967), which is regarded here as a junior synonym of *Hemiselmis* Butcher, 1967 (Butcher 1967). When a post-1960 generic name on which a family name is based is rejected as a junior synonym, the ICZN (Art. 40a) considers that the family name is not to be replaced unless the senior generic synonym is itself the basis of a family name, in which case the Principle of Priority applies to all family names concerned. The senior generic name *Hemiselmis* Butcher, 1967 (Butcher 1967) is the basis of the senior family name Hemiselmidae Butcher, 1967, which should therefore be used instead of Planonephridae.

As far as the genera included in our system are concerned, their names appear to comply with the rules of the ICZN and those of the ICBN as well. *Proteomonas* Hill and Wetherbee, 1986 has a homonym in *Proteomonas* Podlipaev, Frolov and Kolesnikov, 1990 (ICZN), a name of a genus of trypanosomes (Podlipaev et al. 1990). Under the ICZN, the name *Proteomonas* Podlipaev, Frolov and Kolesnikov, 1990 should be changed if it is regarded as a junior homonym of *Proteomonas* Hill and Wetherbee, 1986. This is in line with the essence of recommendation no. 12 in the paper by Hawksworth et al. (1994). (In addition, if trypanosomes are considered as ambiregnal, then they have a standing also under the ICBN - for instance, as Trypanosomatophyceae Silva, 1980, a class of algae. If this view is followed, then the name *Proteomonas* Podlipaev, Frolov and Kolesnikov, 1990 is a junior homonym of *Proteomonas* Hill and Wetherbee, 1986 also under the ICBN, and it should be changed under that Code as well. See ICBN, Art. 54.1).

CONCLUSIONS

The present paper has only addressed the question of classification *within* the cryptomonads themselves. This is commonly perceived by practising taxonomists as being more urgent than the elucidation of the correct position of the cryptomonads within the general systematic framework of the unicellular eukaryotes and their kin. The high-level classification of cryptomonads is currently the object of contrasting views, reflecting the state of flux of our knowledge of protists in general. In that respect, our classification system can be considered as neutral because it can be used within "chromistan" (Cavalier-Smith 1989, Corliss 1994), "protoctistan" (Margulis et al. 1990), "protistan" and "protozoological" (Patterson 1993) contexts, depending on personal preference. Its botanical counterpart (Novarino and Lucas 1993b) can also be used in "phycological" and "botanical" contexts.

An effort has been made to provide a system which reflects possible evolutionary relationships within the cryptomonads and is foreseeably able to accommodate hitherto undescribed genera with little difficulty. It is also hoped that the system will prove to be relatively easy to use. In this respect, it can be noted that the diagnostic character of the families of Cryptomonadida (the position of the flagella) can be observed with the light microscope. As suggested elsewhere (Novarino 1993), this might also be possible in the case of the nucleomorph, on whose presence and position the 3 orders of cryptomonads are based.

From a nomenclatural point of view, in order to avoid "exacerbating the problems" posed by ambiregnal protists (Hawksworth et al. 1994) it is felt that cryptomonads should continue to be treated under both Codes, which is the current practice for ambiregnal protists in general. Consequently, the correct name of suprageneric taxa under each Code should be presented whenever those taxa are referred to, in agreement with the "non-partisan" approach of Patterson and Larsen (1991). Any arbitrary promotion of the usage of only one Code might have negative effects, as noted by Hawksworth et al. (1994, p. 207): "The removal of an entire group from a Code under which it has traditionally been treated would be particularly destabilizing and should be avoided". Generally speaking, our "dual" classification system of cryptomonads is believed to be in agreement with available recommendations and suggestions on ambiregnal protists (e.g. Corliss 1992, Patterson and Larsen 1991, 1992, Hawksworth 1994, Hawksworth et al. 1994), and also with those provisions (e.g. Articles 45.5 and 54.1) which apply to ambiregnal protists in the "Tokyo Code", the latest edition of the ICBN (Greuter et al. 1994). The Tokyo Code seems to have taken into account the general principles underlying the formal revisions of the Codes of nomenclature proposed by Taylor et al. (1987) to deal with ambiregnal protists. It is to be hoped that the next edition of the ICZN, due to be published in 1996 (Hawksworth et al. 1994), will do the same.

Acknowledgements. We should like to dedicate this paper to the memory of the late A. D. Greenwood, as a small tribute to his discovery of the cryptomonad nucleomorph (Greenwood 1974). The reviewers' suggestions and constructive criticism are gratefully acknowledged. Thanks are also due to Drs P. K. Tubbs (International Commission on Zoological Nomenclature, London), and B. Pérez-Uz, D. M^{c}L. Roberts and A. Warren (The Natural History Museum, London), for comments on the original manuscript.

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Received on 14th November, 1994; accepted on 6th February, 1995

Acta Protozoologica (1995) 34: 181 - 185

ACTA
PROTOZOOLOGICA

Molecule Dependent Chemotactic Responses of *Tetrahymena pyriformis* Elicited by Volatile Oils

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Summary. Chemotactic potency of five volatile oils was studied on *Tetrahymena pyriformis* cells. The linalool-acyclic monoterpene alcohol from *Ocimum basilicum* was the only chemoattractant substance. The monocyclic and bicyclic monoterpene rich volatiles from *Mentha piperita* and *Salvia officinalis* - elicited very similar, dose dependent repellent effect. The citronellal - acyclic monoterpene alhedyde - from *Eucalyptus citriodora* had the strongest repellent character of the volatiles tested, but acyclic and monocyclic monoterpene alcohols (citronellol as well as isopulegol) are also present in it. The phenyl propane derivate from *Cinnamomum aromaticum* had also significant repellent effects. In contrast the tested non-volatile oil, Oleum helianthi presented a moderate repellent effect independent to the concentration applied. Our results indicate that effect of signal molecules derived from plants have specific, recognition-dependent effects on the unicellular model cell, *Tetrahymena*.

Key words. Chemotaxis, volatile oils, *Tetrahymena*, signalling.

INTRODUCTION

Volatile oils possessing characteristic scent are obtained by water stream distillation from leaves and/or flowers of several plants. Their role in plants is very obscure as they are stored like inclusions of different tissues and the very scent character (they are volatile at low temperatures) provides them a signal role in nature. Mono- and sesquiterpenes (C_{10} and C_{15}), and their alcohols, aldehydes, ketones etc. are the main components of the distilled, purified products of volatile oils (Petri 1991). Some of their physiological effects (Schilcher 1984) e.g. hyperemic effect in the skin or spasmolytic, stomachic or carminative effects in the intestinal tract were well-known in the ancient ages of medicine. Recent investigations demonstrated their antibacterial effect in Gram negative (*E. coli, Pseudomonas aeruginosa*), and in Gram positive (*Bacillus subtilis, Staphylococcus aeruginosa*) bacteria, and in *Candida albicans* (Jansen et al. 1986). Their fungicide character in *Trichophyton rubrum*, *Trichophyton equizum* was also demonstrated (Dikshit and Husain 1984), and there are references to their antihelminthic (Jain and Jain 1972) and atherosclerosis protective potency (Nikolaevskii et al. 1990).

Chemotaxis is an essential physiological response of animals of all phyla. In vertebrates several basic immunological responses, e.g. inflammation, are thought to be chemotaxis dependent processes (Roitt et al. 1985).

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The origin and character of corresponding chemoattractants is very diverse. In unicellular organisms chemoattraction is more fundamental in nutrition (Levandowsky et al. 1984) and mating (Nobili et al. 1987). In addition unicellular organisms, e.g. *Tetrahymena* respond to hormone-like substances, e.g. insulin, ACTH, T2 (Kőhidai et al. 1994a) and other signal molecules, eg. N-formyl-Noeleucine-Phenylalanine, of vertebrates (Kőhidai et al. 1994b).

Tetrahymena is a popular model cell of molecular and cell biology alike (Csaba 1985). These ciliated protozoans possess almost all pathways of signal transduction e.g. cAMP (Csaba and Lantos 1976, Kovács and Csaba 1987), IP_3 (Kovács and Csaba 1990), Ca^{2+}-calmodulin systems (Kovács and Csaba 1987) which are thought to be essential in higher ranks of evolution. The effect of a great number of signal molecules, e.g. hormones, drugs, inorganic substances was characterized on this cell and the responses elicited were evaluated on different levels of action, e.g. receptor binding (Hegyesi and Csaba 1992), membrane structure (Kőhidai et al. 1986a) and potential (Kőhidai et al. 1986b), metabolic pathways (Kőhidai and Csaba 1985), nuclear level changes (Kőhidai et al. 1985).

The purpose of our present study was to evaluate the chemotactic potential of some volatile oils obtained from different plants on *Tetrahymena* cells. There were two questions to be answered: (1) is there any concentration dependent chemoatractant or repellent potential for *Tetrahymena* of volatile oils from plants? (2) Is there any relation between the supposed chemotactic response evoked and the molecular structure of the substance?

MATERIALS AND METHODS

Tetrahymena pyriformis GL cells, maintained in 0.1% yeast extract containing 1% Bacto tryptone (Difco, Michigan, USA) medium at 28°C were used in the logarithmic phase of growth.

All the volatile oils tested were obtained from the suitable part of the plant by water steam distillation.

Salvia officinalis L. (Lamiaceae): "Folium salviae" is official in Hungarian Pharmacopoeaia (Ph.Hg. VII.) (Végh 1986). Its volatile oil content is min. 1.5 ml/100 g. The oil was obtained from the leaves by us. Characteristic compounds of the oil are eucalyptol 10%, thujone 26% and camphor 15%.

Ocimum basilicum L. (Lamiaceae) (Basil oil): this oil was obtained by us from the flowering herb of *Ocimum basilicum* cultivated in the Botanical Research Institute, Vácrátót (Hungary). The main component of the oil is linalool 40-60% (Lemberkovics et al. 1993).

Mentha piperitha L. (Lamiaceae): Aetheroleum menthae piperitae is official in the Ph.Hg. VII. It is obtained from the herb of the plant; it contains 3-9% of ester in methylacetate, min. 50% of menthol (free and bounded).

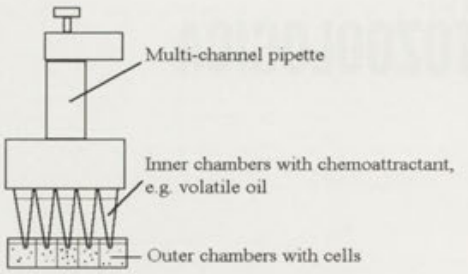


Fig. 1. Setup of capillary chemotaxis assay with a multi-channel pipette

Cinnamomum aromaticum Nees (Lauraceae): Aetheroleum cinnamomi is official in the Ph.Hg. VII. It is obtained from the leaves and branches of the tree. It contains min. 80% of aldehydes in cinnamic aldehyde.

Eucalyptus citriodora, Hook (Myrtaceae): It is originated from Pakistan. The oil is obtained from the leaves of the tree. Characteristic compounds of the oil are: citronellal 60-80%, citronellol and isopulegol (Masada 1976).

Oleum helianthi was applied as negative referent, non-volatile fatty oil. Each oil was tested in a concentration course in range of 10^{-6} - 1 vol%.

The assay of chemotaxis was a modified test of Leick (Leick and Helle 1983). According to this there are two chambers: the outer one is filled with the cells to be tested, the inner one contains the test substance with the chemoattractant or repellent volatile oil. Small capillaries serve as connecting junctions between the two chambers. In our setting tips of a multi-8-channel automatic pipette served as inner chambers (Fig. 1), this way we could minimize the standard error of sampling and the parallels gained were in good correlation. After 15 min incubation with the different concentrations of volatile oils the samples were fixed in 4% formaldehyde containing PBS, (0.05 M phosphate buffer, pH 7.2; 0,9 M NaCl).

The samples were evaluated by Neubauer hemocytometer. Each volatile oil was tested in five replica assays, the figures demonstrate the averages of these results. The statistical analysis was done by SigmaPlot 4.0 and Origin 2.8.

RESULTS

The five tested volatile oils elicited different, concentration dependent chemotactic responses in *Tetrahymena pyriformis* (hereafter we use the plant name which the volatile was obtained from to label the substance origin).

Salvia officinalis and *Mentha piperitha* had very similar profiles in respect of chemotaxis (Fig. 2). Both volatile oils had no chemoattractant character, they were only neutral at 10^{-3} vol% concentration. At the lower ranges the two volatile oils had moderate repel-

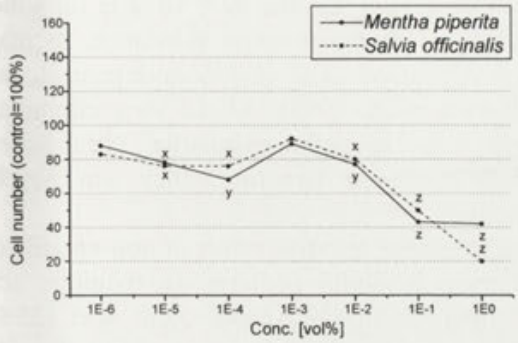


Fig. 2. Chemotactic activity of volatile oils prepared from *Mentha piperita and Salvia officinalis* in *Tetrahymena pyriformis* GL. (x $p<0.05$, y $p<0.01$, z $p<0.001$)

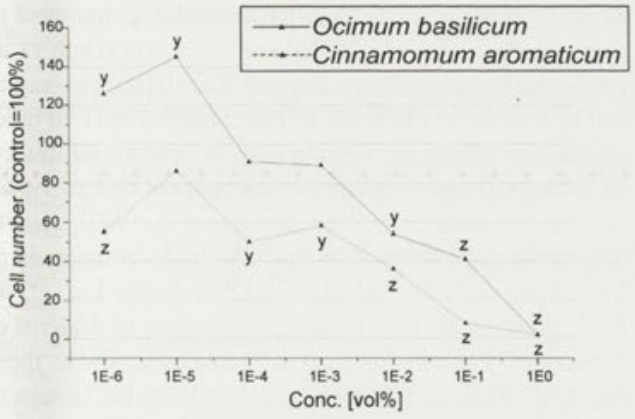


Fig. 3. Chemotactic activity of volatile oils prepared from *Ocimum basilicum* and *Cinnamomum aromaticum* in *Tetrahymena pyriformis* GL. (x $p<0.05$, y $p<0.01$, z $p<0.001$)

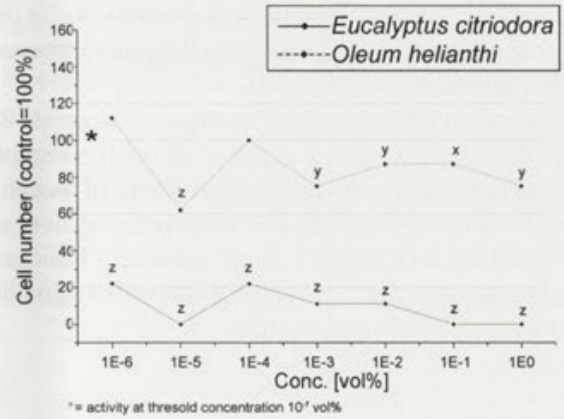


Fig. 4. Chemotactic activity of volatile oils prepared from *Eucalyptus citriodora* and Oleum helianthi in *Tetrahymena pyriformis* GL. (x $p<0.05$, y $p<0.01$, z $p<0.001$)

lent effect while in the higher concentration range (10^{-2}-1 vol%) they had a concentration dependent strong repellent effect.

Cinamomum aromaticum had the second strongest repellent character and it was significant in all concentrations tested. However the higher concentrations had the predominance (Fig. 3).

Ocimum basilicum was the only volatile which could work as an attractant. This positive chemotactic character was present in the low concentrations (10^{-6} - 10^{-5} vol%). The higher concentrations of this oil were also repellent to *Tetrahymena*.

Eucalyptus citriodora had the strongest repellent effect (Fig. 4). In the tested range of concentrations the response was between 11-22%. The well expressed repellent character urge us to find the threshold concentration which was 10^{-7} vol%.

The reference substance Oleum helianthi had significantly different effect compared to volatile oils. While the chemotactic profile of the signal molecules was always concentration dependent, the effect of Oleum helianthi was although slightly negative but roughly independent at the high concentrations (10^{-5} - 1 vol%). This non-signal molecule could induce a marked chemotactic effect only at the lowest (10^{-6} vol%) concentration.

DISCUSSION

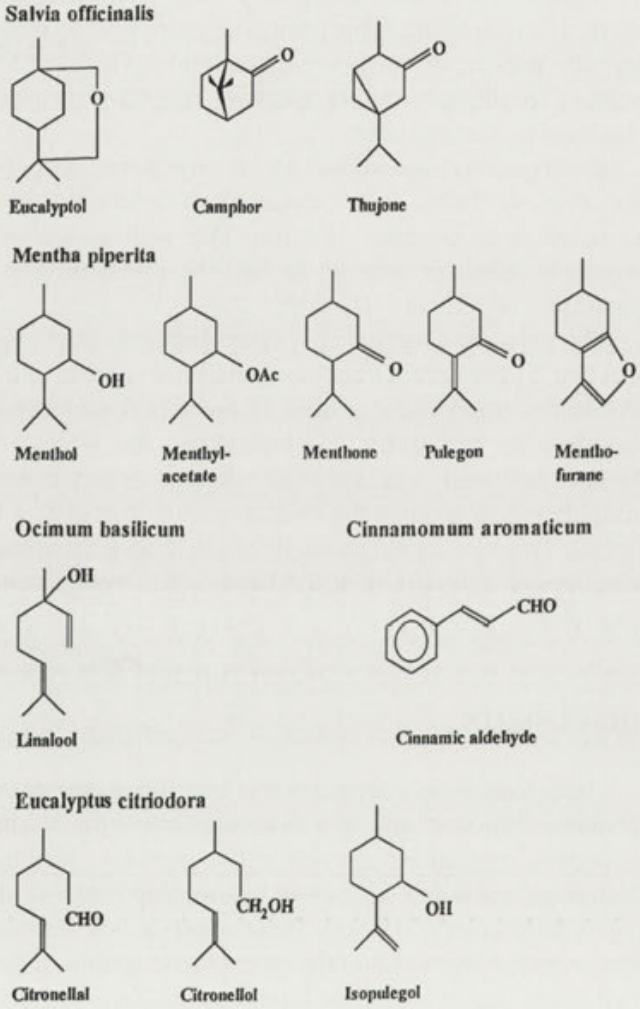
Numerous assays demonstrated that the chemotactic responses elicited and the molecular structure of the attractant molecules, e.g. bioactive peptides, insulin, histamine, serotonin have close relationship (Freer et al. 1979, Kőhidai et al. 1994a). These observations indicate that in the backgrounds of the chemotactic actions of the cell there are well defined interactions between the attractant molecule and the responsible receptor pool of the cell.

However the applied volatile oils are mixtures of some related terpenes (first monoterpenes) - as in general - in each case we can characterize the substance with the 1-3 major components (Table 1).

In the group of monocyclic monoterpenes the essential oil of *Mentha piperita* contains menthol (50-70%) further menthylacetate, menton, pulegon, menthofuran; *Salvia officinalis* has three main components: thuyon (30-40%), cineol (15%) and camphor (8%) (Végh 1986). In both essential oils the non-aromatic ring, the mono- and bicyclic monoterpene skeletons are predominant and this might result similarities in

Table 1

Characteristic components of essential oils investigated



chemotactic capacity. However monocyclic monoterpene alcohol, e.g. isopulegol are also present in *Eucalyptus citriodora* but there are two acyclic monoterpenes (citronellal an acyclic monoterpene aldehyde, and citronellol) (Masada 1976) presented the highest repellent activity.

Not only the two above mentioned monoterpenes provided repellent effect. *Cinnamomum aromaticum* had a more moderate repellent effect. In this volatile oil the predominant component is a phenyl propanederivatecinnamic aldehyde (76%) which has an aromatic structure (Végh 1986).

Ocimum basilicum was the only volatile oil which could serve as a chemoattractant substance. Its main component is linalool, an acyclic monoterpene alcohol (Lemberkovics et al. 1993). All of its predominant components, e.g. citronellol, citronellal, citral have a significantly different structure, they are acyclic monoterpenes.

The different but negative effect of non-volatile oil control Oleum helianthi contains an α-linolic acid (35%), β-linolic acid (13%) and oleic acid (35%). Although these molecules have long chains they did not induce chemotaxis. This negative result indicates that the plasticity of the non-ring structure itself without ketone or alcohol groups is not enough to induce positive chemotactic responses.

The components of the oils used have chemotactic effects in other organisms, too. Citronellol, component of *Ocimum basilicum* is repellent to mole rats (Heth et al. 1992) and larvae of *Aedes aegypti*. Eucalyptol also repellent to *Anopheles* (Schreck and Leonhardt 1991). In other aspect, the effect of volatile oils is always inhibitory, inhibiting enzymes - eudesmol, component of *Eucalyptus globulus* - (Satoh et al. 1992, Nojima et al. 1992); growth of bacteria and yeasts - cinnamic aldehyde, component of *Cinnamomum aromaticum* (Raharivelomanana et al. 1989, Saeki et al. 1989); citral, component of *Melissa* or *Citrus* oils (Végh 1986). This seems to be logical as these negative effects can defend the plants from the attacks of their enemies. However, in the case of *Tetrahymena* both repellent and attractant effects were observed. More repellent effect was observed, however the attractant effect of *Ocimum* was expressed in low concentrations. This demonstrates that *Tetrahymena* is very sensitive to chemical signals and can differentiate between the volatile oils. This differentiation is independent on the general aversion (of other organisms) to these oils.

The structure-dependence of chemotaxis suggests that the elicited responses were realized through a receptor mediated signalling pathway. The sensitivity of receptors is concentration-dependent. The observed negative and positive effects to a physiological response, chemotaxis draws our attention to the evolutionary accord of signalling mechanisms of plants and animals.

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Received on 13th September, 1994; accepted on 17th March, 1995

Acta Protozoologica (1995) 34: 187 - 192

ACTA
PROTOZOOLOGICA

Effects of UV-B Irradiation on Growth, Survival, Pigmentation and Nitrogen Metabolism Enzymes in Cyanobacteria

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Summary. The effects of artificial UV-B irradiation on growth, survival, pigmentation, nitrate reductase (NR), glutamine synthetase (GS) and total protein profile have been studied in a number of N_2-fixing cyanobacterial strains isolated from rice (paddy) fields in India. Different organisms show different effects in terms of growth and survival. Complete killing of *Anabaena* sp. and *Nostoc carmium* occurs after 120 min of UV-B exposure, whereas the same occurs only after 150 min of exposure in the case of *Nostoc commune* and *Scytonema* sp. Growth patterns of the cells treated with UV-B revealed that *Nostoc commune* and *Scytonema* sp. are comparatively more tolerant than *Anabaena* sp. and *Nostoc carmium*. Pigment content, particularly phycocyanin, was severely decreased following UV-B irradiation in all strains tested so far. *In vivo* NR activity was found to increase, while *in vivo* GS activity was decreased following exposure to UV-B for different durations in all test organisms; although complete inhibition of GS activity did not occur even after 120 min of UV-B exposure. SDS PAGE analysis of the total protein profile of the cells treated with UV-B shows a linear decrease in the protein content with increase in UV-B exposure time. Complete elimination of most of the protein bands occurs after 90 and 120 min of UV-B exposure in *Nostoc carmium* and *Anabaena* sp. whereas the same occurs only after 150 min of UV-B treatment in *Nostoc commune* and *Scytonema* sp.

Key words. *Anabaena*, cyanobacteria, glutamine synthetase, nitrate reductase, *Nostoc*, *Scytonema*, ultraviolet radiation.

INTRODUCTION

Cyanobacteria are phylogenetically the oldest group of oxygen evolving photosynthetic prokaryotes, which occupy an important place in both aquatic and terrestrial ecosystems (Newton et al. 1979, Döhler et al. 1986). The members of cyanobacteria are also unique in their cosmopolitan distribution ranging from hot springs to arctic regions, and are therefore expected to face different levels of UV-B (Stanier and Cohen-Bazire 1977). Due to their capacity to utilize atmospheric nitrogen, they occupy a central position in the nutrient cycling. These nitrogen fixing organisms use the enzyme nitrogenase to convert N_2 directly into ammonium (NH_4), a form through which nitrogen enters the food chain. It has been estimated that cyanobacteria fix over 35 million tons of nitrogen annually (Häder et al. 1989) which is thus available for use by higher plants. The role of nitrogen fixing cyanobacteria as a natural biofertilizer, increasing

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the fertility of various soils especially of rice (paddy) fields has been demonstrated by various investigators (Stewart 1980, Kumar and Kumar 1988, Sinha and Kumar 1992).

There is concluding evidence that the solar flux of ultraviolet-B (UV-B, 280-315 nm) has begun to rise over the surface of the earth (Crutzen 1992) due to continuing destruction of the ozone layer by atmospheric pollutants such as chlorofluorocarbons - mainly released from anthropogenic sources (Newton et al. 1979, Caldwell 1981, Worrest and Häder 1989, Madronich 1992). Ozone destruction is believed to intensify and spread to a broader range of latitudes throughout the next century (Toon and Turco 1991). All plant, animal and microbial groups appear to be susceptible to UV-B, but to a highly variable extent. UV-B is a small (less than 1% of total energy) but highly active component of the solar spectrum which has the potential to cause wide ranging effects, including mutagenesis, chronic depression of key physiological processes, and acute physiological stress that ultimately may result in death (Vincent and Roy 1993).

In higher plants, reduction of leaf area, fresh and dry weight, lipid content and of photosynthetic activities have been reported in a number of UV-B sensitive plant species (Biggs et al. 1981, Caldwell 1981, Tevini and Teramura 1989). It has also been reported that UV-B radiation affects many metabolic processes, pigmentation, phycobili protein composition, community composition, nitrogen metabolism and motility of several biological systems (Döhler 1985, Fischer and Häder 1992, Häberlein and Häder 1992, Helbling et al. 1992, Smith et al. 1992).

The aim of the present investigation was to study the effects of UV-B radiation on growth, survival, pigmentation, nitrate reductase, glutamine synthetase and total protein profile of four cyanobacterial strains isolated from Indian rice fields. Cyanobacteria are the dominant microflora of rice fields and it is predicted that any impact of UV-B radiation on nitrogen fixing cyanobacteria will adversely affect the productivity in such habitats (Häder and Worrest 1991).

MATERIALS AND METHODS

Isolation of organisms and growth conditions

Four nitrogen fixing cyanobacterial strains, e.g., *Anabaena* sp., *Nostoc carmium, Nostoc commune* and *Scytonema* sp. were isolated from rice fields near Varanasi, India. Cyanobacterial strains collected from rice fields were thoroughly washed by double distilled water and after mild homogenisation spread on solid agar (1.5%) in Petri dishes of different synthetic media and incubated in light (12 W/m^{-2}) at 20°C. After 10-12 days of incubation minute colonies started appearing on agar plates. Well spread individual colonies were carefully picked up and restreaked on fresh agar plates. Alternatively individual colonies were transferred into 10 ml liquid medium in a test tube and incubated in light. After 7-10 days of incubation, cultures growing in liquid medium were harvested by centrifugation (1500 x g for 10 min) and the resulting pellet was washed, suspended in minimum volume of liquid medium and the filaments were broken with sterilized glass beads by gentle shaking. The homogeneous suspension containing mostly individual cells or short filaments were spread on solid agar medium. Individual cells were marked and after 7-10 days of growth, colonies appearing on agar plates were examined microscopically and retransferred on fresh solid agar plates. Restreaking and subculturing were repeated several times with a view to obtain a single colony free from any contamination. These colonies were picked up and routinely grown in liquid medium. Out of all the media tested, these species show best growth in the medium of Safferman and Morris (1964). This medium was therefore routinely used for the cultivation of all the above strains. Cultures were grown in an autoclaved liquid medium (devoid of any combined nitrogen sources) in Erlenmeyer flasks filled to 40% of their nominal volume and placed in a culture room at 20°C and illuminated with fluorescent or incandescent light of 12 W m^{-2}.

Radiation source

Culture suspensions were transferred to sterile 75 mm Petri dishes and exposed to artificial UV-B produced from a transilluminator (peak at 312 nm, Bachofer, Reutlingen, F.R.G.), the radiation of which extended from 280 to 400 nm at about 5 W m^{-2}. The suspension was gently agitated by a magnetic stirrer during irradiation.

For determining the percent survival, 0.05 ml aliquots were withdrawn at desired time intervals during UV-B exposure and plated on agar plates. Percent survival was scored by colony counts. Similarly, samples were removed at known intervals for measuring growth, nitrate reductase and glutamine synthetase activities and total protein profile. Unless otherwise stated cultures having an initial dry weight of approximately 0.15 mg/ml were used in all experiments.

Protein estimation

Protein concentration was determined by the method of Bradford (1976). Bovine serum albumin was used as the standard.

Nitrate reductase activity

In vivo nitrate reductase activity was estimated by the method of Camm and Stein (1974). The nitrite formed was determined by the diazocoupling method of Lowe and Evans (1964). During UV-B irradiation, 1 ml aliquots were withdrawn at desired intervals and mixed with sulfanilamide. After an interval of 10 min NR dye (α-(N-1)-napthyl ethylenediamine dihydrochloride) was added and the absorbance (O.D.) of the pink color was estimated at 540 nm in a DU 70 spectrophotometer (Beckman, Palo Alto, CA, U.S.A.).

Glutamine synthetase activity

In vivo glutamine synthetase activity was determined by the method of Sampaio et al. (1979) with slight modifications. 1 ml aliquots were withdrawn at desired time intervals during UV-B exposure and mixed with 0.5 ml toluene and incubated for 10 min at 4°C.

The cells were then centrifuged to remove the toluene layer. The pellet was suspended in 0.2 ml washing buffer which contains 50 mM imidazole; 10 mM L-glutamic acid and 5 mM $MgCl_2$, pH 7.0. Thereafter, 0.8 ml of assay mixture (containing 0.1 M imidazole buffer (HCl) pH 7.0; 0.1 M glutamine; 1.0 M potassium arsenate; 0.01 M sodium ADP; 2.0 M hydroxylamine and 0.1 M $MnCl_2$) was added and incubated at 37°C for 30 min. Reactions were terminated by adding 2.0 ml of a stop mixture composed of 10% $FeCl_3$; 24% TCA and 6 N HCl. The absorbance (O.D.) of the pale yellow color was measured at 540 nm in a DU 70 spectrophotometer (Beckman, Palo Alto, CA, U.S.A.).

Absorption spectroscopy

Cells were harvested by centrifugation and immobilized in agar (0.5%, high gel strength) in quartz cuvettes having an optical path length of 10 mm (Hellma, Müllheim, F.R.G., 2 mm thickness). Absorption spectra of these immobilized cells were measured at regular intervals following UV-B exposure in a DU 70 spectrophotometer (Beckman, Palo Alto, CA, U.S.A.).

Gel electrophoresis

SDS polyacrylamide gradient gel electrophoresis was carried out in a vertical system (2001, Pharmacia LKB, Uppsala, Sweden) with gels of 155 x 130 mm, 1.5 mm thick using the method described by Laemmli (1970) with a gradient (5 - 15%) in the resolving gel. Gels were stained with Coomassie brilliant blue R 250 and dried in a gel dryer (Bio-Rad, Richmond, CA).

RESULTS

Growth patterns of four cyanobacterial strains, e.g., *Anabaena* sp., *Nostoc carmium, Nostoc commune* and *Scytonema* sp. were recorded at regular intervals for 7 days in liquid medium by estimating the protein content after 30 and 120 min of UV-B treatment (Fig. 1). None of the UV-B treated forms showed any growth for at least 3 days. Thereafter, the growth was slow and nearly 50% less in 30 min UV-B treated cells in comparison to that of the untreated control in almost all strains. The cells treated under UV-B for 120 min hardly showed any sign of recovery in *Anabaena* sp. and *Nostoc carmium*, whereas *Nostoc commune* and *Scytonema* sp. grew even after 120 min of UV-B treatment.

Figure 2 shows the survival curves (based on colony counts) of all four test organisms following UV-B exposure for different durations. After 30 min of UV-B exposure, there was about 70% and 65% survival in *Anabaena* sp. and *N. carmium*, respectively, whereas 80% survival were recorded in *N. commune* and *Scytonema* sp. After 60 min of UV-B treatment the survival was almost about 50% in *Anabaena* sp. and *N. carmium*, whereas, nearly 60% survival was recorded in *N. commune* and

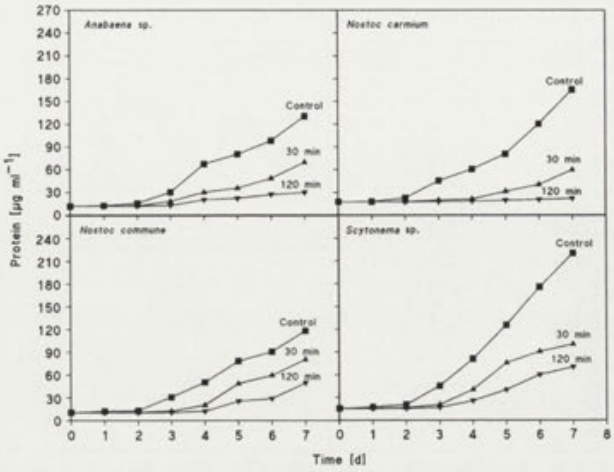

Fig. 1. Growth of the organisms in liquid medium. Exponentially grown cultures were exposed to UV-B at an irradiance of 5 W m^{-2} for 30 and 120 min and thereafter incubated in fluorescent or incandescent light. Growth was measured by estimating protein content at regular intervals for 7 days. Cultures having an initial dry weight of approximately 0.15 mg/ml were exposed to UV-B. The results are representative of three separate experiments. S.D. was consistently less than 10% of means

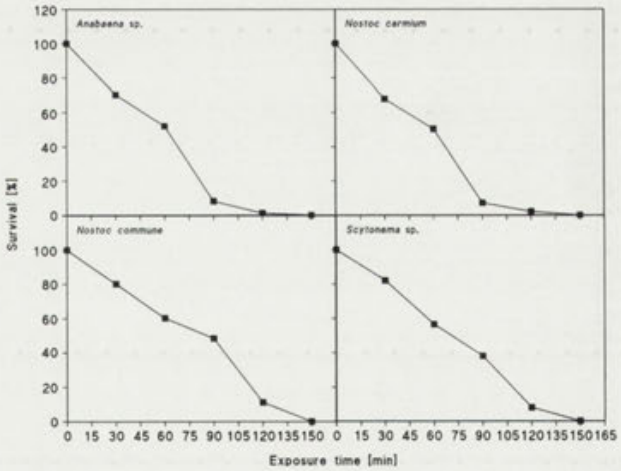

Fig. 2. Percent survival of the organisms after different durations of UV-B exposure. Equal number of cells of all the strains were plated on agar plates after UV-B exposure. The results are based on colony count. Other conditions were as in Fig. 1

Scytonema sp. Complete killing (100%) occurred after 120 min of UV-B treatment in *Anabaena* sp. and *N. carmium*, but in the case of *N. commune* and *Scytonema* sp. the same occurred only after 150 min of UV-B treatment. *Anabaena* sp. and *N. carmium* were found to be relatively more sensitive than *N. commune* and *Scytonema* sp.

Absorption spectra of the cells, immobilized in agar were determined after increasing exposure to UV-B radiation (Fig. 3). Five major peaks at 337, 437, 485,

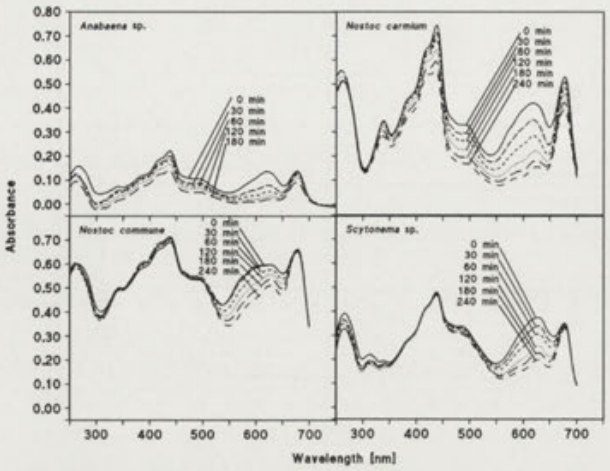

Fig. 3. Absorption spectra of the organisms (immobilized in 0.5% agar) following exposure to different durations of UV-B (for details see Materials and Methods)

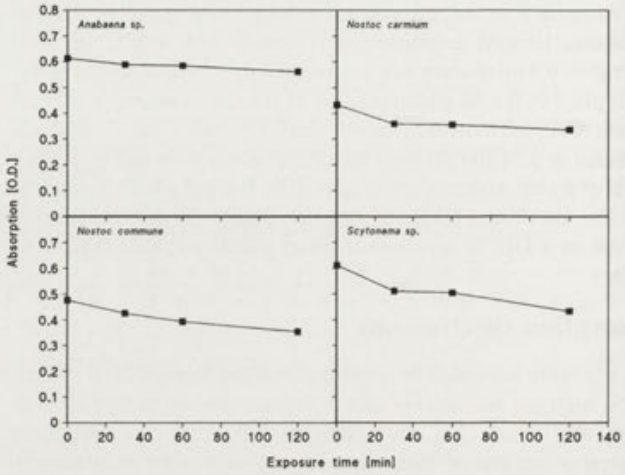

Fig. 5. *In vivo* glutamine synthetase activity of different organisms after increasing exposure to UV-B. Exponentially grown cultures were exposed to UV-B and thereafter aliquots were withdrawn at defined intervals and GS activity was determined as described earlier (see Materials and Methods). Other conditions were as in Fig. 1

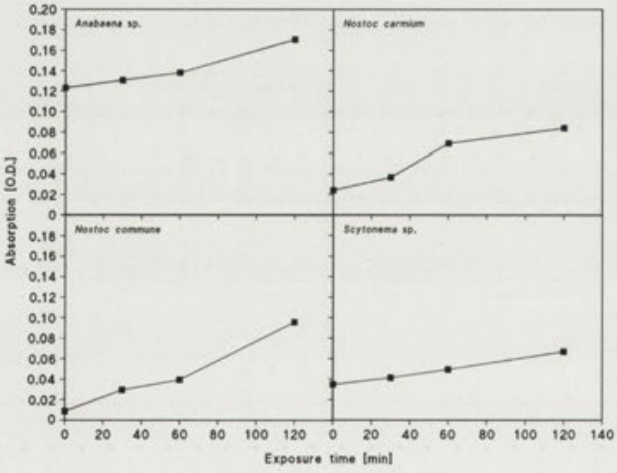

Fig. 4. *In vivo* nitrate reductase activity of different organisms after different durations of UV-B treatment. Exponentially grown cultures were transferred to the medium containing 5 mM nitrate and thereafter exposed to UV-B. At regular intervals aliquots were withdrawn and NR activity was measured instantly. Other conditions were as in Fig. 1

620 and 672 nm were recorded in all the test organisms. In the case of *Anabaena* sp. and *N. carmium* spectra show a steady decline in the absorption with increasing exposure to UV-B radiation, but in comparison to other peaks there was a drastic decline at 620 nm suggesting that phycocyanin is bleached more rapidly than any other pigments such as chlorophyll *a* (437 and 672 nm) or the carotenoids (485 nm). Although, phycocyanin was bleached drastically in *N. commune* and *Scytonema* sp., too, but there was not much effect on chlorophyll *a* and the carotenoids even after 240 min of UV-B exposure.

Figure 4 shows the effect of different durations of UV-B exposure on *in vivo* nitrate reductase activity. The activity increased linearly with the increase in UV-B exposure time in all the strains tested so far. Nitrate reductase activity was significantly higher in *Anabaena* sp. than that of other species.

Figure 5 shows the effect of different durations of UV-B radiation on *in vivo* glutamine synthetase activity. Interestingly, contrary to nitrate reductase activity, glutamine synthetase activity showed a gradual decrease with increase in UV-B exposure time, but there was no complete inactivation even after 120 min of UV-B exposure.

Possible changes in the total protein profile of the test organisms were investigated following the increasing exposure of UV-B. Figure 6 shows the SDS PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) analysis of the whole cell protein after UV-B exposure for different durations in *Nostoc carmium*. Each band shows a gradual decrease in protein content with increase in UV-B exposure time. After 90 and 120 min of UV-B exposure protein bands between the molecular weights of 14.2 and 45 kDa were completely eliminated. There was comparatively less effect between the molecular weights of 55 and 66 kDa even after 120 min of UV-B exposure. Similar observations were also recorded in *Anabaena* sp. (data not shown). *Anabaena* sp.

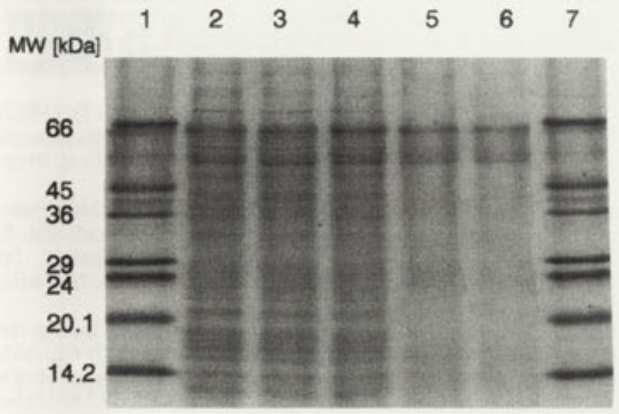

Fig. 6. Vertical SDS PAGE (gradient 5 - 15% T) of *Nostoc carmium* following increasing exposure to UV-B irradiation. Exponentially grown culture was irradiated under UV-B. At known intervals equal amount of cells were withdrawn and subjected to SDS PAGE. Lanes 1 and 7 marker proteins, lane 2 control, lane 3 - 30 min, lane 4 - 60 min, lane 5 - 90 min, lane 6 - 120 min UV-B treated cells

and *N. carmium* seem to be more sensitive in comparison to *N. commune* and *Scytonema* sp., where complete elimination of protein bands occurred only after 150 min of UV-B treatment (data not shown).

DISCUSSION

The present investigations suggest that cyanobacterial strains are sensitive to UV-B but to a variable extent. The discrepancy in their growth behavior and survival of different cyanobacterial strains after UV-B treatment might be due to the presence or absence of cyanobacterial sheath pigments. The filaments of *Nostoc commune* and *Scytonema* sp. are embedded in the mucilaginous sheath which is the site of the pigment called scytonemin which was found to function as a photoprotectant against UV radiation (Garcia-Pichel and Castenholz 1991). An occurrence of some UV protecting pigments and the UV sunscreen role of a mycosporine-like compounds have recently been shown in a number of cyanobacterial strains (Scherer et al. 1988; Garcia-Pichel and Castenholz 1991; Garcia-Pichel et al. 1992, 1993). Inhibitory effects of UV-B on some of the blue-green algae have been reported earlier (Newton et al. 1979, Döhler et al. 1986, Häder et al. 1986). It has been proposed by several workers that cellular constituents absorbing radiation between 280 and 320 nm are destroyed by UV-B radiation, which further affects cellular membrane permeability and protein damage, eventually resulting in the death of the cell (Newton et al. 1979, Caldwell 1981, Tevini and Teramura 1989). 100% killing of the cells exposed to UV-B is possibly due to the inactivation of a number of processes (Caldwell 1981, Häder and Worrest 1991). Our results on pigmentation indicate that the loss in phycocyanin is more drastic than any other pigment such as chlorophyll *a* or carotenoids. This is in accordance with earlier work on cyanobacteria (Donkor and Häder 1991) and cryptophyceae (Häberlein and Häder 1992). It has been shown that strong UV radiation can photo-oxidize and thereby bleach all types of photosynthetic pigments (Häder and Worrest 1991). Even at lower doses, UV-B sometimes causes a depression of chlorophyll *a* and carotenoids via reduced rates of biosynthesis.

UV-B induced stimulation of NR activity has been recorded in the present investigation. This apparent stimulation is also recorded in an angiospermic plant, *Crotalaria juncea* following UV-B exposure (Saralabai et al. 1989). Photoactivation of NR has been reported in *Neurospora crassa* (Roldan and Butler 1980). However, UV-B induced inhibition of the same enzyme has been reported in marine diatoms (Döhler 1990).

UV-B induced inhibition of GS activity has been reported in several marine diatoms (Döhler 1985). Our findings with cyanobacterial strains are in accord with the above finding. Other stresses such as NaCl also inhibit GS activity (Niven et al. 1987). But, there was no complete inhibition even after long hours of UV-B irradiation. To date, it is not well understood to what extent the effects of UV-B on the enzymes of nitrogen metabolism result from a direct impact on enzyme activity or whether enzyme synthesis is affected via RNA damage or to some other as yet unknown reasons.

Results on total protein profile show a linear decrease in protein content with increasing UV-B exposure time. UV-B absorption has a range of more general effects on cellular proteins. UV-induced protein damage can influence the ion permeability of cellular membranes and processes such as nitrogen fixation by cyanobacteria via inactivation of the nitrogenase enzyme (Häder and Worrest 1991). UV-B induced damage of photoreceptor pigments such as those from the presumed photoreceptor of the flagellate *Euglena* or the phycobiliproteins of cyanobacteria and certain flagellates have been reported (Häder and Worrest 1991, Fischer and Häder 1992).

We conclude that continued ozone depletion resulting in increased solar UV-B radiation might adversely affect the cyanobacterial population which in turn will affect the productivity in such habitats. It may further result in

the decline in primary production rates and a change in cyanobacterial community composition because of the differential sensitivity of individual species. Alterations in the species composition may expose the habitat to the development of toxic algal blooms.

Acknowledgements. R. P. S. gratefully acknowledges the DAAD and the Ministry of H. R. D., Govt. of India, for providing financial assistance in the form of a fellowship. This work was supported by the Bundesminister für Forschung und Technologie (project KBF 57) and the European Community (EV5V-CT91-0026) to D.-P. H. The authors are thankful to M. Lebert for extensive useful discussions.

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Received on 2nd December, 1994; accepted on 21st April, 1995

Acta Protozoologica (1995) 34: 193 - 201

ACTA
PROTOZOOLOGICA

Tintinnids in Terra Nova Bay - Ross Sea During Two Austral Summers (1987/88 and 1989/90)

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Summary. Antarctic populations of tintinnids were studied during the first (1987/88) and second (1989/90) Italian expedition in Terra Nova Bay (Ross Sea). We identified 6 genera and 14 species of tintinnids. The community structure was very simple, constituted by the typical Antarctic species. The genus *Cymatocylis* was abundant in the offshore stations, while the species *Codonellopsis gaussi* and *Laackmanniella naviculaefera* were abundant in the stations closer to the coast. The genus *Ptychocylis* was reported for the first time in this area. Most of the tintinnids found had hyaline loricae and showed a great morphological variability. For example, *Cymatocylis drygalskii* ranged in size from 170 to 400 µm long. The geographical distribution and the temporal pattern of tintinnids' abundance appeared in relation with those of chlorophyll *a*, which showed its highest values during the first cruise in the central eastern area of Terra Nova Bay.

Key words. Antarctica, Ross Sea, Tintinnids, geographical distribution, temporal pattern.

INTRODUCTION

In polar seas tintinnids represent a substantial percentage of the total heterotrophic microzooplankton (< 200 µm), which is the key link in the transfer of newly formed organic matter produced by pico- and nano-plankton to larger-size consumers (Hewes et al. 1985).

The first Antarctic studies on the microzooplanktonic fraction date back to the Deutschen Sudpolar Expedition in 1901- 1903. Laackmann (1907), provided the first account of Antarctic ciliates when he described 21 species of tintinnids in detail. These studies were mainly systematic-descriptive and lack all quantitative reference; besides, environmental data were really poor.

Later, Balech (1958, 1971, 1973) described the tintinnids collected in three cruises in subantarctic areas. He reported that *Coxliella cymatiocoides* might be a variant of *C. frigida* and might be considered as the only species among the tintinnids with bipolar distribution, as it was reported in the Arctic seas by Kofoid and Campbell (1929).

Heinbokel and Coats (1986) reported the populations found in the Weddell Sea in November 1983. Their values for tintinnid abundance in the surface waters were always low, with a maximum of 17 ind.dm^{-3}. Yet, they reported that Hentschel (1936) at similar latitudes in the South Atlantic and Littlepage

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(1968) in McMurdo Sound found concentrations of at least 100 ind.dm^{-3}.

Boltovskoy et al. (1989) found, in the Weddel Sea, 14 tintinnid taxa with mean cell numbers of 161 ind.dm^{-3} and a maximum of 859 ind.dm^{-3}. In the ice-covered southern area *Codonellopsis drygalskii* and *Laackmanniella prolongata* were dominant, while in ice-free waters *Cymatocylis gaussi*, *Cd. glacialis* and *Cy. affinis/convollaria* were the main component of the tintinnids.

Alder and Boltovskoy (1991) sampled in the surface layer of the Bellingshausen Sea and the Bransfied Strait. Average values of tintinnids, for the entire area surveyed, were 52 ind.dm^{-3}, and maximum values 589 ind.dm^{-3}. The Bransfield Strait was characterized by the dominance of *Cy. affinis/convallaria*. In the waters around the tip of the Antarctic Peninsula tintinnids were represented by *Cd. balechi* while those of the Bellingshausen Sea were characterized by *Laackmanniella* spp. and *Cy. drygalskii*. In the Weddel Sea, tintinnid carbon values represented 18% of overall microheterotrophic carbon at 0-150 m and, the highest concentration for this group was centered around 25-50 m (Boltovskoy and Alder 1992, Alder and Boltovskoy 1993). In the Weddel-Scotia Confluence area the same authors (1993) reported that tintinnids are concentrated at 50-90 m, with an average of 9.7 ind.dm^{-3}.

The antarctic populations of tintinnids were therefore fairly well known, though data on the area explored by the Italian expedition were lacking completely.

The main purpose of this research was both to produce a systematic description of the commonest organisms and to assess the tintinnids' density distribution in relation to the hydrodynamic features and vis á vis the distribution of phytoplankton biomass. Furthermore the study was focused on the population dynamic and the morphological variability of one of the commonest species: *Cy. drygalskii*.

HYDROLOGICAL FEATURES OF THE STUDY AREA

The study area of the first year (1987-1988) extended approximately between 74° 30' and 75° 30' S and 164° 00' and 166° 00' E corresponding to Terra Nova Bay, where the Italian base is located (Fig. 1), stretching between Cape Washington and the Drygalski Ice Tongue. The maximum depth (> 100 m) was reached in the central part of the Bay bordered by a high and rocky coast and characterized by an extremely steep sea-bottom: the 50 m isobath often lies at 50-100 m from the shore (Brambati et al. 1989). In the second year (1989-90) two coastal stations near the base were periodically sampled. The hydrology of the study area, belonging to the Ross Sea, is characterized during the summer period by the presence of a stable pycnocline and high temperature and salinity gradients. The surface layer, ascribable to Antarctic Surface Water (AASW), having a thickness not greater than 100 m, has temperature values that exceed 0° C. In the coastal area the surface layer is characterized by salinity higher than 34.7 psu and temperature ranging between 1.9°C to 2.1°C. This area is affected by prevailing surface thermal processes. Eastward the offshore surface water is affected by dilution processes due to the ice melting, consequently salinity is lower than 34.6 psu. Below the ASSW a homogeneous water body belonging to the Ross Sea Shelf Water (RSSW) is present. Here the salinity increases slightly towards the bottom with an average temperature of -1.9°C and salinity of 34.9 psu (Boldrin and Stocchino 1990). The surface currents are constituted by two branches: the first incoming from NE in the northernmost part of the Bay and the second from E in the southernmost one (below the latitude of 75° S). The first flux invades the central area and then turns N to constitute the coastal current. The second one, after reaching the Drygalski Ice Tongue, turns N to join the coastal current. Generally the surface circulation is characterized by a clockwise cyclonic stable gyre (Stocchino and Manzella 1991).

MATERIALS AND METHODS

The first campaign took place from 9th January, 1988 to 2nd February, 1988. Samples were collected with sampling bottles at 3 depths (surface, 50 m, and 100 m) for 12 stations in the Terra Nova Bay (Fig. 1).

In the second year of research, two stations in Terra Nova Bay were sampled. The samples were collected at st. Mergellina (M), located at about 250 m from the coast on a bottom 30 m deep (Fig. 1), at surface, 10 m and 25 m, on three dates (27.1., 2.2., 4.2.1990). At st. S. Maria Novella (SMN), which is in the centre of the Bay where the bottom is 500 m deep (Fig. 1), samples were collected at surface, 100 m and 200 m, on six occasions (23.1., 25.1., 30.1., 31.1., 1.2., 8.2.1990).

For each sample, 2 liters of water were collected and immediately filtered with 20 µm mesh, and reduced to 250 cc. Samples were

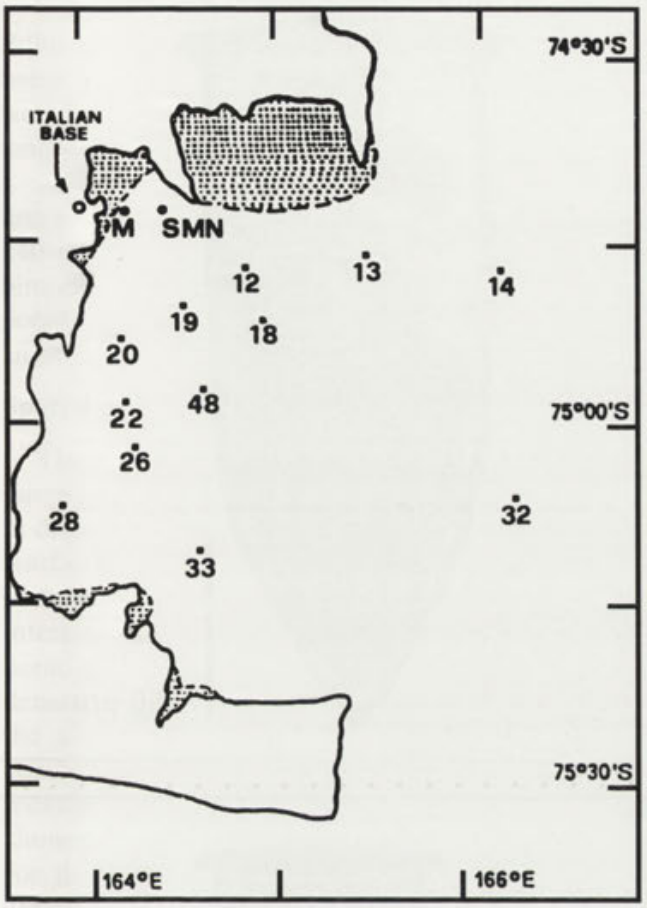


Fig. 1. Study area and sampling stations during the two cruises (1987/88 and 1989/90)

preserved with 4% formaldehyde solution buffered with sodium tetraborate. Subsamples (50-100 cm^3) were then examined in a settling chamber using a Leitz Labovert inverted microscope (x 200), according to the Utermöhl method (1958).

RESULTS

Taxonomical analyses

Following Kofoid and Campbell (1929, 1939), we determined 14 taxa of tintinnids in the coastal area, while in the offshore area 13 taxa were identified, for a total amount of 6 genera and 14 species (Table 1).

Our data are in agreement with Heinbokel and Coats (1986) who found 18 species of tintinnids in the Weddel Sea, mostly belonging to genera *Cymatocylis* and *Laackmanniella*, which are considered characteristic Antarctic genera (Pierce and Turner 1993).

Differences in the taxonomic composition were evident between the two sampled areas: in the nearshore one the dominant species was *Codonellopsis gaussi*, the genus *Cymatocylis* (*Cy. drygalskii* and *Cy. ecaudata*), and *Laackmaniella naviculaefera*, which was occasionally sampled offshore, whilst it seemed to be characteristic of the coastal area at deeper layers like *L. prolongata*, which was dominant also at the 50 m layer in the offshore area. *Ptychocylis* was found for the first time in this area. This genus, not collected during the second year, was previously considered as a boreal endemic (Pierce and Turner 1993). Following Davis (1981), who pinpointed the high morphological variability of the lorica shape of this genus, we prefer not to ascribe the specimens found to any species (Fig.2).

In our samples we found exclusively hyaline genera, except for *L. naviculaefera*, *L. prolongata* and *Cd. gaussi*, which presented a lorica partially agglutinated with diatom frustules.

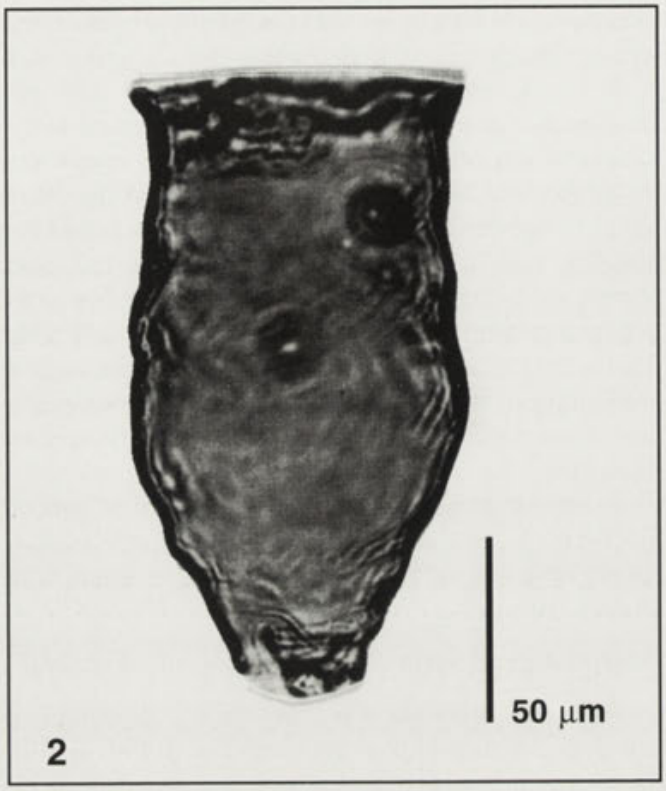
Morphological variability of *Cymatocylis drygalskii*

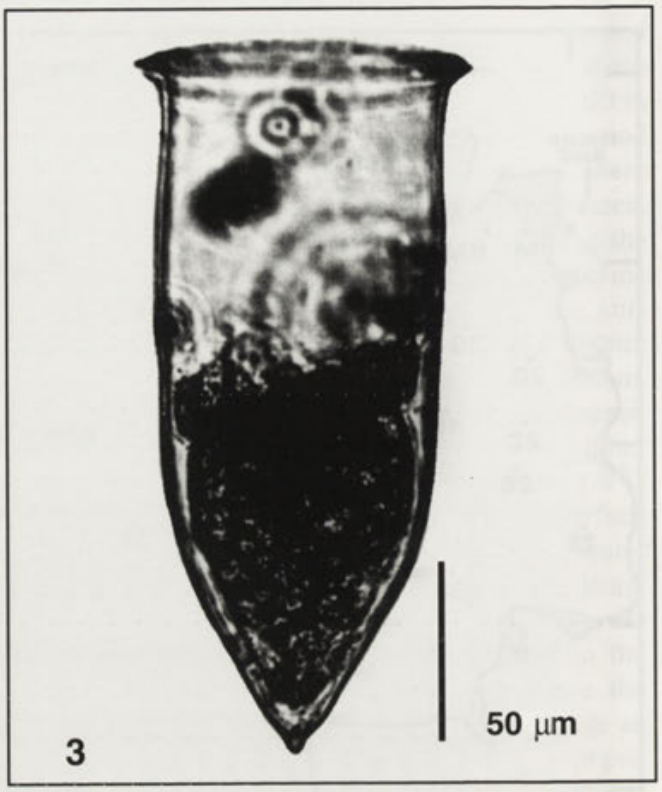
Particular attention was paid to *Cy. drygalskii*, a species determined following Laackmann (1907, 1910). In our samples this species was often a prevalent member of the genus *Cymatocylis*, considered by Schwarz (1964) as an Antarctic endemic. The specimens showed great morphological variability, as already pointed out

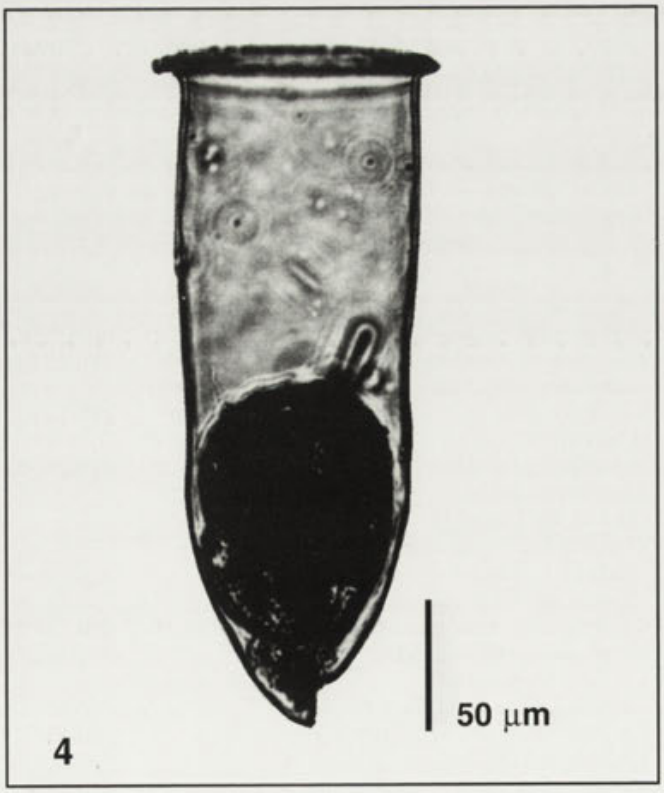
Table 1

List of tintinnid species and minimum and maximum concentration (ind.dm^{-3}) for the three depth layers sampled during the two cruises (1987/88 and 1989/90)

species	1987/88	1989/90
Codonellopsis gaussi (Laackmann)	(0.0-1003.2)	(0.0-155)
Cd. glacialis (Laackmann)	(0.0-73.05)	(0.0-15.38)
Codonellopsis sp. (Jorgensen)	(0.0-65.88)	
Laackmanniella naviculaefera (Laackmann)	(0.0-15.9)	(0.0-12.82)
L. prolongata (Laackmann)	(0.0-535.2)	(0.0-26.31)
Laackmanniella sp. (Kofoid and Campbell)	(0.0-66.25)	
Coxliella frigida (Laackmann)	(0.0-2.4)	
Coxliella sp. (Brandt)		(0.0-2.7)
Cymatocylis conica (Laackmann)	(0.0-2.4)	
Cy. drygalskii (Laackmann)	(3.8-156.35)	(0.0-230.7)
Cy. ecaudata (Kofoid and Campbell)		(0.0-162.78)
Cy. flava (Laackmann)	(0.0-153.9)	
Cy. folliculus (Kofoid and Campbell)		(0.0-12.8)
Cy. glans (Kofoid and Campbell)		(0.0-13.5)
Cy. nobilis (Laackmann)		(0.0-2.56)
Cy. subconica (Kofoid and Campbell)		(0.0-2.63)
Cy. vanhoffeni (Laackmann)	(0.0-16.2)	
Cymatocylis sp. (Laackmann)	(0.0-29.15)	(0.0-7.89)
Ptychocylis sp. (Brandt)	(0.0-4.3)	
Salpingella sp. (Jorgensen)	(0.0-28.5)	(0.0-5.0)







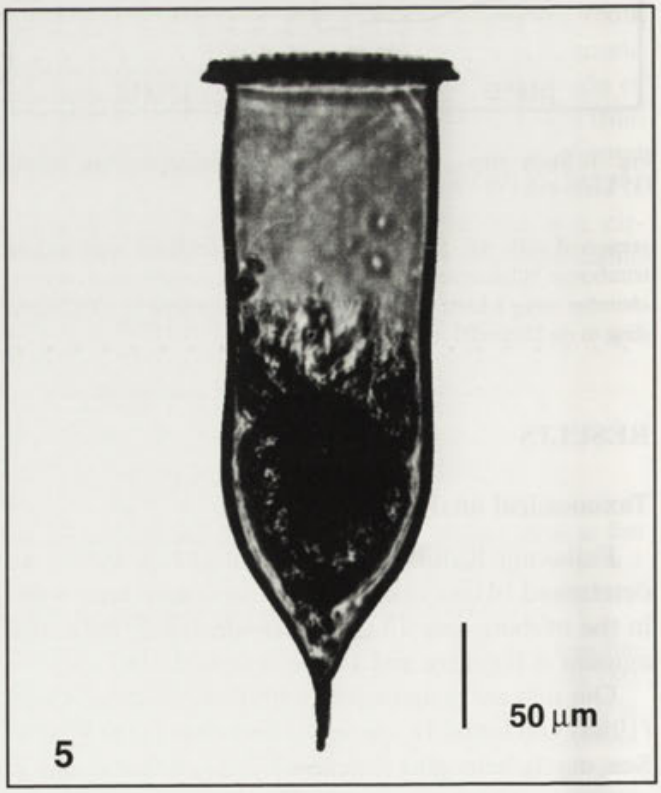


Fig. 2. *Ptychocylis* sp.p.
Fig. 3. *Cymatocylis drygalskii* total length 187.5 µm
Fig. 4. *Cymatocylis drygalskii* total length 257.5 µm
Fig. 5. *Cymatocylis drygalskii* total length 337.5 µm

by van der Spoel (1986), Barria de Cao (1987) and Boltovskoy et al. (1990): the lorica length ranged between a minimum of 168 µm, when the caudal appendix was absent, to a maximum of 396 µm, while the width ranged from 88 to 100 µm (Figs. 3-5).

As regards the length, generally at surface the population showed a unimodal distribution with an average of 240 µm, at intermediate depths the distribution was bimodal at 260 µm and 300 µm, at deepest layers the population had again a unimodal length distribution with an average < 240 µm.

Spatial distribution in the offshore area

The total amount of tintinnids in the offshore area ranged from a minimum of 2.65 ind.dm^{-3} at st. 20 (100 m depth) to a maximum of 1121.25 ind.dm^{-3} at st. 32 (surface). The highest abundance of tintinnids in the surface layer, was centered offshore at st. 32, while at intermediate depths, values were generally more homogeneously distributed with two nuclei of high density at st. 13 and at st. 26. At the greatest depths, the abundance maximum corresponded to the most coastward station (28), with another area of high values located at st. 13 (Fig. 6). At each depth density values showed a distribution pattern similar to that observed for the prevalent currents (Stocchino and Manzella 1991). In particular, the highest density corresponded to the centre of the principal gyres.

Three different patterns of vertical distribution were observed in three distinct zones of the offshore area: in the northernmost part of the sampled network (st. 12, 13, 14 and 19) abundance maxima corresponded to the intermediate depths, like at the central station 26. At the eastern stations (18, 48, 32, 33) maxima were observed near the surface, while at the three most coastward stations (20, 22, 28) maxima seemed to move from the surface to the bottom layers (Fig. 6).

The three areas characterized by different vertical distribution were under the influence of three separate currents: the northernmost of the currents inflowing from the North, the eastern one inflowing from the East and the last being the coastal current (Stocchino and Manzella 1991).

Vertical distribution of chlorophyll *a* (Innamorati et al. 1990) generally showed a pattern similar to that of the tintinnid distribution: in the northern stations, and st. 26, where the maximum of tintinnid abundance corresponded to intermediate layers, the intermediate value of chlorophyll *a* had the same surface value or even higher. Like the tintinnid concentration, the lowest value was observed at st. 14. In most of the eastern stations maxima of chlorophyll *a* appeared at surface, reaching the highest values at st. 32 and st. 48, as observed for tintinnid abundance. Coastal stations 20, 22 and 28, showed a constant decrease both in the surface values of chlorophyll *a* and in the tintinnid abundance (Fig. 6). The mid-depth maxima were due mainly to the abundance of the genus *Laackmanniella*, which prevailed at these layers in the northernmost stations and at st. 26. Surface maxima on the contrary were due to the abundance of the genus *Codonellopsis*, which prevailed at the surface of the central eastern stations, showing an almost complete dominance at st. 32 where it reached the absolute maximum. The sole exception was observed at the southernmost station (33), where the genus *Cymatocylis* prevailed as at coastal stations 20 and 22. At st. 28, the maximum,

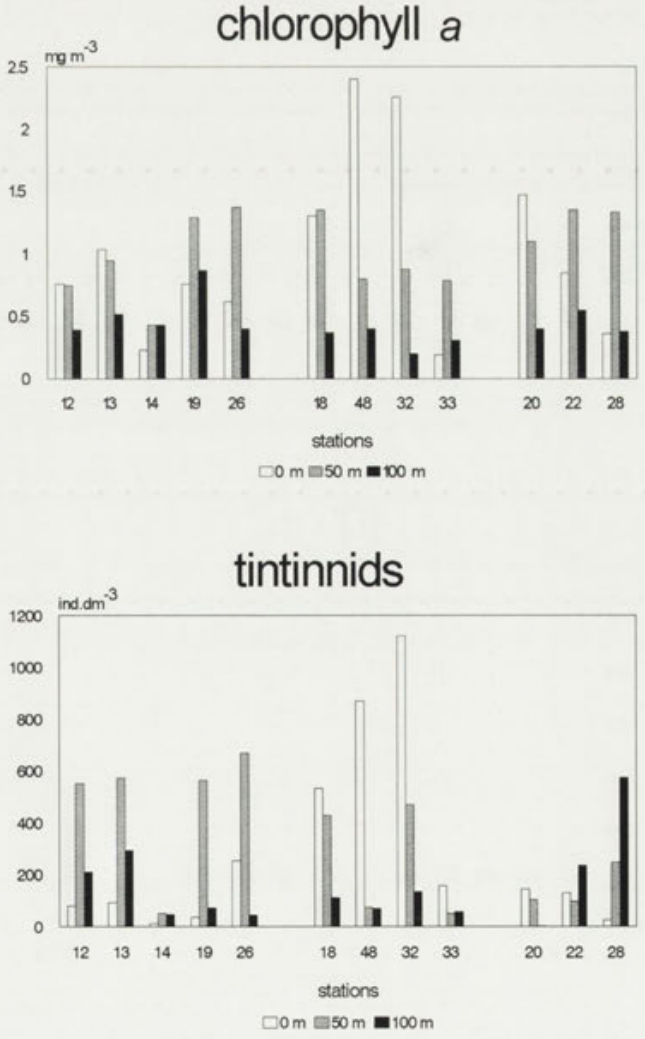


Fig. 6. Vertical distribution of chlorophyll *a* and tintinnid abundance during the first cruise (1987/88)

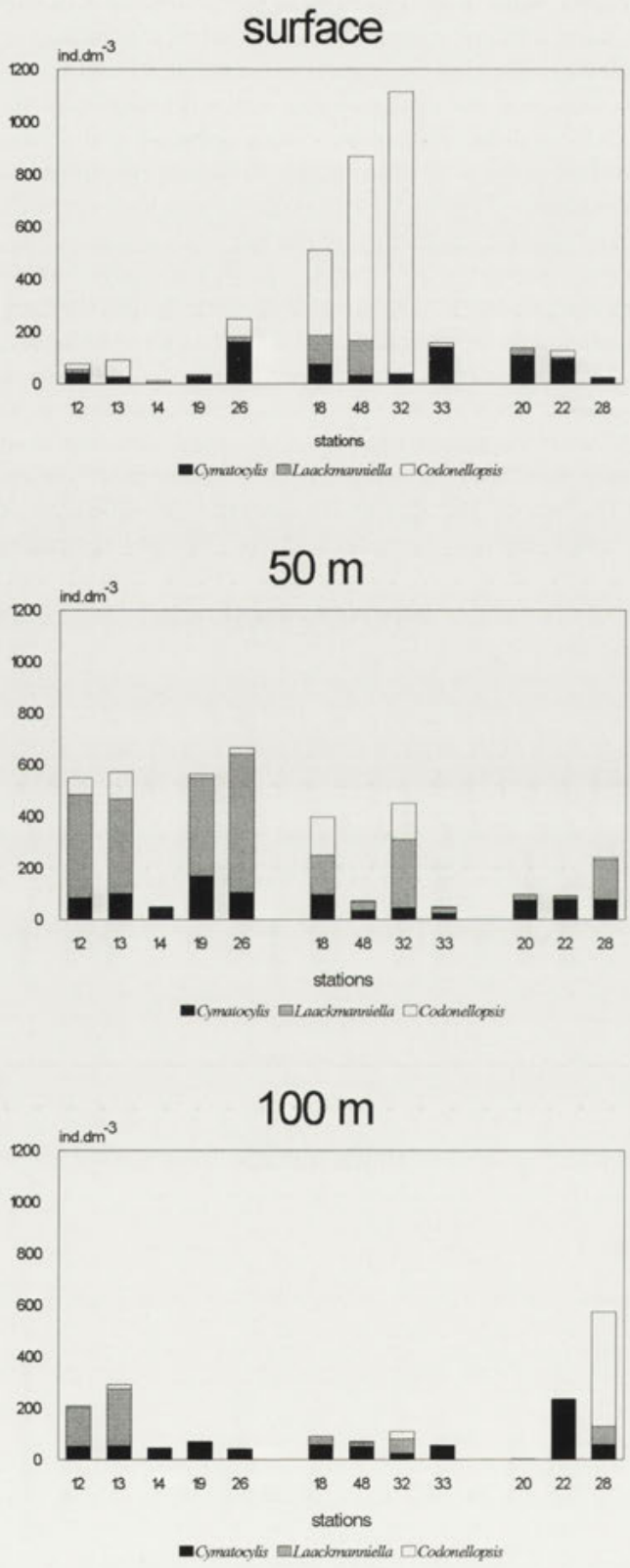


Fig. 7. Vertical distribution of *Cymatocylis*, *Laackmanniella* and *Codonellopsis* during the first cruise (1987/88)

located at the deepest layer, was due to *Codonellopsis* like the surface maxima of the central stations (Fig. 7).

Temporal dynamic

The temporal dynamic of the tintinnid population was followed during the second campaign at two coastal stations. At the most coastward one (st. M) (Fig. 1) quite a constant numeric increase was evident, mainly at the 25 m layer, from a minimum of 32.9 ind.dm^{-3} (0 m; 27.1.90) to a maximum of 476.28 ind.dm^{-3} (10 m; 2.2.90) (Fig. 8).

The dominant species were *Cd. gaussi*, *Cy. ecaudata* and *Cy. drygalskii*. At surface in the first sample only *Cd. gaussi* was present, in the second and third also *Cy. ecaudata* and *Cy. drygalskii* appeared, with an evident increase on the third day. At 10 m and 25 m layers *Cy. ecaudata* was present from the first sample and the increase began on the second day of sampling.

At SMN station (Fig. 1), the increase in total abundance was remarkable only at the surface. At the 100 m and 200 m layers, total abundances were quite constant and very low (from a minimum of 0 ind.dm^{-3} (200 m; 23.1.90) to a maximum of 20 ind.dm^{-3} (200 m; 8.2.90) (Fig. 8).

The dominant species at the surface were the same as reported at st. M: *Cd. gaussi* appeared in the first sample, and *Cy. ecaudata* and *Cy. drygalskii* appeared on 30.1.90. At the 100 m and 200 m depths, the dominant species were *L. prolongata* and *L. naviculaefera*, the latter succeeding the former from 30.1.90.

The chlorophyll *a* temporal trend at st. SMN showed an increase only at the surface, starting from 24th January which was followed six days later by the increase of tintinnid abundance. At st. M chlorophyll *a* values were lower and more constant than those of st. SMN, both as temporal and vertical distribution (Innamorati et al. 1991) (Fig. 8).

DISCUSSION AND CONCLUSION

Differences in species composition between coastal and offshore areas were remarkable: in the coastal stations, three species of tintinnids (*Cd. gaussi*, *Cy. ecaudata*, *Cy. drygalskii*) prevailed at the surface; in the offshore area also *L. prolongata* was abundant while *Cy. ecaudata* was completely absent. The deeper layers (100 and 200 m) at st. SMN, were characterized by *L. prolongata* and *L. naviculeafera*, the latter encountered only at coastal station

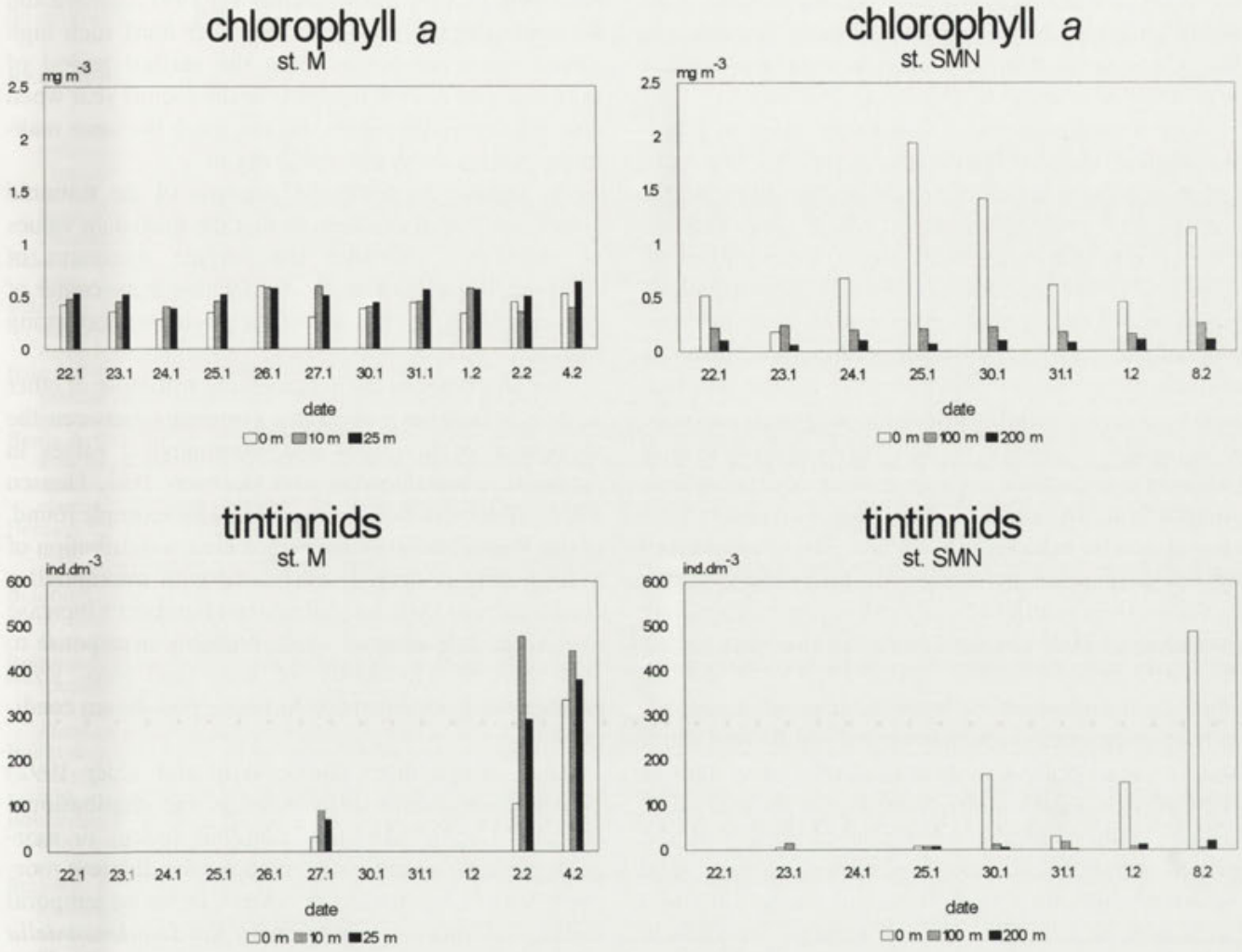


Fig. 8. Chlorophyll *a* and tintinnid temporal pattern at the stations SMN and M during the second cruise (1989/90)

20 in the year before. Furthermore, some species belonging to the genus *Cymatocylis* were sampled only in the most coastward area while the genus *Ptychocylis* was registered only in the offshore area.

The composition of the populations was very monotonous, confirming the peculiarity of the antarctic population of tintinnids (Alder and Boltovskoy and Alder 1992).

In fact, in total, we found only 6 genera: two of these are typically Antarctic (*Laackmanniella*, *Cymatocylis*), four are considered cosmopolitan (*Codonellopsis*, *Coxliella*, *Salpingella*), even if *Cd. gaussi* is restricted to the Southern Ocean, and one (*Ptychocylis*) is considered as a boreal endemic (Pierce and Turner 1993), and was reported for the first time in this area.

The absence of genera with completely agglutinated loricas could be related to the scarcity of available suspended particulate matter, necessary to build up this kind of lorica (Gold and Morales 1976), even though Fabiano et al. (1993) found in the same area a considerable bulk of suspended material. They showed its phytoplankton origin and an evident decrease below the photic zone. It seems that the only kind of detritic material which can be used by tintinnids for building up their lorica is diatom frustules which must prevail among suspended matter after a spring bloom of phytoplankton. The absence or scarcity of non biogenic material could be hypothesized as a justification for the absence of species with agglutinated lorica. Furthermore, as observed by Boltovskoy and Alder (1992), the *Laackman-*

niella genus was generally confined to the deepest layers where empty sinking frustules are more abundant. In fact *Cd. gaussi*, which is a typical surface species, appeared less covered by this kind of frustule.

With regard to tintinnid abundance it seems that it was highest in the offshore area during the first year, and mainly in the zones influenced by the inflows of the eastern and coastal currents, where also chlorophyll *a* concentrations were high (Innamorati et al. 1990). The correspondence between phytoplankton biomass and tintinnid abundance may be considered relevant, in fact, not only the maxima but also the minima corresponded and generally all the distributional patterns (spatial, vertical and temporal) appeared to be related. In general, we can clearly identify at least two areas, as regards both abundance and taxonomic composition. The more productive one consists of the central eastern stations (18, 48 and 32), characterized by the dominance at surface of *Cd. gaussi*, which increases from North to South and reaches its absolute maximum of 1003 ind.dm^{-3} at st. 32. The other area is the north-central consisting of st. 12, 13, 19 and 26, where, at the intermediate layer, the population reaches its maximum values, which never exceed 500 ind.dm^{-3}, mainly due to genus *Laackmanniella*. In these stations chlorophyll *a* values at the same depths showed intermediate values, always lower than at surface in the eastern part. For this reason we believe that the spatial pattern of tintinnid abundance is not due to a random patchiness distribution but is strongly related to hydrodynamic and trophic conditions. Our high values, if compared with the previous recorders (Littlepage 1968; Heinbokel and Coats 1986; Boltovskoy et al. 1989; Alder and Boltovskoy 1991, 1993) - but we reminded tintinnid abundance up to 1200 ind.dm^{-3} founded by Brandini and Kutner (1987) around the Shetland Islands - cannot be interpreted as a casual sampling fact, but as the result of some conditions particularly favourable to the development of these organisms which can reach exceptionally high values. As often hypothesized the structure of the phytoplankton community could affect the abundance of the tintinnid population. The station in which both chlorophyll *a* and their abundance were highest were sampled at the end of the spring phytoplankton bloom (from 28th January to 2nd February). It is well known that this period is characterized by the shift from larger size cells (microphytoplankton) to smaller ones (pico- and nano-plankton) which seemed to be grazed on more efficiently by tintinnids (Burkill et al. 1987, Capriulo et al. 1991, Strom and Welschmeyer 1991, Bernard and Rassoulzadegan 1993). On the other hand such high values were not observed in the earliest period of sampling and did not reappear in the second year when also chlorophyll *a* values did not reach the same maximum, being always below 2 mg m^{-3}.

As regard the temporal patterns of the tintinnid population we also observed that the maximum values of abundance followed the surface maximum of chlorophyll *a* at least at stations located in the center of the Bay (SMN), at the end of the phytoplankton spring bloom.

Our observations are in agreement with those of other authors which have noticed a correlation between the abundance of tintinnids and chlorophyll *a* values in Antarctic areas (Gowing and Garrison 1991, Hansen 1991). Alder and Boltovskoy (1993) for example found, in the Weddel-Scotia Confluence area, a distribution of loricate ciliates strongly correlated with chlorophyll *a* concentrations. Microzooplankton abundances increase toward the late summer - fall, probably in response to an enhanced availability of nano and pico- sized producers, characteristic of Antarctic post-bloom conditions.

The same authors (Boltovskoy and Alder 1992) observed consistent differences in the distributional pattern of some particular tintinnid species or morphotypes such as those observed for the different morphotypes of *Cymatocylis dryglaskii* or for the temporal shifting of species belonging to the *Laackmanniella* genus in our samples, which they ascribed to a peculiar adaptive strategy, particularly related to the composition of the phytoplankton community and consequently to the efficiency of microzooplankton grazing.

In conclusion, the correlation between phytoplankton biomass and composition and tintinnid abundance and species presence appeared to be confirmed by both the spatial and temporal distribution of tintinnid populations. Obviously these two living fractions are under the influence of the current entering the Bay and it appeared that the most productive area is the eastern one, while along the coast the biomass of autotrophic and heterotrophic microplankton showed intermediate values more comparable to those reported in literature for other Antarctic areas.

Acknowledgements. This work was supported by the Italian National Antarctic Research Program. Thanks are due to crew members and especially to the colleagues who collected the samples during the two cruises. The authors would also like to thank Dr. Mara Chemelli for help in microscopic identification, Dr. C. Davis for useful advice

in *Ptychocylis* identification and anonymous reviewers for their helpful comments.

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Received on 4th May, 1994; accepted on 5th January, 1995

Acta Protozoologica (1995) 34: 203 - 209

ACTA
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Observations on the Adhesive Disc of *Trichodina xenopodos* Fantham, 1924 and *T. heterodentata* Duncan, 1977 (Ciliophora: Peritrichida) during Binary Fission

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Summary. Aspects of the ultrastructure of the adhesive disc elements during binary fission and denticle duplication was investigated using a technique to dissolve soft material in *Trichodina xenopodos* Fantham, 1924, a urinary parasite of *Xenopus laevis laevis* (Daudin, 1802), and *T. heterodentata* Duncan, 1977, an ectoparasite of the tadpoles of *X. l. laevis*. The new denticle ring, comprising more or less double the number of denticles in the daughter cells, develops from platelets originating from a band formed by an area on the striated membrane. The central part develops first giving rise to the blade and only after the development of these two elements is well advanced, ray development is initiated. The old ring is resorbed and denticle development is only completed when cells reach maturity.

Key words. Adhesive disc, binary fission, *Trichodina xenopodos*, *T. heterodentata*, scanning electron microscopy.

INTRODUCTION

The ultrastructure of the adhesive discs of *Trichodina xenopodos* Fantham, 1924 and *T. heterodentata* Duncan, 1977 was described in detail by Kruger et al. (1993) using a technique to dissolve soft material and in so doing exposing the underlying denticle elements with the aid of the scanning electron microscope. In the present study the same technique as well as silver impregnated specimens were used to investigate the complex process of duplicating the denticles during binary fission.

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Reproduction of trichodinids has been the subject of study since the previous century. The first account was that of Arlidge (1849) and was followed by many authors who described different processes of reproduction. These include papers of Stein (1854, 1859-1883), Wallengren (1897), Fulton (1923), Diller (1928), Colwin (1936), Padnos and Nigrelli (1942), Lom (1964), Raabe (1964), Kazubski (1965, 1967) and Feng (1985). Although Stein (1859-1883) was the first to describe the process of division in *Trichodina*, Fulton (1923) recognized that the duplication of the denticles and different stages of double denticle rings was part of the process of binary fission. Authors such as Stein (1854) and Ariake (1929) erroneously described these double-ringed specimens as new species.

This paper provides the first account of some aspects of the duplication of denticles during binary fission using scanning electron microscopy.

MATERIALS AND METHODS

Adult specimens of the African clawed toad, *Xenopus laevis laevis* (Daudin, 1802) infected with *T. xenopodos* were collected from localities in the vicinity of Bloemfontein (Kruger et al. 1991) and maintained in the laboratory for a period of three years. Tadpoles of *X. l. laevis* infested with *T. heterodentata* (Kruger et al. 1993) were collected from Zoo Lake in Bloemfontein and maintained in a plastic pond. In both cases the infections of trichodinids remained high and infected specimens were at our disposal at any time throughout the study. Kruger et al. (1991) found that increased hormonal levels in the host bladder stimulated *T. xenopodos* to become active in order for transmission to take place. We noted that under these conditions numerous specimens of trichodinids in different stages of binary fission occurred. We artificially induced reproduction in the hosts by injecting them with human hormones as prescribed by Van Wyk and Du Preez (1984). Specimens of *T. xenopodos* were then collected by dissecting infected toads, whilst *T. heterodentata* was collected by making skin smears from infested tadpoles.

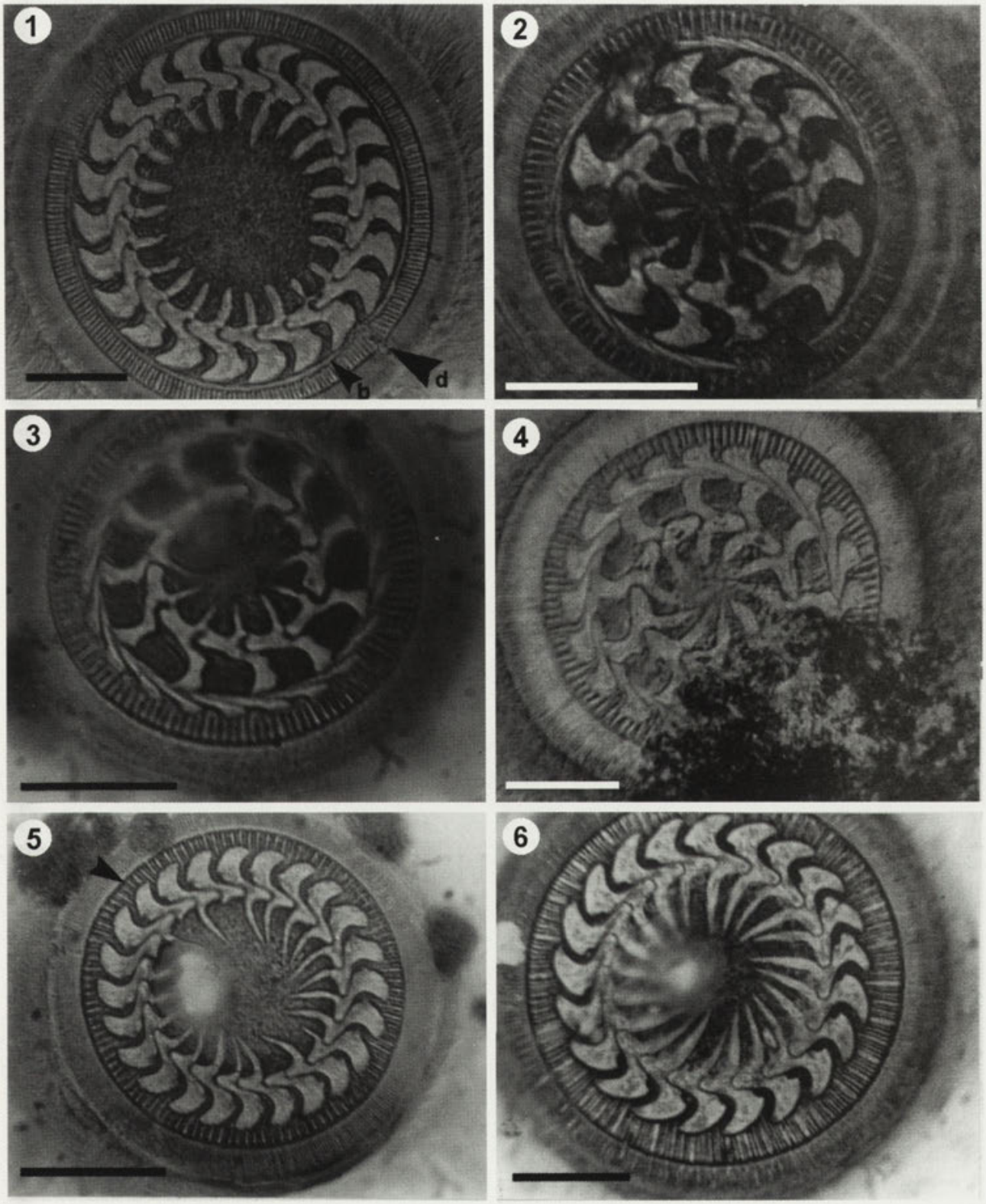
Silver impregnated specimens were prepared as described by Kruger et al. (1991), whilst SEM specimens were prepared as described by Kruger et al. (1993).

RESULTS AND DISCUSSION

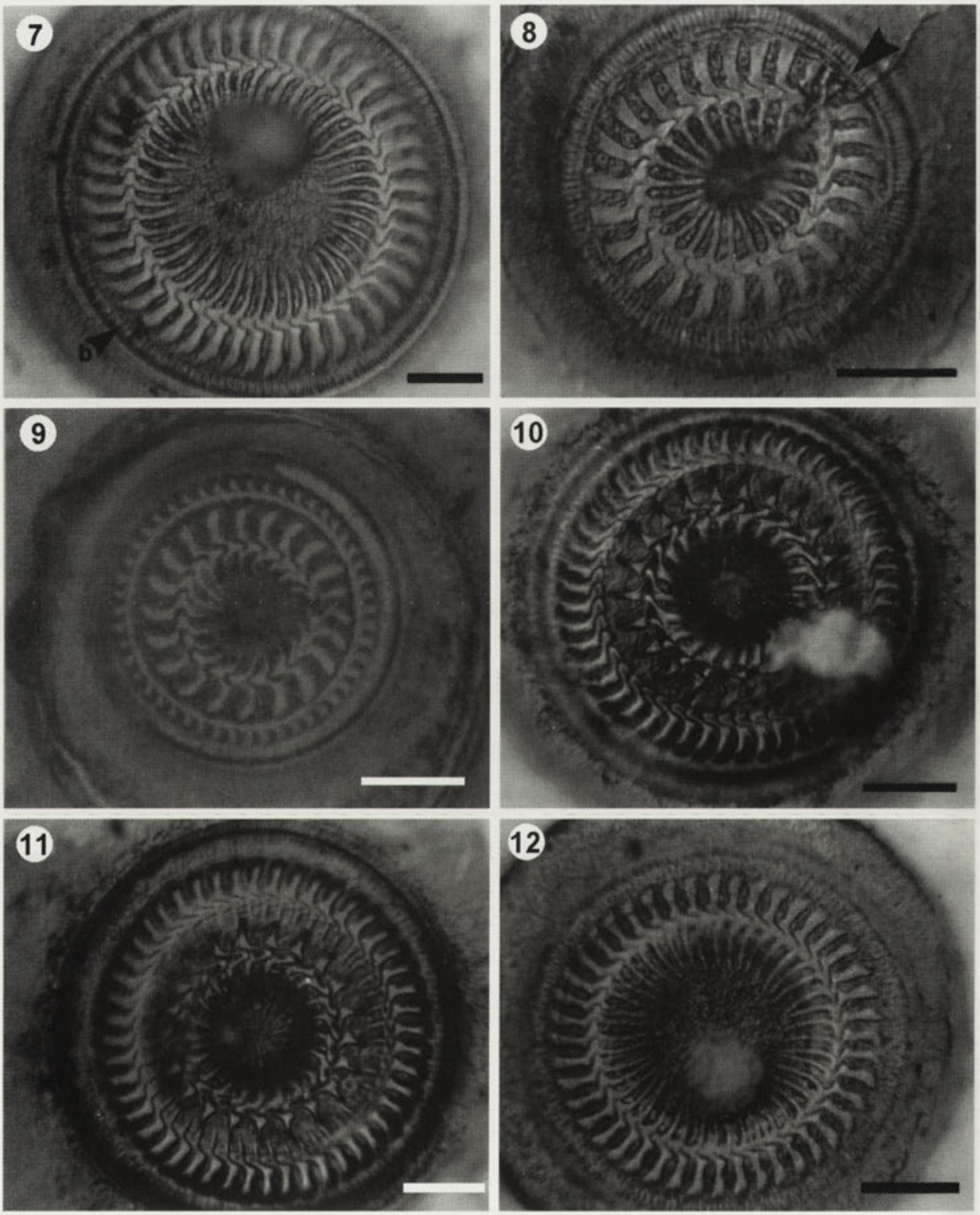
Division in ciliophorans is initiated by the micronucleus followed by a sequence of developments of both the macro- and micronucleus. This process has been described by authors such as Fulton (1923), Diller (1928) and Padnos and Nigrelli (1942). The sequence of events leading to the formation of the new denticle ring as observed in silver impregnated specimens are shown from Figs. 1 to 6 for *T. heterodentata* and Figs. 7 to 12 for *T. xenopodos*. The first sign of division in the adhesive disc is the presence of a distinct band to the distal side of the blades that impregnates in a different way than the rest of the adhesive disc in silver impregnated specimens (Figs. 1, 7). This band has been associated with the formation of the new denticle ring by Wallengren (1897), Diller (1928), Kazubski (1967) and Feng (1985). It is often seen in silver impregnated specimens and even in papers published by different authors and has been noted by Van As and Basson (1986) and Kruger et al. (1993) to occur in large specimens of *T. heterodentata* and *T. xenopodos*. The presence of this band will indicate that the specimen is on the verge of undergoing binary fission (Fig. 1). This band becomes more prominent during fission when the adult cell starts dividing into daughter cells (Fig. 13). Initially the band consists of thickened areas on each of the radial pins adoral to the thatched band that connects the radial pins. Subsequently these areas on several (approximately 6-8) radial pins will fuse to form a platelet (Fig. 14). The denticle ring of the parent cell breaks off at two opposing sides leaving the daughter cells with half the number of denticles as well as radial pins. In both cases the conical parts of the denticle separate from the central parts of the preceding denticles, but fail to join completely in the daughter cells (Figs. 2, 8). In the whole process of development it appears that the old ring in the daughter cell is never joined completely. At this stage the thickened band now consists of elongated platelets clearly overlapping (Fig. 15). In subsequent stages the platelets continue to extend and now appears plaited (Fig. 16). Each of these platelets will develop into a future denticle restoring more or less the number of denticles present in the parent individual.

In the ontogeny of the denticles the platelets will become the central parts from which the blades will develop in a distal direction along with extension of the radial pins. The rays will only develop in a proximal direction some time later. The formation of the central parts of the denticles is the result of the platelets rolling into a funnel. In this process the distal surface will roll in an adoral direction to first form the distal side of the central part (Figs. 3, 9). At this stage the anterior part of the platelet will start extending in a distal direction that will eventually develop into the blade (Fig. 17). The rolling process will continue proximal and distally to form the hollow central part. The distal development of the platelets will continue and only when the blade has almost reached its final shape and size will development of the ray be initiated (Figs. 10, 11). The process until now coincides with a progressive increase in the size of the cell. The original denticle ring maintains its position but loses its shape as seen in silver impregnated specimens (Figs. 4, 11). The altering of the original denticle ring's shape is the result of the resorption process that appears to erode the denticles from the aboral side exposing their tubular nature (Figs. 19, 20). Until now the rays could not develop as not sufficient space was available due to the presence of the original denticles. With the gradual eroding, space is made available for the developing rays (Fig. 20) which continue to develop even after the original denticle ring has been resorbed. The rays are only fully developed when specimens have reached maturity (Figs. 6, 12)

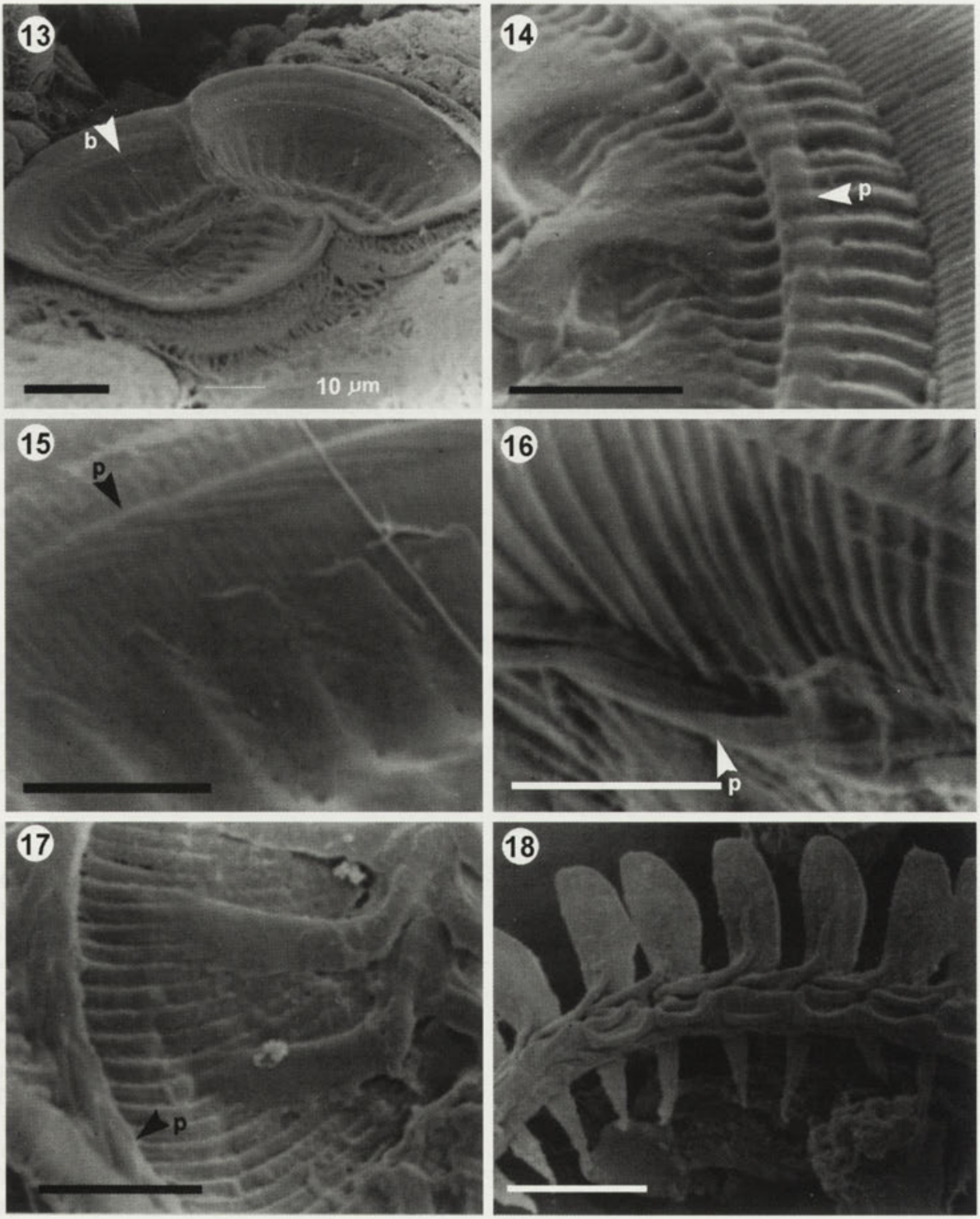
In Fig. 18 an adoral view of the denticles of *T. xenopodos* is shown. In this specimen the rays are already in an advanced stage of development, but not yet completed. The aboral side of the central part is fully formed, whilst as shown in this figure, it is not yet fully develo-



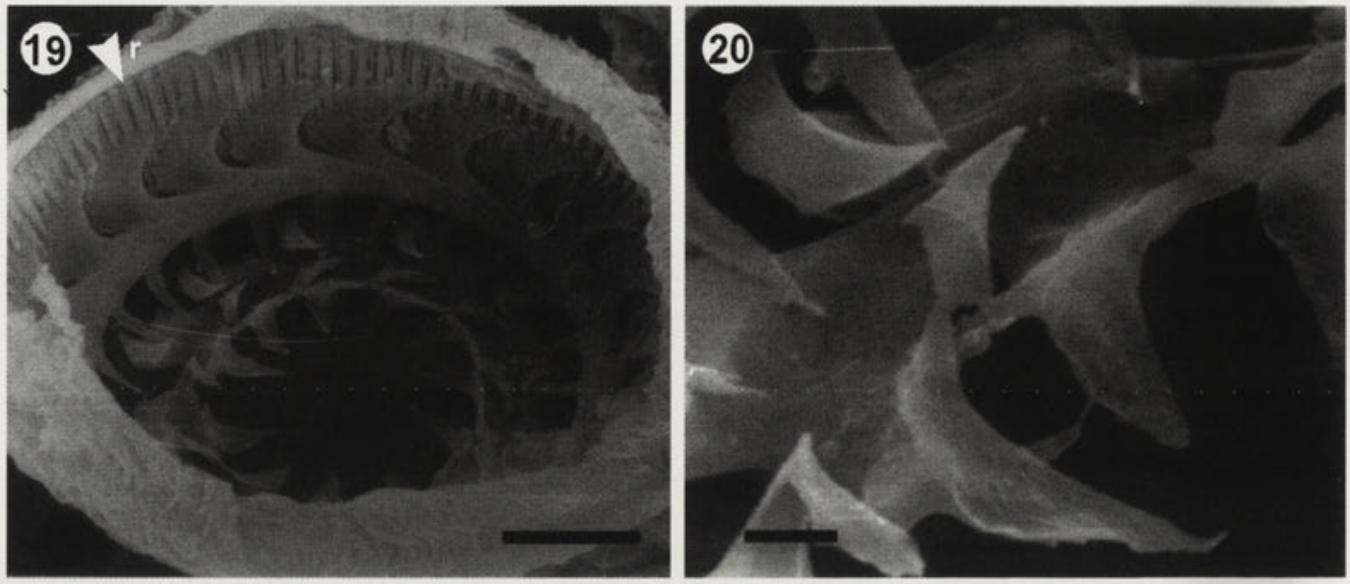
Figs. 1-6. Photomicrographs of silver impregnated specimens of *Trichodina heterodentata* Duncan, 1977. 1 - cell immediately before division with well-developed band, 2 - daughter cell directly after division. Arrow indicates break in denticle ring, 3 - platelets well developed, formation of blades initiated, 4 - blades in process of development whilst old ring is being resorbed, 5 - young specimen with old ring completely resorbed, but rays not yet fully developed. Arrow indicates new radial pin, 6 - mature specimen with rays and radial pins completely developed. b - band, d - division. Bars - 15 μm



Figs. 7-12. Photomicrographs of silver impregnated specimens of *Trichodina xenopodos* Fantham, 1924. 7 - large specimen initiating process of division. Note early development of band that will form platelets, indicated by arrow, 8 - daughter cell directly after division, arrow indicating where denticle ring was broken. Platelets already developed, 9 - daughter cell with platelets starting to form blades, 10 - blades partially developed, ray development initiated, 11 - old denticle ring partially resorbed, but new denticles not yet completely developed, 12 - adult specimen with denticles and radial pins fully developed. b - band. Bars - 15 µm



Figs. 13-18. Scanning electron micrographs of *Trichodina xenopodos* Fantham, 1924 (13, 15-18) and *T. heterodentata* Duncan, 1977 (14) from the clawed toad *Xenopus laevis laevis* (Daudin, 1802). 13 - cell in the process of binary fission, 14 - aboral view of section of adhesive disc showing platelets directly after division into daughter cells, 15 - platelets beginning rolling process to form funnel, 16 - section of adhesive disc with plaited platelets, 17 - section of adhesive disc from aboral view, formation of central part almost completed, 18 - section of developing denticle ring from adoral view, central part not completely closed and rays only partially developed. b - band, p - platelets. Bars - 5 µm (14), 10 µm (13, 15-18)



Figs. 19, 20. Scanning electron micrographs of *Trichodina heterodentata* Duncan, 1977 from the clawed toad *Xenopus laevis laevis* (Daudin, 1802). Aboral view of adhesive disc with advanced development of new denticles and advanced resorption of old ring. r - developing new radial pin. Bars - 15 µm (19), 1 µm (20)

ped adorally. The hollow central part will most likely only be completely closed in adult specimens.

Along with the development of the denticles a completely new striated membrane is formed. The distal development of the blades from the platelets coincides with an extension of radial pins in this direction. In *T. heterodentata* the extension is limited only from the central part in a distal direction, whereas in *T. xenopodos* the growth is in both directions as the striated membrane extends from the tip of the ray to the border membrane. This feature was illustrated by Kruger et al. (1993) in Figs. 17 and 21. In the young daughter cell the radial pins would therefore be newly formed, but extensions of the original radial pins. At some stage before the complete resorption of the old denticle ring, an additional set of radial pins, each placed between existing pins, will develop (Figs. 5, 19). According to Lom (1973) these new radial pins originate from barren kinetosomes found between consecutive radial pins.

The development of the new border membrane and the complex hinge shown by Kruger et al. (1993) is still unknown and could not be followed in the present study.

Coinciding with the denticle development the nuclei undergoes changes to gradually evolve into its horseshoe shape even before resorption of the old ring is completed. During resorption the central part is last to disappear. The consecutive stages of development coincide with a gradual increase in the size of the cell.

CONCLUSIONS

Information emanating from this study clarifies aspects of division previously only theorized on. The old denticle ring does not move to the center of the adhesive disc as suggested by Padnos and Nigrelli (1942), but remains in the same place where it is gradually resorbed. New growth of the radial pins completely replaces the old pins after which a new set develops. This implies that the new thatched band is only completed after the second set of radial pins has developed. The development of the second set of radial pins already starts before the old ring has been resorbed, but is not always visible in silver impregnated specimens. In young specimens directly after completion of the new denticle ring, the radial pins appear broad and in some cases the new radial pins can be seen as a thin line between two radial pins (Fig. 5). This is a useful method of identifying young specimens when describing a species population. The number of denticles in the adult cell is determined by the platelets originating from the radial pins and not by the number of old denticles in the daughter cell. This accounts for the variation in denticle numbers in specimens of the same species population.

With the onset of the development of the new denticle ring, the platelets resemble the structures in the adhesive disc of other families of the Mobilina, such as the Urceolariidae, Leiotrochidae, Trichodinopsidae and Po-

lycyclidae. This confirms the advanced status of the Trichodinidae in mobiline evolution. In the progressive ontogeny of the denticles of the trichodinids in this study, shapes of denticles of other genera can also be distinguished. The shape of the stunted blade of *Hemitrichodina* is reflected in the early development during binary fission as can be seen in Figs. 4, 9. In the more specialized genera such as *Tripartiella, Paratrichodina* and *Trichodinella* the blade anatomy is more complex than shown in the present study, the rays are weakly developed or absent resembling similar stages shown in Figs. 4, 10, 11 of the present study.

Ongoing studies in our laboratory on using the same techniques on representatives of other genera and families will hopefully shed light on the evolution of these complex ciliophorans.

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Received on 16th August, 1994; accepted on 21st March, 1995

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1995) 34: 211 - 216

Trichodinid Ectoparasites (Ciliophora: Peritrichida) of some Fishes from the Bay of Dakar, Senegal (West Africa)

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Summary. Forty two specimens of 11 tidal pool and coastal fish species from the Island of Goreé situated on the coast of Senegal were examined for ectoparasitic trichodinids. Four of these fish species were infested by three known trichodinid species, i.e. *Trichodina jadranica* Raabe, 1958, *T. lepsii* Lom, 1962 and *T. rectuncinata* Raabe, 1958. Comparative descriptions are presented of these trichodinids to establish new host and locality records. Notes on the ultrastructure of the peculiar markings on the blades of *T. rectuncinata* are provided.

Key words. *Trichodina jadranica*, *T. lepsii*, *T. rectuncinata*, marine trichodinids, West Africa.

INTRODUCTION

Various studies on the trichodinid parasites of marine fishes have been published, mostly originating from Eastern Eurasia. From African coastal fish, however, only two studies have so far been conducted, i.e. Fantham (1930) and Maslin-Leny (1988). Fantham (1930) described four species of trichodinids infesting tidal pool fish from southern Africa. These descriptions, however, are not valid since they were described so inadequately that these are impossible to recognise again (Lom and Laird 1969). Maslin-Leny (1988) studied the trichodinids of fish from a coastal lagoon in Benin, West Africa. The fish examined by Maslin-Leny (1988) were mostly euryhaline freshwater species as well as some true estuarine species. The trichodinids recorded in this study were therefore not surprisingly mostly well known freshwater species. Some of these identifications are erroneous and need to be reviewed.

During a visit to Senegal, the authors examined tidal pool fish from the Island of Goreé as well as coastal fish collected in the vicinity of the island. Three known species of trichodinids were identified of which comparative descriptions are presented below.

MATERIALS AND METHODS

Tidal pool fish were collected with the use of hand nets, whilst coastal fish were collected with hand lines from the boat of a local fisherman. Fish specimens were taken live to a temporary laboratory set up in facilities provided by courtesy of the Goreé Institute (Centre for Democracy, Development and Culture in Africa) on the Island of Goreé.

Wet smears were prepared and examined for the presence of trichodinids with the use of a dissecting microscope. Back in the

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laboratory in Bloemfontein air-dried smears were impregnated with silver nitrate in order to study details of the adhesive disc. Values ranging between X and XXX were assigned to each infestation in order to reflect the intensity of an infestation. Information concerning fish specimens collected and trichodinid infestations are provided in Table 1. Some of the infestations identified on wet smears could not be recovered on the dry smears due to either low infestation or inadequate impregnation. All measurements are presented in µm and follow the uniform specific characteristic system proposed by Lom (1958). A detailed description of the denticles is presented in accordance with the method proposed by Van As and Basson (1989). Minimum and maximum values are given, followed in parentheses by the arithmetic mean and standard deviation. In the case of the denticles and radial pins, the mode is given instead of the arithmetic mean. Body diameter is measured as the adhesive disc plus the border membrane. Reference material is in the collection of the authors.

For SEM studies specimens were fixed in 10% buffered neutral formalin made up with seawater. In order to study internal structures of trichodinids, the soft material was removed by dissolving specimens in a nitric acid solution normally used for softening chitin and keratin, washed and dehydrated in ascending concentrations of ethanol, critical point dried and sputter coated with gold and studied in a Jeol Winsem JSM 6400 at 5 kV.

RESULTS AND DISCUSSION

Three known species of trichodinids were identified from collected fish. These are *Trichodina jadranica* Raabe, 1958, *T. lepsii* Lom, 1962 and *T. rectuncinata* Raabe, 1958. Comparative descriptions of these are provided below.

Trichodina jadranica Raabe, 1958 (Figs. 1, 7; Tables 1, 2)

Reference material: 93/11/13-03 (*Balistes punctatus* Gmelin, 1789 from coastal water) in the collection of the authors.

Blade broad, open sickle-shaped, filling most of space between y-axes. Distal blade surface slopes down towards apex. Tangent point small, slightly rounded. Anterior blade surface not distinguishable from distal blade surface. Apex prominent, round, extends past y-axis. Blade apophysis not visible. Posterior blade surface almost triangular, deepest point on same level as apex. Blade connection broad and extended. Posterior projection not visible. Central part robust, sloping downwards to broad rounded tip, fitting tightly into preceding denticle. Central part extends more than halfway past y-axis. Section of central part above x-axis triangular, whilst proximal border in section below almost parallel to x-axis. No indentation in lower part. Ray connection very short, ray extends almost directly from central part. Ray apophysis only visible in some specimens. Rays short, well developed, curved slightly proximally. Tip broad, rounded. Denticles straight, parallel to y-axes. Section of denticle above to section below x-axis more than one (1.2). Denticles fit tightly together with small spaces in-between. Central circle with unevenly distributed granules, impregnates similar to denticle. Tips of rays not in contact with central circle.

Remarks

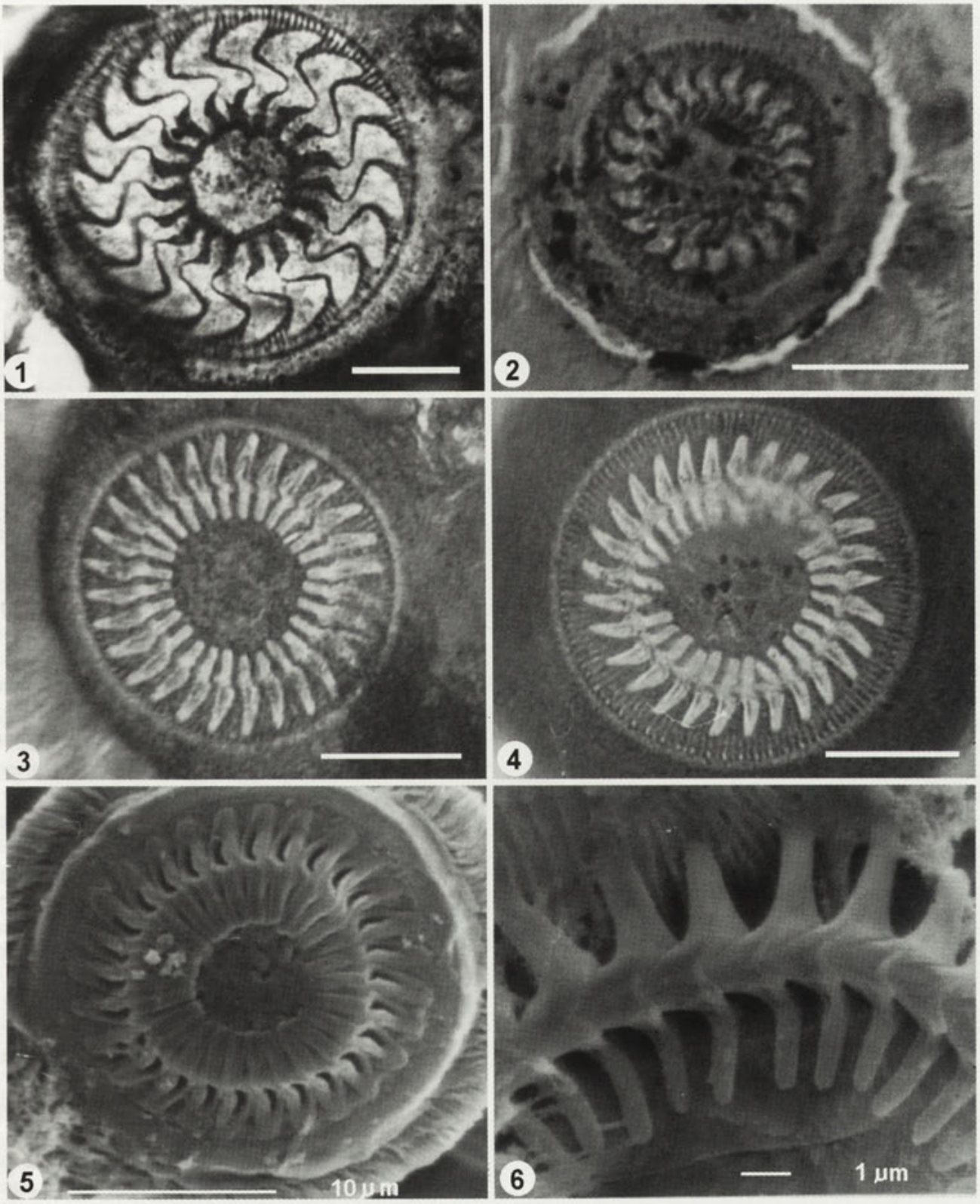
This population corresponds closely to the type population described by Raabe (1958). The overall body and denticle dimensions are almost identical. The only difference is that in the present population the number of denticles are less (17-20), whilst in the population described by Raabe (1958) they vary between 22 to 25. The denticle morphology corresponds closely. There can be little doubt that this population belongs to the same species as that described by Raabe (1958).

Various other populations of trichodinids ascribed to *T. jadranica* have been reported from a long list of host fish from various parts of the world. These include Lom (1970), Stein (1979) and Grupcheva et al. (1989). The present population differs in some aspects with some of these populations and it is not certain that all these populations belong to the same species. In our opinion this species requires comprehensive revision.

T. lepsii Lom, 1962 (Figs. 2, 8; Tables 1, 2)

Reference material: 93/11/13-15 [*Pagrus caeruleostictus* (Valenciennes, 1830) from coastal water] in the collection of authors.

Blade broad, truncated. Distal surface almost parallel to border membrane. Tangent point small, slightly rounded. Anterior blade surface almost parallel to posterior margin. Apex rounded, not very prominent. Blade touches y-axis. Blade apophysis indistinct. Posterior blade surface almost parallel to y-axis with shallow curve. Deepest point close to central part. Blade connection not distinguishable from blade. Posterior projection not visible. Central part short, thin and delicate. Central part extending slightly beyond y-axis. Section of central part above and below x-axis similar. No indentation in section below x-axis. Ray connection not distinguishable. Rays of equal thickness, with slightly swollen rounded tips. Rays curved anteriorly, tips touching and sometimes extending past y +1 axis. No ray apophysis. Section of denticle above to section below x-axis of equal length (1.0). Denticles on the whole loose fitting, with no clear apophyses or indentations connecting different denticle parts.



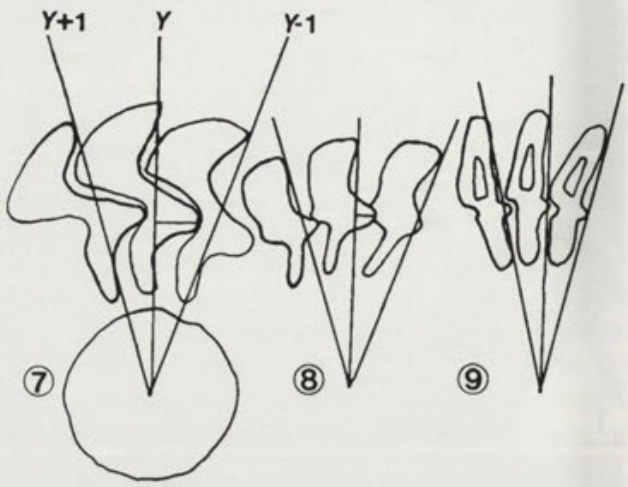
Figs. 1-4. Photomicrographs of silver impregnated specimens of trichodinid species. 1 - *Trichodina jadranica* Raabe, 1958 from *Balistes punctatus*; 2 - *T. lepsii* Lom, 1962 from *Pagrus caeruleostictus*; 3 - *T. rectuncinata* Raabe, 1958 from *Entomacrodus cadenati*; 4 - *T. rectuncinata* from *Parablennius parvicornis*

Figs. 5-6. Scanning electron micrographs of *T. rectuncinata;* 5 - denticles from the aboral side illustrating the cavity in the blade; 6 - denticles from the adoral side clearly showing that the cavity does not extend through the blade. Figs. 1-5 - bar 10 µm, 6 - 1µm

Remarks

Although few individuals were recovered from the material collected, this species can easily be recognised by its unique denticle shape. The present population corresponds closely in terms of denticle morphology to the type population described by Lom (1962) from the gills of *Mugil auratus* in the Rumanian Black Sea Coast. It also corresponds to a population described by Grupcheva et al. (1989) from the gills of *Syngnathus typhleargentatus* from the Baltic Black Sea Coast. The material depicted in the photomicrograph and in the sketch might not resemble a typical *T. lepsii* shape, but that is due to poor impregnation.

In terms of body dimensions the type population is considerably larger with an adhesive disc diameter of 19-27 µm (mean 21) which is above the range of the present population. The present population, however, falls exactly within the range of dimensions given by Grupcheva et al. (1989).



Figs. 7-9. Diagrammatic drawings of the denticles of: 7 - *T. jadranica* Raabe, 1958; 8 - *T. lepsii* Lom, 1962; 9 - *T. rectuncinata* Raabe, 1958

T. rectuncinata Raabe, 1958 (Figs. 3, 4, 9; Tables 1, 2)

Reference material: 93/11/12-08 (*Entomacrodus cadenati* Springer, 1966 from tidal pool fish) in the collection of the authors.

Blade triangular, straight with triangular area which impregnates in a different way than rest of the blade. Distal surface sharply rounded. No clear tangent point as posterior blade surface extends parallel to y-axis. Anterior blade surface slopes sharply downwards. Apex small, but distinct. Blade does not extend past y-axis. Blade apophysis small, not visible in all denticles. No clear blade connection as blade extends almost directly from central part. No posterior projection. Central part small, triangular in shape, does not extend past y-axis. Section of central part above somewhat triangular, section below less so. No indentation visible. Ray connection not distinguishable, ray extends directly from central part. Rays very broad, tapering slightly to rounded blunt points. Ray apophysis only visible in some specimens. Rays generally straight, in some specimens sometimes slanted posteriorly. Section of denticle above to section below x-axis slightly more than one (1.3).

Remarks

Another population of the same species was also collected from *Parablennius parvicornus*. In this population

Table 1

List of host fishes examined in the tidal pools of Goreé and the Bay of Dakar, Senegal (West Africa)

Host	n	n'	Trichodinid species		infestation
Scorpaena stephanica Cadenat, 1993	1	0			
Plectorhinchus mediterraneus (Guichenot, 1850)	1	1	gills	not recovered	X
Pagrus caeruleostictus (Valenciennes, 1830)	1	1	gills	*T. lepsii*	XX
Lithognathus mormyrus (Linnaeus, 1758)	2	2	gills	not recovered	X
Diplodus cervinus (Lowe, 1838)	4	1	skin	not recovered	X
Diplodus sargus cadenati de la Paz, Bauchot et Daget, 1974	2	1	gills	not recovered	X
Parablennius parvicornis (Valenciennes, 1836)	6	5	gills	*T. rectuncinata*	XXX
Entomacrodus cadenati Springer, 1966	18	7	gills	*T. rectuncinata*	XX-XXX
Lipophrys velifer (Norman, 1935)	5	3	gills	not recovered	X
Balistes punctatus Gmelin, 1789	1	1	skin	*T. jadranica*	XX
Chilomacterus reticulatus (Linnaeus, 1758)	1	0			

n - number of fish examined, n' - number of fish infested, not recovered - due to poor impregnation no positive identification could be made of these trichodinids, X - 1 to 5 individuals, XX - 5 to 20 individuals, XXX - 20 to 50 individuals

Table 2

Biometrical data (in µm) of *Trichodina jadranica* Raabe, 1958, *T. lepsii* Lom, 1962 and *T. rectuncinata* Raabe, 1958, collected from the Bay of Dakar, Senegal

Trichodinid species	*Trichodina jadranica* Raabe, 1958	*T. lepsii* Lom, 1962	*T. rectuncinata* Raabe, 1958
Host	*Balistes punctatus*	*Pagrus caeruleostictus*	*Entomacrodus cadenati*
Locality	Bay of Dakar	Bay of Dakar	Tidal Pools
Body diameter	33.0-42.0 (36.5 ± 2.8)	18.5-21.5 (20.0 ± 1.0)	23.5-31.0 (27.8 ± 2.0)
Adhesive disc diameter	27.0-35.5 (31.0 ± 2.5)	15.5-18.5 (16.8 ± 1.3)	20.0-26.0 (23.0 ± 1.8)
Border membrane width	2.0- 3.5 (2.7 ± 0.5)	1.5- 2.0 (1.9 ± 0.2)	1.5- 2.5 (1.9 ± 0.3)
Denticle ring diameter	15.5-21.5 (17.8 ± 1.8)	7.0- 9.5 (8.0 ± 1.0)	11.0-15.0 (12.7 ± 1.0)
Central circle	7.0-11.0 (9.0 ± 1.3)		
Denticle number	17-20 (18)	18-21 (19, 20)	24-28 (26)
Radial pins per denticle	8- 9 (9)	5- 6 (5)	5- 7 (6)
Denticle length	5.5- 7.0 (6.0 ± 0.4)	1.5- 1.5 (1.5 ± 0.0)	1.5- 2.0 (1.9 ± 0.1)
Blade length	3.5- 5.0 (4.2 ± 0.5)	2.0- 2.5 (2.3 ± 0.3)	3.0- 4.0 (3.4 ± 0.4)
Central part width	1.5- 2.5 (2.0 ± 0.2)	0.5- 1.0 (0.8 ± 0.3)	1.0- 1.0 (1.0 ± 0.0)
Ray length	2.5- 3.5 (2.8 ± 0.3)	1.0- 1.5 (1.1 ± 0.2)	2.0- 2.5 (2.1 ± 0.2)
Denticle span	8.5-10.5 (9.3 ± 0.7)	3.5- 4.5 (4.1 ± 0.3)	6.0- 7.0 (6.5 ± 0.4)
n	12	25	8

n - number of specimens measured

the adhesive disc varied from 21.5-28 µm (mean 24), denticle ring 12-15 µm (13), denticle span 6-7.5 µm (mean 6.7) and between 25-29 denticles (mode 26, 27). Both populations are very similar in overall body dimensions and denticle shape and in both cases very little variation in dimensions and denticle shape occurred.

In the original description by Raabe (1958) considerable variation in body dimensions were recorded ranging from 23-50 µm in adhesive disc diameter. Raabe (1958) collected his specimens from different fish species, all with low levels of infestation. From his paper it is not clear whether the morphometric data of different fish species were combined. Grupcheva et al. (1989) describes *T. rectuncinata* from two goby species, a *Crenilabrus* species and four *Blennius* species. Within each of these populations the ranges of dimensions are relatively small, however if the data for the different hosts is combined, the adhesive disc diameter will range from 19-42 µm. *T. rectuncinata* was also recorded by other authors from different hosts. A wide variation in denticle morphology can be found between these different populations. We are in agreement with Grupcheva et al. (1989) that this species requires comprehensive revision.

A consistent feature of the blades in our specimens was a triangular marking in the centre of the blade. This feature can also be seen in some but not all of the micrographs presented by the other authors. A scanning electron microscopic investigation revealed that this marking is in fact a triangular cavity (Fig. 5). This cavity does not penetrate the blade as can be seen from the adoral view in Fig. 6, with the result that the blade within the cavity must be very thin adorally. On this micrograph the robust nature of the adoral side of the central part can be seen. We believe that this cavity in the blade is a consistent feature of the denticle morphology of this species. It was visible in all the silver impregnated specimens in our study, but we concede that in some populations it may not necessarily be visible in all specimens due to differences in impregnation. We suggest that the presence of this triangular blade cavity could serve as a distinguishing taxonomic characteristic. This is not necessarily a feature of this species alone, but may be a characteristic of a species group similar to the group of species with a central circle in common.

Acknowledgments. The authors wish to extend their appreciation to the Director, Mr. André Zaaiman, and the staff of the Goreé Institute for their help and facilities provided for our work in Senegal. Identifications of tidal pool fish was made by Dr. Hans Bath from Germany who's contribution is greatly acknowledged.

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Received on 4th October, 1994; accepted on 18th February, 1995

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1995) 34: 217 - 220

A New Species of *Eimeria* (Apicomplexa) from the Prairie Ringneck Snake, *Diadophis punctatus arnyi* (Serpentes: Colubridae), from Arkansas

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Summary. A new species of coccidian (Apicomplexa, Eimeriidae) is described from the faeces of the prairie ringneck snake, *Diadophis punctatus* arnyi, from Arkansas, USA. Oocysts of *Eimeria kennicotti* sp. n. were found in 2/4 adult snakes and are broadly ellipsoidal, 27.4 x 21.2 (22.4-29.6 x 17.6-23.6) µm, with a thin wall; shape index (length/width) 1.29 (1.13-1.33). A micropyle was absent, an oocyst residuum was present, and a polar granule sometimes present. Sporocysts were elongate, 17.4 x 6.4 (15.2-19.2 x 5.6-7.2) µm, and possessed an indistinct Stieda body; shape index 2.75 (2.44-3.03). Each sporozoite had ellipsoidal anterior and spherical to ellipsoidal posterior refractile bodies.

Key words. *Diadophis punctatus arnyi*, Serpentes, Colubridae, *Eimeria kennicotti* sp. n., Coccidia, Apicomplexa, Eimeriidae.

INTRODUCTION

The prairie ringneck snake, *Diadophis punctatus arnyi* Kennicott, 1859 is a small colubrid that ranges from extreme southwestern Wisconsin and southeastern South Dakota to southcentral Texas and eastern New Mexico (Conant and Collins 1991). This snake inhabits rocky hillsides in open woods, near cedar glade, and native prairie uplands where they feed primarily on earthworms (Fitch 1975). Wacha and Christiansen (1974) described *Eimeria collanuli* from this host in Iowa, and *E. arnyi* was described by Upton and Oppert (1991) from ringneck snakes in Kansas. McAllister et al. (1995) recently reported this latter coccidian from *D. punctatus arnyi* in Arkansas. Herein we describe the oocysts of a third species of eimerian from this host from Arkansas.

MATERIALS AND METHODS

During April, 1993 and May, 1994, 4 adult (3 males and 1 female, mean ±SE snout-vent length 215.3 ±16.5, range 175-256 mm) *D punctatus arnyi* were collected by hand beneath debris in Somervell County, Texas (n = 2), and Polk County, Arkansas (n = 2). Snakes were killed by sodium pentobarbital (Nembutal^{R}) overdose and intestinal contents and feces collected and placed in a 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$) solution. Unsporulated oocysts were allowed to complete development by placing faeces in Petri dishes

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containing a thin layer of $K_2Cr_20_7$ solution for 5 days at room temperature (*ca* 23°C). Sporulated oocysts were then concentrated by flotation in an aqueous sucrose solution (specific gravity 1.30). Measurements and photomicrographs were taken using Nomarski interference-contrast optics and a calibrated ocular micrometer and are reported in micrometers (μm) as means, followed by the ranges in parentheses. Oocysts were 21 days old when measured.

RESULTS

Two (50%) of the snakes were found to be passing unsporulated coccidian oocysts. Upon sporulation, these oocysts were found to represent a previously unreported species, which is described below.

***Eimeria kennicotti* sp. n. (Figs. 1-4)**

Description of oocysts: ellipsoidal, 27.4 x 21.2 (22.4-29.6 x 17.6-23.6) (n = 25), with a smooth, indistinctly bilayered wall *ca* 1.0 thick; shape index (length/width) 1.29 (1.13-1.33). Micropyle absent; large, delicate, spherical oocyst residuum present, 15.6 (12.0-20.0) (n = 15), composed of numerous granules enclosing a vacuolar-like structure, within which were 1-several homogenous globules; polar granule present in approximately 1/3 of the oocysts. Sporocysts elongate, 17.4 x 6.4 (15.2-19.2 x 5.6-7.2) (n = 15); shape index 2.75 (2.44-3.03). Indistinct, blunt Stieda body present at one end of sporocyst, with small, indistinct substieda body. Sporozoites elongate, 15.2 x 3.1 (12.8-18.0 x 2.6-3.4) (n = 10) *in situ*, with transverse striations anteriorly. Sporozoites lie lengthwise, in opposite directions in sporocyst. Each sporozoite with ellipsoidal anterior refractile body, 3.9 long x 2.9 wide (3.2-4.2 x 2.4-3.4) (n = 10) and spherical to ellipsoidal posterior refractile body, 3.2 long x 2.9 wide (2.6-4.0 x 2.4-3.4) (n = 10). Nucleus located between refractile bodies.

Type-host: *Diadophis punctatus arnyi* Kennicott, 1859 "Prairie ringneck snake" (Serpentes: Colubridae). An adult female (snout-vent length 215 mm) collected 29th May, 1994 by L. D. Gage has been deposited in the Arkansas State University Museum of Zoology collection as No. 19782.

Type locality: Polk County, Arkansas, USA, (34° 42'N, 94° 21' W).

Type specimens: phototypes have been deposited in the U.S. National Helminthological Museum, Beltsville, Maryland as USNM No. 84543.

Site of Infection: unknown. Oocysts recovered from feces.

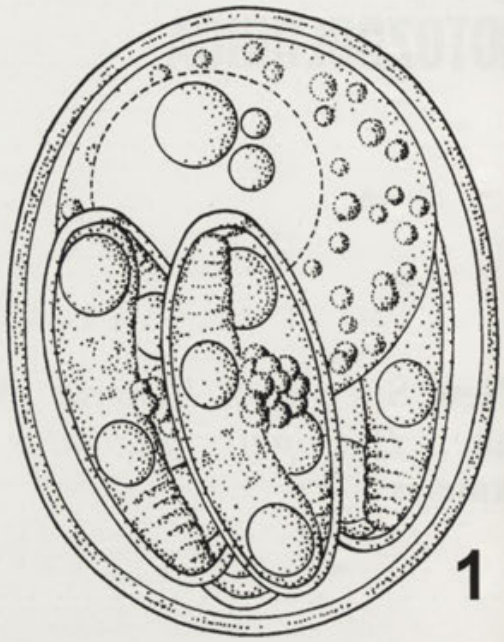


Fig. 1. Composite line drawing of sporulated oocyst of *Eimeria kennicotti* sp. n. from *Diadophis punctatus arnyi*

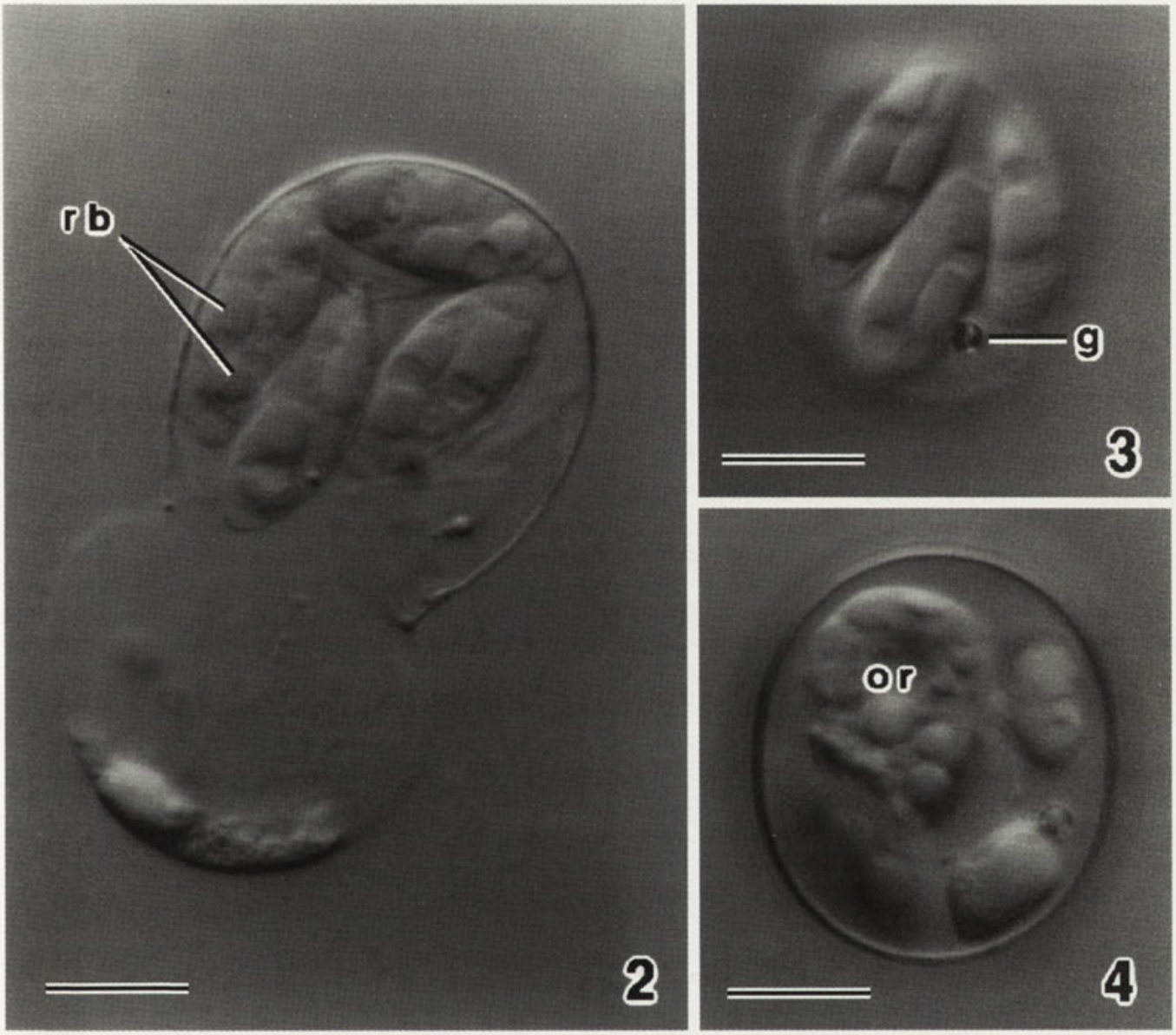
Sporulation: exogenous. Oocysts were passed unsporulated or partially sporulated and completed development within 5 days at 23°C.

Prevalence: 2/4 (50%) snakes were found to be passing oocysts; 2/2 from Polk County, Arkansas and 0/2 from Somervell County, Texas.

Etymology: the specific epithet is given in honor of Robert Kennicott (1835-1866), American herpetologist, who described the host in 1859.

DISCUSSION

Only two species of coccidia have been reported from *Diadophis punctatus arnyi* previously. Oocysts of *Eimeria collanuli* Wacha and Christiansen, 1974 are more elongate, possess smaller sporocysts, and have a smaller oocyst residuum whereas oocysts and sporocysts of *E. arnyi* Upton and Oppert 1991 are smaller and an oocyst residuum is lacking (Wacha and Christiansen 1974, Upton and Oppert 1991). When eimerians from other colubrids are compared (Upton and McAllister 1990), only *E. mikanii* Carini 1933 from *Sybinomorphus mikanii* in Brasil and *E. typhlopisi* Ovezmukhamedov 1968 from *Typhlops ver-*



Figs. 2-4. Nomarski interference-contrast photomicrographs of sporulated oocysts of *Eimeria kennicotti* sp. n. Bars - 10 µm. 2 - collapsed oocyst demonstrating presence of 4 sporocysts, 3 - intact oocyst demonstrating typical arrangement of sporocysts within oocyst, 4 - intact oocyst showing oocyst residuum. Abbreviations: g - polar granule, or - oocyst residuum, rb - refractile bodies of sporozoites

micularis from Russia are similar. Oocysts of both species have considerably smaller sporocysts and fail to possess oocyst residua (Carini 1933, Ovezmukhamedov 1968).

Acknowledgments. We thank the Arkansas Game and Fish Commission and Texas Parks and Wildlife Department for scientific collecting permits No. 775 and SPR-0390-027, respectively, to C.T.M. This is Kansas Agricultural Experiment Station Contribution No. 95-326-J.

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Received on 30th January, 1995; accepted on 9th February, 1995

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1995) 34: 221 - 226

Hirmocystis palorusii sp. n., a New Species of Septate Gregarine from *Palorus ratzeburgii* (Coleoptera: Tenebrionidae)

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Summary. A new cephaline gregarine belonging to the genus *Hirmocystis*, *H. palorusii* sp. n., from a stored grain pest, *Palorus ratzeburgii* (Coleoptera: Tenebrionidae) is described. The epimerite is simple stalkless globule, fresh measures (in µm): (n = 30), length = 7.3, SE ± 0.4, CV% = 33.8, width = 6.6, SE ± 0.4, CV% = 34.8; fixed and stained (n = 30): length = 7.1, SE ± 0.45, CV% = 35.07, width = 6.0, SE ± 0.38, CV% = 34.98. Sporadin is solitary as well as biassociative with hemispherical protomerite and massive deutomerite. The sporadin measures, fresh (n = 30): length = 54.8, SE ± 4.2, CV% = 41.6); fixed and stained (n = 30): length = 54.4, SE ± 4.1, CV% = 41.9; width = 25.8, SE ± 2.8, CV% = 60.85. Caudo-frontal association. Ovoidal gametocysts (n = 30): length = 42.4, SE ± 0.8, CV% = 11.3; width = 53.85, SE ± 1.09, CV% = 11.14) and spherical spores (n = 150) diameter = 4.2, SE ± O.02, CV% = 4.3). The ratio of LP:TL and WP:WD is 1 : 5.2 and 1 : 1.4 respectively.

Key words. *Hirmocystis palorusii* sp. n., *Palorus ratzeburgii*, cephaline gregarine.

INTRODUCTION

Labbé established the genus *Hirmocystis* in 1899. The diagnostic characters of the genus are as follows (cited from Setna and Bhatia 1934): sporadins in associations of 2 to 12 (or more), in linear chains; epimerite a small conical or cylindrical knob; cysts spherical, dehiscence by simple rupture; and spores ovoidal with two coats.

After the establishment of the genus *Hirmocystis* by Labbé 32 species of this genus from various arthropod hosts have been described by many workers throughout the world.

Watson (1916) and Kamm (1922) placed the genus *Hirmocystis* under the family Gregarinidae on the basis of characters like simple epimerite, biassociative sporadins, and insect host. Grassé (1953) separated the genera *Hirmocystis* Labbé; *Hyalospora* Schneider, *Tettigonospora* Smith, *Euspora* Schneider, *Caulocephalus* Bhatia and Setna, *Sphaerocystis* Léger and *Didymophyes* Stein from the family Gregarinidae and placed them in a new family Hirmocystidae. Chakravarty (1959) accepted the revision as proposed by Grassé (1953) but remarked that, "According to the laws of priority in nomenclature the first family name shall stand as it is formed from a valid generic name,

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the name Didymophyidae, therefore, should stand to include under it all the above genera except Sphaerocystis Léger which is put under a new family Sphaerocystidae. Hirmocystidae Grassé is to be regarded as a synonym of Didymophyidae Léger". Chakravarty's (1959) view was accepted by Haldar and Chakraborty (1979) and Sarkar and Haldar (1980).

According to Kudo (1966) the genus *Hirmocystis* should be placed under the family Gregarinidae as the epimerite is simple and symmetrical, sporadins are in association and spores are symmetrical. However, Levine (1979) retained the family Hirmocystidae to include the genera *Hirmocystis* and *Arachnocystis* gen. n. under it and this system of classification is followed in this paper.

In our studies on the cephaline gregarines we have obtained some parasites which are having associative sporadins, simple knob or papilla-like epimerite and gametocysts dehiscing by simple rupture. These parasites were, therefore, identified as belonging to the genus *Hirmocystis* Labbé and could be separated from the genus Gregarina in the absence of sporoducts. The gregarine is described here as a new species for some distinctive characters of its own.

MATERIALS AND METHODS

The host insect *Palorus ratzeburgii* was collected from Kalyani, Nadia, West Bengal and brought alive to the laboratory for investigation. The insects were decapitated, their midgut were carefully removed and teased apart with fine needles. For studying the fresh specimens thin smears were prepared and covered with a cover-slip, the edges of which were sealed with paraffin wax. Measurements of the fresh material were taken. To prepare permanent slide, smears were semidried, fixed in Schaudinn's fluid and subsequently stained with Heidenhain's haematoxylin. Gametocysts were collected from the infected hosts and cultured in moist chamber for sporulation (Sprague 1941). For studying the structure of the spores, spore suspension was taken on a glass slide containing a drop of Lugol's iodine. A coverslip was placed on it and the edges were sealed with paraffin. Observation was done under oil immersion lens. Measurements are given in µm. The following abbreviations have been used: TL - total length, LE - length of epimerite, LP - length of protomerite, LD - length of deutomerite, WE - width of epimerite, WP - width of protomerite, WD - width of deutomerite, TLP - total length of primite; LPP - length of protomerite of primite, WPP - width of protomerite of primite, LDP - length of deutomerite of primite, WDP - width of deutomerite of primite, TLS - total length of satellite, LPS - length of protomerite of satellite, WPS - width of protomerite of satellite, LDS - length of deutomerite of satellite, WDS - width of deutomerite of satellite, LN - length of nucleus, WN - width of nucleus.

RESULTS

Hirmocystis palorusii sp. n. (Figs. 1-15, Table 1)

Host: *Palorus ratzeburgii* (Coleoptera: Tenebrionidae)

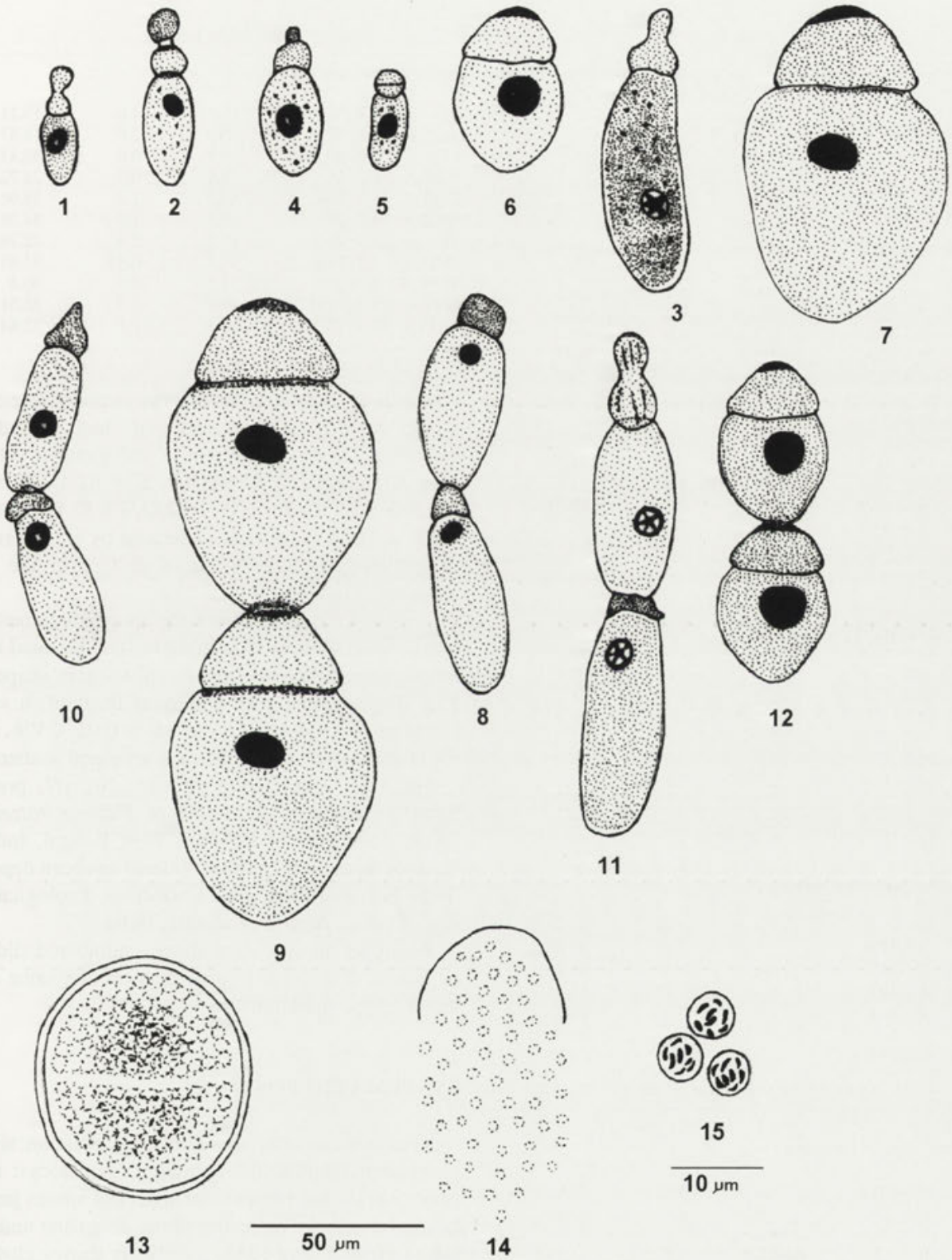
Prevalence: out of 606 hosts examined 207 (34.15%) were found to be infected.

Trophozoite: a large number of small ovoidal trophozoites (Figs. 1-4) are commonly encountered in smear preparations. The epimerite is variously shaped. The most common is a simple stalkless globule seated on the protomerite (Fig. 4). The other types are stalked globule or papilla-like. The junction between the epimerite and the protomerite is not always clearly demarcated. The protomerite is dome-shaped. The septum is thick and convex. Deutomerite is ovoidal and slightly wider at the mid-length. The posterior end is rounded. The pellicle is very thin and epicyteal striations are not observable. Spherical nucleus situated at different places in the deutomerite.

Sporadin: immature are small, cylindrical bodies with subspherical protomerite and cylindrical deutomerite (Fig. 5). But as the sporadins mature they become large obese bodies with a large hemispherical protomerite (Figs. 6, 7). The septum is thick, straight but sometimes very slightly convex. The massive deutomerite is widest near septum. It becomes so much obese that often it is as wide as long. The posterior end is broadly rounded. The nucleus is very large, spherical, and anteriorly placed. The chromatin material is either scattered or sometimes forms a ring, central portion being filled by nucleoplasm. Cytoplasmic granules are large and granulation is heavy in deutomerite but fine and is moderate in protomerite.

Association: firm, caudo-frontal association is observed. Three types of attachments are seen. In the first form, the deutomerite of primite becomes invaginated to receive the protomerite of satellite (Fig. 8); in the second form, the protomerite of satellite is invaginated and deutomerite of primite closely fits in (Figs. 9, 10); in the third form the deutomerite of primite and protomerite of satellite becomes wavy in a complementary manner and fit together (Figs. 11, 12). The pairing partners show no morphological differences in respect of their shape and size.

Gametocyst and spore: gametocysts were obtained from hind gut of 69 heavily parasitized insects. These were shining white in color, ovoidal in shape, double-walled with a clear space in between (Fig. 13). Imma-



Figs. 1-15. Camera lucida drawings of different stages of life cycle of *Hirmocystis palorusii* sp. n. 1-4 - trophozoites of different sizes showed varied epimerite, 5 - a young sporadin, 6-7 - mature obese sporadins, 8-12 - syzygies showing different types of attachments, 13 - a gametocyst with two gametocytes, 14 - cyst dehiscence by simple rupture, 15 - spores each with eight sporozoites

Table 1

The summary of measurements (in µm) of 30 specimens of fresh and 30 specimens of fixed and stained trophozoite, sporadin and syzygy

Measurement	$\bar{x}$	SE ±	CV%
Trophozoite (fresh)			
TL = 19.5-77.0	47.7	2.9	33.8
LE = 2.8-11.4	7.3	0.4	33.8
WE = 2.6- 9.2	6.6	0.4	34.8
LP = 7.4-15.2	8.9	0.4	22.5
WP = 5.6-15.8	9.7	0.4	25.7
LD = 9.4-62.6	31.2	2.2	38.7
WD = 6.4-25.5	13.2	0.7	29.2
Sporadin (fresh)			
TL = 15.3-102.5	54.8	4.2	41.6
LP = 6.7- 23.5	12.4	0.9	41.1
WP = 4.6- 40.0	19.5	2.1	60.51
LD = 8.3- 83.8	42.8	3.4	44.0
WD = 6.8- 62.9	26.1	2.8	60.15
Syzygy (fresh)			
TL = 57.0-162.7	116.0	4.0	19.0
TLP = 42.0- 83.8	58.2	1.8	16.9
LPP = 6.6- 21.0	11.1	0.6	29.7
WPP = 6.4- 17.0	12.4	0.5	23.0
LDP = 33.9- 75.8	49.7	1.7	18.9
WDP = 9.0- 29.8	20.3	1.0	28.6
TLS = 33.5- 79.8	58.0	2.3	22.2
LPS = 4.7- 17.0	7.7	0.5	33.8
WPS = 8.3- 21.4	12.9	0.7	28.7
LDS = 25.5- 77.5	50.4	2.3	25.4
WDS = 11.0- 33.9	20.3	1.2	31.5
Trophozoite (fixed and stained)			
TL = 18.7-75.8	47.4	2.94	33.94
LE = 2.1-10.5	7.1	0.45	35.07
WE = 2.1- 8.3	6.0	0.38	34.98
LP = 6.2-14.6	8.7	0.37	23.33
WP = 4.2-14.6	9.4	0.4	25.31
LD = 8.3-62.4	29.9	2.2	40.36
WD = 6.2-25.0	12.9	0.7	30.38
LP:TL = 1:3.0 - 9.2 ($\bar{x}$ = 5.5); WP:WD = 1:1.0 - 1.8 ($\bar{x}$ = 1.4).			
Sporadin (fixed and stained)			
TL = 14.6-101.9	54.4	4.1	41.9
LP = 6.2- 23.0	12.0	0.9	44.08
WP = 4.2- 39.5	18.6	2.1	61.07
LD = 8.3- 83.2	42.4	3.4	44.48
WD = 6.2- 62.4	25.8	2.8	60.85
LN = 2.1- 12.5	6.1	0.5	49.18
WN = 2.1- 12.5	6.5	0.7	57.38
LP:TL = 1:2.35 - 7.65 ($\bar{x}$ = 4.6); WP:WD = 1:1.0 - 2.17 ($\bar{x}$ = 1.4)			

Table 1(cont.)

Syzygy (fixed and stained)			
TL = 56.2-162.2	115.6	4.0	1911
TLP = 41.6- 83.2	58.0	2.0	1887
LPP = 6.2- 20.8	9.9	0.6	3545
WPP = 6.2- 16.7	11.8	0.5	2372
LDP = 33.3- 75.0	49.2	1.7	1896
WDP = 8.5- 29.1	18.7	1.2	3438
TLS = 33.3- 79.1	57.2	2.4	2276
LPS = 4.2- 16.6	7.3	0.45	3397
WPS = 8.3- 20.8	12.5	0.7	304
LDS = 25.0- 77.0	49.9	2.3	2551
WDS = 10.4- 33.3	19.3	1.1	3284

ture gametocysts are having two equal gametocytes while mature shows scattered large condensed granules. The measurement of the gametocysts are: n = 30; length = 37.9-49.9, $\bar{x}$ = 42.4, SE ± 0.8, CV% = 11.3; width = 48.2-62.O, $\bar{x}$ = 53.85, SE± 1.09, CV% = 11.14. Spores are liberated by simple rupture of gametocysts after 46 h of culture in the moist chamber (Fig. 14).

To study the structure of the spore 30 gametocysts were collected from 20 infected hosts. Liberated spores from cultured gametocysts are spherical in shape (Fig. 15), single-walled with a measurement of: n = 150; diameter = 3.9-4.4, $\bar{x}$ = 4.2, SE ± 0.02, CV% = 4.3. Eight elongated sporozoites are arranged scatterdly.

Holotype: trophozoite on slide No. r/7, prepared from the contents of midgut of *Palorus ratzeburgii* (Wiss.) collected at Kalyani, West Bengal, India by Karnica Saha on July 2nd, 1990 and has been deposited in the National Zoological Collection, Zoological Survey of India, Alinore, Calcutta, India.

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

SYSTEMATIC POSITION

The characters like sporadins in association, simple-globular or papilla-like epimerite, gametocyst dehiscence by simple rupture and spherical spores justifies beyond doubt the inclusion of the gregarine under the genus *Hirmocystis* Labbé, 1899. It shows closeness with *H. lepropi* Haldar and Chakraborty, 1981 in LP:TL and WP:WD values and also in shape of the epimerite and gametocyst, but differs widely in all other morphometric values and other features. General

Table 2

Comparative characters of *Hirmocystis palorusii* sp. n. and its related species. Measure in µm

Characters	*H. ventricosa* (Léger) Labbé, 1899	*H. harpali* Watson, 1916	*H.. lepropi* Haldar and Chakraborty, 1981	*H. lophocateri* Ghose and Haldar, 1989	*H. triboli* Ghose and Haldar, 1989	*H. palorusii* sp. n.
Total lenght	180	100-500	77.5-580	18.9-135	24.3-40.5	18.7-75.8
Epimerite	small cylindro-conical papilla	spherical	subspherical papilla	dome-shape with a neck	tongue-shaped or papilla-like	globular or papilla-like
Protomerite	broadly rounded in front widest in the anterior half, constricted and cylindrical in second half	dome-shaped	ovoid in trophozoite and sporadin	pea-shaped in trophozoites, hemispherical in sporadin	hat-shaped trophozoite and sporadin	dome-shaped in trophozoite and subspherical in sporadin
Sporadin	bi-, triassociative	bi-, tri-, tetrassociative	biassociative	solitary and biassociative	solitary and biassociative	solitary and biassociative
Gametocyst	spherical; 100	not known	oval;150x250 to 200x360	oval; 96.6x38.4	119.7x96.6	ovoidal; 42.4x53.85
Spore	ovoidal; 9x6	not known	ovoidal; 6x4	ovoidal; 5.7x3.3	spherical; 5.5	spherical; 4.2
LP:TL	1:3.5	1:7	1:5.4	1:3.4	1:3.5	1:5.2
WP:WD	1:1.5	1.1.2	1:1.3	1:1	1:1.1	1:1.4
Host	*Tipula obracea,* Pachyrhina pratensis (larva)(Diptera:Tipulidae)	*Harpalus pennsylvanicus erythropus*	*Lepropus* sp. (Coleoptera)	*Lophocateres pusillus* (Coleoptera: Curculionidae)	*Tribolium castaneum* (Coleoptera: Tenebrionidae)	*Palorus ratzeburgii* (Coleoptera: Tenebrionidae)

shape of its sporadin and ovoidal gametocyst resemble that of *H. lophocateri* Ghose and Haldar, 1989 but otherwise it differs in LP:TL, WP:WD, shape of the epimerite, size of the gametocyst, spore attachment and also host from which the parasite has been reported. It resembles *H. harpali* Watson, 1916 and *H. ventricosa* (Legér) Labbé, 1899 in only WP:WD value but differs in all other aspects. *H. triboli* Ghose and Haldar 1989 shows closeness to the described species in shape of the cyst and spore but structural details and all morphometric values build a clear wall of distinction between the two.

The present form differs widely from any previously described species of the genus *Hirmocystis*. As such, it is considered to be a new species and designated as *Hirmocystis palorusii* sp. n. after the generic name of host insect. The comparative characters of *H. lepropi, H. lophocateri, H. triboli, H. harpali, H. ventricosa* and *H. palorusii* have been summarized in Table 2.

Acknowledgement. Authors are grateful to University Grants Commission, New Delhi for financial assistance.

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Received on 26th July, 1994: accept on 5th January,1995

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1995) 34: 227 - 331

Pileocephalus sericornii sp.n., a New Species of Septate Gregarine (Apicomplexa: Sporozoea) from *Lesioderma sericorne* (Coleoptera: Tenebrionidae)

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Summary. An eugregarine parasite belonging to the genus *Pileocephalus, P. sericornii* sp. n., is described from the Indian subcontinent. The trophozoite has a lance or coma-shaped (fresh) epimerite (n = 30; length = 75.1 µm, SE ±4.83, CV% = 35.22; width = 22.6 µm, SE ± 1.57, CV% = 38.14); fixed and stained (n = 30; length = 74.8 µm, SE ± 4.9, CV% = 35.9; width = 22.1 µm, SE ± 1.6, CV% = 39.9); solitary (fresh) sporadins (n = 30; length = 66.7 µm, SE ± 4.0, CV% = 33.1; width = 19.1 µm, SE ± 1.3, CV% = 36.9); fixed and stained (n = 30; length = 66.5 µm, SE ± 4.0, CV% = 33.2; width = 18.8 µm, SE ± 1.4, CV% = 41.0), spherical gametocyst (n = 35; length = 60.88 µm, SE ± 0.56, CV% = 5.4; width = 56.32 µm, SE ± 0.58, CV% = 5.1), and biconical spores (n = 145; length 9.2 µm, SE ± 0.02, CV% = 1.84; width = 6.05 µm, SE ± 0.05, CV% = 5.4). The ratio of LP:TL and WP:WD is 1:6.3 and 1:1.4, respectively.

Key words. *Lesioderma sericorne, Pileocephalus sericornii* sp. n., eugregarine, parasite.

INTRODUCTION

The genus *Pileocephalus* was first established by Schneider (1875). The generic features are as follows - epimerite a lance-shaped or simple cone-like structure; spores biconical. The type species of the genus *Pileocephalus* is *P. chinensis*. The credit of further contribution to this genus goes to Labbé (1899), Léger and Duboscq (1909), Watson (1916), Kamm (1922), Foerster (1938), Semans (1939), Stein (1960), Baudoin (1967), Lipa (1967) and Geus (1969).

In our study on cephaline gregarines of insect pests of West Bengal, India, we have obtained a parasite inhabiting the proventriculus of *Lesioderma sericorne*. The diagnostic characters of the parasite are: (1) - lancet or comma-shaped epimerite; (2) - protomerite is hemispherical and constricted at septum; (3) - deutomerite is widest at shoulder and then tapering more or less sharply; (4) - sporadins solitary; (5) - cyst spherical, dehiscence by simple rupture; (6) - spores biconical.

The combination of these characters places the parasite under the genus *Pileocephalus* Schneider, 1875

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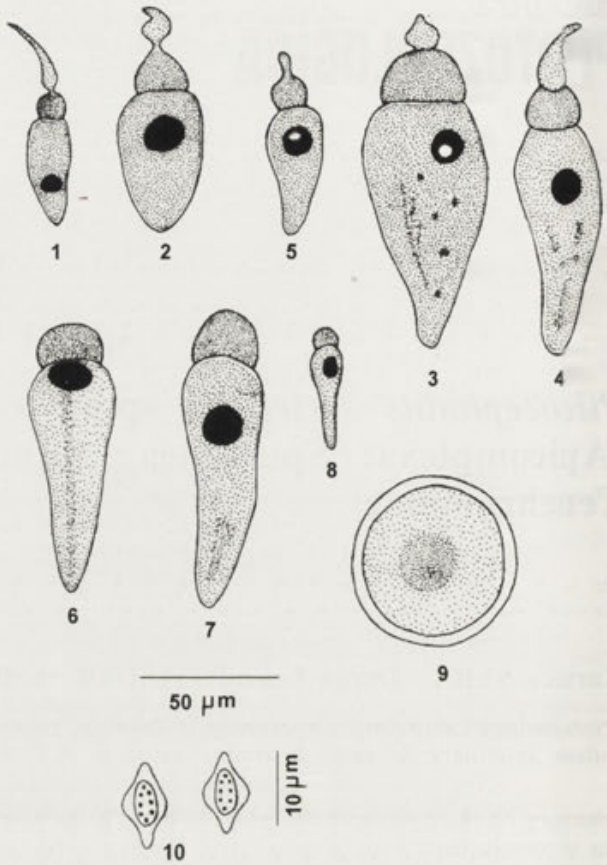
beyond any doubt. The gregarine described here as a new species for some distinctive characters of its own.

MATERIALS AND METHODS

The host insect *Lesioderma sericorne* was collected from Kalyani, Nadia, West Bengal and brought alive to the laboratory for investigation. The insects were decapitated, their proventriculi carefully removed and teased apart with fine needles. For studying the fresh specimen thin smears were prepared and covered with a cover slip, the edges of which were sealed with paraffin wax. Measurements of the fresh material were taken. To prepare permanent slides, smears were semi dried, fixed in Schaudin's fluid and subsequently stained with Heidenhain's hematoxylin. Cysts were collected from the infected hosts and cultured in moist chamber for sporulation (Sprague 1941). For studying the structure of the spores, spore suspension was taken on a glass slide containing a drop of Lugol's iodine. A coverslip was placed on it and the edges were sealed with paraffin. Observation was done under oil immersion lens. All measurements are expressed in µm.

The following abbreviations have been used: TL - total length, LE - length of epimerite, LP - length of protomerite, LD - length of deutomerite, WE - width of epimerite, WP - width of protomerite, WD - width of deutomerite, LN - length of nucleus, WN - width of nucleus.



Figs. 1-10. Camera lucida drawings of different stages of life cycle of *Pileocephalus sericornii* sp. n. 1-5 - trophozoites of different shapes and sizes showing varied epimerite, 6-8 - sporadins of different sizes, 9 - freshly collected gametocyst, 10 - spore with 8 sporozoites

RESULTS

Pileocephalus sericornii sp. n. (Figs. 1-10, Table 1)

Host: *Lesioderma sericorne*

Prevalence: 386 hosts out of 1062 were found infected by theparasite.

Trophozoite: long, widest in middle and tapering in both directions. The epimerite is either like a lancet or like a comma. It may be stalked or stalkless. Upon the stalk is the bulbous portion that is gradually drawn into a long, sharply pointed cone. The apical portion is rarely straight; in most cases it is curved to a side (Figs. 1-4). The shorter form of the epimerite is stalkless and is thick comma-shaped or a short lancet tapering towards the free end (Fig. 5). The protomerite is broadly rounded, slightly wider than high, constricted near septum. The deutomerite is widest at anterior end and broadly rounded posteriorly. The pellicle is thin and epicyteal striations are not observable. Cytoplasmic granules are very few in the epimerite.

Sporadin: solitary (Figs. 6-8), unusually large. The protomerite is large, broadly rounded at the anterior end and is deeply constricted near septum. It is wider than high and broadest near middle. The septum is thick and convex. The deutomerite is widest at shoulder and then gradually tapers to an acute point. The nucleus is large, oval, darkly stained. Cytoplasmic granules are of moderate in size and uniformly distributed throughout the protomerite and the deutomerite.

Gametocyst: freshly collected gametocysts appear as translucent shining dots under a dissecting binocular. The gametocysts are spherical (Fig. 9), double-walled and have a densely granulated sporoplasm. The measurement of the gametocysts are: n = 35; length = 54.2-65.2, $\overline{x}$ = 60.88, SE ± 0.56, CV% = 5.4; width = 52.2-60.2, $\overline{x}$ = 56.32, SE ± 0.58, CV% = 5.1. The gametocysts dehiscence by simple rupture after 72 h of development in the moist chamber.

Spore: observations have been made from 50 gametocysts collected from 35 heavily infected hosts. After culturing the gametocysts in the moist chamber the liberated spores were collected. The spores are biconical in shape (Fig. 10) having the measurement of n = 145; length = 8.9-9.8, $\overline{x}$ = 9.23, SE± 0.02, CV% = 1.84; width = 6.0-6.1, $\overline{x}$ = 6.05, SE ± 0.05, CV% = 5.4.

Holotype: of trophozoite on slide No. GH/10 prepared from the contents of proventriculus of *Lesioderma sericorne* at Kalyani, Nadia, W. B., India by Karnica Saha on April 11, 1990.

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

The holotype and paratype materials have been deposited in the National Zoological Collection, Zoological Survey of India, Alipore, Calcutta, India.

Table 1

The summary of measurements (in µm) of 30 specimens of fresh and 30 specimens of fixed and stained trophozoites and sporadins

Measurement	$\bar{x}$	SE ±	CV%
Trophozoite (fresh)			
TL = 29.3-129.2	75.1	4.83	35.22
LE = 6.4- 25.0	14.9	1.05	38.6
WE = 2.3- 16.9	8.9	0.6	37.4
LP = 4.5- 29.4	12.4	0.85	37.6
WP = 4.5- 34.0	15.9	1.2	40.9
LD = 17.2- 88.5	48.6	3.72	41.96
WD = 6.8- 46.9	22.6	1.57	38.14
Trophozoite (fixed and stained)			
TL = 29.1-128.9	74.8	4.9	35.9
LE = 6.2- 25.0	14.8	1.1	40.9
WE = 2.1- 16.6	8.7	0.6	38.8
LP = 4.2- 29.1	12.2	0.8	38.7
WP = 4.2- 33.3	15.7	0.6	40.2
LD = 16.6- 87.4	48.1	3.6	41.6
WD = 6.2- 45.7	22.1	1.6	39.9
LN = 2.1- 12.5	7.6	0.4	33.4
WN = 2.1- 14.6	8.9	1.0	39.2
LP:TL = 1:4.3-8.9 (6.3); WP:WD = 1:1.0-2.0 (1.4).			
Sporadin (fresh)			
TL = 21.5-105.0	66.7	4.0	33.1
LP = 4.2- 21.2	13.5	0.7	31.1
WP = 4.4- 30.4	15.8	1.1	38.1
LD = 17.4- 83.5	54.0	3.4	34.4
WD = 6.9- 39.0	19.1	1.3	36.9
Sporadin (fixed and stained)			
TL = 20.8-104.0	66.5	4.0	33.2
LP = 4.2- 20.8	13.3	0.8	35.2
WP = 4.2- 29.1	15.2	1.1	41.6
LD = 16.6- 83.2	53.7	3.4	34.7
WD = 6.2- 37.4	18.8	1.4	41.0
LN = 2.1- 10.4	6.7	0.4	33.4
WN = 2.1- 14.6	8.4	0.6	38.4
LP:TL = 1:3.6-6.7 (5.0); WP:WD = 1:0.8-1.7 (1.2)			

SYSTEMATIC POSITION

Solitary sporonts, varied epimerite, gametocyst dehiscence by simple rupture and biconical spores - when a specimen exhibits all these characters, it can, without any hesitation be placed under the family Actinocephalidae Léger 1892. So, the described specimen beautifully displaying all these characters, is definately placed under the said family. Also, with the epimerite being lanceolet and spores biconical, it is without any doubt a member of the genus *Pileocephalus* Schneider, 1875.

The new species is most similar to *P. astaurovi* Lipa, 1967, from *Baicalina spinosa*. There are resemblances in general body shape of sporonts, deutomerite, constriction of protomerite at septum and shape of the gametocyst. However, the epimerite in *P. sericornii* is very much specialized, LP:TL and WP:WD values are different, the size of specimens in description is about half of *P. astaurovi*.

Compared with other species of *Pileocephalus* described to date, *P. sericornii* has minor similarities with a few species. It comes close to *P. chinensis* Schneider 1875 in the shape of the deutomerite and spherical cyst. With *P. heerii* Schneider 1887, the similarities lie in the shape of the epimerite, constriction of protomerite at septum and biconical spores. To list the differences there are many - general shape, acuminate ending of deutomerite, shape of the protomerite, LP:TL and WP:WD values. The presently described species have some resemblances to *P. benli* and *P. nemurae* in the shape of the deutomerite, general shape of sporadin and WP:WD values but differs in all other respects.

Since it has no major similarities with any of the previously described species of the genus *Pileocephalus* it may be regarded as a new species for which the name *Pileocephalus sericornii* sp. n., is proposed after the specific name of the host insect.

The comparative characters of *P. astaurovi, P. chinensis, P. heerii, P. nemurae, P. benli* and *P. sericornii* sp. n. have been summarized in Table 2.

Acknowledgements. Sincere thanks are due to University Grants Commission, New Delhi for financial assistance.

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Table 2

Comparison of characters of *Pileocephalus sericornii* sp. n. with closely related species (measurements in µm)

Characters	*P. chinensis* Schneider, 1875	*P. heerii* Schneider, 1887	*P. astaurovi* Lipa, 1967	*P. benli* Geus, 1969	*P. nemurae* Geus, 1969	*P. sericornii* sp. n
Total length	-	-	up to 230	up to 131	up to 90	29.1-128.9
Epimerite	small unstalked papilla; conoidal at apex	elongated papilla sharply acuminate on a neck in youngs; lanceolet upon a stout neck in mature forms		stalked sharp papilla-like	small unstalked papilla	stalked or unstalked lanceolet or comma shaped
Protomerite	broadly rounded	conical, widest at septum, apex truncate; constriction at septum	broadly rounded wider than long; deep constriction	broadly rounded	broadly rounded widest at the middle	broadly rounded, slightly wider than high; constricted at septum
Deutomerite	widest at shoulder, tapering into broad blunt extremity	conical, ending acuminately, broadest at septum ending in an acute point	widest at shoulder ending in an acute point	widest at shoulder posterior extremity is blunt	widest at middle and ends bluntly	Widest at shoulder and tapering in an acute proximity
Gametocyst	spherical	-	spherical; 500	-	-	spherical; 60.88 x 56.32
Spore	roughly triangular	biconical	-	roughly triangular	-	biconical; 9.2 x 6.05
LP:TL	1:5	1:3	1:2.7-4.1	1:2.38	1:3.7	1:6.3
WP:WD	1:1	1:1	1:1.1-1.4	1:1.27	1:1.5	1:1.4
Host	*Mystacides* sp.	*Phryganea varia*	*Baicalina spinosa*	Lepismatidae	*Perlodes* sp., *Nemura* sp., *Leuctra* sp.	*Lesioderma sericone*
Order of host	Neuroptera	Trichoptera	Trichoptera	Tysanura	Plecoptera	Coleoptera

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Received on 26th January, 1994; accepted on 3rd January, 1995

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1995) 34: 233 - 235

Short communication

Isospora sp. (Apicomplexa: Eimeriidae) in Icterine Warbler (*Hippolais icterina*, Passeriformes: Sylviidae): the Possibility of Parents to Nestlings Transmission

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Summary. The prevalence of *Isospora* sp. in a population of icterine warbler was studied to ascertain the possibility of transmission of oocysts from parents to nestlings. Seventy-two adults and nestlings in 51 nests were examined; *Isospora* sp. oocysts were found in 91.2% of samples from females, 78.9% from males and 35.3% from nestlings. There was no significant difference between the prevalence of infection in females and males; the difference between adults and nestlings was statistically highly significant. The prevalence in nestlings depended on their age (24% in 7-9 days and 50% in 10-12 days old nestlings). From 39 nests with 1 or both parents harboring oocysts, 12 nests contained infected youngs and 27 were infection free; the difference between the average age of nestlings in the infected and uninfected group (9.7 and 9.2 days, respectively) was not significant. As no nests with infection-free parents were found, we could not conclude if nestlings were infected by food contaminated accidentally or directly from their positive parents.

Key words. *Isospora, Hippolais icterina*, icterine warbler, coccidia, transmission, prevalence.

Coccidia of the genus *Isospora* are common parasites of passerine birds; their prevalence in some populations can reach 40% (Scholtyseck and Przygodda 1956, Svobodová 1994). These prevalences are interesting because *Isospora* spp. of passerine birds are monoxenous, oocysts are excreted in feces and the possibility of infection acquired with food seems to be low due to the dispersion of oocysts in the environment. A possible manner of infection is the direct transmission between individuals, e. g., during the nesting period when oocysts could be transmitted to nestlings with food contaminated by feces of infected parents. Przygodda and Scholtyseck (1961) found that the prevalence of infection with *Isospora* spp. is higher in older nestlings of passerine birds; however, they did not examine parent birds.

In 1989-1991, the breeding biology of a population of icterine warbler (*Hippolais icterina*, Passeriformes: Sylviidae) in Central Bohemia, Czech Republic, was studied. Warblers are mainly insectivorous and both females and males feed the young (Hudec 1983, Payevsky 1987). Our attention was directed to

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monoxenous coccidia to ascertain if there is some relation between the oocyst shedding in parents and nestlings.

Warblers were caught in nets using 2 methods: males were attracted by tape replayed song, both sexes were caught near nests with nestlings (Cibulková 1993). Birds were kept in tissue bags (max. 1 h), released, and the feces were collected. Samples from nestlings were collected during controls of nests, feces from siblings being mixed together and representing one sample. Accurate hatching date was detected during previous nest controls. Samples of feces were kept in 2% aqueous potassium bichromate ($K_2Cr_2O_7$) solution for three days at room temperature to allow oocysts sporulation and then stored at 4°C. First microscopical examination of samples was direct; when no oocysts were found further examination involved flotation of the material in 33% aqueous zinc sulfate ($ZnSO_4.7H_2O$) solution.

A total of 72 adult birds and nestlings in 51 nests were examined; 91.2% of samples from females (n=34), 78.9% from males (n=38) and 35.3% from nestlings (n=51) being positive to oocysts of the genus *Isospora* (see Table 1.) Oocysts were subsphaerical, measuring 26.8 x 24.8 μm (19.5-33.8 x 18.0-31.5, n=279; for detailed description see Svobodová 1994). There was no significant difference between the prevalence of infection in adult females and males (G-test, G = 2.15, p>0.05). The prevalence in adults (84.7%) was 2.4 times higher than in nestlings; the difference is highly significant (G-test, G = 32.63, p<0.01). Age dependence of oocyst shedding was proved by division of nestling in 2 age groups, older nestlings being positive more frequently (see Table 2); the difference is significant for one-tailed hypothesis (G-test, G = 3.67, p<0.1). In 39 nests, one or both parents were also examined. All nestlings from these nests had at least one parent positive to *Isospora* oocysts; 12 of the nests contained infected youngs, 27 did not. No family was found with all members being negative, nor a positive nest with negative parents. Average age of infected and uninfected nestlings was 9.7 and 9.2 days, respectively; however, the difference was not significant (Mann-Whitney U-test, Z = -1.25, p=0.21).

Table 1

Prevalence of *Isospora* sp. oocysts in icterine warbler

Year	Females			Males			Nestlings		
	n	+	%	n	+	%	n	+	%
1989	11	9	81.8	8	7	87.5	16	6	37.5
1990	16	16	100	20	13	65.0	23	7	30.4
1991	7	6	85.7	10	10	100	12	5	41.7
Total	34	31	91.2	38	30	78.9	51	18	35.3

n - number of examined, + - number of infected, % - prevalence

Table 2

Prevalence of *Isospora* sp. oocysts in two age groups of nestlings

Age (days)	Number of examined	Number of infected	Prevalence (%)
7-9	29	7	24.1
10-12	22	11	50.0

Coccidia of the genus *Isospora* are very common in this population of icterine warbler, including nestlings. There are probably more mechanisms for the maintenance of *Isospora* in the population of the host. Generally, *Isospora* spp. of birds are transmitted *via* oocyst-contaminated food or water; however, the direct transmission should be considered. The fact that oocysts are not infective before sporulation could decrease the possibility of such transmission; but the process depends on temperature and lasts only 24 h for some passerine species of *Isospora* (Stabler and Kitzmiller 1972, Barré and Troncy 1974). Oocysts adhered to the plumage and other parts of the body of parents could be a source of infection for their youngs. The existence of families with infected parents and negative nestlings may be explained in two ways: (1) the youngs are not (yet) infected; (2) the youngs are infected but not yet shedding oocysts due to the prepatent period, which lasts at least 4-5 days (Stabler and Kitzmiller 1972, Box 1977, Cawthorn and Wobeser 1985, Amoudi 1990).

As no nests with *Isospora*-free parents were found, we cannot determine whether nestlings were infected *via* food contaminated by their parents or accidentally (by oocysts dispersed in the environment).

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Received on 23th March, 1995; accepted 5th April, 1995

ACTA
PROTOZOOLOGICA

Acta Protozoologica (1995) 34: 237 - 238

Book Review

Taxonomische und Ökologische Revision der Ciliaten des Saprobiensystems - Band IV: Gymnostomatea, Loxodes, Suctoria. (Taxonomic and ecological revision of the ciliates of the saprobian system) by W. Foissner, H. Berger, H. Blatterer and F. Kohmann. Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft.

Heft 1/1995, 540 pp.; Price: about DM 100,-; ISBN 3-930253-63-1

This is the fourth and the last part of the remarkable "ciliate atlas" on morphology and ecology of ciliated protozoans used as indicators of water quality.

Ciliates are certainly the most interesting group as potential indicators of organic pollution. They are found in all types of aquatic habitats ranging from the unaffected to the most heavily polluted. The amount of organic matter is perhaps the most important environmental factor that indirectly determines their distribution. Ciliates are particularly important on the more polluted side of the gradient where they are abundantly represented by a large number of morphologically diverse forms. Hence, it is quite natural that ciliated protozoans have got an important place in the saprobian system since its very beginning.

Their wider use in the practice of bioindication, however, has been seriously hindered by two reasons. The first one is that working with ciliates is technically more difficult than with other groups like diatoms or invertebrates. The second and the more important reason is the lack of a comprehensive key enabling accurate species identifications. Since the work of Kahl (1930-35) an immense amount of taxonomic work has been accumulated, however, this important information is scattered among a multitude of scientific journals. Consequently, species identification is extremely difficult if not impossible for anyone not doing research on ciliate taxonomy. The monumental 4-volume "ciliate atlas" by Foissner et al. (1991-95) fills an important gap in ciliate literature and will significantly reduce these difficulties.

This last volume of the series begins with an abundantly illustrated key to all ciliate species treated in volumes I-IV. The key occupies 75 pages and includes all the partial keys known from volumes I-III. It is an original and in my opinion successful attempt to make a practical and easy-to-use key. It will be particularly helpful for beginners and anyone without much experience in ciliate identification.

As the key concerns only selected (indicator) species for an accurate identification the user has to consult the precise diagnoses given in the descriptive part of the book. The diagnoses are accompanied by additional information on similar species (not treated in the atlas) that potentially could be confused. Such approach makes actually feasible the correct determination of species, which significantly distinguishes this book from other guides of indicator species.

The main body of this volume is composed of monographic descriptions of about 60 species representing Gymnostomatea, Suctoria and the genus *Loxodes*. This presentation complements the survey of almost 300 ciliate species with known indicator values covered by the whole atlas. The information on each species is ordered according to the same general scheme: list of synonyms, taxonomy, descriptive diagnosis, note on similar species, and ecological data. All the available taxonomic literature on a given species is thoroughly reviewed.

Each individual description is accompanied by numerous line drawings and micrographs. The amount and the remarkable quality of illustrative material is the most striking feature of the book. Many interesting pictures are reproduced from the older literature, however, most of them are original or taken from previous publications by the same authors. Each detail of ciliate morphology, which can be used for distinguishing species, is fully

illustrated with various techniques. The life aspect is presented with beautiful line drawings as well as phase- and interference-contrast micrographs. Details of ciliary structures are illustrated with a variety of silver methods. Additionally, many scanning electronic micrographs are provided, which help our understanding of ciliate morphology.

Both keys and descriptions are prepared in a way to enable identification *in vivo* whenever possible. This is very important as most often it is impossible to apply staining techniques during routine analyses of samples. It should be stressed that in many cases a live observation may be sufficient for an accurate identification of ciliates provided detailed descriptions of the kind given in this atlas are available.

The ecological sections in each description contain faunistic and autecological data. Information on distribution, abundances and water chemistry data are synthesized from a multitude of scattered literature sources. The amount of literature reviewed is truly amazing. Volume IV itself contains more than one thousand references. The quantity of detailed taxonomic and ecological data accumulated in this atlas makes it a real encyclopedia on ciliated protozoans.

The general part of the book contains a tabular summary of saprobic values of all the species treated in Volumes I-IV. This table contains also the exact location (volume/page) of where the detailed description of a given species can be found. This is very helpful as the index at the end of each book applies only to the species presented in that particular volume. Another table offers a concise summary of basic ecological features (biomass, preferred food, habitat ...) of all species considered in the atlas. The table may be very helpful for a quick ecological interpretation of any species list. In this last volume of the atlas we also find a collection of beautiful graphic tables representing characteristic ciliate communities from a variety of habitats including activated sludge. Readers experienced with monitoring pollution will welcome the table aligning ciliate names from the list of Sladeček et al. (1981) with the names revised in this atlas.

The book is mainly addressed to the people interested in practical use of protozoans in water quality assessment. It will also be very useful in monitoring the function of biological wastewater treatment systems. Without any doubt, the atlas will significantly improve the quality of this kind of analyses. However, the overall significance of this work goes far beyond the problems of bioindication. The quality and the quantity of the information assembled in these four volumes make them absolutely indispensable for any researcher or student of taxonomy and ecology of ciliated protozoans. Perhaps the only significant disadvantage (for some people) will be the fact that the book was written in German. Of course, the authors cannot be blamed for that. In fact they ought to be commended for their well-ordered presentation of the data and the large number of illustrations which make the book accessible even to those with very limited knowledge of German.

Krzysztof Wiąckowski, Kraków, Poland

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ACTA
PROTOZOOLOGICA

1995 AUGUST
VOLUME 34 NUMBER 3