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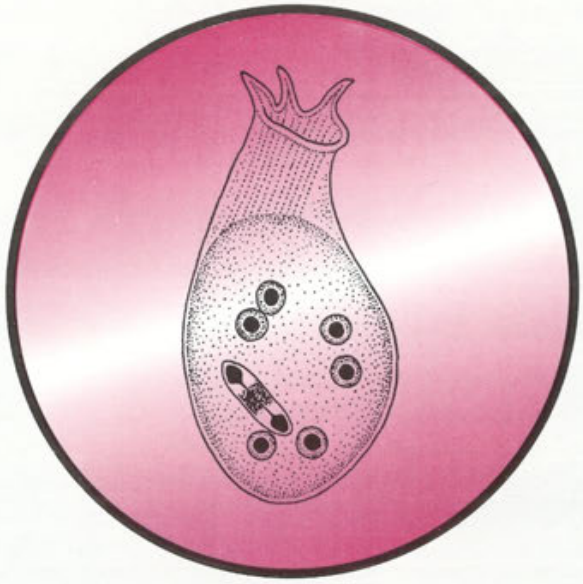

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Is the Origin of *Astasia longa* an Example of the Inheritance of Acquired Characteristics?

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Summary. The possibility of direct transformation of *Euglena gracilis* into *Astasia longa* through induced bleaching of cells of *Euglena* under the effect of such factors as streptomycin, elevated temperature or UV light, was rejected based on available molecular data. *A. longa* has a reduced but functional 73-kb ptDNA which is about twice as small as the 143-kb cpDNA of *E. gracilis*. The function of this genome may be the expression of *rbcL* and of several ORFs. Considering, among others, purported nonphotosynthetic functions of plastids in *Euglena*, and the complex import mechanism of the euglenoid nuclear-encoded chloroplast proteins, it should be supposed that the ptDNA of *A. longa* is localized in the proplastid-like bodies. The size reduction of ptDNA of *Astasia*, associated mainly with the loss of photosynthetic genes, resulted most probably from the intensification of length mutation processes, and could consist of a gradual accumulation of small deletions, as it was demonstrated for ptDNA of *Epifagus virginiana*. The structure, organization and gene contents of cpDNA of the bleached mutants of *E. gracilis* differ drastically from ptDNA of *A. longa*. In some cases the artificial bleaching of *Euglena* may even lead to a total elimination of cpDNA. These facts suggest that the evolution of *A. longa* was independent from the processes of induced bleaching. Furthermore, a detailed comparison between homologous regions of *Astasia* ptDNA and *Euglena* cpDNA indicates that both genomes have evolved from a common ancestor.

Key words: *Astasia longa*, *Euglena gracilis*, *Euglena* bleaching, plastid DNA, gene deletions, genome evolution.

Abbreviations. cpDNA - chloroplast DNA, IR - inverted repeat region, ORF - open reading frame, PFS - paraflagellar swelling, ptDNA - plastid DNA, *rbcL* - RuBisCO large subunit gene, RuBisCO - ribulose-1,5-bisphosphate carboxylase-oxygenase.

INTRODUCTION

Within the last few years a series of papers (e.g. Brooks 1983, Landman 1991, Jablonka et al. 1992), monographs (e.g. Steele 1981) and books (e.g. Lima-de-Faria 1988) have appeared, attempting to resurrect the idea of inheritance of acquired characteristics in contemporary biology. The review article by Landman (1991), besides the numerous examples of "Lamarckian" inheritance, contains a classification systems of various inheritance of acquired characteristics. One of his categories includes systems based on an induced loss of nonessential DNA. The destruction of chloroplasts in *Euglena gracilis* under the effect of streptomycin, first described by Provasoli et al. (1948), is, according to Landman (1991), an example of such a system. When a suspension of green cells of *E. gracilis* var. *bacillaris* is incubated with streptomycin at a concentration of 100 μg ml^{-1} for 4 or more days, a mass loss of chloroplasts takes place in all the cells. The bleached cells of *Euglena* will then transmit their acquired

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character of "the lack of chloroplasts" for at least 9 generations, in the presence of light and on carbon-containing and streptomycin-free media (Provasoli et al. 1948). Besides streptomycin and other antibiotics, a permanent bleaching of *E. gracilis* is induced, for example, by ultraviolet (UV) rays, elevated temperature, high pressure, antihistamines, *o*-methylthreonine and nitrofurans (for reviews see Mego 1968, Schiff and Epstein 1968). According to Provasoli et al. (1948) the induced bleaching of *E. gracilis* is probably a repetition of the process that normally takes place in nature. The process would involve a direct transformation of bleached *E. gracilis* into heterotrophic flagellates of the genus *Astasia*, and especially *A. longa*. This opinion was cited by Landman (1991) who stated that bleached *Euglena* was indistinguishable from *Astasia*. A similar view was adopted by Grant (1991).

Below I will try to answer the following questions: (a) can the artificially bleached *Euglena* cells be distinguished from the *Astasia* cells; (b) do the organization and complexity of chloroplast (cp) DNA from bleached mutants of *E. gracilis* resemble plastid(pt)DNA of *A. longa*?; (c) what factors were responsible for the reduction of Astasia ptDNA? and (d) can such induced bleaching of *E. gracilis* be a model of evolution of *A. longa*?

Astasia longa is not identical with bleached *Euglena gracilis*

The loss of chloroplasts and stigma was assumed by Pringsheim and Hovasse (1948) to be a change that enables a transition from *E. gracilis* to *A. longa*. Those authors thought that *A. longa* was only an apochlorotic race of *E. gracilis*. According to their original opinion, the reasons for bleaching of cells of *E. gracilis* should be sought in mutations combined with an additional effect of some external factors of unknown character. Some time after the publication of the paper by Provasoli et al. (1948), Pringsheim and Pringsheim (1952) also analyzed the possibility of the induced aplastidity. However, only later, in his book "Farblose Algen" did Pringsheim (1963) clearly stress the possibility of the loss of chloroplasts by *E. gracilis* under the effect of some chemical and physical factors (streptomycin, elevated temperature, UV irradiation), also under natural conditions. The view that *A. longa* is identical with bleached *E. gracilis* was for a long time accepted by various authors. For example, in his study of the heterotrophic metabolism in algae, Danforth (1962) states that flagellates of the genus *Astasia* are most probably natural colourless variants of *Euglena*. However, the studies by Blum et al. (1965) revealed that a streptomycin-bleached strain of *E. gracilis* differed from *A. longa* in an array of biochemical, physiological and ultrastructural characters. *Astasia* was much more sensitive to actinomycin D and primycin. Contrary to *A. longa*, *E. gracilis* was able to incorporate the exogenous amino acids, and for this reason its growth was strongly inhibited by *p*-fluorophenylalanine. Transferred to a phosphate-less medium *Euglena* started a synthesis of induced acid phosphatase. *Astasia* was, however, unable to produce that enzyme. Under identical conditions of growth, these two forms also differed in the doubling time (*A. longa*, 11 h; *E. gracilis*, 22 h). Cytological differences between the colourless strain of *Euglena* and *Astasia* included the more prominent overhang of the pellicular ridge in *A. longa* and a different number of pellicle complexes in transverse sections of the cells, amounting to 36 in *Astasia* and 40 in *Euglena*. These and other differences were regarded by Blum et al. (1965) as sufficient to treat *A. longa* as a member of a distinct genus. Their results questioned the view that *A. longa* was identical with bleached *E. gracilis*.

Further cytological differences between the bleached strains of *E. gracilis* and *A. longa* were described at the beginning of 70s. The pellicle outline of bleached *Euglena*, viewed in UV light, is very smooth, while that of *Astasia* is uneven (Rogers and Kimzey 1972). The pellicle in *Astasia* looks as if it has been pulled over paramylon grains. Rogers et al. (1972) found that, contrary to *A. longa*, the bleached *E. gracilis* had dense, paramylon-associated bodies and irregular plate-like paramylon aggregates. However, Kivic and Vesk (1974b), based on their electron microscope studies, were of the opinion that the ultrastructural differences between the cells of *Astasia* and *Euglena* reported by Blum et al. (1965) and Rogers et al. (1972) are not greater than those found between two bleached strains of *E. gracilis*. Thus it appears that the main cytological feature distinguishing the bleached *Euglena* from *Astasia* is the absence of the stigma and paraflagellar swelling (PFS) in the latter (Kivic and Vesk 1974b). The stigma and PFS were found in 18 bleached strains of *E. gracilis*, including some that appear to be aplastidic (Kivic and Vesk 1974b, see also Kronestedt and Walles 1975, Ferrara and Banchetti 1976). Such structures are also present in Pringsheim's laboratory-originated "*A. longa*" (Pringsheim and Hovasse 1948) which is a spontaneous mutant of *E. gracilis*. Though the wild strain of *Astasia* has completely lost the stigma and PFS, the stigma bulge and the flange of the paraflagellar rod have been preserved, which would indicate that the eyespot apparatus was present in the ancestors of *A. longa* (Kivic and Vesk 1974b).

Taken together, although various factors responsible for bleaching result in a drastic reduction of the plastid apparatus in *Euglena*, the factors do not induce any greater changes in the eyespot apparatus. The lack of the stigma and PFS in *A. longa* constitutes a distinct difference between the bleached mutants of *Euglena* and *Astasia*. In this context it should be assumed that a transformation of *E. gracilis* into *A. longa*, induced by a chemical or physical factor, would require not only changes in the plastid apparatus, but also a complete loss of stigma and PFS in *Euglena*. A drastic reduction of PFS is caused by nicotine, which is a strong, reversible inhibitor of carotenoid biosynthesis (Barsanti et al. 1992). However the alkaloid causes no changes in the quantity, size and shape of chloroplasts, and its effect on the stigma is slight; besides, the effect of nicotine on PFS is reversible. Also the theoretical possibility of origin of *Astasia*-like cells of of *E. gracilis* as a result of a simultaneous action of several chemical and/or physical factors (including unknown factor(s) causing a total and irreversible loss of eyespot apparatus) does not imply that these cells would be biochemically and/or genetically identical with the wild-type cells of *A. longa*. Thus, the stated differences - ultrastructural, biochemical and physiological - between the artificially bleached mutants of *Euglena* and *Astasia* tend to negate the possibility of saltational transition from *E. gracilis* to *A. longa* as the result of induced bleaching.

ptDNA of *Astasia* and its reduction pattern

Important information on the evolution of *Astasia* is contained in ptDNA of this colourless, heterotrophic protist. Its circular ptDNA has a size of 73-kb and is approximately 50% smaller than the 143-kb chloroplast genome of *E. gracilis* (Siemeister and Hachtel 1989). Both genomes share a few unique features which provide further evidence of a close evolutionary relationship between *Astasia* and *Euglena* (see Siemeister and Hachtel 1990a, Gockel et al. 1994b). A detailed comparison of the arrangement and organization of genes in *Astasia* ptDNA fragments sequenced hitherto with comparable domains of cpDNA of *E. gracilis* (Siemeister and Hachtel 1990a, Siemeister et al. 1990, Gockel et al. 1994b) suggests that the *Astasia* plastid genome originated as a result of highly specific deletion events from the original chloroplast genome, which was also ancestral to cpDNA of *Euglena*. The absence of sequences homologous to chloroplast genes *psaA*, *psbA*, *psbE*, *atpA* in ptDNA of *A. longa* indicates that the deletions involved most of photosynthetic genes (Siemeister and Hachtel 1989). Besides the intense deletion process, sequence rearrangements took place in *Astasia* ptDNA (Siemeister and Hachtel 1990a, Gockel et al. 1994b).

The plastid genome of *Astasia* is functional. This is shown, among others, by the maintenance of the genes encoding the components of translational apparatus in ptDNA of *A. longa* and their conservative character (Siemeister and Hachtel 1990a; Siemeister et al. 1990; Gockel et al. 1994a, b). One of the functions of the apparatus is the synthesis of the RuBisCO large subunit. Siemeister and Hachtel (1990b) proposed that the activity of RuBisCO could be associated with glycine and serine production in the photorespiratory cycle. Their hypothesis is corroborated by the following facts: (1) a very high homology (97%) of amino acid sequences RuBisCO large subunit of *A. longa* and *E. gracilis*, deduced from *rbcL* nucleotide sequence in both species; (2) the presence of transcripts of *rbcL* gene in the organelle fraction containing *Astasia* ptDNA and (3) the discovery of 53 kDa polypeptide, probably identical with the RuBisCO large subunit (Siemeister and Hachtel 1990b). Besides the biosynthesis of RuBisCO large subunit, another reason for preserving ptDNA and its translational apparatus in *A. longa* may be the expression of genes *ycf13* (*orf456*) and *ycf14* (*orf170*) and several other ORFs (Gockel et al. 1994b). According to Feierabend (1992), these genes do not necessarily have to play specific biosynthetic or metabolic functions, but may be associated with regulatory processes which make the plastid loss impossible. Gockel et al. (1994b) suppose that the protein product of *ycf13* may take part in excision of group III introns.

The intracellular localization of the *Astasia* plastid genome remains uncertain. Studies by Kivic and Vesk (1974a) did not confirm the previous report (Webster et al. 1968) on the presence of plastid-like structures in *A. longa*. However, since Shashidhara and Smith (1991) demonstrated the plastid localization of porphobilinogen deaminase in *A. longa*, the purported absence of proplastid-like bodies in that species may result from the difficulties in distinguishing the vestigial plastids from other vesicles in the cell (see also Kronestedt and Walles 1975). The conclusion is supported, among others, by the occurrence of the plastids in nonphotosynthetic tissues of some species of higher plants (Walsh et al. 1980). The leucoplasts are present in all the phagotrophic, nonphotosynthetic chrysophytes of the kingdom Chromista (Cavalier-Smith et al. 1994). Unexpectedly, parasitic protozoans of the phylum Apicomplexa also contain the vestigial plastids, earlier described as "spherical bodies" and "Hohlzylinders" (Palmer 1992b, Siddall 1992, Hackstein et al. 1995). It should be stressed, as well, that proplastid-like organelles

are not lost by most, and perhaps by all the bleached strains of *Euglena* (Kivic and Vesk 1974a, Parthier and Neumann 1977). Besides, the mere presence of 73-kb ptDNA indicates the occurrence of modified plastids in *A. longa*. In the opposite case we would have to assume a transfer of *Astasia* ptDNA, with all the components of the plastid system of gene expression, into an unknown compartment and on an unknown way.

Other facts also suggest the existence of proplastids in *Astasia*. Higher plant plastids are the place of numerous processes, not associated with photosynthesis, such as biosynthesis of amino acids, fatty acids, heme, nitrite and sulphate reduction, starch metabolism (Weeden 1981, Howe and Smith 1991, Wallsgrove 1991, Emes and Tobin 1993). At least some of these functions are fulfilled also by plastids of Euglenozoa as heme synthesis (Shashidhara and Smith 1991). Thus it is difficult to imagine a process, in which enzymes engaged in these metabolic pathways (all encoded by the nuclear genome and provided with specific transit peptides) could be redirected to other parts of the cell, and maintain their full functionality (Wallsgrove 1991). Also Feierabend (1992) thinks that the presence of plastids in nonphotosynthetic cells and tissues is associated first of all with the availability of this compartment. The total loss of plastid apparatus would be a still more complicated process in Euglenophyceae whose plastids are surrounded by three membranes (for discussion see Whatley 1993) and, compared with higher plants, probably have a much more complex mechanism of protein-import which involves ER or ER-like membranes and the Golgi apparatus (Chan et al. 1990, Shashidhara et al. 1992, Kishore et al. 1993, Shigemori et al. 1994). One can speculate that the successful relocation of euglenoid plastid proteins encoded in the nucleus, most probably into cytosol, would need either the loss of or a change in, at least, two sorts of topogenic sequences: signal-peptide-like domain and transit peptide. This would preclude the transport of those proteins into ER (and Golgi apparatus) and olastids, respectively. However the most difficult task for such a process should be a complete reconstruction of a certain *Euglena* nonphotosynthetic plastid metabolic pathway in a new compartment. It is very likely that transitory stages, with some enzymes already present in the cytosol, and others still remaining in the plastid, would be harmful for the cell. On the other hand, a simultaneous transfer of all the enzymes of an entire metabolic pathway, which would ensure its continuity, does not seem to be very likely. It should be supposed that similar difficulties would occur during the single step relocation of only a few enzymes, is a metabolic pathway was located in two or three compartments (e.g. heme synthesis in *Euglena*, Shashidhara and Smith 1991). In the case of an abrupt, induced loss of plastids in *E. gracilis* the transfer of some nuclear-encoded plastid proteins into cytosol could be done by the inactivation of signal-peptide-like sequences (as a result of their loss or modification) of the mentioned proteins - this would prevent their mistargeting to the endomembrane system. It is unlikely that, for example, the loss of signal-peptide-like swquences by an array of *Euglena* plastid proteins could take place at the same time as the loss of plastids. If in Euglenophyceae not only porphyrin biosynthesis, but also other vital metabolic activities are restricted to plastids, the loss of these organelles by any member of the group would be even more difficult.

The above data indicate that the reduction of the size of *Astasia* ptDNA could not be associated with mass deletions which accompanied induced bleaching (see below) but most probably consisted of a gradual excision of small plastid genome fragments, most often 1-1000 bp long (the so-called length mutation (Palmer 1992a), occurring under strict selection control. Such a process can explain the following facts: (1) distinctly non-random gene deletions, that are reflected in the loss of nearly all photosynthetic genes (except *rbcL*), and retention of the genes for components of the plastid transcriptional and translational systems; (2) the deletions of *orf208* (Siemeister et al. 1990), *psbD*, *chlI*, *psbI*, *petG* (Gockel et al. 1994b) or two tRNA pseudogenes (*ytrnI* and *ytrnW*) (Siemeister and Hachtel 1990a) and conserved *orf456* (*ycf13*) in spite of losing *pbsC* (Gockel et al. 1994b); (3) conservation, by the truncated ptDNA of *A. longa*, of the ability to express the remaining part of genetic information, in spite of the loss of so much DNA; (4) presence of intact and expressed *rbcL* gene, which though not necessary for *Astasia* cells to survive, as evidenced by its absence from at least some bleached strains of *E. gracilis* (Sagher et al. 1976), ensures the existence of the photorespiratory pathway; (5) a very complex pattern of gene organization changes in some segments of the plastid genome of *A. longa*, resulting from a complicated series of deletion events and sequence rearrangements (Gockel et al. 1994b) and (6) the absence of any pseudogenes among the hitherto sequenced genes, except one of the copies of 16S rDNA (Siemeister and Hachtel 1990a). Thus it should be also assumed that the transformation of the plastid apparatus in the ancestors of *Astasia*, including changes in ptDNA, closely resembled the same processes of irreversible but not induced conversion of chloroplasts into plastids, that

took place, among others, in various green algae (*Polytoma*, *Polytomella*) and higher plants (*Cuscuta*, *Conopholis*, *Latharaea*, *Orobanche*). If so, it would be difficult to regard this event in *A. longa* as a peculiarity or an exception.

The presented model of the evolution of *Astasia* plastid genome is confirmed by the studies on ptDNA of *Epifagus virginiana*, a nonphotosynthetic parasitic flowering plant of the family Orobanchaceae. The 70-kb ptDNA of *E. virginiana* contains only 42 genes at least 38 of which encode specific components of the plastid system of gene expression (Wolfe et al. 1992b). Because *Epifagus* plastid DNA has lost nearly all photosynthetic and chlororespiratory genes (some of them are still present as pseudogenes), the function of that genome is different from the role played by ptDNA of *A. longa*. Wolfe et al. (1992b) expressed the opinion that the retention of the plastid genome by *E. virginiana* resulted from a nonbioenergetic function of at least one of the remaining four genes: *accD*, *clpP*, *orf1738* and *orf2216*. A comparative analysis of the gene organization of homologous regions of *Epifagus* ptDNA and tobacco cpDNA (Morden et al. 1991; Wolfe et al. 1992a, b, c) revealed that the origin of plastid DNA of *E. virginiana*, like that of *Astasia* ptDNA, was an outcome of numerous deletions which took place in the entire genome. Most large deletions (at least 27) involving DNA segments of 0.3-11.5 kb (Wolfe et al. 1992b) result most probably from a gradual accumulation of smaller deletions. This is evidenced by the presence of multiple short deletions inside three pseudogenes (*ψatpA*, *ψtrnC* and *ψtrnR*) (Morden et al. 1991) and a prevalence of small (<60>20 bp) deletions in the region of inverted repeat (IR) (Wolfe et al. 1992b). The reduction of ptDNA of another member of the Orobanchaceae, *Conopholis americana*, seems also to be a result of a series of short deletions (Wimpee et al. 1992). Some of the large deletions, several hundred base pairs long, in IR of *E. virginiana* are flanked by short direct repeats (Wolfe et al. 1992b) which suggest that they might result from recombination events. Deletions were found also in cpDNA of tobacco (outgroup sequence was rice cpDNA), but the intensity of the process was much lower compared to the plastid genome of *Epifagus*. For example, 84 deletions of mean length of 33 bp were mapped in IR of *Epifagus* ptDNA, and only 16 deletions of average length of 16 bp were found in IR of tobacco cpDNA (Wolfe et al. 1992b). For this reason Wolfe et al. (1992b) conjecture that the mechanism(s) of deletion between *E. virginiana* and tobacco are the same, the only difference being frequency.

I suggest that the main factor responsible for *Astasia* plastid DNA reduction was, as in *E. virginiana*, a series of small, spontaneous deletions, whose accumulations led to a loss of already nonessential DNA fragments containing single genes or whole gene groups. Besides them, there could also occur large deletions. Like in *Epifagus* the size reduction of the plastid genome of *A. longa* resulted probably from intensified length mutation processes, which govern the quantity of non-coding DNA in chloroplast/plastid genomes (Wolfe et al. 1992b). It can be also supposed that the processes involved the same mechanisms which were discovered in higher plants: slippage-mispairing during DNA synthesis or repair, unequal crossing-over within tandem arrays of repeats and intramolecular recombination between short direct repeats (Palmer 1992a). Thus the evolution of the *Astasia* ptDNA would be of endogenous character and quite independent from the factors causing bleaching of *E. gracilis*.

cpDNA from bleached mutants of *Euglena* and *Astasia* ptDNA

There are also other data excluding the possibility of the origin of *Astasia* by induced bleaching of *Euglena*. Firstly, Hussein et al. (1982), determining the level of chloroplast DNA in artificially bleached strains of *E. gracilis*, found two groups of mutants. One, represented by a few mutants, contained cpDNA in the amounts similar to the wild-type cells and all the wild-type sequences of chloroplast DNA were present, but in non stoechiometric proportions. Another group, including most mutants, had 100-1000 times less cpDNA than the wild-type cells. The characteristic feature of chloroplast DNA of such mutants was a multiple repeat (28-160 times per cell) of sequences representing about 5-12% of the complexity of the wild type genome, which were transcribed as rRNAs. The organization, structure and gene contents of cpDNA from the bleached mutants of *Euglena* differ clearly from the plastid genome of *A. longa*. Contrary to the first group of mutants, as presented above, *Astasia* ptDNA lost about a half of genes occurring in the wild-type chloroplast DNA of *E. gracilis*. In contradistinction to the second group of mutants, the *Astasia* plastid genome is devoid of amplified rDNA. As in the wild-type of *E. gracilis*, rRNA operons in ptDNA of *A. longa* are organized in three tandem repeats and preceded by one additional 16S rRNA gene (Siemeister and Hachtel 1990a, Gockel et al. 1994b). Besides, considering the contents of the RuBisCO large subunit in *Astasia* cells (Siemeister and Hachtel 1990b), it can be speculated that the number of copies of plastid DNA in *A. longa* is close to the number of molecules of cpDNA in etiolated cells of *E. gracilis*. On

the other hand, Hussein et al. (1982) demonstrated that the number of copies of chloroplast DNA in artificially bleached mutants of the second group (2-7 copies) was many times lower than that found in etiolated *Euglena* (210 copies). Possible drastic differences in the number of molecules of cpDNA in the second group of *Euglena* mutants and the number of copies of *Astasia* ptDNA constitute the next important difference between the group of mutants and *A. longa*.

Secondly, Heizmann et al. (1982b) observed that during streptomycin-induced mutagenesis in chloroplast rDNA of *E. gracilis*, *bacillaris* strain, there occurred deletions involving cistrons 1 and 2 (fragments Eco O_{bac} and Eco S_{bac}). The third cistron was correspondingly more conservative and underwent amplification. Similar changes were described also in Z mutants (WZN_2L and $W_{36}ZHD$) and *bacillaris* mutants (Y_1BXD and Y_3BUD). However, there are no data indicating such a pattern of changes in rDNA of the *Astasia* plastid genome (Siemeister and Hachtel 1990a, Gockel et al. 1994b).

Thirdly, electron microscope studies on chloroplast DNA of the bleached mutant Y_3BUD (Heizmann et al. 1982a), which belongs to the first group of *Euglena* mutants, revealed the presence of some large, circular molecules (contour length 42 m), similar to intact wild-type cpDNA. Also numerous minicircular DNA molecules with contour lengths ranging from 0.8 to 8.5 μm were observed (1.9 and 20.2% size of "normal" cpDNA of *E. gracilis*, respectively), which form four classes of average sizes of 1, 2-4, 5 and 8 μm. Hybridization with the wild-type cpDNA and several cloned fragments of chloroplast DNA confirmed the chloroplast nature of at least some of the molecules. These data are in contrast to the results of studies by Siemeister and Hachtel (1989). They found that the 73-kb ptDNA of *A. longa* consists of only two distinct molecular species which are present in approximately equal proportions and differ only in the size of a small fragment (about 0.1 kb).

Fourthly, Conkling et al. (1993) demonstrated that the incubation of *E. gracilis* at a moderately elevated temperature of 33°C (heat-bleaching) brought about a complete loss of chloroplast DNA. The loss of cpDNA began at 60 h, and the DNA was already undetectable after 100 h. According to Conkling et al. (1993) the mass chloroplast DNA loss during the heat-bleaching may be caused by an activation of nucleases in the chloroplast, or an inhibition of cpDNA replication machinery. Because the heat-bleaching causes the complete elimination of *Euglena* chloroplast DNA, it is not responsible for the reduction of ptDNA of *A. longa*. Also there are distinct differences in mechanisms of gene deletions in the evolution of the chloroplast/plastid genomes (see above) and during the process of heat-bleaching.

CONCLUSIONS

In the light of the above facts the induced bleaching of *E. gracilis* cannot be regarded as a model of evolution of *A. longa*. The different structure, organization and complexity of ptDNA of *A. longa* and cpDNA of the bleached mutants of *E. gracilis* constitute a very important genetic difference between both these organisms. In addition the genetic differences are accompanied by ultrastructural, biochemical and physiological differences between the bleached *Euglena* and *Astasia*. The presence of the differences suggests that the artificially bleached mutants of *E. gracilis* and *A. longa* do represent different types of cells. Therefore I think that induced bleaching of *Euglena* and the sequence of changes that has led to the origin of *Astasia* lineage should be regarded as quite distinct processes, while similarities between them are only superficial and consist exclusively of the irreversible loss of photosynthetic abilities, and an associated, permanent transformation of chloroplasts into proplastids. The conclusion is strongly supported by the fact that heat-bleaching leads to a complete elimination of chloroplast DNA. The similarities in the pattern of reduction of *Epifagus* ptDNA and *Astasia* ptDNA suggest that the changes in the plastid apparatus in the ancestors of *A. longa* had a course similar to that in nonphotosynthetic, parasitic higher plants, and thus were not a consequence of interaction with factors evoking bleaching of *Euglena*. Furthermore a comparison of gene organization between homologous segments of ptDNA of *A. longa* and cpDNA of *E. gracilis* indicates that both species have evolved independently from a common ancestor. The evolution of *Astasia* could be initiated by a mutation that had, as its effect, a loss of photosynthetic activity through a cell lineage of an autotrophic species of *Euglena* resembling *E. gracilis*. Such a mutation would be at the same time responsible for the release of the selective pressure on the conserving photosynthetic genes by the ancestors of *Astasia*, and their consequent elimination (except *rbcL*).

It seems that also the loss of cell wall by Gram-positive eubacteria, which Cavalier-Smith (1988) regarded as a crucial step in the origin of eukaryotes, did not proceed according to the scenario proposed by Landman (1991), but resulted most probably from a mutational loss of the ability to produce muramic acid or its incorporation into

peptidoglycan (Cavalier-Smith 1988). Hence the inheritance of acquired characteristics systems proposed by Landman (1991), such as the induced loss of nonessential DNA and heritably stabilized gene expression should be regarded as useless when trying to reconstruct the origin of nonphotosynthetic euglenoids of the genus *Astasia* and the early evolution of the eukaryotic cell. In this context, these systems, at least with respect to both the above phylogenetic events, present themselves mainly as a laboratory peculiarity.

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Faunistics, Taxonomy and Ecology of Moss and Soil Ciliates (Protozoa, Ciliophora) from Antarctica, with Description of New Species, Including *Pleuroplitoides smithi* gen. n., sp. n.

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Summary. Fifty nine moss and soil samples from the maritime and continental Antarctic were investigated for their ciliate fauna using the non-flooded Petri dish method. Collections were made from a variety of biotopes covering most principal soil and vegetation types of the region following a cline from 60°-78°S, i.e. of increasing climatic severity. Sixty four species were found: 51 in region A (Signy Island and Livingstone Island), 16 in region B (Antarctic Peninsula), and 14 in region C (continental Antarctic, viz. Ross Island and South Victoria Land). Twenty nine out of the 64 species were first records for the region, and 5 of them were new species. Mean species number per sample was markedly higher in region A (9.6) than in region B (1.0) and region C (0.9), reflecting a dramatic faunal pauperization with increasing climatic severity and, respectively, decreasing soil fertility. This is strengthened by the observation that all samples from region A contained ciliates, whereas they were lacking in half of the collections from regions B and C. This highly patchy distribution, as yet not found elsewhere, is very likely caused by the severe environment allowing few pioneers to establish permanent populations. As compared with temperate and tropical regions, the Antarctic ciliate species richness is decreased by at least one order of magnitude. The fauna is dominated by *r* - selected, bacteria and fungi feeding colpodids. The most frequent species were *Colpoda ecaudata*, *C. steini*, *C. inflata*, *Pseudocyrtolophosis alpestris*, *Pseudoplatyophrya nana*, and *Cyclidium muscicola*, clearly proving Smith's (1973a) bi-polar biogeography of *Colpoda* to be a methodological artifact. Nine ciliate species are described or redescribed using standard methods: *Pleuroplitoides smithi* sp. n., *Protospathidium serpens*, *Cyclidium glaucoma*, *Notohymena antarctica* sp. n., *Sterkiella thompsoni* sp. n., *Urosomoida granulifera* sp. n., *U. antarctica* sp. n., *Oxytricha lanceolata*, and *Paruroleptus notabilis*. The genera *Pleuroplites* Foissner, 1988 and *Pleuroplitoides* gen. n. (dorsal brush isomorphic and composed of 2 rows with paired, shortened cilia) are united in the family Pleuroplitidae fam. n. (Acropisthiina with extracytostomal extrusome bundle on ventral side; dorsal brush composed of few isomorphic or many heteromorphic kineties).

Key words: Antarctica, biogeography, community structure, faunistics, soil and moss ciliates.

INTRODUCTION

Reliable investigations on soil and moss ciliates from Antarctica are very sparse. The most detailed studies were performed by Smith (1978), who reviewed the older literature and recorded about 50 species, many of which were determined, however, to genus level only. More recently, some small contributions were published, mainly describing new species (Blatterer and Foissner 1988, Ryan et al. 1989, Eigner and Foissner 1993, Foissner 1993).

The present study, which is based on a large collection of samples, is thus the first which provides reliable data on soil and moss ciliates from Antarctica. The results show that Antarctic terrestrial biotopes contain a

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rather diverse, but highly patchily distributed ciliate fauna and unmask Smith's (1973a, 1978) bi-polar biogeography of *Colpoda* as a methodological artifact.

MATERIALS AND METHODS

Samples. Collections were obtained from three regions, all located in the maritime and continental Antarctic, following a cline from 60°-78°S, i.e. of increasing climatic severity (Fig. 1). Collections were made from a great variety of biotopes covering most principal soil and vegetation types of the region. Details, as far as were available, of each site including date of collection, location, dominant associated plant species, and pH are given in the faunistic section of the results. pH was measured in probes rewetted with distilled water for at least five hours. See Smith (1978) and Block (1994) for a concise description of geology, topography, climate, habitats, soils, and vegetation of the area.

Signy Island, South Orkney Islands and Livingstone Island, South Shetland Islands (60°40'-62°38'S, 45°40'-61°04'W; Region A). Samples were obtained by H. G. Smith (Coventry Polytechnic, England) and collected and investigated in 1984 (No. 1-3) and 1985 (No. 4-7). Sample 8 from Livingstone Island was collected in 1981 by R. I. L. Smith and investigated in 1987 (cp. next paragraph).

Antarctic Peninsula (63°-68°S, 55°-69°W; Region B). Samples (No. 9-45) were collected 1981 by R. I. L. Smith, stored at 4°C in sterile polythene bags, and dispatched to me in 1985 by W. Block (British Antarctic Survey). They were inspected for ciliates in 1987. These samples were studied also for nematophagous fungi (Gray and Lewis Smith 1984).

Ross Island and South Victoria Land (77°-78°S, 160°-168°E; Region C). Samples (No. 46-59) were collected by W. Block (British Antarctic Survey) at the turn of years 1984/85. In 1986 they were dispatched and investigated by me. Most of these collections were very small. Thus, several were bulked to some larger samples, uniting similar habitats and locations.

Faunistic methods. All samples obtained were stored at 4°C in Salzburg in the original package and air-dried for four weeks before investigation. Then they were treated with the non-flooded Petri dish method as described by Foissner (1987a, 1992). Briefly, this simple method involves placing 10-50 g of air-dried moss, litter and/or soil in a Petri dish (10-15 cm in diameter) and saturating but not flooding it with distilled water. Such cultures were grown at room temperature and analyzed for ciliates on days 2, 7, 14, 21 and 28 by inspecting about 2 ml each of the run-off. The non-flooded Petri dish method is not perfect, i.e. not all species present can be reactivated from the resting cysts, but probably the most efficient method available. Repeated investigations of some soils showed that 2-5 samples distributed over one year produced 50-80% of the species found in 10 samples investigated

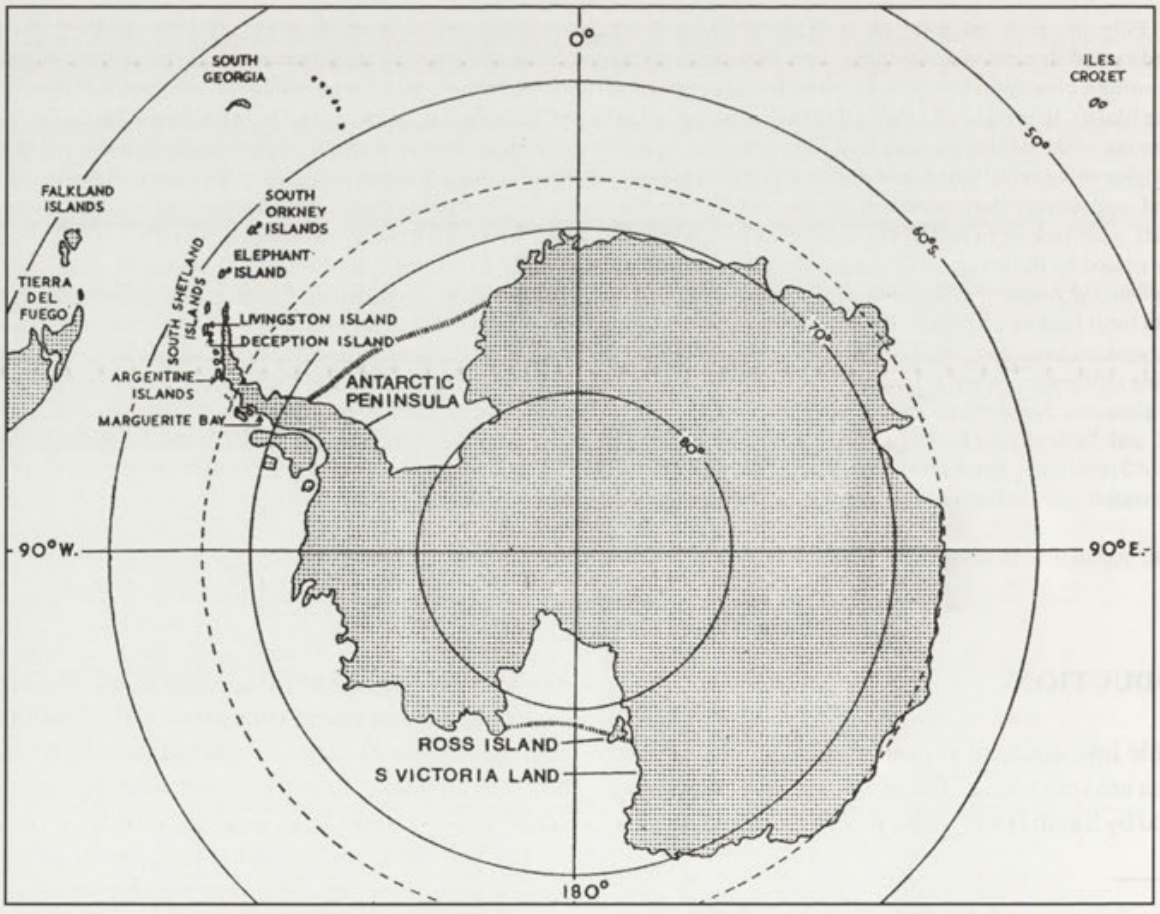


Fig. 1. Sketch map of the Antarctic zone showing sites included in the present study (from Smith 1978, modified)

over two years (Foissner 1987a). Thus, the samples investigated very likely contain more species than shown in Table 1. Furthermore, transportation and storage of the samples may have caused some loss of species.

Identification, terminology and nomenclature are according to literature cited in Foissner (1987a, 1993) and Foissner et al. (1995). Most of the species found were described by myself and my students. Thus, determinations were done on live specimens using a high-power oil immersion objective. However, difficult species were checked in silver slides.

Cytological methods. The species described were studied *in vivo* using a high-power oil immersion objective. The ciliary pattern (infraciliature) was revealed by various silver impregnation techniques, preferably protargol, all described in detail by Foissner (1991). The descriptions are based on raw material obtained with the non-flooded Petri dish method mentioned above, i.e. no pure cultures were set up.

Counts and measurements on silvered specimens were performed at a magnification of x 1000. *In vivo* measurements were conducted at a magnification of x 40-1000. Although these provide only rough estimates it is worth giving such data as specimens usually shrink in preparations or contract during fixation. Standard deviation and coefficient of variation were calculated according to statistics textbooks. Illustrations of live specimens are based on free-hand sketches and micrographs, those of impregnated cells were made with a camera lucida. All figures are orientated with the anterior end of the organism directed to top of page.

Type slides. Two type slides each of the new species described and at least one voucher slide of the species redescribed have been deposited in the Oberösterreichische Landesmuseum in Linz (LI), Austria. The slides contain many protargol impregnated cells, relevant specimens are marked by a black ink circle an the cover glass.

RESULTS AND DISCUSSION

Sample description and ciliates recorded

No. 1: January 1984; Signy Island (60°40'S, 45°40'W); *Drepanocladus* moss. Ciliates recorded: *Colpoda steinii* (very abundant).

No. 2: January 1984; Signy Island (60°40'S, 45°40'W); *Chorisodontium aciphyllum* moss. Ciliates recorded: *Cyclidium glaucoma*, *Gonostomum affine*, *Platyophyra macrostoma*, *Pleuroplitoides smithi*, *Pseudocyrtolophosis alpestris*, *Pseudoplatyophrya nana*.

No. 3: January 1984; Signy Island (60°40'S, 45°40'W); grass sward *Deschampsia antarctica* on loamy soil. Ciliates recorded: *Platyophrya macrostoma*, *Protospathidium serpens*.

No. 4: March 6, 1985; Signy Island (60°40'S, 45°40'W); *Andreaea* moss, pH 4.8. Ciliates recorded: *Acineria uncinata*, *Cyclidium muscicola*, *Fuscheria terricola*, *Hemisincirra gellerti*, *Holosticha sigmoidea*, *Leptopharynx costatus*, *Pseudochilodonopsis mutabilis*.

No.5: March 6, 1985; Signy Island (60°40'S, 45°40'W); grass sward from *Deschampsia antarctica* patch of a sheltered north-facing slope, pH 4.4. Ciliates recorded: *Amphisiella* (?) sp. (very likely a new species but only 1 specimen found), *Blepharisma hyalinum*, *Bresslaua* sp., *Bryometopus pseudochilodon*, *Colpoda inflata*, *C. henneguyi*, *C. lucida*, *C. steinii*, *Cyclidium muscicola*, *Cyrtolophosis mucicola*, *Dileptus alpinus*, *Dimacrocaryon amphileptoides*, *Drepanomonas pauciciliata*, *Enchelys* (?) sp., *Epispathidium terricola*, *Frontonia depressa*, *Gonostomum affine*, *G. kuehnelti*, *Grossglockneria acuta*, *Halteria grandinella*, *Hemisincirra gellerti*, *H. polynucleata* (?), *Holosticha bergeri*, *H. multistilata*, *H. sigmoidea*, *Leptopharynx costatus*, *Nivaliella plana*, *Notohymena antarctica*, *Oxytricha ophistomuscorum*, *Paraenchelys terricola* (with unusually short, 5µm, extrusomes), *Platyophrya vorax*, *Pleuroplites australis*, *Pseudochilodonopsis mutabilis*, *Pseudocyrtolophosis alpestris*, *Pseudoplatyophrya nana*, *Sathrophilus muscorum*, *Sterkiella histriomuscorum*, *Vorticella astyliformis*.

No. 6: March 6, 1985; Signy Island (60°40'S, 45°40'W); *Polytrichum alpestre* and *Chorisodontium aciphyllum* moss, pH 4.0. Ciliates recorded: *Cyclidium muscicola*, *Gonostomum affine*, *Holosticha sigmoidea*, *Pseudocyrtolophosis alpestris* (very abundant).

No 7: March 6, 1985; Signy Island (60°40'S, 45°40'W); *Drepanocladus uncinatus* moss, pH 5.2. Ciliates recorded: *Cyrtolophosis mucicola*, *Leptopharynx costatus*, *Microdiaphanosoma arcuatum* (very abundant), *Opercularia* sp., *Oxytricha ophisthomuscorum*, *Pleuroplitoides smithi*, *Sterkiella thompsoni*.

No. 8: March 28, 1981; Byers Peninsula (Sealer Hill), Livingstone Island, South Shetland Islands (62°38'S, 61°04'W); *Drepanocladus uncinatus* moss, pH 6.9. Ciliates recorded: *Blepharisma hyalinum*, *Colpoda cucullus*, *Cyclidium muscicola*, *Cyrtolophosis acuta*, *C. mucicola*, *Dileptus alpinus*, *Gonostomum affine*, *Paraenchelys terricola*, *Paruroleptus notabilis*, *Pleuroplitoides smithi*, *Pseudocyrtolophosis alpestris*, *Urosomoida granulifera*.

No. 9: March 27, 1981; Mt. Alexander, Hadden Bay, Joinville Island (63°18'S, 55°48'W); *Drepanocladus uncinatus* moss, pH 5.1. Ciliates recorded: *Vorticella astyliformis*.

No. 10: March 27, 1981; Mt. Alexander, Hadden Bay, Joinville Island (63°18'S, 55°48'W); *Polytrichum alpestre* moss, pH 5.8. Ciliates recorded: *Colpoda steinii* (very abundant), *Cyclidium muscicola*.

No. 11: March 28, 1981; Active Sound, Joinville Island (63°25'S, 56°09'W); *Andreaea* sp. moss. No ciliates found.

No. 12: March 26, 1981; Scar Hills, Hope Bay (63°25'S, 57°01'W); gull nest material, mainly *Usnea antarctica* and feathers, pH 6.8. No ciliates found.

No. 13: March 26, 1981; Near Lake Boeckella, Hope Bay (63°24'S, 56°59'W); Adélie penguin guano/mud, pH 6.5. No ciliates found.

No. 14: March 24, 1981; Near Sherrell Point, Astrolabe Island (63°20'S, 58°41'W); *Drepanocladus uncinatus* moss with some soil, pH 6.6. No ciliates found.

No. 15: March 24, 1981; Near Sherrell Point, Astrolabe Island (63°20'S, 58°41'W); *Polytrichum alpestre* moss with some soil, pH 6.1. Ciliates recorded: *Colpoda inflata* (very abundant), *C. steinii*, *Platyophrya vorax*.

No. 16: March 24, 1981; Cape Roquemaurel (63°33'S, 58°57'W); *Drepanocladus uncinatus* moss, pH 5.3. Ciliates recorded: *Nivaliella plana*.

No. 17: March 23, 1981; Andrée Island, Charlotte Bay (64°31'S, 61°30'W); *Brachythecium austro-salebrosum* moss, pH 5.9. Ciliates recorded: *Acineria uncinata*, *Colpoda ecaudata*, *Cyclidium glaucoma*, *Grossglockneria acuta*, *Lamtostyla edaphoni*.

No. 18: March 23, 1981; Andrée Island, Charlotte Bay (64°31'S, 61°30'W); *Bryum argenteum* moss. Ciliates recorded: *Colpoda ecaudata*, *Crytohymena quadrinucleata*.

No. 19: March 23, 1981; Meusnier Point, Charlotte Bay (64°32'S, 61°37'W); *Drepanocladus uncinatus* moss, pH 6.0. Ciliates recorded: *Platyophrya vorax*.

No. 20: March 23, 1981; Meusnier Point, Charlotte Bay (64°31'S, 61°30'W); *Polytrichum alpinum* moss, pH 5.2. No ciliates found.

No. 21: March 23, 1981; Meusnier Point, Charlotte Bay (64°32'S, 61°37'W); *Brachythecium austro-salebrosum* moss, pH 6.7. Ciliates recorded: *Kahlilembus* sp. (single specimen).

No. 22: March 21, 1981; Cuverville Island, north side (64°41'S, 62°38'W); *Polytrichum alpestre* moss, pH 5.7. Ciliates recorded: *Pseudoplatyophrya nana*.

No. 23: March 22, 1981; Gamma Island, Melchior Islands (64°20'S, 63°00'W); *Drepanocladus uncinatus* moss, pH 6.8. Ciliates recorded: *Cyrtohymena candens*.

No. 24: March 19, 1981; Cape Tuxen, Graham Coast (65°16'S, 64°08'W); *Polytrichum alpestre* moss, pH 5.2. Ciliates recorded: *Pseudocyrtolophosis alpestris*, *Pseudoplatyophrya nana*.

No. 25: March 19, 1981; Cape Tuxen, Graham Coast (65°16'S, 64°08'W); *Chorisodontium aciphyllum* moss, pH 4.4. Ciliates recorded: *Pseudocyrtolophosis alpestris*.

No. 26: March 19, 1981; Cape Tuxen, Graham Coast (65°16'S, 64°08'W); *Drepanocladus uncinatus* moss, pH 6.2. No ciliates found.

No. 27: March 20, 1981; Island off Takaki Promontory, Graham Coast (65°33'S, 64°13'W); *Drepanocladus uncinatus* moss, pH 6.6. Ciliates recorded: *Colpoda ecaudata*, *Cyclidium glaucoma*.

No. 28: March 20, 1981; Island off Takaki Promontory, Graham Coast (65°33'S, 64°13'W); *Polytrichum alpestre* moss, pH 4.9. Ciliates recorded: *Pseudocyrtolophosis alpestris* (very abundant).

No. 29: March 15, 1981; Piñero Island (north end) (67°33'S, 67°50'W); *Drepanocladus uncinatus* moss, pH 6.4. No ciliates found.

No. 30: March 14, 1981; Jenny Island (north side), Marguerite Bay (67°44'S, 68°23'W); *Polytrichum alpestre* moss, pH 5.3. Ciliates recorded: *Pseudocyrtolophosis alpestris*.

No. 31: March 14, 1981; Jenny Island (north side), Marguerite Bay (67°44'S, 68°23'W); *Drepanocladus uncinatus* moss, pH 6.2. Ciliates recorded: *Colpoda ecaudata*, *C. steinii*.

No. 32: March 14, 1981; Courtier Island, Dion Islands, Marguerite Bay (67°52'S, 68°43'W); *Drepanocladus uncinatus* moss, pH 6.9. No ciliates found.

No. 33: March 14, 1981; Emperor Island, Dion Islands, Marguerite Bay (67°52'S, 68°43'W); *Prasiola crispa* algal mat on soil (partly Adélie penguin guano), pH 6.0. No ciliates found.

No. 34: March 14, 1981; Emperor Island, Dion Islands, Marguerite Bay; *Prasiola crispa* algal mat on soil (partly on shag guano), pH 6.3. No ciliates found; remoistened sample soon became putrid.

No. 35: March 10, 1981; Lagotellerie Island (north side), Marguerite Bay (67°53'S, 67°24'W); *Drepanocladus uncinatus* moss, pH 5.9. No ciliates found.

No. 36: March 10, 1981; Lagotellerie Island (north side), Marguerite Bay (67°53'S, 67°24'W); *Brachythecium austro-salebrosum* moss, pH 5.8. Ciliates recorded: *Colpoda ecaudata*, *Microdiaphanosoma arcuatum*.

No. 37: March 10, 1981; Lagotellerie Island, Marguerite Bay (67°53'S, 67°24'W); *Bryum algens* moss, pH 5.8. Ciliates recorded: *Colpoda ecaudata*, *C. inflata*.

No. 38: March 10, 1981; Lagotellerie Island (north side), Marguerite Bay (67°53'S, 67°24'W); Adélie penguin guano, pH 7.3. No ciliates found.

No. 39: March 10, 1981; Lagotellerie Island, Marguerite Bay (67°53'S, 67°24'W); *Pohlia nutans*. Ciliates recorded: *Microdiaphanosoma arcuatum*.

No. 40: March 13, 1981; Dismal Island, Faure Islands, Marguerite Bay (68°06'S, 68°50'W); *Drepanocladus uncinatus* moss, pH 5.0. No ciliates found.

No. 41: March 13, 1981; Dismal Island, Faure Islands, Marguerite Bay (68°06'S, 68°50'W); *Andreaea regularis* moss, pH 7.0. Ciliates recorded: *Colpoda inflata* (very abundant).

No. 42: March 12, 1981; Roman Four Promontory, Marguerite Bay (68°13'S, 66°56'W); *Phormidium* sp. (dry cyanobacteria mat which quickly became active when the sample was remoistened; many fungal hyphae and bacteria developed). No ciliates found.

No. 43: March 11, 1981; Refuge Islands, Marguerite Bay (68°21'S, 67°10'W); *Drepanocladus uncinatus* moss. No ciliates were found, but abundant growth of testate amoebae (*Trinema lineare*, *Assulina muscorum*, *Corythion dubium* and *Euglypha* sp.) occurred.

No. 44: March 11, 1981; Refuge Islands, Marguerite Bay (68°21'S, 67°10'W); *Bryum algens* moss, pH 5.9. Ciliates recorded: *Colpoda ecaudata*, *C. inflata*.

No. 45: March 11, 1981; Refuge Islands, Marguerite Bay (68°21'S, 67°10'W); *Cephaloziella varians* moss, pH 7.0. No ciliates found.

No. 46: December 10, 1984; Cape Bird, Ross Island, Keble Valley (168°E, 77°50'S); 4 small samples bulked: dry ridge area, open mineral soil with salt crust, moist; dry ridge area, mineral soil under rock, moist; dry ridge, mineral soil, dry; dry ridge, mineral soil under stone, moist. pH of bulked sample: 9.5. No ciliates found.

No. 47: December 10, 1984; Cape Bird, Ross Island, Keble Valley (168°E, 77°50'S); 5 small samples bulked: dry moss patch (*Bryum* sp.) beside meltstream; wet moss patch (*Bryum* sp.) beside meltstream; algae from flowing meltstream; stream side, soil algal crust between stones; stream side, wet *Bryum* with algal growth. Ciliates recorded: *Homalogastra setosa*, *Lamtostyla perisincirra* (?), *Nassula picta*, *Sphaerophrya terricola*.

No. 48: December 31, 1984; Cape Royds, Ross Island (168°E, 77°50'S); 3 small samples bulked: dry soil from "badlands" near NZARP camp; moist soil from near snow patch at Blue Lake; soil from under *Bryum* moss at Collembola Heights. No ciliates found.

No. 49: December 30, 1984; Garwood Valley, S Victoria Land; 3 small samples bulked: dry soil from stone pavement surface; soil from Polygon edge ("dyke"); wet soil from stone pavement surface. Ciliates recorded: *Pseudoplatyophrya nana*.

No. 50: December 30, 1984; Garwood Valley, S Victoria Land; 3 small samples bulked: "salt" soil from near Garwood glacier; dry soil from "flood plain" of meltstream; rock flour from glacial outflow stream. Ciliates recorded: *Urosomoida antarctica*.

No. 51: December 30, 1984; Garwood Valley, S Victoria Land; 3 small samples bulked: wet *Bryum* moss from edge of meltstream; algae from meltstream; *Bryum* moss from dry area near stream. Ciliates recorded: *Colpoda steinii*, *Leptopharynx costatus*, *Oxytricha lanceolata*, *Vorticella astyliformis*.

No. 52: January 2, 1985; Lake Fryxell, Taylor Valley, S Victoria Land (160°E, 78°S); 3 small samples bulked: damp soil near Burn´s Pool; wet moss and *Nostoc* from near Burn's Pool; dry *Bryum* moss away from meltstream near Burn's Pool. Ciliates recorded: *Colpoda cucullus*, *Drepanomonas sphagni*, *Fuscheria lacustris*, *Homalogastra setosa*, *Oxytricha opisthomuscorum*.

No. 53: January 2, 1985; Lake Bonney, Taylor Valley, S Victoria Land (160°E, 78°S); moist soil and glacial debris with salt from stream side. No ciliates found.

No. 54: January 3, 1985; Lake Vanda, Wright Valley, S Victoria Land (160°E, 78°S); 4 small samples bulked: dry soil from shore of lake; damp granitic soil from shore of lake; very wet soil from shore of lake; clay soil from shore of lake. No ciliates found.

No. 55: January 6, 1985; West Beacon Mountains, S Victoria Land (160°E, 78°S); 2 small samples bulked: soil from near camp site; soil from ridge site. Ciliates recorded: *Colpoda steinii*.

No. 56: January 12, 1985; Cape Crozier, Ross Island (168°E, 77°50'S); 2 small samples bulked: *Prasiola* alga from snow melt area near Post Office Hill; *Prasiola* alga near penguin rookery. No ciliates found.

No. 57: January 13, 1985; Cape Crozier, Ross Island (168°E, 77°50'S); 2 small samples bulked: soil from moss (*Bryum*) patch near snow patch at *Xanthoria* site; soil from *Xanthoria* site, snow melt area. No ciliates found.

No. 58: January 10, 1985; Cape Crozier, Ross Island (168°E, 77°50'S); 4 small samples bulked: *Bryum* moss from *Xanthoria* site; *Bryum* moss and *Xanthoria* (lichen) from *Xanthoria* site; mineral soil and soria from *Xanthoria* site; soil beneath old skua nest near *Xanthoria* site. No ciliates found.

No. 59: January 10 - 12, 1985; Cape Crozier, Ross Island (168°E, 77°50'S); 2 small samples bulked: *Xanthoria* (lichen) from *Xanthoria* site; *Prasiola* alga from near *Xanthoria* site. No ciliates found.

Table 1. Species recorded, their frequency and distribution in 59 moss and soil samples from Antarctica

Species	Region[1]			Total frequency[2] (%)	Habitats[3]						
	A	B	C		I	II	III	IV	V	VI	VII
Acineria uncinata Tucolesco, 1962	+	+	-	3.4	+	-	+	-	+	-	-
Amphisiella (?) sp.	+	-	-	1.7	+	-	-	-	-	-	-
Blepharisma hyalinum Perty, 1849*	+	-	-	3.4	+	+	-	-	-	-	-
Bresslaua sp.	+	-	-	1.7	+	-	-	-	-	-	-
Bryometopus pseudochilodon Kahl, 1932*	+	-	-	1.7	+	-	-	-	-	-	-
Colpoda cucullus (Müller, 1773)	+	-	+	3.4	-	+	-	-	-	+	-
Colpoda ecaudata (Liebmann, 1936)	-	+	-	11.9	-	+	+	-	-	+	-
Colpoda inflata (Stokes, 1884)	+	+	-	8.5	+	-	-	+	+	+	-
Colpoda henneguyi Fabre-Domergue, 1889*	+	-	-	1.7	+	-	-	-	-	-	-
Colpoda lucida Greeff, 1888*	+	-	-	1.7	+	-	-	-	-	-	-
Colpoda steinii Maupas, 1883	+	+	+	11.9	+	+	-	+	-	+	+
Cyclidium glaucoma Müller, 1773	+	+	-	5.1	-	+	+	-	-	+	-
Cyclidium muscicola Kahl, 1931	+	+	-	8.5	+	+	-	+	+	-	-
Cyrtohymena candens (Kahl, 1932)*	-	+	-	1.7	-	+	-	-	-	-	-
Cyrtohymena quadrinucleata (Dragesco & Njiné, 1971)*	-	+	-	1.7	-	+	-	-	-	+	-
Cyrtolophosis acuta Kahl, 1926*	+	-	-	1.7	-	+	-	-	-	-	-
Cyrtolophosis mucicola Stokes, 1885	+	-	-	5.1	+	+	-	-	-	-	-
Dileptus alpinus Kahl, 1932*	+	-	-	3.4	+	+	-	-	-	-	-
Dimacrocaryon amphileptoides (Kahl, 1931)	+	-	-	1.7	+	-	-	-	-	-	-
Drepanomonas pauciciliata Foissner, 1987	+	-	-	1.7	+	-	-	-	-	-	-
Drepanomonas sphagni Kahl, 1931*	-	-	+	1.7	-	-	-	-	-	+	-
Enchelys (?) sp.	+	-	-	1.7	+	-	-	-	-	-	-
Epispathidium terricola Foissner, 1982*	+	-	-	1.7	+	-	-	-	-	-	-
Frontonia depressa (Stokes, 1886)	+	-	-	1.7	+	-	-	-	-	-	-
Fuscheria lacustris Song & Wilbert, 1989*	-	-	+	1.7	-	-	-	-	-	+	-
Fuscheria terricola Berger, Foissner & Adam, 1993*	+	-	-	1.7	-	-	-	-	+	-	-
Gonostomum affine (Stein, 1859)	+	-	-	6.7	+	+	-	+	-	+	-
Gonostomum kuehnelti Foissner, 1982*	+	-	-	1.7	+	-	-	-	-	-	-
Grossglockneria acuta Foissner, 1980	+	-	-	3.4	+	-	+	-	-	-	-
Halteria grandinella (Müller, 1773)	+	-	-	1.7	+	-	-	-	-	-	-
Hemisincirra gellerti (Foissner, 1982)	+	-	-	3.4	+	-	-	-	+	-	-
Hemisincirra polynucleata (?) Foissner, 1984	+	-	-	1.7	+	-	-	-	-	-	-
Holosticha bergeri Foissner, 1987*	+	-	-	1.7	+	-	-	-	-	-	-
Holosticha multistilata Kahl, 1928*	+	-	-	1.7	+	-	-	-	-	-	-
Holosticha sigmoidea Foissner, 1982*	+	-	-	5.1	+	-	-	+	+	-	-
Homalogastra setosa Kahl, 1926*	-	-	+	3.4	-	-	-	-	-	+	-
Kahlilembus sp.	-	+	-	1.7	-	-	+	-	-	-	-
Lamtostyla edaphoni Berger & Foissner, 1987*	-	+	-	1.7	-	-	+	-	-	-	-
Lamtostyla perisincirra (?) (Hemberger, 1985)	-	-	+	1.7	-	-	-	-	-	+	-
Leptopharynx costatus Mermod, 1914	+	-	+	6.7	+	+	-	-	+	+	-
Microdiaphanosoma arcuatum (Grandori & Grandori, 1934)	+	+	-	5.1	-	+	+	-	-	-	-
Nassula picta Greeff, 1888*	-	-	+	1.7	-	-	-	-	-	+	-
Nivaliella plana Foissner, 1980	+	+	-	3.4	+	+	-	-	-	-	-
Notohymena antarctica sp. n.*	+	-	-	1.7	+	-	-	-	-	-	-
Opercularia sp.	+	-	-	1.7	-	+	-	-	-	-	-
Oxytricha lanceolata Shibuya, 1930	-	-	+	1.7	-	-	-	-	-	+	-
Oxytricha opisthomuscorum Foissner et al., 1991*	+	-	+	5.1	+	+	-	-	-	+	-
Paraenchelys terricola Foissner, 1984*	+	-	-	3.4	+	+	-	-	-	-	-
Paruroleptus notabilis Foissner, 1982*	+	-	-	1.7	-	+	-	-	-	-	-
Platyophrya macrostoma Foissner, 1980	+	-	-	3.4	+	-	-	-	-	+	-
Platyophrya vorax Kahl, 1926	+	+	-	5.1	+	+	-	+	-	-	-
Pleuroplites australis Foissner, 1988*	+	-	-	1.7	+	-	-	-	-	-	-
Pleuroplitoides smithi gen. n., sp. n.*	+	-	-	5.1	-	+	-	-	-	+	-
Protospathidium serpens (Kahl, 1930)	+	-	-	1.7	+	-	-	-	-	-	-
Pseudochilodonopsis mutabilis Foissner, 1981*	+	-	-	3.4	+	-	-	-	+	-	-
Pseudocyrtolophosis alpestris Foissner, 1980	+	+	-	13.6	+	+	-	+	-	+	-
Pseudoplatyophrya nana (Kahl, 1926)	+	+	+	8.5	+	-	-	+	-	+	+
Sathrophilus muscorum (Kahl, 1931)	+	-	-	1.7	+	-	-	-	-	-	-
Sphaerophrya terricola Foissner, 1986*	-	-	+	1.7	-	-	-	-	-	+	-

Table 1. (con.)

Sterkiella histriomuscorum (Foissner et al., 1991)	+	-	-	1.7	+	-	-	-	-	-	-
Sterkiella thompsoni sp. n.*	+	-	-	1.7	-	+	-	-	-	-	-
Urosomoida antarctica sp. n.*	-	-	+	1.7	-	-	-	-	-	-	+
Urosomoida granulifera sp. n.*	+	-	-	1.7	-	+	-	-	-	-	-
Vorticella astyliformis Foissner, 1981	+	+	+	5.1	+	+	-	-	-	+	-

[1]See Materials and Methods. [2]All samples, i. e. including those without ciliates. [3]I *Deschampsia antarctica* grass sward (2 sites), II *Drepanocladus uncinatus* and *Drepanocladus* sp. moss (16 sites), III *Brachythecium austro-salebrosum* moss (3 sites), IV *Polytrichum alpinum* and *P. alpestre* moss (8 sites), V *Andreaea* sp. moss (3 sites), VI other mosses (11 sites), VII barren soil (7 sites). *First record for the maritime and continental Antarctic. Other species have been recorded previously by Sudzuki (1964, 1979), Smith (1978) and/or Foissner (1996)

Faunistics and community structure (Tables 1, 2)

Sixty four species were found (Table 1): 51 in region A (Signy Island and Livingstone Island; 8 samples), 16 in region B (Antarctic Peninsula; 37 samples), and 14 in region C (continental Antarctic; 14 samples). These figures are comparable to those reported from islands in the Southern Ocean (Foissner 1996; Table 2) and alpine grassland and shrub soils above the timberline (Foissner 1981a; Table 2), but much smaller than those reported from temperate grassland and lowland sites (Foissner et al. 1985; Table 2) and certain tropical forests where a single sample may contain 80 species (Foissner 1995). However, because the total number of species is an ambiguous measure if sample sizes differ, the mean number of species per sample was calculated (Table 2). This showed that the samples from Signy Island, which is least severe as concerns the climate, contained the highest number of species (9.6), followed by the Antarctic Peninsula (1.0) and the continental Antarctic (0.9). On average, 2.2 species occurred in the Antarctic samples, which was an order of magnitude lower than in alpine (12.3) and temperate (12.7) soils from Austria (Table 2). There is thus a distinct pauperization of the ciliate fauna with increasing latitude and environmental severity, as known also for many other organism groups (Franz 1975). For testate amoebae Smith and Wilkinson (1987) found a loss of 3.3 species for every 1°C drop in mean January temperature.

Of the habitats investigated, those with a grass sward and/or moss contained the richest fauna, whereas few species could be isolated from barren soils, lichens, and

Table 2. Main characteristics of ciliate communities in Antarctic (this study), sub-Antarctic (Gough and Marion Islands), alpine, and temperate terrestrial biotops

Region/Habitat	Number of species	Mean species number per sample[1]	Number of new species	C/P quotient[2]	No. of samples investigated
Region A (Signy Island)	51	9.6	4	1.0	8
Region B (Antarctic Peninsula)	16	1.0	0	2.7	37
Region C (Continental Antarctic)	14	0.9	1	0.8	14
Habitat I [3]	39	-	1	1.1	2
Habitat II[3]	25	1.8	3	1.3	18
Habitat III[3]	7	-	0	3.0	3
Habitat IV[3]	8	1.7	0	2.5	8
Habitat V[3]	8	-	1	0.5	3
Habitat VI[3]	21	2.5	1	1.6	11
Habitat VII[3]	3	0.4	1	2.0	7
Antarctic sites combined	64	2.2	5	0.7	59
Gough Island[4]	39	14.0	2	2.5	7
Marion Island[4]	39	5.7	2	1.4	20
Gough and Marion combined[4]	60	7.8	4	1.4	27
Alpine soil sites in Austria[5]	81	12.3	-[6]	0.8	58
Temperate soil sites in Austria[7]	132	12.7	-[6]	0.5	70

[1]Calculated only if sample size ≥ 7. [2]Ratio of colpodid/polyhymenophorid (hypotrichs, heterotrichs, oligotrichs) ciliates (Lüftenegger et al. 1985). [3]As defined in footnote 3 of Table 1. [4]From Foissner (1996). [5]From Foissner (1981a). [6]Many, because terrestrial ciliates were very insufficiently known at that time. [7]From Foissner et al. (1985)

bird-influenced sites (Tables 1, 2). This is in accordance with previous investigations (Smith 1973b, 1978, Foissner 1996). In many samples only 1-3 species occurred, however, often with great abundances, possibly due to the lack of competition. No ciliates could be isolated from many samples of the Antarctic Peninsula and the continental Antarctic although they consisted of moss and humic soil (e. g. samples 11, 14, 20, 29, 35, 48, 58), which is usually an unfailing indication for the presence of ciliates. I very rarely found such samples in other regions of the world, not even in arid deserts. Thus, the patchy distribution of the ciliates is a conspicuous peculiarity of the Antarctic region and very likely related to the extreme environmental conditions allowing few pioneers to establish stable populations.

Most species with medium and high frequency values (≥ 8%, Table 1) belonged to the class Colpodea (*Pseudocyrtolophosis alpestris*, *Colpoda ecaudata*, *C. steinii*, *C. inflata*, *Pseudoplatyophyra nana*), except for *Cyclidium muscicola*, a very tiny (14-20 µm) hymenostome. All are small to medium-sized bacteria feeders, except for *P. nana*, an obligate fungal sucker, and are widespread in soils worldwide (Foissner 1987a, 1993). The dominance of colpodids was reflected by the high values of the C/P quotient (Table 2), which has been suggested as a measure of biotope extremity (Lüftenegger et al. 1985). Although there was some bias in detail, most quotients were near or above 1, indicating that the habitats investigated favoured *r* - selected "reproducers" (colpodids) rather than *K* - selected "persisters" (polyhymenophorans).

Twenty nine of the 64 species found have been not reported previously from the maritime and continental Antarctic; and most others have been recorded very recently (Foissner 1996), showing our ignorance regarding the Antarctic soil and moss ciliate fauna. Very likely, many other species will be found in other regions of the Antarctic and if a larger sample collective is carefully analysed. However, very few new species were found, indicating a predominance of ubiquists and an absence of endemits.

Smith's bi-polar biogeography of *Colpoda* is a methodological artifact

Colpoda is the most widespread and most abundant of all genera of ciliates in terrestrial biotopes (Smith 1978, Foissner 1987a, 1993). Very likely, this is due to its *r* - selected survival strategy (Lüftenegger et al. 1985, Foissner 1993): it can increase its population density very quickly by multiple division and excyst or encyst within one hour if the environmental variables become favourable or adverse, respectively.

Smith (1973a, 1978) and Smith and Crook (1995) concluded from a detailed literature survey and many original investigations that *Colpoda* is present in Arctic and sub-Antarctic but absent in maritime Antarctic biotopes. As a possible explanation, Smith (1973a) and Smith and Crook (1995) suggested that Antarctic summers are too cold, and each day has too few degree-hours above critical threshold temperature, to permit *Colpoda* to establish permanent populations. He corroborated this hypothesis not only by meteorological data but also by some laboratory experiments indicating that *Colpoda* does not grow and survive at 0°C, which was later supported by Kracht (1982).

Smith's hypothesis of a bi-polar distribution of the genus *Colpoda* is clearly disproven by the present results which show not only the occurrence of several *Colpoda* species in terrestrial biotopes of the maritime and continental Antarctic but also that they are among the three most frequent species found (*Colpoda ecaudata*, *C. steinii*, *Pseudocyrtolophosis alpestris*). Furthermore, active cells of *Colpoda* were observed by Petz (unpubl.) in freshly sampled soils of the continental Antarctic.

How can this discrepancy be explained ? I suggest that it is mainly caused by methodological shortcomings. Smith (1978) used very small quantities (1-2 g) of soil, moss, etc. and inoculated them on agar plates amended with a single strain of bacteria, *Aerobacter aerogenes*. This is very different from the non-flooded Petri dish method applied in this study, where huge amounts of material without additives, except for water, were used. Thus, a more natural biotope was simulated, giving even small populations a fair chance to develop and grow on the natural microflora and microfauna.

Although the field results of Smith must be rejected, his basic idea is very likely correct. It was indeed surprising and most uncommon that several soil and/or moss samples did not contain a single species of *Colpoda* or any ciliate at all (see Sample description and species recorded). This patchy distribution might well be caused by the unfavourable climate allowing few specimens to establish permanent populations, i.e. local extinction possibly occurs frequently. However, this certainly applies not only to members of the genus *Colpoda* but also to most other species, possibly even to a greater extent because they were less frequent (Table 1). Furthermore, such a patchy distribution of the ciliates was also observed in soil and

moss samples from Marion and Gough Island (Foissner 1996).

Description of new and insufficiently known species

Morphometric data shown in Tables 3-8 are repeated in this section only as needed for clarity. All observations are from field material, i.e. not from clone cultures. Thus, it cannot be excluded that similar, but different, species were mixed, although this is unlikely because I excluded all specimens which deviated in at least one prominent character. Certainly, this can generate some bias in the data if used too uncritically. However, I usually exclude only such specimens which have, e.g., a different nuclear structure (very likely often postconjugates), a distinctly deviating ciliary pattern (very likely often injured, regenerating or malformed specimens), or an unusually small size (very likely often degenerating, just excysted or divided specimens). The inclusion of such individuals, which sometimes might belong to another species, would artificially increase variability.

Family Pleuroplitidae fam. n.

Diagnosis: Acropisthiina Foissner and Foissner, 1988 with subapical, i.e. extracytostomal extrusome bundle on ventral side. Dorsal brush composed of few isomorphic or many heteromorphic rows. Type genus: *Pleuroplites* Foissner, 1988.

Comparison with related families: *Pleuroplites* Foissner, 1988 and the new genus *Pleuroplitoides*, described below, are unique among gymnostomatids (= haptorids) in having the extrusomes located not within and/or around the oral basket but in a distinct subapical bundle on the ventral side (Figs. 2, 5). Certainly, this is an extraordinary evolutionary branch which needs to be separated at family level at least. Formerly, I classified *Pleuroplites* with the Trachelophyllidae, simply to avoid establishing a monotypic family (Foissner 1988).

The Pleuroplitidae are very likely related to the Acropisthiina because of distinct homologies in the structure of the oral basket, which is composed of nematodesmata originating not only from the oral dikinetids (as is usual) but also from ciliated oralized somatic monokinetids (Fig. 7). On the other hand, *Pleuroplitoides* (and very likely *Pleuroplites*, too, but this species is so small that details are hardly recognizable light microscopically) has bifurcated nematodesmal bundles (Fig. 7), a character typical of the suborder Lacrymariina (Grain 1984). However, bifurcated nematodesmata are widespread also in prostomatids (e. g. Dragesco et al. 1974, Hiller 1991) and thus possibly a weaker character than the oralized somatic monokinetids.

Genus *Pleuroplitoides* gen. n.

Diagnosis: Pleuroplitidae with isomorphic dorsal brush composed of 2 rows of paired, shortened cilia.

Type species: *Pleuroplitoides smithi* sp. n.

Etymology: composite of *pleuroplites* (laterally armed soldier) and *oides* (similar, to *Pleuroplites*). Masculine gender.

Comparison with related genera: *Pleuroplitoides* is distinguished from *Pleuroplites*, the sole other member of the family, by the structure of the dorsal brush. It consists of 2 rows in *Pleuroplitoides* and more than 3 rows in *Pleuroplites*. Furthermore and more importantly, the rows of *Pleuroplitoides* are isomorphic, i.e. consist of pairs of basal bodies (dikinetids), while those of *Pleuroplites* are heteromorphic, i.e. composed of normal somatic cilia (monokinetids) interspersed between dikinetids with shortened cilia (Foissner 1988).

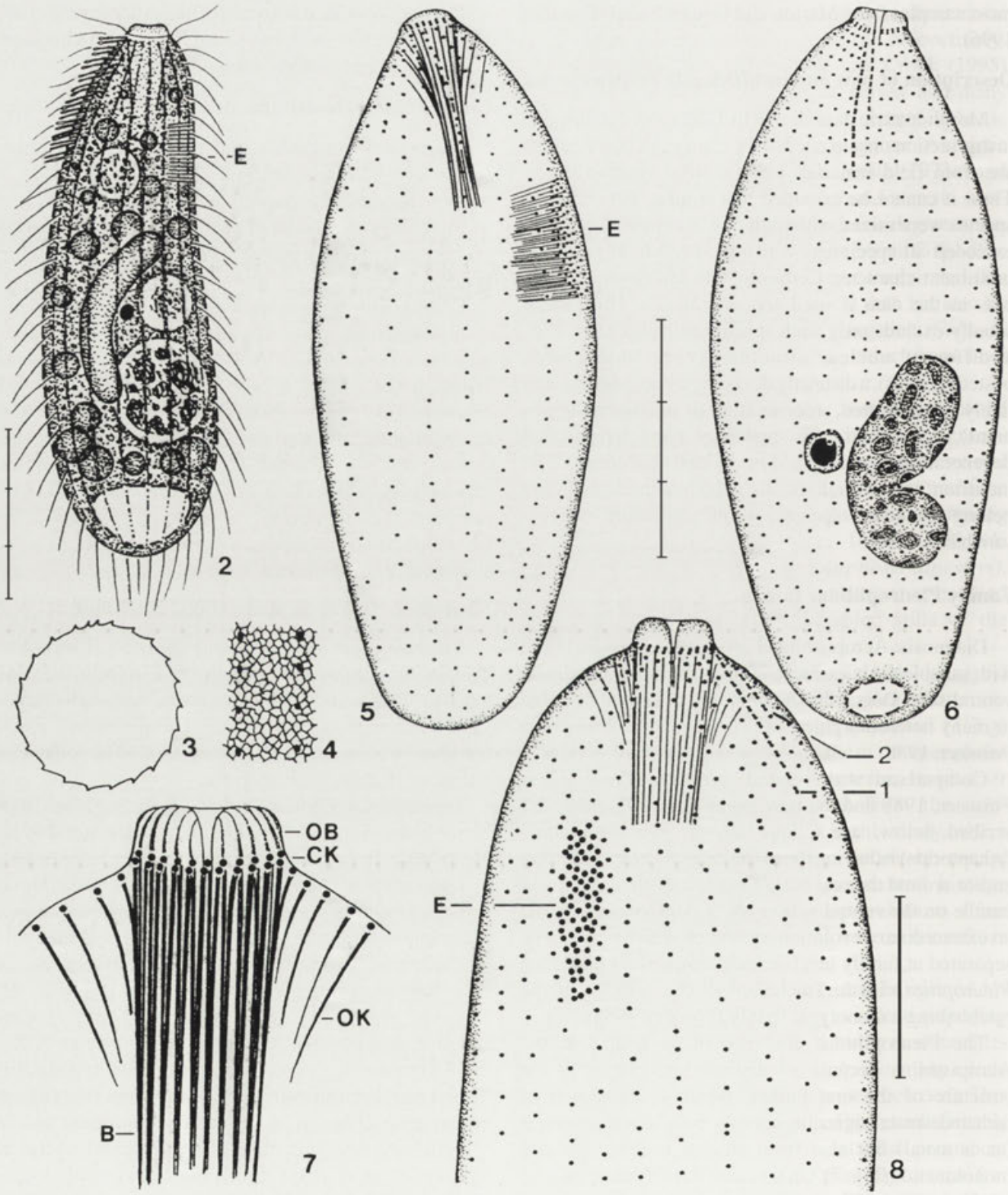
***Pleuroplitoides smithi* sp. n. (Figs. 2-9, Table 3)**

Diagnosis: size *in vivo* about 70-110 x 30-40 µm. Twenty three ciliary rows on average, 2 of them differentiated to brush in anterior quarter. Extrusomes rod-shaped, very slender, about 5 µm long, form elliptical patch between two ventrolateral ciliary rows. Macronucleus reniform, micronucleus globular.

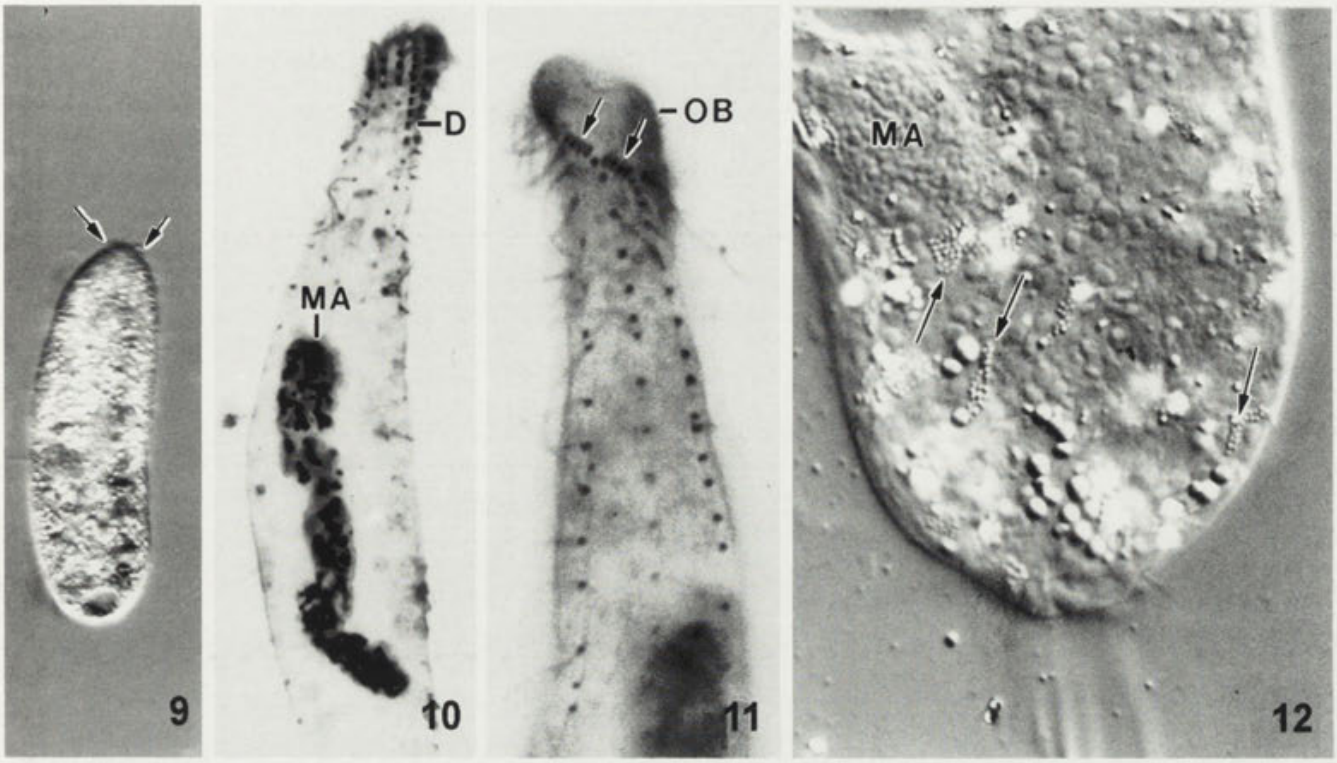
Type location: *Chorisodontium aciphyllum* moss from Signy Island, South Orkney Islands, Antarctica (60°40'S, 45°40'W).

Dedication: named in honour of Professor Dr H. G. Smith (Coventry Polytechnic, England), who undertook many interesting studies on Antarctic soil protozoa.

Description: slightly reniform to bursiform, dorsal side convex, ventral slightly concave (Figs. 2, 9); prepared specimens ellipsoid or pyriform, i.e. inflated in mid-body (Fig. 6) or at posterior end. Anterior end transverse truncate, posterior broadly rounded. Transverse section roundish, with sharp ribs left of ciliary rows (Fig. 3). Macronucleus in or near centre of cell, with many globular and ellipsoidal nucleoli. Micronucleus near macronucleus. Contractile vacuole in posterior end. Fifty to hundred extrusomes in dense, elliptical bundle located ventrolaterally, i.e. about 130° clockwise from dorsal brush, between two slightly widened ciliary rows; individual toxicysts very fine and thus easily overlooked in live cells, distal



Figs. 2-8. *Pleuroplitoides smithi* from life (2-3), after dry silver nitrate (4) and protargol impregnation (5-8). 2 - right lateral view of typical specimen; 3 - anterior polar view; 4 - silverline system; 5-6 - infraciliature of ventral and dorsal side; 7 - fibrillar system of oral apparatus; 8 - dorsolateral view showing extrusome bundle between two somatic ciliary rows. B - oral basket, CK - circumoral kinety, E - extrusome bundle, OB - oral bulge, OK - oralized somatic kinetids, 1-2 - dorsal brush rows. Scale bar division 10 µm



Figs. 9-12. Light micrographs of some of the species described. 9 - Freely moving specimen of *Pleuroplitoides smithi*. Arrows mark inconspicuous oral bulge; 10-11 - *Protospathidium serpens*, dorsal and right lateral views. Arrows mark circumoral kinety composed of isolated dikinetidal fragments; 12 - *Urosomoida granulifera*, surface view of posterior region showing patches of cortical granules (arrows). D - dorsal brush, MA - macronucleus, OB - oral bulge

end however strongly argyrophilic and thus very prominent in prepared specimens (Figs. 1, 5, 8). Cortex flexible, distinctly furrowed by ciliary rows. Cytoplasm usually with many 2-5 µm sized, colourless fat globules and few, large food vacuoles containing residues of ingested ciliates (*Gonostomum affine*). Swims rather slowly by rotation about main body axis.

Cilia 8-10 µm long, rather widely spaced, arranged in longitudinal rows commencing closely underneath circumoral kinety. Dorsal brush cilia very closely spaced, *in vivo* about 4 µm long and slightly inflated at distal end (Fig. 2). Oral bulge very similar to that of *Papillorhabdos*, i.e. flat, inconspicuous and slightly depressed in centre (Figs. 2, 7, 9). Circumoral kinety at base of oral bulge, composed of dikinetids having only posterior basal body ciliated. Oral basket inconspicuous in live cells, but rather distinct in protargol impregnated specimens, composed of bifurcated nematodesmata originating from oral dikinetids. Nematodesmata also originate from 3-5 monokinetids at anterior end of all somatic kineties and extend obliquely to oral basket (Figs. 5, 7, 8).

Silverline system very fine-meshed, as in other gymnostomatids (Fig. 4).

Comparison with related species: *Pleuroplitoides smithi* differs from *Pleuroplites australis* Foissner, 1988, which also occurs in Antarctica (site 5), not only by the generic characters mentioned but also by its larger size (70-100 µm vs. 35-50 µm) and extrusomes (5 µm vs. 2.5 µm) as well as by the reniform (globular in *P. australis*) macronucleus and the higher number of somatic kineties (23 vs. 14). These species are thus easily distinguished even in live condition. However, *P. smithi* is easily confused with *Fuscheria terricola*, also occurring in Antarctica (site 4). This species highly resembles *P. smithi* in all characters, except for the extrusomes, which are located in the centre of the oral bulge, a very useful character for distinguishing *P. smithi* and *F. terricola* in live condition.

Distribution: found at three mossy sites, viz. in samples 2, 7, and 8, but also in a subalpine grassland soil in Austria (Foissner 1987b; designated "genus non det." in Table 1); thus, very likely distributed worldwide, but rare. The unusual location of the extrusomes suggests a special mode of predation.

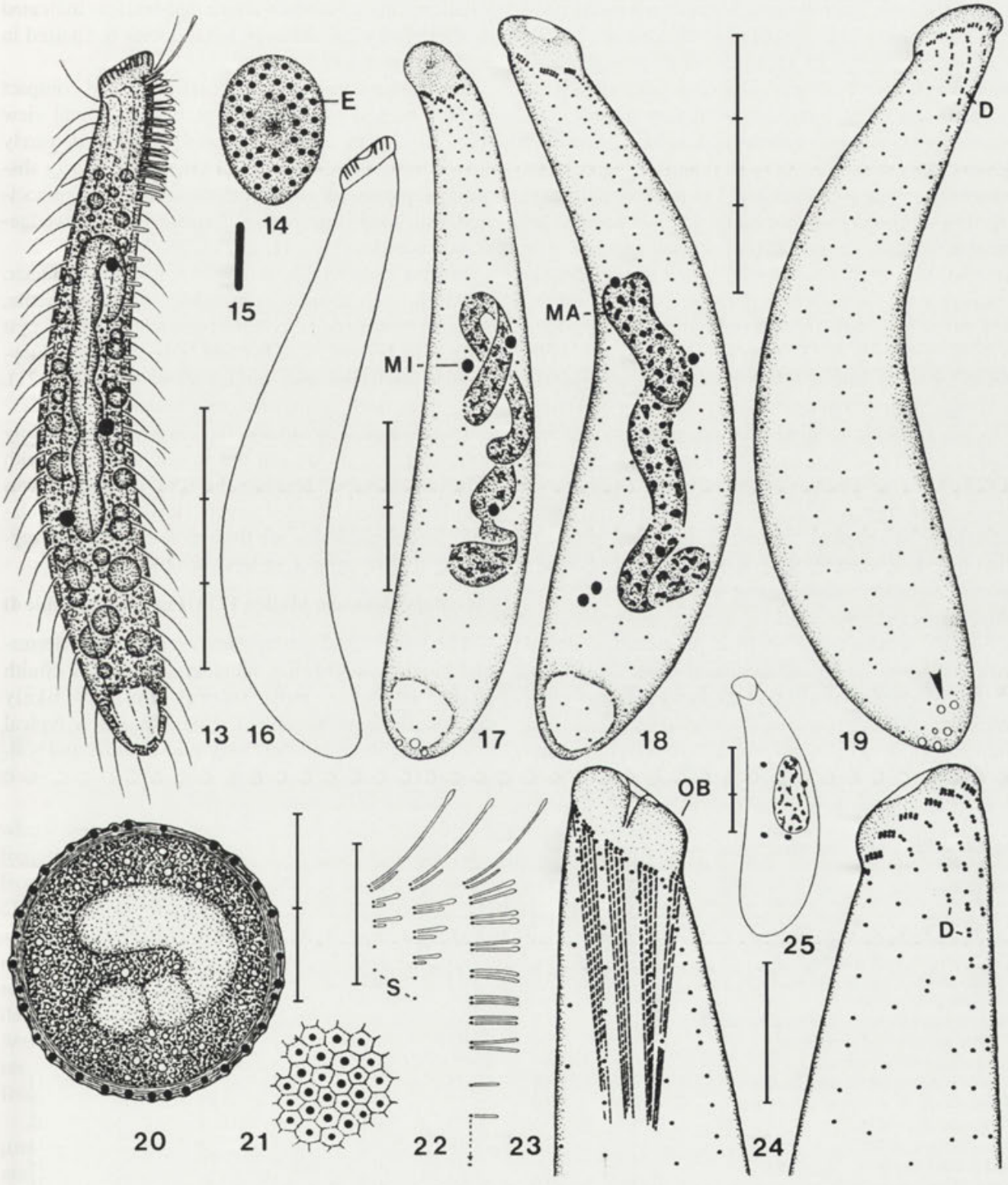
Table 3. Morphometric data from *Pleuroplitoides smithi* (upper line) and *Protospathidium serpens* (lower line)*

Character	$\bar{x}$	M	SD	SD $\bar{x}$	CV	Min	Max	n
Body, length	77.7	74.5	9.0	2.9	11.6	65	90	10
	78.8	77.0	9.0	2.3	11.5	63	95	15
Body, maximum postoral width	31.7	32.0	4.1	1.3	12.9	24	37	10
	18.5	18.0	2.5	0.7	13.7	13	22	15
Distance anterior end to macronucleus	22.9	23.0	5.1	1.6	22.2	17	31	10
	27.9	25.0	6.9	1.8	24.9	20	46	15
Distance anterior end to extrusome patch	14.3	14.0	3.6	1.1	24.9	9	22	10
	-	-	-	-	-	-	-	-
Distance anterior end to end of extrusome patch	26.2	26.0	5.1	1.6	19.4	18	36	10
	-	-	-	-	-	-	-	-
Oral bulge, width at circumoral kinety	6.1	6.0	0.9	0.3	14.4	5	7	10
	7.6	8.0	1.0	0.3	13.0	6	9	15
Chord of macronucleus, respectively, nuclear figure, length	29.5	29.0	5.1	1.6	17.2	22	42	10
	31.3	30.0	6.1	1.6	19.6	20	42	15
Macronucleus, width	9.2	9.0	1.3	0.4	14.3	7	11	10
	5.1	5.0	1.2	0.3	24.3	4	8	15
Micronucleus, diameter	4.9	5.0	0.5	0.2	10.1	4	6	10
	1.7	1.8	0.2	0.1	8.9	1.5	2	15
Macronucleus, number	1.0	1.0	0	0	0	1	1	10
	1.0	1.0	0	0	0	1	1	15
Micronucleus, number	1.0	1.0	0	0	0	1	1	10
	3.3	3.0	1.2	0.3	35.6	2	5	15
Somatic kineties, number in mid-body	23.7	23.0	1.6	0.5	6.6	22	27	10
	11.6	11.0	0.7	0.2	6.4	11	13	15
Basal bodies in a ventral somatic kinety, number	32.1	31.0	5.0	1.6	15.6	25	40	10
	30.7	30.0	5.6	1.4	18.2	20	40	15
Dorsal brush, number of rows	2.0	2.0	0	0	0	2	2	10
	3.5	4.0	-	-	-	3	4	15
Brush kinety 1, length	14.0	14.0	1.5	0.5	10.6	13	18	10
	3.4	3.0	0.6	0.2	18.6	2	4	15
Brush kinety 2, length	6.1	6.0	0.7	0.2	12.1	5	8	10
	4.3	4.0	1.2	0.3	27.3	3	7	15
Brush kinety 3, length	-	-	-	-	-	-	-	-
	10.5	11.0	2.1	0.6	20.3	7	14	15
Brush kinety 4, length	-	-	-	-	-	-	-	
	9.4	10.0	1.6	0.4	16.5	7	11	15
Dikinetids in brush kinety 1, number	14.2	14.0	1.7	0.5	11.9	12	17	10
	1.9	2.0	0.5	0.1	27.7	1	3	15
Dikinetids in brush kinety 2, number	6.4	6.0	1.4	0.5	22.3	5	10	10
	3.0	3.0	0.8	0.2	25.2	2	4	15
Dikinetids in brush kinety 3, number	-	-	-	-	-	-	-	-
	8.5	8.0	1.5	0.4	17.2	7	12	15
Dikinetids in brush kinety 4, number	-	-	-	-	-	-	-	-
	5.2	5.0	0.7	0.2	13.0	4	7	15

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

Protospathidium serpens (Kahl, 1930) Foissner, 1981 (Figs. 10, 11, 13-24, Table 3)

Protospathidium serpens belongs to a group of small spathidiids which are poorly known and thus difficult to identify. The Antarctic population basically matches the original description (Kahl 1930) and the redescription by Foissner (1981b). The population studied by Berger et al. (1984) agrees with Kahl's and Foissner's descriptions in size, shape and number of ciliary rows, but has 15-30 ellipsoidal macronuclear nodules. Thus it might be a race of *P. muscicola* Dragesco and Dragesco-Kerneis, 1979, which, however, has 10-12 ciliary rows, similar to the Antarctic population of *P. serpens*. Obviously, the macronuclear configuration is rather variable in *P. serpens* and some other spathidiids. Thus detailed data are needed from several populations for a reasonable conclusion on the systematic status of *P. serpens*



Figs. 13-25. *Protospathidium serpens* from life (13-16, 20-22) and after protargol impregnation (17-19, 23 - 25). 13 - left lateral view of typical specimen; 14 - frontal view of oral bulge; 15 - extrusome; 16 - broad specimen; 17-19 - infraciliature of ventral, right and left side. Arrowhead marks pores of contractile vacuole; 20-21 - optical section and surface view of resting cyst; 22 - dorsal brush; 23-24 - infraciliature of anterior right and left side (cp. Figs. 10-11); 25 - specimen with ellipsoidal macronucleus. D - dorsal brush, E - extrusomes, MA - macronucleus, MI - micronucleus, OB - oral bulge, S - somatic monokinetids with normal cilia. Scale bar division 10 µm

and *P. muscicola*. The following description is based, if not stated otherwise, on the population found at site 3.

Description (Figs. 10, 11, 13-24, Table 3): size *in vivo* about 70-100 x 12-18 µm. Shape slender, slightly to distinctly sigmoidal, inconspicuously flattened laterally, general appearance thus cylindroid. Macronucleus rather variable, three modifications were found in 79 specimens analyzed: nodular (48 cases; Fig. 17), rod-like (27 cases; Fig. 18), ellipsoidal (4 cases; Fig. 25). Micronuclei of variable position, i.e. not at fixed site and attached to or rather distant from macronucleus. Contractile vacuole terminal, with 3-6 excretory pores. Extrusomes invariably rod-shaped with rounded ends (Fig. 15), 3 µm long in population from site 3, 2 µm in specimens from USA and 2-2.5 x 0.5 µm in Greek population; arranged in 2-3 rough circles around central depression of oral bulge (Fig. 14). Cortex colourless, flexible, in population from Marion Island (Antarctica) with about 5 rows of minute (< 0.3 µm), pale granules between each 2 ciliary rows. Cytoplasm rather hyaline, contains some 1-4 µm sized, colourless fat droplets, obviously digestion products from ingested heterotrophic flagellates and/or bacteria because no ciliates were present when *P. serpens* flourished. Moves slowly.

Somatic infraciliature without peculiarities, dorsal brush, however, highly differentiated (Figs. 10, 13, 19, 22, 24). Anterior cilium of first dikinetid distinctly elongated in each brush row, easily confused with cilia from circumoral kinety segments which, however, lack distal inflation; kinety 3 extends above mid-body, as indicated by shortened cilia, although basal bodies not paired in posterior brush half.

Oral bulge conspicuous, i.e. refractile and compact because packed with extrusomes, oval in frontal view (Fig. 14), centre usually distinctly depressed, rarely almost most flat. Segments of circumoral kinety distinctly separate, adhere to somatic ciliary rows, associated with long, fine nematodesmata forming wedge-shaped bundles (Figs. 11, 17, 18, 23, 24).

Resting cysts 28-32 µm (x = 29.8, n = 8) in diameter, brownish, wall about 2 µm thick, highly refractile, contains conspicuous, compact granules causing cyst surface to become studded and, respectively, honeycombed in lateral and surface view (Figs. 20, 21). Cytoplasm finely granulated, macronucleus tortuous.

Distribution: *Protospathidium serpens* is very likely distributed worldwide and not strictly associated with terrestrial biotopes because the type population was found in a small, flooded trench. However, all populations mentioned above are from mosses and soils, suggesting that *P. serpens* prefers such biotopes.

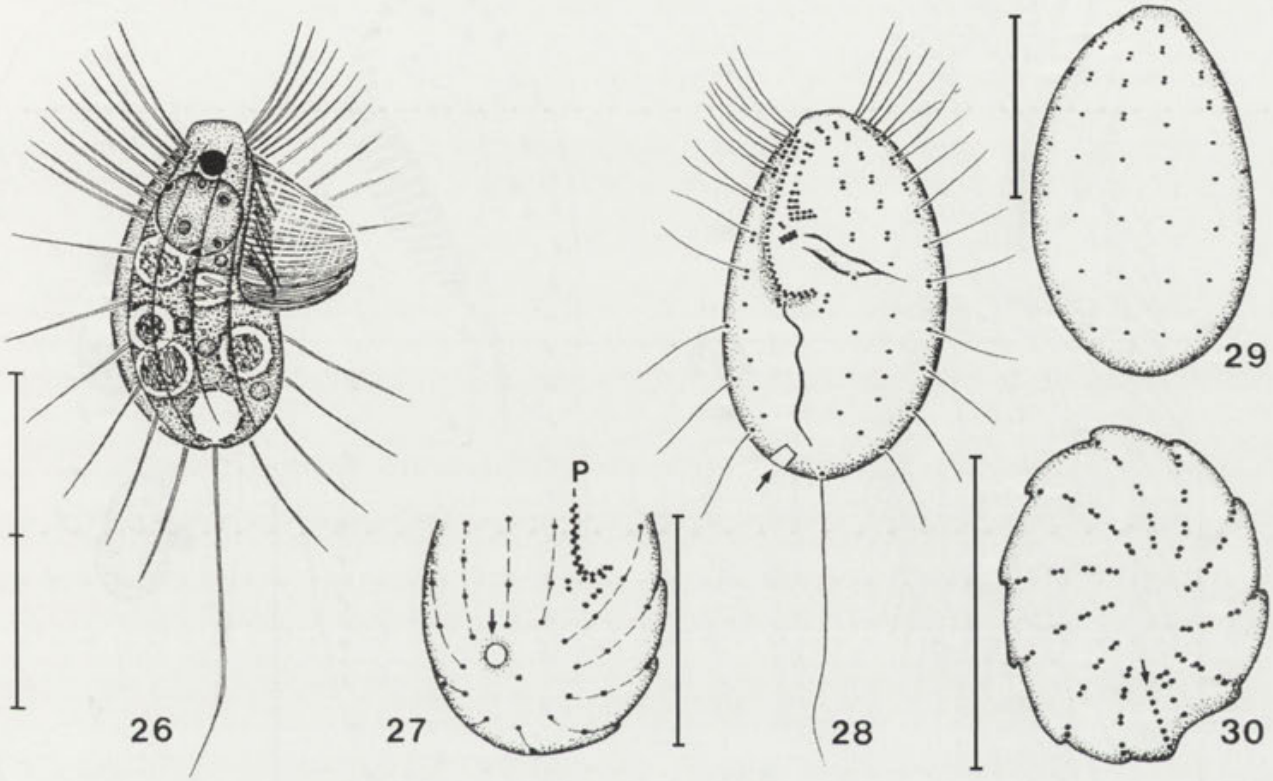
Cyclidium glaucoma Müller, 1773 (Figs. 26-30, Table 4)

This species has been frequently reported from terrestrial biotopes worldwide, including Antarctica (Smith 1978). However, most records are very likely misidentifications because *C. glaucoma* is a typical freshwater ciliate and thus very rare in moss and soil,

Table 4. Morphometric data from *Cyclidium glaucoma*[*]

Character	$\bar{x}$	M	SD	SD $\bar{x}$	CV	Min	Max	n
Body, length	18.2	18.0	1.9	0.6	10.7	15	21	11
Body, width in lateral view	11.5	11.0	1.4	0.4	11.9	10	15	11
Body, width in ventral view	10.0	10.0	1.1	0.3	11.0	8	12	11
Distance anterior end to proximal vertex of paroral membrane	10.4	10.0	0.5	0.2	4.9	10	11	11
Distance anterior end to adoral membranelle 1	1.6	1.5	0.4	0.1	24.0	1	2	11
Distance anterior end to proximal end of adoral membranelle 3	7.2	7.0	0.6	0.2	8.4	6	8	11
Distance anterior end to macronucleus	2.8	3.0	0.3	0.1	12.0	2	3	11
Distance anterior end to excretory pore	17.3	17.0	1.3	0.4	7.8	15	19	11
Macronucleus, length	4.7	5.0	0.5	0.1	9.9	4	5	11
Macronucleus, width	4.3	4.0	0.8	0.2	18.4	3	5	11
Macronuclei, number	1.6	1.0	0.9	0.2	56.5	1	4	14
Somatic kineties, number	10.0	10.0	0.6	0.2	6.3	9	11	11
Kinetids, number in somatic kinety 8	10.4	11.0	0.8	0.2	7.8	9	11	11

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



Figs. 26-30. *Cyclidium glaucoma* from life (26) and after wet silver nitrate impregnation (27-30). 26 - right lateral view of typical specimen; 27 - oblique posterior polar view. Arrow marks pore of contractile vacuole; 28-29 - infraciliature of ventral and dorsal side. Arrow marks pore of contractile vacuole; 30 - anterior polar view. Arrow marks paroral membrane. P - paroral membrane. Scale bar division 10 µm

where *C. muscicola* and *C. terricola* are much more frequent (Foissner 1987a; Table 1). These species differ from *C. glaucoma* by the distinctly subterminal location of the contractile vacuole (Foissner 1995).

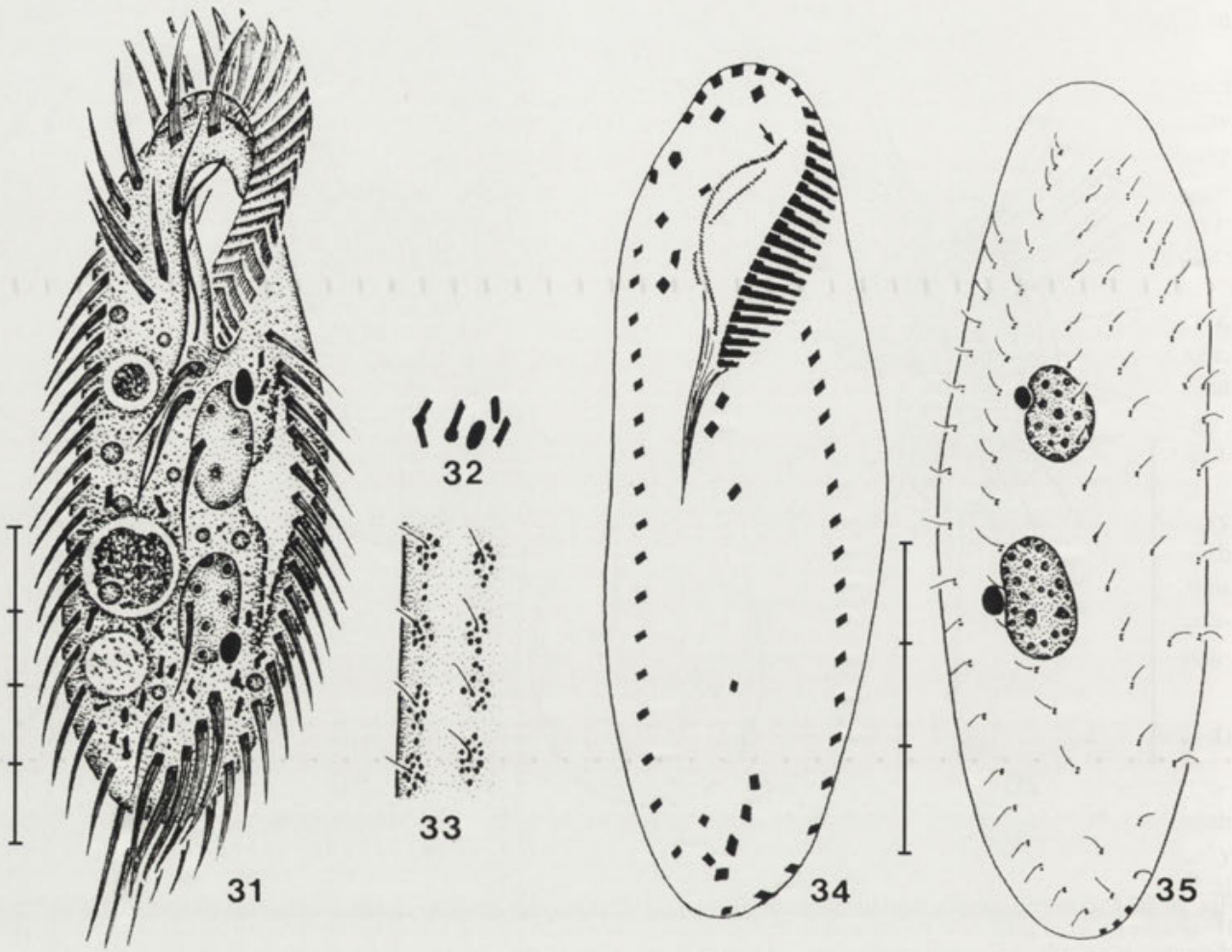
The Antarctic specimens from sites 17 and 27 largely match the freshwater populations studied so far (for review see Foissner et al. 1994). Thus, only a brief description is provided which should, in connection with the detailed morphometry and figures, suffice to recognize and characterize the terrestrial populations from Antarctica.

Size *in vivo* about 17-22 x 8-11 µm, usually 19 x 9 µm. Ellipsoid with narrowed, rather distinctly set off anterior end (frontal plate), laterally slightly flattened (Fig. 26). Macronucleus invariably in anterior third of cell, disintegrated to 2-4 small globules in about half of specimens (Table 4). Micronucleus in anterior indentation of macronucleus, compact and thus conspicuous in live cells, rarely and only faintly impregnates with protargol. Contractile vacuole invariably in posterior end of cell, with single excretory pore (diameter about 1 µm) at end of kinety 2, rarely between kineties 2 and 3 (Fig. 27).

Somatic cilia *in vivo* about 8 µm long, basal bodies paired in anterior third of cell, however, not all dikinetids have both basal bodies ciliated (Fig. 28). Caudal cilium conspicuous, about 20 µm long. Cilia not condensed in posterior half of first kinety left of oral apparatus, unlike some, but not all, freshwater populations (Foissner et al. 1994). Oral structures very much like described by Didier and Wilbert (1981) and Foissner et al. (1994); basal bodies not paired in anterior half of paroral membrane.

Notohymena antarctica sp. n. (Figs. 31-35, Table 5)

Diagnosis: size *in vivo* 80-110 x 30-40 µm. Cortical granules yellow, mainly around cirral bases and dorsal bristles. On average 31 adoral membranelles, 17 right marginal cirri, 18 left marginal cirri, 5 transverse cirri, 3 caudal cirri, and 6 dorsal kineties.



Figs. 31-35. *Notohymena antarctica* from life (31-33) and after protargol impregnation (34-35). 31 - ventral view of typical specimen; 32 - cytoplasmic crystals; 33 - cortical granulation on dorsal side; 34-35 - infraciliature of ventral and dorsal side. Arrow marks hooked anterior portion of paroral membrane, i. e. the genus character. Scale bar division 10 µm

Type location: *Deschampsia antarctica* grass sward from Signy Island, South Orkney Islands, Antarctica (60°40'S, 45°40'W).

Etymology: named after the continent found.

Description: shape prolate ellipsoidal, right side straight or slightly concave, left rather distinctly convex, both ends broadly rounded, flattened laterally up to 3 : 1 (Figs. 31, 34). Flexible like, e. g., *Oxytricha granulifera*. Macronuclear nodules distinctly ellipsoidal (2 : 1), rather close ($\bar{x}$ = 7 µm) together in middle third of body to left of midline. Usually one globular micronucleus attached to each macronuclear nodule. Contractile vacuole in mid-body at left margin, with two inconspicuous collecting canals. Cytopyge in posterior end between transverse cirri and left marginal cirral row; fecal balls contain yellowish globules like those found in cytoplasm. Pellicle colourless, flexible; cortical granules arranged in groups around cirral bases and dorsal bristles (Fig. 33), yellow to yellow-green, give cell yellowish colour at low magnification (≤ x 100), do not stain with protargol. Cytoplasm colourless, contains some 1-4 µm sized, yellowish fat globules and rather many 1-3 µm long crystals, mainly in posterior half (Figs. 31, 32). Feeds on ciliates, heterotrophic flagellates and, possibly, on bacteria. Scrabbles rather fast amongst soil particles.

Marginal cirri about 15 µm long, frontal, transverse and caudal cirri about 20 µm long. Gap between posterior end of marginal rows indistinct because left row extends to midline of cell and indistinctly separate from caudal cirri. Arrangement of ventral cirri oxytrichid, cirral number very constant, i.e. 18, unlike as in *N. australis* (Foissner and O'Donoghue, 1990). Dorsal cilia about 3 µm long *in vivo*,

Table 5. Morphometric data from *Notohymena antarctica* (upper line) and *Sterkiella thompsoni* (lower line)*

Character	$\bar{x}$	M	SD	SD $\bar{x}$	CV	Min	Max	n
Body, length	85.5	87.0	5.8	1.7	6.7	70	91	11
	97.0	97.0	10.3	2.7	10.7	82	115	15
Body, width	30.4	31.0	2.1	0.6	7.0	28	34	11
	40.1	38.0	7.1	1.8	17.7	31	56	15
Anterior somatic end to proximal end of adoral zone, distance	32.4	32.0	2.2	0.7	6.8	28	35	11
	40.3	41.0	3.0	0.8	7.4	35	48	15
Posterior somatic end to posteriormost transverse cirrus, distance	3.0	3.0	0.6	0.2	21.1	2	4	11
	-	-	-	-	-	-	-	-
Distance between macronuclear nodules	6.5	7.0	1.7	0.5	26.3	4	9	11
	-	-	-	-	-	-	-	-
Macronuclear nodules, length	12.9	13.0	2.1	0.6	16.0	11	18	11
	13.1	13.0	3.5	0.9	26.5	9	24	15
Macronuclear nodules, width	7.1	7.0	0.4	0.1	5.6	7	8	11
	8.9	9.0	1.5	0.4	17.0	7	12	15
Micronuclei, length	3.2	3.0	0.3	0.1	10.6	3	4	11
	2.4	2.1	0.4	0.1	16.6	2	3	14
Micronuclei, width	2.8	3.0	0.3	0.1	9.4	2.5	3	11
	1.9	2.0	0.2	0.1	11.6	1.5	2.1	14
Macronuclear nodules, number	2.0	2.0	0	0	0	2	2	11
	3.0	3.0	0.2	0.1	7.5	2	4	120
Micronuclei, number	2.4	2.0	0.7	0.2	28.5	1	3	11
	2.0	1.0	1.7	0.5	84.2	1	6	13
Adoral membranelles, number	30.2	31.0	1.9	0.6	6.4	27	33	11
	34.4	34.0	2.1	0.5	6.0	32	39	15
Right marginal cirri, number	16.8	17.0	1.2	0.4	6.9	15	19	11
	22.3	22.0	1.8	0.5	8.2	19	25	15
Left marginal cirri, number	17.9	18.0	1.3	0.4	7.3	16	20	11
	17.8	17.0	2.2	0.6	12.4	15	24	15
Anterior frontal cirri, number	3.0	3.0	0	0	0	3	3	11
	3.1	3.0	-	-	-	3	4	15
Posterior frontal cirri, number	4.0	4.0	0	0	0	4	4	11
	4.0	4.0	0	0	0	4	4	15
Buccal cirri, number	1.0	1.0	0	0	0	1	1	11
	1.0	1.0	0	0	0	1	1	15
Postoral cirri, number	3.0	3.0	0	0	0	3	3	11
	3.1	3.0	0.6	0.2	19.3	2	5	15
Ventral cirri ahead of transverse cirri, number	2.0	2.0	0	0	0	2	2	11
	2.0	2.0	0	0	0	2	2	15
Transverse cirri, number	5.0	5.0	0	0	0	5	5	11
	5.1	5.0	-	-	-	5	6	15
Caudal cirri, number	3.0	3.0	0	0	0	3	3	11
	2.0	2.0	0	0	0	2	2	15
Dorsal kineties, number	6.0	6.0	0	0	0	6	6	11
	4.0	4.0	0	0	0	4	4	15

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

arranged in 6 rows (Fig. 35): rows 1 and 4 slightly shortened anteriorly, rows 2 and 3 as long as body, row 5 terminates sub-equatorially, row 6 consists of about 5 dikinetids only and ends pre-equatorially.

Oral apparatus and adoral zone of membranelles conspicuous, occupy about 37% of body length. Buccal field rather large and deep, anterior portion semicircularly curved, similar as in *Cyrtohymena*. Paroral and endoral membrane conspicuously curved, intersect optically in mid-portion, paroral distinctly longer than endoral, its distal end hooked (main genus character), both very likely composed of dikinetids (Fig. 34).

Comparison with related species: *Notohymena antarctica* is very similar to *N. australis* (Foissner and O'Donoghue, 1990) Blatterer and Foissner, 1988 as concerns size, shape, and cortical granules. However, it has fewer adoral membranelles and marginal cirri and, more importantly, only 3 caudal cirri. The unusual high

number, viz. 6-8, of caudal cirri in *N. australis* has been confirmed in a German population (Foissner, unpubl.) and is thus a constant character.

Notohymena australis is also easily confused with *Cyrtohymena citrina*, which is very similar in all characters, except for the undulating membrane, which lacks the anteriorly directed hook (Fig. 34). This character, which is rather difficult to recognize, has been confirmed by ontogenetic studies (Voss 1991).

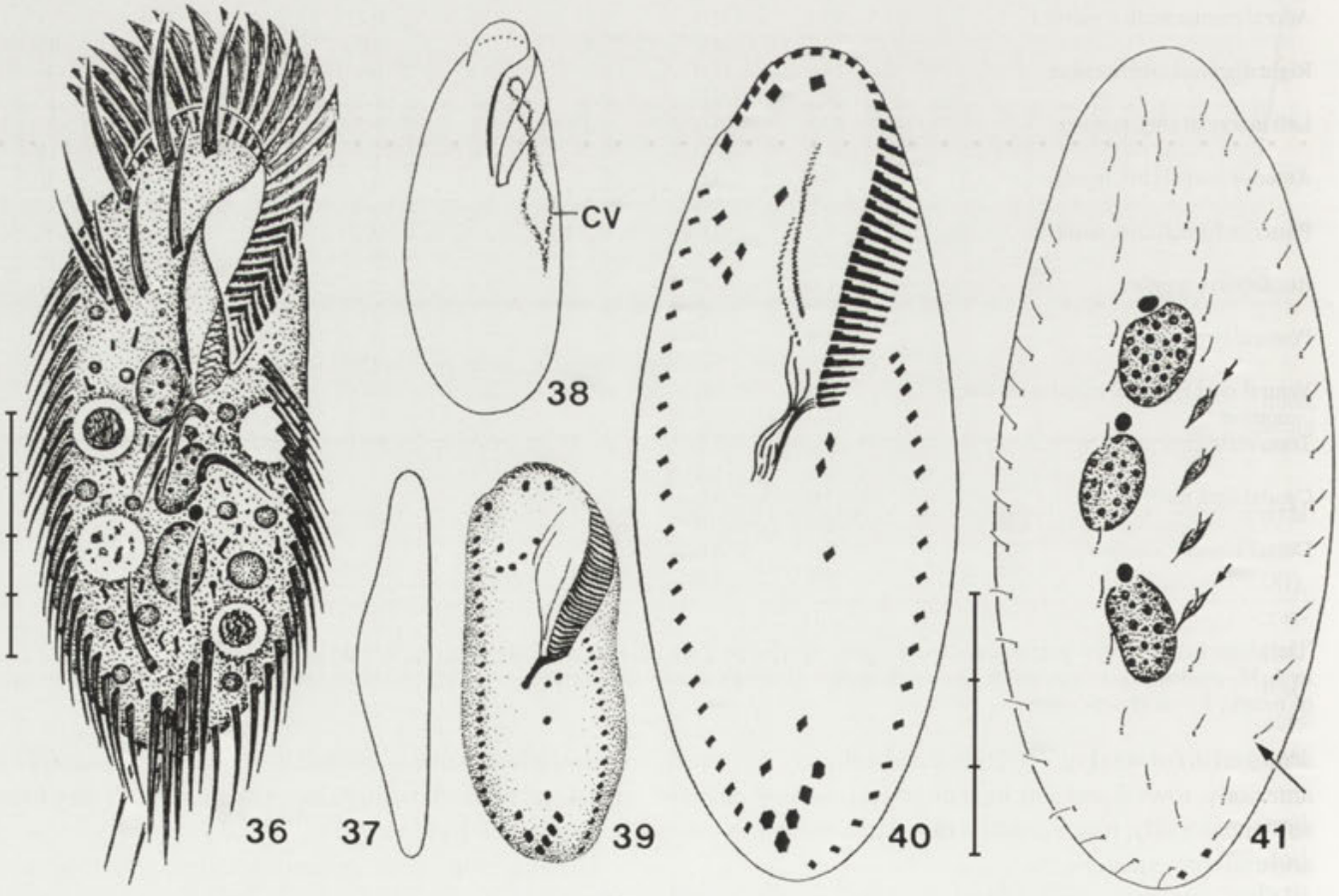
Sterkiella thompsoni sp. n. (Figs. 36-41 , Table 5)

Diagnosis: size *in vivo* about 90-130 x 40-60 µm. Three macronuclear nodules. On average 34 adoral membranelles, 22 right marginal cirri, 17 left marginal cirri, and 5 transverse cirri. Four dorsal kineties with 1 caudal cirrus each associated with kineties 1 and 3.

Type location: *Drepanocladus uncinatus* moss from Signy Island, South Orkney Islands, Antarctica (60°40'S, 45°40'W).

Dedication: named in honour of Jesse C. Thompson Jr. (Roanoke College, Virginia, USA), who provided the first reliable description of this species, but did not establish it as a new taxon because he considered his data as insufficient (see species comparison); an honourable practice which should be applied more often!

Description: usually broadly parallel-sided, rarely slightly bursiform (Fig. 36, 38), anterior end broadly rounded, posterior narrowly rounded and often inconspicuously pointed. Rather rigid like, e. g., *Sterkiella histriomuscorum* and *Stylonychia pustulata*, dorsoven-



Figs. 36-41. *Sterkiella thompsoni* from life (36-38), after wet silver nitrate impregnation (39) and protargol impregnation (40-41). 36-ventral view of typical specimen; 37-38 - lateral and ventral view of oviform specimen; 39 - ventral infraciliature of *Oxytricha* sp. (from Thompson 1972), length about 100µm; 40-41 - infraciliature of ventral and dorsal side. Note that *S. thompsoni* has only three macronuclear nodules, which is its main species character. Fusiform fibre bundles surround dorsal bristles (short arrows). Dorsal kinety 4 is shortened posteriorly (long arrow). CV - contractile vacuole. Scale bar division 10µm

trally flattened up to 2 : 1. Usually 3, very rarely 2 (in 6 out of 120 specimens, Table 5; possibly postdividers) or 4 slightly to distinctly ellipsoidal macronuclear nodules in median of middle third of cell; middle nodule often slightly smaller than anterior and posterior ones. Micronuclei globular, number highly variable, however, most specimens have only one (Table 5). Contractile vacuole in mid-body at left margin, with 2 long collecting canals extending anteriorly and posteriorly, anterior canal often with small dilatations at level of buccal cavity (Fig. 38). Cortex colourless, rigid, without special granules. Cytoplasm colourless, contains many small, yellowish crystals, some 2-6 µm sized, colourless fat globules, and food vacuoles up to 10 µm in diameter. Feeds on bacteria, heterotrophic flagellates, green algae, diatoms and wheat starch. Moves moderately fast, often resting for some time and thus easy to observe.

Anterior frontal cirri, transverse cirri and caudal cirri about 30 µm, other cirri 25 µm long. Marginal rows open at posterior end, gap occupied by caudal cirri right of cell median. Ventral cirral pattern as in other oxytrichids *s. str.* Dorsal cilia *in vivo* about 3 µm long, associated with distinct, oblique fibrillar structures (Fig. 41); arranged in 4 rows which, according to some divisional stages found, originate as follows: row 1 slightly shortened anteriorly and associated with right caudal cirrus; row 2 as long as body; row 3 also extends along whole body length but is associated with left caudal cirrus; row 4 slightly but invariably shortened posteriorly, originates close to right marginal row. Dorsal rows 1-3 simply divide, i.e. none originates by fragmentation as in many other oxytrichids, for instance, *S. histriomuscorum* (Berger et al. 1985).

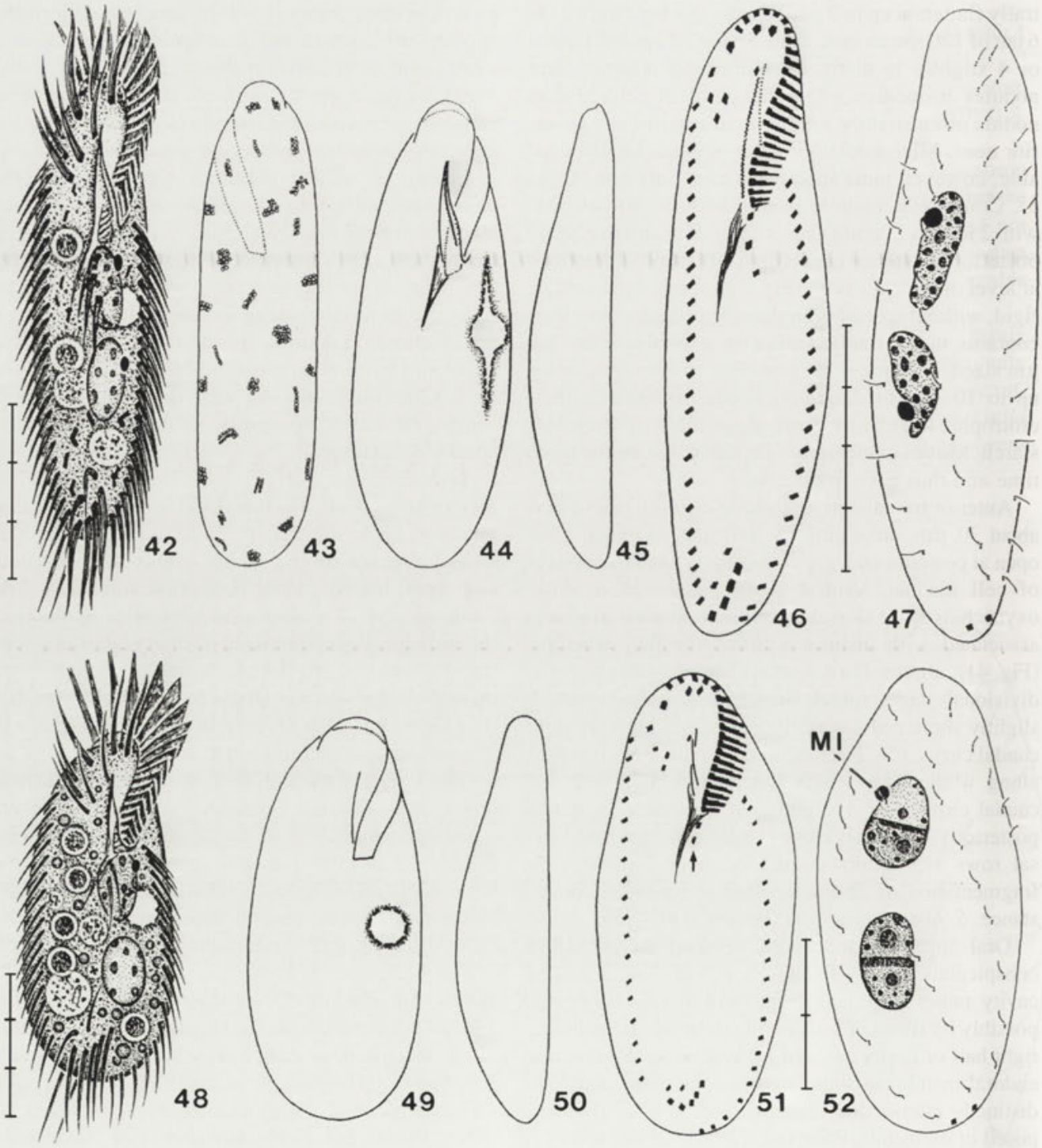
Oral apparatus and adoral zone of membranelles conspicuous, occupy about 42% of body length. Buccal cavity rather large and deep, right margin thickened, possibly by fibres or backwardly directed endoral cilia, right half of cavity covered by hyaline lip. Paroral and endoral membrane slightly curved, inconspicuously to distinctly intersecting optically, both very likely composed of dikinetids. Pharyngeal fibres inconspicuous.

Comparison with related species and generic classification: *Sterkiella thompsoni* differs from its congeners by the unusual number, viz. 3, of macronuclear nodules. All other oxytrichids *s. str.* either have 2, 4 or more nodules. Thus, at first I supposed that *S. thompsoni* could be a teratological population of *S. histriomuscorum* (2 nodules) or *S. cavicola* (4 nodules), especially because some specimens with 2 or 4 macronuclei were found (see description). However, some variability is also apparent in binucleate and quadrinucleate species, when a large number of cells is analysed (Berger and Foissner 1987). Furthermore, the Antarctic population remained constant over 4 weeks and some dividing cells showed that only 3 macronuclear nodules are generated.

Finally, a detailed literature search showed that oxytrichids with 3 macronuclear nodules have been reported several times and, most surprisingly, mainly from Antarctica. Thompson (1972) described and figured (Fig. 39) an *Oxytricha* sp. from a rock pool of the Antarctic Peninsula having the same characteristics as my specimens. Likewise, Sudzuki (1964) mentions an Antarctic *Opisthotricha* with 2-3 macronuclei and a size of 96-120 x 40-80 µm. All these forms are very likely conspecific and *S. thompsoni* is thus obviously widespread in Antarctica.

Seshachar and Kasturi Bai (1963) described an *Oxytricha* sp. from a fish tank in India, which "differs markedly in its nuclear constitution from the other known species of the genus". This still unnamed and very briefly described population has, like *S. thompsoni*, 3 macronuclear nodules propagated through many generations in ordinary laboratory cultures. However, the Indian species is much larger than *S. thompsoni*, viz. 200-450 x 100-150 µm. It is thus very likely that several distinct oxytrichids with 3 macronuclear nodules exist.

The generic classification of *S. thompsoni* is difficult and tentative because it possesses characters of several oxytrichid genera, and *Sterkiella* (formerly *Histriculus*; Foissner et al. 1991) is still vaguely separated from *Stylonychia* and *Oxytricha* (Wirnsberger et al. 1986). However, at least the general appearance, the inflexibility of the body, and the structure of the oral apparatus strongly resemble *S. histriomuscorum* (Foissner 1982, Berger et al. 1985) and *S. cavicola* (Berger and Foissner 1987). On the other hand, the simple dorsal infraciliature of *S. thompsoni* is completely different from that of *Sterkiella* (Berger et al. 1985), *Stylonychia* (Wirnsberger et al. 1986) and *Oxytricha* (Foissner and Adam 1983a), but highly reminiscent of *Urosomoida* (Foissner and Adam 1983b, Ganner et al. 1987) and *Urosoma* (Foissner 1983). Finally, the distinct fibres around the dorsal bristles and the rigidity of the cortex resemble stylonychid oxytrichids and *Stylonychia* which, however, has parallel undulating membranes. To sum up, *S. thompsoni* is a further example of the bewildering and still poorly understood diversity of oxytrichid hypotrichs.



Figs. 42-47. *Urosomoida granulifera* from life (42-45) and after protargol impregnation (46-47). 42 - ventral view of typical specimen; 43 - dorsal view showing cortical granule patches, i. e. the species character; 44-45 - ventral and lateral view of broad specimen; 46-47 - infraciliature of ventral and dorsal side. Scale bar division 10µm

Figs. 48-52. *Urosomoida antarctica* from life (48-50) and after protargol impregnation (51-52). 48 - ventral view of typical specimen; 49-50 - ventral and lateral view of broad specimen; 51-52 - infraciliature of ventral and dorsal side. Note that *U. antarctica* has only two postoral cirri (arrow). MI - micronucleus. Scale bar division 10µm

***Urosomoida granulifera* sp. n. (Figs. 12, 42-47, Table 6)**

Diagnosis: size *in vivo* about 70-100 x 20-30 µm. Cortical granules colourless, about 1 µm in diameter, form small, irregularly arranged patches. On average 23 adoral membranelles, 24 right marginal cirri, 23 left marginal cirri, 4 transverse cirri, and 2 caudal cirri.

Type location: *Drepanocladus uncinatus* moss from Livingstone Island, South Shetland Islands, Antarctica (62°38'S, 61°04'W).

Etymology: *granulifera* (bearing granules) refers to the main species character, viz. the cortical granules.

Description: shape highly variable, slenderly to broadly elliptical or parallel-sided, sometimes slightly fusiform, both ends narrowly to broadly rounded (Figs. 42-44). Flexible like, e. g., *U. agiliformis* and *Oxytricha granulifera*, dorsoventrally flattened up to 2 : 1. Usually 2, very rarely 3 distinctly ellipsoidal macronuclear nodules in middle third of cell to left of midline. Micronuclei ellipsoidal, number highly variable, however, most specimens have 2 (Table 6). Contractile vacuole in mid-body at left margin, with 2 inconspicuous collecting canals extending anteriorly and posteriorly. Cortex colourless, flexible, cortical granules inconspicuous because colourless, minute and sparse, form irregular groups composed of up to 30 granules (Figs. 12, 43). Cytoplasm colourless, contains many small, yellowish crystals. Possibly feeds on bacteria. Movement moderately rapid, scrabbling amongst soil particles.

Anterior frontal cirri, transverse cirri and caudal cirri about 18 µm, other cirri 12 µm long. Marginal rows open at posterior end, gap occupied by posteriormost transverse cirrus and caudal cirri. Ventral and dorsal infraciliature very similar, if not to say identical to that of *U. agiliformis* (cp. Ganner et al. 1987). Dorsal cilia 4 µm long *in vivo*.

Oral apparatus and adoral zone of membranelles also very similar to that of *U. agiliformis*, i.e. inconspicuous, occupy about 34% of body length, bases of largest membranelles *in vivo* about 7 µm wide. Buccal cavity narrow and flat, right half and posterior third of adoral zone covered by hyaline lip. Paroral and endoral membrane almost straight, extend side by side diverging posteriorly. Pharyngeal fibres inconspicuous.

Comparison with related species: the new species is most similar to *U. agiliformis* Foissner, 1982 as concerns size, shape and most morphometric and morphologic characteristics of the infraciliature. However, *U. agiliformis* and other similar oxytrichids discussed by Ganner et al. (1987) lack cortical granules. In this respect, *U. granulifera* resembles *U. agilis* whose granules, however, usually have a yellowish to reddish colour. Furthermore, *U. agilis* has 3 caudal cirri and its posterior portion is always more or less distinctly elongated (Berger and Foissner 1987, Foissner 1982). *Urosomoida minima* Hemberger, 1985 has only 14-15 adoral membranelles and an unusual number, viz. 5, of posterior frontal cirri. *Urosomoida perthensis* Foissner and O'Donoghue, 1990 has 3 caudal cirri and a single micronucleus interposed between the macronuclear nodules.

***Urosomoida antarctica* sp. n. (Figs. 48-52, Table 6)**

Diagnosis: size *in vivo* about 60-75 x 25-35 µm. Two postoral ventral cirri, 2 caudal cirri, 4 dorsal kineties. On average 20 adoral membranelles, 17 right marginal cirri, 19 left marginal cirri, and 5 transverse cirri.

Type location: soil from Garwood Valley, South Victoria Land, Antarctica (about 160°E, 78°S).

Etymology: named after the continent found.

Description: shape broadly elliptical, posteriad usually slightly widened, both ends broadly rounded (Figs. 48, 49). Flexible like, e. g., *Oxytricha granulifera* and *U. granulifera*, and dorsoventrally flattened up to 2 : 1. Macronuclear nodules distinctly ellipsoidal, in middle third of cell to left of midline. Micronuclei slightly ellipsoidal, number rather variable, if only 1 is present it is usually attached to the anterior macronuclear nodule. Contractile vacuole in mid-body near left margin, without distinct collecting canals. Cortex colourless, flexible, without special granules. Cytoplasm colourless, contains many 2-3 µm sized, colourless fat globules, some small vacuoles with yellowish, crystalline content, and many 4-6 µm sized food vacuoles. Feeds on bacteria and, possibly, also on heterotrophic flagellates and small naked amoebae. Moves slowly.

All cirri strikingly thin, anterior frontal cirri, transverse cirri and caudal cirri about 18 µm, other cirri 12 µm long. Marginal rows open widely at posterior end, gap occupied by posteriormost transverse cirri and caudal cirri. Ventral and dorsal infraciliature very similar to that of *U.agiliformis* (cp. Ganner et al. 1987), except for the lacking third postoral cirrus and the normal set of transverse cirri (Fig. 51). Dorsal cilia 3 µm long; kinety 1 shortened anteriorly, kinety 4 terminates pre-equatorially (Fig. 52).

Oral apparatus and adoral zone of membranelles inconspicuous, occupy about 32% of body length, bases of largest membranelles *in vivo* about 6 µm wide.

Table 6. Morphometric data from *Urosomoida granulifera* (upper line) and *U. antarctica* (lower line)*

Character	$\bar{x}$	M	SD	SD $\bar{x}$	CV	Min	Max	n
Body, length	73.9	72.5	9.2	2.7	12.4	59	90	12
	61.6	62.0	5.4	1.5	8.9	52	70	13
Body, width	21.5	21.5	3.1	0.9	14.6	17	29	12
	27.3	28.0	3.3	0.9	12.1	22	33	13
Anterior somatic end to proximal end of adoral zone, distance	24.8	24.5	2.2	0.6	8.7	22	29	12
	19.6	20.0	1.0	0.3	5.3	18	21	13
Distance between macronuclear nodules	2.9	3.0	2.2	0.6	73.8	0	7	12
	5.4	5.0	2.2	0.6	41.2	2	10	13
Macronuclear nodules, length	15.6	15.5	2.5	0.7	16.3	12	20	12
	13.5	13.0	1.6	0.4	11.6	11	17	13
Macronuclear nodules, width	5.7	5.5	0.8	0.2	13.7	5	7	12
	7.6	7.0	1.0	0.3	13.7	6	10	13
Micronuclei, length	3.0	3.0	-	-	-	3	3.5	12
	2.8	2.8	0.3	0.1	11.8	1.8	3	13
Micronuclei, width	2.4	2.2	0.5	0.1	18.7	2	3	12
	2.4	2.5	0.4	0.1	17.3	1.6	2.8	13
Macronuclear nodules, number	2.1	2.0	-	-	-	2	3	12
	2.0	2.0	0	0	0	2	2	13
Micronuclei, number	3.1	2.0	2.1	0.6	67.0	2	8	12
	1.5	1.0	0.7	0.2	45.2	1	3	13
Adoral membranelles, number	23.7	23.0	1.9	0.5	7.9	21	27	12
	19.5	20.0	0.8	0.2	4.0	18	21	13
Right marginal cirri, number	24.1	24.0	3.7	1.1	15.2	16	30	12
	17.0	17.0	1.2	0.3	7.2	14	19	13
Left marginal cirri, number	23.3	23.0	3.2	0.9	13.7	17	29	12
	19.9	19.0	2.1	0.6	10.3	17	24	13
Anterior frontal cirri, number	3.0	3.0	0	0	0	3	3	12
	3.0	3.0	0	0	0	3	3	13
Posterior frontal cirri, number	3.9	4.0	-	-	-	3	4	12
	4.0	4.0	0	0	0	4	4	13
Buccal cirri, number	1.0	1.0	0	0	0	1	1	12
	1.0	1.0	0	0	0	1	1	13
Postoral cirri, number	2.9	3.0	-	-	-	2	3	12
	2.0	2.0	0	0	0	2	2	13
Ventral cirri ahead of transverse cirri, number	2.0	2.0	0	0	0	2	2	12
	2.0	2.0	0	0	0	2	2	13
Transverse cirri, number	3.9	4.0	-	-	-	3	4	12
	5.1	5.0	-	-	-	5	6	13
Caudal cirri, number	2.0	2.0	0	0	0	2	2	12
	2.0	2.0	0	0	0	2	2	13
Dorsal kineties, number	4.0	4.0	0	0	0	4	4	12
	4.0	4.0	0	0	0	4	4	13

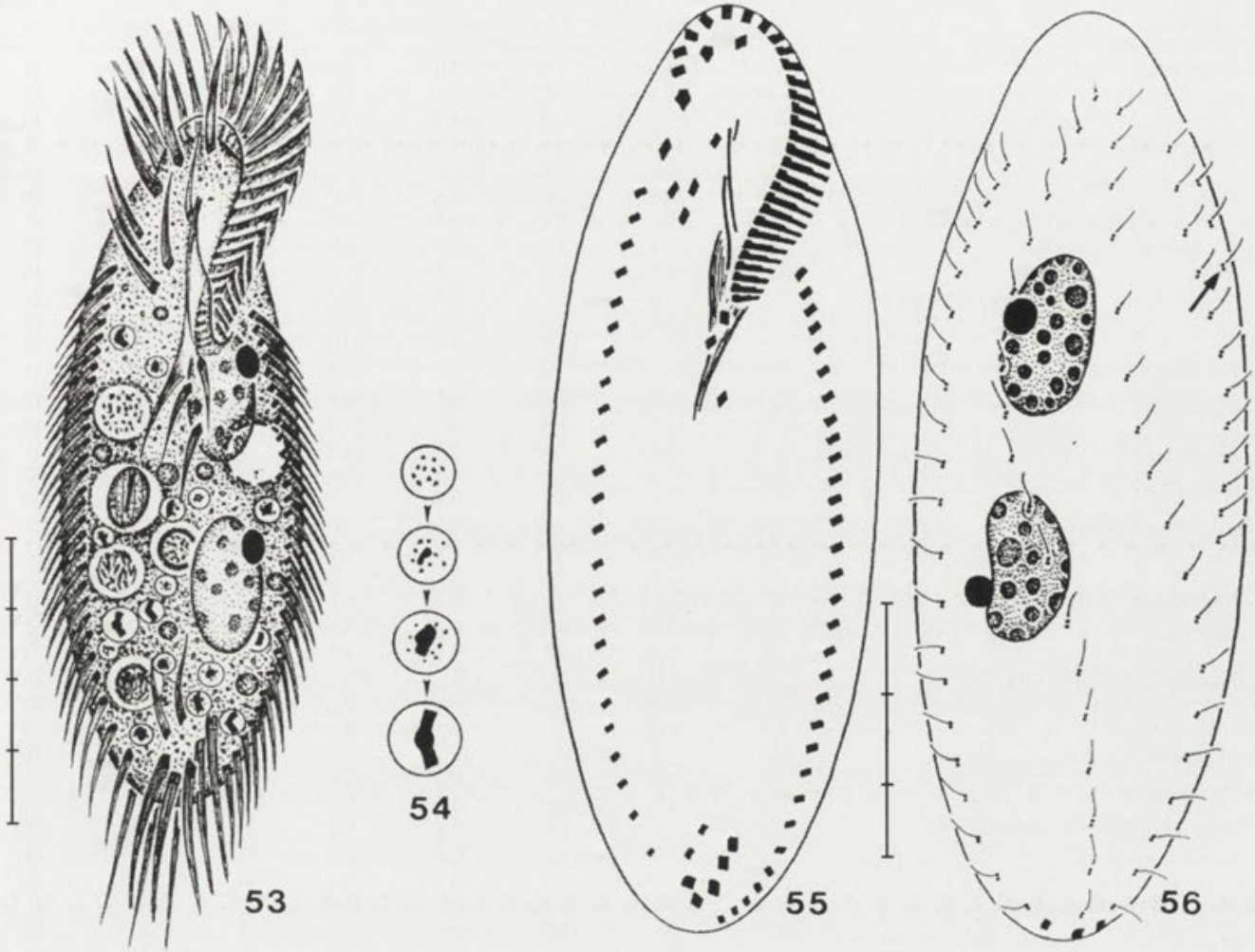
* Data based on protargol-impregnated and mounted specimens from field. Measurements in µm. Abbreviations: CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, n - number of individuals investigated, SD - standard deviation, SD $\bar{x}$ - standard deviation of mean, $\bar{x}$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

Buccal cavity narrow and flat. Paroral and endoral membrane almost straight, slightly intersecting optically. Pharyngeal fibres inconspicuous.

Comparison with related species: the generic classification of *U. antarctica* is uncertain and requires ontogenetic data. The arrangement of the undulating membranes and the full set of transverse cirri indicate that it might belong to *Oxytricha*, whereas the reduced number of postoral and caudal cirri and the simple dorsal infraciliature resemble *Urosomoida*. In any case, this species has a unique combination of characters (see diagnosis) not found in any other known species.

Oxytricha lanceolata Shibuya, 1930 (Figs. 53-56, Table 7)

This species is well-known (Berger and Foissner 1987, 1989) and mentioned in some detail only because it is one of the many examples that geographically widely distant ciliate populations are often sur-



Figs. 53-56. *Oxytricha lanceolata* from life (53-54) and after protargol impregnation (55-56). 53 - ventral view of typical specimen; 54 - development of cytoplasmic crystals; 55-56 - infraciliature of ventral and dorsal side. Arrow marks a short, fifth dorsal kinety found in few specimens. Scale bar division 10 µm

prisingly similar. This is sustained by recent molecular biological data (Bowers and Pratt 1995). The Antarctic population of *O. lanceolata* is morphologically and morphometrically inseparable from the European populations. The median values of the main characters (see below) match exactly (Table 7).

Taking the 3 populations investigated so far, *O. lanceolata* can be characterized as follows: length *in vivo* 80-120 µm (75-110 µm in protargol slides), 2 macronuclear nodules, 2 micronuclei, 23-30 (M = 27-28) adoral membranelles, 25-33 (M = 28-29) right marginal cirri, 25-35 (M = 31-32) left marginal cirri, 5 transverse cirri, 3 caudal cirri, 4 dorsal kineties. The most important character, as compared with the typical oxytrichid ventral cirral pattern and number, is the reduced number of dorsal kineties with kinety 4 distinctly shortened posteriorly. Furthermore, all populations lack special cortical granules, which greatly facilitates *in vivo* separation from an other frequent and rather similar soil species, viz. *O. granulifera*.

Paruroleptus notabilis Foissner, 1982 (Figs. 57-63, Table 8)

Description of Antarctic population: size *in vivo* 90-140 x 15-20 µm. Slenderly ellipsoid to slightly sigmoidal and/or pisciform, i.e. rather distinctly narrowed posteriorly (Figs. 57, 59). Very flexible and dorsoventrally inconspicuously to distinctly (up to 2 : 1)

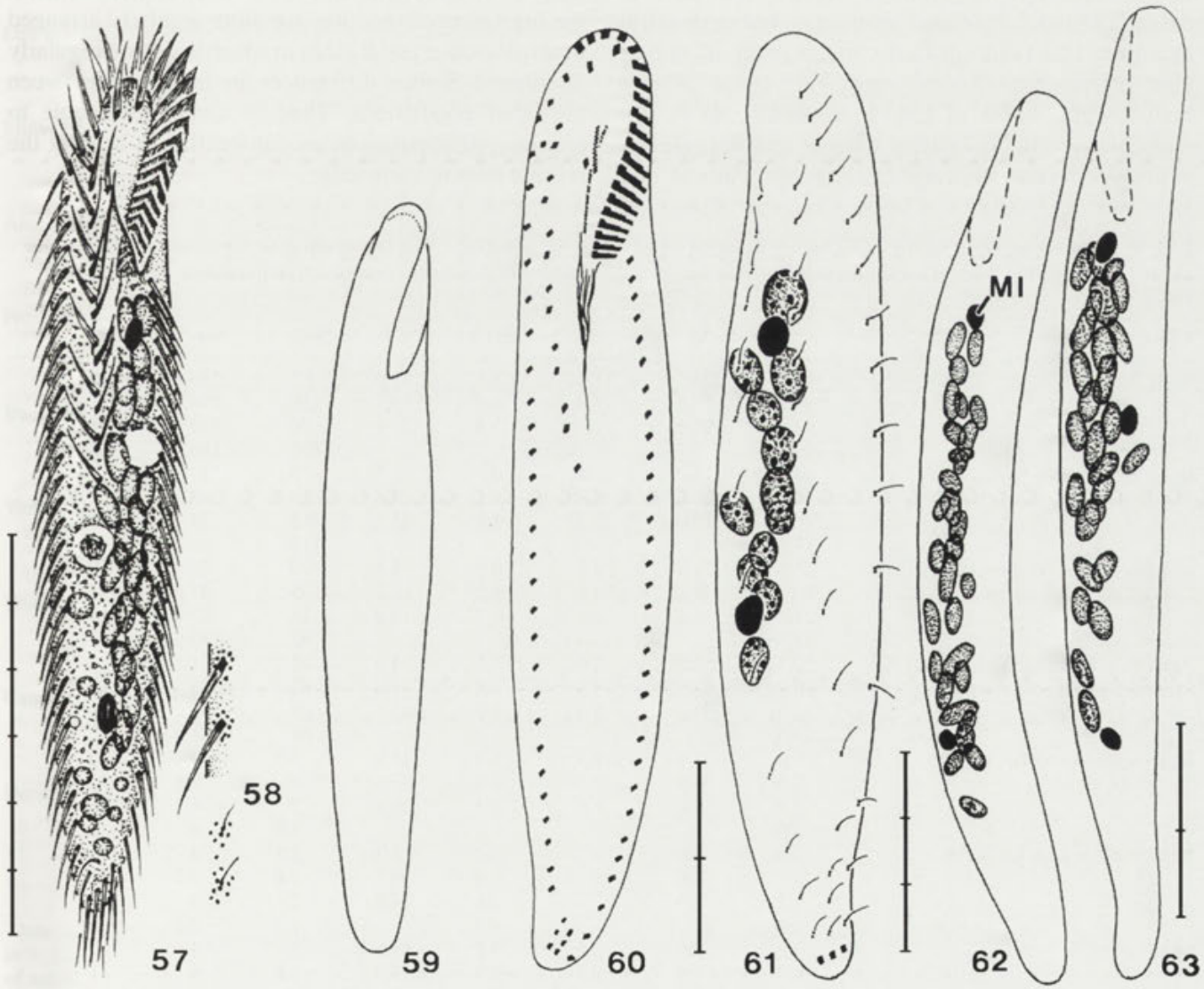
Table 7. Morphometric data from *Oxytricha lanceolata*. Upper line: Antarctic population; middle line: Austrian population (from Berger and Foissner 1987); lower line: population from Madeira, Portugal (from Berger and Foissner 1989)*

Character	$\bar{x}$	M	SD	SD $\bar{x}$	CV	Min	Max	n
Body, length	93.5	91.0	7.1	2.2	7.6	87	110	11
	87.9	89.5	7.3	2.1	8.3	75	100	12
	94.5	96.0	12.2	3.8	12.9	70	108	10
Body, width	34.9	34.0	4.8	1.5	13.8	28	46	11
	30.8	30.0	3.5	1.0	11.4	27	39	12
	34.2	34.5	3.4	1.1	10.0	29	42	10
Anterior somatic end to proximal end of adoral zone, distance	29.4	29.0	1.4	0.4	4.6	27	32	11
	27.5	27.5	1.1	0.3	3.9	25	29	12
	30.1	31.5	3.5	1.1	11.6	24	34	10
Distance between macronuclear nodules	4.5	4.0	1.5	0.5	33.2	2	7	11
	3.9	3.0	3.0	0.9	77.3	1	12	12
	8.5	10.0	2.7	0.7	32.0	3	11	10
Macronuclear nodules, length	16.5	16.0	2.3	0.7	14.2	13	20	11
	15.3	14.5	2.3	0.7	14.9	13	21	12
	13.9	14.5	1.6	0.5	11.5	10	15	10
Macronuclear nodules, width	9.0	9.0	1.3	0.4	14.9	7	11	11
	7.8	7.0	1.1	0.3	13.6	7	10	12
	6.9	7.0	0.9	0.3	12.7	5	8	10
Micronuclei, length	4.0	4.0	0.3	0.1	7.6	3.5	4.5	11
	2.8	2.8	0.2	0.1	5.7	2.5	3	12
	2.8	2.8	0.1	0.1	2.2	2.8	3	10
Micronuclei, width	3.7	3.5	0.2	0.1	6.7	3.5	4.2	11
	2.7	2.7	0.1	0.1	5.3	2.5	3	12
	2.6	2.6	0.2	0.1	6.0	2.4	2.8	10
Macronuclear nodules, number	2.0	2.0	0	0	0	2	2	11
	2.0	2.0	0	0	0	2	2	12
	2.0	2.0	0	0	0	2	2	10
Micronuclei, number	2.0	2.0	0	0	0	2	2	11
	2.1	2.0	0.7	0.2	32.1	1	3	12
	1.8	2.0	0.6	0.2	35.1	1	3	10
Adoral membranelles, number	27.9	28.0	1.4	0.4	5.2	26	30	11
	26.5	27.0	1.5	0.4	5.5	23	28	12
	27.6	28.0	0.8	0.3	3.1	26	29	10
Right marginal cirri, number	28.8	29.0	2.2	0.7	7.6	25	33	11
	28.4	29.0	2.0	0.6	7.1	25	32	12
	28.5	28.0	1.9	0.6	6.7	26	32	10
Left marginal cirri, number	30.0	31.0	2.6	0.8	8.8	25	34	11
	31.4	31.5	2.6	0.8	8.4	27	35	12
	31.7	32.0	3.6	1.1	11.3	25	36	10
Anterior frontal cirri, number	3.0	3.0	0	0	0	3	3	11
	3.0	3.0	0	0	0	3	3	12
	3.0	3.0	0	0	0	3	3	10
Posterior frontal cirri, number	4.6	4.0	1.0	0.3	22.1	4	7	11
	4.0	4.0	0.4	0.1	10.7	3	5	12
	4.0	4.0	0	0	0	4	4	10
Buccal cirri, number	1.0	1.0	0	0	0	1	1	11
	1.0	1.0	0	0	0	1	1	12
	1.0	1.0	0	0	0	1	1	10
Postoral cirri, number	3.0	3.0	0	0	0	3	3	11
	3.0	3.0	0	0	0	3	3	12
	3.0	3.0	0	0	0	3	3	10
Ventral cirri ahead of transverse cirri, number	2.1	2.0	-	-	-	2	3	11
	2.0	2.0	0	0	0	2	2	12
	2.0	2.0	0	0	0	2	2	10
Transverse cirri, number	5.2	5.0	-	-	-	5	6	11
	5.1	5.0	-	-	-	5	6	12
	5.0	5.0	0	0	0	5	5	10
Caudal cirri, number	3.0	3.0	0	0	0	3	3	11
	3.6	3.0	1.3	0.4	36.6	2	6	12
	3.0	3.0	0	0	0	3	3	10

Table 8.(con)

Dorsal kineties, number	4.1	4.0	-	-	-	4	5	11
	4.0	4.0	0	0	0	4	4	12
	4.0	4.0	0	0	0	4	4	10

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



Figs. 57-63. *Paruroleptus notabilis* from life (57-59) and after protargol impregnation (60-63). 57-59 - ventral views of typical specimens; *58 - cortical granulation around cirri and dorsal bristles; 60-61 - infraciliature of ventral and dorsal side of specimen with few macronuclear nodules; 62-63 - variability of nuclear apparatus. MI - micronucleus. Scale bar division 10µm

flattened. Macronuclear nodules globular to ellipsoidal, arranged in 2-3 loose rows mainly in left half of cell (Figs. 61-63). Micronuclei conspicuous because compact and large, *in vivo* up to 7 x 3 µm, usually 1 each near end of macronuclear chain, stain weakly with protargol. Contractile vacuole slightly above mid-body. Cortex flexible, contains tiny (diameter < 0.5 µm), colourless granules mainly around cirral bases and dorsal bristles. Cytoplasm colourless, without crystalline inclusions, posterior portion usually filled with 2-5 µm sized, colourless fat globules. Glides and swims rather slowly.

Somatic and oral infraciliature as described in the other populations, differing mainly in some morphometric characteristics (Table 8). Transverse and caudal cirri distinct; 1 isolated cirrus at end of midventral row (n = 10). Oral apparatus inconspicuous, adoral zone of membranelles occupies only about 24% of body length, bases of largest membranelles 6 µm wide *in vivo*. Buccal cavity narrow and flat, almost entirely covered by very hyaline lip. Paroral and endoral membrane slightly curved, intersect optically, paroral conspicuously short, i.e. about half the length of endoral (Fig. 60). Pharyngeal fibres conspicuous but not surrounded by special structures as in Austrian and Australian populations.

Comparison with other populations (Table 8): as Blatterer and Foissner (1988) mentioned, *P. notabilis* is a difficult species. As yet, four populations have been studied (Table 8). All differ in some important details. The Antarctic specimens resemble the German population best, especially in the inconspicuous cortical granules. However, they have slightly more adoral membranelles, a distinctly higher number of marginal cirri, and fewer macronuclear nodules and dorsal kineties. Furthermore, the macronuclear nodules are more regularly arranged than in the other populations in which they are irregularly distributed. Similar differences are found also between the other populations. Thus, it seems reasonable to consider all populations as conspecific, at least at the present state of knowledge.

Table 8. Morphometric data from *Paruroleptus notabilis*. 1st line: Antarctic population; 2nd line: Australian population (from Blatterer and Foissner 1988); 3rd line: German population (from Berger and Foissner 1987); 4th line: Austrian type population (from Foissner 1982)*

Character	$\bar{x}$	M	SD	SD $\bar{x}$	CV	Min	Max	n
Body, length	103.5	101.5	11.4	3.6	11.0	87	130	10
	158.8	153.0	21.8	6.6	13.7	136	198	11
	93.6	94.0	11.2	3.4	11.9	74	110	11
	163.3	-	-	-	-	150	180	3
Body, width	14.9	15.0	1.4	0.5	9.7	13	17	10
	36.5	38.0	6.9	2.1	18.8	23	46	11
	16.5	15.0	3.9	1.2	23.3	13	25	11
	17.3	-	-	-	-	13	20	3
Anterior somatic end to proximal end of adoral zone, distance	23.8	24.0	2.0	0.6	8.4	20	26	10
	40.4	41.0	4.1	1.2	10.1	35	47	11
	21.2	21.0	2.3	0.7	10.9	18	27	11
	32.7	-	-	-	-	32	33	3
Anterior somatic end to proximal end of midventral row, distance	54.7	54.0	8.0	2.5	14.6	42	71	10
	80.2	83.0	9.3	2.8	11.6	67	95	11
	32.5	32.0	4.3	1.3	13.3	25	39	11
	74.0	-	-	-	-	67	81	2
Macronuclear nodules, length	5.0	5.0	1.3	0.4	24.9	3.5	7	10
	5.0	4.6	1.9	0.6	37.8	3	7.6	11
	4.9	5.0	1.0	0.3	20.4	3	6	10
	5.0	-	-	-	-	3.9	6	3
Macronuclear nodules, width	2.9	3.0	0.2	0.1	7.3	2.5	3	10
	3.1	3.0	0.2	0.1	6.5	2.8	3.5	11
	2.7	2.9	0.4	0.1	14.6	2	3	10
	2.2	-	-	-	-	1.7	2.7	3
Micronuclei, length	3.5	3.5	0.5	0.2	15.1	3	4	10
	4.0	3.9	0.9	0.3	23.0	2.8	6	11
	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Micronuclei, width	2.8	3.0	0.3	0.1	11.8	2	3	10
	2.4	2.3	0.6	0.2	27.2	1.5	3.2	11
	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-

Table 8.(con.)

Macronuclear nodules, number	23.4	24.5	5.5	1.7	23.3	12	30	10
	55.5	55.0	6.9	2.1	12.5	47	69	11
	30.6	31.0	3.1	0.9	10.2	25	35	11
	about 70							3
Micronuclei, number	2.4	2.5	0.7	0.2	29.1	1	3	10
	2.2	2.0	0.8	0.2	34.4	1	4	11
	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Adoral membranelles, number	21.3	21.5	1.6	0.5	7.4	19	23	10
	31.6	31.0	3.2	1.0	10.0	26	36	11
	16.7	17.0	0.7	0.2	3.9	16	18	11
	29.7	-	-	-	-	28	35	3
Right marginal cirri, number	30.5	30.0	4.4	1.4	14.4	24	39	10
	33.5	33.0	3.7	1.1	11.0	28	41	11
	22.5	23.0	2.2	0.7	9.6	18	25	11
	36.3	-	-	-	-	32	42	3
Left marginal cirri, number	30.1	31.0	4.7	1.5	15.7	22	38	10
	39.5	39.0	6.4	1.9	16.3	32	55	11
	24.0	24.0	1.3	0.4	5.3	22	27	11
	39.5	-	-	-	-	32	45	4
Midventral pairs, number	8.0	8.0	1.2	0.4	14.4	6	10	10
	9.5	9.0	-	-	-	7	13	11
	6.5	6.0	-	-	-	4	7	11
	10.0	-	-	-	-	9	10	2
Anterior frontal cirri, number	3.0	3.0	0	0	0	3	3	10
	3.0	3.0	-	-	-	2	4	11
	3.0	3.0	0	0	0	3	3	11
	3.0	-	-	-	-	3	3	3
Frontoterminal cirri, number	2.0	2.0	0	0	0	2	2	10
	2.0	2.0	0	0	0	2	2	11
	2.0	2.0	0	0	0	2	2	11
	2.0	-	-	-	-	2	2	3
Buccal cirri, number	1.0	1.0	0	0	0	1	1	10
	1.0	1.0	0	0	0	1	1	11
	1.0	1.0	0	0	0	1	1	11
	1.0	-	-	-	-	1	1	3
Ventral cirri ahead of transverse cirri, number	1.6	1.5	0.7	0.2	43.7	1	3	10
	1.8	2.0	0.5	0.1	25.8	1	2	12
	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-
Transverse cirri, number	4.1	4.0	0.7	0.2	18.0	3	5	10
	2.9	3.0	0.9	0.2	29.5	1	4	11
	1.7	2.0	0.7	0.2	37.4	0	2	11
	4.2	-	-	-	-	2	6	5
Caudal cirri, number	3.1	3.0	-	-	-	3	4	10
	3.0	3.0	0	0	0	3	3	11
	2.3	2.0	0.7	0.2	28.5	1	3	11
	3.0	-	-	-	-	2	4	3
Dorsal kineties, number	3.0	3.0	0	0	0	3	3	3
	4.0	4.0	0	0	0	4	4	11
	4.0	4.0	0	0	0	4	4	11
	3.0	-	-	-	-	3	3	3

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

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Ultrastructural Survey of Mucocysts Throughout the Life Cycle of *Colpoda cucullus* (Ciliophora, Colpodea)

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Summary. The ultrastructural morphology and organization of mucocysts were surveyed throughout the life cycle of *Colpoda cucullus*, a soil ciliate forming reproductive and resting cysts. Differences in number, distribution and size of these secretory organelles were demonstrated to depend on the developmental stage. By comparison with excysted cells after reproduction, an increase in number of mature mucocysts was observed both in logarithmic and stationary phase growing cells. This finding is consistent with a postulated mucocyst function in secreting material for the cyst wall. Rapid and synchronous mucocyst exocytosis induced by Alcian blue, caused cells in both logarithmic and stationary phases of growth, to become embedded in the respective mucocyst secretions. Unlike cells treated before forming reproductive cysts, almost all the cells treated prior to forming the resting cysts, were unable to survive within a capsule similar to a cyst wall. This difference in behaviour suggests that a complex series of temporally scheduled events is requested for successful resting encystment.

Key words: *Colpoda cucullus*, cyst wall formation, electron microscopy, life cycle, mucocysts.

INTRODUCTION

Mucocysts, as other extrusomes, are widely occurring secretory organelles in Protists. In spite of their functional diversity, mucocyst morphology can be referred to an unique structural model. They are membrane-bound organelles, arising in Ciliates from the endoplasmic reticulum as small vesicles with granular contents which become crystalloid as the organelles increase in size. Precursors of the electron-opaque contents, that are glycoproteinic and mucopolysaccaridic in nature (Wolfe 1988), have been postulated to be synthesized in *Tetrahymena* on ribosomes bound to endoplasmic reticulum close to the Golgi apparatus (Collins and Wilhelm 1981, Kurz and Tiedtke 1993). Mucocyst maturation takes place during transfer from the inner to the cortical cytoplasm where these extrusomes are anchored to the cell surface, releasing asynchronously their contents outside the cell. Synchronous and rapid discharge of mucocyst contents can be induced upon mechanical, electrical or chemical stimulation (Hausmann 1978). The protein content of mucocyst secretion has been biochemically characterized in *T. thermophila* wild-type (Maihle and Satir 1986, Attanoos and Allen 1987) and *bcd1* mutant cells (Cole and Stuart 1991).

As far as mucocyst functions are concerned, a role in food capture has been suggested in *Tetrahymena* (Nilsson 1979, 1981) and in *Peranema trichophorum* (Hilenski and Walne 1983) as well. In these organisms,

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mucocysts may be involved in the release of lysosomal enzymes, and their extruded contents may act as a trap for food particles, thus favouring the food-gathering process. Moreover, discharge of mucocysts seems also to come into play in the ciliate *Ichthyophthirius multifiliis* successfully invading the epithelium of fishes (Ewing et al. 1991). Other proposed functions include defense and protection. From this point of view, mucilage strands on *Peranema trichophorum* pellicle surface would originate from mucocysts (Hilenski and Walne 1983), whereas in Colpodidae (Foissner 1993), as in other encysting ciliates (McArdle et al. 1980), mucocysts may provide material for the formation of cyst walls (Frenkel 1994).

This paper reports an ultrastructural survey of mucocysts throughout the life cycle of *Colpoda cucullus*. Mucocyst morphology and organization are analyzed both in reproductive and resting cysts, as well as in the developmental phases preceding these encysting stages, i.e. in cells growing in logarithmic and in stationary phases, respectively. The question to be answered is the relation between mucocyst features and cyst wall formation, which is assumed to depend on the reproductive or resting function of cysts in the Colpodidae.

MATERIALS AND METHODS

Colpoda cucullus O.F.M. was grown at 27°C in lettuce medium buffered at pH 6.9 and bacterized with *Enterobacter aerogenes*, according to routinely employed culture methods (Franceschi 1957). Logarithmic growth phase was maintained by daily reisolation. Gradual depletion of food supply in left-over cultures effected transition to the stationary growth phase followed by resting encystment.

The examined samples were: cells in logarithmic phase, newly formed daughter cells within the reproductive cyst and after excystment, cells in stationary phase, newly formed and one-day-old resting cysts.

For transmission electron microscopy (TEM), samples were fixed for 45 min at 4°C in 2.5% glutaraldehyde buffered in 0.05 M sodium cacodylate (pH 7.4), and postfixed in 1% OsO_4 with the same buffer for 60 min. After stepwise dehydration through a graded ethanol series, embedding was performed in Epon-Araldite. Thin sections were cut on a Reichert ultramicrotome, double stained with uranyl acetate and lead citrate, and examined with Siemens Elmiskop A II and Philips EM 300 electron microscopes.

For scanning electron microscopy (SEM), cells in logarithmic phase of growth were fixed in Karnovsky's medium and washed in cacodylate buffer (pH 7.2). After stepwise dehydration through a graded ethanol series, samples were critical-point dried using liquid CO_2 in a Bomar apparatus, attached to specimen holders by silver-conducting paint, coated with gold-palladium in a Balzer Union evaporator and observed with a Jeol JMS 5200 electron microscope.

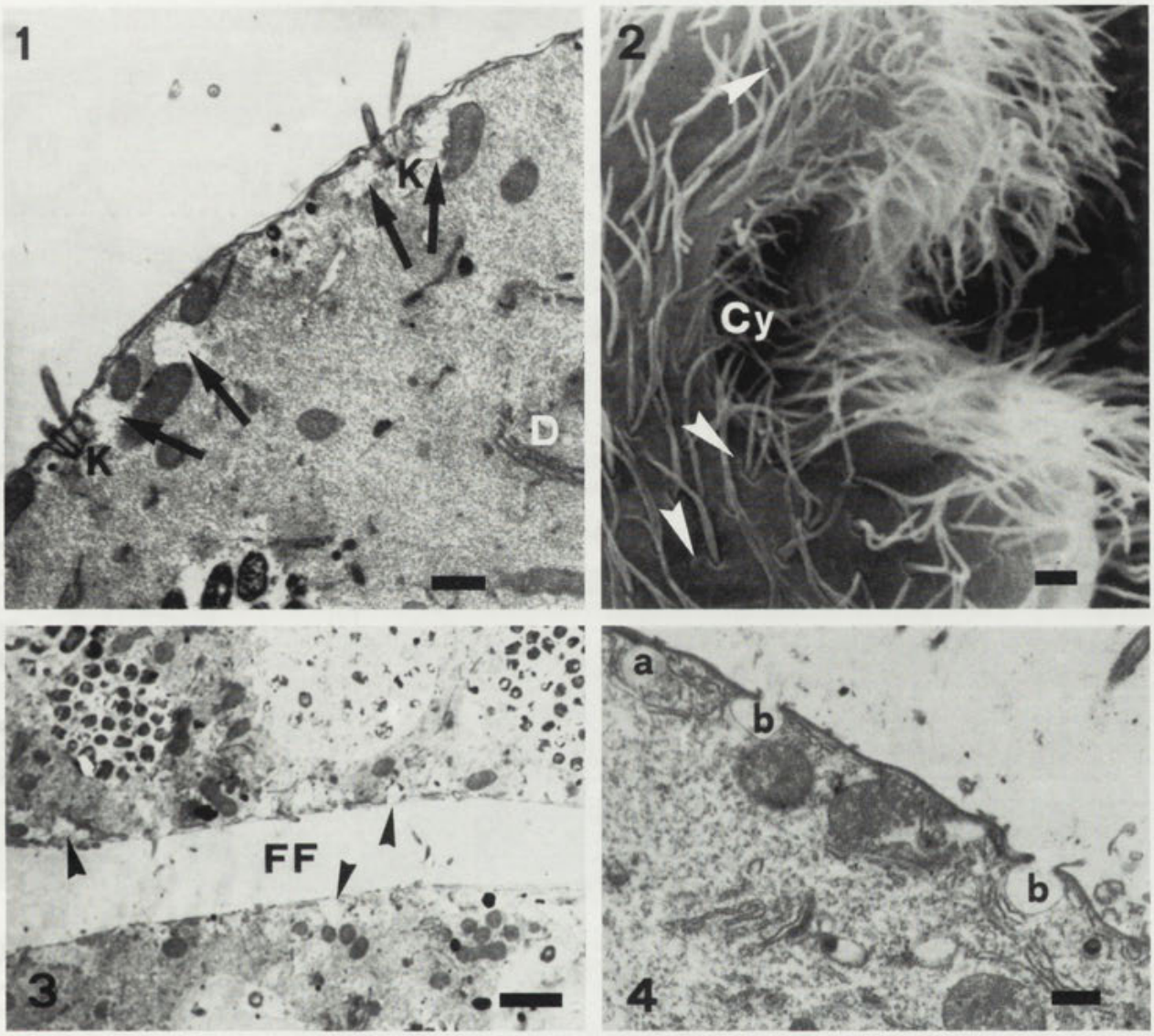
In cells growing in logarithmic and stationary phases, mucocysts were induced to undergo synchronous and rapid exocytosis upon treatment with 1% aqueous solution of Alcian blue, according to Tiedtke's method (1976).

RESULTS

This survey of mucocyst ultrastructure throughout the life cycle of *Colpoda cucullus*, reveals differences in number, distribution and size, depending on the developmental stage. In logarithmic phase, cells examined just before formation of reproductive cysts, show numerous, small, mature mucocysts beneath the cell surface and near paired kinetosomes (Fig. 1). Close to the insertion of paired cilia, small *maculae* are evident and can correspond to the pores of extruding mucocysts (Fig. 2). In the reproductive cysts, a few small mucocysts are found beneath the somatic membrane of newly divided cells (Fig. 3). After excystment following reproduction, mucocysts contiguous to the cell membrane appear to be engaged in the exocytic process (Fig. 4).

In stationary phase, cells analyzed just before forming resting cysts show numerous, large mature mucocysts arranged in clusters below the cell surface (Fig. 5). In newly formed resting cysts, the condensed cytoplasm contains numerous mitochondria, immature and mature mucocysts. The large body just below the cell surface is likely to be due to mature mucocyst fusion (Fig. 6). One day after encysting, the condensed cytoplasm shows the presence of individual mucocysts. The cyst wall is made up of two highly electron-opaque layers differing from each other in structure and thickness. A thin and continuous electron-lucent zone separates the outer layer, with an ill-defined edge and a spongy structure, from the thicker and more compact inner layer (Fig. 7).

When Alcian blue-treated cells in logarithmic and stationary phases are induced to undergo synchronous and rapid mucocyst discharge, an extracellular capsule of mucocyst contents is formed, embedding the still-living organism. This capsule similar to a cyst wall, appears thinner and weakly Alcian blue-positive in cells in logarithmic phase (Fig. 8a), in comparison with cells in stationary phase that show a thicker and more strongly stained capsule (Fig. 8b). Within such capsule, the organisms maintain motility from 2 to 8 h. Moreover, cells in logarithmic phase, treated just before forming reproductive cysts, complete their cell cycle and divide within the capsule, from which the four fission products emerge. Conversely, only 3% of the cells in stationary phase, treated just before forming resting cysts, are able to emerge from their capsule under suitable conditions to excyst, i.e. bacterized medium.

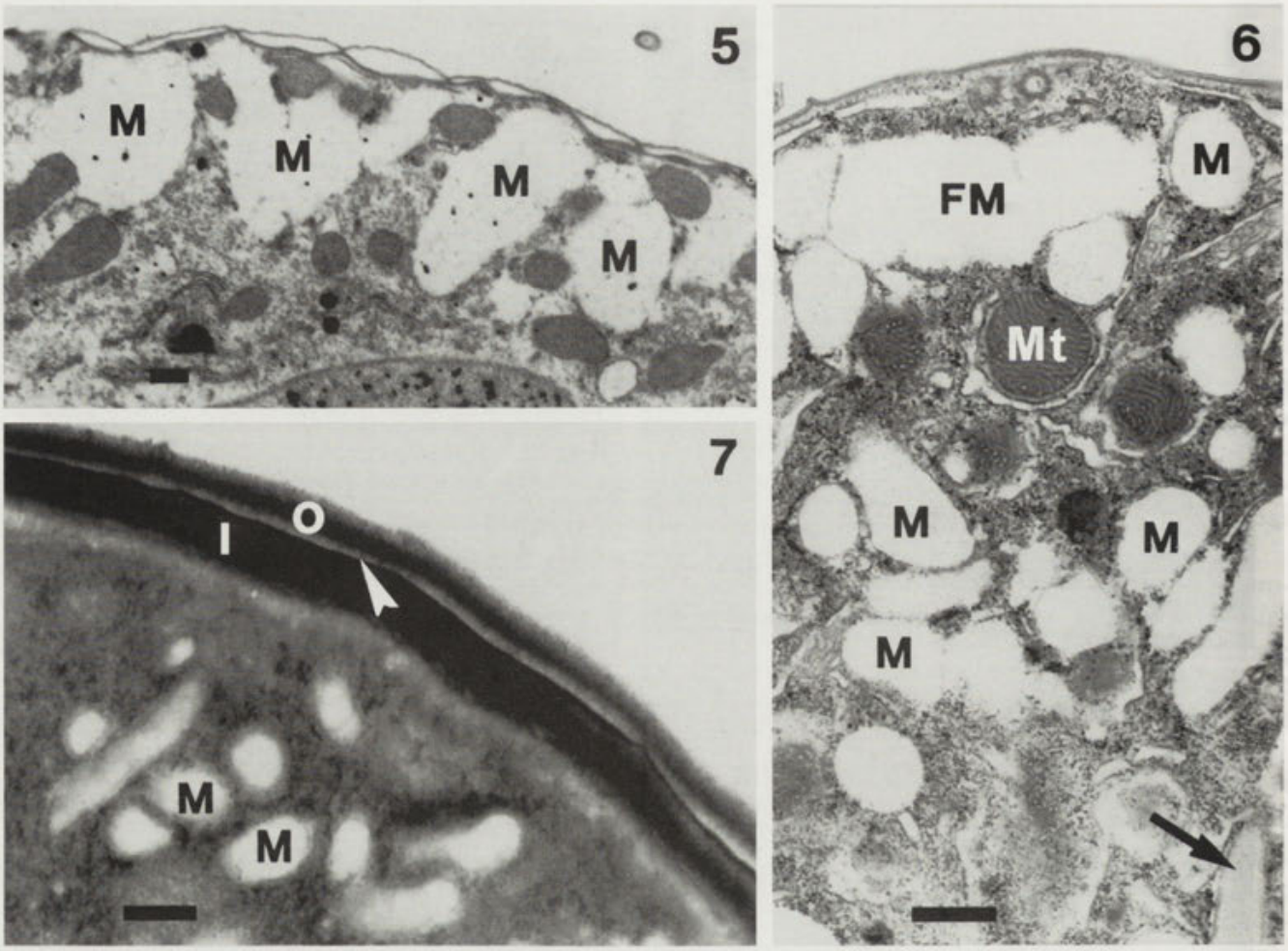


Figs. 1-4. Logarithmic growth phase and reproductive encystment. 1 - in cells analyzed just before forming reproductive cysts, numerous, small, mature mucocysts (arrows) are arranged beneath the somatic membrane, close to paired kinetosomes (K). D - dictyosome-like structures. Bar- 1µm. 2 - SEM right lateral-ventral view. Small *maculae* (arrow-heads) can correspond to the pores of extruding mucocysts. Cy - cytostome. Bar - 2µm. 3 - within the reproductive cyst, a few small mucocysts (arrow-heads) are evident beneath the somatic membrane of two cells separated by the fission furrow (FF). Bar - 2µm. 4 - in the excysted cells, wide cytoplasmic areas divide mucocysts possibly engaged in different phases of exocytosis: (a) before, and (b) during discharge. Bar - 0.25µm

DISCUSSION

A relationship between functional role of mucocysts in encystment and their features, with respect to number and size, has been demonstrated in this survey of developmental cycle in *Colpoda cucullus*. An increase in mature mucocyst number is found in cells in both logarithmic and stationary phases, compared to excysted cells after reproduction. Therefore, an abundance in mucocyst number would characterize the cells just before transition either to reproductive or resting encystment, depending upon the main function of these secretory organelles in encystment and wall formation (Hausmann 1978, Frenkel 1994).

However, the size differences of mature mucocysts observed between the precystic stages of the life cycle (small against very large ones in logarithmic and stationary phases, respectively) may be the result of a lower or higher secretory activity, accordingly. Therefore, this difference in mucocyst size may indicate that cells forming reproductive or resting cysts, are differently engaged in exocytic activity. The amount of mucocyst secretion, in turn, may be related to structural features of the



Figs. 5-7. Stationary growth phase and resting encystment. 5 - in pre-cystic cells, the docking of the numerous and large mucocysts (M) looks like a crown arranged under the somatic membrane. Bar - 0.5µm. 6 - in newly formed resting cysts, condensed cytoplasm contains mitochondria (Mt), immature mucocysts (arrow) and mature mucocysts (M), some of the latter forming a large fusion body (FM). Bar - 0.5 µm . 7 - in one-day-old cysts, numerous, individual mucocysts (M) are scattered throughout the condensed cytoplasm. A thin electron-lucent continuous zone (arrow-head) separates the spongy outer layer (O) from the thicker, more compact inner layer (I) of the cyst wall. Bar - 0.25 µm

cyst walls, thin and one-layered against thick and three-layered, in reproductive or resting cysts, respectively. Nevertheless, the products of mucocyst secretory activity appear to play an essential role in the formation of the outer components of both kinds of cyst walls. Such a role is further supported by synchronous and rapid mucocyst exocytosis induced in Alcian blue -treated cells in logarithmic and stationary phases, causing cells of both populations to become embedded in their mucocyst secretions. However, only the cells treated just before forming reproductive cysts are able to survive. This difference in behaviour suggests that, unlike reproductive encystment, a more complex series of temporal events than the rapid formation of a capsule similar to a cyst wall, is requestedà for successful resting encystment.

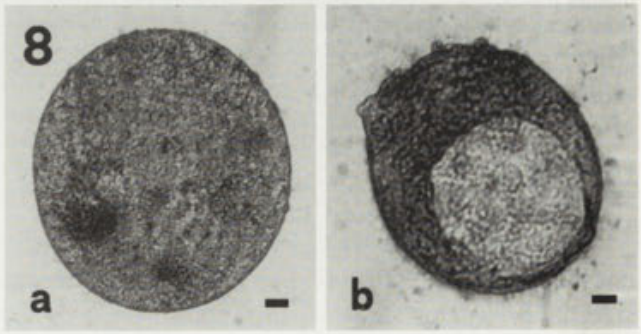


Fig. 8. Effects of Alcian blue treatment on mucocyst exocytosis. Synchronous and rapid mucocyst discharge leads to the formation of a thin and weakly stained capsule, in cells in logarithmic phase of growth (a); whereas, a thick and strongly stained capsule is formed by cells in stationary phase (b). Bar - 10 µm

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In vitro Gametocyte Formation in *Plasmodium falciparum* Isolates Originating from a Small Endemic Malarious Area and Their DNA Profiling with an Oligomer Probe

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Summary. *Plasmodium falciparum* isolates collected from a small endemic area of perennial transmission varied in their ability to form gametocytes *in vitro*, an essential determinant in natural transmission of malaria. Not all the collected isolates got established in continuous cultures. *In vitro* established isolates multiplied better in continuous flow system than in candle jar method. These isolates, in general, show lower rate of multiplication and higher sexual ability to form gametocytes than the isolates kept *in vitro* cultivation in non-malarious areas. Clonal variations within an isolate have been studied with regard to gametocyte production. The clones differed extensively in their ability to produce gametocytes; six of them could not form gametocytes *in vitro*. Genotypic diversity of these isolates and some clones was demonstrated using radiolabelled synthetic 21-oligomer probe, conspicuous restriction fragment length polymorphism was observed in isolates. However, the same probe has been unable to discriminate between phenotypically distinct gametocyte forming and non-gametocyte forming clones.

Key words: Gametocytogenesis, *in vitro* culture, non-gametocyte clones, *Plasmodium falciparum*, RFLP analysis, 21-mer repeat probe.

INTRODUCTION

The ability of *Plasmodium falciparum* to multiply in erythrocytes of man is one of the essential determinants of pathogenicity due to malignant malaria. The capacity to produce gametocytes determines transmission potential of malaria parasites. The two traits of multiplication and gametocyte formation in a parasite isolate can be determined accurately in a system devoid of immune defence against the parasite, for instance in hosts not exposed/immune to malaria parasites or still better *in vitro* culture systems. The two characters show considerable diversity among isolates originating from different geographical regions (Brockelman 1982, Graves et al. 1984, Oduola et al. 1985). We were interested to know the extent of intraspecific diversity existing among isolates procured from a small region of almost perennial transmission. Therefore, we characterized a few isolates and clones, derived from one of the isolates, with regard to these two characteristics using *in vitro* culture methods. Further, restriction fragment length polymorphism (RFLP) in some of these isolates/clones was examined using a synthetic oligonucleotide probe, known to be a repeat sequence occurring in the *P. falciparum* genome. The objective is to look for genomic diversity in phenotypically alike or diverse parasites. This report contains our findings in these regards.

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MATERIALS AND METHODS

Parasites. Six blood samples of confirmed *P. falciparum* cases were obtained from a malaria clinic located in North-West of Delhi. Patients had been residing in close vicinity of this clinic and had not stayed out overnight for last four months. From these samples *in vitro* cultures were initiated by the Petri dish candle-jar method of Trager and Jensen (1976). Each of the established isolates was maintained in continuous culture using human A^+ erythrocytes suspended in RPMI-1640 medium containing 25 mM HEPES, 25 mM sodium bicarbonate, 40 mg/ml gentamicin and 10% heat inactivated pooled AB^+ human serum. This complete medium was replenished daily in the cultures. Subcultures were made on day 5 or 6 to restore the hematocrit to 8% and the parasitemia to below 1%.

***In vitro* multiplication**. Ability of the isolates to multiply in culture was examined using the candle-jar method (Trager and Jensen 1976) and continuous flow technique (Trager 1979, Bhasin and Trager 1984) for three consecutive erythrocytic schizogonic cycles. Giemsa stained slides were prepared at the end of each schizogonic cycle after 48 h and a minimum of 5000 erythrocytes were counted to determine the parasitemia. In candle-jar method parasites were grown either in glass Petri dishes of 50 mm diameter holding 5 ml of cell suspension or in plastic 24-well tissue culture plates holding 0.5 ml medium. For the continuous flow technique 6 ml of culture material was placed in a glass flow vessel, receiving a constant supply of complete medium at the rate of 1 ml/hr and a steady flow of gas mixture (5% oxygen, 5% carbon dioxide and the balance nitrogen). Parasite multiplication rate was calculated by the following formula (Brockelman et al. 1985).

$$\text{Multiplication factor (MF)} = \frac{\text{N (day X)}}{\text{N (day X - 2)}}$$

Where N is the number of asexual parasites per 5000 erythrocytes on day indicated. As the multiplication is exponential at least for the first few cycles in culture, logarithmic transformation is used for parasitic multiplication rate (PMR)

$$\text{PMR} = \log \text{N(day X)} - \log \text{N (day X - 2)}$$

***In vitro* gametocytogenesis**. Microcultures were set up to observe the ability of each isolate to produce gametocytes in culture. Each well was seeded with 0.5 ml of culture material having 8% hematocrit and less than 1% parasitemia. An initial zero hour slide of the seeding material was made to determine the exact parasitemia. The plates were incubated at 37°C in a candle-jar with daily change of medium for eight schizogonic cycles. Thin blood smears were made every 48 h from triplicate wells. Gametocytemia and parasitemia were assessed by counting a minimum of 5000 erythrocytes. The gametocytes were classified as stage II to V (Hawking et al. 1971). Gametocyte forming ability or sexual ability (SA) of parasites was determined by counting the number of stage II gametocytes with respect to 100 rings present on a particular day.

***In vitro* cloning**. Clones from *P. falciparum* isolate FCD-4 were obtained by the limiting dilution method (Rosario 1981). To minimize multiple infection of erythrocytes the stock culture material used for cloning was agitated intermittently or at the most every 10-12 h. Multiple infection by this procedure was always found to be less than 10%. As soon as the parasitemia in this stock culture increased to 1-2%, it was used to initiate cloning. Based on the red cell count made with hemocytometer and parasitemia from Giemsa stained slide, the culture material was so diluted as to obtain a concentration of five infected cells per ml of 1% cell suspension. This diluted material was loaded in aliquots of 100 µl into wells of 96 well plates. Theoretically one infected erythrocyte should be present in every second well. The culture plate was incubated in a candle-jar at 37°C and medium containing 15% serum was replenished daily. On every sixth day each well received medium having additional 1% fresh erythrocyte suspension. On day 18 the contents of each well were split into two adjacent wells of a new 96-well plate and 50 µl of a 2% erythrocyte suspension was added to each well. On day 23, thick smears were made from each well to determine the presence of parasites. All those microcultures which were found to be positive for parasites were regarded as clonal populations and suitably expanded to obtain enough parasites for cryopreservation.

Gametocytogenesis in clones. During the four week period (before the clones were cryopreserved) thin/thick smears were periodically (6 or 8 day following dilution) made from each parasite positive well to determine gametocytemia. Gametocytes were counted in atleast 5000 erythrocytes on five different occasions.

Southern blot hybridization. *Plasmodium falciparum* was grown in sufficient quantity for extraction of parasite DNA by a standard procedure (Tungpradubkul and Panyim 1985). About 4 µg each of DNA from different isolates and clones were individually digested with the required amounts of various restriction enzymes. The DNA samples were digested with *Hind* III and *Alu* I at 37°C for two hours each. Digestion with *Taq* I was carried out at 65°C for one hour. Digested DNA samples were size fractionated on an agarose gel (0.8%) by electrophoresis and transferred to a nylon membrane by Southern blotting (Southern 1975, Sambrook et al. 1989). The membranes were hybridized with a custom synthesized 21-mer γ^{32}P end labeled oligonucleotide probe, which is known to be a specific repeat occurring only in falciparum genome (Aslund et al. 1985), of the following sequence:

5'- AGGTCTTAACTTAACTTG-3'

The probe was synthesized by Structural Biology group of International Centre for Genetic Engineering and Biotechnology, New Delhi. Following hybridization (Sambrook et al. 1989) the membranes were exposed to X-ray films in a cassette with intensifying screen at -70°C for 16 h.

RESULTS

Four out of the six isolates got established in *in vitro* cultures. They were designated as FCD-1, FCD-2, FCD-3 and FCD-4. Isolate FCD-1 took the longest time of four weeks to get adapted to cultures, whereas all other isolates started multiplying (at least by two folds per schizogonic cycle) within 14 days of culture initiation. In general, higher rate of asexual reproduction of parasites was observed in flow vessel technique than candle-jar method (Anova, $p = 0.01$). Table 1 shows the multiplication

Table 1. Parasite multiplication factor (MF)* and multiplication rate (PMR)** of *P. falciparum* isolates grown in flow vessel (FV[1]), multiwell tissue culture plates (MW) and Petri dishes (PD) for three consecutive schizogonic cycles. Starting hematocrit was 8% in all cases

			Erythrocytic schizogonic cycle***					
			First		Second		Third	
Isolate	Parasitemia (zero hour)		MF	PMR	MF	PMR	MF	PMR
FCD-1	FV	0.61	4.77	0.68	1.85	0.27	1.63	0.21
	MW	0.82	3.38±0.017	0.53±0.002	1.65±0.072	0.22±0.019	1.63±0.046	0.21±0.012
	PD	0.40	2.15±0.225	0.33±0.045	2.82±0.319	0.45±0.05	1.41±0.132	0.15±0.042
FCD-2	FV	0.72	4.33	0.64	2.21	0.35	1.35	0.13
	MW	0.42	5.78±0.093	0.76±0.078	2.09±0.153	0.32±0.031	0.86±0.02	-0.07
	PD	0.98	2.27±0.086	0.36±0.017	1.36±0.021	0.13±0.007	0.99±0.046	-0.004
FCD-3	FV	0.57	6.68	0.83	1.64	0.22	1.68	0.23
	MW	0.79	4.00±0.113	0.60±0.±0.012	1.49±0.046	0.17±0.014	1.86±0.021	0.27±0.005
	PD	0.44	3.82±0.01	0.58±0	1.53±0.04	0.19±0.009	1.14±0.04	0.06±0.015
FCD-4	FV	0.68	5.79	0.76	1.80	0.26	1.40	0.15
	MW	0.93	2.12±0.12	0.33±0.024	1.25±0.115	0.10±0.027	1.38±0.246	0.14±0.074
	PD	0.56	2.45±0.107	0.39±0.019	1.36±0.075	0.13±0.024	1.06±0.078	0.03±0.002

[1]One flow vessel has been used for each isolate.
*MF = N (day X) / N(day X-2), where N is the number of asexual parasites per 5000 RBCs on the day indicated.
**PMR = log N (day X) - log N (day X-2).
***Each figure of MF and PMR is calculated from the average asexual parasitemia of triplicate wells (MW) or dishes (PD) ± S.D.

Table 2. Gametocytemia of *P. falciparum* isolates on different days in continuous culture. Starting erythrocyte suspension was 8%

	Percent gametocytemia*				
Isolate	Day 0	Day 4	Day 8	Day 12	Day 14 (16)
FCD-1	0.02	0.10± 0.050	0.21± 0.120	0.66± 0.056	(1.20± 0.688)
FCD-2	0.04	0.05± 0.042	0.51± 0.061	1.68± 0.330	(3.09± 0.251)
FCD-3	0.02	0.33± 0.041	0.95± 0.266	2.73± 0.168	3.78± 0.093
FCD-4	0.04	0.27± 0.133	0.68± 0.060	1.87±0.833	1.49± 0.070

At least 5000 RBCs were examined in Giemsa stained thin film to calculate percent gametocytemia. Results are an average from triplicate wells ± S.D.

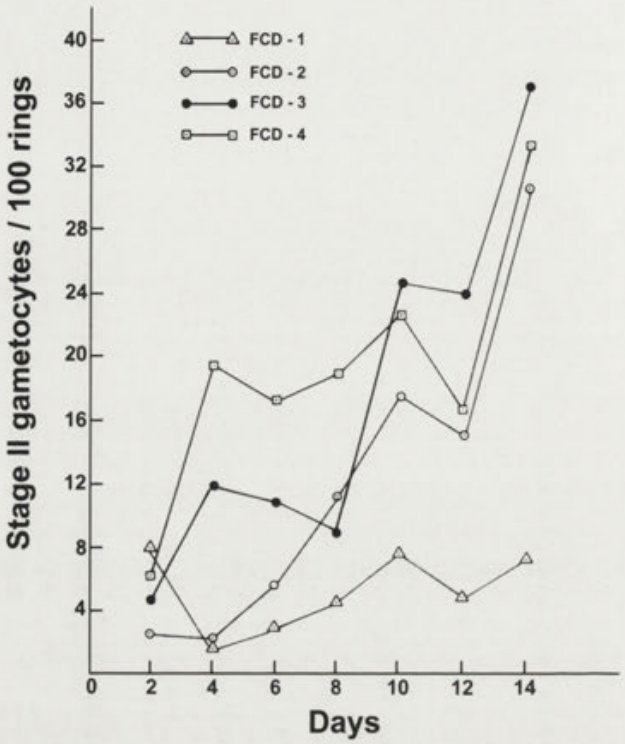

Fig. 1. Sexual ability (SA) of parasites *in vitro*, on different days, of four *P. falciparum* isolates. Each point is an average of triplicate culture

ability of parasites in flow vessels, glass Petri dishes and plastic wells of 24-well tissue culture plates for three consecutive cycles, eight weeks after initiation of isolates in culture. The first schizogonic cycle in each isolate showed the maximum PMR. Parasites multiplied to a lesser extent in each of the subsequent two cycles. No appreciable difference was observed in multiplication ability of all isolates.

Gametocyte formation and their development from stage II to V was observed in all four isolates. Differences in the number of gametocytes formed in each isolate was recorded (Table 2). FCD-1 was found to be the lowest gametocyte producing isolate and FCD-3 the highest. Only a small number of erythrocytic parasites in each schizogonic cycle were recruited to differentiate into the gametocytes, this sexual ability (SA) of parasites is shown in Fig. 1. Isolate FCD-1 showed the lowest SA, with a maximum reaching to 8% in 14 days of continuous culture conditions favoring gametocytogenesis, whereas a much higher SA was observed in the other three isolates ranging between 31 and 37%. Gametocyte formation declined considerably in the following four weeks of continuous cultivation in FCD-1 whereas the other three isolates did not show much change in gametocyte forming ability in the following four months of continuous *in vitro* cultivation.

Clones were derived from isolate FCD-4 on two occasions. The first cloning yielded 27 clones and 40 clones were realized in the next attempt from 72 microcultures set up on each occasion. Of these only 46 were analyzed for their ability to form gametocytes. Six clones were found to be incapable of forming gametocytes *in vitro* (Table 3).

Autoradiograms of DNA samples of isolates/clones digested with *Hind* III and probed with a repeat sequence are given in Fig. 2. On comparison, prominent differences in the organization of repetitive sequences in the form of hybridization bands can be observed in the genomes of FCD-3 and FCD-4 (lane 1 and 2) isolates. Closer examination of band profiles of these autoradiograms reveal minor differences in a few clones of FCD-4 (see arrows in Fig. 2). Similar results were obtained with the other two restriction enzymes (data not presented).

Table 3. Gametocyte forming ability of various clones of FCD-4

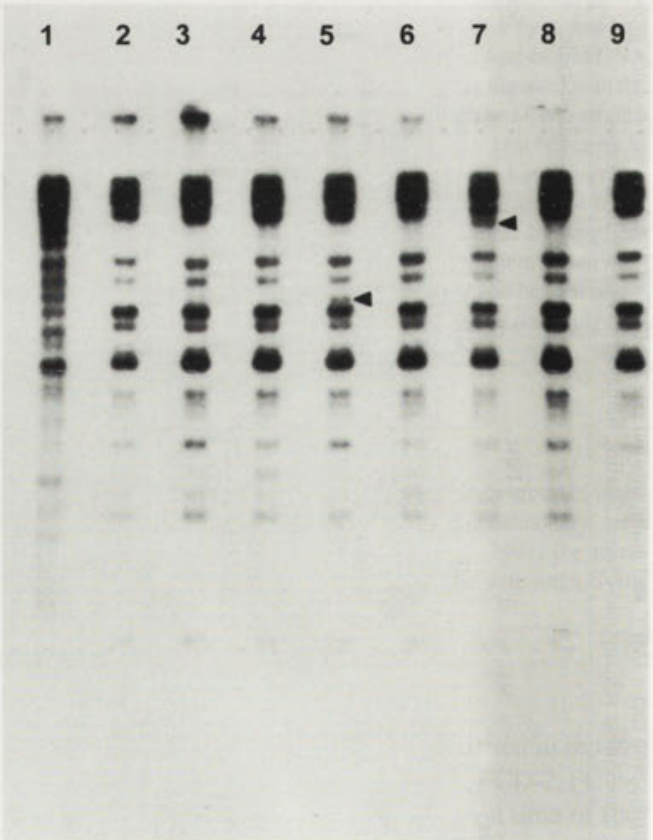

Fig. 2. Autoradiogram of Southern blot hybridized with synthetic radiolabelled oligonucleotide. Four microgram of *P. falciparum* DNA of isolates and clones was digested with restriction enzyme *Hind* III, fragments separated electrophoretically on an agarose gel and Southern transferred to nylon membrane. Arrows indicate the additional bands in clones. Lane 1 - FCD-3, lane 2 - FCD-4, lane 3 - H910, lane 4 - B910, lane 5 - J12, lane 6 - F56, lane 7 - B12, lane 8 - C78, lane 9 - I56

isolate. Clonal parasites were grown in vitro and fresh erythrocytes added every 6/8 day. Forty six clones have been grouped into three categories according to their ability to form gametocytes. Minimum of five observations were made to categorize a clone

Number of clones [code designated]	Category*
6 [B12, C34, D34, E12, F56, J56]	0
[B34, F78, L78]	+
[B56, C910, D910, E56, F1112, F12, H34, H910, I34, J910, M56, N56, N78, N1112, P34, P1112, A56, C56, C78, C1112, E34, E78, F12, I78, I910, J12, K56, M12, A34, A910, B910, D56, E1112, G56, I56, J34, S12]	++

*0 = Non-gametocyte forming mutant clones.
+ - Gametocytes could be observed rarely in thick smears.
++ - Gametocytes observed on 6 or 8 day following dilution in thin smear.

DISCUSSION

All four isolates used in the present study multiplied to a much lesser extent compared to the reported work on other isolates originating from different malarious areas of the world. Jensen and Trager (1977), Trager (1979) obtained an average of at least 6-8 fold and 10 fold increase in parasitemia using the candle-jar or continuous flow methods, respectively, in the first schizogonic cycle employing FCR-3 Gambia isolate. In contrast none of our isolates showed more than a 5 fold multiplication by the candle-jar and 7 fold by the continuous flow method. The differences in the growth rate may be due to the innate genetic diversity or because we have employed sera and blood samples procured from the endemic area for cultivation which might have inhibitory effects on parasite multiplication. The workers in non-malarious areas usually use blood products from local donors which are free from *Plasmodium* inhibitory substances, thus yielding higher growth rates. Human sera from hyperendemic areas of Sudan and other malarious areas have been reported to inhibit the growth of parasites in culture and even retard the intra-erythrocytic development leading to 'crisis forms' (Reese et al. 1981, Jensen et al. 1982, Jensen et al. 1984). Presence of merozoite-invasion-blocking antibody has been reported in the serum of people of malarious areas (Jensen et al. 1984, Wahlin et al. 1984), therefore we used heat inactivated serum for the present studies.

Our results confirm the earlier findings that isolates have markedly individual capacities to form gametocytes (Brockelman 1982, Graves et al. 1984), some isolates are good gametocyte producers over others, FCD-3 for instance formed more gametocytes than other three isolates.

Carter and Miller (1979) introduced a method for estimating conversion rate of asexual parasites to sexual forms at any given time during the culture period based on the following formula:

$$\text{Conversion rate} = \frac{\text{Stage II gametocytes per 100 erythrocytes on day X}}{\text{Rings per 100 erythrocytes on day X-2}}$$

In this formula the erythrocytes have to be enumerated on any day. But in continuous cultures kept for gametocytogenesis there is decline in the haematocrit with each schizogonic cycle. It has been observed that under these conditions, especially after 4-5 schizogonic cycles there is less fluctuation in the number of rings present on different days of continuous culture. Also, the erythrocytes by this time become fragile, dehemoglobinized and are sparsely seen on the thin stained smears made for enumeration of rings or gametocytes. These considerations/constraints prompted us to count stage II gametocytes in relation to 100 rings on a particular day as sexual ability (SA). The term SA has been adopted to differentiate it from Carter and Miller's conversion rate (CR), although not much difference in these two estimates are seen especially after 4-5 schizogonic cycles, the time when peak SA/CR are determined. Because of the simplicity and ease SA estimation found favour over CR with us. The SA estimates of at least three isolates employed here were found to be much higher (31-37%) compared to the CR reported by other laboratories located in non-endemic areas. Carter and Miller (1979) demonstrated CR fluctuations between 5 to 20%, Ponnudurai et al. (1986) between 17-25%. Our study lends support to an earlier observation that sera from endemic areas enhance conversion of asexual parasites to sexual forms (Smalley and Brown 1981). Ono and Nakabayshi (1989) were also able to induce gametocytogenesis *in vitro* using reactive medium prepared from hybridoma cells producing anti-*P. falciparum* antibody.

Clones of some isolates show extreme diversity with regard to their capacity to produce gametocytes *in vitro* (Bhasin and Trager 1984, Graves et al. 1984). A few clones derived form FCD-4 did not form gametocytes at all, in nature these mutants are incapable of being transmitted to another host and thus are of no biological significance to the parasite. However, these mutants are an important study material to understand the process of gametocytogenesis. While some other clones rarely formed gametocytes but the majority of them frequently produced gametocytes *in vitro*

although to a variable extent.

Repetitive sequences have been implicated in the processes of gametocytogenesis and gametogenesis in rodent malaria, *P. berghei* (Dore et al. 1980, Birago et al. 1982). Falciparum genome also consists of about 10% of repetitive sequences (Hough-Evans and Howard 1982). One of the most frequently occurring repeat is 21-mer sequence (Aslund et al. 1985). This probe was chosen for the present study because: this sequence is specific to *P. falciparum* (McLaughlin et al. 1985), not present in genome of other malaria species; highly sensitive, has been successfully used for diagnosis for falciparum malaria (Mucenski et al. 1986); small in size; has been used for RFLP analysis of various isolates (Mucenski et al. 1986). These considerations prompted us to use this sequence as probe. Isolates can be differentiated using a 21-mer synthetic nucleotide probe, however, some clones of an isolate depict only minor differences in band profiles whereas some other clones showing some phenotypic difference can not be differentiated on the basis of banding pattern. Clearly this oligomer can not be employed as probe for clone typing, as *Alu* repeat sequences have been used for human DNA fingerprinting.

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Brazilian Myxosporidians' Check-list (Myxozoa)

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Summary. The characteristics of 52 species from 11 Genera of brazilian myxosporidians (specially early century ones) are given with drawings.

Key words: brazilian Myxozoa, Myxospora, myxosporidian, Myxozoa.

Abbreviations: DS - developmental stage, EM - electron microscopy, IV - iodinophilous vacuole, PC - polar capsule, PF -polar filament, S - spore, Sp - sporoplasm, SEM - scanning electron microscopy.

A great number of the brazilian myxosporidians were described early in this century. There is some difficulties in the revision of the species types, find the original papers and translate some poor descriptions of the valid species. We resume here the following genera (number of species in bracket) of Myxozoa found in brazilian territory: *Agarella* (1), *Ceratomyxa* (4), *Chloromyxum* (2), *Coccomyxa* (1), *Henneguya* (21), *Kudoa* (1), *Leptotheca* (1), *Myxidium* (6), *Myxobolus* (13), *Myxosoma* (1) and *Sphaeromyxa* (1). The brazilian Myxozoa species are listed with summarized original descriptions and copies of the drawings. The host name, locality, site of infection and identification characters for each one of the species are resumed as they appear in original works. The authors did not consider any taxonomic discussion (for this see Lom and Dyková 1992). References are provided with remarks on new hosts. The "?" means uncertain locality, host or infected organ. The usual names of the hosts are given between quotation marks. The measurements are in µm otherwise indicated. All diagrams were redrawn (same scale) from original papers or from sources indicated in the text.

The study of this group of parasites is based on spore morphology. Actually the scanning and electron microscopy studies are the most important helpful techniques to describe and review early descriptions of the myxosporidian species listed here, particular those ones numbered as species 1, 2, etc. The parasite hosts also must be fixed and preserved for further taxonomic considerations and reference.

Genus *Agarella* Dunkerly, 1915

Agarella gracilis Dunkerly, 1915 (Figs. 6-10)

Host: *Lepidosiren paradoxa* Fitzinger, "pirambóia", Pisces.

Locality: Amazon region (Pinto 1928b); Belém City, Pará State (Walliker 1969).

Infected organ: testis (Pinto 1928b); testis and kidney (Walliker 1969).

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Identification characters: DS clusters of up 20 spores in testis. S elongate with two shell-valves prolonged in extended process in the form of a tail, 28.00-35.00 x 13.25-16.50; spore thickness 3.75-4.50; PC four polar capsules, 4.50-7.75 x 1.00-1.50.

References: Dunkerly 1925, Pinto 1928b, Walliker 1969.

Genus *Ceratomyxa* Thélohan, 1892

Ceratomyxa sphaerulosa Thélohan, 1892 (Figs. 1, 2)

Host: *Sphyrna tudes* (L.), Pisces.

Locality: Rio de Janeiro Bay.

Infected organ: gall-bladder.

Identification characters: DS yellowish or green-yellowish, 50-60 to 90-100 x 30-40; S 10-12; Sp 12-15 x 8-9; PC 6-7.

References: Nemeczek 1926, Pinto 1928b, Cunha and Fonseca (host: *S. tiburo*, "agulha") *apud* Pinto 1928b.

Ceratomyxa truncata Thélohan, 1895 (Fig. 3)

Host: "barbeiro" (?), Pisces.

Locality: Brazil.

Infected organ: gall-bladder.

Identification characters: DS polymorphous with long and active lobose like pseudopodes; S 25 x 20-30; PF 45.

References: Cunha and Fonseca (host: "barbeiro") *apud* Pinto 1928b.

Ceratomyxa curvata Cunha and Fonseca, 1918 (Fig. 4)

Host: *Odontaspis americanus* (Shaw), "cação mongonga", Pisces.

Locality: Rio de Janeiro.

Infected organ: gall-bladder.

Identification characters: DS round and hyaline; S semilunar with acute ends, 24 x 12.

References: Cunha and Fonseca 1918c, Nemeczek 1926.

Ceratomyxa hippocampi Cunha and Fonseca, 1918 (Fig. 5)

Host: *Hippocampus punctulatus* (Guich.), "cavalo marinho", Pisces.

Locality: Brazil.

Infected organ: gall-bladder.

Identification characters: DS rare, round and hyaline sometimes with only two spores; S semilunar with round ends, 25 x 7; PF 60.

References: Cunha and Fonseca 1918a (*Coratomyxa hippocampi*), Nemeczek 1926.

Genus *Chloromyxum* Mingazzini, 1890

Chloromyxum leydigi Mingazzini, 1890 (Figs. 11-16)

Host: *Scoliodon terrae-novae* (Richardson), *Raja agassizi* (Mueller and Henle), Pisces.

Locality: Rio de Janeiro.

Infected organ: gall-bladder.

Identification characters: DS polymorphous (oval or round) with pseudopodes; S oval, 6-9 x 6; PC 2-1; PF 20-30.

References: Cunha and Fonseca (host: *S. terrae-novae*, *R. agassizi*, "raia") *apud* Pinto 1928b, Nemeczek 1926.

Chloromyxum sphyrnae Cunha and Fonseca, 1918 (Fig. 17)

Host: *Sphyrna tibura* (L.), "cação cabeça de martelo", Pisces.

Locality: Brazil (Rio de Janeiro ?).

Infected organ: gall-bladder.

Identification characters: S oval, 15 x 13; PC 4.

References: Cunha and Fonseca 1918b, Nemeczek 1926 (host: *S. tudes* (L.)), Pinto 1928b (host: *S. tiburo*).

Genus *Coccomyxa* Léger and Hesse, 1907

Coccomyxa claviforme Cunha and Fonseca, 1919

Host: *Chilomycterus spinosus* (L.), "baiacu", Pisces.

Locality: Brazil.

Infected organ: gall-bladder.

Identification characters: DS round and small; S claviform, 14 x 6; one PC ellipsoid, 6.

Reference: Cunha and Fonseca 1919, Pinto 1925, Nemeczek 1926, Pinto 1928b.

Remark: without drawings.

Genus *Henneguya* Thélohan, 1892

Henneguya linearis (Gurley, 1893) (Figs. 67-68)

Host: *Pimelodus sebae* Cuv. et. Val., *Platystoma fasciatum* L., Pisces.

Locality: South America.

Infected organ: gills.

Identification characters: DS not described. S very marrow, 3 x or 4 x the width.

References: Kudo 1920, Pinto 1928b, Guimarães 1931 (host: *Rhamdia sebae*, *Pseudoplatystoma fasciatum*), Jakowska and Nigrelli 1953, Cordeiro et al.1983/1984.

Henneguya lutzi Cunha and Fonseca, 1918 (Figs. 69 -70)

Host: "pacú", Pisces.

Locality: Pardo River (Mato Grosso State).

Infected organ: gall-bladder.

Identification characters: DS unknown. S ovoid, 11 x 7. IV present, 3 in diameter.

References: Cunha and Fonseca 1918d, Nemeczek 1926 (host: *Pseudopimelodus charus*), Pinto 1928b [host: *P. zungaro* (Humb.)], Guimarães 1931, Jakowska and Nigrelli 1953, Cordeiro et al. 1983/1984.

Henneguya occulta Nemeczek, 1926 (Figs. 71-73)

Host: *Loricaia* sp., "tamboatá", Pisces.

Locality: Rio de Janeiro.

Infected organ: gills.

Identification characters: DS spherical, 75; S 36-46; PC 8; PF 17. Two or three caudal filaments.

References: Nemeczek 1926, Pinto 1928b, Guimarães 1931, Jakowska and Nigrelli 1953, Cordeiro et al. 1983/1984.

Henneguya leporini Nemeczek, 1926 (Figs. 74-75)

Host: *Leporinus mormyrops*, Steind., "piáu", Pisces.

Locality: São Gonçalo das Tabocas River (Minas Gerais State).

Infected organ: urine conductors.

Identification characters: S fusiform, 28-33; PC 5-8.

References: Nemeczek 1926, Pinto 1928b, Guimarães 1931, Jakowska and Nigrelli 1953, Cordeiro et al. 1983/1984.

Henneguya wenyoni Pinto, 1928 (Figs. 76-79)

Host: *Tetragonopterus* sp., "lambari", Pisces.

Locality: São Paulo State.

Infected organ: gills.

Identification characters: DS 2 mm x 1 mm; S elliptical 28-32 x 4.5-6; PC not equal, 3 x 1.5. Bifurcate ends. IV present 2-3 in diameter.

References: Pinto 1928a, b (host: *Astyanax fasciatus* Cuv., "lambari do rabo vermelho"), Guimarães 1931, Jakowska and Nigrelli 1953, Cordeiro et al. 1983/1984.

Hennegyua iheringi Pinto, 1928 (Fig. 80)

Host: *Serrasalmus spilopleura* Kner., Pisces.

Locality: Turvo River, Pirangy City (São Paulo State).

Infected organ: gills(?).

Identification characters: DS unknown. S long with round front end, 22 x 6; PC 3.4 x 2. IV present.

References: Pinto 1928b, Guimarães 1931 (host: *Serrasalmo spilopleura*), Jakowska and Nigrelli 1953, Cordeiro et al. 1983/1984.

Henneguya fonsecai Guimarães, 1931 (Fig. 81)

Host: *Leporinus copelandi* Steind.,"piava", Pisces.

Locality: Paraíba River, Taubaté City (São Paulo State).

Infected organ: fin tissues.

Identification characters: DS spherical, white, 1.5-3 mm; S ovoid, 23-27 x 4.5-5; PC 4-4.2.

References: Guimarães 1931, Jakowska and Nigrelli 1953, Cordeiro et al. 1983/1984.

Henneguya cesarpintoi Guimarães, 1931 (Fig. 82)

Host: *Astyanax fasciatus* Cuv.,"lambarí", Pisces.

Locality: Água Funda (São Paulo State).

Infected organ: gills cavity.

Identification characters: DS unknown; S ovoid, 13-14 x 4-4.5; PC 2.5-2.6 x 0.8. IV not observed.

References: Guimarães 1931, Jakowska and Nigrelli 1953, Cordeiro et al. 1983/1984.

Henneguya bergamini Guimarães, 1931 (Fig. 83)

Host: *Astyanax fasciatus* Cuv.,"lambarí", Pisces.

Locality: Piracicaba River, Piracicaba City (São Paulo State).

Infected organ: body cavity.

Identification characters: DS unknown; S 17-19 x 2 -2.5; IV present, 2 in diameter.

References: Guimarães 1931, Jakowska and Nigrelli 1953, Cordeiro et al. 1983/1984.

Henneguya travassosi Guimarães and Bergamini, 1933 (Fig. 84)

Hosts: *Astyanax fasciatus* (Cuv.), "lambari", *Leporinus copelandi* Steind., "piava", Pisces.

Infected organ: muscles, dorsal tissues.

Locality: Cruzeiro City, Paraiba River (São Paulo State).

Identification characters: DS white small and large ones up to 5 mm. S oval, 25.4-28.8 x 3.8-4.8; PC 3.2-4.2.

References: Guimarães and Bergamini 1933, Jakowska and Nigrelli 1953, Cordeiro et al. 1983/1984.

Henneguya santae Guimarães and Bergamini, 1934 (Fig. 85)

Host: *Tetragnopterus santae* Eigennmann, 1918, Pisces.

Locality: Pinheiros River (São Paulo State).

Infected organ: gills.

Identification characters: DS oval, white-yellowish, 1.00-1.20 x 0.70-0.75 mm; S oval, 8.5-10.6 x 4.9-5.7. IV present.

References: Guimarães and Bergamini 1934, Jakowska and Nigrelli 1953, Cordeiro et al., 1983/1984.

Henneguya visceralis Jakowska and Nigrelli, 1953 (Figs. 86-89)

Host: *Electrophorus electricus* (L.), Pisces.

Infected organ: kidney, liver, heart. mesentery.

Locality: Brazil (fish from New York Aquarium).

Identification characters: DS flat or raised, solid, white cysts. S flattened on one side and slightly convex on the other, 22-24 x 5-6.5; PC occasionally appears asymmetrical, 6.5-8 x 2; PF 44.

References: Jakowska and Nigrelli 1953, Cordeiro et al. 1983/1984.

Henneguya electrica Jakowska and Nigrelli, 1953 (Figs. 90-92)

Host: *Electrophorus electricus* (L.), Pisces.

Infected organ: large electric organs.

Locality: Brazil (fish from New York Aquarium).

Identification characters: DS solid white cysts and yellowish soft cysts. S with nearly symmetrical valves, 35-39 x 6-8; PC approximately one-half of the spore body length, 5-7 x 2; PF 44-50.

References: Jakowska and Nigrelli 1953, Cordeiro et al. 1983/1984.

Henneguya pisciforme Cordeiro, Artigas, Gioia and Lima, 1983 (Figs. 93-98)

Host: *Hyphessobrycon anisitsi* Eigenmann, 1907, "lambari", Pisces, Characidae.

Infected organ: gills.

Locality: Campinas City (São Paulo State).

Identification characters: DS spherical, white-yellowish, 100.87 (91.99-112.03). S biconvex with anterior knob, 20.40 (17.32-23.20) x 6.12 (4.46-6.73); PC unequal, pyriform 4.28 (3.11-6.14) x 1.70 (1.18-2.44). IV present, 2.25 (1,78-3.04).

Reference: Cordeiro et al. 1983/84.

Henneguya theca Kent and Hoffman, 1984 (Fig. 99)

Host: *Eigemannia virescens* (V.), Pisces, Sternopygidae.

Infected organ: brain.

Locality: Brazil.

Identification characters: DS unknown, spores in small nests. S attenuated, anterior convex and spherical in front view, encased in a tight-fitting sheath, 48.0 (40.6-52.6) x 3.5 (3.0-4.1); PC slightly unequal, larger 11.1 (9.8-12.5) x 1.4 (1.0-1.6), smaller 10.4 (8.7-11.7) x 1.4 (1.0-1.6). Tail 23.2 (20.3-24.2). Sheath only: 50.8 (43.7-55.4) x 5.78 (5.5-6.6). PF 19.9 (17.1-23.4). IV present.

References: Cordeiro et al. 1983/1984, Kent and Hoffman 1984.

Henneguya intracornea Gioia, Cordeiro and Artigas, 1986 (Figs. 100-102)

Host: *Astyanax scabripinnis* (Jenyns, 1842), "lambari", Pisces, Characidae.

Infected organ: eye (cornea).

Locality: Atibaia River (São Paulo State).

Identification characters: DS white, round, 356.65 (117.1-707.3). S pyriform, 42.41 (36.53-45.94) x 6.65 (5.64-9.90); PC bottle shape, 8.57 (6.93-9.90) x 2.37 (1.88-3.96); PF 101.88 (75.25-134.60) x 0.96 (0.60-1.49). IV present 3.92 (2.43-5.34).

Reference: Gioia et al. 1986.

Henneguya hoimba Cordeiro and Gioia, 1987 (Figs. 103-104)

Host: *Astyanax fasciatus* Cuv., "lambari", Pisces, Characidae.

Locality: Atibaia River (São Paulo State).

Infected organ: gills.

Identification characters: DS round, white. S ovoid, 24.68 x 7.51; PC 4.44 x 1.88; IV present.

Reference: Cordeiro and Gioia 1987.

Henneguya artigasi Gioia and Cordeiro, 1987 (Figs. 105-107)

Host: *Astyanax scabripinnis* (Jenyns, 1842), "lambari", Pisces, Characidae.

Locality: Campinas City (São Paulo State).

Infected organ: gills.

Identification characters: DS round, white, 400 in diameter. S pyriform, 16.39 x 4.42; PC 3.30 x 1.46; PF 21.

Reference: Gioia and Cordeiro 1987.

Henneguya amazonica Rocha, Matos and Azevedo, 1992 (Figs. 108-109)

Host: *Crenicichla lepidota* Heckel, 1840, Pisces, Cichlidae

Locality: Amazon River, near Belém City (Pará State)

Infected organ: gills

Identification characters: DS oval, 0.05 x 0.15 mm. S ellipsoid with blunt apex, 59.3 (55.0-65.9) x 5.7 (5.2-6.3), bifurcated tail; PC pyriform, 3.3 (2.7-3.6) x 1.5 (1.1-1.9)

Reference: Rocha et al. 1992.

Remarks: EM and SEM. (= *Henneguya* sp. Azevedo and Matos 1989 ?)

Henneguya sp. Jakowska and Nigrelli 1953 (Figs. 110-113)

Host: *Electrophorus electricus* (L.), Pisces.

Infected organ: oral mucosa and upper lip, skin.

Locality: Brazil (fish from New York Aquarium).

Identification characters: DS grey thick cysts (mouth) and white solid cysts (skin). S variable in size and shape, 35-38 x 2.5 occasionally showing asymmetrical PC 3.5.

References: Jakowska and Nigrelli 1953, Cordeiro et al. 1983/1984.

Henneguya sp. Azevedo and Matos, 1989

Host: *Hoplosternum littorale* Hancock, 1828, Pisces, Siluriformes.

Locality: Amazon River, near Belém City (Pará State).

Infected organ: gills.

Identification characters: DS white round to ellipsoidal, 0.4 - 1.0 mm in diameter. S ellipsoidal, 58.7 x 5.3. IV present.

Reference: Azevedo and Matos 1989.

Remarks: EM and SEM.

The following species of *Henneguya* are not included because no complete descriptions or data were given:

H. exilis Kudo, 1929 from scales of *Mugil brasiliensis* (Agassiz, 1829) (= *M. platanus*), Pisces (Rio Grande do Sul State) referred by Mendes (1980).

Henneguya sp. from gills of *Brycon melanopterus* (Cope), "matrinchã", *Pseudoplatystoma fasciatus* (L.), "surubim", *P. tigrinus* (Schomburgk), "caparari", *Plagioscion squamosissimus* (Heckel), "pescada" and *Colossoma macropomum* (Cuv.), "tambaqui", Pisces (Amazonas River) verified by Thatcher (1981).

H. psorospermica Thélohan, 1895 from gills, skin and body cavity of "carpa", "lambari" and "tilápia", Pisces (Irati City, Paraná State) related by Schönhofen et al. (1983).

Henneguya sp. from gills (?) of *Mugil liza* and *M. curema*, Pisces (São Paulo State) observed by Pádua et al. (1983) *apud* Godinho et al. (1988).

Henneguya sp. from eye (cornea) of "lambari", Pisces (Preto River, Minas Gerais State) with DS white, round, 0.2-1.5 mm, S 62.6 x 20.9 referred by Bara and Upegui (1985).

Henneguya sp. from heart and gills of *Mugil liza* Val., Pisces (Rio de Janeiro State) cited by Amato and Freire (1989).

Henneguya sp. from gills of *Pimelodus maculatus* Lacépède, 1803, Pisces (São Paulo State) DS small white; S oval 14.2 x 5.0 with long caudal process, 35.7; PC 5.8 x 1.8, referred by Cordeiro et al. (1989).

Genus *Kudoa* Meglitsch, 1947

Kudoa sp.
Host: *Mugil liza* Val., Pisces.
Infected organ: esophagous.
Locality: Rio de Janeiro State.
Identifications characters: not given.
Reference: Amato and Freire 1989.
Remark: without drawings.

Genus *Leptotheca* Thélohan

Leptotheca chagasi Nemeczek, 1926 (Fig. 114)
Host: *Leptodactylus ocellatus*, Amphibia.
Infected organ: urine ducts.
Locality: Rio de Janeiro State.
Identification characters: DS oval, amoeboid 25-30 x 150. S 15 x 10-11. PC three, 8-8.5.
Reference: Nemeczek 1926 [*Leptotheca (Wardia) chagasi*)].

Genus *Myxidium* Bütschli, 1882

Myxidium immersum (Lutz, 1889) (Figs. 18-25)
(= *Cystodiscus immersus* Lutz, 1889)
(= *Sphaeromyxa immersa* Thélohan, 1895)
(= *Myxidium lindoyense* Carini, 1932)
Host: *Cystignathus ocellatus*, Amphibia, *Bufo agua* (?), Amphibia.
Locality: Brazil.
Infected organ: gall-bladder.
Identification characters: DS round, up to 1 mm in diameter, without ectoplasm or pseudopodes. S broadly fusiform with rounded extremities in front view, rectangular in profile, shell values marked with two longitudinal and 7-9 transverse ridges, 12-14 x 9-10; PC 4 in diameter; PF 50-70.
References: Lutz 1889, Kudo 1920 (host: *B. marinus*, *Leptodactylus ocellatus*), Carini 1932 (DS round, 1 mm in diameter, S oval 11-12 x 7.5-8; PC 4 in diameter; host: *B. marinus*, *Hyla rubra*, *H. nebulosa*, *Leptodactylus ocellatus*, *Paludicula signifera* from Lindoya City, São Paulo State); Kudo and Sprague 1940, Jayasri and Hoffman 1982, Cordeiro et al. 1984 (host: *B. ictericus* Spix from São Paulo State), Delvinquier 1986 (SEM, host: *Physalaemus signiferus*), Delvinquier et al. 1992.

Myxidium striatum (Cunha and Fonseca, 1917) (Fig. 26)
Host: *Menticirrhus americanus* L., "papaterra", *Bairdiella ronchus* Cuv. e Val., "congo ", Pisces.
Locality: Rio de Janeiro City and Grande Island.
Infected organ: gall-bladder.
Identification characters: DS unknown. S ellipsoid, 10-14 x 6-8. Shell with fine striations parallel to the sutural line. PC 4; PF 30.
References: Cunha and Fonseca 1917 (*Myxidium striatus*); Kudo 1920, Nemeczek 1926 (host: *Cynoscyon leiarchus* Cuv. and Val.), Pinto 1928b, Guimarães 1931, Jayasri and Hoffman 1982.

Myxidium fonsecai Penido, 1927 (Figs. 27-28)
Host: *Carapus fasciatus* Pallas, Pisces.
Locality: Porto Esperança (Mato Grosso State).
Infected organ: gall-bladder.
Identification characters: DS unknown. S navette form. One side convex and the other slightly concave. Pyriform PC at each end fuse with the shell valve, 7-9 x 2.5-3; PC 2-3.
References: Penido 1927, Guimarães 1931, Jayasri and Hoffman 1982.

Myxidium cruzi Penido, 1927 (Figs. 29-31)
Host: *Chalcinus nematurus*, "sardinha", Pisces.
Locality: Mato Grosso State.
Infected organ: gall-bladder.
Identification characters: DS round with 2 spores, 15-18. S fusiform with fine longitudinal striations and uninucleate sporoplasm, 17-18 x 5-7; PC pyriform with a visible coiled filament, 5-6 x 2-3.

References: Penido 1927, Pinto 1928b, Guimarães 1931, Jayasri and Hoffman 1982.

Myxidium gurgeli Pinto, 1928 (Fig. 32)

Host: *Acestrorhamphus* sp., "peixe cachorro" or "cigarra", Pisces.

Locality: Mogy-Guassú River (São Paulo State).

Infected organ: gall-bladder.

Identification characters: DS white 7 mm x 5 mm with 200 spores. S fusiform with bluntly round ends. Five longitudinal striations parallel to the sutural line, 14.6 x 8.5; PC 3.4 x 3.

References: Pinto 1928c, Pinto 1928b, Guimarães 1931 (host: *Salminus maxilosus*), Jayasri and Hoffman 1982.

Myxidium cholecysticum Cordeiro and Gioia, 1990 (Fig. 33)

Host: *Astyanax scabripinnis* (Jenyns), "lambari", Pisces, Characidae.

Locality: Alpes farm, Atibaia River basin (São Paulo State).

Infected organ: gall bladder.

Identification characters: DS large, elongate, flattened leaf-like with pseudopodes, 3364.0 (379-9272) x 370.4 (30-967). Pseudopode 154.7 (111-205) x 7.6 (5.7-11.4). S ellipsoid, round ends, 14.1 (12.0-15.9) x 7.8 (6.4-9.6); PC 4.2 (3.4-5.2) x 3.6 (3.0-4.1); PF 55.2 (42.8-60.0).

Reference: Cordeiro and Gioia 1990.

Remarks. The following *Myxidium* species are not included because no complete descriptions or data were given:

Myxidium sp. from gall-bladder of *Bufo paracnemis*, Amphibia (São Paulo State) cited by Gioia et al. 1987.

Genus *Myxobolus* Bütschli, 1882

Myxobolus inaequalis Gurley, 1893 (Figs. 36-37)

Host: *Piramutana blochii* Cuv. and Val., *Synodontis schall* Bl. and Schn., Pisces.

Locality: South American rivers (Brazil, Guiana, Surinam).

Infected organ: skin of head.

Identification characters: DS unknown, stated to form small pustules. S ovoid, 5,2 x 3,3; PC of unequal size.

References: Kudo 1920, Pinto 1928b (host: *Pimelodus clarias*), Guimarães 1931, Walliker 1969, Landsberg and Lom 1991 (host: *P. clarias*).

Myxobolus lutzi Aragão, 1919 (Fig. 38)

Host: *Girardinus januarius*, Pisces.

Locality: Rio de Janeiro State.

Infected organ: testis.

Identification characters: DS not described. Testis tissues destroyed by spore mass. S ovoid, 10 x 7. PF easily extruded.

References: Aragão 1919, Pinto 1928b (host: *Poecila vivipara*, "barrigudinho"), Guimarães 1931 (host: *Poecilia vivipara*), Walliker 1969, Landsberg and Lom 1991 (*M. lutzi* Aragão, 1919, host: *P. vivipara*).

Myxobolus chondrophilus Nemeczek, 1926 (Figs. 39-40)

Host: *Sardinella anchovina* Val., "sardinha", Pisces.

Locality: Rio de Janeiro.

Infected organ: gills.

Identification characters: DS spherical white cysts in gills, 125 x 1000 in diameter. S rounded and slightly narrowed at the anterior end, lenticular in side view, 6 x 4.5; PC 3.

References: Nemeczek 1926, Pinto 1928b, Guimarães 1931, Walliker 1969 (host: *S. anchovia*), Landsberg and Lom 1991 (host: *S. anchovia*).

Myxobolus associatus Nemeczek, 1926 (Figs. 41-42)

Host: *Leporinus mormyrops* Steind., "piau", Pisces.

Locality: São Gonçalo das Tabocas River (Minas Gerais State).

Infected organ: kidney.

Identification characters: DS round or oval cysts, 60 x 80. Spores with two forms, rounded or pointed anterior ends, 15 x 10. PC pyriform and equal in size, 7.

References: Nemeczek 1926, Pinto 1928b, Guimarães 1931 (host: *Salminus maxilosus* Cuv., kidney and liver, Mogy-Guassú River, Emas Falls, São Paulo State), Walliker 1969, Landsberg and Lom 1991.

Myxobolus pygocentris Penido, 1927 (Figs. 43-44)

Host: *Pygocentris piraya* L., "piranha", Pisces.

Infected organ: intestinal contents.

Locality: Porto Esperança City, Paraguay River (Mato Grosso State).

Identification characters: DS unknown. S elliptical, 15-16 x 9-11; PC 9-11 x 3-4.

References: Penido 1927, Pinto 1928b, Guimarães 1931, Walliker 1969 (host: *Serrasalmus piraya* Cuv.), Landsberg and Lom 1991 (host: *S. piraya*).

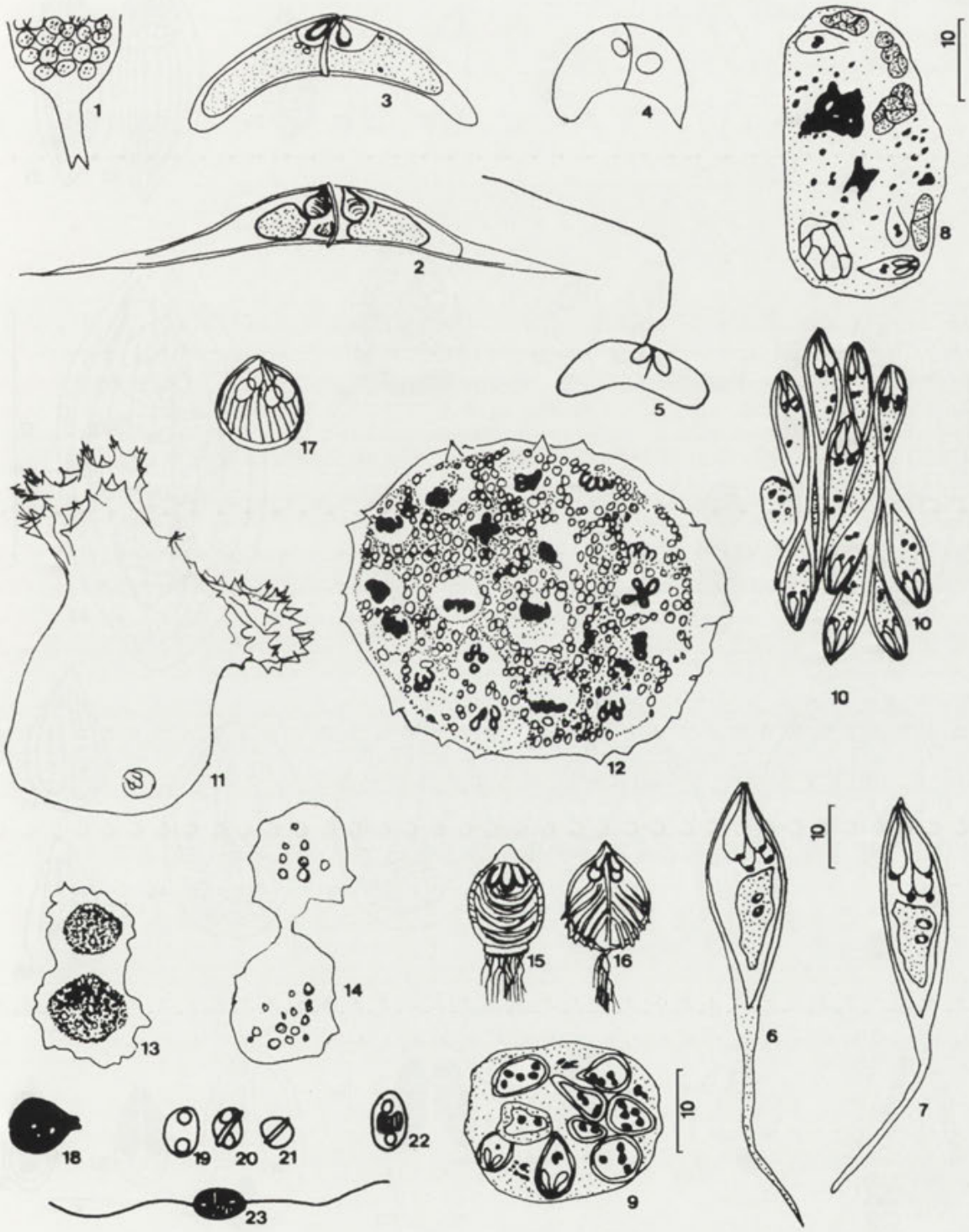
Myxobolus cunhai Penido, 1927 (Figs. 45-46)

Host: *Pygocentris piraya* L., *Pimelodus clarias* L., Pisces.

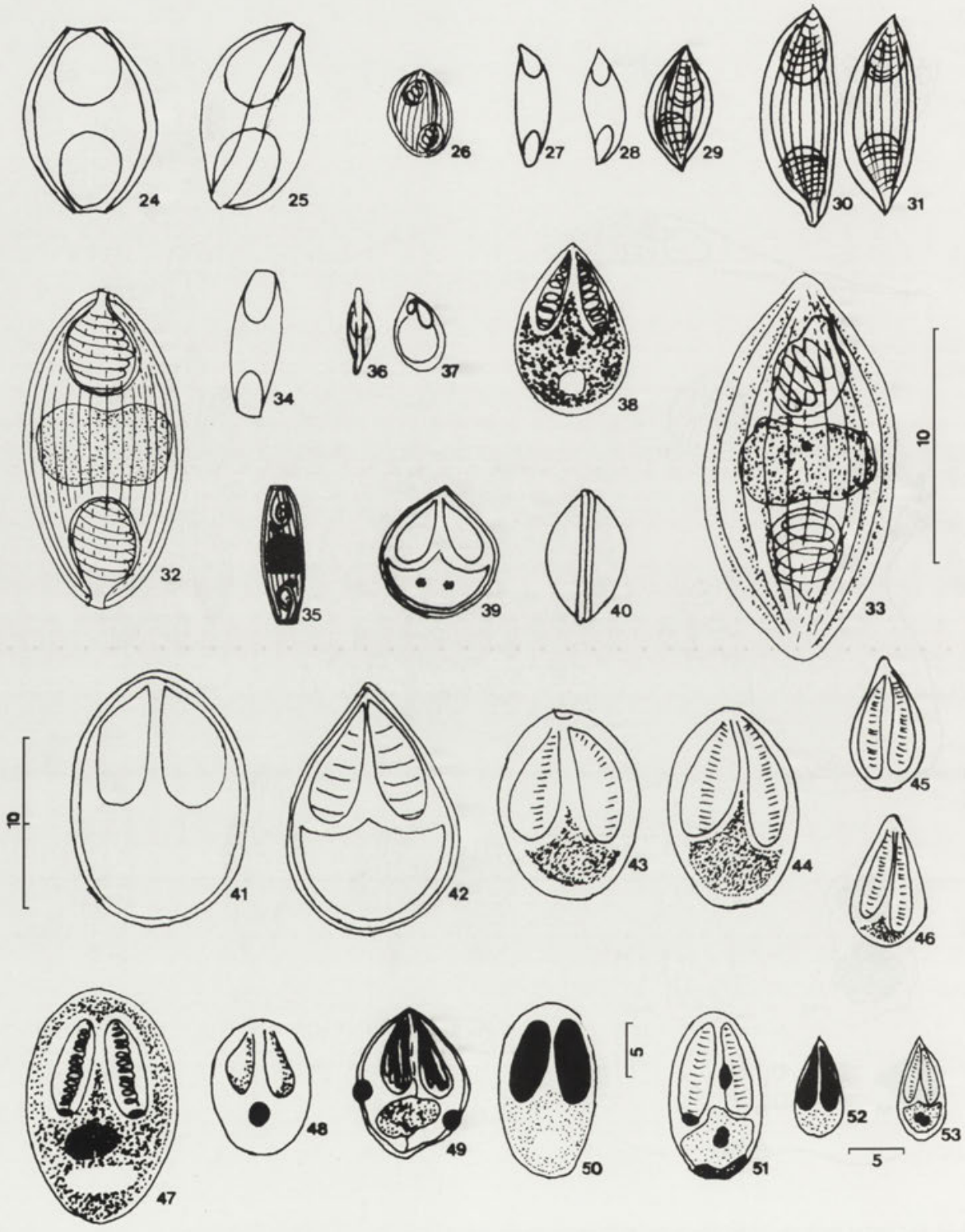
Infected organ: intestinal contents of *P. piraya* and cloaca of *P. clarias*.

Locality: Porto Esperança City, Paraguay River (Mato Grosso State).

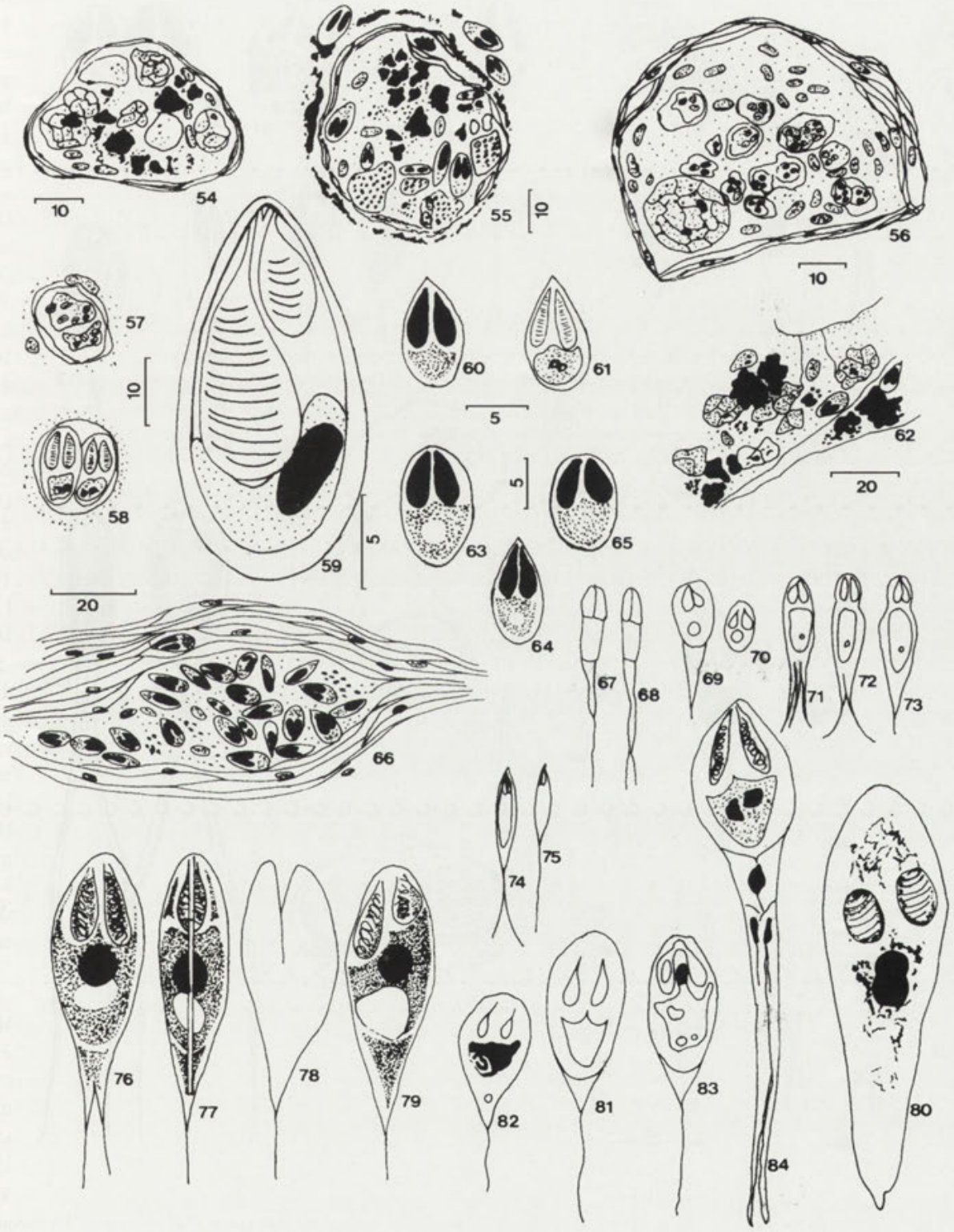
Identification characters: S pyriform, 9-11 x 4-6. PC of unequal size.



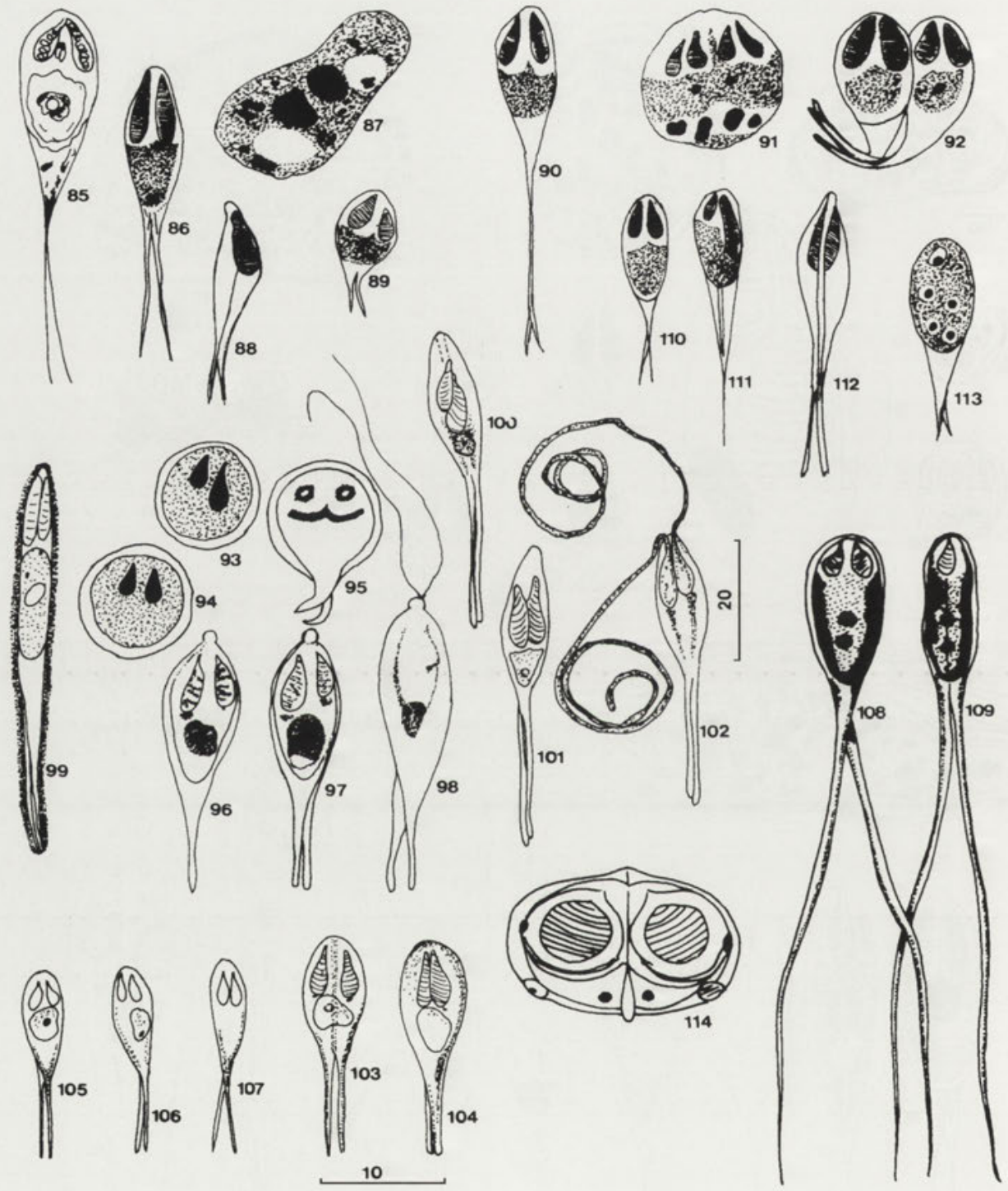
Figs. 1-23. 1 - a part of the trophozoite of *Ceratomyxa sphaerulosa* (Thélohan 1895, Fig. 2, X 750). 2 - fresh spore of *C. sphaerulosa* (Thélohan 1895, Fig. 3, X 750). 3 - spore of *C. truncata* (Thélohan 1895, Fig. 51, X 1500). 4 - spore of *C. curvata*. 5 - spore of *C. hippocampi*. 6-7 - spores of *Agarella gracilis*, Giemsa stain, front view. 8. Immature trophozoite of *A. gracilis*. 9 - late trophozoite of *A. gracilis*. 10. Mature trophozoite of *A. gracilis*. (Figs. 6-10: all from Wallicher 1969, Figs. 15-18). 11-12 - trophozoites of *Chloromyxum leydigi* (Thélohan 1895, Figs. 6-7, X 750). 13-14 - trophozoites of *C. leydigi* in division (Doflein 1898, Figs. 57-58). 15-16 - different views of spores of *C. leydigi* (Thélohan 1895, Figs. 9-10, X 1500). 17 - spore of *C. sphyrnae*. 18 - gall-bladder of *Bufo agua* (?) with trophozoites of *Myxidium immersum*. 19-21 - developmental spores of *M. immersum*. 22 - mature spore of *M. immersum*. 23 - extruded spore of *M. immersum*



Figs. 24-53. 24-25 - spores of *M. immersum* (=*M. lindoyense*, front and lateral view, Carini 1932). 26 - spore of *M. striatum*. 27-28 - spores of *M. fonsecai*. 29-31 - spores of *M. cruzi*. 32 - spore of *M. gurgeli*. 33 - spore of *M. cholecysticum*. 34-35 - spores of *Sphaeromyxa balbianii*. 36-37 - spores of *Myxobolus inaequalis*. 38 - spore of *M. lutzi*. 39-40 - spores of *M. chondrophilus*. 41-42 - spores of *M. associatus*. 43-44 - spores of *M. pygocentris*. 45-46 - spores of *M. cunhai*. 47 - spore of *M. noguchii*. 48 - spore of *M. stokesi*. 49 - spore of *M. kudoi*. 50-51 - macrospore of *M. serrasalmi*. 52-53 - microspore of *M. serrasalmi*



Figs. 54-80. 54 - immature trophozoite of *M. serrasalmi* (spleen). 55 - Trophozoite of *M. serrasalmi*. 56. Trophozoite showing pansporablasts development of *M. serrasalmi1*. 57 - pansporoblast of *M. serrasalmi*. 58 - immature spores of *M. serrasalmi*. 59 - spore of *M. inaequus*. 60-61 - spores of *M.* sp. 1 Walliker 1969. 62 - trophozoite of *M.* sp. 1 in kidney. 63-65 - spores of *M.* sp. 2 Walliker 1969. 66 - trophozoite of *M.* sp. 2. 67-68 - spores of *Henneguya linearis*. 69-70 - spores of *H. lutzi*. 71-73 - spores of *H. occulta*. 74-75 - spores of *H. leporini*. 76-79 - spores of *H. wenyoni*. 80 - spore of *H. iheringi*



Figs. 85-114. 81 - spore of *H. fonsecai*. 82 - spore of *H. cesarpintoi*. 83 - spore of *H. bergamini*. 84 - spore of *H. travassosi*. 85 - spore of *H. santae*. 86 - spore of *H. visceralis*. 87 - disporous pansporoblast of *H. visceralis* (approx. X 4000). 88 - fresh mature spore of *H. visceralis* (approx. X 2000). 89 - abnormally short mature spore of *H. visceralis* (approx. X 2000). 90 - spore of *H. electrica*. 91 - nearly mature disporous pansporoblast of *H. electrica* (approx. X 2000). 92 - young spore in paired condition of *H. electrica* (approx. X 2000). 93-95 - developmental stages of the spore of *H. pisciforme*. 96 - spore of *H. pisciforme*. 97 - spore of *H. pisciforme* with bifurcated caudal process. 98 - extruded spore of *H. pisciforme*. 99 - spore with sheath of *H. theca*. 100-101 - mature spores of *H. intracornea*. 102 - extruded spore of *H. intracornea*. 103-104 - spores of *H. hoimba*. 105-107 - spores of *H. artigasi*. 108-109 - spores of *H. amazonica*. 110-113 - spores of *H.* sp. Jakowska and Nigrelli 1953. 114 - spore of *Leptotheca chagasi*

References: Penido 1927, Pinto 1928b, Guimarães 1931 [host: *P. clarias* (Bloch)?], Walliker 1969 (host: *Serrasalmus piraya* Cuv.), Landsberg and Lom 1991 (host: *S. piraya*, *P. clarius*).

Myxobolus noguchii Pinto, 1928 (Fig. 47)

Host: *Serrasalmo spilopleura* Kner., Pisces.

Infected organ: gills(?).

Locality: Turvo River, Pirangy City, (São Paulo State).

Identification characters: DS unknown. S oval, without striations, 13.6 x 8.5; PC 6.8 x 2.2. IV present.

References: Pinto 1928d, Pinto 1928b, Guimarães 1931, Walliker 1969 (host: *Serrasalmus spilopleura* Kner), Landsberg and Lom 1991 (host: *S. spilopleura*).

Myxobolus stokesi Pinto, 1928 (Fig. 48)

Host: *Pimellodela* sp.(?), Pisces, Siluridae.

Infected organ: subcutaneous tissue of the snout.

Locality: Turvo River, Pirangy City (São Paulo State).

Identification characters: DS sub-cutaneous tumour, 1 mm in diameter. S ovoid, without striations, 8.5-5.3; PC 3.4 x 1.7; IV not visible.

References: Pinto 1928d, Pinto 1928b, Guimarães 1931, Walliker 1969 (host: *Pimelodus* sp.), Landsberg and Lom 1991.

Myxobolus kudoi Guimarães and Bergamini, 1938 (Fig. 49)

Host: *Nematognatha* sp., Pisces.

Infected organ: skin of the body except the fins.

Locality: Emas Falls, Mogi-Guassu River (São Paulo State).

Identification characters: DS sphaerical pearl-white cysts (0.5-1.0 mm in diameter) with a thin and resistant membrane. S 8.5-8.9 x 6.5-7.3; Sp 2.0-2.6 x 2.0-4.0; PC 3.5-4.2 x 1.3-2.0.

References: Guimarães and Bergamini 1938 (work not reviewed), Walliker 1969, data shown above (host: "catfish"), Landsberg and Lom 1991 (host: *Nematognathus* sp.).

Myxobolus serrasalmi Walliker, 1969 (Figs. 50-58)

Host: *Serrasalmus rhombeus* (L.), "piranha", Pisces, Serrasalmidae.

Infected organ: spleen, kidney, liver.

Locality: Negro River, Manaus City (Amazonas State).

Identification characters: S (Macroespore) symmetrically oval, 12.50-18.00 x 7.00-10.00; PC 6.00-9.00 x 2.5-4.0; Microspore piriform, 7.00-9.50 x 3.50-5.00; PC 5.00-7.50 x 1.00-2.00.

References: Walliker 1969, Landsberg and Lom 1991.

Myxobolus inaequus Kent and Hoffman, 1984 (Fig. 59)

Host: *Eigemannia virescens* (V.), Pisces, Sternopygidae.

Infected organ: brain.

Locality: Brazil.

Identification characters: *S pyriform*, 19.8 (15.6-22) x 8.6 (7.8-9.3), thickness 8.0 (7.7-8.5). PC extreme unequal, pyriform and elongate, larger 11.8 (9.4-13) x 3.6 (3.1-3.9), smaller 4.8 (3.9-5.5); PF larger, 191, smaller 22. IV present.

References: Kent and Hoffman 1984, Landsberg and Lom 1991.

Myxobolus sp. 1 (Figs. 60-62)

Host: *Serrasalmus* sp., Pisces, Serrasalmidae.

Infected organ: kidney.

Locality: Preto River, Manaus City (Amazonas State).

Identification characters: DS without cyst wall in sections of kidney. S ovoid with a bluntly pointed anterior end, 9.00-11.00 x 5.00-6.50; PC 5.00-6.00 x 1.50-2.00.

Reference: Walliker 1969.

Myxobolus sp. 2 (Figs. 63-66)

Host: *Colossoma bidens* (Agassiz), Pisces, Characinidae.

Locality: Solimões River (Amazonas State).

Infected organ: connective tissue, spores seen in spleen and liver smears.

Identification characters: DS clusters of spores adjacent to spleen. S oval to ovoid with a pointed anterior end. S 8.00-10.00 x 4.00-7.00; PC 3.50-5.00 x 1.00-2.50.

Reference: Walliker 1969.

Remarks. The following species of *Myxobolus* are not included because no complete descriptions or data were given:

Myxobolus sp. from gills of *Rhamdia quelen*, Pisces related by Splendore 1910.

M. pfeifferi Thélohan, 1895 from bone tumours of *Pogonias chromis* (L.,1766), "miragaia", *Tachysurus barbus* (Lacépède, 1803), "bagre", and *Cynoscium striatus* (Cuv., 1829), "pescada olhuda", Pisces (Rio Grande do Sul State) cited by Mendes 1980.

Myxobolus sp. from liver and dorsal fin of *Colossoma macroponum* (Val.), "tambaqui" and *Brycon melanopterus* (Cope), "matrinchã", Pisces (Amazonas River) stated by Thatcher 1981.

Myxobolus sp. from esophagus, stomach, intestine, liver, gall-bladder, kidney, muscle, gills, ovary and fins of *Mugil liza* Val., Pisces (Rio de Janeiro State) observed by Amato and Freire 1989.

Myxobolus sp. from gills of *Pimelodus maculatus* Lacépède, 1803, Pisces (São Paulo State); S ovoid, 8.2 x 5.6; PC 3.8-1.9 related by Cordeiro et al. 1989.

Genus *Myxosoma* Thélohan, 1892

Myxosoma cerebralis (Hofer, 1903)

Host: *Mugil brasiliensis* (Agassiz, 1829), Pisces.

Infect organ: vertebral column.

Locality: Rio Grande do Sul State.

Identification characters: not given.

Reference: Mendes 1980.

Remarks: Not figured. Lom and Noble 1984 synonymise the genera *Myxosoma* and *Myxobolus*.

Genus *Sphaeromyxa* Thélohan, 1892

Sphaeromyxa balbianii Thélohan, 1892 (Figs. 34-35)

Host: *Scorpena plumieri*, Pisces.

Locality: Brazil.

Infected organ: gall-bladder.

Identification characters: DS round and flat, 3-4 mm; S fusiform with bluntly ends, 15-20 x 5-6; one PC 7 x 4,7; PF 15-20.

References: Cunha and Fonseca (host: *S. plumieri*) *apud* Pinto 1928b, Guimarães 1931.

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Myxobolus molnari sp. n. and *M. mokhayeri* sp. n. (Myxosporea, Myxozoa) Infecting a Mesopotamian fish, *Capoeta trutta* Heckel, 1843

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Summary. A new *Myxobolus* species is described from the gills and another from the fins of *Capoeta trutta* Heckel, 1843 caught in the River Karun in the southwestern part of Iran. The two species differ from each other both in location and in morphology. *Myxobolus molnari* forms plasmodia in the tunica adventitia of blood vessels of the primary gill filaments, directly beneath the endothelium, in a cyst surrounded by connective tissue cells. The plasmodia of *M. mokhayeri* develop in the fins, around the soft fin rays, beneath the stratum spongiosum of the skin and also in cysts surrounded by connective tissue cells.

Key words: *Capoeta trutta*, gills, fins, *Myxobolus*, Myxosporea, new species.

INTRODUCTION

In the years 1993–1994 a faunistic survey comprising several fish species was conducted in the Mesopotamian region of Iran to assess the prevalence of infections caused by myxosporean parasites. Before that survey, the myxosporean fauna of the region were not well known.

From Iranian fishes, Ebrahimzadeh and Nabawi (1975), Ebrahimzadeh and Kaylani (1976) and Moghainemi and Abasi (1992) reported the occurrence of some *Myxobolus* spp. infecting the gills and inner organs of different fish species. Mokhayer (1981) also described *Myxobolus lobatus* from *Barbus brachycephalus*. The occurrence of *Myxobolus mulleri* and *M. oviformis* in freshwater fishes in the neighbouring Iraqi territory was first reported by Herzog (1969). Subsequently, Al-Salim (1986) and Rashid et al. (1989) described the occurrence of *Myxobolus pfeifferi* in different barboid fishes. From the Ponto-Caspian territory of the former Soviet Union, Donec and Shulman (1984) reported 7 *Myxobolus* spp. from *Capoeta* species. Besides the rich myxosporean fauna of the Palaearctic, several *Myxobolus* spp. are known from the Indian Fauna region (Tripathi 1952, Lalithakumari 1969, Haldar et al. 1983).

From barboid fishes (*Barbus* spp.) living in the rivers of the region, Masoumian et al. (1994) have described new *Myxobolus* species that parasitized the gills.

This paper describes two new *Myxobolus* spp., *M. molnari* from the gills and *M. mokhayeri* from the fins of *Capoeta trutta* from River Karun.

MATERIALS AND METHODS

Eight specimens of *Capoeta trutta* (Heckel, 1843), 10–31 cm in length, were used in this study. They were collected between May and October 1994 from River Karum in Khuzestan Province of Southwest Iran.

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Immediately after collection, the fish were transported alive to the laboratory where they were weighed and measured before being killed by transection of the spinal cord. They were then examined for myxosporean parasites under stereo and light microscope.

Spores were obtained from mature cysts in each organ sample. An average of 30 spores were measured using the dimensions recommended by Lom and Arthur (1989). Permanent preparations were made by placing a portion of the spores into glycerin-gelatin and mounting them under a coverslip. The structure of the spore including the spore wall, the suture of the wall, the polar capsules and the iodophilous vacuole was studied by Nomarski interference microscopy. The presence of the vacuole was identified by the use of Lugol's solution.

For histological examinations, infected organs were fixed in 10% buffered formalin, then embedded in paraffin wax, cut into 5 µm thick sections, and stained with haematoxylin-eosin and by Farkas-Mallory's method (Krutsay 1980). Before embedding, both the gill and the fin samples were decalcified in 10% Na-EDTA solution overnight.

RESULTS

Two fish specimens were infected by *Myxobolus* cysts in the primary filaments of the gills, and only one of the fish was infected by *Myxobolus* cysts on the dorsal fin between the soft rays.

Description of the species (based on spores collected from *C. trutta*)

Myxosporea, Bütschli 1881
Bivalvulida, Shulman 1959
Myxobolidae, Thélohan 1892
Myxobolus, Bütschli 1882

Myxobolus molnari sp. n. (Figs. 1, 2)
Host: *Capoeta trutta* (Heckel, 1843)
Locality: River Karun, Ahwaz, Iran.
Site of infection: gills.

Type material: holotype deposited in the collection of the senior author in Veterinary Medical Research Institute, H.A.S., Budapest, Hungary.

Description of the species: the cysts of the parasite were filled with mature spores and could be observed only by stereomicroscope. They appeared as highly refractile, elongated oval formations 0.3 × 1.0 mm in diameter in the middle and basal third of the primary gill filaments, located parallel to the axis of the gill filaments.

The spores are relatively large, ellipsoidal in front view. They are wider at the anterior than at the posterior part. In lateral view, the spores are lemon shaped, with a slight projection at the two ends due to the thickness of the spore wall and the expressed suture. Spore valves are smooth and symmetrical with several edge markings. The wall of the spore seems to be thick, but this thickness is due to the emergence of the sutural line over the surface of the spore. Spore 14.2 (13.3–14.6) µm long, 10.7 (10.4–11.6) µm wide, and 7.4 (6.9–7.8) µm thick. Two polar capsules ellipsoidal in shape, tapering only at the discharging canals of the polar filaments. They are equal in size, 6.8 (6.7–7.2) µm long, 3.9 (3.5–4.2) µm wide. The length of the two polar capsules is slightly less than the half length of the spore. The anterior ends of the polar capsules are set apart to each other. The spore has a distinct intercapsular appendix. Polar filaments closely coiled with 6–7 turns, situated perpendicularly to the longitudinal axis of the capsules. The sporoplasm has a more or less round iodinophilous vacuole which can be seen by using Lugol's solution. There is no mucous envelope or membranaceous envelope on the spore.

Tissue sites: the fixed test material was found to contain half-matured plasmodia and already evacuated cysts (Figs. 3, 4). In the former case, the cysts of the parasite were located in the axis of the primary gill filaments, typically in the basal and middle third of the filaments. The cyst was situated in the tunica adventitia of the centrally running blood vessels of the primary gill filament. Although the cyst exerted lateral pressure on the blood vessel, it only moderately impaired the blood flow, as evidenced by the maintained blood supply of tissues peripheral to the site of cyst development. In the centre of the half-matured plasmodia mature spores could be seen, while along the wall of the plasmodia vegetative nuclei and spore-forming cells were located. Although the developing cyst lifted the secondary filaments, it caused no tissue damage (Fig. 3). After the release of spores, a yellow-staining homogeneous mass surrounded by a connective tissue capsule was left behind in the primary filament (Fig. 4).

Comments: the few *Myxobolus* species hitherto described from fishes of the genus *Capoeta* differ from the species *Myxobolus molnari* sp. n. in their organospecificity and development in the tissues. In addition to this, major differences occur also in the morphology and size of the polar capsules, by which the new species can easily be distinguished from the species *Myxobolus irinae* Daniyarov, 1975; *Myxobolus samgoricus* Gogebashvili, 1966; *Myxobolus tadzhikistanicus* Daniyarov, 1975; and *Myxobolus varicorhini* Dzhalilov and Daniyarov, 1975. Of the species described from the gills of fish species belonging to the genus *Capoeta*, *Myxobolus cristatus* Shulman, 1962 and *Myxobolus kovali* Allamuratov, 1967 substantially differ from *Myxobolus molnari* sp. n. in both size and shape. The maximum spore length of

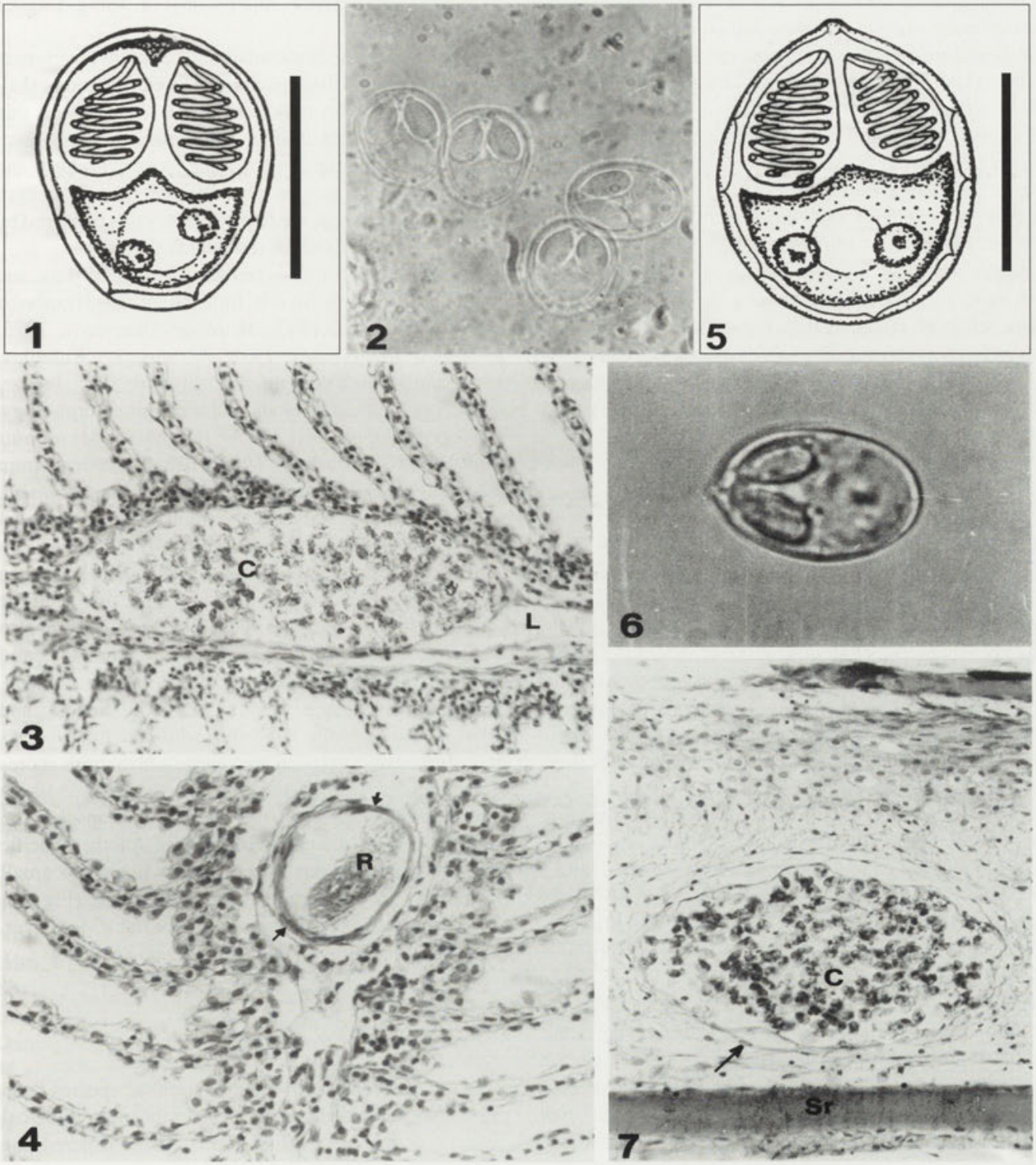

Fig. 1. Drawing of the spore of *Myxobolus molnari* sp. n. Bar - 10 µm
Fig. 2. Light microscopic photograph of the spores of *Myxobolus molnari* sp. n. parasitic in the gills of *Capoeta trutta*. Fresh preparation (X 1440)
Fig. 3. *Myxobolus molnari* sp. n. forms cysts (C) in the centre of the primary gill filament, in the tunica adventitia of the gill blood vessels, directly beneath the endothelium (arrow). L - the lumen of the blood vessel. Haematoxylin and eosin (H.-E.) (X 195)
Fig. 4. After the cyst of *Myxobolus molnari* sp. n. bursts, a homogeneous, yellowish ceroid mass (R) is left behind in the place of the evacuated spores. The mass is surrounded by split-up connective tissue (arrow head). H.-E. (X 290)
Fig.5. Drawing of the spore of *Myxobolus mokhayeri* sp. n.Bar - 10 µm
Fig. 6. Light microscopic photograph of the spore of *Myxobolus mokhayeri* sp. n. parasitic in the fins of *Capoeta trutta*. Fresh preparation (X 2400)
Fig. 7. Cyst (C) of *Myxobolus mokhayeri* sp. n. besides the soft fin ray (Sr), surrounded by connective tissue cells (arrow). H.-E. (X 260)

M. cristatus is about 4 µm less than that of our species and its polar capsules are also smaller, while the spores of *M. kovali* are even smaller in size and their polar capsules are not uniform even within the same spore. The spore measurements of *Myxobolus musajevi* Kandilov, 1963, the species bearing the closest resemblance to the species described by us, come close to those of our species; however, there are differences in the shape of the spore: while the spore of *M. musajevi* is oval, that of *M. molnari* sp. n. is more elongated, slightly wider at its anterior end and tapering posteriorly. The spores of *M. musajevi* have been detected in the gills, kidneys and bile, while *M. molnari* sp. n. is a typical gill parasite as regards its organospecificity.

The species has been named after Dr Kálmán Molnár, an internationally acknowledged Hungarian fish parasitologist.

Myxobolus mokhayeri sp. n. (Figs. 5, 6)
Host: *Capoeta trutta* (Heckel, 1843)
Locality: River Karun, Ahwaz, Iran.
Site of infection: fins, between the soft rays.
Type material: holotype deposited in the collection of the senior author in Veterinary Medical Research Institute, H.A.S., Budapest, Hungary.

Description of the species: the cysts of the parasite, filled with mature spores, could be seen with the unaided eye and appeared as whitish, opalescent formations of elongated oval shape and 0.5 × 1.2 mm in diameter along the soft rays of the dorsal fin.

The spores are relatively large, ovoidal in front view, with a small nipple-like projection on the apical part. In lateral view the spores are elongated ellipsoidal with a thickening at both ends. Suture line well emerged over the surface of the spore. No intercapsular appendix seen. Spore valves symmetrical, smooth and relatively thin. Spores 15.8 (14.4–16.6) µm long, 12.5 (11.6–13.3) µm wide, and 8.6 (8.3–9.1) µm thick. The two polar capsules, ellipsoidal in shape, tapering only at the discharging canals of the polar filaments, are unequal in size. The larger 7.5 (7.7–7.9) µm long, 4.6 (4.1–4.9) µm wide, the smaller 6.6 (5.8–7.5) µm long, 3.6 (3.3–4.1) µm wide. The length of the smaller polar capsule is less than the half length of the spore, while that of the larger is equal with it. The anterior ends of the polar capsules converge toward their pointed ends and open close to each other. Polar filaments closely coiled with 7–9 turns in the larger and 7–8 in the smaller capsules. They are situated perpendicularly to the longitudinal axis of the capsules. The sporoplasm has a more or less round iodinophilous vacuole which can be seen by Nomarski interference microscopy or using Lugol's solution.

Tissue sites: the cyst containing the spores develops in the subcutaneous tissue covering the cartilaginous skeleton of the soft fin rays, where it is located in a thin connective tissue capsule (Fig. 7). In the stratum spongiosum of the skin of the fins, cells having loose cytoplasm and a round nucleus can be observed lateral to the cyst. Towards the skin and the fin rays, the cyst is bordered by loose connective tissue of reticular structure.

Comments: *Myxobolus mokhayeri* sp. n. differs, and is thus easily distinguishable, from the species *Myxobolus cristatus* Shulman, 1962; *M. irinae* Daniyarov, 1975; *M. kovali* Allamuratov, 1967; *M. musajevi* Kandilov, 1963; and *M. tadzhikistanicus* Daniyarov, 1975: in size, shape, organospecificity and polar capsule morphology. The species described by us resembles *Myxobolus samgoricus* Gogebashvili, 1966, a parasite reported from the fins, gills and kidney of fishes of the genus *Capoeta*, but differs from the latter by the small projection present on the apical part of the spore of *M. mokhayeri.* In addition, the spore of *M. mokhayeri* is much larger than that of *M. samgoricus* (9.1-11.0 x 7.8-9.2 µm), and the size and relative ratio of the polar capsules are also different. *Myxobolus varicorhini* Dzhalilov and Daniyarov, 1975 develops in the skin, kidney and spleen, its larger polar capsule is a maximum of 7.1 µm long, and it has an intercapsular appendix, while the new species described by us is a typical fin parasite which forms spores along the soft rays of the fins in a connective tissue capsule, the average length of its larger polar capsule is 7.5 µm, and it has no intercapsular appendix. Another specific feature of the species described by us is the small projection seen on the apical part of the spore (Fig. 6).

The species has been named after Dr Baba Mokhayer, the internationally acknowledged Iranian Professor.

DISCUSSION

In the differentiation of our *Myxobolus* species from previously described species, besides the morphological features (Lom and Arthur 1989) we considered the criteria suggested by Molnár (1994), i.e. that myxosporean parasites are usually characterized by tissue specificity. Thus, in our opinion the species described on the basis of spores found in other organs and tissues of the body are not identical with the species developing in a cyst surrounded by special connective tissue cells of the gills (*M. molnari* sp. n.) and fins (*M. mokhayeri* sp. n.). We

have also adopted Molnár's (1994) view that most myxosporean species are parasites of closely related fish species; thus, in the differentiation we disregarded *Myxobolus* organisms parasitic in genera taxonomically distant from the genus *Capoeta*, even if they were similar in morphology and were developing in the same location.

When describing myxosporeans, a difficulty arises from the fact that, primarily in the case of the histozoic species described earlier, the morphological characters were emphasized and only little attention was paid to determining the exact site of development by histological methods. Most descriptions indicated only the sites where mature spores were found. This is how it could happen that for certain species such as *M. cyprini* Doflein, 1898 almost all organs of the fish were included in the list where the spore-containing small plasmodia were thought to occur (Donec and Shulman 1984). Studying the development of *M. cyprini*, Molnár and Kovács-Gayer (1985) established that the spores developed exclusively in the striated muscle cells, in a large, cyst-like plasmodium. After the plasmodium matures and bursts, the blood stream carries the spores to diverse organs and tissues of the organism. A similar finding was obtained by Baska (1987) for the myxosporean *M. pseudodispar* Gorbunova, 1936. The spores of that species were detected in various organs of the roach and other cyprinids, but Baska's histological examinations proved that the plasmodia of the species form spores in the striated muscle cells. These results have demonstrated that a precise determination of the site and type of development in the tissues is indispensable for the description and differentiation of the species.

The genus *Myxobolus* contains a large number of species. Landsberg and Lom (1991) recorded 444 species, the majority of which were described from Eurasia and North America. From fishes of the *Capoeta (Varicorhinus)* genus indigenous in the area of the former Soviet Union, they mention 8 known *Myxobolus* species (*Myxobolus cristatus* Shulman, 1962; *M. irinae* Daniyarov, 1975; *M. kovali* Allamuratov, 1967; *M. musajevi* Kandilov, 1963; *M. samgoricus* Gogebashvili, 1966; *M. uzbekistanicus* Allamuratov, 1965; *M. tadzhikistanicus* Daniyarov, 1975; *M. varicorhini* Dzhalilov and Daniyarov, 1975).

In their myxosporean identification key, Donec and Shulman (1984) reported the occurrence of 7 *Myxobolus* species in fishes of the genus *Capoeta* (*Capoeta heratensis, C. heratensis steindachneri, C. capoeta*). They synonymized the species *Myxobolus uzbekistanicus* Allamuratov, 1965 with *Myxobolus muelleri* Bütschli, 1882.

To date, no myxosporean parasite has been detected in fishes of the genus *Capoeta* indigenous in the water system of other parts of Central Asia. Studying the myxosporean fauna of Iranian barboid fishes, Masoumian et al. (1994) and Molnár et al. (personal communication) have established that the original fish parasitic myxosporean fauna of the Mesopotamian region markedly differs from that of barbels of the Palaearctic and the Indian Great Fauna Region. The above statement presumably applies also to the genus *Capoeta*.

The species described in this paper are likely to have minor pathological importance, as the observed infections were characterized by a low prevalence and intensity. The parasites did not cause major and irreversible histopathological changes. On completion of their development the spores were released, then during the regeneration of tissues the fish organism eliminated the remnants of the cysts.

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Neothelohanellidae fam. n. and Taxonomic Consideration on the Genera *Neothelohanellus* and *Lomosporus* (Myxozoa: Myxosporea)

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Summary. The status of two genera *Neothelohanellus* Das and Haldar, 1986 and *Lomosporus* Gupta and Khera, 1988 (Myxozoa: Myxosporea) have been reviewed. *Neothelohanellus catlae* and *Lomosporus indicus* the type species of the two respective genera have been retained, whereas *N. krishnagarensis* has been replaced as *Lomosporus krishnagarensis* (Das and Haldar 1986) nov. comb. under the genus *Lomosporus*. Suprageneric taxonomic account of these parasites are made and a new family Neothelohanellidae under the suborder Variisporina Lom and Noble, 1984 has been proposed to include these two genera.

Key words: *Lomosporus*, Myxosporea, Neothelohanellidae fam. n., *Neothelohanellus*, Platysporina, taxonomy, Variisporina.

INTRODUCTION

Lom and Noble (1984) recognised 42 genera in their revised classification of the class Myxosporea Bütschli 1881. Das and Haldar (1986) proposed a new genus *Neothelohanellus* to accommodate two new species from two different major carps. Shortly after, Gupta and Khera (1988) proposed another new genus *Lomosporus* to describe a new species *L. indicus*. This communication discusses the suprageneric status and critically evaluates the validity of those three species under the two genera and proposes a new family Neothelohanellidae to accommodate them in the suborder Variisporina.

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SYSTEMATIC DISCUSSION

The genus *Neothelohanellus* was created by Das and Haldar (1986) and two species were described viz., *N. catlae* the type species from the kidney of *Catla catla* (Ham) and *N. krishnagarensis* from the fatty matrix of brain of *Labeo calbasu* (Ham). The authors have proposed the diagnostic features of the genus to be oval to ellipsoidal spores; a single anterior polar capsule placed either perpendicularly or angularly to the longitudinal axis of the spore; a sporoplasm with an iodinophilous vacuole and histozoic in freshwater fish.

It is evident, therefore, that the salient features of the genus *Neothelohanellus* include two distinct sets of characters: (1) round to ellipsoidal spore and a single anterior polar capsule placed angular to the longitudinal axis of the spore and these fully correspond with the features of *N. catlae* and (2) oblongate spore and a single anterior polar capsule placed perpendicular to the longitudinal axis of the spore and opening of polar capsule is

perpendicular to the longitudinal axis of the spore. These fully conform with the salient features of *N. krishnagarensis*. Furthermore, *N. catlae* has distinct thin, long and thread-like polar filament while in *N. krishnagarensis* the polar filament is thick and short like a ribbon. The nature of the polar filament is not always considered as a generic character. Still, it has got a definite adaptive value and is considered important in the taxonomy of Myxozoa. Moreover, the infection loci of *N. catlae* and *N. krishnagarensis* are different and described from different localities. It is now very apparent that the heterogeneity in the characteristics of the genus *Neothelohanellus* is the result of the intention of the authors to include both the species under the single genus *Neothelohanellus*. Therefore, in order to avoid heterogeneity in the fundamental characters of the genus, some emendations are needed. The heterogeneity can be minimized by separating the two species in two different genera. As *Neothelohanellus catlae* is the type species of the genus *Neothelohanellus*, it must be retained along with some emended diagnostic characters of the genus. The species *N. catlae* Das and Haldar, 1986 seems very close to the genus *Globospora* Lom, Noble and Laird, 1975 except the 'unequal shell valves' which is one of the diagnostic features of the genus *Globospora* and, hence, can not be included in this genus. Therefore, *N. krishnagarensis* Das and Haldar, 1986 is to be separated from *Neothelohanellus* and to be included in a different genus.

Thus, the genus *Neothelohanellus* Das and Haldar, 1986 may be emended as: spores are almost globular, equal symmetrical shell valves meet to form a straight sutural line, a single anterior polar capsule placed angular to the longitudinal axis of the spore and opens almost on the lateral side, thin and long thread-like polar filament and histozoic development in freshwater fish. The type species is *N. catlae* Das and Haldar, 1986.

Gupta and Khera (1988) later established the genus *Lomosporus* for a new myxozoan parasite *Lomosporus indicus* which is the type species and mentioned features such as egg-shaped spore with broader anterior and narrow posterior ends, a single anterior polar capsule placed at right angle to the longitudinal axis of the spore and histozoic development in freshwater fish as characteristics of the genus. Lom and Dyková (1992) synonymized genus *Lomosporus* Gupta and Khera, 1988 with *Neothelohanellus* Das and Haldar, 1986 without going through a detailed analysis and provisionally placed it in the family Myxobolidae. Lom and Dyková (1992) also mentioned that the genus *Neothelohanellus* contains 2 species, however, they did not indicate the names of the two species. Interestingly, Lom and Dyková (1992) mentioned about a species *N. indicus* Gupta and Khera, 1988 which is not in conformity with the International Codes of Zoological Nomenclature. It is to be reiterated that there are two species in the genus *Neothelohanellus* (as proposed by Das and Haldar, 1986) and one described under *Lomosporus*. Lom and Dyková (1992) neither indicated the name of the type species nor clarified to which species *L. indicus* is synonymized. This leads to confusion. The diagnostic features of *Lomosporus* Gupta and Khera, 1988 are exactly similar to those of the *N. krishnagarensis*. Thus, *N. krishnagarensis* is placed in the genus *Lomosporus* and is designated as *L. krishnagarensis* (Das and Haldar, 1986) nov.e comb. Both *L. indicus* and *L. krishnagarensis* differ in their mensural details and sites of infection. So, the genus *Neothelohanellus* contains one species i.e. *N. catlae* and the genus *Lomosporus* includes two species i.e. *L. indicus*, the type species and *L. krishnagarensis* (Das and Haldar, 1986) nov. comb.

SUPRAGENERIC TAXONOMIC ACCOUNT

In their proposition of the genus *Neothelohanellus*, Das and Haldar (1986) neither mentioned the name of the family nor the suborder wherein the described myxosporean could be placed. However, Gupta and Khera (1988) included the genus *Lomosporus* in the family Myxobolidae. Interestingly enough, neither of the authors considered the revised classification of the class Myxosporea Bütschli, 1881 proposed by Lom and Noble (1984). According to them, Myxobolidae is a family of the suborder Platysporina (Kudo, 1919) where polar capsules are present at the apex of the spore. Shulman's conception (1966) of the arrangement of the polar capsule in relation to sutural plane is considered for subordinal level by Lom and Noble (1984). The erection of suborder Variisporina by Lom and Noble (1984) for the myxosporean parasites where polar capsules may occupy various positions of the spore justifies the above mentioned proposition. Thus, the genera *Neothelohanellus* and *Lomosporus* should be placed under the suborder Variisporina because the polar capsule is placed perpendicular or angular to the sutural plane and its orifice is not at the apex of the spore. Obviously, the family Myxobolidae is not the right category for this genus. Lom and Dyková (1992), however, preferred to place *Neothelohanellus* provisionally in the family Myxobolidae (suborder Platysporina) without any discussion.

It was considered that the genus *Neothelohanellus* should be kept under the suborder Platysporina. The argument is that "the genus *Neothelohanellus* represents just a final step in the series: typical *Myxobolus - M. toyami* or *M. anisocapsularis - Thelohanellus - Neothelohanellus*". This statement stems from the use of a prefix 'Neo' before *Thelohanellus* and develops a wrong impression that both *Thelohanellus* and *Neothelohanellus* are taxonomically closely related. It is often found in the literature on the protozoan parasites that a prefix and/or a suffix is used in an existing generic name without consideration of their taxonomic/phylogenetic relationship. This type of naming always leads to confusion and there is no rule in ICZN to avoid such problem. Therefore, appropriate emendation of ICZN rule regarding framing of generic name is suggested. However, the basic difference between the two suborder Platysporina and Variisporina lies in the position of the polar capsule and the site of its opening. It is reiterated that the myxosporean parasites of the genera *Neothelohanellus* and *Lomosporus* fail to satisfy the features stipulated for the suborder Platysporina, i.e, apex of the polar capsule lies solely in the sutural plane of a bilaterally symmetrical spore, but to corroborate with the Variisporina. Therefore, the above placement of the myxosporea in question demands erection of a new family under the suborder Variisporina.

It may very clearly be said that the evolution of myxosporean parasite is based mainly on the structure and arrangement of the polar capsule. This feature is also considered for the classification of this group (cf. Shulmann 1966, Lom and Noble 1984) and justified their scheme of classification considering "arrangement of polar capsules in relation to the sutural plane at subordinal level" (pp. 194). It is proposed that shifting of the arrangement of polar capsule along with the opening reflects gradual evolutionary step from Platysporina to Variisporina (Fig. 1).

There are 10 existing families of the suborder Variisporina viz. Myxidiidae, Ortholineidae, Sinuolineidae, Fabesporidae, Ceratomyxidae, Sphaerosporidae, Chloromyxidae, Auerbachiidae, Alatosporidae and Parvicapsulidae. Both the genera *Neothelohanellus* and *Lomosporus* can hardly be included in any of the existing families, close one being the family Myxidiidae. Myxidiidae have two polar capsules (one is eliminated in the genus *Coccomyxa*) with terminal or slightly lateral foramina and the spores are spindle-shaped, sigmoid or crescentic. So, the relationship of the genera *Neothelohanellus* and Lomosporus with the family Myxidiidae is weak. The family Auerbachiidae possesses one single polar capsule but the spores have asymmetrical, unequal shell valves.

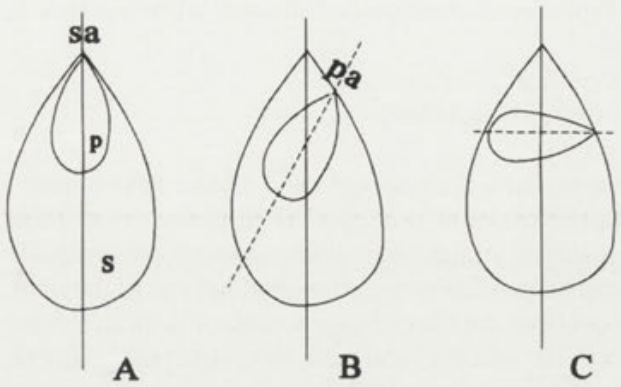

Fig. 1. Schematic diagram of orientation of polar capsule in relation to axis of the spore. A - arrangement in Platysporina, B and C - arrangement in Variisporina (family Neothelohanellidae n. fam.). p - polar capsule, pa - axis of the polar capsule, s - spore, sa - axis of the spore

Hence, the genera *Neothelohanellus* and *Lomosporus* being members of the suborder Variisporina cannot find their place in any of the existing families. Therefore, a new family Neothelohanellidae n. fam. is proposed to include the genera *Neothelohanellus* and *Lomosporus*. The family may be defined as: spores oval, shell valves symmetrical, single polar capsule placed either perpendicular or angular to the longitudinal axis of the spore and the orifice is on the lateral side and histozoic in freshwater fishes.

Thus the two genera *Neothelohanellus* with one species i.e. *N. catlae* and *Lomosporus* with two species i.e. *L. indicus* and *L. krishnagarensis* constitute the new family Neothelohanellidae of the suborder Variisporina Lom and Noble, 1984.

DIAGNOSIS

Suborder Variisporina Lom and Noble, 1984

Family Neothelohanellidae fam. n.

Spores oval, ellipsoidal or globular in valvular view, shell valves symmetrical, straight sutural line, single anterior polar capsule placed either perpendicular or angular to the longitudinal axis of the spore and opening on the lateral side and histozoic in freshwater fishes.

Genus *Neothelohanellus* Das and Haldar, 1986 emend.

Spores are almost globular, equal symmetrical shell valves meet to from a straight sutural line, single anterior polar capsule placed angular to the longitudinal axis of the spore and opens almost on the lateral side, thin and long thread like polar filament, histozoic in freshwater fishes.

Type species: *Neothelohanellus catlae* Das and Haldar, 1986.

Type host: *Catla catla*.

Infection site: kidney.

Genus *Lomosporus* Gupta and Khera, 1988 emend.

Spores oval to ellipsoidal in valvular view, symmetrical shell valves, straight sutural line, a single polar capsule placed perpendicular to the longitudinal axis of the spore and opens on the lateral side, sporoplasm with an iodinophilous vacuole, thick and short ribbon-like polar filament, histozoic in freshwater fishes.

Type species: *Lomosporus indicus* Gupta and Khera, 1988

Host: *Labeo calbasu* (Hamilton).

Infection site: gills and operculum.

Type species: *Lomosporus krishnagarensis* (Das and Haldar, 1986) nov. comb.

Host: *Labeo calbasu* (Hamilton).

Infection site: fatty matrix of brain.

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Short communication

A Polychromatic Action Spectrum for the Inhibition of Motility in the Flagellate *Euglena gracilis*

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Summary. In the present study the effects of simulated solar radiation modified by different cut-off filters on the motility of the flagellate *Euglena gracilis* are investigated and presented in the form of a polychromatic action spectrum. The greatest effectiveness is in the UV-B range of the spectrum, but significant sensitivity was found also in the UV-A and the visible range.

Key words: *Euglena gracilis*, polychromatic action spectrum, simulated solar radiation.

INTRODUCTION

Ozone depletion has been reported since the late sixties; an ozone hole over Antarctica has been discovered in the eighties (Farman et al. 1985). In the last years decreased levels of stratospheric ozone have occurred over the northern hemisphere as well (Gleason et al. 1993, Kerr 1993). Investigations on the effects of enhanced UV-B radiation on individual organisms, populations and whole ecosystems appear to be of great importance (e.g. Acevedo and Nolan 1993, Karentz et al. 1994, Häder et al. 1995). Solar radiation has been shown to affect, among other biological processes, motility and swimming velocity as well as photo- and gravitaxis in the green flagellate *Euglena gracilis* and many other motile phytoplankton algae (e.g. Häder and Häder 1990, 1991; Häder and Liu 1990; Nielsen et al. 1995). *Euglena gracilis* accumulates at specific depths in the water column in order to find favorable conditions for its growth. The basis for this vertical distribution is active movement powered by a flagellum. Like many other motile phytoplankton organisms, *Euglena* is guided by external stimuli, the most important of which are light and gravity (Häder 1988).

Action spectroscopy (i.e. the wavelength dependence of the biological effects on radiation) is an important tool in the field of UV-B-research. With the help of action spectra it is possible to predict the potential biological damage (for a given effect and a given organism) resulting from ozone depletion (Rundel 1983).

The aim of the present study is to investigate the wavelength dependence of the effect of artificial solar-like radiation on the motility of *Euglena gracilis* and to present the data in the form of a polychromatic action spectrum.

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Table 1. Integrated total irradiance and irradiance in the UV-B, UV-A and PAR region of the spectrum of the Höhnle lamp with the different cut-off filters at the respective distance from the lamp

Distance [cm]	Filter	Total 280-700 nm [W/m²]	UV-B 280-315 nm [W/m²]	UV-A 315-400 nm [W/m²]	PAR 400-700 nm [W/m²]
60	WG280	436.3	1.33	88.1	346.8
60	WG295	423.2	1.21	85.1	336.9
60	WG320	432.5	0.46	87.4	344.6
54	WG345	478.1	0.0	80.6	397.5
45	GG400	457.4	0.0	1.6	455.8
45	GG420	409.8	0.0	0.0	409.8

MATERIALS AND METHODS

Organisms and culture conditions

The flagellate, *Euglena gracilis*, strain Z, was precultivated in an organic medium (Starr 1964). For experimental use the cells were transferred to a mineral medium (Checcucci et al. 1976) in which they were grown for 2 weeks prior to the experiments. In both media the cells were grown in static cultures at 20°C in continuous light from mixed daylight and warm tone fluorescent lamps (Osram L36 W/19 daylight 5000 de luxe and Osram L36 W/3L warm white de luxe) at 12 W m^{-2}.

Exposure to radiation

The organisms were exposed to simulated solar radiation from a solar lamp (Dr. Höhnle GmbH, Martinsried, FRG). Spectral irradiance was measured with a spectroradiometer (Optronic model 752, Optronic Laboratories Inc., Orlando, FL, USA, for the emission spectra of the radiation source see Gerber and Häder 1995). During exposure temperature was kept at 20°C in a temperature controlled room.

The cells were exposed in small Petri dishes (5 cm diameter) which were covered with cut-off filters or neutral density filters (WG and GG series, Schott & Gen., Mainz, FRG; UV transmitting neutral density filters from ESCO Products Inc. Oak Ridge, NJ, USA). The cell concentration was 0.6 x 10^6 cells/ml and the volume was 10 ml. As the cut-off filter, mainly the GG-filters, absorb part of the incident radiation, the samples were exposed at variable distances (45 to 60 cm) from the lamp. Integrated irradiance data are shown in Table 1. The cells were exposed for 10 min and motility was determined in the subsequent 10-min interval; then the cells were exposed again, so that exposure and cell counting alternated in 10- min intervals.

Determination of cell numbers and motility

Cell numbers as well the percentage of moving cells were determined with a haematocytometer (Brand, Wertheim, FRG) at a magnification of 160 x. The measurements for the dose- response curves have been carried out at least three times (WG 345: triplicates, WG280: four parallel samples, all other filters: five measurements). As the initial motility of *Euglena* cells was highly variable, motility was normalized to the initial value.

Calculations for the action spectrum

The data were plotted in the form of dose-response curves; subsequently, the linear regression for the second phases (see below) of the dose response curves was calculated and the inverse values of the dose which caused 100% (or 50%) inhibition of motility was plotted against the nominal cut-off value (50% transmission) of the filters. Finally the increments of these data were calculated.

RESULTS AND DISCUSSION

In action spectra in which the action is determined as a function of the cumulative dose (i.e. the dose is increased by prolonged exposure times and not by increasing irradiance of the radiation source), reciprocity should be valid at least during the given exposure interval (Cullen and Lesser 1991). In the case of the inhibition of motility in *Euglena gracilis* (Fig. 1) reciprocity was proven as nearly the same dose (which equals twice the irradiation time in the sample with a 52% neutral density filter compared to the sample irradiated without filter) was needed for partial and complete impairment of motility of the cells.

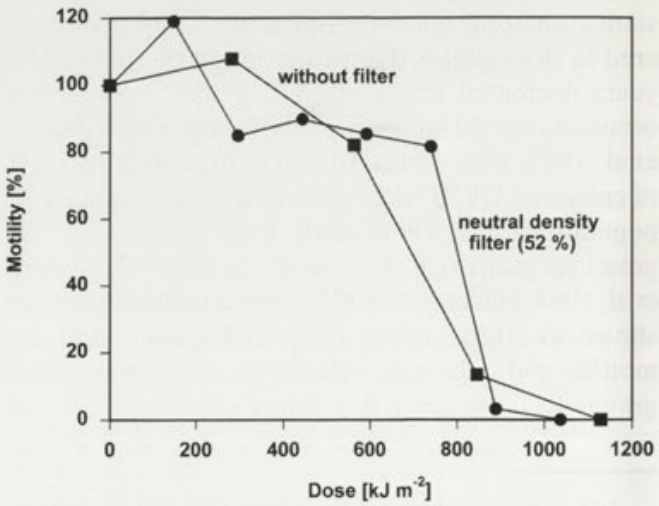

Fig. 1. Motility of *Euglena gracilis* in dependence of the accumulated dose when irradiated without filter (squares) or with a 52% neutral density filter (circles) normalized to the initial value

When *Euglena gracilis* was exposed to simulated solar radiation (Fig. 2) covered with a WG 280 cut-off filter (which cuts off about 10% of the total irradiance but hardly any UV-B radiation, see Table 1), cell motility was impaired very rapidly: after 20-30 min of exposure (about 500 kJ/m^2) the percentage of motile cells had already decreased significantly; after about 50 min of exposure (about 1000 kJ/m^2) all the cells were immotile. Parallel to the occurrence of immotility the cells changed their shape from oblong to round and formed large vacuoles (cf. Gerber and Häder 1995).

When the samples were covered with cut-off filters with increasing cut-off wavelengths the necessary doses to induce inhibition of motility increased. Under a WG 295 filter about 1500 kJ/m^2 was needed for complete immobilization of the organisms and change of cell shape, under WG 320, WG 345, GG 400 and GG 420 filters the critical doses increased to 2000 kJ/m^2, 2500 kJ/m^2, and 4000 kJ/m^2 (both GG-filters), respectively.

The kinetics of inhibition of motility in *Euglena gracilis* seem to consist of two phases: a first phase in which motility decreases to about 50% of the initial value and increases again to about 80-90% and a second phase in which motility decreases rather rapidly until complete immotility (and a change of cell shape) occurs. The second phase takes about 40-60 min in all samples,

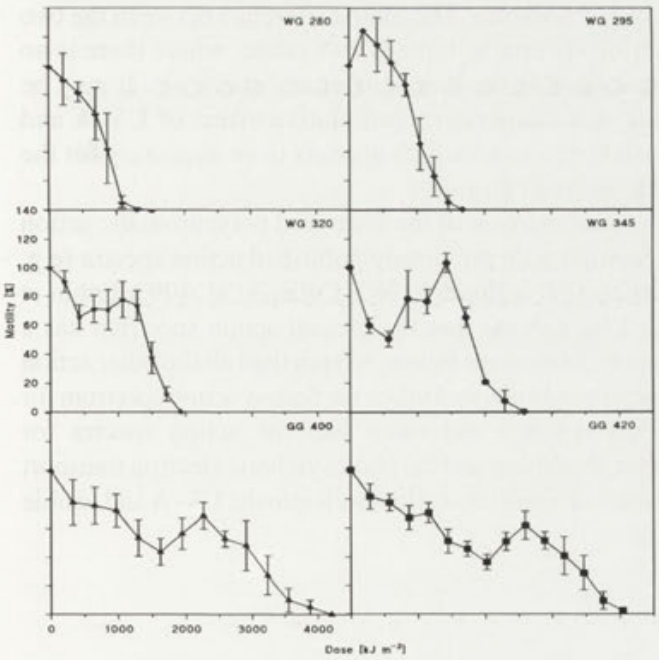

Fig. 2. Effects of filtered simulated solar radiation on the motility (percentage of motile cells normalized to the initial value) of *Euglena gracilis* in dependence of accumulated dose

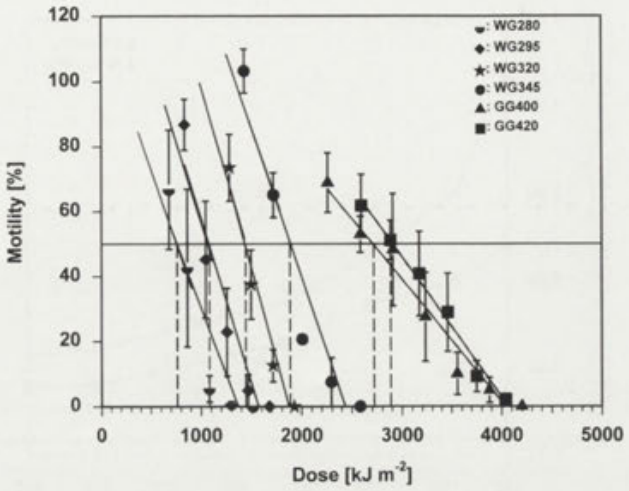

Fig. 3. Linear regression of the second phases of the dose-response curves of Fig. 2. (a) WG 280: lower semi circles, (b) WG 295: diamonds, (c) WG 320: stars, (d) WG 345: circles, (e) GG 400: triangles, (f) GG 420: squares

whereas the length of first phase changes with the cut-off wavelength of the used filter (0 min for the WG 280, 10 min for the WG 295, 50 min for the WG 320 and WG 345 filters).

The course of the first phase indicates the existence of deactivating and (re)activating (repair) processes which seem to overlap. In similar experiments with two *Cryptomonas* species (Gerber and Häder 1993) the dose-response curves are biphasic as well, but in the first phase there is practically no change of cell motility (which does not scatter much in these organisms). This might also be the case in *Euglena*, but motility in *Euglena* scatters very much and the error bars of most of the data points overlap. The constancy of motility in the first phase indicates that the organisms can cope with the accumulated doses during this exposure time or in other words that deactivating and reactivating processes are balanced.

In the second phase the deactivating process is dominating, reactivation is either stopped or outcompeted, which leads to complete immotility of the cells. In this context one has to consider that the impairment of cell motility is not an isolated process: UV-radiation has many other targets in the cell, e.g. the photosynthetic apparatus (Andersson et al. 1992), the membranes (Hada et al. 1993), cytoskeleton proteins (Ekelund 1991), and tubulins (Häder and Brodhun 1991). These targets are damaged or impaired as well and can have direct (tubulins) or indirect effects (lack of energy in photoinhibited cells) effects on cell motility.

As criteria for the action spectrum complete and 50% inhibition of motility were chosen , as the change in cell

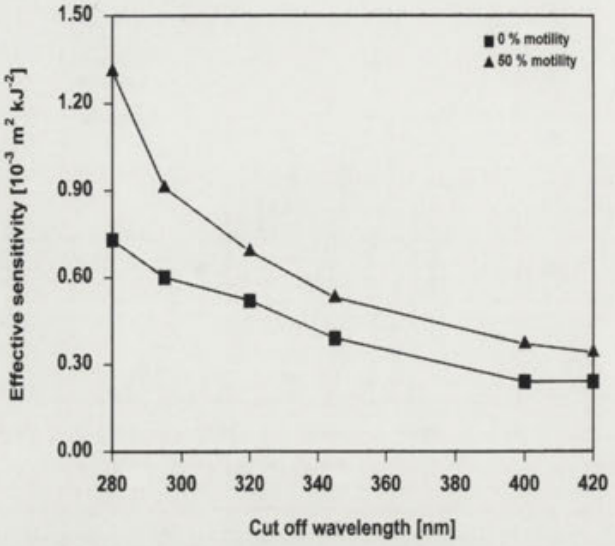

Fig. 4. Polychromatic action spectra for the inhibition of motility in *Euglena gracilis* (50% motility: triangles, 0% motility: squares)

shape occurred together with 0% motility and 50% inhibition and is in the linear range of the inhibition curves. In some of the dose-response curves (WG 345, GG 400, GG 420) 50% motility occurs twice, once in the first phase and once in the second phase, for the action spectrum only the 50% values in the second phases were used. The doses for 50% and 0% motility were determined by linear regression in the second phase (Fig. 3).

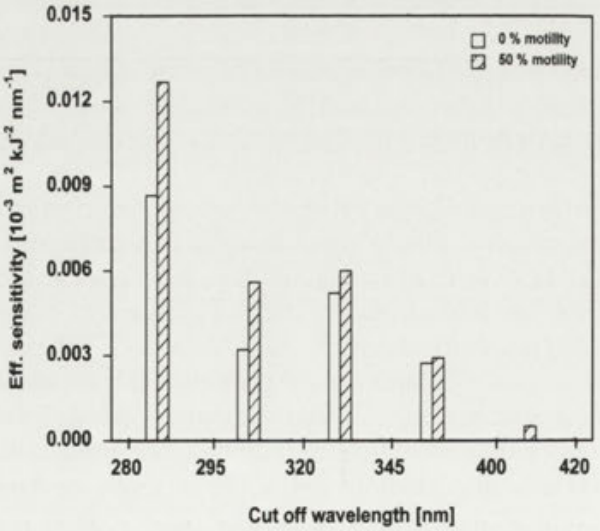

Fig. 5. Incremental polychromatic action spectra for the inhibition of motility in *Euglena gracilis* (50% motility: hatched bars, 0% motility: open bars)

The polychromatic action spectrum for the inhibition of motility in *Euglena* is presented in Fig. 4, an incremental action spectrum in Fig. 5. It can clearly be seen that the sensitivity of the organisms is increasing with decreasing cut-off wavelengths of the filters. This shows that the presence of UV-B radiation (especially short wavelength UV-B) is more damaging to motility of *E. gracilis* than radiation deprived of UV-B. On the other hand the action spectrum also shows that simulated solar radiation deprived of UV-B and UV-A is harmful to (the motility of) the algae as well. A comparison between the slopes of the action spectrum for 0% motility and the action spectrum for 50% motility shows that the UV-B effect is much more pronounced in the 50% motility curve. This might indicate that several processes take place in parallel which finally lead to the inhibition of motility but are due to different biochemical events and show different sensitivity towards UV-B radiation (the earlier processes being more UV-B sensitive than the later ones).

The comparison between a monochromatic action spectrum for the inhibition of motility in *E. gracilis* (Häder and Liu 1990) with the present polychromatic action spectrum shows a similar course in the UV-B range. UV-C radiation was not used here and the peak at 305 nm in the monochromatic action spectrum might be lost in the polychromatic action spectrum due to the lower resolution. The main difference between the two action spectra is in the UV-A range, where there is no inhibition in the monochromatic spectrum. It may be due to a cooperative/cumulative effect of UV-A and visible radiation which appears to be necessary for the inhibition of motility.

A comparison of the measured polychromatic action spectrum with previously published action spectra (e.g. Setlow 1974, Rundel 1983, Cullen et al. 1992, Lubin et al. 1992) shows that the present action spectrum has a much flatter slope below 300 nm than all the other action spectra cited above. Unlike the Setlow action spectrum for DNA damage and more like the action spectra for photoinhibition and the photosynthetic electron transport there is a significant effect well into the UV-A and visible region.

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Book Review

PROTOZOOLOGY by Klaus Hausmann and Norbert Hülsmann with contributors by Hans Machemer, Maria Mulisch and Günther Steinbrück. 2nd edition. Georg Thieme Vrlg. Stuttgart, New York

This is the most recent textbook on those unicellular eukaryotic microorganisms traditionally called Protozoa. The first edition of the book appeared in 1985 and, although it appealed to a more restricted readership being published in German, it enjoyed in parts of Europe where a knowledge of German has been more of a tradition. The quality of the book has been endorsed by its translation into Russian (1988) and Japanese (1989).

The anticipated and greatly desired second edition in English basically follows the plan of the first, but there is considerable improvement of both the format and quantity of information.

The book has three parts. The "Introduction and Overview" contains an scholarly overview of the history of protozoology. Here the reader finds the historical concept of several common terms such as "protozoa", "protoctista", "protista" as well the basic information on the founders of protozoology and their contributions. This part of the book also describes briefly the cellular organisation of protozoa - an item which is further developed in other chapters of the book.

The second and more voluminous part of the book is devoted to Evolution and Taxonomy of Protozoa which not only describes individual protozoan taxa but also expresses the authors' views on the evolution of unicellular prokaryotes and eukaryotes and establishes a rather unconventional systematic arrangement of protozoa, in an attempt to assimilate new opinions on protozoan phylogeny. An impressive number (97) of taxons are defined here, each lower rank taxon being represented by light microscopic and electron microscopic photomicrographs and drawings of excellent quality.

The third part of the book consists of selected topics of general protozoology, some of them being contributed by other specialists. It covers the comparative morphology and physiology of protozoa including a detailed account of protozoan skeletal elements, holdfast organelles, extrusomes, contractile vacuoles, motility, ingestion, digestion, defecation, nuclei and sexual reproduction (Maria Mulisch), morphogenesis and reproduction (Maria Mulisch), molecular biology (Günther Steinbrück). Hans Machemer is author of the section on behaviour of protozoa. The last chapter of the book covers the ecology of protozoa.

A valuable addition to the book is the glossary defining several hundred of basic terms and a detailed bibliography. It lists protozoological journals and periodicals, books and important articles on history of protozoology, general textbooks, monographs on individual groups and species of protozoa, their taxonomy, evolution, morphology and physiology, molecular biology, behaviour and ecology. References are also included for identification keys, protozoological techniques and teaching methods. Thus, even a novice in the field can easily target the most important publications of interest.

In judging the quality of the book, I value its balanced coverage of general and organismal aspects of protozoology. In a relatively limited space of 338 pages a sufficiently exhaustive overview of protozoology is given including some recent achievements in the field of molecular biology and protozoan phylogeny. The organismal part reflects not only the intimate knowledge possessed by both authors of the many protozoa they are dealing with, but also their affection for these wonderful organisms. Let us hope that

this spirit of the book will prove an inspiration for its readers.

Critical comments relate to a few, rather marginal items. It is no wonder, considering the amount of information and number of organisms covered in the book, that there are some inaccuracies in the descriptions of some of the taxa (mostly parasitic) that the authors were less familiar with. The authors were confronted with the same dilemma as are the teachers of protozoology, i.e. how to present to students (or readers) the taxonomy of protozoa, which are a polyphyletic group, "united" only by their unicellular, eukaryotic organisation. Older, conventional schemes, although easy to teach and understand, are in many respects no longer supported by evolutionary data. New schemes, such as that of Cavalier-Smith (1993) and Corliss (1994) and the one in the book by Hausmann and Hülsmann certainly more correspond to evolutionary events of the past and are thought-provoking but are far less easy to grasp by the non-specialist. The authors decided to solve this didactic riddle by presenting an overwiev of older classification schemes. However, in their book, the endeavour to create a phylogenetically supported classification of protozoa has resulted in a large number of forms ("Metakaryota incertae sedis") standing outside the classification. This might cause a problem to newcomers to the field, who do not understand the extreme phylogenetic diversity of these organisms, and who would prefer to have all forms properly classified (even if less accurately in the sense of phylogeny).

It also would have been appropriate if more space had been allocated to views of protozoan phylogeny based on molecular biology. The sole distance matrix tree (p. 461 showing the relationships of just a few organisms only (mostly) ciliates) certainly does not reflect the tremendous contribution that molecular biology has brought to our understanding of the phylogeny of eukaryotic microorganisms. I personally regret that the table showing protozoa as indicators of water quality from the first edition has been omitted. The same is true of the simple key to the most frequently found protozoa in freshwater habitats and some hints about the collection and cultivation of protozoa. Understandably, the authors were motivated for these deletions by their wish to make from the book less the "Taschenbuch" and more a standard textbook. Yet the combination of the "high level" and "low level" science was a distinctive quality of the first edition, serving well the purpose of attracting new "blood" to the field.

Despite these critical comments, the "Protozoology" by Hausmann and Hülsmann is an excellent introductory textbook, honouring the tradition of German protozoology and continuing successfully in the line of the outstanding German protozoology textbooks, such as those of Doflein-Reichenow and Karl Grell. The book is recommended as a "must" for all those who would like to know more the protozoa and understand better these wonderful little organisms.

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