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Review Article

Microsporidia Infection in HIV - Negative Subjects

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Summary. Microsporidia are recognized to cause infections in humans, mostly in immunocompromised patients infected with the human immunodeficiency virus (HIV). These opportunistic parasites are also pathogenic in subjects with immunodeficiency due to other causes than AIDS. Cases of microsporidiosis, some of them lethal, were reported in immunocompromised children and adults, including transplanted patients treated with immunosupressors. Microsporidioses also occur in immunocompetent subjects. Species not found in HIV-infected individuals were shown to cause ocular infections, whereas *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, both identified in AIDS patients, appeared to be also responsible for cases of diarrhoea presented by travellers returning from tropical areas. The finding of *E. bieneusi* in symptomatic as well as in asymptomatic HIV-negative African subjects suggests that microsporidia may be an underestimated cause of intestinal diseases in developing countries. Serological studies showed the occurrence of antibodies directed against *Encephalitozoon* species in European subjects. There is some indication that microsporidia might cause asymptomatic and latent infections in immunocompetent humans.

Key words: human, immunocompetent, immunodeficiency, microsporidia.

INTRODUCTION

Microsporidia (phylum Microspora) are cosmopolite, eukaryotic protists found to parasitize an extremely wide range of animals, from protozoa to humans. Some rare human cases have been reported over the years, but it was not until the emergence of the acquired immunodeficiency syndrome (AIDS) caused by HIV infection, that microsporidiosis became known as an important opportunistic parasite in humans (Desportes-Livage 1996).

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According to the localization of the parasites, clinical signs can consist of gastrointestinal troubles, nephritis, hepatitis, sinusitis, muscle-ache or eye-irritations (Weber *et al.* 1994, Coyle *et al.* 1996). Different manifestations can be presented by patients with disseminated microsporidioses involving most organs (Orenstein *et al.* 1997).

Enterocytozoon bieneusi Desportes *et al.*, 1985, the first species described in AIDS patients was followed by *Encephalitozoon hellem* Didier *et al.*, 1991, *Encephalitozoon intestinalis* Hartskeerl *et al.*, 1995 (Cali *et al.*, 1993) and *Trachypleistophora hominis* Hollister *et al.*, 1996. *Encephalitozoon cuniculi*, a species parasitic in mammals, was also shown to cause microsporidiosis in HIV-infected patients (Hollister *et al.* 1995, Didier *et al.*

1996, Weber *et al.* 1997). Reports have been published the last years referring to *Nosema*-like microsporidia causing myositis (Cali *et al.* 1996), as well as *Pleistophora*-like microsporidia causing severe disseminated infections (Yachnis *et al.* 1996) in AIDS patients.

Thanks to retroviral therapy the number of opportunistic parasite infections, including microsporidiosis, in HIV-positive and AIDS patients is now declining (Goguel *et al.* 1997, Foudraine *et al.* 1998). Curious enough, microsporidian infections also occur in HIV-seronegative persons, and a review of the reported cases is presented below.

IMMUNOSTATUS AND MICROSPORIDIOSIS

As stated by Singh *et al.* (1982), the majority of microsporidia infections reported up to that date appeared to occur in individuals undergoing immunosuppression although the immunostatus of the patients was rarely known.

In 1959 a 9-year-old Japanese boy was reported with fever and convulsions caused by microsporidia. Spores isolated from the cerebrospinal fluid and urine were inoculated into mice that developed a benign microsporidiosis resembling encephalitozoonosis (Matsubayashi *et al.* 1959). This microsporidium was revised by Sprague (1977) and named *Encephalitozoon matsubayashii*. The validity of this species is speculative. Indeed, the parasite might as well correspond to *Encephalitozoon hellem* or *E. intestinalis*, which were later identified in AIDS patients. Only molecular analysis could differentiate these species which exhibit the same development and similar morphological features. Later Weber and Bryan (1994) noted that the reported cutaneous allergy to tuberculin could indicate an impaired immune response in the child.

In 1973, Marcus *et al.* reported one case of human tumor microsporidiosis in a man from South Africa. Clusters of spores were seen in the cells of a widely disseminated pancreatic adenocarcinoma. The species was not identified. The parasite did however induce the development of ascite and hepatosplenomegaly in mice. The authors pointed out the similarities between this microsporidiosis and the pathology induced in small mammals (muridae, hamster) by *E. cuniculi* which infects cells of transplantable animal tumors.

The first well-documented case of an immunocompromised patient with microsporidiosis was an athymic 4-month male infant with hypogammaglobulinemia that died from a disseminated infection (Margileth *et al.* 1973). Tissues contained spores of a microsporidian species named *Nosema connori* by Sprague (1974).

In 1984, antibodies to *E. cuniculi* were detected by serological tests in a 2-year-old boy born in Columbia and living in Sweden (Bergquist *et al.* 1984a). The child suffered convulsive fevers similar to those of the Japanese boy (Matsubayshii *et al.* 1958) and was immunocompromised as indicated by a low T-helper cell count. Spores of the parasite were detected by indirect immunofluorescent assay in the patient's urine.

In 1985, Ledford *et al.* reported a case of microsporidial myositis in a male patient residing in Florida. Although this patient had severe immunodeficiency he remained seronegative for HIV and was still alive four years later (Macher *et al.* 1988). The developmental cycle in sporophorous vesicles (referred to as pansporoblastic) was the characters given by Ledford to place this parasite in the genus *Pleistophora* Gurley, 1893, (Canning and Hazard 1982) created for microsporidia infecting muscles in fish. This case resembles two later reports of infected AIDS patients from Haiti (Chupp *et al.* 1992) and Australia (Field *et al.* 1996a). The ultrastructural study of the Australian microsporidium demonstrated characters that separated it from the genus *Pleistophora sensu stricto* and a new genus was thus created for this species *Trachypleistophora hominis* (Hollister *et al.*, 1996). This species is apparently similar to the microsporidium described by Chupp *et al.* (1992), and the Ledford case from Florida, described above, might also be caused by the same species. Recently a similar parasite was found to cause disseminated microsporidiosis involving most organs in two AIDS-patients from Florida (Yachnis *et al.* 1996).

In 1997, a study was conducted on a series of twelve Tunisien children with innate immunodeficiency. It revealed two cases of intestinal microsporidiosis, one associated with diarrhoea and the other asymptomatic (Aoun *et al.* 1997).

Three cases are hitherto reported from patients immunosuppressed after an organ transplantation. Two patients developed an intestinal microsporidiosis, one after a liver transplant (Sax *et al.* 1995) and the other after a heart-lung transplant (Rabodonirina *et al.* 1996). *Enterocytozoon bieneusi* was identified in stool and duodenal biopsies of the heart-lung transplant recipient (Fig. 1). Both patients received therapy with metroninazole and albendazole respectively, which apparently resolved the infection. The

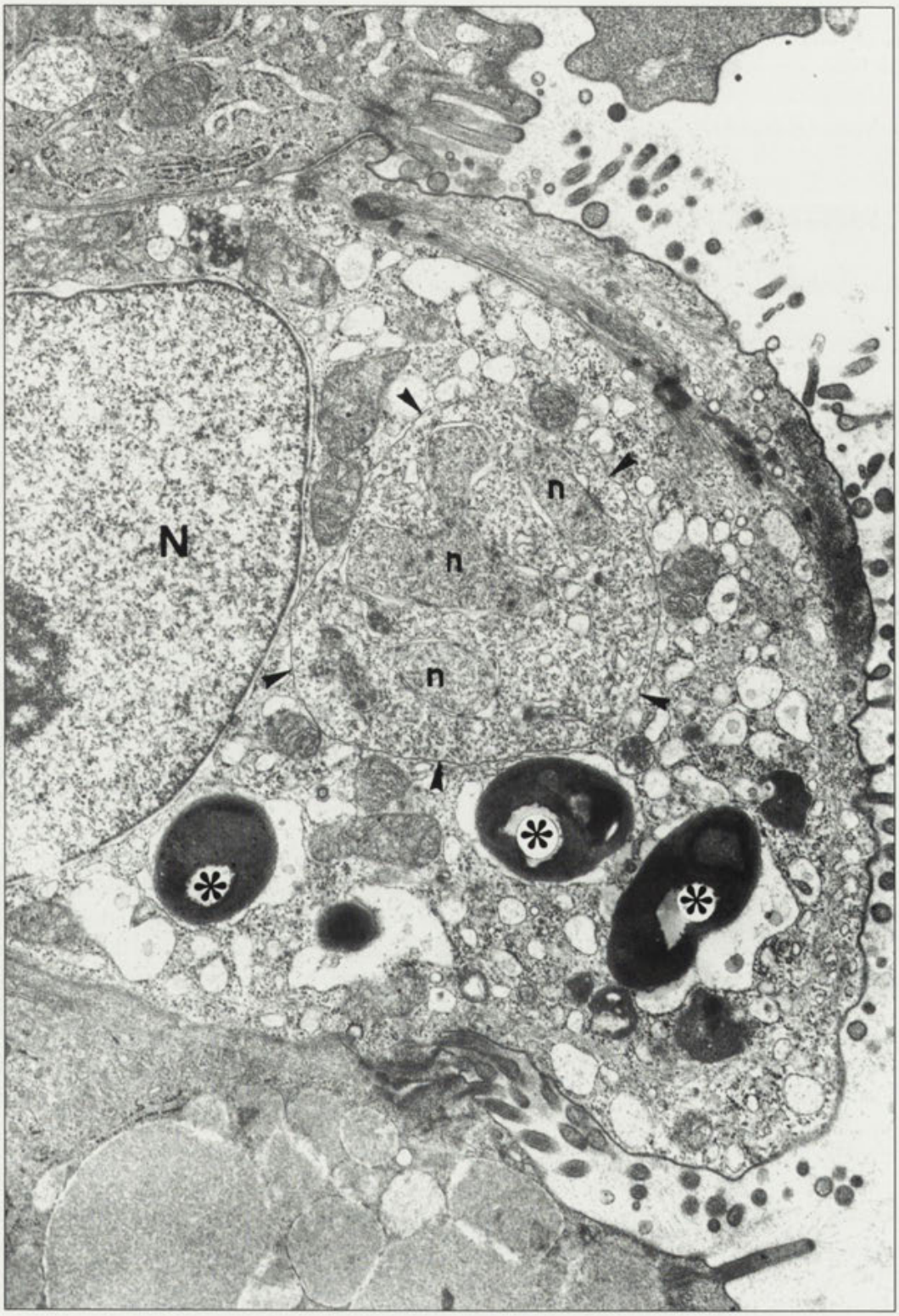

Fig. 1. *Enterocytozoon bieneusi* infection in a heart-lung transplant recipient. Different developmental stages of the parasite are seen in this section of a sloughing enterocyte: a plasmodial stage (arrowheads) containing mitotic nuclei (n) and mature spores (asterisks). N - nucleus of the enterocyte x 16500

third case concerned a patient with chronic myeloid leukemia who developed a pulmonary microsporidiosis after allogeneic bone marrow transplantation. The diagnosis was only made *post mortem*, and the species could not be identified (Kelkar *et al.* 1997).

IMMUNOCOMPETENT INDIVIDUALS

Ocular microsporidiosis

In immunocompetent individuals, microsporidia are known to cause infection of the cornea which is an immunoprivileged site. The first case of corneal microsporidiosis was published by Ashton and Wirasinha (1973). The patient was an 11-year old immunocompetent Tamil boy with prior experience of shock to the now infected eye. Some years later, a 26-year old Botswanan woman underwent enucleation of a blind and painful eye diagnosed as a perforated corneal ulcer caused by microsporidia (Pinnolis *et al.* 1981). The authors designated the responsible microsporidium as *Nosema* Naegeli, 1857 (Sprague 1977). Since no diplokaryotic nuclei were demonstrated and the description was too brief, these microsporidia were later assigned to the genus *Microsporidium* Sprague, 1977 by Canning and Lom (1986). They were named *Microsporidium ceylonensis* and *M. africanum* respectively. Recently, corneal tissues infected with *M. ceylonensis* were reprocessed for electron microscopy (Canning *et al.* in press). The ultrastructural study confirmed that spores were uninucleate and could not be assigned to the genus *Nosema*. In addition the polar tube was anisofilar. For that reason the species could not be placed in the genus *Encephalitozoon* although sporoblasts and spores appeared to be contained in parasitophorous vacuoles.

Additional cases of corneal stroma infections were described in two patients from South Carolina (Davis *et al.* 1990), and a parasite isolated by Shadduck *et al.* (1990) was named *Nosema corneum*. The same year, Bryan *et al.* (1990) published a case from Ohio and they considered the responsible microsporidium to be another species. It was designated as *N. ocularum* Cali *et al.*, 1991 based on the following characters: the size of the spore, the number of coils of the polar filament and the occurrence of diplokarya. Silveira and Canning (1995) studied the development of *Nosema corneum* and proposed a new genus, *Vittaforma* Canning and Silveira, 1995 due to its ultrastructural peculiarities. The name is thus *V. corneae* (Shadduck *et al.*, 1990) Canning and Silveira, 1995.

Interestingly, the keratitis reported in immunocompetent individuals appeared to be caused by species belonging to different genera, whilst most cases of ocular infections presented by AIDS-patients were caused by *Encephalitozoon* species. *E. hellem* Didier *et al.* 1991 was identified in well documented cases. However, *Trachypleistophora hominis*, the species causing myositis, has also been found to disseminate to the cornea in an AIDS patient (Field *et al.* 1996). The infection of the conjunctival and corneal epithelium is the cause of keratocunjunctivitis in all AIDS patients (Cali *et al.* 1991, Weber *et al.* 1994, Field *et al.* 1996) (Table 1).

Traveller's diarrhoea

The first documented cases were reported from Germany and concerned two immunocompetent subjects who were not infected with HIV. Microsporidia spores were detected in the stools of a 26-year old student who developed acute diarrhoea when returning from a 7-week journey through Egypt and Jordan. Ultrastructural study demonstrated a polar filament with the typical arrangement of *E. bieneusi* which consists of 6 coils in two layers (Sandfort *et al.* 1994). The second case was a 3-year-old Turkish girl living in Germany. She developed acute diarrhoea and vomiting after a holiday trip to Turkey where she had resided in a rural area. Spores of *E. bieneusi* were identified in the stool of the child who also was infected with *Cryptosporidium* (Sobottka *et al.* 1995).

Recently, a 15-year old girl returned to France with diarrhea after staying one year in Cameroon (Gangneux *et al.* 1997). Spores were abundant in the stool, and PCR-amplification showed that the species involved was *E. bieneusi*. In all immunocompetent patients described above the diarrhoea was self-limited and resolved without any treatment in a period of two to six weeks. *E. intestinalis* was also detected in fecal samples from diarrheic immunocompetent men who had travelled to Africa and Asia (Raynaud *et al.* 1998).

The cases of microsporidiosis presented by travellers suggest that these infections are endemic in tropical areas. So far, most studies aimed at the detection of microsporidia in patients with HIV infection. There is, however, some indication that these parasite also infect HIV-seronegative patients in subsaharian Africa. Bretagne *et al.* (1993) examined stools of a series of 990 children from Niger (Niamey) presumed to be HIV-seronegative. Of these children, eight were found to be infected with *E. bieneusi* which was associated with diarrhea in six of the cases. Similarly, stool samples from 176 rural children were

Table 1. Species causing ocular infections in HIV-seronegative and in HIV-seropositive humans

Species	Spore size in µm	Number of polar filament coils	HIV serology
Microsporidium ceylonensis Canning and Lom, 1986 (Ashton and Wirnasinha, 1973)	3.5 x 1.5	8-13	-
Microsporidium africanum Canning and Lom, 1986 (Pinnolis *et al.*, 1981)	4.5-5.0 x 2.5-3.0	11-13	-
Nosema ocularum Cali *et al.*, 1991 (Bryan *et al.* 1990)	5.0 x 3.0	9-12	-
Vittaforma corneae Silveira and Canning, 1995 =*Nosema corneum* Shadduck *et al.*, 1990 (Davis *et al.*, 1990)	3.7 x 1.0	5-6	-
Encephalitozoon hellem Didier *et al.*, 1991	2.0-2.5 x 1.0-1.5	6-8	+
Trachypleistophora hominis Hollister *et al.*, 1996	4.0 x 2.4	11	+

examined for microsporidia in Zambia and one sample was found to contain spores of *E. bieneusi*. The child was a 7-month boy, HIV-seronegative and immunocompetent with an asymptomatic infection (Hautvast *et al.* 1997). *E. bieneusi* was also found in three of eleven HIV-negative adult men from Mali. These patients suffered diarrhoea (Maiga *et al.* 1997).

Serological data

Most serological studies aimed for the detection of *Encephalitozoon* species. Indeed antigenic material is easily obtained by *in vitro*-cultivation of these parasites. The first study in this field was performed by Singh *et al.* (1982) in order to survey the prevalence of *Encephalitozoon* (*Nosema*) *cuniculi* in man and domestic as well as laboratory animals. Antibodies were detected by IFA-tests in persons living in tropical areas. Apart from any potential microsporidiosis, the subjects were exposed to filariosis in Malaysia, malaria in Ghana, and tuberculosis in Nigeria. These data suggested a correlation between the pathology of these diseases and a possible impairment of the immune system. Antibodies were also found in sheep and rabbits, and the highest titre came from a laboratory rabbit. There are no indications that the infection was anything but asymptomatic in both humans and animals.

A serological survey was undertaken in Sweden (National Bacteriological Laboratory of Stockholm, 1983) on a large number of sera from patients with different diseases. The results showed interestingly enough that 12% of the sera from Swedes returning from tropical areas reacted positively against *E. cuniculi* spores. It was proposed that the microsporidiosis could be a consequence of a defective immunity caused by malaria in those patients. Such an association between *E. cuniculi* infection and travelling in the tropics was later confirmed by Bergquist *et al.* (1984b). They studied the sera from Swedish homosexual men (a group at high risk for AIDS) and all patients seropositive to *E. cuniculi* had visited a tropical country.

Hollister *et al.* (1991) showed the high prevalence of antibodies to *E. cuniculi* in patients suffering from schistosomiasis, malaria and also from neurological disorders, but not in healthy people. The possibility that the impairment of the immune system caused by tropical diseases render the patients more susceptible to microsporidian infections was also considered by these authors. They

pointed out however, that this interpretation did not account for the infections detected in healthy visitors to the tropics.

A more recent study of van Gool *et al.* (1997) confirmed the occurence of *Encephalitozoon* infections in immunocompetent persons. Antibodies against *E. intestinalis* were found in 8% (24 of 300) of Dutch blood donors and in 5% (13 of 276) of pregnant French women. Only adults were found to have antibodies.

CONCLUDING REMARKS

Microsporidia appear to be relatively common in humans. The opportunistic character of infections caused by these parasites is confirmed by their pathogenicity in patients with AIDS and all other forms of immunosuppression including organ transplantation. Malnutrition is a cause of immunosuppression in children (Parent *et al.* 1994). Such an observation suggests that microsporidia may be also pathogenic in malnourished population. Significantly, *E. bieneusi* infection was asymptomatic in the immunocompetent Zambian child (Hautvast *et al.* 1997), and the diarrhoea caused by this species in immunocompetent travellers was self-limited and resolved without treatment (Sandfort *et al.* 1994, Sobottka *et al.* 1995). First serological data suggested that *E. cuniculi*, the species found in animals, also caused asymptomatic infections in immunocompetent humans. This interpretation should be ascertained since *E. cuniculi* crossreact with antigens from other species later found to infect humans (*E. hellem* and *E. intestinalis*). *E. cuniculi* has been however identified in several AIDS patients (Hollister *et al.* 1995, Didier *et al.* 1996, Field *et al.* 1996), and more interestingly, strains isolated from humans were infective in rabbits (Mathis *et al.* 1997). This study provides the first evidence that animals can be contaminated by humans and the results confirm the interspecific transmission of *E. cuniculi*. The detection of antibodies in a relatively high percentage of immunocompetent and apparently asymptomatic individuals, tends to indicate that microsporidia likewise *Toxoplasma gondii* could be the cause of latent infections. The finding of *E. bieneusi* and *E. intestinalis* in immunocompetent travellers and in HIV-negative individuals from African countries suggest the occurrence of reservoirs of microsporidian infections in tropical areas.

Undoubtedly, molecular methods contribute determinantly to increase our knowledge of microsporidia. Most fascinating are the conclusions of recent studies showing that the amitochondriate phylum Microspora, which was assumed to emerge at the base of the phylogenetic tree, appears to be a sister-group of fungi, both originating from a common mitochondriate ancestor (Germot *et al.* 1997). The isolation and identification of strains in man and in animals will provide information on the zoonotic or human origin of the microsporidian species found in man. In addition PCR amplification provides a powerful tool for large-scale epidemiological surveys.

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ACTA
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Divisional morphogenesis in *Blepharisma americanum*, *B. undulans*, and *B. hyalinum* (Ciliophora: Heterotrichida)

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Summary. Cortical development during cell division of *Blepharisma americanum* Suzuki, 1954, *B. undulans* Stein, 1867, and *B. hyalinum* Perty, 1849 was investigated using protargol impregnation and scanning electron microscopy. Stomatogenesis usually commences in a postoral kinety. Depending on species, 0-3 neighbouring kineties are involved in the anarchic field production. The anterior, non-proliferating portion of kinety 2 is frequently retained as shortened fragment right of the paroral, thus resembling the amphiparakinetal stomatogenic subtype found in stentorids and folliculinids. The oral anlage divides longitudinally to form the right paroral and the left adoral primordium. Differentiation of adoral membranelles proceeds, as is usual, from right to left and, unlike in all other ciliates with true adoral membranelles (hypotrichs, oligotrichs, tetrahymenid hymenostomes), from the centre towards the ends of the primordium. The paroral primordium of the opisthe generates the paroral and an apical, cirrus-like structure, as in *Condylostoma*, which is, however, resorbed when cytokinesis commences. Only the posterior (zigzag) segment of the parental paroral is reorganized. The adoral structures of the proter are maintained in *B. hyalinum*, but reorganized in *B. americanum* and *B. undulans*. The anterior and leftmost part of the adoral zone of membranelles are reorganized *in situ*, while about 5 proximal membranelles arise, possibly only partially, from a unique reorganization anlage developing on the vertex of the buccal cavity. The reorganization anlage was lacking in about one third of the dividers. The asymmetry of the blepharismid somatic infraciliature is caused by a proliferation gradient from anterior right to posterior left. The family status of the Blepharismidae and the subgeneric classification of *Blepharisma* based on nuclear configuration are not supported by the ontogenetic data available; rather blepharismids should remain in the Spirostomidae. Our study strongly suggests that the classical heterotrichs form a natural group distinctly different from other taxa presently assigned to the heterotrichs, such as clevellandellids, armophorids, licnophorids, and odontostomatids. Loxodid karyorelictids and heterotrichs cannot be founded as monophyletic group based on stomatogenic modes, which are markedly different, viz. buccokinetal and parakinetal, respectively. However, both have structures reminiscent to a scutica, viz. a postoral ventral kinetofragment in loxodids and a special reorganization anlage for the parental oral apparatus in heterotrichs.

Key words: ciliates, Heterotrichida, Karyorelictida, ontogenesis, phylogeny.

INTRODUCTION

Recent molecular and ontogenetic evidence indicates that heterotrichs are a melting pot of phylogenetically widely separated organisms. Hirt *et al.* (1995) and Foissner (1996) thus suggested that their complex mouth architectures and cortical ontogenetic processes should be reassessed. While detailed information is available for some heterotrichs *sensu stricto*, viz. *Climacostomum* (Dubochet *et al.* 1979), *Condylostoma* (Bohatier *et al.* 1976), and *Eufolliculina* (Mulisch 1987, Mulisch and Patterson 1987), such data are very incomplete in heterotrichs *sensu lato*,

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such as armophorids, odontostomatids, clevellandellids, and licnophorids (for references, see Foissner 1996). However, ontogenesis of some other classical heterotrichs, viz. *Blepharisma* and *Stentor*, has also never been investigated in detail, though some species belong to the favourites of microsurgeons (Suzuki 1957; Eberhardt 1961, 1962; Wilfert 1972; further references, see Frankel 1989) and comprehensive data are available on the nuclear apparatus during division, reorganization, and conjugation (Young 1939, Weisz 1949, Suzuki 1954, McLaughlin 1957, Tartar 1961, Giese 1973). Thus, we performed a detailed study on *Blepharisma*, using protargol impregnation and scanning electron microscopy. Three species with a different nuclear configuration were chosen to explore the macronucleus-based subgeneric classification proposed by Hirshfield *et al.* (1965). Furthermore, our study should set a solid basis for a detailed comparison of the ontogenesis in heterotrichs and karyorelictids, which are sister groups according to ultrastructural and molecular data (Gerassimova and Seravin 1976, Baroin-Tourancheau *et al.* 1992, Fleury *et al.* 1992, Hammerschmidt *et al.* 1996).

MATERIALS, METHODS AND TERMINOLOGY

Blepharisma americanum Suzuki, 1954 was found in the coastal rain forest near Punta Pirikkihi, about 54 km south of Limon, Carribean Sea coast of Costa Rica, Central America, W 82°40', N 9°40'. It occurred in a soil sample (pH 6) collected on 23.2.1991 on the bank of a small freshwater pond about 20 m distant from the sea shore.

Blepharisma undulans Stein, 1867 was found in an alluvial soil of the Tullnerfeld, Lower Austria [detailed site description in Foissner *et al.* (1985)].

Blepharisma hyalinum Perty, 1849 was found in a mixed forest on the Pfennigberg near Linz, Upper Austria. It occurred in oak litter (0-3 cm) collected on 28.4.1994. The rewetted litter had pH 5.5 ($CaCl_2$). *Blepharisma hyalinum* became abundant one week after rewetting.

All species were isolated from dried, rewetted material with the non-flooded Petri dish method as described by Foissner (1987). Pure cultures of *B. americanum* and *B. undulans* were set up with a few cells from the raw cultures and maintained as described by Lüftenegger *et al.* (1985). Results from *B. hyalinum* are based on raw cultures, where the specimens readily divided.

Species were identified according to Foissner (1989) and Foissner and O'Donoghue (1990). The infraciliature was revealed with protargol as described by Foissner (1991); procedure A was used for *B. undulans* and *B. hyalinum*, and procedure B for *B. americanum*. Preparation for scanning electron microscopy followed the technique described in Foissner (1991). Counts and measurements on silvered specimens were performed at a magnification of x 1000. Standard deviation and coefficient of variation were calculated according to statistics textbooks.

Terminology is according to Corliss (1979) and Foissner (1996). Kinety 1 (K1) is, as usual (Corliss 1979), that which first shows a distinct sign of kinetosome proliferation. However, a definite assigment of the kineties is frequently impossible because several kineties are involved in anlagen production and the number, arrangement, and length of the postoral kineties vary considerably. For example, of 44 early dividers investigated in *B. americanum*, kinety 1 abuts to the proximal oral vertex in 73 % or accompanies the paroral membrane for a short distance in 27%. In *B. hyalinum* even the first bipolar kinety right of the paroral may be sometimes kinety 1 (Fig. 50). Very likely, not a certain kinety but a narrow postoral field is morphogenetically active. Considering these uncertainties and that we could not find any explanatory power of numbering the kineties in *Blepharisma*, we did it only when needed for clarity.

We distinguish between heterotrichs *sensu stricto* (*s. str.*) and heterotrichs *sensu lato* (*s. l.*). The first mainly comprise those united by Stein (1867) and Kahl (1932) in the families Spirostomidae, Condylostomatidae, Stentoridae, and Folliculinidae. Heterotrichs *s. l.* include those later assigned to the heterotrichs by various authors, e.g. the families Clevelandellidae, Nyctotheridae, Sicuophoridae, Epalxellidae, Discomorphellidae, and Mylestomatidae.

RESULTS

Morphostatic cells

The morphology and particularly the infraciliature of *Blepharisma* species are quite uniform and well known (Foissner 1989, Fig. 1). Thus, we provide only a brief description of the key characters and detailed morphometrics (Table 1).

Blepharisma americanum has a moniliform macronucleus with 4-6, rarely up to 9 (Table 1, Repak *et al.* 1977), nodules, and thus belongs to the *Blepharisma s. str.* group according to Hirshfield *et al.* (1965). The terminal nodules are usually slightly enlarged, as in the Japanese type population (Suzuki 1954) and the Australian specimens studied by Foissner and O'Donoghue (1990). Young cultures of *B. americanum* are conspicuously red because the cells have red to pink stripes of cortical granules and a diffusely red-coloured cytoplasm. In aged cultures or in cultures kept in dark, the pigment granules become bluish, almost colourless. The oral apparatus occupies on average 49% of body length (Table 1). The anterior third of the paroral membrane consists of a line of closely spaced basal bodies, while the posterior two thirds consist of zigzagging dikinetids having only the right basal body ciliated. Single long fibres originate from the left basal bodies in the posterior third of the paroral membrane and extend over the buccal cavity to enter the cytopharynx as oral ribs (Fig. 3).

The population of *Blepharisma undulans* used in the present study has been described by Foissner (1989). Briefly, this species has a binodal macronucleus connected by a thin strand; thus it also belongs to the *Blepharisma*

Table 1. Morphometric characteristics from *Blepharisma americanum* (first line), *B. undulans* (second line; from Foissner 1989), and *B. hyalinum* (third line)[1]

Character	x̄	M	SD	SE	CV	Min	Max	n
Body, length	202.7	203.0	34.3	8.6	16.9	151	253	16
	149.4	145.0	13.0	3.4	8.7	133	175	15
	69.9	71.0	5.6	1.4	8.0	61	79	16
Body, maximum width	55.3	49.0	12.9	3.2	23.4	37	79	16
	49.8	49.0	7.5	1.9	15.1	35	63	15
	18.9	19.0	2.0	0.5	10.8	16	24	16
Anterior somatic end to proximal end of adoral zone of membranelles, distance	99.7	102.5	12.5	3.1	12.5	67	115	16
	61.5	61.0	6.1	1.6	9.9	52	75	15
	38.6	39.0	3.2	0.8	8.4	32	43	16
Anterior somatic end to macronucleus, distance	54.0	55.0	11.3	2.8	21.0	35	80	16
	37.7	40.0	5.5	1.4	14.5	30	48	15
	19.8	20.0	2.4	0.6	12.3	15	24	16
Base of longest adoral membranelle, length	5.6	6.0	0.5	0.1	9.2	5	6	16
	4.1	4.0	0.3	0.1	6.2	4	5	15
	3.6	4.0	0.5	0.1	13.8	3	4	16
Macronucleus or macronuclear nodules, length	16.3	16.0	4.5	1.1	27.7	11	27	16
	22.1	22.0	3.1	0.8	13.9	17	28	15
	14.6	14.5	2.4	0.6	16.2	11	19	16
Macronucleus or macronuclear nodules, width	12.5	13.0	2.6	0.6	20.4	8	16	16
	9.6	10.0	2.1	0.5	21.5	7	14	15
	6.2	6.0	0.7	0.2	12.1	5	8	16
Micronuclei, diameter	1.9	2.0	0.1	0.0	7.1	1.5	2	13
	1.7	1.8	0.2	0.1	12.6	1.4	2	15
	1.7	2.0	0.4	0.1	23.2	1	2	12
Macronuclear nodules, number	5.4	5.0	–	–	–	5	6	16
	2.0	2.0	–	–	–	2	2	15
	1.0	1.0	–	–	–	1	1	16
Micronuclei, number[2]	8.2	8.0	1.9	0.2	22.7	4	12	75
	6.6	6.0	1.2	0.3	17.9	5	9	15
	1.1	1.0	0.3	0.1	27.6	1	2	11
Somatic kineties, number postoral	25.8	26.0	1.2	0.3	4.5	24	29	16
	26.1	26.0	1.0	0.3	4.0	25	28	15
	13.4	14.0	0.8	0.2	6.0	12	14	16
Dikinetids in a right lateral kinety, number	100.1	96.5	17.4	4.4	17.3	78	134	16
	140.3	135.0	20.0	5.2	14.3	105	170	15
	46.9	47.0	6.8	1.7	14.4	34	58	16
Adoral membranelles, number	58.9	58.5	2.8	0.7	4.8	55	66	16
	58.4	59.0	2.9	0.7	5.0	53	63	15
	28.0	28.0	1.4	0.3	4.9	26	30	16

[1] Data based on randomly selected protargol - impregnated specimens from pure cultures in exponential growth phase (*B. americanum* and *B. undulans*) or from raw cultures (*B. hyalinum*). Measurements in µm. CV – coefficient of variation in %, M – median, Max – maximum, Min – minimum, n – number of individuals investigated, SD – standard deviation, SE – standard error of the mean, x̄ – arithmethic mean

[2] Due to the small size of the micronuclei in *B. americanum*, inferring with similarly coloured and sized cell inclusions, they were counted in early dividers

s. str. group according to Hirshfield *et al.* (1965). The cortical granules are pink to brick-red. The oral apparatus occupies on average 41% of body length (Table 1) and is structured as described in *B. americanum*.

Blepharisma hyalinum can be easily identified by its small size (about 70 µm), the colourless cortical granules and the single, ellipsoidal macronucleus characterizing the *Compactus* group (Hirshfield *et al.* 1965). The oral apparatus extends about 55% of body length (Table 1). The anterior segment of the paroral membrane, where the basal bodies are arranged in a simple line, is relatively shorter than in the other two species (Fig. 48).

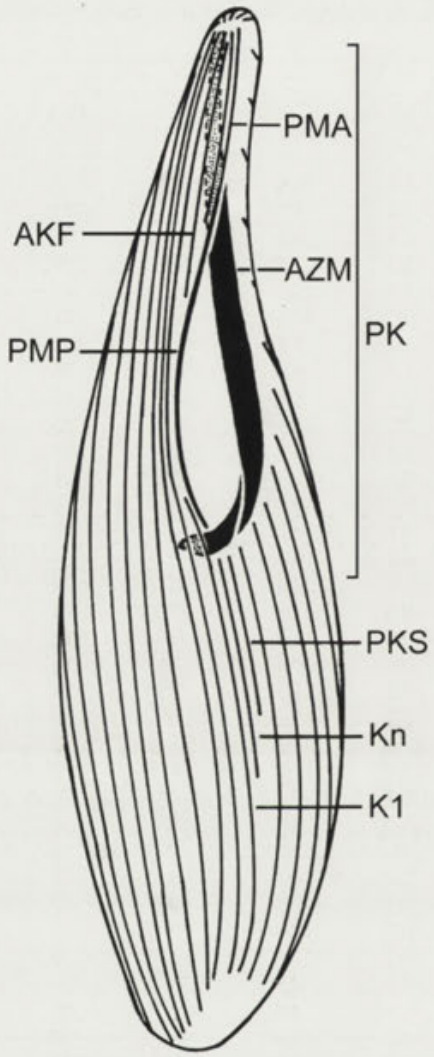


Fig. 1. Descriptive terminology of *Blepharisma*. As concerns K1 see Terminology section. It should be kept in mind that kinety 1 may abut to the proximal oral vertex (73 %) or may accompany the paroral membrane for a short distance as shown in this figure (27%; n - 44 early dividers). AKF - anterior kinety fragment, AZM - adoral zone of membranelles, K1 - stomatogenic (somatic) kinety 1, Kn - somatic kinety n, PK - postoral kineties abutting to posterior and left lateral margin of buccal cavity, PKS - shortened postoral kinety, PMA - anterior portion of paroral membrane composed of a line of single basal bodies, PMP - posterior portion of paroral membrane composed of paired, zigzagging basal bodies

Divisional morphogenesis

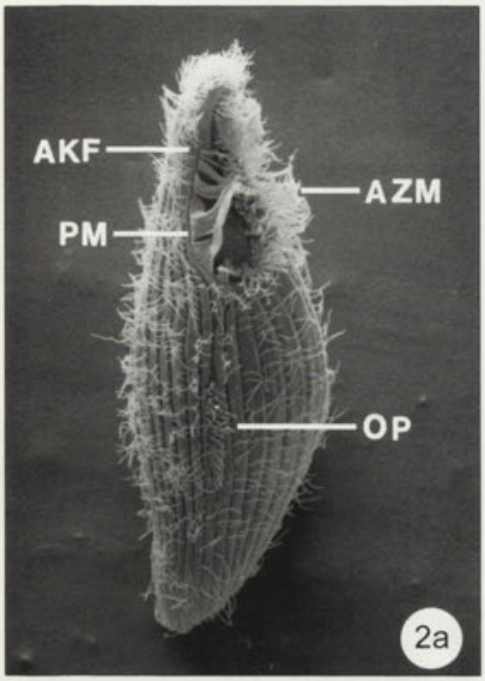
349 dividing specimens of *Blepharisma americanum*, 31 of *B. undulans*, and 46 of *B. hyalinum* were found in the protargol slides. Only few differences could be detected in the ontogenetic processes. Thus, observations and drawings from *B. americanum* (Figs. 2-4, 7-32, 35-47) and *B. hyalinum* (Figs. 33, 34, 48-54) also apply to *B. undulans* (Fig. 6), if not stated otherwise.

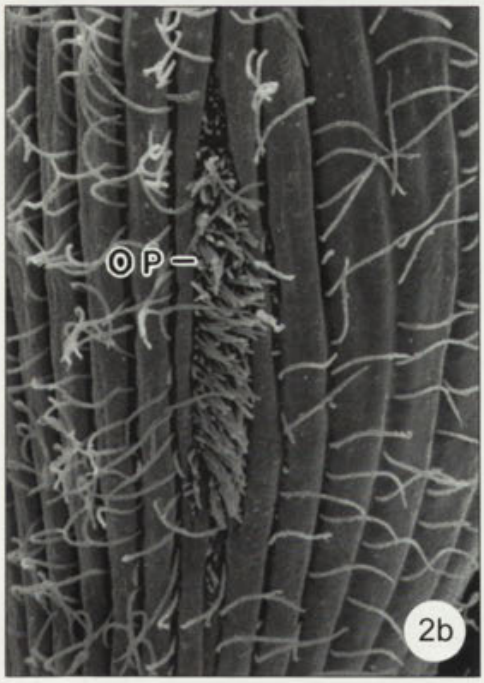
Opisthe stomatogenesis

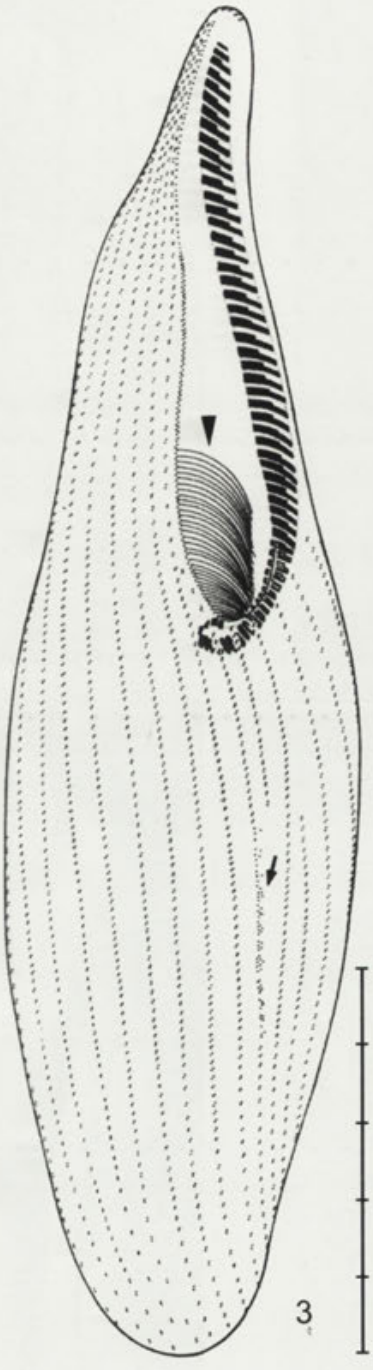
Stomatogenesis commences with the formation of some small groups of basal bodies within and to the left of the middle portion of a postoral kinety (kinety 1 per definition, but see Terminology ; Figs. 2, 3, 6, 15, 33, 48). Next, some neighbouring kineties proliferate basal bodies, at least one kinety but usually two kineties in *Blepharisma americanum* (Figs. 7, 8, 10, 16-18) and *B. undulans*, and rarely none usually one in *B. hyalinum* (Figs. 34, 50). In about 10% of the *B. americanum* specimens (n = 82) kinety n-1 shows some small groups of basal bodies, i.e. four kineties are involved in oral primordium formation. Proliferation proceeds in a sequential fashion, occurring first along "central" kinety 1 and then in kinety 2 and/or n (Figs. 15-17, 33, 34, 48, 50); kinety 2 produces basal bodies earlier than kinety n in 74% of cases (n = 69). Furthermore, proliferation occurs in an oblique fashion, i.e. kineties 2 and n proliferate slightly posteriad and anteriad, respectively, to the elongate field generated by kinety 1 (Figs. 7, 16-18, 34, 50). The elongate anarchic fields, which include the parental basal bodies, soon consist of unoriented kinetosomal pairs (cp. Bohatier 1979, Mulisch and Hausmann 1988), and finally fuse to form a single oral anlage, which progressively becomes larger by continued proliferation of basal bodies (Figs. 18, 51). The anlage develops in a flat groove, which originates by spreading of the furrows in which the stomatogenic kineties extend (Fig. 2b).

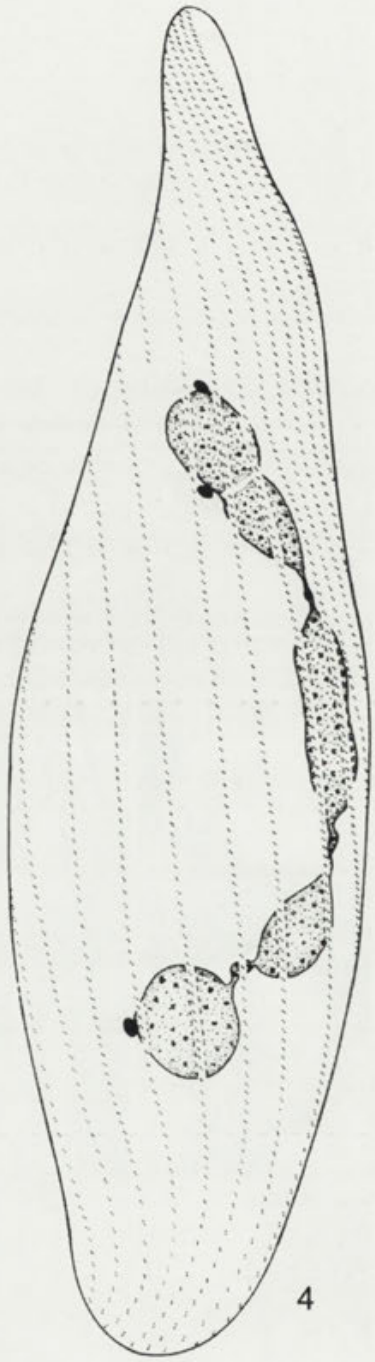
In the next series of events, the adoral membranelles and the paroral membrane are formed. The oral primordium splits longitudinally into two kinetosomal fields of unequal width: the left, which occupies two thirds of the width of the primordium, produces the adoral membranelles, while the narrow right field generates the paroral. Splitting commences centrally and proceeds towards the ends of the anlage (Figs. 8, 10, 18, 19, 51). Likewise, formation of membranelles begins by alignment of dikinetids in the middle part of the primordium and proceeds anteriad and posteriad as well as from right to left (Figs. 8, 10, 18-20, 37, 51, 52). The shortened third row of membranellar kinetosomes is added only when the posterior membranelles, which later form a short spiral, curve to the right (Figs. 11, 13, 21, 53).

The paroral primordium consists of dispersed and probably paired basal bodies (Figs. 8, 18, 19, 51). Subsequently, kinetosomes become more narrowly spaced and form a posterior segment consisting of obliquely (zigzag) arranged dikinetids and an anterior segment made of narrowly spaced kinetids in single file (Figs. 9, 11, 20, 21,

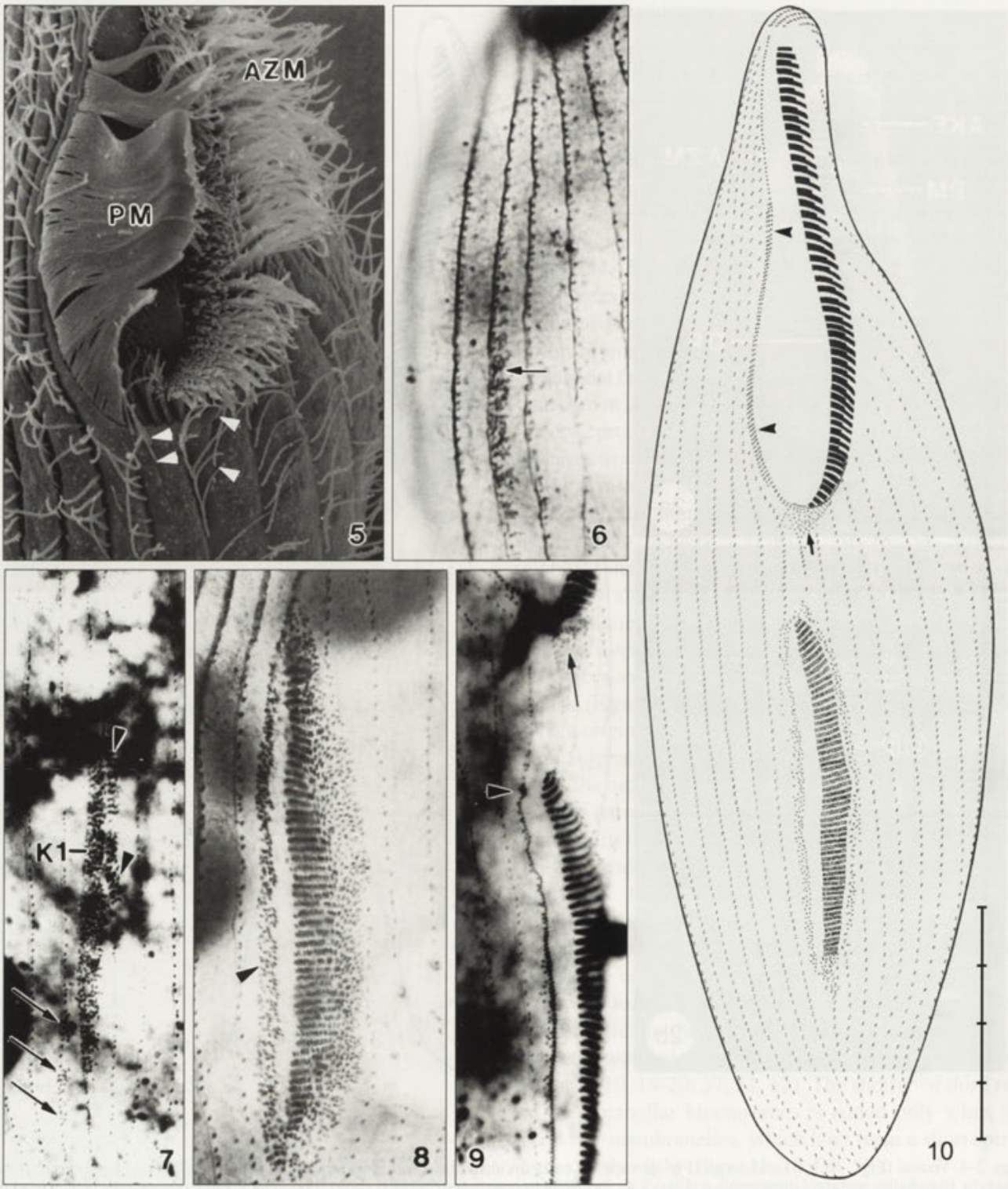




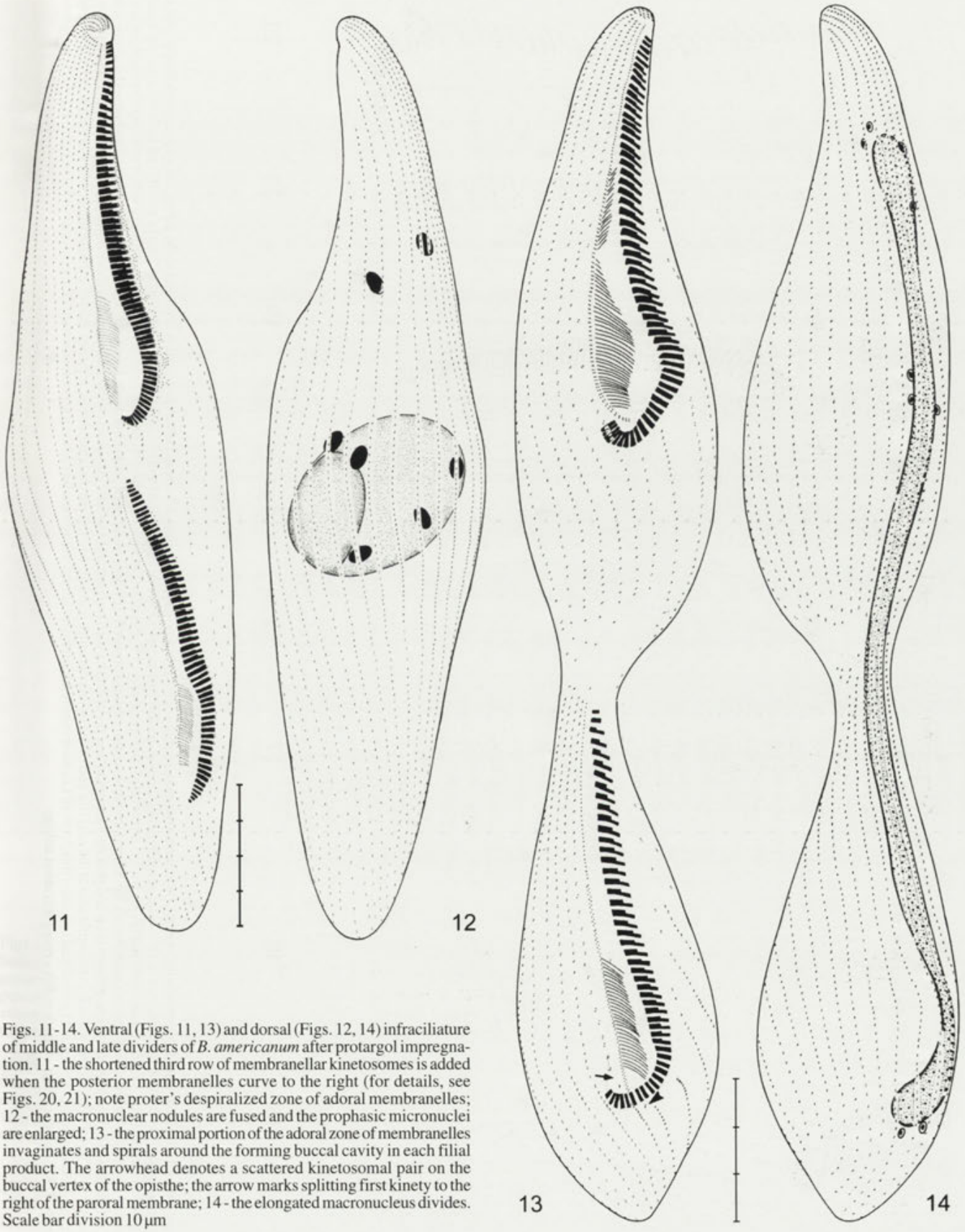




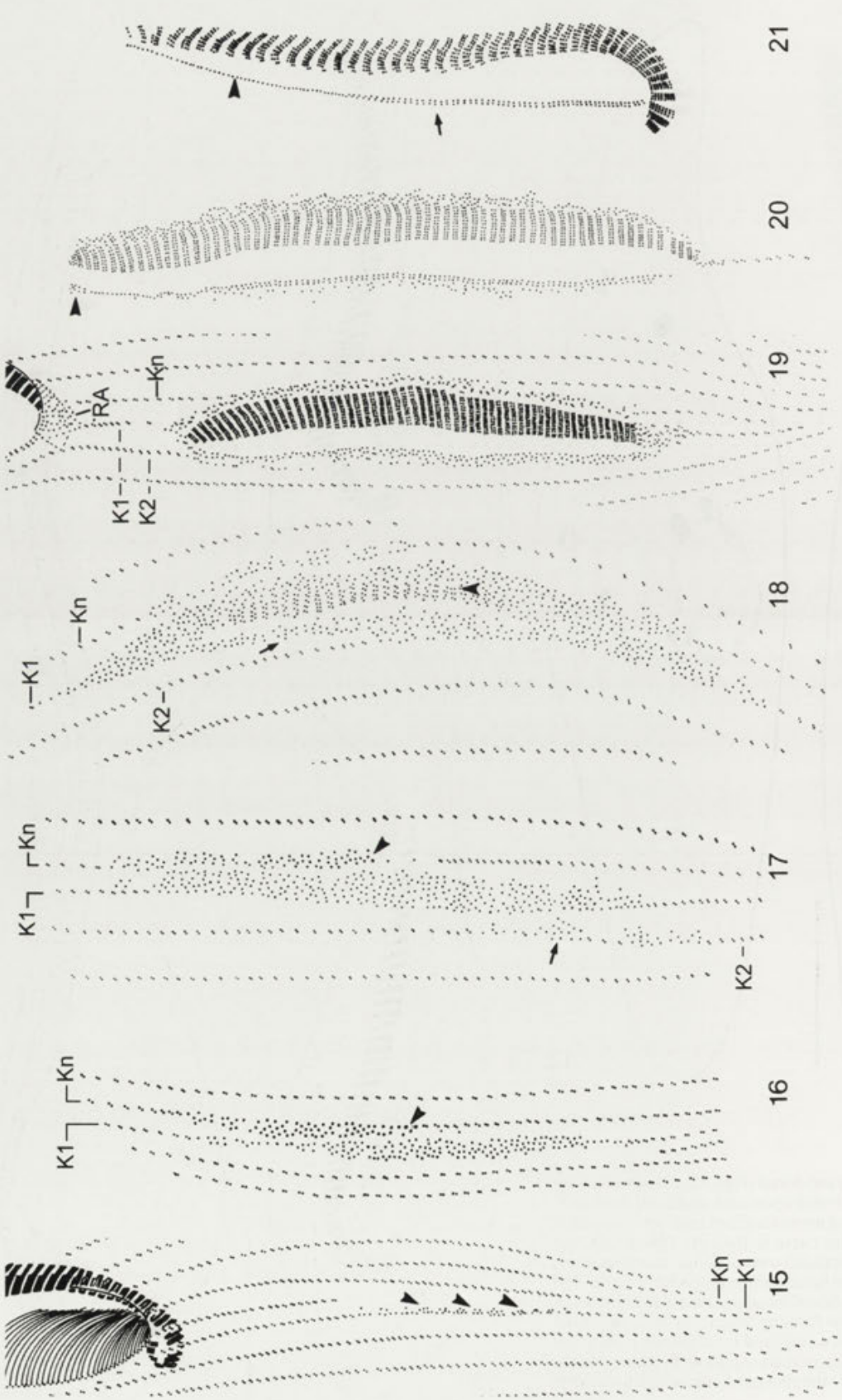
Figs. 2-4. Ventral (Figs. 2a, b, 3) and dorsal (Fig. 4) views of early dividers of *Blepharisma americanum* in the scanning electron microscope (Figs. 2a, b) and after protargol impregnation (Figs. 3, 4). 2a - *Blepharisma americanum* is about 200 µm long, has stripes of red cortical granules, and a moniliform macronucleus with 5 or 6 nodules (Fig. 4). The oral primordium for the opisthe originates subequatorially in a position distinctly apart from the parental oral apparatus; 2b - the oral primordium develops in a flat groove, which originates by spreading of the furrows in which the stomatogenic kineties extend; 3, 4 - ventral and dorsal infraciliature. The anterior third of the paroral membrane is composed of a line of closely spaced basal bodies, while the posterior two thirds consist of zigzagging dikinetids having ciliated only the right basal body. Long fibres (arrowhead) originate from the left basal bodies in the posterior third of the paroral membrane and extend over the buccal cavity to enter the cytopharynx as oral ribs (cp. Fig. 38). Stomatogenesis commences with a proliferation of small groups of basal bodies (arrow) within and to the left of postoral somatic kinety 1; note also the irregular kineties to the left. Some macronuclear nodules are fusing. Scale bar division 10 µm. AKF - anterior kinety fragments, AZM - adoral zone of membranelles, OP - oral primordium, PM - paroral membrane



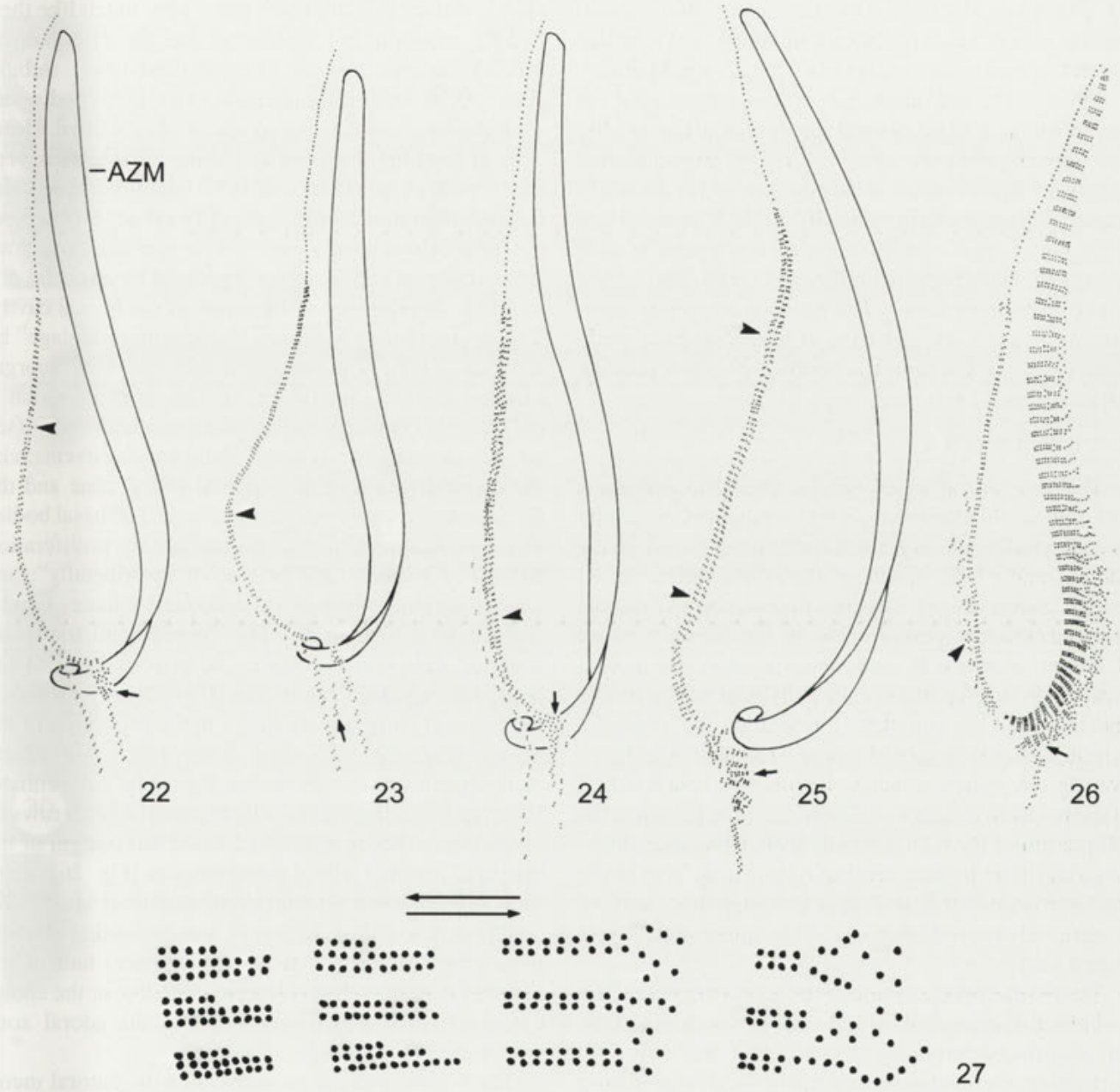
Figs. 5-10. Ventral views of a morphostatic specimen (Fig. 5) and of early (Figs. 6, 7) and middle (Figs. 8-10) dividers of *Blepharisma americanum* (Figs. 5, 7-10) and *B. undulans* (Fig. 6) in the scanning electron microscope (Fig. 5) and after protargol impregnation (Figs. 6-10). 5 - scattered kinetosomal pairs (arrowheads), which have ciliated only one basal body, occur along the buccal vertex; 6 - stomatogenesis commences in postoral kinety 1 (arrow); 7 - kinety 2 proliferates slightly posteriad (arrows) and kinety n slightly anteriad (arrowheads) to the elongated field of basal bodies in kinety 1; 8 - the oral primordium divides longitudinally to form a narrow right paroral (arrowhead) and a broad left adoral anlage; 9 - note cirrus-like patch of basal bodies (arrowhead) at anterior end of newly formed paroral. Arrow marks reorganization anlage of proter; 10 - differentiation of oral structures in the opisthe and reorganization of parental oral apparatus (cp. Figs. 8, 15-21). Arrowheads mark reorganizing paroral membrane; arrow denotes reorganization anlage. In this specimen, kinety 1 abuts to the proximal oral vertex, thus kinety 2 and n have contributed to the oral anlage. Scale bar division 10 µm. AZM - adoral zone of membranelles, K1 - stomatogenic kinety 1, PM - paroral membrane



Figs. 11-14. Ventral (Figs. 11, 13) and dorsal (Figs. 12, 14) infraciliature of middle and late dividers of *B. americanum* after protargol impregnation. 11 - the shortened third row of membranellar kinetosomes is added when the posterior membranelles curve to the right (for details, see Figs. 20, 21); note proter's despiralized zone of adoral membranelles; 12 - the macronuclear nodules are fused and the prophasic micronuclei are enlarged; 13 - the proximal portion of the adoral zone of membranelles invaginates and spirals around the forming buccal cavity in each filial product. The arrowhead denotes a scattered kinetosomal pair on the buccal vertex of the opisthe; the arrow marks splitting first kinety to the right of the paroral membrane; 14 - the elongated macronucleus divides. Scale bar division 10 μm



Figs. 15-21. Details of stomatogenesis in the opisthe of *Blepharisma americanum* after protargol impregnation. 15 - basal bodies originate within and to the left of the middle portion of postoral (stomatogenic) kinety 1 (arrowheads); 16, 17 - some neighbouring kineties proliferate basal bodies in a sequential and oblique fashion, i.e. kineties 2 and n proliferate later than kinety 1 and slightly posteriad (Fig. 17, arrow) and anteriad (Figs. 16, 17, arrowheads), respectively; 18, 19 (detail of Fig. 10) - the oral primordium divides longitudinally to form the narrow right paroral (arrow) and the broad left adoral anlage (arrowhead). Splitting commences centrally and proceeds towards the ends of the primordium. Likewise, formation of membranelles begins by alignment of dikinetids in the middle part of the primordium and proceeds anteriad as well as from right to left; 20 - kinetosomes of the paroral primordium become aligned and a cirrus-like patch of basal bodies (arrowhead) is recognizable at the anterior end of the newly forming paroral; 21 - the paroral membrane is composed of a dikinetidal posterior segment (arrow) and a monokinetidal anterior segment (arrowhead). A short third row of kinetosomes is added to the adoral membranelles when the posterior membranelles curve to the right. K1 - stomatogenic kinety 1, K2 - kinety 2, Kn - kinety n, RA - reorganization anlage



Figs. 22-27. Details of reorganization in the proter oral apparatus of *Blepharisma americanum* after protargol impregnation. 22, 23 - new basal bodies originate very near to the parental paroral membrane concomitantely at various positions (arrowheads). The reorganization anlage for the posterior adoral membranelles originates by proliferation of basal bodies (arrows) within (Fig. 22) and between (Fig. 23) the anterior ends of the postoral kineties; 24, 25 - by further proliferation of basal bodies quadruplets are formed, which separate into two rows of dikinetids. Splitting commences at several sites (arrowheads). The reorganization anlage comprises a small, triangular anarchic field of basal bodies, which abuts to the proximal end of the reorganizing paroral membrane (Fig. 24, arrow). Four to eight adoral membranelles differentiate from the reorganization anlage and replace the proximal parental adoral membranelles (Figs. 25, 26, arrows); 26, 27 - the right dikinetidal row is almost completely resorbed (arrowhead); the left one constitutes the new paroral membrane. The left, two-rowed part of the adoral membranelles is reorganized *in situ*, while the right portion remains unchanged, except of the shortened third row of basal bodies, which is resorbed (Fig. 27). The arrow marks the reorganization anlage, which replaces the posteriormost parental adoral membranelles. The reconstruction of the adoral membranelles takes place very much like their dedifferentiation, but *vice versa*, i.e. the disorganized kinetids arrange to rows and the third row is rebuilt. AZM - adoral zone of membranelles

52, 53). Furthermore, a cirrus-like patch of basal bodies is recognizable at the anterior end of the forming paroral (Figs. 9, 20, 53). This patch consists of 2-3 dikinetids and is resorbed when cytokinesis commences (Figs. 11,

13, 21). Some kinetosomes to the right of the renewed paroral membrane (Fig. 20) are obviously not used for paroral formation and are resorbed during cytokinesis.

Finally, the proximal end of the adoral zone of membranelles invaginates and spirals around the forming cytostome in about one turn (Figs. 13, 38). Some isolated kinetosomal pairs remain at the posterior end of the newly formed oral apparatus in about 40% of the *B. americanum* (Figs. 13, 41, 45-47) and *B. undulans* specimens; in most specimens they disappear during cytokinesis. These pairs, which were never seen in *B. hyalinum*, are highly reminiscent of a scutica and have at least one basal body ciliated (Fig. 5). The same can be observed in reorganizing proters (Figs. 42-44).

Proter reorganization

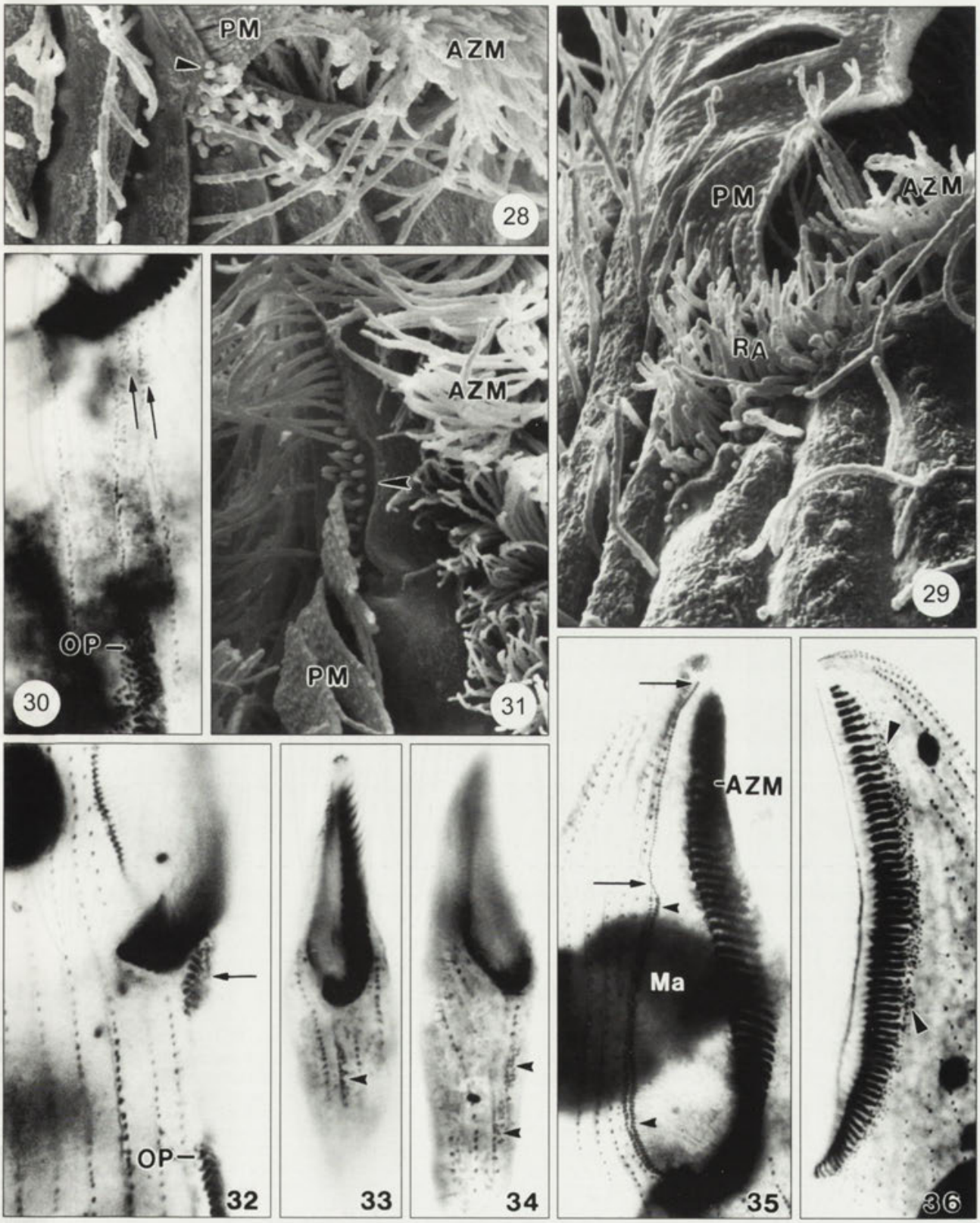
The reorganization of the parental oral apparatus differs in the *Blepharisma* species investigated, especially as concerns the adoral zone of membranelles. While the adoral zone of *B. hyalinum* shows no indication of reorganization, at least in the light microscope, two distinct reorganization processes occur in the adoral zone of *B. americanum* and *B. undulans*, viz. an *in situ* reorganization of the left portion of the individual membranelles and a partial or complete renewal of the proximal membranelles by a special reorganization anlage. However, the reorganization anlage is absent in about one third of the respective stages of *B. americanum*, i.e. when the left margin of the adoral membranelles becomes disordered and the cytopharyngeal spiral unwinds. Very likely, the same is true for *B. undulans*, although this could not be definitely proved because too few appropriate stages were found.

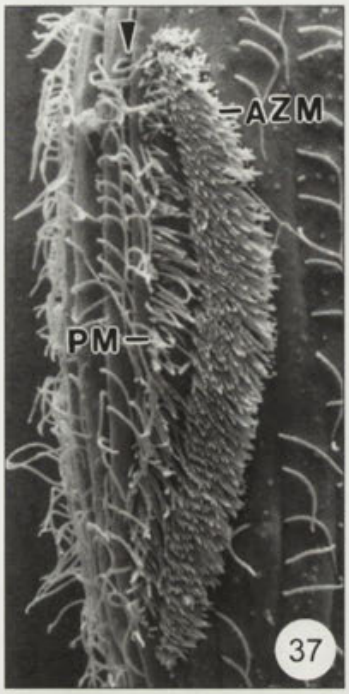
The *in situ* reorganization of the adoral membranelles includes a disorganization and fan-like spreading of the left, two-rowed part of the membranelles, while the right portion remains unchanged, except of the shortened third row of basal bodies, which is resorbed (Figs. 26, 27, 36). The reconstruction of the membranelles, which occurs when the reorganized posterior portion of the adoral zone spiralizes (see below), takes place very much like their dedifferentiation, but *vice versa*, i.e. the disorganized kinetids arrange to rows and the third row is rebuilt (Fig. 27). Whether parental and/or new basal bodies are used for the reconstruction could not be clarified. However, at least the third row of the membranelles is very likely made of newly formed basal bodies because kinetosomes migrating from left to right were never observed.

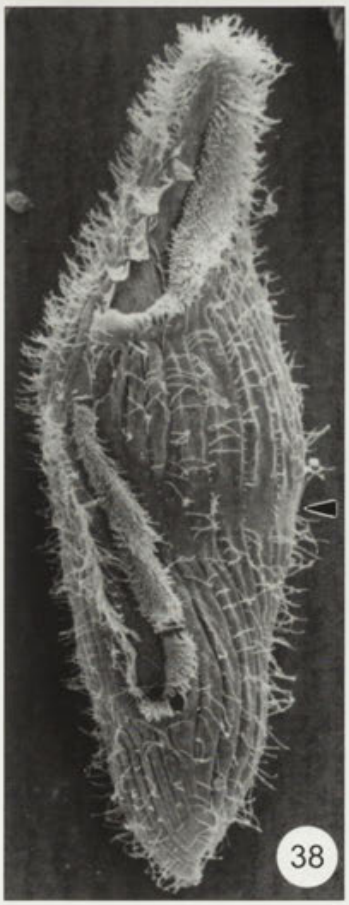
The proximal membranelles of the parental adoral zone are partially or completely reorganized by a special primordium developing on the vertex of the buccal cavity. This primordium was named "Regenerationsanlage" by Eberhardt (1961) and later, more appropriately, "reorganization anlage" (Suzuki 1973). The renewal, which is only loosely coupled with the opisthe stomatogenesis (see below), includes two concomitantly passing events, viz. the despiralization of the parental adoral zone and the formation of the reorganization anlage. The basal bodies of the reorganization anlage originate by proliferation within, to the left and between ("apokinetally"; see Discussion) the anterior ends of somatic kineties 1 and n (Figs. 9, 10, 22-24, 28, 30). They form a small, triangular anarchic field which abuts to the proximal end of the reorganizing paroral membrane (Figs. 24, 29). Probably, the parental paroral participates in the formation of the anlage (Figs. 24, 25, 28), from which 4-8 adoral membranelles or membranellar fragments differentiate. Whether these fragments, which consist of two rows of kinetosomes, become attached to the left margin of the proximal parental adoral membranelles (Fig. 26) or entirely replace some parental membranelles (Figs. 25, 32) could not be clarified. However, some scanning electron micrographs suggest that the right (inner) half of the parental membranelles is retained. Finally, at the end of cytokinesis, the posterior portion of the adoral zone spiralizes again (Fig. 13).

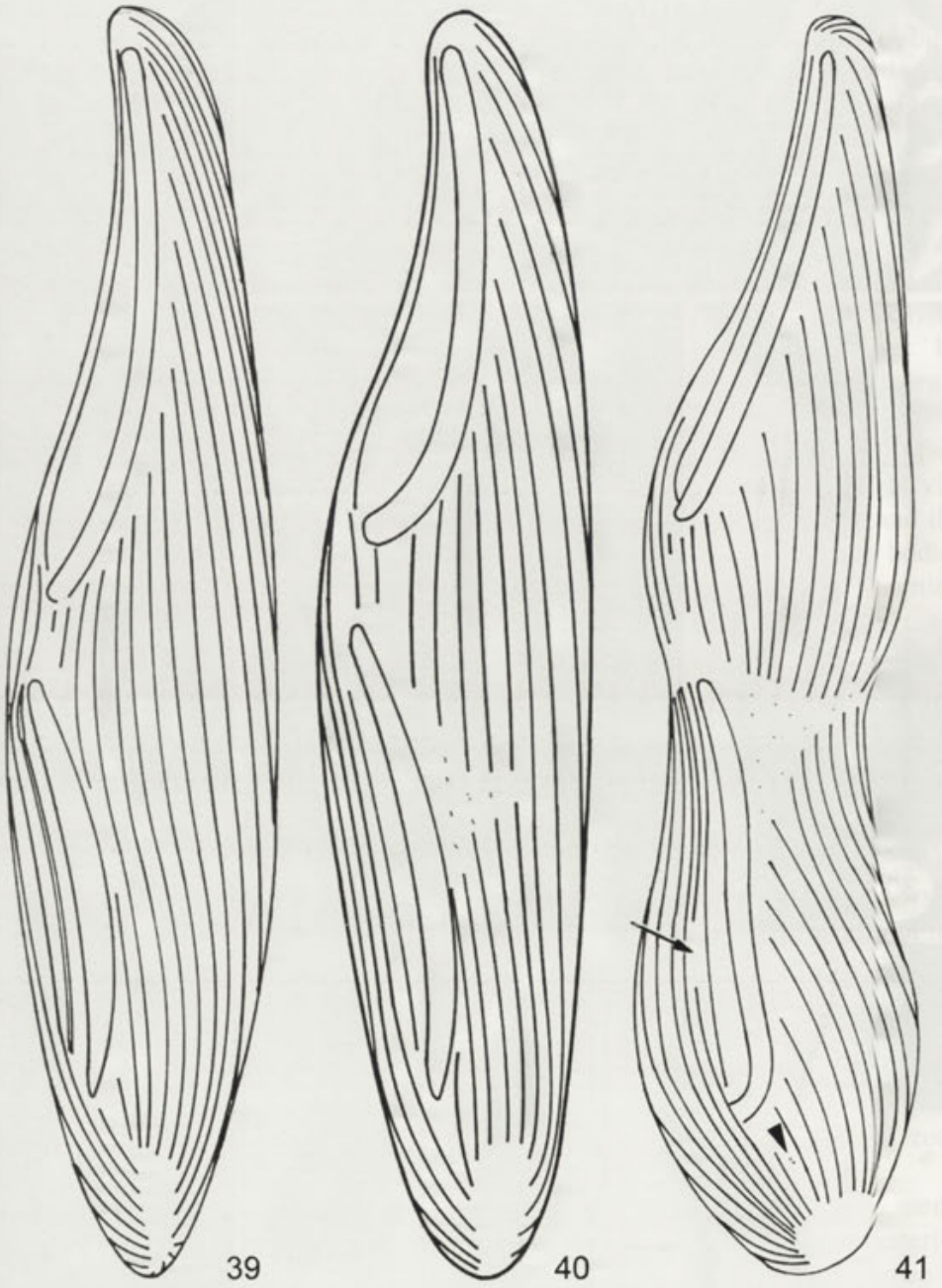
The posterior (zigzag) segment of the paroral membrane is reorganized in all species, while the anterior segment remains unchanged, except in one specimen of *B. americanum*, where it showed some disorder. The

Figs. 28-36. Reorganization of the parental oral apparatus in *Blepharisma americanum* (Figs. 28-32, 35, 36), and early dividers of *B. hyalinum* (Figs. 33, 34) in the scanning electron microscope (Figs. 28, 29, 31) and after protargol impregnation (Figs. 30, 32-36). 28, 29 - the reorganization anlage is a small, triangular field of ciliated basal bodies on the buccal vertex and usually very near to the parental paroral membrane (arrowhead in Fig. 28); 30 - new basal bodies (arrows) for the reorganization anlage originate by proliferation within and/or between the anterior ends of postoral kineties; 31 - the paroral primordium (arrowhead) originates very near to the parental paroral membrane; 32 - up to eight adoral membranelles (arrow) differentiate from the reorganization anlage; 33, 34 - one or two somatic kineties (arrowheads) proliferate basal bodies for the oral primordium in the opisthe of *B. hyalinum*; 35 - the quadruplets of the paroral primordium separate into two double-rows by longitudinal splitting (arrowheads). Note that the anterior, monokinetidal portion of the paroral membrane is not reorganized (arrows); 36 - the left part of the adoral zone of membranelles is reorganized *in situ* (arrowheads) and the third kinetosomal row of the adoral membranelles is resorbed (Fig. 27). AZM - adoral zone of membranelles, MA - macronucleus, OP - oral primordium, PM - paroral membrane, RA - reorganization anlage

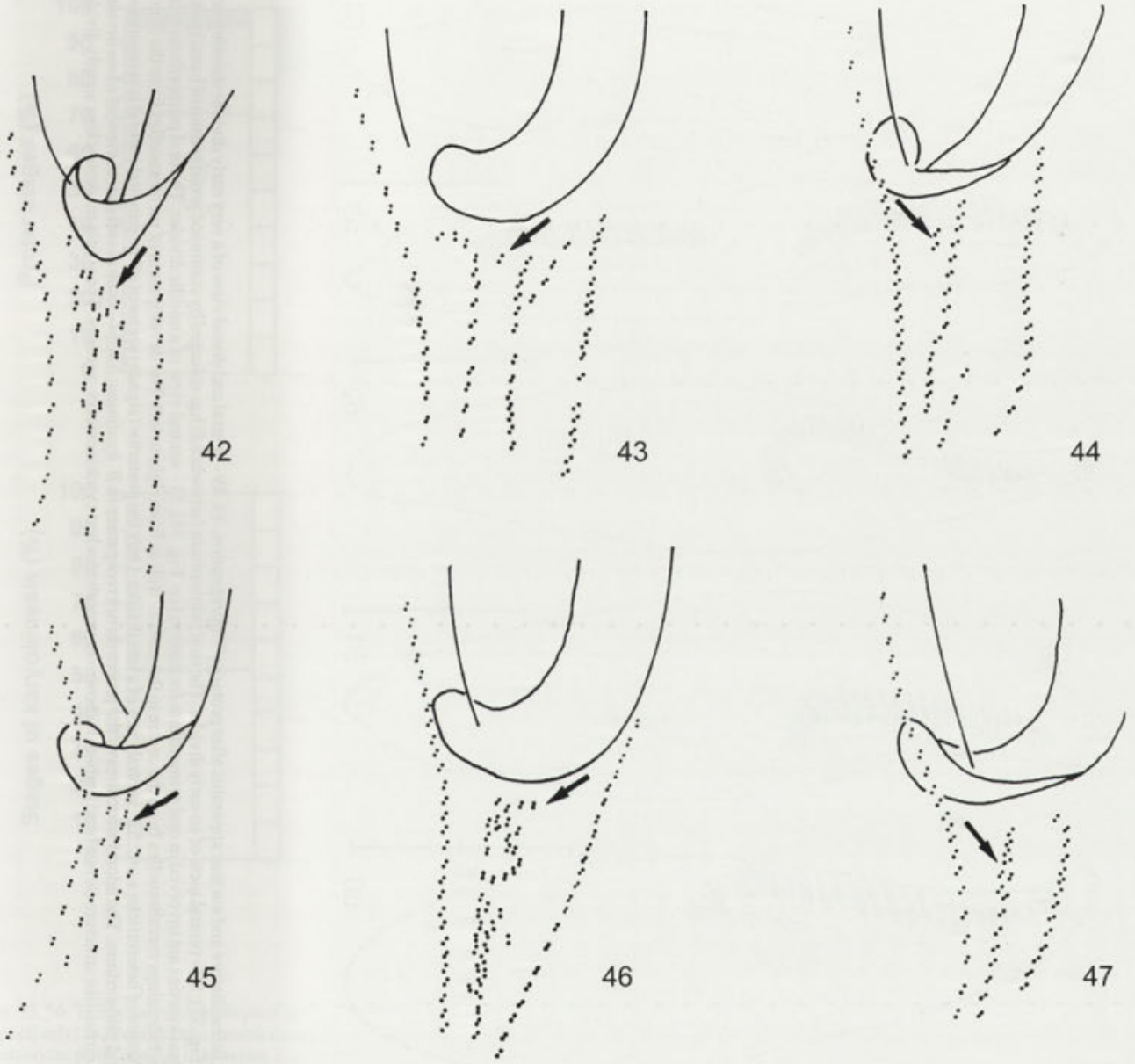






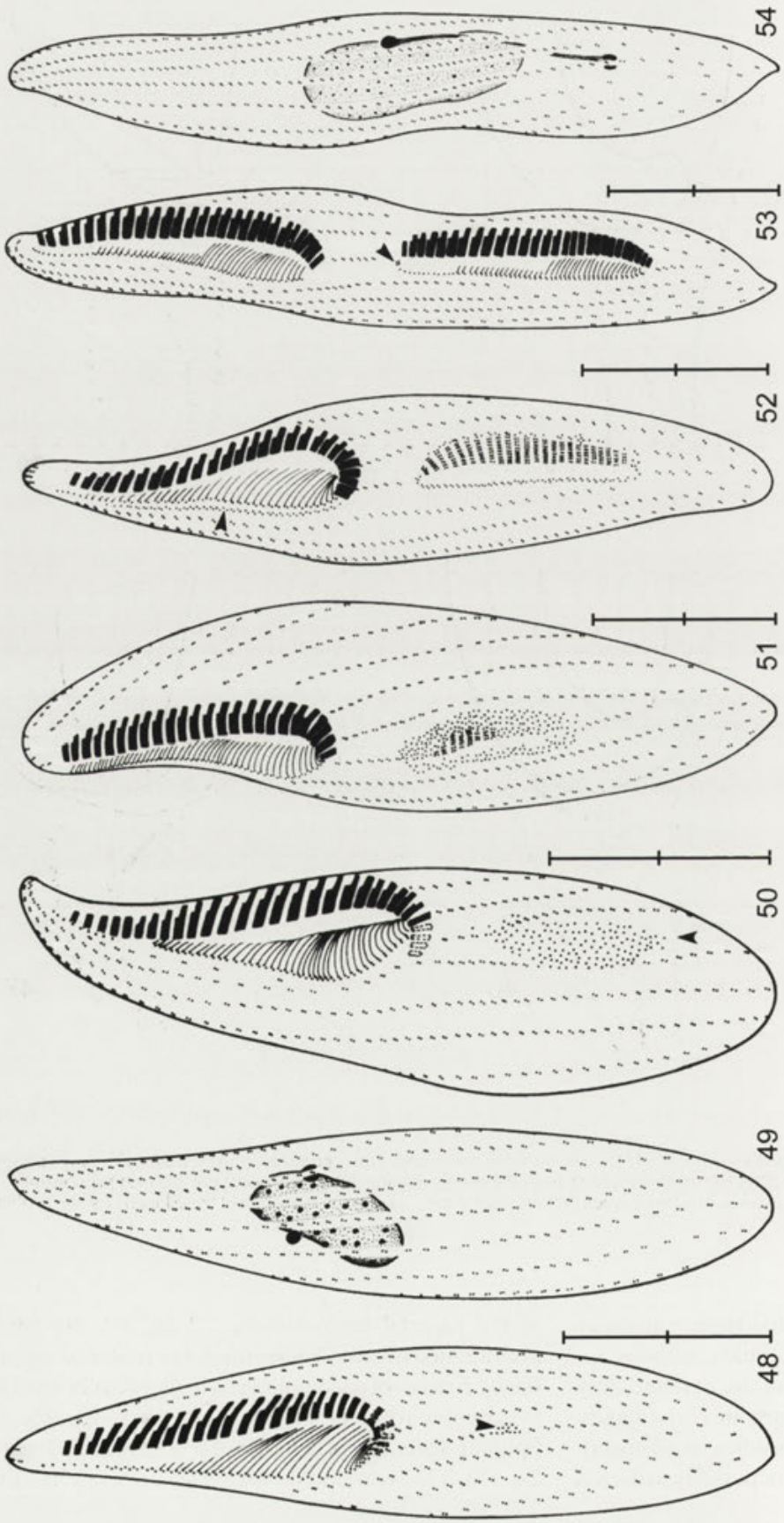


Figs. 37-41. Oral primordium (Fig. 37) and somatic division (Figs. 38-41) of *Blepharisma americanum* in the scanning electron microscope (Figs. 37, 38) and after protargol impregnation (Figs. 39-4). 37 - the anterior portion of stomatogenic kinety 2 (arrowhead), which proliferates only in the posterior region, does not disintegrate and remains as shortened fragment right of the oral apparatus; 38-40 - kinetiesat the left side of the oral primordium separate slightly subequatorially (arrowhead); 41 - during cytokiness the new anterior ends of the left lateral kineties abut to the new oral apparatus, while the rear ends are used in posterior pole formation of the proter. Note splitting (arrow) of the first bipolar kinety to the right of the paroral membrane, resulting in an anterior and a posterior segment, which usually becomes somatic kinety 1 in postdividers. A scattered kinetosomal pair is also visible (arrowhead). Note also that the short kinety fragment right of the paroral can be produced either by kinety 2 (Figs. 37, 41, 52, 53) or kinety3 (Figs. 9, 10, 19), depending on the number and extent of kineties involved in oral primordium formation (see Results)

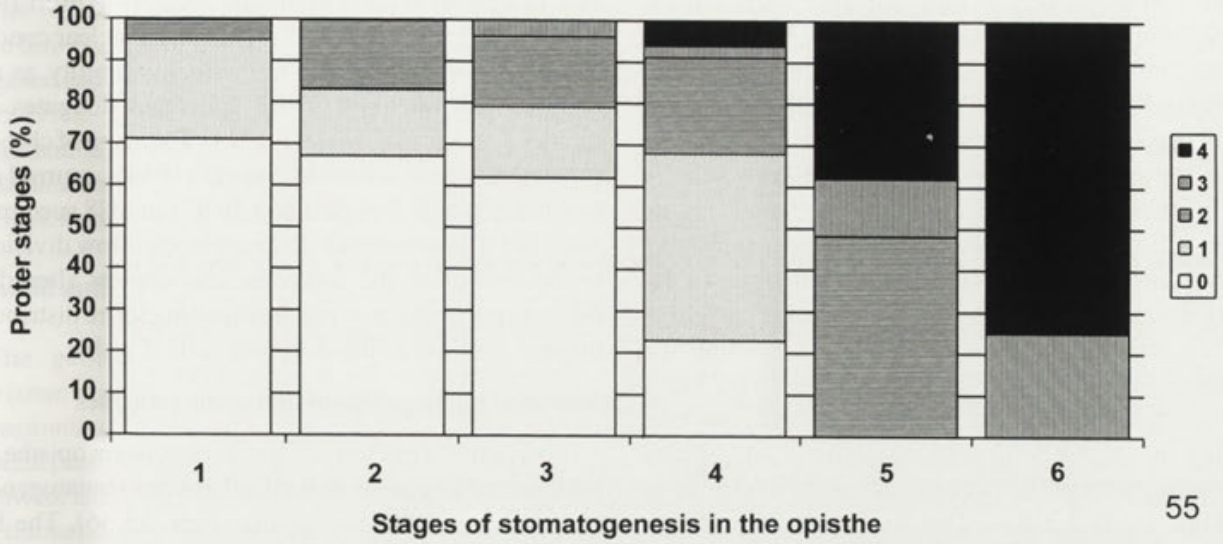


Figs. 42-47. Late proters (Figs. 42-44) and opisthes (Figs. 45-47) of *Blepharisma americanum* after protargol impregnation, showing variability in number and arrangement of kinetosomal pairs (arrows) remaining from the reorganization anlage, respectively, the oral primordium. The kinetosomal pairs, which are resorbed during cytokinesis, are between the postoral kineties. An exact designation of these kineties is impossible for the reasons explained in Terminology

exact sequence of proliferation of individual kinetosomes could not be clarified. However, we could clearly recognize the following details because of the extraordinary quality of the protargol preparations: in early stages, triplets and quadruplets of kinetosomes arise concomitantely at various positions within or possibly to the left of the parental paroral (Figs. 22-24, 31). By further proliferation of basal bodies the entire posterior segment finally consists of quadruplets and longitudinally splits into two double-rows of kinetosomes (Figs. 25, 35, 52). Splitting starts, like proliferation, at several sites (Figs. 10, 24, 35). The right double-row is successively resorbed



Figs. 48-54. Morphogenesis in *Blepharisma hyalinum*, infraciliature and nuclear apparatus after protargol impregnation. 48, 49 - ventral and dorsal view of a very early divider showing oral primordium (arrowhead) close to postoral kinety 1 (cp. Fig. 33); 50 - ventral view of an early divider. The oral primordium (arrowhead) has enlarged by continued proliferation of basal bodies and extends between kinety 1 and n. Usually, two postoral kineties are involved in oral primordium formation (cp. Fig. 34); 51 - ventral view of a middle divider. The oral primordium splits in a narrow right paroral and a broad adoral anlage, which develops membranelles from the centre to the ends as well as from right to left. The development of membranelles from the centre to the ends of the oral primordium is probably a unique feature of heterotrichs *s. str.*; 52 - ventral view of a late divider. Only the posterior (zigzag) segment of the parental paroral is reorganized; the arrowhead denotes resorbing remnants of the paroral primordium. The adoral structures of the proter do not reorganize in *B. hyalinum*; 53, 54 - ventral and dorsal view of a late divider. The paroral primordium of the opisthe shows an apical, cirrus-like structure (arrowhead), which is, however, resorbed when cytokinesis commences. The single macronucleus simply divides (Fig. 54). Scale bar division 10 µm



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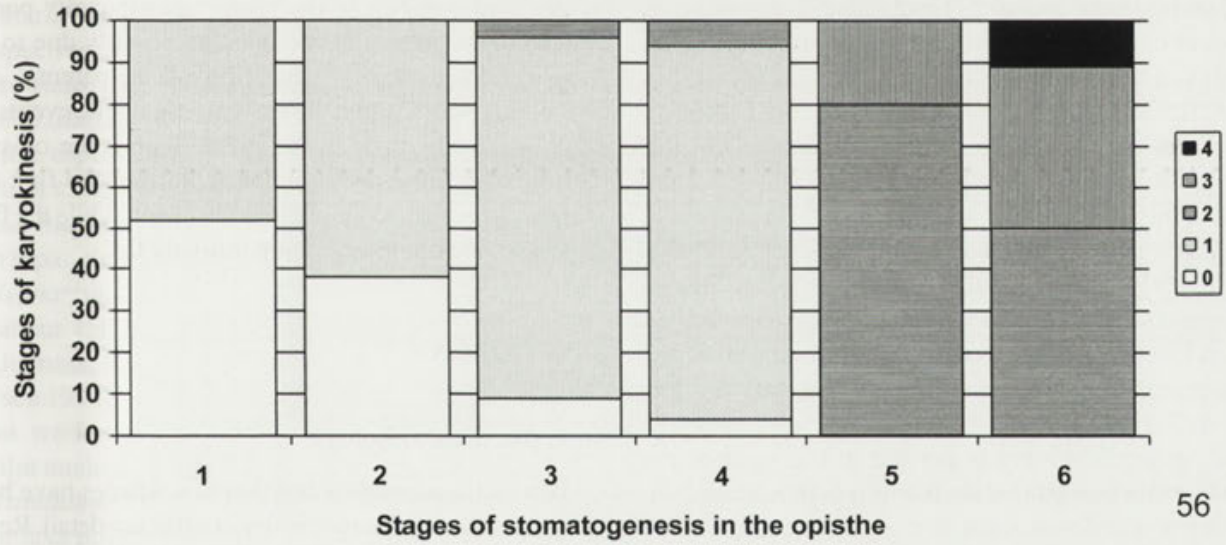

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Figs. 55, 56. Temporal relationships of divisional processes (proter and opisthe stomatogenesis as well as karyokinesis and opisthe stomatogenesis, respectively) in *Blepharisma americanum* (n=290). Stages of stomatogenesis in the opisthe: (1) kinetosome proliferation in kinety 1 (Fig. 15); (2) kinetosome proliferation in kineties 1 and 2 and/or n (Figs. 7, 16, 17); (3) separation of oral primordium in centre of anlage and onset of adoral membranelle formation (Fig. 18); (4) adoral membranelles consist of 2 rows of basal bodies and kinetosomes become more narrowly spaced in the paroral primordium (Figs. 8, 10, 19, 20); (5) third row of kinetosomes is added to the adoral membranelles; new paroral differentiated (Figs. 9, 21, 36); (6) spiralization of the posterior portion of the adoral zone of membranelles and of the paroral membrane. Stages of reorganization in the parental oral apparatus: (0) no reorganization events (Fig. 3); (1) kinetosome proliferation beside kinety 1 and/or the posterior segment of the paroral membrane (Figs. 22, 23, 30); (2) reorganization anlage consists of kinetosomal fields beside and between kineties 1 and n and/or the paroral primordium and the parental paroral are partially separated (Figs. 10, 24, 28); (3) triangular reorganization anlage, parental and newly formed paroral membrane completely separated, reorganization of adoral membranelles (Figs. 11, 25, 29, 35, 36); (4) resorption of parental paroral membrane, spiralization of posterior portion of parental adoral zone of membranelles (Fig. 26). Stages of karyokinesis: (0) no nuclear events (Fig. 49); (1) micronuclei prophasic and/or two or more macronuclear nodules fusing (Fig. 4); (2) micronuclei metaphasic and/or macronuclear nodules fused to an ellipsoidal mass (Fig. 12); (3) micronuclear division and/or one elongated (dividing) macronucleus (Fig. 14); (4) micronuclei and macronucleus divided

(Fig. 26); the left one constitutes the newly built paroral membrane. Oral ribs were always present, however, it could not be clarified whether they persisted or were cryptically reorganized (Figs. 11, 13, 35, 38, 52, 53).

Somatic division

Somatic division commences with an intrakinetal proliferation of basal bodies in some kineties right of the

anterior end of the oral primordium (Fig. 10). This is evident from the close spacing of the dikinetids in this section. The 6-10 kineties adjacent to the left side of the oral primordium do not proliferate, but gradually separate slightly subequatorially at the level of the differentiating opisthe oral apparatus (Figs. 11, 39, 40). The new anterior ends of these kineties converge to the new adoral membranelles during cytokinesis, while their rear ends are used in posterior pole formation of the proter (Figs. 13, 14, 38, 41). In contrast, the right lateral kineties of each filial product remain bipolar during division, except of the first unshortened kinety right of the paroral, which very likely splits during cytokinesis slightly above the cytostome, resulting in a rather long anterior segment and a short posterior segment which becomes K1 in the next generation (Figs. 13, 41, 52, 53). The first bipolar kinety right of the paroral may consist of kinety 2 or 3 depending on the extent of contribution of kinety 2 to stomatogenesis; it may even be different in a dividing cell, i.e. kinety 2 in the proter and kinety 3 in the opisthe (Fig. 53). Extensive proliferation of basal bodies occurs in the postoral kineties of the postdividers. This proliferation, which occurs intrakinetally and/or telokinetally, results in a gradual elongation of the postoral and left lateral kineties. Proliferation may be less intense in one or two postoral kineties resulting in some kind of postoral secant system, formed by short kinety fragments occasionally having a branched appearance in morphostatic specimens and early dividers (Figs. 1, 3, 5, 48).

One shortened kinety is present to the right of the paroral in 33% (n = 105) of the interphase specimens, two such fragments occur in 61% (Figs. 1, 5), and three in 6%. Obviously, at least one (the outermost) of these fragments originates by splitting of the first kinety right of the paroral, as described above. The other fragments, often consisting of 2-3 dikinetids only, are very likely remnants from previous generations, because the anterior portion of stomatogenic kinety 2 often does not disintegrate (Figs. 7, 11, 17, 18, 37, 53). This interpretation, i.e. that one of the fragments originates by splitting of the first unshortened kinety right of the paroral, is supported by the observation that kinety 3 is never (in 82 appropriate stages analyzed) involved in primordium formation; accordingly, only one fragment, that from kinety 2, can remain right of the oral apparatus.

Nuclear division

Nuclear fission matches literature data. The single macronucleus of *B. hyalinum* simply divides (Fig. 54). In the binucleate, respectively monilate species, the nodules fuse to a globular mass in middle dividers (Wilfert 1972; Suzuki 1973; Figs. 4, 12, 35). The macronuclear condensation shows considerable individual variability, as also observed by Suzuki (1973). The mass elongates and divides during cytokinesis (Fig. 14). Extrusion of chromatin material, as observed by Suzuki (1954), occurred also in our strain of *B. americanum*. In 67 out of 88 specimens (76%) of *B. americanum* the micronuclei show divisional processes before the macronucleus appears altered. In 69% of specimens (n = 16) the micronuclei are distributed unequally between the daughter cells (Fig. 14).

Temporal relationships of divisional processes

Fine-tuned temporal relationships between opisthe and proter stomatogenesis as well as between stomatogenesis and nuclear fission are lacking (Figs. 55, 56). The four stages distinguished in the proter are especially poorly related to the events in the opisthe, possibly due to the varying extent of reorganization of the parental oral structures. Specifically, the early stomatogenic events are often more advanced in the proter than in the opisthe. Timing of nuclear division, particularly of the first two stages, also varies to a considerable extent, while the final processes are obviously comparatively fast.

DISCUSSION

Interphase morphology

Few of the numerous *Blepharisma* species have been characterized morphometrically in sufficient detail. Repak *et al.* (1977) measured six limnetic strains of *B. americanum* and found great variability. Our population of *B. americanum* collected from soil, but maintained in pure "limnetic" culture for some years, showed the greatest mean length of all isolates measured so far (Repak *et al.* 1977, Foissner and O'Donoghue 1990, Table 1). This can partially be attributed to the impregnation method used (protargol procedure B, viz. Wilbert's protocol), which often causes some swelling of specimens. Such swelling possibly also accounted for the low number of dikinetids (about 100 *vs.* 173) in the right lateral kineties of our strain compared to that of the smaller Australian pond population studied by Foissner and O'Donoghue (1990). The number of adoral membranelles matched that of most strains studied by Repak *et al.* (1977), while it hardly corresponded to that of the Australian population (55-66 *vs.* 65-100), even showing distinctly different means, i.e. 59 *vs.* 81 (Foissner and O'Donoghue 1990, Table 1).

The soil population of *B. undulans* has, as compared to the limnetic isolates studied by Repak *et al.* (1977) and Dragesco and Dragesco-Kernéis (1991), a larger body size (149x49 *vs.* 111x26 *vs.* 123x33 µm) and more adoral membranelles (58 *vs.* 37 *vs.* 40). The morphometrics of *B. hyalinum* agrees well with that of another soil population investigated by Foissner (1989).

Familial and generic classification

The genera *Blepharisma*, *Anigsteinia*, *Parablepharisma*, and *Pseudoblepharisma* were separated from *Spirostomum* and *Gruberia* and united in a weakly founded [body pyriform or ellipsoid, somewhat anteriorly narrowed, laterally compressed; peristome on left margin; oral dikinetid (= paroral) forward of cytostome] family Blepharismidae by Jankowski in Small and Lynn (1985). Irrespective of the family characters recognized in heterotrichs, the overall similarities in the ontogenesis of *Blepharisma* and *Spirostomum* (Eberhardt 1962; present results), especially the occurrence of a particular reorganization anlage in the proter (very likely present also in *Pseudoblepharisma*, see Grolière 1977), do not support such separation. *Anigsteinia*, though superficially similar to *Blepharisma*, has a quite different nuclear division, viz. the about 150 macronuclear segments do not fuse to a single mass, but to 15-50 nodules dividing amitotically (Larsen 1994); unfortunately, cortical ontogenesis has, as far as we know, not yet been described in this genus. Similar nuclear processes were observed in *Blepharisma* (?) *candidum* (Yagiu and Shigenaka 1956). Therefore, these taxa possibly need a family of their own.

A main character for the intrageneric classification of *Blepharisma* is the shape of the macronucleus, which may be ellipsoidal, vermiform, binodal or multinodal. Such differences, which also occur in other heterotrichs like *Stentor* and *Spirostomum*, were used by Hirshfield *et al.* (1965) to split *Blepharisma* in the subgenera *Compactum*, *Filiformis*, *Halteroides*, and *Blepharisma*. This classification is not supported by our results, which show that, irrespective of the group the species belongs to, divisional processes are almost identical. The number of somatic kineties involved in the formation of the oral primordium is obviously rather variable and related to cell size. Moreover, the extent to which the parental adoral structures are reorganized is different in *B. hyalinum* (none) and *B. bimicronucleatum* (reorganization anlage; unpubl. observations), both belonging to the same (*Compactum*) group.

Comparative morphogenesis in *Blepharisma*

According to Sawyer and Jenkins (1977), the first sign of morphogenetic activity in *Blepharisma japonicum* was the appearance of branched kineties in the area directly subtending the oral apparatus. We also observed branch-like kineties in some dividers of *B. americanum* and *B. undulans*, but not in *B. hyalinum*; they were also absent in the *B. japonicum* population investigated by Dubochet *et al.* (1979), who mention some observations on this species in their paper on *Climacostomum virens*. However, a branch-like kinety pattern occurs also in interphase specimens (see section on somatic division and Foissner and O'Donoghue 1990) and results from previous divisions, where the postoral kineties became divided in more or less long anterior and posterior fragments, some of which did not proliferate basal bodies during and after cytokinesis (Figs. 1, 3, 38, 48, 53). Certainly, branching in the sense of Sawyer and Jenkins (1977) is not related to morphogenesis.

The oral anlage of the opisthe invariably appeared subequatorially in a position distinctly apart from the parental oral apparatus. Furthermore, all basal bodies for the oral primordium originated, depending on species, from one or more parental somatic kineties. Thus, stomatogenesis of *Blepharisma* perfectly matches the parakinetal mode as defined by Corliss (1979) and Foissner (1996). Depending on the number of postoral kineties involved in the formation of the oral anlage, a monoparakinetal (only one, the "director meridian" involved) and a polyparakinetal (two or more involved) subtype have been distinguished (Foissner 1996). The species investigated by us and *B. japonicum* (Sawyer and Jenkins 1977) basically belong to the polyparakinetal subtype, although only one kinety was often involved in the stomatogenesis of *B. hyalinum* and especially *B. bimicronucleatum* (Villeneuve-Brachon 1940 and own unpubl. data). Our observations confirmed Sawyer and Jenkins (1977) in that proliferation proceeded sequentially, occurring first along the centrally located postoral kinety and subsequently in the kineties to the left and right. The oblique proliferation, not mentioned by Sawyer and Jenkins (1977) and Eberhardt (1962), is, however, recognizable in their figures of *B. japonicum* and *B. americanum*, respectively.

Our Figs. 8, 10, 18, and 51 show convincingly that the differentiation of the adoral membranelles commences in the centre of the anarchic field and proceeds in an anterior and posterior direction as well as from right to left. Again,

this contradicts Sawyer and Jenkins (1977), who emphasized that membranelle formation occurred sequentially in an anterior-to-posterior direction in *B. japonicum*. Sawyer and Jenkins (1977) did not provide convincing evidence for their statement and, in our opinion, their Fig. 15 even shows that membranelle formation occurred as in our species. Thus, their assumption of a general gradient of membranelle formation in various groups of ciliates, is groundless. Likewise, Suzuki (1957) did not provide reliable figures for his statement that membranelles develop synchronically in the anarchic field of *B. japonicum*.

The parental oral apparatus of all *Blepharisma* species investigated so far was reorganized during cell division, but to a varying extent. An *in situ* reorganization of the adoral membranelles and a particular reorganization anlage were observed in *B. americanum* (Eberhardt 1962; present results), *B. undulans* (present results), *B. bimicronucleatum* (unpubl. observations), and *B. japonicum* (Sawyer and Jenkins 1977), but not in *B. hyalinum* (present results), at least at the light microscopic level; however, a reorganization anlage was lacking in about one third of the respective stages of *B. americanum*. The facultative appearance of this particular anlage is possibly related to the age of the oral apparatus, i.e. former proters will usually reorganize, while opisthes will not. This would roughly explain the observed ratio. The exact origin of the reorganization anlage was difficult to determine because of the underlying (darkly coloured) adoral membranelle spiral. Eberhardt (1962) and Sawyer and Jenkins (1977) proposed that it is generated by postoral kineties. We observed kinetosomal proliferation beside and between K1 and Kn (Figs. 9, 10, 22, 23, 30), as well as a contact of the anlage with the parental paroral membrane (Figs. 10, 22, 24, 28). We could not clarify whether the contact is due to simple spatial constraints or by a real contribution of the proter paroral to the reorganization anlage.

The posterior zigzag segment of the paroral membrane was completely reorganized in *B. americanum*, *B. undulans*, and *B. hyalinum* (Eberhardt 1962; present study). Sawyer and Jenkins (1977) did not mention such reorganization in *B. japonicum*, but some of their figures (Figs. 18, 19, 21) indicate that it occurred (cp. also Dubochet *et al.* 1979). The anterior portion of the paroral membrane remained intact in all species, as also mentioned by Dubochet *et al.* (1979).

We observed elimination of chromatin during macronuclear division of *B. americanum*, but not in *B. undulans* and *B. hyalinum*. It was reported to occur facultatively in *B. americanum* (Suzuki 1954) and obligatorily in *B. trinodatum* and *B. multinodatum* (Young 1939, Weisz 1949, McLaughling 1957).

Comparative morphogenesis in heterotrichs

Heterotrichs *s. str.* have a parakinetal stomatogenesis, i. e. the oral primordium evolves entirely from parental somatic ciliature. Recently, Foissner (1996) distinguished several parakinetal subtypes in heterotrichs, depending on the number of postoral kineties involved in oral primordium production, the formation of the peristomial ciliature, and the number of oral primordia produced. Most heterotrichs have a polyparakinetal stomatogenesis, i.e. more than one postoral kinety is involved in the formation of the oral anlage (Foissner 1996). The monoparakinetal subtype has been observed in *Blepharisma* spp., *Chattonidium setense* and *Spirostomum teres* (Villeneuve-Brachon 1940; present study). It occurs, at least initially, also in *Nyctotherus ovalis*, *Stentor niger*, and *Pseudoblepharisma crassum*, as well as in regenerating *Condylostoma magnum* (Dragesco 1966, Albaret 1975, Bohatier *et al.* 1976, Grolière 1977). However, it is frequently difficult to ascertain whether one or more postoral kineties are involved in oral primordiumformation, even if a fine-scaled analysis is performed, because the postoral kineties are often narrowly spaced and shortened fragments occur which may fuse with the oral primoridum. Our analysis of *Blepharisma hyalinum* and *B. bimicronucleatum* (unpubl. observations) showed that at least part of the specimens formed the oral primordium from a single postoral kinety. Thus, it is reasonable to assume that a true monoparakinetal stomatogenesis occurs in at least some heterotrichs.

Stomatogenesis is amphiparakinetal if the oral primordium intersects many postoral kineties at two sites (Foissner 1996), as, e.g., in *Fabrea*, stentorids and folliculinids. Accordingly, the oral primordium encloses few to many short, non-proliferating parental somatic kinety fragments, which become somatic ciliary rows on the peristomal field of the opisthe (Pelvat and Haller 1979, Foissner 1996). The oral primordium of *Blepharisma* spp. (present results) and *Pseudoblepharisma crassum* (Grolière 1977) resembles the posterior half of the stentorid primordium in that the anterior portion of stomatogenic kinety 2 is frequently inactive and retained (Fig. 18). Consequently, at least one (if two or three are present; remember that one kinety fragment originates by splitting of kinety 3; see Results) of the shortened kineties right of the paroral is usually a kind of intersected kinety and thus very likely homologous to the somatic peristomial ciliature of stentorids and folliculinids.

The discovery of a transient, cirrus-like structure at the anterior end of the developing paroral membrane was another surprising result of the present study. It is highly reminiscent of *Condylostoma*, which develops several such cirri and retains them as conspicuous ciliary tuft (Bohatier *et al.* 1976). Interestingly, the paroral membrane of *Pseudoblepharisma* and *Gruberia*, two *Blepharisma*-like heterotrichs, is entirely or partially composed of such kinetosomal groups (Grolière 1977).

Folliculinids have a biparakinetal stomatogenic mode, i.e. the proter and opisthe each form an oral anlage independently and amphiparakinetally. The proter anlage is very small and remains in a primordial stage, producing only few membranelles, which are resorbed when the swarmer rebuilds the oral apparatus from a large, normal oral primordium (Mulisch 1987, Mulisch and Patterson 1987). Thus, we aggree with Mulisch and Patterson (1987) that the proter reorganization anlage of *Blepharisma* and *Spirostomum* is likely homologous to the proter anlage occurring in folliculinids.

Another feature linking heterotrichs *s. str.* is the unique formation of membranelles, which proceeds from the centre towards the ends of the primordium. Mulisch (1987), working with the highly specialized folliculinids and having only the incorrect data of Suzuki (1957) and Sawyer and Jenkins (1977) for comparison, supposed that the peculiar adoral zone formation could be a derived trait related to the development of large peristomial wings. This is not supported by our results; rather, the special adoral zone formation, although widely unrecognized (e. g. Pelvat and Haller 1979, Dubochet *et al.* 1979), is likely a common feature of heterotrichs *s. str.* providing a superb autapomorphy for the group. Thus, *Phacodinium* and *Plagiotoma*, which form the adoral zone of membranelles from anterior to posterior, as do hypotrichs, oligotrichs, and hymenostomes, cannot belong to the heterotrichs, as frequently assumed (e.g. Small and Lynn 1985), but should be grouped with the hypotrichs, as also suggested by conventional morphological traits (Fernández-Galiano and Calvo 1992).

A further, admittedly rather general character linking the heterotrichs *s. str.* is the partial or complete reorganization of the parental oral apparatus during division, although data on this are still scant (Foissner 1996). Thus, it is not yet possible to find a meaningful route, if such exists, between families and genera.

In conclusion, numerous and close morphological and morphogenetic relationships exist between heterotrichs *s. str.*, i. e. spirostomid, stentorid and folliculinid ciliates, convincingly arguing for a common ancestor. These "classical" and mostly aerobic heterotrichs form a natural group distinctly different from other taxa, such as clevellandellids, armophorids, licnophorids and odontostomatids, presently assigned to the heterotrichs but showing quite different stomatogenic modes (see Table 2 in Foissner 1996, and References). This is also evident from recent molecular biological data (Hirt *et al.* 1995, Hammerschmidt *et al.* 1996).

Reorganization of the parental paroral membrane in *Blepharisma*: an exception from the rule?

Quite different modes of reorganization of the parental paroral membrane may proposed considering the data available and its diverse organization and degree of dedifferentiation during division. However, Eisler (1989) proposed a general mode for ciliates from very different systematic groups, viz. that the old left row is retained while the right row is newly formed, and that the process invariably involves a longitudinal splitting of the organelle. As concerns our data on *Blepharisma*, one of the reviewers made a very important comment, obviously refering to Eisler's general scheme: "If the paroral of *Blepharisma* is, in principle, organized like in most other ciliates, than proliferation of new kinetosomes automatically should take place towards the right and not towards the left as it is stated. That is due to the orientation of the kinetosomes with their postciliary fibres running to the left towards the cytostome and their direction of proliferation to the right. Therefore, if it would be true, that in *Blepharisma* an existing paroral double-row would produce a new double-row, this newly formed structure would be to the right of the old one and not to the left. But in most ciliates like many hymenostomes, scuticociliates, nassulids, and hypotrichs (for review see Foissner 1996) an existing paroral composed of dyads splits longitudinally during reorganization in the following manner: at first the anterior kinetosomes of the dyads separate from their posterior partners and move to the right. Then, at least the posterior kinetosomes of the dyads get new anterior daughter kinetosomes thus forming a new paroral for the proter. The fate of the former anterior kinetosomes of the dyads is rather different in different ciliate species, they may be resorbed like in *Tetrahymena* (Nelsen 1981), they may form a new kinety like in *Furgasonia* (Eisler 1989) or they may serve as oral anlagen for the opisthe like in scuticociliates (Grolière 1974), but in any case the former posterior kinetosome of the parental paroral dyads are also the posterior kinetosomes of the dyads of the new paroral for the proter. For this reason I cannot believe, that in *Blepharisma*, in contrast to all other ciliates investigated

so far, the parental paroral membrane proliferates towards the left, thus producing a complete new paroral and resorbing the whole old one." Certainly, this argumentation cannot be ignored. On the other hand, our preparations leave no doubt that not the inner left but the outer right row of dikinetids is resorbed. Obviously, transmission electron microscopic investigations are required to solve the problem. Generally, data are still very scant and easily over-interpreted. Eisler (1989), for instance, has given a schematic drawing of the development of the paroral membrane of the proter in *Paraurostyla weissei*, although in the original paper Jerka-Dziadosz (1981) has given only one electron micrograph (p. 89) showing an early stage of a "regenerating promer" (p. 88) and, more importantly, has stated that "Ultrastructural details of reorganization of the anterior preoral membranelles [= inner and outer paroral membrane] have not been followed in detail" (p. 85).

Phylogenetic affinities of heterotrich ciliates

Heterotrichs and karyorelictids represent the earliest branch on molecular ciliate trees (Greenwood *et al.* 1991, Baroin-Tourancheau *et al.* 1992, Fleury *et al.* 1992, Leipe *et al.* 1994, Hammerschmidt et al. 1996). Unfortunately, we know almost nothing about stomatogenesis in karyorelictids (Foissner 1996). However, very recently Bardele and Klindworth (1996) provided convincing evidence that *Loxodes striatus* has a buccokinetal stomatogenesis distinctly different from the parakinetal mode found in heterotrichs *s. str.* Thus, although ultrastructural and molecular characters indicate a close relationship between karyorelictids and heterotrichs (Gerassimova and Seravin 1976, Baroin-Tourancheau *et al.* 1992, Fleury *et al.* 1992, Hammerschmidt *et al.* 1996), they cannot be founded as a monophyletic group with the ontogenetic data available.

Our study suggests, however, that some buccokinetal remnants exist in the heterotrichs *s. str.*, viz. scattered postoral basal bodies (Figs. 5, 13, 41-47) resembling the scutica-like vestiges found in *Protocruzia*, *Loxodes*, and tetrahymenid hymenostomes (Grolière 1974, Puytorac *et al.* 1974, Grolière *et al.* 1980, Bardele and Klindworth 1996, Foissner 1996, and references therein). However, only 40% of our specimens had such scattered dikinetids, and at least some of these were ciliated (Fig. 5), indicating that it is not a classical scuticus, which usually lacks cilia and has a hook-like or whiplash configuration never observed in the *Blepharisma* spp. investigated. This also applies to the scutica-like vestige found in *Tetrahymena*. There is, however, an even more important difference between the classical "hymenostome" scutica and the vestiges mentioned above, viz. they are never included in the formation of the opisthe's oral structures; at best, they play some role in the reorganization of the parental oral apparatus, producing a few posteriormost adoral membranelles. On the other hand, the scutica of some typical scuticociliates, viz. *Paralembus* and *Pseudolembus* is rather inactive, i. e. produces only few oral structures (Grolière 1974).

A further similarity between *Blepharisma* (Figs. 10, 24, 25, 35, 52), *Tetrahymena* (Nelsen 1981) and typical scuticociliates (Grolière 1974) concerns the parental paroral membrane, which is reorganized by longitudinal splitting. However, the parental paroral remnants are completely resorbed in *Blepharisma* and *Tetrahymena*, while they form a major portion of the opisthe oral anlage in the scuticociliates, *viz.* the paroral and the scutica (Grolière 1977, Puytorac *et al.* 1974). This corroborates previous suggestions (Foissner 1996, and References) that the stomatogenic function of the paroral kinety was secondarily transferred to the first somatic (postoral) kinety. Accordingly, the so-called "director meridian" (stomatogenic kinety 1) may be considered a strongly modified scutica or *vice versa*. *Loxodes striatus* would then represent a perfect transitional step because its postoral ventral kinetofragment of 8-10 barren dikinetids is positioned and behaves like a scutica, i. e. is involved in stomatogenesis of the opisthe (Bardele and Klindworth 1996); unfortunately its origin is still unknown.

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An Attempt to Induce Stable Serotype Transformation in the Ciliate *Dileptus anser* with Homologous Immune Serum

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Summary. Effects of homologous immune serum on serotype of the ciliate *Dileptus anser* were studied. The cells were treated for 1 h with γ-globulin fractions of antisera to subclones of two different *D. anser* clones. Each of the subclones was cultivated at 17 or 25°C. The serotype was determined by the standard immobilization reaction of the ciliates treated with an immune serum as well as by an immunofluorescent staining of the cells. It was found that after a short treatment of the ciliates with homologous antiserum there was a gradual change of the cell serotype: during the first 4 days, fraction of cells with original serotype decreased, whereas the increasing number of cells appeared which had a new serotype. Subsequently, the culture gradually restored its initial serotype. The ciliates with such a temporarily appearing serotype responded to the immune serum against the subclone cultivated at the alternative temperature. Most likely, the serotype that appeared after the short treatment with the homologous antiserum and the serotype that appeared as a result of the temperature-induced transformation are the same. No other serotypes were detected after the treatment of the ciliates with homologous immune sera used. Thus, the short-term treatment of the *D. anser* clones studied with homologous antisera resulted only in a temporary change of their serotype; it was impossible to obtain cell lines with the permanently transformed serotype.

Key words: ciliates, *Dileptus anser*, homologous immune serum, immobilization reaction, indirect immunofluorescence, serotype transformation.

Abbreviations: AmD - Actinomycin D, IS - immune serum.

INTRODUCTION

Mechanisms controlling activity of genes for surface proteins, both under normal conditions and during the so-called serotype transformation, seem to be of the greatest interest in the ciliate serotype systems. These mechanisms are the subject of numerous investigations (for the current status of the problem and the main literature, see Bleyman 1996). Nevertheless, the mechanisms responsible for the principle of the "mutual exclusion" of surface antigens remain so far unknown. According to this principle (which is very characteristic of such systems), only one of a series of genes encoding alternative surface proteins is expressed in the given cell at the given moment of time. This principle is closely connected with the phenomenon of serotype transformation, i.e. a "switch-over" of activity from one gene to another (and, accordingly, a replacement of one cell surface protein with

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another), spontaneously or, more often, after some cell treatments. This is often accompanied by phenomenon of "functional inertia" of serotypes, their tendency for inheritance in a series of cell generations. As a result, several different serotypes can be maintained in the clone under the same cultivation conditions (Nanney 1980). Thus, the systems of serotypes in ciliates still remain a very interesting subject of investigation on regulation of gene expression both at the cellular and molecular levels.

It should be emphasized that despite the recent great achievements in molecular-biological studies on the surface antigens and their coding genes in ciliates (Preer 1968, Schmidt 1996), no satisfactory explanation has been offered for the above-mentioned fundamental peculiarities of the systems of serotypes. Moreover, the numerous and various data obtained so far used, with rare exception, only 3-4 species from the *Paramecium aurelia* complex and, to a lesser extent, several other *Paramecium* species and *Tetrahymena thermophila* (Hausmann and Bradbury 1996). This undoubtedly makes it difficult to distinguish general and special phenomena and regularities and stimulates studies using new ciliate species.

The present study is based on the data that are obtained on paramecia and indicate that the serotype transformation can be induced in these ciliates by their short treatment with a homologous antiserum at a non-lethal concentration. Meanwhile, analysis of the extensive literature on this problem confirms the conclusion made by G.H. Beale in 1954: "It is not possible to generalize on the chances of obtaining antigenic transformations by serum treatment" (Beale 1954). Therefore, we considered it worth-while to try exploring this effect on the studied *Dileptus anser*.

MATERIALS AND METHODS

The experiments were carried out on two *D. anser* clones (# 6 and # 5F) that had no serological cross-reactions. Subclones # 6-17, # 6-25, # 5F-17, and 5F-25 isolated from these clones were cultured using a conventional technique: in Prescott's saline, with *Tetrahymena pyriformis* GL as a food (Nikolayeva 1968), at 17°C and 25°C, respectively. Immune sera (IS) IS 6-17, IS 6-25, IS 5F-17, and IS 5F-25 were raised to ciliates of the above subclones. To prepare antigen, ciliates from a mass culture of a large volume, with the cell density about 100 cells/ml, were taken one day after the last feeding. The cells were washed with a sterile medium, separated by centrifugation at 1.5 g for 5 min, and their approximate number in pellet was determined. Immediately before immunization, they were disrupted by pipetting. Protein content was determined using the biuret reaction.

Rabbits were immunized using a schedule of 6 intravenous injections of the corresponding antigen preparation (Sonneborn 1950a). During the whole immunization cycle, approximately 3 mg of protein were administered. Prior to the immunization, normal, preimmune sera were obtained from the same rabbits. These sera were used as controls. Native sera, as well as their γ-globulin fractions obtained by precipitation with 45% saturated $(NH_4)_2SO_4$ were used. The precipitate was washed with buffer solution (0.14 M NaCl in 0.01 M KH_2PO_4; pH 7.4), dissolved in Ringer's solution and dialyzed against the buffer solution.

To find working concentrations of immune sera, survival and multiplication rates were determined in dilepti treated with antiserum diluted to 1:100, 1:500, 1:1000 or 1: 2000 for 1 or 2 h and then washed with fresh culture medium.

The division rate of the ciliates treated with serum was recorded in 50 individual lines for 7 to 8 days. The number of cells that had appeared during the previous day in each line was counted daily. The number of cell divisions occurred was calculated from the mean number of cells in all 50 lines. Then one cell in each line was left for subsequent cultivation.

Survival of dilepti after treatment with the serum at various dilutions was estimated at the end of the experiment from the fraction of individual lines that survived by this time (their initial number usually was 100).

To induce serotype transformation, the ciliates were incubated with the antiserum (which will be called the "inducing" antiserum) for 1 h and then transferred to the usual medium and cultivated as a mass culture (subcloning of cells treated was not performed in these experiments). After this treatment, serotypes of the treated and control ciliates were tested daily for 6-7 days by the standard method, an immobilization of cells with immune sera (Beale 1954); these will be called the "testing" sera. For this test, 5 cells from the subclone tested were placed to each of 10 microaquaria containing equal volumes of the diluted "testing" serum. Thus, the initial sample of cells amounted to 50. In 24 h, the number of immobilized cells was counted in each microaquarium, and the results were summed up. Each series of experiments was performed in triplicates.

Apart from the immobilization experiments, serotypes of ciliates, after treatment with "inducing" homologous serum, were also tested by indirect immunofluorescence method (Coons 1956). Cells were fixed with 96° ethanol for 15-20 min at room temperature and allowed to dry. The fixed cells were treated with the "testing" immune serum in a moist chamber for 30 min at 37°C, washed with the buffer solution and treated with FITC-conjugated goat anti-rabbit IgG (Sigma). After the 30 min incubation, these preparations were washed with the buffer solution, dehydrated, mounted in glycerol and examined under a luminescent microscope (Lumam TM-1, USSR). Intensity of fluorescence was evaluated visually.

Experiments with Actinomycin D (AmD, Sigma) were performed as described earlier (Uspenskaya and Yudin 1996).

RESULTS

Selection of the working concentration of "inducing" immune sera

First of all, it was necessary to determine which antiserum dilution can be used to induce transformation of

the ciliate serotype, i.e. to find such a minimal dilution of the immune serum that the ciliates treated with this serum would suffer as little non-specific damage as possible. Therefore, we first evaluated effects of different concentrations of homologous and heterologous immune sera on survival and division rate of dilepti.

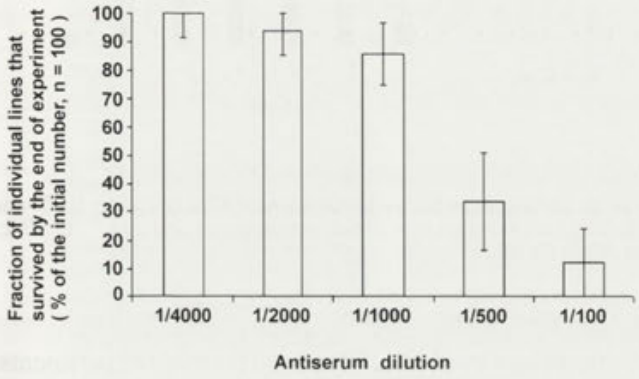

Fig. 1. Effect of homologous immune serum on *Dileptus anser* survival (subclone # 5F-25). Bars on this figure and on the following ones are 95% confidence intervals

In experiments with the subclone # 5F-25 (Fig. 1), the homologous antiserum diluted to 1:100 or 1:500 turned out to be of no use, after the treatment with the antiserum at such concentrations for 1-2 h and subsequent washing, most of the cells (80-90% and 60-70%, correspondingly) remained motionless and died during the next 3-4 days. When dilutions 1:1000 or 1:2000 were used, motility of the treated ciliates after their washing was restored very soon, and only 10-15% of the cells died. No visible effect of the antiserum diluted to 1:4000 was detected.

The division rate of dilepti (Fig. 2) decreased markedly after the treatment of the cells with the homologous serum diluted to 1:500. It never reached its initial level throughout the entire observation period, and subsequently these ciliates died. The dilutions 1:1000 and 1:2000, on the contrary, were not toxic for the cells: they restored very quickly their division rate to the nearly control level.

Therefore, the serum dilutions 1:1000 and 1:2000 were concluded to be the maximal sublethal concentrations of the sera and to be able to affect serotype without any serious non-specific damage of the ciliates. As shown in subsequent experiments, only these concentrations of immune sera did produce the serotype change-over, whereas higher serum dilutions (1:3000 or 1:4000) did not.

Similar tests were performed with preimmune and heterologous immune sera. It was found that treatment of

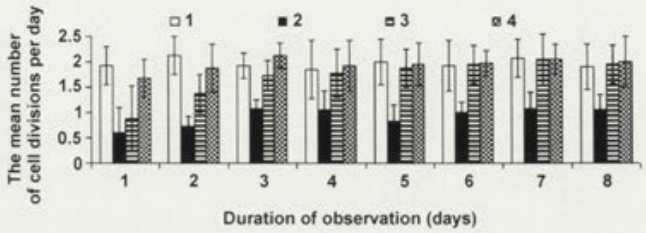

Fig. 2. Effect of homologous immune serum on *Dileptus anser* division rate (subclone # 5F-25). Serum dilutions: 1-control (without serum), 2-1:500, 3-1:1000, 4-1:2000

the ciliates with the heterologous serum at 1:1000 or 1:2000 dilution for 2-3 h with a subsequent washing had no effect on the survival and division rate of the cells during the next 5-6 days. Meanwhile, if undiluted or slightly diluted sera were used, the division rate decreased dramatically, and the delayed death (on the 6th or 7th day) of 70-80% cells occurred.

From these observations, two working dilutions (1:100 and 1:500) were chosen for preimmune and heterologous immune sera, to provide maximum concentrations of the sera which did not still produce any non-specific deleterious effects on the cells (their survival, multiplication rate, etc.).

The effect of "inducing" homologous sera on serotype of ciliates

Several types of these experiments were performed. Firstly, two subclones of the clone # 6 and two subclones of the clone # 5F were used, one subclone of each pair being cultured at 25°, the other, at 17°C. The ciliates were treated with an immune serum at the working concentration, washed and tested for their serotype. Homologous immune sera IS 6-25 and IS 6-17 were used for treating subclones of the clone # 6 as well as IS 5F-25, for subclones of the clone # 5F. Results of the testing of the subclone # 6-25 are presented in Fig. 3. A gradual change of the cell serotype was observed: for the first 4 days the proportion of cells with the initial serotype decreased, whereas the increasing number of cells appeared which did not respond to the antiserum and, hence, had another serotype.

On the 4th day, more than 90% of all ciliates had the changed serotype. After that, the reverse process occurred: the proportion of ciliates with the initial serotype began to gradually increase and by the 7th day the culture restored again the serotype that the cells had prior to the experiment. Similar results were obtained in all subclones

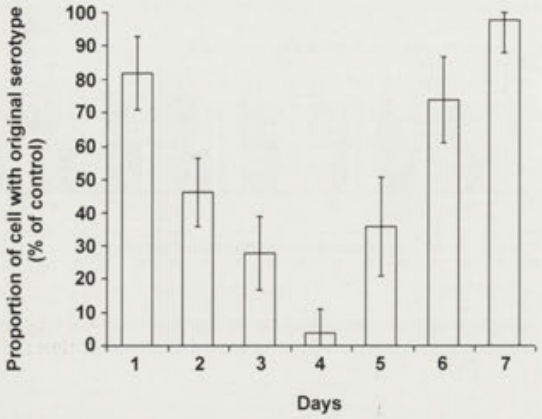

Fig. 3. Change-over of *Dileptus anser* serotype (subclone # 6-25) after treatment with the homologous immune serum IS 6-25 (1:1000) for 1 h

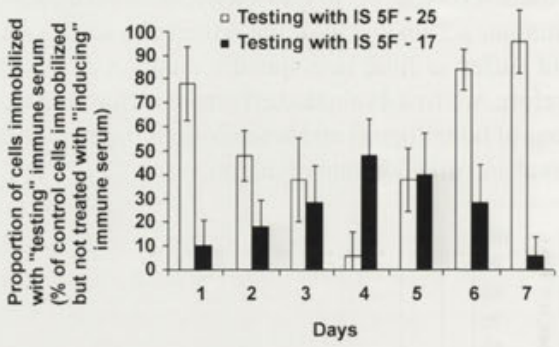

Fig. 4. Testing *Dileptus anser* (subclone # 5F-25) with IS 5F-25 and IS 5F-17 after treatment with the homologous („inducing") IS 5F-25 (1:1000) for 1 h

studied: # 6-17, # 5F-25, and # 5F-17 (data not shown). Thus, in no case steadily transformed cells, i.e. the cells inheriting the new serotype, were observed. Such cells, if ever occurred, had to be extremely rare (see the below data of immunofluorescent analysis).

Secondly, an attempt was made to characterize the new serotype that appeared for a short time in the ciliates treated with the "inducing" homologous immune serum. The same subclones, that were cultivated at the temperatures applied, of the above-mentioned clones of dilepti were used. In this case, the cells "induced" with homologous immune serum were tested in parallel with two "testing" sera. One of these was homologous and the other was raised against a subclone of the same clone but cultured at the different temperature. Figure 4 presents the results of treating the ciliates from the subclone # 5F-25 (i.e. showing „warm" serotype, as it was called) with the „inducing" homologous immune serum and of their subsequent testing with IS 5F-25 and IS 5F-17. Rather unexpectedly, the cells with the temporarily appearing new serotype responded to IS 5F-17. Hence, they are quite likely to have the same serotype which appeared when they were transferred from 25° to 17° (the "cold" serotype, as it was called). In the same way, in the subclone # 5F-17 treated with the homologous serum the cells temporarily appeared that responded to IS 5F-25; most likely, they had the „warm" serotype (the data are not shown).

Apart from testing cells with the immobilization reaction, an immunofluorescence analysis was applied to trace the process of the serotype change-over in the ciliates treated with the homologous immune serum. The subclones # 5F-25, # 5F-17, # 6-25, and # 6-17 as well as all the immune sera available, were used for these experiments. The conclusions made earlier were confirmed when applying the immunofluorescent staining of dilepti: dynamics of the cell luminescence fitted the above-described regularity (Fig. 5). On the 1st day, the cells treated with the homologous immune serum had a very intensive luminescence similar to that in the controls (Figs. 5a, b). Then it gradually decreased and disappeared completely by the 4th day (Fig. 5c). When the luminescent cells were counted in the sample on the 4th day, almost no ciliates with the initial serotype were revealed (typically, only 3 to 5 cells out of 50 observed cells showed a weak luminescence). But if ciliates from the 25°C clone treated with homologous immune serum were tested with the immune serum against the 17°C (which is heterologous for them), a very intensive luminescence was observed on the 4th day of the experiment (Fig. 5d), similar to that of the homologous cells (i.e. the cells of the subclone # 6-17) treated with the same serum. Subsequently (on the 5th and 6th observation days), when the ciliates were tested with the homologous immune serum, their luminescence reappeared and increased rapidly to reach, by the 6th day, the initial and the control levels (Figs. 5a, b, e), which indicates restoration of original the serotype.

Using the same schedule, experiments with the subclone # 6-17 ciliates were performed. Their serotype, as judge from intensity of luminescence, also was found to be gradually replaced by a new serotype which by the 4th day could be easily identified using the immune serum against the subclone # 6-25. However, by the end of the experiment, the original serotype of the culture always returned. Thus, the results of the immobilization reaction and indirect immunofluorescence agreed completely.

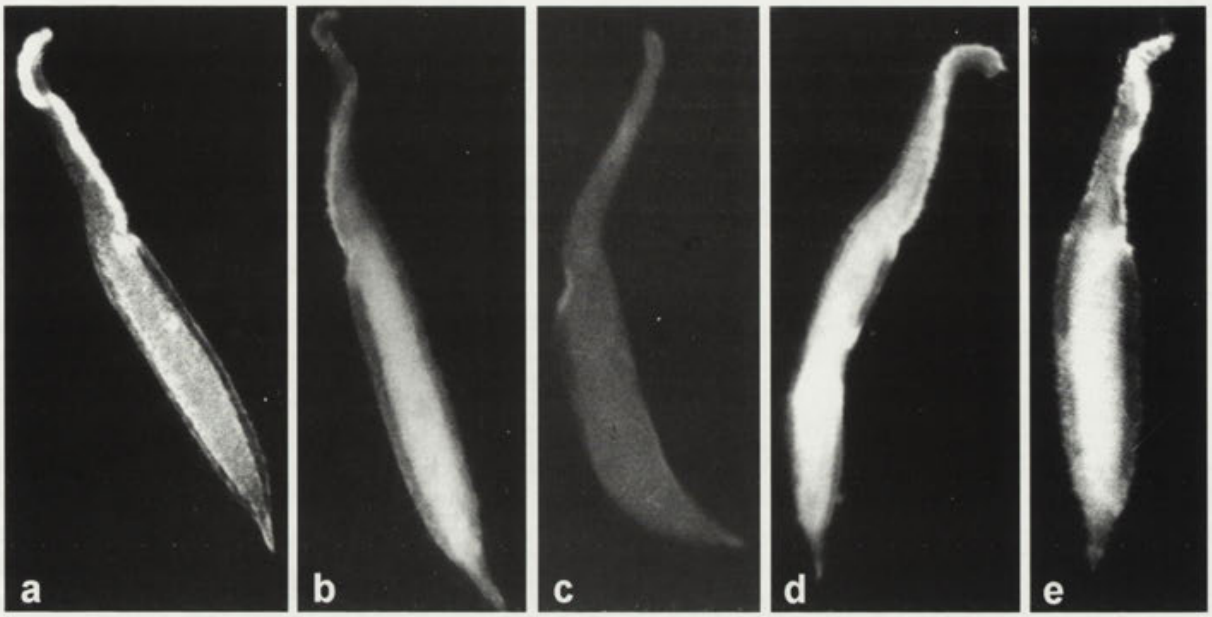

Fig. 5. Immunofluorescence of *Dileptus anser* (subclone # 5F-25) after treatment with the "inducing" homologous immune serum IS 5F-25 (1:1000) for 1 h.a - control, untreated cells tested with the homologous immune serum; b - cells treated with the "inducing" immune serum and tested with the homologous immune serum IS 5F-25 on the 1st day of the experiment; c - the same, on the 4th day, testing with IS 5F-25; d - the same, on the 4th day, testing with IS 5F-17; e - the same, on the 6th day, testing with IS 5F-25. Indirect immunofluorescence, x 150 (Homal)

The Actinomycin D-induced block of the temporary change of serotype caused by homologous immune serum

The effect of a transcription inhibitor, Actinomycin D (AmD, Sigma), on the temporary serotype transformation induced by immune serum was tested in the same ciliate subclones. Previously, in experiments with AmD at various stages of the serotype transformation induced by the change of temperature, we showed that RNA synthesis *de novo* was necessary for the complete serotype change-over but only at early stages of the transformation (the first 1 to 2 days), whereas later the process was controlled at the posttranscriptional level (Uspenskaya and Yudin 1996). We were able to find the AmD concentration with a minimal damaging effect on *Dileptus* cells. At this concentration, AmD may be assumed to act predominantly as an RNA synthesis inhibitor. Such maximal sublethal concentration was found to be 15 μg/ml. It was this concentration that we used in the present experiments.

Subclones # 6-25 and # 6-17 were used for the experiments. AmD was added to the culture medium one day prior to the treatment of dilepti with the "inducing" immune serum and also during the next first day. In all cases, even temporary serotype change-over was not observed after the AmD treatment (data not shown). In other words, the AmD effect was the same as that found in our previous experiments on the serotype transformation induced by the change of temperature (Uspenskaya and Yudin 1996).

Effect of preimmune and heterologous immune sera on ciliate serotype

The experiment was performed on two subclones, # 5F-25 and # 6-25, and two ISs heterologous for them, IS 6-25 and IS 5F-25, correspondingly. The both subclones never cross-reacted with each other. Apart from them, the subclone # D1-25 was also used; it also showed no cross-reactions with the above two subclones. This subclone was tested with the both ISs which were heterologous to it. Besides, the preimmune sera corresponding to each of the immune sera were applied. Otherwise, the scheme of the experiment was the same as that with the homologous immune sera.

In the experiments of this series, after the treatment of the ciliates with both the preimmune and heterologous immune sera, no noticeable, even temporary, change-over of the serotype was revealed (data not shown).

DISCUSSION

The homologous immune serum as a transforming agent is mentioned in nearly all reviews on ciliate serotypes (Preer 1968, 1986; Sommerville 1970; Finger 1974; Sonneborn 1975; Schmidt 1988). However, with accumulation of new experimental data, there is a certain evolution of concepts about the nature of homologous antisera inducing the serotype change. A schematic and somewhat simplified exposure of this change in viewpoints on this problem is as follows. At first, the homologous antiserum was considered merely as one of rather numerous and diverse transformation inducers that were already known at that time (Sonneborn 1950a). Its transforming activity was believed to be due to conjugation of antibodies with corresponding surface antigens. Therefore, it was thought that the serotype change could be induced by homologous antisera applied by themselves (Sonneborn 1947, Sonneborn and Le Suer 1948). When more data were recently reported, some authors were inclined to believe that the antisera did not transform the ciliate serotype directly but merely destabilized it by its transition to an easily modified state (Sonneborn 1950b, Nanney 1980). And whether the serotype is changed after that transition and, if so, to which direction, depends on various factors, primarily on the culture conditions of the ciliates treated with the antiserum. In its extreme form, this viewpoint was expressed, for instance, by G.H. Beale (1957): "The most reasonable interpretation of the serum-induced transformation is that they are brought about by a generalized disturbance of the organism, and not by a specific effect of antibody on antigen".

Accordingly, to change the ciliate serotype in by homologous antisera, this treatment was combined in many studies with some other environmental factors known as transformation inducers. These inducers were most commonly changes in temperature (Dryl 1965, de Seigneux 1981) and, generally, any conditions promoting expression and maintenance of some new serotype. In these studies, it is practically impossible to separate the transforming effect of homologous immune serum itself from other factors.

Several years ago we started studying the serotype system in the ciliate *Dileptus anser*. A polymorphism of this character in natural populations of dilepti was described (Uspenskaya 1988). The process of the serotype transformation induced by a change in the cultivation temperature was characterized in detail. For certain clones we were able to establish rather precisely the highest temperature for the expression of only the serotype that was called "cold", and the lowest temperature that allowed expression of only the serotype that was called "warm". It was found that the shift from the "warm" serotype with "cold", and *vice versa*, took a sufficiently long time, usually from 4 to 6 days. This is convenient for analysis of dynamics of this process both under standard conditions and under effect of various additional factors. During this time, naturally, the ciliates feed and perform usually 4-6 cell divisions (Uspenskaya and Yudin 1992).

In the present study an attempt was made to induce serotype transformation in several *Dileptus* clones after a short treatment with homologous immune sera and subsequent cultivation under the same constant conditions as those before the treatment. We tried to minimize possible damaging effects of antisera on ciliates by choosing such maximal concentrations of the "inducing" sera which do not yet produce any detectable effect on the cell survival and the subsequent division rates of the antiserum-treated ciliates.

It was found that in dilepti from the studied clones the massive and unidirectional serotype change occurred after the treatment for 1 h with the homologous immune sera. Testing with the available antisera showed that most, if not all, changed cells acquired the same new serotype. In the subclones cultured at 17°C, this was the "warm" serotype (which is characteristic of subclones cultured at 25°C and never appears in these clones at 17°C !), whereas in the subclones cultured at 25°C, this was, on the contrary, the "cold" serotype.

We believe that this also accounts for only the temporary, transient character of the serotype changes, as during the entire period of the experiment the subclones remained at constant temperatures that were characteristic of them initially. Meanwhile the new, induced serotype was unable to be fixed and maintained under these, unsuitable to it, temperature conditions. It should be emphasized that in our case, under the constant culture conditions, we could also expect obtaining some other serotypes (which would behave as stable transformants): previously, when studying polymorphism of serotypes in clones of different origin, we observed, under the same culture conditions (both at 17° and at 25°C) a significant amount of clones with no cross-reactions and, hence, with different serotypes (Uspenskaya 1988). Nonetheless, the homologous antisera induced the appearance only of the "warm" (and, correspondingly, of the, "cold") serotype. It is this observation which seems to us the most interesting in the present work. It should be emphasized again that the synthesis and expression of this „temperature" antigen started at the constant and inappropriate temperature.

This might indicate a particularly high sensitivity of the corresponding gene to inducers.

The effect of homologous immune sera as inducers of serotype transformation may be considered sufficiently specific, as neither heterologous immune sera nor preimmune sera caused transformation (see, however, Finger 1974). Besides no severe generalized injures of cells were observed under effect of the dilutions used.

Finally, the results of the experiments with AmD suggest that at early stages of the serotype transformation induced by the homologous antiserum, this process requires the normally occurring transcription, in the same way as the transformation induced by changes of the cultivation temperature (Uspenskaya and Yudin 1996).

Our experiments do not provide information to discuss molecular-biological aspects of the effect of homologous antisera on the ciliate serotype. We can only note that various hypotheses on this subject are proposed and experimentally tested (Finger 1974; Capdeville 1979; Azzouz and Capdeville 1992; Finger *et al.* 1995, 1996).

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Two New Species of *Mantoscyphidia* Jankowski, 1980 (Ciliophora: Peritrichia) Gill Symbionts of Limpets, from South Africa and the Sub-Antarctic Island, Marion

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Summary. In a comprehensive survey of 19 limpet species representing three genera along the South African coast, an ectosymbiotic peritrich was found attached to the gills of specimens of all limpet species examined. This species differs from all the known *Mantoscyphidia* Jankowski, 1980 species based on body morphology and details of the nuclear apparatus and is described as a new species, *M. branchi* sp. n. In a similar investigation of the limpet fauna from a Sub-Antarctic Island in the Southern Ocean, another *Mantoscyphidia* species was found associated with the only limpet, *Nacella delesserti* (Phillips, 1849), occurring on Marion Island. This is described as a new species, *M. marioni* sp. n. These descriptions are based on light and scanning electron microscopy.

Key words: ectosymbiont, *Mantoscyphidia branchi*, *M. marioni*, marine mollusc, scyphidiid peritrich, sessile ciliophoran.

INTRODUCTION

Until the eighties the genus *Scyphidia* Dujardin, 1841 comprised a large number of species from a variety of marine as well as freshwater hosts and habitats. Jankowski (1980) proposed a new system of systematics for the phylum Ciliophora and created three new genera to accommodate some of the *Scyphidia* species, i.e. *Speleoscyphidia* Jankowski, 1980; *Riboscyphidia* Jankowski, 1980 and *Mantoscyphidia* Jankowski, 1980. Later Jankowski (1985) added two more genera to the list i.e. *Scyphidiella* Guhl, 1979 and *Myoscyphidia* Jankowski, 1985. All these genera are separated solely on the basis of the host or substrate on which the peritrich attaches.

The ectosymbiotic peritrichs of the present study conforms in morphological features to those of the family Scyphidiidae Kahl, 1935. Following Jankowski (1985), these ectosymbiotic peritrichs are placed within the genus *Mantoscyphidia* based on their gastropod host.

Within a global context the South African coastline is regarded as a single zoogeographical marine province. The tip of the African continent is the meeting place of two mighty oceans, i.e. the Indian on the east coast and the Atlantic on the west coast. Under the influence of massive sea currents, the South African coast is subdivided into three diverse regions. The west coast is dominated by the cold Benguela Current and the east coast is influenced by the warm Agulhas Current. At the confluence of these two currents, between Cape Point and Cape Agulhas, the coastline is transformed into a third unique coastal region

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(Branch and Branch 1981). Each of these regions sustains a diverse marine fauna and flora, which include 19 different limpet species represented by three genera. Of these, 17 are endemic to the South African zoogeographical marine province. In a comprehensive survey of the symbionts of intertidal invertebrates along the South African coast, an ectosymbiotic peritrich was found associated with the gills of all the limpet species examined. This species differs from all the known *Mantoscyphidia* species based on general body morphology, characteristics of the nuclear apparatus as well as host preference. It is described as a new species based on live observations, Bouin's fixed specimens stained with hematoxylin, Protargol impregnation as well as scanning electron microscopy.

A similar survey was also carried out on intertidal symbionts on the Sub-Antarctic Island of Marion. The endemic limpet *Nacella delesserti* (Phillips, 1849) was also found to host an ectosymbiotic peritrich on the gills, which is described as a new *Mantoscyphidia* species. Due to the unavailability of photomicroscopy facilities on Marion Island, live specimens could not be photographed and measured. This description is based on the study of Bouin's fixed specimens stained with hematoxylin and scanning electron microscopy.

MATERIALS AND METHODS

Specimens of limpets were collected at low tide on the rocky shores of South Africa (Fig. 1). On the west coast (WC) species of *Patella* Linnaeus, 1758 and *Helcion* Montfort, 1810 were collected at Mc Dougall's Bay and at the rocky shores along the Olifants River Mouth. *Patella* and *Helcion* species occurring on the south coast (SC) were collected at De Hoop Nature Reserve, Goukamma Nature Reserve and Keurboom Beach. On the east coast (EC) *Patella* and *Cellana* Adams, 1869 species were collected at Bazley and at the rocky shores of Lake St Lucia. *N. delesserti* was collected on the east coast of Marion Island. The island is situated in the southern Indian Ocean, some 2 300 km south-east of Cape Town, South Africa.

Specimens were taken to a field laboratory where the gills were dissected, placed on a microscope slide, smeared and examined. Live observations were noted and photomicrographs taken of expanded ciliophorans for later measurements in the case of the South African material, as provided in the description below. Wet smears were left to dry for later processing. Additionally, smears were fixed in Bouin's and transferred to 70 % ethanol. Both air dried and Bouin's fixed smears were stained with Mayer's hematoxylin for studying the nuclear apparatus and for obtaining additional body measurements. In order to study details of the infundibulum, air dried smears were impregnated with Protargol using a combined method as described by Lee *et al.* (1985) and Lom and Dykova (1992).

For scanning electron microscopy sections of fresh gills were fixed in 10% buffered neutral formalin. In the laboratory, the gills were washed with tap water, dehydrated through a series of ethanol concentrations and critical point dried. The gills were then mounted on a stub, sputter coated with gold and studied at 5 kV, using a JEOL WINSEM JSM 6400 scanning electron microscope (SEM).

For measurements of live specimens minimum and maximum values are given, followed in parentheses by the arithmetic mean, standard deviation (only in n >9) and number of specimens measured. Measurements based on Bouin's fixed specimens stained with hematoxylin are presented in square brackets. Body length is measured from the scopula to the epistomial disc and body diameter at the widest part of the body. Both macro- and micronuclei's lengths were measured from adoral to aboral. Data given of body striations was obtained with the aid of SEM. The type material is in the collection of the National Museum, Bloemfontein, South Africa.

RESULTS AND DISCUSSION

Mantoscyphidia branchi sp. n. (Figs. 2-14)

Hosts: *Patella aphanes* Robson, 1986; *P. argenvillei* Krauss, 1848; *P. barbara* Linnaeus, 1785; *P. cochlear* Born, 1778; *P. compressa* Linnaeus, 1758; *P. concolor*

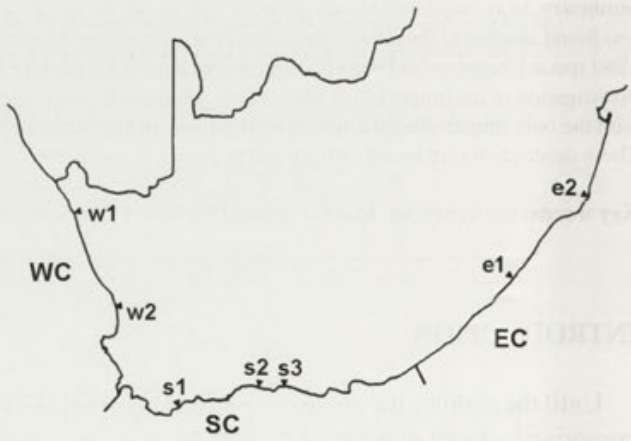


Fig. 1. Map of collection localities along the South African coast line. EC - East Coast, e1 - Bazley, e2 - St. Lucia, SC - South Coast, s1 - De Hoop Nature Reserve, s2 - Goukamma Nature Reserve, s3 - Keurboom Beach, WC - West Coast, w1 - Mc Dougall's Bay, w2 - Olifants River Mouth

Krauss, 1848; *P. granatina* Linnaeus, 1758; *P. granularis* Linnaeus, 1758; *P. longicosta* Lamarck, 1819; *P. miniata miniata* Born, 1778; *P. m. sanguians* Reeve, 1856; *P. obtecta* Krauss, 1848; *P. oculus* Born, 1778; *P. pica* Reeve, 1854; *P. tabularis* Krauss, 1848; *Cellana capensis* (Gmelin, 1791); *Helcion dunkeri* (Krauss, 1848); *H. pectunculus* (Gmelin, 1791) and *H. pruinosus* (Krauss, 1848).

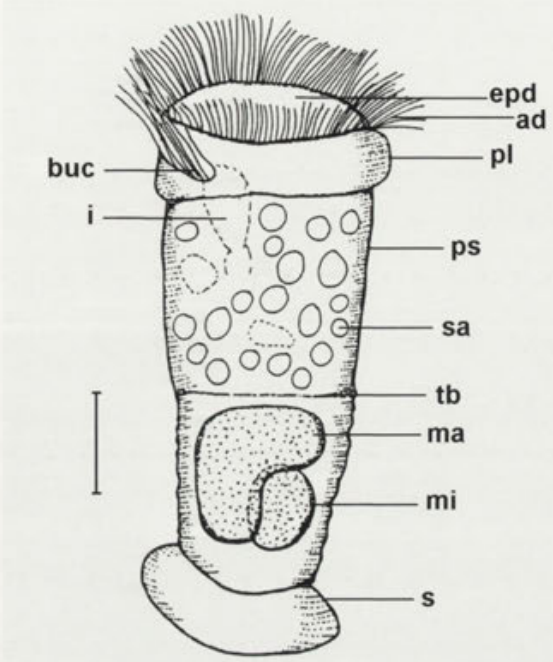


Fig. 2. Microscope projection drawings of *Mantoscyphidia branchi* sp. n. occurring on the gill filaments of *Patella tabularis* Krauss, 1848 collected at Keurboom Beach, South Africa. Scale bar - 10 μm. ad - adoral ciliary spiral, buc - buccal cavity, epd - epistomial disc, i - infundibulum, ma - macronucleus, mi - micronucleus, pl - peristomial lip, ps - pellicle striations, s - scopula, sa - symbiotic algae, tb - telotroch band

Position on host: gills.

Localities: Mc Dougall's Bay and the Olifants River Mouth on the west coast; De Hoop Nature Reserve; Goukamma Nature Reserve and Keurboom Beach on the south coast; Bazley and at the rocky shores of Lake St Lucia on the east coast of South Africa.

Type-specimens: holotype, slide S93/10/15-71 (NMBP 204) in the collection of the National Museum, Bloemfontein, South Africa, Paratype slides S93/10/15-55 (NMBP 205), S93/10/15-65 (NMBP 206), S93/10/15-76 (NMBP 207) in the collection of the authors.

Type host and locality: *P. tabularis*, Keurboom beach (23° 28'S, 34° 0'E).

Etymology: named after Prof. George Branch and his wife Margo, authors of numerous research articles on South African limpets.

Description

Body cylindrical when expanded (Figs. 2-6,10,11), with peristomial area ranging from flattened to arched, depending on body contraction (Figs. 3,7,8). Body length 44-78 μm (65.0±8.8, 14) [45-76 μm (57.1±7.6, 39)], body diameter 17-32 μm (24.2 ±4.4, 14) [20-35 μm (25.3±3.3, 39)]. Telotroch band broad, elevated, situated one third of body length from scopula (Figs. 5,6). Prominent pellicle folds adoral to telotroch band (Figs. 5,6). Scopula very prominent and broad (Figs. 3-5,10), length 5-9 μm (5.7±1.2, 14) [3-9 μm (5.1±1.4, 39)], diameter 15-25 μm (21.5±2.7, 14) [19-31 μm (25.2±3.4, 39)]. Scopula cilia short, densely grouped together (Fig. 9).

Peristome, body and scopula encircled with pellicle striations approximately 0.45 μm apart. Striations evenly spaced and uniform (Fig. 5). Bifurcated pattern found in some individuals on body and scopula. Telotroch band consists of six closely associated striations. Peristomial lip broad, buccal opening large, leading into infundibulum (Figs. 6,8).

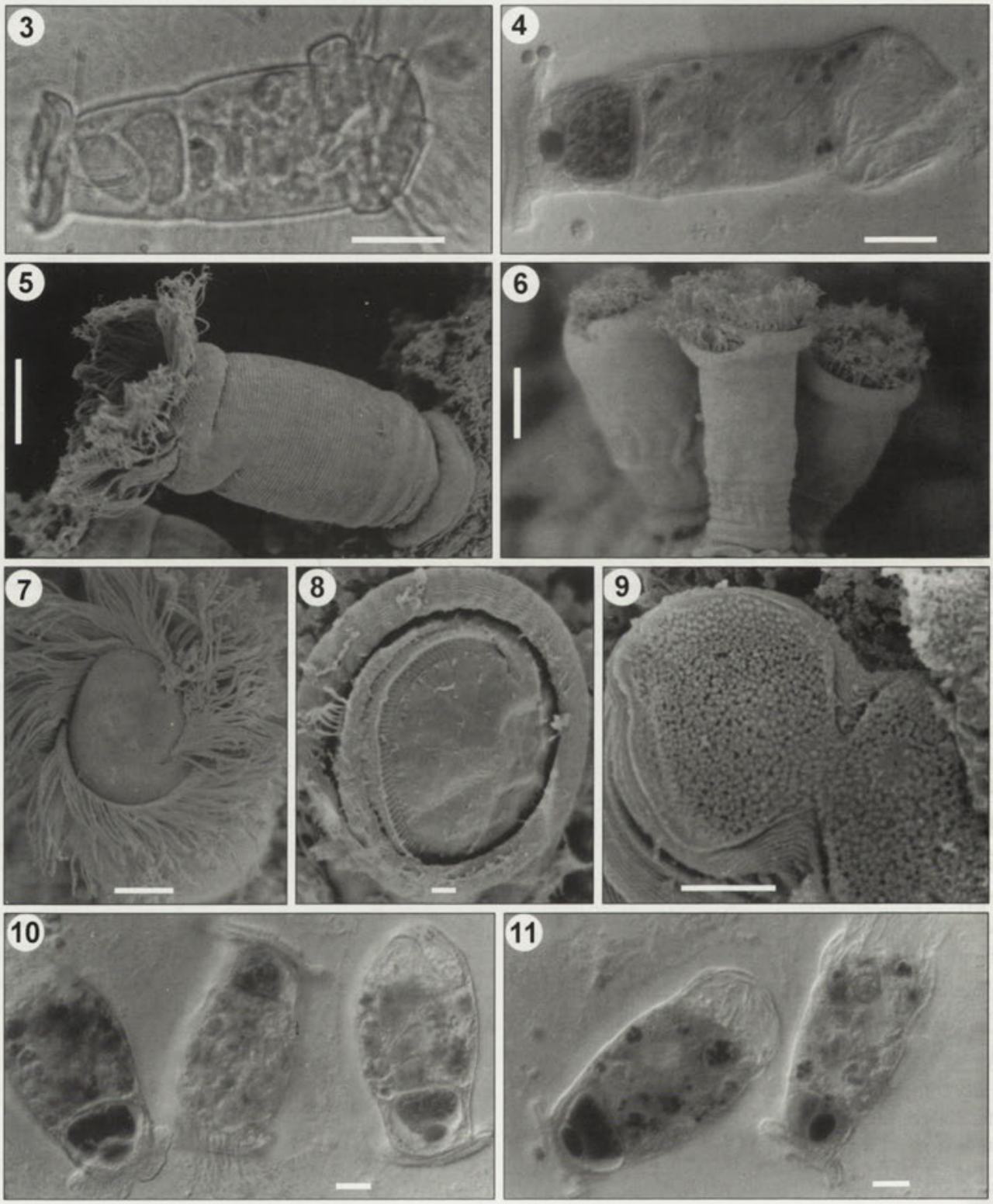
Adoral zone completes spiral of 540° counterclockwise around epistomial disc before plunging into infundibulum (Figs. 7,8,12). Buccal apparatus and haplo- and polykineties start almost at the same point, first seven to ten kinetosomes of haplo- and polykineties barren (Fig. 7). Haplo- and polykineties divided by a pellicle ridge approximately the width of a kinetosome. Polykinety three kinetosomes wide with proximal row of open pores. Haplokinety is a row of dikinetids in which the outer kinetosome of each is ciliated. Polykinety plunges first into infundibulum (Fig. 12). Haplokinety makes another half turn (180°) before plunging into infundibulum. Impregnable structure associated with haplokinety. Inside infundibulum, both haplo- and polykineties complete one turn before reaching cytostome. Cytostome not always clearly visible.

Cytoplasm granular (Figs. 4,10,11). Single contractile vacuole observed in some individuals. A number of food vacuoles in cytoplasm. Symbiotic algae commonly found in cytoplasm, varying in number and size (Figs. 10,11).

Nuclear apparatus (Figs. 2-4,10,11) occupies most of area below telotroch band. Macronucleus large, adorally broad, tapering somewhat aborally, length 12-19 μm (15.7, 4) [6-15 μm (9.5±1.9, 39)], diameter 11-20 μm (14.7, 4) [11-19 μm (14.4±2.0, 39)]. Macronucleus forms indentation, occupied by round to oval-shaped micronucleus, length 7-12 μm (9.5, 2) [3-13 μm (4.9±1.9, 38)], diameter 6-10 μm (8.0, 2) [4-9 μm (6.1±1.3, 38)].

Intraspecific variation

No clear pattern could be observed concerning body length of the different populations (Table 1, Fig. 13). The mean body length of the west coast populations (52 μm) appear to be on the lower side of the spectrum, whilst the south coast population's mean body length is 60 μm, which is exactly the same as that of the mean body length of the east coast populations. Relatively small variations



Figs. 3-11. Photomicrographs of live (3), hematoxylin stained (4,10,11) and scanning electron micrographs (5-9) of specimens of *Mantosyphidia branchi* sp. n. 3 - fully expanded body with arched peristome; 4 - fully expanded body with granular cytoplasm; 5 - fully expanded body, with pellicle striations; 6 - fully expanded body with buccal cavity and pellicle folds adoral to telotroch band; 7 - adoral zone describing 540° spiral; 8- adoral zone with cilia lost; 9 - short densely grouped cilia of scopula; 10, 11 - different forms of body contractions, cytoplasm filled with symbiotic algae, also illustrating form and position of nuclear apparatus. Scale bars - 10 μm (3-6,10,11), 5 μm (7,9), 1 μm (8)

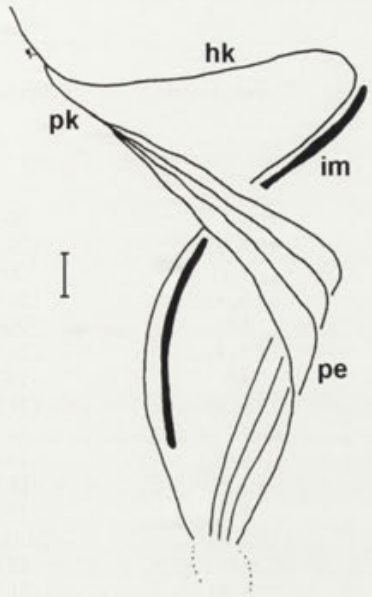


Fig. 12. Buccal infraciliature of *Mantoscyphidia branchi* sp. n occurring on the gill filaments of *Patella tabularis* Krauss, 1848 collected at Keurboom Beach. Scale bar - 1 µm. hk - haplokinety, im - impregnable structure, pe - peniculus, pk - polykinety

in the mean body diameter were observed (Table 1). The mean body diameter of the ectosymbiotic peritrichs collected from all three coastal regions were the same, i.e. 25 µm (Fig. 14).

The body of *M. branchi* is extremely contractile, with fully expanded specimens varying from 40 to 95 µm. During fieldwork live specimens were observed where the same individual showed a reduction in body length, but with the peristome remaining open. In these specimens groups of elevated striations can be seen below the telotroch band, these remain visible in both dried stained specimens, as well as material prepared for SEM (Fig. 6). When the peristome is fully closed, the degree of contraction can also vary. Specimens can be found in a whole range of body contractions on a single smear.

A variety of factors could be responsible for the variation in body length, these include the influence of the different host species on the symbionts, prevailing conditions at the sampling locality, e.g. low temperatures on the west coast versus subtropical conditions on the east coast, or seasonal differences. In our opinion, however, the large variation in body length is most likely due to the extreme contractility and flexibility of the body of this ciliophoran.

Of all the morphological features, the nuclear apparatus is the most consistent in form, position as well as size (Table 2). The macronucleus is shaped in such a way that it leaves a perfect hollow into which the micronucleus snugly fits. When examining specimens under the microscope, the variation in the nuclear apparatus is determined by the side from which the nuclear apparatus is viewed (Figs. 3,4,10,11).

The conventional way of counting the striations was based on the study of silver impregnated specimens. In this study considerable difficulty was experienced with silver impregnation of specimens from the marine environment. This problem has previously also been noted by

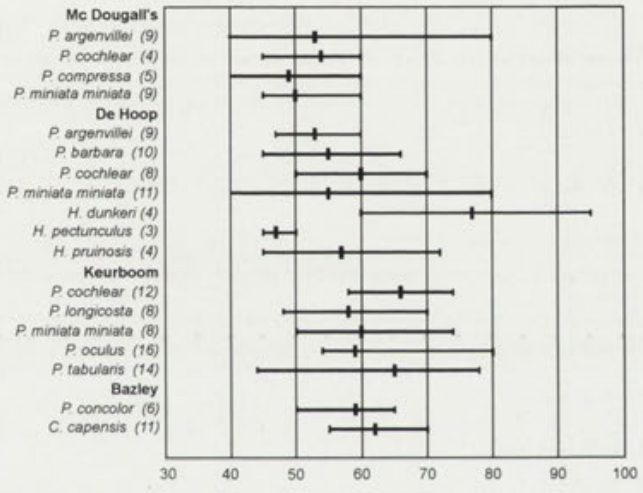


Fig. 13. Comparison in the variation of the body length (µm) of different live populations of fully expanded specimens of *Mantoscyphidia branchi* sp. n. occurring on the gills of limpet species from different localities along the South African coast

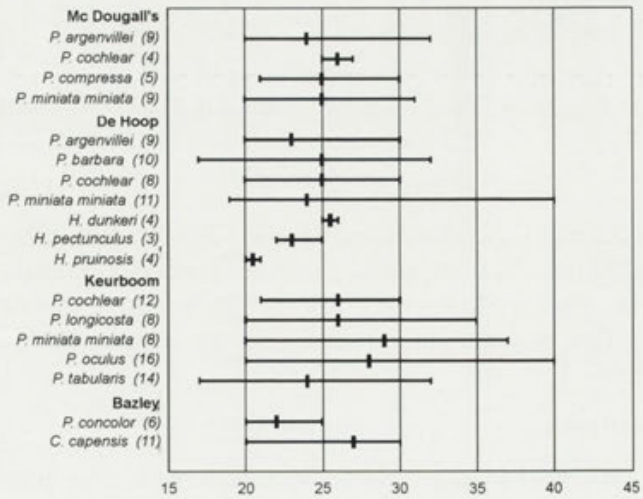


Fig. 14. Comparison in the variation of the body diameter (µm) of different live populations of fully expanded specimens of *Mantoscyphidia branchi* sp. n. occurring on the gills of limpet species from different localities along the South African coast

Table 1. Variation of body measurements (μm) of *Mantoscyphidia branchi* sp. n. of expanded body forms from live observations depending on host and localities. WC - west coast, SC - south coast, EC - east coast

Host Localities	Body length	Body diameter	Scopula length	Scopula diameter
Mc Dougall's (WC)				
P. argenvillei	40-80	20-32	4-9	20-32
	(52.5, 9)	(23.5, 9)	(5.5, 8)	(25.4, 9)
P. cochlear	45-60	25-27	5-5	20-21
	(53.5, 4)	(26.0, 4)	(5.0, 3)	(20.2, 4)
P. compressa	40-60	21-30	4-5	20-31
	(48.6, 5)	(24.8, 5)	(4.7, 4)	(24.6, 5)
P. miniata miniata	45-60	20-31	4-6	18-30
	(50.0, 9)	(24.6, 9)	(5.0, 7)	(21.7, 9)
De Hoop (SC)				
P. argenvillei	47-60	20-30	5-6	13-21
	(52.7, 9)	(22.7, 9)	(5.2, 5)	(18.7, 9)
P. barbara	45-66	17-32	4-6	19-30
	(54.9±6.6,10)	(24.4±4.8,10)	(5.2, 9)	(22.7±3.6,10)
P. cochlear	50-70	20-30	5-6	13-25
	(60.2, 8)	(24.5, 8)	(5.1, 8)	(21.2, 8)
P. miniata miniata	40-80	19-40	4-7	19-31
	(55.0±10.5,11)	(24.4±5.8,11)	(5.2, 8)	(22.5±3.411)
H. dunkeri	60-95	25-26	4-5	24-30
	(77.2, 4)	(25.5, 4)	(4.7, 4)	(27.2, 4)
H. pectunculus	45-50	22-25	5-6	20-31
	(47.0, 3)	(23.0, 3)	(5.3, 3)	(24.0, 3)
H. pruinosus	45-72	20-21	5	20-30
	(56.7, 4)	(20.7, 4)	(1)	(24.0, 4)
Keurboom (SC)				
P. cochlear	58-74	21-30	5-6	20-30
	(65.9±5.5,12)	(25.6,±3.3,12)	(5.5,±0.5,10)	(25.0±4.5,12)
P. longicosta	48-70	20-35	4-6	25-31
	(56.7, 8)	(25.6, 8)	(5.1, 6)	(28.5, 8)
P. miniata miniata	50-74	20-37	5-6	19-32
	(60.3, 8)	(28,7, 8)	(5.3, 7)	(23.0, 7)
P. oculus	45-80	20-40	5-7	23-47
	(58.9±9.7,16)	(27.5±5.2 16)	(5.7±0.7,14)	(32.8±5.3,16)
Bazley (EC)				
P. concolor	50-65	20-25	5-7	19-30
	(58.3, 6)	(22.3, 6)	(5.7, 4)	(25.8, 6)
C. capensis	55-70	20-30	5-6	20-32
	(62.0±6.4,11)	(27.0±3.3,11)	(5.4±0.5,10)	(27.7±3.6,11)

Lom and Laird (1969) and Lom (1970). We found that SEM investigation provided the ideal method of counting the striations (Van As *et al.* 1995). The striations, studied in this way, for three populations are summarised in Table 3.

Remarks

Twelve *Mantoscyphidia* species have so far been found associated with freshwater and marine gastropod hosts, of these four are from freshwater. The first two species, i.e. *M. physarum* (Lachmann, 1856) and *M. limacina* (Lachmann, 1856) were described from freshwater gastropods of the genera *Physa* and *Planorbis*, respectively. Both can be distinguished from *M. branchi* based on their elongated macronuclei. The other freshwater species *M. inclinata* (Lom and Corliss, 1968) from *Cincinnatia* has an oval macronucleus which is located in the upper oral half of the body, whilst no information on the nuclear apparatus of *M. capitis* (Boitsova, 1976) is known.

The first marine species was described as *Cothurnia patellae* Hutton, 1878. Apparently unaware of Hutton's description Cuénot (1891) described *Scyphidia patellae* Cuénot, 1891. Jankowski (1985) placed both species

Table 2. Variation of nuclear measurements (μm) of *Mantoscyphidia branchi* sp. n. of expanded body forms, based on air dried smears stained with hematoxylin, depending on host and localities. WC - west coast, SC - south coast, EC - east coast

Host Localities	Macronucleus length	Macronucleus diameter	Micronucleus length	Micronucleus diameter
Mc Dougall's (WC)				
P. argenvillei	5-12 (8.6±2.0, 33)	8-20 (14.0±3.1, 33)	3-11 (6.1±2.4, 30)	3-14 (7.4±2.1, 30)
P. barbara	6-17 (10.0±2.8, 28)	10-17 (13.1±2.3, 28)	4-9 (5.6±1.5, 25)	4-9 (5.9±1.2, 25)
P. cochlear	5-17 (9.9±2.8, 54)	6-21 (14.4±1.4, 54)	3-12 (6.0±2.2, 52)	3-13 (7.9±2.2, 52)
P. compressa	4-15 (8.3±2.5, 54)	8-17 (12.2±1.9, 54)	3-8 (5.5±1.5, 49)	4-12 (7.1±1.7, 49)
P. granatina	6-21 (10.5±2.9, 46)	10-21 (15.5±3.0, 46)	3-10 (15.5±1.8, 46)	4-15 (8.1±2.6, 45)
P. miniata miniata	6-19 (9.2±2.0, 75)	4-16 (11.8±2.0, 75)	2-11 (5.4±1.8, 69)	3-12 (6.3±2.1, 69)
Papendorp (WC)				
P. argenvillei	5-12 (7.8±2.0, 34)	7-18 (13.0±2.6, 34)	2-8 (4.7±1.5, 34)	3-17 (7.2±2.7, 43)
P. compressa	7-19 (11.9±3.0, 25)	12-22 (18.5±2.5, 25)	4-14 (6.6±2.7, 25)	6-17 (9.5±2.7, 25)
P. granatina	4-19 (9.9±4.0, 24)	7-27 (15.1±4.3, 24)	3-8 (5.0±1.4, 13)	4-11 (7.2±2.4, 13)
De Hoop (SC)				
P. argenvillei	5-13 (9.1±1.6, 84)	6-19 (11.4±1.9, 84)	3-10 (6.1±1.3, 84)	5-11 (8.3±1.4, 84)
P. barbara	5-18 (8.1±1.5, 58)	8-15 (12.0±1.7, 58)	3-9 (5.4±1.4, 56)	5-11 (7.5±1.5, 56)
P. cochlear	4-16 (7.8±2.2, 27)	10-19 (14.0±2.0, 27)	5-11 (6.5±1.6, 27)	6-13 (8.9±1.4, 27)
P. longicosta	4-10 (7.7, 7)	11-18 (13.7, 7)	3-10 (5.2, 7)	3-10 (6.5, 7)
Keurboom (SC)				
P. cochlear	5-10 (7.3, 6)	10-19 (15.5, 6)	2-5 (3.7, 4)	3-10 (6.7, 4)
P. tabularis	6-15 (10.1±2.8, 28)	8-22 (15.2±3.5, 28)	4-11 (7.7±1.6, 25)	6-16 (9.7±2.4, 25)
Bazley (EC)				
P. barbara	6-11 (8.7±1.5, 50)	10-17 (13.0±2.0, 50)	3-6 (3.8±0.8, 45)	2-10 (5.2±1.5, 45)
C. capensis	6-13 (9.0±1.7, 33)	7-18 (12.8±2.2, 33)	3-9 (4.6±1.5, 18)	3-9 (5.8±1.8, 18)

within the genus *Mantoscyphidia* and in order to avoid the confusion of having two species with the same name, he changed Cuénot's *S. patellae* to *M. lusitana* Jankowski, 1985.

Prior to the description of *M. lusitana*, another species, *M. fischeri* (Vayssiére, 1885) was recorded from *Truncatella truncatula*. Madrazo-Garibay and López-Ochoterena (1988) recorded this ectosymbiotic peritrich as well as a number of other species from commercial clams. This paper is probably of little value as they identified ectocommensal scyphidiids, originally described from marine and freshwater gastropods, marine fish as well as freshwater oligochaetes, as occurring internally in marine clams. These records need to be re-evaluated.

Some years later the next marine ectosymbiotic peritrich was described from *Littorina* species from the Adriatic Sea, i.e. *M. littorinae* (Issel, 1918). Raabe (1952) provided a redescription, from the same host, confirming the validity of this species. Kahl (1933) described *M. hydrobiae* (Kahl, 1933) from the tentacles and mouth of a gastropod, *Hydrobia*, subsequently also recorded by Precht (1935) from *H. ventrosa* as well as *Littorina rudis*.

Table 3. Body striations of expanded specimens of *Mantoscyphidia branchi* sp. n. occurring on the gill filaments of *Patella* species of different localities from South Africa

Number of striations:	**Mc Dougall's** *P. argenvillei*	**Papendorp** *P. barbara*	**De Hoop** *P. barbara*
Peristome	6-20 (12.6±4.6, 13)	11-18 (15.2, 5)	9-24 (15.5±4.0, 16)
Adoral to telotroch band	41-78 (65.7±10.7, 13)	45-73 (60.4, 9)	44-79 (57.0±10.1, 18)
Aboral to telotroch band	17-42 (34.8±8.1, 12)	20-41 (31.3, 8)	17-68 (31.0±13.7, 16)
Scopula	6-14 (9.5±3.1, 11)	8-8 (8.0, 2)	5-14 (9.0±2.7, 15)
Total number of striations	74-146 (119.9±22.6, 12)	58-123 (95.1, 8)	77-140 (106.5±20.0, 17)

M. ubiquita (Hirshfield, 1949) was recorded from different limpets (of the genera *Acmaea*, *Lottia* and *Fissurella*) and turbans (*Tegula* species) from the southern Californian coast by Hirshfield (1949). This ectosymbiotic peritrich was also recorded by Lom and Corliss (1968) from San Juan Island limpet off the west coast of the USA. Later Fish and Goodwin (1976) as well as Jamadar and Choudhury (1988) reported *M. ubiquita* from a number of *Littorina* species from the west coast of Wales and India, respectively.

Fish and Goodwin (1976) also described a new species, *M. acanthophora* (Fish and Goodwin, 1976) from topshells (*Gibbula umbilicalis* and *Monodonta lineata*) collected on the west coast of Wales. *M. bengalensis* (Jamadar and Choudhury, 1988) was recorded from an estuarine gastropod, *Cerithidea cingulata*, in India by Jamadar and Choudhury (1988).

M. branchi is the ninth ectosymbiotic peritrich found associated with marine gastropods and is the fourth species from a limpet host. It can be distinguished from all the species based on the body morphology, especially the scopula, characteristics of the nuclear apparatus as well as host preference.

M. branchi differs from the *Mantoscyphidia* species, found on the non-limpet hosts, in the following ways: *M. fischeri* and *M. hydrobiae* have ribbon- and kidney-shaped macronuclei, respectively. *M. littorinae* has an oval to sausage-shaped macronucleus, situated in the aboral part of the body. *M. acanthophora* has a C-shaped macronucleus, which is situated in the middle to adoral part of the body. Another characteristic of this species is the small scopula with cilia. In *M. branchi* the scopula is broad with much shorter cilia which are more densely grouped together. The fifth species, *M. bengalensis* also has a small scopula and a very conspicuous macronucleus, which appears cylindrical and sometimes coiled. In the coiled forms the nucleus fills the whole of the aboral region. A small micronucleus occurs close to the peristome.

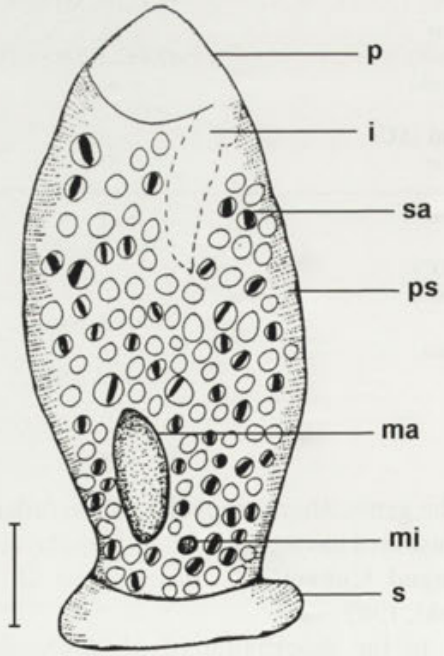
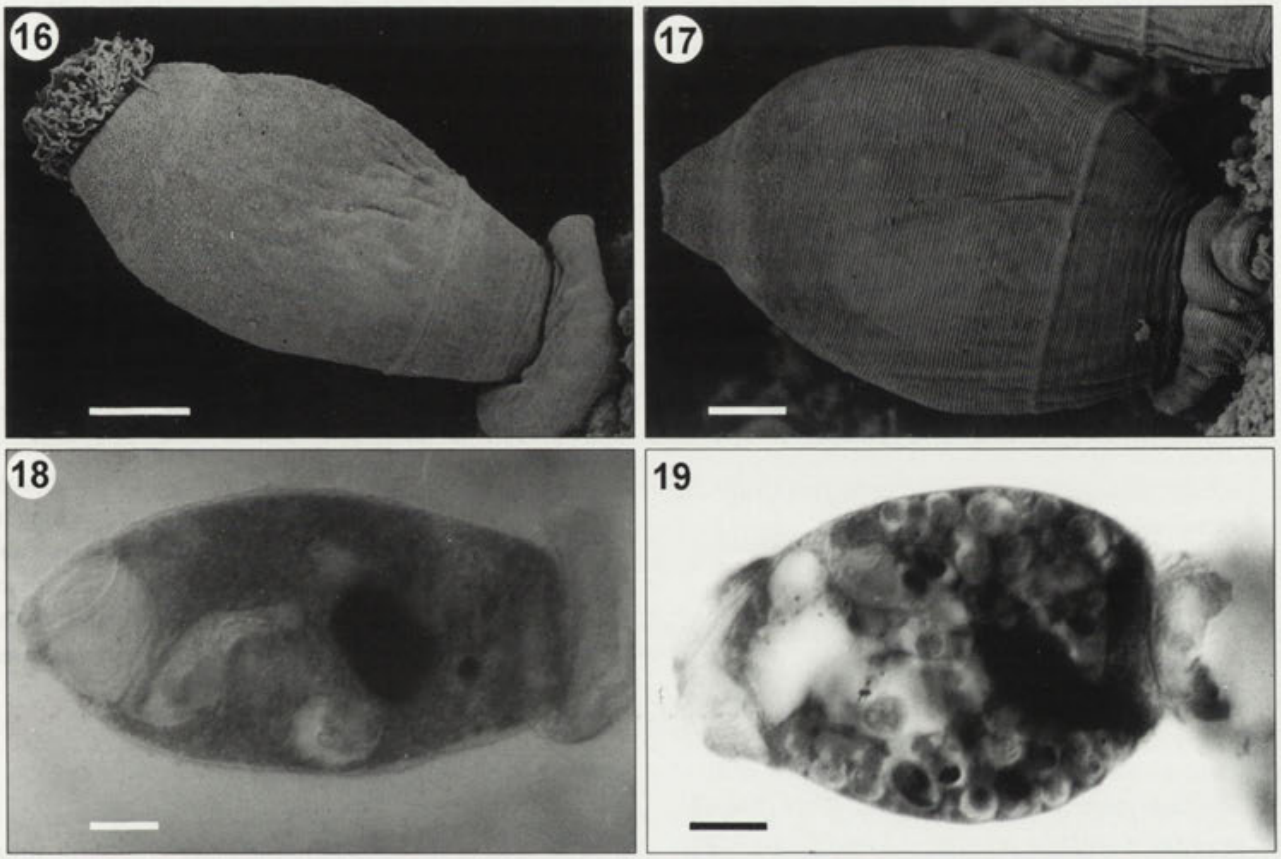


Fig. 15. Microscope projection drawings of *Mantoscyphidia marioni* sp. n. occurring on the gill filaments of *Nacella delesserti* (Phillips, 1849) collected from Marion Island. Scale bar - 20 µm. i - infundibulum, ma - macrocnucleus, mi - micronucleus, p - peristome, ps - pellicle striations, s - scopula, sa - symbiotic algae



Figs. 16-19. Scanning electron micrographs (16,17) and photomicrographs of hematoxylin stained (18,19) specimens of *Mantoscyphidia marioni* sp. n. 16 - expanded body, barrel-shaped; 17 - contracted body form with prominent pellicle striations; 18 - expanded body, barrel-shaped with granular cytoplasm; 19 - contracted body, cytoplasm filled with symbiotic algae. Scale bar - 10 μm

M. branchi differs from the *Mantoscyphidia* species, described from limpets, in the following way. In *M. lusitana*, the macronucleus forms a band (Cuénot 1891), is beaded (Hirshfield 1949) or has a horseshoe-shaped macronucleus adoral to the telotroch band (Madrazo-Garibay and López-Ochoterena 1988). *M. ubiquita* has a sausage-shaped macronucleus, which is situated in the middle part of the body in expanded and partially contracted forms. When individuals are completely contracted the macronucleus occurs closer to the scopula. According to Hirshfield (1949) *M. ubiquita* has two types of micronuclei, depending on the host species. The micronucleus of the ectosymbiotic peritrich from limpet hosts is larger and lies close to the peristome, whilst those from turbans have a small round micronucleus, lying close to the macronucleus. In our opinion these are most likely two different species, which requires further investigation.

Due to the unavailability of the original description of *M. patellae* it is not possible to compare it with *M. branchi*. However, it is highly unlikely that a ectosymbiotic peritrich occurring on New Zealand limpets could be the same as those occurring on endemic South African limpets.

The only mention of an ectosymbiotic peritrich from South Africa was the note by Hodgson *et al.* (1985) recording the occurrence of *M. ubiquita* on different limpets. This paper, however, does not provide enough information for meaningful comparison with the present material. It is highly unlikely that *M. ubiquita*, occurring along the North American coast on a limpets of the genus *Acmaea* (absent from Africa), could also be found on

endemic limpets from southern Africa. We believe that the ectosymbiotic peritrichs they found are in fact the same as *M. branchi*.

***Mantoscyphidia marioni* sp. n. (Figs. 15-19)**

Position on host: gills.

Type specimens: holotype, slide 96/5/7-34 (NMBP 208) in the collection of the National Museum Bloemfontein, South Africa, Paratype, slides 96/5/7-33 (NMBP 209), 96/5/7-27 (NMBP 210) in the collection of the authors.

Type host and locality: *Nacella delesserti* (Phillips, 1849), Boulder Beach, Marion Island (46° 54'S, 37° 45'E).

Etymology: this species is named after Marion Dufresne, whose name was given to the island.

Description

Body cylindrical, somewhat barrel-shaped in expanded forms (Fig. 16), distinctly barrel-shaped in contracted forms (Figs. 15-19). Body length 63-128 µm (87.7±14.8, 49), body diameter 26-69 µm (44.0±11.2, 49). Epistomial disc flattened to arched with broad lip. Elevated pellicle ridges indicate telotroch band position (Figs. 16, 17). Broad prominent scopula, length 5-15 µm (9.3±2.4, 47), diameter 20-57 µm (36.9±9.2, 47) (Figs. 16-18).

Peristome, body and scopula encircled with pellicle striations, 0.5 µm apart (Fig. 17). Number of striations on peristome 11-20 (15.2±2.3, 13), number of striations adoral to telotroch band 45-91 (70.6±11.9, 25), number of striations aboral to telotroch band 12-44 (22.3±8.7, 21), number of striations on scopula 12-34 (18.3±9.1, 25).

Round symbiotic algae (6 µm in diameter) present, distributed throughout whole body, obscuring details of organelles (Figs. 15,19).

Large oval to sausage-shaped macronucleus, length 8-48 µm (20.6±6.6, 49), diameter 5-42 µm (14.8±7.7, 49), very small round micronucleus, length 2-6 µm (3.4±0.9, 33) diameter 2-6 µm (3.9±1.0, 33). Both nuclei occur in middle of body or in aboral region (Figs. 18,19). Micronucleus mostly positioned aborally to macronucleus.

Remarks

This is the first ectosymbiotic peritrich described from the genus *Nacella* Schumacher, 1817. The specimens available in this study did not allow detailed investigation of the infundibulum and adoral spiral as all the specimens were filled with numerous symbiotic algae. The presence of these algae is most likely a specific characteristic of this species. Although no live observations could be made there is still sufficient morphological information available to separate it from all the other known species. In particular information concerning the nuclear apparatus as well as its unique host, i.e. an endemic limpet from an isolated southern ocean island.

The only other species of *Mantoscyphidia* so far recorded from marine gastropods with an oval-shaped macronucleus is *M. littorinae*. Unfortunately, not enough information on *M. littorinae* is known, except for the fact that the latter was recorded from *Littorina* species from eastern Europe. Both ciliophoran species have an oval to sausage-shaped macronucleus with a small round micronucleus found in the middle part of the body and below the telotroch band. *M. marioni* is nearly twice as long as *M. littorinae*, thus the relationship of macronucleus and body size of these two species differs. In the case of *M. littorinae* the macronucleus is large compared to the body size, whilst the macronucleus of *M. marioni* is small in comparison to body size.

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Six New Ciliated Protozoan Species of Trichostomatida, Entodiniomorphida and Suctorida from the Intestine of Wild African Rhinoceroses

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Summary. A survey of ciliated protozoan endocommensals of both black and white wild African rhinoceroses revealed six new species from the caecum and colon in concentrations of $2x10^3$ - $5x10^4$/ml digesta fluid. Two uniformly somatically ciliated species of Trichostomatida, *Helicozoster africanus* with an external ciliated peristomal trough spiralling the entire body-length, was found in both black and white rhinoceroses; while *H. apicalis* with an external ciliated peristomal through spiralling half body - lenght, was found in white rhinoceroses only. Three species of Entodiniomorphida were found in white rhinoceroses only. These were *Rhinozeta pedale* with a foot-like posterior end of the body; *Triplumaria corrugare* with a corrugated triangular posterior terminal tail, and *Lavierella klipdrifi* with a distinctive club-shaped body. One adult species of Suctorida was present in both black and white rhinoceroses, *Allantosoma multisuctores* distinguishable by its numerous ingestory suctorial tentacles scattered uniformly over its entire body. Counts of the total endocommensals stressed the possible role of the colon in the fermentation of the hosts's diet by these Protozoa. In all, nine protozoan families have been demonstrated to be associated with fermentation of the digesta by the nine species of hindgut-fermenting mammals thus far examined by various workers in morphological studies.

Key words: hindgut Protozoa, new species, rhinoceros.

INTRODUCTION

In a survey of ciliated protozoan endocommensals of both black and white wild African rhinoceroses (Mammalia: Rhinocerotida), 48 species of ciliated Protozoa have been recovered from the hindgut and faeces to date. Twenty three of these were new species which have been identified systematically by Buisson (1923), Hoare (1937), van Hoven *et al.* (1987, 1988), and Gilchrist *et al.* (1994). In addition there are the six new species as reported here, large populations of fungi (Teunissen *et al.* 1991), flagellates and anaerobic bacteria (Dehority 1986).

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The six new species belong to three of the four orders of ciliated Protozoa of herbivorous mammals. The holotrich Protozoa are represented by two uniformly somatically ciliated species of Trichostomatida. *Helicozoster* Latteur 1967, *africanus* sp. n. was found in both black rhinoceros (*Diceros bicornis* Linnaeus 1758) and white rhinoceros (*Ceratotherium simum* Burchell 1817); while *H. apicalis* sp. n. was found in white rhinoceros only. Three species belonging to Entodiniomorphida were found in white rhinoceros only. *Rhinozeta pedale* sp. n. with typical upper and lower skeletal plates and short lateral somatic ciliary bands in between, is distinguishable by the foot-like posterior end of the body. *Triplumaria corrugare* sp. n. encased in a cuticular carapace with 3 typical stalked ciliary tufts is distinguishable by its corrugated triangular tail. *Lavierella klipdrifi* sp. n. is covered with a rigid naked striated cuticle and has a tuft of cilia on a retractable peristomal cone similar to that of the type species, but is distinguishable by its distinctive club-shaped body. *Allantosoma multisuctores* sp. n. of the Suctorida is present in the large intestine of both black and white rhinoceroses. The adult form is small solitary with numerous ingestory suctorial tentacles scattered uniformly over the elongate-cycloid body, which distinguishes it from other species of the genus.

MATERIALS AND METHODS

Gastro-intestinal tracts were excised while the carcasses were still warm, between 1 and 2 h after each of the 4 rhinoceroses were shot. Sets of 6 samples from stomach, small intestine, caecum, right ventral ascending colon, right dorsal ascending colon, and descending colon were collected from a slit made in the wall of the gastro-intestinal tract at the sampling point. Of one white rhiniceros, only microscope slides were available for analysis.The pH of the unmixed stomach digesta of one white rhinoceros were determined at different levels using a freshly calibrated Crison pH-meter (Labotec; Industria, South Africa). Otherwise, the digesta were mixed by hand, bailed out with a beaker and strained through a 4 mm-wire sieve. The strained fluid containing the Protozoa was collected. For light microscopy, 25 ml of fluid was added immediately to 25 ml of formalin (14% aq.) (van Hoven *et al.* 1987). For electron microscopy, 2 ml of fluid was added to 10 ml of instantaneous killing prefixative, which contained osmic acid (2% aq.) and $HgCl_2$ (sat. aq. soln.) mixed in the ratio of 5:1 (Parducz 1966) and which prevented retraction of the cilia (Small and Marszalek 1969). Clumping of the Protozoa was obviated by shaking vigorously for 30 s on addition of the preservative.

On reaching the laboratory, formalinized samples were diluted with a mineral solution (Bryant and Burkey 1953) and finally with equal parts of glycerine as stabilising agent (van Hoven 1983). Total counts were made at x 90 magnification with a 0.50 mm-Nageotte counting chamber (W. Schreck, Hofheim, Germany). The different ciliate species, in wet unstained, permanently sealed, slide preparations, were counted at x 400 magnification and converted to a percentage of the total, which was in excess of 200 individuals per host (van Hoven *et al.* 1987). Detailed anatomy was studied at x1000 magnification using an oil immersion objective. Drawings were made with the aid of camera lucida equipment and all measurements of length, width and thickness were made with a calibrated eyepiece micrometer. Thickness was measured by the distance travelled by the vertical stage micrometer between the values observed when the organism comes into and disappears from view under the oil immersion (x1000) objective. Size was measured in micrometers (μm). Lubinsky's terminology (1957, 1958) was used for description of the species, except in the use of motilia in place of caudalia for stalked ciliary tufts occurring anteriorly and posteriorly on the body (Eloff and van Hoven 1980). With the oral end of the organism directed to 12 o'clock, the orientation used was that of Kofoid and MacLennan (1933), Dehority (1985). Light microphotography was carried out with either an Olympus BH2 microscope with a PM-10 AD photomicrographic system or a Reichert Polyvar photomicroscope, each provided with Nomarski-optics for differential interference contrast. An AGFA PAN 25 ASA film was employed in order to obtain well resolved image enlargements.

For scanning electron microscopy (SEM), the prefixed field sample was washed with tap water as soon as possible through stacked geological sieves (125-20). The subsamples from each sieve were fixed for 24 h with Karnovsky fixative (Hayat 1972) and 1% picric acid (v/v aq.) (Bullock 1984) or, where necessary, stored in that fixative. From this stage, standard dehydration protocols were carried out in a holder containing a Nucleopore filter (10-12) under slight negative pressure. During the last change of ethanol, the funnel was removed, and a similar filter was inserted to close the microchamber. After critical-point drying, both filters were removed and mounted on stubs for carbon and gold/palladium coating (2-3 nm). All preparations were viewed with a Cambridge Stereoscan microscope at 5-10 kV.

Since most intestinal ciliated Protozoa are strictly anaerobic and active only at about 39°C, specimens had to be preserved at the site of the kill with instantaneous-acting fixatives incompatible with techniques for study of subpellicular systems. Thus the descriptions presented here exclude subpellicular ciliary morphology.

RESULTS

The stomach and small intestine were void of Protozoa. The stomach was packed tight with ingesta. The pH at the internal surface of the stomach wall was expectedly low, and showed a gradient toward neutrality approaching the centre of the stomach. This gradient in the Pilanesberg white rhinoceros from stomach-wall → halfway to stomach centre → stomach centre was 2.13 → >3 → 6.6. On mixing the stomach contents the pH became 5.5. In the young rhinoceros this would facilitate the passage of ingested inocula of mother's faeces to the hindgut habitat of the acid sensitive intestinal Protozoa.

The distribution of protozoan families and apparent genera and species in the caecum and colon of both black and white rhinoceroses is described in Table 1. The

Table 1. Distribution of families apparent genera and species in the hindgut of both one black and three white rhinoceroses

Family	Black rhinoceros				White rhinoceros			
Genus species	caecum	colon			caecum	colon		
		ventral	dorsal	descending		ventral	dorsal	descending
Buetschliidae								
Blepharosphaera intestinalis	+	+	+	+	+	0	0	0
B. ceratotherii	+	0	+	+	+	+	+	+
B. epsoidalis	0	0	+	+	0	+	0	0
Didesmis ovalis	+	+	+	+	+	0	+	+
D. quadrata	0	+	+	0	0	0	+	0
D. synciliata	0	0	0	0	0	+	+	0
Holophryoides ovalis	0	0	+	0	0	+	+	0
Alloiozona trizona	0	0	0	0	0	+	0	0
Polymorphella ampulla	0	0	+	+	+	+	+	+
Blepharoconus cervicalis	0	0	+	+	0	0	0	0
Paraisotrichidae								
Helicozoster africanus sp. n.	+	+	0	0	+	0	0	0
H. apicalis sp. n.	0	0	0	0	+	0	0	0
Paraisotricha colpoidea	+	+	+	+	+	+	+	0
P. minuta	0	0	+	0	+	+	+	+
Blepharocorythidae								
Charonina odotophora	0	0	0	0	0	+	+	+
C. tenuis	0	0	0	0	0	+	+	+
C. dicerotis	0	0	+	+	0	0	0	0
C. tortuosa	0	0	+	+	0	0	0	0
C. tetragona	0	+	+	+	0	0	0	0
Cycloposthiidae								
Lavierella klipdrifi sp. n.	0	0	0	0	+	+	0	0
L. africana	+	+	0	0	0	0	0	0
Triplumaria hamertoni	+	+	+	+	+	+	+	+
T. selenica	0	0	0	0	0	0	+	0
T. corrugata sp. n.	0	0	0	0	0	0	+	0
Monoposthium vulgaris	0	0	0	0	0	+	+	+
M. brachium	0	0	0	0	0	+	+	+
M. latus	0	0	0	0	0	+	+	+
Cycloposthium bipalmatum	0	0	0	0	0	+	+	0
Diplolophus hydrochoeri	0	0	0	0	0	+	+	0
Arachnodinium noveni	0	0	0	0	0	+	+	+
Phalodinium digitalis	0	0	0	0	0	+	+	+
Prototapirella intestinalis	0	0	0	0	+	0	+	+
Rhinozetidae								
Rhinozeta addoensis	+	0	0	0	+	0	0	0
R. unilaminatus	+	0	0	0	+	0	0	0
R. caecalis	+	+	0	0	0	0	0	0
R. rhinozeta	+	+	+	0	+	0	0	0
R. triciliata	+	+	0	0	+	+	0	+
R. cristata	0	0	0	0	+	+	+	0
R. multiplatus	0	0	0	0	+	0	0	+
R. pedale sp. n.	0	0	0	0	0	0	+	0
Ditoxidae								
Triodinium galea	0	0	0	0	+	+	+	0
Tr. minimum	0	0	0	0	+	+	+	+
Ophryoscolecidae								
Endoralium loxodontae	0	0	+	+	0	+	+	+
Acinetidae								
Allantosoma intestinale	0	0	0	0	+	0	0	0
A. biseriale	0	0	0	0	+	0	+	+
A. multisuctores sp. n.	+	+	+	0	+	+	+	0
Telamodiniidae								
Telamodinium onyx	+	+	0	0	0	0	0	0

Table 2: Protozoan counts, total number of species, new species number of ciliates in the caecum and colon of black (B) and white (W) rhinoceroses

Protozoa	Gut compartments							
	caecum		colon					
			ventral[a]		dorsal[b]		descending	
	B	W	B	W	B	W	B	W
Total counts x 10^3/ml/digesta	362	178(150-233)	266	100(80-120)	259	85(40-130)	31	25(15-35)
Total No. (av.) of species per animal	29	16(11-20)	20	19(13-25)	20	24(23-24)	15	15(9-20)
New species No (av.) of ciliates x 10^3/ml/digesta								
Trichostomatida								
Paraisotrichidae								
Helicozoster africanus sp. n.	50	13 (10-20)	0	0	0	0	0	0
H. apicalis sp. n.	0	30	0	0	0	0	0	0
Entodiniomorhida								
Rhinozetidae								
Rhinozeta pedale sp. n.	0	0	0	0	0	1	0	0
Cycloposthiidae								
Triplumaria corrugata sp. n.	0	0	0	0	0	2(1-2)	0	0
Lavierella klipdrifi sp. n.	0	60(40-80)	0	21(1-40)	0	0	0	1
Suctorida								
Acinetidae								
*Allantosoma multisuctores*n. sp. n.	8	4(2-9)	7	1(0-2)	2	2(0-3)	0	0

Dorsal[b] - small colon; ventral[a] - large colon

numbers and variety of species in the colon were greater than those in the caecum. This was to be expected since food fermentation was initiated and subsequently maintained in the colon as the main locus of fermentation in large herbivorous mammals like rhinoceroses (Hume and Warner 1980).

Protozoan counts x10^3/ml digesta fluid of the proposed new species separately and together with the total number of species in the caecum and colon of 1 black and 2 white rhinoceroses is given in Table 2. The counts of the browsing black rhinoceros outnumbered, by a factor of 2 or 3, those of the grazing white rhinoceroses in every fermentative hindgut compartment. High counts of *Helicozoster* and *Lavierella* were encountered in caecal fluid. Counts x10^3/ml of 50 *H. africanus* for the black rhinoceros, 30 *H. apicalis* for the white rhinoceroses, and 60 *L. klipdrifi* for the white rhinoceroses only. A similar high count of *L. africana* was found for the black rhinoceros only in our study and that of Buisson (1923).

Helicozoster africanus sp. n. (Figs. 1, 8A, 9, 10)

Description (n=33): body ovoid covered with uniformed somatic ciliation (Figs. 8A,9); length 157 ±13.1 µm, width 114±11.0 µm optically measured dorso-ventral thickness top 27 ±7.8 µm, middle 48 ± 9.3 µm, bottom 36 ±11.0 µm. Apical oral-opening (Fig. 10) at termination of elongate peristomal channel. External ciliated peristomal trough (Latteur 1967) (Figs. 8A,9) spiralling the entire body-length and connected anteriorly (Latteur 1967) with peristomal channel leading into cytopharynx. Inner sides of trough bear parallel striations. Outer edges of trough bear dense long flexible cilia (Fig. 10). Under the oil immersion objective (x1000) the entire body covered with 72 close parallel longitudinal ridges (Figs. 8A,9) bearing dense short cilia. Agglomerate of inclusions in posterior third of body (Fig. 8A). Position of round/oval granular macronucleus variable (Fig. 8A); small oval micronucleus recessed in anterior end of macronucleus (Fig.1). Variable number of contractile vacuoles scattered throughout body. Position of cytoproct as a small indention on posterior pole of body.

Type host: *Diceros bicornis* Linnaeus 1758, black rhinoceros.

Other host: *Ceratotherium simum* Burchell 1817, white rhinoceros.

Type locality: black rhinoceros Addo Elephant National Park, Eastern-Cape Province, South Africa (33°-34°S; 25°-26°E).

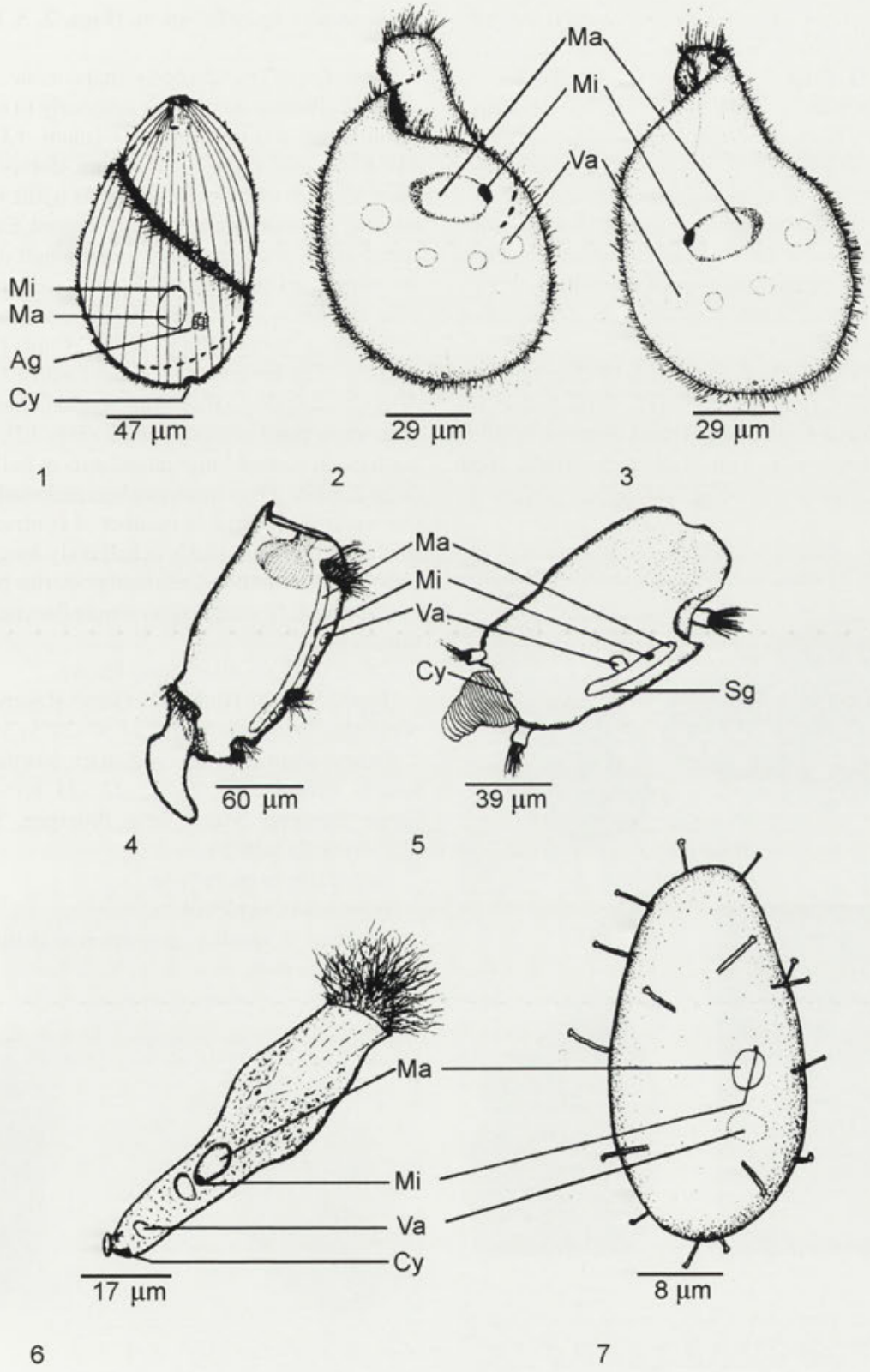


Fig. 1. *Helicozoster africanus* sp. n., ventral view, dotted line indicating length of external trough. Figs. 2,3. *H. apicalis* sp. n. 2 - ventral view, dotted line indicating length of external trough. 3 - dorsal view. Fig. 4. *Rhinozeta pedale* sp. n., ventral view. Fig. 5. *Triplumaria corrugata* sp. n. , ventral view. Fig. 6. *Lavierella klipdrifi* sp. n., ventral view. Fig. 7. *Allantosoma multisuctores* sp. n., ventral view. Ag - agglomerate of inclusions, Cy - cytoproct, Ma - macronucleus, Mi - micronucleus, Sg - skeletal groove, Va - vacuole

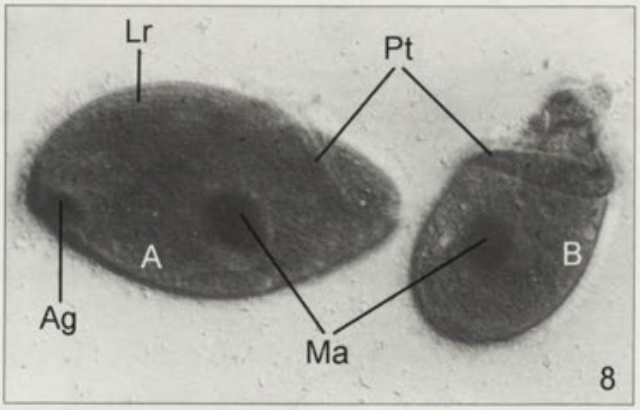
Other localities: white rhinoceros Pilanesberg Game Reserve, North-West Province, South Africa (25°-26°S; 27°-28°E); Ellisras district, Northern Province, South Africa (23°-24°S; 27°-28°E); Hluhluwe Game Reserve, Kwazulu-Natal Province, South Africa (28°-29°S; 31°-32°E).

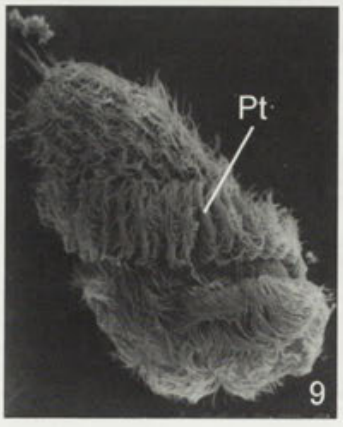
Site of infection: caecum and ventral colon.

Prevalence: Addo black rhinoceros $5x10^4$/ml; Hluhluwe white rhinoceros $2x10^4$/ml; Pilanesberg white rhinoceros $1x10^4$/ml; Ellisras white rhinoceros $1x10^4$/ml.

Etymology: specific name refers to the continent on which hosts were found.

Type materials: black rhinoceros, Addo Elephant National Park, Accession No. 2240485, deposited in the Intestinal Protozoa Collection of the Centre of Wildlife Management, University of Pretoria, Pretoria 0002, South Africa.





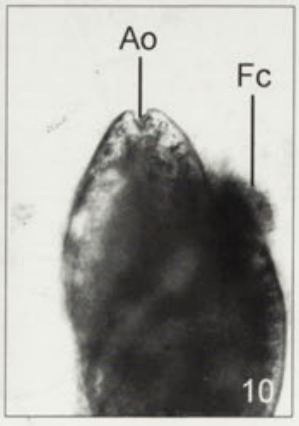


Fig. 8. *Helicozoster* spp. venral view, light Nomarski microphotograph (LNM), x 1250. A - *H. africanus*.; B - *H. apicalis*. Ag - agglomerate of inclusions, Lr - parallel longitudinal ridges, Ma - macronucleus, Pt - external peristomal troughs. Fig. 9. *H. africanus*, scanning electron micrograph (SEM), x 1333. Pt - external peristomal troughs. Fig. 10. *H. africanus* (LMN), x 1250. Ao - apical oral-opening, Fc - long flexible cilia edging external peristomal trough

***Helicozoster apicalis* sp. n. (Figs. 2, 3, 8B, 11, 12)**

Description (n=32): body-shape ovate, with uniform somatic ciliation, narrowing anteriorly to a short column terminating in a rounded apex (diam. 13.3µm); length 118 ±17.1 µm, width 73 ±14.7 µm, dorso-ventral thickness 33 ±7.4 µm. Oral-opening is a slit in tip of apex opening into elongate peristomal channel. External ciliated peristomal trough spiralling anterior half of body-length and connected anteriorly with peristomal channel leading into cytopharynx. Edges of oral-opening slit and of peristomal trough bear dense long flexible cilia (Figs. 11,12). Entire body covered with 72 close parallel ridges bearing dense short cilia. Agglomerate of inclusions in anterior third of body-length (Fig. 12). Oval macronucleus on central longitudinal axis at half body-length (Figs.2,3,8B). Oval micronucleus recessed in one end of macronucleus. Variable number of contractile vacuoles scattered throughout body at half body-length (Figs. 2,3). Cytoproct in small indention in posterior pole of body.

Type host: *Ceratotherium simum* Burchell 1817, white rhinoceros.

Other host: no other hosts known.

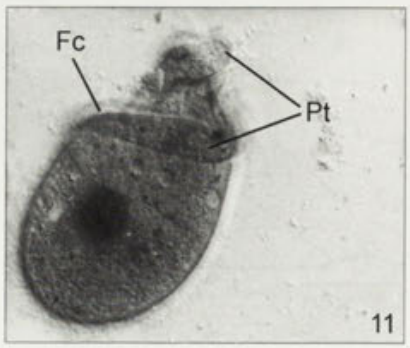
Type locality: Hluhluwe Game Reserve, Kwazulu-Natal Province, South Africa (28°-29°S; 31°-32°E).

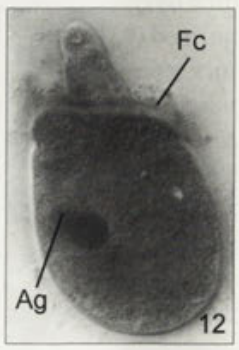
Other localities: Ellisras district, Northern Province, South Africa (23°-24°S; 27°-28°E); Pilanesberg Game Reserve, North West Province, South Africa (25°-26°S; 27°-28°E).

Site of infection: caecum.

Prevalence: $3x10^4$/ml.

Etymology: specific name refers to distinctive anterior peak of organism.





Figs. 11,12. *H. apicalis*, (LNM), x 1250. 11 - ventral view. Fc - long flexible cilia edging external peristomal trough, Pt - external peristomal trough. 12 - dorsal view. Ag - agglomerate of particles, Fc - long flexible cilia edging external peristomal trough

Type materials: white rhinoceros, Accession No. 1130476 (Hluhluwe), deposited in the Intestinal Protozoa Collection of the Centre of Wildlife Management, University of Pretoria, Pretoria 0002, South Africa.

Key to species of *Helicozoster*

The following key is based on body-shape, anterior oral-opening, external peristomal trough.

1 Body-shape ovate terminating in anterior column, external peristomal trough spiralling anterior half of body-length.....*H. apicalis* sp. n.
Body-shape simple ovoid, external peristomal trough spiralling entire body length.....2

2 Anterior oral-opening subterminal .....*H.indicus* Latteur, 1967
Anterior oral-opening terminal.....*H. africanus* sp. n.

***Rhinozeta pedale* sp. n. (Figs. 4, 13)**

Description (n=30): body ovate-longate, dorso-ventrally compressed like the other members of the genus (van Hoven *et al.* 1988), with foot-like posterior end and 2 skeletal plates (Fig. 13); length 228±62.2 µm, width 112 ±39.7 µm, dorso-ventral thickness 23 ±3.0 µm. Oral-opening stretching across entire anterior end of body with characteristic hook-like protrusion on left side (Fig. 13). Adoral zone of cilia borne on retractable cone within peristome. Four semicircular slits forming 4 somatic ciliary bands wrapped partially around the lateral surfaces of body: 3 on the ventral side and 1 on the dorsal side according to Kofoid and MacLennan (1933). First ventral band below oral-opening, second ventral band one half body-length from anterior end of body, third ventral and dorsal bands immediately above foot-like posterior end of body (Fig. 13). All ciliary bands lie across longitudinal axis of body and are housed in the space between 2 thin skeletal plates covering ventral and dorsal surfaces of the body. Cytoproct below posterior end of upper plate. Elongate (173-188 µm) macronucleus beneath ventral edge of upper skeletal plate. Small oval micronucleus recessed in ventral side of macronucleus one-quarter of length of macronucleus from its anterior end. Three or 5 contractile vacuoles on edge of right lateral surface (Fig.4).

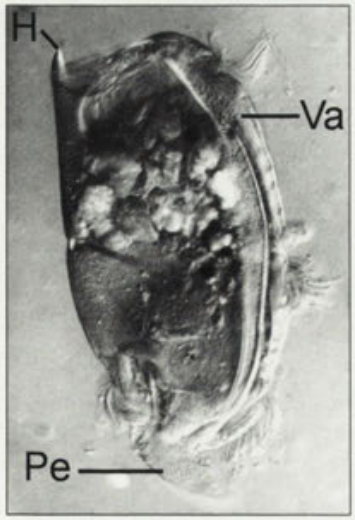


Fig. 13. *Rhinozeta pedale*, ventral view, (LNM), x 1250. H - hook-like protrusion on oral-opening, Pe - foot-like posterior end, Va - contractile vacuoles

Type host: *Ceratotherium simum* Burchell 1817, white rhinoceros.

Other host: no other hosts known.

Type locality: Pilanesberg Game Reserve, North-West Province, South Africa (25°-26°S; 27°-28°E).

Site of infection: dorsal ascending colon.

Prevalence: $1x10^3$/ml.

Etymology: specific name refers to foot-like posterior end of the ciliate.

Type materials: white rhinoceros, Accession No. 4220585 (Pilanesberg) deposited in the Intestinal Protozoa Collection of the Centre of Wildlife Management, University of Pretoria, Pretoria 0002, South Africa.

Key to species of *Rhinozeta*

The following key is based on body-shape, body-size, number of ciliary bands, type of skeletal plate material, cuticular folds (van Hoven *et al.* 1988).

1 With 3 ciliary bands present.....*R. triciliatum* van Hoven *et al.* 1988
With 4 or 5 ciliary bands present.....2

2 With 4 ciliary bands present.....3
With 5 ciliary bands present.....5

3 Small, 94-150 µm long no clear skeletal plate cut-off on left side of body.....*R. rhinozeta* van Hoven *et al.* 1988
Large, 120-331 µm long.....4

4 Posterior end rounded, with clear cuticular folds on the right side and rear of body.....*R. caecale* van Hoven *et al.* 1988
Posterior end foot-like without cuticular folds.....*R. pedale* sp. n.

5 Body largely covered in skeletal plate material.....6
Body partly covered in skeletal plate material.....7
6 Clear lengthwise stripes of skeletal plates.....
.....*R. multiplatum* van Hoven *et al.* 1988
Body mostly uniformly covered by a thin layer of skeletal material.....*R. unilaminatus*
7 Prominent skeletal plate with undulating edge protruding from left body-surface.....
.....*R. cristatum* van Hoven *et al.* 1988
Narrow single plate in the middle of the ventral and dorsal surfaces covering about 50% of each suface...
.....*R. addoense* van Hoven *et al.* 1988

Triplumaria corrugata sp. n. (Figs. 5, 14, 15)

Description (n=34): body-shape ovate-elongate, posterior and terminating in corrugated triangular tail, dorso-ventrally compressed with skeletal rod, and encased in cuticular carapace (Fig. 14); length 153 µm ±23.5, width 79 µm ±12.5, dorso-ventral thickness 22 µm ±3.8. Oral-opening apical (Figs. 14,15). Adoral zone of cilia borne on retractable cone within peristome. Three motilia; one a quarter body-length from anterior end on outside ventral side of body, 2 immediately anterior to corrugated tail on either side of posterior end of body (Fig. 14). Skeletal rod in groove on ventral side of body beneath macronucleus extending full length of body. Cytoproct beneath small raised portion of upper carapace between posterior motilia. Elongate macronucleus posterior to anterior motilium on ventral side of body. Oval micronucleus recessed in dorsal anterior side of macronucleus. Three or more contractile vacuoles in groove on ventral side of body (Fig. 5).

Type host: *Ceratotherium simum* Burchell 1817, white rhinoceros.

Other host: no other hosts known.

Type locality: Pilanesberg Game Reserve, North-West Province, South Africa (25°-26°S; 27°-28°E).

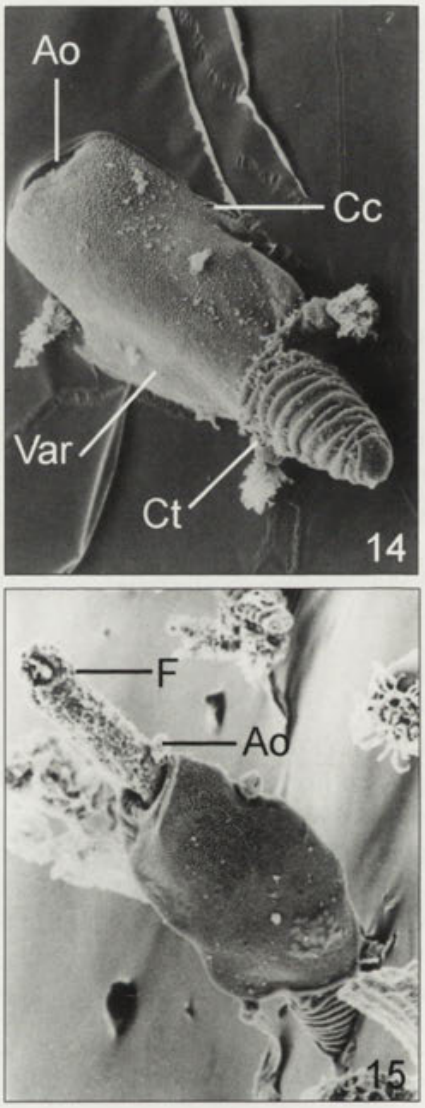
Other locality: Ellisras district, Northern Province, South Africa (23°-24°S; 27°-28°E).

Site of infection: dorsal ascending colon.

Prevalence: Pilanesberg white rhinoceros 0.3×10^3/ml, Ellisras white rhinoceros 1×10^3/ml

Etymology: specific name refers to corrugated tail.

Type material: white rhinoceros, Accession No. 4220585 (Pilanesberg) deposited in the Intestinal Protozoa Collection of the Centre for Wildlife Management, University of Pretoria, Pretoria 0002, South Africa.



Figs. 14,15. *Triplumaria corrugata*, ventral view. 14 - SEM, x 500. Ao - apical oral-opening, Cc - cuticular carapace, Ct - corrugated triangular tail, Var - region of vacuoles. 15 - SEM, x 357. Ao - apical oral-opening, F - piece of vegetation being ingested

Key to species of *Triplumaria*

The following key is based on body-shape, body-size, position of oral-opening.

1 Body long: posterior end rounded and smooth.....2
Body short: posterior end somewhat triangular and corrugated.....*T. corrugata* sp. n.
2 Oral-opening subapical on ventral side of body.....
.....*T. selenica* Latteur *et al.* 1970
Oral-opening apical .....*T. hamertoni* Hoare, 1937

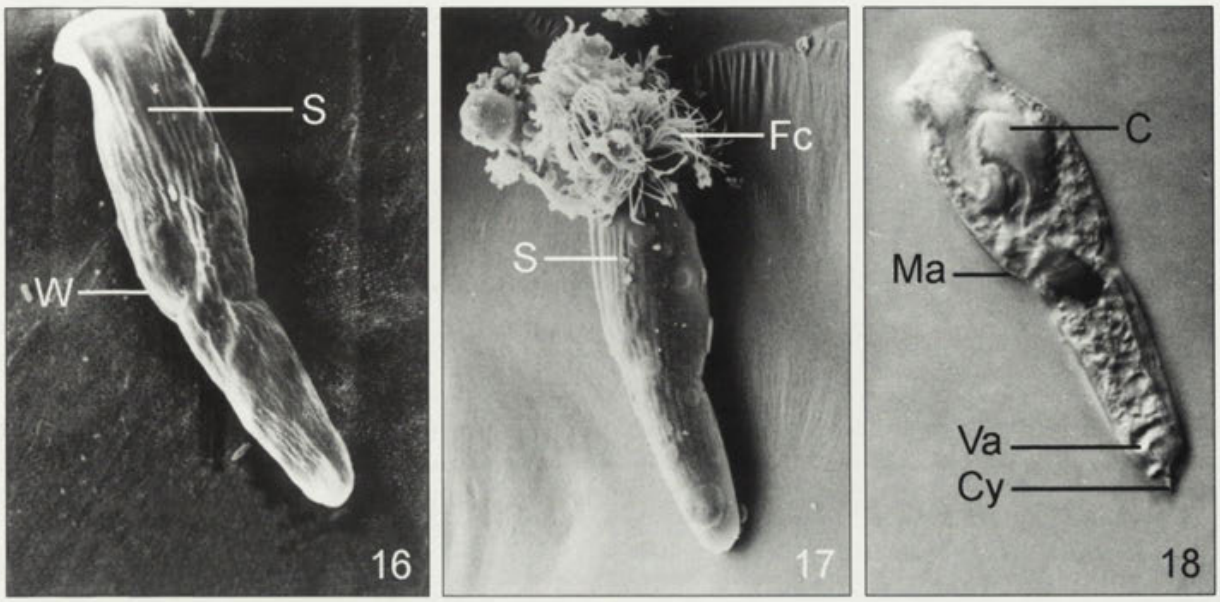


Fig. 16 - 18. *Lavierella klipdrifi*, ventral view. 16 - LNM, x 1250. S - oral cilia of retracted ciliate showing striations of naked cuticle, W - widening at half body-length giving club-shape to body. 17 - SEM, x 867. Fc - tuft of long flexible cilia borne on a cone within peristome, S - cuticular striations. 18 - LNM, x 1250. C - retracted cone within peristome, Cy - covering projecting cytoproct, Ma - macronucleus, Va - contractile vacuoles

***Lavierella klipdrifi* sp. n. (Figs. 6, 16-18)**

Description (n=33): body club-shaped, anterior widening at half body-length to 1.5 times posterior body-width; body covered with rigid naked striated cuticle (Fig.16,17); length 74 µm±8.5, anterior body-width 22 µm ±4.2, anterior body-dorso-ventral-thickness 20 µm ±2.7. Oral-opening apical. Tuft of slender long flexible cilia borne on retractable cone within peristome of oral-opening (Fig. 17). Oval macronucleus in posterior half of body. Oval micronucleus recessed in posterior end of macronucleus (Fig. 6). Position of one or more contractile vacuoles variable. Distinct cytoproct at posterior pole of body (Fig. 6,18).

Type host: *Ceratotherium simum* Burchell 1817, white rhinoceros

Other host: no other hosts known.

Type locality: Pilanesberg Game Reserve, North-West Province, South Africa (25°-26°S; 27°-28°E).

Other localities: Ellisras district, Northern Province, South Africa (23°-24°S; 27°-28°E). Hluhluwe Game Reserve, KwaZulu-Natal Province, South Africa (28°-29°S; 31°-32°E).

Site of infection: caecum, ventral ascending colon.

Prevalence: caecal fluid: Hluhluwe rhinoceros $6x10^4$/ml; Ellisras rhinoceros $4x10^4$/ml; Pilanesberg rhinoceros $8x10^4$/ml. Ventral ascending colon fluid: Pilanesberg rhinoceros $4x10^4$/ml; Ellisras rhinoceros $1x10^3$/ml; Hluhluwe rhinoceros no sample.

Etymology: species name refers to the bottle shape of the ciliate.

Type material: white rhinoceros, Accession No. 4220585 (Pilanesberg), deposited in the Intestinal Protozoa Collection of the Centre for Wildlife Management, University of Pretoria, Pretoria 0002, South Africa.

Key to species of *Lavierella*

The following key is based on body-shape.

1 Body-shape ovate-elongate.....
.....*L. africana* Buisson, 1923
Body-shape club-shaped.....*L. klipdrifi* sp. n.

***Allantosoma multisuctores* sp. n. (Figs. 7, 19)**

Description (n=33 adults): body-shape elongate-cycloid with 20 to 24 ingestory suctorial tentacles scattered uniformly over entire body (Fig. 19); length 35 µm ± 5.3; width 17 µm ±2.6, dorso-ventral thickness 10 µm ±3.0. Distinct sucker borne at end of each tentacle.

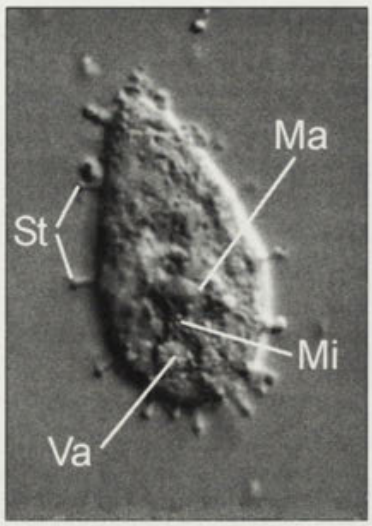


Fig. 19. *Allantosoma multisuctores*, ventral view (LNM) x 1000. Ma - macronucleus, Mi - micronucleus, St - suctorial tentacles, Va - contractile vacuole

Centrally placed spherical macronucleus with spherical micronucleus lying closely along side. Single contractile vacuole. No visible cytoproct (Fig. 7).

Type host: *Disceros bicornis* Linnaeus 1758, black rhinoceros.

Other host: *Ceratotherium simum* Burchell 1817, white rhinoceros.

Type locality: black rhinoceros, Addo Elephant National Park, Eastern-Cape Province, South Africa (33°-34°S; 25°-26°E).

Other localities: white rhinoceros Hluhluwe Game Reserve, KwaZulu-Natal Province, South Africa (28°-29°S; 31°-32°E); Pilanesberg Game Reserve, North-West Province, South Africa (25°-26°S; 27°-28°E); Ellisras district, Northern Province, South Africa (23°-24°S); 27°-28°E).

Site of infection: caecum, ventral and dorsal ascending colon.

Prevalence: caecal fluid: Addo black rhinoceros $8x10^3$/ml, Hluhluwe white rhinoceros $9x10^3$/ml, Pilanesberg white rhinoceros $2x10^3$/ml; fluid of ventral ascending colon: Addo black rhinoceros $7x10^3$/ml, Pilanesberg white rhinoceros $2x10^3$; fluid of dorsal ascending colon: Addo black rhinoceros $2x10^3$/ml, Ellisras white rhinoceros $3x10^3$/ml.

Etymology: species name refers to numerous scattered tentacles with suckers.

Type material: black rhinoceros, Accession No. 2240485 (Addo) deposited in the Intestinal Protozoa Collection of the Centre for Wildlife Management, University of Pretoria, Pretoria 0002, South Africa.

Key to species of *Allantosoma*

The following key is based on the number of tentacles, position of tentacles, body-width, body-length (Imai, 1979).

1 Tentacles confined to each end of body.....2
Tentacles scattered uniformly over entire body surface .....*A. multisuctores* sp. n.

2 One tentacle confined to each end of body.....3
More than one tentacle confined to each end of body 4

3 Body-width 3-7 µm.....*A. lineare* Strelkow, 1939
Body-width 7-11 µm.....
.....*A. brevicornigerum* Hsiung, 1928
Body-width 10-20 µm.....
.....*A. dicornigerum* Hsiung, 1928

4 Body-length 16-35 µm.....5
Body-length 33-67 µm.....6

5 Body-width 7-10 µm.....*A. japonense* Imai, 1979
Body-width 6-17 µm.....
.....*A. biseriale* Strelkow, 1939

6 Body-width 10-20 µm.....
.....*A. cucumis* Strelkow, 1939
Body-width 18-37 µm.....
.....*A. intestinale* Hsiung, 1930

DISCUSSION

In comparing counts of ciliated protozoan endocommensals occurring in the hindgut of rhinoceroses with those of horses and elephants, only those for the 3 grazing white rhinoceroses were used. The higher counts for the black rhinoceros browsing on highly nutritious "spekboom" *Portulacaria afra* were considered exceptional. In fed animals the average total ciliate counts x 10^3/ml digesta in white rhinoceroses, horses (Ozeki *et al.* 1973) and African elephants (Eloff and van Hoven 1980) were respectively, 178, 220, 41 for caecum, 100, 770, 46 for ventral/large colon, 85, 240, no count, for dorsal/small colon. This indicated that the colon probably played an important role in the fermentation of the diet of these 3 groups of large herbivorous mammals as predicted by Hume and Warner (1980).

Of the 48 species recovered to date from the digesta of the rhinoceroses, 15 species (*Blepharosphaera episoidalis*,

Didesmis ovalus, *D. quadrata*, *Holophryoides ovalis*, *Alloiozona trizona*, *Polymorphella ampulla*, *Blepharoconus cervicalis*, *Paraisotricha colpoidea*, *P. minuta*, *Cycloposthium bipalmatum*, *Triadinium galea*, *T. minimum*, *Allantosoma intestinale*, *A. biseriale*, *Telamodinium myx* are in common with those of horses (Hsiung 1930, Strelkow 1939, Adam 1951, Latteur and Dufrey 1967, Ozeki *et al.* 1973), 8 species (*Blepharosphaera intestinalis*, *Paraisotricha colpoidea*, *P. minuta*, *Laverella africana*, *Triplumaria hamertoni*, *T. selenica*, *Cycloposthium bipalmatum*, *Prototapirella intestinalis*) are in common with those of african elephants (Latteur *et al.* 1970, Eloff and van Hoven 1980), and 6 species (*Holophryoides ovalis*, *Blepharosphaera intestinalis*, *B. spisoidalis*, *Didesmis ovalis*, *D. quadrata*, *Paraisotricha colpoidea*) are in common with those of bushpigs (van Hoven and Gilchrist 1991).

These 48 species from rhinoceroses belong to 22 apparent genera belonging to 9 families [Buetschliidae, Paraisotrichidae, Blepharocorythidae, Cycloposthiidae, Ditoxidae, Ophryoscolecidae, Acinetidae, Telamodiniidae (Corliss 1979) and a recently new family Rhinozetidae (van Hoven *et al.* 1988)], 7 more than the 2 Dehority (1986) reported. These families also contain ciliated protozoan species recovered from the intestines of other herbivorous hindgut-fermenting mammals such as zebras, asses, Indian elephants, tapirs, warthogs, guinea pigs, capybaras (Dehority 1986), and bushpigs (van Hoven and Gilchrist 1991).

Only the family Rhinozetidae appeared to be specific for the rhinoceroses as in the case of the Polydiniidae for the elephants and the Troglodytellidae for the anthropoid apes (Dehority 1986). The 25 systematically identified, proposed, and apparent new species are rhinoceros-specific (*Blepharosphaera intestinalis minor*, *Didesmis synciliata*, *Helicozoster africanus*, *H. apicalis*, *Charonina odontophora*, *C. tenuis*, *C. dicerotis*, *C. tortuosa*, *C. tetragona*, *Lavierella klipdrifi*, *Triplumaria corrugata*, *Monoposthium vulgaris*, *M. bracchium*, *M. latus*, *Arachnodinium noveni*, *Phalodinium digitalis*, *Rhinozeta addoense*, *R. unilaminatum*, *R. caecale*, *R. rhinozeta*, *R. triciliatum*, *R. cristatum*, *R. multiplatum*, *R. pedale*, *Allantosoma multisuctores*). Much more detailed research is requred in respect of host specificity of the ciliated Protozoa recovered from the intestines of herbivorous mammals, as well as measured counts of these organisms in the gastro-intestinal tract.

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