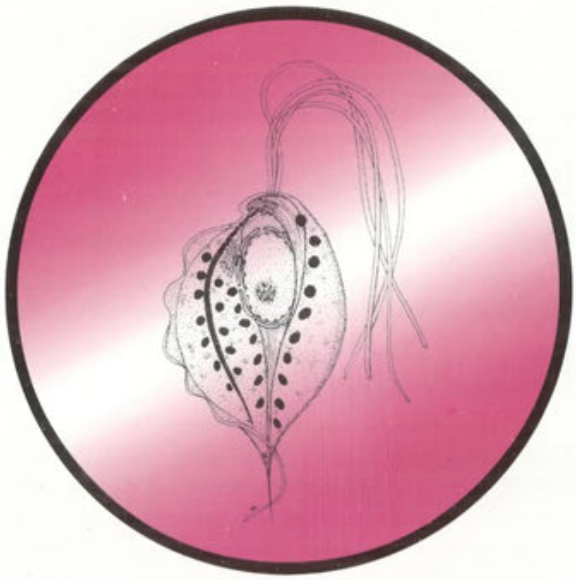
ACTA PROTOZOOLOGICA



NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY
WARSAW, POLAND
1999
VOLUME 38 NUMBER 2
ISSN 0065-1583

Polish Academy of Sciences
Nencki Institute of Experimental Biology
and
Polish Society of Cell Biology

ACTA PROTOZOOLOGICA
International Journal on Protistology

ACTA PROTOZOOLOGICA appears quarterly.

The price (including Air Mail postage) of subscription to ACTA PROTOZOOLOGICA at 1999 is: US $ 180.- by institutions and US $ 120.- by individual subscribers. Limited numbers of back volumes at reduced rate are available. TERMS OF PAYMENT: check, money oder or payment to be made to the Nencki Institute of Experimental Biology account: 11101053-3522-2700-1-34 at Państwowy Bank Kredytowy XIII Oddz. Warszawa, Poland. For matters regarding ACTA PROTOZOOLOGICA, contact Editor, Nencki Institute of Experimental Biology, ul. Pasteura 3, 02-093 Warszawa, Poland; Fax: (4822) 822 53 42; E-mail: jurek@ameba.nencki.gov.pl For more information see Web page **http://www.nencki.gov.pl/public.htm**).

Front cover: *Trichomonas aotus* sp. n. In: F. F. Pindak and M. Mora de Pindak (1998) Diagnostic characteristics of owl monkey *(Aotus trivignatus)* intestinal trichomonads. *Acta Protozool.* **37:** 159-172

This publication is supported by the State Committee for Scientific Research

Desktop processing: Justyna Osmulska, Data Processing Laboratory of the Nencki Institute
Printed at the MARBIS, ul. Kombatantów 60,
05-070 Sulejówek, Poland

Acta Protozool. (1999) 38: 87 - 96

ACTA
PROTOZOOLOGICA

Review Article

Ciliary and Flagellar Activity Control in Eukaryotic Cells by Second Messengers: Calcium Ions and Cyclic Nucleotides

Hanna FABCZAK, Mirosława WALERCZYK, Jerzy SIKORA and Stanisław FABCZAK

Department of Cell Biology, Nencki Institute of Experimental Biology, Warsaw, Poland

Key words: axoneme, Ca^{2+}, calmodulin, cAMP, cGMP, cilium, flagellum, ion channels, protein kinases.

Summary. Extracellular stimuli are converted in eukaryotic cells through signal transduction mechanisms to generate intracellular second messengers such as cyclic nucleotides and Ca^{2+}. These molecular signals, amongst other, may control the ciliary and flagellar locomotor systems by modulating the activity of axonemes, changing the direction and frequency of effective ciliary beating or changing the pattern of flagellar motion. The primary role in regulating the mechanisms of axonemal motility by second messengers is played by processes of phosphorylation and dephosphorylation of axoneme proteins. Ca^{2+} may also regulate the levels of cAMP and cGMP by controlling the activity of cAMP and cGMP cyclases. In addition, Ca^{2+} and cyclic nucleotides may regulate ion channel conductance, thus affecting the cell membrane potential in these cells.

INTRODUCTION

The forces that make the movement of eukaryotic cells possible by means of flagella and cilia are generated by cytoskeletal microtubular systems. The essential element of these structures consists of microtubules which, together with numerous accompanying proteins, form the axoneme - the skeleton of flagella and cilia covered by the ciliary or flagellar membrane. These organelles constitute the motile systems of protozoa, algae, the larvae of some invertebrates and spermatozoa. They are also essential for the movement of eggs along oviducts and mucus in the tracts of respiratory systems. The microtubules within an axoneme are arranged in a way that a central pair of microtubules is surrounded by a ring of peripheral nine microtubular doublets (9 + 2 scheme) (Fig. 1). Each microtubule consists of tubulin heterodimers, known as α- and β-tubulins. The tubulin dimers in central doublets are organized into a complete tubule containing 13 protofilaments per one tubule. The nine peripheral doublets are formed by one complete tubule A and an attached one incomplete tubule B. Each tubule A of these doublets is joined to the central sheath of the axoneme by radial spokes and bears also two dynein arms pointed toward the tubule B of the next doublet. Microtubule doublets are also

Address for correspondence: Hanna Fabczak, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Department of Cell Biology, ul. Pasteura 3, 02-093 Warszawa, Poland; Fax: (4822) 822 53 42; E-mail: hannafab@nencki.gov.pl

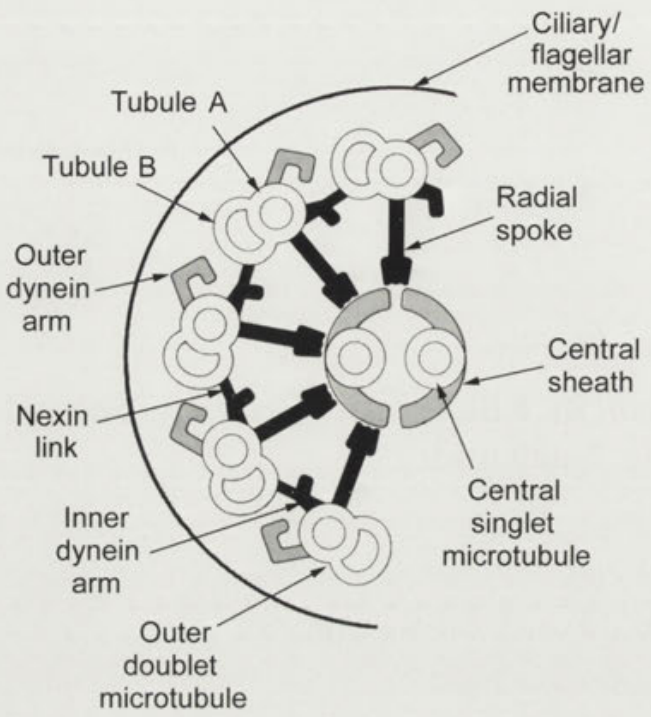


Fig. 1. Schematic illustration of the microtubule arrangement of ciliary or flagellar axoneme

held together by nexin protein links (Stephens 1974, Warner 1974, Omoto 1995). The protein of which the dynein arms consist exhibits Mg^{2+}-dependent ATPase activity. The longitudinal sliding of the outer doublets of microtubules coupled to the hydrolysis of ATP by dynein (Satir 1985, Gibbons 1989), is converted to a bending motion, characteristic for ciliary and flagellar beating, by shear resistance due to some structures such as nexin links, radial spokes and basal bodies. This is an essential feature of the motile behavior of cilium and flagellum (Satir 1985). Movement of flagella can be approximately characterized as oscillation in two dimensions. The motion of cilium is more complex, occurring in two phases, a rapid one-dimensional power stroke affecting the forward movement of the cell and a slower three-dimensional return of the cilium to the initial position. The frequency of ciliary beatings and the direction of effective power stroke of the cilium, as well as flagellar waveform, are modulated when the cell responds behaviorally to environmental stimuli (Sleigh 1974). Thus, both the cilium and flagellum serve as the effector in the complex process of signal transduction. This process starts with the receptor perception of external stimuli and leads over a series of intracellular events to axoneme activity changes.

IMPORTANCE OF Ca^{2+} AND CALMODULIN FOR THE AXONEME FUNCTION

External stimuli recognized by receptors located within the cell membrane affect the pattern of axoneme movement and of cell motile behavior through the action of secondary transmitters like Ca^{2+} and cyclic nucleotides. Studies on the regulation of ciliary movement have been carried out for a long time on ciliate cells and recently on the ciliated respiratory epithelium of higher organisms (Schultz *et al.* 1990; Bonini *et al.* 1991; Salathe *et al.* 1993; Geary *et al.* 1995; Salathe and Bookman 1995). The mechanism of flagellar movement is most often studied in *Chlamydomonas* (Tash 1989; Walczak and Nelson 1994; Habermacher and Sale 1995, 1997) and in spermatozoa (Cook and Babcocks 1993 a, b; Cook *et al.* 1994).

Ciliates

The phenomenon of ciliary reversal has long been known to occur in freshwater ciliates in response to different stimuli such as light, temperature, and chemical or mechanical stimulations (Fabczak and Wood 1980; Ogura and Machemer 1980, Machemer and Deitmer 1985; Nakaoka *et al.* 1987; Van Houten 1988; Fabczak *et al.* 1993 a, b; Kuriu *et al.* 1996). It consists of a transient change in the direction of power stroke of cilia to an opposite one and an increased beat frequency, which results in backward swimming (Eckert 1972, Eckert and Brehm 1979, Preston and Saimi 1990). In natural conditions this phenomenon, for example, occurs when a forward swimming ciliate encounters a stable obstacle. It briefly backs away, tumbles momentarily and changes its swimming direction. Reversal of the ciliary beat in these cells is strictly correlated with the generation of depolarizing receptor potential, which in turn evokes an action potential. The action potential is generated due to the activation of voltage-dependent Ca^{2+} channels located in the ciliary membrane and an influx of Ca^{2+} into the cilium (Dunlap 1977, Tamm 1994). Restoration of the membrane potential (repolarization) following stimulation occurs due to activation of K^+ channels in the plasma membrane (Eckert and Brehm 1979, Bonini *et. al.* 1991), whereas the resting Ca^{2+} level within cell is restored by Ca^{2+}-ATPase existing in the plasma, ciliary and alveolar

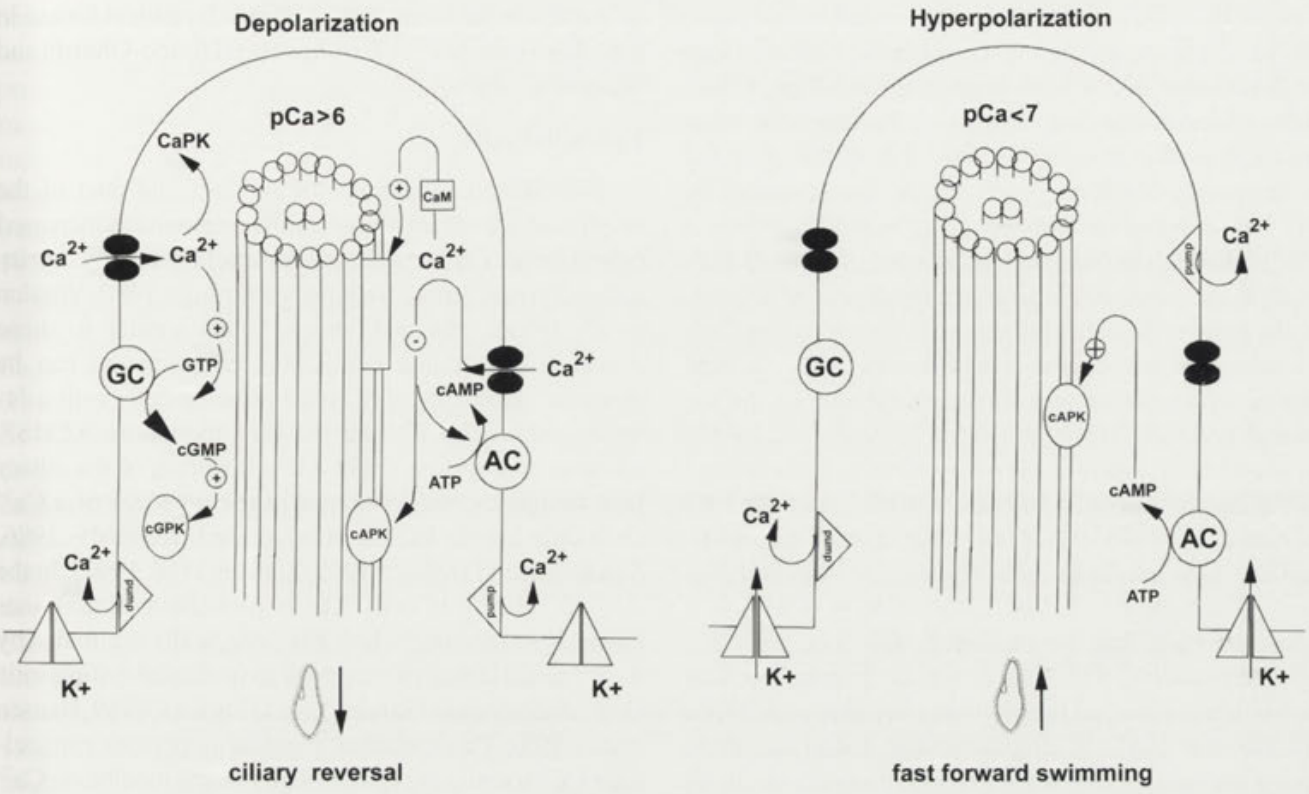


Fig. 2. Diagrams showing cilium responses to the membrane depolarization (A) or its hyperpolarization (B). A - depolarizing stimulus causes an opening of voltage-dependent Ca^{2+} channels in ciliary membrane and influx of extracellular Ca^{2+} into the cilium. The transient increase in ciliary free Ca^{2+} level evokes the change in direction of the power stroke of the cilium and backward (arrow) cell swimming. The increase in Ca^{2+} level within the axoneme may stimulate an activity of Ca^{2+}- dependent protein kinase (CaPK), CaM-dependent protein kinase (CaM-PK), guanylate cyclase (GC) or cGMP-dependent protein kinase (cGPK). An increase in Ca^{2+} concentration inhibits also adenylate cyclase (AC) and cAMP- dependent protein kinase (cAPK) activity. B - the membrane hyperpolarization is accompanied by opening of voltage-dependent K^+ channels in the cell plasma membrane and activation of adenylate cyclase (AC) with resulting increase in intraciliary cAMP level. CyclicAMP activates cAPK and causes in turn the axonemal dynein phosphorylation and increase in the ciliary beat frequency and faster forward cell swimming (arrow). Removal of Ca^{2+} from the cilium is evoked by activation of Ca^{2+} pump located in the ciliary membrane or plasma and alveolar membranes as well

membranes (Doughty and Kaneshiro 1985, Stelly *et al.* 1991).

The depolarization of the cell membrane and increase in intraciliary Ca^{2+} concentration in *Paramecium* is accompanied by ciliary reversal and an increased ciliary beat frequency, i.e. accelerated backward swimming. This seems to indicate that these two phenomena are directly coupled with a rise in Ca^{2+} levels. This view has been confirmed in a series of experiments on Triton-extracted models of *Paramecium* prepared in the presence of Mg^{2+} and EGTA (Nakaoka *et al.* 1984) and behavioral mutants (Kung 1971 a, b; Hinrichsen and Kung 1984). A mutant named pawn fails both to swim backward and to increase its beat frequency upon membrane depolarization. The voltage-clamp experiments carried out on these mutants show that they lack a voltage-dependent Ca^{2+} current (Kung and Eckert 1972, Kung and Naitoh 1973, Eckert and Brehm 1979). A second type of behavioral mutant atalanta, which like pawn cannot move backwards, showed a defect in a downstream element of the signal transduction pathway. On treatment of these cells with depolarizing stimuli, Ca^{2+} enters the atalanta mutant normally but does not induce the reversal of the ciliary power stroke. This altered axonemal response of the atalanta mutant to Ca^{2+} entering was thought to be due to some factors linking Ca^{2+} influx and axoneme function or the axonemal machinery

itself. In this case, the Ca^{2+} entering the cell would cause only an acceleration of the ciliary motion without changing the direction of ciliary beat (Hinrichsen and Kung 1984). These observations indicate that in *Paramecium* those two parameters of axonemal activity, i.e. the direction and the frequency of ciliary power stroke, are regulated by Ca^{2+} but probably in an independent manner.

It is difficult to precisely determine the site of Ca^{2+} action in the axoneme causing an alteration of its activity. On the basis of the data available so far, it seems that these alterations are probably due to processes of Ca^{2+}-dependent phosphorylation and dephosphorylation of the axonemal proteins. This supposition is confirmed by the presence in *Paramecium*, of two immunologically distinct Ca^{2+}-dependent protein kinases, Ca-PK1 and Ca-PK2 (Bonini *et al.* 1991). Ca^{2+} may also control the motile activity of the axoneme indirectly *via* Ca^{2+}-binding proteins (Watanabe *et al.* 1990). Calmodulin (CaM) is a prominent candidate as the signal transducer in Ca^{2+}-regulated motility. This low molecular protein has four Ca^{2+}-binding sites and is ubiquitous in eukaryotes (Klee and Newton 1985). Calmodulin was found to occur, among the other sites, on the external doublets of ciliary microtubules in *Paramecium* (Maihle *et al.* 1981) and *Tetrahymena* (Watanabe *et al.* 1990).

Calmodulin is known not only to play the role of a Ca^{2+}-binding buffer but is also a regulatory protein, which on binding at least three Ca^{2+} cations, undergoes a conformation change and gains an opportunity to change its interaction with target proteins and other substrates. Experiments with ^{125}I-CaM showed that CaM labelled at least 36 polypeptides in nitrocellulose blots of *Tetrahymena* ciliary membrane fraction (Hirano and Watanabe 1985, Hirano-Ohnishi and Watanabe 1988). A similar study of *Paramecium* cilia (Evans and Nelson 1989) identified 9 polypeptides that are labelled by CaM in the presence of Ca^{2+}. Several of those proteins at 63, 96 and 126 kDa appear to be tightly associated with the axoneme. In addition, two axonemal polypeptides of 95 and 105 kDa bind CaM in Ca^{2+}-inhibitable manner. These studies suggest a further level of complexity in the regulation of ciliary function by Ca^{2+} and calmodulin.

Furthermore, the existence of Ca^{2+}-CaM-dependent enzymes, such as CaM - dependent phosphatase, calcineurin (Klumpp *et al.* 1983) or Ca^{2+}-CaM-dependent protein kinase (Watanabe *et al.* 1990) in cilia suggests the involvement of the Ca^{2+}-CaM-complex in phosphorylation and dephosphorylation processes in the axoneme. It appears that β-tubulin, one of the microtubule subunits, is an exclusive substrate of Ca^{2+}-CaM-dependent kinase in the ciliary axoneme of *Tetrahymena* (Hirano-Ohnishi and Watanabe 1989).

Epithelial cells

The ciliated cells from the oviduct and duct of the respiratory system in higher animals respond to increased cytoplasmic Ca^{2+} concentration exclusively by an increased power stroke frequency (Verdugo 1980, Villalon *et al.* 1989, Satir and Sleigh 1990) similar to those observed in atalanta cells. This phenomenon can be observed on treatment of such epithelial cells with solutions containing Ca^{2+} and the Ca^{2+} ionophore A23187, whereas an opposite effect, i.e. a lowering of the ciliary beat frequency, was observed in the presence of a Ca^{2+} chelating agent, EGTA (Girard and Kennedy 1986, Sanderson and Dirksen 1989, Lansley *et al.* 1992). In the ciliated epithelium cells, like in protozoan ciliates, mechanical stimulation, which physiologically is initiated by the presence of mucus, results in an increased cytoplasmic Ca^{2+} concentration (Sanderson and Dirksen 1989, Hansen *et al.* 1995). This stimulation probably permits extracellular Ca^{2+} to enter the cell through plasma membrane Ca^{2+} channels (Satir and Sleigh 1990). Moreover, it has been found that on mechanical stimulation of these cells, Ca^{2+} is released from intracellular stores, e.g. the endoplasmic reticulum (Hansen *et al.* 1995). The time course of this response of epithelial cells is rather slow as compared to the rapid response of ciliate protozoan cells to the mechanical stimulation. On the other hand, Ca^{2+} is known to act directly on the axoneme both in epithelial cells (Verdugo *et al.* 1983) and in ciliate protozoa (Naitoh and Kaneko 1972). Thus, in the case of the epithelial cells, the path of Ca^{2+} entering to the vicinity of the axoneme is much longer than in protozoan cell, where voltage-dependent Ca^{2+} channels located within the ciliary membrane permit Ca^{2+} entry around the axoneme.

Calmodulin has been identified in the ciliated cells of hamster epithelium by immunological procedures (Gordon *et al.* 1982). Evidence for a functional role of calmodulin in control ciliary beat has been stated by observing the effects of calmodulin antagonist (trifluoroperazine, TFP) on ciliary movement of live cells and cellular models of ciliated epithelium (Verdugo *et al.* 1983, DiBenedetto *et al.* 1981 a, b)

Spermatozoa

Involvement of Ca^{2+} on the flagellar motility of spermatozoa, both in sea urchins and mammals, was first

observed in studies on motile cellular models. Similarly as in ciliates, flagellar motion becomes reactivated in the presence of ATP and Mg^{2+}, whereas addition to the medium of Ca^{2+} produces flagellar beat asymmetry and increased curvature of swimming. The cytoplasmic Ca^{2+} level therefore is thought to control locomotor behavior of intact sperm as well (Brokaw 1987, Londeman and Goltz 1988).

Biochemical and immunological evidence showed that calmodulin as in ciliates, is an integral component of the flagella of mammalian and sea urchin spermatozoa (Feinberg *et al.* 1981, Burgers 1982, Gordon 1883, Brokaw and Nakayama 1985, Otter 1989). Such localization suggest its involvement in the control of the flagellar activity and its shape changes during bending. Use of radioactive labelled calmodulin enabled to identify several binding proteins in spermatozoa flagella axoneme, similar to those found in ciliates (Noland *et al.* 1985, Camatini and Casale 1987). Enzymes being under control of calmodulin like myosin light chain kinase and calcineurin like protein were discovered in spermatozoa flagella (Tash and Means 1983). This may suggest that also the flagellar activity might be regulated by means of protein phosphorylation and dephosphorylation processes. Moreover, an identification of substrates for this enzyme, such as cAMP-dependent phosphoproteins, in sperm (Tash *et al.* 1988) and its association with dynein (Tash and Means 1988, Tash 1989) confirm an indispensable role of CaM and Ca^{2+} in the function of the axoneme (Tash 1989).

CYCLIC AMP AND CYCLIC GMP SIGNALLING IN THE AXONEME

Ciliates

Recent studies on the mechanism of ciliary and flagellar motility have shown that, in addition to the above described processes, cyclic nucleotides are also involved in the regulation of axoneme activity. The frequency of ciliary beating in permeabilized cell models of *Paramecium* was also increased by cAMP or cGMP in the presence of ATP and Mg^{2+} (Bonini and Nelson 1988, Bonini *et al.* 1991). It has been demonstrated *in vivo* that conditions causing membrane hyperpolarization, either by a step decrease in external K^+ or step increase in external Ca^{2+}, results in fast forward swimming and an increase of internal cAMP, whereas depolarization and backward swimming are correlated with a decrease in the cAMP level (Bonini *et al.* 1986). Two opposing models have been proposed to describe the signal transduction pathway involving changes in membrane potential correlated with changes in the internal cAMP levels of *Paramecium* (Pech 1995). According to one of those models, the change in membrane potential evoked by hyperpolarizing stimulus leads to an activation of adenylate cyclase and an increase of internal cAMP concentration. This in turn, results in an increase of the beat frequency (Schultz *et al.* 1992, Schultz and Klumpp 1993). The second approach, also based on experimental data, assumes that the stimulus causes an increase in the cytoplasmic cAMP level resulting in hyperpolarization of the cell membrane and an increase of beat frequency (Hennessey *et al.* 1985, Bonini and Nelson 1990). In both models the final effect of stimulation would be an increase in frequency of ciliary beating evoked by an increased cAMP concentration.

Biochemical studies have shown that the phosphorylation of several ciliary proteins including dynein, increases upon addition of cAMP at different levels of Ca^{2+} to permeabilized cells of *Paramecium,* (Travis and Nelson 1988, Hamasaki *et al.* 1991, Bonini and Nelson 1990). In isolated ciliary axonemes from *Paramecium*, a 29 kDa protein was found which co-sediments with 22S dynein and was phosphorylated in response to cAMP (Hamasaki *et al.* 1989). Its phosphorylation was sensitive to the level of Ca^{2+}, which when present at micromolar concentrations, inhibited the phosphorylation. This 29 kDa phosphoprotein is a potential regulator of cAMP-induced fast forward cell swimming. These findings were confirmed by latter studies which showed that phosphorylated preparations of 22S dynein produced faster microtubule motion *in vitro* in microtubule sliding assay than did unphosphorylated preparations of 22S dynein (Hamasaki *et al.* 1991). Direct *in vitro* phosphorylation of dynein polypeptides by purified protein kinase from *Paramecium* was also observed (Walczak and Nelson 1994).

There is accumulating evidence that not only cAMP but also a second cyclic nucleotide cGMP, exerts some of its action directly on the *Paramecium* axoneme. Although in detergent-permeabilized cells, the addition of cAMP or cGMP causes a fast forward swimming, the function of these nucleotides is different in some aspects of motility control (Bonini and Nelson 1988). There are also differences in the Ca^{2+} dependence of adenylate cyclase and guanylate cyclase activities from *Paramecium* estimated *in vitro*. The adenylate cyclase activity is stimulated by lower free Ca^{2+} concentrations and shows inhibition by higher levels (above 5 μM) whereas guanylate cyclase is

stimulated by higher levels of free Ca^{2+} and shows no inhibition by Ca^{2+} up to 100 μM (Gustin and Nelson 1987, Bonini *et al.* 1991). These differences testify that the enzyme activities are regulated separately *in vivo*. It has been also demonstrated that intracellular cGMP levels increased in response to membrane depolarizing stimuli (Majima *et al.* 1986, Schultz *et al.* 1986). This is in contrast to the effect of cAMP, which as mentioned previously, is connected with hyperpolarization of the cell membrane. It was proposed that Ca^{2+} entering the cilia during an action potential *via* the voltage-dependent Ca^{2+} channels elicits ciliary reversal and activates a Ca^{2+}-CaM-dependent guanylate cyclase. Recently it has been shown that the increase in Ca^{2+} concentration elicited independently by the hyperpolarization- or depolarization-activated Ca^{2+} inward currents in somatic membrane or ciliary membrane respectively, leads to an elevation of internal cGMP levels in *Paramecium* cells (Schultz *et al.* 1997). There is compelling evidence that cyclic nucleotide and calmodulin may regulate the behavior of ciliates by direct controlling of the ion channels activity (Hinrichsen *et al.* 1986, Preston *et al.* 1988, Wallen-Friedman *et al.* 1988, Koprowski *et al.* 1997, Kuriu *et al.* 1997).

The cGMP like cAMP, stimulates *in vitro* the phosphorylation of several proteins in isolated cilia from permeabilized cells (Migletta and Nelson 1988). The atalanta mutant can not swim backwards despite the fact that Ca^{2+} enters the mutant normally and the activation of cGMP synthesis after depolarizing stimuli also occurs. Detailed biochemical and immunological studies did not demonstrate any significant difference between proteins from wild and atalanta mutant cells. However, there were distinct differences in the swimming pattern between permeabilized models of wild and mutant atalanta cells reactivated with ATP in the presence of cGMP. The reactivated wild-type models swim in a left-handed helix, whereas atalanta mutants under the same conditions move in a right-handed helix. The phosphoproteins in the wild type cells were very similar to those found in atalanta mutants, except that a 48 kDa protein from atalanta was more phosphorylated (Ann and Nelson 1995). Thus, the ciliary 48 kDa protein may be the part of the mechanism that regulates the orientation of ciliary power stroke.

Epithelial cells

A similar behavioral effect of cyclic nucleotides as in *Paramecium,* was observed in ciliated epithelial cells of the respiratory system where the presence of two guanyl cyclases has been demonstrated; the activity of both enzymes was increased in response to such factors as C-type natriuretic peptide (CNP) or sodium nitroprusside (Geary *et al.* 1993). The action of factors eliciting the increase in cGMP level is also accompanied by an increase in ciliary beat frequency (Jain *et al.* 1993, Geary *et al.* 1995). In addition, recent studies of Sakai *et al.* (1995) have shown that an increase in cGMP level is connected with a rise of the internal Ca^{2+} level in airway epithelium cells. An increase in frequency of power stroke was also observed when an elevation of the cAMP level in ciliated cells of tracheal epithelium was evoked by incubation with 8-Br-cAMP, a membrane-permeable analogue of cAMP (Tamaoki *et al.* 1989, Di Benedetto *et al.* 1991 b). A similar effect is obtained on the treatment of these cells with isoproterenol, an agonist of β-adrenergic receptors, which by activation of a particular enzymatic cascade causes a rise in cAMP concentration (Lansley *et al.* 1992). The increase in ciliary beat frequency upon cAMP elevation in mammalian cells can be blocked by protein kinase inhibitors, suggesting that the phenomenon is mediated by cAMP-dependent phosphorylation events (Di Benedetto *et al.* 1991 b).

A study of cAMP-dependent phosphorylation in ovine tracheal cilia showed that an axonemal protein of 26 kDa is the only polypeptide consistently phosphorylated in a cAMP-dependent manner. The phosphorylation of this protein could be diminished by KT-5720; a highly specific inhibitor of cAMP-dependent protein kinase (cAPK). In tracheal cilia, in contrast to *Paramecium*, addition of Ca^{2+} did not affect the phosphorylation of this protein during treatment with cAMP (Salathe *et al.* 1993). It could be supposed that in this case the phosphorylation is independent of the Ca^{2+} concentration; it seems that it could be explained by other effects of elevated Ca^{2+} levels on the axoneme activity of these cells. Mammalian cilia in contrast to *Paramecium* do not change beating direction upon addition of Ca^{2+} but actually increase ciliary beat frequency. The only recent studies led to the conclusion that the mechanism regulating the frequency of ciliary motion with cAMP and Ca^{2+}-dependent phosphorylation is more complex. The evidence obtained on studies of human nasal respiratory epithelium *in vitro* demonstrates that the regulation of ciliary beat frequency occurs *via* two different phosphorylation cascades, dependent on cAMP or CaM protein kinases (Di Benedetto *et al.* 1991 a, b; Smith *et al.* 1996). The authors suggest that the pathway dependent on CaM kinase, which regulates an intrinsic ciliary beat frequency (observed without external stimuli),

is inhibited by dibutyryl cAMP, which controls the stimulated ciliary beat frequency, but not *vice versa* (Smith *et al.* 1996).

Spermatozoa

Early studies on mammalian and echinoderm sperm demonstrated a correlation between cAMP levels and motility (Tash and Mann 1973, Ishiguro *et al.* 1982). Lindemann (1978) employed detergent-extracted, ATP-reactivated models of bull sperm to show that cAMP increased both the amplitude and the frequency of flagellar beat. The rise in cAMP levels associated with an initiation of flagellar movement was correlated with an increase in the activity of cAPK. Brandt and Hoskins (1980) identified a major soluble 55 kDa protein with the ability to be phosphorylated *in vitro*. Other studies confirm these results and the phosphoprotein involved was named aksokinin (Tash *et al.* 1984). The 55 kDa protein, target of cAMP-dependent phosphorylation, was found to be the regulatory subunit of cAPK type II (Noland *et al.* 1987, Paupard *et al.* 1988).

Autophosphorylation of cAPK is indispensable but insufficient to set spermatozoa in movement. Addition of the pure regulatory subunit by itself, however, was unable to cause stimulation of motility. One conclusion from these results is that the regulatory subunit of cAPK must interact with some other component if it indeed plays a role in motility. Similarly as in the case of the ciliary axoneme, there is ample evidence that phosphorylation and dephosphorylation of dynein could be the key mechanism regulating flagellar motility. It has been shown in the sperm of the tunicate *Ciona* that, the heavy chains and light chain of 18-20 kDa of 20S dynein are phosphorylated after stimulation of kinases in isolated axonemes in the presence of cAMP (Dey and Brokaw 1991). The studies on phosphorylation of the sea urchin sperm 21S outer arm dynein by exogenous cAPK *in vitro* showed that heavy chain and 72, 23, and 18 kDa polypeptides were phosphorylated by cAPK (Tash 1989). The phosphorylation significantly increased the ATP-ase activity of this dynein fraction as well as increased the microtubule sliding rate *in vitro* (Tash and Means 1988). Moreover, the same phosphoproteins that were phosphorylated by cAPK, were dephosphorylated by CaM-dependent phosphatase (Tash 1989, Tash and Means 1988).

The involvement of cAMP in motility regulation of the sea urchin sperm flagellum in response to chemoattractants seems to be closely related to the presence of Ca^{2+} and cGMP. On the basis of results and observation with the use of attractant peptides, which cause prolonged flagellar asymmetry and an elevation of intracellular Ca^{2+}, it seems that the stimulation of the receptor by chemoattractant is followed by an activation of cGMP synthesis and opening of K^+ channels. These leads to the hyperpolarization of the cell membrane, that prevents Ca^{2+} entry and presumably promotes Na^+/Ca^{2+} exchange to lower intracellular Ca^{2+} levels. A negative membrane potential activates Na^+/H^+ exchange to elevate intracellular pH, which terminates cGMP production and K^+ channel activity, thus initiating cAMP synthesis and activation of Ca^{2+} channels, which in turn transiently elevate intracellular Ca^{2+} levels. Return to the resting intracellular Ca^{2+} levels possibly follows an inactivation of Ca^{2+} channels and functioning of Ca^{2+} homeostasis mechanism (Cook and Babcock 1993 a, b; Cook *et al.* 1994).

From the above reported studies it is apparent that the mechanisms regulating ciliary and flagellar motility with the involvement of second messengers, such as Ca^{2+} and cyclic nucleotides are extremely complex (summarized in Fig. 2 for ciliates). These messengers are able to affect the activity of both the cell and ciliary membranes as well as to interact with each other, forming a highly integrated regulatory system.

Acknowledgments. This work was supported in part by grant KBN-6-P203-046-04 and statutory grant to the Nencki Institute of Experimental Biology from the Committee for Scientific Research.

REFERENCES

Ann K.-S., Nelson D. (1995) Protein substrates for cGMP-dependent protein phosphorylation in cilia of wild type and Atalanta mutants of *Paramecium*. *Cell Motil. Cytoskel.* **30:** 252-260

Bonini N.M., Nelson D.L. (1988) Differential regulation of *Paramecium* ciliary motility by cAMP and cGMP. *J. Cell Biol.* **106:** 1615-1623

Bonini N.M., Nelson D.L. (1990) Phosphoproteins associated with cyclic nucleotide stimulation of ciliary motility in *Paramecium*. *J. Cell Sci.* **95:** 219-230

Bonini N.M., Gustin M.C., Nelson D.L. (1986) Regulation of ciliary motility by membrane potential in *Paramecium*: a role for cyclic AMP. *Cell Motil. Cytoskel.* **6:** 256-272

Bonini N.M., Evans T.C., Migletta L.A.P., Nelson D.L. (1991) The regulation of ciliary motility in *Paramecium* by Ca^{2+} and cyclic nucleotides. In: Adv. Second Messenger Phosphoprotein Res. (Eds. P. Greengard and G.A. Robinson), Ravell Press, Ltd., New York. **23:** 227-272

Brandt H., Hoskins D.D. (1980) A cAMP-dependent phosphorylated motility protein in bovine epididymal sperm. *J. Biol. Chem.* **255:** 982-987

Brokaw C.J. (1987) Regulation of sperm flagellar motility by calcium and cAMP-dependent phosphorylation. *J. Cell Biochem.* **35:** 175-184

Brokaw C.J., Nagayama S.M. (1985) Modulation of the asymmetry of sea urchin sperm flagellar bending by calmodulin. *J. Cell Biol.* **100:** 1875-1883

Burgess W.H. (1982) Characterization of calmodulin and calmodulin isotypes from sea urchin gametes. *J. Biol. Chem.* **257:** 1800-1804

Camatini M., Casale A. (1987) Actin and calmodulin coexist in the equatorial regment of ejaculated boar sperm. *Gamete Res.* **17:** 97-105

Cook S.P., Babcock D.F. (1993a) Selective modulation by cGMP of the K^+ channel activated by speract. *J. Biol. Chem.* **268:** 22402-22407

Cook S.P., Babcock D.F. (1993b) Activation of Ca^{2+} permeability by cAMP is coordinated through the pH_i increase induced by speract. *J. Biol. Chem.* **268:** 22408-22413

Cook S.P., Brokaw C.J., Muller C.H., Babcock D.F. (1994) Sperm chemotaxis: egg peptides control cytosolic calcium to regulate flagellar responses. *Develop. Biol.* **165:** 10-19

Dey C., Brokaw C.J. (1991) Activation of *Ciona* sperm motility. Phosphorylation of dynein polypeptides and effects of a tyrosine kinase inhibitor. *J. Cell Sci.* **100:** 815-824

Di Benedetto G., Magnus C.J., Gray P.T.A., Mehta A. (1991a) Calcium regulation of ciliary beat frequency in human respiratory epithelium *in vitro*. *J. Physiol.* **439:** 103-113

Di Benedetto G., Manara-Shediac F.S., Mehta A. (1991b) Effect of cyclic AMP on ciliary activity of human respiratory epithelium. *Eur. Resp. J.* **4:** 789-795

Doughty M.J., Kaneshiro E.S. (1985) Divalent cation-dependent ATP-ase activities in ciliary membranes and other surface structures in *Paramecium tetraurelia*: comparative *in vitro* studies. *Arch. Biochem. Biophys.* **238:** 118-128

Dunlap K. (1977) Localization of calcium channels in *Paramecium caudatum*. *J. Physiol.* **271:** 119-133

Eckert R. (1972) Bioelectric control of ciliary activity. *Science*. **176:** 473-481

Eckert R., Brehm P. (1979) Ionic mechanisms of excitation in *Paramecium*. *Biophys. Bioenerg.* **8:** 353-383

Evans T.C., Nelson D.L. (1989) The cilia of *Paramecium* contain both Ca^{2+}-dependent and Ca^{2+}-inhibitable calmodulin-binding proteins. *Biochem. J.* **259:** 385-396

Fabczak S., Wood D. (1980) Membrane conductance changes elicited by mechanical stimuli. *Microsensory News Letter* **3:** 13

Fabczak S., Fabczak H., Tao N., Song P.-S. (1993a) Photosensory transduction in ciliates. I. An analysis of light-induced electrical and motile responses in *Stentor coeruleus*. *Photochem. Photobiol.* **57:** 696-701

Fabczak S., Fabczak H., Song P.-S. (1993b) Photosensory transduction in ciliates. III. The temporal relation between membrane potentials and photomotile responses in *Blepharisma japonicum*. *Photochem. Photobiol.* **57:** 872-876

Feinberg J., Weinmann J., Weinmann S., Walsh M.P., Harricane M.C., Gabrion J., Demaille J.G. (1981) Immunocytochemical and biochemical evidence for the presence of calmodulin in bull sperm flagellum. *Biochim. Biophys. Acta* **673:** 303-311

Geary C.A., Goy M.F., Boucher R.C. (1993) Synthesis and vectorial export of cGMP in airway epithelium: expression of soluble and CNP-specific guanylate cyclases. *Am. J. Physiol.* **265:** L598-L605

Geary C.A., Davis C.W., Paradiso A.M., Boucher R.C. (1995) Role CNP in human airways: cGMP-mediated stimulation of ciliary beat frequency. *Am. J. Physiol.* **268:** L1021-L1028

Gibbons I.R. (1989) The Dynein ATP-ases. In: Cell Movement (Eds. F.D. Warner, P. Satir and I.R. Gibbons) 3-22

Girard P.R., Kennedy J.R. (1986) Calcium regulation of ciliary activity in rabbit tracheal epithelial explants and outgrowth. *Eur. J. Cell Biol.* **40:** 203-209

Gordon R.E., Wiliams K.B., Puszkin S. (1982) Immune localization of calmodulin in the ciliated cells of hamster tracheal epithelium. *J. Cell Biol.* **95:** 57-63

Gordon M., Morris E.G., Young R.J. (1983) The localization of Ca^{2+} ATP-ase and Ca^{2+} binding proteins in flagellum of guinea pig sperm. *Gamete Res.* **8:** 49-55

Gustin M.C., Nelson D.L. (1987) Regulation of ciliary adenylate cyclase by Ca^{2+} in *Paramecium*. *Biochem. J.* **246:** 337-345

Habermacher G., Sale W.S. (1995) Regulation of dynein-driven microtubule sliding by axonemal kinase and phosphatase in *Chlamydomonas* flagella. *Cell Motil. Cytoskel.* **32:** 106-109

Habermacher G., Sale W.S. (1997) Regulation of flagellar dynein by phosphorylation of a 138-kDa inner arm dynein intermediate chain. *J. Cell Biol.* **136:** 167-176

Hamasaki T., Murtaugh T.J., Satir B.H., Satir P. (1989) *In vitro* phosphorylation of *Paramecium* axonemes and permeabilized cells. *Cell Motil. Cytoskeleton* **12:** 1-11

Hamasaki T., Barkalow K., Richmond J., Satir P. (1991) cAMP-stimulated phosphorylation of an axonemal polypeptide that copurifies with the 22S dynein arm regulates microtubule translocation velocity and swimming speed in *Paramecium*. *Proc. Natl. Acad. Sci. USA* **88:** 7918-7922

Hansen M., Boittano S., Dirksen E.R., Sanderson M.J. (1995) A role for phospholipase C activity but not ryanodine receptors in the initiation and propagation of intercellular calcium waves. *J. Cell Sci.* **108:** 2583-2590

Hennessey T., Machemer H., Nelson D.L. (1985) Injected cyclic AMP increases ciliary beat frequency in conjunction with membrane hyperpolarization. *Eur. J. Cell Biol.* **36:** 153-156

Hinrichsen R.D., Kung C. (1984) Genetic analysis of axonemal mutants in *Paramecium tetraurelia* defective in their response to calcium. *Genet. Res.* **43:** 11-20

Hinrichsen R.D., Burgess-Cassler A. Soltvedt B.C., Hennessey T., Kung C. (1986) Restoration by calmodulin of a Ca^{2+}-dependent K^+ current missing in a mutant of *Paramecium*. *Science* **232:** 503-506

Hirano J., Watanabe Y. (1985) Studies on calmodulin-binding proteins (CaMBPs) in the cilia of *Tetrahymena*. *Exp. Cell Res.* **157:** 441-450

Hirano-Ohnishi J., Watanabe Y. (1988) Target molecules of calmodulin on microtubules of *Tetrahymena* cilia *Exp. Cell Res.* **178:** 18-24

Hirano-Ohnishi J., Watanabe Y. (1989) Ca^{2+}/calmodulin-dependent phosphorylation of ciliary β-tubulin in *Tetrahymena*. *J. Biochem. (Tokyo)* **105:** 858-860

Ishiguro K., Murofushi H., Sakai H. (1982) Evidence that cAMP-dependent protein kinase and protein factor are involved in reactivation of Triton-X100 models of sea urchin and starfish spermatozoa. *J. Cell. Biol.* **92:** 777-782

Jain B., Rubinstein I., Robbins R.A., Leise K.L., Sisson J.H. (1993) Modulation of airway epithelial cell ciliary beat frequency by nitric oxide. *Biochem. Biophys. Res. Comm.* **191:** 83-88

Klee C. B., Newton D. (1985) Calmodulin: an overview. In: Control and Manipulation of Calcium Movement (Ed. J.R. Parrat), Raven Press, New York, 131-146

Klumpp S., Steiner A.L., Schultz J.E. (1983) Immunocytochemical localization of cyclic GMP, cGMP-dependent protein kinase, calmodulin and calcineurin in *Paramecium tetraurelia* *Eur. J. Cell Biol.* **32:** 164-170

Koprowski P., Walerczyk M., Groszyńska B., Fabczak H., Kubalski A. (1997) Modified patch-clamp method for studying ion channels in *Stentor coeruleus*. *Acta Protozool.* **36:** 121-124

Kreimer D.I., Khotimchenko Y.U.S. (1995) Cytoplasm calcium-binding proteins of germ cells and embryos of the sea urchin. *Comp. Biochem. Physiol.* **110A:** 95-105

Kung C. (1971a) Genetic mutation with altered system of excitation in *Paramecium aurelia*. I. Phenotypes of the behavioral mutants. *Z. Vergl. Physiol.* **71:** 142-164

Kung C. (1971b) Genetic mutation with altered system of excitation in *Paramecium aurelia*. II. Mutagenesis, screening and genetic analysis of the mutants. *Genetics* **69:** 29-45

Kung C., Eckert R. (1972) Genetic modification of electric properties in an excitable membrane. *Proc. Natl. Acad. Sci. USA* **69:** 93-97

Kung C., Naitoh Y. (1973) Calcium-induced ciliary reversal in the extracted models of "Pawn", a behavioral mutant of *Paramecium*. *Science* **179:** 195-196

Kuriu T., Nakaoka Y., Oosawa Y. (1996) Cold-sensitive Ca^{2+} influx in *Paramecium*. J. Membr. Biol. **154:** 163-167

Lansley A.B., Sanderson M.J., Dirksen E.R. (1992) Control of the beat cycle of respiratory tract cilia by Ca^{2+} and cAMP. *Am. J. Physiol.* **263:** L232-242

Lindemann C.B. (1978) A cAMP-induced increase in the motility of demembranated bull spermatozoa. *Cell* **13:** 1-18

Londemann C.B., Goltz J.S. (1988) Calcium regulation of flagellar curvature and swimming pattern in Triton X-100 extracted rat sperm. *Cell Motil. Cytoskel.* **10:** 420-431

Machemer H., Deitmer J.W. (1985) Mechanoreception in ciliates. In: Progress in Sensory Physiology. (Eds. R. Hardie *et al.*) Springer, Berlin, Heidelberg, New York, Tokyo. **5:** 81-118

Maihle N.J., Dedman J.R., Means A.R., Chafouleas J.G., Satir B.H. (1981) Presence and indirect immunofluorescent localization of calmodulin in *Paramecium tetraurelia*. *J. Cell. Biol.* **89:** 695-699

Majima T., Hamasaki T., Arai T. (1986) Increase in cellular cyclic GMP level by potassium stimulation and its relation to ciliary orientation in *Paramecium*. *Experientia* **42:** 62-64

Migletta L.A.P., Nelson D.L. (1988) A novel cGMP-dependent protein kinase from *Paramecium*. *J. Biol. Chem.* **263:** 16096-16105

Naitoh Y., Kaneko H. (1972) Reactivated Triton-extracted models of *Paramecium*: modification of ciliary movement by calcium ions. *Science* **176:** 523-524

Nakaoka Y., Tanaka H., Oosawa F. (1984) Ca^{2+}-dependent regulation of beat frequency of cilia in *Paramecium*. *J. Cell Sci.* **65:** 223-231

Nakaoka Y., Kurotani T., Itoh H. (1987) Ionic mechanism of termoreception in *Paramecium*. *J. Exp. Biol.* **127:** 93-103

Noland T.D., Van Eldik L.J., Garbers D.L., Burgess W.H. (1985) Distribution of calmodulin-binding proteins in membranes from bovine epididymal spermatozoa. *Gamete Res.* **11:** 297-303

Noland T.D., Abumrad N.A., Beth A.H., Garbers D.L. (1987) Protein phosphorylation in intact bovine epididymal spermatozoa: Identification of the type II regulatory subunit of cyclic adenosine 3',5'-monophosphate- dependent protein kinase as an endogenous phosphoprotein. *Biol. Reprod.* **37:** 171-180

Ogura A., Machemer H. (1980) Distribution of mechanoreceptor channels in the *Paramecium* surface membrane. *J. Comp. Physiol.* **135:** 233-242

Omoto C.K. (1995) Structural and functional hierarchy of eukaryotic cilia and flagella. *Eur. J. Histochem.* **39:** 85-90

Otter T. (1989) Calmodulin and the control of flagellar movement. In: Cell Movement (Eds. F.D. Warner, P. Satir and I.R. Gibbons), Alan R. Liss, Inc., New York **1:** 289-298

Otter T., Satir B.H, Satir P. (1984) Trifluoperazine-induced changes in swimming behavior of *Paramecium*: evidence for two sites of drug action. *Cell Motil.* **4:** 249-267

Paupard M.-C., Macleod J., Wasco W., Orr G.A. (1988) Major 56,000-dalton, soluble phosphoprotein present in bovine sperms is the regulatory subunit of type II cAMP-dependent protein kinase. *J. Cell. Biochem.* **37:** 161-175

Pech L.L. (1995) Regulation of ciliary motility in *Paramecium* by cAMP and cGMP. *Comp. Biochem. Physiol.* **111A:** 31-37

Preston R.R., Saimi Y. (1990) Calcium ions and the regulation of motility in *Paramecium*. In: Ciliary and Flagellar Membranes (Ed. R.A. Bloodgood), Plenum Publ. Corp. 173-200

Preston R.R., Wallen-Friedman M.A., Saimi Y., Kung C. (1988) Loss of Ca-dependent ion channel functions in two mutants of Paramecium *tetraurelia* with defective calmodulin. *J. Cell Biol.* **107:** 287a

Sakai A., Kondo M., Tamaoki J., Konno K. (1995) Nitric oxide modulation of $Ca2^{+}$ responses in cow tracheal epithelium. *Eur. J. Pharmacol.* **291:** 375-379

Salathe M., Bookman R.J. (1995) Coupling of $[Ca^{2+}]_i$ and ciliary beating in cultured tracheal epithelial cells. *J. Cell Sci.* **108:** 431-440

Salathe M., Pratt M., Wanner A. (1993) Cyclic AMP-dependent phosphorylation of a 26 kD axonemal protein in ovine cilia isolated from small tissue pieces. *Am. J. Respir. Cell Mol. Biol.* **9:** 306-314

Sanderson M.J., Dirksen E.R. (1989) Mechanosensitive and beta-adrenergic control of the ciliary beat frequencies of mammalian respiratory tract cells in culture. *Am. Rev. Respir. Dis.* **139:** 432-440

Satir P. (1985) Switching mechanisms in the control of ciliary motility. In: Modern Cell Biology (Ed. A.R. Liss) **4:** 1-46

Satir P., Sleigh M. (1990) The physiology of cilia and mucociliary interactions. *Ann. Rev. Physiol.* **52:** 137-155

Schultz J.E., Klumpp S. (1993) Cyclic nucleotides and calcium signalling in *Paramecium*. In: Adv. Second Messenger a. Phosphoprotein Res. (Eds. S. Shenolikar and A.C. Nairn), Raven Press, Ltd., New York **27:** 25-46

Schultz J.E., Pohl T., Klumpp S. (1986) Voltage-gated Ca^{2+} entry into *Paramecium* linked to intraciliary increase in cyclic GMP. *Nature* **322:** 271-273

Schultz J.E., Klumpp S., Hinrichsen R.D. (1990) Calcium and membrane excitation in *Paramecium*. In: Calcium as an Intracellular Messenger in Eukaryotic Microbes (Ed. D.H. O'Day), Am. Soc. Microbiol., Washington, D.C. 124-150

Schultz J.E., Klumpp S., Benz R., Schurhoff-Goeters W.J.Ch., Schmid A. (1992) Regulation of adenylyl cyclase from *Paramecium* by an intrinsic potassium conductance. *Science* **255:** 600-602

Schultz J.E., Guo Y.-L., Kleefeld H., Volkel H. (1997) Hyperpolarization- and depolarization-activated Ca^{2+} currents in *Paramecium* trigger behavioral changes and cGMP formation independently. *J. Membr. Biol.* **156:** 251-259

Sleigh M.A. (1974) Patterns of movement of cilia and flagella. In: Cilia and Flagella (Ed. M. A. Sleigh). Academic Press, London and New York 79-92

Smith R.P., Shellard R., Dhillon D.P., Winter J., Mehta A. (1996) Asymmetric interactions between phosphorylation pathways regulating ciliary beat frequency in human nasal respiratory epithelium *in vitro*. *J. Physiol.* **496:** 883-889

Stelly N., Mauger J.P., Keryer G., Claret M., Adoutte A. (1991) Cortical alveoli of *Paramecium*: vast submembraneous calcium storage compartment. *J. Cell Biol.* **109:** 251-258

Stephens R.E. (1974) Enzymatic and structural proteins of the axoneme. In: Cilia and Flagella (Ed. M. A. Sleigh). Academic Press, London and New York 39-76

Tamaoki J., Kondo M., Takizawa T. (1989) Effect of cyclic AMP on ciliary function in rabbit tracheal epithelial cells. *J. Appl. Physiol.* **66:** 1035-1039

Tamm S. (1994) Ca^{2+} channels and signalling in cilia and flagella. *Trends Cell Biol.* **4:** 305-310

Tash J.S., Krinks M., Patel J., Meaus R.L., Klee C.B., Meaus A.R. (1988) Identification, characterization and functional correlation of calmodulin dependent protein phosphatase in sperm. *J. Cell Biol.* **106:** 1625-1633

Tash J.S. (1989) Protein phosphorylation: The second messenger signal transducer of flagellar motility. *Cell Motil. Cytoskel.* **14:** 332-339

Tash J.S., Mann T. (1973) Adenosine 3':5'-cyclic monophosphate in relation to motility senescence of spermatozoa. *Proc. R. Soc. London (Biol.)* **184:** 109-114

Tash J.S., Means A.R. (1983) Cyclic adenosine 3',5'-monophosphate, calcium and protein phosphorylation on flagellar motility. *Biol. Reprod.* **28:** 75-104

Tash J.S., Means A.R. (1988) cAMP- and Ca^{2+}-calmodulin (CaM)-dependent phosphorylation/dephosphorylation pathways regulate dynein. *J. Cell Biol.* **107:** 247a

Tash J.S., Kakar S.S., Means A.R. (1984) Flagellar motility requires the cAMP-dependent phosphorylation of a heat-stable NP-40-soluble 56 kd protein, axokinin. *Cell* **38:** 551-559

Travis S.M., Nelson D.L. (1988) Regulation of axonemal Mg^{2+}-ATPase from *Paramecium* cilia: effects of Ca and cyclic nucleotides. *Biochim. Biophys. Acta* **966:** 84-93

Van Houten (1988) Chemoresponse mechanisms toward the molecular level. *J. Protozol.* **35:** 241-243

Verdugo P. (1980) Ca^{2+}-dependent hormonal stimulation of ciliary activity. *Nature* **283:** 764-765

Verdugo P., Raess B.V., Villalon M. (1983) The role of calmodulin in regulation of ciliary movement in mammalian epithelial cilia. *J. Submicrosc. Cytol.* **15:** 95-96

Villalon M., Hinds T.R., Verdugo P. (1989) Stimulus-response coupling in mammalian ciliated cells. Demonstration of two mechanisms of control for cytosolic $[Ca^{2+}]$. *Biophys. J.* **56:** 1255-1258.

Walczak C.E., Nelson D.L. (1994) Regulation of dynein-driven motility in cilia and flagella. *Cell Motil. Cytoskel.* **27:** 101-107

Wallen-Friedman M.A., Preston R.R., Saimi Y., Kung C. (1988) A possible calmodulin regulatory pathway is revealed in calmodulin mutant of *Paramecium tetraurelia*. *J. Cell Biol.* **107:** 287a

Warner F.D. (1974) The fine structure of the ciliary and flagellar axoneme. In: Cilia and Flagella (Ed. M. A. Sleigh). Academic Press, London and New York 11-37

Watanabe Y., Hirano-Ohnishi J., Takemasa T. (1990) Calcium-binding proteins and ciliary movement regulation in *Tetrahymena*. In: Calcium as an Intracellular Messenger in Eukaryotic Microbes (Ed. D.H. O'Day). Am. Soc. Microbiol., Washington D.C. 124-150

Received on 28th September, 1998

Acta Protozool. (1999) 38: 97 - 103

ACTA
PROTOZOOLOGICA

The Melanin of the Myxomycete *Stemonitis herbatica*

Palaniappan LOGANATHAN and Indira KALYANASUNDARAM

Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai, India

Summary. The melanin pigment from spores of the Myxomycete (true slime mould) *Stemonitis herbatica* was characterized by physical and chemical methods, following extraction in alkali and purification by acid. The infrared (IR) spectra indicated the presence of hydroxyl, carboxyl and carbonyl groups. Patterns of sugar and amino acids were similar to those generally reported for fungal melanins, but the protein and ash content were higher. The ultraviolet (UV), IR and electron spin resonance (ESR) spectra of *S. herbatica* melanin showed a close similarity with those of standard dihydroxyphenylalanine (DOPA) melanin. The nitrogen content and C:H:N ratio were also suggestive of DOPA melanin. Electron micrographs of sectioned spores revealed a two-layered wall, with the melanin deposited in the outer layer, as commonly reported in fungal spores. The general conclusion was that the melanin of *S. herbatica* is similar to fungal melanins in its disposition in the spore walls, and specifically to DOPA melanins formed from tyrosine, in its physico-chemical nature.

Key words: DOPA melanin, melanin characterization, myxomycete, pigment, slime mould, *Stemonitis herbatica*.

INTRODUCTION

Although melanin is widely distributed in the biological world, the actual composition and configuration of the melanin molecule remains obscure. It is generally accepted that even though biological melanin can be broadly classified into a few groups based on biosynthetic pathway (Bell and Wheeler 1986), individual melanins vary widely between species, and even within the same chemical group.

The Myxomycetes or true slime-moulds, have a unique life cycle, with a protozoan-like vegetative phase and fungus-like reproductive phase. Within the group, spore colour still remains a basic criterion in taxonomic classification (Alexopoulos 1978). Previous studies on myxomycete melanin as seen from the literature are limited to the biosynthesis of the pigment during plasmodial differentiation, and the verification of the spore pigment as melanin (Ward and Havir 1957, McCormick *et al.*1970, Chet and Huettermann 1977, Chapman *et al.* 1983). Our earlier studies have established melanin to be a common pigment in the spore walls not only of the traditionally dark-spored orders - the Physarales and the Stemonitales - but also the "bright-spored" groups - Liceales and Trichiales (Loganathan *et al.* 1989, Kalyanasundaram *et al.* 1994).

The fungal melanins are said to be quite diverse in terms of biosynthetic pathways, unlike animal melanins which are said to belong to a homogeneous group referred to as dihydroxyphenylalanine (DOPA) melanin. Considering the doubtful affinities of Myxomycetes, we thought fit to characterize the melanin in at least one species, *Stemonitis herbatica* Peck, belonging to the

Address for correspondence: Indira Kalyanasundaram, Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai - 600 025, India; Fax: 91-44-566693; E-mail: gclib@unimad.ernet.in

order Stemonitales as we have a culture with us that sporulates rhythmically and regularly, to provide spores in sufficient quantity for analysis. The present paper deals with the characterization of melanin in this myxomycete using physical and chemical analyses.

MATERIALS AND METHODS

Organism

The culture of *Stemonitis herbatica* from our culture collection (number: MUBL/IK/SH/5) was used throughout this study.

Culture methods

For maintenance of cultures, the media commonly used in our laboratory namely, 5% carrot extract agar, and 3% rolled oats agar (Indira 1969) were used. In order to obtain sporangia in sufficient quantities for the extraction of melanin, the plasmodia were cultured by a slight modification of the method of Camp (1936) on filter paper sprinkled with powdered oats, in plastic trays of 32 x 26 x 7 cm covered with glass plates.

Chemicals and their sources

Standard DOPA melanin (prepared by the oxidation of tyrosine with hydrogen peroxide) for comparison in spectral studies was procured from Sigma, USA. Buffer (pH 6.8) was prepared from 0.1 M solutions of monobasic sodium phosphate ($NaH_2PO_4 \times 2H_2O$) and dibasic sodium phosphate ($Na_2HPO_4 \times 7H_2O$).

Extraction and purification of melanin

The extraction and purification of melanin from mature sporangia were done by a modification of the method of Ellis and Griffiths (1974) as described in our earlier paper (Loganathan *et al.* 1989). Spores were extracted by boiling in 1 M KOH for 2 h at 100°C, centrifuged, and the supernatant acidified with 1 N HCl to pH 2 to precipitate the pigment. The washed and dried precipitate was regarded as the crude extract. A part of the crude extract was hydrolysed by boiling in 6 N HCl for 2 h at 100°C followed by washing with distilled water. The precipitate was dried in a desiccator, and this is indicated as purified melanin in the biochemical analysis. For all spectral studies, only the purified melanin was used.

Characterization of melanin

Biochemical analyses

The extracted melanin from *S. herbatica* was biochemically analysed.

Total nitrogen content was determined by the micro-kjeldahl method (Nesslerization) of Umbreit and Burris (1972). The total carbon and hydrogen were analysed by decomposition of substratum with explosion-like combustion in a stream of pure oxygen at temperatures between 950° and 1050°C in the presence of an oxidation catalyst (copper oxide) in a Hereaus Elemental Analyser CHN-O-RAPID.

The possible presence of protein in the melanin samples was determined by the method of Bradford (1976). Amino acids were released from the melanin pigment by hydrolysis with 6 N HCl for 22 h in sealed tubes at 110°C. After hydrolysis the insoluble residue was removed by centrifugation and the hydrolysate dried *in vacuuo* over phosphorus pentoxide (P_2O_5) and NaOH. After drying, the pellets were dissolved in distilled water and used for estimation of amino acids. The amino acids were separated and identified by two-dimensional paper chromatography following the procedure of Block *et al.*(1963) with slight modifications (Raju 1976).

The total sugars were determined by the method of Dubois *et al.* (1956) and reducing sugars by the method of Nelson (1944). The ash content was determined by heating at 700°C for 3 h in a muffle furnace.

Spectral studies

The UV spectra were read using a 0.01% solution of melanin prepared in 1 M KOH, in a Beckman DU-40 Spectrophotometer at 200-400 nm.

The IR spectra were read in a Fourier Transform infrared spectrophotometer (FTIR: Bruker IFS 66 V F). The melanin of *S. herbatica* was further compared with standard DOPA melanin by electron spin resonance (ESR) spectra. The powder form (1 mg) of melanin sample was used for ESR assay. The intact spores before pigment extraction and spores after extraction of pigments were checked for ESR signal. The spectra were obtained using a Varian E 112 EPR spectrometer at X-band frequencies of about 9.45 GHz and modulation frequency of 100 KHz, at microwave power 20 mW, and modulation amplitude of 0.5 x 1 mT at room temperature.

Transmission electron microscopy

In order to locate the pigment in the spore wall, spores of *S. herbatica* were subjected to Transmission Electron Microscopy before and after extraction of melanin, by the method described by Mims (1969) with slight modifications. Spores were fixed in 25% glutaraldehyde at 4°C for 2 h and washed with phosphate buffer at pH 7.5, followed by 1% osmium tetroxide for 6 h at room temperature, and washed with buffer. Then the material was dehydrated with acetone and transferred for infiltration, to araldite:acetone (1:1) followed by pure araldite, and embedded in the same at 60°C for 2 h. The ultrathin sections were collected on copper grids and stained in Reynold's lead citrate and uranyl acetate (Reynolds 1963). The grids were observed and photographed in a Philips CM 10 Electron microscope. The elemental analysis (C, H) was made at the Kinetics and Catalysis Centre, and the FTIR and ESR spectra were obtained at the Regional Sophisticated Instrumentation Centre (RSIC), Indian Institute of Technology (IIT), Madras.

RESULTS

Biochemical Analyses

The nitrogen, protein, total sugar, reducing sugar and ash content of the crude and purified melanin from the spores of *S. herbatica* are shown in Table 1.

The statistical analyses showed that there were significant differences in total carbon, nitrogen, protein, ash and sugars, between crude melanin and purified melanin. When compared to crude melanin the purified melanin

Table 1. Biochemical constitution of melanin extracted from *Stemonitis herbatica*

Constituent	Percentage occurrence	
	Crude melanin	Purified melanin
Carbon	31.57[a](0.63)	56.27[b](0.26)
Hydrogen	3.92[a](1.22)	6.06[a](1.27)
Nitrogen	5.72[a](0.48)	3.5[b](0.3)
Ash	6.2[b](0.2)	11.7[a](0.30)
Protein	37.0[a](1.3)	30.0[b](0.9)
Total sugar	11.48[a](1.21)	9.5[b](0.67)
Reducing sugar	6.55[a](0.15)	7.90[b](0.23)
Amino acids	-	10.0

(-) not done
In each horizontal row, means followed by the same letter (a or b) do not significantly differ by Duncan's multiple range test (P = 0.05). Figures in parenthesis represent the standard deviation of mean of triplicated observations

showed higher levels of carbon and ash, whereas the protein and nitrogen values were higher in crude melanin than in purified one.

Totally 12 amino acids were separated and identified from the acid hydrolysate of purified melanin. Aspartic acid, glutamic acid, phenylalanine, glycine and tyrosine were the dominant ones.

Spectral studies

The purified melanin showed a close similarity with that of authentic DOPA melanin by Fourier Transform infrared (FTIR) spectra.

The absorption peaks in the FTIR spectrum of melanin were indicative of H-bonded OH or NH bonds (3300-3700 cm^{-1} and 2924-2930 cm^{-1}), hydroxyl and carbonyl groups (1705-1710 cm^{-1}) and either carboxylated anion or aromatic structures (1620-1650 cm^{-1}) (Fig. 1).

The UV spectrum of melanin from *S. herbatica* showed an absorption peak at 220 nm, which is characteristic of melanin as seen from the standard (Fig. 2).

The electron spin resonance (ESR) measurements were carried out for both authentic DOPA melanin and purified *S. herbatica* melanin. The ESR spectrum of the purified melanin was identical with the spectrum of authentic DOPA melanin, measured similarly (Fig. 3). The g value for the melanin sample was 1.9999 and for authentic DOPA melanin it was 2.0000. There was no hyperfine splitting in the spectra. However, the ESR assay for intact spores before pigment extraction and spores after extraction of pigments showed relatively very weak signals (Fig. 3).

Disposition of Melanin in Spores

The purified melanin pigment comprised about 15% of the dry weight of the spores of *Stemonitis herbatica*.

Electron micrographs of the spores of *S. herbatica* before extraction of pigments showed the spore wall to have at least two clearly demarcated layers, an outer electron dense layer including the protuberances, and an inner electron-transparent layer. Following extraction of melanin, the demarcation of the two layers was not clear, the entire wall showing the electron-dense material in a sparse and diffuse manner (Fig. 4). The conclusion is that melanin occurs in the outer layer of the spore wall, including the ornamentation.

DISCUSSION

Spectral studies

The absorption peaks in the ranges of 220 nm and 240 nm are generally found to be characteristic of UV spectra of melanins (Filip *et al.* 1976, Loganathan *et al.* 1989) and the spectra obtained here from *Stemonitis herbatica* melanin come within this range.

The IR spectrum of *S. herbatica*, showing an absorption pattern indicative of the presence of OH or NH bonds, hydroxyl, carbonyl, and carboxylated anion or aromatic structures is similar to the spectra generally reported for fungal melanins (Bonner and Duncan 1962, Ellis and Griffiths 1974, Filip *et al.*1974, Alviano *et al.* 1991).

Electron spin resonance (ESR) spectroscopy has been widely used for the study of melanin, and the ESR characteristics of this pigment are well established by the earlier workers (Commoner *et al.* 1954, Mason *et al.* 1960, Sealy *et al.* 1982). The quantitative determination of melanin also was made by ESR spectroscopy (Słomiński *et al.* 1994). From the ESR spectra, the dark pigment isolated from spores or vegetative plasmodia of the myxomycetes *Fuligo septica, Physarum nudum* and *P. polycephalum* was identified as melanin (Rakoczy and Panz 1994, Płonka and Rakoczy 1997). In the present study, the ESR spectrum of the isolated pigment showed a close similarity with authentic DOPA melanin.

Biochemical aspects

The values of 56.3%, 6.1% and 3.5% obtained for carbon, hydrogen and nitrogen respectively, are within the range reported for several fungal melanins analysed (Coelho *et al.*1985). According to Bull (1970), synthetic DOPA

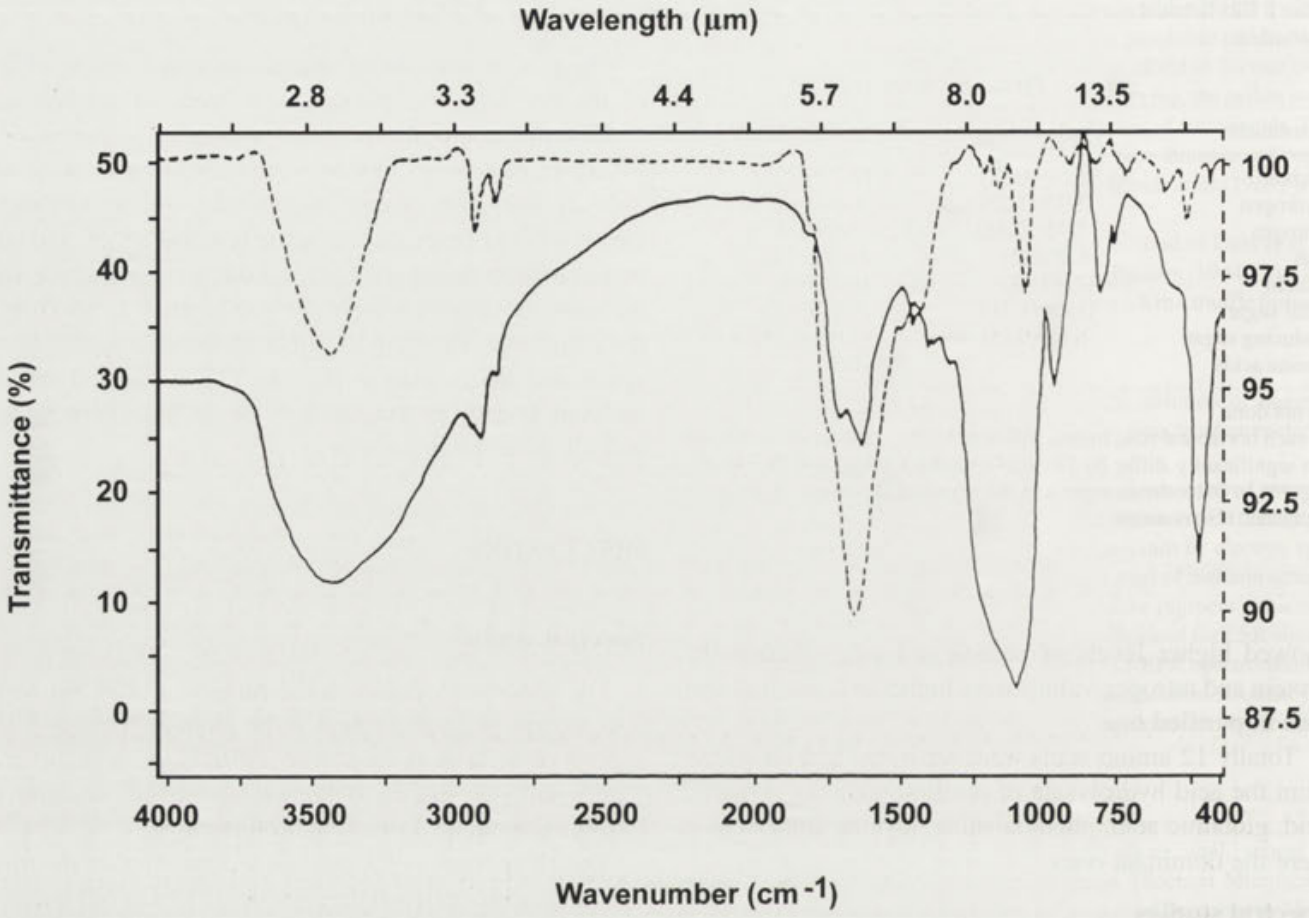


Fig.1. Spectra of melanins by means of Fourier Transform Infrared Spectrophotometer (FTIR: Bruker IFS 66 V F): dotted line - standard; solid line - purified from *Stemonitis herbatica*

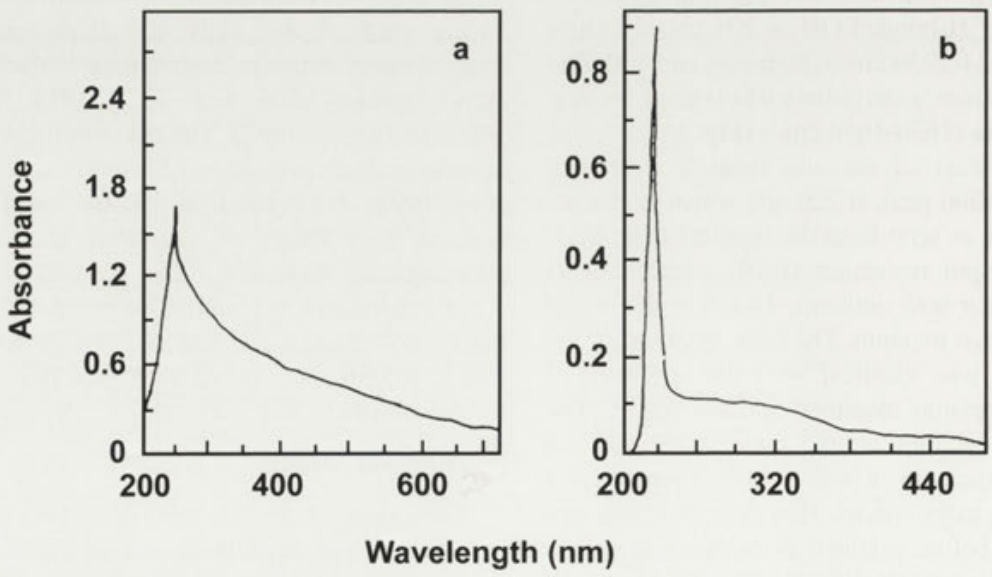


Fig. 2. UV spectra of melanins: a – standard; b - purified from *Stemonitis herbatica*

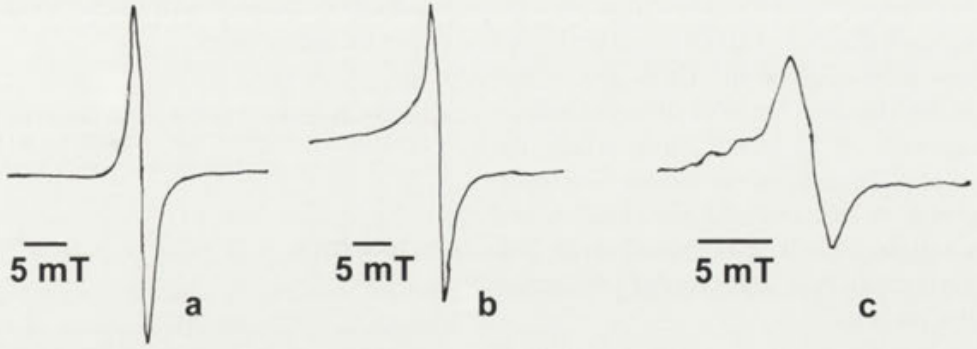


Fig. 3. Electrom spin resonance (ESR) spectra of: a - purified melanin from *Stemonitis herbatica*; b - authentic DOPA melanin; c - spores before extraction of melanin

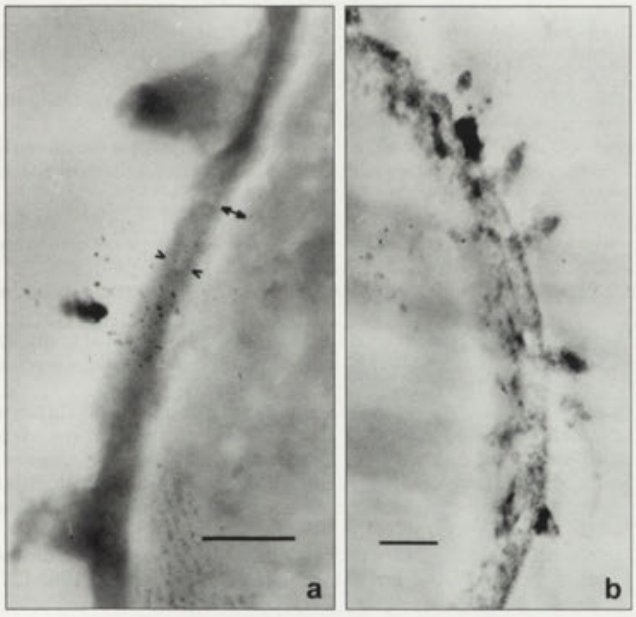


Fig. 4. Transmission electron micrographs of *Stemonitis herbatica* spores: a - before extraction of melanin; b - after extraction of melanin. Arrows indicate inner wall layer, arrowheads indicate outer wall layer. Scale bars - 1 μm

melanin had C-H-N contents of 48.5, 4.7 and 6.3% respectively, while the percentages of C-H-N for the melanin of *Aspergillus nidulans* which also was a DOPA melanin, varied under different growth conditions, ranging from 45-60% C, 5.9-6.7% H and 1.8 to 5.8% N. These ratios compare more favourably with the values obtained in the present study, rather than those reported for the glutaminyl dihydroxy benzene (GDHB) melanin of *Agaricus bisporus* of 57.9% C, 3.8% H and 8.8% N respectively (Rast *et al.* 1981).

The fungal melanins have a higher nitrogen content, and half of the nitrogen released during acid hydrolysis can be identified as amino acids (Martin and Haider 1969). Aspartic acid, glutamic acid, glycine and alanine were the major amino acids in the melanin extracted from some soil fungi (Coelho *et al.* 1985), and a similar pattern was reported for *Fonsecaea pedrosoi* melanin (Alviano *et al.* 1991). In our analysis of myxomycete melanin also the amino acid pattern was fairly similar, with aspartic acid, glutamic acid, phenylalanine and glycine as the major amino acids.

The carbohydrate content reported for fungal melanins varies from 2.1 to 4.8% (Coelho *et al.* 1988). There are exceptions, however, and the high sugar content of 9.5% seen in the melanin of *S. herbatica* is comparable with the carbohydrate content of about 10% in the melanin of the human pathogenic fungus *F. pedrosoi* (Alviano *et al.* 1991).

The results of various analyses, when compared with published reports for fungal melanins, show marked differences only with regard to protein and ash which are considerably higher in the Myxomycete melanin. This could only be regarded as indicative of differences in the nature of complexes formed by the melanin polymer.

The nitrogen content of melanin is said to give some clue to its chemical grouping (Thomas 1955, Piattelli *et al.* 1965). On the other hand, the nitrogen content of melanin may also vary depending upon the species, and the quantity and quality of nitrogen source in the medium (Saiz-Jimenez 1983, Coelho *et al.* 1985). A nitrogen content of 8-10.5% has been reported for the melanin synthesized from tyrosine or DOPA by tyrosinase (Thomas 1955); of 8.8% for the GDHB melanin from *Agaricus bisporus* synthesized from γ-glutaminyl-4-

hydroxybenzene (Rast *et al.* 1981); of less than 1.9% for the dihydroxynaphthalene (DHN) melanin of *Verticillium albo-atrum* (Gafoor and Heale 1971); and less than 1.7% for the catechol melanin of *Ustilago maydis* (Piattelli *et al.* 1965). In the present study, the nitrogen content of 3.5% obtained for the purified melanin, being higher than that reported for catechol and DHN- melanin, suggests that it is neither of these. The higher nitrogen content in the crude sample (5.7%) takes it closer to DOPA melanin.

The general conclusion is that the melanin of *S. herbatica* is a DOPA melanin synthesized from tyrosine through the action of tyrosinase. This has been further confirmed through biosynthetic studies, and the results will be published later. In the order Physarales, Rakoczy and Panz (1994), through their ESR studies on three species, express the view that their melanin is a DOPA melanin.

Disposition of melanin in spores

Melanized fungal walls are generally described in electron microscopical studies as having two distinct wall layers, with the melanin being usually confined to the outer wall layer as electron-dense grains, the inner layer being electron-transparent (Durrel 1964; Tsao and Tsao 1970; Griffiths and Swart 1974; Ellis and Griffiths 1974, 1975; Bell and Wheeler 1986). On this basis, the spore walls of *Stemonitis herbatica* are no different, and apparently contain all the melanin in the outer wall layer.

After alkali extraction of melanin, however, the granules did not disappear but became reduced in density and were sparsely dispersed throughout the wall layers. This suggested that during extraction the pigments might have diffused towards the inner layer and become bound with some wall components. Systematic analyses of myxomycete spore walls have revealed the skeletal material to be a galactosamine polymer (McCormick *et al.* 1970, Chapman *et al.* 1983). In our laboratory, Paramasivan who analysed the spore wall of *S. herbatica* found hexosamine and galactosamine to constitute only 5% of the dry weight of the spore wall. As bound melanin was apparently low in amount, he suggested that it might have been associated with the hexosamines (Paramasivan 1990).

Rakoczy and Panz (1994), using ESR spectroscopy to compare the melanin pigment of three myxomycete species, found the intensities to be much lower in whole spores than in extracted pigment, even though the extraction remained incomplete as in our case. They attribute this, and the variation they found between species, to the presence of substances in the outer spore wall that enclose and mask the melanin.

Acknowledgements. The first author thanks the Council of Scientific and Industrial Research (CSIR), New Delhi, for the award of a Senior Research Fellowship (1990) and Research Associateship (1997).

REFERENCES

Alexopoulos C.J. (1978) The evolution of the taxonomy of the Myxomycetes. In: Taxonomy of Fungi Part I. (Proceedings of the International Symposium on Taxonomy of Fungi, 1973) (Ed. C.V. Subramanian), University of Madras, Madras, India, 1-8

Alviano C.S., Farbiarz S.R., De Souza W., Angluster J., Travassos L.R. (1991) Characterization of *Fonsecaea pedrosoi* melanin. *J. Gen. Microbiol.* **137:** 837-844

Bell A.A., Wheeler M.H. (1986) Biosynthesis and functions of fungal melanins. *Ann. Rev. Phytopathol.* **24:** 411-451

Block T.G., Durrum E.L., Zwieg G. (1963) A Manual of Paper Chromatography and Paper Electrophoresis. Academic Press, New York

Bonner T.G., Duncan A. (1962) Infra-red spectra of some melanins. *Nature* **194:** 1078-1079

Bradford M.M. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilising the principle of protein-dye binding. *Anal. Biochem.* **72:** 248-254

Bull A.T. (1970) Chemical composition of wild-type and mutant *Aspergillus nidulans* cell walls. The nature of polysaccharide and melanin constituents. *J. Gen. Microbiol.* **63:** 75-94

Camp W.G. (1936) A method of culturing myxomycete plasmodia. *Bull. Torrey Bot. Club* **63:** 205-210

Chapman C.P., Nelson R.K., Orlowski M. (1983) Chemical composition of the spore case of the acellular slime mold *Fuligo septica*. *Exp Mycol.* **7:** 57-65

Chet I., Huettermann A. (1977) Melanin biosynthesis during differentiation of *Physarum polycephalum*. *Biochim. Biophys. Acta* **499:** 148-155

Coelho R.R.R., Linhares L.F., Martin J.P. (1985) Amino acid distribution in some soil fungal melanins and of soil humic acids from Brazil. *Pl. Soil* **87:** 337-346

Coelho R.R.R., Linhares L.F., Martin J. P. (1988) Sugars in hydrolysates of fungal melanins and soil humic acids. *Pl. Soil* **106:** 127-133

Commoner B., Townsend J., Pake G.E. (1954) Free radicals in biological materials. *Nature* **174:** 689-691

Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A., Smith F. (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28:** 350-356

Durrell L.W. (1964) The composition and structure of walls of dark fungus spores. *Mycopath. Mycol. Appl.* **23:** 339-345

Ellis D.H., Griffiths D.A. (1974) The location and analysis of melanins in cell walls of some soil fungi. *Can. J. Microbiol.* **20:** 1379-1386

Ellis D.H., Griffiths D.A. (1975) Melanin deposition in the hyphae of a species of *Phomopsis*. *Can. J. Microbiol.* **21:** 442-452

Filip Z., Haider K., Beutelspacher H., Martin J.P. (1974) Comparisons of I.R. spectra from melanins of microscopic soil fungi, humic acids and model phenol polymers. *Geoderma* **11:** 37-52

Filip Z., Semotan J., Kutilek M. (1976) Thermal and spectrophotometric analysis of some fungal melanins and soil humic compounds. *Geoderma* **15:** 131-142

Gafoor A., Heale J. B. (1971) Melanin formation and peroxidase activity in *Verticillium*. *Microbios* **3:** 87-95

Griffiths D.A., Swart H.J. (1974) Conidial structure in two species of *Pestalotiopsis*. *Trans. Br. mycol. Soc.* **62:** 295-304

Indira P.U. (1969) *In-vitro* cultivation of some Myxomycetes. *Nova Hedwigia* **18:** 627-636

Kalyanasundaram I., Menon L., Loganathan P. (1994) Occurrence of melanin in bright-spored Myxomycetes. *Cryptogamie Mycol.* **15:** 229-237

Loganathan P., Paramasivan P., Kalyanasundaram I. (1989) Melanin as the spore wall pigment of some myxomycetes. *Mycol. Res.* **92:** 286-292

Martin J.P., Haider K. (1969) Phenolic polymers of *Stachybotrys atra, Stachybotrys chartarum* and *Epicoccum nigrum* in relation to humic acid formation. *Soil Sci.* **107:** 260-270

Mason H.S., Ingram D.J.E., Allen B. (1960) The free radical property of melanins. *Arch. Biochem. Biophys.* **86:** 225-230

McCormick J.J., Blomquist J.G., Rusch H.P. (1970) Isolation and characterization of a galactosamine wall from spores and spherules of *Physarum polycephalum*. *J. Bacteriol.* **104:** 1119-1125

Mims C.W. (1969) Capillitial formation in *Arcyria cinerea*. *Mycologia* **61:** 784-798

Nelson N. (1944) A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* **153:** 375-380

Paramasivan P. (1990) Some Physiological Aspects of the Life Cycle of *Stemonitis herbatica* Peck. Ph.D. Thesis, University of Madras, Madras

Piattelli M., Fattorusso E., Nicolaus R.A., Magno S. (1965) The structure of melanins and melanogenesis. V. Ustilagomelanin. *Tetrahedron* **21:** 3229-3236

Płonka P.M., Rakoczy L. (1997) The Electron paramagnetic resonance signals of the acellular slime mould *Physarum nudum* plasmodia irradiated with white light. *Curr. Top. Biophysics* **21:** 83-86

Raju P.N. (1976) Changes in the free amino acid pool in nodules of virus infected bean (*Dolichos lablab* L.). In: Proceedings of National Symposium: Nitrogen Assimilation and Crop Productivity (Eds. Y.P. Abrol and S.K. Sinha), Hissar, India, 221-223

Rakoczy L., Panz T. (1994) Melanin revealed in spores of the true slime moulds using the electron spin resonance method. *Acta Protozool.* **33:** 227-231

Rast D.M., Stussi H., Hegnaur H., Nyhlen L.E. (1981) Melanins. In: The Fungal Spore: Morphogenetic Controls (Eds. G. Turian and H. R. Hohl), Academic Press, London, 507-531

Reynolds E.S. (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Sci.* **17:** 208-212

Saiz-Jimenez C. (1983) The chemical nature of the melanins from *Coprinus* sp. *Soil Sci.* **136:** 65-74

Sealy R.C., Hyde J.S., Felix C.C., Menon I.A., Prota G. (1982) Eumelanins and pheomelanins: Characterization by electron spin resonance spectroscopy. *Science* **217:** 545-547

Słomiński A., Paus R., Płonka P., Chakraborty A., Maurer M., Pruski D., Lukiewicy S. (1994) Melanogenesis during the anagen-catagen-telogen transformation of the murine hair cycle. *J. Invest. Dermatol.* **102:** 862-869

Thomas M. (1955) Melanin. In: Modern Methods of Plant Analysis (Eds. K. Paech and M.V. Tracey), Springer-Verlag, Berlin, 661-675

Tsao P. W., Tsao P. H. (1970) Electron microscopic observations on the spore wall and operculum formation in chlamydospores of *Thielaviopsis basicola*. *Phytopathol.* **60:** 613-616

Umbreit W. W., Burris R.H. (1972) Method for nitrogen. In: Manometric and Biochemical Techniques (Eds. W.W. Umbreit, R.H. Burris and J.E. Slautter), Burgess Publishing Company, Minnesota, 259-260

Ward J. M., Havir E. A. (1957) The role of 3:4-dihydroxytoluene, sulfhydryl groups and cresolase during melanin formation in a slime mold. *Biochem. Biophys. Acta* **25:** 440-442

Received on 29th April, 1998; accepted on 11th December, 1998

Acta Protozool. (1999) 38: 105 - 117

ACTA
PROTOZOOLOGICA

Brackish Water *Paramecium* Species and *Paramecium polycaryum*. Morphometrical Analysis and Some Biological Peculiarities

Sergei I. FOKIN and Sergei M. CHIVILEV

Biological Institute of St. Petersburg State University, St. Petersburg, Russia

Summary. Morphometrical analysis of 13 cell attributes in 25 stocks of 5 *Paramecium* species (*P. woodruffi, P. calkinsi, P. nephridiatum, P. duboscqui* and *P. polycaryum*) was carried out to examine the diversity of cell morphology in these ciliates. Classification and ordination of the species data were made on the basis of Braverman recalculation of the Euclidean distance and were graphically represented as dendrogramms and two-dimensional space accounting for similarity/ nonsimilarity of the species. Morphometrical data for the macronuclear and micronuclear morphology also were received. According to the investigated morphological and biological peculiarities and full literature analysis on these five, mainly brackish water species of *Paramecium*, some speculation of their phylogenetic relationships is proposed.

Key words: brackish water species, ciliates, morphometry, *Paramecium calkinsi, P. duboscqui, P. nephridiatum, P. polycaryum, P. woodruffi*, taxonomy.

Abbreviations: BC - buccal cavity, BO - buccal overture, CCV - canals of contractile vacuole, CV - contractile vacuole, Ma - macronucleus, MDS - multidimensional scaling, Mi - micronucleus, PCV - pore of contractile vacuole.

INTRODUCTION

Since discovery of *Paramecium calkinsi* and *Paramecium woodruffi* (Woodruff 1921, Wenrich 1928a), they are mentioned as valid species in all *Paramecium* reviews (Wenrich 1928b; Kalmus 1931; Wichterman 1953, 1986; Vivier 1974), but to date comparatively little other information is available on these brackish water ciliates (Wichterman 1986). Because of the ecological similarity of P. *calkinsi* and *P. woodruffi* (Wenrich 1928a, b), these species are often found together, sometimes with *P. nephridiatum* (Fokin *et al.*, in press) and *P. duboscqui* (Fokin *et al.* 1995). Analysis of native samples shows that *P. woodruffi*, *P. nephridiatum* and, sometimes *P. calkinsi* are species which are not easy to distinguish.

For a long time, *P. duboscqui* (Chatton & Brachon, 1933) was considered a nonvalid species (Wichterman 1953, 1986), but currently its validity is accepted (Fokin 1986, Watanabe *et al.* 1996, Shi *et al.* 1997). However, its morphology and biology have yet to be comprehensively described.

Paramecium nephridiatum (Gelei 1925, 1938), as the result of reviews by Wichterman (1953, 1986), was also considered a nonvalid species. In fact, this was caused by

Address for correspondence: Sergei I. Fokin, Biological Research Institute, St. Petersburg State University, Oranienbaumskoye sch.2, Old Peterhof, St. Petersburg, Russia 198904; E-mail: fokin.invert@pobox.spbu.ru

some significant mistakes in the first description, which were admitted by the author (Gelei 1938). Unfortunately, a second more correct description of this *Paramecium* species is practically unknown. This important article has not been discussed anywhere and is merely listed in several reviews (Vivier 1974; Wichterman 1953, 1986), but without any connection to the taxonomy of *Paramecium*. Very recently we redescribed this species as a valid euryhaline one (Fokin *et al.*, in press).

Paramecium polycaryum which was described from fresh water by Woodruff and Spencer (1923) morphologically (except for nuclear apparatus and cell size) is very similar to *P. calkinsi* (Wichterman 1953, 1986). Jankowski (1969) placed this species in the "*woodruffi*" group. However, the morphology of *P. polycaryum* has not been described sufficiently.

Only Jankowski (1969, 1972) has tried to build up some structure of the relationship between some of the *Paramecium* species (*P. woodruffi*, *P. calkinsi* and *P. polycaryum*). For the most part the data which were the basis for this speculation have not been published.

Morphometrical analysis in combination with some special statistic programs has been used successfully for the *P. aurelia* complex taxonomy (Gates *et al.* 1974, Powelson *et al.* 1975). Meanwhile, nobody has tried to use it for other paramecia.

The aim of this study was an analysis of species-specific traits of brackish water paramecia and *P. polycaryum*, using their morphometric and biological characteristics. These data are the basis for some speculations about relationships inside the group. The data of morphometry with the help of MDS procedures are represented as dendrogramms and two-dimensional space accounting of the intra- and interspecific similarities.

MATERIALS AND METHODS

Paramecium duboscqui stocks and a number of clone cultures of *P. woodruffi*, *P. calkinsi*, *P. nephridiatum* and *P. polycaryum* were use in this study. They were isolated from natural populations in different places, mainly on the coasts of North European Seas (Table 1).

Paramecia were grown on a lettuce medium inoculated with *Enterobacter aerogenes* according to Sonneborn's method (1970) at 20°C.

The morphology of these ciliates was studied at first in a living condition. The cells were immobilized by a device for controlling the cover slide (Skovorodkin 1990) and were observed by phase contrast or DIC optic (Polyvar microscope, Reichert, Austria; Axioskop, Zeiss, Germany).

Cultures were fixed and stained by the Chatton and Lwoff silver impregnation technique (Corliss 1953) on the next day after feeding. Twenty - twenty five properly oriented and well-impregnated specimens were selected from each stock and measured. The 13 attributes, which were used for MDS, are listed in Table 2. The points of the direct measurements are represented in Figs. 1 and 2. The dimensions of the nuclear apparatus (Ma, Mi) were measured as well. For this purpose ciliates were fixed by Bouin's fixative and stained by Feulgen technique.

The measurements of the attributes were carried out using the same microscopes equipped with a micrometer (Carl Zeiss, Germany).

For each stock and for each attribute, the mean, standard deviation, coefficient of variation, minimum value, maximum value were calculated. The mathematical analysis of the data obtained was made by multivariate procedures using the program package developed in the Plymouth Marine Laboratory (PML, Great Britain).

Classification and ordination of species using the full data was made on the basis of Braverman (1965) recalculations of the Euclidean distance. In this case, the transformation of numerical morphological characteristics of *Paramecium* species was made by extracting the 4th power root from the relevant values with for purpose of alignment of data (Clarke and Green 1988). In all cases the secondary matrices were graphically represented as dendrogramms constructed by the using weighed paired group method (Baily 1970).

Ordination of *Paramecium* species was made by MDS (Kruskal and Wish 1978) resulting in the arrangement of the species in two-dimensional space accounting for similarity/nonsimilarity of the species' morphometric traits.

RESULTS

Morphometrical analysis permitted the determination of the main parameters of the cell for five investigated *Paramecium* species (Tables 3, 4; Figs. 3-12).

Paramecium woodruffi (Tables 3-5; Figs. 3, 4)

The average size of the cell in 6 investigated stocks was 165.0 x 52.0 μm. The number of cilia rows varied from 85 to 110 (95 on average). The BO was located, as usual, close to the cell equator (49% of the cell length). The distance from the anterior end of cell to the signal PCV of the anterior CV was approximately 32% of the cell's length. The distance from the signal PCV of the posterior CV to the posterior end of the cell was 18.5% of the cell's length. The size of BC, in average, was 17.5% of the body length. The endoral membrane was composed of 13-18 units (15 on average).

The ovoid or slightly ellipsoidal Ma usually was situated a bit to the anterior end of the cell. Its size varied from 15 x 25 to 25 x 40 μm (26 x 31 μm on average). Paramecia which had the Ma located on the equator line of the cell have also been found.

The number (2-5) of ovoid "endosomal" type Mi had a size between 3 and 6 μm. More often they were located in the anterior part of the cell, but on the opposite side from the Ma. The stock BB2-13 (wo5) had no Mi at all.

Table 1. Origin of the cultures of *Paramecium*

Species	Stock	Number	Origin
P. woodruffi	DV12-21	wo1	Vladivostok district, Russia
	Dz59-22	wo2	Barents Sea, Murmansk district, Russia
	BB1-2	wo3	Baltic Sea, Viborg district, Russia
	BB2-1	wo4	Baltic Sea, Viborg district, Russia
	BB2-13	wo5	Baltic Sea, Viborg district, Russia
	BB3-3	wo6	Baltic Sea, Viborg district, Russia
P. calkinsi	OP1-18	cl1	White Sea, Karelian district, Russia
	AL1-41	cl2	Sevan district, Armenia
	OP1-14	cl3	White Sea, Karelian district, Russia
	DV12-13	cl4	Vladivostok district, Russia
P. nephridiatum	OK-6	nr1	White Sea, Kandalaksha district, Russia
	DZ59-4	nr2	Barents Sea, Murmansk district, Russia
	DZ59-9	nr3	Barents Sea, Murmansk district, Russia
	WS-3	nr4	Baltic Sea, Viborg district, Russia
	DZ59-5	nr6	Baltic Sea, Viborg district, Russia
	WS97-1	nr7	White Sea, Kandalaksha district, Russia
	JZ1-5	nr8	Jerusalem Zoo, Israel
	GH1-1	nr9	North Sea coast, Germany
	WS97-2	nr10	White Sea, Kandalaksha district, Russia
P. duboscqui	DZ19-21	du1	Barents Sea, Murmansk district, Russia
	DZ19-6	du2	Barents Sea, Murmansk district, Russia
	BB2-31	du3	Baltic Sea, Viborg district, Russia
P. polycaryum	TR-1	po1	Toliatti district, Russia
	TR-5	po2	Toliatti district, Russia
	TR-6	po3	Toliatti district, Russia

Table. 2. Description of attributes employed in the analysis of paramecia

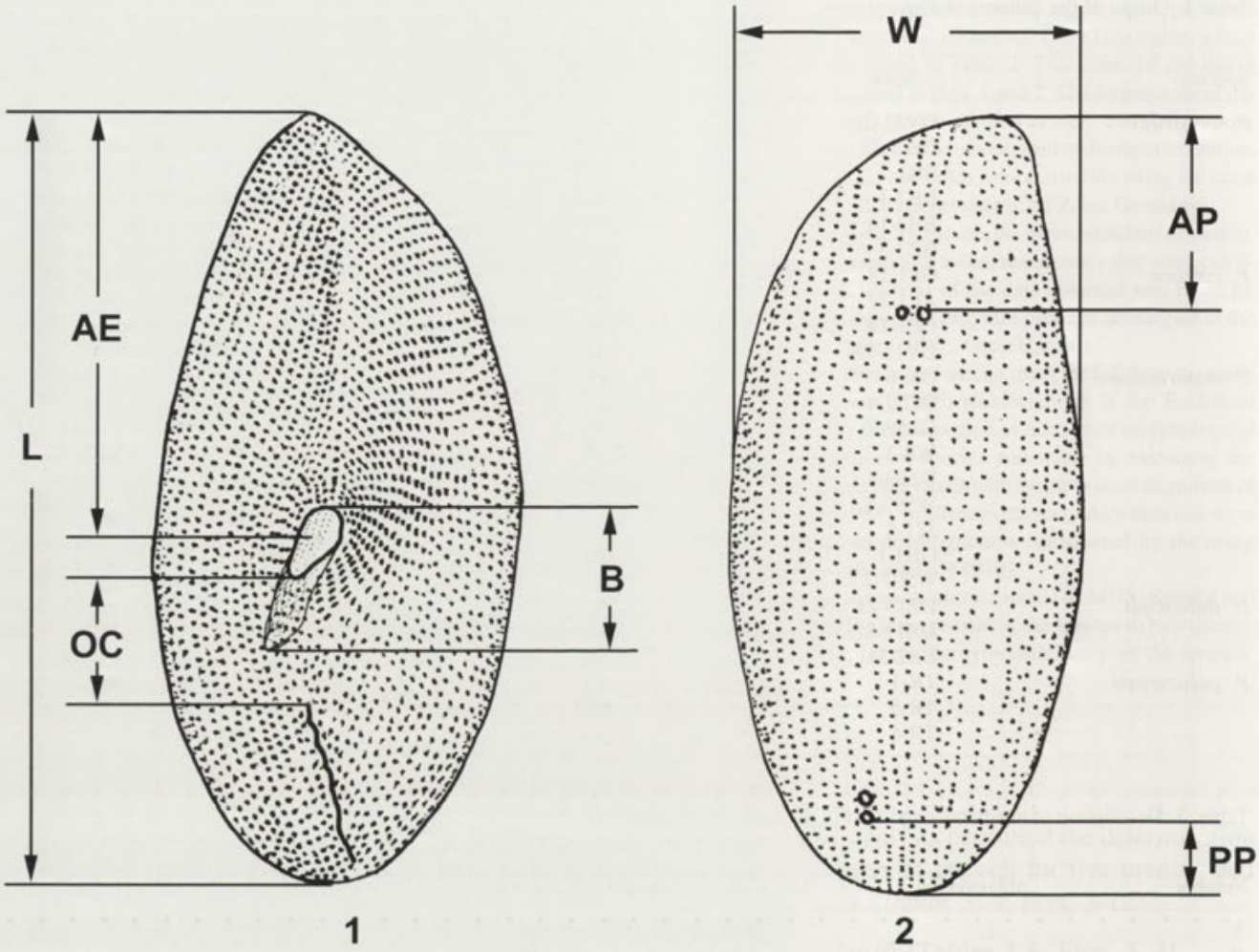
Number	Abbreviation	Description
1	L	Maximum rectilinear length
2	W	Maximum rectilinear width
3	B	Maximum rectilinear buccal cavity
4	AE	Distance from the anterior edge to the equator of the buccal opening
5	OC	Distance from posterior edge of the buccal opening to the anterior edge of cytoproct
6	AP	Distance between the anterior PCV and the edge of the cell
7	PP	Distance between the posterior PCV and the edge of the cell
8	WL	W/L %
9	AEL	AE/L%
10	OCL	OC/L%
11	AP	AP/L%
12	PPL	PP/L%
13	BL	B/L%

PCV- pore of the contractile vacuole

Classification and ordination the morphometric data of the species indicated that all of the stocks formed a cluster with a high degree of similarity: 80% or more (Figs. 13A, B). Inside of the species two groups were found. The first consisted of the stocks which originated from different places: Japan, Barents and Baltic Sea coasts (wo1, wo2 and wo5). Another group was formed by 3 clones originated from a separate population on Berezovyi Island in the Gulf of Finland, Baltic Sea (wo3, wo4, and wo6).

Paramecium calkinsi (Tables 3-5; Figs. 5, 6)

The average size of the cell in 4 the investigated stocks was 115.0 x 38.0 μm. The number of cilia rows was 70-85 (75 on average). The BO was usually located a bit posteriorly from the cell equator (51% of the cell length).



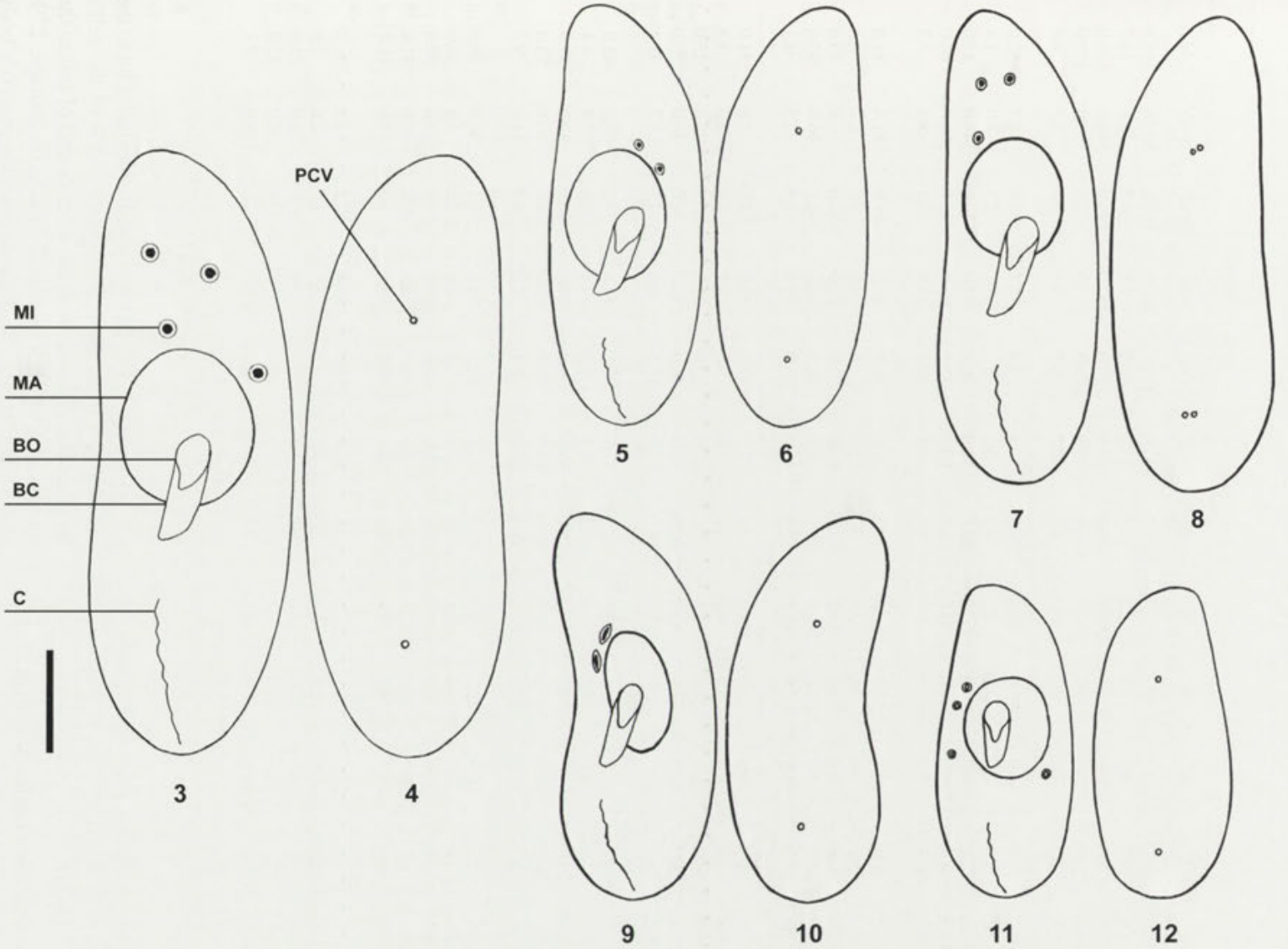
Figs. 1, 2. Ventral (1) and dorsal (2) views of *Paramecium* species. The points of the direct measurements of the cell features. AE - distance from the anterior edge to the equator of the buccal opening; AP - distance between anterior PCV and the edge of the cell; B - maximal buccal cavity length; L - maximum rectilinear length; OC - distance from posterior edge of the buccal opening to the anterior edge of cytoproct; PP - distance between the posterior PCV and the edge of the cell; W - maximum rectilinear width

The endoral membrane was composed of 12-20 units (15 on average). The distance from the anterior end of cell to the single PCV of the anterior CV was around 31% of the cell's length. The distance from the single PCV of the posterior CV to the posterior end of the cell was approximately 17% of the body length. The size of BC, on average, was 21% of the body length.

The ovoid or slightly ellipsoidal Ma was usually located on the cell's equator. The dimensions of the Ma varied from 10 x 18 to 25 x 33 μm (Feulgen preparations). Most often it had a size of approximately 17 x 24 μm.

Two of the investigated stocks of *P. calkinsi* were bimicronucleate and the cells of another two had a variable number of Mi (1-4). The nuclei of all of the stocks manifested "endosomal" type structure. They were spherical in form with a size between 1.7 and 3.4 μm. The Mi generally were located close to the Ma, but sometimes were found in other parts of the cytoplasm as well.

Classification and ordination the morphometric data of the species showed that all four stocks formed a cluster with degree of similarity 69% and more (Figs. 13A, B). Inside of the group the cells of the AL1-41 clone (cl2) had a different size, position of the posterior PCV and body length/ buccal cavity ratio. As a result, the place of this clone on the ordination picture (Fig. 13B) was located quite far from the others.



Figs.3-12. Ventral and dorsal views of 5 *Paramecium* species belong to the "*woodruffi*" group. 3, 4 - *P. woodruffi*; 5, 6 - *P. calkinsi*; 7, 8 - *P. nephridiatum*; 9, 10 - *P. duboscqui*; 11, 12 - *P. polycaryum*. Abbreviations: BC - buccal cavity, BO - buccal overture, C - cytoproct, MA - macronucleus, MI - micronuclei, PCV - pore of contractile vacuole. All drawings are made after paramecia cells morphometry. Scale bar - 30 μm

Table 3. Morphometric characterization of *Paramecium calkinsi* and *Paramecium woodruffi*. AE - distance from anterior end to middle of buccal overture; AP - distance from anterior end to anterior PCV; B - buccal length; Co.V - coefficient of variation; L - body length; M - mean of the species; Max - maximal size of the trait; Min - minimal size of the trait; OC - distance from proximal edge of buccal overture to anterior end of cytoproct; PP - distance from posterior end to posterior PCV; SD - standard deviation; W - body width; $\bar{x}$ - mean of the stock. All measurements in μm

Character	Statistics	*P. calkinsi*					*P. woodruffi*						
		cl1	cl2	cl3	cl4	M	wo1	wo2	wo3	wo4	wo5	wo6	M
L	$\bar{x}$	124.0	94.0	114.5	126.8	115.0	159.2	154.0	178.1	166.9	161.7	160.8	165.0
	SD	6.5	9.7	8.6	12.3	12.8	10.7	16.2	12.0	7.6	6.2	8.6	14.7
	Min	110.0	70.0	80.0	100.0	90.0	140.0	100.0	152.0	152.0	144.0	144.0	139.0
	Max	150.0	120.0	130.0	150.0	137.5	170.0	170.0	200.0	170.0	172.0	180.0	177.0
	Co.V	5.2	10.3	7.5	9.7	11.1	6.7	10.5	6.7	4.5	3.8	5.3	8.9
W	$\bar{x}$	38.3	32.3	41.2	38.6	38.0	50.2	52.4	54.4	52.7	50.6	52.7	52.0
	SD	3.6	3.7	2.6	4.2	3.3	4.8	5.9	5.7	7.7	5.1	4.9	1.4
	Min	32.0	29.0	36.0	32.0	32.0	40.0	44.0	48.0	40.0	40.0	44.0	43.0
	Max	44.0	40.0	44.0	44.0	43.0	60.0	60.0	78.0	65.0	60.0	60.0	64.0
	Co.V	9.3	11.4	6.3	10.8	8.7	9.5	11.2	10.5	14.6	10.1	9.3	2.7
AE	$\bar{x}$	64.6	49.4	62.4	58.1	58.0	79.3	75.0	86.1	81.2	81.6	81.4	81.0
	SD	4.9	5.7	5.4	4.6	5.3	4.7	5.6	5.6	4.3	3.7	6.1	3.3
	Min	52.0	36.0	40.0	52.0	45.0	72.0	68.0	72.0	72.0	76.0	76.0	73.0
	Max	68.0	60.0	70.0	68.0	66.5	92.0	88.0	96.0	88.0	96.0	96.0	93.0
	Co.V	7.6	11.5	8.6	7.9	9.1	5.9	7.5	6.5	5.3	4.5	7.5	4.1
OC	$\bar{x}$	19.7	20.7	26.6	26.4	23.0	34.2	27.7	32.3	33.0	24.3	2.8	31.0
	SD	3.6	2.7	4.7	2.9	3.2	4.8	3.8	5.0	3.1	3.0	3.2	3.5
	Min	14.0	16.0	16.0	24.0	17.5	28.0	24.0	20.0	28.0	20.0	28.0	25.0
	Max	24.0	28.0	36.0	32.0	30.0	40.0	32.0	40.0	36.0	32.0	40.0	37.0
	Co.V	18.3	13.0	17.7	11.0	13.7	14.0	13.7	15.5	9.4	12.3	9.7	11.4
AP	$\bar{x}$	37.7	29.8	40.5	35.4	36.0	41.6	37.7	47.5	49.8	44.2	49.8	45.0
	SD	7.1	5.0	4.1	7.5	3.9	4.3	7.1	2.9	4.6	2.4	5.8	4.4
	Min	32.0	24.0	32.0	28.0	29.0	28.0	36.0	44.0	40.0	40.0	44.0	39.0
	Max	44.0	36.0	48.0	44.0	43.0	48.0	44.0	56.0	60.0	48.0	60.0	53.0
	Co.V	18.8	16.7	10.1	21.2	10.8	10.3	18.8	6.1	9.2	5.4	11.6	9.7
PP	$\bar{x}$	20.0	12.7	21.6	22.3	19.0	33.4	31.3	42.5	39.6	33.1	34.0	36.0
	SD	4.6	2.4	2.6	5.4	3.8	12.5	3.8	4.2	4.3	2.6	5.1	4.0
	Min	12.0	10.0	20.0	16.0	14.5	20.0	28.0	40.0	28.0	28.0	28.0	29.0
	Max	32.0	16.0	28.0	32.0	27.0	60.0	44.0	56.0	44.0	40.0	40.0	47.0
	Co.V	23.0	18.9	12.0	24.2	19.6	37.4	12.1	9.8	10.8	7.8	15.0	11.2
B	$\bar{x}$	28.3	23.9	23.7	21.8	25.0	26.6	29.8	30.0	30.6	27.2	31.0	26.0
	SD	1.9	1.8	2.5	1.5	2.4	2.0	1.8	1.5	2.3	1.2	2.8	1.9
	Min	24.0	20.0	20.0	20.0	21.0	20.0	24.0	28.0	28.0	26.0	28.0	25.0
	Max	30.0	28.0	28.0	24.0	27.5	28.0	32.0	32.0	32.0	30.0	36.0	32.0
	Co.V	6.7	7.6	10.5	6.9	9.7	7.6	6.0	4.9	7.5	4.4	9.0	7.4

The morphometric attributes used distinguished the investigated stocks of *P. woodruffi* and *P. calkinsi* very confidently (Figs. 13A, B).

***Paramecium nephridiatum* (Tables 3-5; Figs. 7, 8).**

The average size of the cell in 9 investigated stocks was 131.0 x 41.0 μm. The number of cilia rows was 68-83 (73 on average). The BO was usually located, a bit to the anterior end from the cell's equator (45% of the cell's length). The distance from the anterior end of cell to the nearest anterior PCV (one of 2-5) was, on average, 27% of the body length. The distance from the posterior body end to the nearest PCV (one of 2-4) of the posterior CV was around 18% of the cell's length. The size of the BC,

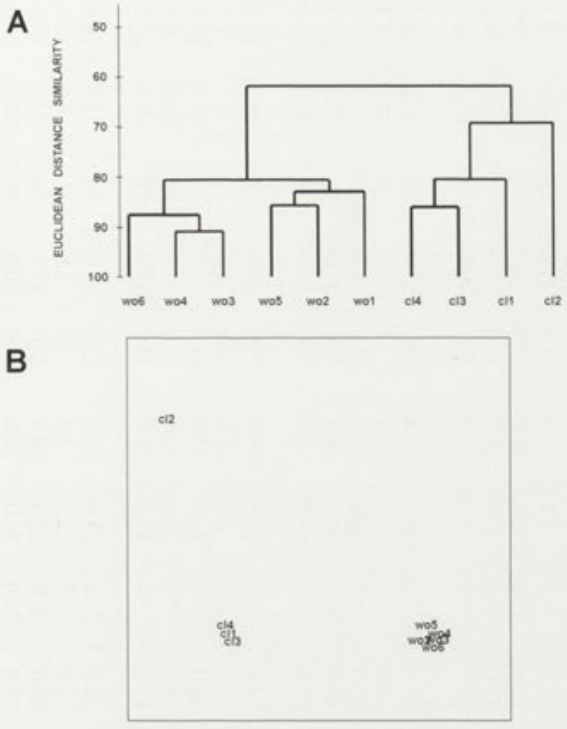


Fig. 13. Similarity of *Paramecium woodruffi* (wo) and *Paramecium calkinsi* (cl) stocks. Dendrogramm (A) for hierarchical clustering and results of non-metric multidimensional scaling (B) by the their morphometric characteristics based on the Euclidean distance measure. A - in %, B - stress = 0.010

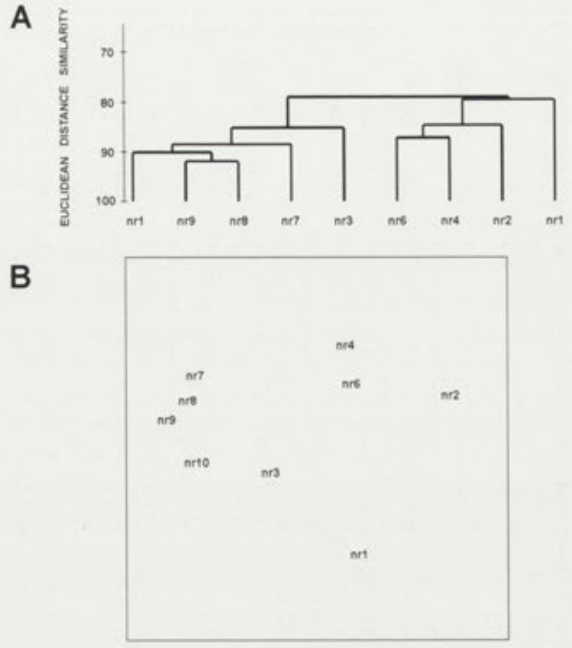


Fig. 14. Similarity of *Paramecium nephridiatum* stocks. Dendrogramm (A) for hierarchical clustering and results of non-metric multidimensional scaling (B) by the their morphometric characteristics based on the Euclidean distance measure. A - in %, B - stress = 0.049

on average, was 20% of the cell's length. The endoral membrane possessed 10-23 units (15 on average).

The ovoid or slightly ellipsoidal Ma was usually, but not always, located in the anterior part of the cell. Their size varied from 14 x 23 to 25 x 30 μm (17 x 25 μm on average).

A number of ovoid Mi (1-6) of "endosomal" type with dimension 1.5-3.8 μm can be found in different parts of the cell, but mainly in anterior section.

Analysis of the morphometric data by MDS showed that the investigated stocks of *P. nephridiatum* formed a cluster with degree of similarity 80% or more (Figs. 14A, B). Inside of the cluster two groups of clones exist. Some of the stocks are practically identical regarding the used attributes (similarity more than 90%).

Paramecium duboscqui (Tables 3-5; Figs. 9, 10)

The average size of the cell in three investigated stocks was 106.0 x 40.0 μm. The number of cilia rows varied from 70 to 90 (74 on average). The BO was located near

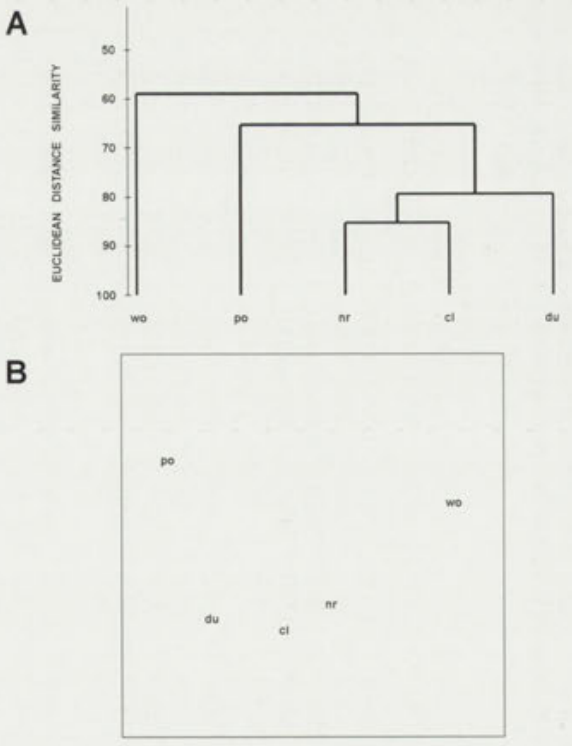


Fig. 15. Similarity of the five *Paramecium* species. Dendrogramm (A) for hierarchical clustering and results of non-metric multidimensional scaling (B) by their morphometric characteristics based on the Euclidean distance measure A - in %, B - stress less than 0.000. *P. polycaryum* (po), *P. duboscqui* (du), *P. nephridiatum* (nr), *P. calkinsi* (cl) and *P. woodruffi* (wo)

Table 4. Morphometric characterization of *Paramecium nephridiatum*, *Paramecium duboscqui* and *Paramecium polycaryum*. AE - distance from anterior end to middle of buccal overture; AP - distance from anterior end to anterior PCV; B - buccal length; Co.V - coefficient of variation; L - body length; M - mean of the species; Max - maximal size of the trait; Min - minimal size of the trait; OC - distance from proximal edge of buccal overture to anterior end of cytoproct; PCV- pore of contractile vacuole; PP - distance from posterior end to posterior PCV; SD - standard deviation; W - body width; $\bar{x}$ - mean of the stock. All measurements in μm

Character	Statistics	*P. nephridiatum*										*P. duboscqui*				*P. polycaryum*			
		nr1	nr2	nr3	nr4	nr6	nr7	nr8	nr9	nr10	M	du1	du2	du3	M	po1	po2	po3	M
L	$\bar{x}$	138.9	121.7	136.6	117.9	124.4	127.5	128.4	132.0	136.1	131.0	101.7	99.8	117.4	106.0	76.0	82.0	93.2	84.0
	SD	6.4	10.1	8.6	14.2	6.3	7.5	3.8	4.3	13.9	6.5	15.4	10.0	10.7	7.9	10.0	5.7	4.0	7.1
	Min	130.0	90.0	110.0	100.0	116.0	114.0	110.0	110.0	120.0	111.0	70.0	75.0	95.0	80.0	65.0	70.0	72.0	69.0
	Max	150.0	135.0	145.0	128.0	132.0	140.0	143.0	149.0	168.0	143.0	120.0	120.0	140.0	127.0	100.0	100.0	100.0	100.0
	Co.V	4.6	8.4	6.6	12.0	5.0	5.8	2.9	3.2	10.2	5.0	15.1	10.0	9.1	7.4	13.1	6.9	4.3	8.5
W	$\bar{x}$	33.0	37.4	40.2	39.0	38.3	46.4	46.4	46.2	45.4	41.0	40.2	40.5	39.2	40.0	37.6	35.6	37.5	37.0
	SD	3.3	3.6	2.9	3.8	3.1	5.2	4.0	7.8	5.6	4.4	3.4	3.3	4.3	5.5	4.1	2.6	3.0	9.2
	Min	28.0	32.0	32.0	32.0	32.0	40.0	42.8	30.0	40.0	34.0	36.0	36.0	28.0	33.0	32.0	32.0	30.0	31.0
	Max	40.0	44.0	44.0	44.0	44.0	57.1	54.3	54.0	56.0	49.0	44.0	44.0	44.0	44.0	44.0	40.0	42.0	42.0
	Co.V	1.0	9.6	7.2	9.7	8.1	11.2	8.6	16.8	12.3	10.6	8.4	8.1	10.9	13.7	10.9	7.3	8.0	24.9
AE	$\bar{x}$	59.1	56.3	59.4	58.8	60.6	60.3	65.6	67.9	62.7	60.0	52.3	52.2	53.7	53.0	35.6	34.5	38.7	36.0
	SD	6.7	5.8	11.8	3.3	3.4	3.5	13.4	4.8	5.1	6.0	3.0	6.1	5.1	7.2	3.1	2.4	10.4	1.8
	Min	48.0	44.0	56.0	56.0	56.0	54.3	60.0	57.1	56.0	54.0	44.0	36.0	48.0	43.0	32.0	30.0	34.0	32.0
	Max	76.0	72.0	68.0	68.0	68.0	68.6	71.4	71.4	71.0	70.0	60.0	60.0	64.0	61.0	42.0	40.0	44.0	42.0
	Co.V	11.3	10.3	19.8	5.6	5.6	5.8	20.4	7.1	8.1	10.0	5.7	11.6	9.5	13.7	8.7	6.9	26.8	4.8
OC	$\bar{x}$	29.3	24.1	26.1	23.0	23.7	27.7	29.7	30.0	29.8	27.0	16.8	18.9	23.8	20.0	23.1	24.2	25.4	24.0
	SD	3.8	3.2	3.3	2.7	3.9	0.0	2.1	1.9	3.2	2.6	1.5	3.5	2.3	5.4	3.9	2.4	4.4	9.3
	Min	24.0	20.0	20.0	18.0	18.0	23.0	26.0	25.5	25.0	22.0	14.0	10.0	16.0	13.0	16.0	16.0	20.0	17.0
	Max	36.0	30.0	32.0	30.0	32.0	31.4	31.4	34.0	37.0	33.0	20.0	20.0	26.0	22.0	28.0	26.0	36.0	30.0
	Co.V	12.9	13.3	12.6	11.7	16.4	0.0	6.7	6.3	10.7	9.5	8.9	18.5	9.7	27.3	16.9	9.9	17.3	38.2
AP	$\bar{x}$	33.9	34.7	38.9	31.1	33.1	35.3	33.9	38.4	37.0	35.0	26.7	29.3	30.9	29.0	23.4	24.1	27.4	25.0
	SD	8.9	4.6	1.8	2.9	3.5	4.0	4.3	3.7	3.5	2.4	2.8	5.0	7.8	1.7	3.1	3.5	4.4	1.7
	Min	24.0	32.0	24.0	28.0	16.0	28.6	28.6	34.0	31.0	26.0	24.0	20.0	28.0	24.0	18.0	18.0	20.0	19.0
	Max	44.0	44.0	44.0	36.0	40.0	40.0	40.0	43.0	43.0	42.0	32.0	36.0	40.0	36.0	26.0	28.0	28.0	27.0
	Co.V	26.2	13.2	4.6	9.3	10.5	11.3	12.7	9.6	9.4	6.8	10.5	17.0	25.2	2.4	13.2	14.5	16.0	6.9
PP	$\bar{x}$	26.0	19.3	22.1	22.4	18.2	18.6	20.9	20.4	23.6	22.0	23.7	17.1	21.7	21.0	12.0	12.0	15.7	13.0
	SD	3.9	3.9	5.1	3.1	4.2	3.6	6.0	3.7	4.2	3.9	1.7	2.1	4.7	2.8	1.8	1.6	1.7	1.7
	Min	24.0	16.0	14.0	16.0	12.0	14.3	14.3	14.0	14.0	15.0	20.0	10.0	12.0	14.0	10.0	10.0	12.0	11.0
	Max	28.0	22.0	36.0	28.0	28.0	23.0	28.6	26.0	29.0	28.0	25.0	20.0	32.0	26.0	16.0	14.0	18.0	16.0
	Co.V	15.0	20.2	23.0	13.8	23.1	19.3	28.7	18.1	17.8	17.4	7.2	12.3	21.6	14.0	15.0	13.3	10.8	13.1
B	$\bar{x}$	26.6	22.4	27.4	26.2	27.5	31.0	29.7	30.0	30.0	27.5	22.6	23.0	21.8	22.0	16.0	16.3	21.8	18.0
	SD	1.5	1.6	1.9	2.6	2.1	1.7	2.1	1.9	1.6	2.6	1.7	2.1	1.5	1.0	2.8	1.3	1.6	2.6
	Min	24.5	20.0	25.0	24.0	24.0	25.4	25.5	28.6	29.0	25.0	20.0	20.0	20.0	20.0	12.0	14.0	20.0	15.0
	Max	28.0	24.5	30.0	30.0	29.0	31.4	34.0	34.3	34.0	31.0	24.0	26.0	24.0	25.0	20.0	18.0	24.0	21.0
	Co.V	5.6	7.1	6.9	9.9	7.6	5.4	7.0	6.3	5.3	9.4	7.5	9.1	6.9	4.5	17.5	7.9	7.3	14.4

Table 5. Morphological and biological features of brackish water *Paramecium* species and *Paramecium polycaryum*

Characters	*P. calkinsi*	*P. woodruffi*	*P. nephridiatum*	*P. duboscqui*	*P. polycaryum*
Body length	60-160(120)**a**	120-210(170)	90-170(130)	70-140(110)	70-130**b**(85)
Body width	20-80(40)	40-80(55)	30-60(41)	30-50(40)	35-60**b**(40)
WL ratio	0.33	0.32	0.29	0.40	0.47
Buccal overture position	51%**c**	50%	47%	49%	44%
Cytoproct position	75%	71%	70%	73%	79%
Number of CCV	8-10(9)	8-16(10)	8-15(10)	0	7-8(7)
Number of PCV	1	1	1-5(2)	1	1-2**d**
Cilia tuft	+	+	+	+	+
Number of cilia rows	70-100(75)	85-110(95)	70-120**f**(73)	70-90(74)	45-90**b**(57)
Number of EM units	12-20(15)	13-18(15)	10-23(15)	12-27(19)	14-28**b**(15)
Ma size	10x18**d**(17x24) 25x33**e**	15x25(26x31) 25x40	14x23(17x25) 25x30	10x23(12x28) 17x35	10x15(13x18) 18x28
Ma position	50%	45%	45%	50%	50%
Ma anlagen number	2	4	4	4	4
Mi size	2-4(3)	3-5(4)	1.5-4(3)	1.2x2.5 (1.5x4) 2.0x5.1	1.4-8**b**(1.6)
Mi number	1-5(2)	0-10(4)	1-7(3)	0-6(2)	0-8(4)
Mi position	Near Ma	Anteriorly	Anteriorly	Near Ma	Near Ma
Mi type	Endosomal	Endosomal	Endosomal	Endosomal	Vesicular
Rotation	Mainly to the right	Mainly to the left	Mainly to the left	To the right	To the left
Endosymbionts	C, Ma	-	C, Ma, PS	PS, Ma	C
Autogamy	+	-	-	-	+
Selfing	+	-	+	-	-
Type of water	F, B, S	F, B, S	F, B, S	F, B, S	F

The table was compiled after literature data and own investigations. All measurements of fixed cells in µm. **a** - mean, **b** - the largest size and numbers were reported by Beran (1990), **c** - distance from the anterior cell's end in % of body length, **d** - minimum, **e** - maximum, **f** - the largest number was reported by Gelei (1938), + - trait's presence, - - trait's absence, B - brackish, C- cytoplasm, CCV - canals of contractile vacule, EM - endoral membrane, F - fresh water, Ma - macronucleus, Mi - micronucleus, PCV - pore of contractile vacuoles, PS - perinuclear space, S - sea water, WL ratio - maximum rectilinear width to length

the cell equator (49% of the cell size, in average). The distance from the anterior end of the cell to the single PCV of the anterior CV was approximately 40% of body length. The distance from the single PCV of the posterior CV to the posterior end of the cell was 19.3% of the cell length. The size of the BC, on average, was also 19.3% of the cell length. The endoral membrane was presented by 12-27 units (19 on average).

The ellipsoidal or bean-shaped Ma was usually also situated on the cell's equator. The dimensions of the Ma varied from 10 x 23 to 17 x 35 µm (12 x 28 µm, mean).

Several spindleform micronuclei (2-4, usually 2) had a "endosomal" type of morphology. The dimension of the nuclei were from 1.7 x 3 to 3 x 5 µm. They were located mainly close to the Ma.

Paramecium polycaryum (Tables 3-5; Figs. 11, 12)

The average size of the cell in the three investigated stocks was 84.0 x 37.0 µm. The number of cilia rows was 45-65 (57 on average). The BO was located significantly to the anterior end of the cell (43% of the cell's length). The distance from the anterior end of cell to the single PCV of the anterior CV was 29% of the body length. The distance from the single PCV of posterior CV to the posterior end was 17.3% of the cell length. The size of the BC, on average, was 21% of the cell length. The endoral membrane was composed of 14-17 units (15 on average). The ovoid or slightly ellipsoidal Ma (from 10 x 15 to 18 x 28 µm) was usually situated close to the cell's equator. Typically the size of the Ma was around 13 x 18 µm.

A number (2-5) of ovoid Mi with size 1.5-2.0 µm were located around the Ma. They manifested "vesicular" type of micronuclear morphology. Classification and ordination the morphometric data for *P. duboscqui* and *P. polycaryum* indicated that they are very easily distinguished from each other and from the other species of the "*woodruffi*" group (Figs. 15A, B).

DISCUSSION

Until now, relatively little information has been available on some of brackish water *Paramecium* species. It is becoming clear since the rediscovery of *P. nephridiatum* (Fokin *et al.*, in press) that a number of studies dedicated to *P. woodruffi* (Agamaliev1983; Jankowsky 1961, 1969; Fokin 1986; 1989a-d, 1997) were, in fact, done on the first species. Since the first description of P. *woodruffi* (Wenrich 1928a; Table 5; Figs. 3, 4), probably only a few articles were connected with the real species (Lieberman 1929, Ammermann 1966, Kościuszko 1986). Mainly, the authors who mentioned *P. woodruffi* (Kahl 1931, 1934; Kalmus 1931; Wichterman 1953, 1986; Vivier 1974; Carey 1992) just repeated the data of Wenrich (1928a, b).

Lieberman (1929) described ciliary arrangement in different species of *Paramecium*. He found that the total number of cilia within a standard area in *P. woodruffi* (144 μm^2) was a bit less than in *P. calkinsi* and much less than in *P. polycaryum*. This article is a rare case when photographs of impregnated subjects were shown. It is important that he used the original cultures of Wenrich (Lieberman, 1929). Also it is clear from these pictures that the position of the BO in the case of *P. woodruffi* is shifted only slightly to the posterior end from the cell's equator. This fact fits with our own observations (Table 3, Fig. 3).

According to our observations, the total number of cilia rows in *P. woodruffi* (95) was higher than for *P. calkinsi* (75) and for *P. polycaryum* (57).

Ammermann (1966) who described two mating types for *P. woodruffi* from brackish water of the North Sea coast did not mention any morphological traits. Kościuszko (1986) has described some morphological characteristics of the paramecia from the Little Caucasus area. Apparently, the samples were taken from fresh water bodies (unpublished materials of the laboratory of Invertebrate Zoology, Biological Research Institute, St. Petersburg, Russia), but the salinity was not measured. The morphological features of the cells fit quite well to *P. woodruffi*. The author indicated the variation of the Mi number for one of these stocks as 1-10. Before this, the largest number of Mi for *P. woodruffi* was mentioned by Dragesco and Dragesco-Kerneis (1986) as 3-9. The number of Mi varied in our stocks from 0 to 5. Therefore, probably, the article of Kościuszko (1986) was the first report about *P. woodruffi* from fresh water.

Our data indicate that the most distinguishing characteristics of *P. woodruffi* are the size of cell and the presence of only one PCV in the each of CV (Figs. 3, 4). Each CV of *P. woodruffi* usually has 8-16 collecting canals with ampoule, but more often in investigated stocks this number was 10 (data not shown). The cells can rotate in both directions during swimming, but more often they rotated to the left.

The species has a binary mating type system; exconjugants of the species have 4 macronuclear anlagen (unpublished data).

Morphometrical analysis using 13 cell attributes can distinguish this species from other brackish water paramecia very confidently (Figs.15A, B).

Paramecium calkinsi (Table 5; Figs. 5, 6) was described at first from fresh water (Woodruff 1921) and then was found in brackish water (Unger 1926, Wenrich and Wang 1928).

This species was intensively studied from the morphological and biological points of view in the1940s and 1950s (Diller 1948; Wichterman 1950; 1951, 1953; Zawoiski 1951; Nakida 1956, 1958; Yusa 1957). Then it was found in fresh, brackish and even sea water (Wichterman 1948, 1950).

During that time only Zawoiski (1951) did some morphometry of the species. He found that the dimension of the cells in a culture is strongly connected with the length of laboratory cultivation. After one month in laboratory conditions *P. calkinsi* cells diminished in size by as much as one-half: from 137 to 70 μm (Zawoiski 1951). We have also found such a tendency, but the diminution was not so strong.

Some distinct traits were found for this species. They are a clockwise direction of spiral during swimming, a more constant in Mi number (2) and formation only 2 macronuclear anlagen during the nuclear reorganization process (Woodruff 1921, Bullington 1925, Diller 1948, Nakida 1956). The endoral membrane was made up of 16 uniformly spaced granules (Yusa 1957). The number of CV canals in *P. calkinsi* cells was reported as 7-8 (Wichterman 1950, 1951; Borror 1972), but we have found 8-10 such structures in 3 of investigated stocks (Fokin, 1986).

Two syngenes with binary mating type system were found so far (Wichterman 1951, 1953). Nakida (1958) noted for *P. calkinsi* the intra-clonal conjugation (selfing). Subsequently we have found that *P. calkinsi* cells do not always rotate to the right (Fokin 1987; Fokin *et al.*, in press). The number of Mi also could be different: 1-5, but a more common number is 2 (Wichterman 1953, Vivier 1974, Fokin 1997).

As a result of morphometric analysis we have found that in investigated stocks the proportions of the cell are different in brackish and in fresh water samples of

P. calkinsi (Table 3; Figs. 13A, B). However, the volume of the material (one fresh water stock- AL1-41 only) is not sufficient for any conclusion.

After redescription of *P. nephridiatum* (Fokin *et al.*, in press) there can be no doubts about the validity of this species as evident from a comparison of the main characteristics (Gelei 1938) and drawings dedicated this *Paramecium* species as well as the results of investigation of DNA patterns for all of the brackish water *Paramecium* species (Fokin *et al.*, in press). It is the fourth species of brackish water euryhaline paramecia wide-spread around the world.

In some traits this species is very similar to *P. woodruffi* (Tables 3, 4). Both contractile vacuoles have collecting canals, the number thus is, usually, greater than 8 (8-14, 10 on average). The nuclear apparatus is located mainly in the anterior part of cell. It consists of one slightly ellipsoid or ovoid Ma and several spherical Mi of "endosomal" type, approximately 3 μm, mainly 3-4 in number.

The species has a binary mating type system. Two mating types have so far been clearly detected. The old macronucleus begins fragmentation before conjugants separation. The new macronuclear anlagen are 4 in number. The selfing takes place in stock cultures in rare cases (Fokin *et al.*, in press).

In contrast to some other species of *Paramecium*, during swimming this species can spiral on its long axis in both directions. Until now the more distinct morphological traits for the paramecia are the size of the cell and the number of CVP (Figs. 3, 4, 7, 8). Typically, both of the CV have more than 1 pore each, usually 2-3.

Using the morphometric analysis of 13 cell attributes this species can be distinguished from other brackish water paramecia very confidently (Figs.15A, B).

Paramecium duboscqui (Table 5; Figs. 9,10) was described from both brackish and fresh water (Chatton and Brachon 1933, Fokin 1986, Fokin and Görtz 1993, Shi *et al.* 1997), but more complete morphological and biological data were reported for fresh water stocks (Watanabe *et al.* 1996, Shi *et al.* 1997). The main traits of the species are: ellipsoid form of the Ma; the number (0-6) of the spindleform Mi (2 on average); CV are vesicle-fed with the tracts of microtubules supporting the walls of the CV; spiral to the right always when swimming (Fokin 1986, 1987, 1997; Shi *et al.* 1997). This species has so far two mating types (syngen 1) and a very unusual reorganization process of the Ma during conjugation (Watanabe *et al.* 1996, Fokin, 1998).

In *P. duboscqui*, the old Ma has one additional division in exconjugants and the 2 pieces of the nucleus are then gradually resorbed in the cytoplasm. The exconjugants have 4 of the Ma anlagen. No fragmentation of the old Ma has been found at all.

Morphometrical analysis has shown that *P. duboscqui* has more similarity with *P. calkinsi* and *P. nephridiatum*. Apparently, this is caused by a similar cell size which was more "heavy" feature for the MDS procedure (Figs. 15A, B). Unfortunately, we have not made a morphometric comparison between fresh and brackish water stocks of *P. duboscqui*.

The first description of *P. polycaryum* (Table 5; Figs. 11, 12) was very poor (Woodruff and Spencer 1923). Some of the morphometric characteristics for the species were mentioned by Wenrich (1928b), Lieberman (1929), Yusa (1957) and, mainly, during the serial investigation of *P. polycaryum* by Japanese authors (Takayanagi 1960; Hayashi and Takayanagi 1961,1962,1964). They also did some biological and cytogenetical studies of the species.

Follow these articles *P. polycaryum* cells have a dimension of 100-115 x 45-50 μm. One round Ma has a size 27-30 x 20-22 μm. The number of small Mi varied from 0 to 8, but 3-4 Mi were typically present more often. The BO was located anteriorly from the cell's equator. The endoral membrane was represented by 14 granules. It should be indicated that the peniculi of *P. polycaryum* usually possess more than 4 rows of kinetosomes each (Opton 1942, Yusa 1957). Our data confirm such a peculiarity which is unique for the "*woodruffi*" group.

The conjugation and autogamy of the species were investigated by Diller (1954, 1958) as well. He found that *P. polycaryum* has 2 mating types (syngen 1) and forms 4 macronuclear anlagen during its conjugation process. The old Ma fragmentation starts after III synkaryon division. Autogamy is a usual type of nuclear reorganization for the species.

Morphogenesis of P. *polycaryum* was studied by Beran (1990). He used only one stock for the study. Some morphological traits of this culture were quite different from other data on *P. polycaryum* (Table 5). These cells had a size of 120-130 x 60 μm (90 cilia rows) with a round Ma (25 μm) and a number of Mi (1-8). The nuclei were compact and had a dimension of 3-8(!) μm. Each of the CV in the cell had several PCV. The number of kinetosomes in the endoral membrane was 28 (!). Unfortunately, we have not had the opportunity to check this culture. Probably, the author worked with some other *Paramecium* (*P. nephridiatum*?).

The morphometric analysis using 13 cell attributes was sufficient for the MDS procedure that distinguished taxonomic species of the "*woodruffi*" group of *Paramecium* very confidently. However, morphometrical data are not enough for any phylogenetic speculation. For such an attempt it is necessary to have some more comparable information on the biological features. With this aim, we have used the data summarized in Table 5 together with other literature and our own materials.

We assumed the following traits as more important for phylogenetic implication on the genus level: system of mating types; existence of autogamy; type of the Ma reorganization during conjugation (time of the old Ma fragmentation, the number of the new Ma anlagen); the morphology of the CV; the morphology and the number of the Mi; the peculiarities of morphogenesis.

It is also useful to make a comparison between endocytobiotic bacterial flora of the paramecia (Diller1948; Fokin 1988, 1989a-d; Fokin and Görtz 1993; Fokin and Sabaneyeva 1993; Fokin *et al.* 1993).

If we try to range the investigated species of the "*woodruffi*" group using these traits we can build up some speculative "tree" of the group. In accordance with such procedure *P. duboscqui* looks more primitive than *P. nephridiatum* and *P. woodruffi*. *P. calkinsi* and *P. polycaryum*, on the contrary, have a "higher" organization than the first 3 species. However, *P. polycaryum* does not feet to the "*woodruffi*" group according to some of its morphological and biological features. Of course, the real phylogenetic relationships must be analyzed by a sequence of the rRNA genes.

Acknowledgments. We are grateful to Drs D. Molloy and T. Kosco for critical comments on the manuscript and to Dr M. Fujishima for his help with the literature.

REFERENCES

Agamaliev F. G. (1983) Ciliates of the Caspian Sea. Systematic, Ecology and Zoogeography."Nauka", Leningrad (in Russian)

Ammermann D. (1966) Das Paarungssystem der Ciliaten *Paramecium woodruffi* and *Paramecium trichium*. *Arch. Protistenkd.* **109:** 139-146

Baily N. (1970) Mathematics in Biology and Medical Sciences. Russian ed. "Mir", Moscow (in Russian)

Beran A. (1990) Mikromorphologische Untersuchungen zur Morphogenese der peniculinen Ciliaten *Frontonia atra* und *Paramecium polycaryum*. Dissertation, Tübingen

Borror A. (1972) Tidal marsh ciliates (Protozoa): morphology, ecology, systematics. *Acta Protozool.* **10:** 29-72

Braverman E. M. (1965) On potential function procedure. *Autom.Telemech.* **21:** 21-26

Bullington W.E. (1925) A study of spiral movement in the ciliate Infusoria. *Arch. Protistenkd.* **50:** 219-274

Carey P. G. (1992) Marine Interstitial Ciliates. An Illustrated Key. Chapman and Hall, London, New York, Madras

Chatton E., Brachon S. (1933) Sur une Paramecie a deux races: *Paramecium duboscqui*, n. sp. *C. R. Soc. Biol.* **114:** 988-989

Clarke K. R. (1993) Non-parametrical multivariate analysis of changes in community structure. *Aust. J. Ecol.* **18:** 1-33

Clarke K. R., Green R.H. (1988) Statistical design and analysis for a biological effects study. *Mar. Ecol. Progr.* **46:** 213-226

Corliss J. O. (1953) Silver impregnation of ciliate protozoa by the Chatton-Lwoff technique. *Stain Technol.* **28:** 97-100

Diller W. F. (1948) Induction of autogamy in single animals of *Paramecium calkinsi* following mixture of two mating types. *Biol. Bull.* **95:** 265

Diller W. F. (1954) Autogamy in *Paramecium polycaryum*. *J. Protozool.***1:** 60-70

Diller W. F. (1958) Studies on conjugation in *P. polycaryum*. *J. Protozool.* **5:** 282-292

Dragesco J., Dragesco-Kerneis A. (1986) Ciliés Libres de l'Afrique Intertropicale. Paris

Fokin S. I. (1986) Morphology of contractile vacuoles in ciliated Protozoa of the genus *Paramecium* (Hymenostomatida, Peniculina) as a species-specific character. *Zool. Zh. (Moscow)* **65:** 5-15 (in Russian with English summary)

Fokin S. I. (1987) Direction of the spiral rotation in paramecia as a species-specific character. In: Current Problems of Protozoology. (Ed. T.V. Beyer) Leningrad, Nauka, 17 (in Russian with English title)

Fokin S. I. (1988) A bacterial symbiont of the macronucleus perinuclear space in the ciliate *Paramecium duboscqui*. *Cytologia (Sankt Petersburg)* **30:** 632-635 (in Russian with English summary)

Fokin S. I. (1989a) Bacterial endobionts of the ciliate *Paramecium woodruffi*. I. Endobionts of the macronucleus. *Cytologia (Sankt-Petersburg)* **31:** 829-844 (in Russian with English summary)

Fokin S. I. (1989b) Bacterial endobionts of the ciliate *Paramecium woodruffi*. II. Endobionts of the perinuclear space. *Cytologia (Sankt-Petersburg)* **31:** 845-850 (in Russian with English summary)

Fokin S. I. (1989c) Bacterial endobionts of the ciliate *Paramecium woodruffi*. III. Endobionts of the cytoplasm. *Cytologia (Sankt-Petersburg)* 31: 964-970. (in Russian with English summary)

Fokin S. I. (1989d) Mate-killer effect of the endobionts of *Paramecium woodruffi*. *Cytologia (Sankt-Petersburg)* **31:** 1085-1088 (in Russian with English summary)

Fokin S. I. (1997) Morphological diversity of the micronuclei in *Paramecium*. *Arch. Protistenkd.* **148:** 375-387

Fokin S. I. (1998) Strategies of the macronuclear endocytobionts of *Paramecium* during the sexual process of the host. *Symbiosis* **25:** 323-342

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

Fokin S. I., Sabaneyeva E.V. (1993) Bacterial endobionts of the ciliate *Paramecium calkinsi*. *Europ. J. Protistol.* **29:** 390-395.

Fokin S. I., Skovorodkin I. N., Sabaneyeva E.V. (1993) Specificity of Holospora-bacterial endonucleobionts of paramecia (Ciliophora).Abstr.IX Int.Congr.Protozool. Berlin, 41

Fokin S. I., Chlebovich V. V., Smurov A. O. (1995) Euryhaline paramecia of the White Sea as a objects for the investigation of cell salinity adaptations. In: Problems of Using and Protection the Natural Resources of the White Sea (Ed. A.F. Alimov). St. Petersburg, 105-106 (in Russian)

Fokin S. I., Stoeck T., Schmidt H. (0000) Rediscovery of *Paramecium nephridiatum* Gelei, 1925 and its characteristics. *J. Euk. Microbiol.* (in press)

Gates M. A., Powelson E. E., Berger J. (1974) Syngenic ascertainment in *Paramecium aurelia*. *Syst. Zool.* **23:** 482-489

Gelei J. (1925) Uj *Paramecium* szeged kornyekerol. *Paramecium nephridiatum* nov.sp. *Allattani Kozl.* **22:** 121-162 (in Hungarian with German summary)

Gelei J. (1938) Beitrage zur Ciliatenfauna der Umgebung von Szeged. VII. *Paramecium nephridiatum*. *Arch. Protistenkd.* **91:** 343-356

Hayashi S., Takayanagi T. (1961) Cytological and cytogenetical studies on *Paramecium*. I. Variation in number of micronuclei. *Ann. Zool. Japan.* **34:** 86-92

Hayashi S., Takayanagi T. (1962) Cytological and cytogenetical studies on *Paramecium polycaryum*. IV. Determination of the mating system based on some experimental and cytological observation. *Jap. J. Zool.* **13:** 357-364 (in Japanese with English summary)

Hayashi S., Takayanagi T. (1964) Cytological and cytogenetical studies on *Paramecium polycaryum*. V. Lethal Interaction in certain stocks. *J. Protozool.* **11:** 128-132

Jankowski A.V. (1961) Conjugation process in a *Paramecium* of infrequent occurrence: *Paramecium woodruffi*. *Dokl. Acad. Sci. USSR* **137:** 989-992 (in Russian)

Jankowski A.V. (1969) A proposal taxonomy of the genus *Paramecium* Hill 1752 (Ciliophora). *Zool. Zh. (Moscow)* **48:** 30-40 (in Russian with English summary)

Jankowski A.V. (1972) Cytogenetic of *Paramecium putrinum* C. et L., 1858. *Acta Protozool.* **10:** 285-394

Kahl K. (1931) Urtier oder Protozoa I: Wimpertiere oder Ciliata (Infusoria), Familie Parameciidae. In: Die Tierwelt Deutschlands und der Angrenzenden Meeresteile (Ed. F. Dahl). Fischer, Jena 289-295

Kahl K. (1934) Ciliate Libera et Ectocomensalia. In: Der Tierwelt der Nord- und Ostsee, (Eds. G.Grimpe and E.Wagler) Akademische Verlagsgesellschaft, Leipzig, 76

Kalmus H. (1931) *Paramecium*, das Pantoffelttierchen, Fischer, Jena

Kościuszko H. (1986) New habitats of *Paramecium woodruffi*- a rare species of the genus *Paramecium*. *Folia Biol.* **34:** 381-386

Kruskal J. B., Wish M. (1978) Multidimensional Scaling. Sage Populations. Bevery Hills, California

Lieberman P. R. (1929) Ciliary arrangement in different species of *Paramecium*. *Trans. Amer. Microscop. Soc.* **58:** 1-7

Nakida A. (1956) Micronuclear behavior during the conjugation of *Paramecium calkinsi*. *Zool. Mag.* **31:** 306-310 (in Japanese with English summary)

Nakida A. (1958) Mating types in *Paramecium calkinsi*. *Zool. Mag.* **33:** 210-213 (in Japanese with English summary)

Opton E. M. (1942) Demonstration of the cytosomic morphology of *Paramecium polycaryum*. *Anat. Rec.* **84:** 485

Powelson E. E., Gates M. A., Berger J. (1975) A biometrical analysis of 22 stocks of four syngens of *Paramecium aurelia*. *Can. J. Zool.* **53:** 19-32

Shi X., Jin M., Liu G. (1997) Rediscovery of *Paramecium duboscqui* Chatton & Brachon, 1933, and a description of its characteristics. *J. Euk. Microbiol.* **44:** 134-141

Skovorodkin I. N. (1990) A device for immobilization of biological objects in the light microscope studies. *Cytologia (Sankt-Petersburg)* **32:** 515-519 (in Russian with English summary)

Sonneborn T. M. (1970) Methods in *Paramecium* research. *Meth. Cell Physiol.* **4:** 241-339

Takayanagi T. (1960) Cytological and cytogenetical studies on *Paramecium polycaryum*. II. Cytological responses of paramecia to mitomycin-C, azan, MEPA and podophyllin. *J. Fac. Sci. Hokkaido Univ. Ser.VI, Zool.* **14:** 453-462

Unger W. B. (1926) The relationship of rhythms to nutrition and excretion in *Paramecium*. *J. Exp. Zool.* **43:** 373-412.

Vivier E. (1974) Morphology, Taxonomy and General Biology of the Genus *Paramecium*. In: *Paramecium*. A Current Survey (Ed. W. J.Van Wagtendonk). Elsevier Sci. Publ. Comp., Amsterdam, London, New York 1-90

Watanabe T., Shi X., Liu G., Jin M. (1996) Cytological study of conjugation and nuclear process in *Paramecium duboscqui* Chatton & Brachon 1933. *Europ. J. Protistol.* **32 (Supp l):** 175-183

Wenrich D. H. (1928a) *Paramecium woodruffi* n.sp. *Trans. Amer. Microscop. Soc.* **47:** 256-261

Wenrich D. H. (1928b) Eight well-defined species of *Paramecium*. *Trans. Am. Microsc. Soc.* **47:** 274-282

Wenrich D. H., Wang C. C. (1928) The occurrence of conjugation in *Paramecium calkinsi*. *Science* **67:** 270-271

Wichterman R. (1948) Mating types and conjugation of four different races of *Paramecium calkinsi* and the effect of X-rays on the mating reaction. *Biol. Bull.* **95:** 271-272

Wichterman R. (1950) The biology of *Paramecium calkinsi* with special reference to ecology, cultivation, structural characteristics and mating-type phenomena. *Proc. Am. Soc. Protozool.* **1:** 11-12

Wichterman R. (1951) The ecology, cultivation, structural characteristics and mating types of *Paramecium calkinsi*. *Proc. Pa. Acad. Sci.* **25:** 51-65

Wichterman R. (1953) The Biology of *Paramecium*. Blakiston, New York, Toronto

Wichterman R. (1986) The Biology of *Paramecium* 2-nd ed. New York, London

Woodruff L. L. (1921) The structure, life history and intrageneric relationships of *Paramecium calkinsi* n.sp. *Biol. Bull.* **41:** 171-180

Woodruff L. L., Spencer H. (1923) *Paramecium polycaryum* sp. nov. *Proc. Soc. Exp. Biol. Med.* **220:** 338-339

Yusa A. (1957) The morphology and morphogenesis of the buccal organelles in *Paramecium* with particular reference to their systematic significance. *J. Protozool.* **4:** 128-142

Zawoiski E. (1951) A statistical analysis of the size of different clones of *Paramecium calkinsi* and the fate of exconjugants. *Pen. Acad. Sci.* **25:** 66-71

Received on 3rd October, 1998; accepted on 26th January, 1999

Acta Protozool. (1999) 38: 119 - 131

ACTA
PROTOZOOLOGICA

Taxonomic Revision of Scale-bearing Heliozoon-like Amoebae (Pompholyxophryidae, Rotosphaerida)

Kirill A. MIKRJUKOV

Department of Zoology and Comparative Anatomy of Invertebrates of Moscow State University, Moscow, Russia

Summary. A taxonomic revision of heliozoon-like filose amoebae assigned to the Rotosphaerida is provided. The presence of spicules is a facultative feature in some species. Twelve species with plate-scales ornamented by several holes are placed in *Pinaciophora* Greeff, 1869. Three species with a single central hole in plate-scales are retained in *Rabdiophrys* Rainer, 1968. Four species lacking apical holes at plate-scales are transferred to a new genus *Rabdiaster* with *R. pertzovi* (Mikrjukov, 1994) as the type-species. Minute hexagonal perforations in tangential periplast elements and the eccentric position of the nucleus is considered as main distinguishing features of the Pompholyxophryidae including genera *Pinaciophora, Rabdiophrys, Rabdiaster* and *Pompholyxophrys* Archer, 1869. The morphology of the plate-scales is used as a taxonomic character of the generic rank. *Lithocolla* Schulze, 1874 and *Belonocystis* Rainer, 1968 are treated as taxa of uncertain position. *Elaeorhanis* Greeff, 1873 is transferred to Nucleariidae; the new marine species *E. tauryanini* with centric diatoms in the cell envelope is described. The terms Cristidiscoidida Page, 1987 and Cristivesiculida Page, 1987 are regarded as junior synonyms of Rotosphaerida Rainer, 1968 and Aconchulinida De Saedeleer, 1934 respectively; the use of the formers is incorrect. The family Nucleariidae Cann et Page is transferred to the order Rotosphaerida uniting now all disciriscitate filose amoebae. A key to 27 pompholyxophryid species is adduced.

Key words: amoebae, *Elaeorhanis tauryanini* sp. n., heliozoa, Nucleariidae, *Pinaciophora,* Pompholyxophryidae, *Rabdiaster* gen. n., *Rabdiophrys,* Rotosphaerida, taxonomy.

INTRODUCTION

History of the question

The order Rotosphaerida was defined by Rainer (1968) as a small group of heliozoon-like amoebae, which he considered as heliozoa. Some familiar species, *Pinaciophora fluviatilis* Greeff, 1869 (Fig. 1 A) and *Pompholyxophrys punicea* Archer, 1869 [syn. *Hyalolampe fenestrata* Greeff, 1869] (Figs. 1 C, 2 D, E) are some of the first organisms described as heliozoa. The eccentric position of the nucleus in spherical cells, together with the presence of an external cell envelope comprised of siliceous elements, led earlier authors to regard these protists as close to or members of the centrohelid heliozoa (Schaudinn 1896, Hartmann 1913, Poche 1913, Tregouboff 1953, Kudo 1954) in spite of the absence of characteristic centrohelidean features such as the centroplast, axonemes in pseudopods (which thus are filopods) and extrusomes. Rainer (1968) pointing out these differences and separated such organisms in another order - the Rotosphaerida at the same rank with two main heliozoan taxa - Centrohelida Kühn, 1926 and

Address for correspondence: Kirill A. Mikrjukov, Department of Zoology and Comparative Anatomy of Invertebrates, Moscow State University, Lenin Hills, Moscow 119899, Russia; E-mail: mikr@1.inv.bio.msu.ru

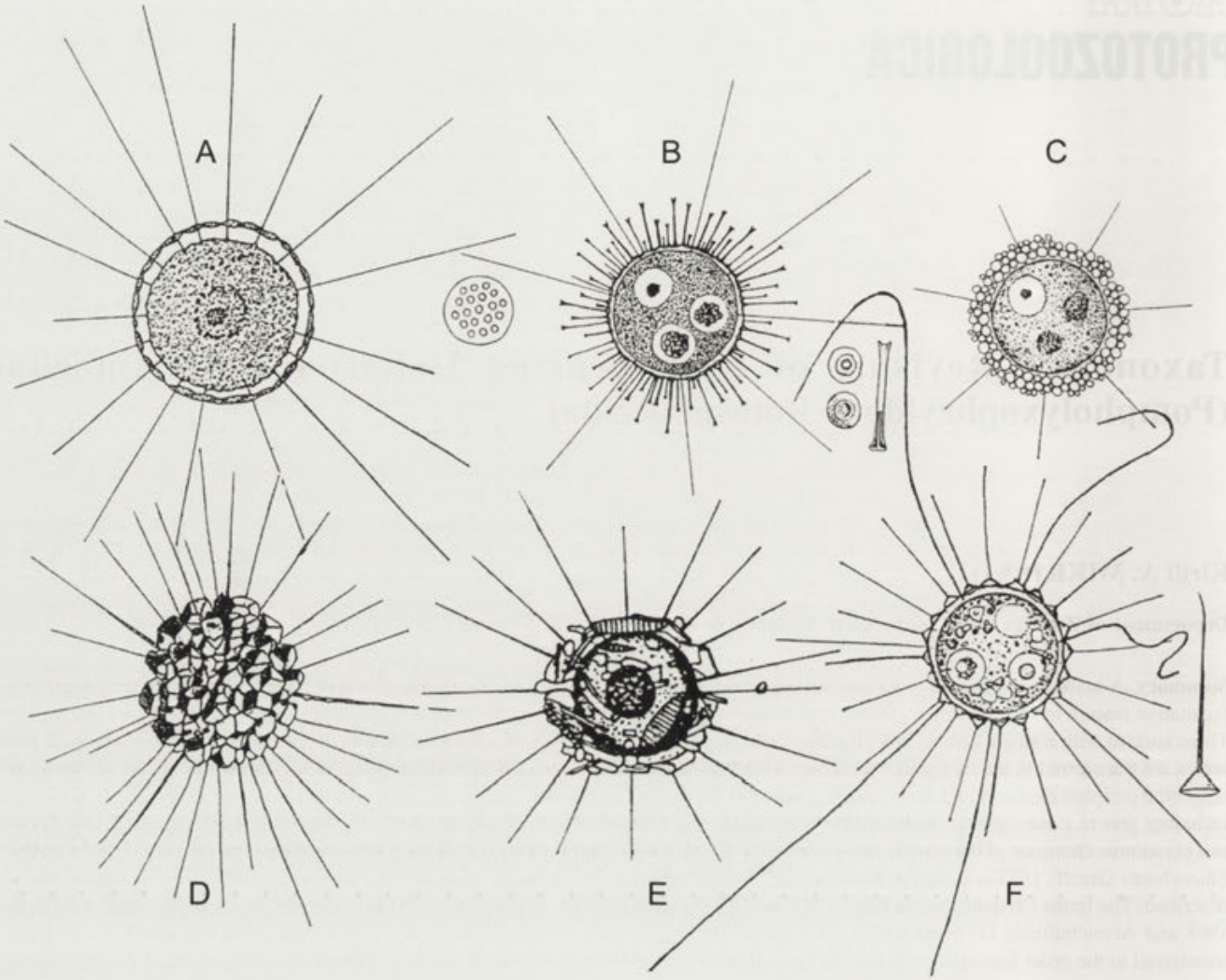


Fig. 1. Line drawings of type-species of rotosphaerid genera. A - *Pinaciophora fluviatilis* (after Greeff 1869); B - *Rabdiophrys anulifera* (after Rainer 1968); C - *Pompholyxophrys punicea* (after Siemensma 1991); D - *Lithocolla globosa* (after Schulze 1874); E - *Elaeorhanis cincta* (after Greeff 1873); F - *Belonocystis tubistella* (after Rainer 1968)

Actinophryida Hartmann, 1913 (Rainer regarded Desmothoracida Hertwig et Lesser, 1874 as "pseudoheliozoa"). Now a polyphyletic nature of heliozoa and sarcodines as a whole is clearly shown (Schulman and Reshetnyak 1980, Patterson 1994, Mikrjukov 1998), and hence all the heliozoan taxa are considered separately in recent systems of protists (e.g. Lee *et al.*, in press).

Initially, the order Rotosphaerida was composed of five genera; they were *Pinaciophora* Greeff, 1869, *Pompholyxophrys* Archer, 1869, *Lithocolla* Schulze, 1874, *Pinaciocystis* Roskin, 1929 and *Rabdiophrys* Rainer, 1969. *Lithocolla* (Fig. 1 D) differed sharply by the apparently exogenous nature of the siliceous elements composing its cell envelope. Four other genera have siliceous periplast elements which seem to be produced by the cell (e. g. are endogenous); they are spherical in *Pompholyxophrys* (Fig. 1 C), tangential plates in *Pinaciophora* [a poorly described genus *Pinaciocystis* was considered as a synonym of *Pinaciophora* (Fig. 1 A)], and tangential plates and radial spicules in *Rabdiophrys* (Fig. 1 B).

Patterson (1985) performed a study of the ultrastructure of *Pompholyxophrys punicea.* This study confirmed the absence of axonemes and extrusomes, and hence the rotosphaerids were definitively shown cannot be regarded as heliozoa but to be a group of filose amoebae. The

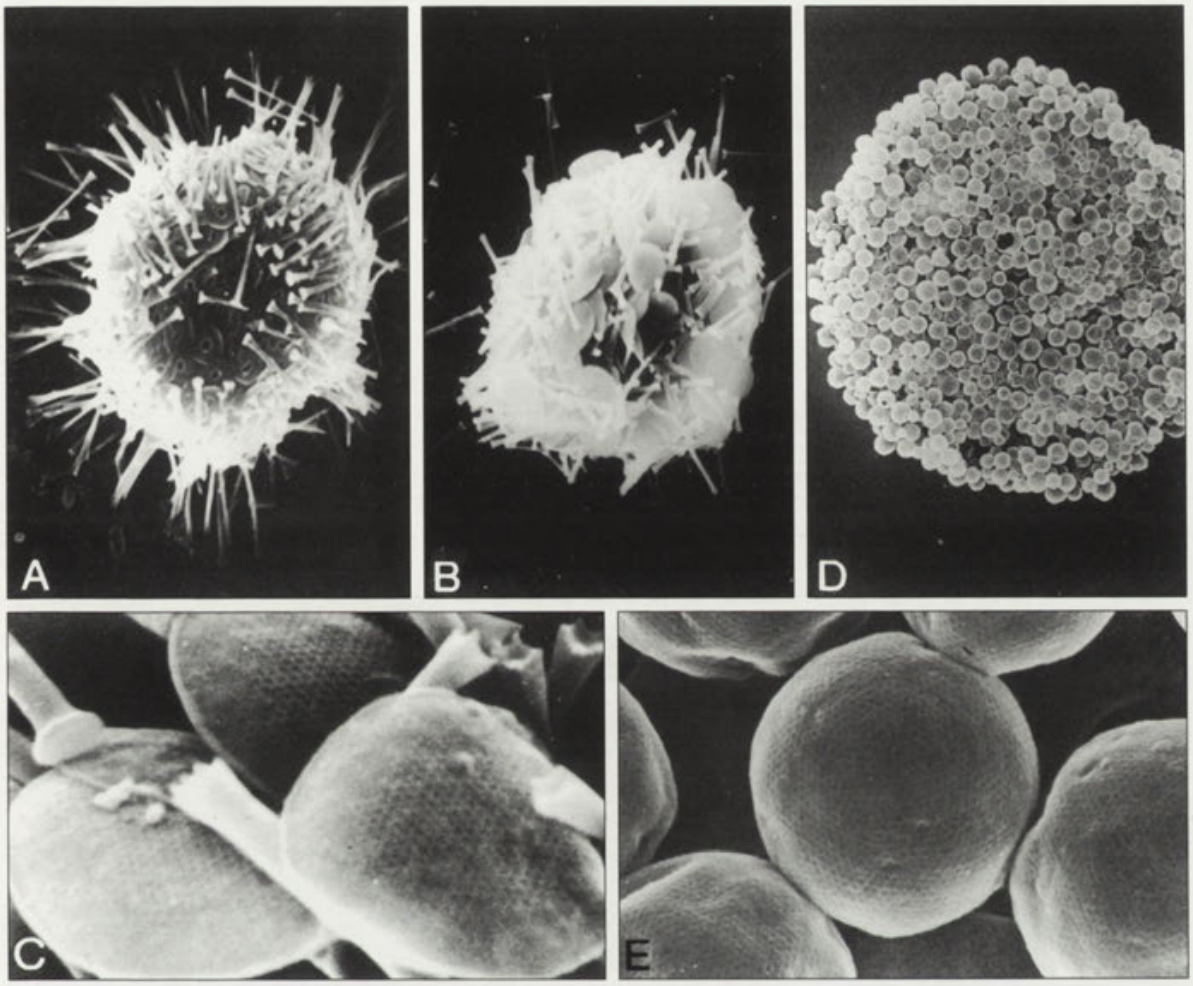


Fig. 2. SEM micrographs of type-species of the genera *Rabdiophrys*, *Rabdiaster* and *Pompholyxophrys*. A - *Rh. anulifera*, x 2000; B, C - *R. pertzovi* and its plate-scales, x 2000 and x 15000; D, E - *P. punicea* and its scales, x 2000 and x 15000. Ornamentation of scales by minute hexagonal pores is well seen in Figs. C and E

discoidal shape of cristae in mitochondria was shown to be identical to that in another filopodiate group of amoebae - the Nucleariidae Cann et Page, 1979, the fine structure of which was well described by Mignot and Savoie (1979), Cann and Page (1979), Patterson (1983), Cann (1986) and Patterson *et al.* (1987). In both groups, the massive discoidal parts of cristae are connected with the inner membrane of mitochondria by a well developed short narrow stalk something similar to that in other discriscitate protistan taxa (see: Page 1985, Patterson and Brugerolle 1988, Triemer and Farmer 1991). Leaning upon the Patterson's (1985) work, Page (1987, 1991) developed a classification for filopodial amoebae. He raised the rank of naked filose amoebae - the order Aconchulinida De Saedeleer, 1934 as in Deflandre (1953), Levine *et al.* (1980), Krylov *et al.* (1980), to subclass, which included two new orders: Cristivesiculatida (uniting tubulocristate families Vampyrellidae Zopf, 1885 and Arachnulidae Page, 1987) and Cristidiscoidida (uniting discriscitate families Nucleariidae Cann et Page, 1979 and Pompholyxophryidae Page, 1987). The rank of the taxon uniting taxa with scales etc. (the rotosphaerids) was reduced to that of the family Pompholyxophryidae Page, 1987 and the taxonomic name Rotosphaerida Rainer was ignored. The Vampyrellid filose amoebae [e. g. *Vampyrella* studied by Hausmann (1977) and *Lateromyxa* studied by Hülsmann (1993)] have mitochondria with tubular cristae; they have elaborate ribosomal arrays, often associated with digestive vacuoles; they have a peculiar style of perforating the algae; they also produce digestion and

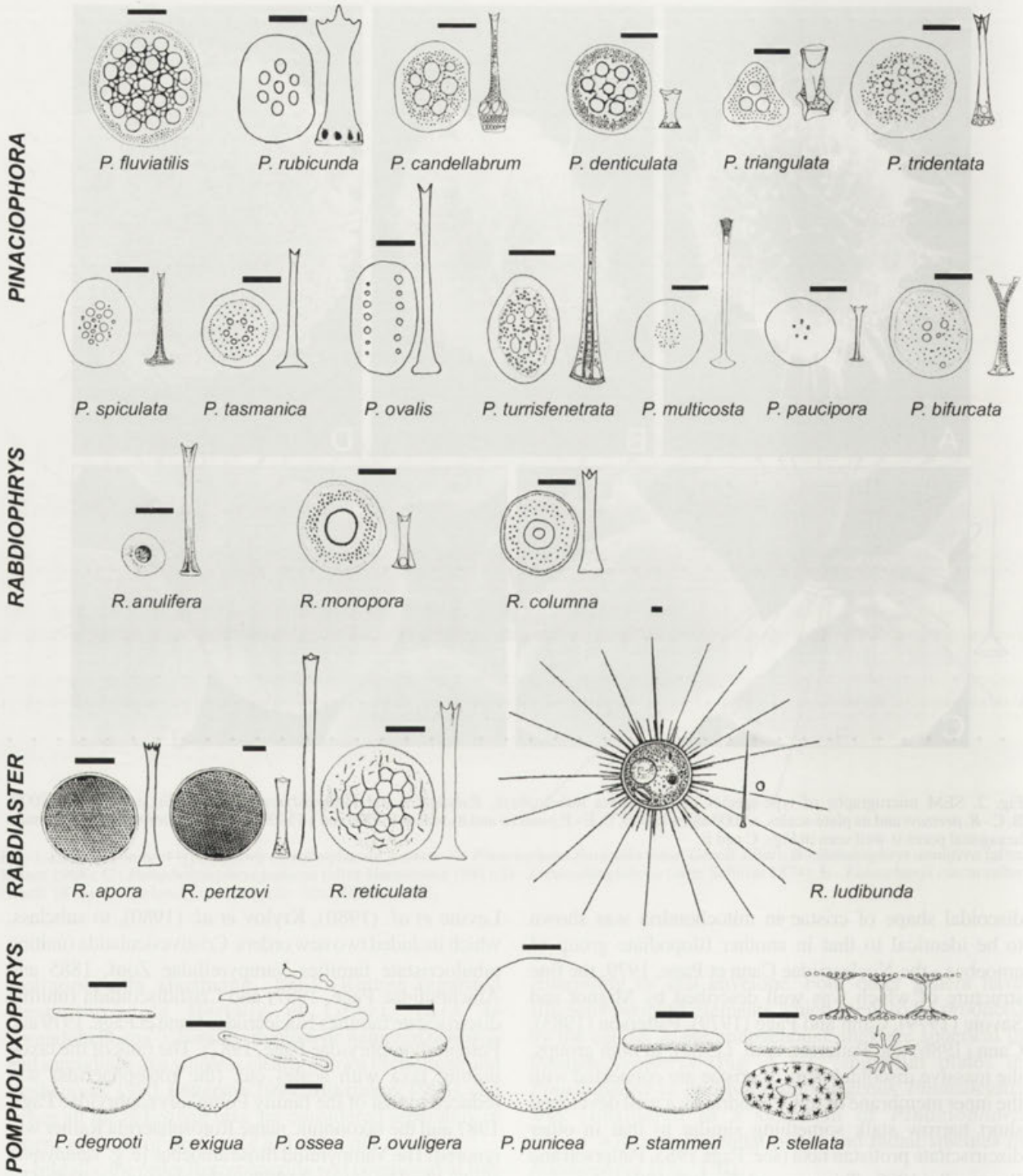


Fig. 3. Schematic line drawings of periplast elements (plate-scales and spicules) of 27 pompholyxophryid species belonging to genera *Pinaciophora*, *Rabdiophrys*, *Rabdiaster* and *Pompholyxophrys* (after Penard 1901; Thomsen 1978; 1979; Croome 1987 a, c; Roijackers and Siemensma 1988; Wujek and O'Kelly 1991; Siemensma 1991; Mikrjukov 1994). Scale bar - 1μm

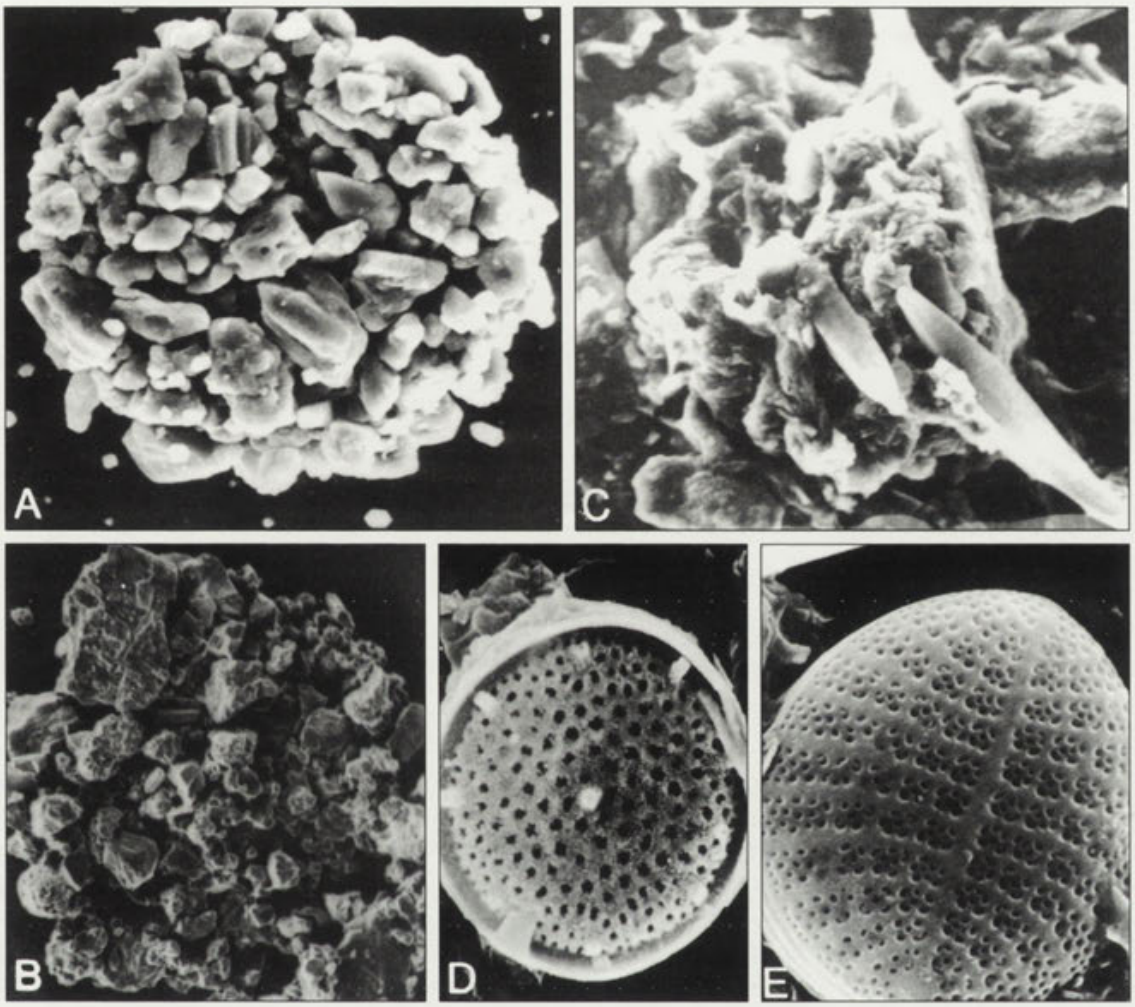


Fig. 4. SEM micrographs of species of *Lithocolla* and *Elaeorhanis*, x 2000. A - freshwater (*Crimea mountains*); B - marine (Tasman Sea) *L. globosa*; C - *E. cincta* (sparse sand grains and pennatophycean diatom shells are seen in the clump of mucus), x 2000; D - centrophycean (*Thalassiosira sp.*); E - pennatophycean (*Cocconeis scutellum* var. *parva*) marine diatoms composing the envelope of *E. tauryanini* sp. n., x 12000

secondary (reproductive) cysts and have an autogamic sexual process in the life cycle (Röpstorf and Hausmann 1992, Röpstorf *et al.* 1993). A giant multinucleate tubulocristate amoeba *Arachnula* studied by Old and Darbyshire (1980) has a commonest shape of a series of delicate threads interspersed between small swellings and forms a large irregular reticulum of several mm; it also produces digestive and secondary cysts. Thus neither vampyrellids nor arachnulids share any common features in the cell structure with disciriscitate filose amoebae.

The pompholyxophryids are little studied, partly because of difficulties in maintaining these protists in cultures. Pompholyxophryids are rare in natural biotopes (e.g. in comparison with the centrohelids). They are recorded with equal frequency in benthic and planktonic samples, they float or roll. Their feeding preferences and food capture mechanisms are unknown; Patterson (1985) reports diatoms in food vacuoles of *P. punicea*. Members of *Pinaciophora* and *Rabdiophrys* are euryhaline whereas the species of *Pompholyxophrys* are exclusively freshwater. The only brackishwater record is of *P. ovuligera* in the Alabama Bay by Jones (1974).

The species and generic taxonomy of pompholyxophryids is now largely based on the siliceous scales and spicules making up the periplast (Roijackers 1988), a contemporary understanding of their biogeography can only be developed from ultrastructural studies. However records supported by ultrastructural observations show

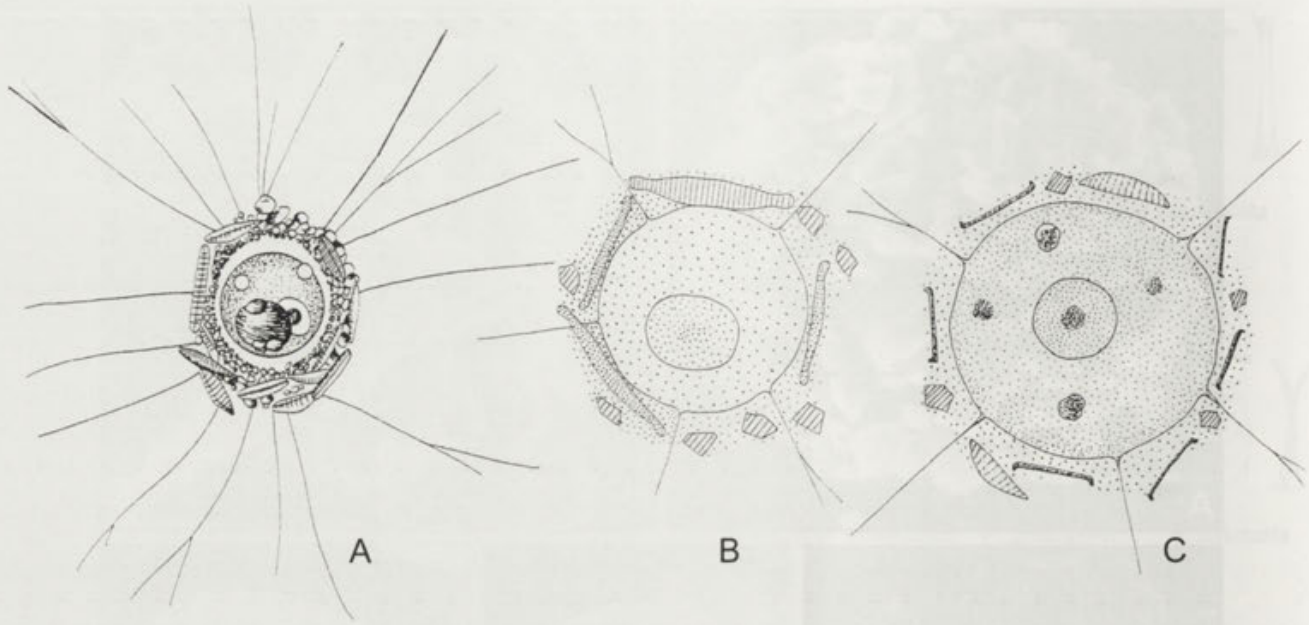


Fig. 5. Line drawings of species of *Elaeorhanis*. A, B - *E. cincta* (after Rainer 1968 and Croome and Mikrjukov 1999); C - *E. tauryanini* sp. n.

their cosmopolitan distribution in the world (Takahashi 1959, 1981; Gaarder *et al.* 1976; Thomsen 1978, 1979; Belcher and Swale 1978; Manton and Sutherland 1979; Nicholls 1983; Wee and Millie 1983; Nicholls and Dürrschmidt 1985; Manton 1986; Croome 1987 a, b, c; Croome *et al.* 1987; Roijackers and Siemensma 1988; Wujek and O'Kelly 1991; Vörs 1992 a, b, 1993; Mikrjukov 1993, 1994; Mikrjukov and Patterson, in press).

Present status

The most recent revision of "cristidiscoidid amoebae" by Roijackers and Siemensma (1988) considers four pompholyxophryid genera: *Lithocolla, Pinaciophora*, *Rabdiophrys* and *Pompholyxophrys*. The single species of *Pinaciocystis* - *P. dubocqi* Roskin, 1929 - is regarded as a junior synonym of *Pinaciophora fluviatilis*. Roijackers and Siemensma (1988) pointed out the absence of mention of the radial spicules in the diagnosis of *Pinaciophora fluviatilis*, whereas all new species of this genus described by Thomsen (1978, 1979), Manton and Sutherland (1979) and Croome (1987 a) are enclosed by periplasts comprised of both tangential plate-scales and radial spicules. However, the type-species of *Rabdiophrys* - *R. anulifera* Rainer, 1968 (Fig. 1 B) also bears radial spicules, and hence Roijackers and Siemensma (1988) transferred all spicule-bearing *Pinaciophora* species to the genus *Rabdiophrys*. Thus the diagnosis of *Pinaciophora* was restricted as "pompholyxophryids with tangential plate-scales only", whilst the *Rabdiophrys* began to include "pompholyxophryids with periplasts composed of both plate-scales and radial spicules". The latter genus included 15 species whilst only two were placed in *Pinaciophora*. Later three species of *Rabdiophrys* were added by Croome (1987 c), Wujek and O'Kelly (1991) and Mikrjukov (1994). However the general structure of tangential plate-scales is almost identical in both genera discussed above which was pointed out by all these authors: they are composed of two (apical and basal) siliceous plates connected by clumps of electron-dense material; the apical plates in the majority of species are ornamented by one or several large rounded holes forming a species-specific appearance of a scale.

The diagnosis of the genus *Pompholyxophrys* was broadened so that it included not only species with spheroid or ovate periplast elements, but also those with discoidal or bone-shaped endogenous siliceous elements of a single type within a species (but excluding plate-scales). The number of species in the genus increased to seven (Siemensma 1991).

The tangential endogenous periplast elements of pompholyxophryids have attracted attention of specialists: it is a texture formed by minute hexagonal perforations (Figs. 2 C, E) of a whole scale surface in *Pompholyxophrys* (Patterson, 1985) or of the apical plate of plate-scales in *Pinaciophora* and *Rabdiophrys* (Roijackers and Siemensma, 1988).

DISCUSSION OF MICROTAXA

We do not agree with Roijackers and Siemensma (1988) and Siemensma (1991) on the taxonomy of two main pompholyxophryid taxa being the genera *Pinaciophora* and *Rabdiophrys* because the latter is very heterogeneous. We recommend that it be separated into four well-defined groups of species (Fig. 3):

(**i**) most members of *Rabdiophrys* have plate-scales perforated by several (usually up to 10) holes which are grouped mostly in the central region of the scale similar to those in scales of *Pinaciophora fluviatilis* (Fig. 3). The scales of *P. rubicunda* (Hertwig et Lesser, 1874) Roijackers et Siemensma, 1988 and those of *R. thomseni* Roijackers et Siemensma, 1988 are almost identical, and these species differ only by the presence of spicules in the latter. Moreover, Thomsen (1978) points out some species now considered as *Rabdiophrys* might have 2-3 spicules only, and hence the presence of spicules could be a facultative feature. Thus there is no clear border between *Pinaciophora* and *Rabdiophrys*. Given the heterogeneity of the genus *Rabdiophrys* we propose to return those species with plate-scales with numerous apical holes (like those in *Pinaciophora*) and (facultative in some species) presence of spicules in periplasts to the genus *Pinaciophora* from which they were taken off by Roijackers and Siemensma (1988),

(**ii**) the type-species of the genus *Rabdiophrys* - *R. anulifera* Rainer, 1968 (Figs. 1 B, 2 A, 3) has plate-scales differing from those in *Pinaciophora* and the most of other species of *Rabdiophrys*. Apical plates of its plate-scales have a single large central hole, which occupies most of the plate surface (this character can be seen by the light microscopy). Two related species [*R. monopora* (Thomsen, 1978) Roijackers et Siemensma, 1988 and *R. columna* (Croome, 1987) Roijackers et Siemensma, 1988] have a similar structure of plate-scalles. We propose to retain these three species in the genus *Rabdiophrys*,

(**iii**) two further species of *Rabdiophrys* lack any holes in the apical plate of plate-scales (excluding minute hexagonal perforations characteristic of all pompholyxophryids). These are: *R. apora* (Croome, 1987) Roijackers et Siemensma, 1988 and *R. pertzovi* Mikrjukov, 1994 (Figs. 2 B, C; 3). *R. ludibunda* (Penard, 1901) Rainer, 1968 (Fig. 3) and *R. rubella* (Penard, 1904) Rainer, 1968 are also devoid of apical holes at plate-scales but have not been studied by electron microscopy,

(**iv**) finally, the plate-scales of *R. reticulata* (Thomsen, 1979) Roijackers et Siemensma, 1988 lack the apical plate. The characteristic reticulate appearance of its scales (when seen with TEM) is replaced by clumps of the electron-dense intercalary material (Fig. 3). We propose to consider both types of species [(**iii**) and (**iv**)] as members of a new pompholyxophryid genus *Rabdiaster* gen. n.

Three other genera are sometimes regarded as pompholyxophryids or rotosphaerids (see: Rainer 1968, Siemensma 1991). They are *Lithocolla* Schulze, 1874, *Elaeorhanis* Greeff, 1873, and *Belonocystis* Rainer, 1968. The fine structure of members of these genera has not yet been studied by electron microscopy, and their firm placement will require new data.

A single species of *Lithocolla* - *L. globosa* Schulze, 1874 (Figs. 1 D; 4 A, B) often occurs in freshwater and marine habitats (Mikrjukov 1994, in press; Mikrjukov and Patterson, in press). It cannot be classified as a pompholyxophryid because of (**i**) the central position of the nucleus, (**ii**) the cell envelope is a dense coat of sand grains [e. g. the exogenous siliceous material (Figs. 4 A, B)] and (**iii**) a characteristic mode of life [this protist moves along the substrate by the extension/withdrawing of its stiff, rod-like filopods (whilst the "pure" pompholyxophryids are more often found floating in interstitial space between particles of a detritus as heliozoa do)]. Poche (1913) considered *Lithocolla* as a member of a separate family Lithocollidae Poche, 1913. The dense sand coat of *Lithocolla* makes a study of its ultrastructure to be very hard, and hence the position of the family Lithocollidae remains *incertae sedis* among protists (as indicated in Patterson 1994).

De Groot (1979) erroneously grouped all testate protists enclosed by an agglutinated shell with two apertures and with two bundles of filopods in *Elaeorhanis cincta* Greeff, 1873, a single species of the genus *Elaeorhanis* Greeff, 1873. This mistake caused some wrong phylogenetic reconstructions considering *Elaeorhanis* to be related to *Diplophrys* Archer (see Patterson 1992) and hence - to labyrinthulids (Dykstra and Portyer 1984) and stramenopiles as a whole (Patterson 1994). However, in the original description, Greeff (1873) points out that *E. cincta* (Fig. 1 E) has a rounded body with filopods radiating in all directions and not gathered in two bundles; it is surrounded by a uniform envelope without any apertures and composed of sand grains and diatom frustules. Further light microscopical observations (Frenzel 1897, Penard 1905, Rainer 1968, Mikrjukov and Croome 1998) have confirmed Greeff's observations (Figs. 5 A, B) and show *E. cincta* to be a cosmopolitan freshwater protist with fine, sometimes branching filopods, a central or slightly excentric position of the nucleus, and a lot of mucus in the envelope which includes sparse sand grains

and pennate diatom shells. Thus the relationships of this protist to *Lithocolla* is not likely. In our recent account (Croome and Mikrjukov 1999) based on light-microscopy and scanning electron microscopy (Fig. 4 C) observations, we consider *E. cincta* as a member of another family of disciriscitate filose amoebae - the Nucleariidae according to the common appearance of these organisms [branching fine filopods, the central nucleus, the cell envelope including a lot of mucus (some species of *Nuclearia* such as the type-species *N. delicatula* Cienkowski, 1865 are surrounded by a mucus sheath)]. However we need a study of the fine structure for the final conclusion on the position of *Elaeorhanis*. The present work includes the description of the second species of the genus - the marine *E. tauryanini* with centric diatoms in the envelope.

Unfortunately we know a little on the genus *Belonocystis* Rainer, 1968 (Fig. 1 F) which was recorded once by its author; the description does not contain any information on the presence of tangential elements in its periplast comprised of well developed radial spicules only. However the presence of tangential elements ornamented by minute hexagonal perforations is the most characteristic taxonomic feature of the pompholyxophryids (Figs. 2 C, E), and hence we cannot regard the genus *Belonocystis* as a member of this family.

DISCUSSION OF THE MACROTAXA

The family Pompholyxophryidae Page, 1987 seems to represent a monophyletic taxon of discoriscitate filose amoebae surrounded by endogenous siliceous artefacts only. We include to this family the genera: *Pinaciophora* Greeff, 1869, *Rabdiophrys* Rainer, 1968, *Rabdiaster* gen. n. and *Pompholyxophrys* Archer, 1869. Tangential periplast elements of these organisms bear fine hexagonal perforations; the nucleus has an eccentric position. The second family of discicristate filose amoebae - the Nucleariidae Cann et Page, 1979 is closely related to pompholyxophryids according to the ultrastructural criteria but differs by the cell surface which is naked or covered with a mucus sheath, and by the central position of the nucleus.

Page (1987) united both families in a new order Cristidiscoidida Page, 1987 pointing out the most important their common ultrastructural feature - the discoidal shape of mitochondrial cristae. Indeed, the great similarity in the fine structure between members of these two families (review by Patterson *et al.* 1987) leads us to regard both groups as two constituent parts of any macrotaxon. However we cannot agree with Patterson *et al.* (in press) who placed all pompholyxophryid genera in the family Nucleariidae together with two nucleariid genera: *Nuclearia* Cienkowski, 1865 and *Vampyrellidium* Zopf, 1885. The production of siliceous artefacts (*i.e.* of the periplast) by the cell is known to cause a significant reorganisation of the cytoplasm and appearance of silica deposition vesicles (Patterson and Dürrschmidt 1986 a, b; Anderson 1994). We regard the ability to produce endogenous siliceous structures as a character a high taxonomic rank (it is a defining feature for a group of four genera, and the lowest rank, which can be used to contain these genera, would be a family). So we agree with Page (1987) that there should be a taxon of the rank of the order to unite naked nucleariids and scale-bearing pompholyxophryids as two families.

However we believe the use of the Page's Cristidiscoidida is unsuitable and incorrect according to two main reasons. Firstly, the term Cristidiscoidida Page, 1987 does not reflect the filose amoeboid nature of organisms named by it, and there are other discicristate taxa e.g. euglenids, schizopyrenids, *Stephanopogon* and others, usually called in common as the discicristates (Patterson and Sogin 1992) or the infrakingdom Discicristata Cavalier-Smith, 1997. Secondly, the order Rotosphaerida as proposed by Rainer (1968) unite filopodiate scale-bearing heliozoon-like amoebae that are well described and has priority as a suprafamilial name for the taxon including the pompholyxophryids. The great similarity of ultrastructural features between the pompholyxophryids and the nucleariids (Patterson *et al.* 1987) is not here regarded as a sufficient reason for the creation of a new order but should be a cause for a revision of the taxonomic position of nucleariids in the general system. Cann and Page (1979) regarded nucleariids at the rank of family (Nucleariidae), and that these naked discicristate filose amoebae or ones covered by mucus should be transferred from their previous position inside the order Aconchulinida De Saedeleer, 1934 to the order Rotosphaerida Rainer, 1968 uniting naked (Nucleariidae Cann et Page, 1979) and scale-bearing (Pompholyxophryidae Page, 1987) discicristate filose amoebae. Prior to this, the aconchulinids contained the tubulocristate filose amoebae: the families Vampyrellidae Zopf, 1887 and Arachnulidae Page, 1987. We therefore regard the term Cristidiscoidida Page, 1987 as conceptually the same as Rotosphaerida Rainer, 1968 whereas the term Cristivesiculatida Page, 1987 advanced for tubulocristate filose amoebae is a junior synonym of

Aconchulinida De Saedeleer, 1934. Thus using the terms Cristidiscoidida and Cristivesiculatida we believe not to be necessary.

Cavalier-Smith (1993, 1996/97) created the order Nucleariidae Cavalier-Smith, 1993 belonging to the subclass Cristidiscoidia Page, 1987 stat. n. Cavalier-Smith, 1993; the latter taxon included also orders Fonticulida Cavalier-Smith, 1993 and Ministeriida Cavalier-Smith, 1996. Any taxonomic remarks to relations of these groups and explanation to the ignorance of mentioning of pompholyxophryids are absent in both contributions. No diagnosis of the order Nucleariida was given. Thus we believe the use of the term Nucleariida to be incorrect too.

DIAGNOSES OF THE TAXA

Order ROTOSPHAERIDA Rainer, 1968

Synonym: Cristidiscoidida Page, 1987.

Diagnosis: discicristate filose amoebae lacking flagella or flagellated stages, not able to eruptive movement.

Composition of 2 families unites 8 genera.

Family 1. NUCLEARIIDAE Cann et Page, 1979

Diagnosis: naked rotosphaerids or those covered by mucus coat. Normally with central nucleus.

Type-genus: *Nuclearia* Cienkowski, 1865.

Composition of 4 genera: *Nuclearia* Cienkowski, 1865 [syn.: *Nuclearina* Frenzel, 1897; *Nuclearella* Frenzel, 1897; *Nucleosphaerium* Cann et Page, 1979; *Heliosphaerium* Frenzel, 1897; *Astrodisculus* Greeff, 1869], *Elaeorhanis* Greeff, 1873 [syn.: *Litosphaerella confirm spelling* Frenzel, 1897; *Estrella* Frenzel, 1897], *Leptophrys* Hertwig et Lesser, 1874, and *Vampyrellidium* Zopf, 1885.

This paper deals with *Elaeorhanis* only. For the taxonomy of other nucleariids see: Patterson (1984), Page (1991).

Genus 1. *Elaeorhanis* Greeff, 1873

Synonym: *Lithosphaerella* Frenzel, 1897; *Estrella* Frenzel, 1897.

Diagnosis: heliozoon-like amoebae surrounded by a mucus sheath, which includes also sand grains and diatom shells. Filopods often branching. Nucleus is central or nearly so.

Type-species: *E. cincta* Greeff, 1873.

Composition of 2 species.

1. *E. cincta* Greeff, 1873 (Figs. 1 E; 4 C; 5 A, B).

Diagnosis: cell body 14-17 (26) μm in diameter, surrounded by mucus sheath about a half as thick as the diameter of the body. One or several contractile vacuoles. Pennate diatom frustules in the mucus sheath. Freshwater.

Distribution: cosmopolitan in fresh waters.

2. *E. tauryanini* sp. n. (Fig. 5 C).

Diagnosis: cell body about 25 μm in diameter, the mucus sheath is about a half of it. No contractile vacuoles. Centric diatom frustules mostly in the envelope. Marine.

Material: samples containing *E. tauryanini* were collected 25.07.1994 by washing of sponges by the hand plankton net at the depth 15-20 m by diving (SCUBA) around of the White sea biological station of Moscow State University (Kandalaksha Bay of the White Sea; 66° 31' N; 33° 07' E; salinity~270).

Observations: are based on the study of 3 cells. Living cells are 23-28 μm in diameter, with mostly stiff filopods, some branch. Cells roll along the bottom of a Petri dish as *E. cincta* does. No contractile vacuoles are observed. The mucus sheath is delicate, homogeneous and not densely covered with frustules of centric diatoms *Thalassiosira* sp. (Fig. 4 D). Only two frustules of pennate diatoms *Cocconeis scutellum* var. *parva* (Fig. 4 E) were observed by SEM of cell coats.

Etymology: the species name is given in honour of A.F. Tauryanin, vice-director and one of the organisers of the White Sea biostation of Moscow University.

Remarks: we regard *E. tauryanini* as a new species of marine filopodial amoebae for following reasons: (**i**) it belongs to the genus *Elaeorhanis* differing from the widespread and similar genus *Lithocolla* by branching pseudopodia and by the abundance of mucus and the presence of diatom frustules in the cell coat; (**ii**) the cosmopolitan freshwater species *E. cincta* was never recorded in marine habitats, has contractile vacuoles, and all its previous records described only pennate diatoms in its mucus sheath.

Family 2. POMPHOLYXOPHRYIDAE Page, 1987

Diagnosis: scale-bearing rotosphaerids with periplasts comprised of one or two types of endogenous siliceous elements; tangential ones are perforated by minute hexagonal pores. Nucleus eccentric.

Type-genus: *Pompholyxophrys* Archer, 1869.
Composition of 4 genera.

Genus 1. *Pinaciophora* Greeff, 1869

Synonym: *Pinacocystis* Hertwig et Lesser, 1874; *Pinaciocystis* Roskin, 1929; *Potamodiscus* Gerloff, 1968.

Diagnosis: scale-bearing, heliozoon-like amoebae with periplasts comprised of tangential plate-scales and (more often) radial spicules. Plate-scales are ornamented by several holes in the apical plate.

Type-species: *P. fluviatilis* Greeff, 1869.

Composition of 13 species: *P. fluviatilis* Greeff, 1869 [syn.: *Pinaciocystis duboscqi* Roskin, 1929; *Potamodiscus kalbei* Gerloff, 1968]; *P. bifurcata* Thomsen, 1978; *P. candelabrum* Thomsen, 1978; *P. denticulata* Thomsen, 1978; *P. multicosta* Thomsen, 1978; *P. ovalis* Croome, 1987; *P. paucipora* Thomsen, 1978; *P. rubicunda* (Hertwig et Lesser, 1874) Roijackers et Siemensma, 1988 [syn. *Rabdiophrys thomseni* Roijackers et Siemensma, 1988]; *P. spiculata* Manton et Sutherland, 1979; *P. tasmanica* Croome, 1978; *P. triangulata* Thomsen, 1978; *P. tridentata* Thomsen, 1978; *P. turrisfenestrata* (Wujek et O'Kelly, 1991) comb. n.

Genus 2. *Rabdiophrys* Rainer, 1968

Diagnosis: scale-bearing, heliozoon-like amoebae with periplasts comprised of tangential plate-scales and radial spicules. Plate-scales with a single large central hole.

Type-species: *R. anulifera* Rainer, 1968 (Fig. 2 A).

Composition of 3 species: *R. anulifera* Rainer, 1968 [syn. *Pinaciophora pinea* Nicholls, 1983]; *R. monopora* (Thomsen, 1978) Roijackers et Siemensma, 1988; *R. columna* (Croome, 1987) Roijackers et Siemensma, 1988.

Genus 3. *Rabdiaster* gen. n.

Diagnosis: scale-bearing, heliozoon-like amoebae with periplasts comprised of tangential plate-scales and radial spicules. Plate-scales lack holes at the apical surface.

Type-species: *R. pertzovi* (Mikrjukov, 1994) comb. n. (Figs. 2 B, C).

Composition of 4 species: *R. pertzovi* (Mikrjukov, 1994) comb. n.; *R. apora* (Croome, 1987) comb. n.; *R. ludibunda* (Penard, 1901) comb. n. [syn. *Acanthocystis rubella* Penard, 1904]; *R. reticulata* (Thomsen, 1979) comb. n.

Genus 4. *Pompholyxophrys* Archer, 1869

Synonym: *Hyalolampe* Greeff, 1869.

Diagnosis: scale-bearing, heliozoon-like amoebae with the periplasts comprised of spherical, ovoid, discoid or bone-shaped elements of a single type within a species. Spicules are absent.

Type species: *P. punicea* Archer, 1869 (Figs. 2 D, E)

Composition of 7 species: *P. punicea* Archer, 1869 [syn.: *Hyalolampe fenestrata* Greeff, 1869]; *P. degrooti* Roijackers et Siemensma, 1988; *P. exigua* (Hertwig et Lesser, 1874) Penard, 1904; *P. ossea* Dürrschmidt, 1985; *P. ovuligera* Penard, 1904; *P. stammeri* (Rainer, 1968) Roijackers et Siemensma, 1988; *P. stellata* Nicholls et Dürrschmidt, 1985.

Key to pompholyxophryid genera and species

1 Tangential elements are plate-scales..... 2
Tangential periplast elements of more complex shape.. .....*Pompholyxoprys* [4]
2 Apical plates of plate-scales are perforated by one or more holes..... 3
Apical plates without holes..... *Rabdiaster* [25]
3 Apical plates with several holes, periplast usually in cludes spicules.....*Pinaciophora* [10]
Apical plates with a single central hole, periplast always comprised of spicules.....*Rabdiophrys* [23]
4 Scales are globular..... 5
Scales are more flattened..... 6
5 Scales are different in size, 1-6 µm; air-dried scales are spherical in outline..... *P. punicea*
Scales are of more or less the same size, up to 1.2 µm; air-dried scales are irregular in outline..... *P. exigua*
6 Scales are elongate, bone- or rod-shaped..... *P. ossea*
Scales are not elongate, but discoid or ovoid..... 7
7 Scales are not strongly flattened, ovoid..... .....*P. ovuligera*
Scales are strongly flattened, not ovoid..... 8
8 Scales are circular or broadly elliptical.... *P. degrooti*
All scales are elliptical, not particularly broad..... 9
9 Scales without intercalary struts..... *P. stammeri*
Scales with intercalary struts..... *P. stellata*
10 Periplasts without spicules..... 11
Periplasts with spicules..... 12
11 Plate-scales with more then 10 large holes..... .....*P. fluviatilis*
Plate scales with 4-8 holes..... *P. rubicunda*

12 Distal holes in plate-scales are comparable with the size of the scale..... 14
Distal pores are very tiny, but not hexagonal; spicules have less then five marginal deticles..... 13
13 The size of holes is very tiny and they are about five in the central area; spicules are 1.5-2.0 μm in length, distally with more then two spines..... .....*P. paucipora*
Pores are tiny, and numerous in the central areas; spicules are 2.5-6.0 μm, with two spines at the apex connected by numerouscostae..... .....*P. multicosta*
14 Plate-scales are circular, ovoid or triangular..... 16
Plate scales are oval or elliptical, spicules are bifurcate..... 15
15 Plate-scales are bilaterally-symmetrical as having two parallel lines of holes..... *P. ovalis*
Holes of various size are gathered irregularly in the centre of the plate-scale..... *P. turrisfenestrata*
16 Plate-scales are triangular..... *P. triangula*
Plate scales are circular or ovoid..... 17
17 Spicules are with a pointed, bifurcate or flared tubular apex..... 18
Spicules are with at least three furcae (denticles) at the apex..... 21
18 A spicule apex is pointed or bifurcate..... 19
A spicule apex is formed by a flared cylindrical shaft without marginal denticles or furcae..... 20
19 Holes in plate-scales are small in number; there is a triangular area between spicules in furcae; the spicule shaft attaches to the base plate by a number of struts ..... *P. bifurcata*
There are about ten large holes gathered in a central circle; spicules are simply bifurcate or have a pointed apex; the base of the spicule s haft without struts ..... *P. tasmanica*
20 Spicules with an elaborated, basket-shaped base..... ..... *P. candelabrum*
A spicule base is a flat base-plate ornamented by a conoid structure, which may be reticulate or formed by concentric rings..... *P. spiculata*
21 Spicules with three denticles at the apex..... .....*P. tridentata*
Spicules are terminating in at least 6 denticles..... 22
22 Two of apical denticles are fused by a thin lamella and elongated or have other elaborated structures..... ..... *P. rubicunda*
Spicule apices lack such elaborated structures..... .....*P. denticulata*
23 Plate scales with two internal connected struts, one-half between the periphery; one large central hole; spicules of two different length classes (with an inter mediate spectrum of spicules)..... .....*R. anulifera*
Spicules of the same size class..... 24
24 Plate scales with a poor hole; spicules with acylindrical shaft flattened distally into the bipartite end..... ..... *R. monopora*
The holes of plate scales has a column-like protrusion at their centre; spicules with a tripartite apex represented by 3 small apical denticles..... .....*R. columna*
25 Spicules with a pointed apex..... *R. ludibunda*
Spicules with apices branching into 3-5 furcae..... 26
26 Distal surface of the upper plate of plate-scales is not reduced, but its texture is arranged as hexagonal minute perforations only..... 27
The upper plate of plate scales is reduced, and hence the texture of the distal surface is formed by the intercalary layer..... *R. reticulata*
27 One type of spicules; their base lacks of a pedestal-like structure..... *R. apora*
Two types of spicules: long and short; short ones possess a pedestal-like structure at its base, ornamented by minute protrusions..... *R. pertzovi*

Acknowledgement. The author thanks Dr. R. Croome for correction of the English, M.A. Gololobova and F.V. Sapozhnikov for their help in definition of marine diatoms, and Dr. A.A. Kotov for his help in preparation of figures.

REFERENCES

Anderson O.R. (1994) Cytoplasmic origin and surface deposition of siliceous structures in Sarcodina. *Protoplasma* **181:** 61-77

Belcher J.N., Swale E.M.F. (1978) Records from England of the heliozoan-like organism *Pinaciophora fluviatilis* Greeff and of its scales, *"Potamodiscus kalbei". Arch. Protistenkd.* **120:** 367-370

Cann J.P. (1986) The feeding behavior and structure of *Nuclearia delicatula* (Filosea, Aconchulinida). *J. Protozool.* **33:** 392-396

Cann J.P., Page F.C. (1979) *Nucleosphaerium tuckeri* nov. gen. nov. sp. - a new freshwater filose amoeba without motile form in a new family Nucleariidae (Filosea: Aconchulinida) feeding by ingestion only. *Arch. Protistenkd.* **122:** 226-240

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

Cavalier-Smith T. (1996/97) Amoeboflagellates and mitochondrial cristae in eukaryote evolution: megasystematics of the new protozoan subkingdoms Eozoa and Neozoa. *Arch. Protistenkd.* **147:** 237-258

Cavalier-Smith T. (1997) A revised 6-kingdom system of life. *Biol. Rev.* **32:** 303-366

Croome R.L. (1987 a) *Pinaciophora columna* n. sp., *P. tasmanica* n. sp. and *P. apora* n. sp., new heliozoans from Australia, and a report of *P. fluviatilis* Greeff from Antarctica. *Arch. Protistenkd.* **133:** 15-20

Croome R.L. (1987 b) Observations of heliozoan genera *Acanthocystis, Raphidiophrys, Clathrulina* and *Pompholyxophrys* (Protozoa, Sarcodina) from Australian freshwaters. *Arch. Protistenkd.* **133:** 237-243

Croome R.L. (1987c) *Pinaciophora ovalis* (Rotosphaerida, Heliozoea), a new species from Australia. *Arch. Protistenkd.* **134:** 343-345

Croome R.L., van der Hoff J., Burton H.R. (1987) Observations of the heliozoean genera *Pinaciophora* and *Acanthocystis* (Heliozoea, Sarcodina, Protozoa) from Ellis Fjord, Antarctica. *Polar. Biol.* **8:** 23-28

Croome R.L., Mikrjukov K.A.(1999) A study of the species composition of heliozoa (Protista) in frozen reservoirs. *Vestnik Mosk. Univ. Biology Ser.* 17 (in Russian)

Deflandre G. (1953) Orders Aconchulina De Saedeleer, Athalamia Haeckel. In: Traité de Zoologie, I (Ed. P.-P. Grassé) Masson et Cie, Paris, 92-96

Dykstra M. J., Porter D. (1984) *Dyplophrys marina,* a new scale-forming marine protist with labyrinthulid affinities. *Mycologia* **76:** 626-632

Frenzel J. (1897) Untersuchungen über die mikroskopische Fauna Argentiniens. I. Die Rhizopoden und Helioamoeben. *Biblthca zool.* **12:** 1-166

Gaarder K.R., Fryxell G.A., Hasle G.R. (1976) *Potamodiscus kalbei* Gerloff, an organism with siliceous scales. *Arch. Protistenkd.* **118:** 346-351

Greeff R. (1869) Über Radiolarien und radiolarienartige Rhizopoden des süßen Wassers. *Arch. f. mikr. Anat.* **5:** 404-505

Greeff R. (1873) Über Radiolarien und radiolarienartige Rhizopoden des süßen Wassers. In: Sitzungsber. Ges. Beförd. Naturw., Marburg, 47-64

Groot A. A. De (1979) Über einige wenig bekannten Rhizopoden. *Hydrobiol. Bull.* **13:** 34-49

Hartmann M. (1913) Rhizopoda. In: Handwörterbuch der Naturwissenschaften, Jena, **8:** 422-446

Hausmann K. (1977) Bakterien und virusahnliche Partiklen im Cytoplasma des Rhizopoden *Vampyrella lateritia. Ann. Sci. Biol. Besseen-Chandesse* **11:** 102-107

Hülsmann N. (1993) *Lateromyxa gallica* n. gen., n. sp. (Vampyrellidae): a filopodial amoeboid protist with a novel life cycle and conspicuous ultrastructural characters. *J. Euk. Microbiol.* **40:** 141-149

Jones E.E. (1974) The Protozoa of Mobile Bay, Alabama. University of South Alabama, Monographs, 1 (1)

Krylov M.V., Dobrovolsky A.A., Issi I.V., Mikhalevich V.I., Podlipaev S.A., Reschetnyak V.V., Seravin L.N., Starobogatov Ya.I., Schulman S.S., Yankowski A.W. (1980) New concepts of the system of unicellular animals. In: Principles of the construction of the macrosystem of the unicellular animals. *Proc. Zool. Inst., Ac. Sci. USSR* **94:** 122-132 (in Russian)

Kudo R. R. (1954) Protozoology. 4 ed. Springfield, Illinois

Lee J.J., Leedale G.F., Patterson D.J., Bradbury P.C. (Eds.) (0000) Illustrated Guide to the Protozoa, 2nd ed. Society of Protozoologists (in press)

Levine N.D., Corliss J.O., Cox F.E.G., Deroux G., Grain J., Honigberg B., Leedale G., Loeblich A.R., Lom J., Lynn D., Merinfeld E.J., Page F.C., Poljansky G., Sprague V., Vavra J., Wallace F.G., Weisser J.A. (1980) A new revised classification of Protozoa. *J. Protozool.* **27:** 37-58

Manton I. (1986) Functional parallels between calcified and uncalcified periplasts. In: Biomineralization in Lower Plants and Animals, (Eds. B.S.C. Leadbeater and R. Riding) Systematic Assoc., Clarendon Press, Oxford, Spec. Vol. **30:** 157-172

Manton I., Sutherland J. (1979) Further observations on *Potamodiscus kalbei* Gerloff = *Pinaciophora* Greeff, with special reference to Alaska and arctic Canada. *Zool. J. Linn. Soc.* **67:** 285-295

Mignot J.-P., Savoie A. (1979) Observations ultrastructurales sur *Nuclearia simplex* Cienkowski (Protozoa, Rhizopodea, Filosia). *Protistologica* **15:** 23-32

Mikrjukov K.A. (1993) On centrohelid and rotosphaerid Heliozoa from the environs of the Vortsjärv limnological station in Estonia. *Proc. Estonian Acad. Sci. Biol.* **42:** 154-160

Mikrjukov K.A. (1994) *Rabdiophrys pertzovi* sp. nov. - a new marine rotosphaerid heliozoon from the White Sea. *Arch. Protistenkd.* **144:** 325-327

Mikrjukov K.A. (1998) On the biology of Heliozoa: the phenomenon of origination of radial forms in benthic sarcodines. *Zool. zhurn. (Moscow)* **77:** 147-157 (in Russian)

Mikrjukov K.A. (0000) On the unity of marine and freshwater fauna of heliozoa: some interesting records of these organisms in the Black Sea and Crimea. *Zool. zhurn. (Moscow)* **78:** (in Russian, in press)

Mikrjukov K.A., Croome R.L. (1998) Observations of heliozoans in ice-covered ponds on Mt. Buffalo. *Victorian Nat.* **115:** 230-232

Mikrjukov K.A., Patterson D.J. (0000) On freshwater and marine heliozoa in Australia. *Rec. Australian Mus.* (in press)

Mikrjukov K.A., Siemensma F.J., Patterson D.J. (0000) The heliozoa. In: Illustrated Guide to the Protozoa, (Eds. J.J. Lee, G.F. Leedale, D.J. Patterson and P.C. Bradbury) 2nd ed. Society of Protozoologists (in press)

Nicholls K.H. (1983) Little-known and new heliozoeans: *Pinaciophora triangula* Thomsen new to North America and a description of *Pinaciophora pinea* sp. nov. *Can. J. Zool.* **61:** 1387-1390

Nicholls K.H., Dürrschmidt M. (1985) Scale structure and taxonomy of some species of *Raphidocystis, Raphidiophrys* and *Pompholyxophrys* (Heliozoea) including description of six new species. *Can. J. Zool.* **63:** 1944-1961

Old K.M., Darbyshire J.E. (1980) *Arachnula impatiens* Cienk., a mycophagous giant amoeba from soil. *Protistologica* **16:** 277-287

Page F.C. (1985) The limax amoebae: comparative fine structure of the Hartmannellidae (Lobosea) and Vahlkampfiidae (Heterolobosea). *Protistologica* **21:** 361-383

Page F.C. (1987) The classification of "naked" Amoebae (Phylum Rhizopoda). *Arch. Protistenkd.* **134:** 199-217

Page F.C. (1991) Klasse Filosea Leidy, 1879. In: Nackte Rhizopoda und Heliozoea. Protozoenfauna. (Eds. F.C. Page and F.J. Siemensma) Gustav Fischer Verlag. Stuttgart - N.Y. **2:** 132-150

Patterson D.J. (1983) On the organization of the naked filose amoeba, *Nuclearia moebiusi* Frenzel, 1897 (Sarcodina, Filosea) and its implications. *J. Protozool.* **30:** 301-307

Patterson D.J. (1984) The genus *Nuclearia* (Sarcodina, Filosea): species composition and characteristics of the taxa. *Arch. Protistenkd.* **128:** 127-138

Patterson D.J. (1985) On the organization and the affinities of the amoeba, *Pompholyxophrys punicea* Archer, based on the ultrastructure examination of individual cells from the wild material. *J. Protozool.* **32:** 241-246

Patterson D.J. (1992) Free-living Fresh-water Protozoa. A Colour Guide. Wolfe Publishing Ltd., Aylesbury, England

Patterson D.J. (1994) Protozoa: evolution and systematics. In: Progress in Protozoology (Eds. K. Hausmann and N. Hülsmann). Proc. IX-th Int. Congr. Protozool., Berlin, 1993. Gustav Fischer Verlag, Stuttgart-Jena-New York, 1-14

Patterson D.J., Brugerolle G. 1988). The ultrastructural identity of *Stephanopogon apogon* and the relatedness of the genus to other kinds of protists. *Europ. J. Protistol.* **23:** 279-290

Patterson D.J., Dürrschmidt M. (1986 a) The formation of siliceous scales by *Raphidiophrys ambigua* (Protista, Centroheliozoa). *J. Cell Sci.* **91:** 33-39

Patterson D.J., Dürrschmidt M. (1986 b) Siliceous structures formed by heliozoa and heliozoon-like amoebae. In: Biomineralization in Lower Plants and Animals (Eds. B.S.C. Leadbeater and R. Riding). Systematic Assoc., Clarendon Press, Oxford, Spec. Vol. **30:** 361-374

Patterson D.J., Sogin M. (1992) Eukaryote origins and protistan diversity. In: The Origin and Evolution of the Cell (Eds. H. Hartman and K. Matsuno). Proc. Conf. on the Origin and Evolution of Prokaryotic and Eukaryotic cells, Shimoda, Japan, 13-46

Patterson D.J., Surek B., Melkonian M. (1987) The ultrastructure of *Vampyrellidium perforans* Surek and Melkonian and its taxo-

nomic position among the naked filose amoebae. *J. Protozool.* **37:** 63-67

Patterson D.J., Simpson A.G.B, Rogerson A., Schuster F. (0000) Amoebae of uncertain affinities. In: Illustrated Guide to the Protozoa (Eds. J.J. Lee, G.F. Leedale, D.J. Patterson and P.C. Bradbury). 2nd edition. Society of Protozoologists (in press)

Penard E. (1901) Sur quelques Héliozoaires des environs de Geneve. *Revue Suisse Zool.* **9:** 279-305

Penard E. (1905) Les Sarcodinés des grands lacs. Geneva

Poche F. (1913) Das System der Protozoen. *Arch. Protistend.* **30:** 125-321

Rainer H. (1968) In: Urtiere, Protozoa; Wurzelfübler, Rhizopoda; Sonnentierchen, Heliozoa (Ed. F. Dahl) Die Tierwelt Deutschlands, Gustav Fischer Verlag, Jena, **56:**

Roijackers R.M.M. (1988) External morphology as taxonomic characteristic in planktonic scale-bearing Chrysophyceae and scaled Heliozoa. *Hydrobiol. Bull.* **22:** 69-73

Roijackers R.M.M., Siemensma F.J. (1988) A study of cristidiscoidid amoebae with description of new species and keys to genera and species (Rhizopoda, Filosea). *Arch. Protistenkd.* **135:** 237-253

Röpstorf P., Hausmann K. (1992) Sexual stages in the life cycle of Vampyrellids (Rhizopoda). *Europ. J. Protistol.* **28:** 355

Röpstorf P., Hülsmann H., Hausmann K. (1993) Karyological investigations on the vampyrellid filose amoeba *Lateromyxa gallica* Hülsmann 1993. *Eur. J. Protistol.* **29:** 302-310

Schaudinn F. (1896) Über das Zentralkorn der Heliozoen, ein Beiträg zur Centrosomenfrage. *Verch. d. Deutsch. Zool. Ges., Bonn.* S. 113-130

Schulze F.E. (1874) Rhizopodenstudien. II. *Arch. mikr. Anat.* **10:** 377-400

Schulman S.S., Reshetnyak V.V. (1980) Does the superclass Actinopoda really exist in the system of Protozoa ? In: Principles of construction the macrosystem of unicellular animals. *Proc. Zool Inst. Ac. Sci. USSR* **94:** 23-41 (in Russian)

Siemensma F. J. (1991) Ordnung Cristidiscoidida Page, 1987. In: Nackte Rhizopoda und Heliozoea. Protozoenfauna. (Eds. F.C. Page and F.J. Siemensma). Gustav Fischer Verlag, Stuttgart/ New York, **2:** 273-284

Takahashi E. (1959) Studies on genera *Mallomonas, Synura* and other plankton in fresh-water by electron microscope. I. *Bull. Yamagata Univ. Agr. Sci.* **3:** 117-151

Takahashi E. (1981) Loricate and scale-bearing protists from Lützow-Holm Bay, Antarctica. I. Species of Acanthoecidae and Centrohelida found at a site selected on the fast ice. *Antarctic Rec.* **73:** 1-22

Thomsen H.A. (1978) On the identity between the heliozoan *Pinaciophora fluviatilis* and *Potamodiscus kalbei*: with the description of eight new species. *Protistologica* **14:** 359-373

Thomsen H.A. (1979) Electron microscopical observations on the brackish-water nannoplankton from the Tvärminne area, SW coast of Finland. *Acta Bot. Fenn.* **11:** 11-37

Tregouboff G. (1953) Classe de Heliozoaires. In: Traité de Zoologie, I (Ed. P.-P. Grassé). Masson et Cie, Paris, 437-489

Triemer R.E., Farmer M. (1991) The ultrastructural organization of the heterotrophic euglenids and its evolutionary implications. In: The Biology of Free-living Heterotrophic Flagellates (Eds. D.J. Patterson and J. Larsen) Syst. Ass., *Oxford Univ. Press, Oxford*, Spec. Vol. **45:** 185-204

Vörs N. (1992 a) Heterotrophic protists (excl. dinoflagellates, loricate choanoflagellates and ciliates). In: Plankton from inner Danish Waters: An analysis of the autotrophic and heterotrophic plankton in Kattegat, HAV 90 Report (Ed. H.A. Thomsen). *Dan. Nat. Agency Envir. Prot.* **11:** 195-249 (in Danish)

Vörs N. (1992 b) Heterotrophic amoebae, flagellates and heliozoa from the Tvärminne area, Gulf of Finland, in 1988-1990. *Ophelia* **36:** 1-109

Vörs N. (1993) Heterotrophic amoebae, flagellates and heliozoa from Arctic marine waters (North West Territories, Canada and West Greenland). *Polar Biol.* **13:** 113-126

Wee J.L., Millie D.F. (1983) A new record of *Pinaciophora fluviatilis* Greeff *sensu* Penard from Laurentian Great Lakes. *J. Great Lakes Res.* **9:** 433-435

Wujek D.E., O'Kelly C.J. (1991) *Rabdiophrys turisfenestrata* n. sp. (Rhizopoda, Pompholyxophryidae) from New Zealand. *Arch. Protistenkd.* **140:** 101-107

Received on 12th August 1998; accepted on March 3rd, 1999

Acta Protozool. (1999) 38: 133 - 144

ACTA
PROTOZOOLOGICA

Characterization of *Paraurostyla coronata* sp. n. Including a Comparative Account of Other Members of the Genus

Seema ARORA, Renu GUPTA, Komal KAMRA and Gulshan R. SAPRA

Department of Zoology, University of Delhi, Delhi, India

Summary. *Paraurostyla coronata* sp. n. is widely distributed in the North Indian fresh water bodies. The cell measures about 200 x 60 µm and exhibits diffused green cytoplasmic pigmentation and pink coloration at the anterior and posterior segments. Dorsally, sub-pellicular pigment granules are arranged in about 15 linear rows as obliquely placed pairs, whereas, at the cell extremities such granules are not packed in any specific design. Seven frontal cirri are arranged in a distinct pattern wherein 6 of them are placed in one row along the AZM shoulder and buccal cirrus positioned away from this row. Cirri on the ventral surface include 6-8 rows, 2 rows of marginal and 6-10 transverse cirri arranged in an oblique pattern. The dorsal surface bears 4 dorsal and 2 dorso-marginal bristle rows. A comparison of combination of various characters and the morphogenetic process reveals that *P. coronata* is distinct from the other described species of the genus.

Key Words: morphogenesis, morphometry, *Paraurostyla coronata*, systematics.

Abbreviations: AZM - adoral zone of membranelles, BC - buccal cirrus, CC - caudal cirri, DK - dorsal kineties, DM - dorso-marginal rows, DP - dorsal primordia, DMP - dorso-marginal primordia, FC - frontal cirri, FVT - fronto-ventral transverse cirri, LMC - left row of marginal cirri, OP - oral primordium, RMC - right row of marginal cirri, TC - transverse cirri, UM - undulating membrane, V- ventral cirri, V_1-V_7 - ventral rows

INTRODUCTION

The ciliate described in the present study was isolated from a fresh water barrage near Delhi (28°34' N, 76°07' E). It depicts the characteristic features of the genus *Paraurostyla* (Borror 1972) showing cirri in numerous longitudinal rows and the absence of midventral rows. While establishing the genus, Borror (1972) included 10 species which were separated primarily by the criteria of morphometry and appearance of live cells. Subsequently one species from this scheme was excluded (Borror 1979). The present study on *P. coronata* reveals that it differs from the other described species in many aspects of appearance, morphometry and morphogenetic details.

MATERIALS AND METHODS

Paraurostyla coronata is widely distributed in the Northern Indian fresh water bodies. The present study was conducted on the clonal cultures of *P. coronata* which was isolated from a water barrage of the river Yamuna at Okhla, New Delhi. Laboratory cultures were maintained at 23 ± 1°C with axenically grown *Chlorogonium elongatum* as the food organism (Ammermann *et al.* 1974).

Address for correspondence: Gulshan R. Sapra, Department of Zoology, University of Delhi, Delhi-110007, India; E-mail: duzdel@del2.vsnl.net.in

Morphometric characterisation was done on randomly selected non-dividing cells of a clonal culture after staining with protargol (Kamra and Sapra 1990). The terminology employed was that of Borror (1972) and Jerka-Dziadosz and Frankel (1969). Data was analysed statistically by linear regression.

RESULTS

Non-dividing cells of *Paraurostyla coronata* measure about 200 x 60 µm in protargol stained preparations. They are bottom dwellers and exhibit crawling movements. A prominent feature of live cells is their diffused green coloured general appearance and pink coloured anterior and posterior extremities. Phase contrast microscopy reveals the arrangement of sub-pellicular pigment granules under the dorsal surface of cells. The granules are arranged in about 15 linear rows and are aligned in pairs lying obliquely in relation to the long axis of the cell. Their packing density appears more towards the central and marginal areas but such configuration could result from the compressed and flattened situation under which the cells are microscopically examined. The pigment granules at the anterior and posterior cell segments are arranged differently, as singlets and randomly packed (Fig. 3).

The cell body is dorsoventrally flattened with its right and left margins running parallel over most of the cell length. Cortical reorganisation is frequently seen even under optimal culture conditions. Encystment is rare whereas cannibalism is quite common. Under optimum growth conditions the generation time of cells is 12 ± 0.5 h. The nuclear apparatus consists of 2 macronuclei and 4 micronuclei. The morphometric data derived from the protargol stained preparations is given in Table 1.

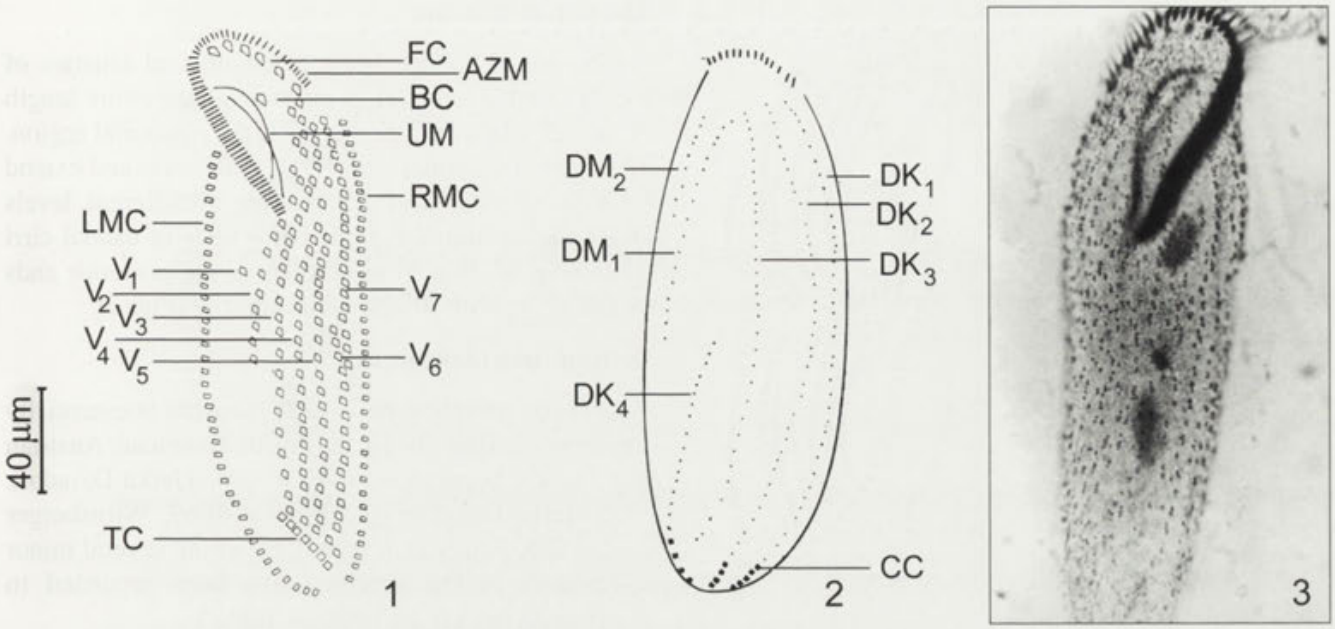
The ventral ciliature

The adoral zone of membranelles (AZM) on the left margin of the ventrally placed peristome spans about 1/3rd of the cell length. On the right side of the peristome are 2 parallel undulating membranes (UMs) which appear crossed in stained preparations due to flattening. There are 7 frontal cirri of which 1 buccal cirrus / FC_1 is at the right

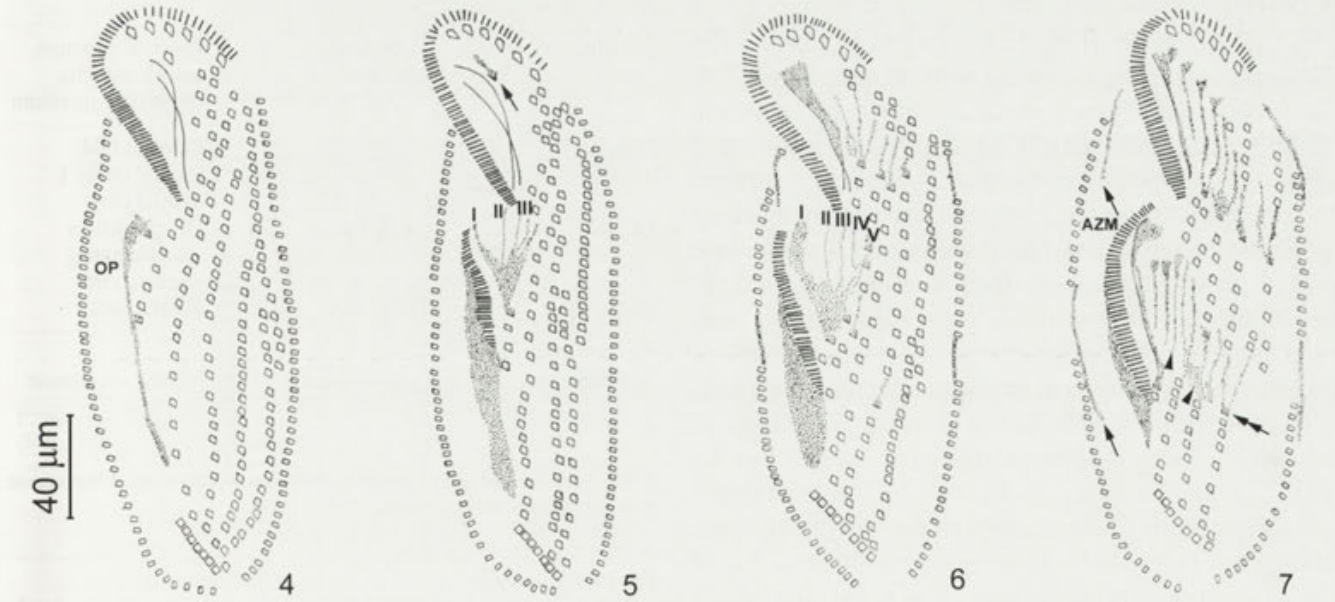
Table 1. Morphometric characterisation of *P. coronata* (data based on protargol impregnated cells)

Character	Mean	S.D.	CV	Minimum	Maximum	n
Body length (µm)	199.6	18.85	9.44	173.4	230.6	25
Body width (µm)	62.4	9.06	14.51	51.2	85.7	25
Macronucleus length (µm)	29.6	8.09	27.33	19.25	45.5	15
Macronucleus width (µm)	10.3	2.03	19.70	7.52	14.0	15
Micronucleus diameter (µm)	3.6	0.48	13.48	2.8	4.2	15
Adoral zonal length (µm)	67.8	3.26	4.84	59.5	73.85	25
Adoral membranelle, number	72	4.04	5.63	65	78	25
Frontal cirri, number	7	0	0	7	7	25
Ventral rows, number	7	0.85	12.76	6	8	25
Ventral cirri, number in:						
V_1	3	1.01	37.07	2	5	20
V_2	17	2.71	16.19	12	22	20
V_3	22	2.54	11.3	19	27	20
V_4	24	2.37	10.07	20	28	20
V_5	24	2.97	12.59	20	28	20
V_6	19	2.71	14.58	15	23	20
V_7	24	2.62	10.92	20	28	20
Right marginal cirri, number	48	3.91	8.05	43	59	25
Left marginal cirri, number	55	4.28	7.77	50	60	25
Transverse cirri, number	8	1.01	12.27	6	10	25
Dorsal rows, number	4	0	0	4	4	25
Dorso-marginal rows, number	2	0	0	4	4	25
Caudal cirri, number	16	1.16	7.1	15	18	20
Caudal cirri, number in:						
DK_1	4	0.48	11.24	4	5	20
DK_2	5	0.56	11.23	4	6	20
DK_4	7	0.81	11.8	6	8	20

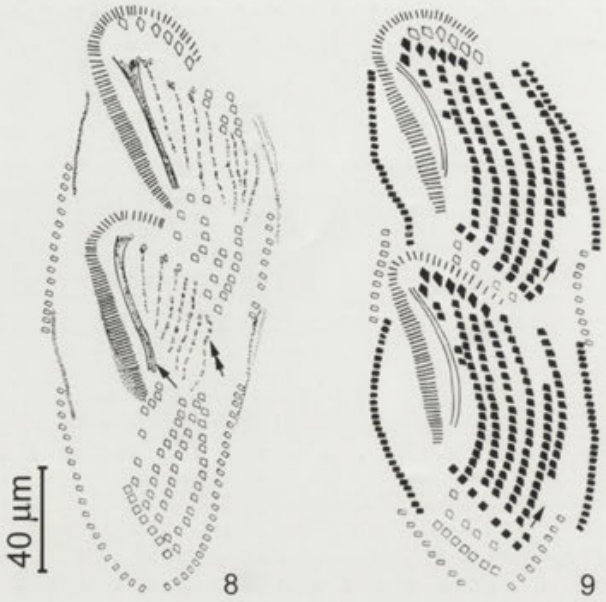
Abbreviations: CV - coefficient of variance, DK_{1-4} - dorsal kineties 1-4, n - number, S.D. - standard deviation, V_1-V_7 - ventral rows of cirri



Figs. 1-3. Line diagrams (1, 2) and photomicrograph (3) of protargol impregnated non-dividing cells of *P. coronata*. 1 - ventral surface, 2 - dorsal surface, 3 - protargol impregnated cell showing arrangement of subpellicular granules. AZM - adoral zone of membranelles, BC - buccal cirrus, CC - caudal cirri, DK_{1-4} - dorsal kineties 1-4, $DM_{1,2}$ - dorso-marginal kineties, FC - frontal cirri, LMC - left row of marginal cirri, RMC - right row of marginal cirri, TC - transverse cirri, UM - undulating membrane, V_1-V_7 - ventral rows



Figs. 4-7. Line diagrams of protargol impregnated cells of *P. coronata* showing morphogenetic events on the ventral surface. 4 - *de novo* formation of oral primordium (OP); 5 - formation of FVT primordia (I-III) by disaggregation of parental cirri (arrow marks a cirrus that is about to be incorporated into a streak); 6 - the UM primordium (I) and the next four primordia (II-V) that are formed in conjunction with OP are now separated from it to its right; 7 - the alignment of new AZM is nearly complete, formation of LMC primordia (arrows) and FVT primordia (arrowheads), two streaks originate from the right most ventral row (double arrow)



Figs.8 - 9. Line diagrams of protargol impregnated cells of *P. coronata* showing morphogenetic events on the ventral surface. 8-stages of primordia split, UM primordia splitted longitudinally (arrow); cirri begin to develop within FVT primordia (double arrow); 9 - dividing cell, arrows show the anterior movement of rightmost ventral row

of the UMs; the rest (FC_{2-7}) are arranged in a somewhat elliptical course lying next to the collar region of the AZM. The frontals are hypertrophied slightly more than the ventrals. Six to 8 (usually 7) longitudinal rows of ventral cirri (V_1 -$V_{6/7/8}$) lie on the right half of the ventral surface. V_{1-3} begins in the apical part of the cell and does not extend up to the posterior tip. The rows (V_4-$V_{6/7}$) commence subapically and extend up to the posterior end of the cell. The right most ventral row ($V_{6/7/8}$) runs more or less parallel to the right margin of the cell. A large gap exists between the posteriormost cirrus and the rest of the cirri in this row. In most cells V_1 is a short row consisting of 2-5 cirri of which the posteriormost cirrus is present in the post oral region. Number of cirri in each of the ventral row is highly variable (Table 1), though the numerical correlation between the total number of ventral cirri and ventral rows is statistically significant (r = 0.99). Six to 10 transverse cirri form an oblique row close to the posterior ends of V_4-$V_{6/7/8}$. Number of ventral cirri and transverse cirri is also directly correlated (r = 0.92). The left marginal cirral row (LMC) rounds off towards the right at the posterior end. The right marginal cirral row (RMC) is not confluent with the LMC row (Fig. 1).

The dorsal ciliature

The dorsal surface bears 6 longitudinal kineties of which 3 on the left (DK_{1-3}) run through the entire length of the cell while the DK_4 begins at the equatorial region. The 2 dorso-marginal rows (DM_{1-2}) are short and extend from the anterior end, terminating at different levels above the equatorial region. Three rows of caudal cirri containing 15-18 cirri are present at the posterior ends of $DK_{1, 2 \text{ and } 4}$ with 3-7 cirri in each row (Fig. 2).

Division morphogenesis

Division morphogenesis of *P. coronata* is essentially similar to that described in the Polish, American, Austrian and French population of *P. weissei* (Jerka-Dziadosz 1965, Jerka-Dziadosz and Frankel 1969, Wirnsberger *et al.* 1985, Fluery *et al.* 1993). However, several minor differences in the process have been recorded in *P. coronata,* which are listed in Table 5.

Morphogenesis in *P. coronata* begins with the kinetosomal proliferation between the LMC and V_1 in the midventral region forming the oral promordium (OP) (Figs. 4, 10). The origin of OP is *de novo* but later basal

Table 2.Parental structures associated with the origin of fronto-ventral transverse cirri primordia in *P. coronata*

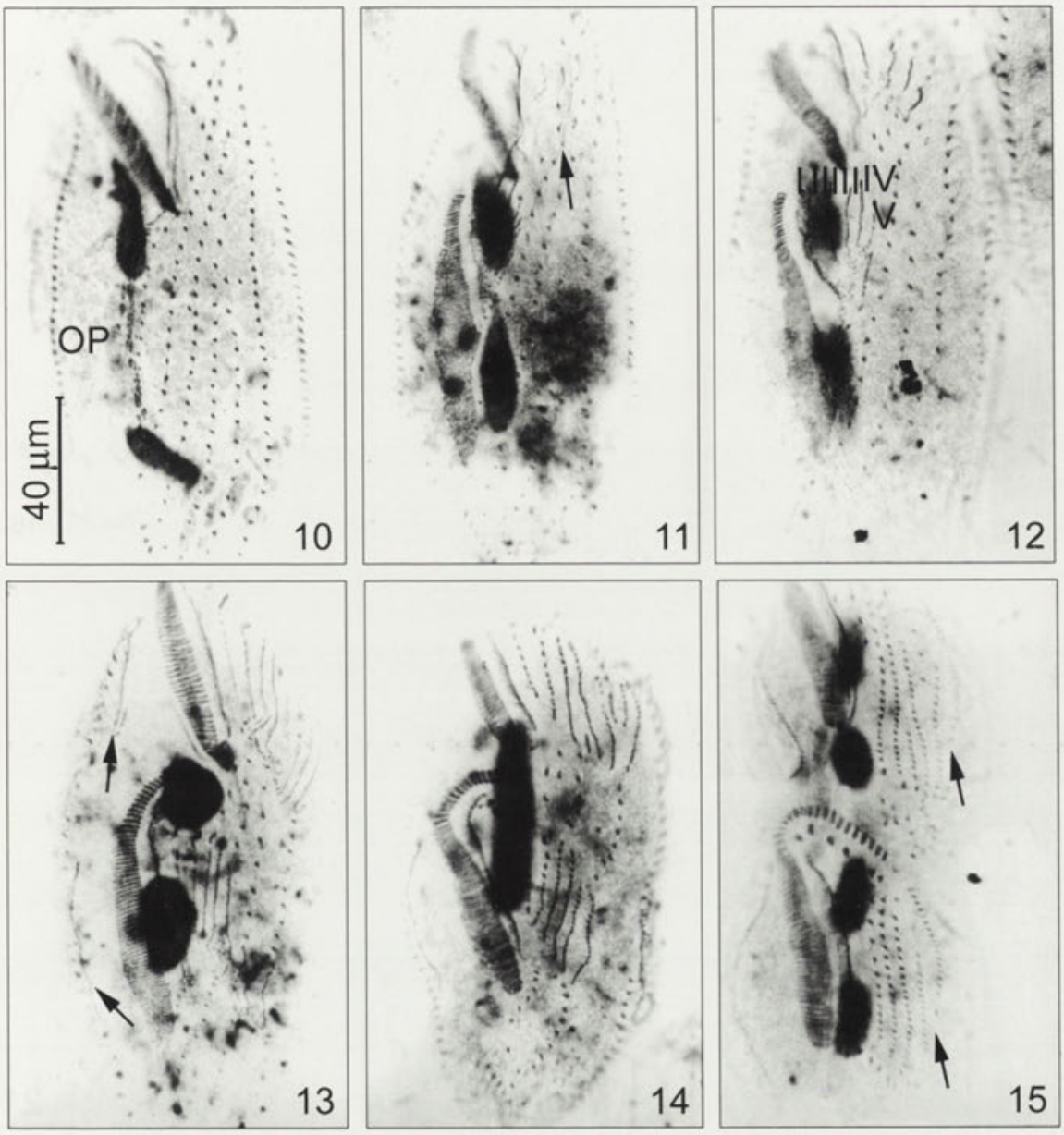
Daughter cell	Primordium number	Parental structure associated with origin of primordium
Proter	I	Parental UM
	II	Frontal cirrus 1
	III – VIII /IX	Ventral cirri
Opisthe	I-III/IV	OP as well as disintegrating ventral cirri
	IV/V – IX	Ventral cirri

Abbreviations: OP - oral promordium, UM - undulating membrane

Table 3. Pattern of cirri formation from the fronto-ventral transverse cirri primordia in *P. coronata*

Primordium number	Cirri formed
I	FC_1
II	FC_2, FC_3, ventral row 1, TC_1
III	FC_4, FC_5, ventral row 2, TC_2
IV	FC_6, FC_7, ventral row, 3, TC_3
V-IX	Ventral rows 4-8, TC_4-TC_8

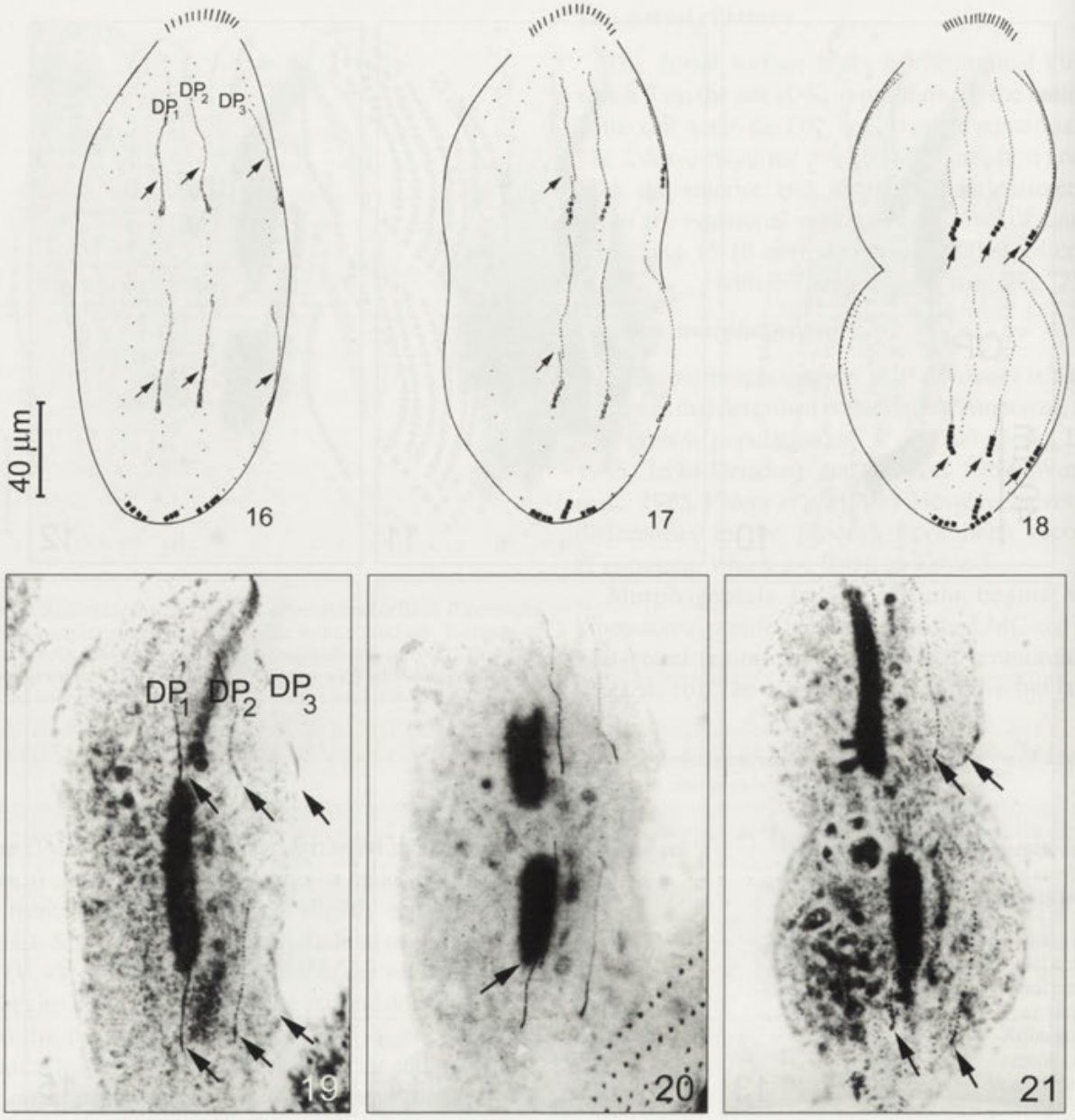
Abbreviations: FC - frontal cirri, TC - transverse cirri



Figs. 10-15. Photomicrographs of protargol impregnated cells of *P. coronata* showing morphogenetic events on the ventral surface (Ref. Figs. 4-9 for description)

bodies from the V_1 and occasionally from the V_2 are incorporated. Subsequently, the membranelles differentiate to form the AZM of the opisthe. While the parental AZM is retained the UMs reorganise to form the new UMs and the frontal cirrus (FC_1) for the proter. The UMs and FC_1 for the opisthe originate in the kinetosomal field near the anterior end of the expanding OP.

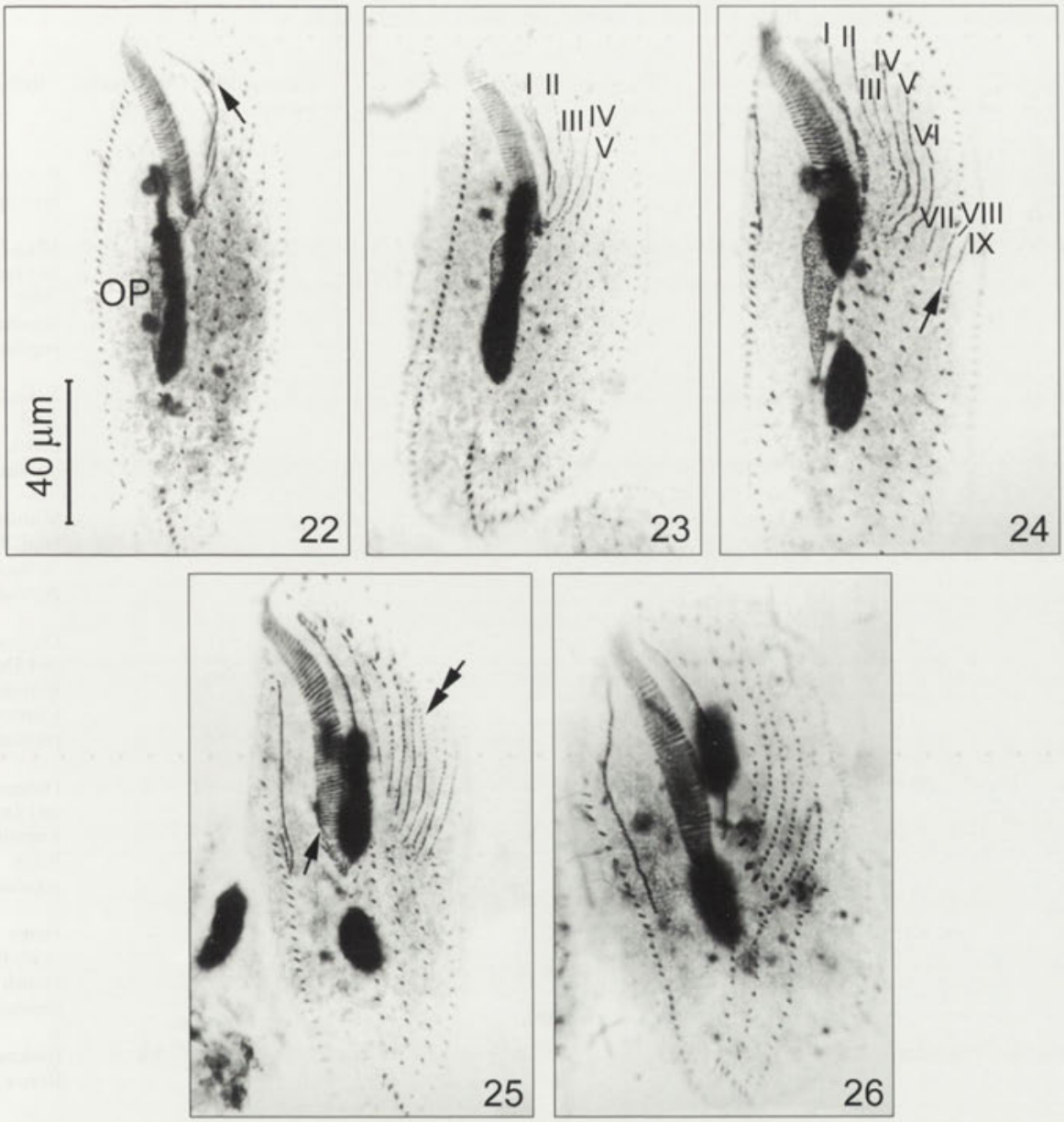
Two sets of 7-10 FVT primordia are formed, one each for the proter and the opisthe. In the proter, the 2nd streak arises as a result of disaggregation of pre-existing frontal cirrus FC_1 (Figs. 5,11). The remaining streaks are formed at the expense of old ventral rows (Figs. 6-8, 12-14). In the opisthe the first 3 and sometimes 4 streaks originate from the expanding OP field (Figs. 6, 12) (Table 2). The



Figs. 16-21. Line diagrams (16-18) and photomicrographs (19-21) of protargol impregnated cells of *P. coronata* showing morphogenetic events on the dorsal surface. 16, 19 - development of DP_{1-3} (arrows) for each daughter cell, 17, 20 - unequal fragmentation of DP_3 to form DK_3 and DK_4 (arrows), 18, 21 - caudal cirri formed by proliferation of 5-6 kinetosomes at the posterior ends of DP_1, DP_2 and DP_4 (arrows). DP - dorsal primordia

remaining streaks develop from the pre-existing ventral cirral rows. Two streaks originate from the 2nd last ventral row (Fig.7). The number of primordia often varies between the primordial sets of proter and the opisthe, resulting in the formation of variable number of ventral rows in the 2 daughter cells. The pattern of cirral differentiation is shown in Table 3.

A pair of marginal streaks (RMCP and LMCP) is formed at two levels in the parental RMC and LMC, one streak each for the proter and opisthe respectively. Four



Figs. 22-26. Photomicrographs of protargol impregnated reorganisers of *P. coronata* revealing changes in the ventral ciliature. 22 - formation of OP and disaggregation of buccal cirrus to form second FVT primordium (arrow). Contribution of kinetosomes by OP to the formation of other FVT primordia; 23 - reorganisation of pre-existing UM, origin of FVT primordia (I-V) from OP and pre-existing ventral rows; 24 - formation of a complete set of FVT primordia (I-IX) and two streaks associated with right most ventral row (arrow); 25 - OP forms membranelles that merge with existing AZM (arrow). Differentiation of cirri in the FVT primordia (double arrow); 26 - reorganised cell. The pre-existing ciliature undergoes resorption

to 6 parental marginal cirri are involved in the formation of each streak. Primordia elongate posteriorly and differentiate into new marginal cirri. Some of the parental marginal cirri are resorbed much later, during cytokinesis (Figs. 7, 8, 13, 14).

Dorsal morphogenesis

Three dorsal primordia (DP_{1-3}) arise for each daughter cell within the parental kineties (DK_{1-3}) (Figs. 16, 19). DP_3 splits into two unequal parts; the smaller component

Table 4. Morphometric comparison of *P. coronata* with other reported species of *Paraurostyla*

Organism	Size (µm)	Frontal cirri, number	Ventral rows, number	Transverse cirri, number	Caudal cirri, number	Dorsal rows, number	Macronuclei, number	Micronuclei, number	Reference
P. coronata	200 x 60	7	6-8	7-10	15-18	6	2	4	Present investigation
P. weissei	-	6	4-6	6-7	18	5	2	4-8	Jerka-Dziadosz and Frankel 1969, American population
P. weissei	200 x 90	8	4-5	8-12	-	5	2	4-5	Jerka-Dziadosz, 1965, Polish population
P. weissei	127 x 48	6	3-5	8	10-18	7	2	4	Wirnsberger *et al.* 1985, Austrian population
P. weissei	190	7	4	7	-	-	-	-	Dragesco and Dragesco-Kernéis 1986, Cameroon population
P. weissei	170	7	4	9	-	-	-	-	Dragesco and Dragesco-Kernéis 1986, Benin population
P. weissei	187 x 47	7	6	6	19	-	-	-	Fleury *et al.* 1993, French population
P. weissei	120-180 x 35-55	7	4	7	-	-	2	3-8	Heckmann 1965, Borror 1972
P. hymenophora	-	6	2	6-7	-	6	2	4	Grimes and L'Hernault 1978
P. dispar	200-250	6-7	4	6-8	-	-	2	2	Kahl 1935
P. viridis	115-175	6	3-4	5	-	-	-	-	Kahl 1935, Foissner *et al.* 1991, Foissner and Berger 1996
P. rubra	-	5	5-6	7-8	-	-	-	-	Kahl 1935

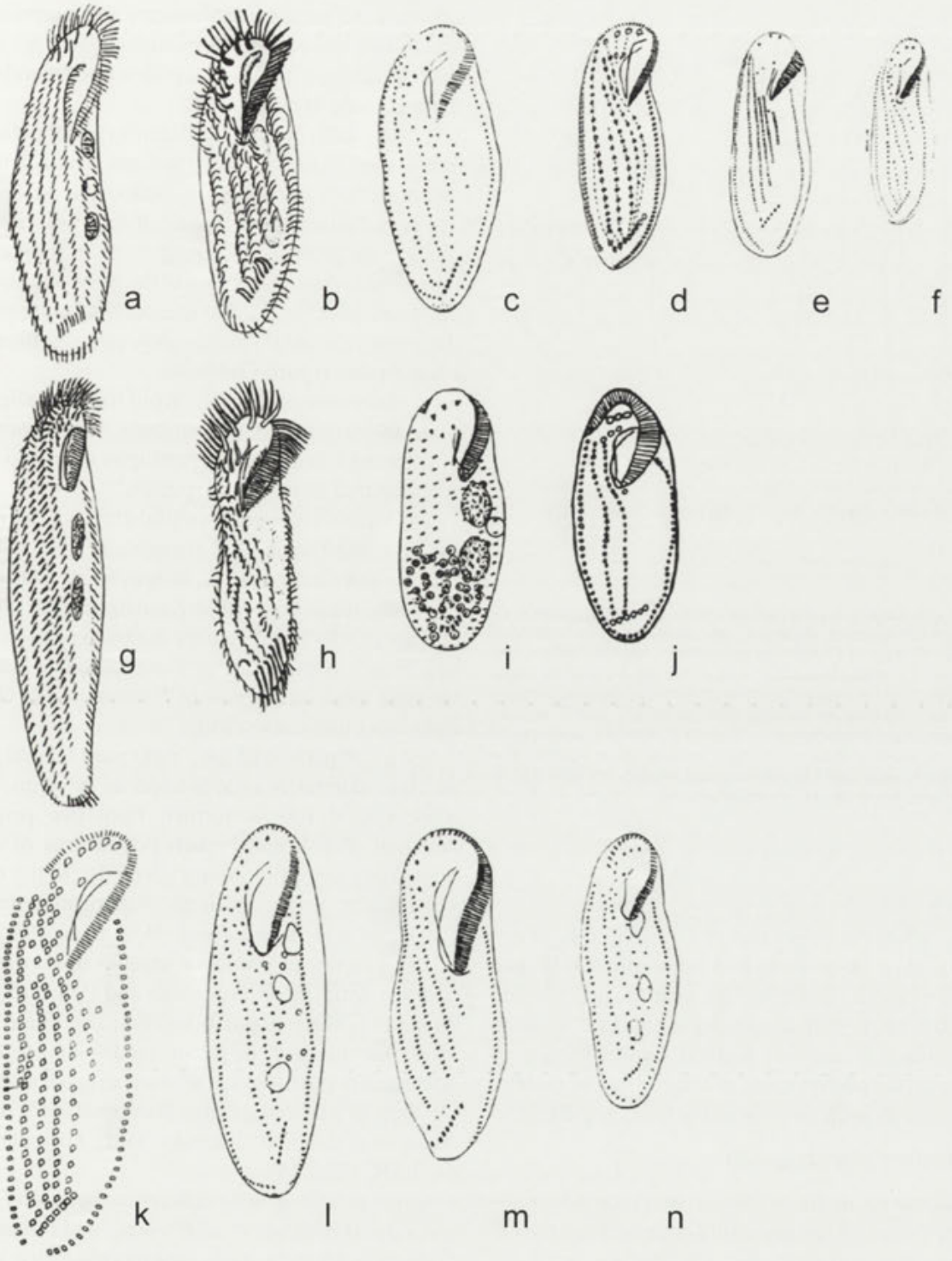


Fig. 27. A comparative description of arrangement of frontal cirri in different species of *Paraurostyla*. In *P. coronata* and African population of *P. weissei* there is a single row of frontal cirri located parallel to the collar region of the AZM and a single buccal cirrus (k-n). In European and American populations of *P. weissei* there are 2 rows of frontal cirri (a-j). a - *P. weissei*, Stein 1859; b - *P. weissei*, Jerka-Dziadosz 1965, Polish population; c - *P. weissei*, Jerka-Dziadosz and Frankel 1969, American population; d - *P. weissei*, Fluery *et al.* 1993, French population; e - *P. weissei*, Borror 1972, American population; f - *P. weissei*, Wirnsberger *et al.* 1985, Austrian population; g - *P. dispar*, Kahl 1935; h - *P. hologama*, Heckman 1965; i - *P. viridis*, Kahl 1935; j - *P. hymenophora*, Grimes and L'Hernault 1978; k - *P. coronata*, present investigation; l - *P. weissei*, Dragesco and Dragesco-Kernéis 1986, Cameroon population; m - *P. weissei*, Dragesco and Dragesco-Kernéis 1986, Benin population; n - *P. weissei*, Dragesco and Dragesco-Kernéis 1986, Cameroon population. The figures have been modified from the original sources a-f x 200, g-n x 300

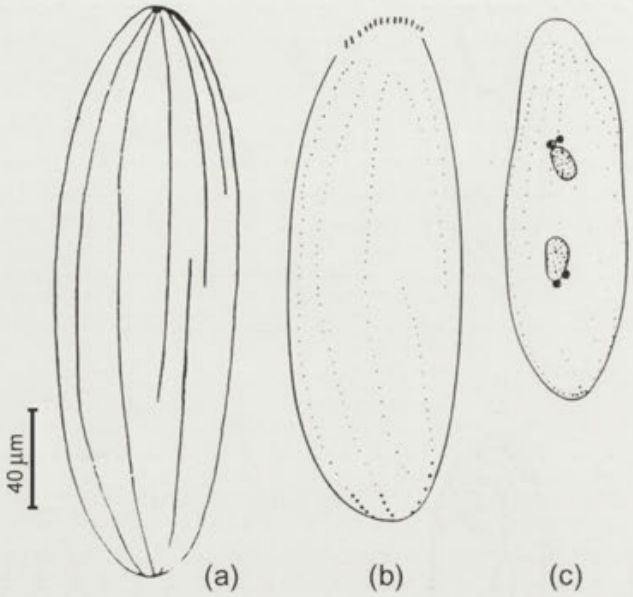


Fig. 28. A comparison of the arrangement of dorsal kineties of *P. coronata* with those of American and Austrian populations of *P. weissei*. a - *P. weissei* (American population, Jerka-Dziadosz and Frankel 1969), 5 dorsal kineties; 3rd and 4th kinety formed by equal splitting of DP_3; b - *P. coronata* (present investigation), 6 dorsal kineties, 3rd and 4th kinety formed by unequal splitting of DP_3; c - *P. weissei* (Austrian population, Wirnsberger *et al.* 1985), 7 dorsal kineties, right most three are formed from the ventral surface (near RMC). The figures have been modified from the original sources and enlarged or reduced in size to bring them to a common scale

forms DK_4 while the larger one gives rise to DK_3 (Figs. 17, 18, 20, 21). Dorso-marginal primordia (DMP_1 and DMP_2) arise on the ventral surface near the anterior end of RMC but later shift to the dorsal surface to form $DM_{1\ and\ 2}$. Caudal cirri are formed by thickening of kinetosome at the posterior end of $DK_{1, 2\ and\ 4}$. The number of caudal cirri in each row vary (Figs. 17, 18, 20, 21).

Reorganization morphogenesis

The process is similar to the division morphogenesis except that a single set of primordia is formed and the OP is smaller. The differentiated OP merges with the reorganising parental AZM (Figs. 22-26).

DISCUSSION

Paraurostyla coronata sp. n. is widely distributed in a variety of habitats in the northern parts of India. The present study on the morphometry and morphogenesis of this ciliate reveals several features which are useful for distinguishing it from the other described members of this genus. These are:

(1) live cells exhibit 2 different types of pigmentation. While most of the organism appears greenish, its anterior and posterior extremities are pink coloured;

(2) a characteristic feature of the FVT cirri arrangement is the presence of a single row of 6 hypertrophied cirri close to the collar region of the AZM and buccal cirrus close to UMs (Fig. 1). A corresponding comparison with the other described species indicates significant differences in this regard (Table 4);

(3) the extent of hypertrophy of frontal is slightly more than the ventral. Consequently, one finds that in *P. coronata* the level of hypertrophy of frontal is far less as compared to the other species;

(4) presence of single ventral cirrus of the row V_1, in the post oral region of *P. coronata* (Fig. 1) is a peculiar feature, not seen in the other species of *Paraurostyla*;

(5) the transverse cirri are arranged in an oblique row and not in a J-shaped manner as observed in the Austrian and French populations of *P. weissei*. The last cirrus of the right most ventral row of *P. coronata* lies close to the right most transverse cirrus;

(6) a comparison of the 2 right most ventral primordia and their derivatives needs a special mention. The right most ventral row is formed from two primordia in Austrian, Polish and French populations of *P. weissei* while only one primordium gives rise to the right most ventral row in *P. coronata*, American population of *P. weissei* and *P. hologama* (Heckmann 1965);

(7) presence of six dorsal kineties in *P. coronata* is in contrast with that of American and Polish (5 DKs) and Austrian (7 DKs) populations (Fig. 28). These variations arise due to their different modes of origin. In the American population of *P. weissei*, an equal splitting of the DP_3 occurs whereas splitting is unequal in *P. coronata*. In Austrian population, 3 streaks (DM_{1-3}) originate close to the RMC (Fig. 28).

Other morphogenetic differences between *P. coronata* and various populations of *P. weissei* and *P. hymenophora* (Table 5) are largely due to their morphometric separation.

Morphometric comparison of *P. coronata* with the other described populations (Table 4) suggests that the African population of *P. weissei* resemble *P. coronata* but in the absence of detailed morphogenetic information for the latter it is difficult to draw any conclusion.

The various species of *P. weissei* described so far differ from each other morphometrically. The reasons for their

Table 5. Morphometric and morphogenetic comparison of *P. coronata* with other described species of *Paraurostyla*

Character	*P. weissei* Austrian population	*P. weissei* American population	*P. weissei* Polish polulation	*P. weissei* French population	*P. hymenophora*	*P. coronata*
Origin of OP	*de novo*	*de novo*	*de novo*	*de novo*	Transverse cirri (TC_6 or TC_7)	*de novo*
Arrangement of transverse cirri	J-shape	In a row	In a row	J-shape	In a row	In a row
No. of dorso-marginal	3	2-3	-	2	-	2
Arrangement of caudal cirri	3-7 cirri in each of $DK_{1,2\ \&\ 4}$	4 cirri in each of $DK_{1,2\ \&4}$	-	4-8 cirri in each of $DK_{1,2\ \&\ 4}$	-	4-8 cirri in each of $DK_{1,2\ \&\ 4}$
Nature of right most ventral row	Composite	Originate from 1 FVT streak	Composite	Composite	Originate from 1 FVT streak	Originate from 1 FVT streak
Number of cirral streaks	8-9	6-7 (5-9)	8	9	6	7-10
Number of frontal cirri involved in streak formation	2	3	-	3	3	1
Number of streaks involved in formation of frontal cirri	4	3	-	3-4	3	4
Differentiation pattern of frontal cirri	1, 2, 2, 1	1, 2, 3	-	1, 2, 2, 2	2, 2, 2	1, 2, 2, 2
Number of streaks involved in ventral row formation	6-7	4-5	-	6	2	6-8

Abbreviations: DK - parental kineties, FVT - fronto-ventral transverse cirri, OP - oral primordium

clubbing into a single species are questionable. In our opinion, the *P. weissei* complex should be separated into two groups on the basis of number, arrangement and degree of hypertrophy of frontal cirri since it is one of the stable and distinguishing characters of the genus (Fig. 27).

Acknowledgement. Authors wish to express their sincere gratitude to the anonymous reviewer of this paper who gave his forthright constructive suggestions for improvement and modification of the manuscript. The work was supported by the Junior and Senior Research Fellowship to S.A. from the University Grants Commission.

REFERENCES

Ammermann D., Steinbrück G., Von Berger L., Hennig W. (1974) The development of the macronucleus in the ciliated protozoan *Stylonychia mytilus*. *Chromosoma* **45:** 401-429

Borror A.C. (1972) Revision of the order Hypotrichida (Ciliophora, Protozoa). *J. Protozool.* **19:** 1-23

Borror A.C. (1979) Redefinition of the Urostylidae (Ciliophora, Hypotrichida) on the basis of morphogenetic characters. *J. Protozool.* **26:** 544-550

Dragesco J., Dragesco-Kérneis A. (1986) Cilies libres de l'Afrique intertropicale. *Faune trop.* **26:** 435-438

Fluery A., Guyander H.L., Iftode F., Laurent M., Bornens M. (1993) A scaffold for basal body patterning revealed by a monoclonal

antibody in the hypotrich ciliate *Paraurostyla weissei*. *Dev. Biol.* **157:** 285-302

Foisnner W., Berger H. (1996) A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes and waste waters, with notes on their ecology. *Fresh Wat. Biol.* **35:** 375-482

Foissner W., Blatterer H., Berger H., Kohmann F. (1991) Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems - Band I. Cyrtophorida, Oligotrichida, Hypotrichia, Colpodea - Informationsberichte des Bayer. *Landesamtes Wasswirt.* **1/91:** 258-264

Grimes G.W., L'Hernault S.W. (1978) The structure and morphogenesis of the ventral ciliature in *Paraurostyla hymenophora*. *J. Protozool.* **25:** 65-74

Heckmann V. K. (1965) Totale Konjugation bei *Urostyla hologama* n. sp. *Arch. Protistenk.* **108:** S55-62

Jerka-Dziadosz M. (1965) Morphogenesis of ciliature in division of *Urostyla weissei* (Stein) *Acta Protozool.* **3:** 345-353

Jerka-Dziadosz M., Frankel J. (1969) An analysis of the formation of ciliary primordia in the hypotrich ciliate *Urostyla weissei*. *J. Protozool.* **16:** 612-637

Kahl A. (1935) Urtiere oder Protozoa I: Wimpertiere order Ciliata (Infusoria) 4 Peritricha und Chonotricha. *Tierwelt Dtl.* **30:** 564-568

Kamra K., Sapra G. R. (1990) Partial retention of parental ciliature during morphogenesis of the ciliate *Coniculostomum monilata* (Dragesco and Njiné, 1971). Njiné, 1978 (Oxytrichidae, Hypotrichida). *Eur. J. Protistol.* **25:** 264-278

Stein F. (1859) Der Organismus der Infusionsthiere nach eigenen Forschungen in systematischer Reithenfolge bearbeitet I. Abtheilung. Algemeiner Theil und Naturges chichchte der hypotrichen Infusionsthiere. W. Engelmann, Leipzing, 206

Wirnsberger E., Foissner W., Adam H. (1985) Cortical patterns in non-dividers, dividers and reorganizers of an Austrian population of *Paraurostyla weissei* (Ciliophora, Hypotrichida): A comparative morphological and biometrical study. *Zool. scripta* **14:** 1-10

Received on 15th June, 1998; accepted on 15th January, 1999

Acta Protozool. (1999) 38: 145 - 153

ACTA
PROTOZOOLOGICA

Ortholinea gadusiae sp. n. and *Sphaeromyxa opisthopterae* sp. n. (Myxozoa: Myxosporea) from the Clupeid Fish of the Bay of Bengal, West Bengal, India

Nirmal K. SARKAR

Department of Zoology, Rishi Bankim Chandra College, Naihati, West Bengal, India

Summary. Two new Myxosporean (Myxozoa) species *Ortholinea gadusiae* sp. n. (Ortholineidae Lom and Noble, 1984) and *Sphaeromyxa opisthopterae* sp. n. (Sphaeromyxidae Lom and Noble, 1984) have been described from the urinary bladder of *Gadusia chapra* (Hamilton-Buchanan) and gall bladder of *Opisthopterus tardoore* (Cuvier) respectively from the coastal water of the Bay of Bengal near Digha, West Bengal, India.

Key words: Bay of Bengal, Clupeidae, gall bladder, Myxosporea, *Ortholinea gadusiae* sp. n., *Sphaeromyxa opisthopterae* sp. n., urinary bladder.

INTRODUCTION

The Clupeids (Teleostei) are highly palatable food fish available throughout India during June to November of the year. These fish are often parasitised by myxosporean protozoa (Chakravarty 1943, Sarkar and Mazumder 1983, Sarkar 1984) in the gall bladder, urinary bladder and kidney tubules. During this investigation two new myxosporeans of the genera *Sphaeromyxa* Thelohan, 1892 and *Ortholinea* Shulman, 1962 has been isolated, studied and described in this paper.

Address for correspondence: Nirmal K. Sarkar, Department of Zoology, Rishi Bankim Chandra College, Naihati 743165, West Bengal, India

MATERIALS AND METHODS

The necropsies were done of frozen fish (Clupeidae), collected from the landing places of coastal water of the Bay of Bengal near Digha, West Bengal, India. The myxosporean parasites were studied in detail at 1500x from wet smears treated with Lugol's iodine solution and from dry smears stained with Giemsa after fixation in absolute methanol. Various concentrations of potassium hydroxide solutions (2-10%) and a saturated solution of urea were used to extrude the polar filaments of the spores. The India ink method (Lom and Vavra 1963) was employed to detect the mucous envelope of the spore. The illustrations were drawn

with the aid of a camera lucida. The description of the myxosporeans follows the recommendations of Lom and Arthur (1989).

OBSERVATIONS

Ortholinea gadusiae sp. n. (Figs. 1 A-J)

Large number of plasmodia were observed in the content of the urinary bladder. These plasmodia showed various type of shapes and sizes. The shapes of the plasmodia were oval, spherical or elongated rectangular while the sizes varied from 24.0 x 14.0 to 57.5 x 37.5 µm with an average of 31.75 x 20.0 µm.

The mature spores were oval, spherical or triangular in shape. The anterior ends of the spores were broad, flat or slightly convex while the posterior ends were narrow and round. The suture was thin, and bent but never sinuous. The ends of the spore were always round. The two shell-valves were smooth and symmetrical and had an incon-

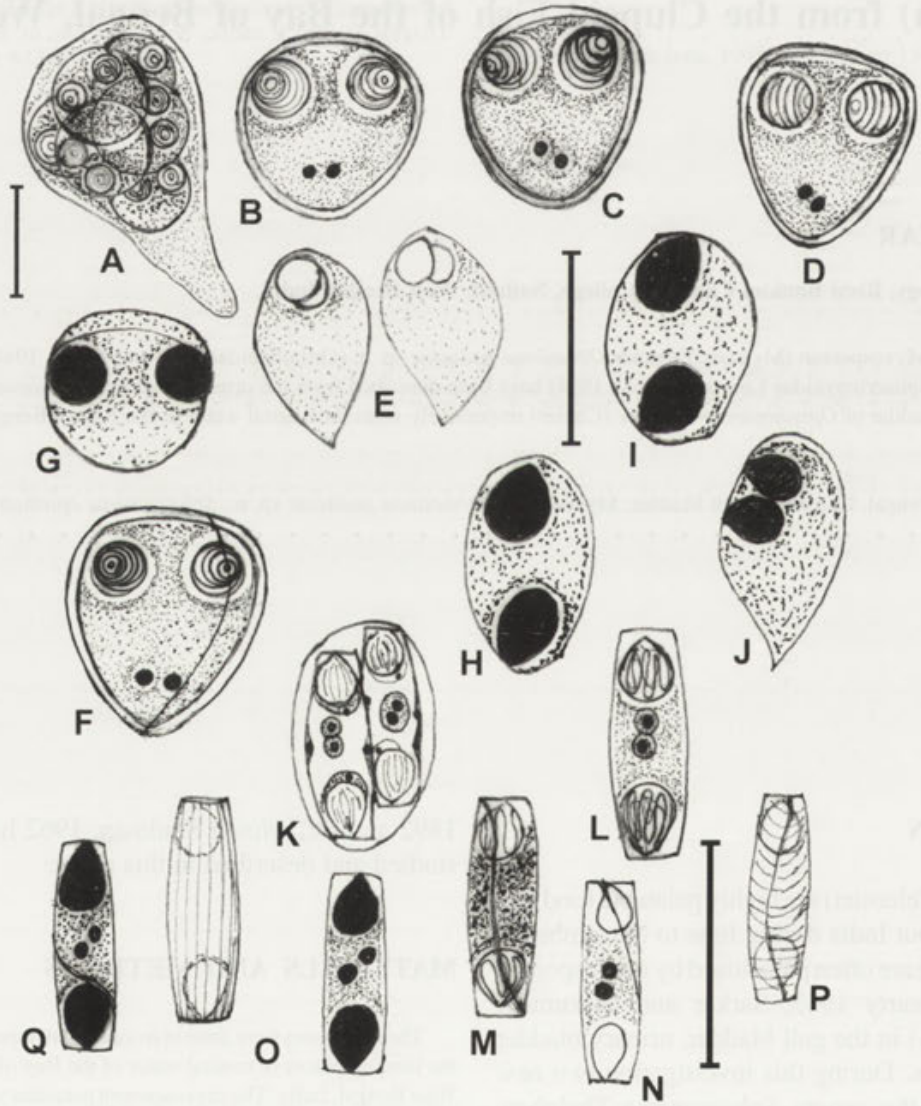


Fig. 1. Plasmodium and spores. A-J - plasmodium and spores of *Ortholinea gadusiae* sp. n. A - polysporic plasmodium, B-D - spores in valvular view, E - spores in side view, F - spore in oblique view showing thin and bent suture, G - spore in sutural view, H, I - spores in top view, J - abnormal spore, K-Q - plasmodium and spores of *Sphaeromyxa opisthopterae* sp. n., K - developing disporic plasmodium, L - spore in valvular view, M, N - spores in sutural view, O - spore with longitudinally striated shellvalve, P - spore with suture and lateral ornamentations of shellvalve, Q - spore with suture across the width. A-F and K-O - Lugol's iodine; G-J and P, Q - Giemsa. Scale bars - 10 µm

Table 1. Morphometric comparison of the present species with the other *Ortholinea* spp.

Parasite [Host]	Plasmodium	Spore	Polar capsule	Infection locus	Locality
Ortholinea divergens (Thelohan, 1895) Shulman, 1962 [*Blennius pholis* L., *Crenilabrus melops*]	A few spores in each plasmodium, surface with small protubarances; 65.0 x 55.0 µm	Round to oval, shellvalve with faint striations 10.0 x 12.0x 8.0 µm	Pyriform, openings widely separated, 4.0 µm long	K / UB	Mt/AtO, PcO
O. polymorpha (Davis, 1917) Lom & Dykova, 1992 [*Opsanus tau*]	Elongated, polysporic, lobose pseudopodium; 20.0-40.0 x 50.0-80.0 µm	Subspherical with ridges 6.5 x 11.0 µm in diameter	Pyriform 4.4 x 2.4 µm; 3-6 turns of polar filament	UB	Marine, off Beaufort region, USA
O. orientalis (Shulman & Shulman-Albova, 1953) Shulman, 1966 [*Clupea harengus, Eleginus navaga*]	Round or irregular with lobopodia; 2-10 spores in each plasmodium	Round or oval with one end pointed, shell-valve smooth, 7.5-8.5 µm in diam.	Subspherical, almost touching each other; 2.2-3.0 µm or 3.0-4.2 µm or 8.0-11.5 µm	UB	PcO, W. S.
O. gobiusi Naidenova, 1968 [*Gobius ophiocephalus*] cited from Lom & Dykova, 1992	Disporic	Ovoid, posteriorly pointed, 20-30 fine ridges; 8.8 x 7.1 µm	Spherical 1.9 µm in diam.	UB	Black Sea
O. umdulans (Meglitsch, 1970) Aurther & Lom, 1985 [*Caulopsetta scapha*]	Round, one to many lobopods, polysporic	Spherical or subspherical suture undulated, 20 fine striations; 8.3 x 7.4 x 6.3 µm	Pyriform with wide neck, distinct polar filament; 2.9 x 2.2 µm	UB	Marine off Wellington & Nepier, New Zealand
O. *irregularis* (Kabata,1962) Arthur & Lom, 1985 [*Drepanopsetta platissoides* Malmgreen]	Subglobular or oval, with or without pseudopodium; 15.0-20.0 x 20.0-25.0 µm, disporic	Pyriform or highly irregular, suture more sinuous, shellvalve smooth and unornamented, widest diam., at the centre of the longitudinal axis; 8.0-11.0 x 6.0-9.0 µm	Round, axis of capsule parallel, 2.2 µm in diam.	UB	North Sea
O. australis Lom *et al.* 1992 [*Acanthopagrus australis*]	Round, flattened, polysporic; 10.0 x 60.0 µm (rarely 1 mm)	Subcircular, suture with circular and 5-9 longitudinal ridges; 8.7-8.0 x 6.2-7.3 µm	Oval, anteriorly pointed, 3-4 turns of polar filament; 3.7 x 2.9 µm	GB	Marine, N.S.W.

Table 1 (con.)

O. striateculus Su & White, 1994 [*Leptatherina presebyteroides*]	Unknown	Subspherical or oval, sutural ridge straight, 18-20 striations on shellvalve; 10.1 x 10.0 µm	Pyriform, equal, intwrcapsular ridge; 5-7 turns of polar filament; 3.5 x 2.5 µm	UT	Marine, off Tasmania, Australia
O. percotti (Dogiel & Achmerov, 1960) Aurther & Lom, 1985 [*Percottus glehni*]	Small, 0.5 mm (largest) polysporic	Round, flattened anteriorly, suture broad, thick with a constriction at the centre	Oval or spherical 3.0 µm in diam.	GL FN	Amur River
O. indica Sarkar, 1997 [*Microspinosa cuja* (Ham.)] (in press)	Variable in shape, pansporoblast formation disporic and polysporic	Elongated oval or egg shaped suture thick; 7.38 x 6.17µm	Spherical, or oval, open inopposite direction, 3-4 turns polar filament	UB	Sundarban Biosphere
O. fluviatilis Lom & Dykova 1995 [*Tetradon fluviatilis*]	Monosporic and polysporic, surface with curved sinuous villi-like projections, squat shaped, 20 µm elongated, 11.0 x 50.0 µm	Anterior end flat, wide and posterior end narrow, 8.3 x 7.8 µm; suture slightly undulated; shellvalve with ridges	Subspherical 4-6 turns of polar filament, 3.1 µm (2.8-3.3 µm)	UT	South East Asia
O. gadusiae sp. n. [*Gadusia chapra*]	Oval or triangular with one lobopodium, polysporic; 31.75 x 20.0 µm	Spherical or oval, no striations; 10.8 x 9.2 x 8.0 µm	Spherical, equal (rarely uneuql), 4-6 turns of polar filament; 3.0 µm or 2.8 x 2.0 µm	UB	Marine, the Bay of Bengal, India

Abbreviations: AtO - Atlantic Ocean, FN - fin, GB - gall bladder, GL - gill, K - kidney, Mt - Mediteranean Sea, N. S. W. - New South Wells, PcO - Pacific Ocean, UB - urinary bladder, UT - ureter, W.S. - White Sea

spicuous longitudinal ridge in the middle. A pair of polar capsules was found at the broader end. These capsules were round and equal in the valvular view but the capsules appeared unequal in sutural view due to their reverse opening. The each capsule had 4-5 coils of polar filament. The extra-capsular spore cavity was filled with fine granular mass of sporoplasm containing one or two nuclei. The sporoplasm was devoid of iodinophilous vacuole. There was no mucous envelope around the spore.

Measurements: based on 50 fresh spores from frozen hosts (treated with Lugol's iodine), range given with mean value in the parentheses (in µm).

Spore: length 9.0-11.7 (10.8), width 9.0-9.9 (9.2), thickness 7.2-9.0 (8.0).

Polar capsule: diameter 2.3-3.2 (3.0) or length 1.8-3.0 (2.8), width 1.0-2.5 (2.0)

Site of infection: urinary bladder.

Incidence: 1/28 (3.6 %).

Pathogenicity: not apparent.

Host: *Gadusia chapra* (Cuvier).

Period of infection: September - October, 1997.

Locality: coastal water of the Bay of Bengal near Digha, West Bengal, India.

Material: syntypes on slide No. MXOL - 10, stained with Giemsa; deposited to the Department of Zoology, Rishi Bankim Chandra College, Naihati, West Bengal, India.

Sphaeromyxa opisthopterae sp. n. (Figs. 1 K-Q)

Trophic forms or plasmodia were observed in the bile of the gall bladder. These plasmodia were small, oval to spherical in shape. The largest plasmodium was 50.0 x

Table 2. Morphometric comparison of *Sphaeromyxa opisthopterae* sp. n. with its related *Sphaeromyxa* spp.

Parasite [Host]	Plasmodium	Spore	Polar capsule	Site of infection	Locality
Sphaeromyxa balbiani Thelohan, 1892 [*Motella tricirrata*]	Flattened, discform; 3.0-4.0 mm (largest)	Fusiform with truncate ends, longitudinally striated shell valve; 15.0 x 5.0 µm	Broadly pyriform; 7.0 x 4.7 µm	GB	Roscoff Napoli Beaufort
S. sabrazesi Lavarel & Mesnil, 1900 [*Hippocampus brevarostris*]	Disc form, lobose pseudopodia, polysporic; 2.0 mm in diam.	Cylindrical, bent in arch form, truncated; smooth shellvalve or with faint striations; 28.0 x 4.3 µm	Cylindrical; 9.0-10.0 x 3.0 µm	GB	Mt Sea
S. minuta Polyanski, 1955 [*Hippocampus hippoglossus*]	Unknown	Straight, cylindrical, no widening at the middle, truncate ends; 13.57-16.2 x 2.7-3.6 µm	Pyriform; 2.7-4.5 µm	GB	Bt Sea
S. magna Zukov, 1964 [*Liparis gibbus*]	Cookie shaped, 4.0 mm (largest)	Straight, no widening in the middle, truncate ends; shellvalve with ridgbed surface; 23.0 x 6.4 µm	Oval, 8.5 x 4.0 µm	GB	PcO, Bt Sea
S. nesogobii Su & White, 1994 [*Nesogobius* sp.]	Unknown	Elongate, fusiform, arcute, round to truncate ends; shellvalve smooth; 20.0-23.7 µm (21.7 µm) x 6.3-7.9 µm (6.6 µm)	Pyriform; 6.8-7.9 µm (7.6 µm) x 3.1-3.3 µm (3.2 µm)	GB	Off Tasmania, Australia
S. opisthopterae sp.n. [*Opisthopterus tardoore*, Cuvier]	Irregular, mostly oval to spherical; 48.0 x 18.0 µm (largest)	Elongated funsiform with sharply truncated ends, suture elongated 'S'-shaped, 6-8 longitudinal striations, shellvalve ornamented with horizontal ridges in relation to suture; 9.0-12.74 µm (11.5 µm) x 3.3-4.5 µm (3.9 µm)	Oval to broadly pyriform; 2.63-3.8 µm (3.6 µm) x 1.8-3.0 µm (2.8 µm)	GB	The Bay of Bengal (coastal water)

Abbreviations: Bt - Barents Sea, GB - gall bladder, Mt - Mediteranean Sea, PcO - Pacific Ocean

Table 3. A comparative study of the present species with the other *Sphaeromyxa* spp. recorded from India.

Parasite [Host]	Plasmodium	Spore	Polar capsule	Infection site	Locality
Sphaeromyxa theraponi Tripathi, 1951 [*Therapon jurboa*]	Unknown	Arched, suture more or less 'S'- shaped-shell, valve thin and smooth, no sutural ridge; 19.8 x 5.4 µm	Small, pyriform; 7.2 x 3.7 µm	GB	Canning, South 24 Parganas, West Bengal, India
S. pultai Tripathi, 1951 [*Odontamblyopus rubicandus*]	Circular, mono or disporous; 12.6-13.9 µm and 19.0-27.3 µm	Long, slightly truncated ends, shellvalve thin and smooth, suture almost parallel to longitudinal axis of the spore; no sutural ridge; 28.8-30.0 x 5.0-5.5 µm	Long, pyriform and truncated ends	GB	Hooghly River, North-24 Parganas, West Bengal, India
S. dighae Sarkar & Majumder, 1983 [*Hitsa ilisha*]	Disporous	Large and broad, bent in the middle (140° angle), shellvalve smooth with terminal bend, arched in sutural view; 23.82 x 3.33 µm	Ellipsoidal, subterminal; polar filament ribbon like, 4-5 coils; 8.43 x 2.32 µm	GB	Digha, coastal water of the Bay of Bengal, West Bengal, India
S. hareni Sarkar, 1984 [*Tachysurus platystomus* (Day)]	Unknown	Nearly fusiform, bent, elongated 'S'-shaped suture, shellvalve smooth; 27.55 x 7.8 x 5.14 µm	Ovoid to ellipsoidal, coiling of the polar filament parallel to longitudinal axis of spore; 9.34 x 4.3 µm	GB	Digha, coastal water of the Bay of Bengal, West Bengal, India
S. ganapatii Kalvati & Baidehi, 1991 [*Therapon jarboa*]	Pansporoblast formation; 20.8-22.6 x 9.6-10.4 µm	Crescent shaped, rounded ends, sutural ridge not distinct; shellvalve smooth; 17.5 x 4.8 µm (16-19 x 4-4.8 µm)	Pyriform with drawn out anterior ends, 5-6 coils of polar filament; 5.6 x 1.68 µm (4.0-6.4 x 1.6-1.8 µm)	GB	Chilka Lake, Oryssa, India

Table 3 (con.)

S. opisthopterae sp. n. [*Opisthopterus tardoore,* Cuvier]	Irregular, mostly oval to spherical; 48.9 x 18.0 µm (largest)	Elongated fusiform with sharply truncated ends, suture elongated 'S' - shaped, 6-8 longitudinal striations, shell valve ornamented with horizontal ridges in relation to suture; 9.0-12.74 µm (11.5 µm) x 3.3-4.5 µm (3.9 µm)	Oval to broadly pyriform; 2.63-3.8 µm (3.6 µm) x 1.8-3.0 µm (2.8 µm)	GB	The Bay of Bengal (coastal water) West Bengal, India

Abbreviation: GB - gall bladder

20.0 µm in dimension (mean 31.75 x 14.75 µm). These were disporic and polysporic in nature.

The mature spores were small, elongated or almost fusiform with sharply truncated ends. The suture was very thin, slightly ridged and elongated 'S' - shaped. The two shellvalves were symmetrical and longitudinally striated. Besides, each shellvalve had much faint ornamentation. The two polar capsules were almost oval or broadly pyriform, situated one on either ends of the spore. The polar filament in each capsule was folded longitudinally. The extra-capsular spore cavity was filled with monokaryotic or dikaryotic sporoplasm. There was no iodinophilous vacuole in the sporoplasm and no mucous envelope around the spore.

Measurements: based on 30 fresh spores (treated with Lugol's iodine) from frozen hosts; range given with mean in the parentheses (in µm).

Spore: length 9.0-12.74 (11.5), width 3.28-4.55 (3.9).

Polar capsule: length 2.63-3.82 (3.6), width 1.8-3.0 (2.82).

Site of infection: gall bladder.

Incidence: 3/28 (10.1%)

Pathogenicity: not apparent.

Host: *Opisthopterus tardoore* (Cuvier) - Clupeidae.

Period of infection: November - December, 1997.

Locality: coastal water of the Bay of Bengal near Digha, West Bengal, India.

Material: syntypes on slide No. MXSRX - 23, stained with Giemsa; deposited to the Department of Zoology, Rishi Bankim Chandra College, Naihati, West Bengal, India.

DISCUSSION

Shulman (1962) transferred the then known myxosporean species *Sphaerospora divergens* Thelohan, 1895 to a new genus *Ortholinea* and considered *Ortholinea divergens* (Thelohan, 1895) as type species. The diagnostic features of the genus *Ortholinea* are mono to polysporic plasmodium, spherical or subspherical spore, anteriorly flattened; straight suture parallel to the sutural plane, pointed posteriorly; subspherical to pyriform polar capsules; coelozoic in the urinary system of marine fish (Lom and Noble 1984). Since most of the above features conform with the features of the first myxosporean species presented here, the species is assigned to the genus *Ortholinea* Shulman, 1962.

This *Ortholinea* genus includes 10 species to-date (Shulman 1962 1966; Naidenova 1968; Arthur and Lom 1985; Lom and Dykova 1992, 1995; Lom *et al.* 1992; Su and White 1994; Sarkar in press). The present *Ortholinea* sp. show similarity either in shape or in size of its spore with the spores of *Ortholinea divergens* (Thelohan, 1895) Shulman 1962, *O. polymorpha* (Davis, 1917) Lom and Dykova 1992, *O. undulans* (Meglitsch 1970) Arthur and Lom 1985, *O. gobiusi* Naidenova 1968, *O. australis* Lom *et al.* 1992. However, the smooth surface of shellvalve of the spore of the present species is different from the spore of the above mentioned *Ortholinea* spp. having ridged or faintly striated surface of the shellvalve. Moreover, the spores of *O. orientalis* (Shulman and Shulman-Albova, 1953) Shulman 1966 (dimension of spore - 7.5 x 8.5 µm, polar

capsules - 2.2-3.0 µm), and *O. fluviatilis* Lom and Dykova, 1995 (dimension of spore 8.3 x 7.8 µm, polar capsules 2.8-3.3 µm) are smaller than the dimensions of the spores of the present species. Further, the spore of *O. irregularis* (Kabata, 1962) Arthur and Lom, 1985 (dimension of spore 8.0-11.0 x 6.0-9.0 µm) is irregular in shape and its sinuous suture is different from definite shape and bent suture of the present species. The other similar species viz., *O. australis* Lom *et al.* 1992 and *O. striateculus* Su and White 1994 also have spores with slightly sinuous suture and larger dimensions of spore and therefore, are different from the bent suture and smaller dimensions of the spore of the present *Ortholinea* sp. The present species is also different from the only Indian species i.e., *Ortholinea indica* Sarkar, (in press) by larger dimensions of the plasmodium, spore and polar capsule. The present species, is, therefore, considered as new species and designated as *Ortholinea gadusiae* sp. n. after the name of its host.

The other myxosporean species obtained from the gallbladder of *Opisthopterus tardoore* is placed under *Sphaeromyxa* Thelohan, 1892 as this species possesses salient features of *Sphaeromyxa* such as fusiform spore with truncated or sliced ends, polar filament short and thick and coelozoic in the gall bladder of marine fish (Lom and Noble 1984). Of the thirty one species of *Sphaeromyxa* Thelohan 1892, (Lom and Dykova 1992, Su and White 1994), the spores of *S. balbiani* Thelohan, 1892, *S. sabrazesi* Lavaren and Mesnil 1900 (cited from Lom and Dykova 1992), *S. minuta* Polyanski 1955, and *S. magna* Zukov 1964 show similarity with the spore of the present species by their truncated ends and by the site of infection (gall bladder). However, all the above mentioned *Sphaeromyxa* spp. have much larger plasmodia and spores than that of the present *Sphaeromyxa* sp. Moreover, the spore of *S. sabrazesi* is bent on its long axis. Hence the present species is distinct from the other species mentioned above. Furthermore, five *Sphaeromyxa* spp. are known to-date from India. These are *S. theraponi* Tripathi 1951, *S. pultai* Tripathi 1951, *S. dighae* Sarkar and Mazumder 1983, *S. hareni* Sarkar 1984 and *S. ganapatti* Kalavati and Baidehi 1991. But none of these have sharply truncated ends of the spores and therefore are different from the present species. Again, a recently described species *Sphaeromyxa nesogobi* Su and White, 1994 shows similarity in the ends of the spore of the species in study. However, the ends are bluntly truncated in former species while the ends are sharply truncated in the present species. Moreover, the spore of *S. nesogobi* is larger in dimension (21.7 x 6.6 µm). Therefore, *Sphaeromyxa* sp. in study is considered as a new species and designated as *Sphaeromyxa opisthopterae* sp. n. after the name of its host.

Acknowledgements. Author is grateful to the Teacher-in-Charge, Dr. A.K. Roy and Prof. S. Roy Chaudhury, H.O.D. of Zoology, Rishi Bankim Chandra College, Naihati, West Bengal, India for Laboratory facilities. Thanks are due to Dr. K. Goswami and authorities of the Computer Section for their cooperation.

REFERENCES

Arthur J.R., Lom J. (1985) *Sphaerospora arii* n. sp. (Mysoxporea: Sphaerosporidae) from the kidney of a longnose skate (*Raja rhina* Jordon and Gilbert) from the Pacific Ocean off Canada. *Can. J. Zool.* **63:** 2902-2906

Chakravarty M. (1943) Studies on Myxosporidia from common food fishes of Bengal. *Proc. Indian Acad. Sci.* **18B:** 21-35

Davis H.S. (1917) The Myxosporidia of the Beaufort region. A systematic and biologic study. *Bull. Fish.* **35:** 199-244

Kalavati C., Baidehi J. (1991) Two new species of Myxosporidians from fishes of Chilka Lake: genus *Sphaeromyxa* Thelohan and genus *Zschokkella* Auerbach. *U. P. J. Zool.* **11:** 146-150

Kabata Z. (1962) Five new myxosporidia from marine fishes. *Parasitology* **52:** 172-186

Lavaren A., Mesnil F. (1900) Sur une Myxosporidie des voies biluaires del 'Hippocampo (*Sphaeromyxa sabrazesi* nov. sp.). *C. R. Soc. Biol.* **52:** 380-382

Lom J., Vavra J. (1963) Mucous envelops of spores of the Subphylum Cnidospora (Doflein, 1901). *Vest. Cs. Spol. Zool.* **27:** 4-6

Lom J., Noble E.R. (1984) Revised classification of the class Myxosporea Bütschli, 1881. *Folia Parasitol.* **31:** 193-205

Lom J., Arthur J.R. (1989) A guideline for the preparation of species descriptions in Myxosporea. *J. F. Dis.* **12:** 151-156

Lom J., Dykova I. (1992) Protozoan Parasites of Fishes. Elsevier Science Publication, Netherlands

Lom J., Dykova I. (1995) New species of the genera *Zschokkella* and *Ortholinea* (Myxozoa) from the South - east Asian teleost fish *Tetradon fluviatilis*. *Folia Parasitol.* **42:** 161-168

Lom J. Rohde K., Dykova I. (1992) Studies on protozoan parasites of Australian fishes. I. New species of the genera *Coccomyxa* Leger et Hesse 1907, *Ortholinea* Shulman, 1962 and *Kudoa* Meglitsch 1947 (Myxozoa: Myxosporea). *Folia Parasitol.* **39:** 289-306

Meglitsch P.A. (1970) Some coelozoic Myxosporidia from New Zeland fishes, family Sphaerosporidae. *J. Protozool.* **17:** 112- 115

Naidenova N.N. (1968) *Ortholinea gobiusi* n. sp. from *Gobius ophiocephalus* of the Black Sea. *Biol. Morya* **14:** 60-62 (in Russian)

Polyanski Yu.I. (1955) Data on fish parasitology of the northern Seas of USSR. Fish parasites of Barents Sea. *Trudy Zool. Inst. ANSSSR* **19:** 5-170 (in Russian)

Sarkar N. K. (1984) A new myxosporidian *Sphaeromyxa hareni* sp. n. (Myxozoa: Myxidiidae) from an Indian marine teleost *Tachysurus platystomus* (Day). *Acta Protozool.* **23:** 183-186

Sarkar N.K. (0000) Some new Myxosporidia (Myxozoa: Myxosporea) of the genera *Myxobolus* Butschli, 1882, *Unicapsula* Davis 1924, *Kudoa* Meglitsch 1947, *Ortholinea* Schulman 1962 and *Neoparvicapsula* Gaevskaya, Kovaleva and Shulman 1982. *Proc. Zool. Soc. Calcutta* (in press)

Sarkar N. K., Mazumder S. (1983) Myxosporidian *Sphaeromyxa dighae* n. sp. (Myxozoa: Myxidiidae) from the gallbladder of *Hilsa Ilisha* (Clupeidae). *Acta Protozool.* **22:** 257-260

Shulman S.S. (1962) Myxosporidia. In: Opredelitel Parazitov Presnovodnyh Ryb SSSR. Izd. ANSSSR, Moskow, Leningrad 47-130 (in Russian)

Shulman S.S. (1966) Myxosporidian Fauna of the SSSR. Emerind Publisher, New Delhi. 631 (English Trans.)

Su Xiao-Qun, White R.W.G. (1994) New Myxosporeans (Myxozoa: Myxosporea) from marine fishes of Tasmania, Australia. *Acta Protozool.* **33:** 251-259

Tripathi Y. (1951) Studies on parasites of Indian fishes. I. Protozoa: Myxosporidia together with a checklist of parasitic protozoa described from Indian fishes. *Rec. Indian Mus.* **50:** 63-89

Received on 29th June, 1998: accepted on 20th November, 1998

Acta Protozool. (1999) 38: 155 - 159

ACTA
PROTOZOOLOGICA

Short Communication

The Ciliated Protozoa of the Pitcher Plant *Sarracenia purpurea*

Ignacio ROJO-HERGUEDAS and José L. OLMO

Departamento de Microbiología III. Facultad de Biología, Universidad Complutense de Madrid, Madrid, Spain

Summary. The ciliated protozoa diversity found inside the digestive liquid of pitcher plant *S. purpurea* is described. The samples examined come from 9 countries belonging to 3 continents. A total of twenty six ciliates species have been identified. The most common species were: *Cyclidium glaucoma*, *Tetrahymena pyriformis* and species belonging to the genus *Colpoda*. These ciliate species are found frequently in a variety of freshwater ecosystems such as holes in trees or pools of water that accumulate in the natural basins formed by the leaf rosettes of bromeliads. Results confirm the possible ubiquity and cosmopolitan nature of the ciliated protozoa. Finally, the functional role of ciliates in the pitcher plant is discussed.

Key words: ciliated protozoa, pitcher plant, *Sarracenia purpurea*.

INTRODUCTION

The insectivorous (carnivorous) plants live within nutrient-poor environments such as bogs or heaths, the impoverished soil of forest openings and occasionally on marl, the crumbly clay soil associated with weathered limestone. Despite being phototrophic, the carnivorous plants have evolved the capacity of supplementing their nutrition by capturing and digesting animal prey. *Sarracenia purpurea* is 1 of the 9 North American species of the pitcher-plant genus, with a natural distribution extending north of Florida to British Columbia.

Address for correspondence: Ignacio Rojo-Herguedas or José L. Olmo, Departamento de Microbiología III. Facultad de Ciencias Biológicas, UCM, 28040 Madrid, Spain; Fax + 34 91 394 4964; E-mail: rojohi@eucmax.sim.ucm.es; jl.olmo@wpo.nerc.ac.uk

The pitcher of *S. purpurea* is a deformed leaf differentiated into a special organ for trapping insects. Insects are attracted to the pitcher by colour and honeydew secreted from the lip region. On falling into the pitcher, insects are trapped and then digested (Heslop - Harrison 1978). The liquid within the pitcher contains rainwater, digestive enzymes secreted by the plant and a microbial community. This microbial community is involved in the degradation of prey and includes bacteria, flagellates, rhizopods, ciliates, larvae of the Diptera and other aquatic organisms (Hegner 1926, Addicot 1974, Bradshaw and Creelman 1984).

Investigations of ciliated protozoa from pitcher plants and their functional role within these plants are very few (Hegner 1926). The objective of the present work is to reveal ciliate diversity in the pitcher liquid and to discuss the functional role of ciliates in the digestive processes of *S. purpurea*.

MATERIALS AND METHODS

Eighty three samples of pitcher liquid from Australia, Germany, Spain, United Kingdom, Czech Republic, Slovak Republic, France, Canada and USA were analysed. Samples from USA and Canada were collected from their natural environment. All other samples were obtained from greenhouse plants.

A 5-10 ml sample of pitcher liquid was collected from each of the plants using a sterile syringe. Samples were placed in sealed sterile vials for transport. Details of leaf age culture temperature and pesticides applied were recorded.

The samples were analysed when they were delivered and throughout the following two weeks.

Cysts were observed in some samples. These samples were cultivated in Petri dishes and at room temperature (14-20 °C). The only enrichment culture technique employed was the addition of one or two wheat grains per dish, with the purpose of activating the excystment.

Identification was carried out by live observations with bright field and phase contrast microscopy. The infraciliature of ciliates was revealed by two silver staining techniques: pyridinated silver (Fernández-Galiano 1994) and protargol (Wilbert 1975, Foissner 1991).

Principal taxonomic works used were: Kahl (1930-1935), Small and Lynn (1985), Dragesco and Dragesco-Kernéis (1986), Curds (1982), Curds *et al.* (1983), Foissner (1993), Foissner *et al.* (1991, 1992, 1994, 1995) and references in each of these.

RESULTS AND DISCUSSION

Of the 83 samples of pitcher liquid from *Sarracenia purpurea* studied, only 44 showed ciliated protozoa. These samples belonged to older leaves, without pesticide treatment.

Previous studies of ciliates in natural samples have shown that many species are typically rare or cryptic (Finlay *et al.* 1996, Fenchel *et al.* 1997, Finlay and Esteban 1998). The species inventory obtained for pitcher liquid from *Sarracenia purpurea* could have been more extensive if different enrichment culture techniques had been used or the samples had been observed for longer than two weeks.

The identified species were: *Colpoda inflata, C. cucullus, C. steinii, C. aspera, Chilodonella uncinata, Cinetochilum margaritaceum, Platyophrya vorax, Cyrtolophosis mucicola, Lacrymaria olor, Tetrahymena pyriformis, T. rostrata, Uroleptus lacteus, Histriculus muscorum, Euplotes affinis, Drepanomonas revoluta, Cyclidium glaucoma, Ctedectoma acanthocryptum, Frontonia acuminata, Paramecium putrinum, Stylonychia pustulata, Oxytricha* sp., *Halteria grandinella, Leptopharynx costatus, Urotricha farcta, Vorticella infusionum, Vorticella* sp. and *Spirostomum teres* (Table 1).

The most commonly found group was hymenostomatids, especially *Cyclidium glaucoma* and *Tetrahymena pyriformis*. This was followed by the colpodids, represented by the genus *Colpoda*, and then, to a lesser extent, the hypotrichids, nassulids, heterotrichids and peritrichids.

In addition to the ciliates, other species of protozoa such as *Chilomonas* sp., *Chlamydomonas* sp., *Euglena* sp., naked amoebae and testate amoebae were identified.

The majority of ciliates species identified were reselected species that can form resistant cysts and feed on bacteria. These features are characteristic of most small organisms, moreover they are conducive to cosmopolitan distribution and ubiquity (Fenchel 1993, Finlay *et al.* 1996, Finlay *et al.* 1999).

The young leaves of *Sarracenia purpurea* are sterile whilst closed but become colonised with a variety of aquatic organism on maturity.

The ciliated protozoa found within the pitcher of *S. purpurea* have several possible routes of entry, which can include aerial or prey transportation and import from the soil immediately surrounding the plant. These methods of colonisation are commonly associated with pitcher plants and feature widely in protozoan distribution. Liquid samples originating from different continents show marked similarities in species composition of protozoa. Thus, adding further weight to the theory that ciliates are ubiquitous and thus likely to be found in the world wherever a suitable habitat exists that will support their population growth (Finlay *et al.* 1999).

In principle, a habitat as unusual and little studied as pitcher liquid, should be a source of new species, as has been suggested for some anaerobic freshwater habitats (Esteban *et al.* 1993). However, these results show that species found in pitcher liquid are similar to those in other freshwater ecosystems such as holes within trees (Maguire 1971), or pools of water that accumulate in the natural basins formed by the leaf rosettes of bromeliads (Laessle 1961). Furthermore, the results show that the pitcher of *S. purpurea* supports a freshwater ecosystem, where the prey-catch provides nutrients for a varied microbial community.

Sarracenia purpurea may secrete digestive enzymes (Hepburn *et al.* 1927), however, the main form of prey digestion stems from degradation by bacterial inhabitants (Juniper *et al.* 1989). The bacterial activities increase

Table 1. Ciliate species found in the liquor of *Sarracenia purpurea* from nine countries

Taxa / Country	Ger.	Fra.	Aus.	Spa.	U.K.	Can.*	USA*	Czech Rep.	Slov. Rep.
Chilodonella uncinata (Ehr., 1838) Strand, 1928			+						
Cinetochilum margaritaceum (Ehr., 1831) Perty, 1849					+				
Colpoda aspera Kahl, 1926	+	+							
C. cucullus (O.F.M., 1773) Gmelin, 1790			+	+					
C. inflata (Stok., 1884) Kahl, 1931				+					
C. steiini Maupas, 1883		+	+	+					+
Cyclidium glaucoma O.F.M., 1773	+	+	+	+	+	+	+	+	+
Cyrtolophosis mucicola Stok., 1885	+	+							
Drepanomonas revoluta Pen., 1922						+			
Euplotes affinis (Duj., 1841) Kahl, 1932				+					
Frontonia acuminata (Ehr., 1833) But., 1889		+							
Halteria grandinella (O.F.M., 1773) Duj., 1841		+							
Histriculus muscorum (Kahl, 1932) Corliss, 1960			+		+				
Lacrymaria olor (D.M.F., 1786) B.Saint.Vic. 1824									+
Leptopharynx costatus Mermord, 1914	+					+			+
Oxytricha sp.			+						
Paramecium putrinum C. & L., 1859	+								
Platyophrya vorax Kahl, 1926	+								
Spirostomum teres C. & L., 1858				+					
Stylonychia pustula (O.F. M., 1773) Ehr., 1835	+								
Tetrahymena pyriformis (Ehr., 1830) Lwoff, 1947	+	+	+				+	+	
T. rostrata (Kahl, 1926) Corliss, 1960		+	+						
Uroleptus lacteus Kahl, 1935		+				+			
Urotricha farcta C. & L., 1859	+			+					
Vorticella infusionum Duj., 1841					+				
Vorticella sp.						+			
Samples	6	6	23	13	1	7	14	6	7

*- samples from natural environment plants

organic and mineral nutrient availability for the host plant. In addition, they are responsible for production of organic matter (Hepburn *et al.* 1920, 1927).

The ciliated protozoa graze upon bacteria of the pitcher plant, probably fulfilling a similar function as in other freshwater ecosystems: regulating and modifying the size and character of the bacterial community, accelerating turnover of microbial biomass and nutrients and the direct excretion of nutrients (Fenchel 1977, 1987; Coleman *et al.* 1978; Bamphorth 1980; Caron and Goldman 1990).

Similarly, the abundance of ciliate protozoa in *Sarracenia purpurea* pitcher is controlled by Diptera larva: *Wyemyia smithii* and *Metriocnemus knabi* (Cochram-Stafira and Ende 1998). Aside from regulating the population, the larvae accelerate the breakdown of prey and the rate of ammonia production in pitcher of *Sarracenia purpurea* (Bradshaw and Creelman 1984).

Bacteria, protozoa and Diptera larva activities are very likely to contribute to the availability of nitrogen, phosphorus, sulphur and other elements in the pitcher plants. This offers the carnivorous plant special advantages, particularly in nutrient poor environments. Pitcher plants are therefore able to thrive in places where other plants that are noncarnivorous can not colonise.

Conclusion

The ciliate biota of pitcher liquid from *Sarracenia purpurea* are similar irrespective of the origin of the host plants and consist of some common freshwater and soil ciliates which also frequently occur in e.g. water in holes of trees or in bromeliads. Therefore, there is not a specific ciliate fauna in this specialised habitat.

Acknowledgements. We wish to thank all researchers and amateur growers of *Sarracenia purpurea*, who kindly provided samples of pitcher liquor: Siegfried Hartmeyer and Andreas Laudensack (Gesellschaft für Fleischfressende Pflanzen., Germany); Sébastien Duvéré (Association Francaise d' Amateurs de plantes carnivores, France); Joly Stephane and Jean-Philippe Rosello (Dionee Societé, France); Brian Denton and Christopher Walkden (Australian Carnivorus Plants Society, Australia); Madelaine Groves, Mike Marsh and J. Keesing (Royal Botanic Gardens Kew, U.K.); Joe Cumbe (U.S.A.); Liane Cochram-Stafira Dept. Ecology and Evolution, University of Chicago, U.S.A); Terre Golembiewski, (University of Wisconsin Whitewater, U.S.A.); Jeff Stein (Botany and Plant Pathology Undergraduate, Michigan State University, U.S.A), Alan Bickell (Canada); Jan Flísek (Czech Republic); Ondrej Stevko (Darwiniana Society, Slovak Republic); Jardín Botánico de la Orotava, Tenerife; (Spain) Invernadero de la Arganzuela, Madrid; (Spain) and Gordon Wells (Spain).

REFERENCES

Addicott, J.F. (1974) Perdition and prey community structure: An experimental study of the effect of mosquito larvae on the protozoan communities of pitcher plants. *Ecology* **55:** 475-492

Bamphorth S. S. (1980) Terrestrial protozoa. *J. Protozool.* **27:** 33-36

Bradshaw W. E., Creelman R. A. (1984) Mutualism between the carnivorous purple pitcher plant and its inhabitants. *Am. Mid. Nat.* **112:** 294-304

Caron D. A., Goldman J. C. (1990) Protozoan nutrient regeneration. In: Ecology of Marine Protozoa (Ed. G. M. Capriulo). Oxford University Press, New York, 283-306

Cochram-Stafira L., von Ende C. N. (1998) integrating bacteria into food webs: Studies with *Sarracenia purpurea* inquilines. *Ecology* **79:** 880-898

Coleman D. C., Cole C. V., Hunt H. W., Klein D. A. (1978) Trophic interactions in soil as the affect energy and nutrient dynamics. I. Introduction. *Microb. Ecol.* **4:** 345-349

Curds C. R. (1982) British and Other Freshwater Ciliated protozoa. Part 1. Ciliophora: Kinetofragminophora. Cambridge University Press. London

Curds C. R., Gates M. A., Roberts D. Mc L. (1983) British and Other Freshwater Ciliated Protozoa. Part 2. Ciliophora: Oligohymenophora, and Polyhymenophora. Cambridge University Press. Cambridge

Dragesco J., Dragesco-Kernéis A. (1986) Ciliés Libres de l'Afrique Intertropicale. ORSTOM. Paris

Esteban G., Finlay B. J., Embley T. M. (1993) New species double the diversity of anaerobic ciliates in a Spanish lake. *FEMS Microbiol. Lett.* **109:** 93-100

Fenchel T. (1977) The significance of bacterivorous protozoa in the microbial community of detrital particles. In: Aquatic Microbial Communities (Ed. J. Jr. Cairns). Garland Publishing, Inc., New York, 529-544

Fenchel T. (1987) Ecology of Protozoa. Science Tech. Publishers, Madison/Springer-Verlag, Berlin

Fenchel T. (1993) There are more small than large species? *Oikos* **68:** 375-378

Fenchel T., Esteban G. F., Finlay B. (1997) Local versus global diversity of microorganism: cryptic diversity of ciliated protozoa. *Oikos* **80:** 220-225

Fernández-Galiano D. (1994) The ammoniacal silver carbonate method as a general procedure in the study of protozoa from sewage (and others) waters. *Wat. Res.* **28:** 495-496

Finlay B. J., Esteban F. G. (1998) Planktonic ciliate species diversity as an integral component of ecosystem function in a freshwater pond. *Protist* **149:** 155-165

Finlay B. J., Esteban F. G., Fenchel T. (1996) Global diversity and body size. *Nature* **383:** 132-133

Finlay B. J., Esteban F. G., Olmo J.L., Tyler P. A. (1999) Global distribution of free-living microbial species. *Ecography* **22:** 138-144

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

Foissner W. (1993) Colpodea (Ciliophora). G. Fischer, Stuttgart, Jena, New York

Foissner W., Blatterer H., Kohmann F. (1991) Taxonomische und ökologische Resvision der Ciliaten des Saprobiensystems. I: Cyrtophorida, Oligotrichida, Hypotrichia, Colpodea. Munich: Bayer. Ladesamtes für Wasserwirtschaft

Foissner W., Beger H., Kohmann F. (1992) Taxonomische und ökologische Resvision der Ciliaten des Saprobiensystems. II: Peritrichia, Heterotrichida, Odontostomatida. Munich: Bayer. Ladesamtes für Wasserwirtschaft

Foissner W., Beger H., Kohmann F. (1994) Taxonomische und ökologische Resvision der Ciliaten des Saprobiensystems. III: Hymenostomata, Prostomatida, Nassulida. Munich: Bayer. Ladesamtes für Wasserwirtschaft

Foissner W., Blatterer H., Kohmann F. (1995) Taxonomische und ökologische Resvision der Ciliaten des Saprobiensystems. IV: Gymnostomatea, Loxodes, Suctoria. Munich: Bayer. Ladesamtes für Wasserwirtschaft

Hegner R. W. (1926) The protozoa of the pitcher plant (*Sarracenia purpurea*). *Biol. Bull.* **50:** 271-276

Hepburn J. S., Jones F. M., Jhon E. Q. St. (1920) The absorption of nutrients and allied phenomena in the pitchers of the Sarraceneaceae. *J. Franklin Inst.* **189:** 147-184

Hepburn J. S., Jones F. M., Jhon E. Q. St. (1927) The biochemistry of the American pitcher plants. *Wagner Free Inst. Sci.* **11:** 1-50

Heslop-Harrison Y. (1978) Carnivorous plants. *Scient. Am.* **238:** 104-115

Juniper B. E., Robins R. J., Joel D. M. (1989) The carnivorous plants. Academic Press, London

Kahl A. (1939-1935) Urtiere oder Protozoa. I. In: Die Tierwelt Deutchlands, (Ed. F. Dahl). Jena: G. Fischer, 1-886

Leassle A. M. (1961) A micro-limnological study of Jamaican Bromeliads. *Ecology* **42:** 499-571

Maguire B. Jr. (1971) Phytotelmata: biota and community structure determination in plants-held waters. *A. Rev. Ecology Syst.* **2:** 439-464

Small E.B., Lynn D. H. (1985). Phylum Ciliophora Doflein, 1091. In: An Illustrated Guide to the Protozoa, (Eds. J.J. Lee, S. H. Hutner and E. C. Bovee), Society of Protozoologists, Kansas, 393-575

Wilbert N. (1975) Eine verbesserte Technik der Protargolimpregnation für Ciliaten. *Mikrokosmos* **6:** 171-179

Received on 23rd December, 1998; accepted on 10th March, 1999

INSTRUCTIONS FOR AUTHORS

ACTA PROTOZOOLOGICA publishes original papers on experimental or theoretical research in all fields of protistology with the exception of faunistic notices of local character and purely clinical reports. Short communications, as well as longer review articles may also be submitted. Contributions should be written in English. Submission of a manuscript to ACTA PROTOZOOLOGICA implies that the contents are original and have not been published previously, and are not under consideration or accepted for publication elsewhere. There are no page charges except colour illustration. Names and addresses of suggested reviewers will be appreciated. In case of any question please do not hesitate to contact Editor. Authors should submit papers to:

Miss Małgorzata Woronowicz
Managing Editor of ACTA PROTOZOOLOGICA
Nencki Institute of Experimental Biology,
ul. Pasteura 3
02-093 Warszawa, Poland
Fax: (4822) 822 53 42
E-mail: jurek@ameba.nencki.gov.pl

Extensive information on ACTA PROTOZOOLOGICA is now available *via* internet. The address is: **http://www.nencki.gov.pl/public.htm**

Organization of Manuscripts

Submissions

Please enclose three copies of the text, one set of original of line drawings (without lettering!) and three sets of copies with lettering, four sets of photographs (one without lettering). In case of photographs arranged in the form of plate, please submit one set of original photographs unmounted and without lettering, and three sets of plates with lettering.

The ACTA PROTOZOOLOGICA prefers to use the author's word-processor disks (3.5" and 5.25" format IBM or IBM compatible, and MacIntosh 6 or 7 system on 3.5" 1.44 MB disk only) of the manuscripts instead of rekeying articles. If available, please send a copy of the disk with your manuscript. Preferable programs are Word or WordPerfect for Windows and DOS WordPerfect 5.1. Disks will be returned with galley proof of accepted article at the same time. Please observe the following instructions:

1. Label the disk with your name: the word processor/computer used, e.g. IBM; the printer used, e.g. Laserwriter; the name of the program, e.g. Word for Windows or WordPerfect 5.1.
2. Send the manuscript as a single file; do not split it into smaller files.
3. Give the file a name which is no longer than 8 characters.
4. If necessary, use only italic, bold, underline, subscript and superscript. Multiple font, style or ruler changes, or graphics inserted the text, reduce the usefulness of the disc.
5. Do not right-justify and use of hyphen at the end of line.
6. Avoid the use of footnotes.
7. Distinguish the numerals 0 and 1 from the letters O and l.

Text (three copies)

The text must be typewritten, double-spaced, with numbered pages. The manuscript should be organized into Summary, Key words, Abbreviations used, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables and Figure Legends. The Title Page should include the full title of the article, first name(s) in full and surname(s) of author(s), the address(es) where the work was carried out, page heading of up to 40 characters. The present address for correspondence, Fax, and E-mail should also be given.

Each table must be on a separate page. Figure legends must be in a single series at the end of the manuscript. References must be listed alphabetically, abbreviated according to the World List of Scientific Periodicals, 4th ed. (1963). Nomenclature of genera and species names must agree with the International Code of Zoological Nomenclature, third edition, London (1985) or International Code of Botanical Nomenclature, adopted by XIV International Botanical Congress, Berlin, 1987. SI units are preferred.

Examples for bibliographic arrangement of references:

Journals:

Häder D-P., Reinecke E. (1991) Phototactic and polarotactic responses of the photosynthetic flagellate, *Euglena gracilis*. *Acta Protozool.* **30:** 13-18

Books:

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

Articles from books:

Allen R. D. (1988) Cytology. In: *Paramecium*, (Ed. H.-D. Görtz). Springer-Verlag, Berlin, Heidelberg, 4-40

Zeuthen E., Rasmussen L. (1972) Synchronized cell division in protozoa. In: Research in Protozoology, (Ed. T. T. Chen). Pergamon Press, Oxford, **4:** 9-145

Illustrations

All line drawings and photographs should be labelled, with the first author's name written on the back. The figures should be numbered in the text as Arabic numerals (e.g. Fig. 1). Illustrations must fit within either one column (86 x 231 mm) or the full width and length of the page (177 x 231 mm). Figures and legends should fit on the same page. Lettering will be inserted by the printers and should be indicated on a tracing-paper overlay or a duplicate copy.

Line drawings (three copies + one copy without lettering)

Line drawings should preferably be drawn about twice in size, suitable for reproduction in the form of well-defined line drawings and should have a white background. Avoid fine stippling or shading. Computer printouts of laser printer quality may be accepted, however *.TIF, *.PCX, *.BMP graphic formats on disk are preferred.

Photographs (three copies + one copy without lettering)

Photographs at final size should be sharp, with a glossy finish, bromide prints. Photographs grouped as plates (in size not exceeding 177 x 231 including legend) must be trimmed at right angles accurately mounted and with edges touching and mounted on firm board. The engraver will then cut a fine line of separation between figures. Magnification should be indicated. Colour illustration on transparent positive media (slides 60 x 45 mm, 60 x 60 mm or transparency) are preferred.

Proof sheets and offprints

Authors will receive one set of page proofs for correction and are asked to return these to the Editor within 48-hours. Fifty reprints will be furnished free of charge. Orders for additional reprints have to be submitted with the proofs.

Indexed in Chemical Abstracts Service, Current Contents (Agriculture, Biology and Environmental Sciences), Elsevier BIOBASE/Current Awareness in Biological Sciences, LIBREX-AGEN, Protozoological Abstracts. POLISH SCIENTIFIC JOURNALS CONTENTS - AGRIC. & BIOL. SCI. data base is available in INTERNET under URL (UNIFORM RESOURCE LOCATOR) address: http://ciuw.warman.net.pl/alf/psjc/ any WWW browser; in graphical operating systems: MS Windows, Mac OS, X Windows - mosaic and Netscape programs and OS/2 - Web Explorer program; in text operating systems: DOS, UNIX, VM - Lynx and WWW programs.

ACTA
PROTOZOOLOGICA

REVIEW ARTICLES

ORIGINAL ARTICLES

SHORT COMMUNICATION

1999 MAY
VOLUME 38 NUMBER 2