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

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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
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, Preston and Saimi 1990), whereas the resting Ca$^{2+}$ level within cell is restored by Ca$^{2+}$-ATPase existing in the plasma, ciliary and alveolar

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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. Cyclic AMP 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 (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 1971a, 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.
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-PKI 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* (Maible 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 protozoa 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 Mg²⁺, whereas addition to the medium of Ca²⁺ produces flagellar beat asymmetry and increased curvature of swimming. The cytoplasmic Ca²⁺ 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 Ca²⁺ 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 Mg²⁺ (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 Ca²⁺, 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 adenylyl 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 Ca²⁺ 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 Ca²⁺, 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 (Walezak 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 Ca²⁺ dependence of adenylyl cyclase and guanylyl cyclase activities from *Paramecium* estimated *in vitro*. The adenylyl cyclase activity is stimulated by lower free Ca²⁺ concentrations and shows inhibition by higher levels (above 5 μM) whereas guanylyl cyclase is
stimulated by higher levels of free Ca\(^{2+}\) and shows no inhibition by Ca\(^{2+}\) up to 100 \(\mu\)M (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 (Majita 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 nucleotides and calmodulin may regulate the behavior of cilia 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 (Tamaki et al. 1989, Di Benedetto et al. 1991b).

A similar effect is obtained on the treatment of these cells with isoproterenol, an agonist of \(\beta\)-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. 1991b).

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. 1991a, 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 aksookinin (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. 1982). The 55 kDa protein, target 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).

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 chemotacticants 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.

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The Melanin of the Myxomycete *Stemonitis herbatica*

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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
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$_2$PO$_4$ x 2H$_2$O) and dibasic sodium phosphate (Na$_2$HPO$_4$ x 7H$_2$O).

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 vacuo over phosphorus pentoxide (P$_2$O$_5$) 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 Black 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 Reynolds' 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*

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Crude melanin</th>
<th>Purified melanin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>31.57±(0.63)</td>
<td>56.27±(0.26)</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>3.92±(1.22)</td>
<td>6.06±(1.27)</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>5.72±(0.48)</td>
<td>3.54±(0.3)</td>
</tr>
<tr>
<td>Ash</td>
<td>6.2±(0.2)</td>
<td>11.7±(0.30)</td>
</tr>
<tr>
<td>Protein</td>
<td>37.0±(1.3)</td>
<td>30.0±(0.9)</td>
</tr>
<tr>
<td>Total sugar</td>
<td>11.48±(1.21)</td>
<td>9.55±(0.67)</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>6.55±(0.15)</td>
<td>7.90±(0.23)</td>
</tr>
<tr>
<td>Amino acids</td>
<td>-</td>
<td>10.0</td>
</tr>
</tbody>
</table>

(±) 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.

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 (Stomfiski 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 (Rakoczyn and Panz 1994, Plonka and Rakoczyn 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

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-glutaminyldihydroxybenzene (GDHB) melanin of *Agaricus bisporus* of 57.9% C, 3.8% H and 8.8% N respectively (Rast et al. 1981).
hydroxybenzene (Rast et al. 1981); of less than 1.9% for the dihydroxynaphthalene (DHN) melanin of *Verticillium albo-astrum* (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 (Durrell 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.

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

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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. woodruffii*, *P. calcinsi*, *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 dendrograms 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 calcinsi*, *P. duboscqui*, *P. nephridiatum*, *P. polycaryum*, *P. woodruffii*, 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 calcinsi* and *Paramecium woodruffii* (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. calcinsi* and *P. woodruffii* (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. woodruffii*, *P. nephridiatum* and, sometimes *P. calcinsi* 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
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 polyphasis* which was described from fresh water by Woodruff and Spencer (1923) morphologically (except for nuclear apparatus and cell size) is very similar to *P. calcinisi* (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. polyphasis*). 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. polyphasis*, using their morphometrical 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 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). *Paramecium* 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

<table>
<thead>
<tr>
<th>Species</th>
<th>Stock</th>
<th>Number</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. woodruffi</em></td>
<td>DV12-21</td>
<td>wo1</td>
<td>Vladivostok district, Russia</td>
</tr>
<tr>
<td></td>
<td>Dz59-22</td>
<td>wo2</td>
<td>Barents Sea, Murmansk district, Russia</td>
</tr>
<tr>
<td></td>
<td>BB1-2</td>
<td>wo3</td>
<td>Baltic Sea, Viborg district, Russia</td>
</tr>
<tr>
<td></td>
<td>BB2-1</td>
<td>wo4</td>
<td>Baltic Sea, Viborg district, Russia</td>
</tr>
<tr>
<td></td>
<td>BB2-13</td>
<td>wo5</td>
<td>Baltic Sea, Viborg district, Russia</td>
</tr>
<tr>
<td></td>
<td>BB3-3</td>
<td>wo6</td>
<td>Baltic Sea, Viborg district, Russia</td>
</tr>
<tr>
<td><em>P. calkinsi</em></td>
<td>OP1-18</td>
<td>cl1</td>
<td>White Sea, Karelian district, Russia</td>
</tr>
<tr>
<td></td>
<td>AL1-41</td>
<td>cl2</td>
<td>Sevan district, Armenia</td>
</tr>
<tr>
<td></td>
<td>OP1-14</td>
<td>cl3</td>
<td>White Sea, Karelian district, Russia</td>
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<tr>
<td></td>
<td>DV12-13</td>
<td>cl4</td>
<td>Vladivostok district, Russia</td>
</tr>
<tr>
<td><em>P. nephridiatum</em></td>
<td>OK-6</td>
<td>nr1</td>
<td>White Sea, Kandalaksha district, Russia</td>
</tr>
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<td>DZ59-4</td>
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<td>WS97-1</td>
<td>nr7</td>
<td>White Sea, Kandalaksha district, Russia</td>
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<td></td>
<td>DZ1-5</td>
<td>nr8</td>
<td>Jerusalem Zoo, Israel</td>
</tr>
<tr>
<td></td>
<td>GH1-1</td>
<td>nr9</td>
<td>North Sea coast, Germany</td>
</tr>
<tr>
<td></td>
<td>WS97-2</td>
<td>nr10</td>
<td>White Sea, Kandalaksha district, Russia</td>
</tr>
<tr>
<td><em>P. duboscqui</em></td>
<td>DZ19-21</td>
<td>du1</td>
<td>Barents Sea, Murmansk district, Russia</td>
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<tr>
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<td>DZ19-6</td>
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<td>BB2-31</td>
<td>du3</td>
<td>Baltic Sea, Viborg district, Russia</td>
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<tr>
<td><em>P. polycaryum</em></td>
<td>TR-1</td>
<td>po1</td>
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</tr>
<tr>
<td></td>
<td>TR-5</td>
<td>po2</td>
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</tr>
<tr>
<td></td>
<td>TR-6</td>
<td>po3</td>
<td>Toliatti district, Russia</td>
</tr>
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</table>

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

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

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).
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.
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; X - mean of the stock. All measurements in μm.

<table>
<thead>
<tr>
<th>Character</th>
<th>Statistics</th>
<th><em>P. calkinsi</em></th>
<th><em>P. woodruffi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cl1 cl2 cl3 cl4 M</td>
<td>wo1 wo2 wo3 wo4 wo5 wo6 M</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>124.0 94.0 114.5 126.8 115.0</td>
<td>159.2 154.0 117.1 169.9 161.7</td>
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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,
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

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Fig. 13. Similarity of *Paramecium woodruffi* (wo) and *Paramecium calcinsi* (cl) stocks. Dendrogram (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. Dendrogram (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

Fig. 15. Similarity of the five *Paramecium* species. Dendrogram (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. calcinsi* (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; x - mean of the stock. All measurements in µm.

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http://rcin.org.pl
Table 5. Morphological and biological features of brackish water *Paramecium* species and *Paramecium polycaryum*

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<td>F, B, S</td>
<td>F, B, S</td>
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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 vacuole, 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 Paramecium nephridiatum (Fokin et al., in press) that a number of studies dedicated to P. woodruffi (Agamaliev 1983; 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 μm²) 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. 13A, 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 the 1940s 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 slightly to the posterior end from the cell's equator. This fact fits with our own observations (Table 3, Fig. 3).

Twosyngenes with binary mating type system were found so far (Wichterman 1951, 1953). Nakida (1958) noted for P. calkinsi the intra-clonal conjugation (sealing). Subsequently we have found that P. calkinsi cells do not always rotate to the right (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. woodruffii (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 (Option 1942, Yusa 1957). Our data confirm such a peculiarity which is unique for the "woodruffii" 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(1) 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 Mi 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 (Diller, 1948; 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.

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

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Summary. A taxonomic revision of heliozoan-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 former is incorrect. The family Nucleariidae Cann et Page is transferred to the order Rotosphaerida uniting now all disciricate 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 heliozoan-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...
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 and 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 disciriscitate 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 Dejandre (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 disciriscitate families Nucleariidae Cann et Page, 1979 and Pompholyxophryidae Page, 1987). The rank of the taxon uniting taxa with scales etc. (the rotothroplids) 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 pompholyxophyid species belonging to genera Pinaciophora, Rabdiophrys, Rabdiaster and Pompholyxophrys (after Penard 1901; Thomsen 1978; 1979; Croome 1987 a, c; Reijackers and Siemensma 1988; Wujek and O’Kelly 1991; Siemensma 1991; Mikrjukov 1994). Scale bar - 1 μm
Taxonomic revision of heliozoon-like amoebae

Fig. 4. SEM micrographs of species of *Lithocolla* and *Elacorhanis*, 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 (Röpstorff and Hausmann 1992, Röpstorff et al. 1993). A giant multinucleate tubulocrystal 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 discirriscitate 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

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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. dubocqui* Roskin, 1929 - is regarded as a junior synonym of Pinaciophora flaviatilis. Roijackers and Siemensma (1988) pointed out the absence of mention of the radial spicules in the diagnosis of Pinaciophora flaviatilis, 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 “pompholyxophyrids with tangential plate-scales only”, whilst the Rabdiophrys began to include ”pompholyxophyrids 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 pompholyxophryid 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. thomensii* 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-scales. 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. perzovi* 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 [(iiii) and (iiv)] 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/withdrawal 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
disciristbate filose amoebae - the Nucleariidae according
to the common appearance of these organisms [branching
tine 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 sur-
rrounded 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. How-
ever 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 disciristbate filose
amoebae surrounded by endogenous siliceous artefacts
only. We include to this family the genera: Pinaciophora
Greeff, 1869, Rabdiophrys Rainer, 1968, Rabioclaster gen.
n. and Pompholyxophrys Archier, 1869. Tangential peri-
plast elements of these organisms bear fine hexagonal
perforations; the nucleus has an eccentric position. The
second family of disciristbate filose amoebae - the
Nucleariidae Cann et Page, 1979 is closely related to
pompholyxophryids according to the ultrastructural crite-
rria 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 im-
portant 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 endog-
enos 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 disciristbate taxa e.g. Euglenids,
schizopyrenids, Stephanopogon and others, usually called
in common as the disciristates (Patterson and Sogin
1992) or the infrakingdom Disciristata 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 disciristbate filose amoebae or ones covered
by mucus should be transferred from their previous
position inside the order Aconchulinida De Saedeleer,
1987 to the order Rotosphaerida Rainer, 1968 uniting
naked (Nucleariidae Cann et Page, 1979) and scale-
bearing (Pompholyxophryidae Page, 1987) disciristate
filose amoebae. Prior to this, the aconchulinids contained
the tubulocristate filose amoebae: the families Vampyrellidium
Zopf, 1887 and Arachnulididae Page, 1987. We therefore
regard the term Cristidiscoidida Page, 1987 as conceptu-
ally 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.

**DIAGNOSIS 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. NUCLEARIIIDAE 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.: Lithosphaerella 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. POMPHOLYXOPHYRIDAE 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.
128 K. A. Mikrjukov

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. 


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). 


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).

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. degrootii* 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
2 Tangential periplast elements of more complex shape................................................. *Pompholyxophrys* [4]
3 Apical plates of plate-scales are perforated by one or more holes.................. 4
4 Apical plates without holes................................................................. *Rabdiaster* [25]
5 Apical plates with several holes, periplate usually in cludes spicules...................................... *Pinaciophora* [10]
6 Scales are globular................................................................. 5
7 Scales are more flattened............................................................. 6
8 Scales are different in size, 1-6 μm; air-dried scales are spherical in outline.......... *P. punicea*
9 Scales of more or less the same size, up to 1.2 μm; air-dried scales are irregular in outline........ *P. exigua*
10 Scales are elongate, bone- or rod-shaped ....... *P. ossea*
11 Scales are not elongate, but discoid or ovoid ....... 7
12 Scales are not strongly flattened, ovoid............................................. *P. ovuligera*
13 Scales without intercalary struts.............................................. *P. stammeri*
14 Scales with intercalary struts............................................. *P. stellata*
15 Periplasts without spicules................................. 11
16 Periplasts with spicules............................................................. 12
17 Plate-scales with more then 10 large holes.............................................. *P. fluviatilis*
18 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 denticles. 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 numerous costae.

P. multicosta
Plate-scales are circular, ovoid or triangular. 16
Plate scales are oval or elliptical, spicules are bifurcate.

P. ovalis
Plate-scales are bilaterally-symmetrical as having two parallel lines of holes.

P. turrisfenestrata
Holes of various size are gathered irregularly in the centre of the plate-scale.

P. triangula
Plate-scales are triangular.

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 shaft without struts.

P. tasmanica
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
Spicules with three denticles at the apex.

P. tridentata
Spicules are terminating in at least 6 denticles.

P. rubicunda
Spicule apices lack such elaborated structures.

P. denticulata

P. bifurcata

P. tasmanica

P. candelabrum

P. spiculata

P. tridentata

P. rubicunda

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 mediator spectrum of spicules).

R. anulifera
Spicules of the same size class.

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
24 Plate scales with a poor hole; spicules with acylindrical shaft flattened distally into the bipartite end.

P. bifurcata

P. tasmanica

P. multiflora

P. ovalis

P. turrisfenestrata

P. triangula

P. bifurcata

P. tasmanica

P. candelabrum

P. spiculata

P. tridentata

P. rubicunda

P. denticulata

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Taxonomic revision of heliozoon-like amoebae


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http://rcin.org.pl
Characterization of *Paraurostyla coronata* sp. n. Including a Comparative Account of Other Members of the Genus

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Summary. *Paraurostyla coronata* sp. n. is widely distributed in the North Indian fresh water bodies. The cell measures about 200 x 60 \( \mu \)m 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 cirrus, 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, V1-V7 - 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).
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 macro-nuclei 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 / FC1 is at the right

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

<table>
<thead>
<tr>
<th>Character</th>
<th>Mean</th>
<th>S.D.</th>
<th>CV</th>
<th>Minimum</th>
<th>Maximum</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length (μm)</td>
<td>199.6</td>
<td>18.85</td>
<td>9.44</td>
<td>173.4</td>
<td>230.6</td>
<td>25</td>
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<tr>
<td>Body width (μm)</td>
<td>62.4</td>
<td>9.06</td>
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<td>51.2</td>
<td>85.7</td>
<td>25</td>
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<tr>
<td>Macronucleus length (μm)</td>
<td>29.6</td>
<td>8.09</td>
<td>27.33</td>
<td>19.25</td>
<td>45.5</td>
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<tr>
<td>Macronucleus width (μm)</td>
<td>10.3</td>
<td>2.03</td>
<td>19.70</td>
<td>7.52</td>
<td>14.0</td>
<td>15</td>
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<tr>
<td>Micronucleus diameter (μm)</td>
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<td>0.48</td>
<td>13.48</td>
<td>2.8</td>
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<tr>
<td>Adoral zonal length (μm)</td>
<td>67.8</td>
<td>3.26</td>
<td>4.84</td>
<td>59.5</td>
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<td>Adoral membranelle, number</td>
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<td>4.04</td>
<td>5.63</td>
<td>65</td>
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<td>25</td>
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<tr>
<td>Frontal cirri, number</td>
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<td>0</td>
<td>7</td>
<td>7</td>
<td>25</td>
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<tr>
<td>Ventral rows, number</td>
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<td>0.85</td>
<td>12.76</td>
<td>6</td>
<td>8</td>
<td>25</td>
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<tr>
<td>Ventral cirri, number in:</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>V1</td>
<td>3</td>
<td>1.01</td>
<td>37.07</td>
<td>2</td>
<td>5</td>
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</tr>
<tr>
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<td>2.71</td>
<td>16.19</td>
<td>12</td>
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<td>V4</td>
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<tr>
<td>V7</td>
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<td>2.62</td>
<td>10.92</td>
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<td>20</td>
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<td>Right marginal cirri, number</td>
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<td>8.05</td>
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<td>Left marginal cirri, number</td>
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<td>7.77</td>
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<td>Transverse cirri, number</td>
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</tr>
<tr>
<td>Dorsal rows, number</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>Dorso-marginal rows, number</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>Caudal cirri, number</td>
<td>16</td>
<td>1.16</td>
<td>7.1</td>
<td>15</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Caudal cirri, number in:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DK1</td>
<td>4</td>
<td>0.48</td>
<td>11.24</td>
<td>4</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>DK2</td>
<td>5</td>
<td>0.56</td>
<td>11.23</td>
<td>4</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>DK3</td>
<td>7</td>
<td>0.81</td>
<td>11.8</td>
<td>6</td>
<td>8</td>
<td>20</td>
</tr>
</tbody>
</table>

Abbreviations: CV - coefficient of variance, DK1-4 - dorsal kineties 1-4, n - number, S.D. - standard deviation, V1-V7 - ventral rows of cirri
Paraurostyla coronata sp. n.

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 - dorsal kinetics I-4, DM - dorso-marginal kinetics, FC - frontal cirri, LMC - left row of marginal cirri, RMC - right row of marginal 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)

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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.

The dorsal ciliature

The dorsal surface bears 6 longitudinal kineties of which 3 on the left (DK1,2) run through the entire length of the cell while the DK3 begins at the equatorial region. The 2 dorso-marginal rows (DM1,2) 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 DK1,2 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 V1, in the midventral region forming the oral promordium (OP) (Figs. 4, 10). The origin of OP is de novo but later basal

<table>
<thead>
<tr>
<th>Daughter cell</th>
<th>Primordium number</th>
<th>Parental structure associated with origin of primordium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proter I</td>
<td>FC1</td>
<td>Parental UM</td>
</tr>
<tr>
<td>Proter II</td>
<td>FC1, FC2, ventral row 1, TC1</td>
<td>Frontal cirrus 1</td>
</tr>
<tr>
<td>Opisthe I</td>
<td>FC1, FC2, ventral row 2, TC1</td>
<td>Vental cirri</td>
</tr>
<tr>
<td>Opisthe II</td>
<td>FC1, FC2, ventral row, 3, TC1</td>
<td>OP as well as disintegrating ventral cirri</td>
</tr>
<tr>
<td>Opisthe III</td>
<td>Ventral rows 4-8, TC1</td>
<td>Vental cirri</td>
</tr>
</tbody>
</table>

Abbreviations: OP - oral promordium, UM - undulating membrane

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

<table>
<thead>
<tr>
<th>Primordium number</th>
<th>Cirri formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>FC1</td>
</tr>
<tr>
<td>II</td>
<td>FC1, FC2, ventral row 1, TC1</td>
</tr>
<tr>
<td>III</td>
<td>FC1, FC2, ventral row 2, TC1</td>
</tr>
<tr>
<td>IV</td>
<td>FC1, FC2, ventral row, 3, TC1</td>
</tr>
<tr>
<td>V-IX</td>
<td>Ventral rows 4-8, TC1, TC2</td>
</tr>
</tbody>
</table>

Abbreviations: FC - frontal cirri, TC - transverse cirri

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

<table>
<thead>
<tr>
<th>Primordium number</th>
<th>Cirri formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>FC1</td>
</tr>
<tr>
<td>II</td>
<td>FC1, FC2, ventral row 1, TC1</td>
</tr>
<tr>
<td>III</td>
<td>FC1, FC2, ventral row 2, TC1</td>
</tr>
<tr>
<td>IV</td>
<td>FC1, FC2, ventral row, 3, TC1</td>
</tr>
<tr>
<td>V-IX</td>
<td>Ventral rows 4-8, TC1, TC2</td>
</tr>
</tbody>
</table>

Abbreviations: FC - frontal cirri, TC - transverse cirri
bodies from the V₁ and occasionally from the V₂ 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 (FC₁) for the proter. The UMs and FC₁ 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 FC₁ (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<sub>1</sub> (arrows) for each daughter cell. 17, 20 - unequal fragmentation of DP, to form DK<sub>3</sub> and DK<sub>4</sub> (arrows). 18, 21 - caudal cirri formed by proliferation of 5-6 kinetosomes at the posterior ends of DP<sub>1</sub>, DP<sub>2</sub>, and DP<sub>4</sub> (arrows). DP - dorsal primordia.

remaining streaks develop from the pre-existing ventral cirral rows. Two streaks originate from the 2<sup>nd</sup> 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
Paraurostyla coroncita sp. n. 139

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*_3) arise for each daughter cell within the parental kineties (*DK*_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*

<table>
<thead>
<tr>
<th>Organism</th>
<th>Size (μm)</th>
<th>Frontal cirri, number</th>
<th>Ventral rows, number</th>
<th>Transverse cirri, number</th>
<th>Caudal cirri, number</th>
<th>Dorsal rows, number</th>
<th>Macronuclei, number</th>
<th>Micronuclei, number</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. coronata</em></td>
<td>200 x 60</td>
<td>7</td>
<td>6-8</td>
<td>7-10</td>
<td>15-18</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>Present investigation</td>
</tr>
<tr>
<td><em>P. weissei</em></td>
<td></td>
<td>6</td>
<td>4-6</td>
<td>6-7</td>
<td>18</td>
<td>5</td>
<td>2</td>
<td>4-8</td>
<td>Jerka-Dziadosz and Frankel 1969, American population</td>
</tr>
<tr>
<td><em>P. weissei</em></td>
<td>200 x 90</td>
<td>8</td>
<td>4-5</td>
<td>8-12</td>
<td>-</td>
<td>5</td>
<td>2</td>
<td>4-5</td>
<td>Jerka-Dziadosz, 1965, Polish population</td>
</tr>
<tr>
<td><em>P. weissei</em></td>
<td>127 x 48</td>
<td>6</td>
<td>3-5</td>
<td>8</td>
<td>10-18</td>
<td>7</td>
<td>2</td>
<td>4</td>
<td>Wirnsberger <em>et al.</em> 1985, Austrian population</td>
</tr>
<tr>
<td><em>P. weissei</em></td>
<td>190</td>
<td>7</td>
<td>4</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Dragesco and Dragesco-Kernéis 1986, Cameroon population</td>
</tr>
<tr>
<td><em>P. weissei</em></td>
<td>170</td>
<td>7</td>
<td>4</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Dragesco and Dragesco-Kernéis 1986, Benin population</td>
</tr>
<tr>
<td><em>P. weissei</em></td>
<td>187 x 47</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Fleury <em>et al.</em> 1993, French population</td>
</tr>
<tr>
<td><em>P. weissei</em></td>
<td>120-180 x 35-55</td>
<td>7</td>
<td>4</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>3-8</td>
<td>Heckmann 1965, Borror 1972</td>
</tr>
<tr>
<td><em>P. hymenophora</em></td>
<td>-</td>
<td>6</td>
<td>2</td>
<td>6-7</td>
<td>-</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>Grimes and L'Hernault 1978</td>
</tr>
<tr>
<td><em>P. dispar</em></td>
<td>200-250</td>
<td>6-7</td>
<td>4</td>
<td>6-8</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>Kahl 1935</td>
</tr>
<tr>
<td><em>P. viridis</em></td>
<td>115-175</td>
<td>6</td>
<td>3-4</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Kahl 1935, Foissner <em>et al.</em> 1991, Foissner and Berger 1996</td>
</tr>
<tr>
<td><em>P. rubra</em></td>
<td>-</td>
<td>5</td>
<td>5-6</td>
<td>7-8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Kahl 1935</td>
</tr>
</tbody>
</table>
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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*, Wiersberger 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.
forms DK₃, while the larger one gives rise to DK₄ (Figs. 17, 18, 20, 21). Dorso-marginal primordia (DMP₁ and DMP₂) arise on the ventral surface near the anterior end of RMC but later shift to the dorsal surface to form DM₁ and 2'. Caudal cirri are formed by thickening of kinetosome at the posterior end of DK₁,₂ 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 V₁ 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 DP₁ occurs whereas splitting is unequal in *P. coronata.* In Austrian population, 3 streaks (DM₁,₃) 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

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

<table>
<thead>
<tr>
<th>Character</th>
<th>Austrian population</th>
<th>American population</th>
<th>Polish population</th>
<th>French population</th>
<th>P. hymenophora</th>
<th>P. coronata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin of OP</td>
<td>de novo</td>
<td>de novo</td>
<td>de novo</td>
<td>de novo</td>
<td>Transverse cirri (TC₆ or TC₇)</td>
<td>de novo</td>
</tr>
<tr>
<td>Arrangement of transverse cirri</td>
<td>J-shape</td>
<td>In a row</td>
<td>In a row</td>
<td>J-shape</td>
<td>In a row</td>
<td>In a row</td>
</tr>
<tr>
<td>No. of dorso-marginal</td>
<td>3</td>
<td>2-3</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Arrangement of caudal cirri</td>
<td>3-7 cirri in each of DK₁₂ &amp; 4</td>
<td>4 cirri in each of DK₁₂ &amp; 4</td>
<td>-</td>
<td>4-8 cirri in each of DK₁₂ &amp; 4</td>
<td>-</td>
<td>4-8 cirri in each of DK₁₂ &amp; 4</td>
</tr>
<tr>
<td>Nature of right most ventral row</td>
<td>Composite</td>
<td>Originate from 1 FVT streak</td>
<td>Composite</td>
<td>Composite</td>
<td>Originate from 1 FVT streak</td>
<td>Originate from 1 FVT streak</td>
</tr>
<tr>
<td>Number of cirral streaks</td>
<td>8-9</td>
<td>6-7 (5-9)</td>
<td>8</td>
<td>9</td>
<td>6</td>
<td>7-10</td>
</tr>
<tr>
<td>Number of frontal cirri involved in streak formation</td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Number of streaks involved in formation of frontal cirri</td>
<td>4</td>
<td>3</td>
<td>-</td>
<td>3-4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Differentiation pattern of frontal cirri</td>
<td>1, 2, 2, 1</td>
<td>1, 2, 3</td>
<td>-</td>
<td>1, 2, 2, 2</td>
<td>2, 2, 2</td>
<td>1, 2, 2, 2</td>
</tr>
<tr>
<td>Number of streaks involved in ventral row formation</td>
<td>6-7</td>
<td>4-5</td>
<td>-</td>
<td>6</td>
<td>2</td>
<td>6-8</td>
</tr>
</tbody>
</table>

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).

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

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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.

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

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

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


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

<table>
<thead>
<tr>
<th>Parasite [Host]</th>
<th>Plasmodium</th>
<th>Spore</th>
<th>Polar capsule</th>
<th>Site of infection</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sphaeromyxa balbiani</em> Thelohan, 1892 [<em>Motella tricirrata</em>]</td>
<td>Flattened, disciform; 3.0-4.0 mm (largest)</td>
<td>Fusiform with truncate ends, longitudinally striated shell valve; 15.0 x 5.0 µm</td>
<td>Broadly pyriform; 7.0 x 4.7 µm</td>
<td>GB</td>
<td>Roscoff, Napoli, Beaufort</td>
</tr>
<tr>
<td><em>S. sabrazesi</em> Lavarel &amp; Mesnil, 1900 [<em>Hippocampus brevarostris</em>]</td>
<td>Disc form, lobose pseudopodia, polysporic; 2.0 mm in diam.</td>
<td>Cylindrical, bent in arch form, truncated; smooth shell valve or with faint striations; 28.0 x 4.3 µm</td>
<td>Cylindrical; 9.0-10.0 x 3.0 µm</td>
<td>GB</td>
<td>Mt Sea</td>
</tr>
<tr>
<td><em>S. minuta</em> Polyanski, 1955 [<em>Hippocampus hippoglossus</em>]</td>
<td>Unknown</td>
<td>Straight, cylindrical, no widening at the middle, truncate ends; 13.57-16.2 x 2.7-3.6 µm</td>
<td>Pyriform; 2.7-4.5 µm</td>
<td>GB</td>
<td>Bt Sea</td>
</tr>
<tr>
<td><em>S. magna</em> Zukov, 1964 [<em>Liparis gibbus</em>]</td>
<td>Cookie shaped, 4.0 mm (largest)</td>
<td>Straight, no widening in the middle, truncate ends; shell valve with ridged surface; 23.0 x 6.4 µm</td>
<td>Oval. 8.5 x 4.0 µm</td>
<td>GB</td>
<td>PeO, Bt Sea</td>
</tr>
<tr>
<td><em>S. nesogobii</em> Su &amp; White, 1994 [<em>Nesogobius</em> sp.]</td>
<td>Unknown</td>
<td>Elongate, fusiform, arcuate, round to truncate ends; shell valve smooth; 20.0-23.7 µm (21.7 µm) x 6.3-7.9 µm (6.6 µm)</td>
<td>Pyriform; 6.8-7.9 µm (7.6 µm) x 3.1-3.3 µm (3.2 µm)</td>
<td>GB</td>
<td>Off Tasmania, Australia</td>
</tr>
<tr>
<td><em>S. opisthopterae</em> sp.n. [<em>Opisthopterus tardoire, Cuvier</em>]</td>
<td>Irregular, mostly oval to spherical; 48.0 x 18.0 µm (largest)</td>
<td>Elongated fusiform with sharply truncated ends, suture elongated 'S'-shaped, 6-8 longitudinal striations, shell valve ornamented with horizontal ridges in relation to suture; 9.0-12.7 x (11.5 µm) x 3.3-4.5 µm (3.9 µm)</td>
<td>Oval to broadly pyriform; 2.63-3.8 µm (3.6 µm) x 1.8-3.0 µm (2.8 µm)</td>
<td>GB</td>
<td>The Bay of Bengal (coastal water)</td>
</tr>
</tbody>
</table>

Abbreviations: Bt - Barents Sea, GB - gall bladder, Mt - Mediterranean Sea, PeO - Pacific Ocean
Table 3. A comparative study of the present species with the other *Sphaeromyxa* spp. recorded from India.

<table>
<thead>
<tr>
<th>Parasite [Host]</th>
<th>Plasmodium</th>
<th>Spore</th>
<th>Polar capsule</th>
<th>Infection site</th>
<th>Locality</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sphaeromyxa</em></td>
<td></td>
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<tr>
<td><em>theraponi</em> Tripathi, 1951 [<em>Therapon jurboa</em>]</td>
<td>Unknown</td>
<td>Arched, suture more or less ‘S’-shaped shell, valve thin and smooth, no sutural ridge; 19.8 x 5.4 μm</td>
<td>Small, pyriform; 7.2 x 3.7 μm</td>
<td>GB</td>
<td>Canning, South 24, Parganas, West Bengal, India</td>
</tr>
<tr>
<td><em>S. pulai</em> Tripathi, 1951 [<em>Odontamblyopus rubicandus</em>]</td>
<td>Circular, mono or disporous; 12.6-13.9 μm and 19.0-27.3 μm</td>
<td>Long, slightly truncated ends, shell valve thin and smooth, sutural almost parallel to longitudinal axis of the spore; no sutural ridge; 28.8-30.0 x 5.0-5.5 μm</td>
<td>Long, pyriform and truncated ends</td>
<td>GB</td>
<td>Hooghly River, North-24, Parganas, West Bengal, India</td>
</tr>
<tr>
<td><em>S. dighae</em> Sarkar &amp; Majumder, 1983 [<em>Hisia ilisha</em>]</td>
<td>Disporous</td>
<td>Large and broad, bent in the middle (140° angle), shell valve smooth with terminal bend, arched in sutural view; 23.82 x 3.33 μm</td>
<td>Ellipsoid, subterminal; polar filament ribbon-like, 4-5 coils; 8.43 x 2.32 μm</td>
<td>GB</td>
<td>Digha, coastal water of the Bay of Bengal, West Bengal, India</td>
</tr>
<tr>
<td><em>S. hareni</em> Sarkar, 1984 [<em>Tachysurus platystomus</em> (Day)]</td>
<td>Unknown</td>
<td>Nearly fusiform, bent, elongated ‘S’-shaped suture, shell valve smooth; 27.55 x 7.8 x 5.14 μm</td>
<td>Ovoid to ellipsoidal, coiling of the polar filament parallel to longitudinal axis of spore; 9.34 x 4.3 μm</td>
<td>GB</td>
<td>Digha, coastal water of the Bay of Bengal, West Bengal, India</td>
</tr>
<tr>
<td><em>S. ganapatii</em> Kalvari &amp; Baldehi, 1991 [<em>Therapon jarrow</em>]</td>
<td>Pansporoblast formation; 20.8-22.6 x 9.6-10.4 μm</td>
<td>Crescent shaped, rounded ends, sutural ridge not distinct; shell valve smooth; 17.5 x 4.8 μm (16-19 x 4-4.8 μm)</td>
<td>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)</td>
<td>GB</td>
<td>Chilka Lake, Orissa, India</td>
</tr>
</tbody>
</table>
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 shell valves were symmetrical and longitudinally striated. Besides, each shell valve 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 iodonilophilous 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.


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 shell valve 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 shell valve. 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 a new species and designated as Ortholinea gadustiae 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 Sarkar, 1892, S. sabrazesi Lavaren and Mesnil 1900 (cited from Lom and Dykova 1992), S. minuta Polanskyi 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 sp. have much larger plasmidia 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 sp. are known to-date from India. These are S. theraponi Tripathi 1951, S. pultrai Tripathi 1951, S. dighae Sarkar and Mazumder 1983, S. harenii 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.

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Lavaren A., Mesnil F. (1900) Sur une Myxosporide des voies biliaires del' Hippecampo (Sphaeromyxa sabrazesi nov. sp.). C. R. Soc. Biol. 52: 380-382
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The Ciliated Protozoa of the Pitcher Plant *Sarracenia purpurea*

Ignacio ROJO-HERGUEDAS and José L. OLMO

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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 glaucum*, *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.

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 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), Cords (1982), Cords 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. cecullus*, *C. steinii*, *C. aspera*, *Chilodonella uncinata*, *Cinetochilum margaritaceum*, *Platyophrya vorax*, *Cyltrophosis mucicola*, *Lacrimarya olor*, *Tetrahymena pyriformis*, *T. rostrata*, *Uroleptus lacteus*, *Histriculus muscorum*, *Euplotes affinis*, *Drepanomonas revoluta*, *Cyclidium glaucoma*, *Ciedectoma acanthocryptum*, *Frontonia aquinata*, *Paramecium putrinum*, *Stylonychia pustulata*, *Oxytricha sp.*, *Halteria grandinella*, *Leptopharynx costatus*, *Urotricha farcta*, *Vorticella infusionum*, *Vorticella* sp. and *Spirostrum 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...
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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: *Wyethia smithii* and *Metrocenmus knabi* (Cochramp-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.

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