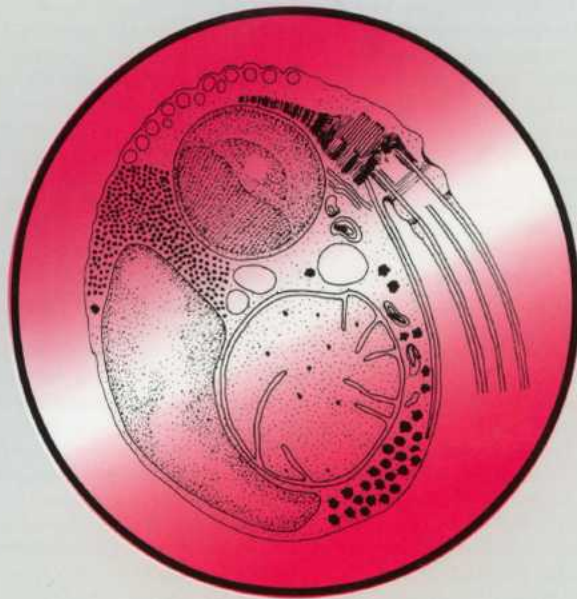


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# ACTA

# PROTOZOOLOGICA



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## **GEORGE I. POLJANSKY (1904-1993)** **100 Anniversary of Birthday**

Professor George Ivanovich Poljansky was born in March 15, 1904 in St. Petersburg, Russia. The biologists and protozoologists all over the world have still remembered him as a splendid researcher and man. The 100 anniversary of his birthday was celebrated in March 2004 at the Institute of Cytology, Russian Academy of Sciences, in St. Petersburg. Workers of the Institute, very often previous students of Professor, organized of a day session. The organization of the meeting at this place is of special significance. Professor Poljansky, more than a half of the century before, was actively engaged in organizing the Institute of Cytology. For 30 years he headed the Laboratory of Cytology of Unicellular Organisms, holding simultaneously the post of Vice-Director of the Institute. He was emotionally connected with this place working as a scientist, but especially as a teacher. He had over 60 graduated students and was the author or co-author of over 300 scientific papers, cooperating with scientists from Russia and other countries.

Moreover he was actively engaged in the activities of different societies and organizations in Russia and abroad. He was the honorary member of The Zoological Society and The Protozoological Society in France, the American Society of Protozoology and Czechoslovak Zoological Society. Very often his activity was awarded; he obtained a bronze medal of the France Zoological Society, Rudolph Luckart Medal of The Parasitological Society in

Germany, the Mikołaj Kopernik Medal of Polish Academy of Sciences. His contributions to biological sciences and especially to protozoology was also noticed and awarded in Russia.

Professor Poljansky was the editor and member of the Editorial Boards of many scientific journals in Russia and in other countries, including Polish *Acta Protozoologica*. We, as the editors and biologists, very often availed ourselves of his help and advice. His knowledge and additionally the fancy to our journal were for us of a special value.

Professor Poljansky participated in many congresses, symposia and scientific meetings. Last time he attended Warsaw in October 1987 as the honorary guest of the session organized on the 100 anniversary of Leon Cienkowski death. Professor Poljansky, during his appearance, presented a lot of interesting facts concerning Cienkowski's activity in Russia. The picture, which begins this recollection, derived exactly from this meeting.

The death of Professor Poljansky, of note biologist and friendly and gallant men was a severe loss. It was a splendid idea to recollect, on the occasion of Professor Poljansky 100 anniversary of birthday, himself and his achievements.

Anna Wasik  
Nencki Institute of Experimental Biology  
Warszawa, Poland

## Effects of Salt Concentration and Bacteria on Encystment Induction in Ciliated Protozoan *Colpoda* sp.

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**Summary.** The promoting or suppressing elements for cyst formation (encystment) of *Colpoda* sp. was examined. Encystment was promoted by an increase in concentration of ions such as  $\text{Ca}^{2+}$ ,  $\text{Na}^+$  and  $\text{K}^+$  contained in the surrounding medium, which may be detected by ciliates as an environmental signal for forthcoming desiccation, and was suppressed by the presence of bacteria in the surrounding media. When the surrounding media contained both low concentrations (1 mM) of salts and bacteria ( $10^7$  cells/ml), encystment triggered by cations was completely canceled. However, a gradual increase up to finally 8 mM in salt concentration accomplished by a natural evaporation of the surrounding saline solutions invalidated the encystment-suppression effect by bacteria.

**Key words:** *Colpoda*, encystment, environmental signals, desiccation.

### INTRODUCTION

During the processes of cyst formation (encystment) and excystment in protozoans, overall morphogenetic reconstruction of the cell occurs (Grimes 1973, Matsusaka 1979, Delgado *et al.* 1987, Foissner and Foissner 1987, Matsusaka *et al.* 1989, Martín-González *et al.* 1992, Delmonte Corrado *et al.* 1996). These processes are believed to be controlled by unknown intracellular signaling chains leading to gene expression initiated by environmental signals (Hirukawa *et al.*

1998, Suizu and Matsuoka 1998, Villalobo *et al.* 2001), and these processes are excellent model systems for elucidating the molecular mechanism of cellular-level morphogenesis. One of the strategies for the molecular mechanism of the encystment or excystment is to isolate and characterize receptors that are activated by environmental signals. To date, only a certain receptor-like molecule responsible for encystment has been isolated in a soil amoeba and named "encystment-stimulating protein" (ESP) (Yang and Villemez 1994). However, the kind of environmental signal actually activating the receptor (ESP) has not been determined. The environmental signals that directly activate the receptors are still not understood, although in many protozoans, several environmental changes (desiccation, starvation, accumulation of metabolic wastes) inducing encystment or ex-

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cystment have been listed (Rastogi *et al.* 1973, Yonezawa and Takahashi 1990, Gutiérrez *et al.* 2001, Tomaru 2002); for example, in the case of encystment induced by starvation, several factors including (1) discontinuation of nutrient supply, (2) absence of food vacuoles, (3) absence of substances released from bacteria in surrounding medium are candidates for signals to activate certain receptors.

In the case of *Colpoda* employed in the present study, most of the cells suspended in a fresh saline solution (1 mM CaCl<sub>2</sub>, 1 mM KCl, 0.1 mM Tris-HCl, pH 7.1) without bacteria are transformed into cysts, although a simple starvation of the cells suspended in 0.1 mM Tris-HCl buffer without any other salts does not induce a prominent cyst formation (Watoh *et al.* 2003). Increased osmolality of the surrounding medium resulting from the addition of 5 mM mannitol (corresponding to the osmolality of the solution containing 1 mM CaCl<sub>2</sub> and 1 mM KCl) does not induce encystment (Watoh *et al.* 2003). *Colpoda* cells that are transferred from culture medium into the saline solution probably detect an increased ionic concentration as a signal for forthcoming desiccation. On the other hand, such an induction of encystment is completely invalidated by the presence of bacteria (10<sup>7</sup> cells/ml) in the surrounding medium (Watoh *et al.* 2003). A desiccation signal should have precedence over other environmental signals, because desiccation is invariably lethal for vegetative cells. Presumably much higher concentrations of salts are needed to overcome the encystment-suppression effect by bacteria. The present study demonstrated that a gradual concentration of the surrounding media containing salts invalidated the encystment-suppression effect by bacteria.

## MATERIALS AND METHODS

*Colpoda* sp. was isolated from cysts adhered to dried fallen leaves in the field, and cultured in an infusion of dried cereal leaves (0.1 %) inoculated with bacteria (*Enterobacter aerogenes*) at 23°C in the dark. Bacteria were cultured on agar plates containing 1.5% agar, 0.5% polypeptone, 1% meat extract and 0.5% NaCl. Prior to encystment induction, cultured vegetative cells were rinsed 3 times in each test solution by transferring the cells into fresh test solutions using a thin glass pipette, and 50-60 cells were subsequently suspended in 1.5 ml of each test solution. The rate of encystment was expressed as the percentage of total number of tested cells (50-60 cells). Columns and attached bars shown in Figs. 1- 4 correspond to the means of 4 identical measurements (50-60 cells per measurement) and standard errors. Each series of measurements was performed using the cells obtained from each batch culture at about 25°C

under fluorescent room lighting (0.1~ 0.5 W/m<sup>2</sup>). The density of bacteria was spectroscopically determined in a diluted bacterial suspension, the value of which had been calibrated by comparing the cell density obtained by counting the colonies on plates and the value of optical density at 600 nm (OD<sub>600</sub>).

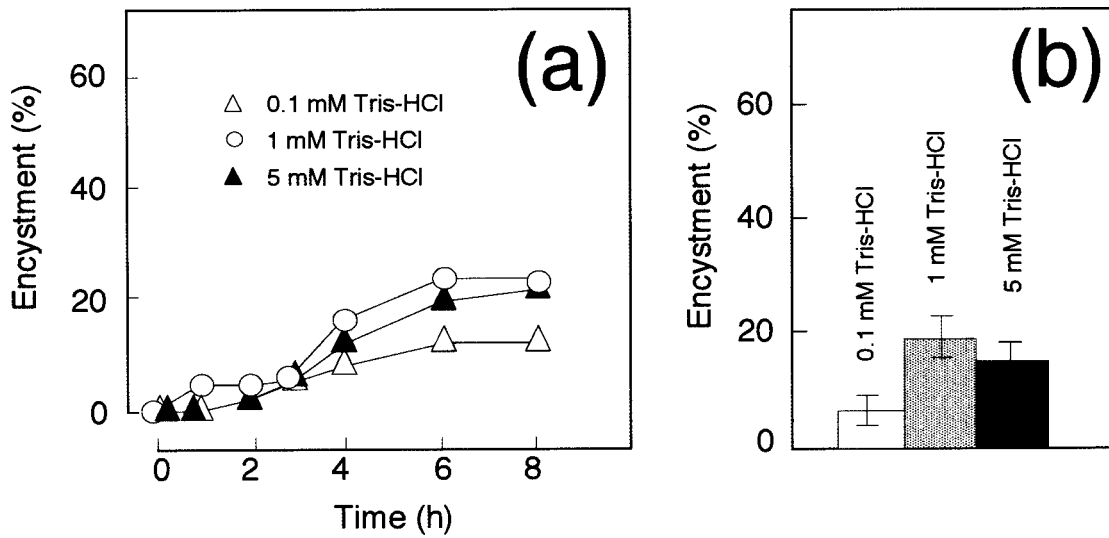
## RESULTS AND DISCUSSION

### Encystment induction

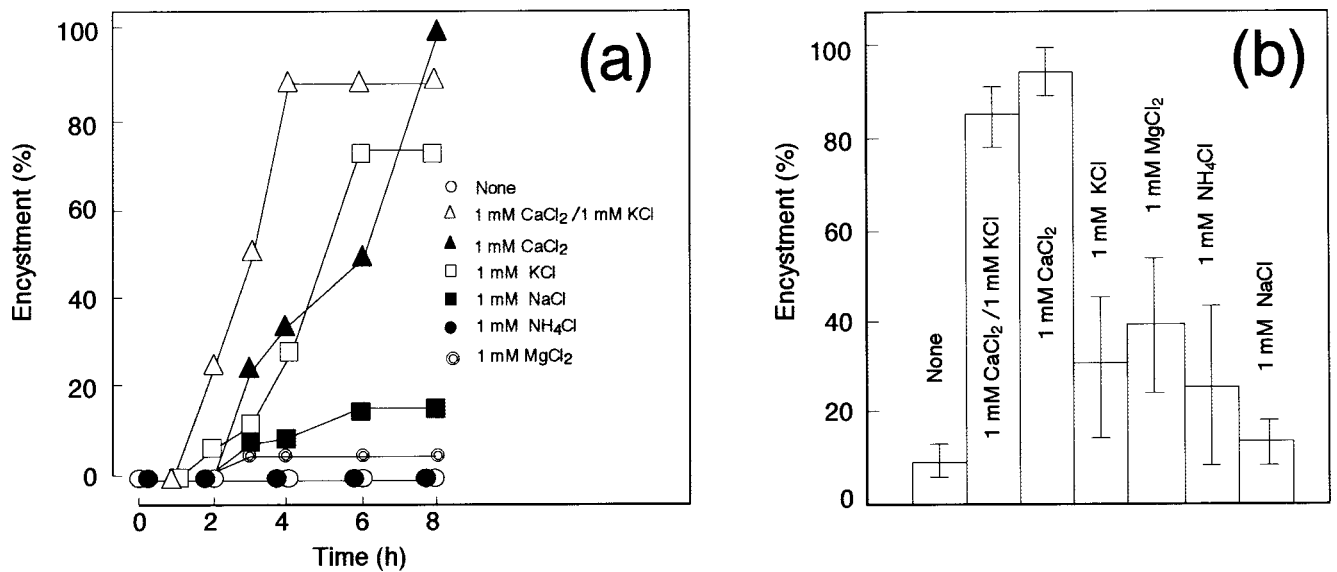
**Effect of Tris-HCl:** When cultured *Colpoda* cells were suspended in saline solution containing 1 mM CaCl<sub>2</sub>, 1 mM KCl and 0.1 mM Tris-HCl (pH 7.2), most of the cells encysted. This implies that certain ions contained in this saline solution may have induced encystment. Therefore, we examined first whether Tris-HCl is effective for initiating cyst formation (Fig. 1). When the cells were transferred into 0.1 mM Tris-HCl buffer (pH 7.2), a few of the cells encysted (Fig. 1). Such a slight induction of encystment may not have been due to Tris<sup>+</sup> or Cl<sup>-</sup>, because some of the cells also encysted even when the cells were suspended in distilled water (Fig. 4d). Although at concentrations of Tris-HCl greater than 1 mM, the mean values of encystment rates tended to increase, there was no significant difference among the 3 series of different concentrations of Tris-HCl ranging from 0.1 mM to 5 mM ( $p > 0.05$ , Kruskal-Wallis test) (Fig. 1). In consequence, Tris-HCl does not have a prominent effect on the induction of encystment at least below 5 mM.

**Effects of low concentrations of Ca<sup>2+</sup>:** Among several saline solutions at 1 mM concentration, the solutions containing CaCl<sub>2</sub> showed prominent encystment induction (Fig. 2); in this experiment, only the encystment rate induced in the solution containing CaCl<sub>2</sub> is significantly different from that induced in the medium containing only 0.1 mM Tris-HCl (Fig. 2; see the column labeled "None") ( $p < 0.05$ , Mann-Whitney test). The fact that 5 mM Tris-HCl buffer (containing 4.5 mM Cl<sup>-</sup>) (Fig. 1) and 1 mM MgCl<sub>2</sub> solution were not effective (Fig. 2) implies that the effect of CaCl<sub>2</sub> may be not attributed to Cl<sup>-</sup> but to Ca<sup>2+</sup> at least in these concentrations.

It is possible that encystment induction in some of the cells suspended in Tris-HCl buffer without other any salts or suspended in distilled water is mediated by Ca<sup>2+</sup> contamination in the solution. If so, the encystment of the cells should be completely suppressed in medium in which free Ca<sup>2+</sup> is eliminated by EGTA. If the concentration of free Ca<sup>2+</sup> contaminating the medium is as-



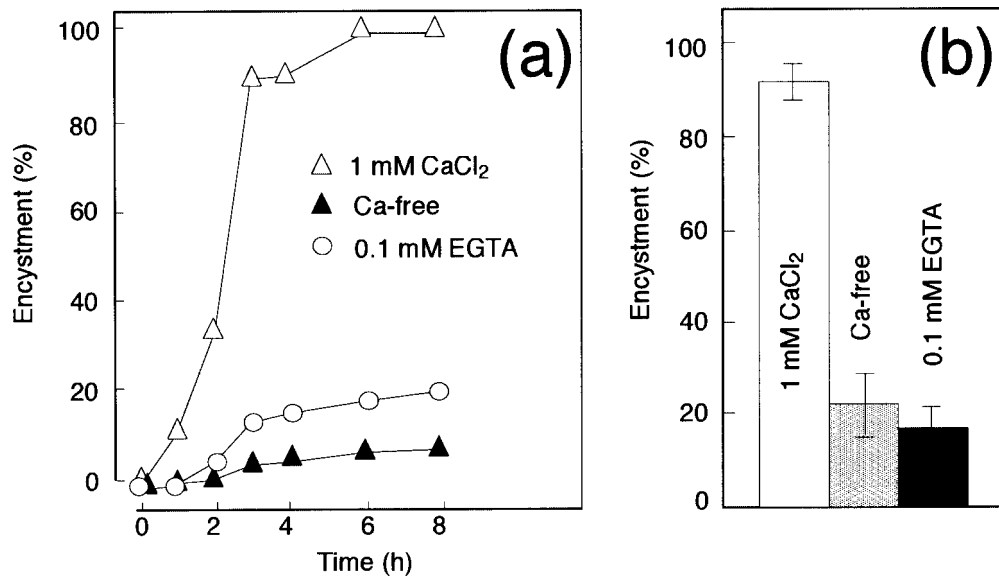
**Fig. 1.** Effects of various Tris-HCl concentrations on the induction of *Colpoda* encystment. **a** - typical time course of encystment; **b** - the rate of encystment (%) at 8 h after induction.



**Fig. 2.** Effects of various ions (salts) on the induction of *Colpoda* encystment. Every solution contained 0.1 mM Tris-HCl (pH 7.2) and various salts. **a** - typical time course of encystment; **b** - the rate of encystment (%) at 8 h after induction.

summed to be  $10^{-6}$  M, the addition of 0.1 mM EGTA (final concentration) reduces the free  $\text{Ca}^{2+}$  concentration to about  $10^{-9}$  M. A few cells encysted in spite of the addition of 0.1 mM EGTA (Fig. 3); there is no significant

difference between  $\text{Ca}^{2+}$ -free medium (without addition of  $\text{CaCl}_2$ ) and the medium containing EGTA ( $p > 0.05$ , Mann-Whitney test). It is likely that, in the solution containing no promoting or suppressing factor for en-



**Fig. 3.** Effect of elimination of Ca<sup>2+</sup> contamination in the surrounding medium on encystment of *Colpoda*. Every solution contained 0.1 mM Tris-HCl (pH 7.2). **a** - typical time course of encystment; **b** - the rate of encystment (%) at 8 h after induction.

cystment, the determination of whether encystment is triggered is unsettled.

### Encystment induction and suppression

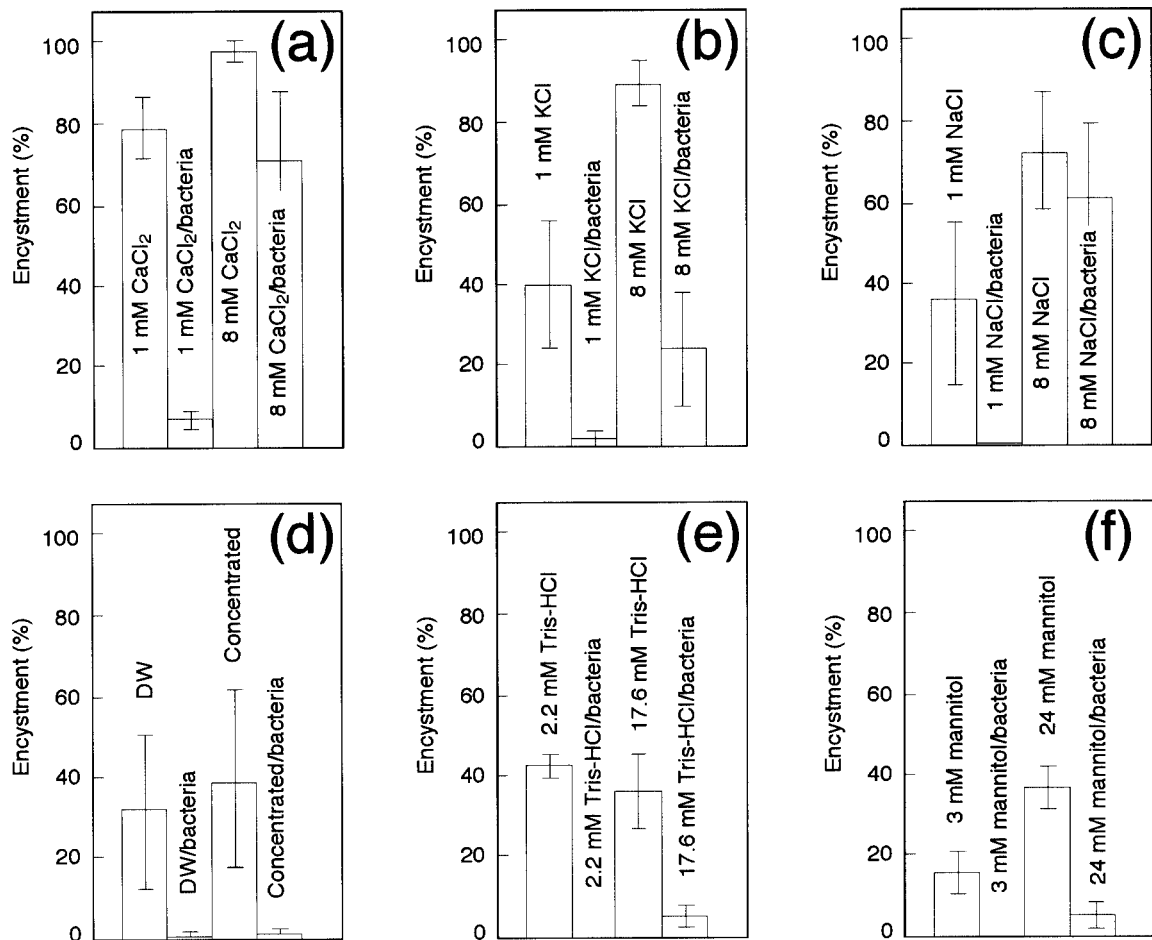
**Effects of ions concentrations and bacteria:** In the saline solutions containing CaCl<sub>2</sub>, KCl or NaCl gradually concentrated up to 8 mM by natural evaporation, encystment was prominently promoted (Fig. 4). The results indicate that *Colpoda* cells detect the rise of concentration of cations or anions in the surrounding medium as the signal to encyst because of forthcoming desiccation. The induction of cyst formation by 1 mM salt concentration was severely repressed by the presence of bacteria (10<sup>7</sup> cells/ml) in the surrounding medium (Fig. 4); however, a gradual rise in the concentration of CaCl<sub>2</sub> overcame the bacterial effect (Fig. 4a), despite the fact that the bacterial density increased concomitantly with the increase in concentration of the CaCl<sub>2</sub> solution. There was a significant difference between the encystment rate of the cells suspended in the solution containing both 1 mM CaCl<sub>2</sub> and bacteria, and the rate in the concentrated medium (8 mM CaCl<sub>2</sub>) with bacteria ( $p < 0.05$ , Mann-Whitney test). Concentrated NaCl solution also overcame the bacterial effect (Fig. 4c); there was a significant difference between the solution containing both 1 mM NaCl and bacteria, and

the concentrated solution with bacteria ( $p < 0.05$ , Mann-Whitney test). In the case of KCl solution (Fig. 4b), no marked effect was observed ( $p > 0.05$ , Mann-Whitney test). As shown in Fig. 4d, the evaporation of distilled water suspending the cells up to 1/8 volume had no effect. This result suggests that the encystment-promoting effect of concentrated NaCl or KCl solution is not attributable to the concentration of contaminated Ca<sup>2+</sup> in the solutions. In concentrated MgCl<sub>2</sub> solution, a large number of cells were killed.

Tris-HCl buffer at 2.2 mM (pH 7.2) contains 2 mM Cl<sup>-</sup> (identical molar concentration with Cl<sup>-</sup> produced by ionization of 1 mM CaCl<sub>2</sub>), and the buffer concentrated up to 1/8 volume (17.6 mM Tris-HCl) contains 16 mM Cl<sup>-</sup>. Concentration of Tris-HCl up to 1/8 volume did not have a marked encystment-promoting effect (Fig. 4e). This result suggests that neither Cl<sup>-</sup> nor Tris<sup>+</sup> is involved in the promotion of encystment, and that the encystment-promoting effect of salts such as CaCl<sub>2</sub>, NaCl or KCl might be attributed to cations produced by ionization of these salts.

Mannitol solution at 5 mM whose osmolality is approximately equivalent to that (5 mOsm) containing 1 mM CaCl<sub>2</sub> and 1 mM KCl did not promote encystment (Watoh *et al.* 2003). In addition, the fact that Tris-HCl buffer concentrated up to 17.6 mM equivalent to *ca* 33.6



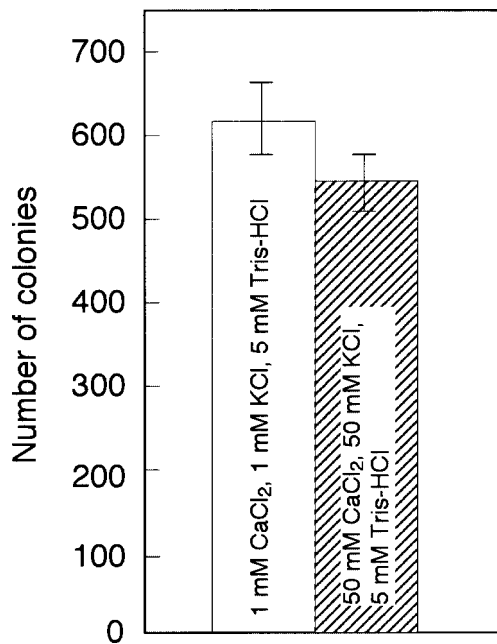


**Fig. 4.** Encystment-promoting effect by concentrated media and dominance between these effects and the encystment-suppressing effect by bacteria ( $10^7$  cells/ml). The rate of encystment was expressed as the percentage of total number of tested cells (50-60 cells) at *ca* 7 h after the *Colpoda* cells were suspended in the solutions. **a-c** - the 1 mM saline solutions; **d** - distilled water (DW); **e** - 2.2 mM Tris-HCl (pH 7.2); **f** - 3 mM mannitol. Solutions with or without bacteria ( $10^7$  cells/ml) were gradually concentrated up to 1/8 volume for *ca* 7 h at room temperature by natural evaporation to finally produce concentrations of 8 mM saline solutions (a-c), 17.6 mM Tris<sup>+</sup> and 16 mM Cl<sup>-</sup> (e) and 24 mM mannitol solution (f), respectively. The media did not contain Tris-HCl buffer except for the solution used in Fig. 4 (e).

mOsm does not have an encystment-promoting effect (Fig. 4e) implies that the encystment-promoting effect of concentrated saline solutions may not be responsible for the increased osmolality of the surrounding media. In order to confirm that, we examined the effect of highly concentrated mannitol solution. As shown in Fig. 4f, a gradual concentration of 3 mM mannitol solution to produce a final molar concentration of 24 mM whose osmolality is approximately equivalent to that of 8 mM CaCl<sub>2</sub> solution did not induce a number of cyst formation. However, there was a significant difference between the encystment rate of the cells suspended in 3 mM mannitol and the rate in its concentrated medium

(24 mM mannitol) ( $p < 0.05$ , Mann-Whitney test). Judging from the statistical analysis, we cannot completely eliminate the effect of osmolality on the promotion of encystment.

The use of some concentrated saline solutions such as CaCl<sub>2</sub> or NaCl solution invalidated the encystment-suppressing effect of bacteria (Figs 4a, c). This is not an issue of the viability of bacteria in such concentrated media, because bacteria were still alive even in the highly concentrated saline solution (Fig. 5); there was no significant difference between low concentration of saline solution (1 mM CaCl<sub>2</sub>, 1 mM KCl and 5 mM Tris-HCl, pH 7.2) and highly concentrated solution (50 mM



**Fig. 5.** Effect of concentration of saline solution on viability of bacteria. Living bacteria ( $10^7$  cells/ml) were suspended overnight in saline solution containing 5 mM Tris-HCl (pH 7.2), 1 mM CaCl<sub>2</sub> and 1 mM KCl (open column) or solution containing 5 mM Tris-HCl (pH 7.2), 50 mM CaCl<sub>2</sub> and 50 mM KCl (shaded column). The bacterial suspensions were then 1000-times diluted, and 50  $\mu$ l of the suspension was transferred onto the agar plate to spread. After 1-day incubation at room temperature, the colonies of each plate were counted. Columns and attached bars correspond to the means of 4 identical measurements and standard errors.

CaCl<sub>2</sub>, 50 mM KCl and 5 mM Tris-HCl, pH 7.2) ( $p > 0.05$ , Mann-Whitney test).

In conclusion, *Colpoda* detects increased concentration of cations such as Ca<sup>2+</sup>, Na<sup>+</sup> or K<sup>+</sup> in the external medium as the environmental signal for forthcoming desiccation and promptly initiates cyst formation, and Ca<sup>2+</sup> is most effective among these cations. Such a desiccation signal would have precedence over other encystment-suppressing elements derived from bacteria.

## REFERENCES

- Delgado P., Calvo P., Torres A. (1987) Encystment in the hypotrichous ciliate *Paraurostyla weissei*: ultrastructure and cytochemistry. *J. Protozool.* **34**: 104-110
- Delmonte Corrado M. U., Chessa M. G. and Pelli P. (1996) Ultrastructural survey of mucocysts throughout the life cycle of *Colpoda cucullus* (Ciliophora, Colpodea). *Acta Protozool.* **35**: 125-129
- Foissner I., Foissner W. (1987) The fine structure of the resting cysts of *Kahliaella simplex* (Ciliata, Hypotrichida). *Zool. Anz.* **218**: 65-74
- Grimes G. W. (1973) Differentiation during encystment and excystment in *Oxytricha fallax*. *J. Protozool.* **20**: 92-104
- Gutiérrez J. C., Callejas S., Borniquel S., Benítez L., Martín-González A. (2001) Ciliate cryptobiosis: a microbial strategy against environmental starvation. *Int. Microbiol.* **4**: 151-157
- Hirukawa Y., Nakato H., Izumi S., Tsuruhara T., Tomino S. (1998) Structure and expression of a cyst specific protein of *Acanthamoeba castellanii*. *Biochim. Biophys. Acta* **1398**: 47-56
- Martín-González A., Benítez L., Gutiérrez J. C. (1992) Ultrastructural analysis of resting cysts and encystment in *Colpoda inflata*: II. Encystment process and a review of ciliate resting cyst classification. *Cytobios* **72**: 93-106
- Matsusaka T. (1979) Effect of cycloheximide on the encystment and ultrastructure of the ciliate, *Histiculus*. *J. Protozool.* **26**: 619-625
- Matsusaka T., Noguchi O., Yonezawa F. (1989) Cortical morphogenesis during encystment in a ciliate, *Euplotes encysticus* Yonezawa, 1985. *Europ. J. Protistol.* **24**: 133-137
- Rastogi A. K., Sagar P., Agarwala S. C. (1973) Role of riboflavin and certain amino acids in the excystment of *Schizopyrenus russelli*. *J. Protozool.* **20**: 453-455
- Suizu F., Matsuoka T. (1998) Biochemical analysis of cyst formation in unicellular *Blepharisma*. *Cytobios* **96**: 157-163
- Tomaru A. (2002) Encystment-inducing factors in the ciliate *Euplotes elegans*. *Zool. Sci.* **19**: 741-746
- Villalobo E., Moch C., Perasso R., Baroin-Tourancheau A. (2001) Searching for excystment-regulated genes in *Sterkiella histriomuscorum* (Ciliophora, Oxytrichidae): a mRNA differential display analysis of gene expression in excysting cells. *J. Eukaryot. Microbiol.* **48**: 382-390
- Watoh T., Yamaoka M., Nagao M., Oginuma K., Matsuoka T. (2003) Inducing factors for encystment and excystment in ciliated protozoan *Colpoda* sp. *Jpn. J. Protozool.* **36**: 105-111 (in Japanese)
- Yang S., Villemez C. L. (1994) Cell surface control of differentiation in *Acanthamoeba*. *J. Cell. Biochem.* **56**: 592-596
- Yonezawa F., Takahashi T. (1990) Encystment-inducing substance in cell-free fluid from the culture of the ciliate *Euplotes encysticus* grown in bacterized inorganic medium. *Zool. Sci.* **7**: 1157a

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## ***Uroleptopsis* Kahl, 1932 (Ciliophora: Hypotricha): Morphology and Cell Division of Type Species, Redefinition, and Phylogenetic Relationships**

**Helmut BERGER**

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**Summary.** The morphology and the morphogenesis of the marine hypotrich *Uroleptopsis citrina* Kahl, type of *Uroleptopsis* Kahl, were investigated using live observation and protargol impregnation. The results are used for a redefinition of *Uroleptopsis* and a phylogenetic analysis of the Pseudokeronopsidae. Each of the many macronuclear nodules of *U. citrina* divides individually as in *Pseudokeronopsis* spp., that is, the nodules do not fuse to a single mass. Consequently, *Uroleptopsis*, *Pseudokeronopsis*, and *Thigmokeronopsis* - which shows an intermediate type of macronuclear division - are united to the Pseudokeronopsidae. *Uroleptopsis* has three autapomorphies, namely the lack of transverse cirri, the formation of two cirri from the undulating membrane anlage, and a gap in the adoral zone. *Uroleptopsis ignea* has midventral rows which are the autapomorphy for the new subgenus *Uroleptopsis (Plesiouroleptopsis)*. *Uroleptopsis (Uroleptopsis)*, which contains the four other *Uroleptopsis* species, lacks a buccal cirrus in the ordinary position right of the paroral; in non-dividers this cirrus is part of the posterior bow of the bicorona. In *U. citrina* some cirral anlagen of the middle portion of the midventral complex do not form an ordinary cirral pair, but only a single midventral cirrus. *Keronopsis tannaensis* Shigematsu is transferred to *Uroleptopsis* because it lacks transverse cirri and has likely a bicorona. *Keronopsis multiplex* Ozaki and Yagiu is synonymized with *U. roscoviana* (Maupas). A key to the five *Uroleptopsis* species is provided and the terminology for urostylids is actualized. The *Uroleptopsis citrina* population from the Adriatic Sea is designated as neotype because (i) no preparations are available of the original type population, and (ii) synonymy with *Pseudokeronopsis rubra* and some other species was proposed by Borror (1979) and Borror and Wicklow (1983).

**Key words:** Adriatic Sea, key to species, neotypification, phylogeny, Protozoa, Pseudokeronopsidae, revision, terminology, *Uroleptopsis (Plesiouroleptopsis)* subgen. n., *Uroleptopsis tannaensis* comb. n., Urostylidae.

### **INTRODUCTION**

Kahl (1932) established *Uroleptopsis* because the lack of transverse cirri in some holostichid species prevailed their classification in *Holosticha (Keronopsis)*. *Uroleptopsis* was accepted until 1979, when Borror has put it - together with *Trichototaxis* - into the

synonymy of *Keronopsis sensu lato*. Later, he synonymized it with *Pseudokeronopsis* (Borror and Wicklow 1983). In 1990, Mihailowitsch and Wilbert described *P. ignea* which lacks transverse cirri. Thus, Foissner (1995) transferred it to *Uroleptopsis*. However, the resurrection of Kahl's genus by Foissner was not accepted by Eigner (2001).

Recently I found the type species *U. citrina* in the Adriatic Sea and could study its morphology and morphogenesis. The data indicate that *Uroleptopsis* is as well defined as many other genera of hypotrichs. Thus, it should not be synonymized with *Pseudokeronopsis*

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which is very likely the sister group of *Uroleptopsis* according to Hennigian argumentation.

## MATERIALS AND METHODS

**Sampling and culture.** *Uroleptopsis citrina* was found in a sample which I collected on the sandy littoral of the northern Adriatic Sea ahead the campground Pra' delle Torri (45°34'N 12°49'E) near the Italian village of Duna Verde on 25.05.2002. The sample contained mainly sand and seagrass run ashore. It was transported to Salzburg in a 1-litre bottle. In the laboratory raw cultures were established using Petri dishes 15 cm across filled with sea water from the sample site. Some squashed wheat grains were added to support microbial growth. The species grew also well in artificial sea water (30‰; Biosal, Aqualine Buschke, Berg, Germany).

**Morphological methods.** Cells were studied in life using, inter alia, a high-power oil immersion objective and differential interference contrast optics. Live measurements were made at magnifications of 125-1250×. Although live values are more or less rough estimates, it is worth giving such data as specimens usually contract during fixation or shrink in preparations. The infraciliature was revealed with the protargol method according to protocol A in Foissner *et al.* (1999). Counts and measurements on prepared specimens were performed at a magnification of 1250×. Illustrations of live specimens are based on micrographs and freehand sketches, while those of prepared cells were made with a camera lucida. Five neotype slides (accession nos. 2004/301-305) of protargol preparations are deposited in the Oberösterreichische Landesmuseum in Linz (LI), Austria. Since the slides contain a high number of individuals only specimens illustrated have been marked by a ring. Further specimens from the neotype population are in the 5 neotype slides of *Amphisiella annulata* which are deposited in the same museum (accession nos. 2003/146-150).

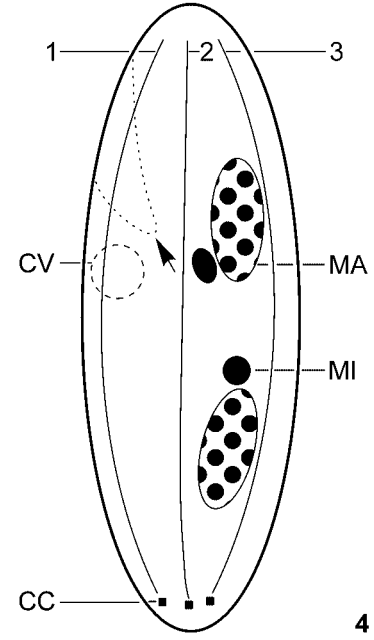
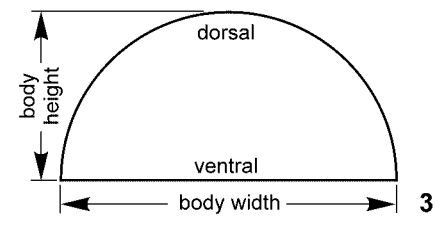
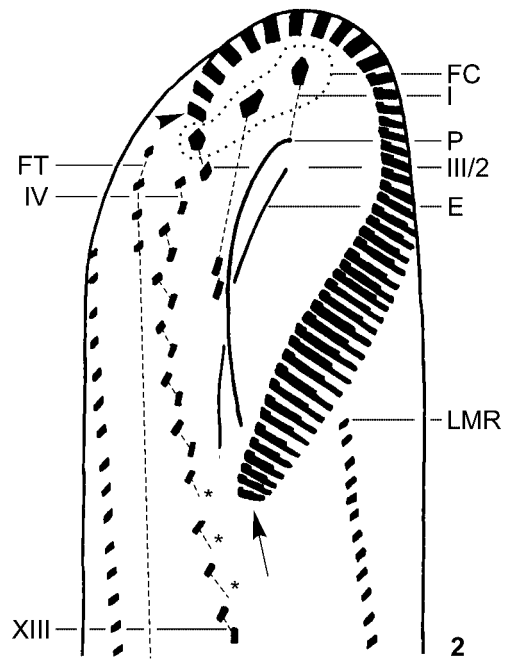
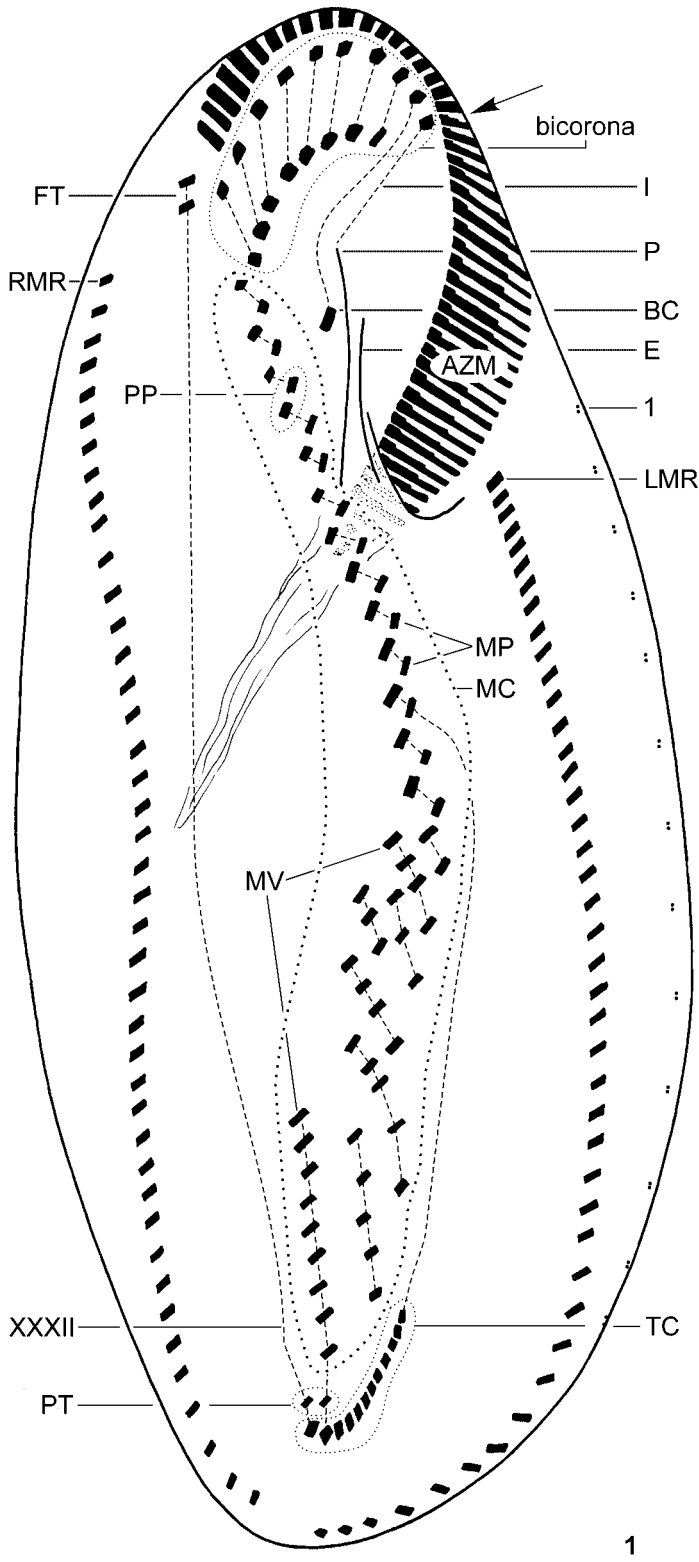
The morphometric data shown in Table 1 are repeated only as needed for clarity. All observations are from specimens of raw cultures, that is, not from cloned individuals. Consequently it cannot be excluded that similar species are mixed, although this is very unlikely because specimens which deviate in at least one important character are excluded. Certainly, this can generate some bias in the data if applied to uncritically. However, I usually excluded only such individuals which have, for example, a distinctly deviating cirral pattern (very likely often injured, regenerating, or malformed specimens) or

an unusually small size (very likely often degenerating or just divided specimens). The inclusion of such specimens would artificially increase variability.

Specimens of the neotype population have been sent to Prof. Martin Schlegel (Leipzig University, Germany) for molecular biology analysis. Results will be published elsewhere.

**Terminology and nomenclature.** The terminology for the supposed autapomorphy of the urostyleids, the midventral cirri, is rather confusing since the term has not been used uniformly. The expression midventral cirri was introduced by Borror (1972) as follows: "Between the right and left marginal cirri in members of the Holostichidae is a double row of cirri that often is arranged in a zigzag position. The midventral cirri arise from a longitudinal series of transverse streaks in *Urostyle cristata*, ...". However, this term was not used in all subsequent papers on urostyleids. For example, Buitkamp (1977) designated the two rows formed by the zigzagging cirri as ventral rows. Hemberger (1982) and Foissner (1982) basically accepted Borror's expression and designated the two rows as right and left midventral row (note, that each of these two midventral rows originates from many anlagen whereas, for example, a marginal row originates from a single anlage!). In several urostyleid genera (e.g., *Bakuella*, *Keronella*) not only cirral pairs but also more or less long rows are formed by the midventral anlagen. Wiackowski (1985) summarized both the cirral pairs and the cirral rows under the term midventral cirri. By contrast, Song *et al.* (1992) confined the expression midventral row to the zigzagging arranged cirral pairs and designated the cirral rows in the posterior body portion as ventral rows. In 1994, Eigner introduced two terms for these cirral rows in the posterior body portion of some taxa, namely (i) short midventral row composed of 3-4 cirri, and (ii) long midventral row composed of more than four cirri. According to Eigner's terminology, for example, a *Bakuella* species has (i) a "midventral row" (composed of zigzagging cirral pairs), (ii) one or more "short midventral rows", and (iii) one or more "long midventral rows". Since the midventral row mentioned under (i) can also be either short or long, the terms introduced by Eigner are somewhat misleading. In addition, the left cirrus of several cirral pairs is lacking in non-dividers of *Uroleptopsis citrina* further complicating the terminology (see below). To overcome these terminological problems, the various structures are designated as shown in Figs 1-4. The new generic term is "midventral complex" which can be composed of various structures. For example, in *Holosticha* species the complex consists of midventral pairs only, whereas in *Bakuella* it is composed of midventral pairs and midventral rows. In species with three enlarged frontal cirri,

**Figs 1-4.** Terminology of urostyleid ciliates. **1** - infraciliature of ventral side of a species with a bicorona. Arrow marks approximate border between distal and proximal portion of adoral zone of membranelles where in some taxa, for example, *Uroleptopsis citrina*, a more or less distinct break (gap) occurs. Frontal-midventral-transverse cirri which originate from the same anlage are connected by a broken line (for the sake of clarity only the leftmost transverse cirrus and the two rightmost transverse cirri are connected with the corresponding midventral pair, respectively, midventral rows); **2** - infraciliature of ventral side of a species with three frontal cirri. Arrow marks proximal end, arrow head distal end of adoral zone of membranelles. Asterisks mark anlagen which eventually produce only a single midventral cirrus; **3** - schematic cross section showing dorsoventral flattening. **4** - infraciliature of next adoral side, nuclear apparatus, and contractile vacuole. Arrow marks proximal end of adoral zone of membranelles. AZM - adoral zone of membranelles; BC - buccal cirrus; CC - caudal cirri; CV - contractile vacuole; E - endoral; FC - frontal cirri; FT - frontoterminal cirri (= migratory cirri); LMR - left marginal row; MA - macronuclear nodule with nucleoli; MC - midventral complex; MI - micronucleus; MP - midventral pairs; MV - midventral rows; P - paroral; PP - pseudo-pair (composed of rear [= left] cirrus of an anlage and front [= right] cirrus of next anlage, that is, the cirri of a pseudo-pair do not originate from the same anlage); PT - pretransverse ventral cirri; RMR - right marginal row; TC - transverse cirri; I - first (= leftmost) frontal-midventral-transverse cirral anlage; III/2 - cirrus behind right frontal cirrus (according to Wallengren's 1900 terminology); IV - this anlage produces the first midventral pair in species with three frontal cirri; XIII - thirteenth anlage; XXXII - last (= rightmost) frontal-midventral-transverse cirral anlage (number varies among species and often within species); 1, 2, 3, ... - dorsal kineties (kinety 1 is the leftmost one).



the distinction between the frontal cirri and the midventral complex is straightforward (Fig. 2). In taxa with a bicorona - for example, *Keronella* and *Uroleptopsis* - it is sometimes difficult to define the beginning of the midventral complex (Fig. 1). However, usually the cirri of the anterior corona and even those of the posterior are slightly to distinctly larger than the midventral cirri and often at least slightly set off from them. The dorsoventral flattening of a hypotrich is expressed as the ratio of body width to body height (Fig. 3).

For authorship and date of scientific names, see Berger (2001). Usually, the taxa Pseudokeronopsinae and Pseudokeronopsidae discussed in the chapter on phylogenetic relationships are categorized as subfamily and family, as indicated by the defined endings -inae and -idae (for example, Borror and Wicklow 1983, Lynn and Small 2002). I do not use categories above the obligatory genus because they are useless in phylogenetic analysis (for details on this matter and the resulting conflicts with nomenclature, see Ax 1995, 1999 and Wägele 2001). However, to avoid inflation, I retain these names.

## RESULTS AND DISCUSSION

### Morphology and morphogenesis of type species

#### *Uroleptopsis citrina* Kahl, 1932 (Figs 5-28, 35-42; Table 1)

1932 *Uroleptopsis citrina* sp. n. - Kahl, *Tierwelt Dtl.*, **25**: 543, Fig. 87 (Fig. 13; original description; no type material available).

1933 *Uroleptopsis citrina* Kahl 1932 - Kahl, *Tierwelt N.- u. Ostsee*, **23**: 107, Fig. 16.12 (guide to marine ciliates).

1950 *Uroleptopsis citrina* K. - Kudo, *Protozoology*, p. 672 (textbook).

1972 *Uroleptopsis citrina* Kahl, 1932 - Borror, *J. Protozool.*, **19**: 11 (revision).

**Nomenclature:** No derivation of the name is given in the original description. The species-group name *citrina* (Latin adjective; lemon-yellow) obviously refers to the yellow colour of this species.

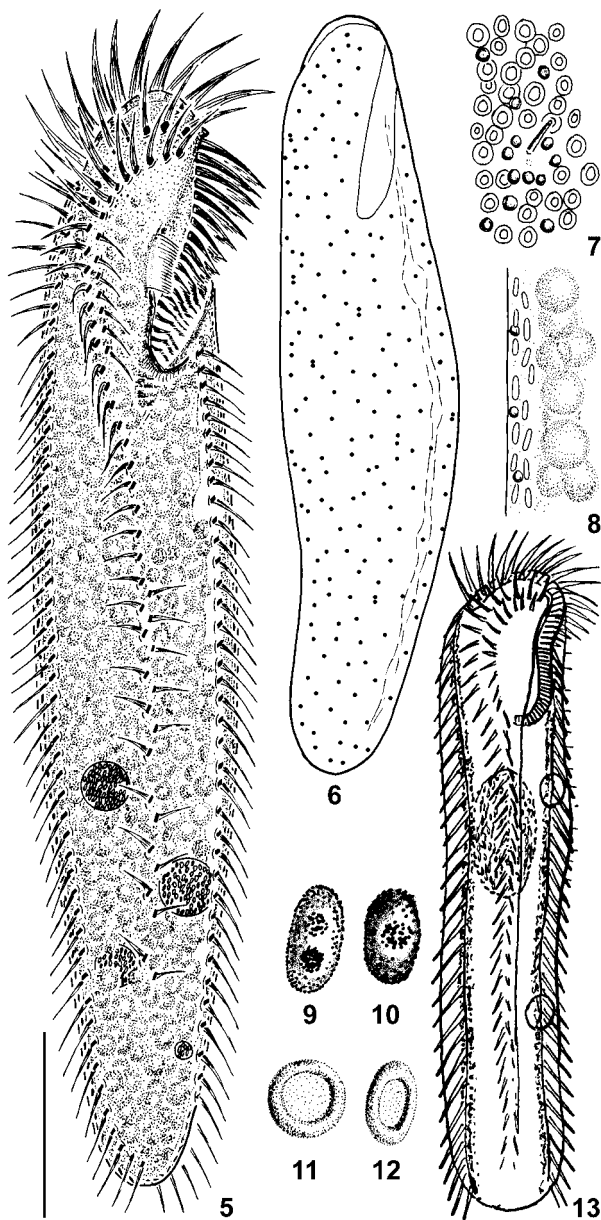
**Improved diagnosis** (based on original data only): Size around  $200 \times 40 \mu\text{m}$  in life. Body outline elongate. Cortical granules yellow, ring-shaped arranged around dorsal bristles and scattered. Underneath cell surface a 2-3  $\mu\text{m}$  wide seam formed by ring-shaped structures. On average 39 adoral membranelles (29 proximal, 10 distal); each 7 cirri in anterior and posterior corona; anterior and posterior portion of midventral complex each composed of about 7 cirral pairs, middle portion composed of about 10 right cirri only; 39 left and 48 right marginal cirri. Usually 2 frontoterminal cirri and invariably 3 dorsal kineties. Marine.

**Morphology** (Figs 5-12, 14-19, 35-42; Table 1): At first data from the Adriatic population are provided. For

a brief characterization of Kahl's population, see last paragraph of the morphology chapter.

Size of Adriatic population usually  $160-220 \times 30-50 \mu\text{m}$ , body length : width ratio about 5 : 1 in life, 3.8 : 1 on average in protargol preparations (Table 1). Body outline elongate-elliptical to almost band-shaped; at left anterior corner a minute process likely causing break in adoral zone (Figs 5, 35). Body about 1.5-2.0 : 1 flattened dorso-ventrally, very flexible, rather resistant against cover-glass pressure, not distinctly contractile. Pellicle slightly to distinctly crenulated along cirral rows. Nuclear apparatus masked by cytoplasmic inclusions, therefore very difficult to recognize in life without staining (Figs 5, 35, 38). Macronuclear nodules scattered throughout cytoplasm, usually ellipsoidal (length : width ratio about 2 : 1 on average in protargol preparations; Table 1), sometimes globular or dumbbell-shaped, with one or few nucleoli (Figs 9, 10); specimen shown in Figs 14-16 with about 100 macronuclear nodules. Micronuclei globular, difficult to distinguish from globular macronuclear nodules in protargol preparations and therefore difficult to count; number likely around five. Contractile vacuole difficult to recognize in freely motile specimens; slightly squeezed cells show distinct lacunar system with several dilatations near left body margin (Figs 5, 6). Cortical granules difficult to recognize, although of ordinary size (0.8-1.2  $\mu\text{m}$  across) and yellow colour; variable number of granules ring-shaped arranged around dorsal bristles, but also scattered over whole cell (Figs 6-8, 36, 37). Yellow colour of specimens basically not caused by cortical granules but due to diffuse colour of cytoplasm. Anterior and posterior body end often more distinctly yellow than remaining body portions. Underneath cell surface a distinct, about 2-3  $\mu\text{m}$  wide seam formed by numerous ring-shaped structures of unknown function; individual structures colourless, 1.5-2.0  $\mu\text{m}$  across (Figs 7, 8, 11, 12, 36-38). Cytoplasm usually packed with fatty-shining globules 2-4  $\mu\text{m}$  across (Figs 5, 8, 35, 38). Food vacuoles 3-10  $\mu\text{m}$  across, contain bacteria. Movement without peculiarities, that is, moderately fast gliding showing great flexibility.

Adoral zone of membranelles occupies 30% of body length on average (Table 1), bipartite by inconspicuous break (gap) about there where zone turns from ventral body surface to dorsal side of frontal scutum (Figs 5, 14, 17-19, 39, 41). Gap usually distinct in protargol preparations, on average 3  $\mu\text{m}$  wide, separates zone into about 10 distal and about 30 proximal membranelles. Proximal portion of adoral zone in most specimens roughly in

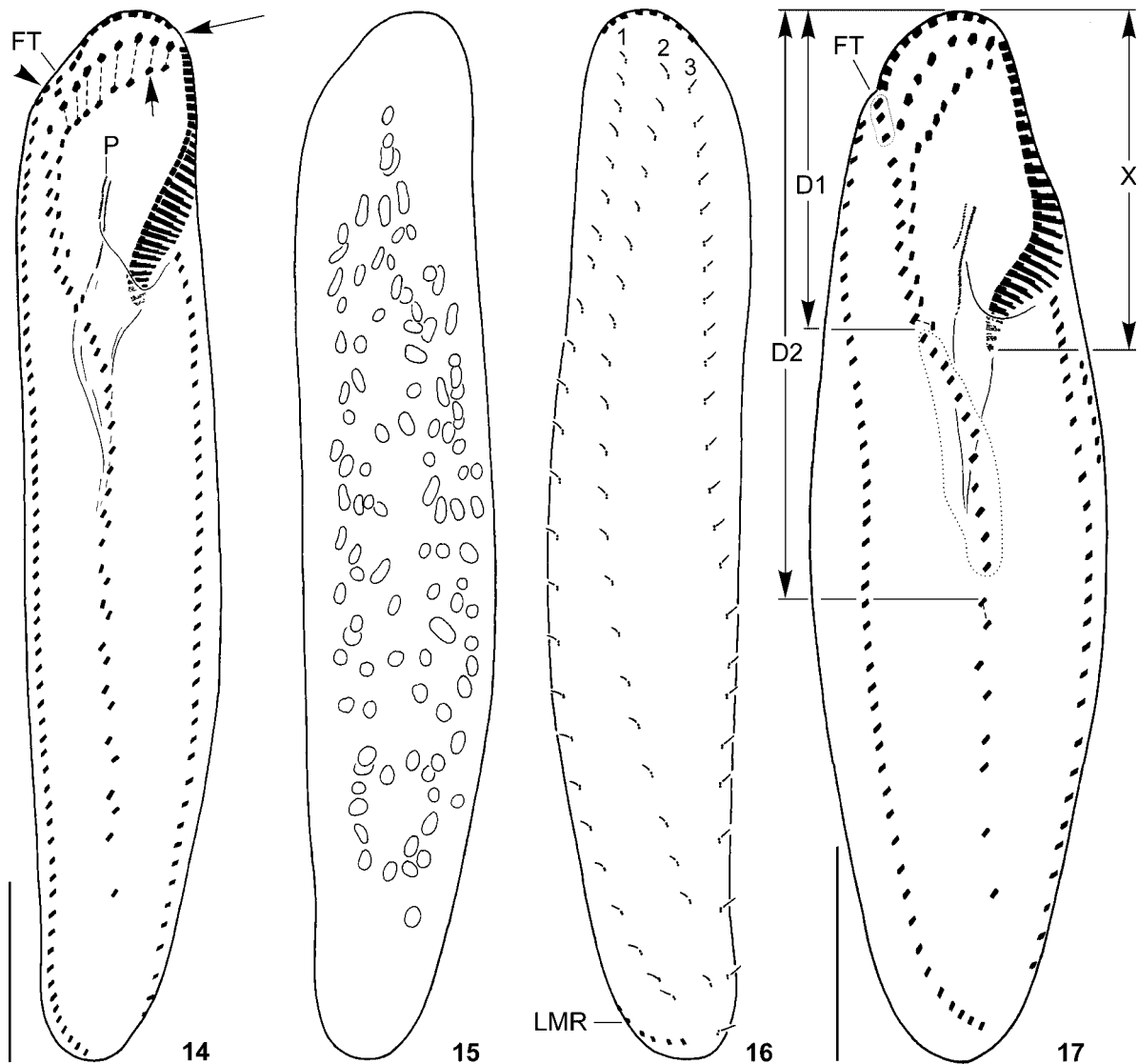


**Figs 5-13.** *Uroleptopsis citrina*. Neotype population (5-12) and Kahl's population (13) from life (5-8, 11-13) and after protargol impregnation (9, 10). **5** - ventral view of a representative specimen; **6** - ventral view of a slightly squeezed specimen showing contractile vacuole system and arrangement of cortical granules; **7** - top-view showing ring-shaped structures (1.5-2.0 µm across) and cortical granules (0.8-1.0 µm across) which form more or less distinct circles around base of dorsal bristles; **8** - 2-3 µm wide seam formed by the ring-shaped structures; **9, 10** - macronuclear nodules have few or one nucleoli; **11, 12** - the ring-shaped structures are reminiscent of the erythrocytes of mammals; **13** - ventral view (from Kahl 1932), individual size not indicated. Scale bar 30 µm.

*Gonostomum* pattern, that is, extends along left body margin, performs more or less abrupt right bend and slight clockwise rotation to plunge into the buccal cavity;

four-rowed portion of middle membranelles often slightly set off which is sometimes even recognizable in life in that this right portion (or only some cilia) forms a separate ciliary bundle (Fig. 14); width of membranelles increases rapidly up to 10 µm from proximal end to level where left marginal row commences. Distal portion of adoral zone extends onto right body margin to 7% of body length on average (Table 1). In several specimens proximal portion of adoral zone sigmoidally curved (Fig. 19), almost as illustrated by Kahl (1932; Fig. 13). Buccal area very narrow in life, of ordinary size in protargol preparations possibly due to inflation of buccal cavity (Figs 5, 14, 35). Buccal lip distinctly curved and thickened at vertex (Figs 5, 41). Paroral and endoral begin about at same level, that is, at about 14% of body length (Figs 14, 41; Table 1). Paroral short (9 µm on average in protargol preparations; Table 1), straight, composed of 6-8 µm long, likely zigzagging arranged cilia, on anterior portion of buccal lip, optically not intersecting with endoral which is also more or less straight, but about twice as long as paroral. Pharyngeal fibres inconspicuous in life, clearly recognizable after protargol impregnation, of ordinary length and structure, extend obliquely backwards, with long, fine structures (cilia of endoral?) beating inside.

Cirral pattern and number of cirri of usual variability, except for number of cirral pairs in anterior and posterior portion and number of single midventral cirri in middle portion of midventral complex which vary rather strong (Figs 14, 17-19; Table 1). Cirri of bicorona 12-15 µm long, remaining cirri about 12 µm. Frontal ciliature conspicuous because of the bicorona type; anterior and posterior corona composed of each seven cirri on average due to two peculiarities, namely (i) the formation of two cirri from anlage I, and (ii) the buccal cirrus (= cirrus II/2) is formed, but does not migrate posteriorly into the ordinary position (for details, see morphogenesis). Only one out of at least 31 specimens with eight cirri in anterior and only seven cirri in posterior corona. Cirri of anterior corona slightly larger than those of posterior, base of most (all?) coronal cirri of polygonal outline. Bicorona not very distinctly set off from anteriormost cirral pair of midventral complex. No cirrus immediately right of paroral, that is, "buccal cirrus" lacking. Usually two, rarely three frontoterminal cirri in ordinary position, namely near distal end of adoral zone (Figs 14, 17-19). Midventral complex composed of 39 cirri on average; due to a morphogenetic peculiarity separated in three more or less clearly recognizable portions (Figs 14, 17-19): (i) anterior portion composed of seven midventral



**Figs 14-17.** *Uroleptopsis citrina*. Neotype population after protargol impregnation. **14-16** - infraciliature of ventral and dorsal side and nuclear apparatus of neotype specimen. Long arrow marks gap in adoral zone, short arrow marks cirrus II/2 (= "buccal cirrus") which is not right of the paroral in non-dividers. Arrowhead denotes anterior end of right marginal row. Broken lines connect cirral pairs of bicorona. **17** - infraciliature of ventral side of a specimen with 3 frontoterminal cirri. The short cirral row left of the left marginal row is likely a remnant of the parental left marginal row. Middle portion of midventral complex, which is composed of single midventral cirri, is encircled. D1 - distance between anterior body end and last midventral cirral pair of anterior portion of midventral complex; D2 - distance between anterior body end and first midventral pair of posterior portion of midventral complex; FT - frontoterminal cirri; LMR - left marginal row; P - paroral; X - length of adoral zone of membranelles; 1-3 - dorsal kineties. Scale bars 30  $\mu$ m.

pairs on average; (ii) middle portion made of about 10 single cirri forming a more or less continuous (not zigzagging) row; and (iii) posterior portion composed of around seven cirral pairs whose cirri are usually wider separated than those of the anterior portion; length of these three portions highly variable. Midventral complex terminates at 84% of body length on average; rearmost cirri must not be misinterpreted as transverse cirri which

are lacking (checked in many hundred specimens). Right cirri of midventral pairs usually composed of  $2 \times 4$  basal bodies, left cirri often made of  $2 \times 3$  basal bodies. Right marginal row commences close to frontoterminal cirri, curves leftwards to about cell midline at posterior end, and usually terminates about 4  $\mu$ m ahead rear cell end. Left marginal row begins in ordinary position, that is, slightly ahead proximal end of adoral zone, extends onto



**Table 1.** Morphometric data on *Uroleptopsis citrina*<sup>a</sup>

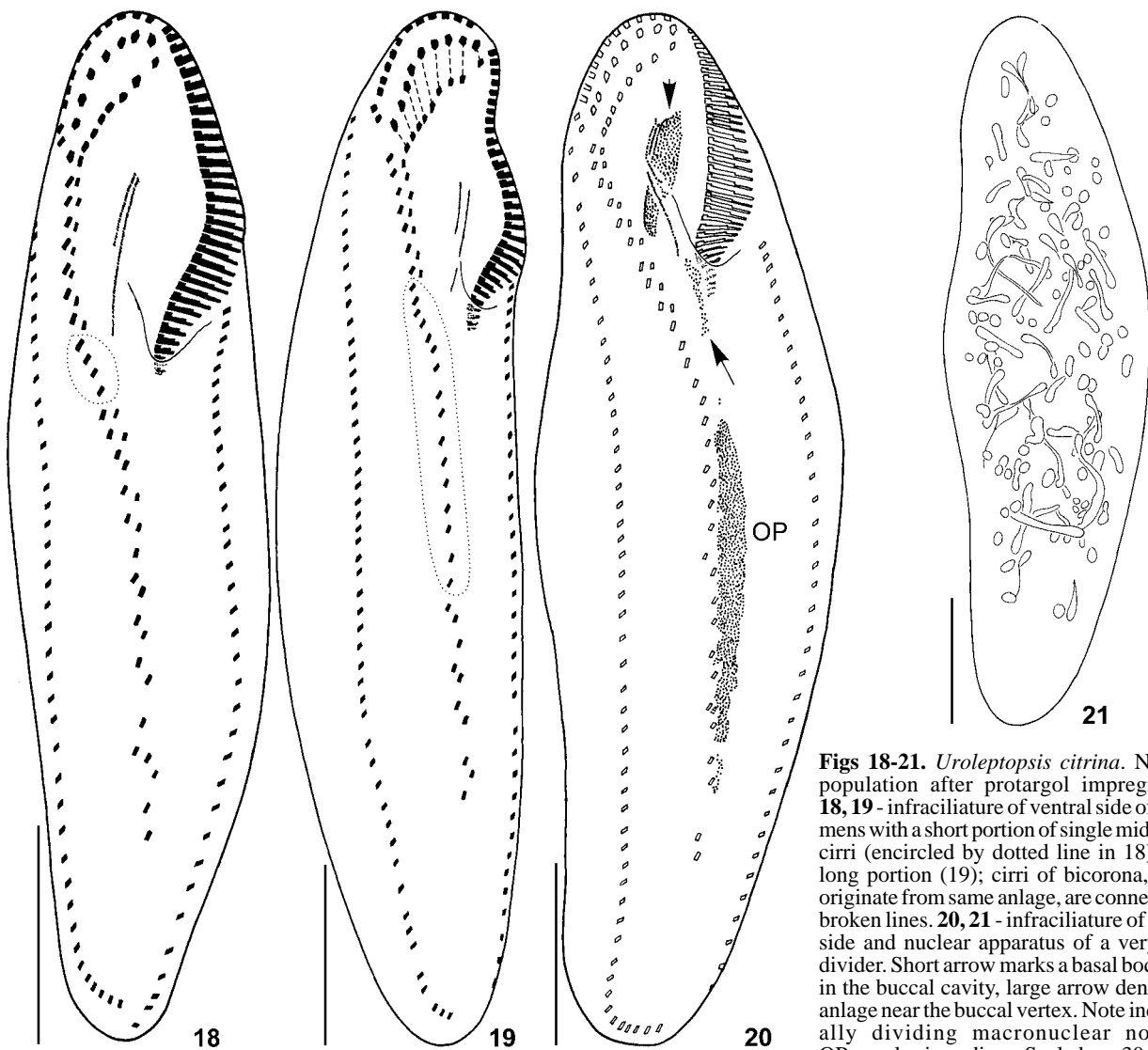
Characteristics	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Body, length	155.0	154.0	19.9	3.6	12.9	99.0	188.0	31
Body, width	41.0	42.0	7.2	1.3	17.5	26.0	57.0	31
Body length : width, ratio	3.8	3.8	0.6	0.1	14.6	3.0	5.2	31
Adoral zone of membranelles, length <sup>b</sup>	46.2	46.0	5.0	0.9	10.9	32.0	54.0	31
Adoral zone, relative length (%)	29.9	29.3	1.9	0.3	6.5	27.0	35.0	31
Anterior body end to distal end of adoral zone, distance	11.3	11.0	2.6	0.5	22.8	5.0	18.0	31
Anterior body end to rear end of gap in adoral zone, distance	4.0	4.0	1.9	0.3	47.3	1.0	9.0	31
Gap in adoral zone, length	3.1	3.0	1.1	0.2	34.1	0.8	6.0	31
Anterior body end to paroral, distance	22.3	22.0	3.3	0.6	14.8	15.0	29.0	31
Paroral, length	8.9	9.0	1.6	0.3	17.7	6.0	12.0	31
Anterior body end to first midventral cirral pair, distance	18.4	18.5	3.3	0.6	17.9	11.0	24.0	30
Distance 1 <sup>c</sup>	41.5	40.0	7.6	1.4	18.4	24.0	68.0	31
Distance 2 <sup>c</sup>	72.8	72.0	17.2	3.1	23.6	46.0	112.0	31
End of midventral complex to rear body end, distance	25.6	24.0	6.3	1.1	24.6	14.0	38.0	31
Anterior body end to right marginal row, distance	14.7	15.0	3.5	0.6	23.6	8.0	21.0	29
End of right marginal row to rear body end, distance	4.0	4.0	1.6	0.3	40.4	1.0	6.0	31
Antermost macronuclear nodule, length	4.7	4.0	1.3	0.2	27.3	3.0	8.0	31
Antermost macronuclear nodule, width	2.4	2.5	0.5	0.1	21.7	1.5	3.0	31
Antermost micronucleus, diameter	2.5	2.5	0.3	0.1	13.1	1.5	3.0	28
Adoral membranelles, total number	39.4	39.0	4.0	0.7	10.2	29.0	47.0	31
Proximal adoral membranelles, number	29.5	29.0	2.8	0.5	9.5	22.0	35.0	31
Distal adoral membranelles, number	9.9	10.0	1.4	0.2	13.8	7.0	12.0	31
Anterior corona, number of cirri	7.5	7.0	1.2	0.2	15.2	6.0	10.0	31
Posterior corona, number of cirri	7.5	7.0	1.2	0.2	15.3	6.0	10.0	31
Frontoterminal cirri, number	2.3	2.0	0.5	0.1	20.5	2.0	3.0	31
Midventral pairs in anterior portion of midventral complex, number	7.1	7.0	2.0	0.4	27.6	4.0	15.0	31
Single midventral cirri, number	10.3	11.0	4.5	0.8	43.5	4.0	18.0	31
Midventral pairs in posterior portion of midventral complex, number	6.8	5.5	3.3	0.6	48.2	2.5	14.0	31
Midventral cirri, total number	39.2	38.0	6.4	1.2	16.4	26.0	53.0	31
Left marginal cirri, number	38.9	38.0	6.1	1.1	15.7	28.0	49.0	31
Right marginal cirri, number	47.7	48.0	6.3	1.1	13.3	34.0	63.0	31
Dorsal kineties, number	3.0	3.0	0.0	0.0	0.0	3.0	3.0	31
Dorsal kinety 1, number of bristles	25.2	25.0	4.3	0.8	16.9	14.0	32.0	30

<sup>a</sup> Measurements in  $\mu\text{m}$ . Data based on mounted, protargol-impregnated, and randomly selected specimens. CV - coefficient of variation in %, M - median, Max - maximum, Min - minimum, n - number of specimens investigated, SD - standard deviation, SE - standard error of arithmetic mean,  $\bar{x}$  - arithmetic mean. <sup>b</sup> See Fig. 17. <sup>c</sup> Distance 1 and 2, see Fig. 17.

dorsolateral surface posteriorly where it ends about in midline; marginal rows thus optically more or less confluent (Figs 14, 16). Dorsal cilia 2-3  $\mu\text{m}$  long in life, arranged in three bipolar kineties (Figs 16, 40; Table 1). Caudal cirri lacking.

Brief description of Kahl's population (Fig. 13): body 150-250  $\mu\text{m}$  long; body length : width ratio 5-6 : 1; anteriorly flattened, very flexible, more or less acontractile. Many macronuclear nodules. Invariably a single large globular to ellipsoidal mass in cell centre, stains with methyl green; according to Kahl (1932) this is a kind of mycetom, that is, cytoplasm packed with bacteria; Kahl stated that the constancy of this feature has to be

checked. Contractile vacuole near left margin at about 33% of body length, rarely visible; at about 66% obviously a second vacuole, which, however, is not contractile. Cortex packed with pale yellow oval ring-shaped protrichocysts about 1.5  $\mu\text{m}$  across; smaller, solid, lemon-yellow protrichocysts (= cortical granules) near the cirral and bristle rows, form difficult-to-recognize rings around dorsal bristles; these lemon-yellow granules cause the colour of the cell. Oral apparatus occupies 16-20% of body length; adoral zone curiously shaped, narrow, turns rightwards proximally and ends in a short cytopharynx. Distinct buccal lip as well as buccal field and undulating membrane (paroral) lacking; however, sometimes the

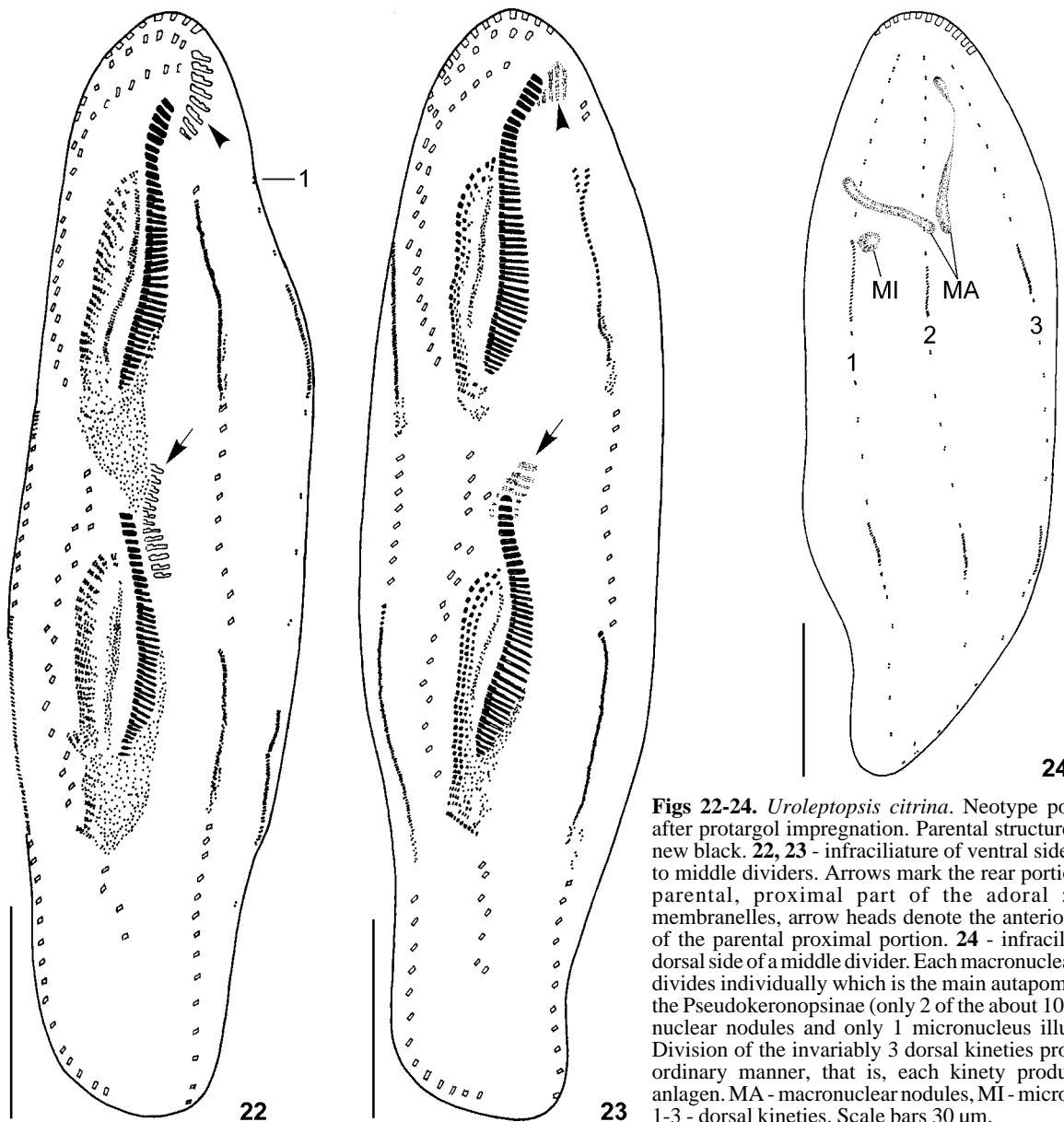


**Figs 18-21.** *Uroleptopsis citrina*. Neotype population after protargol impregnation. **18, 19** - infraciliature of ventral side of specimens with a short portion of single midventral cirri (encircled by dotted line in 18) and a long portion (19); cirri of bicorona, which originate from same anlage, are connected by broken lines. **20, 21** - infraciliature of ventral side and nuclear apparatus of a very early divider. Short arrow marks a basal body field in the buccal cavity, large arrow denotes an anlage near the buccal vertex. Note individually dividing macronuclear nodules. OP - oral primordium. Scale bars 30  $\mu$ m.

oral seam extends slightly rightwards. Marginal rows separated posteriorly; left row commences near middle portion of proximal part of adoral zone. Cirri of ventral rows (= midventral complex) finer than those of bicorona, making the impression of a single row.

**Cell division** (Figs 20-28, 42): This part of the life cycle proceeds basically as in *Pseudokeronopsis* (Wirnsberger 1987) and in some details also as in *Thigmokeronopsis* (Petz 1995). Consequently I mention only relevant deviations from *Pseudokeronopsis* which is very likely the sister group of *Uroleptopsis*. A very early stage and one or two stages between those shown in Figs 20, 22 are lacking in the sequence presented (Figs 20-28).

**Stomatogenesis:** As in *Thigmokeronopsis* and *Pseudokeronopsis*, the anlage for the new adoral zone of the proter is formed left of the endoral, likely on the "roof" of the buccal cavity (Fig. 20). Distinctly later, the newly formed adoral zone with many differentiated membranelles, the undulating membranes anlage, and the many oblique frontal-midventral cirral anlagen are recognizable (Fig. 22). In this and some later stages the new adoral zone is more or less longitudinally arranged about in cell midline (Figs 22, 23). Meanwhile, the parental adoral zone is successively resorbed. In the stages shown in Figs 22, 23 the disintegrating proximal portion of the parental adoral zone is divided with one part near the anterior cell end and the second about in

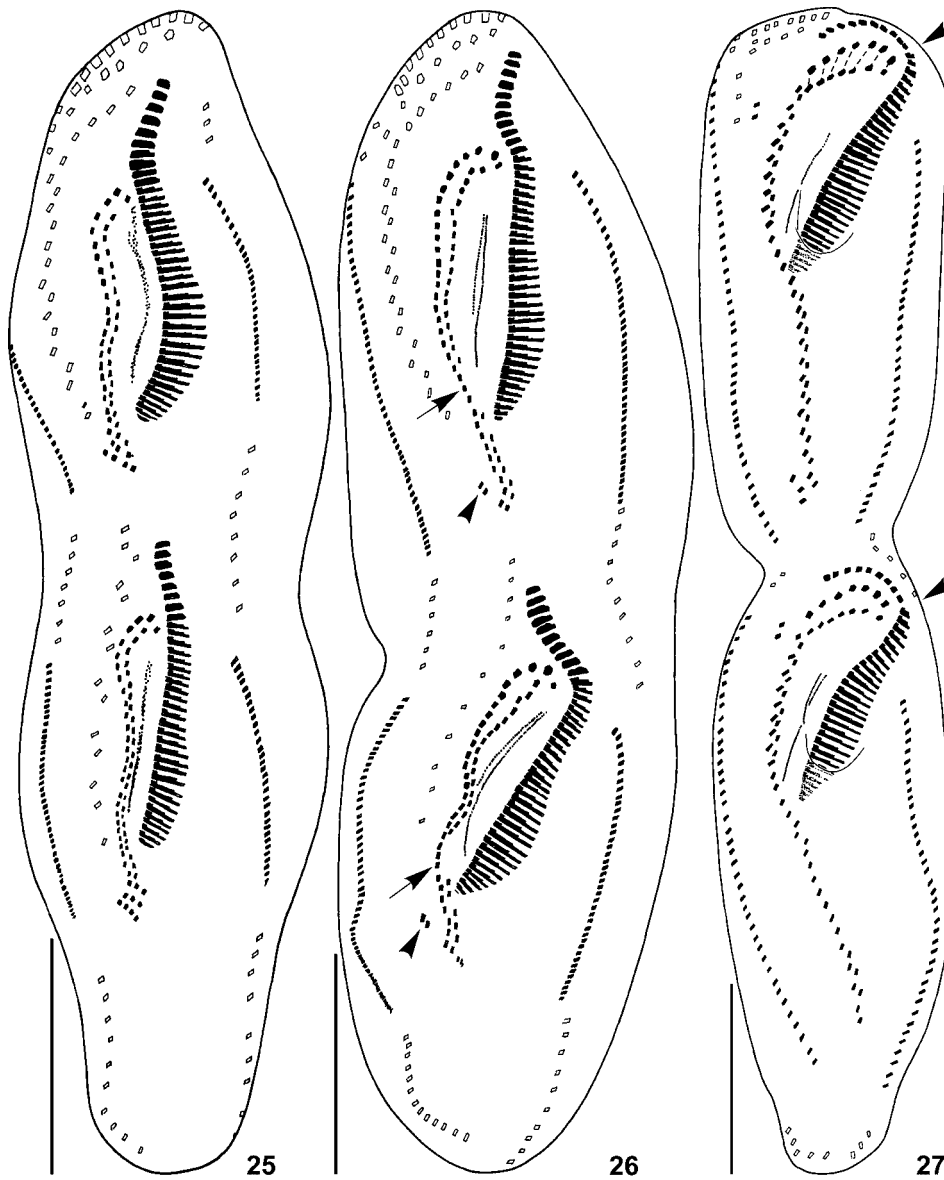


**Figs 22-24.** *Uroleptopsis citrina*. Neotype population after protargol impregnation. Parental structures white, new black. **22, 23** - infraciliature of ventral side of early to middle dividers. Arrows mark the rear portion of the parental, proximal part of the adoral zone of membranelles, arrow heads denote the anterior portion of the parental proximal portion. **24** - infraciliature of dorsal side of a middle divider. Each macronuclear nodule divides individually which is the main autapomorphy of the Pseudokeronopsinae (only 2 of the about 100 macronuclear nodules and only 1 micronucleus illustrated). Division of the invariably 3 dorsal kineties proceeds in ordinary manner, that is, each kinety produces two anlagen. MA - macronuclear nodules, MI - micronucleus, 1-3 - dorsal kineties. Scale bars 30  $\mu$ m.

mid-body. The break in the new adoral zone occurs obviously rather late, that is, shortly before or after the separation of the proter and the opisthe (Fig. 27).

The oral primordium of the opisthe originates immediately left of the midventral complex about in the middle body region (Fig. 20). Obviously no parental midventral cirrus is incorporated in the formation of the primordium, which agrees with the data on *Pseudokeronopsis* and *Thigmokeronopsis*. Later, the adoral membranelles organize in a posteriad direction and the primordium for the undulating membranes splits into the endoral and paroral (Figs 22, 23, 25-27).

**Development of frontal, midventral, and frontoterminal cirri:** Since some early stages are lacking, the origin of the frontal-midventral cirral anlagen of both the proter and the opisthe remains unknown. In the stage shown in Fig. 22, almost the complete number of anlagen is clearly recognizable in both filial products. In early to middle dividers the differentiation of cirri begins (Figs 22, 23). From anlage I, which forms the undulating membranes, two cirri originate (Figs 25-27). This is an important difference to *Pseudokeronopsis* and *Thigmokeronopsis* where, as usual, only one cirrus is formed. In the stage shown in Fig. 25, the full set of



**Figs 25-27.** *Uroleptopsis citrina*, neotype population. Infraciliature of ventral side after protargol impregnation. Parental structures white, new black. **25** - middle divider. Note that all frontal-midventral cirral anlagen have produced at least two cirri, including anlagen I and II; **26** - middle to late divider. Note that in some midventral cirral anlagen the left cirrus of the pairs is resorbed (arrows). Arrowheads denote new frontoterminal cirri which start to migrate anteriorly; **27** - very late divider (dorsal side, see Fig. 28). Arrows mark site where the gap in the adoral zone is formed. Note that the number of single midventral cirri is rather different in proter (5) and opisthe (about 13). Cirri of bicorona, which originate from same anlage, are connected by broken line. Scale bars 30  $\mu$ m.

frontal cirri, which form the bicorona, and midventral cirri, which form the midventral complex, is recognizable. However, a somewhat later stage shows that about in the third quarter of the midventral complex the left cirrus of each pair disappears (Fig. 26). This loss explains the curious pattern of the midventral complex of interphasic specimens (Figs 14, 17-19). More or less simultaneously the anterior two cirri of the rightmost

(= posteriormost) anlage begin with the migration to near the distal end of the adoral zone where they form the frontoterminal cirri (Figs 26, 27).

**Development of marginal rows and dorsal kineties:** The new marginal rows and dorsal kineties originate in ordinary manner, that is, two primordia each develop within the parental rows and kineties (Figs 22-28). No caudal cirri are formed (Figs 16, 28).

**Nuclear apparatus:** The nuclear apparatus divides as in *Pseudokeronopsis* (for example, Wirnsberger 1987), that is, the many macronuclear nodules divide individually (Figs 21, 24). The micronuclei behave like those of other hypotrichs (Fig. 28).

**Occurrence and ecology:** *Uroleptopsis citrina* is a benthic, marine species. Kahl (1932) found it in the German city of Kiel “not rare” in an aquarium where it was sluggishly borrowing in the mesosaprobic debris. Kahl (1932) did not state from where the material in the aquarium was. However, in his guide to marine ciliates (Kahl 1933) he wrote “in alten Kieler Kulturen und Aquarien nicht selten” (= not rare in old cultures from Kiel and in aquaria) so that we can conclude that he found it (inter alia? exclusively?) in the Baltic Sea at the coast of which the city of Kiel is located.

I found *U. citrina* in the littoral of the northern Adriatic Sea at a water temperature of about 20°C (further details, see materials and methods). Due to the neotypification (see below), this sample site becomes the new type locality. It occurred, inter alia, together with *Amphisiella annulata* (see Berger 2004), *Pseudoamphisiella* sp., and some euplotids. There exist some records from the Bulgarian coast of the Black Sea which are, however, not substantiated by illustrations or morphological data (for review, see Detcheva 1992).

**Systematics:** Kahl (1932) described *U. citrina*, type of *Uroleptopsis*, rather detailed from life (Fig. 13). Subsequently it was listed in several reviews, but no additional data have been provided. Borror and Wicklow (1983) classified it as one of several junior synonyms of *Pseudokeronopsis rubra* which is hardly comprehensible because *U. citrina* is yellow, has no buccal cirrus right of the paroral, lacks transverse cirri, and has invariably three dorsal kineties; in contrast, *Pseudokeronopsis rubra* is red, has an ordinary buccal cirrus and distinct transverse cirri, and usually six dorsal kineties (Wirnsberger *et al.* 1987).

The population from the northern Adriatic Sea very closely resembles the type population from the Baltic. They agree in the following features: (i) marine habitat; (ii) size; (iii) nuclear apparatus; (iv) contractile vacuoles; (v) yellow cortical granules and ring-shaped structures underneath cell surface; (vi) narrow buccal field; (vii) bicorona and long midventral complex basically composed of cirral pairs (the lack of some left cirri in the middle region of the midventral complex is very difficult to recognize without silver impregnation and was therefore possibly overlooked by Kahl although he even wrote

that the two ventral rows nearly make the impression of a single row); (viii) lack of a buccal cirrus in ordinary position; (ix) lack of transverse cirri; (x) three dorsal kineties.

Differences concern (i) the oral apparatus; (ii) a so-called “Mycetom” (a plasma-region containing many bacteria) described by Kahl (1932); (iii) the distance between the rear end of the marginal rows; (iv) the body shape; and (v) the colour. The gap in the adoral zone of the Adriatic population is clearly recognisable only in protargol preparations; thus, it is unknown whether or not this feature was present in Kahl’s population who did not have the advantage of silver impregnation. Further, Kahl did not see a buccal field and a paroral. The buccal field is obviously very narrow in Kahl’s population and therefore one can also say that it is lacking. The lack of the paroral in his specimens is much more difficult to explain because he described this structure in many other hypotrichs; possibly he overlooked it because it is indeed rather inconspicuous. The mycetom was obviously present in all specimens studied by Kahl; however, he wrote that the constancy of this feature has to be checked. Possibly it was a kind of parasitism. In the specimens of Kahl’s population the marginal rows are distinctly separated posteriorly (Fig. 13). In contrast, they optically almost overlap in the specimens from the Adriatic Sea (Figs 14, 16, 18). Since this feature is difficult to recognize in life it must not be over-interpreted. Kahl’s specimen is more or less band-shaped whereas the specimens from the Adriatic Sea are usually elongate elliptical in outline. Possibly, Kahl’s specimens did not have their natural outline due to the mycetom (see above) whose effect on the cell is not known. According to Kahl, the yellow colour of *U. citrina* is due to the yellow cortical granules. However, in my population the number of these granules is usually too low to cause such a distinct colour. In contrast, the yellow colour of the Adriatic population is mainly caused by a diffuse colour of the cytoplasm which is often very distinct in the marginal areas.

**Neotypification:** No type or voucher slides are available from any *U. citrina* population. As mentioned above, Borror and Wicklow (1983) have synonymized it with *Pseudokeronopsis rubra*, a proposal which is certainly incorrect (see also chapter History of *Uroleptopsis*). To avoid such a misclassification in future it seems wise to define *U. citrina* objectively by the designation of a neotype (ICZN 1999, Foissner 2002). The neotypification of *P. rubra* was already done

by Wirnsberger *et al.* (1987). According to Article 75.3 of the ICZN (1999), the designation of a neotype is only valid when seven particulars are published: (i) as mentioned above, the systematic status of *U. citrina* is somewhat unclear because it was synonymized with the rather common *Pseudokeronopsis rubra* in the last revision on urostylelids (Borror and Wicklow 1983). (ii) for a differentiation of *U. citrina* from related taxa, see next chapter. (iii) the neotype specimen (Figs 14-16), respectively, neotype population from the Adriatic Sea is described in detail (see above); thus, recognition of the neotype designated is ensured. (iv) it is generally known that no type material is available from species described by Kahl. Further, there is no indication that Detcheva, who recorded it from the Black Sea, made permanent preparations, let alone designated a neotype. (v) there is strong evidence that the neotype is consistent with *U. citrina* as originally described by Kahl (1932). For a detailed comparison, see previous chapter. The differences discussed must not be over-interpreted and are possibly due to some minor misobservations by Kahl (1932). Further, it cannot be excluded that Kahl's specimen were slightly influenced by the so-called mycetom, which was possibly a kind of parasitism. (vi) unfortunately, the neotype does not come from very near the original type locality (northern Adriatic Sea against Baltic Sea near the German city of Kiel; distance about 1000 km). However, both sites are marine habitats from the Holarctic region. As generally known, many ciliates - especially marine ones which live in a comparatively homogenous medium - are cosmopolitans (Patterson *et al.* 1989) so that this point should not be over-interpreted. For a more detailed discussion of this problem, see Foissner *et al.* (2002, p. 44) and Foissner (2002). A detailed description of the new type locality, that is, the sample site of the neotype population, is given in the chapter materials and methods. (vii) the slide containing the neotype specimen and nine slides containing some further specimens, including those depicted in the present paper, of the neotype population are deposited in the Biologiezentrum des Oberösterreichischen Landesmuseums in Linz (LI), Austria.

**Comparison with similar species:** For a separation of *U. citrina* from the other *Uroleptopsis* species, see the key below. According to Kahl (1932) and my experience, *Uroleptopsis citrina* is very easily confused with *Pseudokeronopsis flava* which is also yellow. However, this species has usually two transverse cirri, four dorsal kineties, one buccal cirrus in ordinary position, and lacks a break in the adoral zone (Wirnsberger

*et al.* 1987). Protargol impregnation is therefore recommended to check these features.

### Redefinition of *Uroleptopsis* Kahl, 1932

1932 *Uroleptopsis* gen. n. - Kahl, *Tierwelt Dtl.*, **25**: 543 (original description). Type species (by original designation on p. 543): *Uroleptopsis citrina* Kahl, 1932.

1933 *Uroleptopsis* Kahl 1932 - Kahl, *Tierwelt N.- u. Ostsee*, **23**: 107 (guide to marine ciliates).

1950 *Uroleptopsis* Kahl - Kudo, *Protozoology*, p. 672 (textbook).

1972 *Uroleptopsis* Kahl, 1932 - Borror, *J. Protozool.*, **19**: 11 (revision).

1974b *Uroleptopsis* Kahl - Stiller, *Fauna Hung.*, **115**: 62 (revision).

1982 *Uroleptopsis* Kahl, 1932 - Hemberger, *Dissertation*, p. 120 (revision).

1992 *Uroleptopsis* Kahl, 1930-5 - Carey, *Marine interstitial ciliates*, p. 187 (guide).

**Redefinition:** Basing on the new data on the type species and the data on *U. ignea* (Mihailowitsch and Wilbert 1990; see below), *Uroleptopsis* can be redefined by the combination of the following three autapomorphies (A) and several more or less young plesiomorphies: gap in adoral zone (A). Transverse cirri absent (A). Frontal-midventral cirral anlage I forms two cirri (A). Two arched rows (= bicorona) of frontal cirri. Buccal cirrus in ordinary position, that is, right of paroral. 2 or more frontoterminal cirri. Midventral complex basically composed of midventral pairs. 1 left and 1 right marginal row. Caudal cirri absent. Many macronuclear nodules which divide individually. Parental adoral zone completely replaced during morphogenesis, that is, proter gets totally new adoral zone. Living in saline waters.

For a discussion of the autapomorphies, see chapter phylogenetic relationships. Possibly this ground plan has to be changed when new data on the other *Uroleptopsis* species became available.

**Nomenclature:** No derivation of the genus-group name is given in the original description. *Uroleptopsis* is likely a composite of the name of the hypotrich genus *Uroleptus* and the Greek suffix *-opsis* (looking like). Probably, the name should indicate the resemblance of *Uroleptus* and *Uroleptopsis* species. Feminine gender because ending with *-opsis* (ICZN 1999, Article 30.1.2). *Uroleptosis* in Borror and Wicklow (1983, p. 123) is an incorrect subsequent spelling.

**History of *Uroleptopsis*:** Kahl (1932) established *Uroleptopsis* because the lack of transverse cirri in some species prevailed their classification in *Holosticha* (*Keronopsis*) (nowadays most species of this subgenus are classified in *Pseudokeronopsis*). Kahl described

one new species, the type *U. citrina*, and transferred two species to *Uroleptopsis*, namely *Oxytricha viridis* and *Uroleptus roscovianus*. *Uroleptopsis* was accepted - beside the workers listed in the synonymy - for example, by Corliss (1977, 1979), Tuffrau (1979, 1987), and Tuffrau and Fleury (1994). By contrast, Borror (1979) has put it - together with *Trichototaxis* - into the synonymy of *Keronopsis* (*sensu lato*). Later, he synonymized it with *Pseudokeronopsis* because he classified the type species *U. citrina* as junior synonym of *P. rubra*, the type species of *Pseudokeronopsis* (Borror and Wicklow 1983). Borror argued - however, without convincing evidence - that some *P. rubra* populations also lack transverse cirri so that the presence or absence of these cirri cannot be used to define groups. I suggest that he mixed, certainly erroneously, species with and without transverse cirri. In addition, Borror and Wicklow (1983) obviously overlooked that due to this synonymy *Pseudokeronopsis* Borror and Wicklow, 1983 would become invalid, or better its establishment would have been superfluous because it would be the junior synonym of *Uroleptopsis* Kahl, 1932.

In 1990, Mihailowitsch and Wilbert described *Pseudokeronopsis ignea* which lacks transverse cirri. Thus, Foissner (1995) transferred this species to *Uroleptopsis*. However, this resurrection of Kahl's genus by Foissner was not accepted by Eigner (2001) who distinguished two patterns of transverse cirri formation. According to Eigner, *Pseudokeronopsis ignea* has transverse cirri (Figs 29, 30). However, his interpretation of the morphogenetic data of *P. ignea* is not comprehensible for me. I agree with Mihailowitsch and Wilbert (1990) and Foissner (1995) that this species does not have transverse cirri as defined usually. Thus I accept the decision of Foissner to exclude Mihailowitsch and Wilbert's species from *Pseudokeronopsis* and to put it into *Uroleptopsis*.

The presence or absence of distinct cirral groups is widely used to establish genera or subgenera. Examples are *Tachysoma* (caudal cirri absent against present in many 18-cirri oxytrichids; for review, see Berger 1999) and *Australothrix* (transverse cirri lacking against present in many urostylids; Blatterer and Foissner 1988). Consequently it seems logically to accept *Uroleptopsis*, inasmuch as it shows - beside the lack of transverse cirri - two further apomorphies, namely (i) two frontal cirri originating from the frontal-midventral anlage I against single cirrus in almost all other hypotrichs, and (ii) a gap in the adoral zone of membranelles. *Uroleptopsis* is therefore as well defined as many other genera of

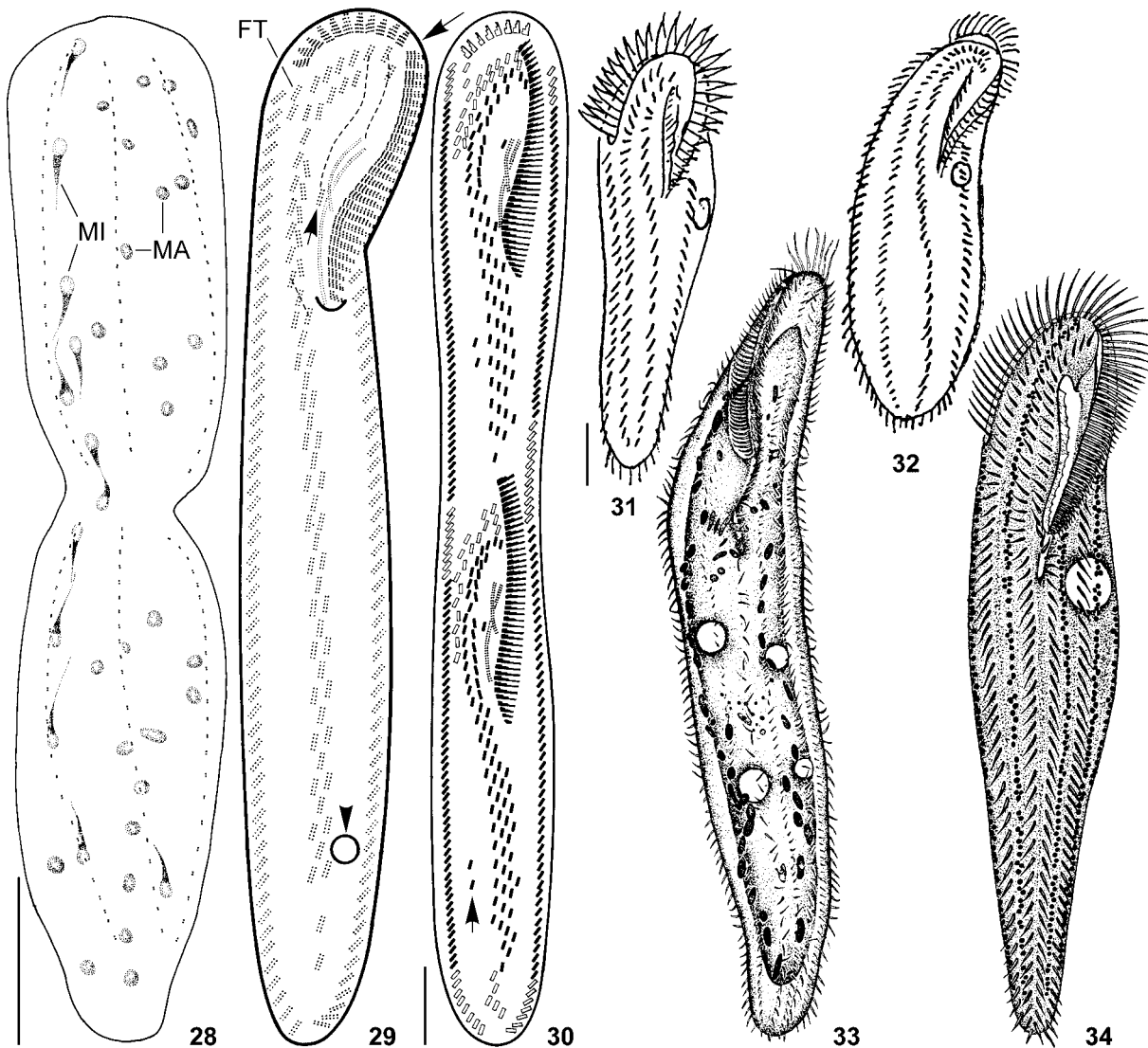
hypotrichs and should not be synonymized with *Pseudokeronopsis*.

**Species included in *Uroleptopsis*:** (i) *Uroleptopsis citrina* Kahl, 1932 (Figs 5-28, 35-42); (ii) *U. roscoviana* (Maupas, 1883) Kahl, 1932 (Fig. 31); (iii) *U. viridis* (Pereyaslawzewa, 1886) Kahl, 1932 (Fig. 32); (iv) *U. tannaensis* (Shigematsu, 1953) comb. nov. (Fig. 33); (v) *U. ignea* (Mihailowitsch and Wilbert, 1990) Foissner, 1995 (Figs 29, 30).

As already mentioned in the history section, Kahl (1932) transferred *Oxytricha viridis* and *Uroleptus roscovianus* to *Uroleptopsis*. Both species, which need detailed redescription, have a more or less distinct bicorona and lack transverse cirri and a buccal cirrus which is in the ordinary position. Thus Kahl's combinations can be accepted. *Holosticha* (*Keronopsis*) *multiplex* Ozaki and Yagiu, 1943 is very similar to *Uroleptopsis roscoviana* in shape, colour, and cirral pattern (Figs 31, 34). Although *H. multiplex* is smaller (body length 70-160 µm against 190-220 µm) I suppose that they are synonymous. Ozaki and Yagiu described their species in the subgenus *Keronopsis* without mentioning the corresponding genus. I suspect that they used Kahl's (1932) system where *Keronopsis* is classified as subgenus of *Holosticha*.

*Keronopsis tannaensis* is described after Haidenhein's haematoxylin preparations (Fig. 33). Shigematsu (1953) stated that this species lacks transverse cirri. A bicorona is neither mentioned nor clearly illustrated. However, the original classification in *Keronopsis* implies that the frontal cirral pattern must be more or less of the bicorona type. Jankowski (1979) transferred it to *Holosticha*. However, this genus has, inter alia, three enlarged frontal cirri and distinct transverse cirri. Borror and Wicklow (1983) synonymized *K. tannaensis* with *Pseudokeronopsis decolor* which has - like its congeners - transverse cirri. All combinations proposed so far would make these taxa (*Keronopsis*, *Holosticha*, *Pseudokeronopsis*) in-homogenous. I transfer it to *Uroleptopsis* because this results in the lowest number of contradictions: *Uroleptopsis tannaensis* (Shigematsu, 1953) comb. nov. (basonym: *Keronopsis tannaensis* Shigematsu, 1953). Detailed redescription needed.

Mihailowitsch and Wilbert (1990) recognised the main features of *Pseudokeronopsis ignea* which separate it from other *Pseudokeronopsis* species, namely the presence of midventral rows and the lack of transverse cirri. They argued that these characters could be used to establish a genus. However, they refrained from this act



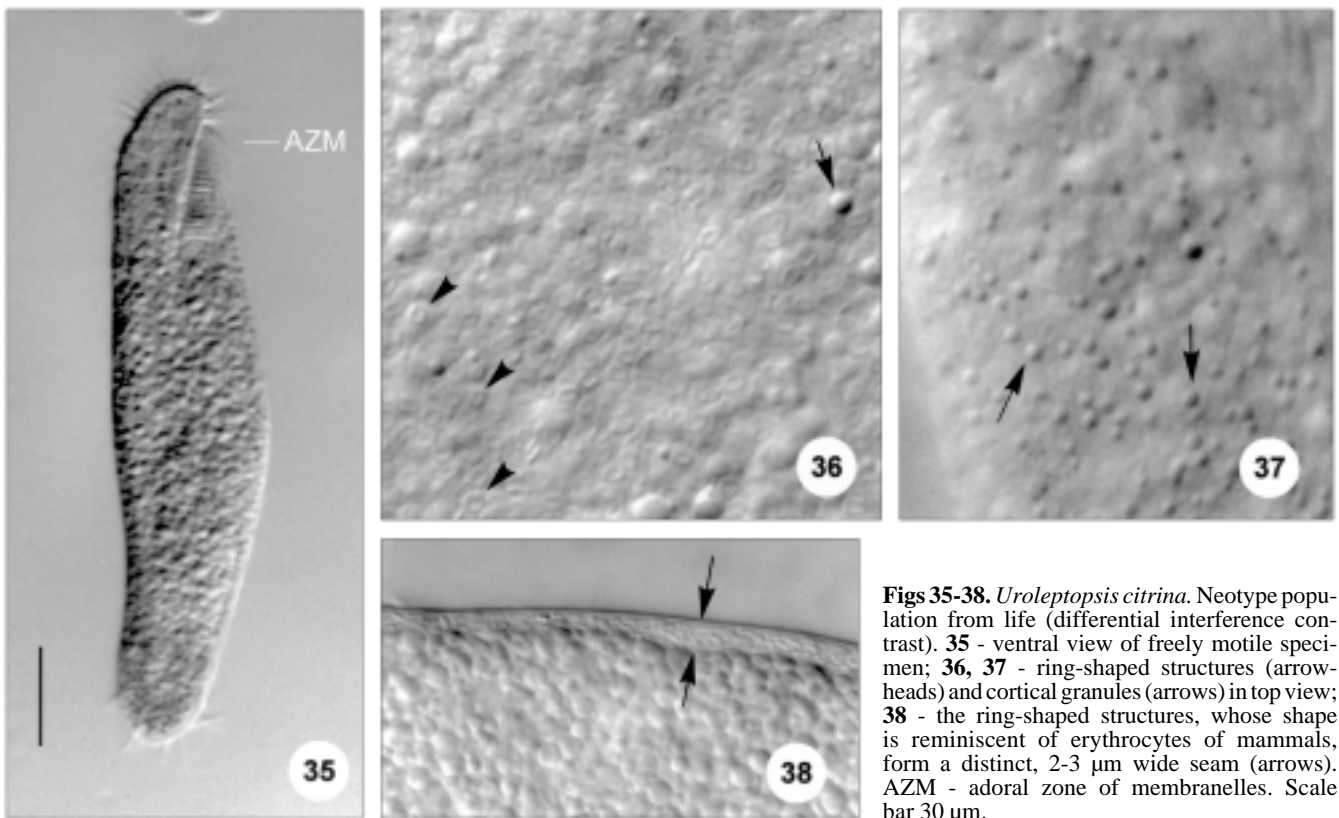
**Figs 28-34.** *Uroleptopsis citrina* (28, neotype population) and other *Uroleptopsis* species (29-34) after protargol impregnation (28-30), from life (31, 32, 34), and Haidenhain's haematoxylin preparation (33). **28** - infraciliature of dorsal side and nuclear apparatus of a late divider (ventral side of same specimen, see Fig. 27); **29, 30** - *Uroleptopsis ignea* (from Mihailowitsch and Wilbert 1990), infraciliature of ventral side of a morphostatic specimen (body length 405  $\mu\text{m}$  according to scale bar; 262-353  $\mu\text{m}$  according to their Tabele 6) and a late divider (parental structures white, new black). Long arrow in (29) marks gap in adoral zone, short arrow marks buccal cirrus; arrowhead denotes contractile vacuole. Broken lines connect cirri of anlagen I, which also forms 2 cirri, and II. Arrow in (30) marks new frontoterminal cirri of opisthe. Note that the major portion of the midventral complex is composed of midventral rows each made of usually 3 cirri (anteriormost row marked by broken line). **31** - *Uroleptopsis roscoviana* (after Maupas 1883 from Kahl 1932), ventral view; **32** - *Uroleptopsis viridis* (after Pereyaslawzewa 1886 from Kahl 1932), ventral view, size not indicated. **33** - *Uroleptopsis tannaensis* (from Shigematsu 1953), dorsal view showing nuclear apparatus, contractile vacuoles, cirral pattern and dorsal kineties, and adoral zone of membranelles (individual size not indicated). Shigematsu wrote "ventral view" explaining that he did not distinguish between the infraciliature of the ventral and dorsal side; **34** - *Keronopsis multiplex* (from Ozaki and Yagiu 1943), ventral view, 70-160  $\mu\text{m}$ . I suppose that this is a synonym of *U. roscoviana*. FT - frontoterminal cirri; MA - macronuclear nodules; MI - dividing micronuclei. Scale bars 30  $\mu\text{m}$ .

because Borrer and Wicklow (1983) stated a high variability of the feature transverse cirri in many urostyloid genera. Foissner (1995) discussed Borrer and Wicklow's (1983) decision to reject *Uroleptopsis*. He resurrected Kahl's genus and simultaneously transferred *P. ignea* to

*Uroleptopsis*. As already mentioned, Eigner (2001) did not accept the resurrection.

**Species misplaced in *Uroleptopsis*:** Three further species have been assigned to *Uroleptopsis*. However, as discussed below, they do not fit into the ground plan





**Figs 35-38.** *Uroleptopsis citrina*. Neotype population from life (differential interference contrast). **35** - ventral view of freely motile specimen; **36, 37** - ring-shaped structures (arrowheads) and cortical granules (arrows) in top view; **38** - the ring-shaped structures, whose shape is reminiscent of erythrocytes of mammals, form a distinct, 2-3  $\mu\text{m}$  wide seam (arrows). AZM - adoral zone of membranelles. Scale bar 30  $\mu\text{m}$ .

of *Uroleptopsis* and therefore belong elsewhere. *Uroleptopsis multiseta* Dragesco, 1970 has around seven cirral rows. They are longitudinally arranged and widely spaced and therefore do not form a midventral pattern. Dragesco (1970) himself suggested that this species possibly belongs to a new genus, *Plesiotricha*; however, he did not formally transfer it to his own genus. Later, he classified it in *Kahliella* (Dragesco and Dragesco-Kernéis 1986). Due to this act it became a secondary homonym of *Kahliella multiseta* Dragesco, 1970 and therefore the species-group name of *U. multiseta* had to be replaced: *Kahliella microstoma* Dragesco and Dragesco-Kernéis, 1986. For a more detailed discussion of this nomenclatural problem, see Foissner (1987a).

Borror (1972) transferred *Paraholosticha ovata* to *Uroleptopsis*. Stiller (1974a) obviously overlooked this act because she transferred it to *Uroleptopsis* too. This species has, inter alia, only two macronuclear nodules, lacks a distinct midventral complex, and lives in freshwater. All these features strongly indicate that it does not belong to *Uroleptopsis*. Probably it is a junior synonym of *Paraholosticha muscicola* (Berger 2001).

A further species transferred to *Uroleptopsis* is *Uroleptus kahli* Grolière, 1975 because Jankowski (1979) mentioned "*Uroleptopsis kahli*, *ibidem*" under the heading *Perisincirra* Yankowskij, 1978 for which Grolière's species is the type. I do not understand the word *ibidem* ("the same reference" or "in the same place") in this context because neither Grolière (1975) nor Yankowskij (1978) mentioned a combination of *Uroleptus kahli* with *Uroleptopsis*. I therefore assume that Jankowski (1979) made the combination with *Uroleptopsis*, possibly par lapsus. Anyhow, the classification of Grolière's species in *Uroleptopsis* is incorrect because recently we found that *Perisincirra* is a valid group (Foissner *et al.* 2002).

**Comparison of *Uroleptopsis citrina* and *U. ignea*:** *Uroleptopsis citrina* and *U. ignea* are the sole *Uroleptopsis* species whose morphology and morphogenesis are described by modern methods. Thus they can be compared thoroughly. They share the following synapomorphies: lack of transverse cirri, two frontal cirri originate from anlage I, gap in adoral zone. The following two conspicuous differences exist: (i) the type species lacks a buccal cirrus in the ordinary position, that is, right

of the paroral (present in *U. ignea*), and (ii) the pattern of the midventral complex. In *U. citrina* the middle portion of the midventral complex is composed of the right cirri of the cirral pairs only (Fig. 14); the anterior and posterior portion consist of ordinary midventral pairs although, very rarely, short midventral rows occur at the end of the complex. In contrast, the anterior portion of the midventral complex of *U. ignea* is composed of midventral pairs, while the middle and posterior portion consist of midventral rows (Figs 29, 30). A more detailed analysis of the first difference shows that the buccal cirrus is not lacking in *U. citrina*, but it does not migrate posteriorly into the ordinary position; it is - in contrast to *U. ignea* - part of the bicorona even in non-dividers. This difference is of course very conspicuous and sufficient to establish a new subgenus or even genus. As mentioned above, all other species assigned to *Uroleptopsis* are not described by modern methods. However, the data available indicate that they lack a buccal cirrus in the ordinary position, that is, they are obviously more similar to *U. citrina* than to *U. ignea*. Thus, I establish a new subgenus for *U. ignea* (see below). A subgenus has the advantage that the binomen including the authorship of the species does not change again.

Interestingly, the species now assigned to *Uroleptopsis* (*Uroleptopsis*) are from marine habitats, whereas *U. ignea* - the sole species belonging to the other subgenus - was discovered in an inland saltwater. Further studies will show whether this ecological separation is confirmed or not.

***Uroleptopsis* (*Uroleptopsis*) Kahl, 1932 stat. n.**

1932 *Uroleptopsis* gen. n. - Kahl, *Tierwelt Dtl.*, **25**: 543 (original description). Type species (by original designation on p. 543): *Uroleptopsis citrina* Kahl, 1932.

**Diagnosis:** *Uroleptopsis* with cirrus II/2 (= buccal cirrus) not in ordinary position right of paroral, but in line with cirri of posterior corona.

**Nomenclature:** For genus-group names the principle of co-ordination applies. Thus, a name established for a taxon at either rank in the genus-group is deemed to have been simultaneously established by the same author for a nominal taxon at the other rank in the group; both nominal taxa have the same type species (ICZN 1999, Article 43.1). Consequently, Kahl (1932) is the author and *U. citrina* Kahl, 1932 the type species of the present subgenus, which is also termed the nominotypical subgenus (ICZN 1999, Article 44.1).

**Remarks:** At least in the type species, cirrus II/2 (= buccal cirrus) is formed during cell division and present in interphasic specimens; however, it does not migrate posteriorly in the ordinary position immediately right of the paroral (Figs 14, 27). *Uroleptopsis citrina* has a further highly interesting feature, namely the lack of the left cirrus in some cirral pairs of the middle region of the midventral complex. Unfortunately, we do not know whether or not this character is also present in the other species assigned to *U. (Uroleptopsis)*. Thus, this feature - which could be a second autapomorphy for *U. (Uroleptopsis)* - is not included in the characterization.

**Species included:** *Uroleptopsis* (*Uroleptopsis*) *citrina* Kahl, 1932; *U. (Uroleptopsis) viridis* (Pereyaslawzewa, 1886) Kahl, 1932; *U. (Uroleptopsis) roscoviana* (Maupas, 1883) Kahl, 1932; *U. (Uroleptopsis) tannaensis* (Shigematsu, 1953) comb. nov. (foundation of new combination, see above).

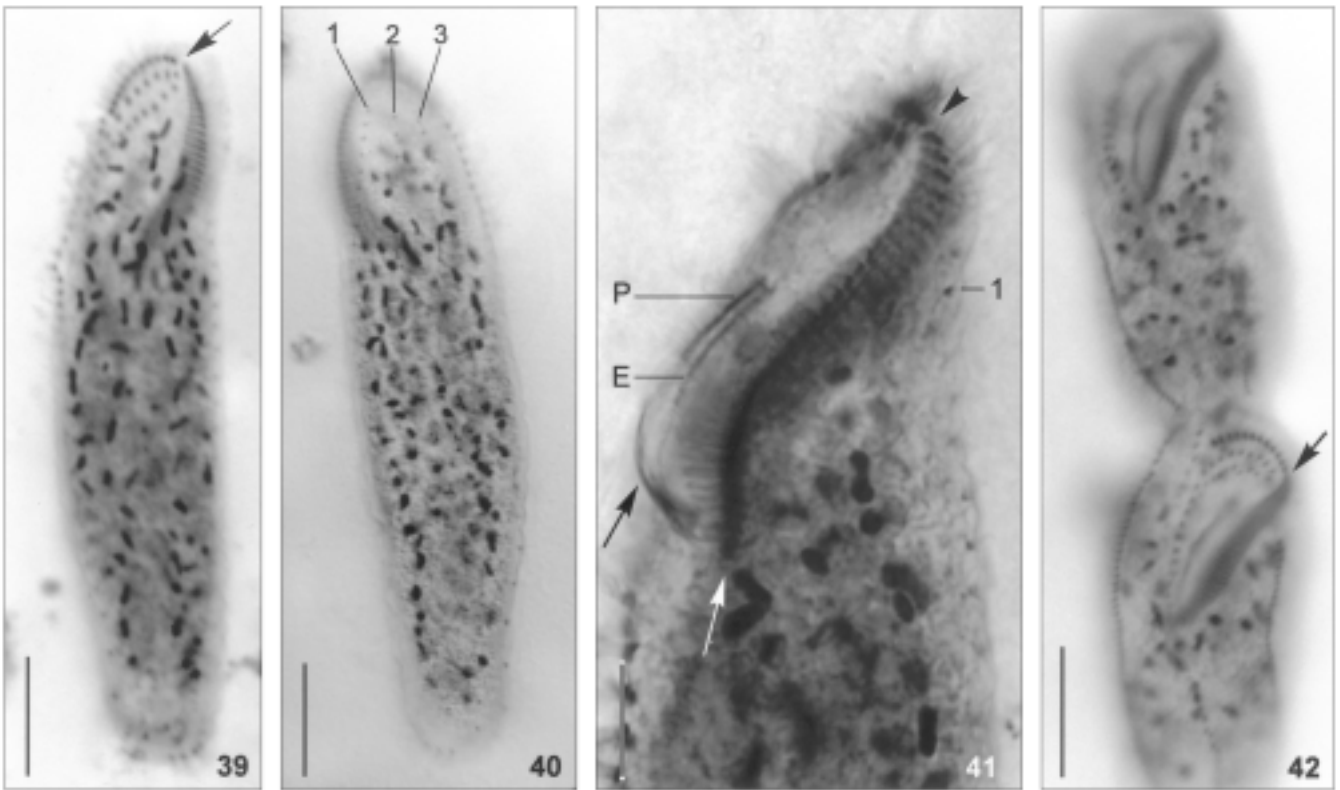
***Uroleptopsis* (*Plesiouroleptopsis*) subgen. n.**

**Diagnosis:** *Uroleptopsis* with midventral complex composed of midventral pairs and midventral rows.

**Type species:** *Pseudokeronopsis ignea* Mihailowitsch et Wilbert, 1990.

**Nomenclature:** *Plesiouroleptopsis* is a composite of the Greek *plesió* (near, neighbouring; Hentschel and Wagner 1996) and the existing generic name *Uroleptopsis* (see there for derivation). *Plesiouroleptopsis* alludes to the fact that the type species, *Pseudokeronopsis ignea*, has a buccal cirrus in ordinary position which is the plesiomorphic character state. In contrast, the type species of *U. (Uroleptopsis)*, *Uroleptopsis citrina*, has no cirrus immediately right of the undulating membranes which is interpreted as apomorphic state. The subgenus *Plesiouroleptopsis* has, like *Uroleptopsis*, feminine gender because ending with *-opsis* (ICZN 1999, Article 30.1.2).

**Remarks:** As already mentioned, *Pseudokeronopsis ignea* was transferred to *Uroleptopsis* by Foissner (1995). It is the sole species in *Uroleptopsis* with a buccal cirrus in the ordinary position which is very likely the plesiomorphic state. By contrast, the midventral rows are a novelty within the Pseudokeronopsidae and therefore the autapomorphy for this species/subgenus. Midventral rows are not a very complex feature. They originate in that simply more than two cirri per streak are produced. Consequently, their convergent evolution in



**Figs 39-42.** *Uroleptopsis citrina*. Infraciliature of neotype population after protargol impregnation. **39, 40** - ventral and dorsal side. Arrow marks gap in adoral zone of membranelles. **41** - left lateral view of oral apparatus showing adoral zone with gap (arrowhead), undulating membranes, buccal lip (black arrow), nuclear apparatus, and dorsal kinety 1. White arrow marks proximal end of adoral zone of membranelles; **42** - ventral side of a very late divider. At this stage the gap in the adoral zone is not yet recognizable (arrow). E - endoral; P - paroral; 1-3 - dorsal kineties. Scale bars 30  $\mu$ m (39, 40, 42) and 10  $\mu$ m (41).

other urostylelids - for example, *Bakuella* and *Holostichides* - is not a great surprise.

**Species included:** Only the type species - *Uroleptopsis (Plesiouroleptopsis) ignea* (Mihailowitsch and Wilbert, 1990) Foissner, 1995 - is included. For further discussion, see same chapter at genus section.

**Key to *Uroleptopsis* species**

Note that only two of the five species listed below are described after protargol preparations. Thus, for the remaining three species the features concerning the cirral pattern are rather uncertain. Further, size and shape as well as the nuclear apparatus do not allow a clear separation. Consequently, I use the colour of the cells as key character, which is admittedly a difficult feature, especially for beginners. Please be sure that the colour of your specimens is a real colour and not due to a badly adjusted optics of your microscope! However, before you use the key, you must be sure that your

specimens belong to an *Uroleptopsis* population. Thus, identification needs both protargol impregnation (cirral pattern) and live observation (colour). Only very experienced workers have a chance to identify these species correctly exclusively after live observation.

- 1. Buccal cirrus present, that is, cirrus arranged right of paroral (Fig. 29).....*Uroleptopsis (Plesiouroleptopsis) ignea* - Buccal cirrus lacking, that is, no cirrus immediately right of paroral (e.g., Fig. 14).....*Uroleptopsis (Uroleptopsis) 2*
- 2. Cells more or less colourless (Fig. 33).....*Uroleptopsis tannaensis* - Cells light green, rose-carmine, or yellow.....3
- 3. Cells rose-carmine (Figs 31, 34).....*Uroleptopsis roscoviana* - Cells yellow or light green.....4
- 4. Cells yellow (Figs 5, 13).....*Uroleptopsis citrina* - Cells light green (Fig. 32).....*Uroleptopsis viridis*

### Phylogenetic relationships

Borror and Wicklow (1983) - basically using the system proposed by Wicklow (1981) - united *Pseudokeronopsis* (including *Uroleptopsis* as synonym; see above) and *Thigmokeronopsis* in the Pseudokeronopsidae. As unifying features they mentioned the presence of a bicorona and the far posteriorly extending distal end of the adoral zone of membranelles. Eigner and Foissner (1992) also considered *Thigmokeronopsis* and *Pseudokeronopsis* as sister groups. However, they could not provide a synapomorphy because of the lack of appropriate data on *Thigmokeronopsis*.

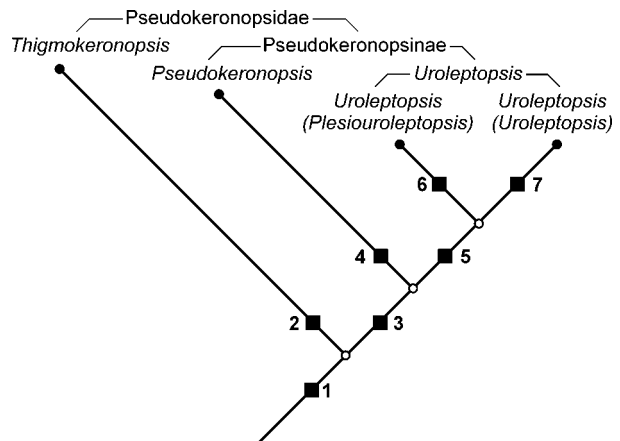
I agree with this grouping but provide a different and more detailed foundation of the relationships (Fig. 43). The diagram proposed is the most parsimonious one of three possible arrangements of *Pseudokeronopsis*, *Thigmokeronopsis*, and *Uroleptopsis*.

### Pseudokeronopsidae (Fig. 43, autapomorphy 1)

(i) The many macronuclear nodules fuse to some parts during division.

**Remarks:** This state is realized in *Thigmokeronopsis*, at least in *T. crystallis* and *T. antarctica*, where the many nodules fuse to several parts during morphogenesis (Petz 1995). Unfortunately, nothing is known about the fate of the macronucleus during this part of the life cycle in the type species *Thigmokeronopsis jahodai* (Wicklow 1981). The plesiomorphic state of this feature is that all (two to many) nodules fuse to a single mass during division. This state is present in all non-euplotid hypotrichs, except the pseudokeronopsids. In *Metaurostyloopsis marina* the macronuclear nodules fuse to a single mass too (Song *et al.* 2001). However, the mass is not globular or has another compact shape as in most species, but is a strongly branched structure. Interestingly, the formation of the proter's adoral zone proceeds very similar (identical?) as in the pseudokeronopsids (see next paragraph). Thus, *Metaurostyloopsis* could be the sister group of the Pseudokeronopsidae because the macronucleus already shows the tendency not to fuse to a compact, globular mass.

So far I do not know a second autapomorphy for the Pseudokeronopsidae. The formation of a new adoral zone for the proter, as described for *Thigmokeronopsis*, *Uroleptopsis*, and *Pseudokeronopsis*, is possibly a candidate. However, a more or less identical mode is



**Fig. 43.** Diagram of phylogenetic relationships within the Pseudokeronopsidae. Autapomorphies (black squares 1-7): **1** - the many macronuclear nodules fuse to some parts during division. **2** - thigmotactic field of cirri; anlagen of marginal rows and dorsal kineties originate de novo. **3** - each of the many macronuclear nodules divides individually. **4** - four or more dorsal kineties. **5** - cirral anlage I forms two cirri; transverse cirri lacking; gap in adoral zone. **6** - some midventral cirral anlagen produce midventral rows. **7** - buccal cirrus not in ordinary position; some midventral cirral anlagen produce finally only one cirrus. For detailed description and discussion of autapomorphies, see text.

described for *Metaurostyloopsis marina* (Song *et al.* 2001) and *Holosticha multistilata* (Hemberger 1982). In both species, the macronuclear nodules fuse to a single mass so that they cannot be included in the Pseudokeronopsidae (see, however, previous paragraph for *Metaurostyloopsis*). Consequently this type of stomatogenesis is either a plesiomorphy in the ground plan of the Pseudokeronopsidae or this feature evolved convergently. Song *et al.* (1997) also described a total new formation of the proter's adoral zone for *Pseudoamphisiella lacazei*. However, in this species the corresponding oral primordium originates obviously behind the parental adoral zone and not in the buccal cavity as in the pseudokeronopsids. Further, the macronuclear nodules fuse to a single mass so that it cannot be included in the Pseudokeronopsidae. In the other urostylids, few parental adoral membranelles (for example, *Bakuella*; Song *et al.* 1992, Eigner and Foissner 1992) to many (for example, in *Urostyla grandis*; Ganner 1991) are reorganized. This partial reorganisation is obviously distinctly different from the total new formation discussed above.

Pseudokeronopsid species have a bicorona, a type of frontal ciliature also present in other urostylid taxa, for example, *Bicoronella* (Foissner 1995), *Keronella*

(Wiackowski 1985), *Pseudourostyla* (Jerka-Dziadosz 1964, Borror 1972), and *Neokeronopsis* (Warren *et al.* 2002). This distribution indicates that the bicorona is a plesiomorphy for the Pseudokeronopsidae, although one cannot exclude that such a frontal ciliature evolved convergently.

Borror and Wicklow (1983) characterized the Pseudokeronopsidae, *inter alia*, by the far posteriorly extending distal end of the adoral zone. In fact, this feature applies to *Pseudokeronopsis* and *Thigmokeronopsis*, and to a somewhat smaller extent also to *Uroleptopsis* (Figs 17-19). However, this characteristic is also known from other urostylids, for example, *Notocephalus* (Petz *et al.* 1995) and *Pseudoamphisiella* (Song *et al.* 1997), which certainly do not belong to the Pseudokeronopsidae. Since the branching pattern outside the Pseudokeronopsidae is unknown, we do not know whether this distal elongation is a symplesiomorphy or a convergence.

#### ***Thigmokeronopsis* (Fig. 43, autapomorphies 2)**

(i) Thigmotactic field of cirri.

**Remarks:** *Thigmokeronopsis* species have a more or less large field of thigmotactic cirri (= left postoral ventral files according to Petz 1995) between the midventral complex and the left marginal row (Wicklow 1981, Petz 1995). Such a group of cirri is lacking in other hypotrichs strongly indicating that this is an autapomorphy of *Thigmokeronopsis* (Wicklow 1981, Eigner and Foissner 1992).

(ii) Anlagen of marginal rows and dorsal kineties originate *de novo*.

**Remarks:** Generally, new marginal rows and dorsal kineties originate within the parental structures. By contrast, in all three *Thigmokeronopsis* species the new marginal rows and dorsal kineties occur distinctly left or right of the parental structures (Wicklow 1981, Petz 1995).

#### **Pseudokeronopsinae (Fig. 43, autapomorphy 3)**

(i) More or less each of the many macronuclear nodules divides individually.

**Remarks:** So far, this feature is only described for several *Pseudokeronopsis* populations (for example, Gruber 1884, Rühmekorf 1935, Wirnsberger 1987) and for *Uroleptopsis citrina* and *U. ignea* (present paper, Mihailowitsch and Wilbert 1990). The plesiomorphic state is the partial fusion still present in *Thigmokeronopsis* (see Pseudokeronopsidae). It is more parsimonious to assume that the individual division has evolved *via* the

intermediate state still realized in *Thigmokeronopsis* than directly from the common mode where all nodules fuse to a single mass.

Here the ring-shaped structures, which form a distinct seam in *U. citrina*, have to be discussed (Figs 7, 8, 11, 12, 36-38). These structures have also been described for some *Pseudokeronopsis* populations (Prowazek 1900, Hu and Song 2001, Song *et al.* 2002), but not for *Thigmokeronopsis* (Wicklow 1981, Petz 1995). Thus, one could suggest that this feature is an autapomorphy for the Pseudokeronopsinae. However, these structures are also described for two holostichid species (Song and Wilbert 1997, Gong *et al.* 2001) which certainly are not members of the Pseudokeronopsidae. Thus, this feature cannot be used as phylogenetic marker at the present state of knowledge.

Interestingly enough, many *Uroleptopsis* and *Pseudokeronopsis* species have a more or less distinct colour whereas all three *Thigmokeronopsis* species are colourless. However, currently too few data are available to assess the meaning of this feature for phylogenetic analysis.

#### ***Pseudokeronopsis* (Fig. 43, autapomorphy 4)**

(i) Four or more dorsal kineties present.

**Remarks:** I do not know if the increase of the number of dorsal kineties from three to four or more is in fact a good autapomorphy for *Pseudokeronopsis*. Anyhow, it is interesting that all *Thigmokeronopsis* species and both *Uroleptopsis* species investigated in detail have invariably three dorsal kineties. This indicates that three kineties is the plesiomorphic state within the Pseudokeronopsidae. In contrast, *Pseudokeronopsis* species have four or more bristle rows (Foissner 1984, Wirnsberger *et al.* 1987, Hu and Song 2001, Song *et al.* 2002). Only *P. flava* has very rarely three kineties (Wirnsberger *et al.* 1987), which, however, must not be over-interpreted.

Eigner and Foissner (1992) used the feature "Parental basal bodies not involved in formation of ciliary structures of daughters". Likely they meant the frontal-midventral-transverse-cirri because the formation of the marginal rows and dorsal kineties proceeds in ordinary manner in *Pseudokeronopsis*, that is, within the parental structures (Wirnsberger 1987). In contrast, the parental midventral complex is obviously not involved in primordia formation. However, the data on *Thigmokeronopsis* (Petz 1995) and *Uroleptopsis* (Mihailowitsch and Wilbert 1990, present paper) indicate that this feature also applies to these taxa. Thus, it could

be an autapomorphy of the Pseudokeronopsidae. However, it is very difficult to decide whether or not parental cirri are definitely involved in primordia formation. Consequently this feature is not used further although it cannot be excluded that it is a useful marker in future when more morphogenetic data on urostylids are available.

***Uroleptopsis* (Fig. 43, autapomorphies 5)**

(i) Cirral anlage I forms two cirri.

**Remarks:** Within the pseudokeronopsids this feature only occurs in *Uroleptopsis*. In *Pseudokeronopsis*, *Thigmokeronopsis*, and most other hypotrichs, the anlage I, which produces the undulating membranes, forms only the left frontal cirrus. This state must be therefore considered as plesiomorphic. *Bicoronella costaricana* and *Caudiholosticha sylvatica* have 1-7 cirri behind the left frontal cirrus indicating that in these two species anlage I also produces more than one cirrus (Foissner 1982, 1995; Berger and Foissner 1989; Berger 2003). *Caudiholosticha sylvatica* has three frontal cirri indicating that it is not closely related to the pseudokeronopsids. In contrast, *Bicoronella costaricana* has a bicorona so that it could be a near relative of the pseudokeronopsids. However, morphogenetic data are needed to show the fate of the macronuclear nodules and the origin of the supernumerary cirri behind the left frontal cirrus.

(ii) Transverse cirri lacking.

**Remarks:** This feature was the main reason for Kahl (1932) to separate *U. citrina* from *Holosticha* (*Keronopsis*) species (now *Pseudokeronopsis*). The loss of the transverse cirri occurred undoubtedly several times independently within the hypotrichs. Other urostylid taxa which lack transverse cirri are, for example, *Australothrix* spp. (Blatterer and Foissner 1988), *Eschaneustyla* (Eigner 1994, Foissner *et al.* 2002), and *Holostichides* spp. (Foissner 1987b). However, in *Eschaneustyla* the macronuclear nodules fuse to a single mass during cell division and *Australothrix* and *Holostichides* lack, in addition, a bicorona strongly indicating that none of them is a member of the Pseudokeronopsidae.

(iii) Gap in adoral zone.

**Remarks:** Within the pseudokeronopsids, *Uroleptopsis citrina* and *U. ignea* are the sole species which have a distinct break in the adoral zone. The gap

is not very distinct in life and therefore I assume that it has been overlooked in the three other *Uroleptopsis* species which are not yet described after protargol impregnation. This break, which is likely the site where the zone turns from ventral to dorsal, is possibly homologous with the interruption separating the anterior (= outer, = collar) membranelles from the ventral (= inner, = buccal) membranelles of the oligotrichs (Petz and Foissner 1992, Foissner *et al.* 1999). In most hypotrichs, this transition site is inconspicuous because not marked by a gap. Very likely such a distinct division evolved several times independently, for instance, in *Holosticha* (e.g., Petz *et al.* 1995, Berger 2003), *Afrothrix*, *Erniella*, *Etoschothrix* (for review, see Foissner *et al.* 2002), or *Parabirojimia* (Hu *et al.* 2002).

***Uroleptopsis* (*Plesiouroleptopsis*) (Fig. 43, autapomorphy 6)**

(i) Some midventral cirral anlagen produce midventral rows.

**Remarks:** A midventral complex composed of cirral pairs is undoubtedly the plesiomorphic state. Consequently the formation of more than two cirri within a streak has to be interpreted as autapomorphy of *U. ignea*, the sole species belonging to the present subgenus. Midventral rows occur in other urostylid taxa too, for example, *Bakuella*, *Eschaneustyla*, *Holostichides*, *Keronella*, *Paragastrostyla* (Wiackowski 1985, Song and Wilbert 1988, Song 1990, Song *et al.* 1992, Eigner 1994). However, in none of these taxa the macronuclear nodules divide individually so that we must postulate the convergent evolution of midventral rows. A midventral row is not a very complex feature because it is produced by a simple increase of the number of cirri formed within an anlage. Thus, the convergent evolution of midventral rows is nothing unusual.

***Uroleptopsis* (*Uroleptopsis*) (Fig. 43, autapomorphies 7)**

(i) Buccal cirrus not in ordinary position.

**Remarks:** For review on the confusing terminology of this cirrus, see Berger and Foissner (1997) and Berger (1999). The plesiomorphic state is the presence of a single cirrus (= cirrus II/2 according to Wallengren's 1900 terminology) in ordinary position which is immediately right of the paroral. Some taxa have two or more buccal cirri but only few lack such a cirrus, for example,

*Paragastrostyla* and *Periholosticha* (Hemberger 1985). However, in these taxa cirrus II/2 is indeed lacking while it is present, but not in the ordinary position in *U. citrina*. As already mentioned above, it is part of the posterior corona in interphasic specimens (Figs 14, 27). A similar situation is realized in *Neokeronopsis spectabilis*, a huge urostylid freshwater species with prominent transverse cirri, only two macronuclear nodules which fuse during morphogenesis, and fragmentation of dorsal kinety 3 (Warren *et al.* 2002, own observations). In this species cirrus II/2 is also in line with the posterior corona, but the long undulating membranes are arranged more or less as in *Cyrtohymena* so that cirrus II/2 is simultaneously positioned immediately right of the anterior portion of the paroral.

(ii) Some midventral cirral anlagen finally produce only one cirrus.

**Remarks:** This is obviously a real novelty within the urostylids. The plesiomorphic state is that a frontal-midventral anlage produces finally two cirri, a so-called midventral pair (the rearmost anlagen, of course, produce three to four cirri if they also form a transverse cirrus, respectively, a pretransverse ventral cirrus and a transverse cirrus; Figs 1, 2). At first, two cirri are produced in *U. citrina* too (Fig. 25). However, somewhat later the left cirrus of the corresponding pairs is obviously resorbed so that only the right cirrus remains in the interphasic specimen (Fig. 26). Interestingly enough, this resorption is confined to the middle portion of the midventral complex. This section of the complex therefore looks almost like a midventral row. It is unknown whether or not this feature is present in the other species assigned to *U. (Uroleptopsis)*. It could be that this feature is only an autapomorphy of *U. citrina*. A very similar resorption of cirri is described for *Psammocephalus faurei* by Wicklow (1982) indicating a convergence.

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## REFERENCES

- Ax P. (1995) Das System der Metazoa I. Ein Lehrbuch der phylogenetischen Systematik. Fischer, Stuttgart, Jena, New York
- Ax P. (1999) Das System der Metazoa II. Ein Lehrbuch der phylogenetischen Systematik. Fischer, Stuttgart, Jena, New York
- Berger H. (1999) Monograph of the Oxytrichidae (Ciliophora, Hypotrichida). *Monographiae biol.* **78**: i-xii, 1-1080
- Berger H. (2001) Catalogue of Ciliate Names 1. Hypotrichs. Verlag Helmut Berger, Salzburg
- Berger H. (2003) Redefinition of *Holosticha* Wrzesniewski, 1877 (Ciliophora, Hypotricha). *Europ. J. Protistol.* **39**: 373-379
- Berger H. (2004) *Amphisiella annulata* (Kahl, 1928) Borror, 1972 (Ciliophora: Hypotricha): morphology, notes on morphogenesis, review of literature, and neotypification. *Acta Protozool.* **43**: 1-14
- Berger H., Foissner W. (1989) Morphology and biometry of some soil hypotrichs (Protozoa, Ciliophora) from Europe and Japan. *Bull. Br. Mus. nat. Hist. (Zool.)* **55**: 19-46
- Berger H., Foissner W. (1997) Cladistic relationships and generic characterization of oxytrichid hypotrichs (Protozoa, Ciliophora). *Arch. Protistenk.* **148**: 125-155
- Blatterer H., Foissner W. (1988) Beitrag zur terricolen Ciliatenfauna (Protozoa: Ciliophora) Australiens. *Stapfia* **17**: 1-84
- Borror A. C. (1972) Revision of the order Hypotrichida (Ciliophora, Protozoa). *J. Protozool.* **19**: 1-23
- Borror A. C. (1979) Redefinition of the Urostylidae (Ciliophora, Hypotrichida) on the basis of morphogenetic characters. *J. Protozool.* **26**: 544-550
- Borror A. C., Wicklow B. J. (1983) The suborder Urostylina Jankowski (Ciliophora, Hypotrichida): morphology, systematics and identification of species. *Acta Protozool.* **22**: 97-126
- Buitkamp U. (1977) Die Ciliatenfauna der Savanne von Lamto (Elfenbeinküste). *Acta Protozool.* **16**: 249-276
- Carey P. G. (1992) Marine Interstitial Ciliates. An Illustrated Key. Chapman and Hall, London, New York, Tokyo, Melbourne, Madras
- Corliss J. O. (1977) Annotated assignment of families and genera to the orders and classes currently comprising the Corlissian scheme of higher classification for the phylum Ciliophora. *Trans. Am. microsc. Soc.* **96**: 104-140
- Corliss J. O. (1979) The Ciliated Protozoa. Characterization, Classification and Guide to the Literature. 2nd ed. Pergamon Press, Oxford, New York, Toronto, Sydney, Paris, Frankfurt
- Detcheva R. B. (1992) Catalogi Faunae Bulgaricae 1 Protozoa, Ciliophora. Academia Scientiarum Bulgaricae, Sofia
- Dragesco J. (1970) Ciliés libres du Cameroun. *Annls Fac. Sci. Univ. féd. Cameroun (Numéro hors-sériés)*: 1-141
- Dragesco J., Dragesco-Kernéis A. (1986) Ciliés libres de l'Afrique intertropicale. Introduction à la connaissance et à l'étude des ciliés. *Faune tropicale* **26**: 1-559
- Eigner P. (1994) Divisional morphogenesis and reorganization in *Eschaneustyla brachytone* Stokes, 1886 and revision of the Bakuellinae (Ciliophora, Hypotrichida). *Europ. J. Protistol.* **30**: 462-475
- Eigner P. (2001) Divisional morphogenesis in *Uroleptus caudatus* (Stokes, 1886), and the relationship between the Urostylidae and the Parakahliliellidae, Oxytrichidae, and Orthoamphisiellidae on the basis of morphogenetic processes (Ciliophora, Hypotrichida). *J. Euk. Microbiol.* **48**: 70-79
- Eigner P., Foissner W. (1992) Divisional morphogenesis in *Bakuella pampinaria* nov. spec. and reevaluation of the classification of the urostylids (Ciliophora, Hypotrichida). *Europ. J. Protistol.* **28**: 460-470
- Foissner W. (1982) Ökologie und Taxonomie der Hypotrichida (Protozoa: Ciliophora) einiger österreichischer Böden. *Arch. Protistenk.* **126**: 19-143
- Foissner W. (1984) Infraciliatur, Silberliniensystem und Biometrie einiger neuer und wenig bekannter terrestrischer, limnischer und mariner Ciliaten (Protozoa: Ciliophora) aus den Klassen Kinetofragminophora, Colpodea und Polyhymenophora. *Stapfia* **12**: 1-165
- Foissner W. (1987a) Miscellanea nomenclatorica ciliatea (Protozoa: Ciliophora). *Arch. Protistenk.* **133**: 219-235
- Foissner W. (1987b) Neue und wenig bekannte hypotriche und colpode Ciliaten (Protozoa: Ciliophora) aus Böden und Moosen. *Zool. Beitr. (N.F.)* **31**: 187-282
- Foissner W. (1995) Tropical protozoan diversity: 80 ciliate species (Protozoa, Ciliophora) in a soil sample from a tropical dry forest

- of Costa Rica, with descriptions of four new genera and seven new species. *Arch. Protistenk.* **145**: 37-79
- Foissner W. (2002) Neotypification of protists, especially ciliates (Protozoa, Ciliophora). *Bull. zool. Nom.* **59**: 165-169
- Foissner W., Berger H., Kohmann F. (1999) Identification and ecology of limnetic plankton ciliates. *Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft* **3/99**: 1-793
- Foissner W., Agatha S., Berger H. (2002) Soil ciliates (Protozoa, Ciliophora) from Namibia (Southwest Africa), with emphasis on two contrasting environments, the Etosha region and the Namib Desert. Part I: Text and line drawings. Part II: Photographs. *Denisia* **5**: 1-1459
- Ganner B. (1991) Zur Taxonomie einiger Ciliaten (Protozoa) aus Fließgewässern. Dissertation Universität Salzburg
- Gong J., Song W., Hu X., Ma H., Zhu M. (2001) Morphology and infraciliature of *Holosticha bradburyae* nov. spec. (Ciliophora, Hypotrichida) from the Yellow Sea, China. *Hydrobiologia* **464**: 63-69
- Grolière C-A. (1975) Descriptions de quereques ciliés hypotriches de tourbières a sphaignes et des étendues d'eau acides. *Protistologica* **11**: 481-498
- Gruber A. (1884) Über Kern und Kernteilung bei den Protozoen. *Z. wiss. Zool.* **40**: 121-153
- Hemberger H. (1982) Revision der Ordnung Hypotrichida Stein (Ciliophora, Protozoa) an Hand von Protargolpräparaten und Morphogenesedarstellungen. Dissertation Universität Bonn
- Hemberger H. (1985) Neue Gattungen und Arten hypotricher Ciliaten. *Arch. Protistenk.* **130**: 397-417
- Hentschel E. J., Wagner G. H. (1996) Zoologisches Wörterbuch. Tiernamen, allgemeinbiologische, anatomische, physiologische Termini und Kurzbiographien. Gustav Fischer Verlag, Jena
- Hu X., Song W. (2001) Morphological redescription and morphogenesis of the marine ciliate, *Pseudokeronopsis rubra* (Ciliophora: Hypotrichida). *Acta Protozool.* **40**: 107-115
- Hu X., Song, Warren A. (2002) Observations on the morphology and morphogenesis of a new marine urostyleid ciliate, *Parabirojimia similis* nov. gen., nov. spec. (Protozoa, Ciliophora, Hypotrichida). *Europ. J. Protistol.* **38**: 351-364
- ICZN (The International Commission on Zoological Nomenclature) (1999) International Code of Zoological Nomenclature. International Trust for Zoological Nomenclature, London
- Jankowski A. W. (1979) Revision of the order Hypotrichida Stein, 1859. Generic catalogue, phylogeny, taxonomy. *Trudy zool. Inst., Leningrad* **86**: 48-85
- Jerka-Dziadosz M. (1964) *Urostyla cristata* sp. n. (Urostyleidae, Hypotrichida): the morphology and morphogenesis. *Acta Protozool.* **2**: 123-128
- Kahl A. (1932) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. *Tierwelt Dtl.* **25**: 399-650
- Kahl A. (1933) Ciliata libera et ectocommensalia. *Tierwelt N.- u. Ostsee Lieferung 23 (Teil II. c.)*: 29-146
- Kudo R. R. (1950) Protozoology. 3rd ed. Thomas Publisher, Springfield
- Lynn D. H., Small E. B. (2002) Phylum Ciliophora Doflein, 1901. In: An Illustrated Guide to the Protozoa, 2nd ed. (Eds J. J. Lee, G. F. Leedale, P. Bradbury). Society of Protozoologists, Lawrence **1**: 371-656
- Maupas E. (1883) Contribution a l'étude morphologique et anatomique des infusoires ciliés. *Archs Zool. exp. gén., 2<sup>e</sup> Série* **1**: 427-664
- Mihailowitsch B., Wilbert N. (1990) *Bakuella salinarum* nov. spec. und *Pseudokeronopsis ignea* nov. spec. (Ciliata, Hypotrichida) aus einem solebelasteten Fließgewässer des östlichen Münsterlandes, BRD. *Arch. Protistenk.* **138**: 207-219
- Ozaki Y., Yagi R. (1943) Studies on the marine ciliates of Japan, mainly from the Setonaikai (the inland sea of Japan). III. *J. Sci. Hiroshima Univ. Series B, Div. I. Zoology* **10**: 21-52
- Patterson D. J., Larsen J., Corliss J. O. (1989) The ecology of heterotrophic flagellates and ciliates living in marine sediments. *Progr. Protistol.* **3**: 185-277
- Pereyaslawzewa S. (1886) Protozoaires de la mer Noire. *Zap. novoross. Obshch. Estest.* **10**: 79-114
- Petz W. (1995) Morphology and morphogenesis of *Thigmokeronopsis antarctica* nov. spec. and *T. crystallis* nov. spec. (Ciliophora, Hypotrichida) from Antarctica sea ice. *Europ. J. Protistol.* **31**: 137-147
- Petz W., Foissner W. (1992) Morphology and morphogenesis of *Strobilidium caudatum* (Fromental), *Meseres corlissi* n. sp., *Halteria grandinella* (Müller), and *Strombidium rehwaldi* n. sp., and a proposed phylogenetic system for oligotrich ciliates (Protozoa, Ciliophora). *J. Protozool.* **39**: 159-176
- Petz W., Song W., Wilbert N. (1995) Taxonomy and ecology of the ciliate fauna (Protozoa, Ciliophora) in the endopagial and pelagial of the Weddell Sea, Antarctica. *Stapfia* **40**: 1-223
- Prowazek S. (1900) Protozoenstudien II. *Arb. zool. Inst. Univ. Wien* **12**: 243-300
- Rühmekorf T. (1935) Morphologie, Teilung und Hungerformen von *Keronopsis*. *Arch. Protistenk.* **85**: 255-288
- Shigematsu A. (1953) Two new marine ciliates. *J. Sci. Hiroshima Univ., Series B. Div. I. Zoology* **14**: 47-50
- Song W. (1990) Morphologie und Morphogenese des Bodenciliaten *Periholosticha wilberti* nov. spec. (Ciliophora, Hypotrichida). *Arch. Protistenk.* **138**: 221-231
- Song W., Wilbert N. (1988) *Parabakuella typica* nov. gen., nov. spec. (Ciliata, Hypotrichida) aus dem Edaphon eines Standortes in Qingdao, China. *Arch. Protistenk.* **135**: 319-325
- Song W., Wilbert N. (1997) Morphological studies on some free living ciliates (Ciliophora: Heterotrichida, Hypotrichida) from marine biotopes in Qingdao, China, with descriptions of three new species: *Holosticha warreni* nov. spec., *Tachysoma ovata* nov. spec. and *T. dragescoi* nov. spec. *Europ. J. Protistol.* **33**: 48-62
- Song W., Wilbert N., Berger H. (1992) Morphology and morphogenesis of the soil ciliate *Bakuella edaphoni* nov. spec. and revision of the genus *Bakuella* Agamaliyev & Alekperov, 1976 (Ciliophora, Hypotrichida). *Bull. Br. Mus. nat. Hist. (Zool.)* **58**: 133-148
- Song W., Warren A., Hu X. (1997) Morphology and morphogenesis of *Pseudoamphisiella lacazei* (Maupas, 1883) Song, 1996 with suggestion of establishment of a new family Pseudoamphisiellidae nov. fam. (Ciliophora, Hypotrichida). *Arch. Protistenk.* **147**: 265-276
- Song W., Petz W., Warren A. (2001) Morphology and morphogenesis of the poorly-known marine urostyleid ciliate, *Metaurostylopsis marina* (Kahl, 1932) nov. gen., nov. comb. (Protozoa, Ciliophora, Hypotrichida). *Europ. J. Protistol.* **37**: 63-76
- Song W., Wilbert N., Warren A. (2002) New contribution to the morphology and taxonomy of four marine hypotrichous ciliates from Qingdao, China (Protozoa: Ciliophora). *Acta Protozool.* **41**: 145-162
- Stiller J. (1974a) Ergänzungen der von Fauré-Fremiet vorgenommenen Neuordnung der hypotrichen Ciliaten. *Annl. hist.-nat. Mus. nat. hung.* **66**: 129-133
- Stiller J. (1974b) Járólábacsok csillósok - Hypotrichida. *Fauna Hung.* **115**: 1-187
- Tuffrau M. (1979) Une nouvelle famille d'hypotriches, Kahliliellidae n. fam., et ses conséquences dans la repartition des Stichotrichina. *Trans. Am. microsc. Soc.* **98**: 521-528
- Tuffrau M. (1987) Proposition d'une classification nouvelle de l'ordre Hypotrichida (Protozoa, Ciliophora), fondée sur quelques données récentes. *Annl. Sci. nat. (Zool.)*, 13<sup>e</sup> Série **8** (years 1986-1987): 111-117
- Tuffrau M., Fleury A. (1994) Classe des Hypotricha Stein, 1859. *Traite de Zoologie* **2**: 83-151
- Wägele J-W. (2001) Grundlagen der phylogenetischen Systematik. Pfeil, München
- Wallengren H. (1900) Zur Kenntnis der vergleichenden Morphologie der hypotrichen Infusorien. *Bih. K. svensk. VetenskAkad. Handl.* **26**: 1-31
- Warren A., Fyda J., Song W. (2002) The morphology of the poorly-known freshwater urostyleid ciliate *Neokeronopsis spectabilis* (Kahl, 1932) nov. gen. nov. comb., (Ciliophora: Urostyleidae), with notes on its morphogenesis. *Europ. J. Protistol.* **38**: 195-206
- Wiackowski K. (1985) The morphology and morphogenesis of *Keronella gracilis* n. gen., n. spec. (Hypotrichida, Ciliophora). *Protistologica* **21**: 81-91



- Wicklow B. J. (1981) Evolution within the order Hypotrichida (Ciliophora, Protozoa): ultrastructure and morphogenesis of *Thigmokeronopsis jahodai* (n. gen., n. sp.); phylogeny in the urostylina (Jankowski, 1979). *Protistologica* **17**: 331-351
- Wicklow B. J. (1982) The Discocephalina (n. subord.): ultrastructure, morphogenesis and evolutionary implications of a group of endemic marine interstitial hypotrichs (Ciliophora, Protozoa). *Protistologica* **18**: 299-330
- Wirnsberger E. (1987) Division and reorganization in the genus *Pseudokeronopsis* and relationships between urostylids and oxytrichids (Ciliophora, Hypotrichida). *Arch. Protistenk.* **134**: 149-160
- Wirnsberger E., Larsen H. F., Uhlig G. (1987) Rediagnosis of closely related pigmented marine species of the genus *Pseudokeronopsis* (Ciliophora, Hypotrichida). *Europ. J. Protistol.* **23**: 76-88
- Yankovskij A. V. (1978) The revision of the system of Polyhymenophora class (Spirotrichia). *Tezisy Dokl. zool. Inst. Akad. Nauk SSSR* **1978**: 39-40

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## Redescription of *Cryptobia helicis* Leidy, 1846 (Kinetoplasta: Bodonea: Cryptobiidae), Disposition of Flagellates Mistakenly Assigned to This Species, and Description of a New Species from a North American Pulmonate Snail

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**Summary.** *Cryptobia helicis* Leidy, 1846, the type species of the genus, is redescribed on the basis of material from the seminal receptacle of *Neohelix albolabris albolabris*, the first of three host pulmonate snails mentioned by Leidy. The flagellate from *Triodopsis tridentata*, the second host mentioned by Leidy, is very different, and is described as *C. innominata* sp. n. The *Cryptobia* in *Anguispira alternata*, the third host designated by Leidy, is also distinct, but cannot properly be described until more material is available. The extensively studied cryptobias in *Helix pomatia* and *H. aspersa aspersa*, long considered to be *C. helicis*, are very different from true *C. helicis* and *C. innominata*. The name *C. helicogena*, originally proposed as *Trypanoplasma helicogena* Kühn, 1911, is applicable to the flagellate in *H. pomatia*, and tentatively also to the one in *H. aspersa aspersa*.

**Key words:** Bodonea, *Cryptobia helicis*, *C. innominata* sp. n., *C. helicogena*, Kinetoplasta, taxonomy.

### INTRODUCTION

The description of *Cryptobia helicis* by Leidy (1846) was based on material taken from the seminal receptacle of three species of terrestrial pulmonate snails collected in the vicinity of Philadelphia, Pennsylvania, USA. Two of the snails, *Neohelix albolabris albolabris* and *Triodopsis tridentata* (referred to by Leidy as *Helix albolabris* and *H. tridentata*), belong to the family Polygyridae; the third, *Anguispira alternata* (also referred to as a species of *Helix*), is a member of the family Endodontidae. Soon afterward Leidy (1847a) requested the permission of the Academy of Natural

Sciences of Philadelphia to replace *Cryptobia* with *Cryptoicus*, because a similar name, *Cryptobium*, had previously been used for a genus of beetles.

Leidy published three other papers dealing with *C. helicis*, one of them (1847b) being nearly the same as his first. In the text of this paper, however, he used *Cryptoicus* in place of *Cryptobia*, and he enlarged the list of hosts, mentioning *Bulimus decollatus* (now called *Rumina decollata*) and "several other species of *Helix*, viz. *Helix elevata* and *H. thyroidus*" (now called *Mesodon elevatus* and *M. thyroidus*). The genus *Rumina* is in the family Achatinidae, and *Mesodon*, like *Neohelix* and *Triodopsis*, is in the family Polygyridae. The next paper (Leidy 1851) gave the name of the flagellate as *Bodo helicis*, a change proposed by Diesing (1850), and listed both *Cryptobia helicis* and *Cryptoicus helicis* as synonyms. The last mention of *Cryptobia* by

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Leidy (1856) again merely listed *Bodo helicis* with its synonyms.

Diesing (1850) was the first author to have encountered a *Cryptobia* in a European snail. He found the flagellate to which he referred as *Bodo helicis* in *Helix nemoralis* (family Helicidae) at Vienna. Soon afterward, Keferstein and Ehlers (1860) reported on "Infusorien" occurring in the seminal receptacle of *Helix pomatia*. Their illustrations, like those of Leidy, show clearly that the organisms were flagellates. After the start of the twentieth century, various European authors became interested in cryptobias. Friedrich (1909), Jollos (1911), Bělař (1916, 1926), and Schindera (1922) published accounts of the structure, division, and other aspects of the biology of what all of them called *Trypanoplasma helicis*, and which all of them obtained from the seminal receptacle of *H. pomatia*. Kühn (1911) also used *Trypanoplasma* in place of *Cryptobia*. He examined a wide variety of terrestrial and freshwater snails, thereby enlarging the list of known hosts. In addition, he named and briefly described five species: *T. helicogenae* (from *Helix pomatia*, at that time assigned to the subgenus *Helicogena*); *T. tacheorum* (from *Tachea nemoralis*, *T. hortensis*, and *T. austriaca*); *T. desertorum* (from *H. desertorum* and *H. desertorum* var. *hasselquitzki*); *T. rupestre* (from *H. cingulata*); and *T. limnorum* (from *Lymnaea stagnalis* and *L. palustris*). He thought the species in *H. aspersa* was perhaps *T. helicogenae*, and that the flagellate in *H. arbustorum* was possibly *T. tacheorum*. Flagellates found in *H. lactea*, *H. muralis*, and *H. virgata* var. *variabilis minor* were not assigned to species. His opinion with respect to the status of the flagellate from *H. aspersa* will be dealt with in a later portion of this paper.

Kühn's (1911) descriptions and illustrations of species are too sketchy to firmly establish the individuality of any of them. Matthey (1923), who studied flagellates from several species of European snails and who also used the genus name *Trypanoplasma*, took a more conservative approach, pointing out that the criteria Kühn used for distinguishing species, primarily dimensions of the body and relative lengths of the flagella, were superficial. Matthey had noted that these characters varied in flagellates from one species of snail to another, but thought the variations might be due to physiological conditions in the seminal receptacles of different hosts. He suggested that Kühn's species might better be treated as subspecies of "*T. helicis*," then went on to say that even this seemed to be unnecessarily precise.

The type species of *Trypanoplasma* is *T. borreli* Laveran and Mesnil (1901), first found in the blood of *Scardinius erythrocephalus* and later in various other freshwater fishes. At least for flagellates from the seminal receptacle of pulmonate snails, however, *Cryptobia* has priority (Crawley 1909), and it has sometimes been applied to flagellates from the blood of fishes and amphibians as well as to those in snails. Brugerolle *et al.* (1979) pointed out that in spite of the remarkable similarity of flagellates from fishes and those from snails (the similarity is evident in electron micrographs as well as in intact specimens studied with the light microscope) the blood parasites are typically transmitted by leeches, within which there are stages rather different from those in the blood. They proposed, therefore, that the two genera not be synonymized. They even went a step further, suggesting that separate subfamilies (Cryptobiinae, Trypanoplasmatinae) be established. Woo and Wehnert (1983), however, found that "*Cryptobia*" *salmositica* can be transmitted from one rainbow trout to another without involvement of an intermediate host. Nevertheless, recent studies of kinetoplast DNA and RNA of various bodonid flagellates, including *Trypanoplasma borreli* and the *Cryptobia* from *Helix pomatia* (Lukeš *et al.* 1998, 2002; Doležel *et al.* 2000; Simpson *et al.* 2002) strongly indicate that *Trypanoplasma* and *Cryptobia* belong at least in separate genera.

My cytological study (Kozloff 1948) of what I assumed to be Leidy's *Cryptobia helicis* was based on material from the European *Helix aspersa aspersa*, now well established in some areas of North America. A *Cryptobia* found in *Monadenia fidelis*, a helicid snail native to the Pacific northwest region of North America, appeared to be similar, but this host was not so readily available as *H. aspersa aspersa* and my protargol impregnations of its flagellate parasites were not particularly good. At that time I had not seen material from any of the three snails in which Leidy found the parasites on which he based his description of *C. helicis*. Later, however, I was able to study flagellates from *Triodopsis tridentata* and *Anguispira alternata* collected near Ithaca, New York, and also from a snail identified as *Neohelix albolabris*, collected at Chapel Hill, North Carolina. The latter has recently been named *N. solemi* (Emberton 1988), on the basis of penis morphology and certain shell characters. The parasites in each of these three host species were different, and none of them was the same as the flagellate taken from *H. aspersa aspersa*. Thus it became clear that the most intensively

studied cryptobias, those from *Helix pomatia* and *H. aspersa aspersa*, must have another name. Unfortunately, my attempts to make satisfactory protargol impregnations of the cryptobias from *T. tridentata* and *A. alternata* failed. It was not until recently that parasitized *N. albolabris albolabris* and additional *T. tridentata* became available to me. Attempts to obtain more parasitized specimens of *Anguispira alternata* have not been successful.

Pyne (1959, 1960, 1967) was the first to describe the ultrastructure of the *Cryptobia* from *Helix pomatia*. A few years later, Brugerolle *et al.* (1979), for their comprehensive ultrastructural study of flagellates belonging to the *Bodo-Cryptobia-Trypanoplasma* group, used material from both *H. pomatia* and *H. aspersa* (almost certainly subspecies *aspersa*). They reported no differences between flagellates from these two host snails. Another study of ultrastructure of a *Cryptobia* is that of Current (1980), concerned to a large extent with the interesting way in which the flagellate is attached to cells in the wall of the seminal receptacle. Current's material came from a polygyrid snail collected in Nebraska and identified as *Triadopsis multilineata* (correctly *Triadopsis multilineata*), recently assigned to *Webbhelix* (Emberton 1988). The flagellate in this snail is probably much closer to the ones from the first two polygyrids examined by Leidy than to those occurring in *H. pomatia*, *H. aspersa aspersa*, and other European snails of the family Helicidae.

Another complication related to the problem under discussion here is that the genus name *Cryptobia* has been used for certain flagellates parasitizing the gills or digestive tract of fishes, and for a few other organisms found in various vertebrate and invertebrate hosts. Not all of them are necessarily close to flagellates of the *Cryptobia-Trypanoplasma* complex. Brugerolle *et al.* (1979) studied the ultrastructure of three species from fishes, one living on the gills of a freshwater fish, two inhabiting the digestive tract of marine fishes, and found the morphology of these flagellates to be similar to that of species of *Cryptobia* and *Trypanoplasma*. In reconstructions of the ultrastructure of *Cryptobia* from species of *Helix*, Brugerolle *et al.* (1979) showed the cytostome to be located much farther posteriorly than it is in the two fish cryptobias whose structure they also illustrate. Current (1980), however, showed the cytostome of the species of *Cryptobia* he found in *Webbhelix multilineata* also to be relatively far forward.

The purposes of this paper are to establish the identity of *Cryptobia helicis* from *Neohelix albolabris*

*albolabris*, to distinguish it from the flagellate in *Helix pomatia* and *H. aspersa aspersa*, to suggest a name by which the flagellate in these European snails can be called, and to describe the species parasitizing *Triadopsis tridentata*. It is not likely that any flagellates of fishes or of invertebrates other than pulmonate snails will have a bearing on the systematics of *C. helicis* and related flagellates that have been mistakenly assigned to it, so no further consideration will be given to them.

This study is based entirely on light-microscope observations. These, unfortunately, do not reveal details of some important structures that can be seen in electron micrographs. Nevertheless, techniques of staining and impregnation bring out enough details to make slide preparations useful for distinguishing species that are decidedly different, which is the case with the flagellates that will be dealt with here

## MATERIALS AND METHODS

To prepare cryptobias for light microscopy, the most useful single method is impregnation with activated silver albumose (protargol). When it works successfully, it brings out the flagella and nucleus, as well as the shape of the cell, and sometimes what I described as the "aciculum" (Kozloff 1948). For fixation prior to impregnation, Hollande's fluid and 5% glutaraldehyde (not buffered) were used; the former gave best results. Staining with iron hematoxylin after both fixatives stained the kinetoplast, which is not impregnated by protargol, and the endosome and peripheral chromatin of the nucleus. Phase-contrast optics were helpful in making measurements of body form and flagella of living flagellates.

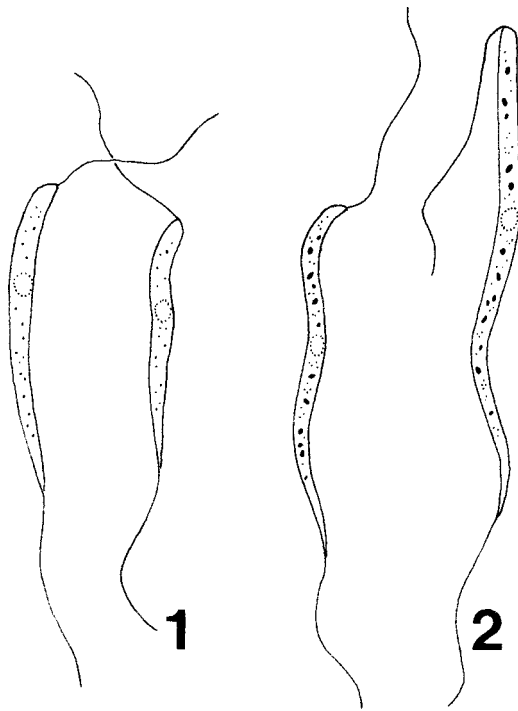
The specimens of *Helix aspersa aspersa* for my 1948 paper were collected in Berkeley and Oakland, Alameda County, California, USA; the slide preparations are still good. Additional specimens of *H. aspersa aspersa* were collected recently in El Cerrito, Alameda County. Fixed smears of the contents of the seminal receptacle of *H. pomatia* were sent to me from near České Budějovice, Czech Republic. A parasitized *Neohelix albolabris albolabris* was found near Newport, Perry County, Pennsylvania, USA and specimens similar to it, but now assigned to *N. solemi*, were collected in Chapel Hill, Orange County, North Carolina, USA. *Triadopsis tridentata* was found in Westmoreland County, Pennsylvania, USA.

## RESULTS

### Redescription of *Cryptobia helicis* Leidy, 1846 (Figs 1, 3-6)

**Host:** *Neohelix albolabris albolabris* (Say, 1816) (Polygyridae).

Because Leidy listed "*Helix*" *albolabris* first among the three hosts in which he reported the presence of



**Figs 1-2.** 1 - *Cryptobia helcis* Leidy from *Neohelix albolabris albolabris*; drawing of living specimens. 2 - *Cryptobia innominata* sp. n. from *Triodopsis tridentata*; drawings of living specimens.

*C. helcis*, the flagellate parasitizing this snail has been chosen as the type species of the genus. There is another good reason for doing this: Leidy's drawing of a mass of flagellates showed the body of most individuals to be comparatively plump and closer to the shape of specimens from *N. albolabris albolabris* than to the shape of those from *Triodopsis tridentata* and *Anguispira alternata*. We do not know, however, whether Leidy diluted the contents of the seminal receptacle with water or with a saline solution, or how accurately he intended to draw the parasites he observed.

**Locality:** Philadelphia, Philadelphia County, Pennsylvania, USA.

**Redescription:** Neotype slide deposited in the United States National Museum, Washington D. C. (USNM no. 1021509), this is a smear preparation, impregnated by the protargol method, containing numerous individuals, considered to be syntypes, from the seminal receptacle of one *N. albolabris albolabris* collected near Newport, Perry County, Pennsylvania. Another slide (USNM no. 1021510) is a hematoxylin-stained preparation from the same host snail.

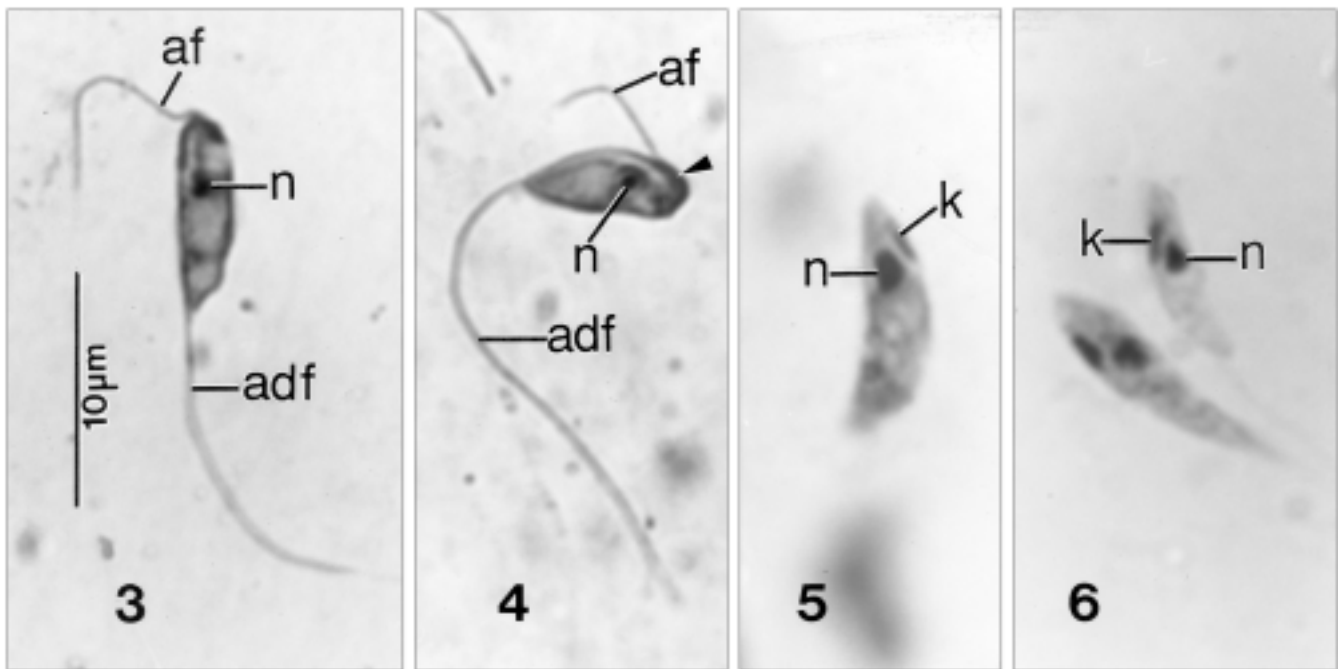
Living specimens from the type collection, selected to show extremes of size, measured from 11.25 by 1.25  $\mu\text{m}$  to 27 by 2.25  $\mu\text{m}$ . The body (Fig. 1) was usually 10 to 12 times as long as wide, but occasionally proportionately longer or shorter individuals were observed. The anterior end was rounded and the posterior end typically tapered to a point. Flagellates fixed in Hollande's fluid and impregnated with protargol (Figs 3, 4) are usually much shorter than living specimens and only 5 to 7 times as long as wide; furthermore, the posterior end is much like the anterior end in being rounded.

In freshly made smears, the free flagellum of medium and large specimens is usually about half to two-thirds as long as the body, rarely as long as or slightly longer than the body. It is more often directed to one side or posteriorly than anteriorly. The adherent flagellum does not become free until it reaches the posterior end of the body; the free portion is usually two-thirds to fully as long as the body. Flagellar length of small specimens was not studied extensively, because if they were products of recent divisions the lengths of the two flagella, in proportion to each other and to the length of the body, would be atypical for at least a short time.

During binary fission of the *Cryptobia* from *Helix aspersa aspersa*, only one of the two flagella of the parent, either the anterior or adherent one, is conferred upon each new individual; the complementary flagellum is neofomed and for a time is therefore much shorter than it normally is in a fully differentiated individual.) In protargol-impregnated specimens (Figs 3, 4), the proportionate lengths of the flagella are comparable to those of living specimens. The adherent flagellum, moreover, rarely becomes free until it has reached the posterior end of the body.

The nucleus (Figs 1, 3-6) is typically located anterior to the middle of the body, sometimes near the middle. In protargol preparations (Figs 3, 4), it is most commonly a vesicular structure with a central nucleolus, but sometimes the nucleus as a whole is rather darkly impregnated. Iron hematoxylin generally stains the entire nucleus rather intensely (Figs 5, 6).

The kinetoplast, as stained by hematoxylin (Figs 5, 6), is about 2 to 2.5  $\mu\text{m}$  long and usually more or less rodlike, but sometimes thickest near its anterior or posterior end; its posterior portion presumably has a mitochondrion joined to it, but this has not been distinguished. Nothing unequivocally comparable to the distinctive "aciculum" seen in protargol impregnations of the *Cryptobia* from *Helix pomatia* and *H. aspersa aspersa* has been identified, but a slightly crescentic peripheral darkening



**Figs 3-6.** *Cryptobia helcis* Leidy, photomicrographs of specimens fixed in Hollande's fixative. **3, 4** - specimens impregnated with protargol; **5, 6** - specimens stained with iron hematoxylin. adf - adherent flagellum, af - anterior flagellum, k - kinetoplast, n - nucleus. The arrowhead in Fig. 4 indicates a darkened area that may correspond to the "aciculum" of *C. helcigenae*.

in the same part of some specimens (Fig. 4) perhaps indicates such a structure. The cytoplasm of living flagellates has small granular inclusions, but these are not conspicuous in specimens stained with hematoxylin or impregnated by the protargol method.

Flagellates in several *Neohelix solemi* collected at Chapel Hill, Orange County, North Carolina, USA ranged in size from 12 by 1.25  $\mu\text{m}$  to 25 by 2.25  $\mu\text{m}$ , and the appearance of living, stained, and protargol-impregnated flagellates conformed to my redescription of *C. helcis*. If there is a difference that can be detected by light microscopy, it has eluded me, and I tentatively consider the *Cryptobia* in *N. solemi* to be morphologically identical to *C. helcis* from *N. albolabris albolabris*.

**Description of *Cryptobia innominata* sp. n. (Figs 2, 7-12)**

**Host:** *Triodopsis tridentata*.

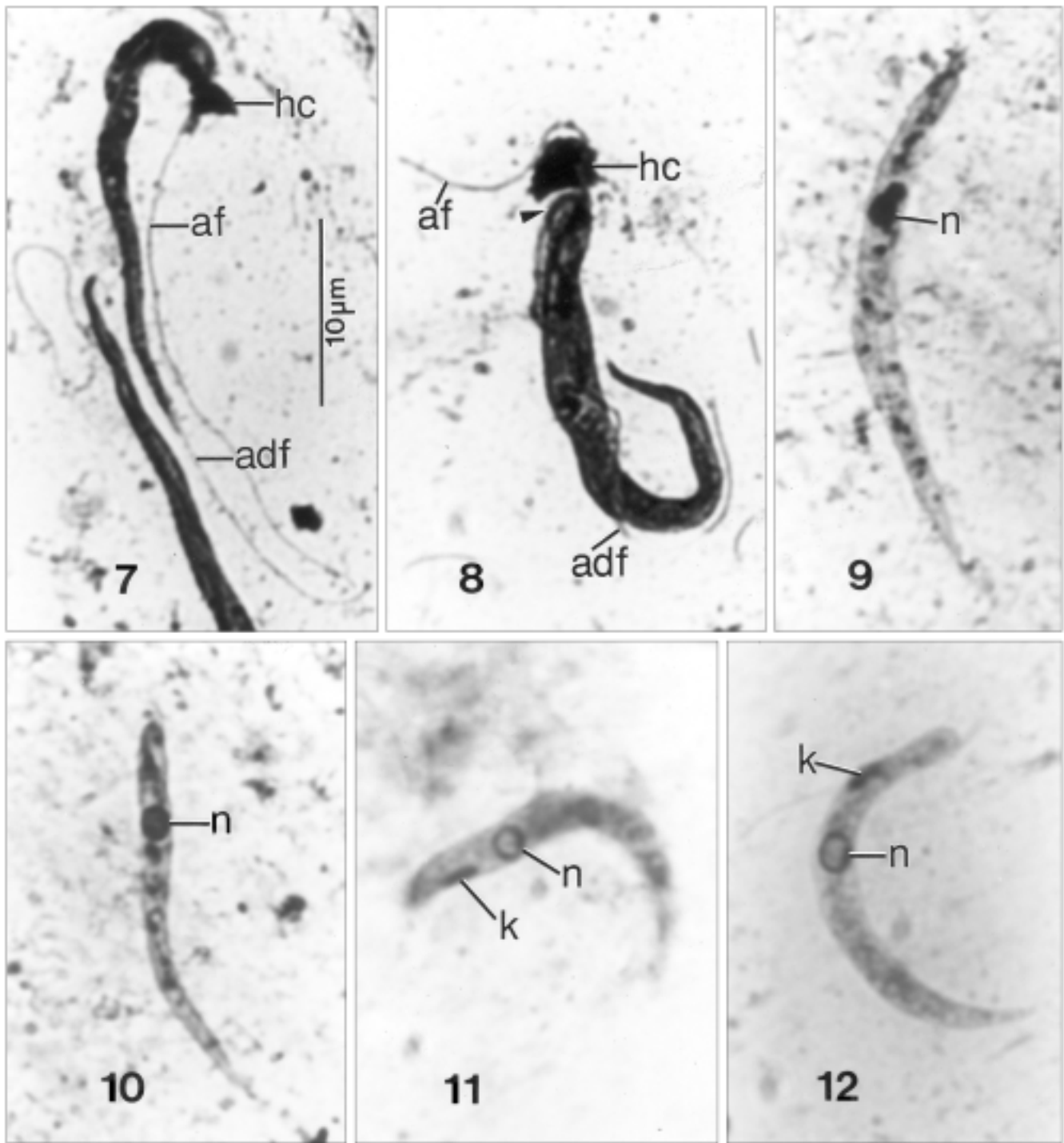
**Type locality:** Powder Mill Nature Reserve, Westmoreland County, Pennsylvania, USA.

**Etymology:** The name *innominata*, meaning "not named," alludes to the fact that this species, apparently never restudied, had been assumed to be identical with *C. helcis* from *Neohelix albolabris albolabris*.

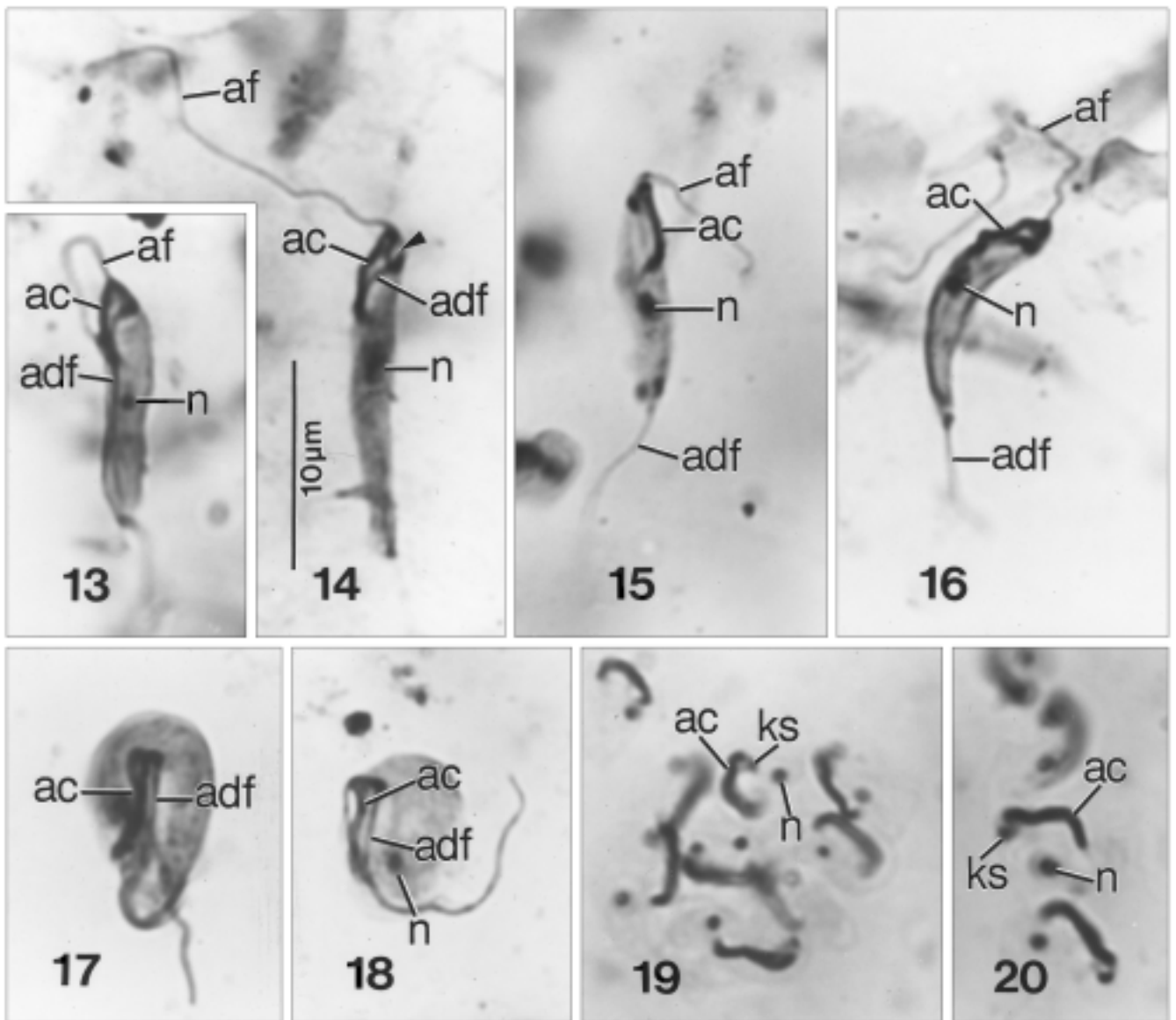
**Description:** Holotype slide deposited in the United States National Museum, Washington D. C. (USNM 1021511); this is a smear preparation containing numerous specimens (syntypes) impregnated by the protargol method. Another smear impregnated with protargol (USNM 1021512) and one stained with iron hematoxylin (USNM 1021513) have also been deposited.

This species, in general, is considerably larger than *C. helcis*, and its shape is typically more slender and more variable (Figs 2, 7-12). Numerous living specimens, measured at random in fresh smears from two snails, ranged in size from 22.4 by 1.35  $\mu\text{m}$  to 50.5 by 2.25  $\mu\text{m}$ . Vigorous serpentine movements of the body are characteristic. After fixation, specimens become proportionately shorter and wider than they were when alive.

The proportionate length of the flagella varies considerably, as is to be expected in view of the fact that one or the other is retained during division and the other is neoformed. In general, however, the anterior flagellum ranges from about one-fourth the length of the body (Fig. 8) to decidedly longer than the body (Fig. 7). In smears that have been stained or impregnated, as well as in fresh preparations, the proximal portion of the anterior



**Figs 7-12.** *Cryptobia innominata* sp. n., photomicrographs of specimens fixed in Hollande's fluid. **7-10** - specimens impregnated with protargol; **11, 12** - specimens stained with iron hematoxylin. adf - adherent flagellum, af - anterior flagellum, hc - cell from host tissue, k - kinetoplast, n - nucleus. The arrowhead in Fig. 8 indicates a darkened area that may correspond to the "aciculum" of *C. helicogena*.

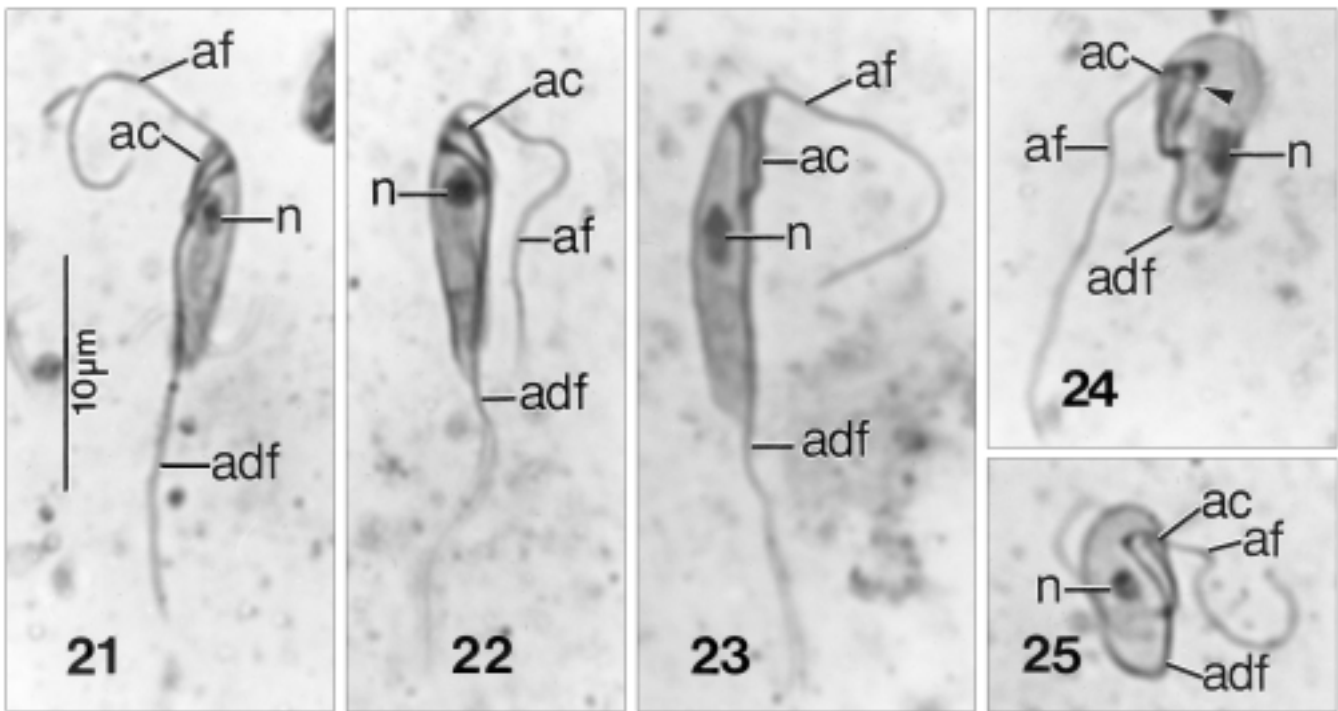


**Figs 13-20.** *Cryptobia helicogena* from *Helix pomatia*, fixed in Hollande's fluid and impregnated with protargol. **13-16** - elongated individuals; **17-20** - somewhat rounded forms; **19, 20** - only the aciculum and nucleus are distinct, but the kinetosomes from which the flagella originate are so heavily impregnated that they cannot be distinguished. ac - "aciculum," adf - adherent flagellum, af - anterior flagellum, ks - kinetosomes, n - nucleus. The arrowhead in Fig.14 is directed at what appears to be a connective between the anterior portion of the "aciculum" and proximalmost part of the adherent flagellum.

flagellum is often seen to be embedded in a fragment of a cell, presumably one that was part of the epithelial lining of the seminal receptacle (Figs 7, 8). The adherent flagellum usually extends well beyond the posterior end of the body (Fig. 7). In living specimens, it typically does not separate from the body until it reaches the posterior end, but fixation often causes a considerable portion of it to become detached.

The nucleus is usually located anterior to the middle of the body. In hematoxylin-stained smears, the chromatin forms a distinct peripheral layer (Figs 11, 12); a nucleolus is rarely evident. In protargol-impregnated preparations, the contents of the nucleus are usually darkened rather uniformly (Figs 9, 10). The kinetoplast of *C. innominata* occupies a position similar to that of *C. helcis*. The cytoplasm of living specimens contains





**Figs 21-24.** *Cryptobia* cf. *helicogenae* from *Helix aspersa aspersa*, fixed in Hollande's fluid and impregnated with protargol. **21-23** - elongated individuals; **24, 25** - somewhat rounded forms. The arrowhead in fig. 24 is directed at what appears to be a connective between the anterior part of the "aciculum" and the proximalmost part of the adherent flagellum. ac - "aciculum," adf - adherent flagellum, af - anterior flagellum, n - nucleus.

numerous conspicuous inclusions, and these are especially distinct in lightly impregnated specimens (Figs 9, 10). Perhaps at least some of them correspond to the microbodies, food vacuoles, and symbiotic bacteria noted in other species of *Cryptobia* by Brugerolle *et al.* (1979) and Current (1980).

In protargol preparations, a few specimens (Fig. 8) exhibit a darkly impregnated area that could conceivably be comparable to the "aciculum" of the cryptobias from *Helix pomatia* and *H. aspersa aspersa*. This will be discussed later.

#### **Names that may be applied to species of *Cryptobia* in European pulmonate snails**

Now that *C. helicis* parasitizing *Neohelix albolabris albolabris* has been redefined and the flagellate observed by Leidy in *Triodopsis tridentata* has been described, it is necessary to decide what to call the cryptobias found in *Helix pomatia* and *H. aspersa aspersa*. The name *C. helicogenae* (Kühn, 1911) (described as *Trypanoplasma helicogenae*) can confidently be applied to the flagellate from *H. pomatia*,

which, as "*C. helicis*," was the object of cytological studies by Friedrich (1909), Jollos (1911), Bělař (1916, 1926), Schindera (1922), Pyne (1959, 1960), and Brugerolle *et al.* (1979), and also used in a molecular study of the kinetoplast (Lukeš *et al.* 1998, 2002; Doležel *et al.* 2000; Simpson *et al.* 2002). Because he had not studied flagellates from the hosts Leidy had examined, Kühn (1911) wisely treated genuine *C. helicis* as a *species inquirenda*. He did not designate a type specimen or a type locality for *C. helicogenae*, but stated that parasitized specimens of *H. pomatia* were collected at Gutenstein, Germany and at Budapest, Hungary.

So far as I have been able to determine, the *Cryptobia* from *H. aspersa aspersa* is morphologically identical to *C. helicogenae*. Protargol impregnations reveal the "aciculum" of *C. helicogenae* from *H. pomatia* (Figs 13-18) just as clearly as they do that of specimens from *H. aspersa aspersa* (Figs 21-25); in many specimens from both hosts, in fact, the aciculum, kinetosomal mass, and nucleus are often conspicuous when the flagella and outlines of the cells show faintly or not at all (Figs 19, 20). Another distinctive minor detail often seen in protargol

preparations of flagellates from *H. pomatia* and *H. aspersa aspersa* is what appears to be a delicate connective between the anterior portion of the aciculum and the proximalmost part of the adherent flagellum (Figs 14, 24). I am unable to reconcile this with any elements shown in the spatial reconstruction published by Brugerolle *et al.* (1979).

A neotype slide of *C. helicogenae*, bearing numerous protargol-impregnated specimens that can be considered to be syntypes, has been deposited in the United States National Museum, Washington D. C. (USNM no. 1021514), together with a second slide from the same collection (no. 1021515). The host snail was *Helix pomatia* found near České Budějovice, Czech Republic. Two slides of protargol-impregnated smears from *Helix aspersa aspersa* collected in Oakland, Alameda County, California, USA have also been deposited (USNM nos. 1021516a, 1021516b).

## DISCUSSION

What I described as the “aciculum” of *C. helicogenae* from *H. aspersa aspersa* is almost certainly the bundle of microtubules that reinforce the pharynx (see Brugerolle *et al.* 1979, p. 202, Fig. 7). In his study of the *Cryptobia* from *Webbhelix multilineata*, Current (1980, p. 286, Fig. 17) showed a relatively short but similar microtubular complex. The somewhat crescentic argentophilic deposit seen in the anterior part of the body of some *C. helicis* (Fig. 4) and *C. innominata* (Fig. 8) perhaps indicates a comparable structure.

The systematics of flagellates in the genus *Cryptobia* is certain to become extremely difficult as more pulmonate snails are examined for the presence of these organisms. Living specimens from a single host cannot be characterized by much more than size, shape, and proportionate lengths of the flagella. In stained and impregnated preparations, the position and appearance of the nucleus and kinetoplast may be the only helpful features, unless there are other discrete structures, such as the “aciculum” of *C. helicogenae*.

Relatively few snails have, up to now, been examined for the presence of cryptobias. These flagellates have, however, been found in several families of stylommatophoran and basommatophoran pulmonates. Because they are transmitted from one host to another during copulation, it is probable that cryptobias in species of snails that are reproductively isolated are in some

way, morphological or molecular, distinct. The possibility that *C. helicogenae* in *H. pomatia* is slightly different from the flagellate in *H. aspersa aspersa* has already been mentioned. Because the two species of *Helix* do not mate, it is likely that their flagellates have been isolated for a long time. Even *H. aspersa aspersa* and *H. aspersa maxima*, as recognized by Chevallier (1977) are not likely to mate in nature, although there has been some success with attempts to induce copulation in captivity (Gomot-de Vaufleury and Borgo 2001).

In the literature of protozoology, it is too often assumed that parasites living in closely related hosts are identical if they appear to be similar. Future investigators dealing with cryptobias should certainly specify, in papers dealing with similar flagellates from more than one host species, which parts of a description and which figures apply to material from a particular host. Thus “*Cryptobia helicogenae* from *Helix pomatia*” will clearly designate the flagellate from the snail that has provided material for all molecular studies until now, and also for early cytological work and that of Brugerolle *et al.* (1979); “*C. cf. helicogenae* from *C. aspersa aspersa*” is the correct way to indicate the flagellate whose cytology has been studied by Kozloff (1948) and in part by Brugerolle *et al.* (1979). It is important, however, to remember that *C. helicis*, not *C. helicogenae*, is the type species of the genus. We may perhaps assume, for the time being, that the molecular attributes of *C. helicogenae* are typical of all cryptobias known to occur in pulmonate snails of North America and Europe. Nevertheless, some of these parasites may have evolved along different lines, and thorough systematic work on similarities and differences between genera requires that the type species be studied.

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John Slapcinsky sent several fully mature *Anguispira alternata* from each of two localities in Illinois, and James Atkinson provided specimens from Michigan. It was disappointing to find no flagellates in any of these, or in those I collected in Indiana. *Triodopsis fallax*, collected in North Carolina by Sue Brady and in Tennessee by

Edward Crawford, Jr. were parasitized by a *Cryptobia* somewhat similar to *C. innominata*. This material was valuable because it enabled me to experiment with procedures of staining and silver impregnation and to test various batches of protargol, and thus helped me to make more satisfactory permanent preparations of the species with which this report is primarily concerned. A period of study in the Department of Zoology, University of North Carolina, where I was able to work on *C. helicis* from *N. solemi*, was made possible by a fellowship from the John Simon Guggenheim Memorial Foundation.

## REFERENCES

- Bělař K. (1916) Protozoenstudien. II. *Arch. Protistenkd.* **36**: 241-296
- Bělař K. (1926) Der Formwechsel der Protistenkerne. *Ergebn. Fortschr. Zool.* **6**: 1-420
- Brugerolle G., Lom J., Nohynkova E., Joyon L. (1979) Comparaison et évolution des structures cellulaires chez plusieurs espèces des bodonidés et cryptobiidés appartenant aux genres *Bodo*, *Cryptobia* et *Trypanoplasma* (Kinetoplastida, Mastigophora). *Protistologica* **15**: 197-221
- Chevallier H. (1977) La variabilité de l'escargot petit gris *Helix aspersa* Müller. *Bull. Mus. Natn. Hist. Nat. Paris, Zool.* **311**: 425-442
- Crawley H. (1909) The priority of *Cryptobia* Leidy 1846 over *Trypanoplasma* Laveran and Mesnil 1901. *Bull. U. S. Bur. Anim. Industr., Dept. Agric.* **119**: 16-20
- Current W. I. (1980) *Cryptobia* sp. in the snail *Triadopsis multilineata* (Say): fine structure of attached flagellates and their mode of attachment to the spermatheca. *J. Protozool.* **27**: 278-287
- Diesing C. M. (1850) *Systema Helminthum*, vol. 1. Vienna (Vindobonae)
- Doležel D., Jirků M., Maslov D. A., Lukeš J. (2000) Phylogeny of bodonid flagellates (Kinetoplastida) based on small-subunit rRNA gene sequences. *Int. J. Syst. Evol. Microbiol.* **50**: 1943-1951
- Emberton K. C. (1988) The genitalic, allozymic, and conchological evolution of the eastern North American Triodopsinae (Gastropoda: Pulmonata: Polygyridae). *Malacologia* **28**: 159-273
- Friedrich L. (1909) Über Bau und Naturgeschichte des *Trypanoplasma helicis* Leidy. *Arch. Protistenk.* **14**: 493-450
- Gomot-de Vaufleury A., Borgo R. (2001) Experimental hybridization between two sub-species of snails (*Helix aspersa aspersa* and *Helix aspersa maxima*): consequences for fertility, survival and growth. *Invert. Reprod. Devel.* **40**: 217-226
- Jollos V. (1911) Bau und Vermehrung von *Trypanoplasma helicis*. *Arch. Protistenk.* **21**: 103-110
- Keferstein W., Ehlers E. (1860) Beiträge zur Kenntnis der Geschlechtsverhältnisse von *Helix pomatia*. *Zeitschr. wiss. Zool.* **10**: 251-270
- Kozloff E. N. (1948) The morphology of *Cryptobia helicis* Leidy, with an account of the fate of the extranuclear organelles in division. *J. Morphol.* **83**: 253-273
- Kühn M. (1911) Die Trypanoplasmen und deren Verbreitung in einheimischen und ausländischen Schnecken. *Schr. phys.-ökon. Ges. Königsberg.* **52**: 63-89
- Laveran A., Mesnil F. (1901) Sur les flagellés à membrane ondulante des poissons (genres *Trypanosoma* Gruby et *Trypanoplasma* n. gen. C. r. Acad. Sci., Paris, **133**: 670-675
- Leidy J. (1846) Description of a new genus and species of Entozoa. *Proc. Acad. Nat. Sci. Philadelphia* **3**: 100-101
- Leidy J. (1847a) (Note recording the request of Leidy to change the name of *Cryptobia* to *Cryptoicus*.) *Proc. Acad. Nat. Sci. Philadelphia* **3**: 239
- Leidy J. (1847b) Description of a new genus and species of Entozoa. *Cryptobia helicis*. *J. Acad. Nat. Sci. Philadelphia, ser. 2*, **1**: 67-68
- Leidy J. (1851) Corrections and additions to former papers on helminthology published in the proceedings of the Academy. *Proc. Acad. Nat. Sci. Philadelphia* **5**: 284-290
- Leidy J. (1856) A synopsis of entozoa and some of their ectocongeners. *Proc. Acad. Nat. Sci. Philadelphia* **8**: 42-58
- Lukeš J., Jirků M., Avliakulov N., Benada O. (1998) Pankinetoplast DNA structure in a primitive bodonid flagellate, *Cryptobia helicis*. *EMBO J.* **17**: 838-846
- Lukeš J., Guilbride D. L., Votypka J., Zikova, A., Benne, R., Englund, P. T. (2002). Kinetoplast DNA network: evolution of an improbable structure. *Eukaryotic Cell* **1**: 495-502
- Matthey R. (1923) Contribution à l'étude de *Trypanoplasma helicis* Leidy. *Rev. Suisse Zool.* **30**: 425-456
- Pyne C. K. (1959) L'ultrastructure de *Cryptobia helicis* (flagellé, fam. Bodonidae). *C. r. Acad. Sci. Paris* **248**: 1410-1413
- Pyne C. K. (1960) L'ultrastructure de l'appareil basal des flagellés chez *Cryptobia helicis* (flagellé, Bodonidae). *C. r. Acad. Sci. Paris* **250**: 1912-1914
- Pyne C. K. (1967) L'ultrastructure des flagellés des familles Trypanosomidae et Bodonidae. *Ann. Sci. Nat., Zool., ser. 12*, **9**: 345-524
- Schindera M. (1922) Beiträge sur Biologie, Agglomeration und Züchtung von *Trypanoplasma helicis* Leidy. *Arch. Protistenk.* **45**: 200-240
- Simpson A. G. B., Lukeš J., Roger A. J. (2002) The evolutionary history of kinetoplastids and their kinetoplasts. *Mol. Biol. Evol.* **19**: 2071-2083
- Woo P. T. K., Wehnert S. D. (1983) Direct transmission of a hemoflagellate, *Cryptobia salmositica* (Kinetoplastida: Bodonina) between rainbow trout under laboratory conditions. *J. Protozool.* **30**: 334-337

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## Morphological Variability of Testate Amoebae (Rhizopoda: Testacealobosea: Testaceafilosea) in Natural Populations

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**Summary.** Morphometric investigation of 24 species of testate amoebae (*Centropyxis sylvatica* (Deflandre) Thomas, *Cyclopyxis eurystoma* v. *parvula* Deflandre, *C. puteus* Thomas, *Hyalosphenia papilio* Leidy, *H. elegans* Leidy, *Schoenbornia humicola* (Schönborn) Decloitre, *Diffflugia acuminata* Ehrenberg, *D. corona* Wallich, *D. gramen* Penard, *D. labiosa* Wailes, *D. lanceolata* Penard, *D. limnetica* (Levander) Penard, *D. lithophila* Penard, *D. lobostoma* Leidy, *D. oblonga* Ehrenberg, *D. parva* Ogden, *D. pyriformis* Perty, *D. urceolata* Carter, *Phryganella acropodia* (Hertwig et Lesser) Hopkinson, *Assulina muscorum* Greef, *Tracheleuglypha acolla* Bonnet, Thomas, *Trinema lineare* Penard, *Wailesella eboracensis* (Wailes) Deflandre, *Difflogiella oviformis* f. *fusca* (Penard) Bonnet et Thomas) is conducted. Variability of natural populations of testate amoebae is expressed either in the changes of the shell size (correlated), or in the changes of the size of separate parameters (non correlated), resulting in a significant broadening of phenotypic spectrum in populations. The size of shell aperture demonstrates the greatest variability. The character of variability (its amplitude and correlativity) differs not only in different species, but also in different populations of the same species. Three main types of size variability in testate amoebae populations are established. There are monomorphic populations, polymorphic populations with correlated characters and populations with a high phenotypic plasticity, non-correlated characters and the elements of discreteness. A scheme of the close related species analysis is proposed.

**Key words:** morphology, morphometry, Rhizopoda, testate amoebae.

### INTRODUCTION

Testate amoebae are unicellular predominantly asexual organisms. At present only a minor part of testate amoebae is known to possess different forms of sexual reproduction (Schönborn 1966). In some species (*Trinema lineare*, *Corythion dubium*, *C. orbicularis*,

*C. delamarei*) asexual binary fission is alternated with isogamic copulation of trophozoids during the life cycle (Sukhanova and Yudina 1990, Yudina and Sukhanova 2000). In a testate amoeba *Arcella vulgaris* meiotic stages in the reproduction cycle have been observed (Raikov *et al.* 1989, Mignot and Raikov 1992).

The applicability of the “biological species” concept for testate amoebae, as well as for the rest predominantly asexual protists, is questionable. Delimitation of panmyxic reproductively isolated systems turns out to be difficult. However, this does not mean that asexual organisms are “extraspecific forms of life”. All living

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things in the recent biosphere are thought to be organized in species, but there are species of various types, differing from each other (Zavadsky 1968, Poljansky 1992). At present the species is defined not as a genetically closed, but as a genetically stable system. Thus, in asexual forms, a species may be considered as a system of close biotypes (groups of individuals phenotypically resembling each other), possessing closely related genotypes, inhabiting a common distribution area and bound by a common evolutionary fate (Yablokov and Yusufov 1998).

In the diagnoses of testate amoebae species morphological criteria traditionally predominate. Recently a "morphospecies" concept has been proposed as a basic one for protists (Finlay *et al.* 1996, Finlay 1998). However, in the case of testate amoebae as well as other protists (e.g. diatoms) the difficulty in the application of this concept lies in their great morphological variability, which forms continuous network, which is not clearly defined into taxonomic units.

Biometric analysis is the most important part of phenotypic analysis of testate amoebae, as organisms with few morphological characters. Testacean systematics as well as foraminiferan or radiolarian taxonomy is based on morphological features of external structures. Some protistologists (Couteaux 1976) consider this approach to be incorrect, postulating that systematics should be based on the morphology of cellular structures. However, this standpoint does not agree with the data on genetically controlled shell construction (Netzel 1972a, b, c; Ogden 1979a, b; Anderson and Cowling 1994; Anderson 1995). Protozoologists working in the fields of taxonomy, biology and ecology of testate amoebae consider shell morphology to be as important as the features of cellular structures (Anonymous 1977). Most of the subsequent descriptions of new species have been based on shell morphology only. Cytological analysis, as a rule, amounted to description of pseudopodia shape (Chardez 1990, 1991, 1994). Rarely it was used in differential diagnosis (Ogden and Meisterfeld 1989, Ogden 1991). The investigation of clones during describing new species or redescribing old ones is also difficult to carry out in practice.

The analysis of morphological variability of testate amoebae began in the first quarter of the XX<sup>th</sup> century with the studies of morphological variability of *Diffflugia corona* clones (Jennings 1916), longtime modifications and variability of testaceans from the genus *Arcella* (Hegner 1919, Reynolds 1923, Jollos 1924) and *Centropyxis* (Root 1918). Concurrently biometric ap-

proach to the analysis of variability of testacean natural populations was developing (Bassine 1929, Zinger 1935, Hoogenraad and de Groot 1937). Later, the relations between morphological features of testate amoebae and environmental parameters were established (Schönborn 1962, 1983; Heal 1963; Chardez and Leclercq 1963). Biometric approach has been extensively applied in the last two decades. It yielded detailed information on the variability of natural populations and laboratory cultures of some testacean species (Schönborn *et al.* 1983, 1987; Lüftenegger *et al.* 1988; Schönborn and Peschke 1988, 1990; Wanner 1988, 1991, 1994a, b, 1995; Schönborn 1990, 1992; Lüftenegger and Foissner 1991; Wanner and Meisterfeld 1994; Wanner *et al.* 1994). In some cases it was used for comparative geographical analysis (Bobrov *et al.* 1995) and redescription of closely related species (Foissner and Korganova 1995, 2000).

Recently Wanner (1999) reviewed the data on variability in testate amoebae and the influence of environmental conditions upon their morphology. It was emphasized that for understanding the phenomena in question a detailed biometric analysis of variability of natural populations is necessary, as well as laboratory experiments on clones. All this makes possible to reveal different aspects of adaptive mechanisms of testate amoebae, including their reaction to environmental factors and their combinations.

The aim of the present study was to perform a detailed analysis of intra- and interpopulation variability in natural populations of 24 testacean species inhabiting different biotopes (soils, sphagnum bogs and freshwater habitats) on the basis of original and literature data.

## MATERIALS AND METHODS

Morphological investigation of 24 testate amoebae species has been conducted.

The samples containing testate amoebae were taken in different geographical areas of Russia and from different biotopes: freshwater, soil and bogs: *Centropyxis sylvatica* (Deflandre) Thomas - Central Forest Biosphere Nature Reserve, Tverskaja province, mixed spruce forest, podzolic brown soil. *Cyclopyxis eurystoma* v. *parvula* Deflandre - Central Forest Biosphere Nature Reserve, Tverskaja province, mixed spruce forest, podzolic brown soil. *Cyclopyxis puteus* Thomas - Primorsky kray, Sikhote-Alin reserve, *Pinus sibirica* and larch forest with *Ledum*, dark colored skeleton soil. *Hyalosphenia elegans* Leidy - Malozemelskaia shrub tundra (Pechora River basin), microcommunity *Sphagnum angustifolium*. *Hyalosphenia papilio* Leidy Population 1 - Malozemelskaia shrub tundra (Pechora River basin), peat, depth 330-335 cm. *Hyalosphenia papilio* Leidy Population 2 - Malozemelskaia shrub tundra (Pechora River basin), peat,

depth 340-345 cm. *Hyalopshenia papilio* Leidy Population 3 - Malozemelskaia shrub tundra (Pechora River basin), microcommunity *Sphagnum balticum*. *Hyalopshenia papilio* Leidy Population 4 - Tomskaya oblast', fen, microcommunity *Sphagnum capillifolium*. *Hyalopshenia papilio* Leidy Population 5 - Malozemelskaia shrub tundra (Pechora River basin), microcommunity *Sphagnum angustifolium*. *Schoenbornia humicola* (Schönborn) Decloitre Population 1 - Khabarovskiy kray, coast of Okhotsk Sea, spruce forest, brown soil. *Schoenbornia humicola* (Schönborn) Decloitre Population 2 - Khabarovskiy kray, coast of Okhotsk Sea, spruce forest with *Hylocomium*, *Dicranum*, *Rhytidiadelphus*, brown soil. *Schoenbornia humicola* (Schönborn) Decloitre Population 3 - Novgorodskaya oblast', pine forest, podzol soil. *Schoenbornia humicola* (Schönborn) Decloitre Population 4 - Karelia, pine forest, podzol soil. *Diffugiella oviformis* f. *fusca* (Penard) Bonnet et Thomas - Kirovskaya province, mesotrophic-oligotrophic swamp, microcommunity *Sphagnum girgensonii*. *Phryganella acropodia* (Hertwig et Lesser) Hopkinson - archipelago Komandor Islands, Bering Island, dry shrub rich in herbs with heath tundra, brown skeleton soil. *Wailesella eboracensis* (Wailes) Deflandre Population 1 - Malozemelskaia shrub tundra (Pechora River basin), microcommunity *Sphagnum flexuosum*. *Wailesella eboracensis* (Wailes) Deflandre Population 2 - Malozemelskaia shrub tundra (Pechora River basin), microcommunity *Sphagnum balticum*. *Assulina muscorum* Greef - Malozemelskaia shrub tundra (Pechora River basin), microcommunity *Sphagnum nemorum*. *Tracheleuglypha acolla* Bonnet et Thomas - Central Forest Biosphere Nature Reserve, Tverskaja province, mesotrophic swamp. *Trinema lineare* Penard - archipelago Komandor Islands, Bering Island, dry shrub rich in herbs with heath tundra, brown skeleton soil. *Diffugia pyriformis* Perty - Penza region, pond, debris. *Diffugia oblonga* Ehrenberg - Penza region, boggy lake. *Diffugia acuminata* Ehrenberg - Penza region, pond, debris. *Diffugia parva* Ogden - Penza region, boggy lake. *Diffugia labiosa* Wailes - Penza region, Sura River, debris. *Diffugia lanceolata* Penard - Penza region, pond, debris. *Diffugia lithophila* Penard - Penza region, Sura River, sandy sediments. *Diffugia urceolata* Carter - Penza region, Sura River, sandy sediments. *Diffugia limnetica* (Levander) Penard - Penza region, pond, debris. *Diffugia corona* Wallich - Penza region, Sura River, sandy sediments. *Diffugia gramen* Penard - Penza region, Sura River, sandy sediment. *Diffugia lobostoma* Leidy - Penza region, pond, debris.

Morphometrical measurements were conducted by means of a light microscope under the magnification of 400 $\times$ . Statistical analysis was performed with the help of STATISTICA 5.5A Software.

## RESULTS

Analysis of variation coefficients provides general information on the degree of variability of characters in populations (Table 1). It appears to vary for different characters in different populations. Some species (*Hyalosphenia elegans*, *Wailesella eboracensis*, *Diffugia lithophila*, *D. oblonga*, *Cyclopyxis puteus*, *Diffugiella oviformis*) are characterized by a minor degree of variability. Others (*Diffugia acuminata*,

*D. labiosa*, *D. lanceolata*, *D. urceolata*, *Schoenbornia humicola*, *Trinema lineare*), on the contrary, are characterized by great morphometrical variability. The greatest variation coefficients are revealed for shell aperture diameter, lesser ones - for the shell length.

On the basis of our own results and literature data (Schönborn *et al.* 1983; Wanner and Funke 1986; Schönborn *et al.* 1987; Balik 1988; Lüftenegger *et al.* 1988; Schönborn and Peschke 1988, 1990; Wanner 1988; Schönborn 1990, 1992; Lüftenegger and Foissner 1991; Dekhtyar 1993, 1994; Bobrov *et al.* 1995) variation coefficients of morphometrical parameters in 75 testacean species from 133 populations have been analysed. Results, represented in Fig. 5, show that in some populations variation coefficients may reach 40 %. However, in an overwhelming majority of cases coefficients do not exceed 20 % (for aperture size) and 15 % (for length, breadth and depth of the shell). In general, the size of the pseudostome is characterized by greatest variability.

The results of the comparison of variation coefficients in different taxonomical testacean groups are shown in Table 2. It turned out that filose amoebae are more variable in size than lobose ones (Mann-Whitney U test:  $p < 0.001$ ). On the other hand, there are no differences in terms of degree of morphometrical variability between freshwater, bog and soil testate amoebae as well as between different life forms (simple acrostomia, compressed acrostomia, centrostomia, plagiostomia).

Characteristics, reflecting correlation between all morphological parameters, are more significant in morphometrical analysis. Pearson correlation coefficients between the main morphometrical characters of testate amoebas are represented in Table 3. It shows that there are species with highly correlated characters. However, there are species that possess non-correlated characters. For some species low values of correlation coefficients between the length and the breadth of the shell were noted. Pseudostome diameter is very often weakly correlated with the rest of the parameters. Low values of correlation coefficients suggest that some of the shell characteristics (size of the aperture, for instance) change independently of others. These characters appear to serve as tools of adaptation to variable environmental constraints. In other words, adaptation to environmental conditions is realized rather through a wider phenotypic spectrum than through a simple proportional decrease or increasing in the size of the shell. Such tendencies are pronounced in a greater or lesser degree in different species.

**Table 1.** Biometric characterisation of the investigated testacean species. Numbers designate features as described in Figs 1-4. Measurements in  $\mu\text{m}$ .  $\bar{x}$  - arithmetic mean; M - median; SD - standard deviation; SE - standard error of mean; CV - coefficient of variation in %; Min - minimum; Max - maximum; n - number of specimens investigated.

Character	$\bar{x}$	M	SD	SE	CV	Min	Max	n
<i>Centropyxis sylvatica</i>								
1	115.2	119.5	10.14	1.43	8.8	78.0	128.0	50
2	124.6	128.0	10.70	1.51	8.6	90.0	136.0	50
3	68.8	70.0	7.77	1.10	11.3	48.0	95.0	50
1:2	1.1	1.1	0.10	0.01	8.9	1.0	1.7	50
<i>Cyclopyxis eurystoma v. parvula</i>								
1	35.3	33.0	8.26	0.99	23.4	25.0	67.0	70
2	15.0	14.0	5.30	0.63	35.3	8.0	47.0	70
3	13.8	13.0	4.82	0.58	34.9	7.2	22.0	70
1:2	0.4	0.4	0.05	0.01	12.5	0.3	0.7	70
<i>Cyclopyxis puteus</i>								
1	148.1	142.0	12.74	1.88	8.6	130.0	180.0	46
2	124.2	124.0	2.95	0.48	2.4	120.0	132.0	38
3	39.8	40.0	2.63	0.39	6.6	34.0	46.0	46
1:2	0.8	0.9	0.07	0.01	8.3	0.7	1.0	38
<i>Diffflugia acuminata</i>								
1	204.9	198.0	33.32	4.97	16.3	150.0	294.0	43
2	79.8	77.5	13.89	2.07	17.4	58.5	126.0	44
3	43.7	42.7	8.62	1.30	19.7	33.0	75.0	44
1:2	0.4	0.4	0.06	0.01	15.9	0.3	0.6	43
<i>Diffflugia lanceolata</i>								
1	152.8	155.0	16.30	1.77	10.7	87.5	189.5	85
2	65.7	65.0	9.86	1.08	15.0	40.5	84.5	84
3	36.1	35.5	7.47	0.82	20.7	21.0	54.0	82
1:2	0.4	0.5	0.07	0.01	15.5	0.31	0.5	84
<i>Diffflugia corona</i>								
1	166.1	165.0	12.54	1.58	7.5	141.0	210.0	63
2	159.9	159.1	9.83	1.24	6.1	132.5	184.5	63
3	67.9	69.5	12.90	2.28	19.0	8.5	86.1	32
1:2	1.0	1.0	0.06	0.01	6.1	0.8	1.1	62
<i>Diffflugia labiosa</i>								
1	206.9	202.0	24.92	3.72	12.1	155.5	260.5	45
2	130.4	133.5	15.05	2.24	11.5	96.0	159.6	45
3	59.8	59.6	9.73	1.67	16.3	40.5	76.5	34
1:2	0.6	0.64	0.07	0.01	10.5	0.5	0.8	45
<i>Diffflugia limnetica</i>								
1	104.2	102.7	9.29	1.03	8.9	84	130.5	82
2	85.3	84.5	10.82	1.19	12.7	60.5	11.5	82
3	33.4	32.5	6.14	0.94	18.4	23.5	51.5	43
1:2	0.8	0.8	0.08	0.01	9.6	0.7	1.0	82
<i>Diffflugia lobostoma</i>								
1	159.0	162.5	15.81	3.10	9.9	124.5	185.5	26
2	138.3	139.1	15.50	3.04	11.2	110.5	163.5	26
3	45.9	46.5	6.60	1.41	14.4	35.5	57.5	22
1:2	0.9	0.86	0.07	0.01	8.4	0.7	1.0	26
<i>Diffflugia gramen</i>								
1	68.8	69.0	6.92	1.09	10.1	57.0	85.5	40
2	51.5	49.9	8.58	1.36	16.7	39.5	77.5	40
3	23.3	2.5	5.40	1.08	23.2	15.9	36.0	25
1:2	0.8	0.7	0.08	0.01	10.9	0.6	0.9	40
<i>Diffflugia lithophila</i>								
1	116.3	114.5	7.23	1.75	6.2	100.5	132.5	17
2	93.7	93.5	5.01	1.21	5.4	86.5	108.0	17
3	43.3	42.5	3.84	0.99	8.9	35.5	48.5	15
1:2	0.8	0.8	0.06	0.01	7.0	0.7	0.9	17

Table 1 (contd.)

<i>Diffflugia urceolata</i>								
1	248.8	245.5	28.43	3.22	11.4	189.0	341.1	78
2	192.4	192.1	38.06	4.31	19.8	100.5	275.5	78
3	114.8	114.0	18.11	2.15	15.8	77.5	148.5	71
1:2	0.8	0.78	0.11	0.01	13.7	0.4	1.0	78
<i>Diffflugia oblonga</i>								
1	238.2	236.5	14.82	2.00	6.2	207.0	287.5	55
2	118.3	118.5	7.87	1.06	6.7	97.5	140.5	55
3	48.1	46.5	7.32	1.07	15.2	36.0	65.5	47
1:2	0.5	0.5	0.03	0.00	6.8	0.4	0.6	55
<i>Diffflugia pyriformis</i>								
1	274.3	272.6	21.95	4.48	8.0	239.5	334.5	24
2	148.7	148.5	19.28	3.86	13.0	114.5	187.5	25
3	51.5	49.1	9.80	2.04	19.0	33.5	70.5	23
1:2	0.6	0.6	0.07	0.01	12.7	0.4	0.8	25
<i>Diffflugia parva</i>								
1	155.3	155.5	12.51	2.25	8.1	128.5	177.5	31
2	72.4	70.5	10.12	1.82	14.0	51.5	90.0	31
3	28.2	27.0	4.94	0.89	17.5	18.5	39.5	31
1:2	0.5	0.36	0.07	0.01	14.3	0.4	0.6	31
<i>Hyalosphenia elegans</i>								
1	98.8	100.0	4.14	0.59	4.2	85.0	105.0	50
2	50.0	50.0	2.58	0.37	5.2	44.0	56.0	50
3	19.9	20.0	1.13	0.16	5.7	17.0	23.0	50
1:2	0.5	0.5	0.03	0.00	5.2	0.4	0.6	50
<i>Hyalosphenia papilio</i> Population 1								
1	124.9	125.0	5.93	0.84	4.7	115.0	140.0	50
2	83.1	84.0	5.51	0.78	6.6	65.0	97.0	50
3	37.0	38.0	1.77	0.25	4.7	33.0	40.0	50
1:2	0.7	0.7	0.05	0.01	7.1	0.5	0.8	50
<i>Hyalosphenia papilio</i> Population 2								
1	124.1	125.0	5.07	0.72	4.1	113.0	138.0	50
2	85.8	86.5	4.48	0.63	5.2	75.0	94.0	50
3	37.5	38.0	2.39	0.34	6.4	33.0	43.0	50
1:2	0.7	0.7	0.04	0.01	5.7	0.6	0.8	50
<i>Hyalosphenia papilio</i> Population 3								
1	103.5	102.0	7.34	1.04	7.1	88.0	122.0	50
2	67.9	67.0	5.14	0.73	7.6	58.0	80.0	50
3	29.7	23.0	5.19	0.74	9.8	23.0	37.0	50
1:2	0.7	0.7	0.04	0.01	5.7	0.6	0.7	50
<i>Hyalosphenia papilio</i> Population 4								
1	99.2	98.0	4.09	0.58	4.1	91.0	115.0	50
2	65.6	65.0	4.73	0.67	7.2	55.0	78.0	50
3	27.8	27.0	1.72	0.24	6.2	25.0	32.0	50
1:2	0.7	0.7	0.04	0.01	5.7	0.6	0.8	50
<i>Hyalosphenia papilio</i> Population 5								
1	118.3	121.0	9.65	0.96	8.2	95.0	137.0	100
2	71.4	72.5	5.98	0.60	8.4	60.0	85.0	100
3	33.0	35.0	5.16	0.52	15.7	24.0	43.0	100
1:2	0.6	0.6	0.03	0.00	4.8	0.52	0.69	100
<i>Schoenbornia humicola</i> Population 1								
1	57.2	56.0	3.73	0.53	6.5	45.0	65.0	50
2	34.7	35.0	2.73	0.39	7.9	28.0	40.0	50
3	18.6	18.0	2.10	0.30	11.3	13.0	23.0	50
1:2	0.6	0.6	0.04	0.01	6.7	0.5	0.7	50
<i>Schoenbornia humicola</i> Population 2								
1	35.0	35.0	3.99	0.56	11.4	26.0	45.0	50
2	21.2	22.0	2.52	0.36	11.9	15.0	25.0	50
3	9.9	10.0	1.34	0.19	13.5	8.0	13.0	50
1:2	0.6	0.6	0.05	0.01	7.7	0.5	0.7	50



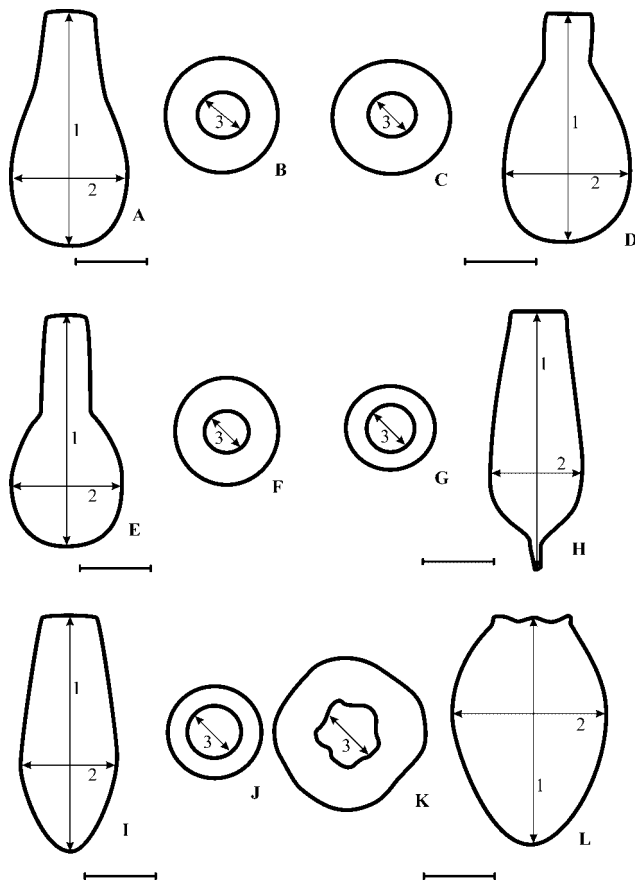
Table 1 (contd.)

<i>Schoenbornia humicola</i> Population 3								
1	38.1	38.0	1.74	0.25	4.6	33.0	42.0	50
2	24.6	25.0	1.05	0.15	4.3	22.0	27.0	50
3	10.5	10.0	1.31	0.19	12.5	7.0	14.0	50
1:2	0.7	0.7	0.03	0.01	4.3	0.6	0.7	50
<i>Schoenbornia humicola</i> Population 4								
1	37.9	37.0	1.79	0.25	4.7	35.0	45.0	50
2	22.7	23.0	1.24	0.18	5.5	20.0	27.0	50
3	9.3	9.0	1.20	0.59	12.9	7.0	38.0	50
1:2	0.6	0.6	0.03	0.01	5.0	0.5	0.7	50
<i>Diffugiella oviformis</i>								
1	21.4	22.0	2.12	0.30	9.9	16.0	27.0	50
2	17.8	17.0	1.87	0.26	10.5	15.0	21.0	50
3	11.2	11.0	1.56	0.22	13.9	8.0	13.0	50
1:2	0.8	0.8	0.07	0.01	8.1	0.7	0.9	50
<i>Phryganella acropodia</i>								
1	39.5	40.0	1.80	0.25	4.6	34.0	44.0	50
2	19.8	20.0	1.12	0.16	5.7	17.0	23.0	50
3	19.6	19.0	1.67	0.24	8.5	17.0	22.0	50
1:2	0.5	0.5	0.01	0.00	3.0	0.4	0.5	50
<i>Waillesella eboracensis</i> Population 1								
1	26.4	26.0	1.60	0.23	12.6	23.0	29.0	50
2	14.5	14.0	0.97	0.14	12.1	12.0	17.0	50
3	5.5	5.5	0.58	0.08	23.2	5.0	7.0	50
4	5.5	5.0	0.58	0.08	24.0	5.0	7.0	50
5	9.7	10.0	0.56	0.08	9.3	8.0	11.0	50
6	11.2	11.0	0.93	0.13	8.4	9.0	15.0	50
<i>Waillesella eboracensis</i> Population 2								
1	27.2	27.0	3.43	0.49	12.6	23.0	48.0	50
2	15.0	15.0	1.80	0.25	12.0	13.0	26.0	50
3	5.6	5.0	1.31	0.18	23.2	5.0	14.0	50
4	5.5	5.0	0.50	0.07	9.2	5.0	6.0	50
5	9.8	10.0	0.91	0.13	9.3	5.0	11.0	50
6	11.6	12.0	0.99	0.14	8.5	9.0	14.0	50
<i>Assulina muscorum</i>								
1	45.1	45.0	3.53	0.35	7.8	32.0	50.0	100
2	34.0	35.0	3.47	0.35	10.2	18.0	42.0	100
3	12.9	13.0	2.10	0.21	16.3	5.0	16.0	100
1:2	0.8	0.8	0.07	0.01	9.0	0.5	1.0	100
<i>Tracheleuglypha acolla</i>								
1	41.8	42.0	2.37	0.34	5.7	38.0	46.0	50
2	26.6	27.0	3.44	0.49	12.9	21.0	34.0	50
3	8.8	9.0	1.41	0.20	16.1	6.0	11.0	50
<i>Trinema lineare</i>								
1	28.6	30.0	5.04	0.80	17.6	21.0	40.0	40
2	11.8	11.0	2.76	0.44	23.5	8.0	18.0	40
3	6.8	7.0	1.68	0.26	24.8	4.0	11.0	40
1:2	0.4	0.4	0.05	0.01	13.0	0.3	0.6	40

Bivariant frequency distribution diagrams (Fig. 6) allow us to estimate not only the degree of polymorphism in testate amoebae populations, but also the discreteness of total variability. In this way it is possible to distinguish monomorphic populations (for example, *Cyclopyxis eurystoma* v. *parvula*), polymorphic populations with correlated characters (*Diffugia urceolata*, *D. corona*, *Assulina muscorum*) and populations with a high pheno-

typic plasticity, non-correlated characters and the elements of discreteness (*Diffugiella oviformis*, *Waillesella eboracensis*).

Populations of the same species in different biotopes may differ from each other not only as to size (Table 4) but also as to the degree of correlation between characters (Tables 5, 6). This phenomenon may be considered as an adaptation mechanism in testate amoebae.

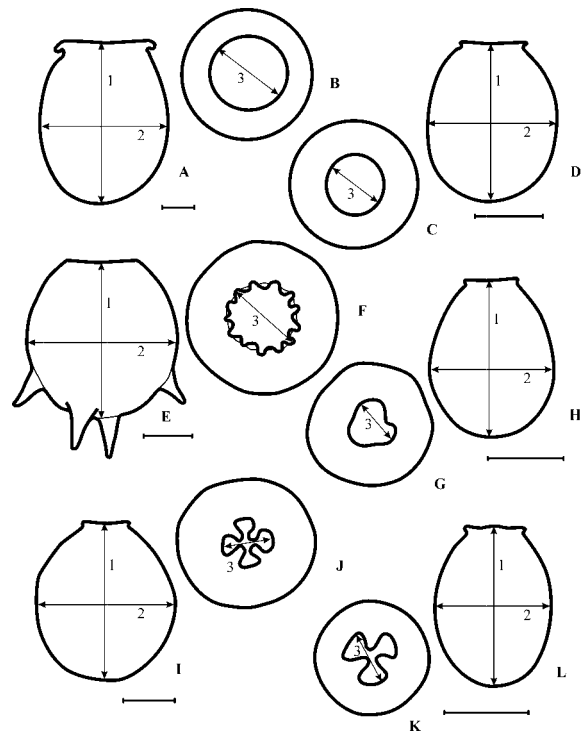


**Fig. 1.** Outline of shells and position of measured axis. **A, B** - *Diffflugia oblonga*; **C, D** - *D. pyriformis*; **E, F** - *D. parva*; **G, H** - *D. acuminata*; **I, J** - *D. lanceolata*; **K, L** - *D. labiosa*. 1 - shell length, 2 - shell breadth, 3 - aperture diameter. Scale bars 50  $\mu$ m.

These variants of variability occur in species with all types of shell (organic, with xenosomes or idiosomes), species representing various life forms, inhabiting freshwater bodies, sphagnum bogs, soils, species from different phyletic branches (Filosea, Lobosea).

## DISCUSSION

The analysis of the variability of testate amoebae in natural populations reveals several general patterns: (a) the variability in natural populations of testate amoebae is expressed either in the changes of the shell size (correlated) or in the changes of the size of separate characters (non correlated). Frequently one character can be decreased in size, and another - to be enlarged. This results in a significant broadening of phenotypic

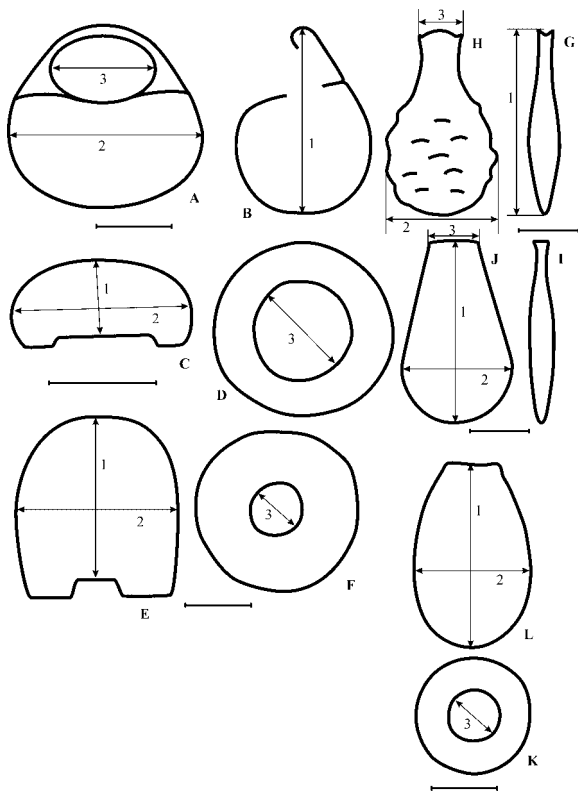


**Fig. 2.** Outline of shells and position of measured axis. **A, B** - *Diffflugia urceolata*; **C, D** - *D. lithophila*; **E, F** - *D. corona*; **G, H** - *D. limnetica*; **I, J** - *D. lobostoma*; **K, L** - *D. gramen*. 1 - shell length, 2 - shell breadth, 3 - aperture diameter. Scale bars 50  $\mu$ m.

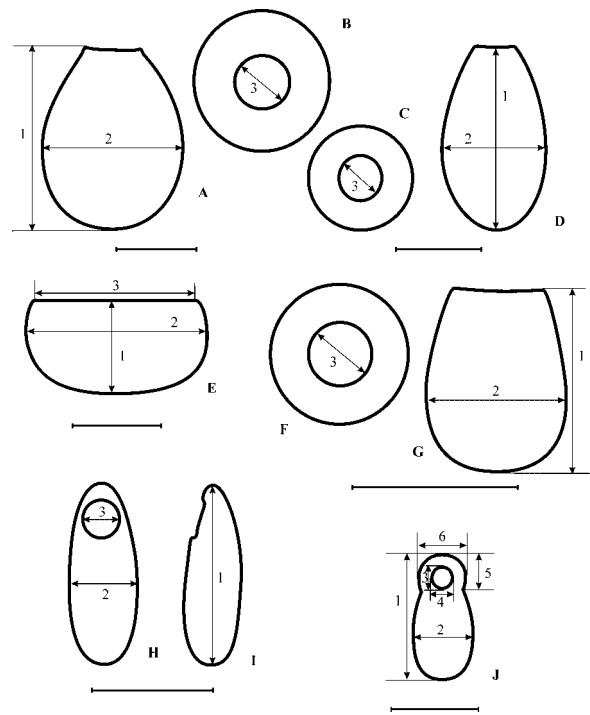
spectrum in populations; (b) the size of the pseudostome is the most variable character. Moreover, this character, as a rule, changes independently from the others. Taking into account that the aperture of the shell is the main way of the organism's interaction with the environment, variations of its size are likely to be adaptive; (c) the pattern of variability (degree and correlativity) differs not only in different species but in different populations of the same species. It might be supposed that the variability of populations is due to the environmental conditions of the habitat where the population is formed.

A pronounced polymorphism of testate amoebae has been recorded by many researches. For example, Schönborn (1992) has shown the phenospectra of *Trinema complanatum* in litter and humus soil horizons of a spruce forest to include 6 morphological variations in each horizon. Only a part of morphological variants were common for both horizons.

The results of the analysis of variability in natural populations of testate amoebae are supplemented by the



**Fig. 3.** Outline of shells and position of measured axis. **A, B** - *Centropyxis sylvatica*; **C, D** - *Cyclopyxis eurystoma v. parvula*; **E, F** - *Cyclopyxis puteus*; **G, H** - *Hyalosphenia elegans*; **I, J** - *Hyalosphenia papilio*; **K, L** - *Schoenbornia humicola*. 1 - shell length, 2 - shell breadth, 3 - aperture diameter. Scale bars 50  $\mu\text{m}$  (A, B, E-J); 20  $\mu\text{m}$  (C, D, K, L).



**Fig. 4.** Outline of shells and position of measured axis. **A, B** - *Assulina muscorum*; **C, D** - *Tracheleuglypha acolla*; **E, G** - *Diffugiella oviformis*; **H, I** - *Trinema lineare*; **J** - *Waillesella eboracensis*. **A-I** - 1 - shell length, 2 - shell breadth, 3 - aperture diameter. **J** - 1 - shell length, 2 - shell breadth, 3 - aperture length, 4 - aperture breadth, 5 - length of "head region", 6 - breadth of "head" region. Scale bars 20  $\mu\text{m}$ .

investigations of clonal cultures. The question to be solved is whether individual variability in testacean populations is due to genotypic features or to environmental impact (trophic interactions, abiotic factors, etc.).

In laboratory experiments on *Trinema lineare* and *Euglypha laevis* clones Schönborn (1992) has shown that in stable controlled conditions the variation coefficients of morphometrical parameters considerably grows with the time of conducting the clonal culture. Consequently, individual variability is likely to be genetically conditioned, and it is not always directly adaptive. In other words, in a genotype spontaneous size fluctuations within certain intervals are incorporated. In concrete biotopes the selection is for a total phenotypic spectrum, i.e., from all possible morphological and morphometrical variations of a species those individuals are represented in the population that possess the advantages of a better adaptation to concrete conditions. Localities being heterogeneous, in a population there may be several pheno-

types, adapted to different microlocalities. However, there will not always be a uniform sample of phenotypes in a homogeneous locality, either. In the experiments of Schönborn (1992), individual variability was recorded even in stable conditions of clonal cultures. Thus, species in natural populations possess an extremely complex phenotypic spectrum. It is expressed differently in different species. If we take this into account, it becomes even more surprising that individual variability in the majority of species is rather low and the species is stable.

The results of the experiments of Schönborn (1992) could be also explained in another way. Deterioration of conditions in the culture in the course of experiments (changes of a trophic regime, accumulation of metabolites in the growing culture, an increasing population density) could result in the expansion of the morphological spectrum of the population. This hypothesis could be supported by an increase in the number of organisms of

**Table 2.** Variation coefficients in different testate amoebae groups. n - number of characters investigated,  $\bar{x}$  - arithmetic mean, Min - minimum, Max - maximum, SD - standard deviation.

Life - forms	n	$\bar{x}$	Min	Max	SD
Simple acrostomia	75	12.2	3.2	29.8	5.77
Compressed acrostomia	152	11.4	2.3	35.2	6.40
Centrostomia	38	13.4	2.4	35.3	9.18
Plagiostomia	91	13.5	2.5	35.3	6.96
Biotope inhabited	n	$\bar{x}$	Min	Max	SD
Freshwater	66	12.0	3.1	26.2	5.78
Sphagnum	62	10.5	2.3	32.1	7.04
Soil	256	12.6	2.4	35.3	6.78
Class	n	$\bar{x}$	Min	Max	SD
Lobosea	266	11.3	2.3	35.3	6.18
Filosea	114	14.0	2.5	35.3	7.45

**Table 4.** Statistical significance (Student's t-test) of shell measurements between populations of *Schoenbornia humicola* and populations of *Hyalosphenia papilio*. P1-P4 - populations (see Materials and Methods).

	<i>Schoenbornia humicola</i>			<i>Hyalosphenia papilio</i>		
	P1	P2	P3	P1	P2	P3
P2	***			P2	NS	
	***				***	
P3	***	***		P3	***	***
	***	***			***	***
P4	***	***	NS	P4	***	***
	***	***	***		***	***

1st line - shell length; 2nd line - shell width. \*\*\* P < 0.001; NS - not significant.

**Table 3.** Pearson correlation coefficients between shell measurements of the investigated testate amoebae species. L - length of the shell, B - breadth of the shell, Dp - aperture diameter; \*\*\* P < 0.001; \*\* 0.01 > P > 0.001; \* 0.05 > P > 0.01; + 0.1 > P > 0.05; NS - not significant.

Species	L-B	L-Dp	B-Dp
<i>Centropyxis sylvatica</i>	0.71***	0.46**	0.51***
<i>Cyclopyxis eurystoma</i> v. <i>parvula</i>	0.89***	0.74***	0.68***
<i>Cyclopyxis puteus</i>	0.31+	0.42**	0.31+
<i>Diffflugia acuminata</i>	0.47**	0.51**	0.83***
<i>Diffflugia corona</i>	0.46**	-0.05 <sup>NS</sup>	-0.06 <sup>NS</sup>
<i>Diffflugia labiosa</i>	0.69***	0.60***	0.70***
<i>Diffflugia lanceolata</i>	0.46***	0.32**	0.78***
<i>Diffflugia limnetica</i>	0.48**	0.29+	0.70***
<i>Diffflugia lobostoma</i>	0.76***	0.01 <sup>NS</sup>	-0.10 <sup>NS</sup>
<i>Diffflugia gramen</i>	0.83***	0.51**	0.73***
<i>Diffflugia lithophila</i>	0.17 <sup>NS</sup>	0.32 <sup>NS</sup>	-0.03 <sup>NS</sup>
<i>Diffflugia oblonga</i>	0.40**	0.35**	0.13 <sup>NS</sup>
<i>Diffflugia pyriformis</i>	0.72***	0.62**	0.48*
<i>Diffflugia parva</i>	0.27 <sup>NS</sup>	0.40*	-0.06 <sup>NS</sup>
<i>Diffflugia urceolata</i>	0.78***	0.79***	0.87***
<i>Hyalosphenia elegans</i>	0.41**	0.04 <sup>NS</sup>	0.43***
<i>Hyalosphenia papilio</i> Population 5	0.84***	0.84***	0.85***
<i>Schoenbornia humicola</i> Population 2	0.80***	0.48***	0.55***
<i>Difflogiella oviformis</i>	0.67***	0.64***	0.57***
<i>Phryganella acropodia</i>	0.89***	0.84***	0.85***
<i>Wailesella eboracensis</i> Population 1	0.45**	0.26 <sup>NS</sup>	0.27 <sup>NS</sup>
<i>Assulina muscorum</i>	0.60***	0.49***	0.78***
<i>Tracheleuglypha acolla</i>	0.82***	0.64***	0.66***
<i>Trinema lineare</i>	0.82***	0.84***	0.83***

**Table 5.** Correlation coefficients between shell measurements of 2 populations *Wailesella eboracensis* from *Sphagnum* in Malozemel shrubby tundra. 1st line - Population 1, 2nd line - Population 2. Statistical significance (Student's t-test) between 2 populations  $P < 0.05$ .

2	3	4	5	6	Character
0.45	0.26	0.26	0.14	0.13	1
0.86	0.89	0.09	0.51	0.00	
	0.27	0.33	0.29	0.29	2
	0.86	0.00	0.57	-0.04	
		0.97	0.33	0.04	3
		0.00	0.57	-0.09	
			0.37	0.09	4
			0.45	0.36	
				0.26	5
				0.59	

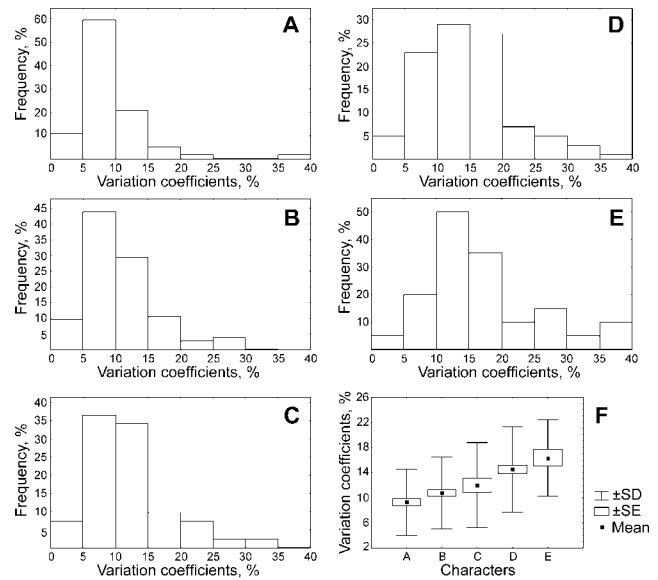
smaller size closer to the end of the experiment. In the same investigation (Schönborn 1992), the experiments with clonal strains of large and small individuals were carried out. Our calculations on the basis of Schönborn's (1992) data have shown that in clonal strains of large individuals the average values of shell size remain the same in the course of the experiment, and only occasionally are a little reduced. As for the minimum values, they are reduced by 14.4-25.9 %, whereas the maximal ones, by 0-17.6 %. In other words, in populations, which have initially consisted of large individuals, the number of smaller organism increases. In clonal strains of small testaceans the average size was increasing with time in the majority of experiments. The increase of the average size took place first of all is due to the growth of the number of large amoebae (in one of the clones the shell dimensions increased more than by 50 %), and not due to the elimination of small testate amoebae.

Thus, individual variability in experimental conditions depends on the duration of the experiment and thus on varying conditions of the medium, and also on the phenotype of the mother cell chosen for the clonal culture.

Later Wanner (Wanner 1994a, b; Wanner and Meisterfeld 1994; Wanner *et al.* 1994) in a series of laboratory experiments with clones and natural populations of *Cyclopyxis kahli*, *C. eurystoma* v. *parvula*, *Euglypha strigosa*, *E. rotunda*, *Trinema lineare*,

**Table 6.** Correlation coefficients between shell measurements of 4 populations *Hyalosphenia papilio* and 4 populations *Schoenbornia humicola* from different biotopes. L - length of the shell, B - breadth of the shell, Dp - aperture diameter.

Population	L:B	L:Dp
<i>H. papilio</i> Population 1	0.21	0.23
<i>H. papilio</i> Population 2	0.21	0.33
<i>H. papilio</i> Population 3	0.70	0.65
<i>H. papilio</i> Population 4	0.52	0.55
<i>Sch. humicola</i> Population 1	0.61	0.45
<i>Sch. humicola</i> Population 2	0.80	0.48
<i>Sch. humicola</i> Population 3	0.50	0.38
<i>Sch. humicola</i> Population 4	0.41	0.05



**Fig. 5.** Frequency distribution of variation coefficients in shell length (A), shell breadth (B), shell depth (C), aperture length (D) and aperture breadth (E). Mean variation coefficients of different characters (F). SD - standard deviation, SE - standard error of mean. The differences between variation coefficients are significant (Mann-Whitney U test:  $p < 0.05$ ) for all character combinations (exception B vs. C).

*T. enchelys* has shown the influence of light intensity, temperature, fertilising and pesticides, and the nature of food upon morphometrical characteristics of testate amoebae. The analysis of the Wanner's data (Wanner 1994a) has shown that the variability of different morphological parameters in clonal cultures varied, preserving its values practically irrespective of experimental conditions. For example, at *Cyclopyxis kahli* the varia-

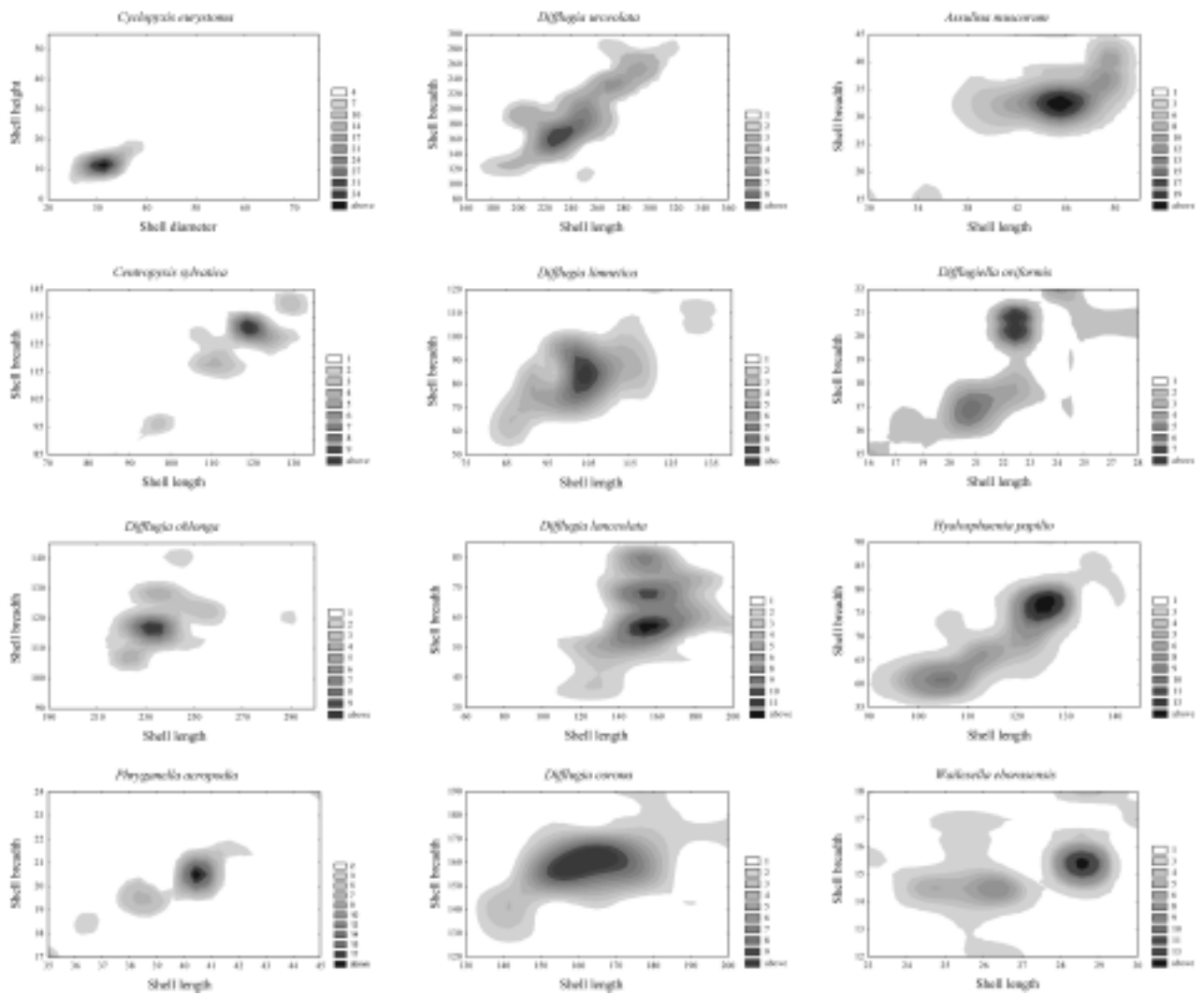


Fig. 6. Bivariant frequency distribution of characters in different testate amoebae species.

tion coefficient of the pseudostome diameter in almost all variants of experiments was almost three times higher than the coefficient of variation of the shell diameter. The degree of variability of various morphological characters is most likely to be determined by the species genotype. So the species realize its adaptive strategy by means of the most variable characters and due to variability of these ones the species can be polymorphic or polytypic.

Summing up, all available data indicate to a rather high variability of testate amoebae. The existing morphological types are grouped around a limited number of adaptive peaks, formed under the influence of ecological

factors. However, these peaks are often weakly expressed, their discreteness is frequently insignificant, there being a lot of transitional forms. Thus the space of morphometrical characters as a whole is characterized by both continuity and discreteness. In different cases (different biotopes or different complexes of closely related species) it is possible to distinguish discrete units with a varying accuracy. Nevertheless, the systematicists have traditionally tried to designate the extreme forms as taxonomical units (subspecies or forms) irrespective of whether there are clear boundaries between them. The imprecision of differential diagnoses stems from the features of the object classified (asexual protists with

continuous variability). All this entails great difficulties in the diagnostics of testacean species and in the applicability of the "species" concept for them.

Recently the "morphospecies" concept (Finlay *et al.* 1996, Finlay 1998) has been suggested with reference to protists. The morphological criterion is put forward as a predominant one. On the one hand, this makes possible to avoid numerous difficulties associated with the separation of genetically isolated systems of organisms, not differing morphologically (syngenes), and, on the other hand, this is a rather convenient approach for the ecologists, as morphology to a great extent reflects the characteristics of the ecological niche occupied by an organism. However, the problems in connection with asexual protists are arisen. The notion of "species aggregates" has been offered (Finlay 1998) for clusters of species, which are morphologically indiscernible or form a continuum of morphologically overlapping forms. However, such an approach is likely to result in the loss of some ecological information reflected in the total morphological variability of the object or the number of transitional forms (distinguished as subspecies or forms). A high level of morphological variability within the population can indicate the heterogeneity of the biotope, where several morphs, including transitional ones, can exist simultaneously. A narrow phenospectrum, on the contrary, is more likely to arise in a homogeneous microbiotope or under severe abiotic conditions.

On the other hand, the account of all morphological diversity, not divided into discrete units, introduces significant difficulties into systematics and unambiguous species diagnosis. The problem is also complicated by the incompleteness of the descriptions of many taxa.

Earlier it was proposed to define the species of testate amoebae as wide phenetic clusters in which specimens are linked to each other through intergradation (Medioli and Scott 1983). However, there arises a question of what value of phenotypic variability can be accepted as infraspecific. In the work of Medioli and Scott (1983) an extreme degree of "lumping" can be seen. They have divided all diversity within Arcellinida into 14 species.

Detailed study of different species complexes presented recently by Foissner and Korganova (1995, 2000) is an excellent way of clarifying the problems outlined.

We propose the following scheme of the close related species analysis: (a) Revealing morphological variability of populations from various localities, and also variability within groups of closely related species; (b) A detailed

biometric analysis of variability within the complex of close species studied and the evaluation of the possibility to distinguish more or less discrete units; in case of impossibility of unambiguous differentiation of taxa in the species complex demonstrating a high degree of polymorphism, the description of extreme variants and the indication of continuity of changes; (c) Redescription or establishing the synonymy of poorly described species; (d) Taking in account the environmental constraints determining morphological variability.

As a result of such research we will obtain a clearer understanding of the characteristics of phenotypic space of testate amoebae natural populations at a morphological level. From the systematic point of view it would help to outline the borders between species of testate amoebae more clearly. The adaptive strategy of testate amoebae is diverse. Its mechanisms are different at different species, and the knowledge of its variants realised would help to build the hierarchy of morphological characters as to their significance in different taxa.

A research like this could increase our knowledge of the causes and factors producing changes in shell morphology in ecological and paleoecological investigations. It would considerably expand the interpretation possibilities of rhizopode analysis, as it has already been shown for the rhizopode population of oligotrophic moors (Bobrov *et al.* 1999).

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## REFERENCES

- Anonymous (1977) Communiqué du groupement d'études sur les Thecamoebiens. *Protistologica* **13**: 333-334
- Anderson O. R. (1995) Effects of caffeine treatment on the fine structure, silica secretion, and shell morphology of the testate amoeba *Assulina muscorum*. *J. Morphol.* **225**: 251-260
- Anderson O. R., Cowling A. J. (1994) The fine structure of the euglyphid testate amoeba *Assulina muscorum* (Rhizopoda: Euglyphidae) with observations of growth rate in culture, morphometrics, and siliceous scale deposition. *Eur. J. Protistol.* **30**: 451-461
- Balik V. (1988) *Centropyxis moldavica* sp.n. (Rhizopoda, Testacea), the new species of soil testacea from Czechoslovakia. *Acta Protozool.* **27**: 161-164
- Bassine F. (1929) Biometrische Untersuchungen an *Arcella vulgaris*. *Arch. Russ. Protistol.* **8**: 12-24 (in Russian with German Summary)
- Bobrov A. A., Yazvenko S. B., Warner B. G. (1995) Taxonomic and ecological implications of shell morphology of 3 testaceans (Protozoa, Rhizopoda) in Russia and Canada. *Arch. Protistenk.* **145**: 119-126

- Bobrov A. A., Charman D. J., Warner B. G. (1999) Ecology of testate amoebae (Protozoa: Testacea) on Peatlands in Western Russia with special attention to niche separation in closely related taxa. *Protist* **150**: 125-136
- Chardez D. (1990) Contribution a la connaissance des Thecamoebiens aquatiques du Tyrol allemand (Rhizopoda, Testacea). *Acta Protozool.* **29**: 153-156
- Chardez D. (1991) Note sur *Diffflugia humilis* sp. n. (Protozoa: Rhizopoda: Testacea). *Acta Protozool.* **30**: 45-47
- Chardez D. (1994) Contribution a l'etude biogeographique de *Centropyxis carinata* Chardez, 1964. *Acta Protozool.* **33**: 195-198
- Chardez D. and Leclercq J. (1963) Variabilite des populations d' *Euglypha strigosa* (Ehrenberg) Leidy en fonction de l'habitat (Rhizopoda, Testacea). *Bull. Inst. Agron. et Stat. Rech. Gembloux.* **31**: 21-27
- Couteaux M.-M. (1976) Dynamisme de l'equilibre des Thecamoebiens dans quelques sols climaciques. *Mem. Mus. Nat. Hist. Nat., Ser. A, Zool.* **96**: 1-183
- Dekhtyar I. N. (1993) New species of the family Diffugiidae (Lobosea, Rhizopoda) with remarks on validity of the genus *Protocucurbitella*. *Zool. Zh.* **72**: 5-15 (in Russian with English summary)
- Dekhtyar I. N. (1994) The new and rare species of testate amoebae of the families Arcellidae, Centropyxidae, Lesquereusiidae and Hyalospheniidae (Rhizopoda, Testacealobosia). *Zool. Zh.* **73**: 3-14 (in Russian with English summary)
- Foissner W., Korganova G. A. (1995) Redescription of 3 testate amebas (Protozoa, Rhizopoda) from a naucasian soil - *Centropyxis plagiostoma* Bonnet and Thomas, *Cyclopyxis kahli* (Deflandre) and *Cyclopyxis intermedia* Kufferath. *Arch. Protistenk.* **146**: 13-28
- Foissner W., Korganova G. A. (2000) The *Centropyxis aerophila* complex (Protozoa: Testacea). *Acta Protozool.* **39**: 257-273
- Finlay B. J. (1998) The global diversity of protozoa and other small species. *Intern. J. Parasitol.* **28**: 29-48
- Finlay B. J., Corliss J. O., Estban G., Fenchel T. (1996) Biodiversity at the microbial level: the number of free-living ciliates in the biosphere. *Quart. Rev. Biol.* **71**: 221-237
- Jennings H. S. (1916) Heredity, variation and results of selection in the uniparental reproduction of *Diffflugia corona*. *Genetics* **1**: 407-534
- Heal O. W. (1963) Morphological variations in certain Testacea (Protozoa, Rhizopoda). *Arch. Protistenk.* **106**: 351-368
- Hegner R. W. (1919) Heredity, variation and the appearance of diversities during the vegetative reproduction of *Arcella dentata*. *Genetics* **4**: 95-150
- Hoogenraad H. R., de Groot A. A. (1937) Biometrische Untersuchungen an Süßwasser-rhizopoden. (Rhizopoden und Heliozoen aus dem Süßwasser der Niederlande. VI.). *Arch. Hydrobiol.* **31**: 101-132
- Jollos V. (1924) Untersuchungen uber Variabilität und Vererbung bei *Arcella*. *Arch. Protistenk.* **49**: 307-374
- Lüftenegger G., Foissner W. (1991) Morphology and biometry of twelve soil testate amoebae (Protozoa, Rhizopoda) from Australia, Africa and Austria. *Bull. Brit. Mus. Nat. Hist. (Zool. Ser.)* **57**: 1-16
- Lüftenegger G., Petz W., Berger H., Foissner W., Adam H. (1988) Morphologic and biometric characterisation of twenty-four soil testate amoebae (Protozoa, Rhizopoda). *Arch. Protistenk.* **136**: 153-189
- Medioli F. S., Scott D. B. (1983) Holocene Arcellacea (Thecamoebians) from Eastern Canada. *Cushman Found. Spec. Publ.* **21**: 5-63
- Mignot J. P., Raikov I. B. (1992) Evidence for meiosis in the testate amoeba *Arcella*. *J. Protozool.* **39**: 287-289
- Netzel H. (1972a) Die Bildung der Gehäusewand bei der Thekamobe *Centropyxis discoides* (Rhizopoda, Testacea). *Z. Zellforsch. Mikrosk Anat.* **135**: 45-54
- Netzel H. (1972b) Morphogenese des Gehäuses von *Euglypha rotunda* (Rhizopoda, Testacea). *Z. Zellforsch. Mikrosk Anat.* **135**: 63-69
- Netzel H. (1972c) Die Scalenbildung bei *Diffflugia oviformis* (Rhizopoda, Testacea). *Z. Zellforsch. Mikrosk Anat.* **135**: 55-61
- Ogden C. G. (1979a) Siliceous structures secreted by members of the subclass Lobosia (Rhizopoda: Protozoa). *Bull. Brit. Mus. Nat. Hist. (Zool. Ser.)* **36**: 203-207
- Ogden C. G. (1979b) Comparative morphology of some pyriform species of *Diffflugia* (Rhizopoda). *Arch. Protistenk.* **122**: 143-153
- Ogden C. G. (1991) The biology and ultrastructure of an agglutinate testate amoeba *Diffflugia geosphaira* sp. nov. (Protozoa, Rhizopoda). *Eur. J. Protistol.* **140**: 141-150
- Ogden C. G., Meisterfeld R. (1989) The taxonomy and systematics of some species of *Cucurbitella*, *Diffflugia* and *Netzelia* (Protozoa: Rhizopoda), with an evaluation of diagnostic characters. *Eur. J. Protistol.* **25**: 109-128
- Poljansky G. I. (1992) Protozoology and the problem of species. *J. Protozool.* **39**: 177-180
- Raikov I. B., Karadzhan B. P., Kaur R., Mignot J. P. (1989) Nuclear fine-structure at interphase and during encystment in 2 forms of the testacean *Arcella vulgaris*. *Eur. J. Protistol.* **24**: 369-380
- Reynolds B. D. (1923) Inheritance of double characteristics in *Arcella polypora*. *Genetics* **8**: 477-496
- Root F. M. (1918) Inheritance in the asexual reproduction of *Centropyxis aculeata*. *Genetics* **3**: 173-206
- Schönborn W. (1962) Die Ökologie der Testaceen im oligotrophen See, dargestellt am Beispiel des Grossen Stechlinsees. *Limnologica* **1**: 111-182
- Schönborn W. (1966) Beschalte Amoben (Testaceae). Wittenberg, Lutherstadt
- Schönborn W. (1983) Modifikabilität und Evolutionstrends bei Protozoen. *Biol. Rdsch.* **21**: 225-235
- Schönborn W. (1990) Analyse subfossiler Protozoenschalen der Sedimente eines kleinen sauren Waldsees (Kleiner Barsch-See, nordische DDR). *Limnologica* **21**: 137-145
- Schönborn W. (1992) Adaptive polymorphism in soil-inhabiting testate amebas (Rhizopoda) - its importance for delimitation and evolution of asexual species. *Arch. Protistenk.* **142**: 139-155
- Schönborn W., Peschke T. (1988) Biometric studies on species, races, ecophenotypes and individual variations of soil-inhabiting testacea (Protozoa, Rhizopoda), including *Trigonopyxis minuta* n.sp. and *Corythion asperulum* n.sp. *Arch. Protistenk.* **136**: 345-363
- Schönborn W., Peschke T. (1990) Evolutionary studies on the *Assulina - Valkanovia* complex (Rhizopoda, Testaceafilosia) in sphagnum and soil. *Biol. Fertil. Soils* **9**: 95-100
- Schönborn W., Foissner W., Meisterfeld R. (1983) Licht- und rasterelektronenmikroskopische Untersuchungen zur Schalenmorphologie und Rassenbildung bodenbewohnender Testaceen (Protozoa, Rhizopoda) sowie Vorschläge zur biometrischen Charakterisierung von Testaceen-Schalen. *Protistologica* **19**: 553-566
- Schönborn W., Petz W., Wanner M., Foissner W. (1987) Observations on the morphology and ecology of the soil-inhabiting testate amoeba *Schoenbornia humicola* (Schonborn, 1964) Decloitre, 1964 (Protozoa, Rhizopoda). *Arch. Protistenk.* **134**: 315-330
- Sukhanova K. M., Yudina T. A. (1990) The ecology and life cycle of the fresh water testacean rhizopod *Trinema lineare* Penard. In: Ecology of Marine and Freshwater Free-living Protozoa, (Ed. K. M. Sukhanova). Nauka, Leningrad, 133-142 (in Russian with English summary)
- Wanner M. (1988) Biometrische und rasterelektronenmikroskopische Untersuchungen an Testaceen-Schalen (Protozoa, Rhizopoda). *Arch. Protistenk.* **136**: 97-106
- Wanner M. (1991) Zur Morphologie von Thekamoben (Protozoa, Rhizopoda) in suddeutschen Wäldern. *Arch. Protistenk.* **140**: 45-66
- Wanner M. (1994a) Effects of light, temperature, fertilizers and pesticides on shell size of the common freshwater and soil species *Cyclopyxis kahli* (Rhizopoda, Testacealobosia). *Limnologica* **24**: 333-338
- Wanner M. (1994b) Biometrie der Schalen von *Cyclopyxis kahli* Deflandre (Rhizopoda, Testacealobosia) - eine Möglichkeit zur Bioindikation? *Verh. Dtsch. Zool. Ges.* **87**: 317
- Wanner M. (1995) Biometrical investigations of terrestrial testate amoebae (Protozoa: Rhizopoda) as a method for bioindication. *Acta Zool. Fennica* **196**: 267-270



- Wanner M. (1999) A review on the variability of testate amoebae: methodological approaches, environmental influences and taxonomical implications. *Acta Protozool.* **38**: 15-29
- Wanner M., Funke W. (1986) Rasterelektronenmikroskopische Untersuchungen an *Edaphonobiotus campascooides* (Protozoa, Testacea). *Arch. Protistenk.* **132**: 187-190
- Wanner M., Meisterfeld R. (1994) Effects of some environmental factors on the shell morphology of testate amebas (Rhizopoda, Protozoa). *Eur. J. Protistol.* **30**: 191-195
- Wanner M., Esser S., Meisterfeld R. (1994) Effects of light, temperature, fertilizers and pesticides on growth of the common freshwater and soil species *Cyclopyxis kahli* (Rhizopoda, Testacealobosia), interactions and adaptations. *Limnologica* **24**: 239-250
- Yablokov A. V., Yusufov A. G. (1998) The Theory of Evolution. Higher School, Moscow (in Russian)
- Yudina T. A., Sukhanova K. M. (2000) Cell biology and life cycle of the testate amoeba *Corythion delamarei*. *Cytology* **42**: 613-623
- Zavadsky K. M. (1968) Species and Speciation. Nauka, Leningrad (in Russian)
- Zinger Y. A. (1935) Variability of size and shell sculpture in *Arcella*. *Bull. Stat. Biol. Bolchevo* **7-8**: 191-194 (in Russian with German summary)

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## Shell Morphology, Biometry and Distribution of Some Marine Interstitial Testate Amoebae (Sarcodina: Rhizopoda)

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**Summary.** The morphology and biometry of eight marine interstitial testate amoebae (*Centropyxiella lucida*, *Cyphoderia littoralis*, *Messemyriella filosa*, *Ogdeniella elegans*, *O. maxima*, *Pomoriella valkanovi*, *Pseudocorythion acutum* and *Rhumleriella filosa*) were studied by light microscopy and by scanning electron microscopy. Their size frequency distributions were analysed and all studied species was defined as a size-monomorphic. All of them are characterized by a well-expressed main-size class and by a small size range of the shell breadth. Regarding to the shell length *P. acutum* and *O. elegans* are characterized by a not well-expressed main-size class in favour of subsidiary classes, but all species have a shell length ranges in close limits. The data about the shell ultrastructure of the species *M. filosa*, *O. elegans*, *O. maxima* and *R. filosa* are reported for the first time in the literature.

**Key words:** biometry, distribution, marine testate amoebae, morphology, shell structure.

### INTRODUCTION

The marine interstitial testate amoebae form a specific taxocenose in the marine sand supralittoral. They were found and described during the last three decades. 129 species of testate amoebae from the orders Arcellinida and Gromida have been established in the studied seas and oceans so far. They belong to three ecological categories: obligatory psammobionts (79 species), psammophiles (11 species), and psammoxenes (39 species) (Golemansky 1969, 1980, 1994a).

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The morphological investigations and taxonomic descriptions of the marine interstitial testate amoebae have been accomplished mainly by using light microscopy on limited number of specimens so far. Because of that, the information about two important taxonomic criteria - the ultrastructure and biometry of their tests is scanty and incomplete. Golemansky (1979a) and Golemansky and Ogden (1980) published the first data about the shell ultrastructure of 3 marine psammobiotic testate amoebae. Later Golemansky and Coûteaux (1982), Ogden and Coûteaux (1986, 1989), Anderson *et al.* (1996) and Golemansky and Todorov (1996) enlarged the list of the studied interstitial testate amoebae by using SEM and further contributed to the information about their shell structure and morphological variability. To date, the shell

ultrastructure of 18 species of marine interstitial testate amoebae is studied as a result of the cited contributions.

The aim of the present publication is to further enrich the data about the shell ultrastructure of some unstudied or little known marine interstitial testate amoebae and to make a biometric analysis of their morphological variability. Since, there is not enough data regarding the geographical distribution of the studied testate amoebae in protozoological literature, we consider that more detailed information about some rare and little known species in the World Ocean will be useful.

## MATERIALS AND METHODS

The material for the present study was collected from several localities in sandy beaches of Black Sea (Bulgaria), North Sea (Sweden), and Atlantic Ocean (Brazil) during the period from November 1999 till March 2002. Samples were taken from the humid zone of supralittoral, near the waterline, in a depth of 0-50 cm in the sand. Information relevant to each sample is given with the species description.

The biometric characterization of the species was made according to Schönborn *et al.* (1983). The following parameters were calculated: arithmetic mean ( $\bar{x}$ ); median (M); standard deviation (SD); standard error of the mean (SE); coefficient of variation in % (CV); extreme values (Min and Max); number of examined individuals (n). Size measurements of shells were made by light microscopy at 400 × magnification.

For scanning electron microscopy the shells were isolated, cleaned by several washes with distilled water, mounted directly on stubs and air-dried. The shells were coated evenly with gold in vacuum coating unit. Microphotographs were obtained by using Phillips SEM 515, operating at 25 kV.

## RESULTS AND DISCUSSION

### *Centropxyiella lucida* Golemansky, 1971 (Figs 1-4)

**Shell morphology:** The shell is colourless, elliptical in outline, usually having almost parallel lateral margins and rounded apertural and aboral regions (Figs 1, 2). It is slightly flattened dorso-ventrally and shell height is about 2/3 to 3/4 of the shell breadth. By cross-section is oval and appears to taper evenly along most of the shell length. The aperture is normally circular, large, and situated sub-terminally at the flattened ventral side of the shell. The aperture is surrounded by small collar that not reach to the maximum breadth of the shell (Figs 1, 3).

The shell is composed of small to medium flattish pieces of quartz (xenosomes) so arranged that the shell surface is smooth (Figs 2, 4). The flattened ventral side

is made of smaller flat particles, whilst the dorsal surface and apertural collar are made from larger flattish and rarely angular xenosomes (Figs 1-4).

**Biometry:** Table 1 shows that the coefficients of variation of all characters are low and shell measurements are rather constant. The values of the shell breadth and the diameter of aperture correspond well to those of the original description of species based on a material from Pacific (Golemansky 1971), but our specimens from North Sea are smaller in length and more flattened (62-72  $\mu\text{m}$  vs. 70-81  $\mu\text{m}$  and 23-28  $\mu\text{m}$  versus 30-36  $\mu\text{m}$ , respectively).

The analysis of the size frequency distribution indicates that *C. lucida* is a size-monomorphic species characterized by a comparatively well-expressed main-size class and by a small size range (Fig. 5). Figure 5a shows that 98% of all specimens measured have a shell length 62-69  $\mu\text{m}$  and 2% only have a shell length bigger than 69  $\mu\text{m}$ . The frequency analysis of the shell breadth shows that the specimens of our population are constant by this character (more than 55% of them have a shell breadth 40  $\mu\text{m}$ , and all specimens measured are in limits of 37-45  $\mu\text{m}$ ).

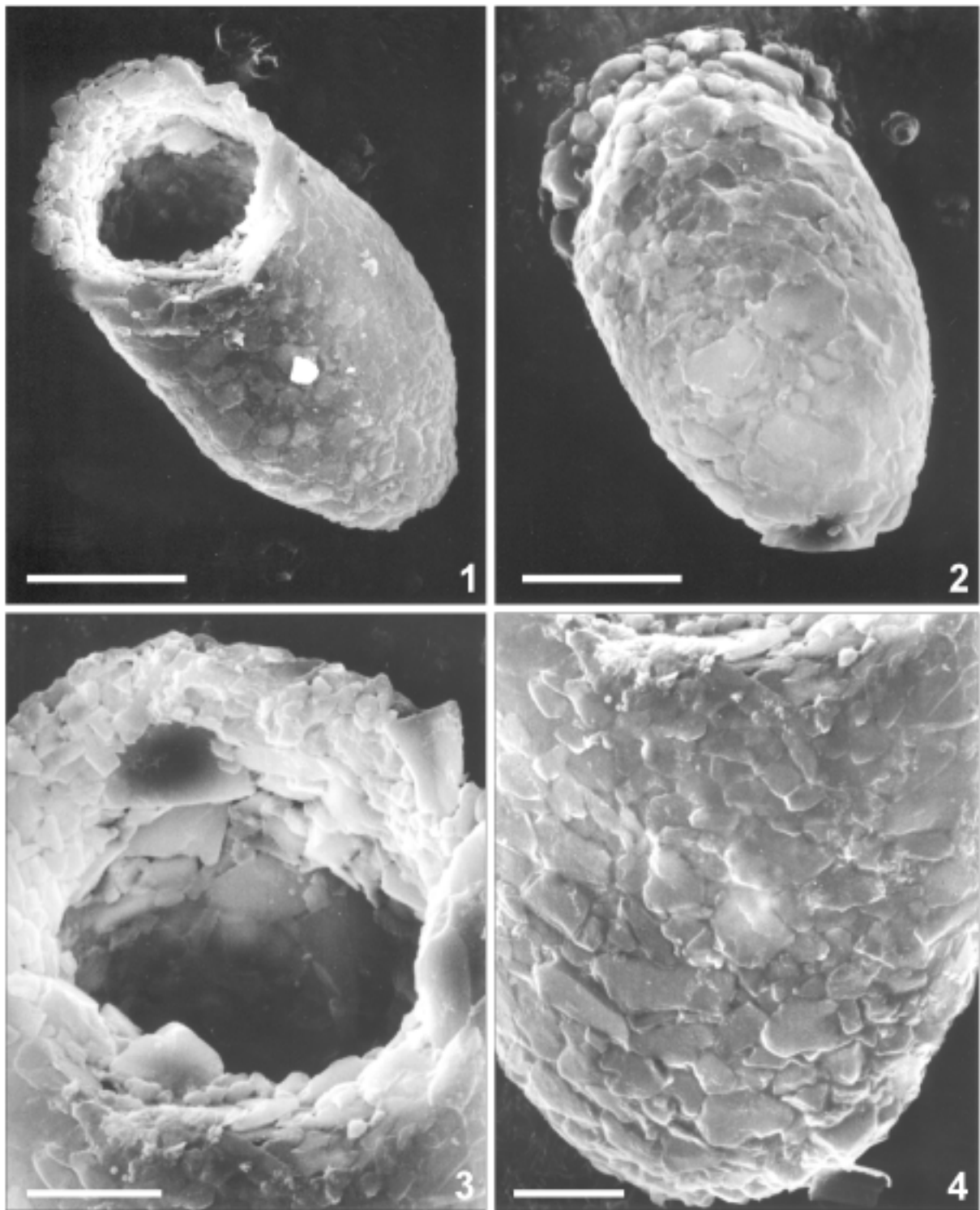
**Material:** *C. lucida* was isolated from samples collected at a sandy beach, 4 m above the high-water mark gathered at Tjarno Biological station, Reserve Saltö, North Sea (Sweden), in August 2000.

**Geographical distribution:** Pacific: Nanaimo /Canada/ (Golemansky 1971); Tokyo and Tshigaki /Japan/ (Sudzuki 1977, 1979); Black Sea: Sunny Coast /Bulgaria/ (Golemansky 1974, 1980); Atlantic: Roscoff /France/ (Golemansky 1992); North Sea: Kattgat /Sweden/ (Golemansky 2002).

### *Pomoriella valkanovi* Golemansky, 1970 (Figs 6-9)

**Shell morphology:** The shell is colourless, transparent, elongate pyriform in ventral view, and circular in cross-section. In lateral view the anterior region is elongated and curved, with a short neck (Figs 6-8). The aperture is circular, bordered by organic cement and is situated through angle to the longitudinal axis of the shell (Figs 8, 9).

The shell is composed of approximately forty oval shell plates arranged without overlapping, in four longitudinal rows, and joined by an abundance of organic cement (Figs 6, 8, 9). The shell plates are of two different types: bigger elongate-oval, about 8-10  $\mu\text{m}$  in length and 5-7  $\mu\text{m}$  in width in the aboral region, and smaller oval, about 3.5-4 × 1.5-2  $\mu\text{m}$  in the apertural region of the shell.



**Figs 1-4.** *Centropyxiella lucida*. **1** - ventral view; **2** - dorsal view; **3** - apertural view; **4** - posterior end of the shell, covered by plate "xenosomes". Scale bars 20  $\mu\text{m}$  (1, 2); 10  $\mu\text{m}$  (3, 4).

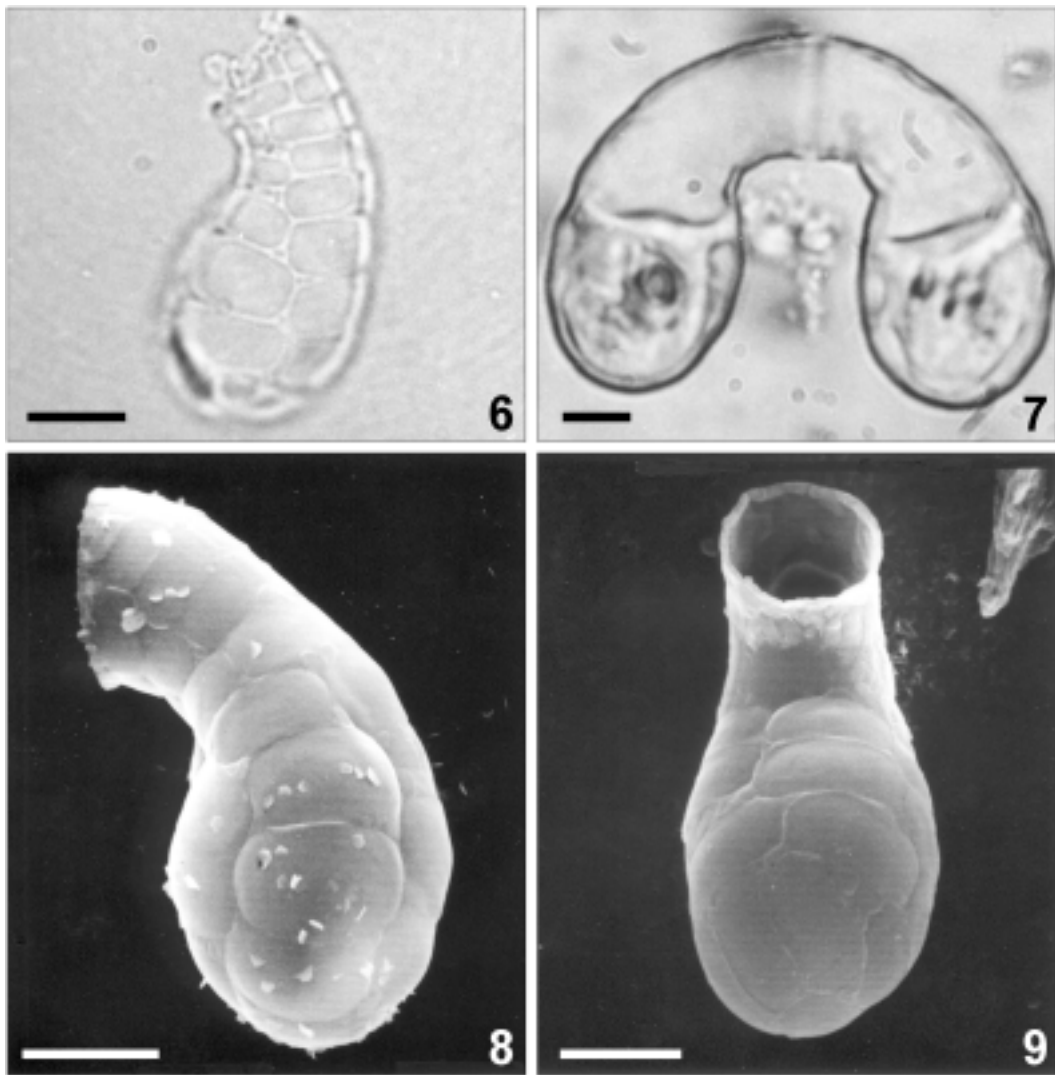
**Table 1.** Biometric characterization of the investigated testacean species.  $\bar{x}$  - arithmetic mean; M - median; SD - standard deviation; SE - standard error of the mean; CV - coefficient of variation in %; Min - minimum; Max - maximum; n - number of examined individuals (measurements in  $\mu\text{m}$ ).

Character	$\bar{x}$	M	SD	SE	CV	Min	Max	n
<i>Centropxyiella lucida</i>								
Length	65.9	66	2.2	0.3	3.3	62	72	51
Breadth	40.1	40	1.6	0.2	4.0	37	43	51
Height	25.6	26	1.3	0.2	4.9	23	28	51
Diameter of aperture	24.4	25	0.9	0.1	3.8	21	26	51
Diameter of collar	33.9	34	1.6	0.2	4.7	31	37	51
<i>Pomoriella valkanovi</i>								
Length	43.4	43	2.1	0.2	4.8	39	50	75
Diameter of shell	21.1	21	0.8	0.1	3.9	20	23	75
Diameter of aperture	12.1	12	0.3	0.1	2.8	11	13	75
<i>Rhumbleriella filosa</i>								
Length	42.9	43	1.0	0.2	2.3	41	45	22
Breadth	32.4	32	0.7	0.1	2.0	31	34	22
Height	30.8	31	0.8	0.2	2.6	30	33	22
Large axis of aperture	13.5	14	0.5	0.1	3.7	13	14	22
Small axis of aperture	6.3	6	0.4	0.1	7.2	6	7	22
<i>Messemvriella filosa</i>								
Length	49.6	50	2.1	0.3	4.3	45	52	43
Breadth	22.3	22	0.8	0.1	3.8	21	24	43
Height	20.7	21	0.9	0.1	4.3	19	23	43
Diameter of aperture	9.8	10	1.0	0.1	9.9	9	12	43
Diameter of collar	20.0	20	1.2	0.2	6.2	17	22	43
<i>Cyphoderia littoralis</i>								
Length	49.5	50	1.3	0.2	2.6	47	53	44
Diameter of shell	22.5	23	1.4	0.2	5.9	20	25	44
Diameter of aperture	8.5	8	0.5	0.1	6.3	8	10	44
<i>Pseudocorythion acutum</i>								
Total length	72.3	72	3.1	0.5	4.3	66	79	36
Diameter of shell	24.8	25	0.4	0.1	1.6	24	25	36
Diameter of collar	23.3	24	1.5	0.3	6.4	20	25	36
Length of spine	9.2	9	1.7	0.3	18.3	7	12	36
<i>Ogdeniella elegans</i>								
Length	53.9	54	2.4	0.5	4.5	48	58	23
Breadth	24.7	25	1.6	0.3	6.5	21	28	23
Height	15.5	16	0.5	0.1	3.3	15	16	23
Large axis of aperture	11.2	11	0.4	0.1	3.4	11	12	23
Small axis of aperture	6.8	7	0.6	0.1	9.5	6	7	23
Diameter of collar	24.5	24	2.3	0.5	9.3	22	30	23
<i>Ogdeniella maxima</i>								
Length	59.3	59	2.0	0.4	3.3	56	63	28
Breadth	43.7	44	1.7	0.3	3.8	40	46	28
Height	22.8	23	0.9	0.2	3.9	21	25	28
Length of neck	16.6	16	1.4	0.3	8.3	15	20	28
Diameter of collar	26.8	27	1.3	0.2	4.8	25	29	28

Golemansky (1970a) noted in the original description of the species that the shell plates are rectangular, square or polygonal, sometimes rounded at the corners. Later Golemansky and Ogden (1980) using SEM found out that the shell plates are not rectangular, square or polygonal but are usually rounded at the corners. Our

observations confirm the description of Golemansky and Ogden (1980).

**Biometry:** The studied population is quite constant in its characters - the coefficients of variation range from 2.8 for the diameter of aperture to 4.8 for the shell length (Table 1). The obtained values correspond to the original



**Figs 6-9.** *Pomoriella valkanovi*. **6** - lateral view; **7** - two specimens after asexual division of the animal; **8** - lateral view, showing different shape and size of the organic plates and abundant organic cement between the plates; **9** - ventral view, showing well visible organic rim around the aperture. Scale bars 10  $\mu\text{m}$ .

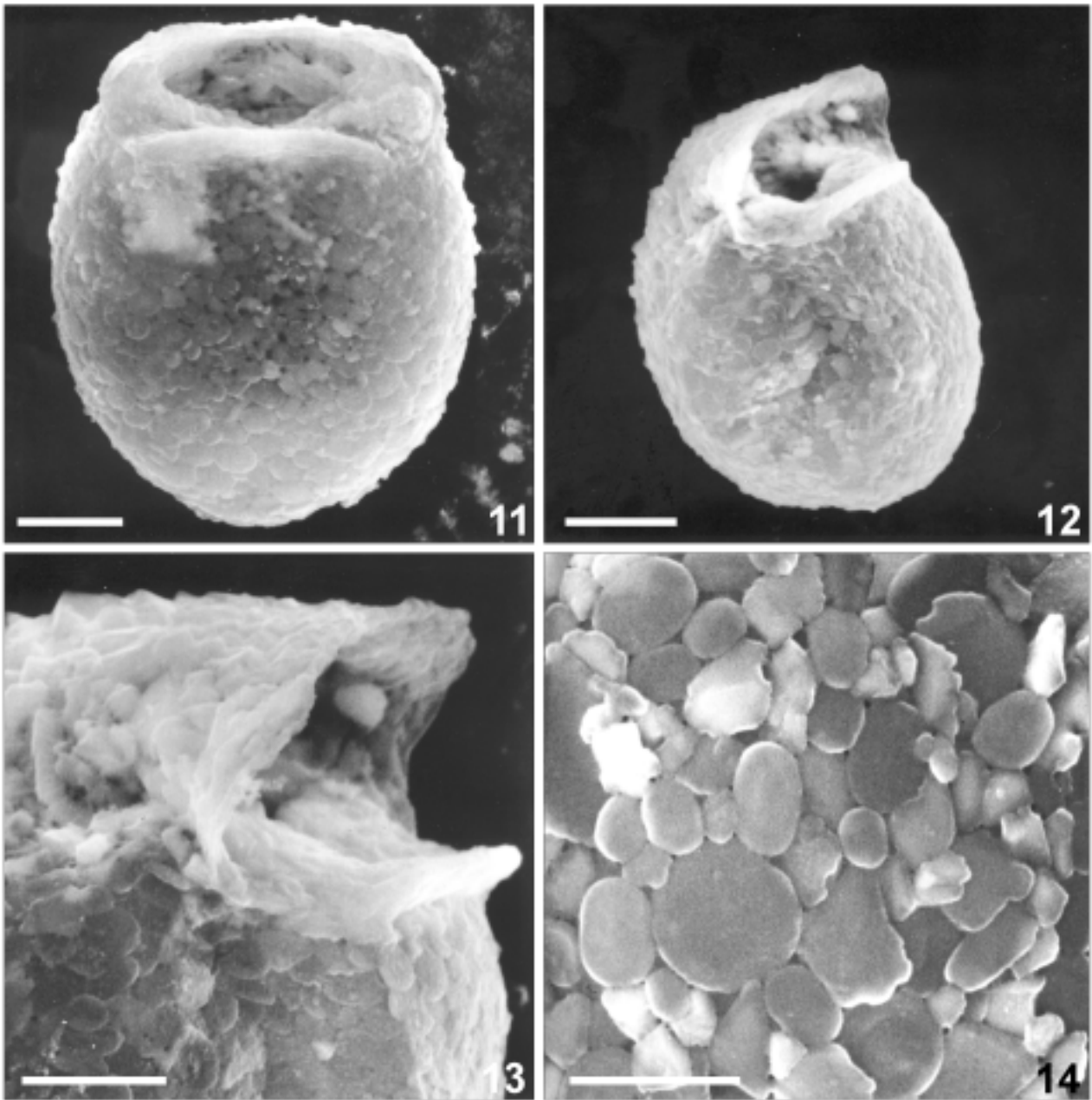
description (Golemansky 1970a) and those of Golemansky and Ogden (1980).

The analysis of the size frequency distribution indicates that *P. valkanovi* is a size-monomorphic species, characterized by a main-size class and a small size range (Fig.10). Figure 10a shows that 97% of all measured specimens have a shell length 40-47  $\mu\text{m}$ . Only 1.3% has shell length smaller than 40  $\mu\text{m}$  and 1.3% is bigger than 47  $\mu\text{m}$ . The frequency analysis of the shell diameter of *P. valkanovi* shows that in all specimens measured this character is very constant and ranges in close limits -

between 20 and 23  $\mu\text{m}$  (Fig. 10b). For identification at species level it can be used as an important taxonomic criteria.

**Material:** The studied specimens were isolated from samples collected at a sandy beach, 3-4 m above the high-water mark gathered at Nessebar, Black Sea Coast (Bulgaria), in March 2002.

**Geographical distribution:** Black Sea: Sunny Coast, Ropotamo, Pomorie, Nessebar /Bulgaria/ (Golemansky 1969, 1970a, 1974, 1980); Baltic Sea: Zelenogorsk, Komarovo, Repino /Russia/, Talin /Estonia/ (Golemansky



**Figs 11-14.** *Rhumbleriella filosa*. **11** - ventral view; **12** - view of  $\frac{3}{4}$ , with well visible aperture; **13** - lateral view in the region of the aperture, showing the flexible organic membrane bordered aperture; **14** - detail of the dorsal structure of the shell, composed of plates oval and elliptic idiosomes. Scale bars 10  $\mu\text{m}$  (11, 12); 5  $\mu\text{m}$  (13); 2  $\mu\text{m}$  (14).

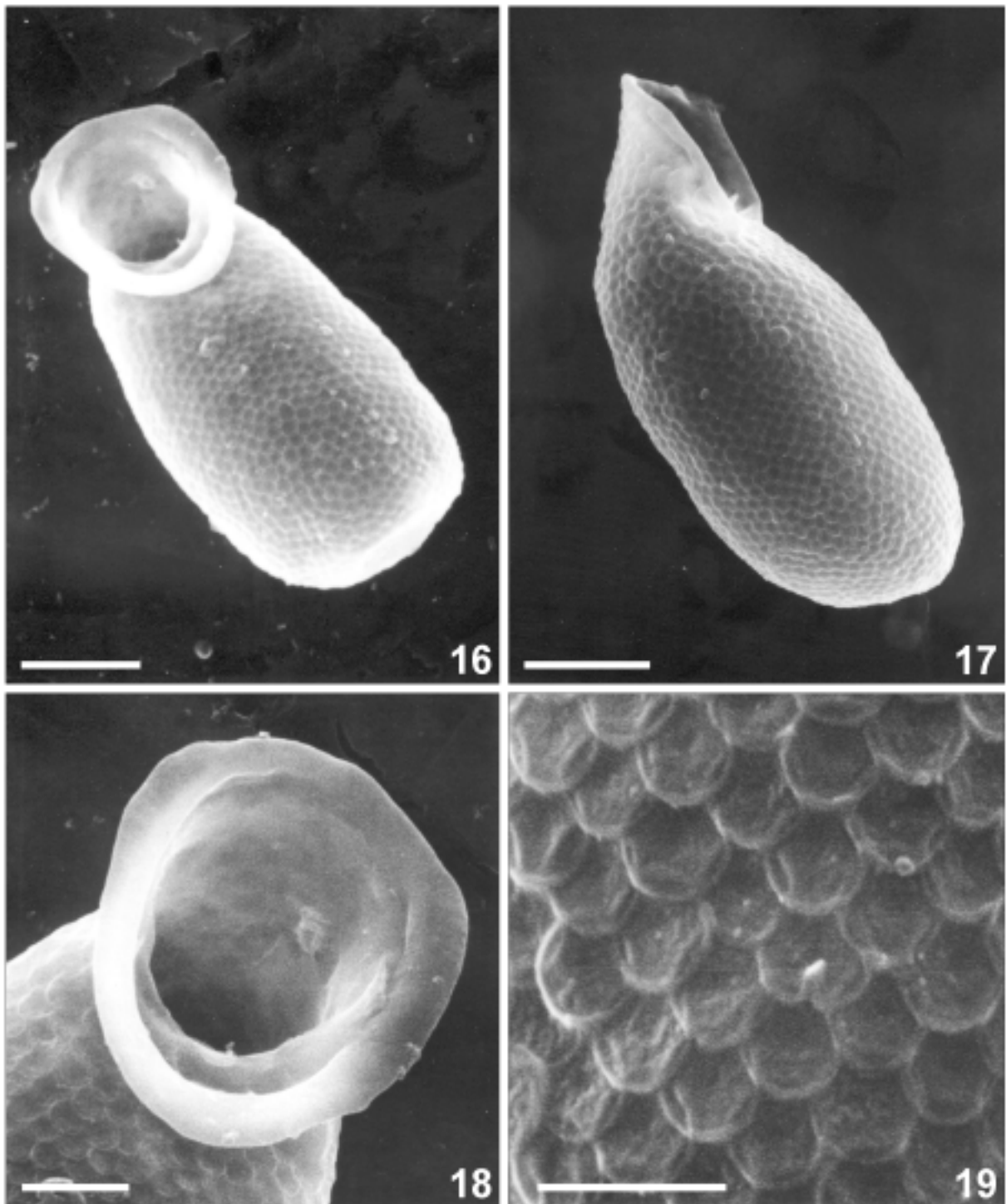
1980, 1983, 1998a); Atlantic: Roscoff /France/ (Golemansky 1992); Aegean Sea: Perea /Greece/ (Golemansky 1994b); Marmora Sea: Jalova /Turkey/ (Golemansky 1998c).

***Rhumbleriella filosa* Golemansky, 1970 (Figs 11-14)**

**Shell morphology:** The shell is colourless, translucent, a type of simple cryptostomy, ovoid in ventral and

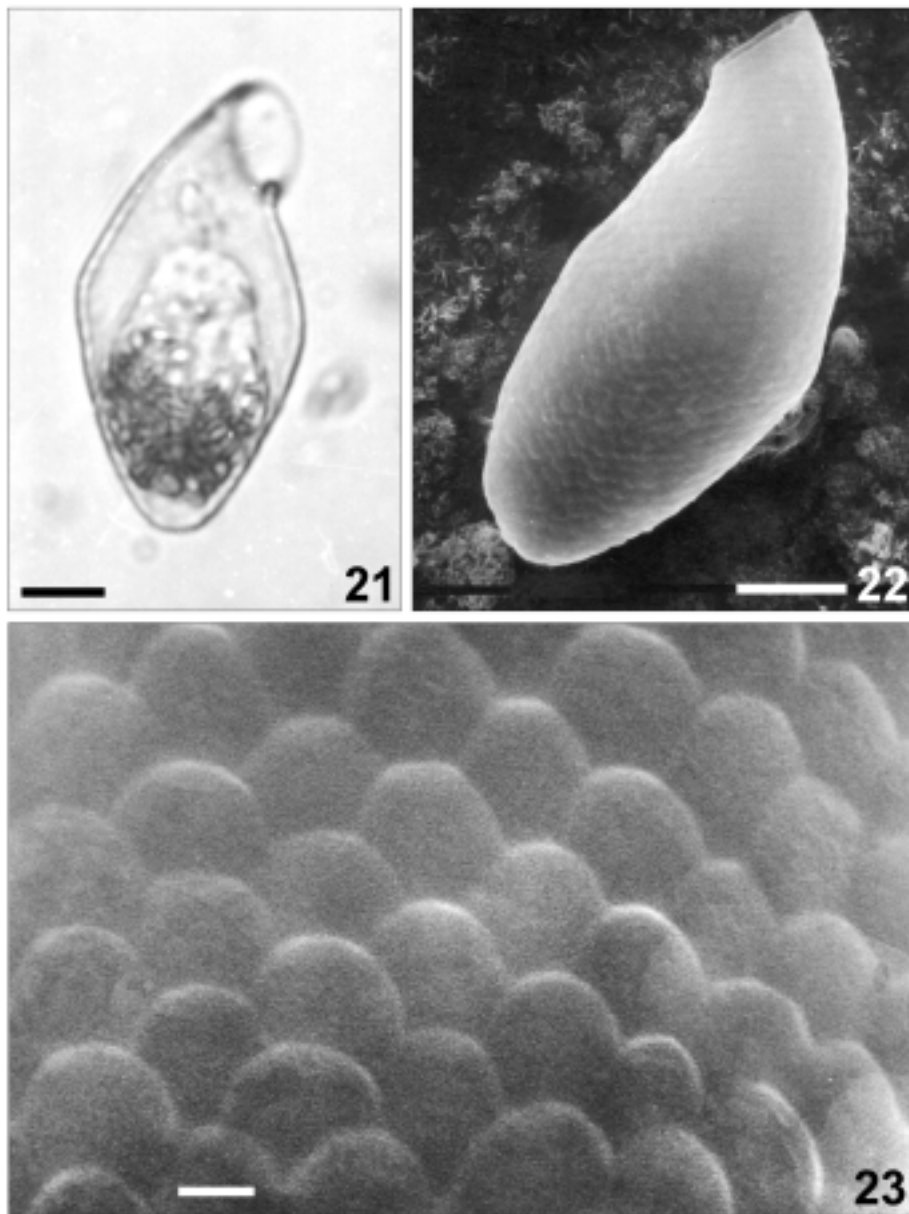
dorsal views. In lateral view it is hemispherical, with a bulging dorsal side and flattened ventral side, rounded at both ends, and with a maximum height near the middle of the length. The aperture is sub-terminal, invaginated, oblique, oval, easily visible, and surrounded by a flexible endogenous membrane (Figs 11-13).

Our study by SEM shows that the shell of *R. filosa* has a comparatively thin wall and is easily fragile. It is



**Figs 16-19.** *Messemvriella filosa*. **16** - ventral view; **17** - lateral view; **18** - apertural view, showing the recurved organic membrane surrounding the aperture; **19** - shell structure, showing the overlapping silicious plates (idiosomes) on the shell surface. Scale bars 10  $\mu\text{m}$  (16, 17); 5  $\mu\text{m}$  (18, 19).





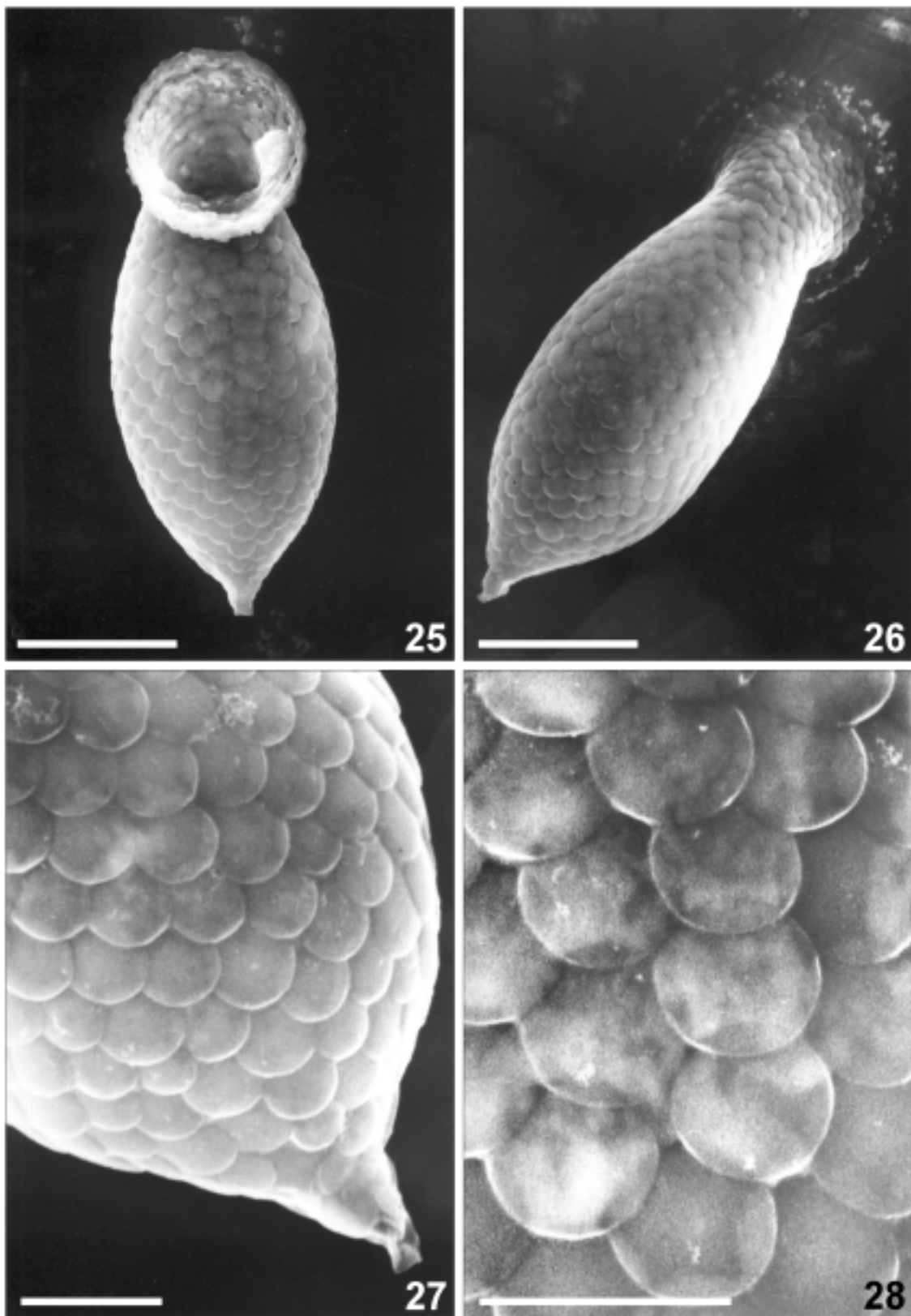
**Figs 21-23.** *Cyphoderia littoralis*. **21** - lateral view; **22** - lateral view; **23** - shell structure, showing the imbricated siliceous plates (idiosomes) on the shell surface. Scale bars 10  $\mu\text{m}$  (21, 22); 1  $\mu\text{m}$  (23).

composed mainly of a mixture of small to medium circular or oval, flattened, siliceous shell plates, which are mixed with isolated flattish pieces of quartz dorsally (Fig. 14). The shell surface is smooth, with well-defined outline (Figs 11, 12).

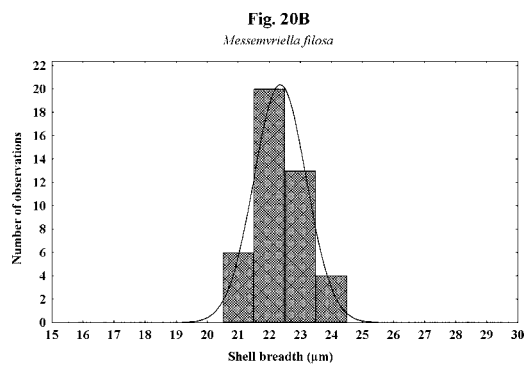
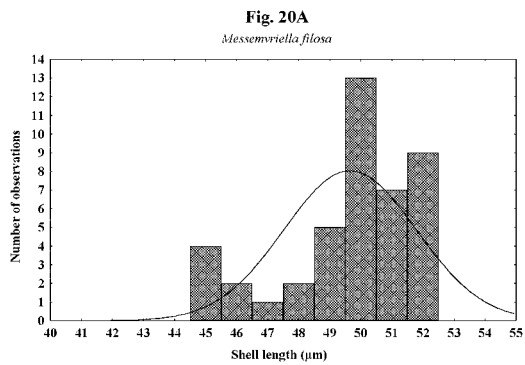
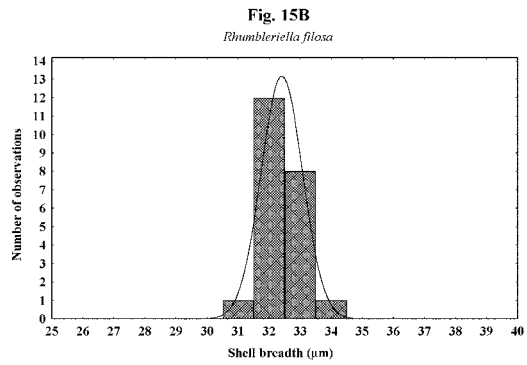
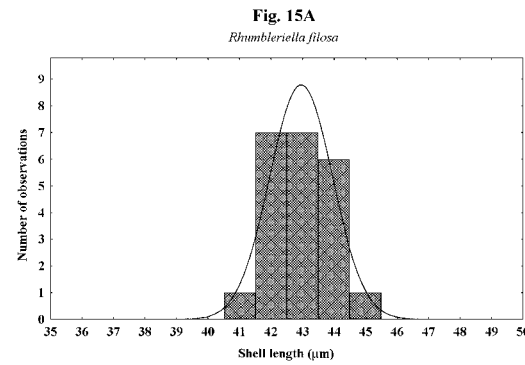
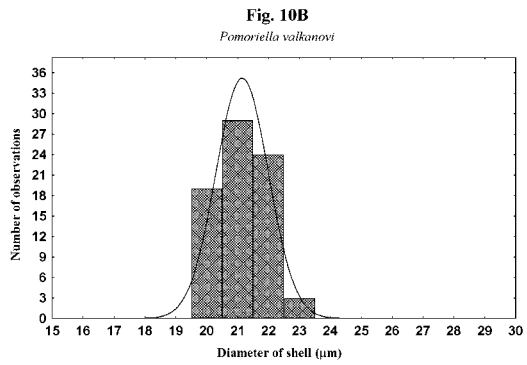
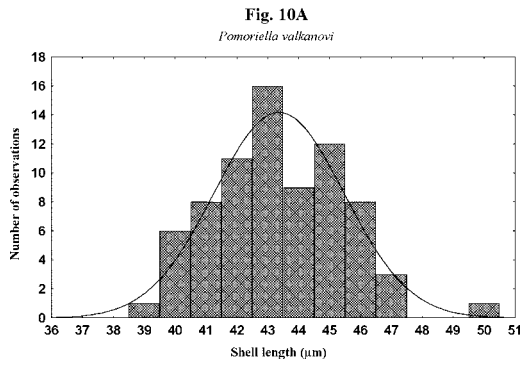
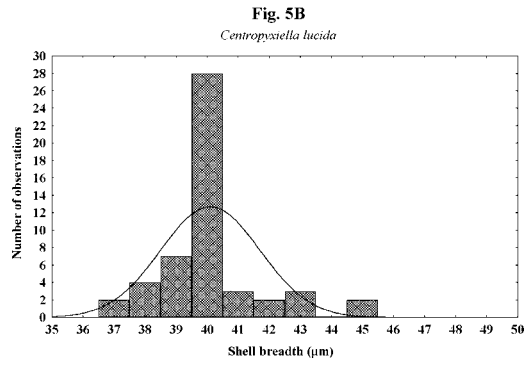
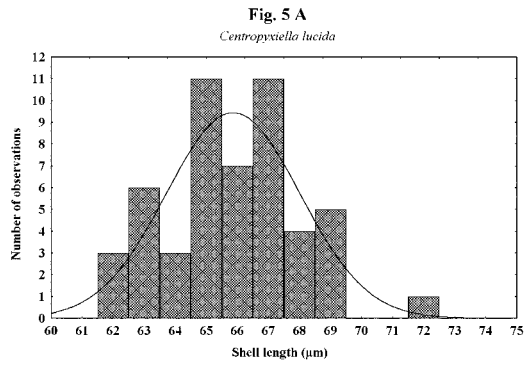
**Biometry:** The coefficients of variation of all characters are low (between 2.0 and 7.2) and show that the

shell measurements are rather constant (Table 1). As a whole our values correspond well to the original description (Golemansky 1970b), but the individuals of the North Sea are smaller than those of the Black Sea population.

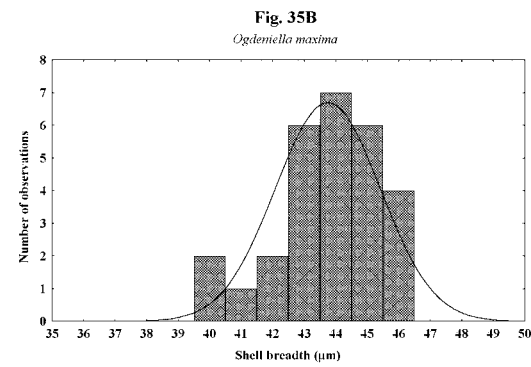
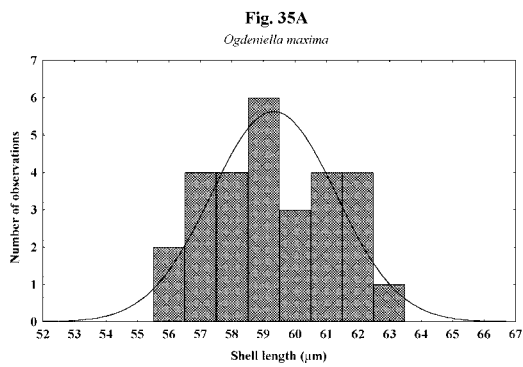
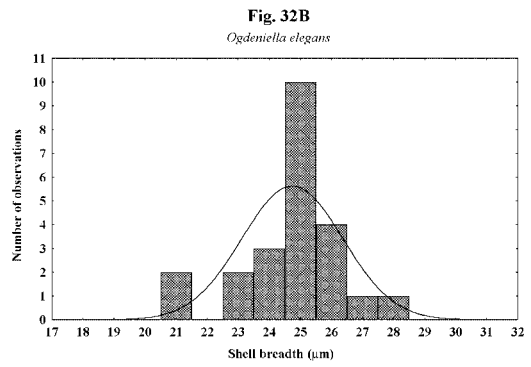
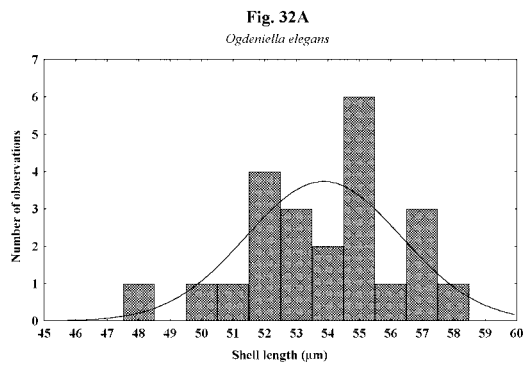
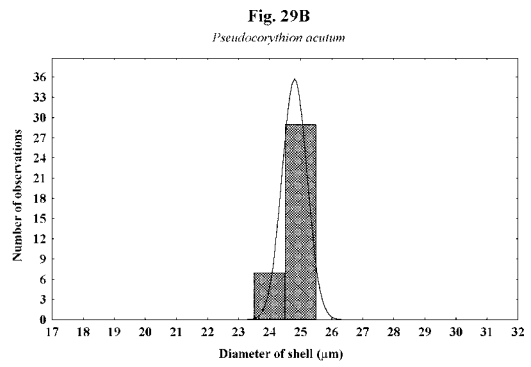
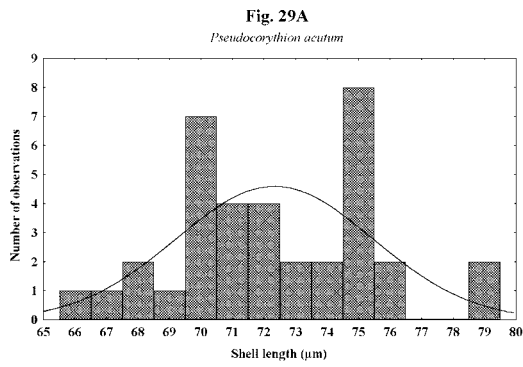
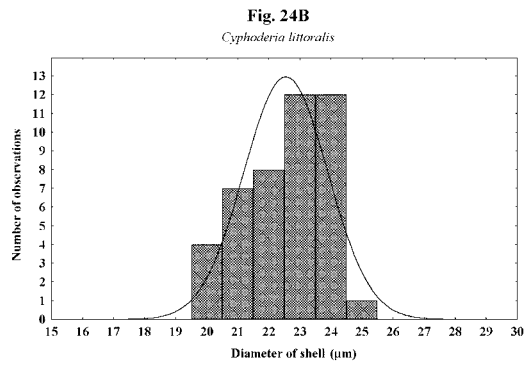
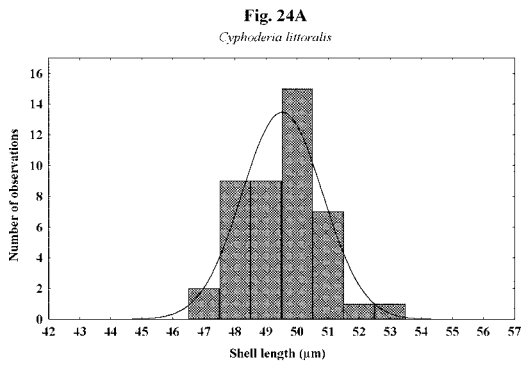
The analysis of the size frequency distribution indicates that *R. filosa* is a size-monomorphic species, characterized by a main-size class and a small size range



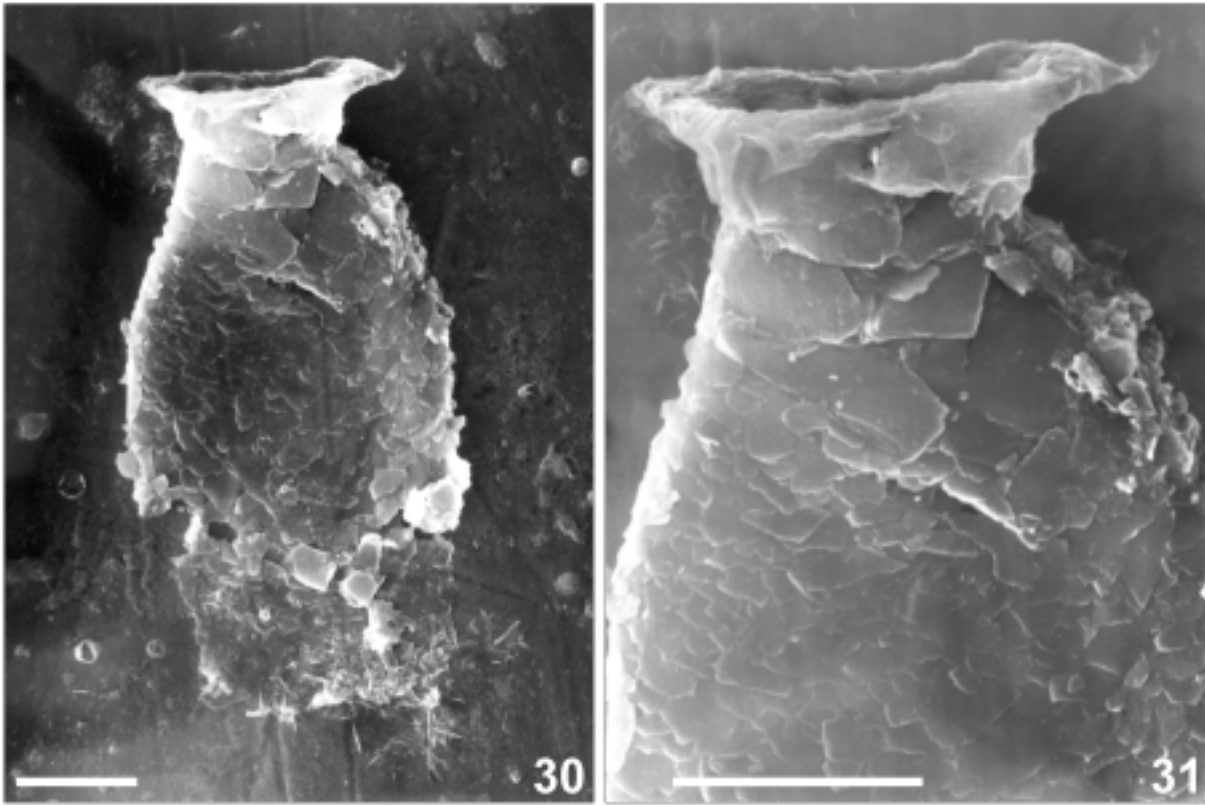
**Figs 25-28.** *Pseudocorythion acutum*. 25 - ventral view; 26 - dorsal view; 27 - view of posterior end of the shell, with small spine; 28 - detail of the shell structure, composed of round idiosomes. Scale bars 20  $\mu\text{m}$  (25, 26); 5  $\mu\text{m}$  (27, 28).



**Figs 5, 10, 15, 20.** Histograms showing the size frequency of the shell length (A) and the shell breadth (B) of: **5** - *Centropysiella lucida*; **10** - *Pomoriella valkanovi*; **15** - *Rhumbleriella filosa*; **20** - *Messemvriella filosa*.



**Figs 24, 29, 32, 35.** Histograms showing the size frequency of the shell length (A) and diameter of shell (B) of: **24** - *Cyphoderia littoralis*; **29** - *Pseudocorythion acutum*; **32** - *Ogdeniella elegans*; **35** - *Ogdeniella maxima*.



**Figs 30-31.** *Ogdeniella elegans*. **30** - broad lateral view of a specimen, with irregular posterior end; **31** - detail of the shell structure. Scale bars 10  $\mu\text{m}$ .

(Fig. 15). All specimens examined of our population have a shell length 41-45  $\mu\text{m}$  and a shell breadth 31-34  $\mu\text{m}$ .

**Material:** The specimens were taken from samples collected at a sandy beach, 4 m above the high-water mark gathered at Tjarno Biological station, Reserve Saltö, North Sea (Sweden), in August 2000.

**Geographical distribution:** Black Sea: Alepou, Galata /Bulgaria/ (Golemansky 1970b); North Sea: Oostende /Belgium/ (Chardez 1977) and Kattegat /Sweden/ (Golemansky 2002); Mediterranean: Var, Brusac /France/ (Decloître 1975); Atlantic: Gironde /France/ (Chardez and Thomas 1980) and Abidjan /Ivory Coast/ (Sudzuki 1983); Pacific: Taiwan (Sudzuki 1986).

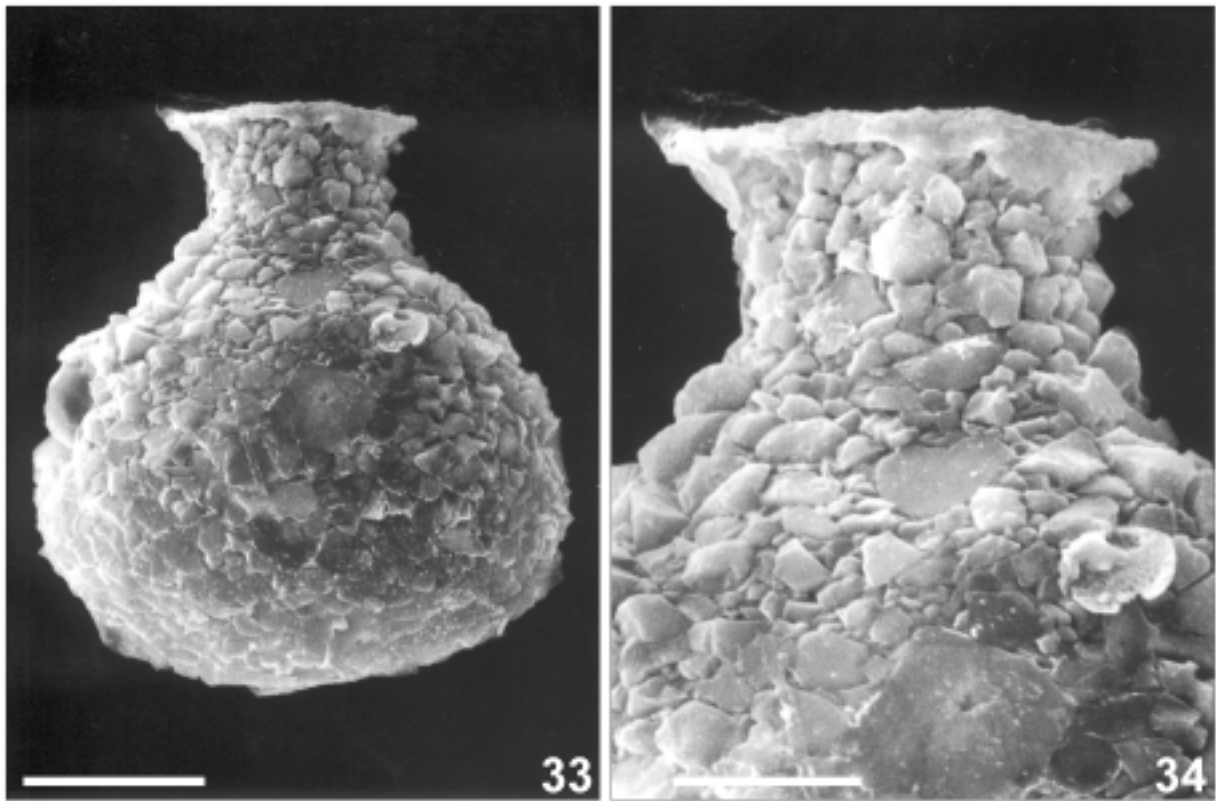
***Messemvriella filosa* Golemansky, 1973 (Figs 16-19)**

**Shell morphology:** The shell is colourless, transparent, ovoid-elongate in ventral view, and circular or slightly oval in cross-section. Anteriorly there is a large circular collar around the aperture, and aborally the shell is rounded (Figs 16-18). The collar has the same diameter as the maximum breadth of the shell. In lateral view, the shell is feebly bulging dorsally, with almost plane

ventral side (Fig. 17). The surface of the test is composed of numerous small, circular shell plates that are arranged to overlap each other and organic cement is not seen between the plates (Fig. 19). The diameter of the shell plates varied between 2 and 2.5  $\mu\text{m}$ . The collar is entirely organic, without shell plates and usually has a recurved rim, formed by a flexible organic membrane (Figs 16, 18). In the original description Golemansky (1973a) notes that the aperture is oblique, while most of the specimens examined in the present material have the aperture and the apertural collar parallel to the main axis of the test (Fig. 17).

**Biometry:** The coefficients of variation of all characters are less than 10% and show that shell measurements of the Black Sea population are moderately variable (Table 1). Our values correspond well to those of the original description (Golemansky 1973a) and those of the South-West Atlantic population (Golemansky 2000).

The analysis of the size frequency distribution indicates that *M. filosa* is a size-monomorphic species characterized by a small size range and by a compara-



**Figs 33-34.** *Ogdeniella maxima*. **33** - broad lateral view; **34** - detail of the shell structure. Scale bars 20  $\mu\text{m}$  (33); 10  $\mu\text{m}$  (34).

tively well-expressed main-size class (Fig. 20). The measurements of all specimens of our population are in close limits - the shell length ranges from 45 to 52  $\mu\text{m}$  and the shell breadth is in limits of 21-24  $\mu\text{m}$ .

**Material:** The specimens were isolated from samples collected at a sandy beach, gathered at Rio de Janeiro, "Copacabana", Atlantic (Brazil), in November 1999.

**Geographical distribution:** Black Sea: Nessebar and Sunny Coast /Bulgaria/ (Golemansky 1973a); Japan Sea: Sidžun /North Korea/ (Golemansky 1979b); Baltic Sea: Hanko /Finland/ (Golemansky 1998a); Mediterranean: Ischia /Italy/ (Golemansky 1998b); Atlantic: Copacabana /Brazil/ (Golemansky 2000); North Sea: Kattegat /Sweden/ (Golemansky 2002).

***Cyphoderia littoralis* Golemansky, 1973 (Figs 21-23)**

**Shell morphology:** The shell is colourless, transparent, retort-shaped, with a characteristic enlargement in the middle of the shell length, and tapering at both ends, circular or oval in cross-section. Anteriorly, there is a short curved neck, terminated by a circular or slightly oval, oblique aperture (Figs 21, 22). A small organic

collar surrounds the aperture rarely. The shell is composed of numerous circular, flattened, siliceous shell plates, which overlap each other and are arranged in diagonal rows. The organic cement is usually not seen between the plates (Fig. 23).

**Biometry:** The coefficients of variation of all characters are low (between 2.6 and 6.3) and show that the specimens of our population are rather constant in their characters (Table 1). Our values correspond well to the original description of the species (Golemansky 1973b).

The analysis of the size frequency distribution indicates that *C. littoralis* is a size-monomorphic species, characterized by a main-size class and a small size range. Figure 24 shows that about 90% of all specimens measured have a shell length of 48-51  $\mu\text{m}$  and a shell diameter of 21-24  $\mu\text{m}$ .

**Material:** The specimens were isolated from samples collected at a sandy beach, 4 m above the high-water mark gathered at Tjarno Biological station, Reserve Saltö, North Sea (Sweden), in August 2000.

**Geographical distribution:** Cosmopolitan. Observed by many authors in different localities from Baltic Sea,

Black Sea, Mediterranean, North Sea, China Sea, Aegean Sea, Atlantic, Pacific and Indian Ocean.

***Pseudocorythion acutum* (Wailes, 1927) Valkanov, 1970 (Figs 25-28)**

**Shell morphology:** The shell is colourless, transparent, ovoid-elongate, slightly flattened laterally, anteriorly there is a large circular collar surrounding the aperture and posteriorly there is a small pointed spine (Figs 25-27). The collar has usually the same diameter as the shell body and runs smoothly into the bodyline (Figs 25, 26). The shell is composed of numerous circular shell plates, which overlap each other and are arranged in diagonal rows (Figs 27, 28). The organic cement is usually not seen between the plates. The body plates are bigger, with diameter about 3  $\mu\text{m}$ , while the plates that cover the apertural collar and the posterior spine are smaller, between 1.0 and 1.5  $\mu\text{m}$  in diameter (Figs 25-27).

**Biometry:** Coefficients of variation of the characters measured (except the length of spine) are low (between 1.6 and 6.4) and show that shell measurements are relatively constant (Table 1). The length of spine is more variable (CV=18.3) and range between 7 and 12  $\mu\text{m}$ . The specimens of our population are bigger than those of the Wailes (1927) and those of the Antarctic population (Golemansky and Todorov 1999).

The analysis of the size frequency distribution indicates that *P. acutum* is a size-monomorphic species characterized by a comparatively small size range (Fig. 29). Figure 29a shows that our specimens are characterised by a not well-expressed main-size class of the shell length, but all of them are in limits of 66-79  $\mu\text{m}$ . On the other hand the specimens of our population are very constant by the shell breadth and all of them have shell breadth 24-25  $\mu\text{m}$  (Fig. 29b).

**Material:** The specimens were isolated from samples collected at a sandy beach, 4 m above the high-water mark gathered at Tjarno Biological station, Reserve Saltö, North Sea (Sweden), in August 2000.

**Geographical distribution:** Cosmopolitan. Observed by many authors in numerous localities from Pacific, Atlantic, Black Sea, North Sea, Mediterranean, Baltic Sea, Japan Sea, Aegean Sea, Marmara Sea and Antarctic Sea.

***Ogdeniella elegans* (Golemansky, 1970) Golemansky, 1982 (Figs 30, 31)**

**Shell morphology:** The shell is colourless, transparent, ovoid, with thin walls and exceptionally fragile. Anteriorly there is a short neck with a large circular

collar surrounding the aperture. In ventral view the posterior end is rounded, lanceolate or irregular (Figs 30, 31). The collar has the same diameter as the maximum breadth of the body. In lateral view the shell is flattened, about 1/2 to 3/5 of the shell breadth, lanceolate and tapering posteriorly.

So far, the shell structure of *O. elegans* has not being studied by scanning electron microscopy and light microscopy bases all available data on investigation. In the original description of the species (Golemansky 1970a) and later in the revision of this genus Golemansky (1982) noted that the shell is composed of the endogenous, polygonales and transparent plates, rarely mixed with the xenosomes, situated mainly on the neck and on the anterior part of the shell. Our study on the shell structure of *O. elegans* by scanning electron microscopy shows that the whole shell surface is covered with a mixture of small to medium flattish pieces of quartz, so arranged to overlap each other, and to give a relatively smooth and regular outline. The shell has a thin wall and is extremely fragile. It usually collapses when is air-dried in preparation for scanning electron microscopy.

**Biometry:** The coefficients of variation of all characters are less than 10% and show that shell measurements are moderately variable (Table 1). Our values correspond well to those of the original description (Golemansky 1970a) but our individuals are bigger compare to those from the Baltic Sea (Golemansky 1973b).

The analysis of the size frequency distribution indicates that *O. elegans* is a size-monomorphic species, characterized by a small size range and by a comparatively well-expressed main-size class (Fig. 32). Figure 32a shows that 83% of all specimens measured have a shell length 52-57  $\mu\text{m}$  and all of them are in limits of 48-58  $\mu\text{m}$ . Figure 32b shows that the shell breadth of the our specimens ranges from 21 to 28  $\mu\text{m}$ , and about 3/4 of them have a shell breadth 24-26  $\mu\text{m}$ .

**Material:** The specimens were isolated from samples collected at a sandy beach, 4 m above the high-water mark gathered at Tjarno Biological station, Reserve Saltö, North Sea (Sweden), in August 2000.

**Geographical distribution:** Cosmopolitan. Observed in more than 25 localities from Black Sea, Baltic Sea, Mediterranean, Aegean Sea, Marmara Sea, Atlantic and Pacific Ocean.

***Ogdeniella maxima* (Golemansky, 1970) Golemansky, 1982 (Figs 33, 34)**

**Shell morphology:** The shell is colourless, robust, ovoid or circular, with a distinct short neck, slightly

flattened laterally. Anteriorly there is a large circular collar surrounding the aperture, and posteriorly the shell is rounded. The diameter of the collar is about 2/3 of the maximum breadth of the test (Figs 33, 34).

The shell surface is composed mainly of small to medium pieces of quartz so arranged to give a relatively regular outline. It is noteworthy that the characteristic large "xenosomes" situated in aboral region of the shell, and mentioned by Golemansky (1970c, 1982) as a typical feature of *O. maxima*, are missing in all specimens of our population. Investigation of the shell structure by SEM shows that the whole shell surface is covered by mixture of small and medium pieces of quartz. Chitinous parts (free of "xenosomes") are missing on the shell surface, in comparison to those indicated by Golemansky (1970c) in the original description and drawings of the species, based on light microscopy.

**Biometry:** The coefficients of variation of the measured characters (except length of neck) are low (from 3.3 to 4.8) and show that shell measurements are rather constant (Table 1). Our values are smaller than those of the original description of species (Golemansky 1970c) but correspond well to those of the Atlantic population (Golemansky 1992).

The analysis of the size frequency distribution indicates that *O. maxima* is a size-monomorphic species, characterized by a well-expressed main-size class and by a small size range. Figures 35 a, b show that in all measured specimens the shell length and the shell breadth range in close limits - 56-63 µm and 40-46 µm, respectively.

**Material:** The specimens were isolated from samples collected at a sandy beach, 4 m above the high-water mark gathered at Tjarno Biological station, Reserve Saltö, North Sea (Sweden), in August 2000.

**Geographical distribution:** Black Sea: Pomorie /Bulgaria/ (Golemansky 1970c); North Sea: Douve /England/ (Chardez 1986) and Kattegat /Sweden/ (Golemansky 2002); Pacific: Tokyo /Japan/ (Sudzuki 1977); Atlantic: Roscoff /France/ (Golemansky 1992); Marmara Sea: Jalova /Turkey/ (Golemansky 1998c).

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## REFERENCES

- Anderson O. R., Rogerson A., Hannah F. (1996) A description of the testate amoeba *Ovulina parva* gen. n. sp. n. from coastal marine sediments. *J. Biol. Ass. U. K.* **76**: 851-865
- Chardez D. (1977) Thécamoebiens du Mésopsammon des plages de la Mer du Nord. *Rev. verv. Hist. Nat.* **416**: 18-34
- Chardez D. (1986) Thécamoebiens des plages de la Mer du Nord. *Acta Protozool.* **25**: 375-378
- Chardez D., Thomas R. (1980) Thécamoebiens du Mésopsammon des plages de Lacanau et Leporge - Ocean (Gironde - France). *Acta Protozool.* **19**: 277-285
- Decloître L. (1975) Thécamoebiens observés dans la zone littorale et supralittorale au Brusac à Six-Fours-les-Plages. *Ann. Soc. Sci. Nat. & Archéol. de Toulon et du Var* 171-180
- Golemansky V. (1969) Sur une biocénose thécamoebienne peu connue des eaux souterraines littorales des mers. *Progress in Protozoology, III. Leningrad, Nauka*, 194
- Golemansky V. (1970a) Rhizopodes nouveaux du psammon littoral de la Mer Noire (Note préliminaire). *Protistologica* **6**: 365-371
- Golemansky V. (1970b) *Chardezia caudata* gen. n. sp. n. et *Rhumbleriella filosa* gen. n. sp. n. - deux thécamoebiens nouveaux du psammon littoral de la Mer Noire (Rhizopoda: Testacea). *Bull. Inst. Zool. & Musée de Sofia* **32**: 121-125
- Golemansky V. (1970c) Thécamoebiens (Rhizopoda, Testacea) nouveaux des eaux souterraines littorales de la Mer Noire. *Acta Protozool.* **8**: 41-46
- Golemansky V. (1971) Taxonomische und zoogeographische Notizen über die thekamöbe Fauna (Rhizopoda, Testacea) der Küstengrundgewässer der sowjetischen Fernostküste (Japanisches Meer) und der Westküste Kanadas (Stiller Ozean). *Arch. Protistenk.* **113**: 235-249
- Golemansky V. (1973a) *Messemvriella filosa* n. gen. n. sp. - une nouvelle thécamoebienne psammobionte (Rhizopoda: Testacea) des eaux souterraines littorales de la Mer Noire. *Zool. Anz., Leipzig* **190**: 302-304
- Golemansky V. (1973b) Deuxième contribution à la connaissance des thécamoebiens (Rhizopoda, Testacea) du psammal littoral de la Mer Baltique. *Bull. Inst. Zool. & Musée de Sofia* **38**: 49-60
- Golemansky V. (1974) Sur la composition et la distribution horizontale de l'association thécamoebienne (Rhizopoda, Testacea) des eaux souterraines littorales de la Mer Noire en Bulgarie. *Bull. Inst. Zool. & Musée de Sofia* **40**: 195-202
- Golemansky V. (1979a) *Cyphoderia compressa* n. sp. (Rhizopoda: Arcellinida) - un nouveaux thécamoebien psammobionte du supralittoral des mers. *Acta Protozool.* **18**: 429-434
- Golemansky V. (1979b) Thécamoebiens psammobionts du supralittoral coréen de la Mer Japonaise et description de deux nouvelles espèces - *Rhumbleriella coreana* n. sp. et *Amphorellopsis conica* n. sp. *Acta zool. Bulg.* **12**: 5-11
- Golemansky V. (1980) La faune thécamoebienne interstitielle du psammal supralittoral des mers. Sofia, DSc Thesis. (in Bulgarian)
- Golemansky V. (1982) Révision du genre *Ogdeniella* nom. n. (= *Amphorellopsis* Golemansky 1970) (Rhizopoda, Gromida) avec considérations sur son origine et évolution dans le milieu interstitiel. *Acta zool. Bulg.* **19**: 3-12
- Golemansky V. (1983) Interstitial thecamoebas (Rhizopoda, Arcellinida and Gromida) of the Soviet Finn Bay coast. *Acta zool. Bulg.* **23**: 3-8 (in Bulgarian)
- Golemansky V. (1992) Thécamoebiens interstitiels (Rhizopoda: Arcellinida, Gromida et Monothalamida) du supralittoral français de l'Atlantique dans la région du Roscoff (Bai de Morlaix). *Acta zool. Bulg.* **45**: 3-14
- Golemansky V. (1994a) On some ecological preferences of marine interstitial testate amoebas. *Arch. Protistenk.* **144**: 424-432



- Golemansky V. (1994b) Revue sur les thécamoebiens interstitiels (Protozoa: Testacea) du littoral grec de la Mer Egeé. *Biologia Gallo-Hellenica* **22**: 269-278
- Golemansky V. (1998a) Interstitial testate amoebae (Rhizopoda: Arcellinida and Gromiida) from the Finish coast of the Baltic Sea and summary checklist of the interstitial testate amoebae in the Baltic Sea. *Acta Protozool.* **37**: 133-137
- Golemansky V. (1998b) Interstitial testate amoebae (Rhizopoda: Testacea) from the Italian coast of the Mediterranean Sea. *Acta Protozool.* **37**: 139-143
- Golemansky V. (1998c) Interstitial testate amoebae (Rhizopoda: Testacea) from the sand supralittoral of the Eastern Marmorean coast (Turkey). *Acta zool. Bulg.* **50**: 3-7
- Golemansky V. (2000) Marine interstitial rhizopods (Rhizopoda: Arcellinida, Gromida and Foraminiferida) from the South-West Atlantic (Region of Rio de Janeiro) and description of *Psammolagynis atlantica* gen. n., sp. n. *Acta zool. Bulg.* **52**: 3-12
- Golemansky V. (2002) Interstitial and benthic marine rhizopods (Protozoa: Rhizopoda) from the strait of Kattegat (Swedish coast). *Acta zool. Bulg.* **54**: 7-14
- Golemansky V., Coûteaux M. -M. (1982) Etude en microscopie électronique a balayage de huit espèces de thécamoebiens interstitiels du supralittoral marin. *Protistologica* **18**: 473-480
- Golemansky V., Ogden C. (1980) Shell structure of three littoral species of testate amoebae from the Black Sea (Rhizopodea: Protozoa). *Bull. Br. Mus. Nat. Hist. (Zool.)* **38**: 1-6
- Golemansky V., Todorov M. (1996) Interstitial Rhizopods (Rhizopoda: Testacea & Foraminiferida) from the Antarctic Region of Chile and Valparaiso in the Pacific. In: Bulgarian Antarctic Research. Life Sciences, (Eds. V. Golemansky, N. Chipev). Sofia, *Pensoft Publ.* 62-69
- Golemansky V., Todorov M. (1999) First report of the interstitial testate amoebae (Protozoa: Testacea) in the marine supralittoral of the Livingston Island (Antarctic). In: Bulgarian Antarctic Research. Life Sciences. II, (Eds. V. Golemansky, N. Chipev). Sofia, *Pensoft Publ.* 43-47
- Ogden C. G., Coûteaux M. -M. (1986) The nature of the shell of *Alepiella tricornuta*, a marine testate amoeba (Sarcodina: Rhizopoda). *Protistologica* **22**: 213-220
- Ogden C. G., Coûteaux M. -M. (1989) Interstitial marine rhizopods (Protozoa) from littoral sands of the east coast of England. *Europ. J. Protistol.* **24**: 281-290
- Schönborn W., Foissner W., Meisterfeld R. (1983) Licht- und rastelelektronenmikroskopische Untersuchungen zur Schalenmorphologie und Rassenbildung bodenbewohnender Testaceen (Protozoa: Rhizopoda) sowie Vorschläge zur biometrischen Charakterisierung von Testaceen-Schalen. *Protistologica* **19**: 553-566
- Sudzuki M. (1977) Protozoans in the marine beach interstices. II. Taxonomy and ecology of testacea from a sandy beach island recently constructed. *Jap. J. Protozool.* **8**: 23
- Sudzuki M. (1979) Psammobiont Rhizopoda and Actinopoda from marine beaches of Japan. *Acta Protozool.* **18**: 293-304
- Sudzuki M. (1983) Protozoans in the marine beach interstices. IX. Psammobiont testacea from Abidjan, Côte d'Ivoire, West Africa. *Jap. J. Protozool.* **16**: 8
- Sudzuki M. (1986) Protozoans in the marine beach interstices. XII. Psammobiont testacea from Taiwan (Formosa). *Jap. J. Protozool.* **19**: 32-33
- Valkanov A. (1970) Beitrag zur Kenntnis der Protozoen des Schwarzen Meeres. *Zool. Anz.* **184**: 241-290
- Wailes G. H. (1927) Rhizopoda and Heliozoa from British Columbia. *Ann. & Mag. Nat. Hist.* **20 (Ser. 9)**: 153-156

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## A New Species of *Ellobiophrya* Chatton *et* Lwoff, 1923 (Ciliophora: Peritrichia) Attached to *Mantoscypthidia* Jankowski, 1980 (Ciliophora: Peritrichia) Species

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**Summary.** Surveys carried out along the coast of South Africa revealed the presence of a secondary symbiont of the genus *Ellobiophrya* Chatton *et* Lwoff, 1923 found attached to the narrow basal part adoral to the scopula of *Mantoscypthidia spadiceae* Botes, Basson *et* Van As, 2001 and *M. midae* Botes, Basson *et* Van As, 2001 occurring on the gills of *Haliotis spadicea* Donovan, 1808 and *H. midae* Linnaeus, 1758, respectively. *Mantoscypthidia branchi* Van As, Basson *et* Van As, 1998 found on the gills of *Cymbula* H. *et* A. Adams, 1854 and *Scutellastra* H. *et* A. Adams, 1854 species respectively, had the same ellobiophryid species attached to the narrow part adoral to the scopula. This ellobiophryid differs from all the known *Ellobiophrya* species with respect to morphology of the body, features of the nuclear apparatus, and host preference and is therefore described as a new species, *Ellobiophrya maliculiformis* sp. n.

**Key words:** *Ellobiophrya maliculiformis* sp. n., *Mantoscypthidia*, marine mollusc, scyphidiid peritrich, secondary symbiont.

### INTRODUCTION

Representatives of the family Ellobiophryidae Chatton *et* Lwoff, 1929 attach to the host by means of a scopula that has been adapted to form a ring-like cinctum or caudal process (Clamp 1982). Only parts of the scopula are included in the bouton of the cinctum, as the remainder of the scopula (the principal part) is found in the usual location and secretes the embryonic stalk linking the two daughters that result from binary fission.

Currently the family comprises two genera, i.e. *Ellobiophrya* Chatton *et* Lwoff, 1923 and *Caliperia* Laird, 1953. All of the known species of the genus *Ellobiophrya* were found associated with fish, bivalves or bryozoan hosts from marine habitats. *Ellobiophrya donacis* Chatton *et* Lwoff, 1923 was described from the gill filaments of the bivalve *Donax vittatus* (Chatton and Lwoff 1923, 1928, 1929). Nearly sixty years later Clamp (1982) described *E. conviva* from the tentacles of the ectoprocts *Bugula neritina* and *B. turrita*. Another species, *E. oblida* (Naidenova *et* Zaika, 1969) occurs on the marine fish *Proterorhinus marmoratus*. It was originally described as *Clausophrya oblida* by Naidenova and Zaika (1969), but was placed within the genus *Ellobiophrya* by Clamp (1982).

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*Caliperia longipes* Laird, 1953 and *C. brevipes* Laird, 1959 were both described from the gill filaments of marine fishes (Laird 1953, 1959). This genus is characterised by a non-contractile skeletal rod within the arms of the cinctum and by not having the cinctal arms bonded to one another at the tips. Clamp and Bradbury's (1997) observations, however, revealed that the cinctal arms of *C. brevipes* are linked by a bouton and that the cytoskeletal structure within them has the fine structure of a myoneme. These characteristics place *C. brevipes* in the genus *Ellobiophrya*. This species was renamed as *E. brevipes* (Laird, 1959) with *C. longipes* the sole remaining species in the genus (Clamp and Bradbury 1997). According to Clamp, the genus *Caliperia* may not exist at all, and if *C. longipes* could be recollected someday, it may also turn out to be an *Ellobiophrya* (Clamp, personal comm.)<sup>1</sup>.

The ellobiophryid found in this study belongs to the genus *Ellobiophrya*, based on the morphology of the cinctum and the presence of a bouton. The same *Ellobiophrya* species was attached around the body of various scyphidiid peritrich hosts adoral to the scopula. The hosts were populations of *Mantoscypthidia spadiceae* Botes, Basson *et al.* 2001, *M. midae* Botes, Basson *et al.* 2001 and *M. branchi* Van As, Basson *et al.* 1998, which occur on the gills of *Haliotis spadicea* Donovan, 1808, *H. midae* Linnaeus, 1758 and different limpet species, respectively (Van As *et al.* 1998, Botes *et al.* 2001). This ellobiophryid differs from the known species with respect to morphological features of the body, characteristics of the nuclear apparatus, and host preference and is described as a new species.

## MATERIALS AND METHODS

South African haliotids, i.e. *Haliotis spadicea* (Venus Ears) and *H. midae* (Perlemoen) were collected from infratidal pools on the rocky shores along the south coast of South Africa. The haliotids hosted two scyphidiid peritrich species, *Mantoscypthidia spadiceae* and *M. midae*. *Mantoscypthidia branchi* was found on the gills of all the limpet species collected from the rocky shore along the south, west and east coast of South Africa. Gills were dissected, placed on a microscope slide, smeared, and examined using a compound microscope. Live specimens of ellobiophryids were observed and photomicrographs were taken of ellobiophryids found associated with *Mantoscypthidia spadiceae* and *M. midae* for the purpose of measur-

ing body dimensions. The species is described from the type population, found attached to the host *Mantoscypthidia spadicea*. Additional data and measurements from the other host populations, namely *M. midae* and *M. branchi*, are given in Table 1.

Additionally, wet smears were fixed in Bouin's fluid, transferred to 70% ethanol and stained with Heidenhain's Iron, Mayer's and Harris' Hematoxylin for studying the nuclear apparatus and for measuring body dimensions. In order to study details of the infundibulum, Bouin's-fixed smears were stained with protargol, initially using a combined method as described by Lee *et al.* (1985) and Lom and Dykova (1992). This method proved rather unsuccessful, as the ellobiophryids had many symbiotic algae and inclusions, which obscures the position of the infraciliature. Clamp's "quick method" (Clamp, personal communication) which is an adaptation of the method of Wicklow and Hill (1992), gave the best results. A brief summary of the method is: Bouin's-fixed smears were transferred to 70% ethanol, then 50%, 30% and distilled water: followed by bleaching in 0.5% potassium permanganate for 5 min and washed in distilled water; transferred to 5% oxalic acid for 5 min and washed for 10 min; some slides were placed in 1% protargol solution for 10-15 min at 67-70°C, with copper sheets and others for a period of 12-24 h at room temperature; transferred to 1% hydroquinone (in 5% sodium sulfite) for 7-8 min, washed briefly; transferred to 0.5% gold chloride for 15 s, washed briefly; transferred to 2% oxalic acid for up to 3 min; remove and washed for 5 min; transferred to 5% sodium thiosulfate for 5 min and washed in distilled water for 5 min; slides were dehydrated in 30, 50, 70, 95, 100% ethanol; transferred to xylene and mounted using Canada Balsam.

For scanning electron microscopy (SEM), gills were fixed in 4% and 10% buffered neutral formalin. In some cases, gills were fixed in Parniczak and 2.5% glutaraldehyde. In the laboratory in Bloemfontein, the specimens were cleaned by washing gills in tapwater, dehydrated in a series of ethanol concentrations and critical-point dried. Gills bearing ellobiophryids attached to mantoscypthidians were mounted on stubs, sputter-coated with gold and studied at 5kV and 10kV, using a JOEL WINSEM JSM 6400 scanning electron microscope.

For measurements of live specimens, minimum and maximum values are given, followed in parentheses by the arithmetic mean, standard deviation and number of specimens measured. Measurements based on Bouin's-fixed specimens stained with hematoxylin are presented in square brackets. Body length is measured from the epistomial disc to the cinctum and body diameter at the widest part of the body. Description of pellicular striations was done from specimens viewed by SEM. The type material is in the collection of the National Museum, Bloemfontein, South Africa.

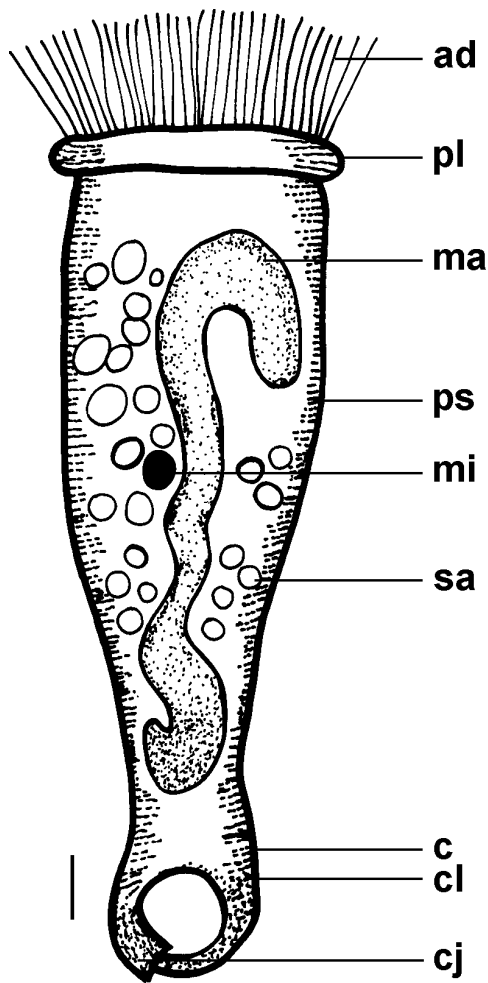
## RESULTS AND DISCUSSION

### *Ellobiophrya maliculiformis* sp. n. (Figs 1-13)

**Type host and locality:** *Mantoscypthidia spadiceae* Botes, Basson *et al.* 2001, attaches to narrow basal part adoral to the scopula; De Hoop Nature reserve, south coast (34°28'S, 20°30'E) of South Africa.

**Other hosts and localities:** *M. midae* Botes, Basson *et al.* 2001 and *M. branchi* Van As, Basson *et al.*

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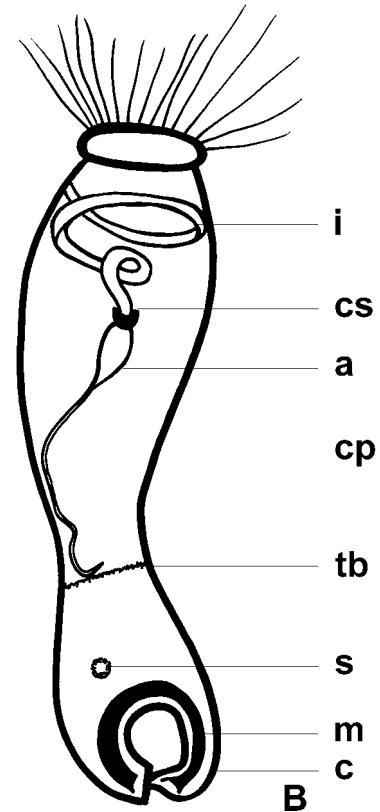
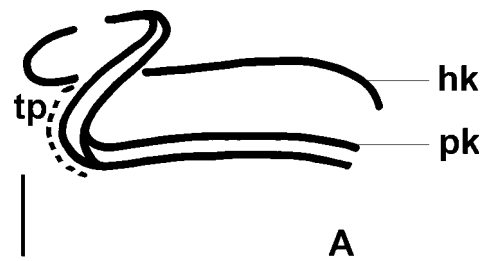


**Fig. 1.** Microscope projection drawing of *Ellobiophrya maliculiformis* sp. n. found as a secondary symbiont on *Mantoscaphidia spadiceae* Botes, Basson *et al.* Van As, 2001; *M. midae* Botes, Basson *et al.* Van As, 2001 and *M. branchi* Van As, Basson *et al.* Van As, 1998 occurring on the gills of *Haliotis spadicea* Donovan, 1808; *H. midae* Linnaeus, 1758 and *Scutellastra barbara* (Linnaeus, 1758) collected along the south coast of South Africa. ad - adoral ciliary spiral, c - cinctum, cj - cinctal junction, cl - cinctal limb, ma - macronucleus, mi - micronucleus, pl - peristomial lip, ps - pellicle striations, sa - symbiotic algae. Scale bar 10  $\mu$ m.

As, 1998, De Hoop Nature reserve, south coast (34°28'S, 20°30'E) and Papendorp, Olifants River mouth, west coast of South Africa (31°40'S, 18°15'E).

**Type specimens:** Holotype, slide 98/04/11-04 (NMBP 282), Paratype slides 98/04/04-05 (NMBP 283), 97/04/05-04c (NMBP 284), in the collection of the National Museum, Bloemfontein, South Africa.

**Etymology:** Named after the mode of attachment adoral to scopula of the hosts, which resembles handcuffs.



**Fig. 2.** Diagrams illustrating the infraciliature of *Ellobiophrya maliculiformis* sp. n. found as a secondary symbiont on *Mantoscaphidia spadiceae* Botes, Basson *et al.* Van As, 2001, *M. midae* Botes, Basson *et al.* Van As, 2001 and *M. branchi* Van As, Basson *et al.* Van As, 1998 occurring on the gills of *Haliotis spadicea* Donovan, 1808, *H. midae* Linnaeus, 1758 and *Scutellastra barbara* (Linnaeus, 1758) collected along the south coast of South Africa. A - haplo- and polykinetids. B - infundibulum. a - ampulla, c - cinctum, cp - cytopharynx, cs - cystostomal sphincter, hk - haplokinety, i - infundibulum; m - myoneme, pk - polykinety, s - scopula, tb - telotroch band, tp - third polykinetid. Scale bars 10  $\mu$ m.

**Description**

Trophont conical, elongate, tapering aborally towards scopular region (Figs 1, 3, 4, 9-12). Length of body 50-125  $\mu$ m (78.5  $\pm$  15.1, 40) [60-98  $\mu$ m (70.2  $\pm$  17.5, 43)],

**Table 1.** Body measurements ( $\mu\text{m}$ ) of live observations (A) and hematoxylin-stained specimens (B-F) of *Ellobiophrya maliculiformis* sp. n. from *Mantoscyphidia midae* Botes, Basson *et Van As*, 2001 and *M. branchi* Van As, Basson *et Van As*, 1998 occurring on the gills of haliotid (A,B) and different limpet (C-F) species from the south coast of South Africa.

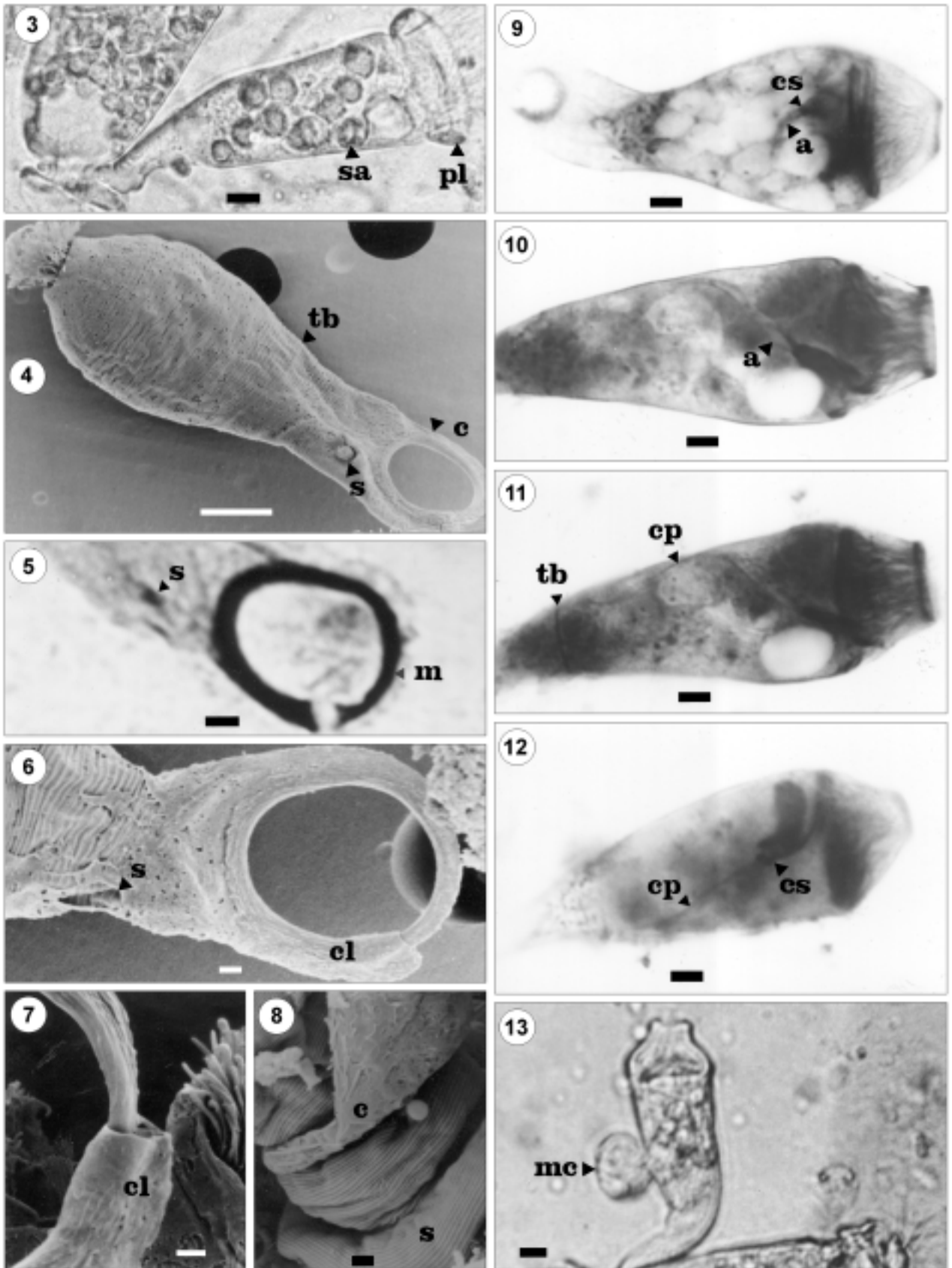
Mollusc host	A ( <i>Haliotis midae</i> )	B ( <i>H. midae</i> )	C ( <i>Scutellastra barbara</i> )	D ( <i>S. argenvilli</i> )	E ( <i>S. cochlear</i> )	F ( <i>Cymbula compressa</i> )
Ciliate host	<i>M. midae</i>	<i>M. midae</i>	<i>M. branchi</i>	<i>M. branchi</i>	<i>M. branchi</i>	<i>M. branchi</i>
Body length	60.0-85.0 (72.9 $\pm$ 8.4, 20)	43.0-93.0 (61.9 $\pm$ 13, 35)	45.0-65.0 (56.5 $\pm$ 6.4, 9)	45-83 (62.6 $\pm$ 11.9, 18)	40-70 (56.5 $\pm$ 9.8, 12)	51-70 (60.0, 5)
Body diameter	15.0-25.0 (20.1 $\pm$ 2.4, 20)	13.0-29.0 (23.1 $\pm$ 3.9, 35)	20-31 (26.3 $\pm$ 3.6, 9)	15-30 (21.3 $\pm$ 3.7, 18)	13-26 (18.6 $\pm$ 4.1, 12)	18-39 (29.4, 5)
Outer cinctum diameter	12.0-15.0 (13.5 $\pm$ 2.1, 2)	-	11	9-17 (12.6, 5)	13-16 (14.5, 4)	-
Inner cinctum diameter	-	-	1	2-10 (6, 5)	5-10 (7.2, 4)	-
Limb diameter	-	1.0-6.0 (2.2 $\pm$ 1, 30)	2-4 (2.3, 8)	2-6 (3.1 $\pm$ 1.1, 17)	2-5 (3.4 $\pm$ 0.9, 11)	2-3 (2.8, 5)

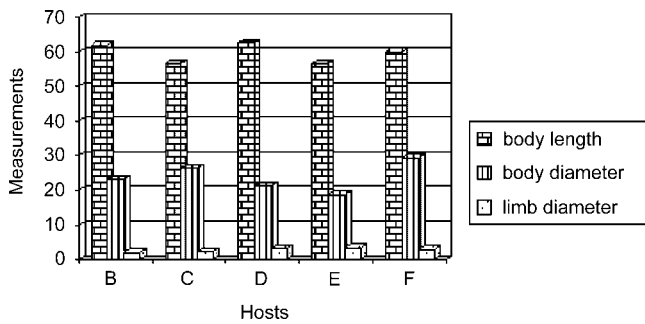
**Table 2.** Body striations of *Ellobiophrya maliculiformis* sp. n. found attached to *Mantoscyphidia spadiceae* Botes, Basson *et Van As*, 2001 and *M. branchi* Van As, Basson *et Van As*, 1997 occurring on the gill filaments of *Haliotis spadicea* Donovan, 1808 and *Scutellastra barbara* (Linnaeus, 1758)\* respectively from the south coast of South Africa.

	Number of striations Host <i>M. spadiceae</i>	Number of striations Host <i>M. branchi</i>
Peristome	8-22 (14.2 $\pm$ 4.6, 10)	7-9 (8.0, 3)
Peristome to cinctum	54-118 (86.7 $\pm$ 17.3, 10)	66-96 (78.6 $\pm$ 8.8, 13)
Total number of striations	62-140 (100.9 $\pm$ 20.5, 10)	66-116 (82.4 $\pm$ 12.8, 13)

\* A new phylogenetic classification for the patellid limpets was suggested by Ridgway *et al.* (1998), grouping the patellid limpets in four monophyletic genera, namely *Helcion* Montfort, 1810; *Cymbula* H. *et A.* Adams, 1854; *Scutellastra* H. *et A.* Adams, 1854 and *Patella* Linnaeus, 1758, with the latter genus not occurring in South Africa. All limpets were formerly placed in the genus *Patella*.

**Figs 3-13.** Scanning electron micrographs (**4, 6-8**) and photomicrographs of live (**3, 13**) and protargol stained specimens (**5, 9-12**) of *Ellobiophrya maliculiformis* sp. n. occurring as a secondary symbiont on *Mantoscyphidia spadiceae* Botes, Basson *et Van As*, 2001, *M. midae* Botes, Basson *et Van As*, 2001 and *M. branchi* Van As, Basson *et Van As*, 1998 on the gills of *Haliotis spadicea* Donovan, 1808, *H. midae* Linnaeus, 1758 and *Scutellastra barbara* (Linnaeus, 1758) collected along the south coast of South Africa. **3** - live specimen of *E. maliculiformis* with protruding adoral cilia attached to *M. spadiceae*; **4** - detached *E. maliculiformis*, upper part of the body partially contracted; **5** - bifurcated structure at the tip of the myoneme in the cinctum, scopula indicated by arrow; **6** - cinctum, scopula indicated by arrow; **7** - one limb of cinctum tapers, fitting into the cinctal junction of the shorter, broader limb; **8** - attachment of cinctum around *Mantoscyphidia spadiceae*; **9-12** - protargol-stained specimens; **13** - microconjugant attached to ellobiophryid associated with *M. spadiceae*. a - ampulla, c - cinctum, cl - cinctal limb, cp - cytopharynx, cs - cytostomial sphincter, m - myoneme, mc - microconjugant, pl - peristomial lip, s - scopula, sa - symbiotic algae, tb - telotroch band. Scale bars 10  $\mu\text{m}$  (3, 4, 9-13), 1  $\mu\text{m}$  (5-8).





**Fig. 14.** Comparison in the variation of body length, body diameter and cinctal diameter of different populations of *Ellobiophrya maliculiformis* sp. n. found attached to *Mantoscyphidia midae* Botes, Basson *et al.* 2001 (B) and *M. branchi* Van As, Basson *et al.* 1998 (C-F) associated with haliotid (B) and limpet species (C-F) collected along the south coast of South Africa.

diameter of body 15–30  $\mu\text{m}$  ( $20.5 \pm 3.7$ , 40) [16–37  $\mu\text{m}$  ( $23.9 \pm 5$ , 43)]. Peristome with broad, striated peristomial lip (Fig. 3); zig-zag striations present on peristome in contracted specimens. Prominent peristomial sphincter (Figs 10, 11). Striations on peristome not always visible. Body striated; 101 striations on average, spaced 0.5  $\mu\text{m}$  apart. Striations evenly spaced and uniform (Fig. 4, Table 2).

Trochal band narrow, slightly elevated, one quarter length of body from cinctum, not always clearly visible (Figs 4, 11). Cinctum flattened with two cytoplasmic cinctal limbs of uneven thickness forming closed circle (Figs 4–8). One limb tapers, fitting into cinctal junction of shorter somewhat broader limb, both limbs terminate at bouton (Fig. 7). Limb that tapers forms bifurcated structure at tip of its myoneme (Fig. 5). Scopula is typical, but nonfunctional, except when it participates in secretion of larval stalk that links two daughters after fission (Figs 2B, 4–6).

Oral infraciliature of *E. maliculiformis* divisible into peristomial part and infundibular part as in other sessile peritrichs. Adoral zone completes spiral of 360° counter-clockwise around epistomial disc, with haplo- and polykinety starting almost at same point. Peristomial part consists of outer band of kinetosomes (polykinety) and inner band of kinetosomes (haplokinety) which parallel one another for entire length before plunging into infundibulum (Fig. 2A).

Haplokinety and polykinety run together around peristome and separate before plunging into infundibulum. Polykinety joined by additional polykinetids after entering infundibulum. P1 and P2 were positively observed in

most of the specimens. The third polykinetid, which is normally very short, was observed only in few specimens, running parallel and closely associated with polykinety from the lip of opening up to first turn within infundibulum. Inside infundibulum, polykinetids and haplokinety make one turn (360°–400°) each on opposite walls, before reaching cytostome.

Conspicuous cytostomial sphincter visible at end of infundibulum which constricts area between infundibulum and cytostome (=ampulla) (Figs 9–11). Ampulla tubular when empty and slightly bulbous when filled with food (Figs 9–12). Ampulla merges with cytopharynx that is very small in diameter throughout its length, recurving slightly just adoral to trochal band (Figs 2B, 11, 12).

Symbiotic algae present throughout cytoplasm, varying in number and size, obscuring position and shape of nuclear apparatus (Fig. 3). Micronucleus fusiform, but not always visible. Macronucleus coiled and sausage-shaped, extending throughout body. Prominent sections of nucleus visible in adoral and aboral sides (Fig. 1).

Reproduction is by means of conjugation and binary fission followed by telotroch formation. *Ellobiophryids* in various stages of binary fission were observed as well as individuals with attached microconjugants (Fig. 13), which confirms the first record of conjugation (Fig. 13) in the genus *Ellobiophrya* (Botes *et al.* 2001). Live observations of conjugation were made in two instances in populations on *M. midae*, four times in populations on *M. spadiceae* (Fig. 13), and twice in populations on *M. branchi*.

Binary fission and the subsequent formation of telotrochs were observed in *ellobiophryid* populations associated with all three hosts. After binary fission one daughter individual becomes a telotroch and the other remains a trophont attached by the parental cinctum to the host. As in other *ellobiophryids*, the telotroch is attached during development to the trophont daughter by a short, rigid stalk that passes between the scopulas of the two individuals (Bradbury and Clamp 1991). The telotroch is slightly asymmetric, as is the case in other *Ellobiophrya* species.

A larval stalk was identified during an observation of telotroch formation in a live specimen of *Ellobiophrya maliculiformis* attached to *M. midae*. The telotroch was attached to the trophont daughter by this short stalk, and the trochal band of cilia was in the process of differentiating, but was not beating yet. The parent's peristome was open, with cilia creating a feeding current. This telotroch was found on a gastropod host that had been collected 8–10 h beforehand and was observed

for a period of 55 min before it separated from the parent and swam away. The aboral end (scopula) that was attached to the embryophore of the parent ellobiophryid became broader after separation.

A telotroch-like individual was also observed attached to the body of a trophont of *E. maliculiformis*. It was attached to the middle region of the trophont, and it may have been a microconjugant that had just attached in preparation for conjugation, rather than a telotroch that was preparing to separate from the other daughter. This telotroch had a short, stalk-shaped structure which attached it to the trophont, but it was not attached to the scopula, as would have been the case in a developing telotroch. The apparent stalk may have been a slender cytoplasmic connection because a larval stalk is expected to be linked to the scopula (embryophore) of the trophont daughter.

### Intraspecific variation

Body measurements of live observations and hematoxylin-stained material of *E. maliculiformis* are summarised in Table 1. The effect of contraction on the body length of live specimens versus hematoxylin-stained specimens is as follows: in the *M. spadiceae* populations there was a 27% body contraction between live observations and those stained with hematoxylin. The length and diameter of the body varied among different populations of *E. maliculiformis*. The average body length of ellobiophryid populations on *M. midae* was 61.9 µm. The average body length of populations found associated with *M. branchi*, ranged between 56.5 and 62.6 µm (Table 1).

Ellobiophryids from *M. branchi*, *M. spadiceae* and *M. midae* had the same body form. The ratio of body length to diameter in hematoxylin-stained specimens of *E. maliculiformis* found on *M. midae* and *M. branchi* is as follows: 2.68 (*M. midae*), 2.15 (*M. branchi*), 2.94 (*M. branchi*), 3.04 (*M. branchi*) and 2.04 (*M. branchi*). Also no significant differences could be found in the diameter of the cinctal limbs of different populations (see Table 1 and Fig. 14).

Live specimens of *E. maliculiformis* from *M. spadiceae* were extremely contractile, with body length ranging between 50 and 125 µm. The body of *M. branchi* is also extremely contractile, with fully expanded specimens varying from 40 µm to 95 µm. Van As *et al.* (1998) observed during fieldwork that the same individual of *M. branchi* could achieve a reduction in body length with the peristome remaining open. In these specimens, groups of elevated striations can be seen

aboral to the telotroch band. When the peristome of *M. branchi* is fully closed, the degree of contraction can also vary. Specimens of *M. branchi* can be found in a whole range of body contractions on a single smear. Live ellobiophryids were able to contract to half of their fully extended body length.

Although the nuclear apparatus of all the populations were mostly obscured by algal inclusions, there were no great differences in the shape of the macronucleus. It is coiled and stretches throughout the body, much the same as those of *E. conviva*, *E. oblida* and *E. brevipes* (Clamp and Bradbury 1997).

The only difference between the ellobiophryid populations of *M. spadiceae* and *M. branchi* was that the latter had slightly fewer body striations (Table 2). This could be due to the fact that *E. maliculiformis* specimens found associated with *M. spadiceae* has a greater body length. The *M. spadiceae* population had a prevalence of 35.4% of ellobiophryids associated with the scyphidiid peritrichs, and the *M. midae* population had a prevalence of 34.3%, whilst those ellobiophryids found associated with *M. branchi* had a prevalence of 17%.

### Remarks

This is the first record of an ellobiophryid from Africa and the first found associated with another ciliophoran host in the marine environment. Other records of peritrichs found in a symbiotic association with peritrichs, are that of *Epistylis lwoffii* Fauré-Fremiet, 1943 which attached to the epistylidid *Apiosoma piscicola* (Blanchard), which in turn is found on the skin of freshwater fish (Fauré-Fremiet 1943, Clamp 1982) and *E. colisarum* (Foissner *et Schubert*, 1977) attaching to an epistylidid which lives symphoriontly on a freshwater fish, *Colisa fasciata* (Anabantoidei: Belontiidae) (Foissner and Schubert 1977).

In comparing *E. maliculiformis* with other species of *Ellobiophrya*, it shows the most resemblance to *E. oblida* in respect to body form. In both *E. oblida* and *E. maliculiformis*, the expanded peristome is wider in diameter than the rest of the body, and the peristomial lip is everted. *Ellobiophrya oblida* is, however, a much larger species than *E. maliculiformis* and has a different host and site preference as it occurs on the skin of marine fish. The position of the scopula of *E. maliculiformis* differs from the other species of *Ellobiophrya* in that it is located much nearer to the cinctum. The cinctum of *E. maliculiformis* is also asymmetrical, with uneven limbs. The longer thinner limb fits into the junction of the shorter much broader limb. The limb diameter of the populations varies be-



**Table 3.** Summary of the taxonomic characteristics of the species of the family Ellobiophryidae: *Ellobiophrya donacis* Chatton *et* Lwoff, 1923, *E. conviva* Clamp, 1982, *E. oblida* (Naidenova *et* Zaika, 1969), *E. brevipes* (Laird, 1959), *E. maliculiformis* sp. n. and *Caliperia longipes* Laird, 1953. Ma - macronucleus, Mi - micronucleus.

Species	<i>E. donacis</i>	<i>E. conviva</i>	<i>E. oblida</i>	<i>E. brevipes</i>	<i>E. maliculiformis</i>	<i>C. longipes</i>
Host	<i>Donax vittatus</i> Marine bivalve mollusc	<i>Bugula neritina</i> , <i>B. turrita</i> Marine ectoprocts (Bryozoa)	<i>Proterorhinus marmoratus</i> Marine fish	<i>Raja erinacea</i> Marine fish	<i>Mantoscyphidia spadiceae</i> , <i>M. midae</i> , <i>M. branchi</i> , Scyphidid peritrichs	<i>Oliverichthus melobesi</i> , <i>Ericentrus rubrus</i> , Marine fishes
Position on host	Gill filaments	Ciliated tentacles around mouth	Skin	Gills	Narrow basal part adoral to scopula of host	Gills
Collection locality	Morgat, France	North Carolina, USA	Black Sea	New Brunswick, Canada	South coast of South Africa	Wellington, New Zealand
Body length (µm)	50 (100)	46.2	180	60.2 (54.5)	50-125 (78.5)	31.2-68.4 (51.5)
Body diameter (µm)	40 (30)	26.8	36.5	34.6 (35.7)	15-30 (20.5)	24.0-52.6 (38.8)
Body and nuclei	Body subcylindrical, elongate, tapers towards oral pole Ma - compact and spherical, Mi - fusiform	Body subcylindrical, elongate slightly, tapers towards aboral pole Ma - cylindrical, length of soma, Mi - fusiform/oval	Body cylindrical, subconical, tapers towards aboral pole Ma - cylindrical, Mi - fusiform	Body cylindrical, elongate, subconical, tapers towards aboral pole, Ma - cylindrical, long and flat, Mi - fusiform	Body conical, elongate, tapers towards aboral pole, Ma - coiled, sausage-shaped, Mi - fusiform	Body cylindrical, tapers towards aboral pole, Ma - cylindrical, Mi - fusiform/lenticular
Cinctum	Limbs joined, bouton, no internal rod myoneme, contractile	Limbs joined (cemented at tips), bouton, no internal rod, contractile	Limbs joined, bouton, no internal rod, myoneme	Limbs joined, bouton, no internal rod, myoneme	Limbs of uneven thickness, fitting into junction, bouton, no internal rod, myoneme	Limbs not joined, no bouton, 5-6 µm
Ampulla shape	Narrow and lanceolate	Wide and bulbous	Not described	Long, slender, tapering smoothly into cytopharynx, small in diameter, elongate, almost tubular, narrow, lanceolate when not filled	Tubular when empty, bulbous when filled, merges with cytopharynx	Resembles pipette bulb, cytostomal sphincter between infundibulum and ampulla

Table 3 (contd.)

Cytopharynx	Not described	Elongate, ends near aboral end of macronucleus	Not described	Long sineuous tube discharging near posterior part of macronucleus	Small, recurving just adoral to trochal band	Large and funnel-shaped, ends blindly posterior at macronucleus
Expanded peristome	Not described	Argentophilic cytostomal sphincter, not prominent, uneverted peristomal lip, subequal in diameter	Argentophilic cytostomal sphincter not described, widest at peristome with thickened peristomal lip	Argentophilic cytostomal sphincter at entrance to peristome, uneverted peristomal lip	Conspicuous cytostomal sphincter at end of infundibulum	Argentophilic cytostomal sphincter present peristomal disc invaginated
Extent of infundibulum	Does not extend far beyond peristome	Approximately a third of distance from peristome to sphincter	Extends approximately a third of distance from peristome to cinctum	Short, ends at ampulla, quarter of distance from peristome to aboral end of body	Approximately a third of distance from peristome to cinctum	Haplo- and polykinety make one and one quarter turns before plunging in
Pattern of infundibular kinetids	Not described	Rows in P2 end aborally far short of junction of P1 with polykinety	Not described	Rows in P2 extend aborally almost to junction of P1 with polykinety	Not clearly visible due to algal inclusions	Not described
Pellicular striations	Closely spaced, faint striae	Prominent transverse striae	Closely spaced, faint striae	Closely spaced, faint striae	Evenly spaced and uniform	Smooth pellicle (no annuli)
Inclusions	Cytoplasmic inclusions (type 1 and 2)	Greenish areas in body (diatoms/algal cells)	Not described	Not described	Symbiotic algae, throughout cytoplasm, obscuring body features	Greenish, yellowish spherules (algal origin)
Larval stalk and embryophore	Well developed larval stalk and cylindrical embryophore	Temporary stalk in telotroch, embryophore present (shorter and not as thick as in <i>E. donacis</i> )	Not described	Short, straight, rigid stalk, larger in diameter than <i>E. conviva</i> , less conspicuous than in <i>E. donacis</i>	Short, rigid stalk, embryophore present	Not described

tween 2.2 and 3.4  $\mu\text{m}$  (see Table 1). Table 3 represents a summary of the taxonomic characteristics of all species of the family Ellobiophryidae. This summary was compiled from Chatton and Lwoff (1923, 1928, 1929), Clamp (1982), Bradbury and Clamp (1991), Clamp and Bradbury (1997), and also includes the summarized characteristics of *E. maliculiformis*.

Up to four specimens of *E. maliculiformis* were observed attached adoral to the scopula of a single scyphidiid peritrich. Some ellobiophryids were attached to the peristomial region or even in the region of the telotroch band of the host's body, gripping it where the nuclear apparatus is situated (Fig. 8). Two ellobiophryids were observed attached between the macro- and micro-nuclei of a single *Mantoscypthidia spadiceae*. In cases where the ellobiophryids were attached to the peristomial region of the host its buccal cavity was probably obstructed. The ellobiophryids might interfere with the host's feeding. Ellobiophryids attached to the nuclear region of the host's body might have an influence on reproductive processes, possibly interfering with division.

The present ellobiophryid has a distinctive host situation, as all other *Ellobiophrya* species are found attached to the gills or skin of an animal host. It is interesting to note that the two haliotid species, namely *Haliotis spadicea* and *H. midae*, each have a different mantoscypthidian species occurring on the gills, namely *M. spadiceae* and *M. midae*, whilst in contrast all seventeen limpets species have only one species of mantoscypthidian, i.e. *M. branchi*, and all three *Mantoscypthidia* species had the same species of ellobiophryid attached adoral to the scopulas, i.e. *Ellobiophrya maliculiformis*.

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## REFERENCES

- Botes H., Basson L., Van As L. L. (2001) Two new species of *Mantoscypthidia* Jankowski, 1980 (Ciliophora: Peritrichia) gill symbionts of *Haliotis* Linnaeus, 1758 (Mollusca: Archaeogastropoda) from the south coast of South Africa. *Acta Protozool.* **40**: 131-140
- Botes H., Van As L. L., Basson L., Van As J.G. (2001) Reproductive processes of *Ellobiophrya* sp., hypersymbiont of scyphidiid peritrichs. *Proc. Microsc. Soc. south. Afr.* **31**: 76
- Bradbury P.C., Clamp J. C. (1991) The fine structure of the cinctum of *Ellobiophrya conviva* (Ciliophora: Peritricha). *J. Protozool.* **38**: 312-320
- Chatton E., Lwoff A. (1923) Un cas remarquable d'adaptation: *Ellobiophrya donacis* n. g., n. sp., péritriche inquilin des branchies de *Donax villatus* (Lamellibrance). *C. R. Soc. Biol.* **88**: 749-752
- Chatton E., Lwoff A. (1928) La reproduction D'*Ellobiophrya donacis* Ch. Et Lw., peritriche inquilin des branchies de *Donax vittatus* (Lammelibranche). *Bull. Soc. Zool. Fr.* **53**: 268-272
- Chatton E., Lwoff A. (1929) Contribution a l'étude de l' adaptation *Ellobiophrya donacis* Ch. et Lw. Péritriche vivant sur les branchies de l'acéphale *Donax vittatis* Da costa. *Bull. Biol. Fr. Belg.* **63**: 321-349
- Clamp J. C. (1982) *Ellobiophrya conviva* n. sp., a commensal of marine ectoprocts and a revision of the family Ellobiophryidae (Ciliophora: Peritricha). *J. Protozool.* **29**: 149-156
- Clamp J. C., Bradbury P. C. (1997) Redescription of *Ellobiophrya brevipes* (Laird, 1959) n. comb. (Ciliophora: Peritrichia) and the fine structure of its pellicle and cinctum. *J. Euk. Microbiol.* **44**: 374-382
- Fauré-Fremiet E. (1943) Commensalisme et adaptation chez une vorticellide: *Epistylis lwoffii* n. sp. *Bull. Soc. Zool. Fr.* **68**: 154-157
- Foissner W., Schubert G. (1977) Morphologie der Zooide und Schwärmer von *Heteropolaria colisarum* gen. nov., spec. nov. (Ciliata: Peritrichida), einer symphorionten Epistylididae von *Colisa fasciata* (Anabantoidei: Belontiidae). *Acta Protozool.* **16**: 231-247
- Laird M. (1953) The Protozoa of New Zealand intertidal zone fishes. *Trans. R. Soc. NZ.* **81**: 79-143
- Laird M. (1959) *Caliperia brevipes* n. sp. (Ciliata: Peritricha) epizoic on *Raja erinacea* Mitchell at St Andrews, New Brunswick. *Can. J. Zool.* **37**: 283-287
- Lee J. J., Hunter S. H., Bovee E. C. (1985) Illustrated Guide to the Protozoa. Society of Protozoology, Lawrence, USA
- Lom J., Dykova I. (1992) Protozoan Parasites of Fishes. Elsevier, Amsterdam
- Naidenova N. N., Zaika, W. E. (1969) Two new species of Protozoa from the fishes of the Black Sea. *Parazitologiya* **3**: 97-101 (in Russian)
- Ridgway S. A., Reid D. G., Taylor J. D., Branch G. M., Hodgson A. N. (1998) A cladistic phylogeny of the family Patellidae (Mollusca: Gastropoda). *Philos. Trans. R. Soc. Lond.* **353**: 1654-1671
- Van As L. L., Basson L., Van As J. G. (1998) Two new species of *Mantoscypthidia* Jankowski, 1980 (Ciliophora: Peritrichia) gill symbionts of limpets, from south Africa and the Sub-Antarctic Island, Marion. *Acta Protozool.* **37**: 101-111
- Wicklow B. J., Hill B. F. (1992) A short procedure for protargol staining. In: Protocols in Protozoology, (Eds J. J. Lee, A. T. Soldo). The Society of Protozoologists, Lawrence, Kansas, C-5.1 - C-5.3

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## *Trypanosoma (Herpetosoma) grosi kosewiense* subsp. n., the Parasite of the Yellow-Necked Mouse *Apodemus flavicollis* (Melchior, 1834)

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**Summary.** Yellow-necked mice, *Apodemus flavicollis*, were examined for the presence of *Herpetosoma* trypanosomes at the Mazurian Lakeland, Poland. Bloodstream trypomastigotes were used for light microscopy investigations. In the host's blood, slender and stout trypomastigote stages of *Trypanosoma grosi kosewiense* subsp. n. were observed. The body length of the slender form ranged from 16.27 to 28.05  $\mu\text{m}$ , mean 23.00  $\mu\text{m}$ , and width from 0.79 to 2.56  $\mu\text{m}$ , mean 1.43  $\mu\text{m}$ . The free flagellum is 3.66-12.79  $\mu\text{m}$  long, mean 7.59  $\mu\text{m}$ . The kinetoplast is particularly large. Stout forms have a characteristic broad and short body and is 10.78-23.54  $\mu\text{m}$  long, mean 14.74  $\mu\text{m}$ , and 1.18-2.78  $\mu\text{m}$  width, mean 1.76  $\mu\text{m}$ . In contrast to the slender form of *T. grosi kosewiense* and many other trypanosomes, the broadest size of its body falls midway of the PN distance, not at the nuclear level. The free flagellum is 5.06-11.86  $\mu\text{m}$  long. It is easy to notice the wide range of morphometric parameters. The results of light microscopy morphological description and morphometrical values of *Trypanosoma grosi kosewiense* subsp. n. were compared with those of *Trypanosoma grosi* Laveran *et* Pettit, 1909 parasitizing the wood mouse, *A. sylvaticus*, and revealed that trypanosomes parasitizing the yellow-necked mouse are different in many investigated parameters.

**Key words:** *Apodemus flavicollis*, morphology, *Trypanosoma (Herpetosoma) grosi kosewiense* subsp. n.

### INTRODUCTION

*Herpetosoma* trypanosomes occurring in West and Middle Europe are not very well known. The first records about trypanosomes parasitizing European small rodents were made in the beginning of the 20th century (Thiroux 1905, Laveran and Pettit 1909). Later these trypanosomes were investigated only in the U. S. A and in some Western-European countries (Davis 1952-53, Krampitz 1959, Šebek 1975, Molyneux 1976, Šebek *et*

*al.* 1980, Noyes *et al.* 2002). The studies regarded morphology, primarily; few publications concerned the results of ecological and biochemical investigations. So far, in Europe about 15 species of *Herpetosoma* trypanosomes, parasitizing rodents and insectivores have been recorded. However, new species are constantly being described, as well as new localities of known trypanosomes (Karbowski and Siński 1996, Karbowski *et al.* 1998a,b; Macdonald *et al.* 1999). For the description of trypanosomes, Hoare (1972) recommended the following criteria: the host species, the vector species, the morphology and the biology of the trypanosome. Since then, ultrastructural and biochemical criteria were added. On account of the great variability within the *Herpetosoma* subspecies, and incomplete knowledge

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about these parasites, their recognition is difficult on the basis of morphological features only. Presently, for biochemical description, isoenzyme electrophoresis (Mohamed *et al.* 1987), lectin affinity (Maraghi *et al.* 1989) as well as 18S rRNA genotyping (Noyes *et al.* 2002) have been applied. Because a single criterion is insufficient for a majority of trypanosomes (Maraghi *et al.* 1989), the combination of various features (morphological, biological, biochemical) is recommended for the description and recognition of these parasites.

Until recently *Herpetosoma* trypanosomes were not investigated in Poland. Only in the last few years records have appeared about trypanosome infections of the bank-vole, *Clethrionomys glareolus*, and voles from genus *Microtus* (Karbowski and Siński 1996, Bajer *et al.* 1998, Karbowski *et al.* 1998b). Trypanosomes infecting yellow-necked mice *Apodemus flavicollis* are practically unknown; however, *Trypanosoma grosi*, infecting the wood mouse, *Apodemus sylvaticus*, is well described in England and Germany (Krampitz 1959, Hoare 1972, Noyes *et al.* 2002).

The *Herpetosoma* trypanosomes are not very pathogenic and make no visual pathological effects to their hosts. The metacyclic forms of trypanosomes infecting mammals penetrate the skin through, or *per os*-when the host swallows the infected vector (Maraghi *et al.* 1995). This way of infection with trypanosomes was showed experimentally by Calvo *et al.* (1992) and Maraghi *et al.* (1995).

## MATERIALS AND METHODS

**Animals.** Yellow-necked mice *Apodemus flavicollis* were investigated in Kosewo Górne, Mazurian Lakeland, Poland, from May 1997 until June 1998. The animals were trapped monthly, using live traps containing oats as bait. After collection of material, the animals were numbered by toe clipping and released where they were trapped. Thus, many individuals were investigated repeatedly during the following months. In summary, 127 yellow-necked mice were examined.

**Detection of trypanosomes infection and morphometric measurements.** Blood samples were obtained from the tail tip of investigated animals. The trypanosomes were detected by centrifugation in microhaematocrit tubes (8 min, 6200 g). They accumulated above the WBCs fraction, and their movements were observed using a light microscopy, using magnifications 10 x 10 and 10 x 20 (eyepiece x objective). Smears were made from the fraction with trypanosomes, as well as from non-centrifuged blood. The smears were fixed in absolute methanol (10 min) and stained with Giemsa's stain, diluted 1:15 in 0.2 M phosphate buffer, pH 7.2 (60 min). Giemsa's reagent stains the cytoplasm of *Herpetosoma* trypanosomes light blue, the nucleus and kinetoplast a violet or purple-blue colour. Because the quality of staining with Giemsa's reagent was poor in the case of

smears made from the centrifuged blood, the commercial kit Hemacolor (Kruszewicz 1994) was used.

For the measurements of parasites, the "Analysis" software in combination with a video camera and the Olympus BX50F4 microscopy was used. This method allows obtaining results accurate to 0.01  $\mu\text{m}$ . Stained blood smears were analyzed at a magnification of 1200x. The nomenclature of morphometrics parameters of trypanosomes was that commonly used by other authors (Hoare 1972, Matthews *et al.* 1977, Kingston *et al.* 1992).

Typical trypomastigote forms were selected for measurements. Poorly stained, damaged or deformed individuals were ignored. The data obtained were compared with similar data given by Krampitz (1959), for *Trypanosoma grosi* Laveran *et Pettit*, 1909, parasitizing the *Apodemus sylvaticus*, related to the *Apodemus flavicollis*.

**In vitro cultures.** The blood of infected mice was obtained by heart puncture and heparinized. Earlier the animals were anesthetized. The blood was inoculated into 4 ml of Veal Infusion Medium (VIM) and into 4 ml of Schneider's *Drosophila* Medium (SDM). The media were supplemented with 20% of foetal calf serum. Moreover, VIM medium also contained 0.2 mg/ml of vitamin B<sub>12</sub>, 100 mg/ml of streptomycin and 100 units/ml of penicillin (Kingston 1973, Morton and Kingston 1976, McHolland-Raymond *et al.* 1978). SDM contained an addition of 100 mg/ml of gentamycin and 0.2 mg/ml of vitamin B<sub>12</sub> (Mohamed and Molyneux 1987). The cultures were incubated at 26°C and checked every second day, using light microscopy, magnification 1200x.

## RESULTS

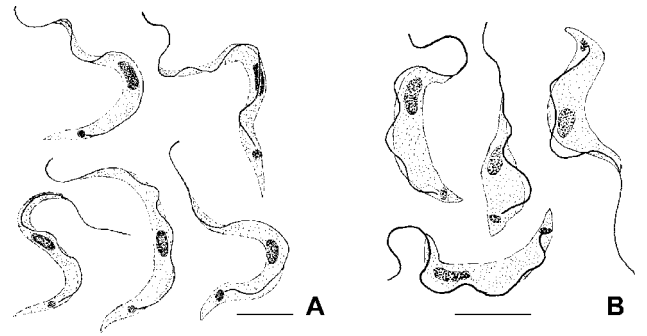
Infection with *Trypanosoma grosi kosewiense* subsp. n. was discovered in 6 yellow-necked mice of the 127 caught. Morphometric measurements were made on 240 specimens of trypomastigotes, obtained from 4 heavily infected hosts.

**Morphological characterization.** The trypanosomes in the blood of the yellow-necked mouse occur as trypomastigote forms. Two different morphological forms were observed. A slender form is typical for *Trypanosoma lewisi* group. It has a narrow, long body, with a sharpened posterior end. The length of the body is 16.27-28.05  $\mu\text{m}$ , mean 23.00  $\mu\text{m}$ , the width is 0.79-2.56  $\mu\text{m}$ , mean 1.43  $\mu\text{m}$ . The free flagellum is 3.66-12.79  $\mu\text{m}$  long, mean 7.59  $\mu\text{m}$ . The undulating membrane is weakly developed, however always visible. The nucleus is oval in shape and is located in the middle or in the anterior part of the body, parallel to the long axis. It is 1.42-3.71  $\mu\text{m}$  long, mean 2.68  $\mu\text{m}$ . The kinetoplast is particularly large. It is visible on the blood smears as oval in shape, 0.35-1.46  $\mu\text{m}$  in diameter, mean 0.87  $\mu\text{m}$ , or rod-shaped, 0.42 x 0.75 up to 1.18 x 2.07  $\mu\text{m}$  in size, mean 0.69 x 1.02  $\mu\text{m}$ . It is located near the posterior end of the body (Figs 1, 2A). A wide range of morphometrics parameters (Table 1) was readily noted.

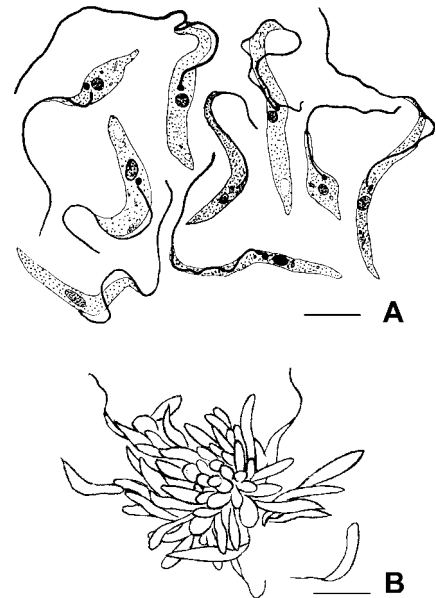


**Fig. 1.** Photomicrographs of bloodstream trypomastigotes of *Trypanosoma grosi kosewiense* subsp. n., from the yellow-necked mouse, *Apodemus flavicollis*, in the Mazurian Lakeland, Poland. Scale bar 10  $\mu$ m.

Stout forms (Fig. 2B) have a characteristic broad and short body. The posterior end is more or less sharp or blunt. The cell is 10.78-23.54  $\mu$ m long, mean 14.74  $\mu$ m, and 1.18-2.78  $\mu$ m in width, mean 1.76  $\mu$ m. In contrast to the slender form of *T. grosi kosewiense* and many other trypanosomes, the broadest size of its body occurs in the middle of the PN distance, not at the nuclear level. The free flagellum is 5.06-11.86  $\mu$ m long, mean 8.56  $\mu$ m. The undulating membrane is well developed. The nucleus, slightly oval or round in shape, is shifted to the anterior end of body. If the nucleus is oval, it is placed parallel, oblique, or in extremely broad individuals, perpendicular to the long axis of the body. The nucleus is 1.64-3.35  $\mu$ m long, mean 2.38  $\mu$ m. The kinetoplast is oval or rod-shaped, 0.25 x 0.30  $\mu$ m to 1.27 x 1.34  $\mu$ m in size. It is located nearer to the posterior end of the body in stout forms, than in the slender forms. Similarly to the slender forms, there is a wide range of morphometric param-



**Fig. 2.** Drawings of Giemsa-stained typical bloodstream trypomastigotes of *Trypanosoma grosi kosewiense* subsp. n., from the yellow-necked mouse, *Apodemus flavicollis*. **A** - slender forms; **B** - stout forms. Scale bars 10  $\mu$ m.



**Fig. 3.** The *Trypanosoma grosi kosewiense* subsp. n., culture forms seen in Schneider's medium. **A** - epimastigotes and promastigotes; **B** - the rosettes of epimastigotes. Scale bars 10  $\mu$ m.

eters visible. The data obtained for trypanosomes isolated from the yellow-necked mouse *Apodemus flavicollis* were compared with the same parameters of *Trypanosoma grosi*, presented by Krampitz (1959) and Hoare (1972) (Table1).

**The morphology of *T. grosi kosewiense* subsp. n. in *in vitro* cultures.** *T. grosi kosewiense* subsp. n., isolated from the blood of yellow-necked mouse, grew well in Schneider's medium, and weaker in VIM medium. The trypanosomes occur in *in vitro* cultures in the

**Table 1.** Dimensions (in  $\mu\text{m}$ ) of slender and stout forms of *Trypanosoma grosi kosewiense* subsp. n. from Mazurian Lakeland compared with *T. grosi* Laveran *et* Pettit, 1909, description by Krampitz (1959).

Parameter		PK	KN	PN	NA	BL	FF	No
<i>T. grosi kosewiense</i> f. slender	mean	3.87 $\pm$ 0.65*	8.34 $\pm$ 0.79	12.21 $\pm$ 1.97	10.79 $\pm$ 1.98	23.00 $\pm$ 2.31	7.59 $\pm$ 1.35	160
	range	2.32-5.78	5.73-11.10	8.73-15.57	5.84-15.42	16.27-28.05	3.66-12.79	
<i>T. grosi kosewiense</i> f. stout	mean	2.08 $\pm$ 0.66	7.30 $\pm$ 1.42	9.37 $\pm$ 1.67	5.37 $\pm$ 1.14	14.74 $\pm$ 2.29	8.56 $\pm$ 1.51	80
	range	0.93-4.01	3.98-11.12	5.71-15.06	3.49-8.88	10.78-23.54	5.06-11.86	
<i>T. grosi</i> (Krampitz 1959)	mean	1.90	7.30	9.20	12.30	21.5	4.55	
	range	1.00-2.50	6.00-9.00	-	10.00-17.50	-	1.5-7.6	

Parameter		L	N	W	NI	KI	FF:BL	No
<i>T. grosi kosewiense</i> f. slender	mean	30.59 $\pm$ 2.23	2.68 $\pm$ 0.36	1.43 $\pm$ 0.30	1.17 $\pm$ 0.25	1.47 $\pm$ 0.09	3.15 $\pm$ 0.74	160
	range	23.69-36.17	1.42-3.71	0.79-2.56	0.70-1.95	1.23-1.75	1.54-6.04	
<i>T. grosi kosewiense</i> f. stout	mean	23.33 $\pm$ 2.66	2.38 $\pm$ 0.34	1.76 $\pm$ 0.30	1.80 $\pm$ 0.39	1.30 $\pm$ 0.11	1.77 $\pm$ 0.44	80
	range	17.88-31.85	1.64-3.35	1.18-2.78	0.94-2.68	1.14-1.74	1.04-3.11	
<i>T. grosi</i> (Krampitz 1959)	mean	23.80	-	-	0.74	1.26	4.72	-
	range	21.00-28.00	1.50-3.00	-	-	-	-	

Data in italic-unpublished (calculated here on the base of previous data); \*-standard deviation; PK-posterior end to kinetoplast; KN-kinetoplast to nucleus center; PN-posterior end to nucleus center; NA-nucleus centre to anterior end; BL-body length; L-total length; FF-free flagellum length; N-nucleus length; No-number of individuals investigated; W-width of body on the nucleus level, excluding the undulating membrane; and indices: nuclear index  $NI = PN / NA$ ; kinetoplastic index  $KI = PN / KN$ ; flagellar index  $FF:BL (= BL/FF)$ . Index  $NI = 1$  demonstrates the central position of the nucleus in the body;  $NI < 1$  demonstrates the position of the nucleus nearer the posterior end of the body;  $NI > 1$  demonstrates the position of the nucleus nearer anterior end of the body. Analogous- $KI = 2$  demonstrates the location of the kinetoplast midway between the nucleus and the posterior end of the body;  $KI < 2$  the location of the kinetoplast nearer to the posterior end;  $KI > 2$  nearer to the nucleus (Keymer 1967, Hoare 1972). Index  $FF:BL$  defines the proportion of the free flagellum to the length of body.

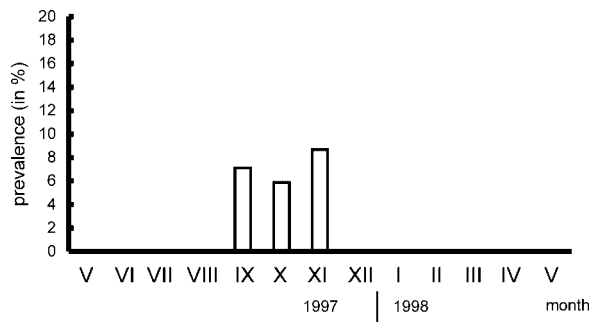
epimastigote and promastigote forms. They are motile for about 10 weeks, later symptoms of degeneration are observed, and the cultures are lost. In the lag phase of culture, rosettes occur. In Schneider's medium they are bigger and more numerous than in VIM medium. After 2-3 weeks, free epi- and promastigote forms appear. At this point the density of trypanosomes reached  $5 \times 10^6$  of individuals in 1 ml of medium. After 4-5 weeks the size of trypanosomes diminished, the range of dimensions is smaller, and finally the trypanosomes disappeared.

The body length of epimastigotes (Fig. 3A) is 13.00-25.00  $\mu\text{m}$ . The nucleus is oval in shape, the distance of the nucleus center to the anterior end of body is 3-10  $\mu\text{m}$ , and most often was no greater than 5  $\mu\text{m}$ . The

distance of the nucleus center from posterior end is 8-15  $\mu\text{m}$ . The kinetoplast is oval and is easily observed stained on slides. The free flagellum is 5-15  $\mu\text{m}$  long. The epimastigotes were often connected in rosette form with the anterior ends (Fig. 3 B).

The shape, body length of promastigotes, and length of flagellum and organelles' size are similar to those found in epimastigotes. Some promastigotes are characterized by a great swelling in the middle of body (Fig. 3A).

**The prevalence, seasonal dynamics and intensity of infection.** The infection of yellow-necked mice with trypanosomes was detected in August, September and October 1997. The prevalence of infection reached



**Fig. 4.** The dynamics of infection of yellow-necked mouse *A. flavicollis* with *Trypanosoma grosi kosewiense* subsp. n., during the period May 1997–May 1998.

8.7% (Fig. 4). In other months the infection with trypanosomes was not detected. The intensity of infection was very variable, from 150 trypanosomes up to 300 000 in 1 ml of blood. Both morphological forms of trypanosomes were recovered in the blood of 5 of 6 investigated mice. Their proportions were about 20:1 (slender/stout form), and were similar in each host. The last (sixth) mouse had a low intensity of infection, with slender forms only. The infected mice were caught again in the few following months, however in no individuals was the infection with trypanosomes detected for the second time.

## DISCUSSION

**Morphology.** So far, the trypanosomes parasitizing in yellow-necked mouse, *A. flavicollis*, were seldom found and were recognized originally as *T. grosi* Laveran *et* Pettit, 1909 (Krampitz 1959; Šebek 1960, 1975; Hoare 1972, Šebek *et al.* 1980). However, these recognitions were supported by the similarity of hosts, because the peculiar host of *T. grosi*, the wood mouse *A. sylvaticus* is a close relative to *A. flavicollis*. Other criteria were not considered at the time. The results of our investigations show that trypanosomes parasitizing the yellow-necked mouse are bigger than *T. grosi*. This phenomenon is evident in every investigated parameter. Indices NI and KI show that the nucleus is located closer to the body centre, and the distance between the kinetoplast and the posterior end of the body is longer. The morphological differences between trypanosomes parasitizing *A. sylvaticus* and *A. flavicollis* also were noted by

Šebek (1960). Moreover, by the polyphyletic character of *Trypanosoma grosi* parasitizing wood mice (*Apodemus sylvaticus*) in Great Britain was demonstrated (Noyes *et al.* 2002).

The characteristic feature of this parasite is the pleomorphism of trypomastigota forms living in the host's blood, which manifests itself with two morphological forms: slender and stout. This pleomorphism wasn't observed in other rodent's trypanosomes, but is known in *T. (Herpetosoma) rangeli* Tejera, 1920 and in many trypanosomes from the *Trypanozoon* subgenus (Hoare 1972, Urdaneta-Morales 1983). The reason for pleomorphism of *T. grosi kosewiense* is not clear at the present. In many cases the morphological differentiation is in correlation with the age of trypanosomes. However, the dividing forms, described in *T. grosi* (Krampitz 1959) and in other trypanosomes parasitizing rodents from the family Muridae, were absent in the blood of trypanosome infected yellow-necked mice.

**Conclusions.** In spite of the described differences between *T. grosi kosewiense* subsp. n., parasitizing *Apodemus flavicollis*, and *Trypanosoma grosi* Laveran *et* Pettit, 1909, there are some counterarguments against a description of a new species. The differences in the morphology may be induced by the peculiarity of their hosts. The variability of morphometric parameters, induced by different hosts was described in other species of trypanosomes (Mathew *et al.* 1992, Dávila *et al.* 1998), however, the change of host has no influence on the location of dividing forms in his body. It was mentioned above, that dividing forms of *T. grosi kosewiense* subsp. n. were not revealed in the peripheral blood of *A. flavicollis*.

The second counterargument is the close relationship between *A. sylvaticus* and *A. flavicollis*, which implicates the possibility of cross infections between these hosts. It is necessary to note that the wood mouse and the yellow-necked mouse live in different habitats. The yellow-necked mice prefer deciduous and coniferous forests, and choose well sheltered and moist habitats. Wood mice favour warm and dry habitats (Pucek 1984). In the case of overlapping areas, the wood mouse is gradually pushed out from the areas attended by yellow-necked mouse individuals (Hoffmeyer 1973). Consequently, the interactions between individuals of the different species are too rare and short, for the efficient exchange of ectoparasites and cross infections with trypanosomes. It is also important that the wood mouse is more numerous than the yellow-necked mouse in the



western Europe, and the yellow-necked mouse is more numerous in the east (Pucek 1984). These factors can have an influence on the morphology and biology of trypanosomes parasitizing these closely related rodents.

In view of the above arguments and counterarguments, we can recognize trypanosomes found in the yellow-necked mouse *A. flavicollis* as a new subspecies, *Trypanosoma (Herpetosoma) grosi kosewiense* subsp. n.

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## REFERENCES

- Bajer A., Bednarska M., Pawełczyk A., Konopka E., Karbowski G., Siński E. (1998) Blood parasites in a wild rodent community of Mazury Lakes District, Poland. *Wiad. Parazytol.* **44**: 426
- Calvo M. M. de L., Nogueira B., Alejandro R. (1992) La vía oral: una puerta de acceso para *Trypanosoma cruzi*. *Rev. Latinoam. Microbiol.* **34**: 39-42
- Dávila A. M. R., Ramirez L., Silva R. A. M.S. (1998) Biometrical alterations of *Trypanosoma evansi* isolate in laboratory rodents. *Vet. Parasit.* **76**: 149-52
- Davis B. S. (1952-53) Studies on the trypanosomes of some California mammals. *Univ. Calif. Publ. Zool.* **57**: 145-250
- Hoare C. A. (1972) The Trypanosomes of Mammals. Blackwell Scientific Publications, Oxford
- Hoffmeyer I. (1973) Interaction and habitat selection in the mice *Apodemus flavicollis* and *A. sylvaticus*. *Oikos* **24**: 108-116
- Karbowski G., Siński E. (1996) Occurrence and morphological characteristics of a *Trypanosoma evotomys* strain from North Poland. *Acta Parasit.* **41**: 105-107
- Karbowski G., Wita I., Czaplinska U., Polishchuk I. K. (1998 a) *Trypanosoma (Herpetosoma)* sp. in the social vole (*Microtus socialis*) for first time in Ukraine. *Wiad. Parazytol.* **44**: 466
- Karbowski G., Wita I., Rychlik L., Gadomska K. (1998 b) The morphology of *Herpetosoma* trypanosomes in small rodents in Poland. *Wiad. Parazytol.* **44**: 467
- Keymer I. F. (1967) *Trypanosoma (Megatrypanum)* spp. Hoare, 1964 of ruminants. *Vet. Rec.* **80**: 286
- Kingston N. (1973) Trypanosomes from elk (*Cervus canadensis*) in Wyoming. *J. Parasit.* **95**: 1132-1133
- Kingston N., Bobek B., Perzanowski K., Wita I., Maki L. (1992) Description of *Trypanosoma (Megatrypanum) stefanskii* sp. n. from Roe Deer (*Capreolus capreolus*) in Poland. *J. Helminth. Soc. Wash.* **59**: 89-95.
- Krampitz H. E. (1959) Über das europäische Waldmastrypanosom, *Trypanosoma grosi* Laveran et Pettit 1909 (Protomonadina, Trypanosomidae). *Z. ParasitKde* **19**: 232-258
- Kruszewicz A.G. (1994) Związek parazytologii z ornitologią. Część II. Przegląd systematyczny pasożytów i możliwości diagnozowania inwazji. *Notatki orn.* **35**: 421-430 (in Polish)
- Laveran A., Pettit A. (1909) Sur le trypanosome du mulot *Mus sylvaticus* L. *C. R. Soc. Biol., Paris* **67**: 564-565
- Macdonald D. W., Anwar M., Newman C., Woodroffe R., Johnson P. J. (1999) Inter-annual differences in the age-related prevalences of *Babesia* and *Trypanosoma* parasites of European badgers (*Meles meles*). *J. Zool. (London)* **247**: 65-70
- Maraghi S., Molyneux D. H., Wallbanks K. R. (1989) Differentiation of rodent trypanosomes of the subgenus *Herpetosoma* by lectins. *Trop. Med. Parasit.* **40**: 273-278
- Maraghi S., Wallbanks K. R., Molyneux D. H. (1995) Oral transmission of trypanosomes of the subgenus *Herpetosoma* from small mammals. *Parasit. Res.* **81**: 693-695
- Mathew C. J., Nedunchellian S., Venkataraman K. S. (1992) Biometrical observations on different strains of *Trypanosoma evansi*. *Vet. Parasit.* **43**: 143-145
- Matthews M. J., Kingston N., Morton J. K. (1977) *Trypanosoma cervi* Kingston and Morton, 1975 from mule deer, *Odocoileus hemionus*, in Wyoming. *J. Wildl. Dis.* **13**: 33-39
- McHolland-Raymond L. E., Kingston N., Trueblood M. S. (1978) Continuous cultivation of *Trypanosoma theileri* at 37 C in bovine cell culture. *J. Protozool.* **25**: 388-394
- Mohamed H. A., Molyneux D. H. (1987) *In vitro* cultivation of *Herpetosoma* trypanosomes in insect cell tissue culture media. *Parasit. Res.* **73**: 9-14
- Mohamed H. A., Molyneux D. H., Scott C. M. (1987) Isoenzyme characterization of trypanosomes of the subgenus *Herpetosoma*. *Parasitology* **94**: 39-48
- Molyneux D. H. (1976) Biology of trypanosomes of the subgenus *Herpetosoma*. In: Biology of the Kinetoplastida (Eds. W. H. R. Lumsden, D. E. Evans). Academic Press, London, **1**: 285-325
- Morton J. K., Kingston N. (1976) Further studies on trypanosomes in game animals in Wyoming. *J. Wildl. Dis.* **12**: 233-236
- Noyes H. A., Ambrose P., Barker F., Begon M., Bennet M., Bown K. J., Kemp S. J. (2002) Host specificity of *Trypanosoma (Herpetosoma)* species: evidence that bank voles (*Clethrionomys glareolus*) carry only one *T.(H.) evotomys* 18S rRNA genotype but wood mice (*Apodemus sylvaticus*) carry at least two phylogenetic parasites. *Parasitology* **124**: 185-190
- Pucek Z. (1984) Keys to Vertebrates of Poland. Mammals. PWN, Warszawa
- Šebek Z. (1960) Některé nové poznatky o krevních parazitech našich hmyzožravců a myšovitých. *Vlastiv. Sb. Vysočiny, Odd. Ved přír.* **75-80** (in Czech)
- Šebek Z. (1975) Blutparasiten der wildlebenden Kleinsäuger in der Tschechoslowakei. *Folia Parasit. (Praha)* **22**: 11-20
- Šebek Z., Sixl W., Stünzner D., Valová M., Hubálek Z., Troger H. (1980) Zur Kenntnis der Blutparasiten wildlebender Kleinsäuger in der Steiermark und im Burgenland. *Folia Parasit. (Praha)* **27**: 295-301
- Thiroux A. (1905) Recherches morphologiques et expérimentales sur *Trypanosoma duttoni* (Thiroux). *Annl. Inst. Pasteur* **19**: 565-572
- Urdaneta-Morales S. (1983) Pleomorphism in trypomastigotes of *Trypanosoma cruzi* from blood and cell culture. *Trop. Med. Parasit.* **34**: 225-228

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## A New Coccidian Parasite, *Isoospora samoensis*, from the Wattled Honeyeater (*Foulehaio carunculata*) from American Samoa

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**Summary.** A new species of *Isoospora* is described from the feces of the wattled honeyeater, *Foulehaio carunculata* from American Samoa. Numerous oocysts of similar morphology were found in a single adult wattled honeyeater. Sporulated oocysts are ovoid,  $28.9 \times 26.1$  ( $25-32 \times 23-30$ )  $\mu\text{m}$ , with a smooth, colorless, bilayered wall; the inner wall is slightly thicker and darker than the outer wall. The average shape index is 1.1. No micropyle or oocyst residuum are present but the oocyst contains one or two ovoid polar granules. Sporocysts are ovoid,  $17.1 \times 10.9$  ( $16-18 \times 10-11$ )  $\mu\text{m}$  with a smooth single layered wall and an average shape index of 1.6. The Stieda body is broad, dome-like with a rather rectangular-shaped substieda body. Within the sporocyst is a large amorphous residuum composed of coarse granules and 4 randomly arranged, sausage-shaped sporozoites with a subspherical, posterior refractile body and a centrally located nucleus.

**Key words:** Coccidia, *Isoospora samoensis* sp. n.

### INTRODUCTION

As one of the most widely distributed birds of Fiji, Tonga, and Samoa, the wattled honeyeater (*Foulehaio carunculata*) may be found in any habitat from montane forest to mangroves along the seaward edge (Watling 1982). An endemic bird to the region and common inhabitant of suburban gardens, *F. carunculata*

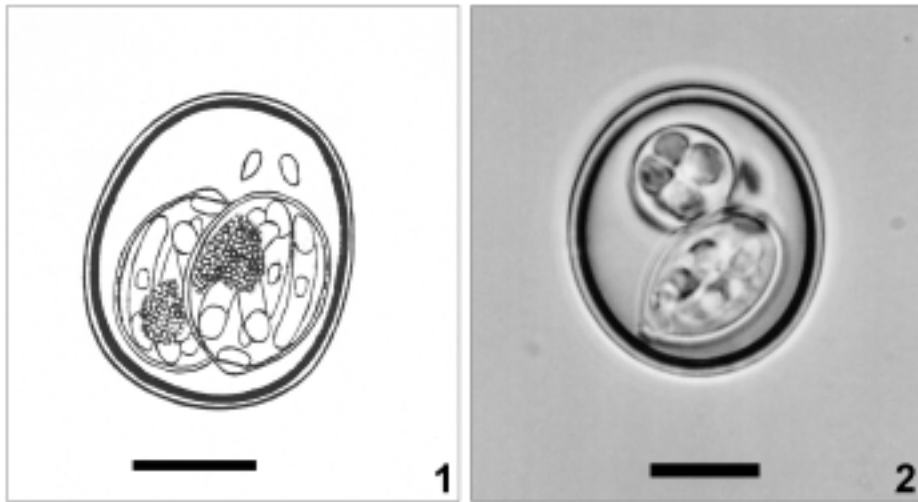
is a medium-sized bird with drab olive-green plumage. It has a paler underside, which may appear scaled, a fine, slightly down-curved bill, and a conspicuous yellow wattle below its eye. Although drab in appearance, this bird is an attractive songster. Its down-curved bill, distinctive feeding behavior, and voice distinguish it from other birds (Watling 1982).

*Foulehaio carunculata* eats primarily nectar but also fruit and a fair proportion of spiders and insects during breeding season. It also kills lizards and geckos, which it sometimes eats.

No prior studies have reported coccidian parasites in *F. carunculata* or in any other species in the genus. This

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**Figs 1, 2.** *Isospora samoensis* sp. n. from *Foulehaio carunculata*. **1** - composite line drawing of sporulated oocyst; **2** - photomicrograph of sporulated oocyst. Scale bars 10 µm.

paper describes a new coccidian species found in the Wattled Honeyeater, *F. carunculata*.

## MATERIALS AND METHODS

One *F. carunculata* was captured and a fecal sample was obtained on November 28, 2001 from the island of American Samoa in the South Pacific. The sample was sent to the second author's laboratory for examination and was received on July 15, 2002. Procedures for preserving fecal material and for measuring and photographing oocysts were described in McQuiston and Wilson (1989). All measurements are presented in µm with size ranges in parentheses following the means. Oocysts were approximately 8 months old when examined, measured, and photographed.

## RESULTS

### *Isospora samoensis* sp. n. (Figs 1, 2)

**Description of oocysts:** Oocysts ovoid,  $28.9 \times 26.1$  (25-32, SD=2.09  $\times$  23-30, SD=1.94) (N=26) with a smooth, bilayered wall; the inner wall slightly thicker and darker than the outer wall. The shape index (length/width) is 1.1 (1-1.3, SD=0.08). Micropyle and oocyst residuum are absent, but one or two ovoid polar granules are present. Sporocyst ovoid,  $17.1 \times 10.9$  (16-18, SD=0.59  $\times$  10-11, SD=0.48) (N= 13) with a smooth single layered wall; shape index 1.6 (1.5-1.7, SD=0.10).

The Stieda body is broad, dome-like with a rather rectangular-shaped substieda body. The sporozoites are sausage shaped with a slightly ovoid, posterior refractile body and a smaller, centrally located nucleus. The sporozoites are randomly placed in the sporocyst with a large, amorphous residuum composed of coarse granules.

**Type host:** *Foulehaio carunculata* (Gmelin, 1788) wattled honeyeater (Passeriformes: Meliphagidae).

**Type specimens:** A phototype series and buffered formalin-preserved sporulated oocysts of *Isospora samoensis* sp. n. are deposited in the Harold W. Manter Laboratory of Parasitology, University of Nebraska State Museum, Lincoln, Nebraska 68588, accession no. HWML 45410 (syntypes) and HWML 45411 (phototypes).

**Type location:** American Samoa, Tau village on Tau Island: 14°14'01"S, 169°30'52"W

**Prevalence:** 1/1 was infected with *Isospora samoensis*.

**Sporulation time:** Unknown, oocysts were partially sporulated when received at the laboratory and became fully sporulated after exposed to air for several days.

**Site of infection:** Unknown, oocysts found in feces.

**Etymology:** *samoensis* means found on the island of Samoa.

**Remarks:** The only bird sampled was passing hundreds of oocysts. All the oocysts appeared morphologically similar and unique to the host.

The oocysts appeared unusually sensitive to osmotic pressure. Many oocysts buckled within 1-2 h after floating in Sheather's sugar solution.

## DISCUSSION

The isosporan coccidia are protozoan parasites with a direct life cycle and no intermediate hosts. Oocysts develop endogenously, are passed in the feces, and sporulate in the environment. Birds become infected when they ingest the sporulated oocysts while feeding.

It is unusual to find coccidian oocysts in the feces of a fruit eating avian host. McQuiston (2000) sampled 655 South American passerine birds representing 190 species and compared the prevalence of coccidian parasites with host diet, habitat and behavior. Only 1.1% of birds whose primary diet was fruit were passing coccidian oocysts. However, the study also reported that hosts whose diet included ground insects had a prevalence rate of coccidia of 22.8%. Since wattled honeyeaters forage on the ground for spiders and insects to supplement their diet, this is most likely the mechanism for transmission of the coccidian parasite.

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## REFERENCES

- McQuiston T. E. (2000) The prevalence of coccidian parasites in passerine birds from South America. *Trans. Ill. State Acad. Sci.* **93**: 221-227
- McQuiston T. E., Wilson M. (1989) *Isoospora geospizae*, a new coccidian parasite (Apicomplexa: Eimeriidae) from the small ground finch (*Geospiza fuliginosa*) and the medium ground finch (*Geospiza fortis*) from the Galapagos Islands. *Syst. Parasitol.* **14**: 141-144
- Watling D. (1982) *Birds of Fiji, Tonga, and Samoa*. Millwood Press, Wellington, New Zealand

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## New Species of *Odonaticola* Sarkar *et* Haldar, 1981 (Apicomplexa: Conoidasida) from Dragonflies (Insecta: Odonata) in West Bengal, India

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**Summary.** Descriptions of four new species of the genus *Odonaticola* Sarkar *et* Haldar, 1981 (Apicomplexa: Conoidasida) from dragonflies (Insecta: Odonata) in the family Libellulidae in West Bengal are presented. These include: *Odonaticola bradinopyga* sp. n. from *Bradinopyga geminata*; *O. aspinosa* sp. n. from *Crocothemis servilia servilia*; *O. abhoytura* sp. n. from *Pantala flavescens* and *O. amojya* sp. n. from *C. s. servilia*.

**Key words:** Apicomplexa, Conoidasida, Odonata, *Odonaticola abhoytura* sp. n., *O. amojya* sp. n., *O. aspinosa* sp. n., *O. bradinopyga* sp. n.

### INTRODUCTION

Ever since Sarkar and Haldar (1981) established the genus *Odonaticola* (Apicomplexa: Conoidasida) from *Brachythemis contaminata* (Fabricius, 1793), it has been a fascinating object of research to protozoan taxonomists. The genus has a wide-ranging distribution. It has been reported from Japan, diverse localities in the Indian subcontinent and also has been observed in odonates of Thailand and Indonesia (personal observa-

tion D. P. Haldar). Amoji and Kori (1992) revised the genus and listed 13 species of *Odonaticola*. However, they missed the name of *O. pantalae* Prema *et* Janardanan, 1991 from *Pantala flavescens* (Fabricius, 1798). Prasad and Janardanan (1994) added another species *O. neurothemisi*, and Haldar and Biswas (2002) described three more new species in the genus. Hoshide and Janovy (2002) published a SEM account of the nucleus of *O. polyhamatus*. The study of gregarines from odonates assumes further importance since the hosts are highly beneficial and wonderful predators for many known and unknown insect pests.

Keeping this in mind we continued our study on the gregarine parasites of odonates and have discovered four more new species which have been described in this communication.

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The species of gregarines placed in *Odonaticola* differ principally in: (1) the number of petaloid spines on the epimerite; (2) length of the neck; (3) measurements and ratios of the body parts; (4) host species; and (5) geographical distribution. In all cases the body of the host is enormously long and a heavy load of parasites is present in the gut of infected animals. It may be extremely difficult for the hosts to fly easily and thus they may fall prey to a diversity of predators. Because ecologically the odonates are powerful determining factors in preserving the balance of life in ponds, rivers, lakes and their surroundings (Tillyard 1917), any decrease in their numbers may prove to be detrimental. It is, therefore, time to think about their gradual decline in numbers and to carry out research on appropriate natural conservation methods so that these beautiful creatures do not disappear altogether.

## MATERIALS AND METHODS

Odonates do not survive in captivity, hence the digestive tracts were examined immediately after they were collected from the fields of Kalyani, Chandannagar, Abhoypur and Karimpur in the districts of Nadia and Hooghly (23-25°N and 88-89°E). Rest of the employed methods have been followed after Haldar and Biswas (2002) as elaborated hereunder.

Dragonflies were decapitated and then dissected under a dissecting Olympus No. 1116 binocular. The entire gut was carefully taken out and placed on a glass slide with a little drop of 0.5% saline solution. The gut was gently teased with needles for the parasites to come out of the gut lumen. The presence of parasites within the gut was observed by an Olympus CH2 phase contrast microscope in living condition. Thin smears were prepared on glass slides and the slides containing gregarines were fixed in Schaudinn's fluid for 20 min. Fixed slides were kept in 70% alcohol overnight to remove the excess mercuric chloride. Slides were then mordanted overnight in 3% iron alum and stained with Heidenhain's haematoxylin for 20 min. Differentiation of staining was done with 1% iron alum and slides were then thoroughly washed under running tap water, dehydrated in ascending grades of alcohol, cleared in xylene and mounted in DPX.

Gametocyst were collected from faecal matter or hindgut of infected hosts. For observing sporulation, the technique of Sprague (1941) with slight modifications has been followed.

For normal development of eugregarine cysts, highly successful results were obtained by incubating them at room temperature in moist chambers constructed in the following manner.

Two pieces of blotting papers were placed on both inner surfaces of the chamber and cysts were placed in a drop of normal saline (0.5%) on a glass slide and kept within moist chambers for at least 24 h. Cysts were observed from time to time under the microscope; mode of dehiscence of cysts was observed in this condition. Cysts ruptured to release innumerable oocysts with or without filaments; liberated oocysts were removed from the moist chamber at regular intervals and stages of development of sporozoites were also noted.

A drop of oocyst suspension was taken on a glass slide and a drop of Lugol's iodine solution was added to it; developmental stages of sporozoites were observed under oil immersion lens. Figures of stained specimens were drawn with the aid of a camera lucida using a YOMA microscope, measurements were taken using an ocular micrometer calibrated with a stage micrometer.

The following abbreviations are used: DG - diameter of gametocyst; LD - length of deutomerite; LE - length of epimerite; LEN - length of epimerite neck; LN - length of nucleus; LO - length of oocyst; LP - length of protomerite; TL - total length; WD - width of deutomerite; WE - width of epimerite; WN - width of nucleus; WO - width of oocyst; WP - width of protomerite. The ratios used are the ratio of the length of protomerite to total length (LP: TL) and the ratio of the width of protomerite to the width of deutomerite (WP: WD).

Primary types are deposited in the following collections: PLUK - Protozoology Laboratory, University of Kalyani, West Bengal, India; HWML - Harold W. Manter Laboratory of Parasitology, University of Nebraska, Lincoln, Nebraska, USA.

## RESULTS

### *Odonaticola* Sarkar *et* Haldar, 1981

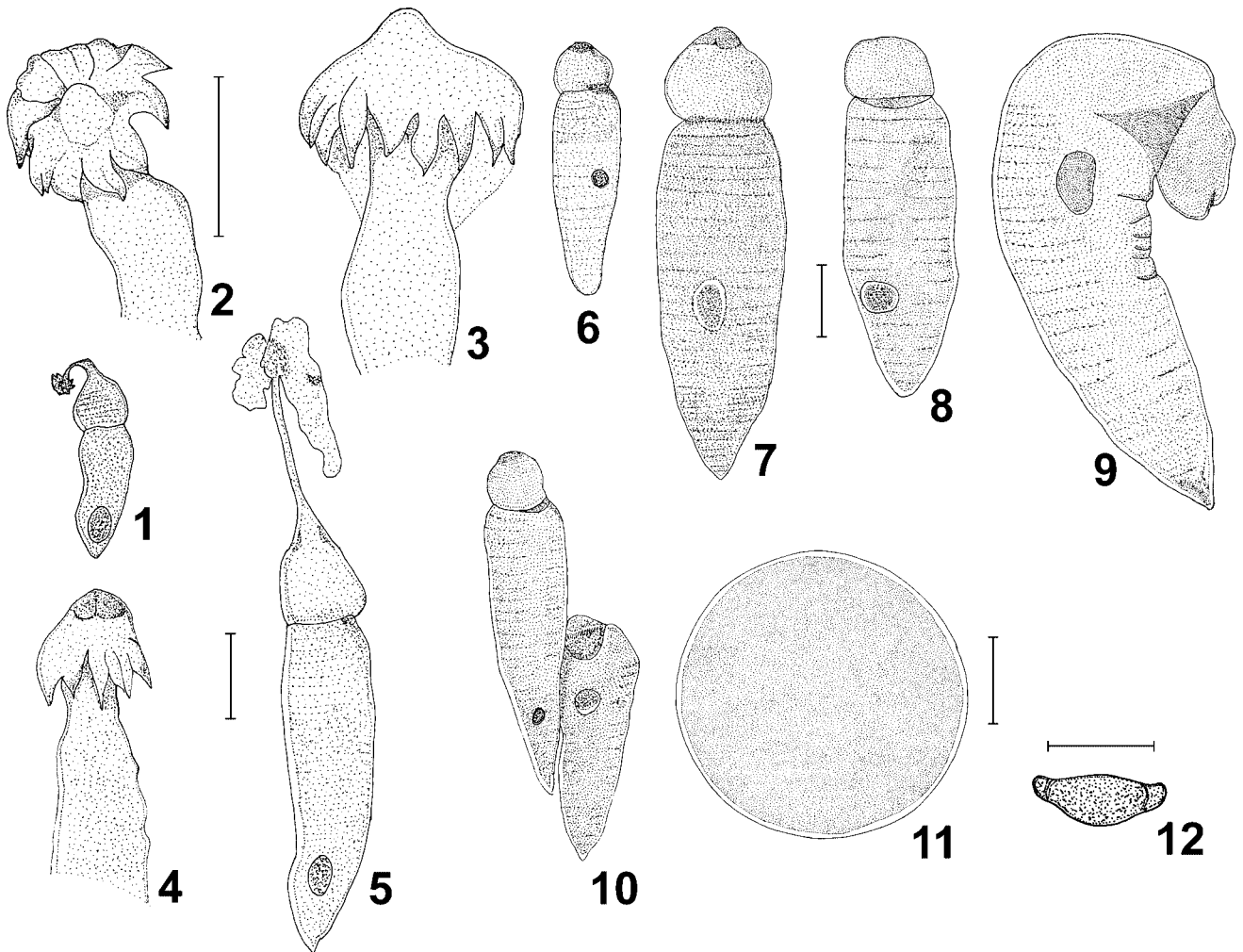
**Diagnosis:** The gregarines parasitizing odonates are very large and are placed in the genus *Odonaticola* (Actinocephalidae: Menosporinae) based on the following diagnostic features: epimerite hat-shaped; marginal spines petaloid; neck long; sporadins solitary; gametocysts dehisce by simple rupture; and, oocysts smooth, boat-shaped.

**Type species:** *Odonaticola hexacantha* Sarkar *et* Haldar, 1981; by original designation.

**Remarks:** The septate gregarines (Apicomplexa: Conoidasida) are endoparasitic protozoans commonly found in the digestive tracts of various terrestrial arthropod hosts. Previously it was believed that flying insects such as dragonflies (Order Odonata) were less heavily parasitized by protozoan parasites, particularly by eugregarines, than other ground dwelling terrestrial insects. However observations made during the past few years have revealed the presence of a number of eugregarine protozoan parasites in their midgut (Haldar 1992).

### *Odonaticola bradinopyga* sp. n. (Figs 1-12)

**Trophozoite:** Epimerite consisting with long slender neck and hat- or umbrella-shaped knob at apex; edge of knob drawn into 10-12 petaloid lobes, each lobe gradually tapered to sharp tip and marked with central longitudinal line; protomerite conical; deutomerite cylindrical-conical or fusiform; cytoplasm densely granulated and



**Figs 1-12.** *Odonaticola bradinopyga* sp. n. trophozoites: **1** - entire view; **2, 3** - epimerite fronto-lateral view; **4** - epimerite lateral view; **5** - epimerite attached to gut tissue of host sporadins; **6** - early stage; **7-9** - various shapes, sizes and positions; **10** - sidewise association. **11** - gametocyst, advanced stage; **12** - oocyst. Scale bars 10  $\mu\text{m}$  (**12**); 20  $\mu\text{m}$  (**2, 3**); 100  $\mu\text{m}$  (**1, 4-10**); 200  $\mu\text{m}$  (**11**).

lodges an ovoidal nucleus; nucleus position variable from mid body to periphery.

**Sporadin:** Characteristically solitary; protomerite more or less bell-shaped; deutomerite cylindro-conical in shape; cytoplasm densely granulated and with numerous transverse striations of myoneme fibrils; body highly flexible.

**Association:** Sidewise between two morphologically variable sporadins.

**Gametocyst:** Colour milky white, covered by thin ectocyst. After 88 h, ectocyst detaches from cyst wall followed by noticeable appearance of some notches on its surface; after 96 h cyst wall bursts by simple rupture and oocysts liberated.

**Oocyst:** Boat-shaped when single; triangular-shaped when in clusters of three.

**Measurements** (in  $\mu\text{m}$ ) (n = 20):

Trophozoite: TL = 333-1029 (595); LE = 36-246 (134.9); WE = 14-72(31.7); LP = 58-130(83.3); WP = 65-196 (113.2); LD = 188-681(376.7); WD = 58-188(124.1); LEN = 58-217 (128.3); WN = 22-51(35.3); LN = 36-72 (48.9)

Sporadin: TL = 222-1405 (666.9); LP = 71-174 (113.4); WP = 75-188 (131.9); LD = 151-1261 (553.5); WD = 75-232 (158.4); LN = 27-72 (52.9); WN = 18-58 (40.1); DG = 656-670; LO = 12-13; WO = 6; LP: TL = 1: 3.8-9.4; WP: WD = 1: 0.8-2.1

**Taxonomic summary**

**Type material:** Catalog No.: 1-14. 13 slides containing syntypes have been deposited at the PLUK; 1 slide containing hapantotype has been deposited at the HWML, Lincoln, Nebraska, U.S.A; Catalog No.: HWML 45347

**Type locality:** India, West Bengal, Kalyani in Nadia district, Kalyani University campus.

**Host:** Dragonfly *Bradinopyga geminata* (Rambur, 1842) (Insecta: Odonata: Anisoptera: Libellulidae)

**Location in host:** Midgut.

**Incidence of infection:** Of 100 odonate hosts examined 44 (44.5%) were found to be infected.

**Etymology:** The specific epithet is derived from the genus of host from which the gregarine was collected.

**Remarks:** For the first time in India, *Bradinopyga geminata* (Rambur, 1842) has been recorded to host a species of the genus *Odonaticola*. In *Odonaticola bradinopyga* the epimerite consists of a long slender neck with a spherical hat-shaped structure at its apex. The tip looks like an umbrella, in which the edge is drawn out into 10-12 petaloid lobes that are at the tips and marked with a central longitudinal line. Other species described by Sarkar and Haldar (1981) with a hat-shaped epimerite are characterized by variable number of petaloid spines, namely: *O. hexacantha*, the type species of the genus has 6; *O. longicollara* has 7; *O. rodgii* has 8; and *O. orthetri* has several petaloid spines. Six petaloid spines also are present in *O. neurothemisi* Prasad and Janardanan, 1994 although it differs from the type species in other features. Oocysts are boat-shaped in all the previously described species along with two short projections on the sides in *O. hexacantha* and triangular when in clusters of three in the present species. The sporadins are long enough in *O. longicollara* and *O. rodgii* and resemble the newly described species. In addition, the hosts and the collection localities are also different in all the species. Hence we consider this gregarine to represent a new species.

***Odonaticola aspinosa* sp. n. (Figs 13-22)**

**Trophozoite:** Epimerite blunt umbrella-like with long slender neck; characteristic petaloid spines absent; protomerite hemispherical; deutomerite almost spherical; nucleus large spherical with distinct endosome.

**Sporadin:** Solitary; protomerite cylindrical in smaller forms; deutomerite almost spherical with rounded nucleus. Association: biassociative forms rarely found; two associating partners differ greatly in morphology. Gametocyst: large, milky white spherical bodies; two unequal game-

toocytes covered by thin uneven ectocyst; oocysts liberate by simple rupture after 48 h.

**Oocyst:** Boat shaped.

**Measurements** (in  $\mu\text{m}$ ) (n= 20):

Trophozoite: TL = 291-728 (441.7); LE = 93-198 (133.7); WE = 7-52 (24.4); LP = 43-156 (93.6); WP = 57-125 (75.8); LD = 114-374 (214.5); WD = 100-218 (161.2); LN = 16-88 (40.2); WN = 21-43 (33.6)

Sporadin: TL = 191-426 (255.7); LP = 70-100 (76.0); WP = 41-80 (54.6); LD = 92-348 (173.8); WD = 101-165 (189.1); LN = 21-64 (41.5); WN = 21-50 (36.4); DG = 329; LO = 6; WO = 4; LP: TL = 1: 1.9-8.0 (3.7); WP: WD = 1: 1.6-2.7 (2.2)

**Taxonomic summary**

**Type material:** Catalog No.: 1-14. 13 slides containing syntypes have been deposited at the PLUK; 1 slide containing hapantotypes has been deposited at the HWML, Lincoln, Nebraska, U.S.A; Catalog No.: HWML 16519

**Type locality:** India, West Bengal, Hooghly district, Chandannagar.

**Host:** Dragonfly *Crocothemis servilia servilia* (Drury, 1773) (Insecta: Odonata: Anisoptera: Libellulidae)

**Location in host:** Midgut.

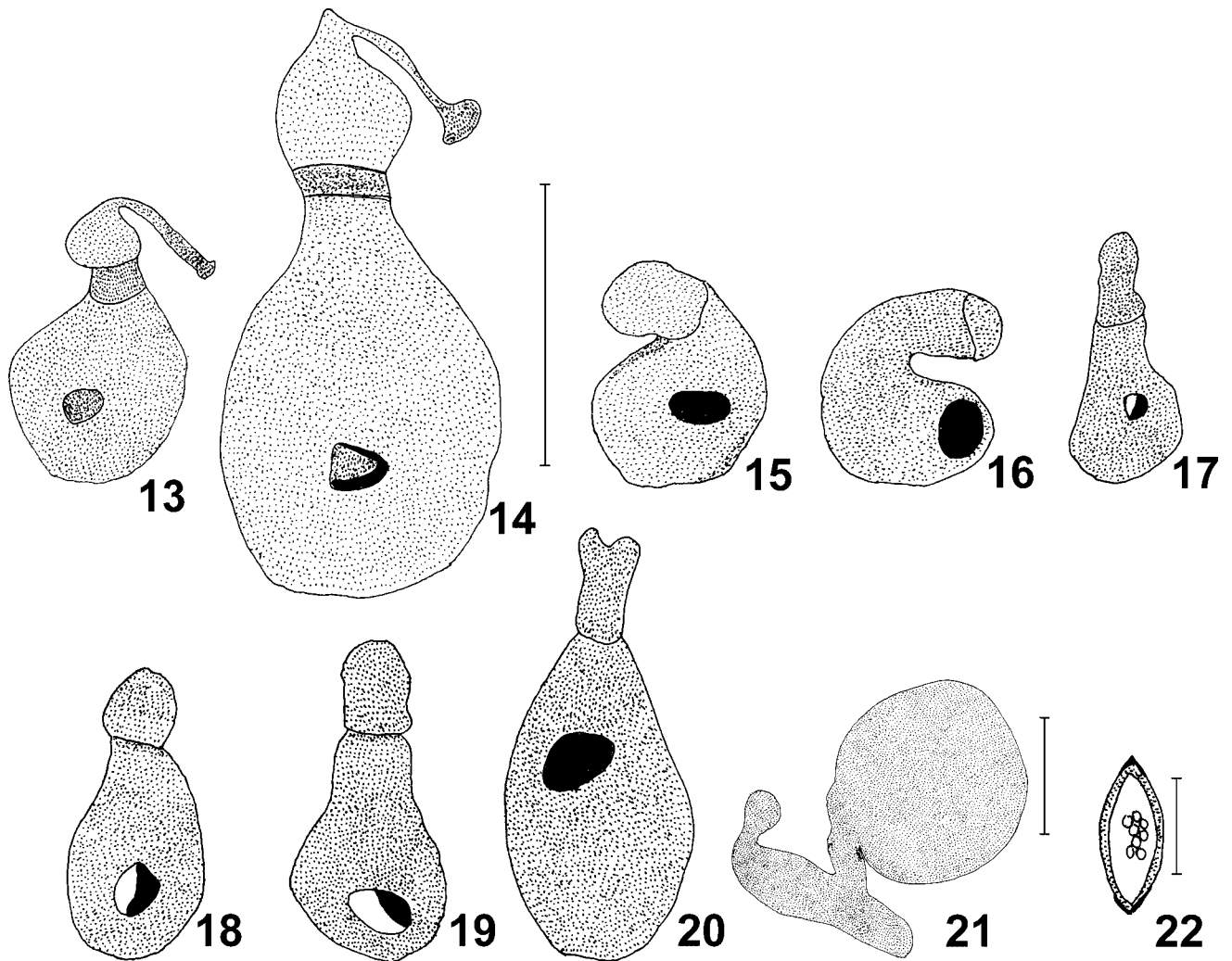
**Incidence of infection:** Of 958 dragonfly hosts examined 33 (3.4 %) were found to be infected.

**Etymology:** Due to the absence of characteristic petaloid spines, the name *aspinosa* has been proposed for it.

**Remarks:** Based on having solitary sporadins, umbrella-like epimerite with long slender neck, simple rupture of gametocysts and boat-shaped oocysts, the present species justifies being placed in the genus *Odonaticola*.

The newly described species resembles *Odonaticola crocothemis* Kori *et* Amoji, 1983 in maximum length of trophozoite and shape of oocysts but can be separated based on differences in the shape of the epimerite, gametocyst and statistical measurements. In both cases the host insect is *Crocothemis servilia servilia* (Drury). The presently described species resembles *O. magnus* (Hoshide, 1958) (= *Hoplorhynchus magnus*) nov. comb. in having an umbrella-like epimerite, boat-shaped oocysts which vary in dimensions but is dissimilar in shape and length of sporadin, diameter of gametocyst and other morphometric values. The average length of trophozoites in *O. elliptica* Sarkar, 1981 and the newly described form are nearly identical besides other similarities like spherical shape of gametocyst with thin cyst wall and boat-shaped oocysts; both also infect the same host.





**Figs 13-22.** *Odonaticola aspinosa* sp. n. **13** - young trophozoite; **14** - mature trophozoite; **15-17** - early stage sporadin; **18-20** - mature sporadin; **21** - gametocyst dehiscing by simple rupture; **22** - oocysts with eight sporozoites. Scale bars 10  $\mu$ m (22); 200  $\mu$ m (13, 14); 200  $\mu$ m (15-21).

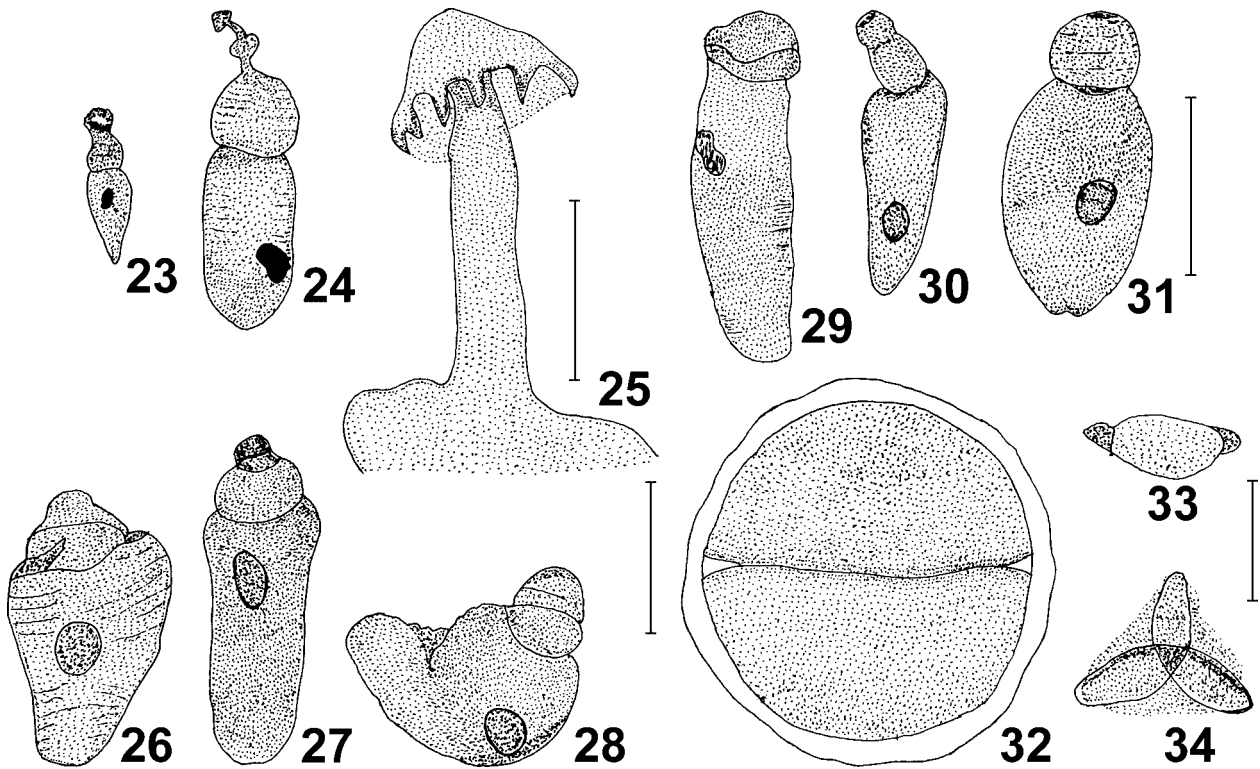
However the two species differ in the structure of epimerite which is knob-like with 10 petaloid recurved spines at its periphery with a long neck, a bell- or dome-shaped protomerite and a long slender deutomerite in *O. elliptica* and the epimerite is umbrella-like with a long slender neck, devoid of any petaloid spines in the present form, a feature that at once distinguishes it from *O. elliptica*; the shape and size of the protomerite and deutomerite are also different in the two species.

Based on differences in a number of features with other species inhabiting the same host, we designate *Odonaticola aspinosa* as a new species.

#### *Odonaticola abhoypura* sp. n. (Figs 23-34)

**Trophozoite:** Epimerite bud-like with short neck; drawn into 7-11 petaloid recurved spines placed at noticeable distances from each other; protomerite globular; deutomerite cylindro-conical; epimerite in fully grown trophozoites with a swollen bulbous portion at the middle of neck; nucleus ovoid or slightly crescentic; cytoplasm densely granular with crosswise striations.

**Sporadin:** Solitary; protomerite globular, conical, rectangular or cylindrical; deutomerite ellipsoid, tubular or cylindro-conical; nucleus ovoid, ellipsoid or bilobed, posi-



**Figs 23-34.** *Odonaticola abhoypura* sp. n. 23 - young trophozoite; 24 - mature trophozoite; 25 - epimerite, lateral view; 26-29 - early sporadin; 30, 31 - mature sporadin; 32 - early gametocyst; 33 - single oocyst; 34 - cluster of oocysts. Scale bars 10  $\mu$ m (33, 34); 20  $\mu$ m (23-25); 200  $\mu$ m (26-32).

tion variable; larger cytoplasmic granules and crosswise myonemmal striations present.

**Association:** Biassociative forms rarely found; partners differ greatly in shape and size.

**Gametocyst:** Rounded or slightly ovoid; double-walled enclosing two unequal gametocytes; dehisces by simple rupture at 72 h.

**Oocyst:** Boat-shaped when liberated singly, triangular-shaped when in clusters of three.

**Measurements** (in  $\mu$ m) (n=20):

Trophozoite: TL = 200-404 (336.7); LE = 40-120 (64.4); WE = 15-71 (39.6); LP = 29-89 (51.4); WP = 44-111 (85.5); LD = 131-280 (220.9); WD = 58-138 (108.4); LN = 33-53 (45.3); WN = 18-35 (26.6); LEN = 15-98 (61.4)

Sporadin: TL = 200-1492 (510.6); LP = 49-188 (92.7); WP = 67-362 (130.7); LD = 151-1318.0 (417.9); WD = 71-536 (200.0); LN = 35-130 (58.1); WN = 20-87 (37.4); DG = 524 x 508; LO = 13; WO = 6; LP: TL = 1: 2.7-10.5; WP: WD = 1: 0.5-3.0

#### Taxonomic summary

**Type material:** Catalog No.: 1-14. 13 slides containing syntypes have been deposited at the PLUK; 1 slide containing hapantotypes has been deposited at the HWML, Lincoln, Nebraska, U.S.A; Catalog No.: HWML 45351

**Host:** *Pantala flavescens* (Fabricius, 1798) (Insecta: Odonata: Anisoptera: Libellulidae)

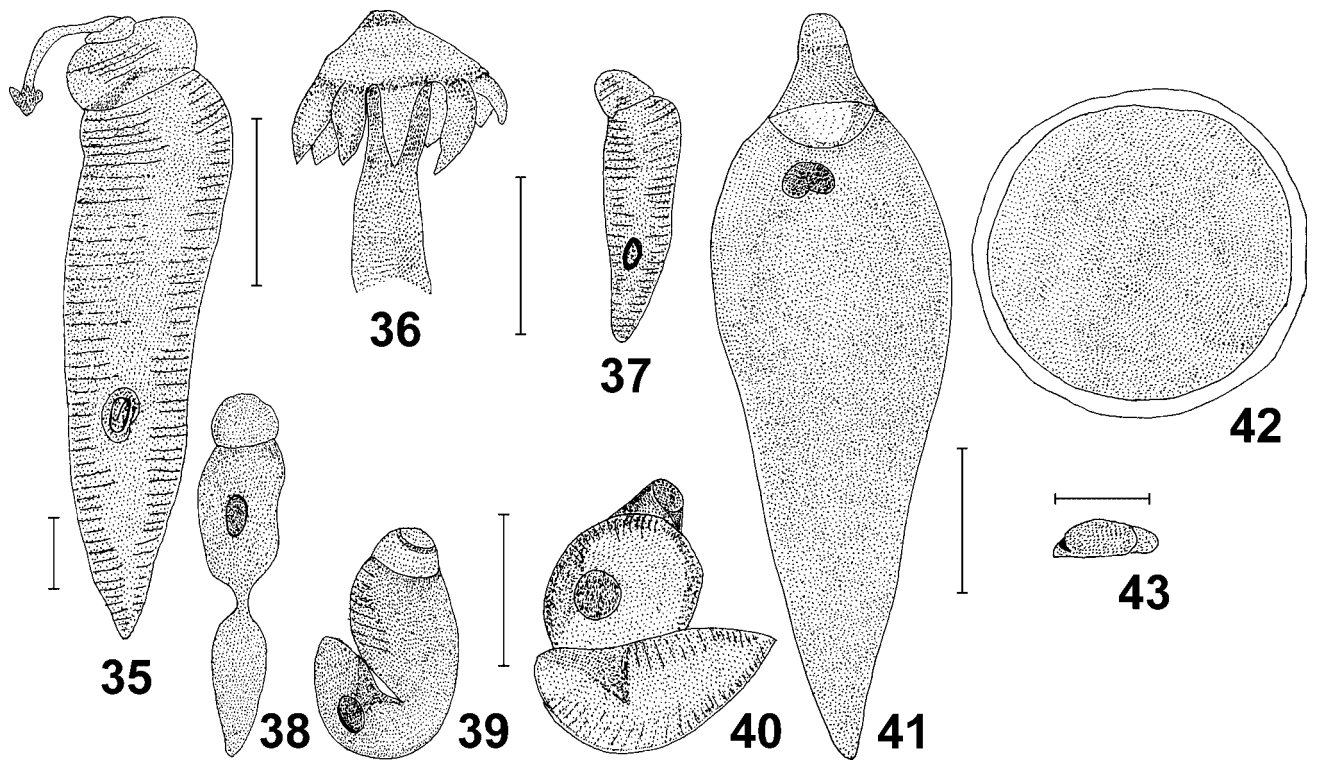
**Type locality:** India, West Bengal, Nadia district, Abhoypur (Karimpur)

**Location in host:** Midgut.

**Incidence of infection:** Of 55 dragonfly hosts examined 15 (25.4 %) were infected.

**Etymology:** The species is named after the locality where the host dragonflies were obtained.

**Remarks:** In 1991, Prema and Janardanan reported a new species *Odonaticola pantalae* from the dragonfly *Pantala flavescens* (Fabricius, 1798) in Kerala. Interestingly the gregarine under report obtained from



**Figs 35-43.** *Odonaticola amojya* sp. n. **35** - mature trophozoite; **36** - epimerite details; **37, 38** - sporadins different shapes and sizes; **39, 40** - bending position; **41** - mature sporadin; **42** - gametocyst, freshly formed; **43** - oocyst. Scale bars 10  $\mu$ m (43); 20  $\mu$ m (36); 100  $\mu$ m (37, 38); 200  $\mu$ m (35, 39-42).

the same host about 2000 km apart from Kerala shares all the basic characters with *O. pantalae*, for which its inclusion under the genus *Odonaticola* is beyond doubt. The epimerite is hat-shaped with 7-11 petaloid spines and a long slender neck in the gregarine under report. The size of the trophozoite and sporadin is much smaller in our new species. In *O. pantalae* the spherical gametocyst is provided with a thick ectocyst and its oocysts are boat-shaped having two short projections at each pole, while in the present form, gametocysts are either round or ovoid and oocysts do not bear any spines. Thus we designate this gregarine as a new species of the genus *Odonaticola* for which the name *O. abhoyapura* sp. n is proposed.

***Odonaticola amojya* sp. n. (Figs 35-43)**

**Trophozoite:** Epimerite with long slender straight or bent neck and conical umbrella- or hat-shaped triangular knob at apex; edge drawn into 10 petaloid recurved spines at periphery by which parasites seem to stick to gut epithelium of host; protomerite more or less dome-shaped; deutomerite long fusiform; cytoplasm granular;

nucleus ovoid at variable positions; transverse myoneme fibrils clearly discernable; pellicle highly flexible.

**Sporadin:** Solitary; slender when young bearing scars of just-detached epimerite at top; short rudimentary epimerite retained in a few sporadins; protomerite bell- or dome-shaped; deutomerite long slender; densely granular, traversed by transverse myonemmal lines; position of nucleus variable; very large sporadins bearing short slender nipple-like protomerite attached with gigantic posteriorly tapering deutomerite characteristically observed. Association: rarely biassociative; partners differ greatly in shape and size. Gametocyst: creamy white; globular, covered by transparent ectocyst; dehisce by simple rupture at 72 h.

**Oocyst:** Appear boat-shaped when in clusters of three; with eight sporozoites.

**Measurements** (in  $\mu$ m) (n=20)

Trophozoite: TL = 497-927 (758.3); LE = 71-232 (159.0); WE = 13-22 (17.8); LP = 80-116 (92.2); WP = 130-160 (139.4); LD = 311-638 (507.0); WD = 145-181 (163.8); LN = 65-72 (69.1); WN = 43-51 (48.4); LEN = 55-181 (133.4)

**Table 1.** Comparative characters of the four species that have been described here under the genus *Odonaticola* (measurements in  $\mu\text{m}$ ).

Character	<i>O. bradinopyga</i> sp. n.	<i>O. aspinosa</i> sp. n.	<i>O. abhoypura</i> sp. n.	<i>O. amojya</i> sp. n.
Trophozoite	595	441	337	758
Epimerite	umbrella-like; edge drawn into 10-12 petaloid spines	umbrella-like with blunt edge	bud-like, circular; edge drawn into 7-11 petaloid spines	conical umbrella-like; edge drawn into 10 petaloid spines
Sporadin	667 Protomerite bell-shaped; deutomerite cylindro-conical	442 Protomerite cylindrical; deutomerite almost spherical	511 Protomerite lobular, rectangular, cylindrical or conical; deutomerite ellipsoidal, tubular or cylindro-conical	874 Protomerite dome-shaped, slender or nipple-like; deutomerite slender and gigantic
Gametocyst	663 Milky-white; covered by thin ectocyst; dehisce by simple rupture	328 Milky-white; covered by thin ectocyst; dehisce by simple rupture	524 × 508 Milky-white; slightly ovoidal in outline; double-walled; dehisce by simple rupture	1008 Creamy-white; covered by thin ectocyst; dehisce by simple rupture
Oocyst	12 × 6 Boat-shaped when single; triangular when in clusters of three	10 × 4 Boat-shaped when single; triangular when in clusters of three	13 × 6 Boat-shaped when single; triangular when in clusters of three	12 × 4 Boat-shaped when single; triangular when in clusters of three
LP: TL (Trophozoites)	1:7	1: 4	1: 6.6	1: 8.2
WP: WD (Trophozoites)	1:1	1: 2	1:1	1:1
Host	<i>Bradinopyga geminata</i>	<i>Crocothemis s. servilia</i>	<i>Pantala flavescens</i>	<i>Crocothemis s. servilia</i>

Sporadin: TL = 493-2000 (873.5); LP = 87-246 (134.5); WP = 58-290 (137.6); LD = 391-1753 (739.0); WD = 101-580 (193.8); LN = 43-116 (68.8); WN = 36-72 (48.9); DG = 901-1116; LO = 12-13; WO = 4; LP: TL = 1:3.5-10.0; WP: WD = 1: 0.7-3.3

#### Taxonomic summary

**Type material:** Catalog No.: 1-14. 13 slides containing syntypes have been deposited at the PLUK; 1 slide

containing hapantotypes has been deposited at the HWML, Lincoln, Nebraska, U.S.A; Catalog No.: HWML 45349

**Type locality:** India, West Bengal, Nadia district, Karimpur

**Host:** Dragonfly *Crocothemis servilia servilia* (Drury, 1773) (Insecta: Odonata: Anisoptera: Libellulidae)

**Location in host:** Midgut.

**Incidence of infection:** Of 89 dragonfly hosts examined 25 (28.1 %) were infected.

**Etymology:** The species is named in honour of the eminent odonatologist Prof. S. D. Amoji of Karnatak University, India

**Remarks:** In the literature three species of *Odonaticola* are reported from the common dragonfly *Crocothemis servilia servilia* (Drury). *Odonaticola amojya* sp. n differs from the others harboured by the same host in having a conical epimerite with 10 petaloid recurved spines and a long neck. In *O. magnus* the epimerite is umbrella-shaped with 6-7 recurved hooks and a long neck; *O. elliptica* has a conical umbrella-like epimerite with many curved spines and a neck elliptical in outline; and *O. crocothemis* has a hood-shaped epimerite with several downwardly directed laminated filaments with a central spine and a long neck. In addition *O. amojya* has a globular gametocyst with transparent ectocyst and boat-shaped oocysts. Although *O. magnus*, *O. crocothemis*, *O. elliptica* and *O. amojya* share a common host, the structural and morphometric variations as well as different localities of collection of the host insect justify the designation of a new species.

The comparative characters of *Odonaticola* new species described from the dragonfly *C. s. servilia* have been compiled in Table 1.

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## REFERENCES

- Amoji S. D., Kori S. S. (1992) Revision of the genus *Odonaticola* Sarkar et Haldar, 1981 (Apicomplexa: Gregarinasina). *Acta Protozool.* **31**: 169-171
- Haldar D. P. (1992) Some aspects of protozoan parasites in odonates of India. Proc. IV South Asian Symposium on Odonatology, Allahabad, India. Abstracts: 1
- Haldar D. P., Biswas S. (2002) On the structure and life history of three new species of the septate gregarines (Apicomplexa: Conoidasida) from odonates of West Bengal. In: Current Trends in Odonatology. (Ed. A. Kumar) Daya Publishing House, Delhi. 357-377
- Hoshide H. (1958) Studies on the cephaline gregarines of Japan. (II). (3) Description of the members belonging to the families Didymophyidae, Actinocephalidae, Acanthosporidae, Stylocephalidae, Dactylophoridae. *Bull. Fac. Educ. Yamaguchi Univ.* **8**: 35-101
- Hoshide K., Janovy J. Jr. (2002) The structure of the nucleus of *Odonaticola polyhamatus* (Gregarinea: Actinocephalus), a parasite of *Mnais strigata* (Hagen) (Odonata: Calopterygidae). *Acta Protozool.* **41**: 17-22
- Kori S. S., Amoji S. D. (1983) On the life history of a new actinocephalid gregarine, *Odonaticola crocothemis*, sp. nov. from the midgut of an odonate insect, *Crocothemis servilia* (Drury). *The Ind. Zoologist* **7**: 155-158
- Prasadanan P. K., Janardanan K. P. (1994) On a new species of actinocephalid gregarine (Apicomplexa: Cephalina) from the dragonfly *Neurothemis fluvia* (Drury) of Kerala, India. *Fraseria* **1**: 7-10
- Prema S., Janardanan K. P. (1991) Morphology and life cycles of two new species of cephaline gregarines (Apicomplexa: Cephalina) from odonate insects in Kerala, India. *Acta Soc. Zool. Bohemoslov.* **55**: 60-64
- Sarkar N. K. (1981) On some actinocephalid gregarines (Apicomplexa: Eugregarinida) of odonate insects from West Bengal, India. *Proc. Ind. Acad. Sci. (Anim. Sci.)* **90**: 649-657
- Sarkar N. K., Haldar D. P. (1981) Observations on four new species of actinocephalid gregarines (Protozoa: Sporozoa) under a new genus *Odonaticola* from odonate insects. *Arch. Protistenk.* **124**: 288-302
- Sprague V. (1941) Studies on *Gregarina blattarum* with particular reference to the chromosome cycle. *Ill. Biol. Monogr.* **18**: 5-57
- Tillyard R. J. (1917) The Biology of Dragonflies. Cambridge University Press, Cambridge

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## Nicotine Affects Behaviour, Morphology and Cortical Cytoskeleton of *Amoeba proteus*

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**Summary.** Nicotine, a natural alkaloid of tobacco leaves and roots, is the main factor for human tobacco addiction. To assess its toxic effect on motile cells we have made series of experiments on free-living *Amoeba proteus*. It was found that nicotine changed cell morphology, inhibited locomotion, as well it led to degradation of actin cytoskeleton and finally to cell destruction. Obtained results suggest necrosis not the apoptosis as the mechanism of cell death, however many phenomena concerning structure of cytoskeleton and physical cell disintegration suggest that process might to be active.

**Key words:** *Amoeba proteus*, cytoskeleton, nicotine.

### INTRODUCTION

Nicotine ( $C_5H_4N$ )CH(CH<sub>2</sub>)<sub>3</sub>N(CH<sub>3</sub>), a natural alkaloid of tobacco leaves and roots, is the main factor for human tobacco addiction (Dani *et al.* 2001), however other substances present in tobacco smoke may also contribute to the damaging effects of smoking. The result of tobacco addiction, long-term cigarette smoking, is a leading cause of premature death because of its contribution to the development of clinical cardiovascular disease and cancer, and is probably one of the most important public health issue of our time. That is why a

great number of laboratories examine the effect of nicotine on living organisms and try to clear up the molecular bases of its activity.

The lipophilic nature of nicotine results in high solubility in membrane lipids and fast influx into cells. It is easily absorbed in human airways and can affect epithelial and blood cells. It was found that tobacco smoke may affect the function of the immune system, first of all the leukocyte cells. Polymorphonuclear leukocytes are the first line of the host defence against acute bacterial infections.

Stimulated leukocytes are highly motile and capable of pseudopodia formation and cytoplasmic flow. In the case of infection they migrate along the chemotactic gradient at a rate of 10-15  $\mu$ m/min to the site of inflammation, where they phagocytose bacteria and dead cells remnants. Both migration and phagocytosis

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require the ability for efficient and properly regulated reorganization of the cytoskeleton.

The effect of tobacco smoke components and nicotine solution on the morphology and function of leukocytes has been described by many authors (i.e., Eichel and Shahrik 1969, Nouri-Shirazi and Guinet 2003, Sikora *et al.* 2003). Oral leukocytes are immediately affected by toxic agents from cigarettes. Leukocytes treated with nicotine (pure nicotine and tobacco smoke) rounded up, lost the ability to form pseudopods and ceased migration (Eichel and Shahrik 1969), however they remained strongly attached to the surface (Blue and Janoff 1978, Corberand *et al.* 1980). Their cytoplasm became motionless and cellular organelles exhibited only Brownian movement (Eichel and Shahrik 1969). As a result, phenomena which are related to cell motility, i. e. the chemotactic response (Bridges *et al.* 1977) and phagocytosis (Blue and Janoff 1978, Ryder 1994) were also disturbed.

It was also found that nicotine inhibits apoptosis, turning off well known mechanisms of protection against pathological changes in cells (Wright *et al.* 1993, Maneckjee and Minna 1994, Hakki *et al.* 2001).

Leukocytes crawl using a common mechanism of locomotion, frequent for other animal tissue cells (e.g. embryonic cells, lymphocytes, invasive tumour cells) and the only form of locomotion for free-living amoebae. This type of cells, contrary to fibroblasts or keratinocytes, does not form tension-loaded cytoskeletal structures. Unfortunately, most of the tissue cells which normally migrate by means of amoeboid movement have to be studied in primary cultures, since their immortal cell lines are non-motile. Transformation strongly changes cell physiology, and the organization of the cell cytoskeleton, which influences the cell's response to toxins. The evident similarity of locomotion of leukocytes and amoebae as well as data concerning morphological and cytological changes in leukocytes treated with nicotine stimulated us to examine the effect of this alkaloid on *Amoeba proteus* cells.

*A. proteus* is known for years as a perfect model for examination of cell motility (Grębecki 1994, 2001a, b) because of its size and the rate of migration. Both cells move relatively fast, and cover roughly a cell length distance in a minute (300  $\mu\text{m}/\text{min}$  for *A. proteus*, 10–15  $\mu\text{m}/\text{min}$  for leukocytes) (Bray 1992).

The purpose of this study was to determine the effect of nicotine solution on the morphology, locomotion, adhesion, and actin cytoskeleton localization of *A. proteus*.

Finally we tried to define the nature of changes observed in amoebae after treatment with nicotine; trying to determine whether they are necrotic or apoptotic.

## MATERIALS AND METHODS

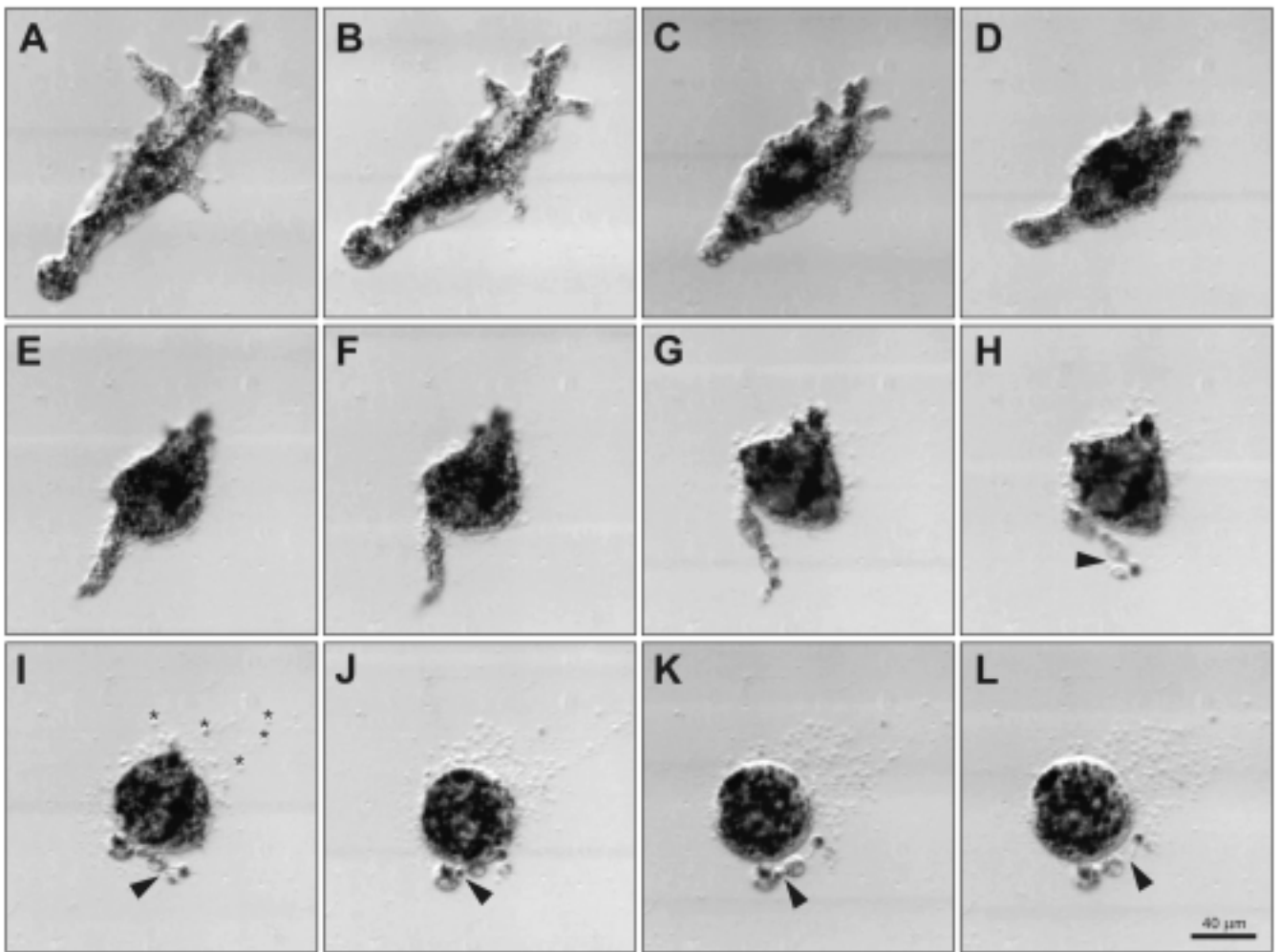
*Amoeba proteus* of strain A (Jeon and Lorch 1973) was taken for examination. The cells were grown in glass dishes in Pringsheim medium and fed twice a week with *Tetrahymena pyriformis*. Experiments were performed at room temperature on the third day after feeding. Amoebae were treated with nicotine at a final concentration of  $10^{-3}$  M (Sigma Aldrich). After addition of nicotine the cells were observed for 10 to 60 min under a PZO Biolar microscope equipped with Pluta Differential Interference Contrast (DIC) and images were recorded using a C2400 Hamamatsu camera system and NV 8051 Panasonic time-lapse recorder. For scanning electron microscopic (SEM) observations the cells were fixed in 3.5% paraformaldehyde, dehydrated through a graded series of ethanol and acetone, dried by the  $\text{CO}_2$  critical point technique and coated with gold. SEM observations were performed in a Jeol 1200 EX transmission electron microscope with an ASID 19 scanning attachment, operating at 80 KV.

Fluorescence microscopy was used for studies of actin distribution. *Amoeba proteus* cells were fixed in 3.5% paraformaldehyde solution and then actin filaments were stained with 1% FITC conjugated phalloidin (Sigma-Aldrich). Specimens were observed with a Nikon Diaphot inverted microscope using  $\times 10$  0.26NA and  $\times 20$  0.4NA Nomarski DIC objectives. Images were collected with a Retiga 1300 (QImaging Inc.) cooled CCD digital camera and transmitted *via* a FireWire interface to a PC computer. For image acquisition and processing AQM Advance 6.0 software (Kinetic Imaging plc) was used.

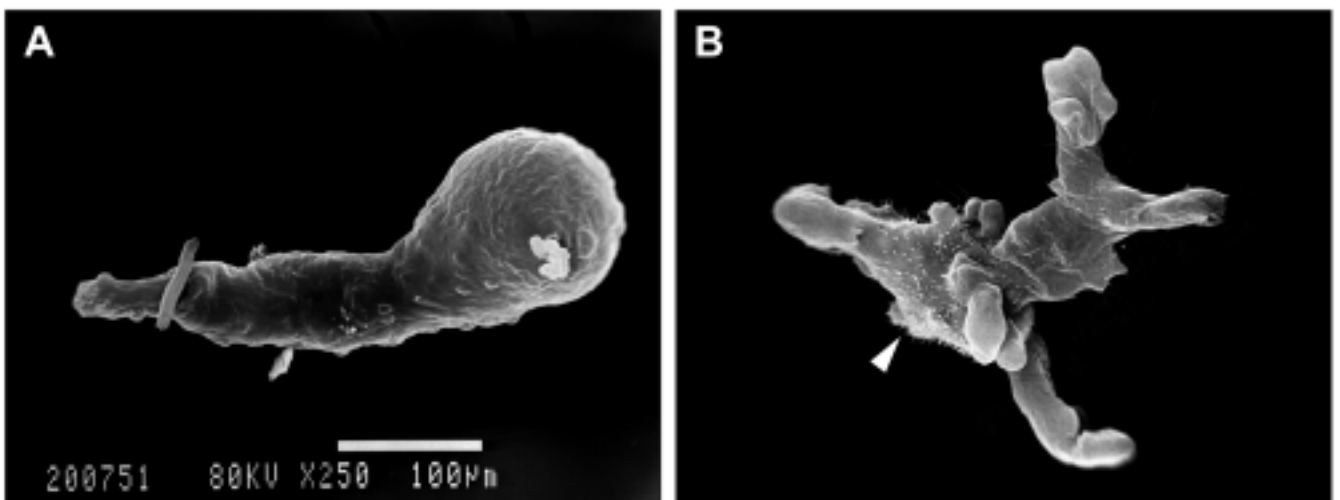
The cell viability was measured with propidium iodide (Sigma-Aldrich) according to Bielak-Żmijewska *et al.* 2000. To establish the nature of cell death DNA fragmentation test was performed as described by Bielak-Żmijewska *et al.* (2000) and nuclear morphology was studied on cells stained with Hoechst 33258 (Sigma-Aldrich) (concentration  $10^{-3}$ M) in fixed cells (Bielak-Żmijewska *et al.* 2000).

## RESULTS

Locomoting *Amoeba proteus* cells were treated with nicotine solution at a final concentration of  $10^{-3}$  M. This concentration was chosen after a series of preliminary experiments because it was the lowest concentration that evoked morphological changes (data not shown). The cells responded to the alkaloid immediately after treatment. They rapidly contracted, lost polarity and detached from the substratum (Figs 1 A–D). As seen on Fig. 2A, the surface of nicotine-treated *A. proteus* is destitute of any adhesive structures (minipodia or rosette-contacts), that are normally present on the ventral surface of untreated migrating amoebae (Fig. 2B, ar-

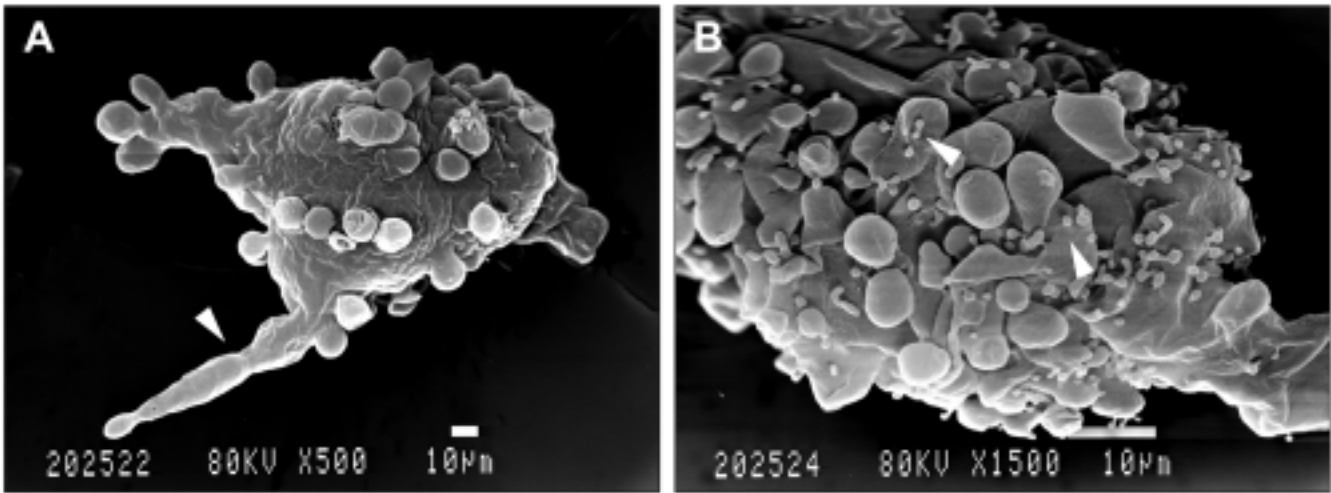


**Figs 1 A-L.** Consecutive stages (DIC records from 0 to 30 min) of changes evoked by nicotine action on *Amoeba proteus*. arrowhead - site of autotomy; asterisks - blebs separation.



**Fig. 2.** *A. proteus* treated with nicotine solution (A) and untreated cell (B), arrowhead shows adhesive minipodia on cell surface.





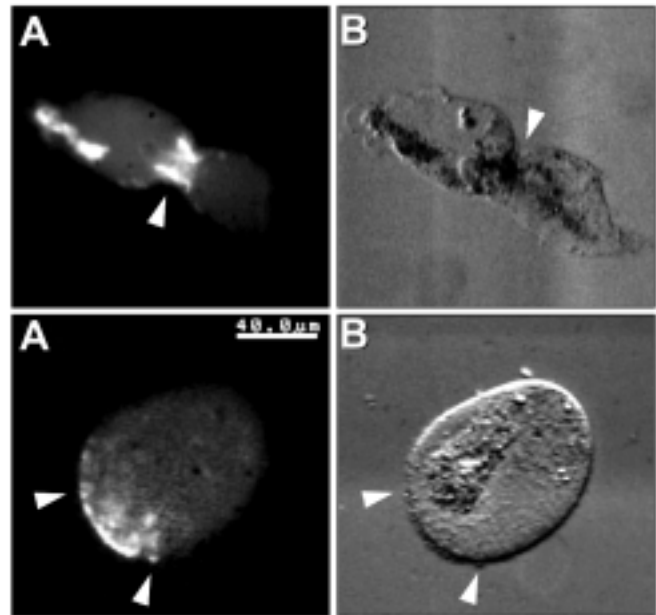
**Fig. 3.** *A. proteus* with intensive blebbing and long protrusion (A - arrowhead) and small blebs on bleb surface (B - arrowheads).



**Fig. 4.** Autotomic blebs on amoeba surface.

rowhead shows minipodia). During the first stage of reaction a great variety of cell shapes were formed.

Immediately after treatment amoebae undergo intensive blebbing (Fig. 3A), sometimes leading to formation of secondary blebs on the bleb surfaces (Fig. 3B, arrowheads). Some of the cells form large, elongated projections (Figs 1 E-G, 3A, arrowhead). Finally cell contraction leads to autotomy (Figs 1 H-L, site of autotomy marked by arrowhead). Blebs gradually separate and form numerous small vesicles (Figs 1I, asterisks; 4). The irreversibility of these processes means that exposure of *A. proteus* to this nicotine solution leads to the total destruction of the cell.



**Fig. 5.** F-actin distribution in *A. proteus* treated with nicotine. **A** - phalloidin staining; **B** - Nomarski DIC image (arrowheads - F-actin concentrated in a form of contractile ring).

**Fig. 6.** F-actin in blebbing *A. proteus*. **A** - phalloidin staining; **B** - Nomarski DIC image (arrowheads - zone of blebs formation).

Staining of F-actin with fluorescently labelled phalloidin in amoebae treated with nicotine solution shows that the actin cytoskeleton is concentrated at sites of autotomy, forming structures similar to the contractile ring of a dividing cell (Fig. 5, arrowhead). In blebbing cells actin filaments were localized in areas underlying zones of bleb formation (Fig. 6, arrowheads).

The final result of treatment of *A. proteus* with nicotine solution was cell death. We have performed a series of experiments to determine the character of this process. *In vivo* propidium iodide staining showed that the membrane became perforated in nicotine, the usual symptom of cell death. Since nicotine treatment is said to significantly influence apoptotic death of tissue cells, we decided to check whether the death of nicotine-treated *A. proteus* is apoptotic or necrotic. We have not noticed any differences in morphology of nuclei between control and treated cells and no DNA fragmentation was seen in examined cells. These results suggest that nicotine-induced cell death is necrotic in character.

## DISCUSSION

It is well known that nicotine caused changes in the development and function of the human immune system (Buisson and Bertrand 2002, Middlebrook *et al.* 2002, Nouri-Shirazi and Guinet 2003) and leads to tumour development. It modulated the microcirculation, cell proliferation, membrane transport, metabolism of cytotoxic drugs and hormonal milieu in mammals (Berger and Zelle 1988). It was also found that the ability of cells to enter apoptosis is influenced by nicotine. Some authors postulated (Maneckjee and Minna 1994, Hakki *et al.* 2001) that this alkaloid inhibits apoptotic death of pathologically disordered cells preventing their elimination, whilst others (Berger and Zeller 1988, Yamamura *et al.* 1998) claim the opposite, finding nicotine to be an apoptosis promotor.

*Amoeba proteus* cells exposed to nicotine solution undergo a series of behavioural and morphological changes: cell contraction, formation of elongated protrusions and intensive blebbing, which finally lead to the total destruction of the cells.

The process of blebbing is often observed as a consequence of the action of various extracellular factors. Blebs are hemispherical protrusions at the cell surface, usually forming very fast and characterized by a lack of organelles within them. Blebbing was observed in various cells including Walker 256 carcinosarcoma cells (Keller *et al.* 2002) or fibroblasts (Pletushkina *et al.* 2001). We have observed a similar response in *A. proteus* cells under the influence of nicotine. Harris (1990) suggested that blebs in tissue cells appeared as the consequence of plasma membrane detachment from the cortical actin layer. He also proposed that later

F-actin may repolymerize under the plasma membrane, permitting bleb retraction. In *A. proteus*, blebbing is irreversible and actin concentrates in the bleb base, promoting vesicle detachment. Irreversible blebbing followed by vesicle detachment may lead to cell membrane loss and may be at least partially responsible for the strong toxic effect of nicotine on *A. proteus*, leading to the cell death.

It is known that the process of blebs formation was also noticed physiologically in locomoting cells (Harris 1990, Keller 2000), during mitosis (Laster and MacKenzie 1996) and apoptosis (Laster and MacKenzie 1996). The complexity of this common process was summarized by Keller *et al.* (2002) "It can be induced by compromising actin polymerization (Harris 1990, Cunningham 1995) or cross-linking of actin filaments (Cunningham 1995, Janmey *et al.* 1992), by disassembly of microtubules (Keller and Zimmermann 1986, Keller and Egli 1998, Pletushkina *et al.* 2001), by deformation or suction pressure (Schütz and Keller 1998), or by lysophosphatidic acid, which induces phosphorylation of myosin light chains through activation of Rho (Hagmann *et al.* 1999)". Bleb localization might correspond to the sites of adhesive structures observed in locomoting cells. Their appearance in these places may be connected to the uneven distribution of actin prior to the experiment (Grębecka *et al.* 1997). It might be true only for the first steps of the process because later stages of autotomy are very fast and difficult to study by means of immunocytochemistry.

We hope that this image of active cell death will contribute to a better understanding of the response of the body's immune system to nicotine. This may be especially important for leukocytes from the oral cavity, the first component of the immune system coming into contact with nicotine in the smoker's body, which may also undergo strong morphological changes. The experiments performed suggest necrosis rather than apoptosis as the mechanism of the actual cell death, although many phenomena concerning the structure of the cytoskeleton and physical disintegration of the cell suggest that the process might be active. The best interpretation we can propose for the fate of amoebae after nicotine application is deregulation of the cell motility machinery in such a way that, in spite of propelling cell movement, its modified and intense contractile activity causes cell disintegration and death.

Even if the results obtained do not lead to an optimistic conclusion concerning the possibility to diminish toxic

activity of nicotine in the smoker's body, a deeper understanding of its toxicity can be an argument against using any form of tobacco.

## REFERENCES

- Berger M. R., Zeller W. J. (1988) Interaction of nicotine with anticancer treatment. *Klin Wochenschr.* **11(Suppl.)**: 127-133
- Bielak-Żmijewska A., Koronkiewicz J., Skierski J., Piwocka K., Radziszewska E., Sikora E. (2000) Effect of curcumin on the apoptosis of rodent and human nonproliferating and proliferating lymphoid cells. *Nutrition Cancer* **38**: 131-138
- Blue M.-L., Janoff A. (1978) Possible mechanisms of emphysema in cigarette smokers. *Am. Rev. Resp. Disease* **117**: 317-325
- Bray D. (1992) Cell Movements. Garland Publishing, INC., New York, London
- Bridges R. B., Krall J. H., Huang T. H., Chancellor M. B. (1977) Effect of tobacco smoke on chemotaxis and glucose metabolism of polymorphonuclear leukocytes. *Infect. Immun.* **15**: 115-123
- Buisson B., Bertrand D. (2002) Nicotine addiction: the possible role of functional upregulation. *Trends Pharmacol. Sci.* **23**: 130-136
- Corberand J., Laharrague P., Nguyen F., Dutan G., Fontanilles A. M., Gleizes B., Gyrard E. (1980) *In vitro* effect of tobacco smoke components on the functions of normal human polymorphonuclear leukocytes. *Infect. Immun.* **30**: 649-655
- Cunningham C. C. (1995) Actin polymerization and intracellular solvent flow in cell surface blebbing. *J. Cell. Biol.* **129**: 1589-1599
- Dani J. A., Ji D., Zhou F. M. (2001) Synaptic plasticity and nicotine addiction. *Neuron* **31**: 349-352
- Eichel B., Shahrik H. (1969) Tobacco smoke toxicity: loss of human oral leukocyte function and fluid-cell metabolism. *Science* **166**: 1424-1427
- Grębecka L., Pomorski P., Grębecki A., Łopatowska A. (1997) Adhesion-dependent F-actin pattern in *Amoeba proteus* as a common feature of amoebae and the metazoan motile cells. *Cell. Biol. Int.* **21**: 565-573
- Grębecki A. (1994) Membrane and cytoskeleton flow in motile cells with emphasis on the contribution of free-living amoebae. *Int. Rev. Cytol.* **148**: 37-80
- Grębecki A., Grębecka L., Wasik A. (2001a) Minipodia, the adhesive structures active in locomotion and endocytosis of amoebae. *Acta Protozool.* **40**: 235-247
- Grębecki A., Grębecka L., Wasik A. (2001b) Minipodia and rosette contacts are adhesive organelles present in free-living amoebae. *Cell Biol. Intern. Rep.* **25**: 1279-1283
- Hagmann J., Burger M. M., Dagan D. (1999) Regulation of plasma membrane blebbing by cytoskeleton. *J. Cell. Biochem.* **73**: 488-499
- Hakki A., Pennypacker K., Eidzadeh S., Friedman H., Pross S. (2001) Nicotine inhibition of apoptosis in murine immune cells. *Exp. Biol. Med.* **226**: 947-953
- Harris A. K. (1990) Protrusive activity of the cell surface and the movements of tissue cells. In: Biomechanics of Active Movement and Division of Cells. (Ed. N. Akkas) NATO ASI Series, Springer-Verlag, Berlin, Heidelberg, H42, 249-291
- Janmey P. A., Lamb J. A., Ezzell R. M., Hvidt S., Lind S. E. (1992) Effects of actin filaments on fibrin clot structure and lysis. *Blood* **80**: 928-936
- Jeon K. V., Lorch I. J. (1973) Strain specificity in *Amoeba proteus*. In: The Biology of Amoeba. (Ed. K. W. Jeon.) Academic Press, New York and London, 549-567
- Keller H. (2000) Redundancy of lamellipodia in locomoting Walker carcinosarcoma cells. *Cell Motil. Cytoskeleton* **46**: 247-256
- Keller H. (2002) Mechanism of protrusion and cell locomotion. *JSME Intern. J., Ser. C* **45**: 843-850
- Keller H., Eggli P. (1998) Protrusive activity, cytoplasmic compartmentalization, and restriction rings in locomoting blebbing Walker carcinosarcoma cells are related to detachment of cortical actin from the plasma membrane. *Cell Motil. Cytoskeleton* **41**: 181-193
- Keller H., Zimmermann A. (1986) Shape changes and chemokinesis of Walker 256 carcinosarcoma cells in response to colchicine, vinblastine, nocodazole and taxol. *Invasion Metastasis* **6**: 33-43
- Keller H., Rentsch P., Hagmann J. (2002) Differences in cortical actin and dynamics document that different types of blebs are formed by distinct mechanism. *Exp. Cell Res.* **277**: 161-172
- Laster S. M., MacKenzie J. M. Jr. (1996) Bleb formation and F-actin distribution during mitosis and tumor necrosis factor-induced apoptosis. *Microsc. Res. Tech.* **34**: 272-28
- Maneckjee R., Minna J. D. (1994) Opioids induce while nicotine suppresses apoptosis in human lung cancer cells. *Cell Growth Differ.* **5**: 1033-1040
- Middlebrook A. J., Martina C., Chang Y., Lukas R. J., DeLucka D. (2002) Effects of nicotine exposure on T cell development in fetal thymus organ culture: arrest of T cell mutation. *J. Immunol.* **169**: 2915-2924
- Nouri-Shirazi M., Guinet E. (2003) Evidence for the immunosuppressive role of nicotine on human dendritic cell functions. *Immunology* **109**: 365-330
- Pletushkina O. J., Rajfur Z., Pomorski P., Oliver T. N., Vasiliev J. M., Jacobson K. A. (2001) Induction of cortical oscillations in spreading cells by depolymerization of microtubules. *Cell. Motil. Cytoskeleton* **48**: 235-44
- Ryder M. J. (1994) Nicotine effects on neutrophil F-actin formation and calcium release: implications for tobacco use and pulmonary diseases. *Exp. Lung Res.* **20**: 283-296
- Schütz K., Keller H. (1998) Protrusion, contraction and segregation of membrane components associated with passive deformation and shape recovery of Walker carcinosarcoma cells. *Eur. J. Cell Biol.* **77**: 100-110
- Sikora L., Rao S. P., Spiramarao P. (2003) Selectin-dependent rolling and adhesion of leukocytes in nicotine-exposed microvessels of lung allografts. *Am. J. Physiol. Lung Cell Mol. Physiol.* **285**: L654-L663
- Yamamura M., Amano Y., Sakagami H., Yamanaka Y., Nishimoto Y., Yoshida H., Yamaguchi M., Ohata H., Momose K., Takeda M. (1998) Calcium mobilization during nicotine-induced cell death in human glioma and glioblastoma cell lines. *Anticancer Res.* **18**: 2499-502
- Wright S. C., Zhong J., Zheng H., Larrick J. W. (1993) Nicotine inhibition of apoptosis suggests a role in tumor promotion. *FASEB J.* **7**: 1045-1051

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## BOOK REVIEW

**Protistology by Klaus Hausmann, Norbert Hülsmann and Renate Radek with contributions by Hans Machemer, Maria Mulisch and Günter Steinbrück, 3<sup>rd</sup> edition, E. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller) Stuttgart, Germany; ISBN 3-510-65208-8 hardcover € 64.-/ U.S. \$ 78.-, and ISBN 3-510-65209-6 € 49.-/ U.S. \$ 59.- student edition.**

Unicellular organisms with the eukaryotic type of cell organization, known today as protists have been at the forefront of biology since their discovery by Anthony van Leeuwenhoek at the end of 17th century. Their seemingly simple cellular organization, possibility of cultivation of many of them in simple media, their wide occurrence in nature made them in the past the classical experimental material of biologists of many demoninations.

Even in contemporary biology, preoccupied with molecular, biochemical and developmental aspects of organisms, protists have not lost their standing as key organisms in many areas of biological and medical research. The origin of the eukaryotic cell, the history of its evolution into representatives of today's five generally recognized kingdoms of organisms (Protozoa, Chromista, Plantae, Animalia and Fungi) is the area where protists play a basic role. The relatively recent discovery by methods of molecular biology of nanosized protists as widely distributed organisms now calls for their morphological characterization, evolutionary and taxonomic affiliation and for the evaluation of their importance in ecosystems. These two examples (and many others could be mentioned) confirm that today protistology is not a mere remnant of its past glory but an integral part of modern biology. Several animal and human diseases caused by protists are still with us in the 21st century (malaria, toxoplasmosis, amebiasis, leishmaniasis, trypanosomiasis, etc), a reality stressing the practical importance of protists.

Although it is evident that protists are organisms deserving study, it is paradoxically difficult to find a textbook on protists which would be modern and comprehensive. Modern informations on protists are scattered throughout specialized publications and journals in many fields of biology. The last textbook before that considered in this review was published nine years ago, a long time if one considers the precocious development of today's biology. Thus the "Protistology" by Klaus Hausmann, Norbert Hülsmann and Renate Radek and their colleagues is a very welcomed addition to biological literature. It is in fact the only presently available textbook intended to introduce the reader (be it a student, an advanced scientist or an amateur in the best sense of the word) to the world of protists. Although the present volume is the third edition of a highly successful textbook of the past, it is a novelty as it covers not only protozoa dealt with in the preceding editions ("Protozoologie" in 1985 and "Protozoology" in 1995), but also the single celled representatives of other organismal kingdoms.

The book is organized in three parts: a "general part", a "core part" (evolution and taxonomy of protists) and a third part (selected topics of protistan organization, evolution, morphogenesis, molecular biology, behavior and ecology). A glossary and an extensive list of bibliographical references to journals, textbooks, monographs and important articles on individual protist groups are a much welcomed addition to the book.

The general part covers the cellular organization of protists and contains a short but very informative chapter on the historical aspects of protist research and its contribution to biology in general.

When updating the former Protozoology from 1995 and transforming it into the "core part" of Protistology of 2003, the authors had a not an easy task. Not only it was necessary to include information on many unicellular organisms not previously covered (e.g. green algae, some chromists, fungal protists), but to arrange them into a kind

of system which would be “user’s friendly” and phylogeny oriented. For that purpose the authors have selected a consensus tree based on combined protein sequences and thus having chance to reflect true relationships among organisms. The phylogenetically related protistan groups are presented in the book as phyla of a single organismal realm, protista. Thus the division of organisms into traditional kingdoms is avoided, reflecting perhaps the modern thinking that protists, although a polyphyletic assemblage, nevertheless represent a kind of entity by virtue of their size and level of organization. Each phylum is then presented by description of several of its representative organism, accompanied by photomicrographs and schematic drawings.

The third part, “Selected Topics of General Protistology” is an assemblage of diverse topics as “Comparative Morphology and Physiology of Protists”, “Nuclei and Sexual Reproduction”, “Morphogenesis and Reproduction”, selected molecular biology topics, “Behavior of Protists” and Ecology of Protists.

The main character, permeating the whole book is the illustration material of high quantity and quality consisting of photomicrographs and drawings (many of them by the authors). I consider this as an outstanding paedagogic feature of the book, a feature perhaps reflecting the tradition of German protistology which produced in the past some outstanding and still usable textbooks as e.g. the classical Doflein Reichenow’s “Lehrbuch der Protozoenkunde” and the “Protozoology” (1973) by Karl Grell.

The “Protistology” documents the intimate knowledge of authors of their subject and undoubtedly their fascination with protists and their structural beauty. Because of this one can criticize only a few items in “Protistology”. Due to the very rapid progress of biology, some quite recent developments are not included in the book, as e.g. the finding of mitochondrial remnants in microsporidia and in *Giardia*. Also it is evident from simple enumeration of items of the book section “Selected topics of General Protistology” that the coverage of some of these items is rather superficial (especially as molecular biology is concerned), as each item could be well the subject of a whole book. On the contrary, the inclusion in this part of the book of the very detailed chapter “Behavior of Protists” seems superfluous. An important monograph on microsporidia (“The Microsporidia and Microsporidiosis”; eds. M. Wittner and L.M. Weiss; ASM Washington, 1999) is not included in the bibliography.

In summary, I consider the “Protistology” by Hausmann, Hülsmann and Radek as the contemporary best and probably the only available comprehensive introductory book for those interested in the biology of unicellular eukaryotes. It is possible in these days to be a top “specialist” (but not a “connoisseur”!) of an organism without actually seeing it under the microscope. However, contrary to such minimalist approach, I believe that a certain amount of “organismal knowledge” belongs to a scientific culture and the pride of being a scientist. It is fortunate that the “Protistology” is here, ready to open its pages to anybody interested in the realm of protists.

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