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New contributions to the marine benthic ciliates from the Antarctic area, including description of seven new species (Protozoa, Ciliophora)

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Abstract

Twenty-six marine benthic ciliates including seven new species were isolated from King George Island, Antarctic. The morphology and taxonomy of 19 of them are described in the present paper: *Aegyriana paroliva*, *Amphileptus* sp., *Amphisiella antarctica* nov. spec., *Condylostoma* cf. *magnum*, *Dysteria parovalis* nov. spec., *Folliculina* ? sp., *Hartmannula* cf. *angustipilosa*, *Hemigastrostyla szabo* nov. spec., *Heterostentor coeruleus*, *Holosticha* sp., *Intranstylum antarcticum* nov. spec., *Metaurostylopsis rubra*, *Orthodonella shenae*, *Philasterides* cf. *armatalis*, *Pithites pelagicus* nov. spec., *Pleuronema coronatum*, *Strombidium apolatium* nov. spec., *Telotrochidium* sp., and *Thigmokeronopsis magna* nov. spec. Based on the new observations, an improved diagnosis for the genus *Aegyriana* is suggested: dorsoventrally flattened Dysteriidae with tail-shaped podite, which is positioned subcaudally in a glabrous region within somatic kineties; oral structure in two parts: ca three close-set fragments on right and one preoral kinety on left; left postoral kineties shortened posteriorly and continuous with right ones, leaving no median gap or suture; cytopharyngeal rods dominant. According to the new understanding and information obtained, a refined diagnosis of the genus *Hemigastrostyla* is also suggested: hypotrich with *Oxytricha*-like cirral pattern; eight to ten frontal (including one buccal) and five to seven ventral cirri; five transverse and three caudal cirri; no right-lateral anlagen of dorsal kineties occurring and the old adoral zone will be partly replaced by newly formed structure during morphogenesis; dorsal cilia located in small pits, fibre system highly developed; marine habitat.

Keywords: Antarctic, benthic ciliates, new species, taxonomy and morphology

Introduction

In 2000–2001, the present authors investigated and reported 22 benthic ciliated protozoans collected from the Antarctic area, including six new species and two new genera (Song and Wilbert 2002). The current work is based on a new research project undertaken early 2002 and has confirmed the estimation that in this area there are still many taxa that remain unknown or should be studied further by modern methods, as has been noticed by many previous researchers (Fenchel and Lee 1972; Thompson 1972; Thompson and Croom

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1978; Corliss and Snyder 1986; Agatha et al. 1990, 1993; Valbonesi and Luporini 1990a, 1990b; Wilbert et al. 1993; Petz 1994, 1995; Petz et al. 1995; Foissner 1984, 1986; Wiencke et al. 1998; Song et al. 1999; Song and Wilbert 2000, 2002).

In February to April 2002, a new taxonomic survey on the periphytic ciliates in the same area as that surveyed 2 years ago was carried out. As a result, at least 26 species have been isolated and observed (Table I), of which 19 species are morphologically or morphometrically described here.

Materials and methods

Organisms and preparation

Specimens were collected (February to April 2002) from the periphyton on rocks and tidal pools in the littoral zone of Potter Cove, King George Island (62°14'S, 58°40'W), as in the previous investigation (Song and Wilbert 2002).

Specimens were either observed immediately with a pre-cooled microscope and photographed by a video camera or maintained for days in the laboratory. Wheat grains were added to the medium to provide bacterial food. Protargol and nitrate silver impregnations according to Wilbert (1975) and Song and Wilbert, respectively, were applied to reveal the infraciliature and the silverline system.

Table I. Name of 26 benthic species occurring in the sampling area during the 2002 survey.

Species name	Morphologically described (+) or not described (-) in the present work
<i>Aegyriana paroliva</i> Song and Wilbert, 2002	+
<i>Amphileptus</i> sp.	+
<i>Amphisiella antarctica</i> nov. spec.	+
<i>Chlamydonella</i> sp.	-
<i>Condylostoma</i> cf. <i>magnum</i> Spiegel, 1926	+
<i>Diophrys oligothrix</i> Borror, 1965	-
<i>Diophrys scutum</i> Dujardin, 1842	-
<i>Dysteria parovalis</i> nov. spec.	+
<i>Euplotes balteatus</i> (Dujardin, 1841)	-
<i>Folliculina</i> ? sp.	+
<i>Hartmannula</i> cf. <i>angustipilosa</i> Deroux and Dragesco, 1968	+
<i>Hemigastrostyla szaboi</i> nov. spec.	+
<i>Heterostentor coeruleus</i> Song and Wilbert, 2002	+
<i>Holosticha</i> sp.	+
<i>Holosticha diademata</i> (Rees, 1884)	-
<i>Intranstylum antarcticum</i> nov. spec.	+
<i>Litonotus</i> sp.	-
<i>Metaurostylopsis rubra</i> Song and Wilbert, 2002	+
<i>Orthodonella shenae</i> Song and Wilbert, 2002	+
<i>Philasterides</i> cf. <i>armatalis</i> Song, 2000	+
<i>Pithites pelagicus</i> nov. spec.	+
<i>Pleuronema coronatum</i> Kent, 1881	+
<i>Strombidium apolatium</i> nov. spec.	+
<i>Telotrochidium</i> sp.	+
<i>Thigmokeronopsis magna</i> nov. spec.	+
<i>Vorticella</i> sp.	-

Counts and measurements on stained specimens were performed at a magnification of $\times 1250$. Drawings were made with the help of a camera lucida. Terminology and systematics basically follow Corliss (1979).

Ecological features

The water temperature in the period of sampling was about 1°C , salinity about 33‰.

Deposition of slides and type materials

Holotypes and paratypes (for new species) as well as voucher slides (for known taxa) in the form of permanent slides using either protargol or silver nitrate impregnations are deposited in the Oberösterreichische Landesmuseum, Linz, Austria.

Results

Order PLEUROSTOMATIDA Schewiakoff, 1896

Genus *Amphileptus* Ehrenberg, 1830

Amphileptus sp.

(Figures 1A, 12A)

We identified this organism from a protargol-impregnated slide ($n=4$). Since no living observation was able to be carried out, that is, the morphology in vivo and the position/

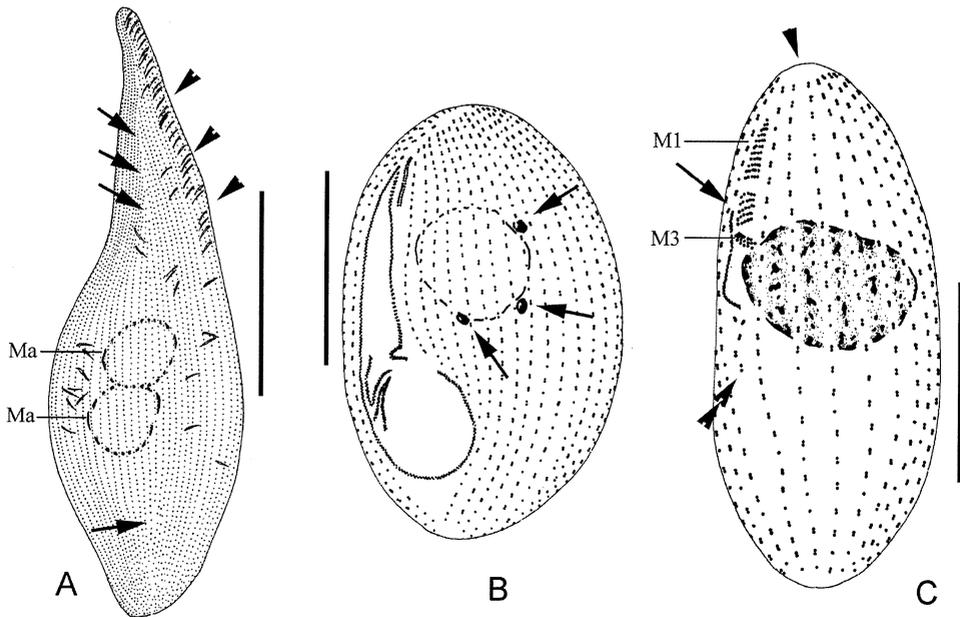


Figure 1. (A) *Amphileptus* sp., (B) *Pleuronema coronatum* and (C) *Philasterides* cf. *armatalis* after protargol impregnation. (A) Right side view of infraciliature, arrows mark the suture formed by some shortened kineties, arrowheads indicate the extrusomes; (B) ventral-left view, to show the general appearance of infraciliature and nuclear apparatus (arrows mark the micronuclei); (C) left side view, arrowhead marks the apical plate; arrow indicates the anterior end of paroral membrane, double-arrowheads mark the scuticula. Ma, macronucleus; M13, membranelle 1, 3. Scale bars: $70\ \mu\text{m}$ (A); $30\ \mu\text{m}$ (B); $15\ \mu\text{m}$ (C).

number of the contractile vacuoles as well as other diagnostic characters remain unclear, this organism has to be hence treated as an unknown form here.

Description

Cells after protargol impregnation generally form-constant (Figure 1A), ca 200 μm in length, which seems not to have evident (?) tail. Two ellipsoid macronuclear nodules, large and in mid-body position, arranged closely together. No micronucleus detected. Extrusomes (in protargol-impregnated specimens) rod-shaped, slightly curved, about 8–10 μm long; densely distributed in oral region and scattered in other parts of body (Figures 1A, 15A).

Infraciliature typical of genus. On right side, ca 50 densely ciliated somatic kineties forming a conspicuous suture in mid-body (arrows in Figure 1A) whereas on left side probably over 12 (? not clearly detected) loosely ciliated kineties (including perioral kinety), all of which seem to extend along whole length of cell. Dorsal brosse composed of “numerous” basal body pairs and extending posteriorly to about half of cell length.

Remarks

Most studies using modern methods on this genus have been carried out on freshwater forms (Fryd-Versavel et al. 1975; Foissner 1984, 1986; Dragesco and Dragesco-Kernéis 1986; Song and Wilbert 1989. Considering the cell size, general appearance after impregnation and the habitat, the present organism is similar to the large marine form, *Amphileptus marinus* (Kahl 1931) which was recently redescribed by Song et al. (2003). The latter has, however, conspicuously lower number of right somatic kineties (ca 50 versus 20–27). It possibly represents an undescribed form, but further information is required.

Order NASSULIDA Jankowski, 1967

Genus *Orthodonella* Bhatia, 1936

Orthodonella shenae Song and Wilbert, 2002

(Figure 2E–G; Table II)

This species was newly established by the present authors (Song and Wilbert 2002), but unfortunately, was assigned incorrectly to the order Cyrtophorida (Corliss 1979).

The current population resembles that previously described by Song and Wilbert (2002) very well except that it exhibits more variability in size and shape of cells: from 52 to 126 μm in length after impregnation (Table II). In addition, two pores for each of two contractile vacuoles are found on the dorsal side in at least two specimens (Figure 2F, arrows). As an additional contribution, we also supply here the statistical data that was previously lacking (Table II).

Order CYRTOPHORIDA Fauré-Fremiet in Corliss, 1956

Genus *Dysteria* Huxley, 1857

Dysteria parovalis nov. spec.

(Figures 2A–D; Table III)

Diagnosis

Marine oval *Dysteria*, in vivo 50–80 \times 40–60 μm ; constantly with nine ventral kineties in right field, of which three extend to anterior cell end; ca six to eight short fragments of

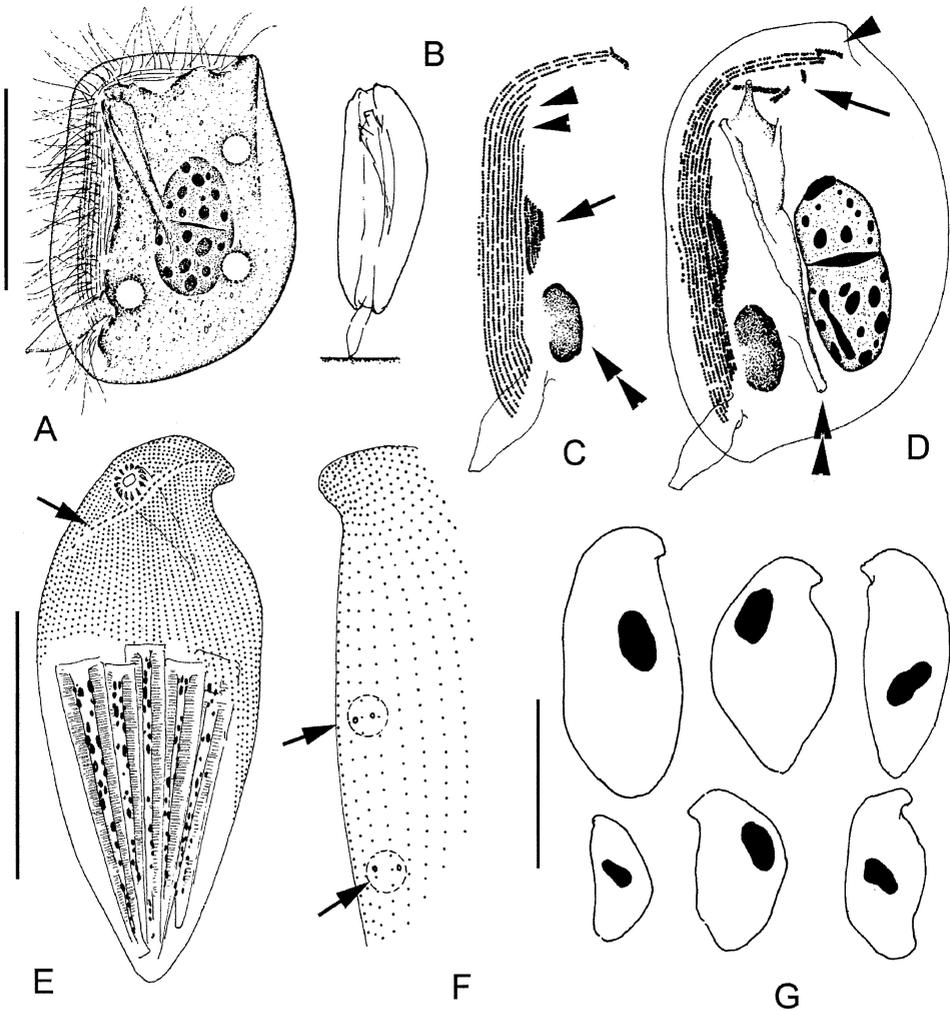


Figure 2. (A–D) *Dysteria parovalis* nov. spec. and (E–G) *Orthodonella shenae* from life (A, B) and after protargol impregnation (C–G). (A) Lateral view of a typical specimen; (B) ventral view, to show the flattened body shape; (C) somatic infraciliature, arrowheads mark the shortened ventral kineties; arrow indicates the fragment-like left kineties, while double-arrowheads mark the argentophilic structure; (D) left view, to show the general appearance of ciliature and nuclear apparatus; arrow marks the oral structure; double-arrowheads indicate the posterior end of cytopharyngeal fibres; arrowhead points to the terminal fragment; (E) ventral view, to show a cell full of large diatoms, arrow marks the synhymenium; (F) dorsal view, to show the pores of contractile vacuoles (arrows); (G) to demonstrate different sizes of cells after fixation. Scale bars: 50 μm (A, E); 40 μm (D); 70 μm (G).

kineties in left equatorial field; oral ciliature consisting of three double-rowed fragments; macronucleus oval; three contractile vacuoles.

Etymology

Composite of *par* (similar to) and the species name *ovalis*, indicating that this species is similar to the well-known form *Dysteria ovalis*.

Table II. Morphometric characterization of *Orthodonella shenae*.

Character	Min	Max	Mean	SD	SE	CV	<i>n</i>
Body length	52	126	95.4	23.46	5.87	24.6	16
Body width	23	54	40.8	10.68	2.67	26.1	16
Number of somatic kineties	50	57	52.7	2.98	1.13	5.6	7
Length of macronucleus	24	37	30.1	4.36	1.09	14.4	16
Width of macronucleus	12	20	16.2	2.76	0.69	17.1	16
Number of macronuclei	1	1	1	0	0	0	>50
Number of contractile vacuole pores	2	4	–	–	–	–	3
Number of cytopharyngeal rods (nematodesmata)	13	14	13.3	0.46	0.16	3.5	8

All data are based on protargol-impregnated specimens. Measurements are in μm .

Min, minimum; Max, maximum; Mean, arithmetic mean; SD, standard deviation; SE, standard error of mean; CV, coefficient of variation in %; *n*, number of specimens.

Table III. Morphometric characterization of *Dysteria parovalis* nov. spec.

Character	Min	Max	Mean	SD	SE	CV	<i>n</i>
Body length	56	79	65.3	5.52	1.38	8.5	16
Body width	41	53	47.4	3.63	0.91	7.7	16
Number of right somatic kineties ^a	9	9	9	0	0	0	11
Number of left kinety fragments	ca 6–7	–	–	–	–	–	
Number of kineties extending dorsally anterior to the cytostome	3	3	3	0	0	0	11
Length of macronucleus	28	40	34.7	3.83	0.96	11.0	16
Width of macronucleus	13	17	14.8	1.56	0.39	10.5	16
Number of macronuclei	1	1	1	0	0	0	>50
Length of podite	12	17	13.6	1.40	0.53	10.3	7

All data are based on protargol-impregnated specimens. Measurements in μm .

^aComplete kineties extending along ventral side.

Description

Mostly about $70 \times 50 \mu\text{m}$ in vivo. Body bilaterally flattened by about 1:2–3. From side view, cell broadly oval and quadrilateral with posterior portion often slightly wider than anterior; apical end considerably truncated while posterior end obliquely truncated (Figure 2A). No furrows or ridges detected. Podite about $15 \mu\text{m}$ long, posteriorly positioned on narrow ventral side (Figure 2B). Cytoplasm colourless, containing numerous tiny granules but no food vacuoles recognizable. Cytostome ventrally positioned at anterior end, diagonally oriented, with inconspicuous nematodesmata (cytopharyngeal rods) extending dorso-caudally (Figure 2D). Three contractile vacuoles detected (to be confirmed as pulsation was not observed) two near dorsal margin, the third one near base of podite (Figure 2A). Macronucleus oval, about in mid-body, ca $25 \times 15 \mu\text{m}$ in vivo in size, characteristically dimorphic (Figure 2D).

Movement genus-typical, slowly crawling over substratum and slightly thigmotactic.

Infraciliature as shown in Figure 2D: when viewed from lateral aspect, “right” field of ciliature composed of nine densely spaced, slightly fragmented kineties of variable length,

of which three extend to dorsal anterior end, while other six are shortened anteriorly (Figure 2C). To left of distal end of these kineties, one fragment of kineties is always present as a double-rowed structure. In mid-body, about six to eight short rows of densely packed basal bodies forming left equatorial field (Figure 2C, arrow). Equatorial fragment consisting of about 10 basal bodies. One large argentophilic patch-like “gland” positioned subcaudally, about 10 μm in length, always present near the base of podite (Figures 2C, double-arrowheads; 15J, arrows). Oral ciliature composed of three fragment-like kineties (Figure 2D, arrow).

Remarks

The new species is characterized by the combination of the following criteria: broadly oval shape, without grooves or ridges, three contractile vacuoles (?), nine ventral kineties, large cell size and Antarctic habitat.

Till now, about 11 *Dysteria* species have been described using modern methods: *D. calkinsi*, *D. ovalis*, *D. pusilla*, *D. cristata*, *D. armata*, *D. monostyla*, *D. antarctica*, *D. brasiliensis*, *D. procera*, *D. magna*, and *D. derouxi* (Deroux 1965, 1976). The former five species have considerably smaller size (usually smaller than 40 μm in length), fewer ventral kineties (three to six), thus can be clearly separated from *D. parovalis* (Fauré-Fremiet 1965; Gong et al. 2002, 2003a, b, 2004; Song and Wilbert 2002).

Dysteria monostyla and *D. antarctica* can be identified by only five ventral kineties (versus nine in *D. parovalis* Petz et al. 1995; Gong et al. 2002). *Dysteria brasiliensis* can be recognized by the presence of the long caudal spine and also fewer ventral kineties (consistently five; Song and Packroff 1997).

Dysteria derouxi is large (75–110 μm long in vivo), has constantly eight ventral kineties (versus nine in *D. parovalis*) and has two ventrally located contractile vacuoles (Gong and Song 2003b), hence easily separated from *D. parovalis*.

Compared with the new species described here: *Dysteria procera* can be identified by having only three ventral kineties (versus nine in *D. parovalis*) (Gong and Song 2003a); *D. magna* is an extremely large form (150 \times 100 μm versus 50–80 \times 40–60 μm for *D. parovalis*) with broadly rectangular body shape and constantly eight ventral kineties (versus nine in *D. parovalis*) (Gong and Song 2003a).

Genus *Aegyriana* Deroux in Song and Wilbert, 2002

In original description, the oral structure was insufficiently and incorrectly interpreted as it is difficult to detect because of its extremely anterior position (Deroux 1970; Song and Wilbert 2002). With the current population we had the opportunity to make further observations, so that an improved definition can be given here.

Improved diagnosis

Dorsoventrally flattened Dysteriidae with tail-shaped podite, which is positioned subcaudally in a glabrous region within the somatic kineties; oral ciliature in two parts: ca three close-set fragments on right and one kinety on left; left postoral kineties shortened posteriorly and continuous with right ones, leaving no median gap or suture; cytopharyngeal rods prominent.

Aegyriana paroliva Song and Wilbert, 2002
(Figures 3A–E, 12B, C, E, F)

Redescription

Body generally less variable (less flexible) than in original population, generally oval in shape with conspicuous snout-like projection on anterior left (Figure 3A, B). Cells in median area distinctly thickened (Figure 3B). Cytoplasm dark grey due to numerous globules and food vacuoles (Figure 3C). Food vacuoles often large (about 5–10 μm across), full of green or reddish algae or flagellates, but not many diatoms (Figure 3C). No contractile vacuoles observed.

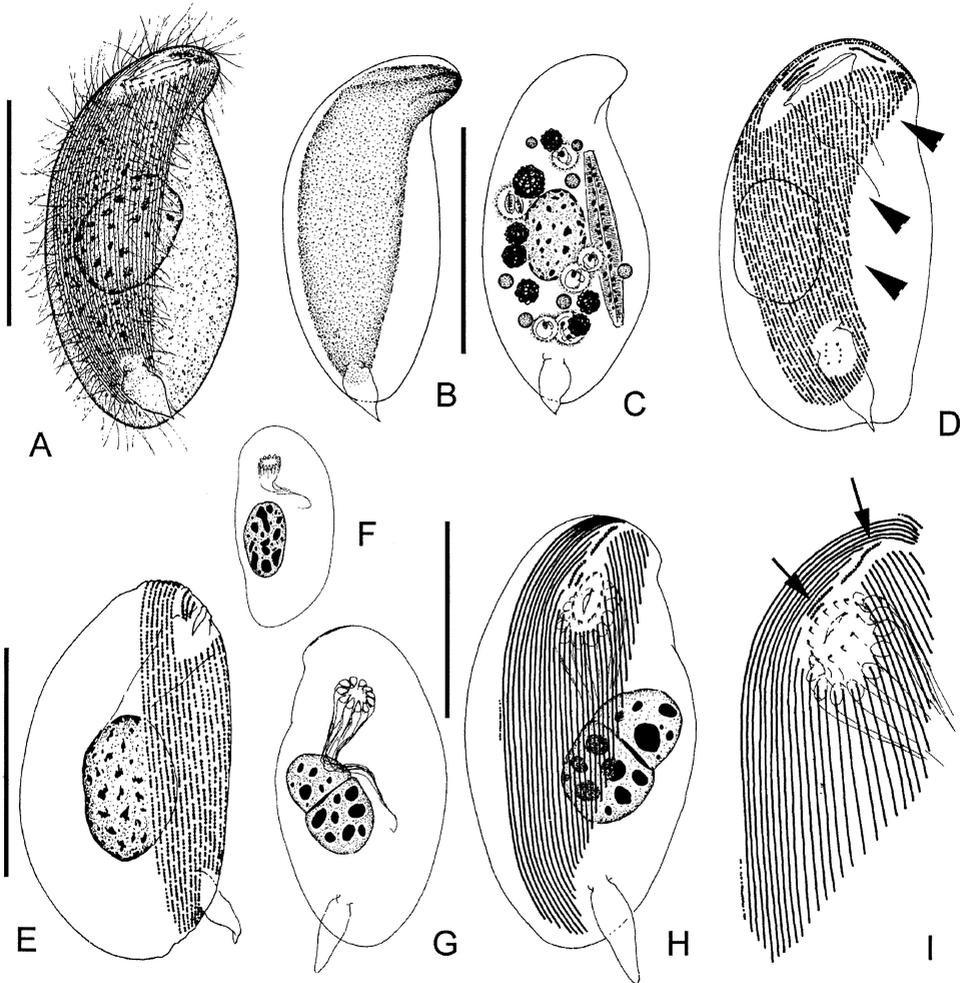


Figure 3. (A–E) *Aegyriana paroliva* and (F–I) *Hartmannula* cf. *angustipilosa* from life (A–C) and after protargol impregnation (D–I). (A) Ventral view, to show a typical specimen; (B) ventral view, to demonstrate the thickened portion in central area; (C) schematic display, to show a cell with all kinds of food that it contains; (D) ventral view of infraciliature, arrowheads mark the shortened kineties on left side; (E) lateral view, to show the flattened body shape and the macronucleus; (F, G) dorsal view, to show the cytopharyngeal structure and nuclear apparatus, note that the macronucleus in some cases is conspicuously dimorphic (F); (H) ventral view, to show the general arrangement of somatic kineties; (I) detailed portion of ventral view, arrows mark the oral kineties (oral apparatus). Scale bars: 50 μm (A, B, E); 40 μm (H).

Ciliation basically matching original description well, preorally about six to seven kineties arched to left margin of cell with some anterior-most kineties consistently shifted dorsally (Figure 3D). Perioral kineties (oral apparatus) clearly in two groups: right one three-rowed and fragment-like, separated from the single left kinety; all structures consisting of densely arranged dikinetids. On ventral side, general arrangement of kineties similar to that described previously (Song and Wilbert 2002) with all postoral kineties terminating anteriorly at buccal region and forming dominant buccal area (Figure 3D). However, 18–20 kineties on left posteriorly shortened gradually leftwards (Figures 3D, 15E, arrowheads and arrows). About nine kineties at posterior end “interrupted” by podite and hence forming large glabrous area, on the right of which there are about eight kineties, and ca four on the left (Figure 3D).

Genus *Hartmannula* Poche, 1913

***Hartmannula* cf. *angustipilosa* Deroux and Dragesco, 1968**
(Figures 3F–I, 12G; Table IV)

We failed to observe some critical features in vivo such as contractile vacuoles and other living characters for this Antarctic form. Although many well-impregnated specimens have been obtained. Its identification remains uncertain and we can here only tentatively describe it as a population of *Hartmannula angustipilosa* Deroux and Dragesco, 1968.

Description

Body size about 60–90 × 30–50 μm in vivo, mostly long oval with inconspicuous snout-shaped projection on anterior left (Figure 3H). Dorsoventrally distinctly flattened, ventral side flat, dorsal vaulted. Podite about 15 μm long. Cytostome prominent, sub-apically located in a longitudinal to oblique orientation; pharyngeal basket consisting of about 15 cytopharyngeal rods, extending slightly leftwards and posteriorly (Figure 3G). Dimorphic macronucleus large and oval, positioned in mid-body and containing several large nucleoli (Figure 3G, H). Micronucleus not detected.

About nine rightmost kineties extending preorally, with anterior portion curved to left margin; one terminal fragment positioned on anterior-left margin of cell. All other kineties (ca 18–26 in number) terminating around cytostome, leaving narrow glabrous buccal area. In addition to these normal kineties, there is one fragment-like equatorial right kinety with about 15 basal bodies in mid-body (Figure 3H, I).

Table IV. Morphometric characterization of *Hartmannula* cf. *angustipilosa*.

Character	Min	Max	Mean	SD	SE	CV	<i>n</i>
Body length	63	86	74.5	6.88	1.91	9.2	13
Body width	36	52	44.1	4.77	1.32	10.8	13
Number of somatic kineties	28	37	32.3	2.59	0.72	8.0	13
Length of podite	14	20	16.7	2.50	1.02	15.0	6
Length of macronucleus	26	34	29.2	2.81	0.70	9.6	11
Width of macronucleus	12	23	17.5	3.39	0.85	19.3	11
Number of macronuclei	1	1	1	0	0	0	>20

All data are based on protargol-impregnated specimens. Measurements in μm.

Oral ciliature consisting of (constantly) three short rows of dikinetids, which are obliquely (and preorally as well) positioned: one is anteriorly located and two posteriorly (Figures 3I, 12G, arrows).

Remarks

Deroux and Dragesco (1968) described three populations under the name *Hartmannula angustipilosa*, these are remarkably different in size and numbers of somatic kineties. The Antarctic form resembles the largest population in both body shape, size and the number of kineties. We speculate that the three populations in the original description might represent different species. Further information is required.

Genus *Pithites* Deroux and Dragesco, 1968

Pithites pelagicus nov. spec.

(Figures 4, 13 A–G; Table V)

Diagnosis

Large-sized marine *Pithites* with slightly dorsoventrally flattened body shape, about $50\text{--}80 \times 35\text{--}50 \mu\text{m}$ in vivo; oral ciliature consisting of ca seven fragments; seven to eight somatic kineties on right side, of which four rightmost ones extend dorsally around the cytostome; eight left kineties; equatorial and terminal fragments present; one contractile vacuole subcaudally positioned left of median; one ellipsoid dimorphic macronucleus; about 15 cytopharyngeal rods.

Etymology

The Latin word pelagic (planktonic, free swimming), indicating that this organism is a planktonic form.

Description

In vivo mostly $50\text{--}60 \times 40 \mu\text{m}$ in size. Body shape rather stable, oval to cordiform when viewed from ventral side, with posterior end more or less narrowed. Dorsoventrally flattened by about 2:3, conspicuously asymmetrical: ventral side flat, dorsal vaulted (Figure 4B). Pellicle thin and conspicuously notched in apical area on dorsal side, where the somatic kineties are arranged. When viewed from ventral side, one conspicuous shallow subcaudal cavity located near meridian of cell, which renders the associated area transparent and bright (Figure 4A).

Cytoplasm colourless to slightly greyish, often containing numerous tiny granules. Cytostome prominent, apically located. Cytopharynx typical of genus, long and extending posteriorly; pharyngeal basket consisting of about 9–12 cytopharyngeal rods (Figure 4H). One large contractile vacuole subcaudally positioned (Figure 4A, arrowhead). Food vacuoles not detectable. One macronucleus irregularly ellipsoid and positioned in mid-body, containing large nucleoli (Figure 4E). Micronuclei not detected.

Cilia about $7 \mu\text{m}$ long, densely arranged. Movement fast and jerky when swimming and attempting to attach to substratum by the apical area. Another form of locomotion is a circular movement on the bottom of Petri dish as shown in Figure 4C.

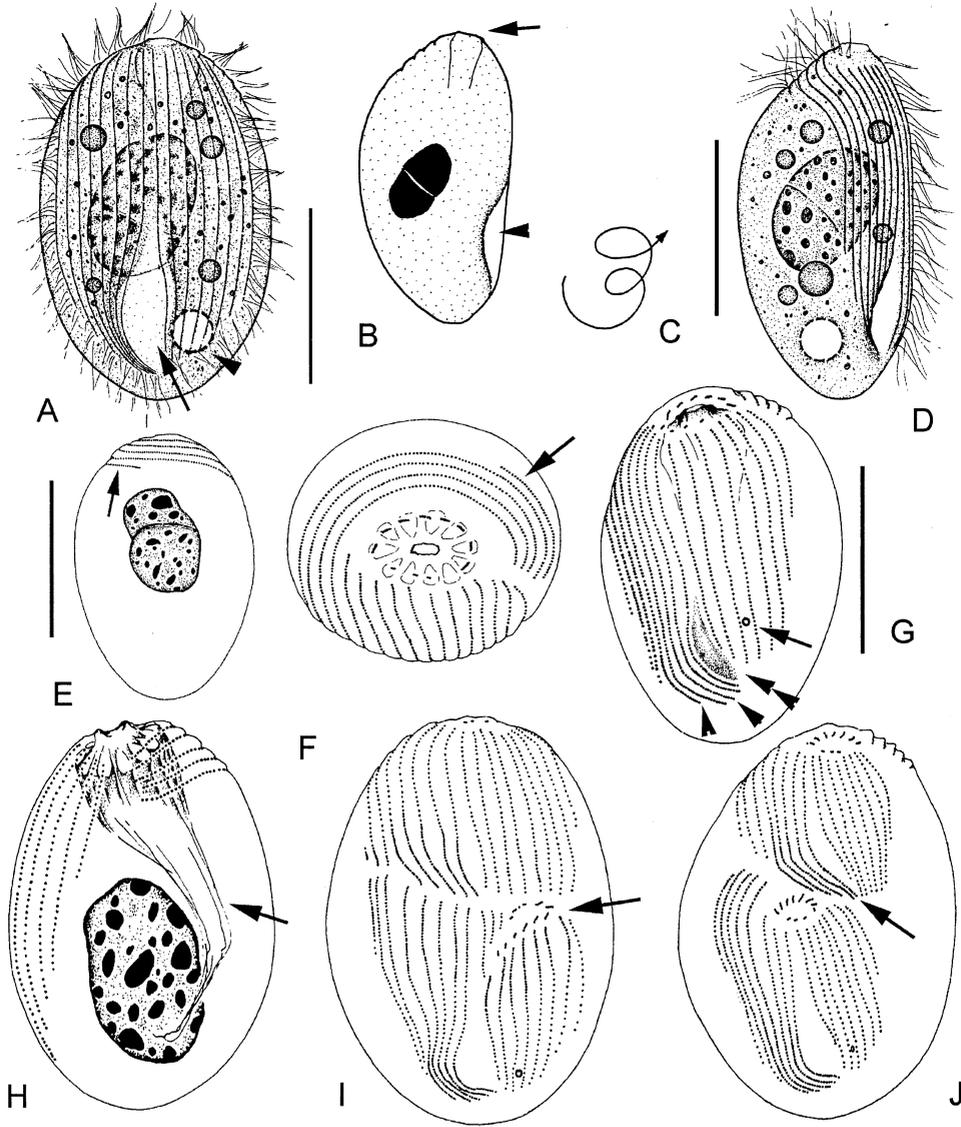


Figure 4. *Pithites pelagicus* nov. spec. from life (A–D) and protargol impregnation (E–J). (A) ventral view of a typical specimen, arrowhead marks the contractile vacuole, arrow indicates the subcaudal cavity; (B) lateral view, arrow indicates the cytostome, arrowhead marks the subcaudal cavity; (C) pattern of movement; (D) lateral view, to show the body shape; (E) left lateral view, to show the orientation of the cytopharynx (arrow); (F) apical view, arrow points to the terminal kinety (“dorsal brush”); (G) ventral view, to show the general infraciliature, arrowheads mark the densely ciliated part of left kineties, arrow points to pore of contractile vacuole, while the double-arrowheads mark the subcaudal cavity; (H) dorsal view, arrow marks the terminal kinety; (I, J) ventral view of cells in division, arrow in I marks the newly forming buccal apparatus, while in J it indicates the densely ciliated portion of kineties. Scale bars: 35 μm (A, D, E); 30 μm (G).

Infraciliature as shown in Figure 4E–G: four densely ciliated rightmost kineties extending preorally, curved on to dorsal side and terminating at left margin, parallel to which one terminal fragment is positioned at anterior left end (Figure 4F, arrow). About 12 postoral

Table V. Morphometric characterization of *Pithites pelagicus* nov. spec.

Character	Min	Max	Mean	SD	SE	CV	n
Body length	56	81	69.3	7.24	1.81	10.4	16
Body width	36	54	42.9	5.50	1.37	12.9	16
Number of somatic kineties on right side	7	8	7.7	0.47	0.11	6.1	20
Number of somatic kineties on left side	8	8	8	0	0	0	14
Number of kineties extending dorsally to left side of cell ^a	4	4	4	0	0	0	14
Length of macronucleus	22	41	35.3	5.91	1.48	16.8	16
Number of perioral fragments	7	7	7	0	0	0	6
Number of contractile vacuole pores	1	1	1	0	0	0	13

All data are based on protargol-impregnated specimens. Measurements in μm .

^aTerminal fragment not counted.

kineties arranged longitudinally around cytostome. Of these, almost always eight kineties on right of subcaudal cavity, with the posterior ends considerably more densely ciliated than the remainder (Figure 4G, arrowheads), and ca eight kineties on left, which are gradually shortened posteriorly. Contractile vacuole pore always located between third and fourth kineties (Figure 4G, arrow).

Oral ciliature consisting of ca seven short fragment-like dikinetids, mostly anterior to cytostome (Figure F). Telokinetal stomatogenesis; overview of very early stages as in Figure 4I, J.

Remarks

Only one known species in this genus has been reported, namely the type species, *Pithites vorax* Deroux and Dragesco, 1968. The new species can be distinguished from the former by its larger size ($30\text{--}35 \times 16\text{--}20$ versus $50\text{--}80 \times 35\text{--}50 \mu\text{m}$ in *P. pelagicus*), higher number of somatic kineties (six left and five to seven right versus eight and seven to eight, respectively) and lack of the thread-like structure present in the caudal region of *Pithites vorax* (Deroux and Dragesco 1968).

Order SCUTICOCILIATIDA Small, 1967

Genus *Philasterides* Kahl, 1931

Philasterides cf. *armatalis* Song, 2000

(Figure 1C)

Only a few cells found in two protargol-impregnated slides, so that it can be described only at infraciliature level. According to the oral structure and general somatic infraciliature, it seems to be an undescribed member of the genus *Philasterides*. However, it has to be treated as an unknown species until further information is obtained.

Description

Cells about $40 \times 20 \mu\text{m}$ long after protargol impregnation; body shape long oval to slender, circular in cross-section; apical end widely pointed with small apical plate surrounded by somatic kineties (Figure 1C, arrowhead); posterior end generally rounded. Ventral surface

slightly indented around buccal area, dorsally convex. Buccal field about 50% of cell length. One large oval macronucleus centrally located, $12 \times 16 \mu\text{m}$ in size; position of micronucleus uncertain, possibly adjacent to macronucleus.

About 22 somatic kineties longitudinally arranged, extending over entire length of body and mainly composed of dikinetids throughout. Each row with about 28 (paired or single) basal bodies.

Buccal apparatus consisting of three well-developed membranelles (M1–3) and one short paroral membrane which extends anteriorly to about the middle of membranelle 2. Membranelle 1 (M1) long and consisting of about 11 transverse rows of kinetosomes; membranelle 2 (M2) about half as long as M1 with ca five rows; membranelle 3 (M3) smaller, close to M2 with only three transverse rows. Paroral membrane likely not bipartite (? impossible to detect since cells lying on side). Scutica (Sc) with several pairs of basal bodies, sparsely arranged, posterior to cytostome (Figure 1C, double-arrowheads).

Remarks

This organism differs from the marine form *P. armatalis* Song, 2000 in structure of scutica, buccal apparatus, smaller body size and lower in having fewer ciliary rows (see Song 2000).

Genus *Pleuronema* Dujardin, 1836

***Pleuronema coronatum* Kent, 1881**

(Figures 1B, 13H, M)

This species was also identified from protargol-impregnated specimens (with numerous individuals). All morphometrical data correspond to the previous descriptions perfectly (Dragesco 1968; Song and Wilbert 2002). Hence only some extra data will be supplied here.

Micronuclei almost always several in number and closely adjacent to the rounded, large macronucleus. The Antarctic population has on average 40 somatic kineties, of which about five are shortened posteriorly on the left of the buccal field (Figure 1B).

Order *Peritrichida* Stein, 1859

Genus *Intranstylum* Fauré-Fremiet, 1904

The genus *Intranstylum* is a relatively rarely reported taxon, most of its members having been only superficially described using classical methods (Kahl 1935; Stiller 1971). As accepted by previous researchers, it is recognized by: (1) the colonial form of all congeners and (2) the extremely reduced or degenerated spasmoneme, which may connect all zooids but has no contractile function, thus the stalk is non-contractile.

In our recent publication (Song and Wilbert 2002), one *Zoothamnium* sp. with abnormal spasmoneme was described from the same biotope as in the current investigation. However, after rechecking the slides, we believe it was a misidentification and should be conspecific with the population found this time. With reference to the large size, body shape, appearance of colony, the habitat and the poorly developed myoneme system, this form should represent an undescribed *Intranstylum*.

Intranstylum antarcticum nov. spec.
(Figures 5A–C, 13I–L)

Diagnosis

Large-sized marine *Intranstylum* ca $100 \times 60 \mu\text{m}$ in vivo with ellipsoidal body shape, smooth pellicle and thick, single-layered peristomial border; one contractile vacuole located apically; macronucleus C-shaped, transversely positioned. Colony large, with about 20 or more zooids; stalk smooth, with regular, dichotomous branching; myoneme long with branches of different thickness.

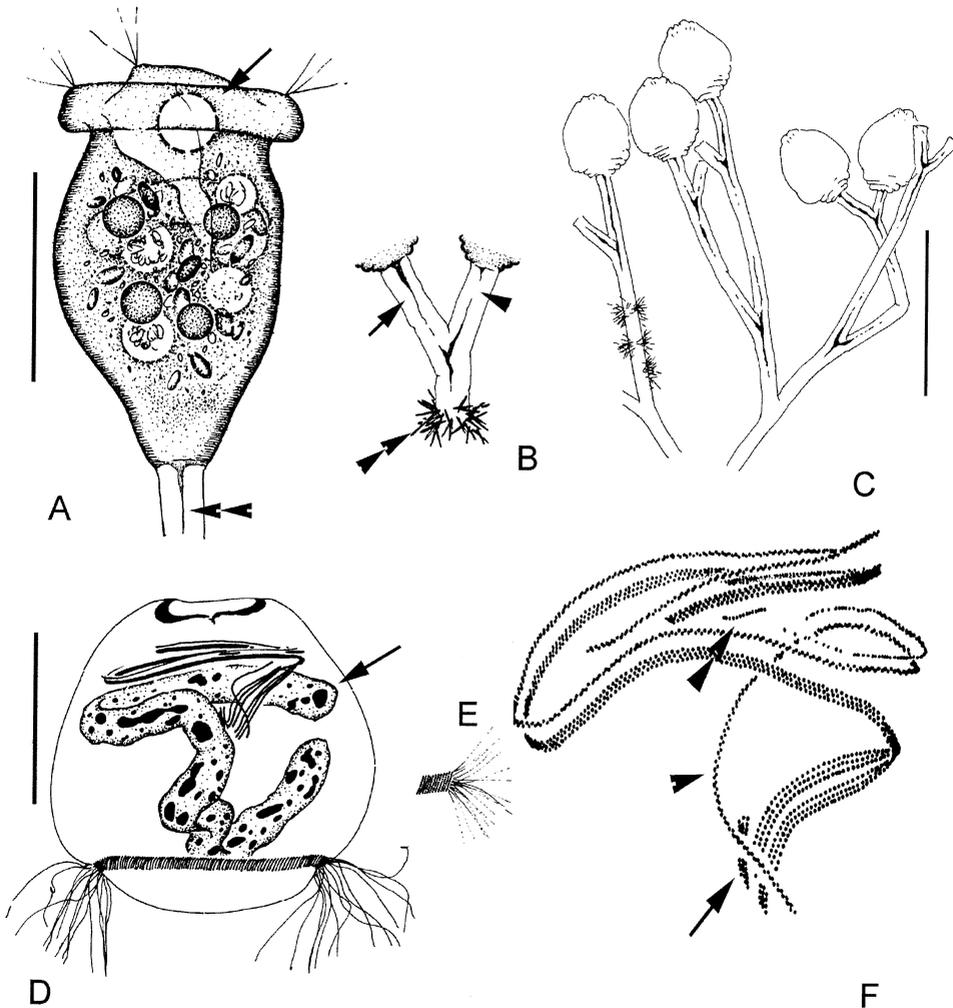


Figure 5. (A–C) *Intranstylum antarcticum* nov. spec. and (D–F) *Telotrochidium* sp. from life (A–C) and protargol impregnation (D–F). (A) Typical specimen, arrow marks the peristome, double-arrowheads mark the reduced spasmoneme; (B) portion of stalk, arrow marks the myoneme, note the place where the myoneme is broken (arrowhead); double-arrowheads indicate the bacteria attached to the stalk; (C) to show the general stalk arrangement; (D) general view, to show the macronucleus (arrow) and infraciliature; (E) detailed part of aboral ciliary wreath; (F) buccal apparatus, arrow marks the peniculus 3, arrowhead indicates the haplokinety, double-arrowheads point to the epistomial membrane. Scale bars: $50 \mu\text{m}$ (A); $200 \mu\text{m}$ (C); $30 \mu\text{m}$ (D).

Description

Zooids in similar size, in vivo about 100 μm long with thick, single-layered peristomial border; body form-constant, slender and elongated vase-shaped, only slightly constricted below peristomial collar; peristomial disc large and flattened (Figure 5A). Cells not very sensitive to mechanical stimuli. When contracted, zooids usually globular in shape with distinctly truncated or folded plate at both cell ends. Pellicle smooth when observed at low magnification, fine transverse striations recognizable only under high magnification, with no visible granules or any other pellicular structure. Cytoplasm colourless or slightly greenish, usually containing many food vacuoles, which are oval or irregularly shaped and measure about 3–6 μm in length (Figure 5A). Contractile vacuole large, apically located. Macronucleus C-shaped, thick and transversely positioned.

Colony large (probably over 500 μm in length), having about 20 (probably more ?) zooids and a regular dichotomously branching stalk. Stalk with smooth surface, about 10 μm thick. Myoneme system consisting of long spasmoneme within stalk, which is about as long as the body length, relatively poorly developed as described in previous report (Song and Wilbert 2002 under the name of *Zoothamnium* sp.). In the present population, spasmoneme also varied in length (20–100 μm) and in different parts of the stalk thickness (Figure 5B). No differentiation of macro- and microzooids.

Remarks

Though numerous cells/colonies were found, no information about the infraciliature is available due to over-staining of the impregnation. Hence the comparison to known congeners can be carried out only on the basis of observations in vivo.

This new species can be recognized by the combination of the following characters: large cell size and dichotomous by branched stalk, long and poorly developed spasmoneme, generally oval to ellipsoidal body shape of zooids and the marine habitat (Kahl 1935; Stiller 1971; Song et al. 2003).

Genus *Telotrochidium* Kent, 1881
***Telotrochidium* ? sp.**
 (Figure 5D–F)

Only several cells were found on protargol-stained slides, thus no further identification can be carried out. Here only a brief description is given based on the impregnated specimens.

Description

Cells oval to barrel-shaped, about 60 \times 40 μm after protargol impregnation (Figure 5D). Macronucleus vermiform, strongly twisted (Figure 5D, arrow), while micronucleus not detected.

Aboral ciliary wreath consisting of broad band of kineties, which are obliquely and densely arranged and each of which consists of more than 10 basal bodies (Figure 5D). No scopula recognizable. Buccal ciliature as shown in Figure 5F, epistomial membrane near distal end of polykinety (double-arrowheads in 5F), while haplokinety (arrowhead in 5F) considerably shifted anteriorly; peniculus 3 distinctly short (arrow in 5F).

Remarks

As noticed by other researchers, sometimes it is difficult to separate the swimmers of other stalked peritrichs and the genus *Telotrochidium*, hence it is not completely ensured that there is no misidentification of the present organism concerned. We await further observations.

Order *Heterotrichida* Stein, 1859**Genus *Folliculina* Lamark, 1816*****Folliculina* ? sp.**

(Figure 6)

Several individuals were observed and impregnated, but no cells with lorica have been found (really not present?). Since the genus identification depends largely on the shape or structure of the lorica, the definition of the genus is, therefore, questionable.

Description

Body shape in vivo as shown in Figure 6A. Total length of cell about 250 μm long in vivo, shape relatively constant (not very sensitive to disturbance); two ear-shaped wings wide and relatively long (about 120 μm in length), generally transparent except the border area where membranelles arranged (Figure 6A, B). Cytoplasm colourless, but mostly dark grey to black due to numerous food vacuoles (with algae or diatoms) and lipid granules, especially under lower magnification. Macronucleus globular, about 30 μm across with many large nucleoli (Figure 6C).

This organism seems to be a typical benthic form: always using its caudal area to attach to surfaces such as debris.

About 110 somatic kineties, which are typical of folliculinids, consisting of densely arranged dikinetids, each with ca 7 μm long cilia. Membranelles along margin of two wings, extending into deep buccal cavity (Figure 6C, arrowheads). Paroral membrane mostly multi-rowed with irregularly arranged basal bodies, parallel to adoral zone (Figure 6C, arrow).

Genus *Condylostoma* Bory, 1824***Condylostoma* cf. *magnum* Spiegel, 1926**

(Figure 7A–C)

This organism was only found from protargol-impregnated slides and no in vivo data (e.g. concerning the body shape and size) are available. With reference to the infraciliature, it is extremely similar to the well-known species, *Condylostoma magnum* Spiegel, 1926, but differs from the latter, however, in having fewer somatic kineties (ca 30 versus 47–56) (Dragesco 1996; Song and Wilbert 1997), which indicates that these two forms might not be conspecific. As a document contribution to studies in the future, a brief description is supplied here.

Description

Cells about 250 μm long after impregnation, no tail present although caudal end might be conspicuously pointed (Figure 7A). Buccal field probably relatively small (about one-sixth

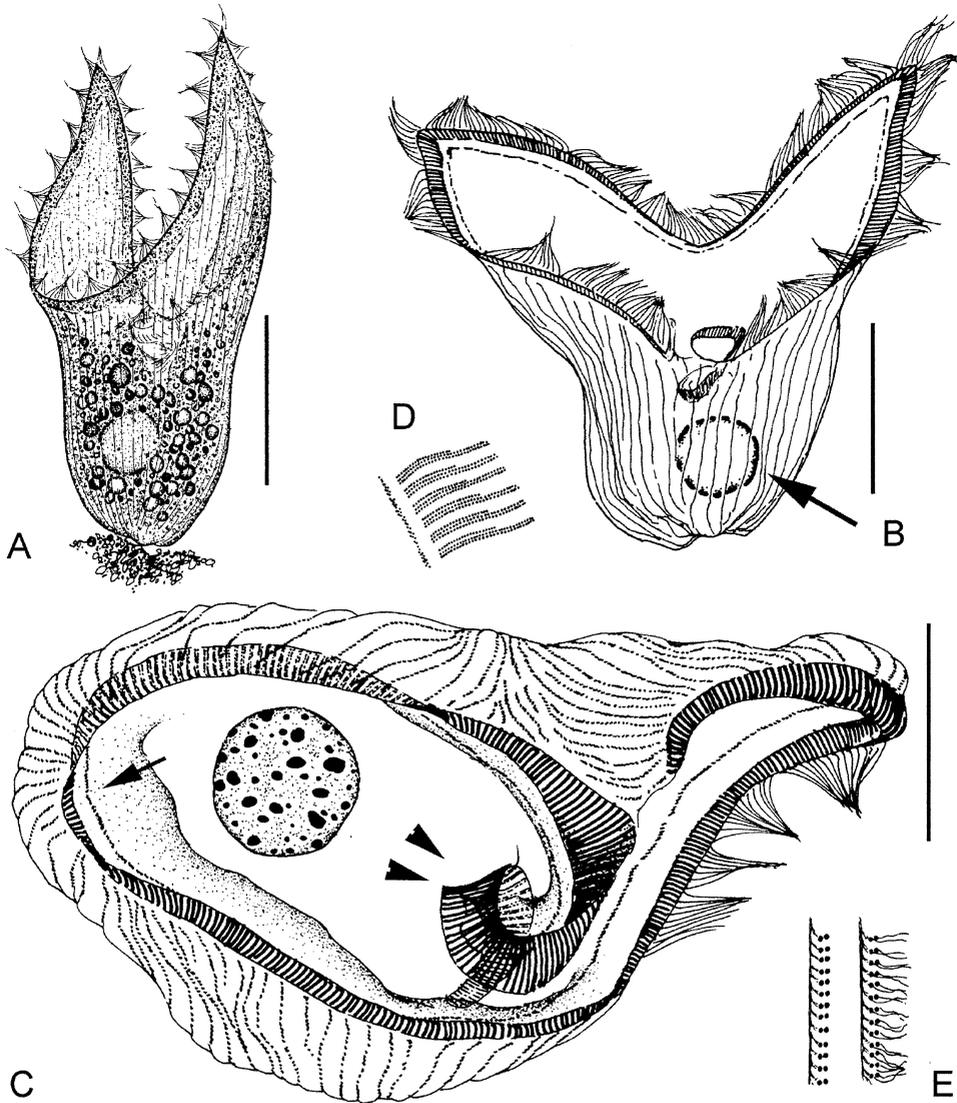


Figure 6. *Folliculina*? sp. from life (A) and after protargol impregnation (B–E). (A) A typical specimen, note that it is attached to substratum; (B) lateral view, arrow marks the macronucleus; (C) apical view, arrow marks the paroral membrane, arrowheads indicate proximal end of adoral-zone membranelles, which extends deeply into the buccal cavity; (D) details of membranelles and paroral membrane; (E) somatic kineties, to show the fibres associated with dikinetids. Scale bars: 100 μm (A); 50 μm (B); 20 μm (C).

of body length after fixation) (Figure 7C). Macronucleus moniliform with ca 10 ellipsoid nodules, lying slightly to right of main body axis (Figure 7B).

Somatic kineties composed of paired basal bodies as in other congeners, both ciliated with relatively short cilia. About 30 somatic kineties, some of which terminate subcaudally.

Adoral zone of membranelles conspicuous, consisting of more than 120 membranelles with proximal portion extending spirally into dominant buccal cavity. Paroral membrane developed, on right of dominant buccal cavity, terminating posteriorly near cytopharynx (Figure 7C). The number of cirri-like membranelles at distal end of adoral zone (at least

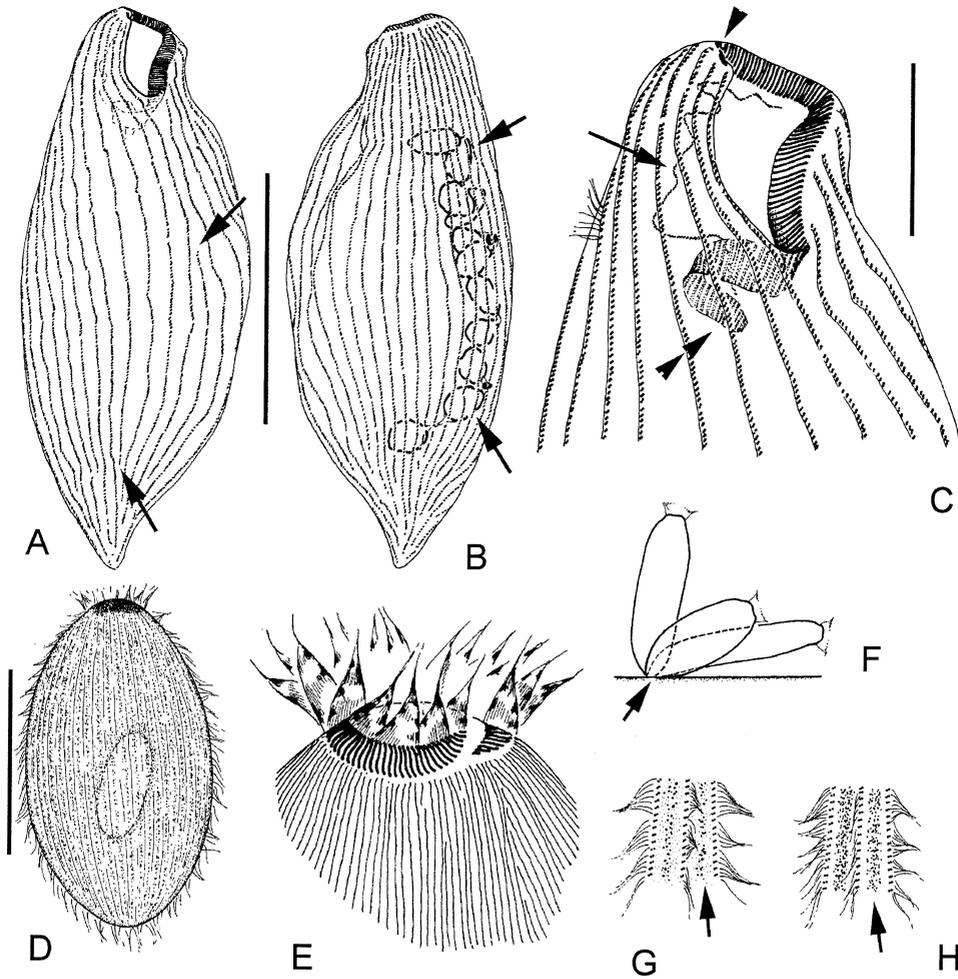


Figure 7. (A–C) *Condyllostoma* cf. *magnum* and (D–H) *Heterostentor coeruleus* from life (D–F) and after protargol impregnation (A–C, G–H). (A) Ventral view, arrows mark the shortened kineties; (B) dorsal view, arrows mark the macronuclear nodules; (C) anterior portion of ventral view, arrow marks the paroral membrane, arrowhead and double-arrowheads point to the distal and proximal ends of adoral zone of membranelles, respectively; (D) a slightly contracted individual; (E) anterior portion, to show the appearance of the membranelles; (F) diagram of movement while attached to the bottom, arrow indicates the caudal region used as attachment area; (G, H) details of pellicle, arrows mark the pigments (cortical granules). Scale bars: 100 μm (A, B); 30 μm (C); 80 μm (D).

two) not exactly determined because of the invagination and contraction of related portion (Figure 7C, arrowhead).

Genus *Heterostentor* Song and Wilbert, 2002
***Heterostentor coeruleus* Song and Wilbert, 2002**
 (Figures 7D–H, 14J–N)

The present population resembles the form described previously (Song and Wilbert 2002), so a complete redescription is unnecessary. Only the main features found to vary from the 2002 population are included here.

Description

Body often more slender oval than elongated cylindrical, dorsoventrally only slightly flattened, length to width about 1:2–3 when moving freely, but 1:3–5 when attached to substratum by posterior end of cell and contracting from time to time (Figure 7F). Dark blue pigments beneath pellicle arranged in at least two patterns: sparsely distributed between ciliary rows with larger granules or conspicuously densely distributed (Figure 7G, H). In anterior cell end (around adoral zone of membranelles), these pigments/granules render the cell dark blue.

Numerous membranelles in adoral zone which, in vivo, appear to be clustered into about 20 groups of membranelles ca 25 μm long and extend forwards (Figure 7E).

Order STROMBIDIIDA Jankowski, 1980**Genus *Strombidium*** Claparède and Lachmann, 1859

This well-known, species-rich genus has typical family characters: adoral zone of membranelles generally consisting of collar and buccal parts and two somatic kineties: circularly arranged girdle/equatorial kinety and the short, subcaudally positioned ventral kinety, although the former may have a small gap, i.e. not be completely continuous. Unlike *Strombidium*, the related genus *Spirostrombidium* established by Jankowski (1978) is characterized by the differently arranged somatic kineties, i.e. the girdle (equatorial) kinety does not encircle the equatorial area, but is opened and extends spirally to the caudal region, often very close and parallel to the short ventral kinety. Thus, some members of this genus seem to possess only one somatic kinety when observed superficially (Petz et al. 1995; Song and Packroff 1997; Song et al. 1999, 2000).

Based on the pattern of the girdle kinety, the most critical feature to separate the two genera is the posterior end of the equatorial kinety which extends to the caudal area and is parallel to the ventral one versus terminates anterior to the proximal end of ventral kinety.

According to this definition, the new form we obtained should be classified within the genus *Strombidium*.

Strombidium apolatum nov. spec.

(Figures 8, 14A–I; Table VI)

Diagnosis

Cordiform *Strombidium* in vivo about $50 \times 40 \mu\text{m}$; ca 13 collar and seven buccal membranelles not continuous; girdle and ventral kinety with ca 45 and 60 basal body pairs, respectively; single elongate to sausage-like macronucleus; extrusomes evenly distributed along the somatic kineties; marine habitat.

Etymology

Composite of *apo* (derived from) and the species name *latum*, indicating that this species is different from the congener *S. latum*.

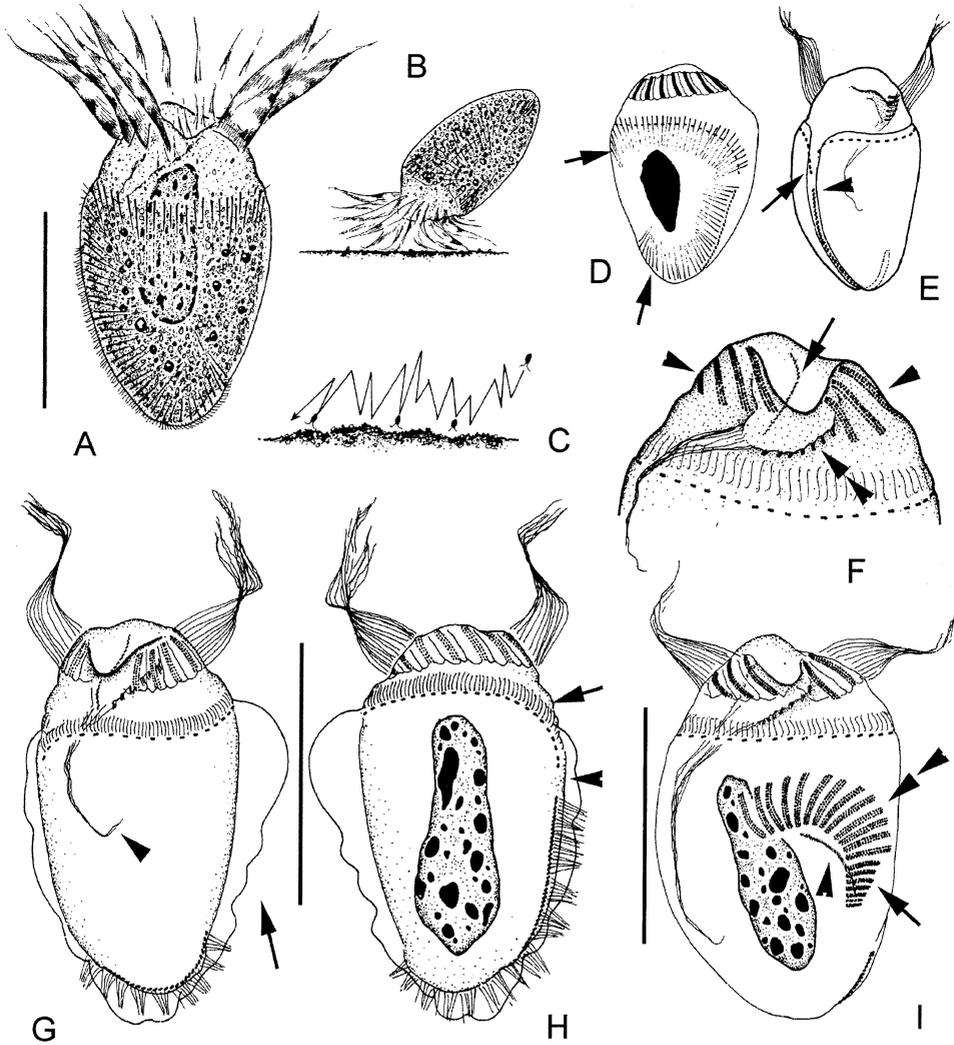


Figure 8. *Strombidium apolatatum* nov. spec. from life (A–C) and after protargol impregnation (D–I). (A) Ventral view of a typical specimen; (B) to show a cell attached to the bottom; (C) pattern of movement; (D) dorsal view, to show the arrangement of extrusomes (arrows); (E) ventral–lateral view, to show the girdle and ventral kinety, which almost meet at equatorial area (arrow and arrowhead, respectively); (F) anterior portion of ventral view, to show the buccal structure: arrow marks the paroral membrane, double-arrowheads indicate the buccal membranelles, arrowheads point to the collar membranelles; note the highly developed cytopharyngeal fibres extending backwards; (G) ventral view, arrow marks the subpellicular platelet layer; (H) dorsal view, arrow marks the equatorial girdle, arrowhead points to the gap between girdle and ventral kinety; (I) an individual in early divisional stage, double-arrowheads mark the newly formed collar membranelles, while arrow indicates the buccal ones; note the paroral membrane (arrowhead). Scale bar: 30 μm .

Description

Size about 40–60 \times 30–45 μm *in vivo*, after fixation ca 35–50 μm in length; cell ratios about 3:2 for length/width and ca 4:3 for dorsoventral flattening. Body shape consistently cordiform, often slightly asymmetrical when viewed ventrally; anteriorly broadly rounded, posteriorly slightly pointed (Figure 8A). Buccal cavity shallow, about one-quarter of cell

Table VI. Morphometric characterization of *Strombidium apolatium* nov. spec.

Character	Min	Max	Mean	SD	SE	CV	<i>n</i>
Body length	31	49	42.1	5.38	1.62	12.8	11
Body width	25	38	33.1	4.67	1.41	14.1	11
Number of collar membranelles	12	14	12.6	0.79	0.30	6.3	7
Number of buccal membranelles	6	8	7.3	0.82	0.33	11.1	6
Number of basal bodies in ventral kinety	ca 60	–	–	–	–	–	–
Number of basal bodies in ventral kinety	ca 45	–	–	–	–	–	–
Length of macronucleus	23	32	28.6	2.51	0.76	8.8	11
Width of macronucleus	7	10	8.8	0.98	0.30	11.1	11

All data are based on protargol-impregnated specimens.

length. Transparent subpellicular platelet layer in posterior three-quarters of cell (Figure 8G, arrow), although the polygonal platelets often found in other congeners were not detected here. Equatorial girdle in anterior two-thirds length, ca 2 µm wide, immediately ahead of subpellicular platelet layer (Figure 8H, arrow); extrusomes densely and uniformly distributed, inserting along girdle (Figure 8D, arrows).

Cytoplasm tightly packed with dark globules (especially directly below the cell surface) and lipid droplets, rendering cells dark to almost black at low magnification. Macronucleus elongated to sausage-like (but sometimes irregularly shaped after fixation), about 25–30 × 7–10 µm in size, with many large nucleoli (Figures 8H, 14D, H). Micronucleus not detected.

Movement fast and almost without pause, hectically to and fro on the debris (Figure 8C).

Adoral zone spirally around peristomial field, cilia of most membranelles about 25–30 µm long, always extending anteriorly (Figure 8A). Twelve to fourteen collar membranelles (CM), bases ca 7 µm in length and conspicuously longer than those in buccal zone. The buccal zone (BM) consists of six to eight membranelles and is positioned in the shallow buccal cavity, hence conspicuously separated from the CM (Figure 8F). Pharyngeal fibres highly developed, ca 20 µm long and extending obliquely to right side (Figure 8F, G).

Girdle kinety (equatorial kinety) composed of about 45 dikinetids (*n*=5), running along the edge of subpellicular platelet layer, begins at right margin, turns to dorsal side and then curves slightly towards the posterior (but clearly separated from the ventral kinety; Figure 8H, arrowhead). Ventral kinety more densely ciliated than girdle one, containing ca 60 basal body pairs, extending from right body margin subcaudally to the left and terminating laterally at posterior end of girdle kinety at level of about anterior third of cell length (Figure 8H). Cilia in girdle kinety about 2 µm long, while in ventral one ca 5 µm in length (Figure 8G, H).

Remarks

The genus *Strombidium* is a species-rich taxon containing more than 30 nominal species (Kahl 1932; Maeda and Carey 1985; Lynn et al. 1988; Montagnes et al. 1988, 1990; Montagnes and Taylor 1994; Montagnes and Lynn 1991; Lynn and Gilron 1993; Petz et al. 1995). With respect to the subpellicular platelet layer which covers almost two-thirds of cell length, the flattened body shape, the shallow buccal cavity, densely arranged cilia in ventral

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kinety, the laterally positioned ventral kinety and the sausage-like macronucleus, this species clearly differs from all other known members of this genus (Lynn et al. 1988; Montagnes et al. 1988, 1990; Montagnes and Lynn 1991; Petz et al. 1995; Song and Packroff 1997; Song et al. 2000).

Order HYPOTRICHIDA Stein, 1859
Genus *Thigmokeronopsis* Wicklow, 1981
Thigmokeronopsis magna nov. spec.
 (Figures 9, 15J–M, O; Table VII)

Diagnosis

Large flexible marine *Thigmokeronopsis* about 150–300 × 50–80 μm in vivo; ca 65 adoral membranelles extending to about one-third of cell length; 12–16 left postoral cirral rows forming thigmotactic field; 60 left and 70 right marginal cirri, about 43 and 15 pairs of cirri in midventral and frontal rows, respectively; one buccal, ten transverse and two fronto-terminal cirri; three dorsal kineties; no caudal cirri; more than 150 macronuclear nodules; one contractile vacuole positioned in about mid-body.

Description

Size mostly about 200 × 60 μm, body very flexible and variable in shape but generally slender and elongate, with anterior part distinctly narrower than posterior; anteriorly narrowly rounded, posteriorly tapered; buccal field wide, about 30% of cell length (Figure 9A). Pellicle thin, no cortical granules observed although cytoplasm usually brownish to dark brown in colour (due to food?) as observed under low magnifications. Cytoplasm with many tiny lipid droplets and food vacuoles containing mainly small and large pennate diatoms or small protozoans. Contractile vacuole on left of body and near equatorial level (Figure 9A, H). More than 150 macronuclear nodules scattered throughout the cell (Figure 9F), spherical to ellipsoid, usually with one to several large nucleoli (Figure 9G).

Movement slow, usually three different modes observed: (1) crawling on bottom of Petri dish or debris, making small circular movement (Figure 9I); (2) attached by thigmotactic field to the bottom while the raised anterior part of body makes (slow) left–right movements (Figure 9J); or (3) when disturbed, relatively faster, crawling around irregularly. When swimming in water, moves slowly with no special features (Figure 9B).

Buccal field large and deep. Adoral zone of membranelles extending far on to right side. Paroral and endoral membranes about equal in length, slightly bent in posterior portion, optically appearing to intersect (Figure 9K). One buccal cirrus at about mid-level of undulating membranes.

About 15 frontal cirri forming bicorona, which are not clearly distinguished from the midventral cirri posteriorly although the anterior-most (usually three) cirri are conspicuously enlarged (Figure 9K). Two midventral rows distinctly separated (so that the cirri are not arranged in typical zig-zag pattern), terminating near posterior rightmost transverse cirri (Figure 9E, K). Left postoral cirral field (=thigmotactic field) consisting of about 13 (in maximum width) densely packed longitudinal rows, in each of which the cirri are basically loosely arranged (Figure 15K). Bases of cirri in this thigmotactic field mostly smaller than other “normal” ones and more or less ovoid in shape (Figures 9K, 15K).

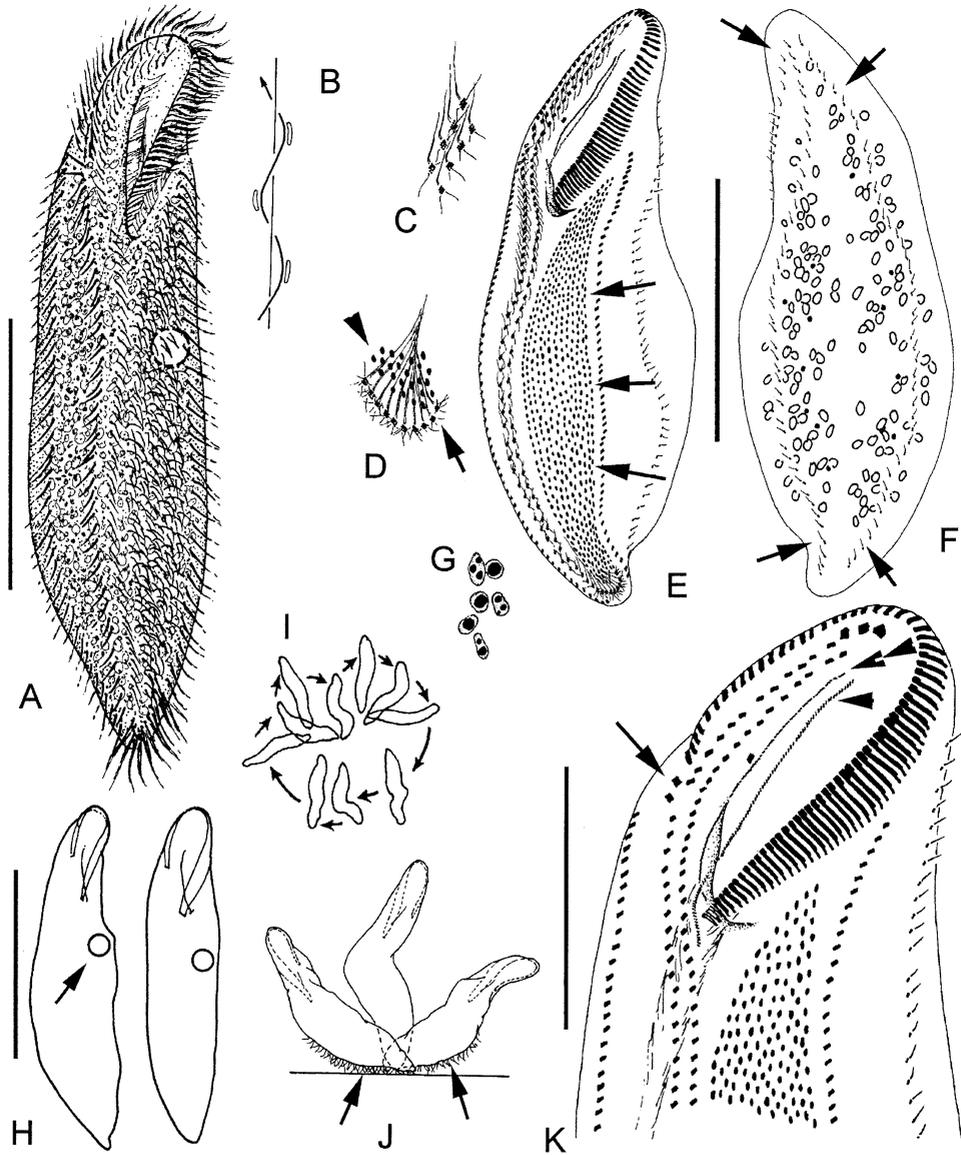


Figure 9. *Thigmokeronopsis magna* nov. spec. from life (A, B, H–J) and after protargol impregnation (C–G, K). (A) A typical specimen, ventral view; (B) schematic, to show the pattern of movement when swimming; (C) four pairs of midventral cirri, to demonstrate the fibre system associated with the cirri; (D) details, to show transverse (arrow) and thigmotactic cirri (arrowhead); (E) ventral view, arrows mark the thigmotactic area; (F) dorsal view of the same cell as in (E), to show the distribution of macronuclei, arrows mark the dorsal kineties; (G) several macronuclear nodules; (H) two different body forms, arrow points to the contractile vacuole; (I) diagram showing the pattern of movement while on the bottom; (J) the third type of movement: cells firmly attached to the bottom, arrows mark the thigmotactic area; (K) anterior portion of ventral side, arrowhead marks the paroral membrane, double-arrowheads indicate the endoral membrane, arrow points to the frontoterminal cirri. Scale bars: 100 μm (A); 50 μm (E, F); 150 μm (H); 40 μm (K).

Table VII. Morphometric characterization of *Thigmokeronopsis magna* nov. spec.

Character	Min	Max	Mean	SD	SE	CV	<i>n</i>
Body length	135	255	202.2	34.42	7.69	17.0	20
Body width	71	91	81.7	6.34	1.91	7.8	11
Length of buccal field	68	90	78.7	7.53	1.88	9.6	16
Number of adoral membranelles	59	71	64.9	4.83	1.61	7.4	9
Number of buccal cirri	1	1	1	0	0	0	>20
Number of frontoterminal cirri	2	2	2	0	0	0	13
Number of cirral pairs in frontal rows	12	16	14.0	1.48	0.43	10.6	12
Number of cirral pairs in midventral rows	39	46	43.3	3.84	1.11	7.7	12
Number of cirri in left marginal row	55	63	59.5	2.95	0.93	5.0	10
Number of cirri in right marginal row	64	76	70.5	3.75	1.19	5.3	10
Number of transverse cirri	9	13	10.2	1.34	0.39	13.2	12
Number of cirral rows in thigmatotactic area ^a	12	16	13.5	1.38	0.40	10.2	12
Number of dorsal kineties	3	3	3	0	0	0	13

All data are based on protargol-impregnated specimens. Measurements in μm .

^aCounted in maximum area.

Transverse cirri slightly enlarged and arranged in J-shape, joining thigmatotactic field (Figures 9D, E, 15L). Marginal rows not confluent posteriorly. Dorsal kineties constantly three in number, densely ciliated (ca 3 μm long, Figure 15J); without caudal cirri.

Remarks

Till now, four species within the genus *Thigmokeronopsis* have been described: *T. jahodai*, *T. antarctica*, *T. rubra*, and *T. crystallis* (Wicklow 1981; Petz; Hu et al. 2004). *Thigmokeronopsis antarctica* is separated from the new species by having several buccal cirri, shortened midventral rows, only one thigmatotactic cirral row and no transverse cirri. *Thigmokeronopsis magna* differs from *T. crystallis* in possessing many more cirral rows in the thigmatotactic field (12–16 versus 4–7) (Wicklow 1981; Petz 1995).

The new species is distinguished from the type species, *Thigmokeronopsis jahodai* Wicklow, 1981, in that it has: (1) three dorsal kineties (versus four in *T. jahodai*); (2) one buccal cirrus (versus two in *T. jahodai*); (3) more cirri in the midventral rows (ca 100 versus 50) (Wicklow 1981).

Thigmokeronopsis rubra Hu et al., 2004 is a colourful (brick-red) species with two kinds of distinct cortical granules and thus can be clearly separated from *T. magna*. In addition, the former possesses fewer adoral membranelles (33–43 versus ca 65) and fewer thigmatotactic cirral rows (7–11 versus 12–16) (Hu et al. 2004).

Genus *Holosticha* Wrzesniowski, 1877

Holosticha sp.

(Figure 10A–C)

In view of its body size, the extremely shortened midventral rows with only a few pairs of cirri and the presence of large “extrusomes” (cortical granules?), this small *Holosticha* possibly represents an unknown form. Unfortunately, only a small number of protargol-impregnated individuals were observed on a permanent slide and no other necessary

information, e.g. features observable in life or biometrical data, is available. Further studies are required in order to determine the identity of this taxon.

Description

Body shape in vivo unknown; after impregnation, broadly oval, about $65 \times 40 \mu\text{m}$ in size; buccal field about two-fifths of cell length (Figure 10A). Distal end of adoral zone evenly bent towards right side with ca 17 membranelles, the longest bases of adoral membranelles

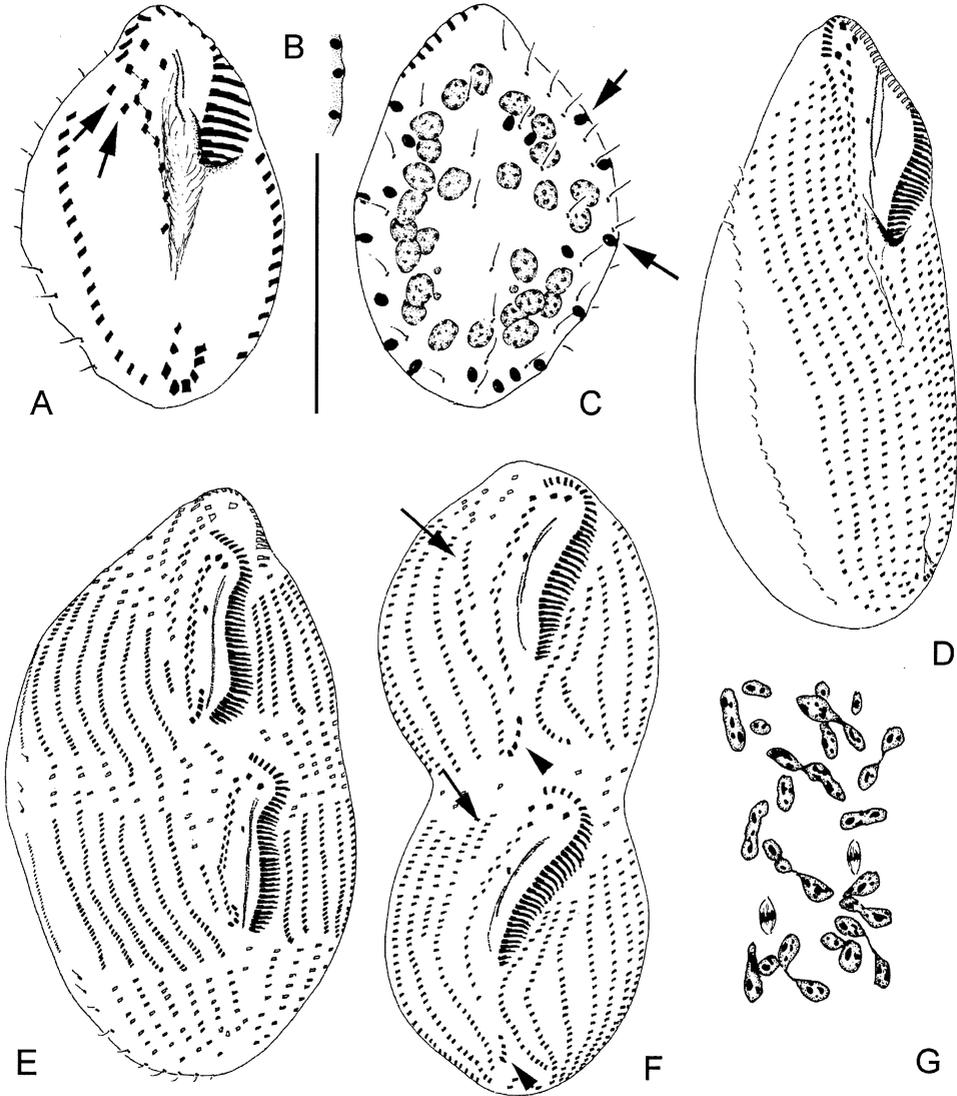


Figure 10. (A–C) *Holosticha* sp. and (D–G) *Metaurostylopsis rubra* after protargol impregnation. (A) Ventral view, arrows mark the fronto-terminal cirri; (B) detailed portion, to show the extrusomes; (C) dorsal view, arrows mark the extrusomes; (D) ventral view, to show general structure of ciliature; (E, F) cells in middle and late divisional stage, arrows in (F) mark the fronto-terminal cirri, arrowheads indicate transverse cirri; (G) macronuclei. Scale bar: $40 \mu\text{m}$.

about 8–10 μm long. Paroral and endoral membranes short and straight, pharyngeal fibres over 20 μm long (Figure 10A). About 30 macronuclear nodules distributed throughout the cell, each oval in shape and ca 4 μm long; four globular micronuclei recognizable, which are relatively large (3 μm across). On dorsal side, always several argentophilic extrusome-like structures (cortical granules ?), which are about 3 μm in length, vase-shaped with rounded posterior end and positioned along dorsal kineties (Figure 10B, C).

Three frontal cirri, only slightly enlarged and difficult to separate from the midventral cirri; one buccal cirrus near anterior end of undulating membranes; two fronto-terminal cirri between distal end of adoral zone of membranelles and anterior end of right marginal row. Midventral rows atypical and very short, consisting of only three to four pairs of cirri, posterior to which there is a single row with three to five cirri extending to about midway along the cell (Figure 10A). Six to seven enlarged transverse cirri forming J-shape, immediately anterior to which two small cirri are often observed. Right marginal row consisting of ca 17 cirri, left row with about 14; the two rows are widely separated at posterior cell end.

Four dorsal kineties, each with about 6–15 cilia (cilia about 5 μm long), no caudal cirri (Figure 10C).

Genus *Metaurostylopsis* Song, Petz and Warren, 2001

***Metaurostylopsis rubra* Song and Wilbert, 2002**

(Figures 10D–G, 15A–I, N)

The current population matches the original description very well. We thus supply here only some information derived from recent observation of its morphological features and the morphogenetic process.

Description

Body often more oval than originally reported, size in vivo about $150 \times 50 \mu\text{m}$. Eight left and six right marginal rows on average; adoral zone about one-third of body length with 45 membranelles; about 12 pairs of cirri in midventral rows, single ventral row extending to about posterior third of cell length with ca 13 cirri; ca 10 fronto-terminal, one buccal, four frontal and five transverse cirri (Figures 10D, 15E, F, I). Constantly three dorsal kineties, no caudal cirri.

On the basis of the morphogenetic stages obtained from the present population, formation of the ciliature in *Metaurostylopsis rubra* can be summarized as follows: (1) The parental oral apparatus is entirely renewed by the proter's oral primordium. (2) Two sets of fronto-ventral-transverse cirral anlagen give rise to the buccal, fronto-terminal, transverse and anterior most frontal cirri, as well as to the ventral and the midventral rows in both divisional parts. (3) The leftmost frontal cirrus develops from the undulating membrane primordium in both proter and opisthe. (4) Streak II–III generates the other frontal and the buccal cirri. (5) Streaks IV to n-1 give rise to the midventral rows. (6) The short ventral row posterior to the midventral rows originates also from the anlage n-1 (the last but one). (7) The fronto-terminal cirri are formed by the last streak of the fronto-ventral-transverse anlagen. (8) Two anlagen develop within each marginal cirral row, each of which forms a separate marginal row. (9) One anlage develops within each of the three parental dorsal kineties in both dividers. (10) No caudal cirri are differentiated.

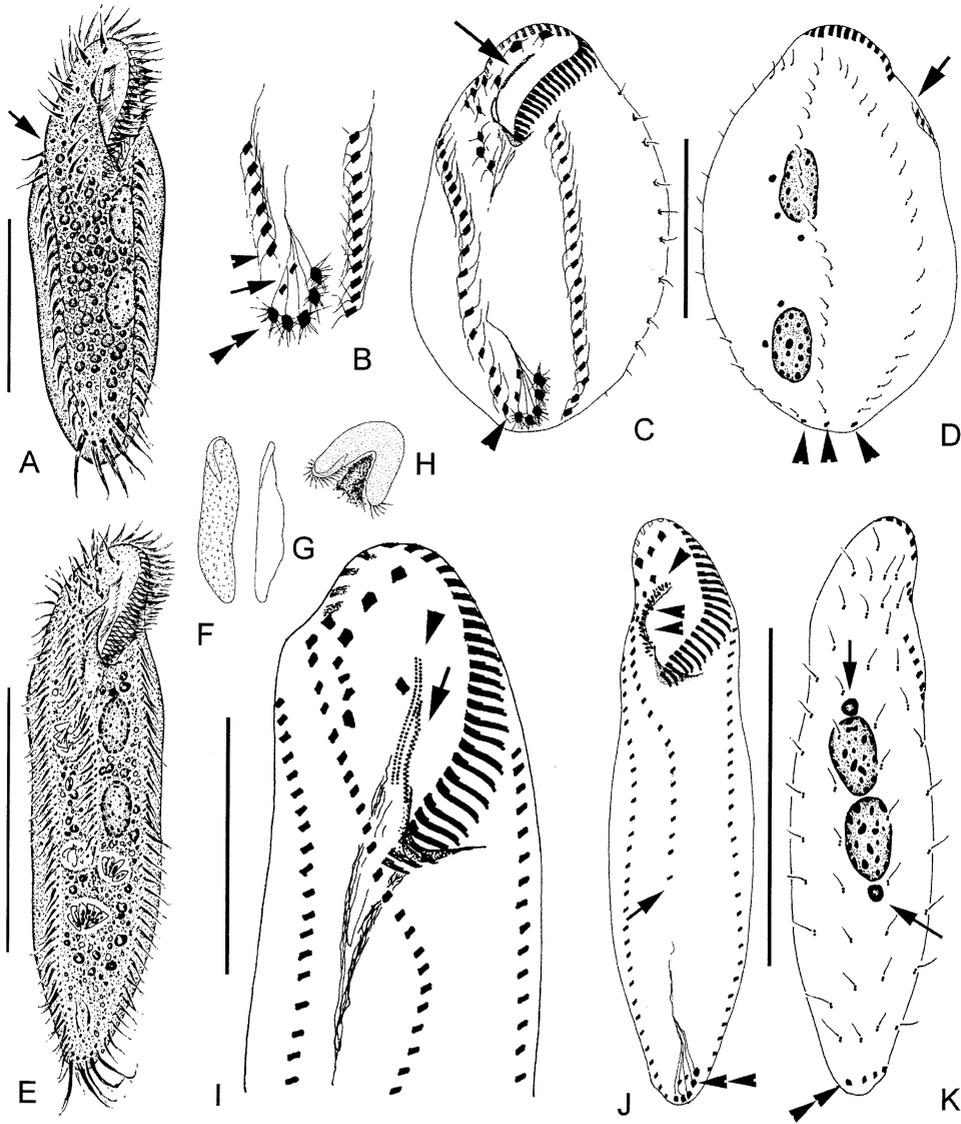


Figure 11. (A–D) *Hemigastrostyla szaboi* nov. spec. and (E–K) *Amphisiella antarctica* nov. spec. from life (A, E–H) and after protargol impregnation (B–D, I–K). (A) A typical specimen, arrow marks the groove, where the marginal cirri are positioned; (B) posterior portion of ventral side, arrow marks the two small ventral cirri, double-arrowheads indicate the transverse cirri, arrowhead points to the posterior end of right marginal row, note that there is a conspicuous gap between the transverse and right marginal cirri; (C) ventral view, arrow marks the undulating membranes, while the arrowhead indicates the position where the right marginal row and transverse cirri join together; (D) dorsal view, arrow indicates the anterior end of right marginal row, while arrowheads mark the caudal cirri; (E) ventral view of a typical individual; (F) dorsal view, to show the cortical granules; (G) lateral view, to demonstrate the flattened body shape; (H) to show the flexible body; (I) anterior portion of ventral side, arrowhead indicates the double-rowed paroral membrane, arrow marks the endoral membrane; (J) ventral view, arrowheads mark the extrusomes, which are positioned along undulating membranes, arrow indicates the posterior end of ventral row, while double-arrowheads mark the transverse cirri; (K) dorsal view, arrows mark the micronuclei, double-arrowhead indicates the caudal cirri. Scale bars: 50 μm (A, C); 80 μm (E); 30 μm (I); 60 μm (J, K).

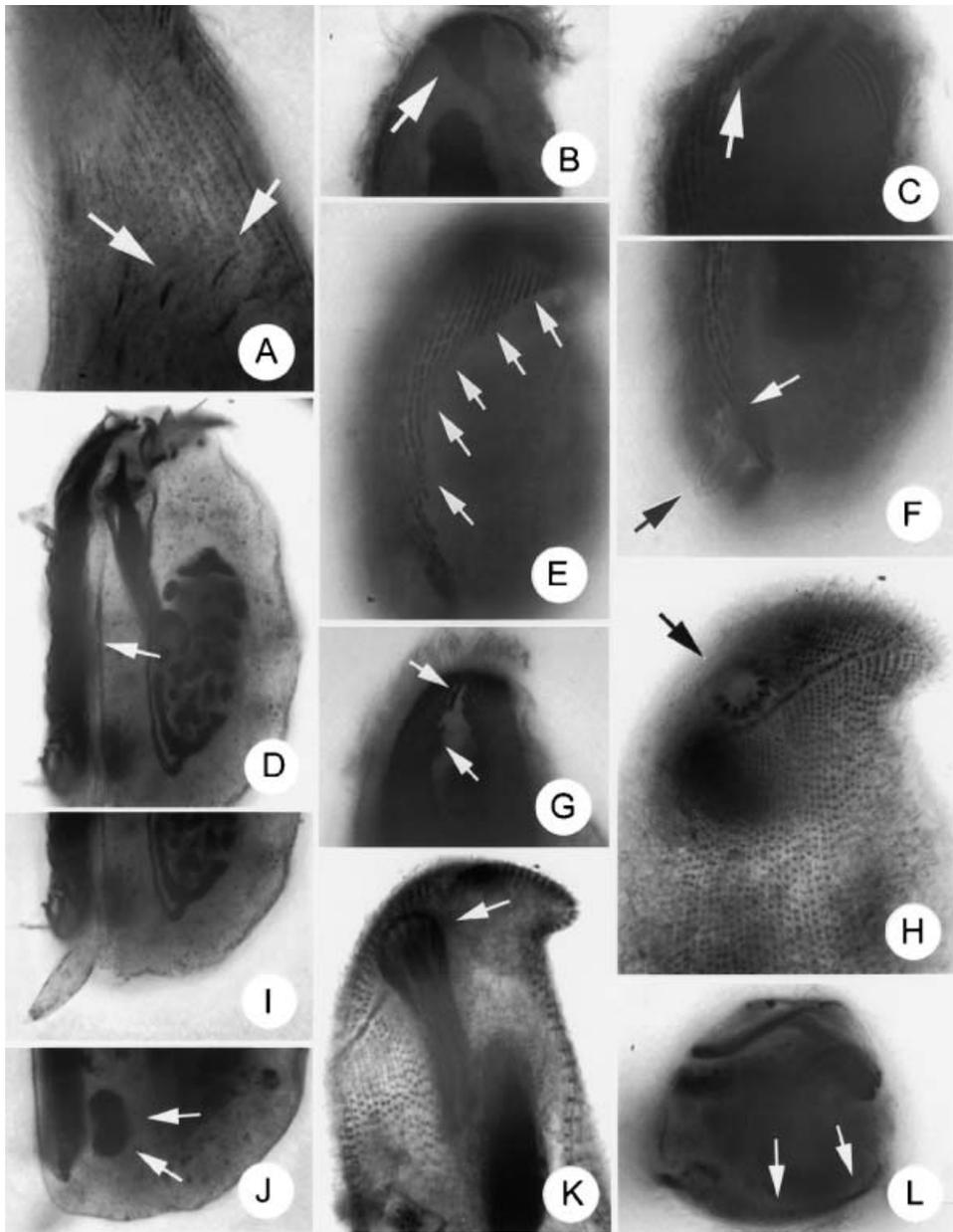


Figure 12. Photomicrographs of (A) *Amphileptus* sp., (B, C, E, F) *Aegyriana paroliva*, (D, I, J) *Dysteria parovalis* nov. spec., (G) *Hartmannula* cf. *angustipilosa*, (H, K) *Orthodonella shenae*, and (L) *Telotrochidium* sp. after protargol impregnation. (A) Infraciliature of anterior portion; arrows indicate the extrusomes; (B) ventral view of anterior portion; arrow indicates the cytopharynx; (C) ventral view of oral area, arrow marks the three-rowed perioral kineties; (D) left side, arrow marks the shortened left somatic kineties; (E) ventral view, arrows indicate the shortened kineties; (F) ventral view of caudal portion, white arrow indicates the glabrous area, whereas the black arrow points to the podite; (G) ventral view, arrows mark the perioral kineties; (H) ventral view of anterior part, arrow indicates the cystome; (I) posterior portion, to show the podite; (J) the same specimen but focusing at different level, to show the argyrophilic patch (gland ?); (K) ventral view, to show the highly developed cytopharynx (arrow); (L) lateral view, arrow marks the broad aboral wreath of cilia.

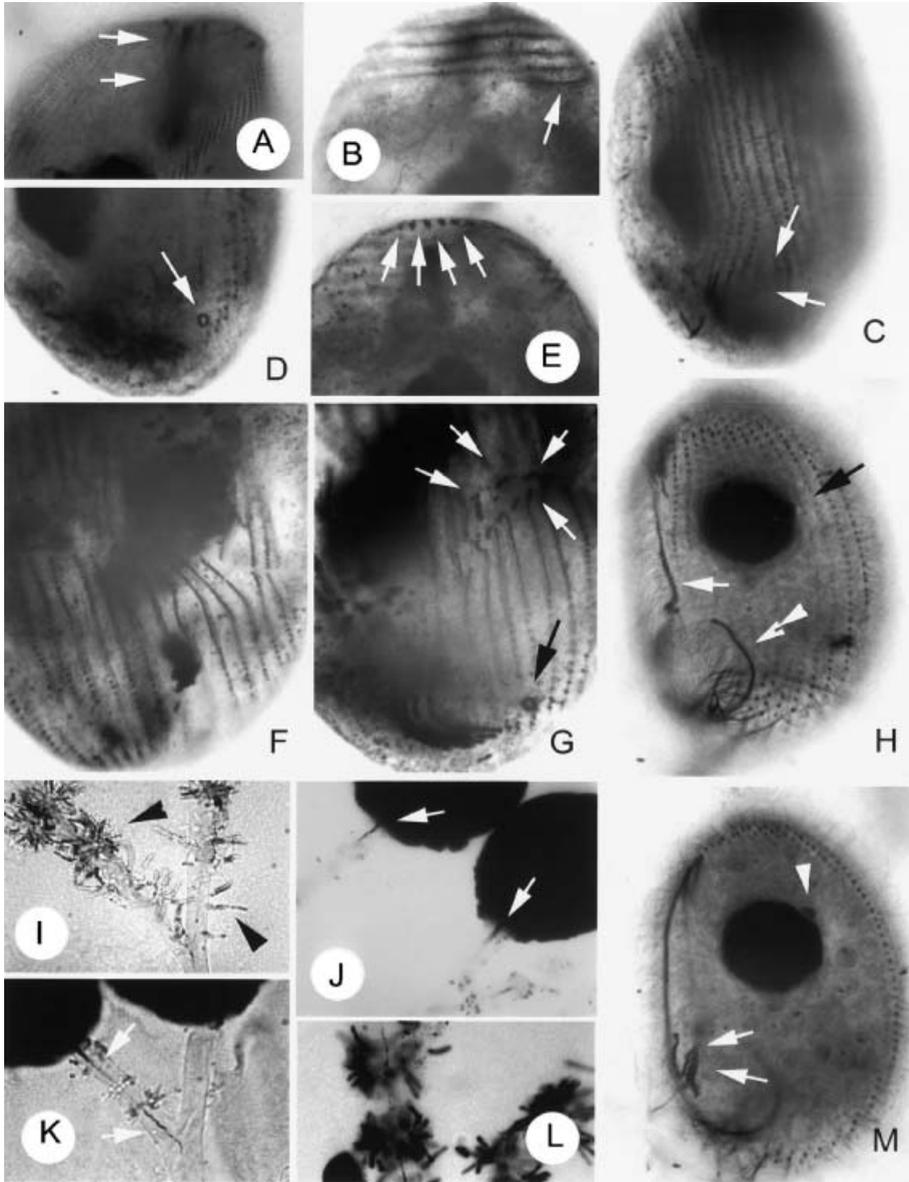


Figure 13. Photomicrographs of (A–G) *Pithites pelagicus* nov. spec., (H, M) *Pleuronema coronatum* and (I–L) *Intranstylum antarcticum* nov. spec. after protargol impregnation. (A) Lateral view of the anterior portion; arrows mark the cytopharynx; (B) dorsal side view (looking from ventral to dorsal) of anterior portion, arrow indicates the terminal fragment (“dorsal brush”); (C) ventral view, arrows mark the gap between left and right somatic kineties; (D) ventral view, to show the contractile vacuole pore (arrow); (E) ventral view of apical area, arrows mark the fragment-like perioral kineties; (F) ventral view of a specimen in division; (G) ventral view at a later stage of division, white arrows mark the newly formed oral apparatus in the opisthe, while the black arrow indicates the contractile vacuole pore; (H) left-lateral view, the arrowheads point to the posterior end of paroral membrane, the black arrow indicates whereas the macronucleus; (I, L) stalk, arrowheads in (I) mark the attached diatoms (?); (J) two zoids, arrows indicate the reduced spasmoneme; (K) to show the spasmoneme (arrow); (M) left-lateral view, arrowhead marks the micronucleus, arrows point to the membranelle 3.

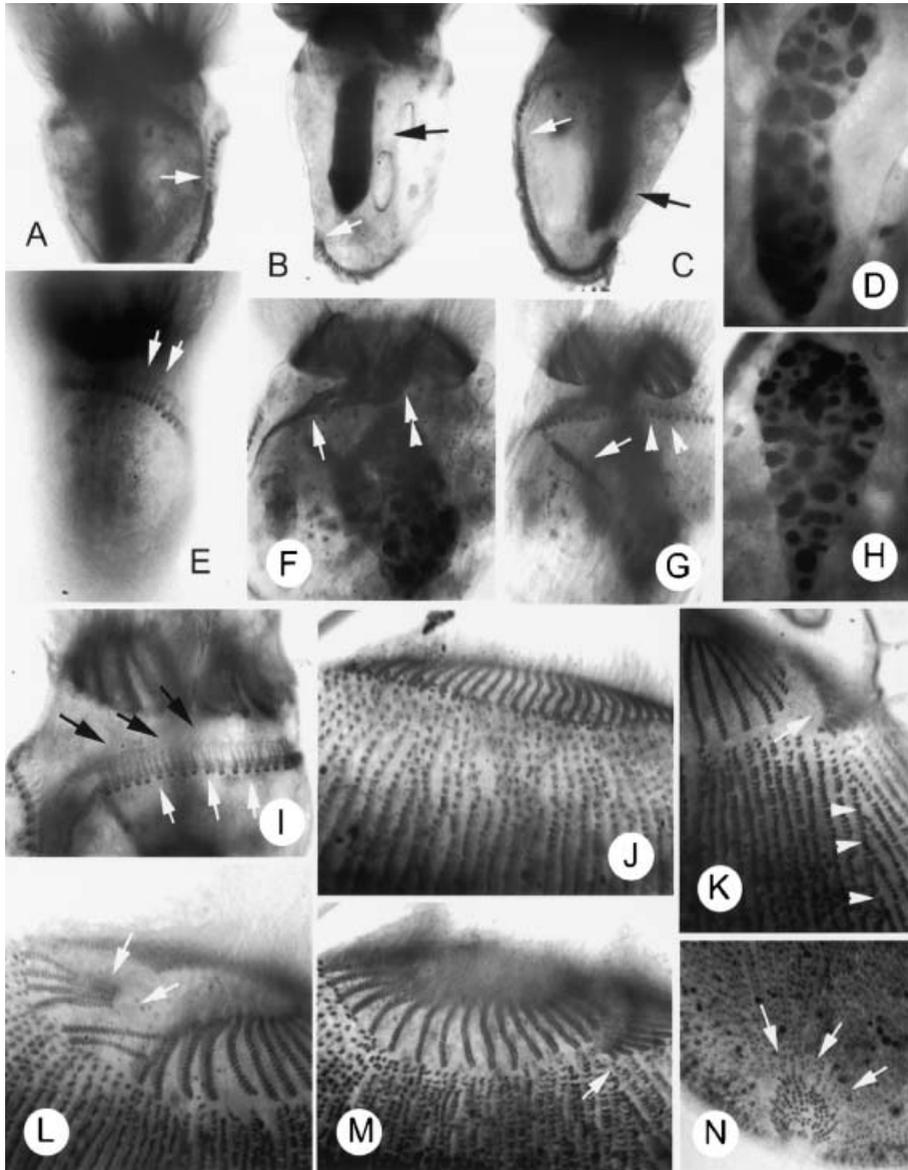


Figure 14. Photomicrographs of (A–I) *Strombidium apolatium* nov. spec. and (J–N) *Heterostentor shenae* after protargol impregnation. (A) Dorsal view, to show the gap between the equatorial and ventral kinety (arrow); (B) dorsal view, black arrow marks the macronucleus, whereas the white one indicates the end of ventral kinety; (C) ventral view, white and black arrows point to the kinety-gap and macronucleus, respectively; (D, H) macronucleus; (E) dorsal view, to show the equatorial kinety and the girdle (arrows); (F) ventral view, arrowheads mark the inconspicuous buccal membranelles, whereas the arrow points to the cytopharynx; (G) ventral view, focusing at surface level, arrow indicates the newly formed oral primordium, arrowheads mark the equatorial kinety; (I) ventral view, to show the girdle (black arrows) and the equatorial kinety (white arrows); (J) dorsal side of anterior portion, to show the adoral zone of membranelles; (K, M) ventral view, arrow indicates the proximal end of adoral zone of membranelles, while the arrowheads in (K) mark the suture, where some kineties are shortened; (L) to show some irregularly arranged membranelles (arrows); (N) caudal view, to show the thigmotactic area, where the basal bodies are densely arranged (arrows).

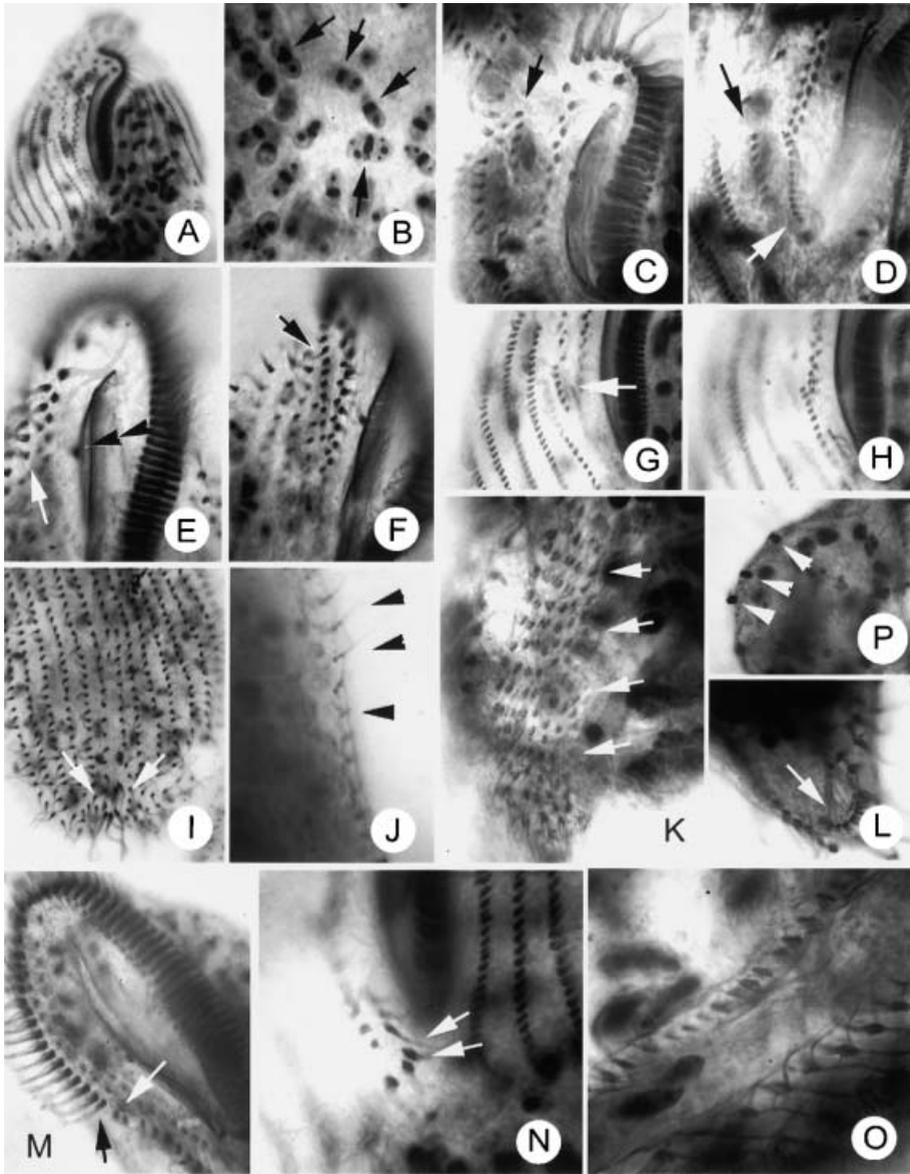


Figure 15. Photomicrographs of (A–I, N) *Metaurostylopsis rubra*, (P) *Holosticha* sp. and (J–M, O) *Thignokeronopsis magna* nov spec. after protargol impregnation. (A) Ventral view of anterior portion in late divisional stage; (B) to show the macronuclei in replication stage (arrow marks the replication band); (C, D) detailed portion of the opisthe, black arrows in (C) and (D) indicate the fronto-terminal cirri; white arrow in (D) points to the ventral row; (E, F) buccal field, white arrow in (E) marks the fronto-terminal row, while double-arrowheads indicate the buccal cirrus; black arrow in (F) points to the long fronto-terminal row; (G, H) ventral view of proter, arrow in (G) marks the fronto-terminal cirral row; (I) ventral view, to show the general pattern of ventral, marginal and transverse cirri; (J) portion of dorsal side, to show the dorsal kineties (arrowheads); (K) ventral view, to show the thigmotactic area, note that the cirri are densely but irregularly arranged (arrows); (L) ventral view of caudal part, arrow indicates the transverse cirri; (M) ventral view of buccal region, white arrow indicates the two fronto-terminal cirri, whereas the black one marks the distal end of adoral zone of membranelles; (N) ventral view in a late divider, to show the newly formed transverse cirri (arrows); (O) to show the fibres connecting to the marginal cirri; (P) dorsal view, to show the large extrusomes (arrowheads).

Thus the morphogenetic process is very similar to its congener, *Metaurostylopsis marina* (Kahl, 1932) Song et al., 2001 (Song et al. 2001), though some details of this new species remain unknown because certain divisional stages have not yet been observed.

Genus *Hemigastrostyla* Song and Wilbert, 1997

This genus was established by Song and Wilbert (1997), which was defined originally by (1) generally having an *Oxytricha*-like cirral pattern; (2) usually possessing slightly cephalized body shape; and (3) presence of two “extra” ventral cirri.

On the basis of the new understanding subsequently obtained (Song and Hu 1999), however, the diagnosis of this genus should be slightly amended and some new points need to be added: (1) no right-lateral anlagen of dorsal kineties occurs during morphogenesis; (2) the proximal portion of the old adoral zone (AZM) will be replaced by newly formed structure; (3) 8–10 frontal (including one buccal), five to seven ventral cirri; (4) highly developed fibre system which is associated with cirri; (5) inflexible body shape; (6) dorsal cilia located in small pits.

According to this new definition, the organism found in the present investigation should be assigned as a new species of the genus *Hemigastrostyla*.

Hemigastrostyla szaboi nov. spec. (Figure 11A–D, 16G–L; Table VIII)

Diagnosis

Medium-sized marine *Hemigastrostyla* in vivo about 100–150 × 30–50 μm with elongated body shape; ca 27 adoral membranelles, eight (including one buccal) frontal, five ventral, five transverse and three caudal cirri; left and right marginal row each with 23 cirri on average, three dorsal kineties; two macro- and two to six micronuclei.

Dedication

We dedicate this species to the Hungarian protozoologist, Dr Andras Szabo, in recognition of his contributions to the study of ciliates.

Description

Cells generally inflexible; body shape distinctly slender with both ends only slightly narrowed, ratio of cell length to width about 3:1 (Figure 11A); buccal field narrow, about one-third to two-fifths of body length; dorsoventrally flattened 1:2. Cell margins generally parallel but in anterior portion often slightly swelling outward. Ventral side conspicuously grooved because of the presence of marginal rows (Figure 11A, arrow).

Pellicle thin; no cortical granules recognizable (not carefully observed). Cytoplasm often containing numerous light-reflecting globules (3 μm across), which render the cell completely dark, especially in posterior portion of body. Food vacuoles basically not detectable. No contractile vacuole detected. Two macronuclei, elongate and often conspicuously separated from each other (Figures 11D, 16K); two to five oval micronuclei near macronuclei (Figure 16G, arrows).

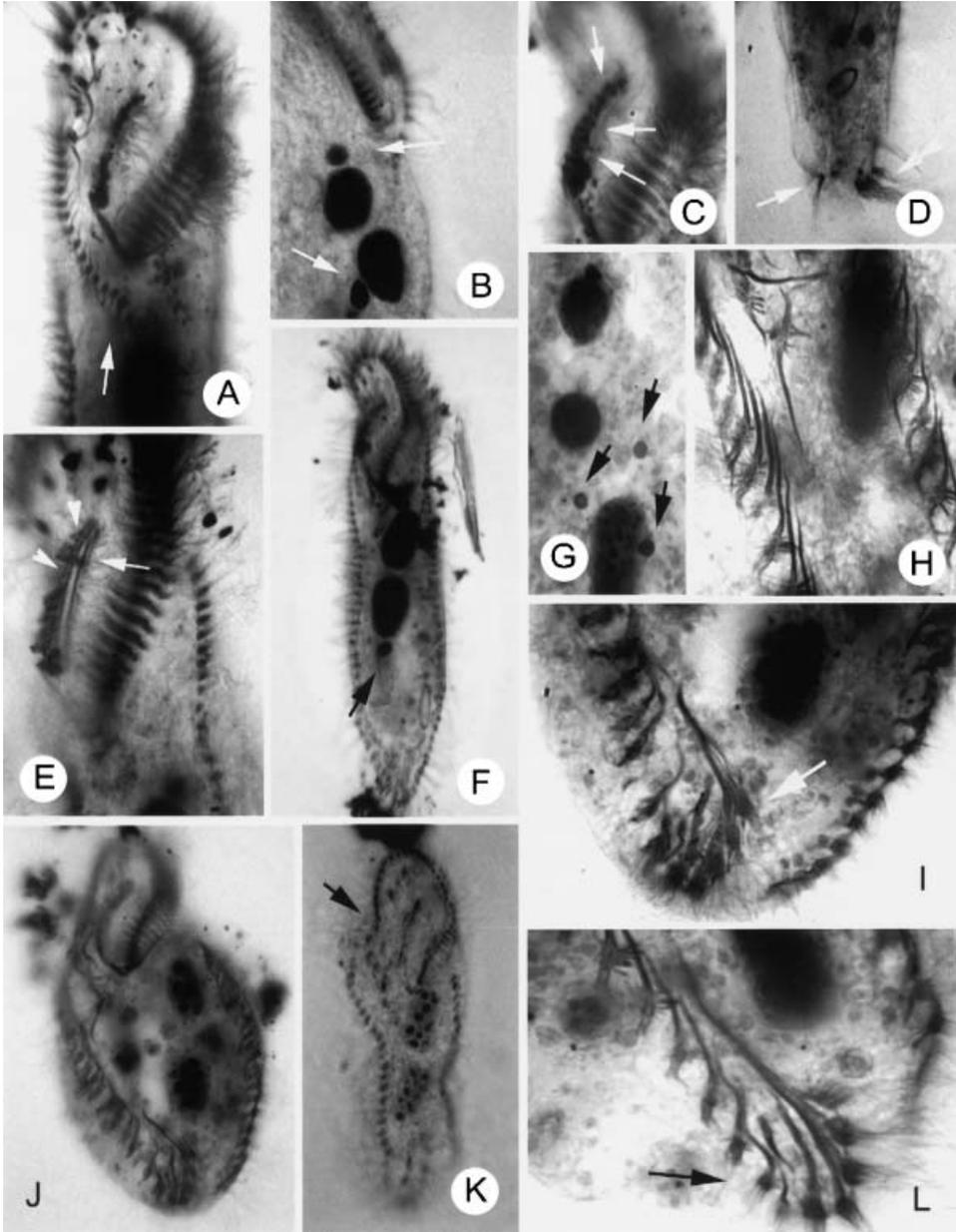


Figure 16. Photomicrographs of (A–F) *Amphisiella antarctica* nov. spec. and (G–L) *Hemigastrostyla szaboi* nov. spec. after protargol impregnation. (A) Ventral view of buccal region, to show the ventral row (arrow); (B) macro- and micronuclei (arrows); (C, E) buccal area, to show the argentophilic granules along the undulating membranes (arrows and arrowheads); (D) caudal portion, to show the transverse and caudal cirri; (F) ventral view, arrow marks the micronucleus; (G) nuclear apparatus, arrows indicate the micronuclei; (H) to show the fibres associated with the marginal cirri; (I) ventral view of posterior cell end, to show the transverse cirri, note that there is no gap between right marginal row and the transverse cirri; (J) ventral view, to show the general appearance of infraciliature; (K) ventral view, to show a slender form, arrow indicates the distal end of adoral zone of membranelles; (L) ventral view of caudal region, arrow marks the transverse cirri.

Table VIII. Morphometric characterization of *Amphisiella antarctica* nov. spec. (upper line) and *Hemigastrostyla szaboi* nov. spec. (lower line).

Character	Min	Max	Mean	SD	SE	CV	n
Body length	112	181	139.6	17.43	3.90	12.5	20
	93	139	113.8	13.9	3.49	12.3	16
Body width	23	46	32.8	6.82	1.53	20.8	20
	38	45	42.0	2.49	0.75	5.9	11
Length of buccal field	34	52	41.9	5.58	1.25	13.3	20
	31	40	36.3	2.85	0.71	7.8	16
Number of adoral membranelles	25	32	28.9	2.18	0.54	7.5	16
	25	29	26.8	1.39	0.49	5.2	8
Number of frontal cirri ^a	8	12	9.6	1.32	0.44	16.5	9
	8	8	8	0	0	0	16
Number of cirri in the ventral row (upper line)	21	36	29.3	3.26	0.94	11.1	12
	5	5	5	0	0	0	16
Number of ventral cirri (lower line)	5	5	5	0	0	0	16
Number of cirri anterior to transverse cirri	2	2	2	0	0	0	>20
	5	5	5	0	0	0	>20
Number of transverse cirri	5	6	5.1	0.34	0.09	6.7	16
	39	56	44.4	5.19	1.29	11.7	13
Number of cirri in left marginal row	21	26	23.4	1.81	0.60	7.7	9
	42	55	46.2	4.42	1.23	9.6	13
Number of cirri in right marginal row	18	28	23.1	3.95	1.32	17.1	9
	4	4	4	0	0	0	13
Number of dorsal kineties ^b	3	3	3	0	0	0	11
	4	4	4	0	0	0	12
Number of caudal cirri	3	3	3	0	0	0	8
	12	23	17.3	3.18	0.71	18.4	20
Length of macronucleus	13	22	18.0	2.68	0.67	14.9	16
	7	17	11.8	2.93	0.66	24.8	20
Width of macronucleus	7	13	9.3	1.96	0.47	21.1	16
	2	2	2	0	0	0	>30
Number of macronuclei	2	2	2	0	0	0	>30
	2	2	2	0	0	0	>30
Number of micronuclei	2	2	2	0	0	0	>30
	2	6	3.7	1.35	0.41	36.2	11

All data are based on protargol-impregnated specimens. Measurements in μm .

^aIncluding one buccal cirrus; ^bonly the complete rows counted.

Movement relatively slow, crawling without pause on debris.

Buccal field narrow, adoral zone of membranelles (AZM) extending to about one-third of body length, bases of membranelles up to $8 \mu\text{m}$ long, cilia ca $15 \mu\text{m}$ long in vivo. Distal end of adoral zone of membranelles bending considerably posteriad at right margin. Paroral and endoral membranes about equally long, parallel to each other. Pharyngeal fibres short and conspicuous after protargol impregnation (Figure 11C).

Frontal area constantly eight cirri, anterior two to three of which are distinctly enlarged, while others are smaller (Figure 11C). Single buccal cirrus (counted among frontal cirri in Table VIII), situated beside mid-point of undulating membranes. Three postoral ventral cirri anteriorly located, immediately beneath the cytostome level (Figure 11C), while two pretransverse ventral cirri close to five terminally positioned thick transverse cirri. Right marginal row terminating subcaudally anterior to rightmost transverse cirrus, which often gives an appearance of confluence of right marginal cirri and TC (Figure 16L, arrow). Cirri

in both marginal rows rather large and densely spaced; anterior portions of right marginal row often extending on to dorsal side. At posterior end, the two marginal rows widely separated. All cirri associated with strongly impregnated fibre system (Figures 11B, 16I, L).

Dorsal kineties constantly three, cilia about $3\ \mu\text{m}$ long, positioned in small pit as recognized after impregnation. Three caudal cirri located caudally on cell margin (Figure 11D, arrowheads).

Remarks

The genus *Hemigastrostyla* previously contained only two species (Borror 1963; Song and Wilbert). This new organism can be separated from the closely related *H. enigmatica* (Dragesco and Dragesco-Kernéis 1986) Song and Wilbert by having fewer frontal and buccal cirri (eight versus consistently more than eight in the latter), no “extra” ventral cirri (versus present), fewer dorsal kineties (three versus five) and the body shape (non-cephalized versus cephalized) (Dragesco and Dragesco-Kernéis 1986; Song and Wilbert 1997). Differences from *H. stenocephala* (Borror 1963) Song and Wilbert 1997, are that the new species has conspicuously shorter dorsal cilia (ca 3 versus $16\ \mu\text{m}$ in length in *H. stenocephala*), fewer frontal and ventral cirri (13 versus 17), less slender body shape and no extra ventral cirri (versus two in *H. stenocephala*) (Borror 1963).

Genus *Amphisiella* Gouret and Roeser, 1888

Amphisiella antarctica nov. spec.

(Figures 11E–K, 16A–F; Table VIII)

Diagnosis

Slender marine *Amphisiella* $120\text{--}200 \times 30\text{--}50\ \mu\text{m}$ in vivo with narrowed caudal portion. Eight to twelve frontal and five transverse cirri; single ventral row extending to posterior third of cell length with about 29 cirri on average; two small ventral cirri close to transverse cirri; 25–32 adoral membranelles; 39–56 left and 42–55 right marginal cirri; four dorsal kineties and four caudal cirri; two macro- and two micronuclei. Cortical granules large and sparsely distributed on dorsal side.

Description

In vivo mostly about $160 \times 40\ \mu\text{m}$ in size. Cells flexible, slightly contractile and often more or less distorted in middle portion (even twisted while gliding on debris, Figure 11H); dorsoventrally flattened by about two-thirds. Body shape distinctly slender with posterior portion distinctly narrowed; ratio of cell length to width about four to one (Figure 11E), buccal field narrow and inconspicuous, about one-quarter of body length; cell margins generally parallel but often curved outward in middle portion. Dorsal side slightly uneven, irregularly bulging (Figure 11G).

Pellicle soft and thin; “cortical granules” colourless and large (about $1\ \mu\text{m}$), not grouped and sparsely distributed on dorsal side (Figure 11F). Cytoplasm greyish or colourless, often containing numerous light-reflecting globules ($2\text{--}5\ \mu\text{m}$ across), which render the cell dark under low magnification. Contractile vacuole not observed. Food vacuoles several to many, always containing small diatoms or flagellates (Figure 11E). Two elongate macronuclei, ca

$20 \times 15 \mu\text{m}$ in size in vivo, slightly separated from one another near left side of body; constantly two large ($4\text{--}5 \mu\text{m}$ across), globular micronuclei, adjacent to macronuclei (Figures 11K, 16B).

Movement relatively slow, crawling without pause on debris. When swimming, spirally rotating about the longitudinal axis.

Buccal apparatus as shown in Figure 11I. Adoral zone of membranelles (AZM) extending to about 25% of cell length, longest base of membranelles about $7 \mu\text{m}$ long; distal end of AZM bending only slightly posteriad at right. Paroral membrane two-rowed, parallel to zig-zagging endoral membranes. Along undulating membranes always some densely packed argentophilic granules (extrusomes ?) recognizable (Figure 11J, arrowheads). Pharyngeal fibres conspicuous after protargol impregnation, about $30 \mu\text{m}$ long (Figure 11I).

Four enlarged frontal cirri followed by usually five smaller ones posteriorly; single ventral row consisting of 21–36 cirri extends to about posterior third of cell length. Always two small cirri anterior to five enlarged transverse cirri, which are positioned almost completely at posterior end of cell (Figure 11J) and are connected by five fibres about $40 \mu\text{m}$ long. The two marginal rows are widely separated posteriorly and terminate near transverse cirri (Figure 11J).

Four dorsal kineties loosely ciliated, cilia about $5 \mu\text{m}$ long, comprising ca 10 pairs of basal bodies each. Four caudal cirri at margin of cell (Figure 11K).

Remarks

Considering the body shape, size, habitat and general appearance, at least two marine morphotypes should be compared with the new species described here: *Amphisiella thiophaga* (Kahl, 1928) Kahl, 1932 and *A. annulata* (Kahl, 1928) Kahl, 1932. The new species differs from the former in having a narrowed caudal portion (versus broadly rounded in *A. thiophaga*) and a relatively shorter ventral row of cirri that terminates far away from the transverse cirri (versus extends completely to the transverse cirri) (Kahl 1932).

Compared with *A. annulata*, the new species can be distinguished by body shape (with narrowed posterior end versus broadly rounded), lack of ring-like granules (“Ringkugeln”) in cytoplasm (versus present in the former species) and probably the presence of conspicuous cortical granules (versus absence ?; none were mentioned in original description).

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