

The Diversity of Scuticociliates (Protozoa, Ciliophora): a Report on Eight Marine Forms Found in Coastal Waters of China, with a Description of One New Species

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Summary. Eight marine scuticociliates, *Pseudoplatynematum denticulatum* (Kahl, 1933) nov. comb., *Protocyclidium sinica* nov. spec., *Histiobalantium marinum* Kahl, 1933, *Porpostoma notata* Möbius, 1888, *Philaster hiatti* Thompson, 1969, *Parauronema longum* Song, 1995, *Uronemella parafilificum* Gong *et al.*, 2007, and *Paranophrys magna* Borror, 1972, collected from Chinese coastal waters, were investigated using live observations and silver impregnation methods. Investigations of a Chinese population of *Platynematum denticulatum* (Kahl, 1933) reveal that it has a highly strengthened pellicle and distinct spines and thus corresponds well with the definition of *Pseudoplatynematum* Bock, 1952. A new combination, *Pseudoplatynematum denticulatum* (Kahl, 1933) nov. comb., is therefore proposed and an improved species diagnosis is supplied. *Protocyclidium sinica* nov. spec. is characterized by: small body size with buccal field approximately 60% of body length; extrusomes present; 13 or 14 somatic kineties; somatic kinety 1 comprising approximately 24 densely arranged kinetids; somatic kinety n shortened posteriorly; single macronucleus. Additional information is documented on the morphology of six other species of scuticociliates based on the China populations.

Key words: Infraciliature, marine ciliates, new combination, new species, *Protocyclidium*, *Pseudoplatynematum*, Scuticociliate.

INTRODUCTION

The ciliates in the subclass Scuticociliatia are common members of ecosystems in habitats worldwide and

they often act as symbionts or even pathogens of aquatic animals (Foissner *et al.* 2009; Lobban *et al.* 2011; Miao *et al.* 2009, 2010; Wang *et al.* 2009a, b). However, recent investigations have demonstrated that the diversity of this group has not been well documented (Fan *et al.* 2009, 2011; Gao *et al.* 2010; Lobban *et al.* 2011; Pan *et al.* 2010; Zhang *et al.* 2010). Moreover, many species need to be reinvestigated using modern methods as they have not been redescribed since the

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original reports (Fan *et al.* 2010; Guggiari and Peck 2008; Kahl, 1935; Pan *et al.* 2010; Song and Wilbert 2000a, b). Because of their small size and the high degree of similarity in the infraciliature among many scuticociliates, species circumscription and identification relies on a combination of characters observed both *in vivo* and following silver impregnation (Foissner 1995; Foissner *et al.* 1982, 1994, 2009; Small and Lynn 1985; Song and Wilbert 2000a, b, 2002).

During a survey of the ciliate fauna in the coastal waters of China, eight scuticociliates were isolated from various habitats and investigated. One isolate represented a typical species of *Pseudoplatynematum* Bock, 1952, but had previously been wrongly assigned to the genus *Platynematum* Foissner *et al.*, 1994. A *Cyclidium*-like species proved to be a new member of the genus *Protocyclidium* Alekperov, 1993. The remaining six species were all previously known, but new information is here documented for each based on the China populations.

MATERIALS AND METHODS

Pseudoplatynematum denticulatum nov. comb. was collected from the surface of sandy littoral sediments at the No. 1 bathing beach, Qingdao, northern China (36°03'18"N; 120°20'37"E), on 29th April 2009 when the water temperature was 19°C and the salinity 32‰. *Histiobalantium marinum* was collected from the same beach as the former on 28th April 2010 when the water temperature was 12°C and the salinity 29‰. In each case sand and seawater were taken from the top 5 cm sand layer.

Protocyclidium sinica nov. spec. was collected from a mariculture pond in Daya Bay, southern China (22°43'23"N; 114°35'41"E), on 30th November 2009 when the water temperature was 22°C and the salinity 31‰. *Porpostoma notata* was isolated from water near the bottom of a mariculture pond at Weifang, northern China (37°05'49"N; 119°29'59"E), on 6th May 2009 when the water temperature was 22°C and the salinity 28‰. Numerous dead bodies of crab larvae had been deposited at the bottom of this pond when it was drained.

Philaster hiatti was collected from Dapeng Bay, Shenzhen, southern China (22°36'14"N; 114°24'32"E), on 18th August 2007 when the water temperature was 27°C, the salinity 32‰ and the pH 8.3.

Parauronema longum was isolated from a harbor at Qingdao, northern China (36°04'28"N; 120°18'46"E) on 11th March 2009 when the water temperature was 11°C, the salinity 30‰. Glass slides were fixed to a frame and immersed in the harbor water for about 10 days to allow colonization to occur.

Uronemella parafilificum and *Paranophrys magna* were collected from a small puddle in a drying aqueduct near mariculture ponds in Weifang, northern China (37°05'49"N; 119°29'59"E), on 30th May 2009 when the salinity was 85‰.

Protocyclidium sinica, *Porpostoma notata*, *Philaster hiatti*, *Uronemella parafilificum* and *Paranophrys magna* was each isolated from seawater samples collected using sampling bottles.

For a summary of the sampling locations and dates see Table 3.

Cells were isolated and observed *in vivo* using differential interference contrast microscopy. The protargol (Wilbert 1975) and Chatton-Lwoff wet silver-nitrate (Song and Wilbert 1995) methods were used in order to reveal the infraciliature and argyrome (silverline pattern), respectively. Drawings of stained specimens were made with the help of a camera lucida. Measurements were made under 100–1250 × magnification. Classification and terminology follow Lynn (2008).

RESULTS AND DISCUSSION

Family Cinetochilidae Perty, 1852

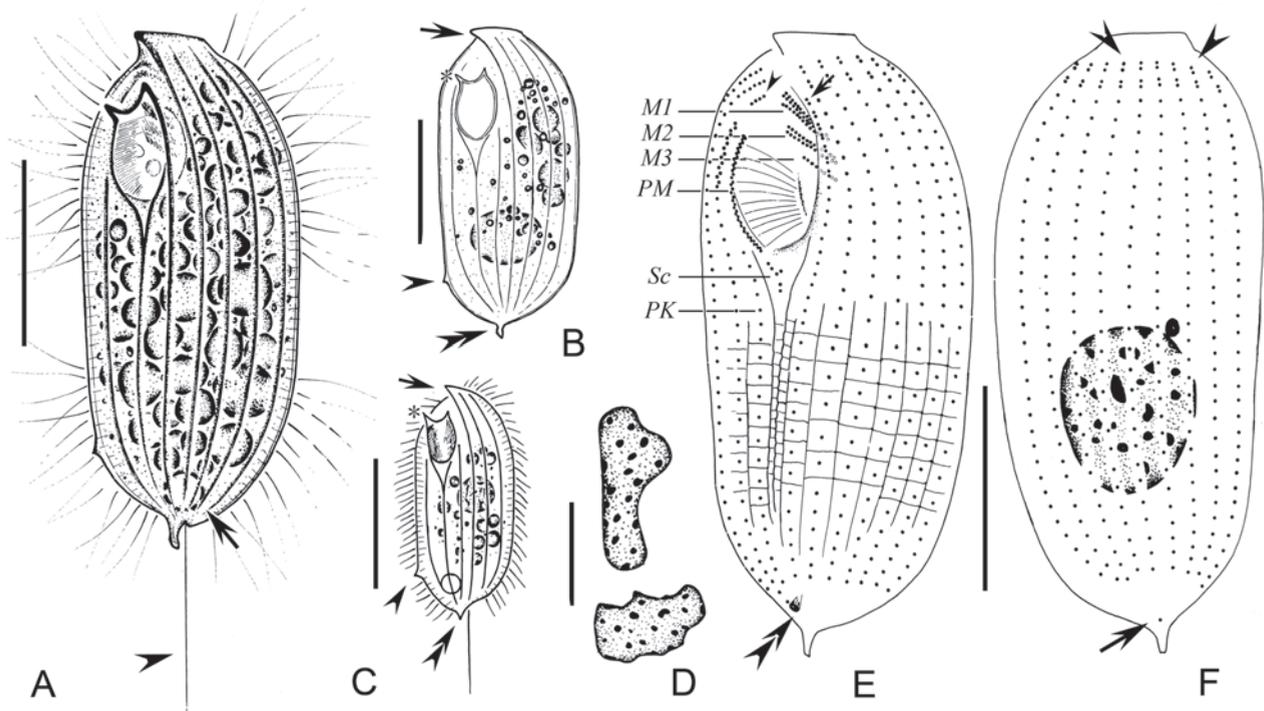
Pseudoplatynematum denticulatum (Kahl, 1933) nov. comb. (Figs 1, 2; Table 1)

Pseudoplatynematum denticulatum (Kahl, 1933) was originally assigned by Kahl (1933) to the genus *Platynema* Kahl, 1931. This genus, however, was invalid because the name was preoccupied so Kahl (1935) substituted the name *Platynematum* and recombined the constituent species. However, this was also an invalid genus until Foissner *et al.* (1994) selected a type species. The genus *Pseudoplatynematum* was established by Bock (1952) and a new diagnosis was recently supplied (Fan *et al.* 2010). The present study revealed that the China population of *P. denticulatum* possesses all the diagnostic characters of the genus *Pseudoplatynematum*. Hence, a new combination, *Pseudoplatynematum denticulatum* (Kahl, 1933) nov. comb., is proposed.

Basionyms: *Platynema denticulatum* Kahl, 1933; *Platynematum denticulatum* (Kahl, 1933) Kahl, 1935; *Platynematum denticulatum* (Kahl, 1933) Foissner *et al.*, 1994.

Since the original description based on a German population of this organism comprises only brief summary of its living characters, a redescription and improved diagnosis are here supplied based on an investigation of specimens from the China population, both *in vivo* and following silver staining.

Improved diagnosis: Elongate-elliptical *Pseudoplatynematum*, 45–60 × 18–25 μm *in vivo*, dorsoventrally flattened about 2 : 1; four spines, one each at anterior end, posterior end, buccal field, and right posterior of body. Buccal field extending to 25% of body length; single contractile vacuole located at posterior end of



Figs 1A–F. *Pseudoplatynematum denticulatum* (Kahl, 1933) nov. comb. from life (A–C) and after staining with protargol (D) and silver nitrate (E–F). **A** – ventral view of a typical individual, arrowhead indicates the long caudal cilium, arrow depicts the contractile vacuole; **B** – ventral view, to show the spines at the anterior end (arrow), posterior end (double-arrowhead), right posterior (arrowhead) of the body and buccal field (asterisk); **C** – after Kahl (1933); to show the same spines as those shown in (B); **D** – shape variants of macronucleus; **E** – ventral view showing the infraciliature and part of the argyrome, double-arrowhead refers to the contractile vacuole pore, arrowhead indicates the anterior fragment of somatic kinety 1, arrow marks the shortened somatic kinety n; **F** – dorsal view of infraciliature, arrowheads mark the two dikinetids in the anterior end of each somatic kinety, arrow indicates the kinetosome of the caudal cilium. M1–3 – membranelles 1–3, PK – postoral kinety, PM – paroral membrane, Sc – scutica. Scale bars: 20 μm .

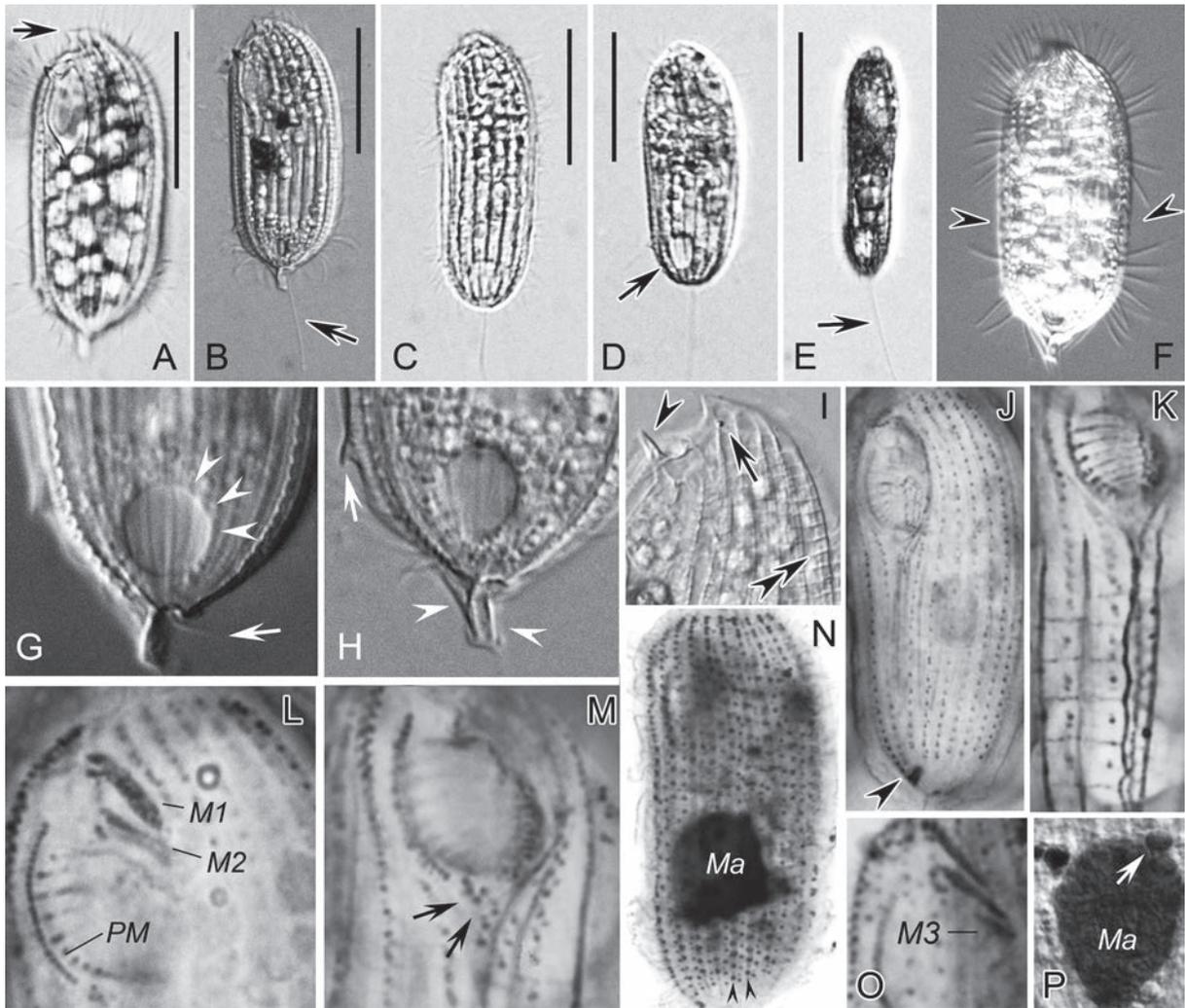
cell; membranelle 1 three-rowed, membranelle 2 two-rowed, membranelle 3 single-rowed; 17–19 somatic kineties; one postoral kinety; marine habitat.

Deposition of voucher specimens: Two voucher slides with protargol- and silver nitrate-impregnated specimens respectively are deposited in the Laboratory of Protozoology, Ocean University of China (registry numbers: FXP-2009042901-01; FXP-2009042901-02). A voucher slide with protargol-impregnated specimens is deposited in the Natural History Museum (NHM), UK, with registration number NHMUK 2011.5.20.1.

Description: Body approximately $50 \times 18 \mu\text{m}$ *in vivo*, elongate-elliptical in outline, right and left cell margins nearly parallel, dorsoventrally flattened 2 : 1 (Figs 1A, B, 2D, E). Buccal field depressed, extending to 25% of body length. Pellicle conspicuously strengthened and with several longitudinal ridges (Figs 1A, 2A, I). Four spines constantly present, one

quadrangular spine at anterior end of cell, one thorn-like spine at right anterior edge of buccal field, one rhombic spine at posterior end of cell, and one triangular spine at right posterior side of body (Figs 1A, B, 2A, G–I). Extrusomes arranged in rows alongside pellicular ridges (Fig. 2I). Endoplasm hyaline and colourless, containing numerous refractile granules. Macronucleus, approximately $12 \times 9 \mu\text{m}$, globular or sometimes irregular in shape (Fig. 1D). Contractile vacuole located at posterior end of cell, approximately $8 \mu\text{m}$ in diameter during diastole (Figs 1A, 2D, G). Somatic cilia $6 \mu\text{m}$ long *in vivo*, although usually undetectable in middle portion of body (Figs 1A, 2F). Caudal cilium approximately $30 \mu\text{m}$ long, located in a surface depression near rhombic caudal spine (Figs 1A, 2G).

Swims continuously and rapidly rotating about main body axis.



Figs 2A–P. *Pseudoplatynematum denticulatum* (Kahl, 1933) nov. comb. from life (A–I) and after staining with silver nitrate (J–M) and protargol (N–P). **A, B, C** – ventral view of three individuals, arrow in (A) marks the spine at the anterior end, arrow in (B) indicates the caudal cilium; **D, E** – ventral (D) and lateral (E) view of another individual, arrow in (D) refers to the contractile vacuole, arrow in (E) depicts the caudal cilium; **F** – arrowheads indicate that the cilia in the middle portion of body are usually undetectable *in vivo*; **G** – posterior portion of body, arrow marks the caudal cilium inserted in a concave depression near the caudal spine, arrowheads depict the contractile vacuole; **H** – posterior portion of body, arrow marks the right posterior spine, arrowheads indicate the rhombic caudal spine; **I** – anterior part of body, arrow marks the quadrangular spine at the anterior end of cell, arrowhead indicates the thorn-like spine at the buccal field, double-arrowhead refers to the extrusomes. **J** – ventral view of argyrome, arrowhead depicts the contractile vacuole pore; **K** – part of the argyrome; **L** – buccal apparatus; **M** – arrows depict the scutica; **N** – to show the extrusomes between the ciliary rows (arrowheads); **O** – membranelle 3; **P** – showing macronucleus (Ma) and micronucleus (arrow). M1–3 – membranelles 1–3, Ma – macronucleus, PM – paroral membrane. Scale bars: 25 μ m.

Infraciliature as shown in Figs 1E, F. Seventeen to 19 somatic kineties, anterior ends of which are usually composed of two dikinetids, and one postoral kinety (Fig. 1E). Several kinetids in anterior part of somatic kinety 1 slightly separated from the others and forming a fragment; kinetids at level of paroral membrane tightly arranged in zig-zag pattern (Figs 1E, 2M). Somatic kinety n starting anteriorly at lower level than

others and containing seven or eight dikinetids in anterior region (Fig. 1E). Membranelles 1–3 attached to inner wall of oral cavity and oriented obliquely to main body axis; membranelle 1 three-rowed, membranelle 2 two-rowed, membranelle 3 single-rowed; paroral membrane starting anteriorly at level of membranelle 2; scutica composed of six kinetosomes (Figs 1E, 2L, M, O).

Table 1. Morphometric characterization of *Pseudoplatynematum denticulatum* (Kahl, 1933) nov. comb. (upper line), *Protocyclidium sinica* nov. spec. (middle line), and *Histiobalantium marinum* Kahl, 1933 (lower line). Data based on protargol-impregnated specimens. Measurements in μm . CV – coefficient of variation in %, n – number of specimens investigated, Max – maximum, Mean – arithmetic mean, Min – minimum, SD – standard deviation.

Characters	Min	Max	Mean	SD	CV	n
Body, length	43	57	48.4	3.5	3.1	25
	23	30	27.1	2.4	9.0	20
	46	65	53.9	4.8	8.9	22
Body, width	15	20	17.4	1.4	8.0	25
	16	22	19.2	1.6	8.3	20
	20	38	29.6	4.8	16.4	22
Buccal field, length	11	14	12.4	0.8	4.9	25
	14	17	15.4	0.9	6.1	20
	25	37	31.6	2.9	9.2	22
Somatic kineties, number	17	19	18.0	0.4	2.3	25
	13	14	13.7	0.5	3.4	20
	29	42	36.6	3.2	8.8	22
Kinetids in somatic kinety 1, number*	–	–	–	–	–	–
	22	26	23.9	1.1	4.8	20
Macronucleus, length	9	20	11.4	2.6	22.6	25
	8	11	9.3	0.8	8.5	20
	–	–	–	–	–	–
Macronucleus, width	5	11	8.6	1.7	19.6	25
	6	10	7.6	1.2	15.8	20
	–	–	–	–	–	–

* Dikinetids counted as single kinetal units.
– Data unavailable.

Argyrome composed of rectangular meshes; more than 10 oral ribs converge towards cytostome which lies at posterior end of buccal field; contractile vacuole pore located near posterior end of somatic kinety 1 (Figs 1E, 2J, K).

Discussion: Our isolate corresponds well with the descriptions in Kahl (1933) and Kahl (1935) in terms of the habitat and general morphology, especially the four spines and their positions on the body (Figs 1B, C). Hence, the identity of this organism is not in doubt.

Hitherto, the genus *Pseudoplatynematum* comprised four species only one of which, namely *P. dengi*, has been investigated using modern methods. Nevertheless, each of these can be clearly separated from *P. denticu-*

latum by various morphological features visible *in vivo* and, in the case of *P. dengi*, by the infraciliature.

Pseudoplatynematum loricatum differs from *P. denticulatum* by: (i) its larger body size *in vivo* ($60\text{--}70 \times 20\text{--}24 \mu\text{m}$ vs. $45\text{--}60 \times 18\text{--}25 \mu\text{m}$); (ii) having more somatic kineties ($20\text{--}24$ vs. $17\text{--}19$); (iii) the number, size and shape of the spines at the anterior end of the cell (around 20, small and triangular vs. single, large and quadrangular). *Pseudoplatynematum parvum* can be separated from *P. denticulatum* by: (i) the presence (vs. absence) of a caudal spine; (ii) two (vs. one) spines present at right posterior side; (iii) having a small, triangular (vs. large, quadrangular) spine at the anterior end (Bock 1952). *Pseudoplatynematum dengi* can be separated from *P. denticulatum* by: (i) larger body size and different body outline *in vivo* ($70\text{--}80 \times 16\text{--}22 \mu\text{m}$, elongate and slightly curved vs. $45\text{--}60 \times 18\text{--}25 \mu\text{m}$, elliptical); (ii) spine absent (vs. present) in posterior right of body; (iii) paroral membrane bipartite (vs. continuous); (iv) somatic kinety n-1 with more than ten (vs. two) anterior dikinetids (Fan *et al.* 2010).

Family Cyclidiidae Ehrenberg, 1838

Protocyclidium sinica nov. spec. (Fig. 3; Table 1)

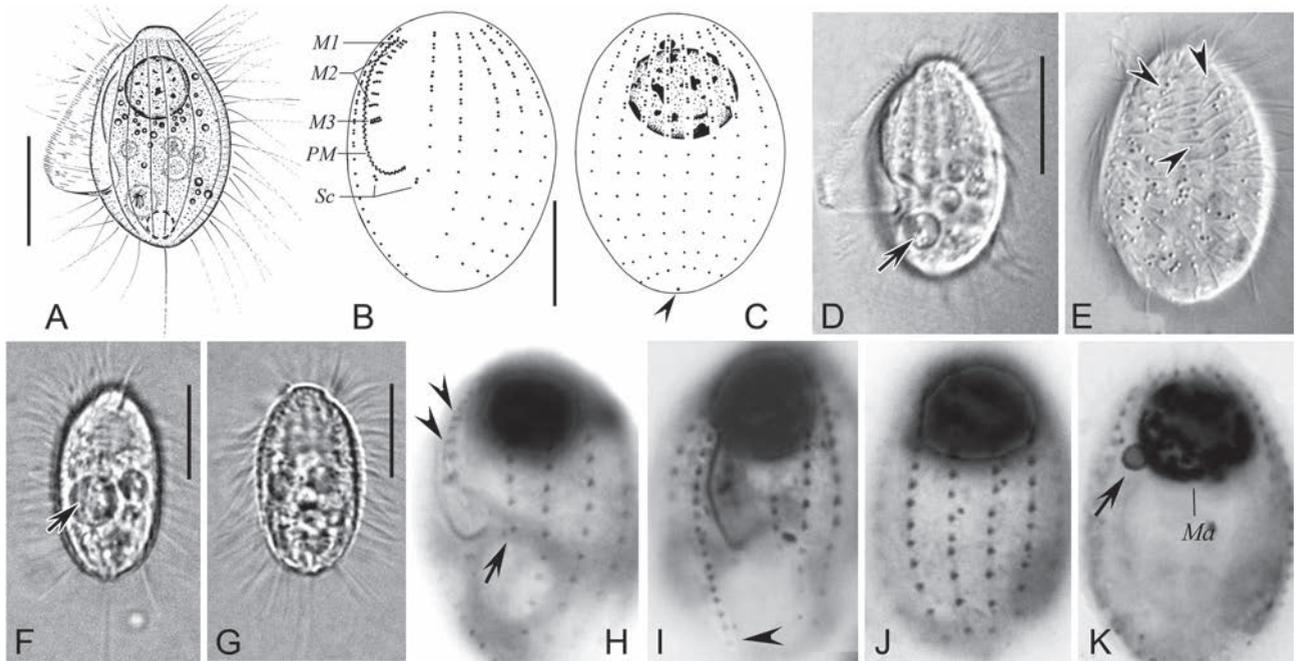
Diagnosis: Body $20\text{--}35 \times 12\text{--}20 \mu\text{m}$ *in vivo*; buccal field about 60% of body length; extrusomes present; 13 or 14 somatic kineties, somatic kinety 1 comprising approximately 24 basal bodies, somatic kinety n shortened posteriorly terminating at level of paroral membrane; single macronucleus; marine habitat.

Type locality: A mariculture pond in Daya Bay, southern China ($22^{\circ}43'23''\text{N}$; $114^{\circ}35'41''\text{E}$).

Deposition of slides: The holotype slide with protargol-impregnated specimens is deposited in the Laboratory of Protozoology, OUC, China (registration number: JJM-2009-1130-01). Another protargol preparation is deposited as a paratype slide in the collection of the NHM, UK, with registration number NHMUK 2011.5.20.2.

Etymology: The name *sinica* recalls the fact that this species was first found from China.

Description: Body usually $20\text{--}35 \times 12\text{--}20 \mu\text{m}$ *in vivo*, ellipsoidal with a conspicuous apical plate about 1/3 body width (Figs 3A, D, G). Pellicle smooth, with conspicuous extrusomes arranged in rows between somatic kineties (Fig. 3E). Buccal field about 60% of body length, prominent paroral membrane on its right side. Somatic cilia and cilia of paroral membrane about $12 \mu\text{m}$ long, caudal cilium about $20 \mu\text{m}$ long. Cytoplasm colourless, typically with many food vacuoles (Figs 3D, F). Single globular macronucleus, approxi-



Figs 3A–K. *Protocyclidium sinica* nov. spec. from life (A, D–G) and after staining with protargol (B, C, H–K). **A** – ventral view of a typical individual; **B**, **C** – ventral (B) and dorsal (C) view of infraciliature, arrowhead shows the kinetosome of the caudal cilium; **D** – ventral view, arrow indicates a food vacuole; **E** – dorsal view, arrowheads refer to the extrusomes; **F**, **G** – two typical individuals, arrow marks a food vacuole; **H** – arrow marks the short somatic kinety n, which ends near the posterior part of the paroral membrane, arrowheads show the horizontally oriented fragments of membranelle 2; **I** – arrowhead depicts the densely arranged kinetids in somatic kinety 1; **J** – to show the arrangement of kinetids in the dorsal ciliary rows; **K** – to show the macronucleus (Ma) and micronucleus (arrow). M1–3 – membranelles 1–3, Ma – macronucleus, PM – paroral membrane, Sc – scutica. Scale bars: 15 μ m.

mately $9 \times 8 \mu\text{m}$ in diameter; one micronucleus, approximately $2 \mu\text{m}$ across (Fig. 3K). Contractile vacuole located near posterior end of cell, approximately $5 \mu\text{m}$ in diameter (Fig. 3A).

Membranelle 1 comprises two longitudinal rows; membranelle 2 composed of about seven horizontally oriented rows; membranelle 3 small, two-rowed. Paroral membrane gently curved, extending to about 60% of body length. Scutica comprises four kinetosomes arranged in two groups, located near posterior end of paroral membrane. Thirteen or 14 somatic kineties; somatic kinety 1 conspicuously long, comprising approximately 24 kinetids, posterior 10 of which are usually monokinetids (Figs 3B, C, I). Somatic kinety n markedly shorter than others, terminating posteriorly at level of buccal vertex (Figs 3B, H). All other somatic kineties (somatic kineties 2 to n-1) about equal length, each with approximately 15 kinetids comprising densely arranged dikinetids in anterior 1/3 and loosely spaced monokinetids in posterior 2/3 (Figs 3G, H).

No silver nitrate preparations of sufficient quality were obtained to allow observation of the argyrome or contractile vacuole pore.

Discussion: The genus *Protocyclidium* was established by Alekperov (1993) and updated by Foissner *et al.* (1994). The genus is characterized mainly by the arrangement of the oral membranelles, i.e. membranelles 2 and 3 barely separated and forming a ciliary field composed of almost equidistant, transversely oriented rectangles that increase in width from anterior to posterior. It comprises five species, most of which were transferred from the poorly defined genus *Cyclidium* (Alekperov 1993, Foissner *et al.* 1994).

The type species, *Protocyclidium terrenum* Alekperov, 1993, can be clearly distinguished from *P. sinica* by: (i) the elongated macronucleus accompanied with three micronuclei (vs. globular macronucleus accompanied with one micronucleus); (ii) fewer somatic kineties (about 11 vs. 14); (iii) the soil (vs. marine) habitat (Alekperov 1993).

Protocyclidium sinica can be separated from *P. muscicola* (Kahl, 1931) Foissner *et al.*, 2002 by having more somatic kineties (13–14 vs. 9–10), more kinetids in somatic kinety 1 (24 on average vs. about 13, counted from figure), and its marine (vs. soil) habitat (Foissner 1995).

Protocyclidium sinica is distinguished from *P. terricola* (Kahl, 1931) Foissner *et al.*, 2002 by: (i) kinetids arranged more densely in somatic kinety 1 than of other kineties (vs. arrangement of kinetids in somatic kinety 1 similar to that of other kineties); (ii) somatic kinety n distinctly shorter in *P. sinica* than *P. terricola*; (iii) marine (vs. soil) habitat (Foissner *et al.* 2002).

Protocyclidium sinica differs from *P. sphagnetorum* (Šrámek-Hušek, 1949) Foissner *et al.*, 2002 by its oval body outline (vs. body elongate with ratio of length to width about 2 : 1) and in having 13 or 14 (vs. 17–19) somatic kineties (Foissner *et al.* 2002, Grolière 1973).

Protocyclidium sinica can be clearly separated from *P. citrullus* (Cohn, 1866) Foissner *et al.*, 2002 by having more kinetids in somatic kinety 1 (about 24 vs. 14, counted from figure) and the arrangement of kinetids in the dorsal somatic kineties with the anterior 1/3 of each kinety comprising dikinetids and the posterior 2/3 comprising seven or eight monokinetids in *P. sinica* whereas in *P. citrullus* the anterior 1/2 comprises dikinetids and the posterior 1/2 about 3 monokinetids (Song and Wilbert 2002).

Family Histiobalantiidae de Puytorac and Corliss in Corliss, 1979

Histiobalantium marinum Kahl, 1933 (Fig. 4; Table 1)

Although this species was redescribed by Dragesco (1960) and Wilbert (1986), some details of its infraciliature are still not clear. Furthermore, the form described by Wilbert (1986) seems to be another species. Thus, a detailed redescription based on the Qingdao population is supplied.

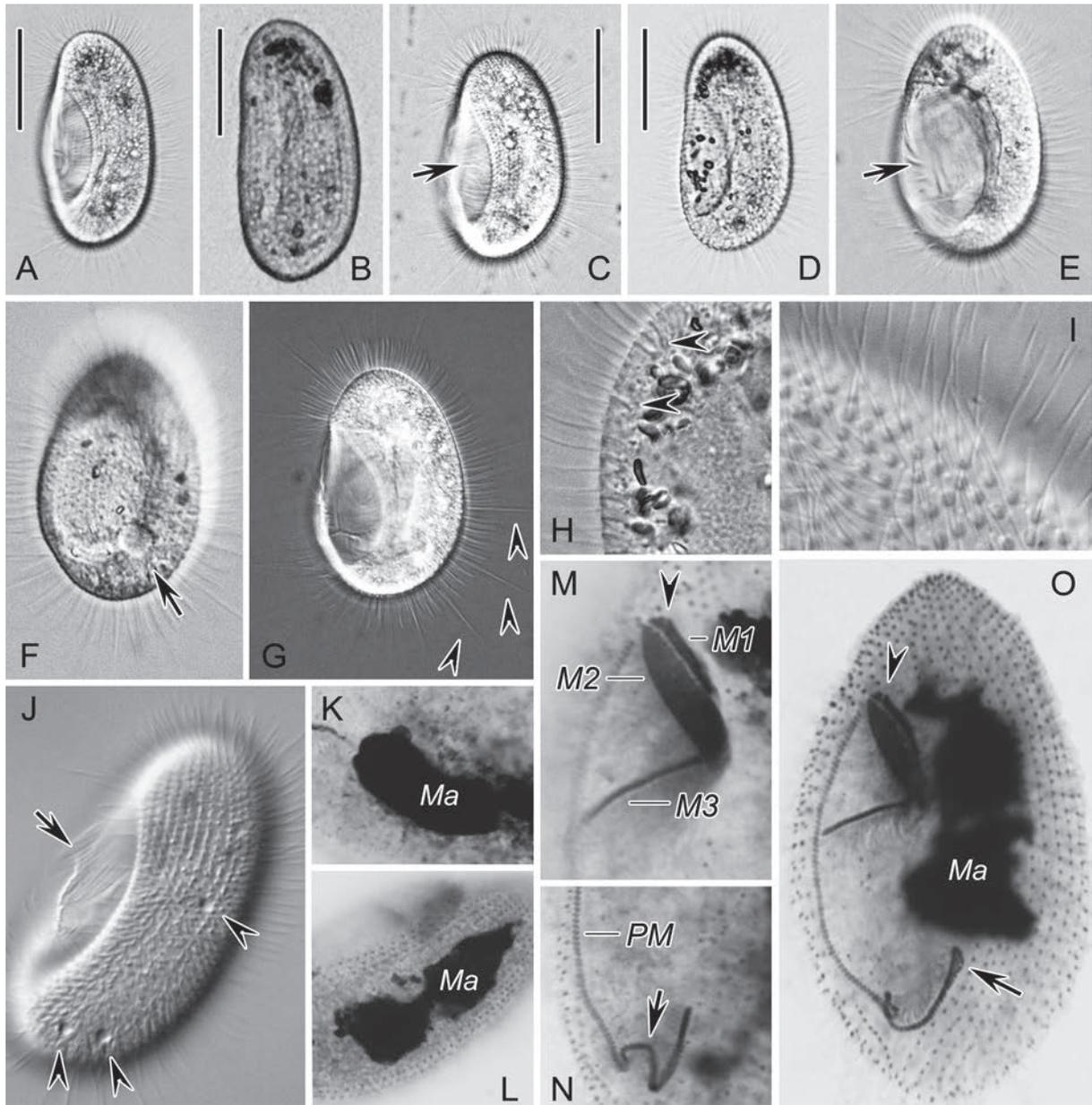
Description: Body 65–80 × 38–45 µm *in vivo*, outline broadly oval in ventral view (Figs 4A–E). Buccal field prominent and expandable, about 60% of body length (Figs 4A, C, E); buccal cavity deep and large, occupying about 2/3 of body width when fully extended (Fig. 4E). Cytoplasm transparent and containing many crystal granules (< 5 µm). Three to five small contractile vacuoles ventral-laterally positioned, usually 3 µm in diameter (Fig. 4J); a large contractile vacuole dorsally located near posterior end of cell, about 8 µm

across during diastole (Fig. 4F). Two kinds of somatic cilia: type 1 with “normal” cilia, 10–12 µm long; type 2 with longer (20–25 µm long) cilia that radiate out from body (Fig. 4G). No caudal cilia recognizable. Pellicle with numerous concave depressions from which cilia emerge (Fig. 4I). Extrusomes about 5 µm long, densely packed beneath pellicle (Fig. 4H).

Swims moderately fast and rotating about main body axis. Cells can also adhere tightly to a substrate.

Infraciliature as shown in Figs 4M–O. Twenty-nine to 42 somatic kineties, with evenly arranged monokinetids; postoral and preoral kineties absent (Fig. 4O). Three obliquely oriented membranelles arranged in a V-shape (Fig. 4M). Membranelle 1 three-rowed, about 6 µm long, composed of two parts, a small triangular or trapeziform anterior portion and a longer posterior portion (Fig. 4M). Membranelle 2 large, about 10 µm long, composed of seven or eight ciliary rows that are slightly curved and gradually increase in length from right to left. Membranelle 3 two-rowed, 9 µm long on average (Fig. 4M). Cilia of membranelles up to 30 µm long. Paroral membrane prominent, extending to 60% of body length, with anterior part straight and posterior part characteristically flexible; basal bodies in posterior end of paroral membrane sometimes scattered (Fig. 4O). Macronucleus irregularly shaped, usually composed of two conjoined parts (Figs 4K, L, O). Two micronuclei (n = 5).

Discussion: The China population corresponds well with those described by Kahl (1933) and Dragesco (1960) in terms of body size and locomotion. Wilbert (1986) also described a population of *Histiobalantium marinum* from Ontario Lake, however, we consider that this was probably a misidentification. As indicated by the brief description by Kahl (1933), and the comparison with *H. marinum* in Dragesco (1960), *H. marinum* var. *major* Kahl, 1935 is a similar form but with a larger body size and smaller buccal field. According to the description and figures in Wilbert (1986), the Ontario population has: (i) a body length of about 60–102 µm after fixation, which is much larger than that of the China population (45–60 µm); (ii) a buccal field that extends to about 50% of body length (vs. 60% in the China population), and; (iii) about 30 somatic kineties on the dorsal side, counted from the figures, (vs. less than 20 in the China population). Hence, the Ontario population is probably *H. marinum* var. *major* rather than *H. marinum*.



Figs 4A–O. *Histiobalantium marinum* from life (A–J) and after staining with protargol (K–O). **A–E** – different individuals with peristome extended to different extents, arrows show the buccal cavity; **F** – dorsal view, arrow indicates the contractile vacuole on the dorsal side in the posterior third of the cell; **G** – ventral view, arrowheads show the long somatic cilia; **H** – arrowheads depict the extrusomes; **I** – cell surface, to show the concave depressions where the somatic cilia are inserted; **J** – ventral side, arrowheads refer to the small contractile vacuoles in the ventral-lateral region, arrow indicates one of the oral membranelles; **K, L** – to show the macronucleus; **M, N** – detail of the oral structure, arrow shows the flexible part of the paroral membrane, arrowhead marks the fragment in membranelle 1; **O** – general view of ventral infraciliature, arrowhead marks the fragment in membranelle 1, arrow depicts the scattered basal bodies near the posterior end of the paroral membrane. M1–3 – membranelles 1–3, Ma – macronucleus, PM – paroral membrane. Scale bars: 30 μ m.

Family Philasteridae Kahl, 1931

Porpostoma notata Möbius, 1888 (Fig. 5; Table 2)

This species was redescribed by Song (2000) based on a population from northern China, hence only a brief description of the new population is documented here.

Description: Body 100–180 × 20–50 µm *in vivo*, length to width ratio 3 : 1–5 : 1; anterior portion pointed. Buccal cavity depressed near anterior 2/5 of body. Numerous food granules in cytoplasm when initially isolated (Figs 5A–C, F, G). Extrusomes slender, approximately 4 µm long, easily detectable *in vivo* and after silver nitrate impregnation (Figs 5E, K). Contractile vacuole located at posterior end of cell, approximately 10 µm across (Figs 5A, B). Single caudal cilium approximately 20 µm long (Fig. 5I). Membranelles 1–3 hardly separated. Membranelle 1 starting from the apex of body, and composed of 10–23 parts that gradually widen posteriorly (Figs 5D, L, N). Cilia of membranelles approximately 10 µm in length. Paroral membrane inconspicuous, L-shaped (Fig. 5O). On average, 55 somatic kineties forming a suture at anterior part on dorsal side (Fig. 5M). Macronucleus band-like and twisted, occupying most of the body length (Fig. 5J).

Philaster hiatti Thompson, 1969 (Fig. 6; Table 2)

The species is well-known since Thompson (1969) first reported its infraciliature and Coats and Small (1976) investigated its morphogenesis. The China population conforms well with the previous studies apart from small differences in body size. Therefore, a brief redescription based only on the China population is supplied here.

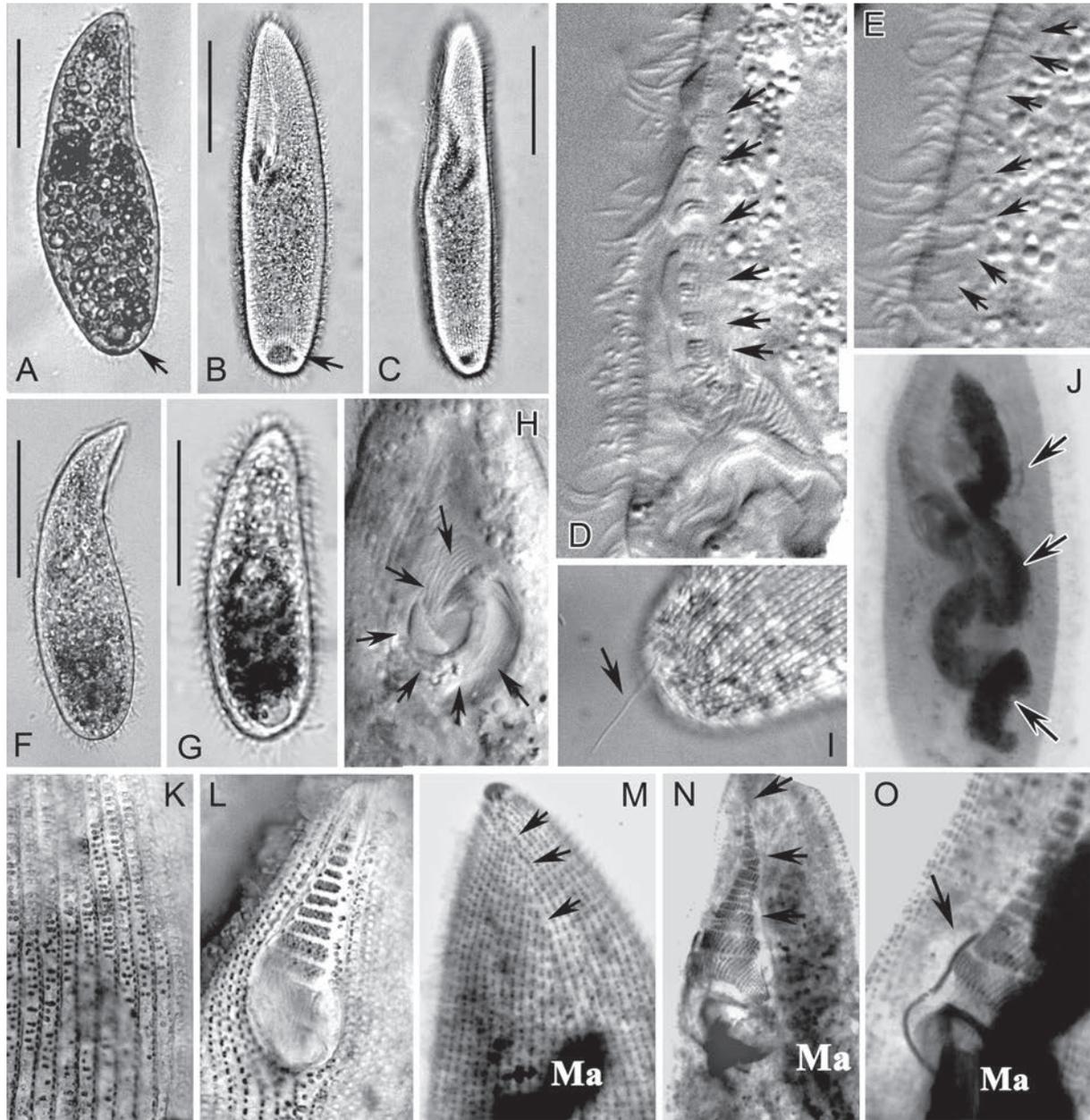
Description: Body 65–95 × 15–25 µm *in vivo*, usually about 70 × 20 µm, elongate with anterior part slightly curved to one side, posterior end rounded, anterior end slightly pointed (Fig. 6C). Buccal field occupying 40% of body length, forming a deep and wide cavity posteriorly (Figs 6F, G). Pellicle slightly ridged (Fig. 6E). Cell reddish or slightly dark in appearance (Figs 6A–D). Colourless oil droplets 2–3 µm across, crystal granules about 2 × 1 µm, and small red granules < 1 µm across, distributed in cytoplasm (Fig. 6H). Single elliptical macronucleus, about 20 × 10 µm, located in centre of cell. Somatic cilia 6–7 µm long, single caudal cilium 10–13 µm long. Contractile vacuole 5 µm in diameter, located at posterior end of cell. Swims fast with anterior end sweeping from side to side; sometimes stationary with only anterior end adhering to substrate.

Table 2. Morphometric characterization of *Porpostoma notata* Möbius, 1888 (first line), *Philaster hiatti* Thompson, 1969 (second line), *Paraaronema longum* Song, 1995 (third line), *Uronemella parafilificum* Gong *et al.*, 2007 (fourth line), and *Paranophrys magna* Borror, 1972 (fifth line). Data based on protargol-impregnated specimens. Measurements in µm. CV – coefficient of variation in %, n – number of specimens investigated, Max – maximum, Mean – arithmetic mean, Min – minimum, SD – standard deviation.

Characters	Min	Max	Mean	SD	CV	n
Body, length	84	140	115.5	15.5	13.4	18
	46	95	67.7	12.4	18.3	21
	64	105	84.9	9.3	11.0	17
	24	32	38.2	2.7	9.7	15
	47	66	56.9	6.1	10.7	20
Body, width	20	52	32.5	6.8	20.8	18
	14	25	18.1	3.1	17.0	21
	24	37	30.4	3.3	10.8	17
	16	25	19.1	2.5	13.1	15
	25	40	32.2	5.1	16.0	20
Buccal field, length	16	60	46.0	9.8	21.4	18
	20	45	29.2	6.1	20.9	21
Somatic kineties, number	–	–	–	–	–	–
	–	–	–	–	–	–
	21	28	23.8	1.9	8.1	20
	50	63	55.8	4.4	7.9	18
	29	33	31.2	1.1	3.5	21
Kinetids in somatic kinety 1, number*	19	20	19.7	0.5	2.4	17
	16	17	16.9	0.3	1.5	15
	24	28	25.9	1.6	6.2	20
	–	–	–	–	–	–
	–	–	–	–	–	–
Macronucleus, length	40	60	50.2	5.2	10.4	17
	18	21	19.0	1.2	6.6	10
	41	49	44.0	2.9	6.6	10
	6	10	85.4	11.7	13.8	18
	10	25	18.6	5.0	26.7	21
Macronucleus, width	15	25	19.9	3.5	17.4	17
	6	10	8.1	1.3	16.5	15
	13	24	17.2	2.0	17.8	20
	–	–	–	–	–	–
	5	15	9.2	2.6	28.5	21
	7	12	9.5	1.2	13.0	17
	6	10	7.8	1.6	20.1	15
	10	19	14.1	2.1	15.0	20

* Dikinetids counted as single kinetal units.

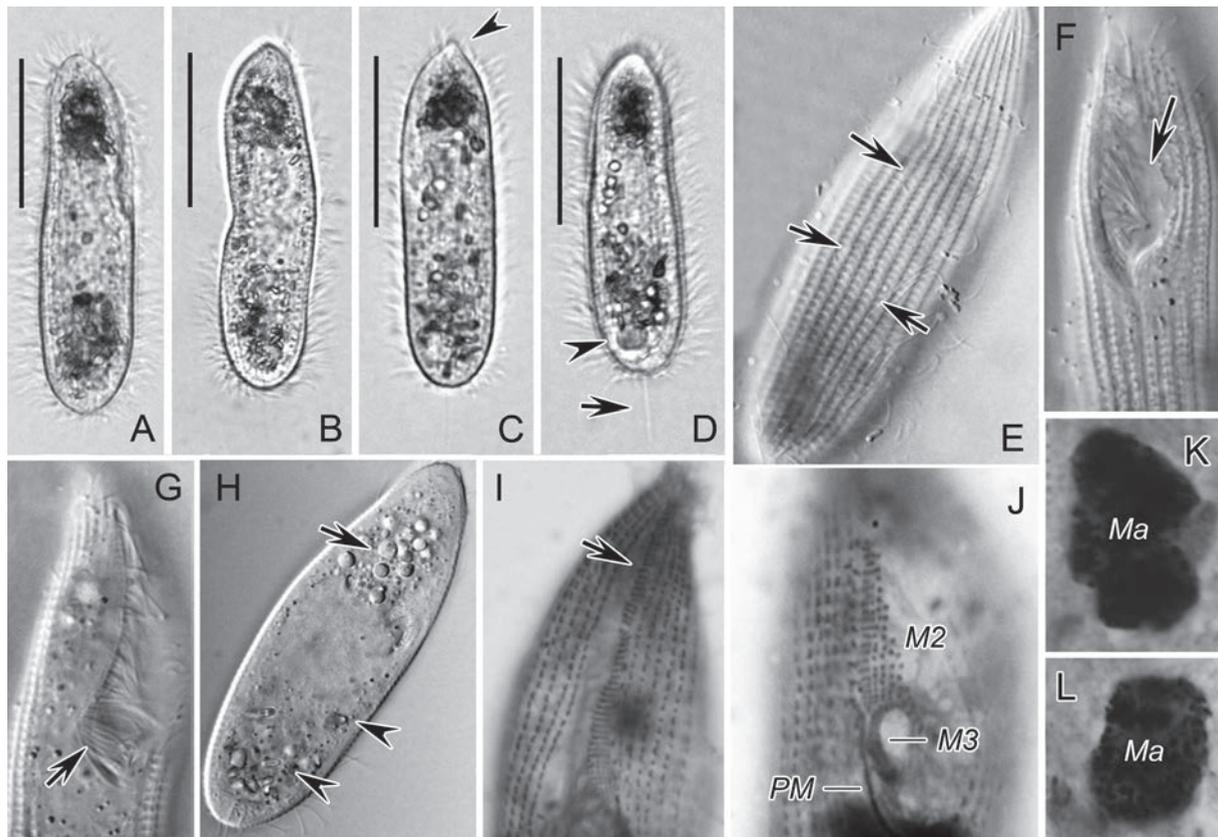
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Figs 5A–O. *Porpostoma notata* *in vivo* (A–I) and after staining with protargol (J, M–O) and silver nitrate (K, L). **A, B, F, G** – to show different body shapes, arrows in (A) and (B) show the contractile vacuole; **C** – lateral view; **D** – detail of buccal field, arrows mark the numerous transverse fragments that make up membranelle 1; **E** – arrows refer to the extrusomes; **H** – oral field, arrows mark the buccal cavity; **I** – posterior part of cell, arrow shows the caudal cilium; **J** – arrows show the band-like and slightly twisted macronucleus; **K** – apical view of extrusomes after silver nitrate impregnation; **L, N** – to show the component parts of membranelle 1; **M** – dorsal anterior portion of the cell, arrows mark the suture; **O** – arrow refers to the paroral membrane. Ma – macronucleus. Scale bars: 50 μ m.

Twenty-one to 33 somatic kineties, with evenly spaced dikinetids. Membranelle 1 long and narrow, composed of approximately 25 horizontally oriented kinety rows; membranelle 2 wider than membranelle 1, forming a finger-like structure pointing posterior left; membranelle 3

small, located beneath membranelle 2 and longitudinally oriented. Paroral membrane starting anteriorly near right posterior of membranelle 2 and close to membranelle 3, evenly curved (Figs 6I, J). Single macronucleus, ellipsoidal or irregularly shaped (Figs 6K, L).



Figs 6A–L. *Philaster hiatti* from life (A–H) and after protargol impregnation (I–L). **A–D** – different individuals, arrowhead in (C) shows the pointed anterior end, arrowhead in (D) marks the contractile vacuole, arrow indicates the caudal cilium; **E** – arrows mark the longitudinal ridges; **F, G** – anterior part of body, arrow in (F) refers to the buccal cavity, arrow in (G) marks the cilia of membranelle 2; **H** – cytoplasmic granules, arrow depicts the large colourless granules, arrowheads show the small red granules; **I** – anterior part of buccal field, arrow indicates the component parts of membranelle 1; **J** – posterior part of the buccal structure, to show membranelles 2 and 3 and the paroral membrane; **K, L** – macronucleus. M2, 3 – membranelles 2, 3, Ma – macronucleus, PM – paroral membrane. Scale bars: 35 μm .

Table 3. Sampling locations and dates for the eight species.

Species	Sampling location	Sampling date
<i>Pseudoplatynematum denticulatum</i>	Qingdao, northern China	29 April 2009
<i>Histiobalantium marinum</i>	(36°03'18"N; 120°20'37"E)	28 April 2010
<i>Protocyclidium sinica</i>	Daya Bay, southern China (22°43'23"N; 114°35'41"E)	30 November 2009
<i>Porpostoma notata</i>	Weifang, northern China (37°05'49"N; 119°29'59"E)	6 May 2009
<i>Philaster hiatti</i>	Dapeng Bay, southern China (22°36'14"N; 114°24'32"E)	18 August 2007
<i>Parauronema longum</i>	Qingdao, northern China (36°04'28"N; 120°18'46"E)	11 March 2009
<i>Uronemella parafilificum</i>	Weifang, northern China	30 May 2009
<i>Paranophrys magna</i>	(37°05'49"N; 119°29'59"E)	

Family Uronematidae Thompson, 1964

***Parauronema longum* Song, 1995 (Fig. 7; Table 2)**

Description: Body 60–100 × 25–45 μm *in vivo*, elongate with a small, truncated apical plate (Figs 7A–E). Buccal field extending to 40% of body length, with a narrow opening (Fig. 7F). Cilia of paroral membrane about 8 μm long (Fig. 7K). Pellicle forming longitudinal ridges between somatic kineties (Fig. 7J). Extrusomes slender, approximately 4 μm in length (Fig. 7G). Cytoplasm containing numerous crystal granules (Fig. 7K). Single ovoid macronucleus accompanied by a single micronucleus, located near body center (Fig. 7I). One contractile vacuole located at posterior end of cell, about 10 μm across during diastole (Fig. 7H). Contractile vacuole pore located near posterior end of somatic kinety 2 (Fig. 7L). Somatic cilia 10–12 μm long. Single caudal cilium, approximately 20 μm long (Fig. 7H).

Infraciliature and argyrome both conform closely with the original description (for details, see Song 1995).

Discussion: Song (1995) established this species based on populations from mariculture ponds in Qingdao, China. Our new population has a much larger body size than the original (60–100 μm vs. 33–52 μm in length). Nevertheless, we believe the two are conspecific because of their close similarity in other living characters and infraciliature.

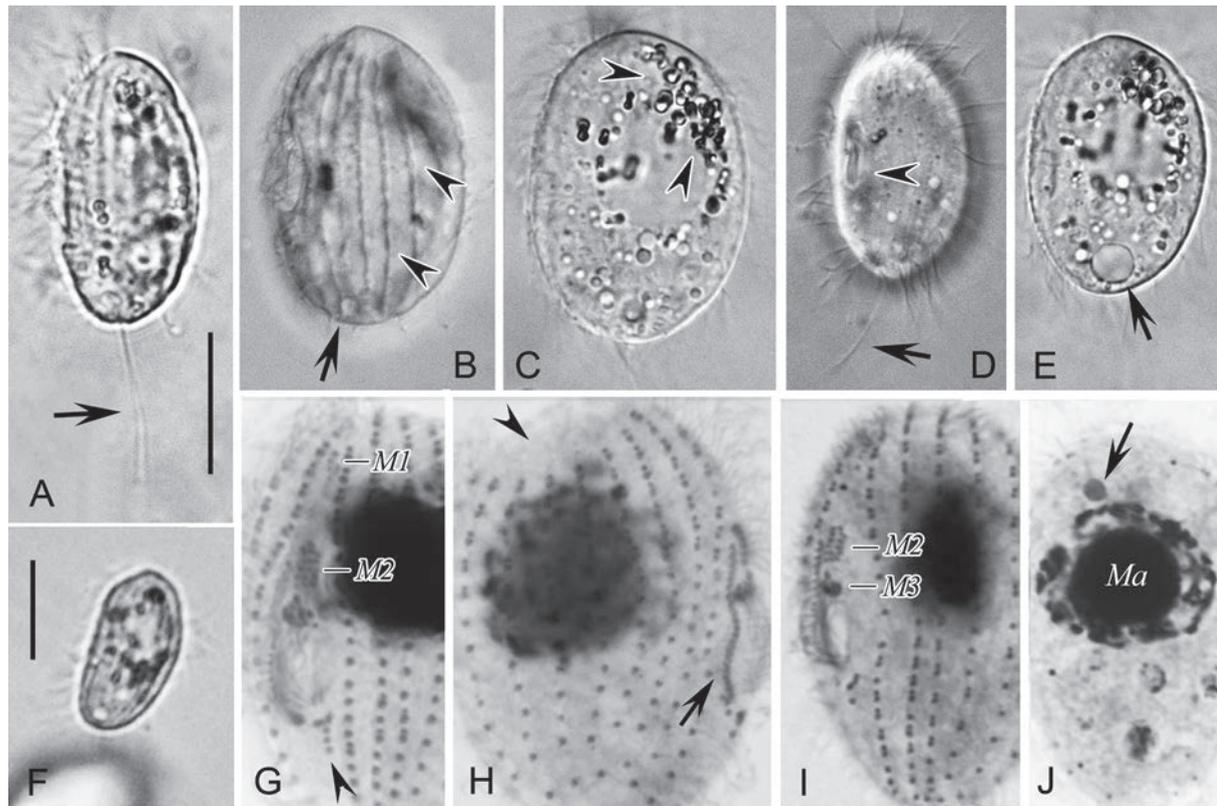
Family Uronematidae Thompson, 1964

***Uronemella parafilificum* Gong *et al.*, 2007 (Fig. 8; Table 2)**

Description: Body 20–35 × 12–20 μm *in vivo*, kidney-shaped with obliquely oriented truncated apical plate (Figs 8A, F). Buccal field in mid-body region (Fig. 8D). Pellicular ridges present between ciliary rows



Figs 7A–L. *Parauronema longum* *in vivo* (A–H, J, K) and after staining with protargol (I) and silver nitrate (L). A–E – showing different individuals; F – ventral view, arrows mark the buccal field; G – arrowheads show the extrusomes; H – posterior of cell, arrow and arrowhead indicate the contractile vacuole and the caudal cilium, respectively; I – to show the macronucleus (Ma) and micronucleus (arrowhead); J – cell surface, to show the pellicular ridges; K – arrowhead refers to the cilia of the paroral membrane, arrows indicate the crystal granules in the cytoplasm; L – arrow shows the location of contractile vacuole pore. Ma – macronucleus. Scale bars: 50 μm.



Figs 8A–J. *Uronemella paraflificum* from life (A–F) and after staining with protargol (G–J). **A, F** – two typical individuals, arrow in (A) shows the caudal cilium; **B** – arrow indicates the contractile vacuole during systole, arrowheads mark the pellicular ridges; **C** – to show the crystal granules (arrowheads); **D** – showing the buccal field (arrowhead) and caudal cilium (arrow); **E** – to show the contractile vacuole during diastole (arrow); **G** – detail of the buccal structure and scutica (arrowhead); **H** – arrow refers to the paroral membrane, arrowhead depicts the truncated anterior end of the cell; **I** – membranellae 2 and 3; **J** – showing the macronucleus and micronucleus (arrow). M1–3 – membranellae 1–3, Ma – macronucleus. Scale bars: 15 μ m.

(Fig. 8B). Cytoplasm contains many dumbbell-shaped or spherical crystals (Fig. 8C). Multiple caudal cilia present in freshly collected specimens (three individuals were examined), but only one caudal cilium was present after two days in culture; length of caudal cilia 20–25 μ m (Fig. 8A). Contractile vacuole located at posterior end of cell, 8 μ m in diameter during diastole (Figs 8B, E). Sixteen or 17 somatic kineties, anterior third of each composed of dikinetids; somatic kineties 1 and n comprising on average 19 and 23 kinetids respectively (Figs 8G–I). Membranelle 1 single-rowed with six kinetosomes; membranelle 2 two- or three-rowed; membranelle 3 small. Scutica Y-shaped, comprising three pairs of kinetosomes (Figs 8G–I). Macronucleus located in anterior half of body, accompanied by single micronucleus (Fig. 8J). Swims rapidly; sometimes ro-

tates while attached to substratum, or to other individuals, via the caudal cilium.

Discussion: *Uronemella paraflificum* is separated from *U. flificum* mainly by having fewer somatic kineties (16–19 vs. 21–23), and it was reported to have multiple (about 4) caudal cilia *in vivo* in the original description (vs. one caudal cilium in *U. flificum*), although only one caudal cilium complex was found in silver impregnated specimens of *U. paraflificum* (Gong *et al.* 2007, Song and Wilbert 2002). Cells with multiple (morphotype I) and single (morphotype II) caudal cilia were both observed in our study. Considering the strong similarity of the living morphology (except for the caudal cilia) between morphotypes I and II, and the conformity of the infraciliature between morphotype II and the previously reported population of *U. paraflifi-*

cum (Gong *et al.* 2007), the two morphotypes appear to be conspecific, thus demonstrating that *Uronemella parafilificum* has a variable number of caudal cilia in its life history. However, there is also the possibility that two species were present in the original culture and that during the two day cultivation period, the one with a single caudal cilium outgrew the one with multiple caudal cilia. Gene sequence data are probably required in order to determine which of these possibilities is true.

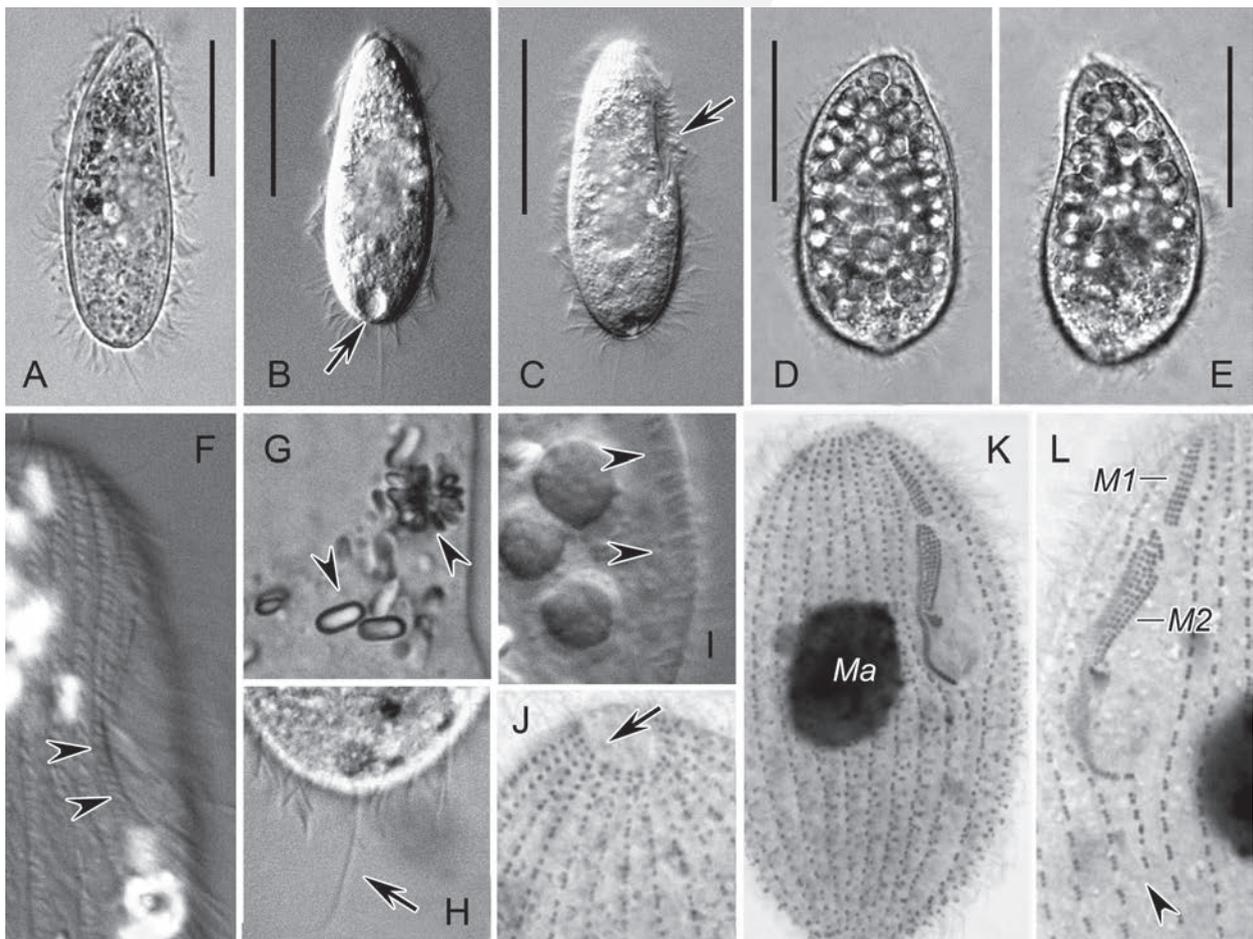
Family Uronematidae Thompson, 1964

***Paranophrys magna* Borrer, 1972 (Fig. 9; Table 2)**

Description: Body 45–70 × 15–25 µm *in vivo*, cylindrical in shape, ratio of body length to width 2–3 : 1 (Figs 9A–C). Buccal field shallow, occupying one-third

of body length (Figs 9C, F). Cytoplasm colourless, containing numerous granules when well-fed (Figs 9D, E). Crystals scattered throughout body, sometimes in clusters (Fig. 9G). Extrusomes small, about 1.5 µm long (Fig. 9I). Single caudal cilium approximately 20 µm long (Fig. 9H). Contractile vacuole located at posterior end of cell, pulsating at ca. 10 s intervals (Fig. 9B).

Infraciliature as shown in Figs 9K, L. Twenty-two to 28 somatic kineties, 44 kinetids in somatic kinety 1 on average. Membranelle 1 with three longitudinal rows; membranelle 2 wider but about equal in length to membranelle 1; membranelle 3 small. Anterior half of paroral membrane closely associated with membranelle 2. Scutica composed of three to five pairs of kinetisomes arranged in a row.



Figs 9A–L. *Paranophrys magna* from life (A–I) and after staining with protargol (J–L). A–C – slender individuals, arrow in (B) shows contractile vacuole, arrow in (C) depicts the buccal field; D, E – actively feeding individuals; F – arrowheads mark the depressed buccal field; G – the crystals that some times cluster (arrowheads); H – caudal cilium; I – arrowheads show the extrusomes; J – arrow marks the small, bald apical plate; K – general view of ventral infraciliature; L – detail of buccal apparatus, arrowhead refers to the scutica. M1–2 – membranelles 1, 2, Ma – Macronucleus. Scale bars: 30 µm.

Discussion: The new population was isolated from a drying puddle with salinity about 85‰. Nevertheless, its morphology corresponds closely with that of populations isolated from natural marine waters (Borror 1972, Song and Wilbert 2000b). Thus, *Paranophrys magna* is very likely a euryhaline species.

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