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Notes on Two Marine Ciliates from the Yellow Sea, China: *Placus salinus* and *Strombidium apolatum* (Protozoa, Ciliophora)

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Abstract The living morphology and infraciliature of two rare marine ciliates, *Placus salinus* Dietz, 1964 and *Strombidium apolatum* Wilbert and Song, 2005, collected from the coastal waters near Qingdao, China, were investigated by *in vivo* observation and protargol impregnation technique. The improved diagnosis for *Placus salinus* is as follows: medium-sized marine *Placus*, *in vivo* (50–60) μm × (30–40) μm; cell elliptical to barrel-shaped; 28–31 somatic kineties; single macronucleus usually ellipsoid and one micronucleus located in the indention of the macronucleus; one contractile vacuole posteriorly positioned. *Strombidium apolatum* is characterized by: marine *strombidium* (40–60) μm × (30–45) μm *in vivo*, cordiform in shape with somewhat pointed posterior end and conspicuous apical protrusion; extrusomes prominent, about 15 μm in length and evenly arranged along the circle kinety; about 16 collar and 5–6 buccal membranelles; one elongate macronucleus and one micronucleus; circle and ventral kineties consisting of about 53 and 45 dikinetids respectively.

Key Words marine ciliates; *Placus salinus*; *Strombidium apolatum*

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1 Introduction

Planktonic ciliates are ubiquitous in ocean surface water and mostly a major component of planktonic food webs (Lynn and Montagnes, 1988). These protists serve as an important link between smaller unicellular organisms and those at higher trophic levels in marine microbial system (Pierce and Turner, 1992). Being small and fragile, many of these organisms have been only rather superficially investigated, which were usually based largely on living observations (Kahl, 1932; Maeda and Carey, 1985). Because of this reason, 'numerous' ambiguities concerning the identification of species have accumulated in the last century and most nominal species need to be redefined or re-investigated using both living observation and modern staining methods (Busch, 1930; Kahl, 1932).

During faunistic surveys on marine ciliates in coastal waters of North China, two planktonic ciliates, *Placus salinus* Dietz, 1964 and *Strombidium apolatum* Wilbert and Song, 2005, were isolated and studied using the protargol impregnation method. The results are documented here.

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2 Materials and Methods

P. salinus. Two populations were collected from mollusk and shrimp culturing waters near Qingdao in April 2002 and April 2004 respectively. The ecological characters of the two sampling sites are: salinity 32 and 30, pH 8.0–8.4, and water temperature about 12 ℃ and 25 ℃ respectively.

S. apolatum. Two populations were isolated from the littoral area off Qingdao in April and July 2003. The ecological characters for the sampling sites: salinity was about 30, pH ranged 7.8–8.0, and water temperatures were about 13 ℃ and 22 ℃.

Individuals were observed *in vivo* using an oil immersion objective (× 1 250) and differential interference contrast microscope. The infraciliature was revealed by protargol staining method according to Wilbert (1975). Drawings of impregnated specimens were made with the help of a camera lucida at × 1 250 magnification. Drawings of living specimens were based on *in vivo* observation and photomicrographs. Terminology is mainly according to Corliss (1979) and Petz *et al.* (1995).

Voucher slides of protargol-impregnated specimens have been deposited in the collection of the Laboratory of Protozoology, OUC, China.

3 Results

3.1 *Placus salinus* Dietz, 1964

Although this species has been redescribed several times since it was originally reported by Dietz (1964) (Borror, 1972; Lei *et al.*, 1999; Hu *et al.*, 2003), useful information is still lacking. Therefore, a full redescription is provided here according to new observations on the Qingdao populations.

Improved diagnosis Medium-sized marine *Placus*, *in vivo* (50−60) μm × (30−40) μm; cell elliptical to barrel-shaped; 28−31 somatic kineties; single macronucleus usually ellipsoid and one micronucleus located in the indention of the macronucleus; one contractile vacuole posteriorly positioned.

Morphology of Qingdao *Placus salinus* populations (Figs.1 and 2; Table 1) Cell about (50−60) μm × (30−40) μm *in vivo*. Body shape constant, elliptical to barrel-shaped when viewed bilaterally with anterior end somewhat truncated and posterior end broadly rounded (Figs.1 A, B, C; 2 A, B). Bilaterally about 2∶3 flattened and length to width ratio about 2∶1, depending on the number of the ingested preys. Pellicle meridionally ribbed along kineties, structured as in its other congeners (*e.g.* Foissner, 1972) (Fig.2 C, arrowheads). Oral opening about 1/3 of body width, located in the center of anterior pole, elongate elliptical in top view and inconspicuous *in vivo* (Figs.1 A, B, C; 2 A, B). Subapical cavity (about 6 μm in length) (defining ventral side), which contains slightly longer cilia (about 10 μm long), located right underneath the adoral organelles (Figs.1 A, double-arrowheads; 2 I, arrows). Somatic cilia about 5 μm long, distributed posteriad to the circumoral dikinetid and longitudinally arranged.

Table 1 Morphometric characterization of *Placus salinus*

Character	Min	Max	Mean	SD	SE	CV	n
Body length	52	72	55.0	8.51	2.83	15.5	9
	41	70	57.3	9.94	3.31	17.3	9
Body width	32	48	42.4	6.52	2.47	15.4	7
	36	58	44.1	7.57	2.68	17.1	8
Macronucleus, number	1	1	1	0	0	0	10
	1	1	1	0	0	0	9
Macronucleus, length	17	33	26.4	5.09	1.93	19.3	7
	16	20	17.2	1.30	0.43	7.6	9
Macronucleus, width	12	18	16.1	2.04	0.76	12.6	7
	10	15	12.3	1.41	0.47	11.5	9
Somatic kineties, number	28	31	30.4	1.17	0.37	3.9	10
	29	31	30.4	1.01	0.34	3.3	9
Subapical cavity, length	5	8	6.4	1.01	0.34	15.7	9
	–	–	–	–	–	–	–
Apex to beginning of subapical cavity, distance	10	16	13.5	3.16	1.12	23.4	8
	–	–	–	–	–	–	–
Oral basket rod, number	–	–	–	–	–	–	–
	30	38	31.9	3.08	1.16	9.7	7
Oral basket rod, length	–	–	–	–	–	–	–
	29	43	38.8	4.30	1.52	11.1	8

Notes: Population I, the upper line; Population II, the lower line. Data based on protargol impregnated specimens. CV: coefficient of variation; Max: maximum; Mean: arithmetic mean; Min: minimum; n: sample number; SD: standard deviation; SE: standard error of the mean. Measurements in μm. '–' Data unavailable.

Cytoplasm colourless but often rather dark in posterior half at low magnification due to presence of ingested algae including diatoms (Figs.1 A, C). In some specimens, a few food vacuoles (up to 20 μm in diameter) often recognizable (Fig.2 A), frequently replete with other ciliates. Extrusomes (Ex) (about 2 μm long *in vivo*) rod-like, regularly and densely arranged along somatic ciliary rows (Fig.1 F, arrowheads). One contractile vacuole (CV) about 6 μm in diameter, terminally located, pulsating with long period (often over 10 min) (Figs.1 A, B, C; 2 A, arrow).

Macronucleus (Ma) usually ellipsoidal, located near body center, containing numerous globular nucleoli (about 2−4 μm in diameter) (Fig.2 G, arrows); single spherical micronucleus about 2 μm in diameter attached to macronucleus.

Cells always attach to the substrate motionlessly or crawl very slowly; when disturbed, the organisms swim rapidly for several seconds and then return to the substrate.

Oral basket trumpet-shaped, composed of 30−38 fine rods (about 38 μm in length) which extend to almost mid-body (Fig.1 A, inset). Circumoral dikinetid (CO) composed of 30−38 close-set dikinetids, each associated with an oral basket rod (Figs.1 E, G; 2 H, arrowheads). Adoral organelles (brosse, AO), which

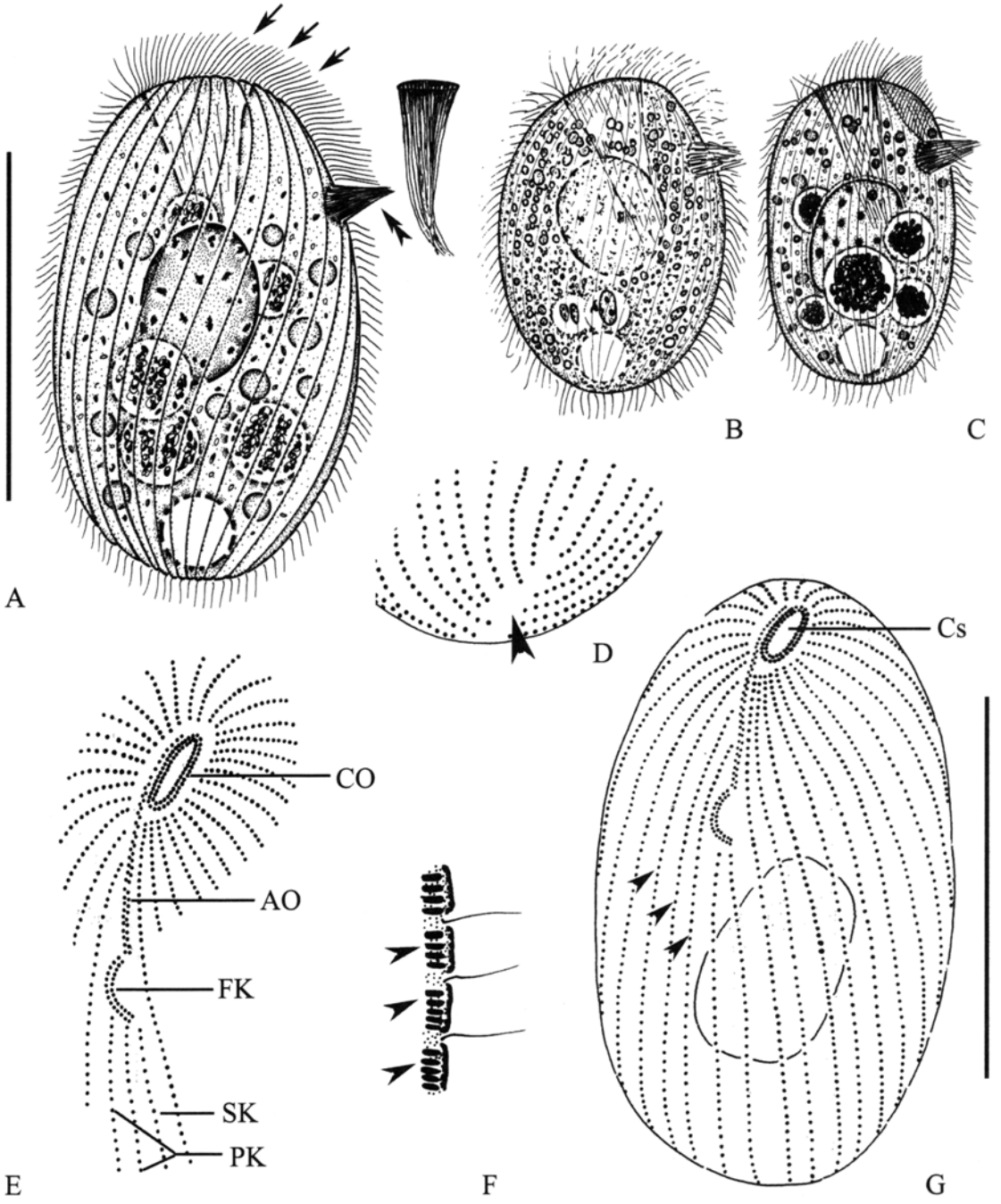

Fig.1 *Placus salinus* from life (A, B, C, F) and after protargol impregnation (D, E, G). A. A typical cell, inset marks the structure of cytopharynex, arrows denote the adoral organelles and double-arrowhead indicates the subapical cavity; B–C. Two living cells showing the different body shapes; D. Subcaudal view of infraciliature, arrow marks the suture in the caudal area; E. Detailed anterior portion of infraciliature; F. Optical section of cortex, arrowheads indicate extrusomes; G. Frontoventral view of the infraciliature, arrowheads denote the shortened somatic kineties. Abbreviations: AO: adoral organelles (brosse); CO: circumoral dikinetid; Cs: cytostome; FK: fragment kinety; PK: postcavity kineties; SK: somatic kinety. Scale bars: 40 μm (A, G).

extends from subapical cavity anteriad, along right side of oral opening, are prominent with densely arranged cilia (about 7–8 μm long) and composed of basal body pairs (Figs.1 A, arrows, E, G; 2E, arrowheads). On average 30 (28–31) longitudinal somatic kineties, each composed of densely packed monokinetids (Figs.1 G, 2D); all somatic kineties extending almost complete length of cell and forming suture at posterior end of cell (Figs.1D, arrowhead; 2J, arrowheads). Usually 2–4 somatic kineties at the right of subapical cavity slightly shortened, terminating at adoral organelles (Figs.1G, arrowheads; 2E, double-arrowheads); 2 or 3 kineties extending from posterior portion to subapical cavity (named postcavity

kineties, PK) (Figs.1 E, G). Subapical cavity located behind adoral organelles, composed of 2 C-shaped rows of ciliated basal bodies (named fragment kineties, FK) (Figs.1 E, G; 2 E, arrow, F, arrows).

3.2 *Strombidium apolatum* Wilbert and Song, 2005

This species has been recently reported by Wilbert and Song from the Antarctic (Wilbert and Song, 2005). Basically, our populations correspond well with the original description regarding the basic morphology. Hence, we believe that they should be conspecific. Because some information was lacking in the original description, an additional contribution with statistical data is provided here.

Description of Qingdao *Strombidium apolatum* populations (Figs.3 and 4; Table 2) Cell (40−60) μm × (30−45) μm *in vivo* and (30−60) μm × (20−35) μm after protargol impregnation. Body shape constant, usually elongate obconic; broadest always at 'shoulder' area of the cell (slightly behind anterior collar membranelles) with caudal end more or less pointed and dorsoventrally 4:3 flattened (Figs.3 A, C; 4 A,

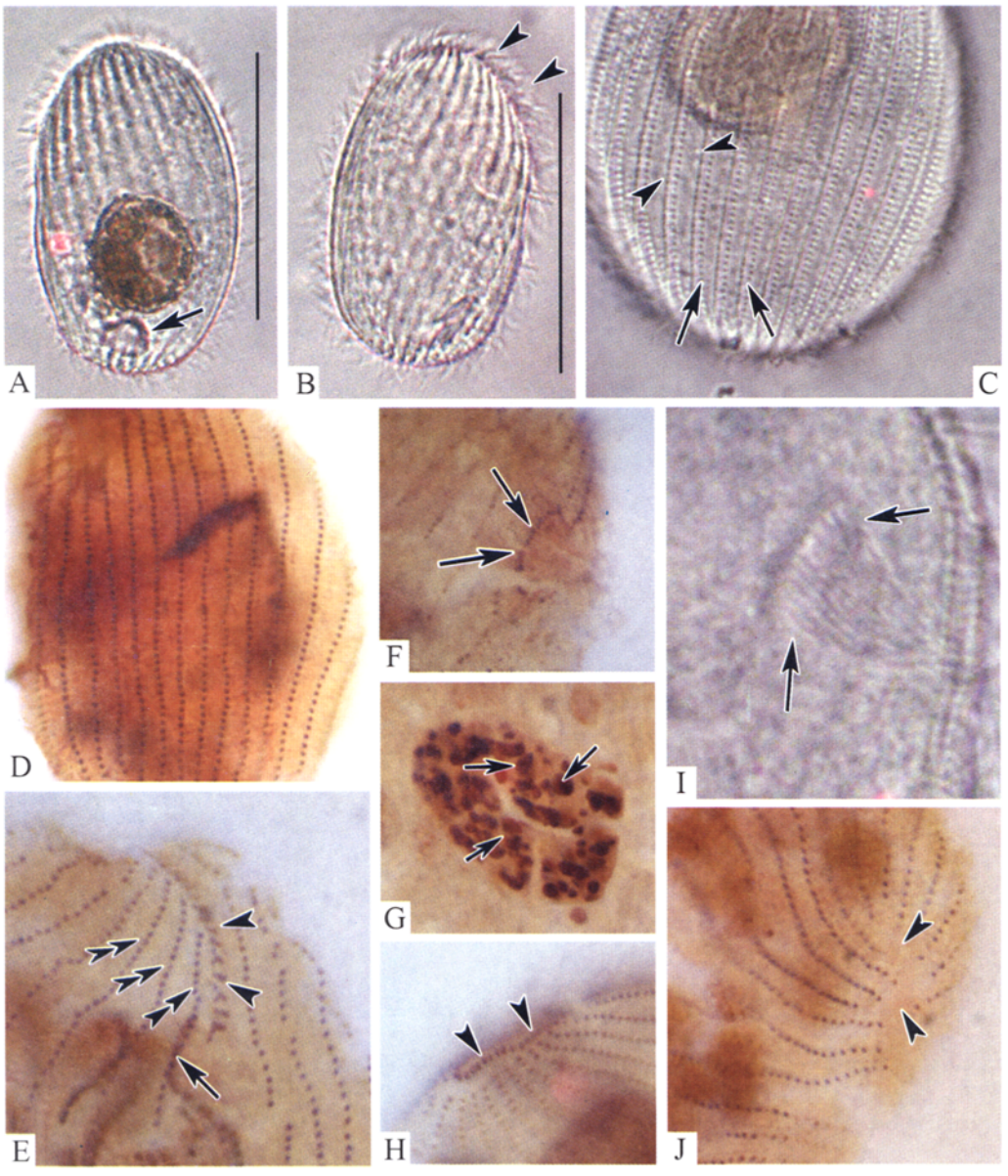

Fig.2 *Placus salinus* from life (A−C, I) and after protargol impregnation (D−H, J). A. A typical individual, arrow indicates the contractile vacuole; B. Right side view of the cell, arrowheads mark the adoral organelles; C. A slightly pressed specimen showing somatic kineties (arrows) and ribs (arrowheads); D. General view of cell showing the densely arranged basal bodies in somatic kineties; E. Ventral view of the detailed anterior portion of the cell, arrow indicates the fragment kineties, arrowheads mark the adoral organelles and double-arrowhead denotes the shortened somatic kineties; F. Ventral view of cell showing the fragment kineties (arrows); G. To show the macronucleus, arrows indicate the small, spherical nucleoli; H. Anterior portion of the cell, arrowheads mark the circumoral dikinetid; I. Arrows mark the long cilia in the subapical cavity; J. To show the suture in the caudal area (arrowheads). Scale bars: 40 μm (A, B).

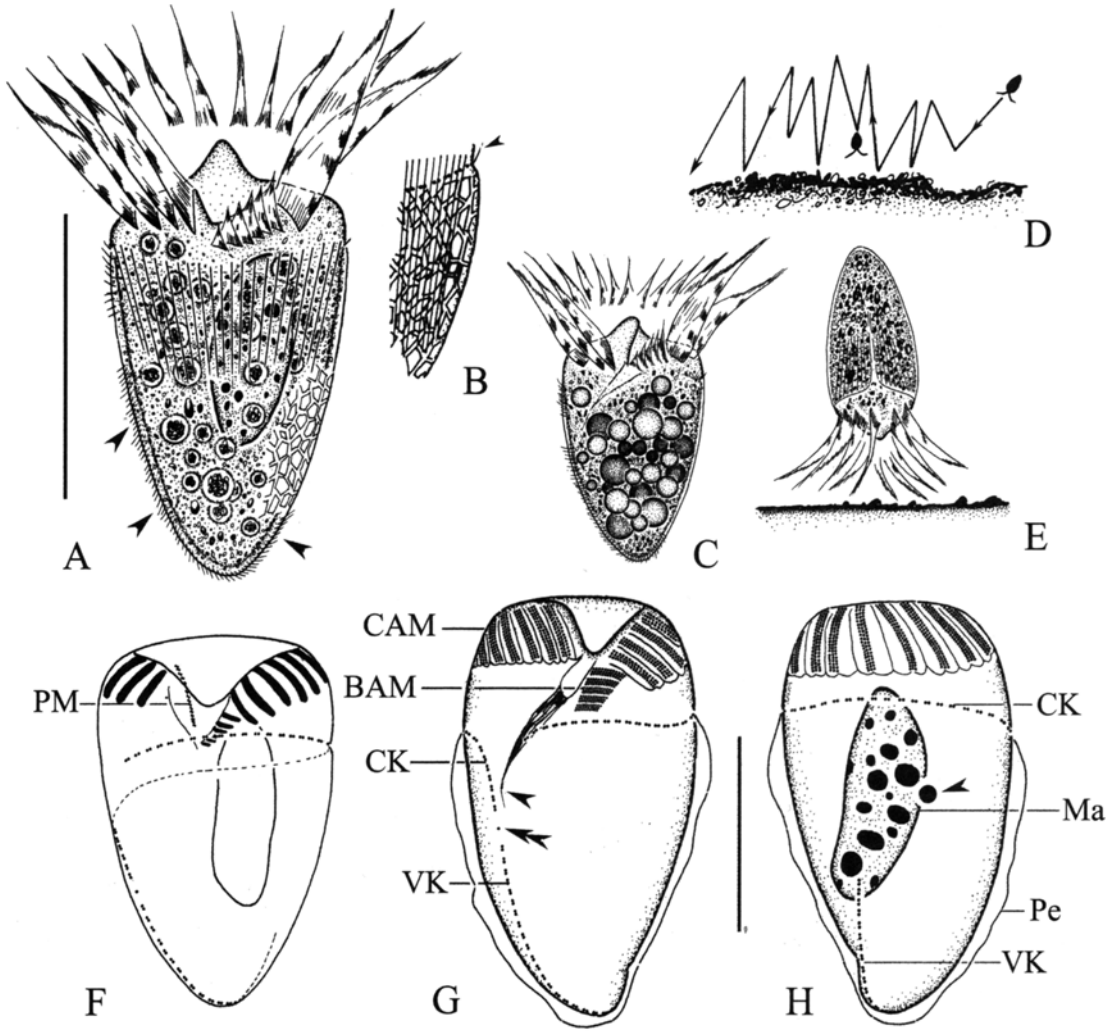

Fig.3 *Strombidium apolatum* from life (A–E) and after protargol impregnation (F–H). A. Ventral view of a typical individual, arrowheads show cilia in the ventral kinety; B. Portion of perilemma and the extrusomes *in vivo*, arrow indicates cilia in the circle kinety; C. Ventral view demonstrating different body shape and the ingested food vacuoles in the cytoplasm; D. Pattern of locomotion; E. To show a cell attaching to the substrate; F. To show the arrangement of somatic kineties; G, H. Infraciliature of ventral and dorsal sides, arrowhead in G denotes cytopharyngeal fibres, double-arrowhead indicates the single basal body connecting circle and ventral kineties, arrowhead in H marks micronucleus. Abbreviations: BAM: buccal membranelles; CAM: collar membranelles; CK: circle kinety; Ma: macronucleus; Pe: perilemma; PM: paroral membrane; VK: ventral kinety. Scale bars: 50 μm (A); 30 μm (F, G).

B). Subpellicular platelet layer (perilemma) located in posterior 3/4 of the cell with numerous polygonal platelets (3–6 μm across) (Figs.3 A, B; 4 G). Apical protrusion conspicuous *in vivo* but undetectable after impregnation (Figs.3 A; 4 A, double-arrowheads). Buccal cavity shallow, extending to about 1/5 of cell length. Cilia of most membranelles about 25–30 μm long, extending anteriorly as shown in Fig.3 A.

Cytoplasm colorless, usually with numerous food vacuoles (about 6–8 μm across) containing algae including diatoms, which render cells almost dark grey at low magnification and yellowish to brownish at high magnification (Figs.3 A, C; 4 A, B, arrows). Extrusomes prominent, about 15 μm long, longitudinally oriented and not in bundles, inserting along the circle kinety (Figs.3 A; 4 D, arrows). One macronucleus near mid-body, ellipsoidal or spindle-shaped, usually (19–35) μm × (7–16) μm after protargol impregnation and containing numerous nucleoli (about 2–4 μm in diameter) (Figs.3 H, 4 K). One micronucleus (about 4 μm across), closely attached to macronucleus and hardly impregnated by protargol (Fig.3 H, arrowhead).

Locomotion very fast with its body upside down, hectically to and fro on the debris and rotating around longitudinal body axis simultaneously (Figs.3 D, E). When disturbed, the organism will swim away immediately in wild spirals.

Oral apparatus occupies anterior end of cell, consisting of a paroral membrane (PM) on inner wall of buccal lip and a membranellar zone. Membranellar zone distinctly opens ventrally, bipartited into collar membranelles (CAM, consisting of 14–19 membranelles) and buccal membranelles (BAM, comprising 5–6

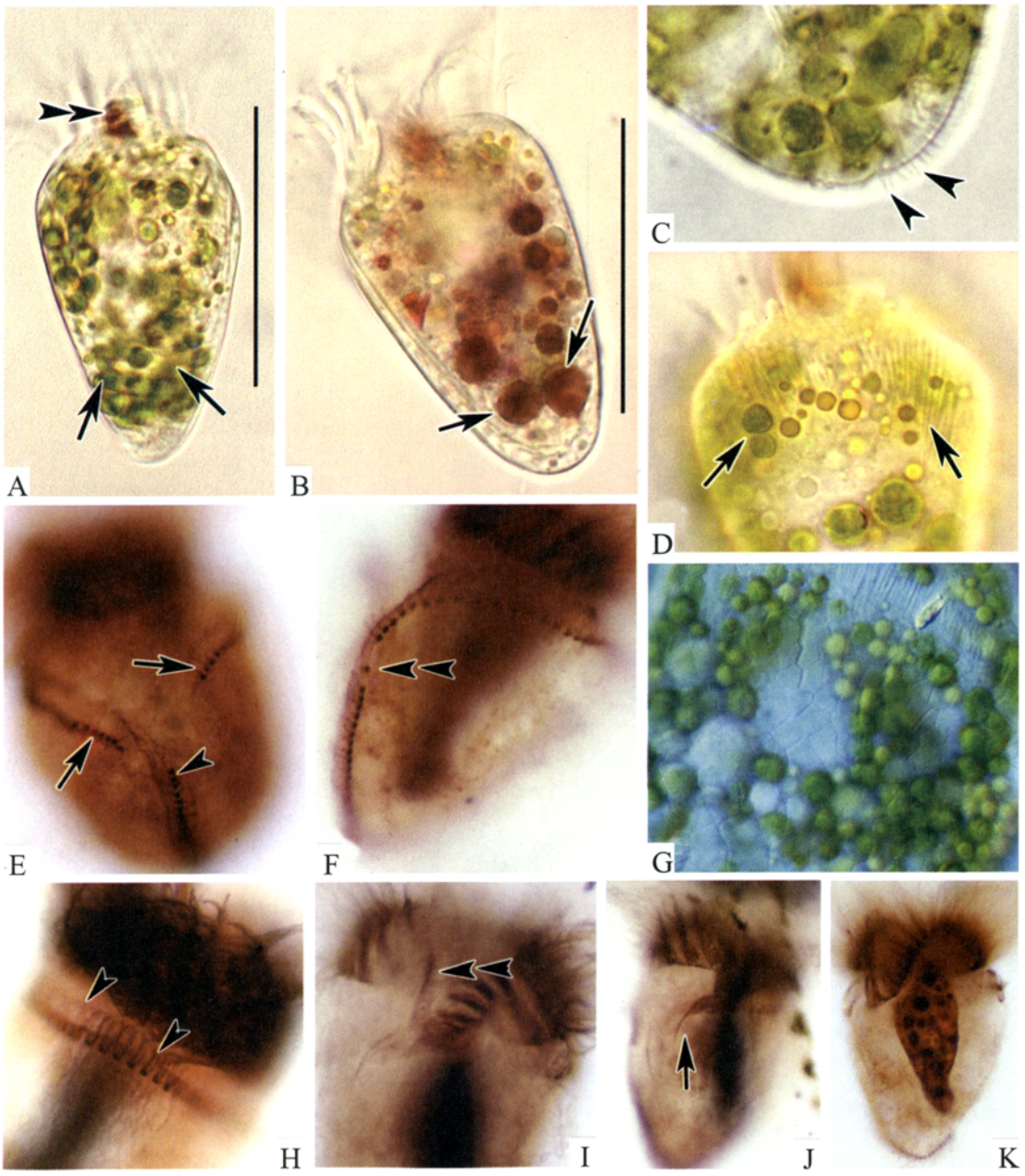

Fig.4 Photomicrographs of *Strombidium apolatum* from life (A–D, G) and after protargol impregnation (E–F, H–K). A, B. Two typical individuals showing cytoplasm filled with ingested yellowish algae (A, arrows) and brownish diatoms (B, arrows), double-arrowhead marks the apical protrusion; C. Posterior view of cell, arrowheads indicate cilia of the ventral kinety; D. Ventral view of cell showing the densely arranged extrusomes (arrows); E. Right side of cell, arrows mark circle kinety and arrowhead indicates ventral kinety; F. Right side of the cell, double-arrowhead marks the single basal body connecting ventral and circle kinety; G. Details of pellicle showing the numerous polygonal platelets; H. Dorsal view showing cilia in circle kinety (arrowheads); I. Apical view of the cell, double-arrowhead indicates the paroral membrane; J. Ventral view of cell showing pharyngeal fibres extending to right side of the cell; K. To show macronucleus. Scale bars: 40 μm (A, B).

membranelles); these two portions are not separated but bases of the former are much longer than those of the latter (Figs.3 F, G, H). Paroral membrane on right wall of buccal cavity, extending to center of apical protrusion (Figs.3 F; 4 I, double-arrowheads). Cytopharyngeal fibres prominent, up to 15 μm long and extending obliquely to right side of cell (Figs.3 G, arrowhead; 4 J, arrow).

Both circle and ventral kineties composed of dikinetids, each with about 3 μm long cilia (Figs.3 A, C, arrows; 4 C, H, arrowheads). Circle kinety (CK, composed of 40–69 dikinetids) located at the edge of subpellicular platelet layer, beginning at right margin, turning to dorsal side and then curved slightly to posterior direction but clearly separated from the ventral kinety (Figs.3 F, G, H; 4 E, arrows). Ventral kinety (VK) more densely-ciliated than circle kinety, containing 25–49 basal body pairs, extending from right margin subcaudally onto the left and terminating laterally at posterior end of circle kinety at level of about

Table 2 Morphometric characterization of *Strombidium apolatum*

Character	Min	Max	Mean	SD	SE	CV	n
Body length	32	59	43.4	7.95	1.99	18.3	16
	40	60	49.9	5.81	1.12	11.6	27
Body width	20	36	28.8	4.84	1.21	16.8	16
	26	36	30.4	2.74	0.53	8.9	27
Collar membranelles, number	15	19	16.4	1.41	0.49	8.6	8
	14	17	15.4	0.87	1.21	5.6	17
Buccal membranelles, number	5	6	5.6	0.52	0.16	9.2	10
	5	6	5.8	0.49	0.14	9.0	11
Basal body pairs in circle kinety, number	46	66	52.8	5.45	1.51	10.3	13
	40	69	53.6	6.34	1.22	11.8	27
Basal body pairs in ventral kinety, number	25	38	30.5	4.36	1.26	14.3	12
	28	49	36.6	5.40	1.06	14.8	26
Macronucleus, number	1	1	1	0	0	0	18
	1	1	1	0	0	0	15
Micronucleus, number	1	1	1	0	0	0	4
	1	1	1	0	0	0	6
Macronucleus, length	22	35	27.2	3.91	0.95	14.4	17
	19	35	27.6	3.42	0.66	12.4	27
Macronucleus, width	8	16	11.2	2.01	0.52	17.9	15
	7	14	9.5	1.85	0.36	19.5	27

Notes: Population I, the upper line; Population II, the lower line. Data based on protargol-impregnated specimens. CV: coefficient of variation; Max: maximum; Mean: arithmetic mean; Min: minimum; n: sample number; SD: standard deviation; SE: standard error of the mean. Measurements in μm.

anterior 1/3 of cell length (Figs.3 F, G, H; 4 E, arrowhead). Usually one basal body detected which connects both circle and ventral kineties (Figs.3 G, 4 F, double-arrowheads).

4 Discussion

To date, approximately 10 nominal *Placus*-species in both freshwater and marine habitats have been described, of which 5 have been investigated using modern techniques: *P. striatus* Cohn, 1866; *P. luciae* (Kahl, 1926) Kahl, 1930; *P. salinus* Dietz, 1964; *P. longinucleatus* Song and Wilbert, 1989 and *P. antarcticus* Petz *et al.*, 1995 (Cohn, 1866; Sauerbrey, 1928; Kahl, 1930; Tucolesco, 1962; Dietz, 1964; Burkovsky, 1970; Song and Wilbert, 1989; Petz *et al.*, 1995).

Morphologically, *P. striatus* should be closely related to *P. salinus*; nevertheless, it differs from *P. salinus* in having less, spiral somatic kineties (14–16 vs. 29–31 and not spiral) (Kahl, 1930).

P. salinus is separated from *P. longinucleatus* by the habitat (marine vs. freshwater), shape of macronucleus (ellipsoidal vs. worm-shaped) and number of somatic kineties (29–31 vs. 19–21) (Song and Wilbert, 1989).

P. luciae exhibits a similar size and body shape to *P. salinus*. However, it can be distinguished by the freshwater habitat (vs. marine) and fewer somatic kineties (16–23 vs. 29–31) (Foissner, 1972).

Compared with *P. antarcticus*, *P. salinus* is much smaller (50–60 vs. 85–160 μm in length) and has fewer somatic kineties (29–31 vs. 36–40) (Petz *et al.*, 1995).

Our populations of *Strombidium apolatum* correspond well with the original description except the following combined features: 1) perilemma composed of numerous polygonal platelets (vs. perilemma transparent and no platelets found), 2) one micronucleus (vs. absent in the original description), and 3) fewer buccal membranelles (5–6 vs. 6–8). We think all these differences mentioned above are just population-dependent, and we believe that the identification of the Qingdao form is unquestionable.

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