Acta Protozool. (2007) 46: 229–245

ACTA
PROTOZOOLOGICA

Two New Ciliates from the North China Seas, *Schizocalyptra aeschtae* nov. spec. and *Sathrophilus holtae* nov. spec., with New Definition of the Genus *Sathrophilus* (Ciliophora, Oligohymenophora)

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Summary. The living morphology, infraciliature, and silverline system of two marine ciliates, *Schizocalyptra aeschtae* nov. spec. and *Sathrophilus holtae* nov. spec. collected from sandy beaches near Qingdao, north China seas, were investigated. *Schizocalyptra aeschtae* is diagnosed as follows: 75–200 × 30–80 μm *in vivo*, bilaterally flattened about 1:2; about 15 prolonged cilia in posterior half of body; 60 somatic kineties on average; bases of membranelle 1 (M1) and M2b short; M3 about the same length as M2a; paroral membrane with 6–12 fragments at its posterior part; 2 to numerous macronuclear nodules in different shapes and sizes; one large contractile vacuole terminally located; marine habitat. *Sathrophilus holtae* nov. spec. is characterized by: *in vivo* 35–70 × 20–40 μm, cylindrical in outline; dorsoventrally flattened, with a conspicuously long caudal bristle; 21 somatic kineties on average; M1 three-rowed and bipartite with first row conspicuously long and separated from the other two; M2 two-rowed and L-shaped; ratio of lengths of M2 to M3 about 3:1; paroral membrane extending to above level of M2; one contractile vacuole pore at the end of SK1; marine habitat. Since no diagnosis according to modern investigation is available for the genus *Sathrophilus*, a new definition is supplied: dorsoventrally flattened, elongated Cinetochilidae with *Tetrahymena*-like buccal apparatus and bipartite M1; paroral membrane slightly curved, terminating anteriorly to about level of M1/M2; buccal field above the equatorial level; postoral kineties and groups of kinetosomes (scutica) present; having a distinctly long caudal bristle and one conspicuous, cilia-free apically plate. The systematic position of *Sathrophilus* is briefly discussed which, based on the morphological and morphogenetic characters of *Sathrophilus holtae*, appears to be a loxocephalid rather than a scuticociliate or a hymenostomatid. In addition, a synonym is recognized, i.e. *Paradexiotricha*, and a new combination is suggested: *Sathrophilus puytoraci* (Grolière 1975) nov. comb. [basionym: *Paradexiotricha puytoraci* Grolière, 1975].

Key words: Scuticociliates, Loxocephalida, morphology, morphogenesis, new species, new combination.

INTRODUCTION

Scuticociliates are mostly small, free-living or parasitic/commensal ciliates with great species diversity, which are, for a long time, considered to be a mono-

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phyletic assemblage (Kahl 1931, Raabe 1970, Foissner *et al*. 1994, Petz *et al*. 1995, Song 2000). Free-living marine scuticociliates have been frequently found in eutrophic water and sediments with high abundance (Kahl 1931; Borror 1963; Agamaliev 1968; Song 2000; Song and Wilbert 2000, 2002). Since these ciliates are generally small in size and show similar living features, species identification is largely based on the infraciliature after silver impregnations, especially features of oral structures (Corliss 1979, Song 2000).

The genera *Schizocalyptra* and *Sathrophilus* are rarely found in marine habitats (Dragesco 1968, 2002; Grolière and Detcheva 1979; Fernandez-Leborans and Novillo 1994). Morphologically, *Schizocalyptra* is similar to the well-known *Pleuronema* in that it possesses a prominent buccal apparatus with bipartite membranelle 2, but differs in its fragmented (*vs.* continuous) paroral membrane (PM). To date, only two species have been described from marine habitats (Fernandez-Leborans and Novillo 1994, Dragesco 1968).

Sathrophilus has long been regarded as a scuticociliate (Corliss 1979) and is characterized by its *Tetrahymena*-like buccal apparatus and the long caudal bristle. About 14 nominal species have been assigned to this genus, and only five of which have been examined following silver impregnations, namely *S. arenicolus*, *S. hovassei*, *S. marinum*, *S. muscorum* and *S. vernalis* (Grolière 1973, Grolière and Detcheva 1979, Foissner *et al*. 1982, Dragesco 2002; Fig. 8).

In this paper we describe two new species, belonging to the above mentioned genera. As a further contribution, an improved definition of the genus *Sathrophilus* is presented and its systematic position is discussed.

MATERIALS AND METHODS

***Schizocalyptra aeschtae* nov. spec.** One population was collected on 22 April 2005 from a sandy beach on the Yellow Sea coast near Qingdao (36°08′N; 120°43′E) of northern China. Temperature about 15ºC, salinity 30‰.

***Sathrophilus holtae* nov. spec.** Two populations were collected: (1) on 14 July 2003 from sandy littoral sediments near Tianjin, on the Bohai Sea coast of northern China (39°10′N; 117°10′E) (salinity 30‰, temperature 18ºC); (2) on 12 May 2006 from a sandy beach on the Yellow Sea coast near Qingdao, China (salinity 32‰, temperature 19ºC).

Cells were observed *in vivo* using bright field and differential interference contrast microscopy. The infraciliature was revealed by protargol (Wilbert 1975) and silver nitrate impregnation (Song and Wilbert 1995) methods. Drawings of living cells were based on free-hand sketches and photomicrographs, while those of silver-impregnated cells were made using a camera lucida. Terminology is mainly according to Corliss (1979).

RESULTS

Order Scuticociliatida Small, 1967
Family Pleuronematidae Kent, 1881
Genus *Schizocalyptra* Dragesco, 1968

***Schizocalyptra aeschtae* nov. spec. (Figs 1–3; Table 1)**

Diagnosis: 75–200 × 30–80 μm *in vivo*, bilaterally flattened about 1:2; *vs.* 15 prolonged cilia in posterior half body; 60 somatic kineties on average; M1 and M2b short; M3 about the same length as M2a; paroral membrane with 6–12 fragments at its posterior part; 2 to numerous macronuclear nodules in different shape and size; one large contractile vacuole terminally located; marine habitat.

Dedication: We dedicate this species to our eminent colleague, Dr. Erna Aescht, Biologiezentrum des Oberösterreichischen Landesmuseums, Austria, in recognition of her contributions to the study of ciliates.

Type Location: Sandy beach on the Yellow Sea coast near Qingdao (36°08′N; 120°43′E), China.

Deposition of Slides: One holotype slide with protargol impregnated specimens is deposited in the Natural History Museum, London, UK with registration number 2007:4:22:1 and one paratype slide with silver nitrate impregnated specimens is deposited in the Laboratory of Protozoology, OUC (slide number: 2005042201-2).

Description: *In vivo* body usually 80 × 40 ~ 160 × 80 μm. Oval in outline, bilaterally flattened about 1:2, ventral margin concave, with the middle more curved than other regions, dorsal margin conspicuously convex (Figs 1A, D, E; 2A–E, H). One contractile vacuole, about 25 μm in diameter, caudally positioned (Figs 1A, D, E; double-arrowheads in Fig. 2H). Extrusomes spindle-shaped, about 4 μm long, densely arranged in the cortex beneath the crenellated pellicle (Fig. 2C; arrow in Fig. 2F; arrowhead in Fig. 2J). Cytoplasm colourless to grayish, usually with many crystals and globular or ellipsoidal food vacuoles (6–10 μm across) containing bacteria and diatoms (Figs 1A, E; 2G, H; 3E).

Number and shape of macronuclear nodules highly variable from 2 to 61, mostly about 10–20 in number (Fig. 1G). Macronuclear nodules usually spherical, oc-

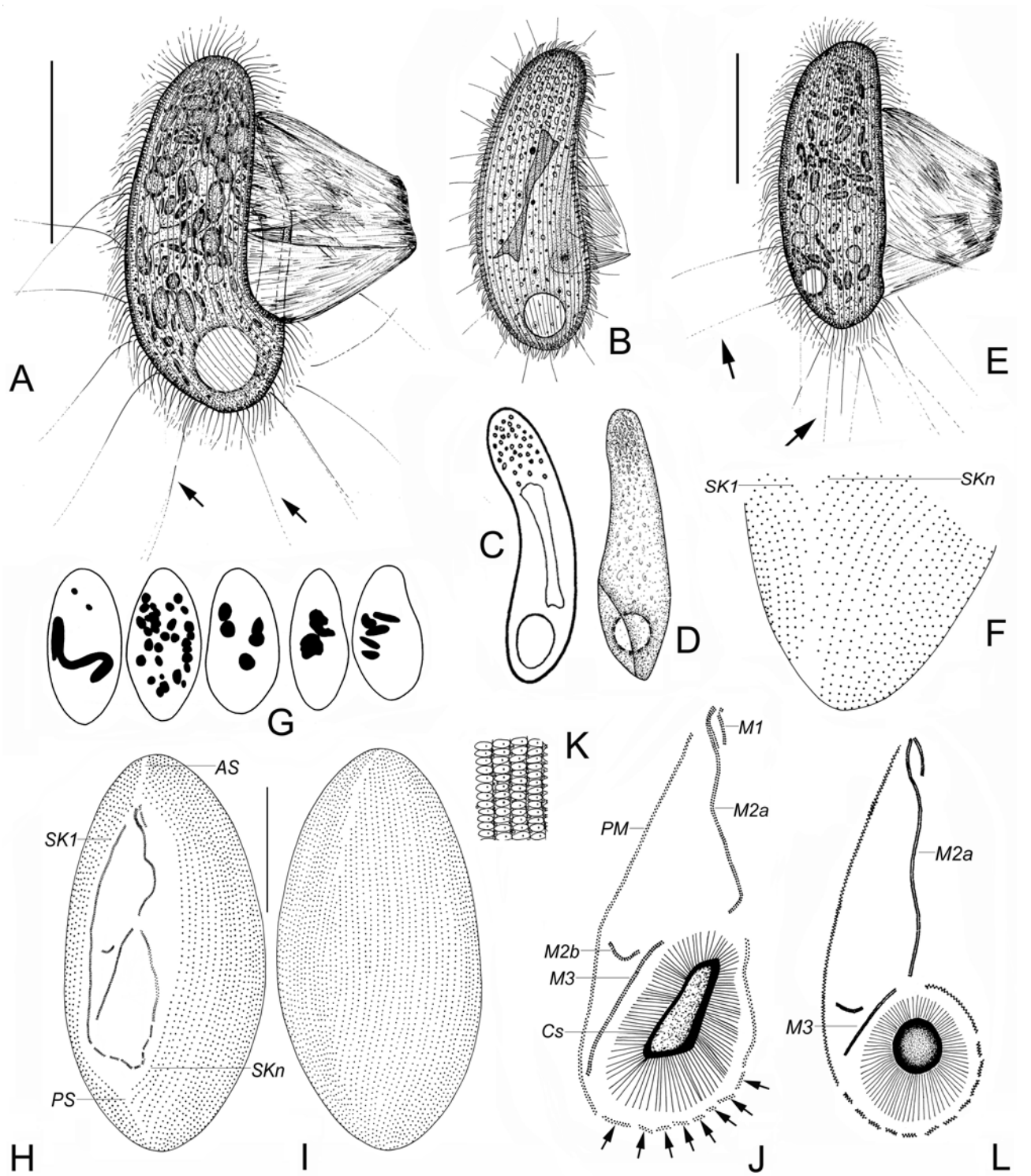

Fig. 1. *Schizocalyptra aeschtae* nov. spec. from life (**A**, **D**, **E**), after silver nitrate (**K**) and protargol impregnations (**F–J**). **B**, **C**, **L** – *S. magna* Dragesco, 1968 (after Dragesco 1968). **A**, **B**, **E** – right side views, arrows mark the prolonged cilia; **C**, **D** – ventral views; **F** – posterior portion, to note SK1 and SKn; **G** – showing the variation of macronuclear nodules; **H**, **I** – ventral and dorsal view, to show the general infraciliature; **J**, **L** – buccal apparatus, arrows mark the fragmented PM; **K** – silverline system. AS – anterior suture; Cs – cytostome; M1, M2a, M2b and M3 – membranelles 1, 2a, 2b and 3; PM – paroral membrane; PS – postoral suture; SK1, n – somatic kinety 1 and n. Scale bars: 40 μm (H); 50 μm (A, E).

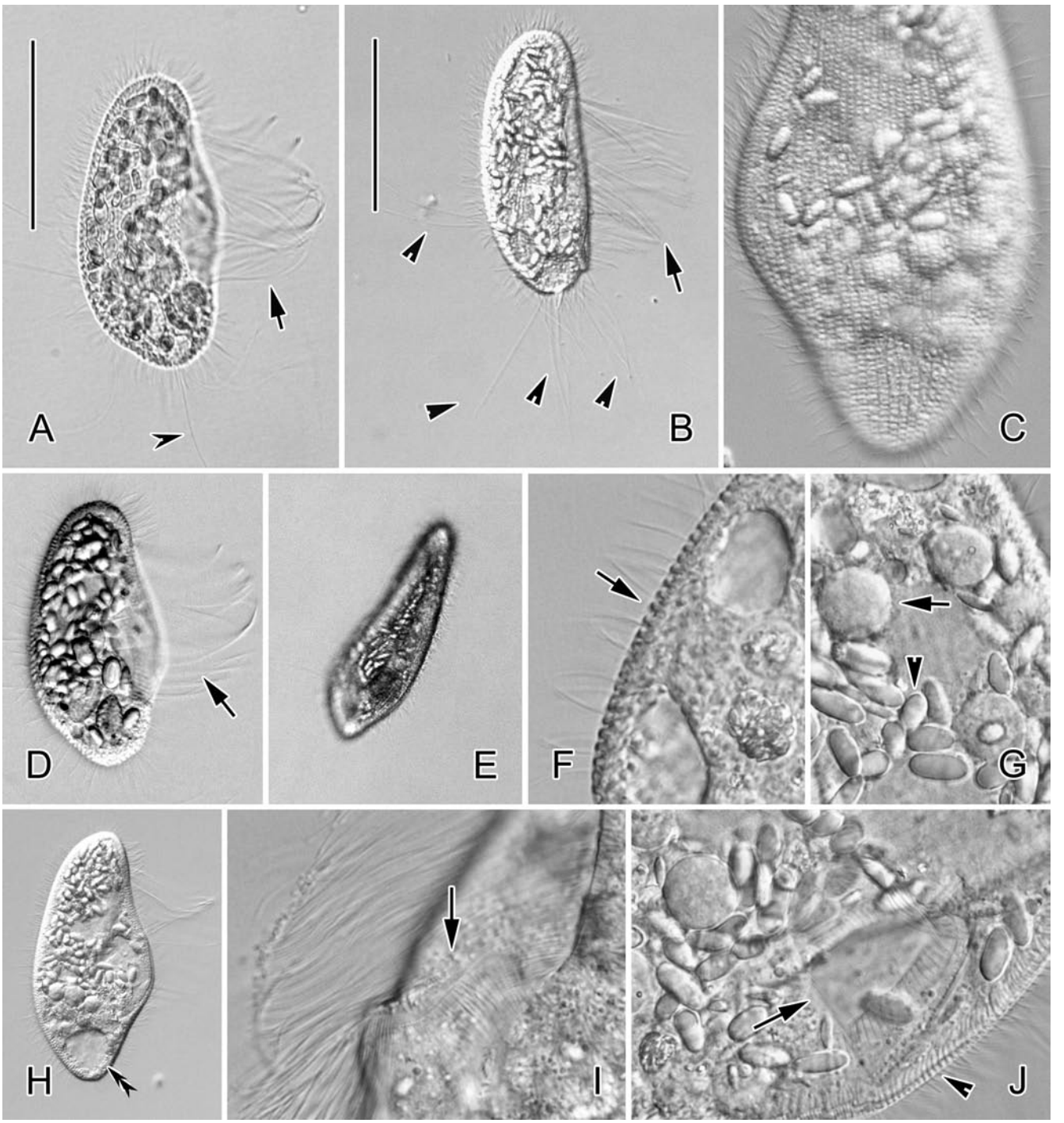

Fig. 2. Photomicrographs of *Schizocalyptra aeschtae* nov. spec. from life (**A**–**J**). **A**, **B**, **D**, **H** – general views, showing the conspicuous membranelles (arrows), the prolonged cilia (arrowheads) and the contractile vacuole (double-arrowheads); **C** – left side view, to show densely arranged extrusomes; **E** – ventral view; **F** – arrow showing the crenellated pellicle; **G** – showing the spherical (arrow) and ellipsoidal (arrowhead) food vacuoles; **I** – arrow indicates the membranelle 2b; **J** – cytostome, arrow indicates the buccal opening and arrowhead marks extrusomes. Scale bars: 50 μm.

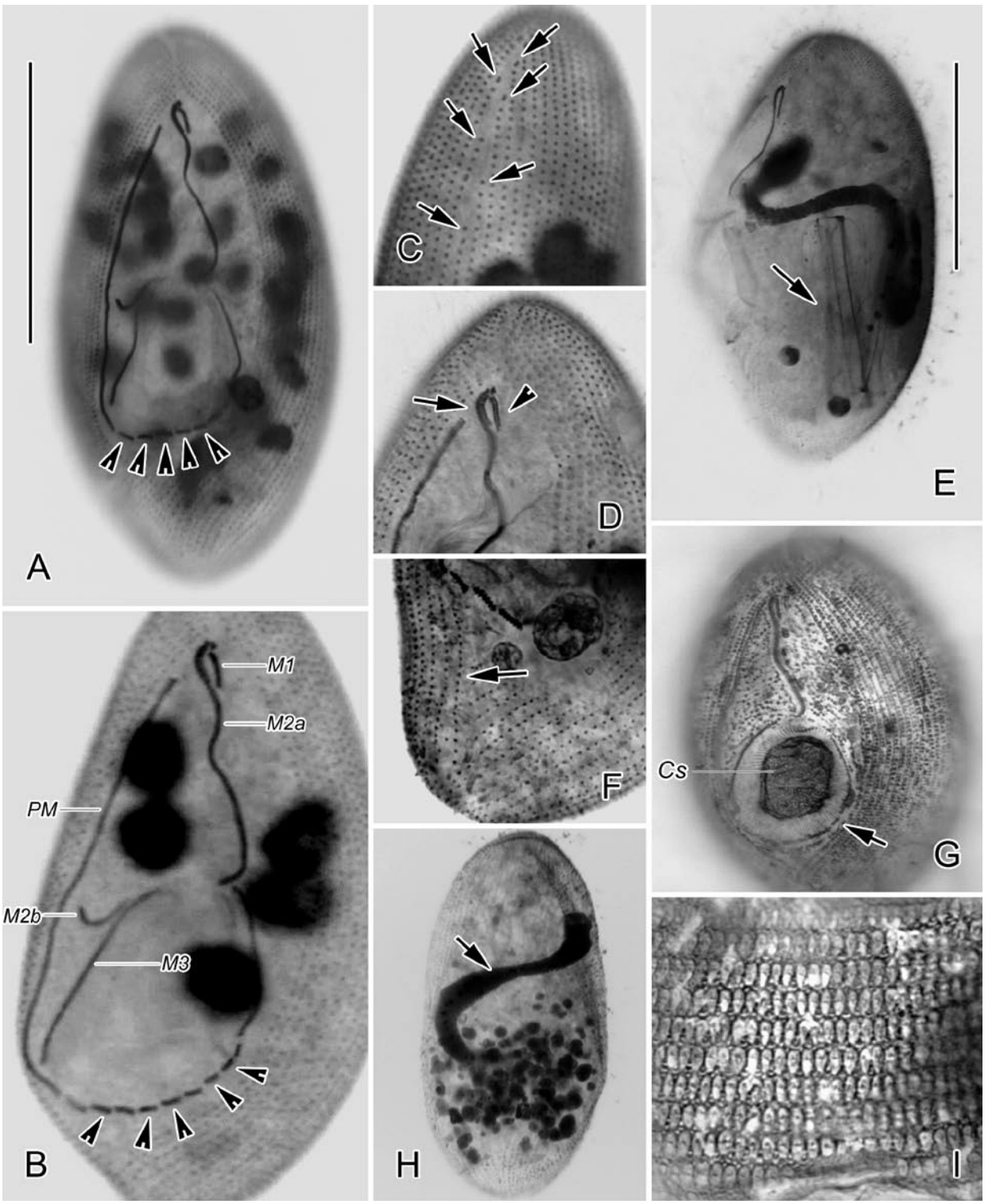

Fig. 3. Photomicrographs of *Schizocalyptra aeschtae* nov. spec. after protargol (**A–F**, **H**) and silver nitrate impregnations (**G**, **I**). **A**, **B** – ventral views, arrowheads mark the fragments in the PM; **C** – anterior portion of dorsal side, arrows indicate the anterior suture; **D** – anterior ventral view, to note that the anterior 1/7 of M2a (arrow) is 3-rowed and M1 (arrowhead) is 2-rowed; **E** – showing the ingested diatom (arrow); **F** – posterior portion, arrow depicts SK1; **G** – ventral view of an individual, arrow indicates one of the fragments in the PM; **H** – macronuclear nodules, arrow marks the ribbon-like macronuclear nodule; **I** – silverline system. Cs – cytostome; M1, 2a, 2b, 3 – membranelle 1, 2a, 2b, 3; PM – paroral membrane. Scale bars: 60 μm.

Table 1. Morphometric characterization of *Schizocalyptra aeschtae* nov. spec. Data based on protargol impregnated specimens. Abbreviations: CV – co-efficiency of variation in %, Max – maximum, Mean – arithmetic mean, Min – minimum, n – number of individuals examined, SD – standard deviation.

Characters	Min	Max	Mean	SD	CV	n
Body length in µm	76	188	130.0	33.35	25.70	25
Body width in µm	32	72	59.0	11.28	19.00	26
Length of buccal area in µm	60	104	78.0	12.95	16.63	23
Width of buccal area in µm	20	36	28.0	5.58	20.16	16
Number of somatic kineties	43	70	58.0	6.99	42.27	23
Number of fragments in paroral membrane	6	12	8.0	1.28	42.63	22
Number of macronuclear nodules	2	61	15.4	13.25	88.33	30

casionally elongate or band-like with many to "numerous" small nodules (Figs 1G; 3H).

Somatic cilia about 10 µm long *in vivo*. About 15 prolonged cilia *ca* 30–50 µm long, distributed over posterior 1/3 to 2/3 of cell (Figs 1A, E, arrows; 2A, B, arrowheads). Cilia in paroral membrane conspicuously long, about 40 µm in length (Figs 2A, B, D, arrows).

In Petri dishes, cells continuously swim in water for long periods, occasionally resting on the bottom with ventral side downwards; swims moderately slowly while rotating around the long axis of the cell, swimming in an asymmetric trace. This species was kept in the water from the type locality at room temperature (*ca* 20°C) for up to a week, but cultures could not be successfully established.

Infraciliature as shown in Figs 1H, I, both anterior suture (AS) and postoral suture (PS) conspicuous (Figs 1F, H, I), and extend to the dorsal side (Figs 1I; 3C, arrows). Approximately 60 somatic kineties (SK) with monokinetids. SK1, parallel to buccal field and extends to about 90% of body length (Figs 1F, H; 3F, arrow). SKn reaches *ca* 85% of body length (Figs 1F, H).

Buccal apparatus typical of the genus, about 2/3 of body length (Figs 1A, E, H, J; 2J; 3A, B, G). Membranelle 1 (M1) short and inconspicuous, composed of 2 rows of basal bodies (Figs 1J; 3D, arrowhead). Anterior part of membranelle 2 (M2a) generally posteriorly straight, anterior end distinctly 3-rowed (Figs 1J; 2I; 3A, B, D); posterior part of M2 (M2b) slightly curved and clearly separated from M2a, locating right to anterior third of membranelle 3 (Figs 1J; 2I; 3A, B); M3 composed of 2 rows of basal bodies, about the same length as M2a (Fig. 1J); paroral membrane (PM) prominent, about 2/3 of the body length, with 6–12 fragments at posterior portion (Figs 1J, arrows; 3A, B, arrowheads; arrow in Fig. 3G). Reticulate silverline system (Figs 1K; 3I).

Comparison: To date, only two species of *Schizocalyptra* have been reported: *S. magna* Dragesco, 1968 and *S. marina* Fernandez-Leborans and Novillo, 1994 (Dragesco 1968, Fernandez-Leborans and Novillo 1994).

With reference to the body shape and general structure of the oral apparatus, *Schizocalyptra aeschtae* closely resembles *S. magna* (Figs 1A–E, J, L) (Dragesco 1968). However, the former differs in having fewer somatic kineties (43–70 *vs.* 64–110), smaller body size (80–160 µm *vs.* 150–300 µm long), more macronuclear nodules (2–61 *vs.* 1) and a lower ratio of length of M2a: M3 (1:1 *vs.* about 3:1). In addition, the prolonged cilia of *S. magna* are distributed over the entire cell, whereas they are found only in the posterior half of the cell in *S. aeschtae* (Figs 1A–E, J, L; Table 2).

Schizocalyptra marina was reported by Fernandez-Leborans and Novillo (1994) based on an examination of specimens impregnated by the silver carbonate method. It is characterized by: (1) large size (220–279 µm long); (2) 3–4 macronuclear nodules; (3) on average 66 somatic kineties; (4) ratio of length of M2a to M3 about 3:1. Unfortunately, information on diagnostic features of the living cell are lacking, e.g., the position of contractile vacuole(s) and the presence/absence

Table 2. Comparison of *Schizocalyptra aeschtae* nov. spec. with related congeners.

Characters	*S. aeschtae*	*S. magna*	*S. marina*
Body length *in vivo* (μm)	75–200	150–300	220–279
Distribution of prolonged cilia	posterior 1/3 to 2/3	all over the cell	–
Number and shape of macronuclear nodules	2 to 61, band-shaped or spherical	1, bowknot-shaped	3–4 elongate ovoid (?)
Number of somatic kineties	43–70 (mean 58)	64–110 (mean 82)	65–68 (mean 66)
Number of fragments in PM	6–12	11	–
Ratio of M2a:M3 length	1:1	3:1	3:1
Data source	present work	Dragesco 1968	Fernandez-Leborans and Novillo 1994

– Data not available.

of the prolonged cilia in the caudal area. In addition, the infraciliature is insufficiently known. Thus, further studies on this organism are needed. Compared with our new species, however, *S. marina* shows significant differences both in the ratio of M2a:M3 (3:1 *vs.* 1:1 in *S. aeschtae*) and in the body size *in vivo* (220–279 μm *vs.* 150–200 μm long in *S. aeschtae*) (Table 2) (Fernandez-Leborans and Novillo 1994).

A key to the identification of known species of *Schizocalyptra* is here supplied:

1 Single macronuclear nodule ..... *S. magna*
1 Two or more macronuclear nodules 2
2 Ratio of length of M2a: M3 about 1:1, body < 200 μm long *in vivo* ..... *S. aeschtae*
2 Ratio of length of M2a: M3 about 3:1; body > 220 μm long *in vivo* ..... *S. marina*

Order Loxocephalida Jankowski, 1980
Family Cinetochilidae Perty, 1852
Genus *Sathrophilus* Corliss, 1960

Syn. *Saprophilus* Stokes, 1887
***Paradexiotricha* Grolière, 1975**

Hitherto, the genus definition of *Sathrophilus* has never included features revealed by silver impregnation. We therefore supply an improved definition with data presented here.

Improved diagnosis for *Sathrophilus*: Dorsoventrally flattened, elongated Cinetochilidae with *Tetrahymena*-like buccal apparatus and bipartite M1; paroral membrane terminating anteriorly to about level of M1/M2; buccal field above the equatorial level; postoral kineties and scutica present; having a distinctly long caudal bristle and one conspicuous, cilia-free apical plate.

Remarks and comparison: The genus *Sathrophilus* Corliss, 1960 replaced the invalid taxon *Saprophilus* Stokes, 1887, because the latter name was preoccupied (Corliss 1960).

Grolière (1975) established the genus *Paradexiotricha* and distinguished it from *Sathrophilus* mainly by its crochet-shaped M1 and M2 and slenderer body shape (length to width about 3:1). Furthermore, the caudal bristle is not recognizable in one species, i.e., *Paradexiotricha puytoraci* Grolière, 1975. However, the general infraciliature, especially the pattern of buccal apparatus, is the same as that in *Sathrophilus*, these characters should be used at the species-level rather than genus-level in our opinion. Thus, *Paradexiotricha* is regarded as a junior synonym of *Sathrophilus* and a new combination is suggested: *Sathrophilus puytoraci* (Grolière 1975) nov. comb.

The monotypic genus *Sphenostomella* Jankowski, 1980 has only one known species, *Sphenostomella hovassei* (Grolière 1975) Jankowski, 1980. It differs from *Sathrophilus* in its long and developed M3 and the frontally positioned buccal cavity, the anterior end of which is beneath the conspicuous cilia-free apical plate (Grolière 1975, Small and Lynn 1985, Lynn and Small 2002).

Sathrophilus can be separated from the closely-related genus *Cinetochilum* in the slenderer body shape, less-sculptured cell surface, presence of single caudal

cilium (*vs.* several in the latter), postoral kinety(ies), conspicuous apical cilia-free area, anteriorly positioned buccal field as well as the conspicuous scutica (Foissner *et al*. 1994).

This genus differs from similar genera, such as those of the *Dexiotricha-Dexiotrichides*-complex, in the combination of the following aspects: (1) body dorsoventrally flattened (*vs.* circular in cross section in other genera) and (2) M1 longest of the three membranelles (*vs.* M1 shorter than M2 in other genera) (Lynn and Small 2002, Song *et al*. 2003).

***Sathrophilus holtae* nov. spec. (Figs 4–7; Table 3)**

Diagnosis: Marine *Sathrophilus*, 35–70 × 20–40 µm *in vivo*, elliptical in outline, dorsoventrally flattened about 5:3; 21 somatic kineties on average; M1 three-rowed and bipartite with first row conspicuously long and separated from the other two; M2 two-rowed; ratio of lengths of M2 to M3 about 3:1; paroral membrane extending to above M2 level; contractile vacuole pore located caudally at the end of SK1.

Dedication: We dedicate this species to Dr. Portia A. Holt, managing editor of the Journal of Eukaryotic Microbiology, for her contributions to protozoology.

Type Location: Sandy beach on the Bohai Sea coast near Tianjin (39°10′N; 117°10′E), China.

Deposition of Slides: One holotype slide with protargol impregnated specimens is deposited in the Natural History Museum, London, UK with registration number 2007:5:12:1. One paratype slide with silver nitrate impregnated specimens (slide number: 2006051201-2) is deposited in the Laboratory of Protozoology, OUC, Qingdao, China.

Description: Size *in vivo* usually about 50 × 25 µm. Body conspicuously rigid, shape rather constant, dorsoventrally flattened *ca* 5:3 (Figs 4A; 5A–B, I, L–M). When viewed from ventral side, body elliptical in outline, length to width about 2–2.5:1 (Figs 5A–D, I–K); buccal area about 1/3 even 2/5 of cell length, located slightly right of cell median (Figs 4A; 5D–G, J, arrows; 7F). Pellicle slightly crenellated (Figs 4A; 5A, B, L, M). Extrusomes bar-shaped, about 4 µm long, positioned between ciliary rows (arrowheads in Figs 4B–C; 5C; 6A–B, D, E–F; 7H–I). Cytoplasm transparent, often containing many food vacuoles (Figs 4A; 5A–B; 6H). Macronucleus oval, located near body center, accompanied by a small micronucleus (Figs 6B–C, H, arrow; 7E, arrow). Single contractile vacuole *ca* 5 µm in diameter, sub-caudally positioned on ventral side (Figs 4A; 5B, K–M, arrowheads).

Cilia about 7 µm long, evenly distributing but often undetectable in middle portion of cell *in vivo* (Figs 4A; 5A–B, I; 7B, arrowheads). Single caudal bristle *ca* 20–30 µm in length (Figs 4A; 5H, M, arrows). Cilia of buccal apparatus about 5 µm long (Fig. 4A).

Movement mainly by rotating along the long axis of the body when swimming or gliding along substrate, to which it sometimes attaches and remains motionless for a short period.

Infraciliature as shown in Figs 4B–C, F–G, I; 7H–I: 18–23 somatic kineties, mostly consisting of monokinetids, extending over the whole cell length, each kinety starting anteriorly with a pair of basal bodies in both populations examined (Figs 4B–C, F–G, I; small arrows in Figs 7H–I). Number of basal bodies in SK differs greatly in the two populations (e.g., up to 14 in Tianjin population *vs.* up to 27 in Qingdao population). Anterior ends of ventral somatic kineties forming a conspicuous preoral cilia-free area (apical plate or "suture", PS) (Figs 4F; 7B, G). Invariably having one postoral kinety (PK, Fig. 4F), to its left, often 2 groups (rows) of basal bodies (scutica) near the cytostome (Fig. 4B, I; double-arrowheads in Fig. 4F). Somatic kinety 1 (SK1) distinctly bipartite, allowing space(s) for curvature of PM (Figs 4B, F, I; 6L, arrows). Kinetosomes in anterior part of SK1 densely arranged with one basal body pair at its anterior end (Figs 4B, arrow, F, I, arrowheads; 6C, K, arrows; arrowhead in Fig. 7A). SKn terminates anteriorly at about level of M2 with 4–6 dikinetids densely arranged in anterior portion (Fig. 4F, arrow; double-arrowheads in Figs 6G, K; 7A, C, H, double-arrowheads).

Arrangement of buccal apparatus unique: M1 transversely oriented, composed of three rows of about 15 basal bodies each, the anteriormost row, of which is conspicuously longer, curved and separated from the other two and intersects with SKn-1 (Figs 4B, F, H–I; 6A, C, K; 7A, G–H; arrow in Fig. 7C); M2 parallel to M1, two-rowed, each with about 12 basal bodies, the rightmost of which turns posteriad in some specimens (Figs 4F, H; 6A, C; 7C, G–H); M3 much shorter and smaller, two or three-rowed, composed of about 10 basal bodies in total (Figs 4B, F, H–I; 6C; 7C, G–H). PM slightly curved on right margin of buccal cavity, basal bodies arranged in zig-zag pattern, anteriorly terminating between M1 and M2 (Figs 4B, F, H–I; 6A, C; 7A–C, G). Scutica usually composed of 10 basal body pairs, arranged usually in two to several irregular rows (Figs 4B, E–F, I; arrows in Figs 7A, H).

Silverline system connects basal bodies as shown in Figs 4D; 7D, arrows. Contractile vacuole pore (CVP)

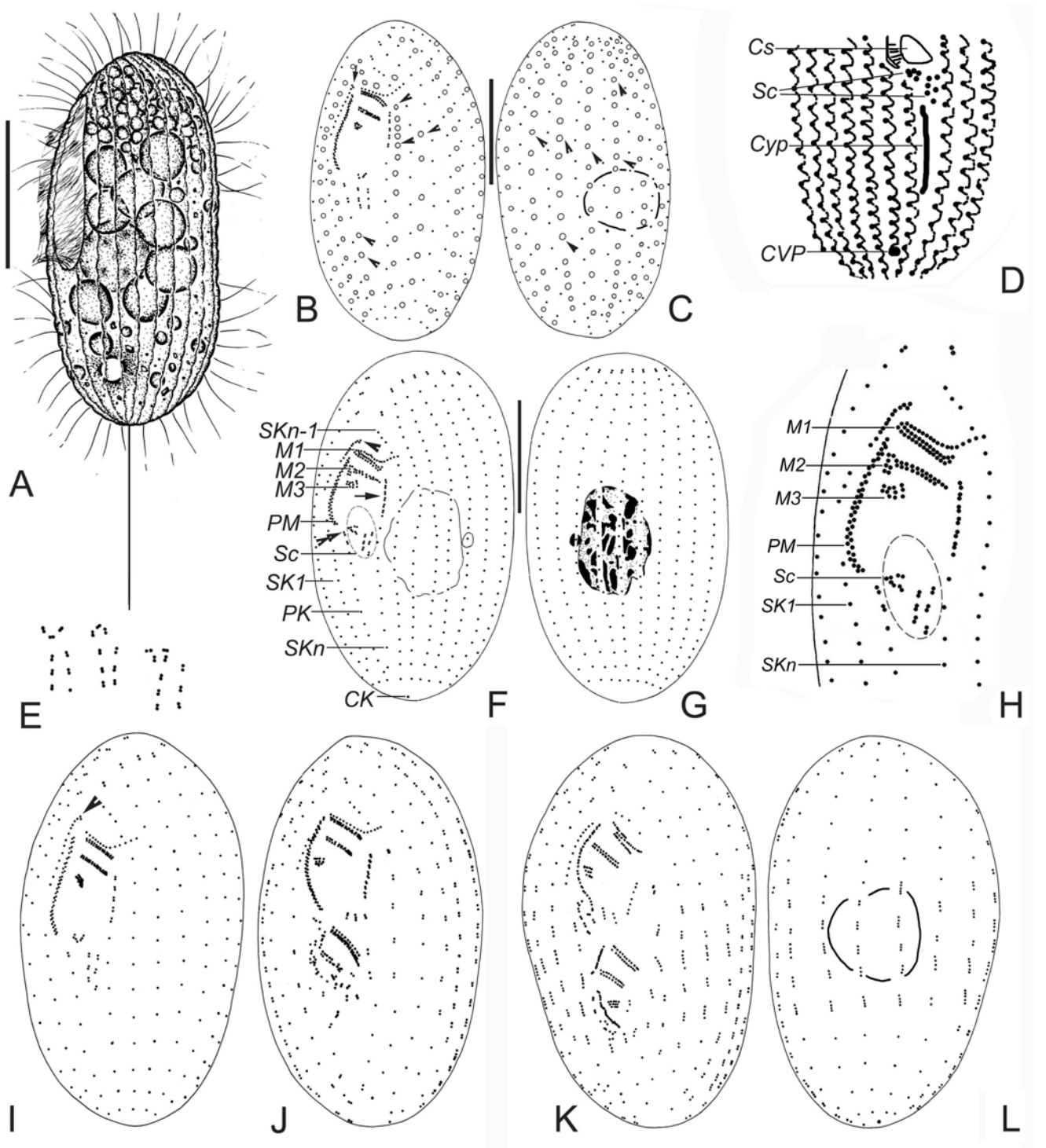

Fig. 4. *Sathrophilus holtae* nov. spec. from life (**A**), after protargol (**B**, **C**, **E–L**) and silver nitrate impregnations (**D**). **A** – left-ventral view, note that cilia are inconspicuous in the mid-body; **B**, **C** – ventral and dorsal views, note the extrusomes between kineties (arrowheads) and the basal body pair at the anterior end of SK1 (arrow); **D** – posterior portion of ventral side; **E** – different arrangements of basal bodies in scutica (from 3 individuals); **F**, **G**, **I** – the infraciliature, note the anterior parts of SK1 (arrowheads in F and I), SKn (arrow in F), PK (double-arrowheads); **H** – buccal apparatus; **J** – ventral side of an individual in middle stage of morphogenesis; **K**, **L** – ventral and dorsal sides of an individual in mid-to-late stage of morphogenesis. CK – caudal kinetosome; Cs – cytostome; CVP – contractile vacuole pore; Cyp – cytoproct; M1–3 – membranelles; PK – postoral kinety; PM – paroral membrane; Sc – scutica; SK1, n–1 and n – somatic kineties 1, n–1 and n. Scale bars: 15 μm (B, F); 20 μm (A).

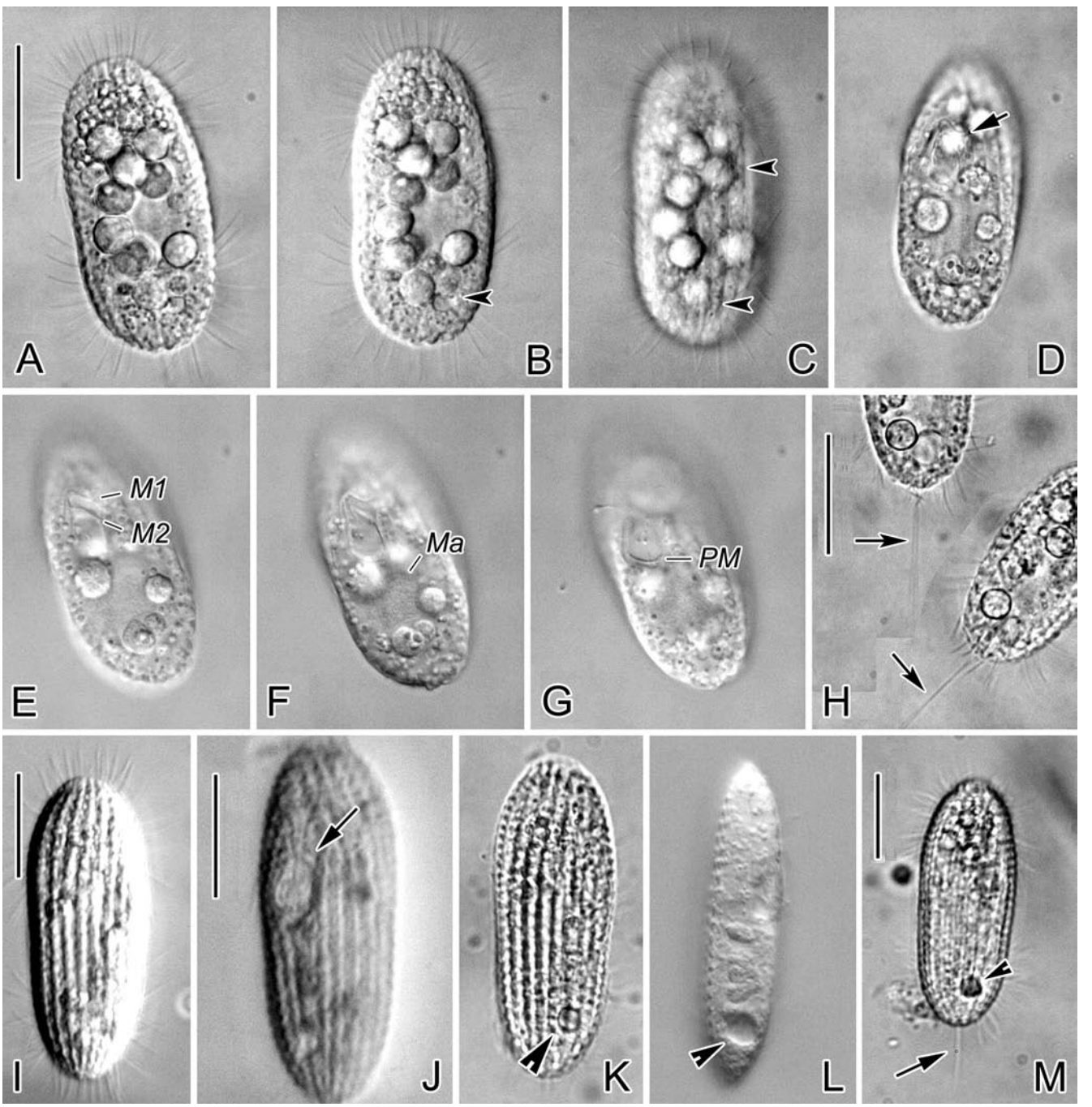

Fig. 5. Photomicrographs of the Tianjin population (**A–H**) and Qingdao population (**I–M**) of *Sathrophilus holtae* nov. spec. from life. **A**, **B**, **I**, **M** – typical cells, note that cilia are inconspicuous in the middle portion of cells, arrowheads mark the contractile vacuole, arrow indicates the caudal bristle; **C** – to show extrusomes (arrowheads); **D**, **J** – to indicate the buccal cavity (arrows); **E**, **F**, **G** – focusing on buccal apparatus; **H** – arrows mark the caudal bristle; **K**, **L** – right (K) and ventral views, arrowheads indicate the contractile vacuole. M1–2 – membranelles 1 and 2; Ma – macronucleus; PM – paroral membrane. Scale bars: 20 μm.

located at posterior end of SK1 and cytopyge (Cyp) slit-like, positioned posterior to scutica (Figs 4D; 7D).

Morphogenesis: Two individuals in mid-late divisional stages were observed and showed three morphogenetic features: (1) stomatogenesis is of, basically, the parakinetal mode (hence similar to that of hymenostomatids) rather than the buccokinetal mode (typical of scuticociliates), i.e., the oral apparatus of the opisthe

Table 3. Morphometric characterization of *Sathrophilus holtae* nov. spec. Data based on protargol impregnated specimens (Tianjin population, upper line; Qingdao population, lower line).

Characters	Min	Max	Mean	SD	CV	n
Body length in µm	38	60	48.3	5.54	11.50	51
	56	66	61.6	2.64	4.29	20
Body width in µm	18	35	26.7	3.90	14.60	51
	26	38	27.9	2.94	10.54	20
Length of buccal cavity in µm	11	17	14.0	1.61	11.50	20
	14	18	14.9	1.37	9.19	20
Number of somatic kineties	18	23	21.6	1.03	4.80	50
	19	20	19.5	0.51	2.62	17
Macronucleus, length in µm	7	20	12.2	2.61	21.40	44
	14	20	15.4	1.87	12.14	19
Macronucleus, width in µm	5	13	10.6	1.91	18.00	44
	6	14	10.1	1.94	19.21	19
Number of basal bodies in the scutica	18	20	19.3	1.00	5.17	9
	20	22	20.4	0.80	3.93	21
Number of basal bodies in the anterior part of somatic kinety 1	8	13	10.7	1.19	11.10	36
	11	13	13.0	0.44	3.41	20
Number of dikinetids in the anterior end of somatic kinety 1	1	1	1	0	0	51
	1	1	1	0	0	20
Number of dikinetids in the anterior part of somatic kinety n	4	6	5.0	0.25	5.00	32
	6	6	6	0	0	20

is entirely formed in the posterior area near the SK1, perhaps deriving from the scutica (Fig. 4J); (2) the parental PM is dedifferentiated to form the new scutica (possibly also the postoral kinety?) for the proter; (3) the formation of the densely ciliated portion of the somatic kineties in the equatorial area is initiated at the late morphogenetic stages (Figs 4K–L).

These features are similar to those of *Dexiotrichides pangi* Song *et al*., 2003, and hence an intermediate pattern between that of scuticociliates and *Tetrahymena* (Song *et al*. 2005).

Comparison with related congeners: Among the 14 nominal species of *Sathrophilus*, two (i.e. *S. marinum* and *S. arenicolus*) should be compared with our new species considering their general morphology, infraciliature and habitat (Grolière and Detcheva 1979, Dragesco 2002; Table 4; Fig. 8D, F).

Morphologically, *Sathrophilus holtae* is similar to *S. marinum* Grolière and Detcheva, 1979 in terms of its body shape and size and the number of somatic kineties (Grolière and Detcheva 1979; Fig. 8F; Table 4). However, these two taxa can be clearly separated by: (1) the structure of M1 (3 rows in bipartite pattern *vs.* 3 rows packed together and of equal length in *S. marinum*, see Fig. 8F); (2) the number of postoral kineties (1 *vs.* 2 in *S. marinum*); (3) ratio of length of buccal area to body length (*ca* 1:4 *vs.* 2:5); (4) the number and arrangement of basal body pairs in the scutica (many pairs in 2 ir-

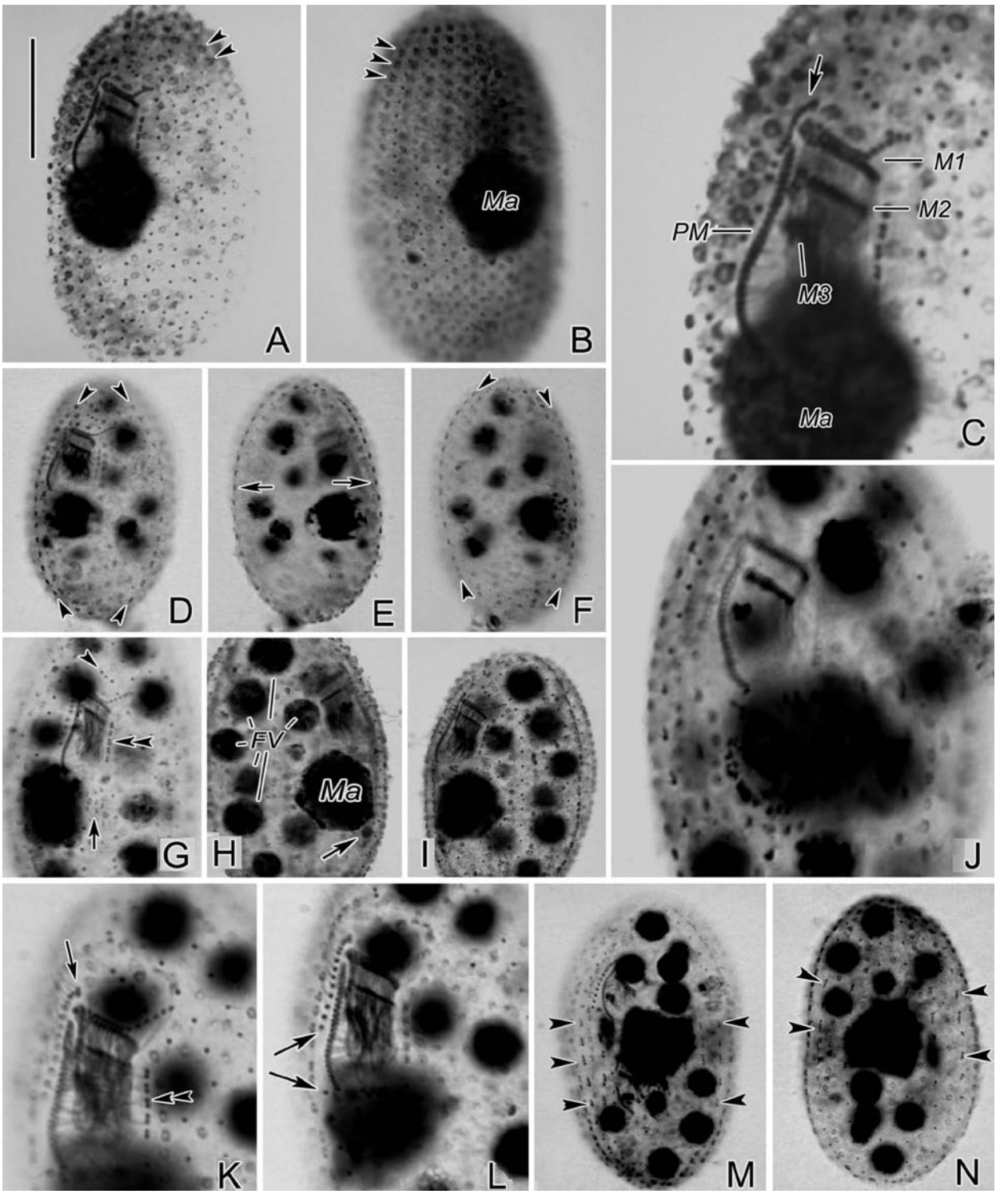

Fig. 6. Photomicrographs of *Sathrophilus holtae* nov. spec. (Tianjin population) after protargol impregnation (**A**–**N**). **A**, **B** – ventral and dorsal views, arrowheads mark the extrusomes between somatic kineties; **C** – oral portion of cell, arrow indicates the anterior part of SK1; **D**, **E**, **F** – arrowheads and arrows indicate the extrusomes; **G**, **I**, **K**, **L** – ventral views of the anterior part of cells, arrowhead depicts the anterior end of SKn-1, double-arrowheads mark the anterior end of SKn, arrow in G marks the scutica, arrow in K indicates the anterior end of SK1, arrows in L depict the bipartite SK1; **H** – food vacuoles and macronucleus, arrow indicates the micronucleus; **J**, **M**, **N** – cells in morphogenesis, note the duplicating kinetosomes in somatic kineties (arrowheads). FV – food vacuoles; M1–3 – membranelles 1–3; Ma – macronucleus; PM – paroral membrane. Scale bar: 15 μm.

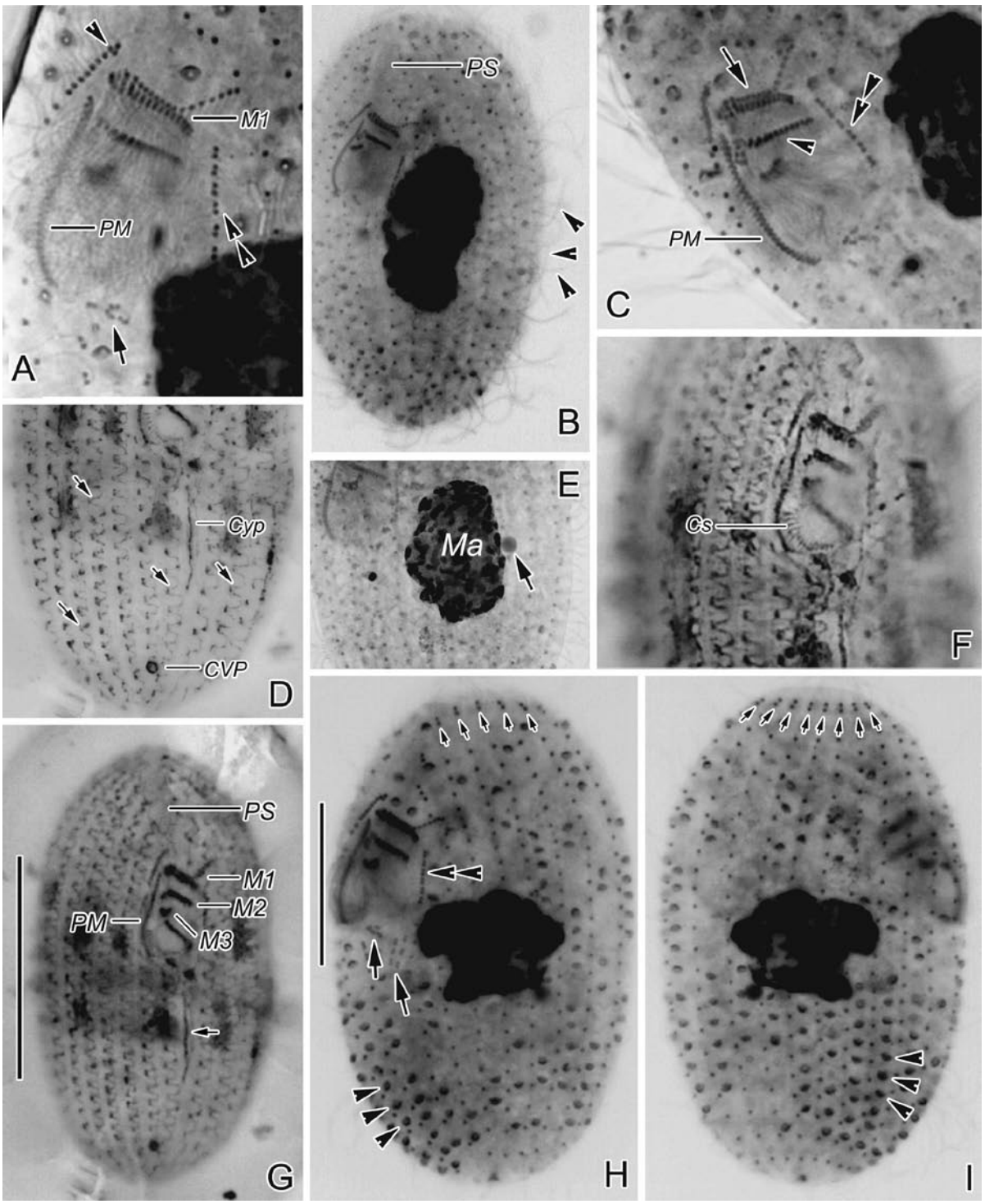

Fig. 7. Photomicrographs of *Sathrophilus holtae* nov. spec. (Qingdao population) after protargol (**A–C**, **E**, **H**, **I**) and silver nitrate impregnations (**D**, **F**, **G**). **A** – to show the oral apparatus, note the anterior parts of SK1 (arrowhead), SKn (double-arrowheads) and the scutica (arrow); **B** – to indicate cilia in the middle part of the cell (arrowheads); **C** – oral apparatus, note M1 (arrow), M2 (arrowhead) and the anterior part of SKn (double-arrowheads); **D** – posterior ventral portion of a cell, arrows mark the silverline system; **E** – to show Ma and Mi (arrow); **F** – anterior ventral portion of a cell; **G** – ventral view of a cell, arrow marks the cytopyge (Cyp); **H**, **I** – to show the infraciliature, note the extrusomes (arrowheads), the basal body pairs at the end of each somatic kinety (small arrows), the scutica (large arrows) and the anterior portion of SKn (double-arrowheads). Cs – cytostome; CVP – contractile vacuole pore; Cyp – cytopyge; M1–3 – membranelles 1–3; Ma – macronucleus; Mi – micronucleus; PM – paroral membrane; PS – preoral suture. Scale bars: 20 μm (H), 30 μm (G).

regular rows *vs.* 4 pairs in one transversely positioned row in *S. marinum*) (Grolière and Detcheva 1979; Figs 4F, H; 8F; Table 4).

Compared with *Sathrophilus arenicolus* Dragesco, 2002, our new species can be recognized by clearly different structure of M1, having fewer somatic kineties (18–23 *vs.* 24–29), and in having more basal bodies in the scutica (*ca* 20 *vs. ca* 6) that are arranged in conspicuously different patterns (compare Figs 4E–F, 8A, D; Table 4) (Dragesco 2002).

Three species, viz. *Sathrophilus hovassei*, *S. vernalis* and *S. muscorum*, are, more or less, also similar to *S. holtae* and have been described using silver staining methods (Dragesco and Grolière 1969; Grolière 1973, 1975; Foissner *et al*. 1982; Fig. 8C, E, G–J; Table 4). Compared with the former two species, *S. holtae* can be distinguished by having conspicuously fewer somatic kineties (18–23 *vs.* 30–31 in *S. hovassei*, 31–34 in *S. vernalis*), a much larger ratio of M2 to M3 length (around 3 *vs.* < 1) and a marine (*vs.* freshwater) habitat. Similarly, *S. holtae* can readily be separated from *S. muscorum* which is a terrestrial (*vs.* marine) form possessing 14–16 somatic kineties (*vs.* 18–23 in *S. holtae*) and 12 basal bodies in the scutica (*vs.* about 20 in *S. holtae*) (Foissner *et al*. 1982; Fig. 8C, E; Table 4).

The infraciliatures of *Sathrophilus agitatus* Stokes, 1887, *S. chlorophagus* Kahl, 1931, *S. elongatus* Vuxanovici, 1962, *S. granulatus* Czapik, 1968, *S. mobilis* Kahl, 1926, *S. ovatus* Kahl, 1926, *S. oviformis* Kahl, 1926 and *S. putrinus* Kahl, 1926 have yet to be described (Stokes 1887; Kahl 1926, 1931; Vuxanovici 1962; Figs 8B, K–Q; Table 4). Nevertheless, *S. holtae* can be distinguished from these taxa with the combination of the following living features: (1) ratio of buccal field: body length; (2) body shape and size, and (3) marine habitat (*vs.* terrestrial or freshwater biotopes).

Systematic position of the genus *Sathrophilus*: Because of the absence of both morphogenetic and molecular data, the systematic position of *Sathrophilus* has never been seriously questioned since Corliss (1979) placed it into the order Scuticociliatida. Based on its general infraciliature, *Sathrophilus* could be an intermediate form between typical scuticociliates and *Tetrahymena*-like hymenostomatids, although according to the characters of its buccal apparatus it is more like a hymenostomatid.

Without further description, Jankowski (1980) established a new order Loxocephalida under the subclass Scuticostomata to conclude the taxa which are traditionally considered as scuticociliates assigned in the families Loxocephalidae, Cinetochilidae and Urozonidae. This new order is arranged to be parallel to other typical scuticociliates, e.g., philasterids.

Based on the 18S rRNA gene sequence data of *Cardiostomatella vermiforme* and *Dexiotrichides pangi*,

Table 4. Comparison of *Sathrophilus holtae* nov. spec. with related congeners.

Characters	*S. holtae*	*S. arenicolus*	*S. marinum*	*S. muscorum*	*S. hovassei*	*S. vernalis*
Body shape	cylindrical	cylindrical	cylindrical	oval	cylindrical	cylindrical
Body length *in vivo* (μm)	35–70	50–70	40–50	25–40	60	71–122
Number of somatic kineties	18–23	24–29	20–22	14–16	30–31	31–34
Ratio of buccal area: body length	1/4–1/3	1/4	*ca* 2/5 *	1/3	1/4–1/3	*ca* 1/4 *
Number of postoral kineties	1	–	2	1	2–3*	2–3
Number of basal bodies in scutica	20	5	8	12	*ca* 12 *	12–18
Position of contractile vacuole	sub-terminal	terminal	–	sub-terminal	sub-terminal	–
Habitat	marine	marine	marine	freshwater	freshwater	freshwater
Data source	present work	Dragesco 2002	Grolière and Detcheva 1979	Foissner *et al*. 1982	Foissner *et al*. 1982	Grolière 1973

* Data from line drawings.

– Data not available.

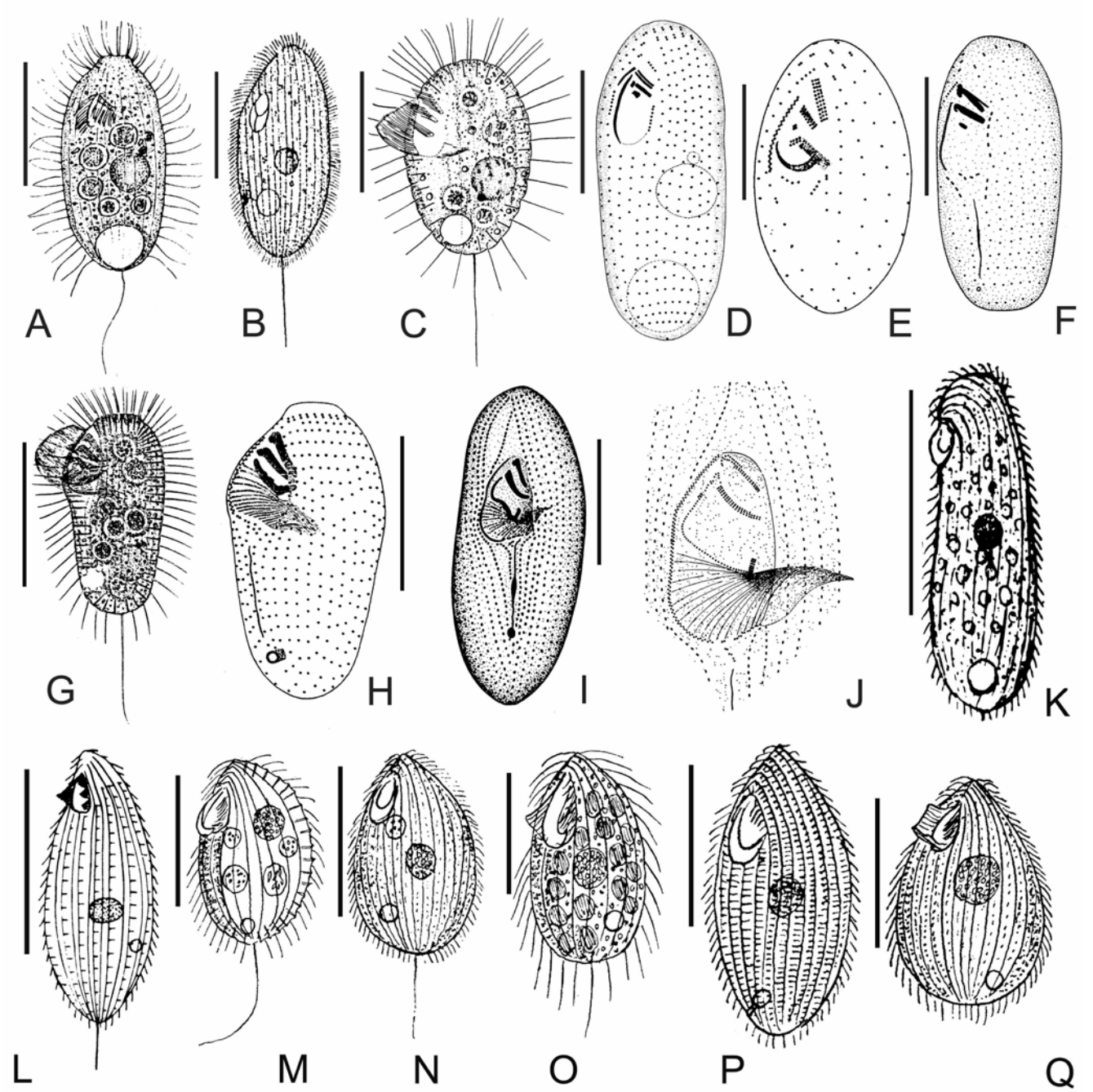

Fig. 8. Ventral side views of some *Sathrophilus* species from life (**A–C**, **G**, **K–Q**) and after protargol impregnation (**D–F**, **H–J**). **A**, **D** – *S. arenicolus* Dragesco, 2002; **B** – *S. agitatus* Stokes, 1887; **C**, **E** – *S. muscorum* (Kahl, 1931) Corliss, 1960; **F** – *S. marinum* Grolière and Detcheva, 1979; **G**, **H** – *S. hovassei* Grolière, 1975; **I**, **J** – *S. vernalis* Dragesco and Grolière, 1969, ventral side view (I) and buccal apparatus (J); **K** – *S. elongatus* Vuxanovici, 1962; **L** – *S. granulatus* Czapik, 1968; **M** – *S. putrinus* Kahl, 1926; **N** – *S. ovatus* Kahl, 1926; **O** – *S. chlorophagus* Kahl, 1931; **P** – *S. mobilis* Kahl, 1926; **Q** – *S. oviformis* Kahl, 1926. A, D, I after Dragesco (2002), B after Stokes (1887), F after Grolière and Detcheva (1979), C, E, G, H after Foissner *et al*. (1982), J after Grolière (1973); K after Vuxanovici (1962), L after Czapik (1968), M, N, P, Q after Kahl (1926), O after Kahl (1931). Scale bars: 15 µm (E), 20 µm (B, C, F, P), 25 µm (D), 30 µm (A, H, M–O, Q), 40 µm (I, K, L), 45 µm (G).

Li *et al*. (2006) suggested that the *Dexiotricha-Dexiotrichides*-complex should represent an independent order Loxocephalida (they incorrectly re-established it as a new order, however) which is systematically positioned between hymenostomatids and scuticociliates, hence moved from the scuticociliates. According to this new arrangement, Loxocephalida contains all taxa with the pattern of *Tetrahymena*-similar buccal as well as somatic ciliature, including *Sathrophilus* and related genera (Li *et al*. 2006). This understanding is unquestionably supported by both the stomatogenetic and molecular studies on a loxocephalid, *Dexiotrichides pangi* Song *et al*., 2003 by Song *et al*. (2005).

With reference to the process of binary fission revealed in the present work, *Sathrophilus* is generally similar to *Dexiotrichides pangi*, hence, an intermediate mode between hymenostomatids and scuticociliates, i.e., the old buccal apparatus is dedifferentiated to form the PM anlagen which gives rise to the new PM and scutica (thus partly similar to that of scuticociliates) while the formation of the new oral primordium exhibits a parakinetal mode, which means that only the scutica contribute to the formation of the new oral primordium, hence slightly similar to that of tetrahymenids. This result confirms again that *Sathrophilus* should be transferred from scuticociliates and supports the independent position of loxocephalids.

Acknowledgements. This work was supported by the National Science Foundation of China (Projects No. 40676076, 30500057 and 30430090), a grant from the Center of Excellence in Biodiversity, King Saud University and the Darwin Initiative Programme (Project No. 14-015) which is funded by the UK Department for Environment, Food and Rural Affairs. We thank Dr. Dapeng Xu and Ms. Chen Shao for suggestions on an early version of this manuscript and Ms. Ping Zhu and Ms. Xiaowen Zhu for their help during sampling.

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Received on 10th May, 2007; revised version on 10th July, 2007; accepted on 10th July, 2007