

Hapantotypification and Morphological Redescription of the Marine Planktonic Ciliate, *Spirostrombidium cinctum* (Kahl, 1932) Petz, Song *et al.* Wilbert, 1995 (Ciliophora: Oligotrichida)

Dapeng XU and Weibo SONG

Laboratory of Protozoology, KLM, Ocean University of China, Qingdao, China

Summary. The living morphology and infraciliature of a poorly-known marine planktonic ciliate, *Spirostrombidium cinctum* (Kahl, 1932) Petz, Song *et al.* Wilbert, 1995, collected from coastal mollusc farming ponds near Qingdao, north China, are investigated based on observations of live and protargol impregnated specimens. Since modern studies on *S. cinctum* are unavailable, an improved diagnosis based on the Qingdao population is given: medium-sized marine *Spirostrombidium*, *in vivo* about $55 \times 40 \mu\text{m}$; dorsoventrally *ca* 2:3 flattened; cell asymmetric barrel-shaped with inconspicuous apical protrusion; on average 25 anterior and 14 ventral membranelles; 3-4 posteriorly-directed thigmotactic membranelles; one macronucleus and one micronucleus; extrusomes prominent, about 6-10 μm long, arranged along somatic kineties; extra, girdle and ventral kinety consisting of about 17, 62 and 23 dikinetids, respectively.

Key words: infraciliature, marine planktonic ciliate, Oligotrichida, *Spirostrombidium cinctum*.

INTRODUCTION

Oligotrich ciliates are ubiquitous in the ocean surface and usually dominate planktonic ciliate communities (Lynn and Montagnes 1988, Lynn *et al.* 1988). Their ecology, especially of the vegetative stage, has been frequently studied since they serve as an important link between smaller unicellular organisms and those of higher trophic levels in the marine microbial food webs

(*e.g.* Pierce and Turner 1992). Being small and fragile, many of these organisms have been only rather superficially investigated, which were usually based on living observations (Fauré-Fremiet 1924, Kahl 1932, Maeda and Carey 1985). Because of this, numerous ambiguities concerning the identification of taxa have accumulated in last century and hence, most nominal species need to be re-investigated using modern methods. This is especially true for the marine forms, which were relatively poorly considered as revealed in some recent studies (Lynn *et al.* 1988, Montagnes *et al.* 1990, Martin and Montagnes 1993, Petz *et al.* 1995, Song *et al.* 2000).

In the present paper, one oligotrich ciliate, *Spirostrombidium cinctum*, isolated from coastal waters off Qingdao, is redescribed from life and after

Address for correspondence: Weibo Song, Laboratory of Protozoology, KLM, Ocean University of China, Qingdao 266003, P. R. China; Fax [+86]-532-2032283; E-mail: wsong@ouc.edu.cn

protargol impregnation. The results are documented here.

MATERIALS AND METHODS

Ciliates were collected with 20 µm mesh plankton nets from some mollusc-culturing ponds in the Jiaozhou Bay near Qingdao, China (36°08'N; 120°43'E). The water temperature was ~15°C and the salinity ~33‰.

Specimens were observed *in vivo* with phase contrast and differential interference contrast microscopy. The infraciliature was revealed by protargol impregnation (Wilbert 1975). Counts, drawings (with help of a Camera Lucida) and measurements were performed at a magnification of ×1250. Terminology is mainly according to Agatha *et al.* (2005) and Corliss (1979).

RESULTS

Order Oligotrichida Bütschli, 1887

Family Strombidiidae Fauré-Fremiet, 1969

Genus *Spirostrombidium* Jankowski, 1978

Spirostrombidium cinctum (Kahl, 1932) Petz, Song *et* Wilbert, 1995 (Figs 1-3, Table 1)

To the authors' knowledge, this species has never been investigated using modern methods. Hence we supply an improved diagnosis based on an examination of the Qingdao population.

Improved diagnosis: Medium-sized marine *Spirostrombidium*, *in vivo* about 55 × 40 µm; dorsoventrally *ca* 2:3 flattened; cell asymmetric barrel-shaped with inconspicuous apical protrusion; on average 25 anterior and 14 ventral membranelles; 3-4 posteriorly-directed thigmotactic membranelles; one macronucleus and one micronucleus; extrusomes prominent, about 6-10 µm long, arranged along somatic kineties; extra, girdle and ventral kinety consisting of about 17, 62 and 23 dikinetids, respectively.

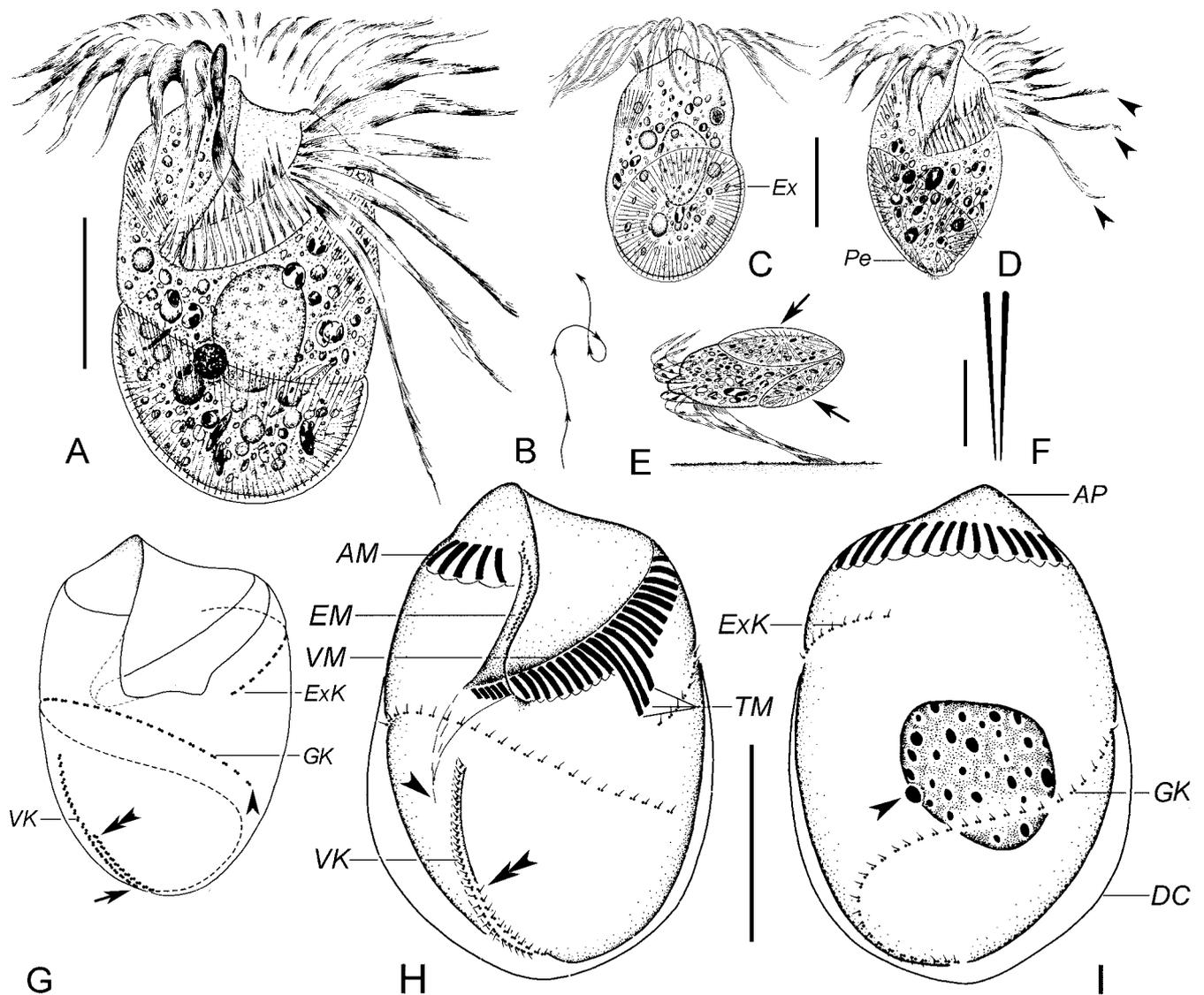
Deposition of slides: Since no silver impregnated specimens have previously been preserved, one neotype (registration number 2005:24:17) is deposited in the Natural History Museum, London and a second slide (registration number 03102901) is deposited in the Laboratory of Protozoology, Ocean University of China.

Description of the Qingdao population: Cells *in vivo* mostly about 45-65 × 30-45 µm. Body shape generally invariable, slightly asymmetric and barrel-shaped with posterior end widely rounded; when viewed from ventral side, broadest mostly at the equatorial area (Figs 1A, 2A). Dorsoventrally flattened and thickness:width about 2:3 (Figs 1D, 2D). Apical protrusion (collar) inconspicuous *in vivo*, which may disappear or be undetectable after fixation (Figs 1A; 2A, D, arrowhead). Buccal cavity relatively deep and prominent, extending obliquely towards right body side and terminating at about 2/5 of cell length (Figs 1A, 2A). Locomotion

Table 1. Morphometric characterization of *Spirostrombidium cinctum*. Data based on protargol-impregnated specimens. All measurements in µm. Abbreviations: Max - maximum, Mean - arithmetic mean, Min - minimum, n - number of specimens, SD - standard deviation.

Character	Min	Max	Mean	SD	n
Body length	40	58	47.3	3.71	23
Body width	27	44	32.4	4.07	23
Distance from apex to cytostome*	13	28	20.4	3.13	22
No. of anterior membranelles**	23	28	25.5	1.55	15
No. of ventral membranelles	12	15	13.7	1.11	13
No. of thigmotactic membranelles	3	3	3.0	0.00	15
No. of dikinetids in girdle kinety	51	77	62.4	6.67	15
No. of dikinetids in ventral kinety	18	29	22.8	3.26	16
No. of dikinetids in extra kinety	13	19	16.8	1.80	12
No. of macronucleus	1	1	1.0	0.00	23
No. of micronucleus	1	1	1.0	0.00	4
Macronucleus length	12	22	16.8	2.84	22
Macronucleus width	8	18	13.2	2.28	22

*Measured from anteriormost to the posterior end of buccal zone of membranelles; **Including the thigmotactic membranelles.

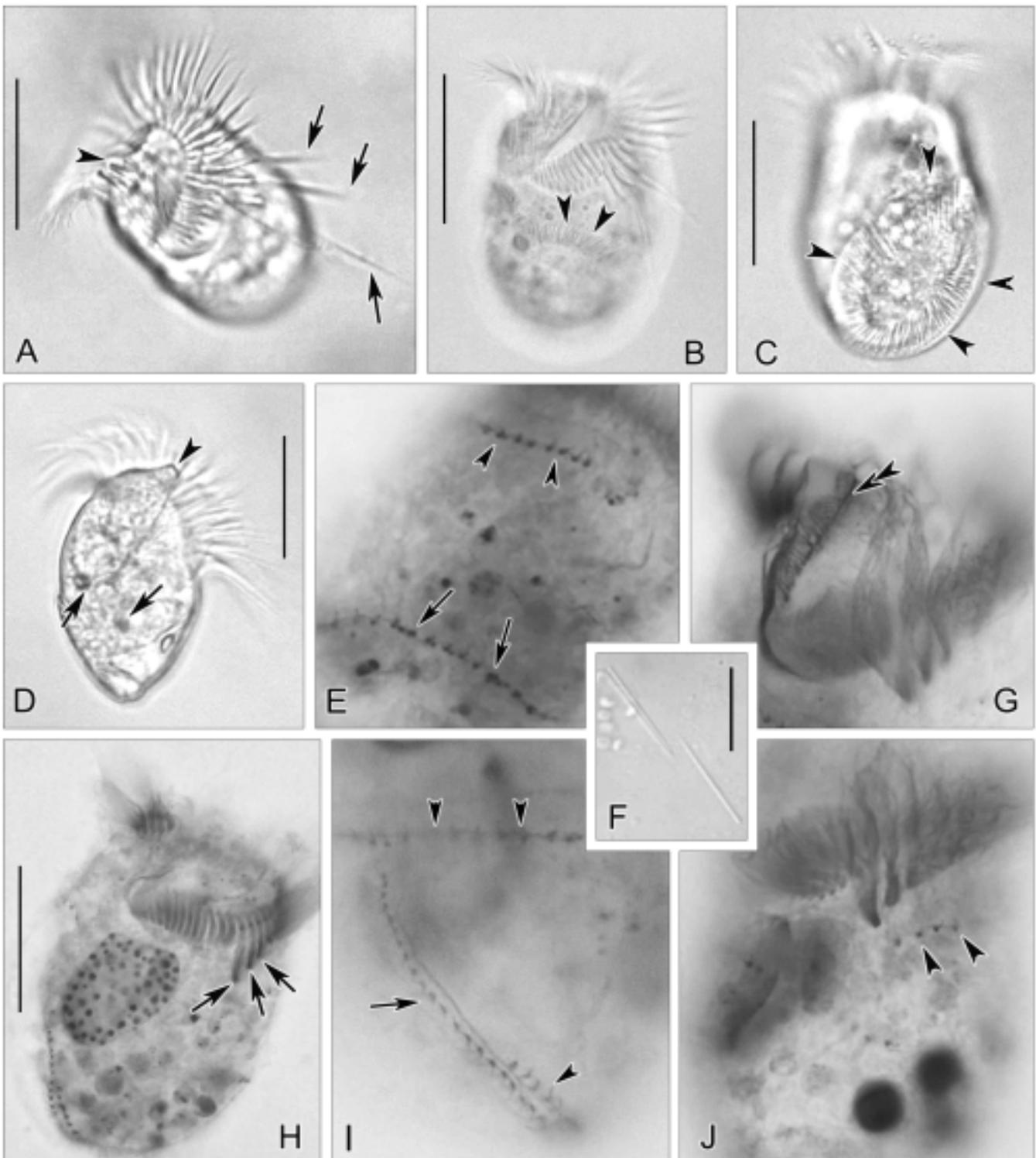


Figs 1A-I. *Spirostrombidium cinctum* from life (A-F) and after protargol impregnation (G-I). **A** - a representative specimen; **B** - swimming trace; **C** - dorsal view; **D** - lateral view, arrowheads to show the three thigmotactic membranelles; **E** - to show the creeping state, note the cell attached to the substrate with its three thigmotactic membranelles, arrows mark the bipartited subpellicular platelet layer; **F** - extrusomes; **G** - pattern of somatic ciliature, to show the beginning (arrowhead) and the end of the girdle kinety (double arrowheads), arrow to indicate the end of the ventral kinety; **H, I** - ventral and dorsal view of ciliary pattern, arrowhead in (H) indicates pharyngeal fibres, in (I) marks the micronucleus and double arrowheads in (H) show the end of the girdle kinety. AM - anterior membranelles; AP - apical protrusion; DC - distended cell surface; EM - endoral membrane; Ex - extrusomes; ExK - extra kinety; GK - girdle kinety; Mi - micronucleus; TM - thigmotactic membranelles; VK - ventral kinety; VM - ventral membranelles. Scale bars: 20 μm (A, C, D, H, I); 5 μm (F).

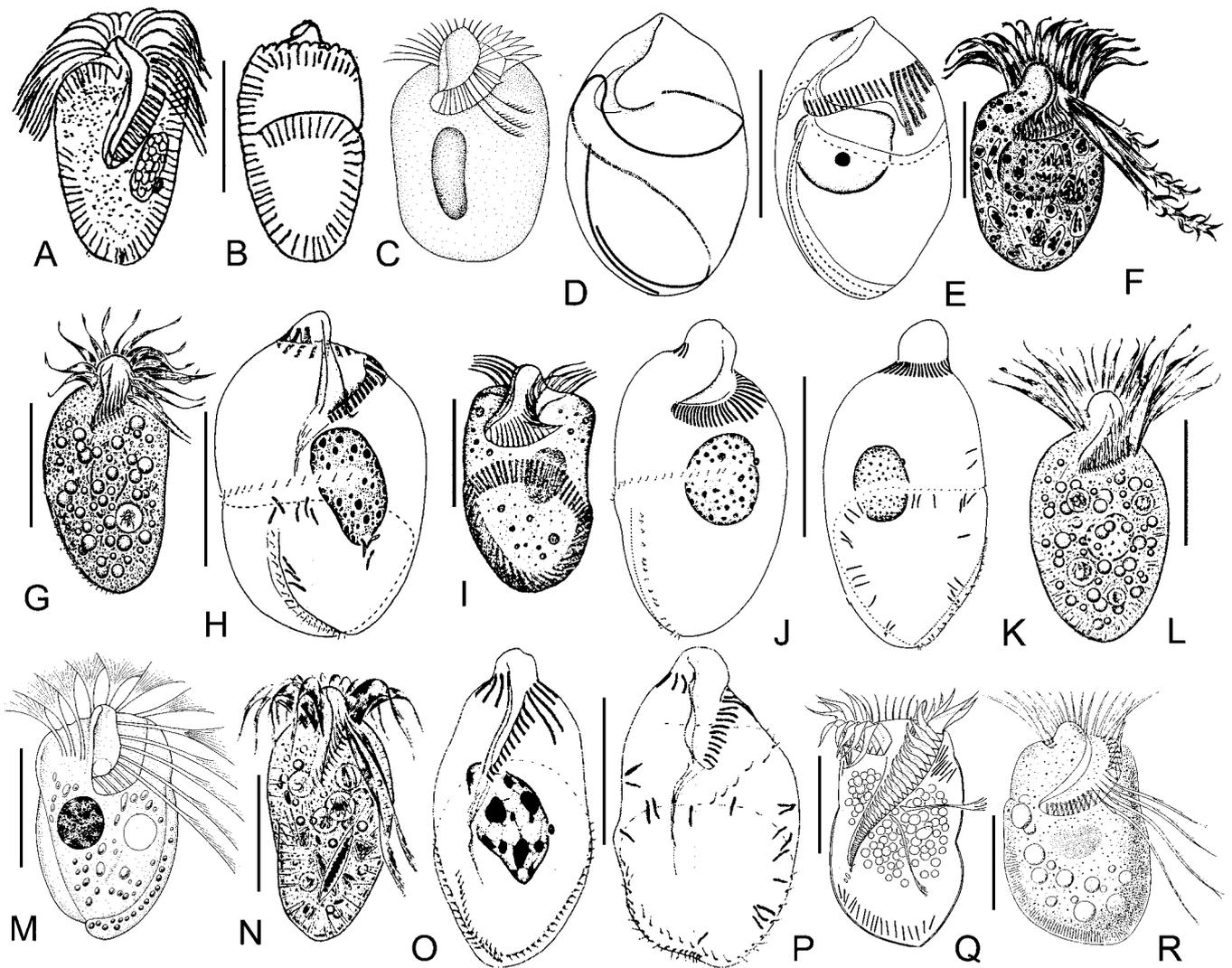
with two patterns: moderately fast and irregular when swimming (Fig. 1B), or very fast when crawling on the debris, using its 3 thigmotactic membranelles for attachment with ventral side down (Fig. 1E).

Cilia of most anterior membranelles about 20 μm long, stretching anteriorly as shown in Figs 1A and 2A. The

three thigmotactic membranelles about 28 (range 25-30), 35 (range 33-37), 42 (range 40-45) μm in length, respectively, which are shortened from proximal to distal ones and always directed posteriorly like three tails (Figs 1D, arrowheads; 2A, arrows). Bases of three thigmotactic membranelles about 7-11 μm in length, connecting



Figs 2A-J. *Spirostrombidium cinctum* from life (A-D, F) and after protargol impregnation (E, G-J). **A** - a typical specimen, the apical protrusion (arrowhead) and the three thigmotactic membranelles (arrows); **B, C** - ventral and dorsal view, the arrangement of extrusomes (arrowheads); **D** - lateral view, the apical protrusion (arrowhead) and the ingested algae (arrows); **E** - dorsal view, the extra kinety (arrowheads) and girdle kinety (arrows); **F** - extrusomes after cell bursts; **G** - apical view, the endoral membrane (double arrowheads); **H** - arrows indicate the three thigmotactic membranelles; **I** - right side view, to demonstrate the girdle (arrowheads) and ventral kinety (arrow); **J** - the extra kinety. Scale bars: 40 μ m (A-D); 8 μ m (F); 20 μ m (H).



Figs 3A-R. Comparison of some closely related species. **A, B** - *Spirostrombidium cinctum* (from Kahl 1932); **C** - *Spirostrombidium urceolare* (from Maeda and Carey 1985); **D-F** - *Spirostrombidium urceolare* (from Lei *et al.* 1999); **G, H** - *Spirostrombidium platum* (from Song and Packroff 1997); **I** - *Spirostrombidium pseudocinctum* (from Wang 1934); **J-L** - *Spirostrombidium echini* (from Song *et al.* 1999); **M** - *Strombidium elegans* (from Maeda and Carey 1985); **N-P** - *Strombidium elegans* (from Song *et al.* 2000); **Q** - *Strombidium latum* (from Fauré-Fremiet 1950); **R** - *Strombidium clavellinae* (from von Buddenbrock 1922). Scale bars: 30 μm (A, B, D-F, H, J-N, R); 50 μm (G, Q); 20 μm (I, O, P).

ventral membranelles (length of bases not the same, range 3-6 μm) and other anterior ones (bases about 6 μm).

Cell fragile, highly sensitive to pressure of cover-slip and easy to burst; pellicle rather delicate with thin and distinct subpellicular platelet layer covering the posterior 1/2 of body, but no polygonal cortical platelets detected

(Figs 1A, C; 2C). This structure is obliquely positioned and seems in two parts: ventral one is smaller (Fig. 1A) while the one on dorsal side is dominant (Figs 1C, E, arrows; 2C). Cytoplasm colourless to grayish, sometimes yellow-green due to ingested algae (Figs 1A; 2D, arrows). Extrusomes prominent and acicular-shaped (*ca* 10 μm long), evenly arranged along somatic kineties,

Table 2. Morphometric comparison between *Spirostrombidium cinctum* and morphologically similar species. 1 - *Spirostrombidium cinctum*; 2 - *Spirostrombidium echini*; 3 - *Spirostrombidium urceolare*; 4 - *Spirostrombidium platium*; 5 - *Spirostrombidium pseudocinctum*; 6 - *Strombidium latum*; 7 - *Strombidium clavellinae*; 8 - *Strombidium elegans*. Measurements in μm .

Characters	1	2	3	4	5	6	7	8
Cell length (<i>in vivo</i>)	45-65	50-70	70-80	70-100	-	110-170	70-80	30-50
Body shape	barrel-shaped	conical to ovoid	widely oval	conical	elongate elliptical	widely oval	widely oval	slim-conic
Length of buccal field: body length	2/5	<1/4	ca 1/3	ca 1/3	ca 1/3	ca 2/3	1/4-1/3	1/3-1/2
No. of ventral membranelles	12-15	15-18	13-16	8-11	14-17	ca 15*	ca 10*	8-10
No. of anterior membranelles	23-28	19-24	20-22	15-18	26-29	>20*	ca 25*	12-15
No. of thigmotactic membranelles	3-4	absent	3	absent	absent	2	4	2
No. of dikinetids in girdle kinety	51-77	>70	87-121	-	-	-	-	37-44
No. of dikinetids in ventral kinety	18-29	ca 15*	38-49	absent	-	-	-	9-21
Habitats	marine	marine (endo-commensal)	marine	marine (meso-saprobic)	marine	marine	marine	marine (meso-saprobic)
Data resource	Present work	Song <i>et al.</i> (1999)	Lei <i>et al.</i> (1999)	Song and Packroff (1997)	Petz <i>et al.</i> (1995)	Fauré-Fremiet (1950)	von Buddenbrock (1922)	Song <i>et al.</i> (2000)

*Assumed from the illustrations; - Data unavailable or structure difficult to judge (or doubtful if present).

but not in bundles (Figs 1A, C, D; 2B, C, arrowheads). Contractile vacuole not observed.

Macronucleus ovoid to ellipsoidal, centrally located, contains numerous small globular nucleoli (about 2 µm across) (Figs 1I, 2H). Single spherical micronucleus about 3 µm in diameter, closely attached to macronucleus (Fig. 1I, arrowhead).

All somatic kineties composed of dikinetids, one basal body of each with a short cilium (about 2-3 µm long) (Figs 1H, I). Girdle kinety consisting of about 62 (51-77) widely spaced dikinetids, extending from left/mid ventral side transversely across ventral and dorsal side, curving posteriad along left margin, across posterior pole and terminates subcaudally on right ventral-lateral area (Figs 1G, H, double arrowheads; 2E, arrows; 2I, arrowheads). Ventral kinety, which is composed of about 23 (18-29) densely arranged dikinetids, extends anteriad from posterior pole, parallel to the distal end of girdle kinety and terminates at equatorial level (Figs 1G, H; 2I, arrow). Thus, girdle kinety and ventral kinety both with inverse orientation. Ventral kinety positioned mainly in the right ventral-lateral area (Figs 1H; 2I, arrow), but in some specimens, ventral kinety could shifted almost to the right side of the cell (Fig. 2H). One fragment-like "extra" kinety positioned around "shoulder" area of left side of the cell and composed of about 17 (13-19) sparsely arranged dikinetids (Figs 1H, I; 2E, J, arrowheads).

Oral apparatus consisting of a single endoral membrane on inner wall of buccal lip and a membranellar zone (Figs 1H, 2H). Membranellar zone distinctly opens ventrally, bipartited into an anterior portion of about 25 (23-28) membranelles and a ventral portion of about 14 (12-15) membranelles, which are all composed of 3 kinety rows. Endoral membrane extending to center of protrusion (Figs 1H; 2G, double-arrowheads). Pharyngeal fibres about 10 µm long (Fig. 1H, arrowhead).

DISCUSSION

Comparison with related species

Our population corresponds well with Kahl's original description regarding the basic morphology (viz. the size, dorsoventrally flattened body shape, the distribution of extrusomes, behavior etc.) (Kahl 1932). Kahl also mentioned that 3 or 4 pointed membranelles were found in his population rather than "thigmotactic membranelles" (Figs

3A, B). We suppose that this could be due to a misinterpretation since these 3 thigmotactic membranelles might be sometimes less prominent like some individuals in our observations (Fig. 1D, arrowheads), hence it is reasonable to identify our population to be conspecific with Kahl's.

In 1995, Petz *et al.* (Petz *et al.* 1995) - without knowledge of its infraciliature - transferred *Strombidium cinctum* Kahl, 1932 into the genus *Spirostrombidium*. The pattern of somatic ciliature revealed by the present study corresponds well with the new diagnosis for *Spirostrombidium* (Petz *et al.* 1995, Agatha 2004), thus the new combination made by Petz *et al.* is confirmed.

In 1973, Hartwig found *S. cinctum* in the sand of North Sea around the Island of Sylt (Hartwig 1973). His organism corresponded well with Qingdao population considering the cell shape (oval), size (40-50 µm) and habitat (marine). The cell shape, habitat, distribution of extrusome of the Mexican population reported by Aladro-Lubel resembled Qingdao population except that the former is a little smaller (31.5-35 × 21 *vs.* 45-65 × 30-45 µm) (Aladro-Lubel 1984).

To date, four species in *Spirostrombidium* with flattened body shape inhabiting marine habitat have been reported, all of which have been studied using protargol impregnation method: *Spirostrombidium pseudocinctum* (Wang, 1934) Petz, Song *et* Wilbert, 1995; *Spirostrombidium urceolare* (Stein, 1867) Lei, Xu *et* Song, 1999; *Spirostrombidium platum* (Song *et* Packroff, 1997) Song, Wilbrt *et* Warren, 1999; *Spirostrombidium echini* Song, Wilbrt *et* Warren, 1999 (Wang 1934, Song and Packroff 1997, Lei *et al.* 1999, Song *et al.* 1999). The key morphological features of these taxa are given in Table 2. *Spirostrombidium pseudocinctum* (Wang, 1934) Petz, Song *et* Wilbert, 1995 is similar to *S. cinctum* in terms of cell size, habitat, number of anterior and ventral membranelles, but the former can be distinguished by: (1) the arrangement of extrusomes which are distributed only in ventral side (*vs.* present also in dorsal side), (2) absence of thigmotactic membranelles (*vs.* present), and (3) lacking of the extra kinety (*vs.* present) (Fig. 3I) (Wang 1934, Petz *et al.* 1995).

Spirostrombidium urceolare (Stein, 1867) Lei, Xu *et* Song, 1999 resembles *S. cinctum* in the presence of 3 thigmotactic membranelles, similar number of anterior and ventral membranelles. However, the former can be recognized by the following combined features: (1) larger size (70-80 *vs.* 45-65 µm), (2) extrusomes widely

arranged (*vs.* densely arranged along somatic kineties), (3) absence of an extra kinety (*vs.* present), and (4) evidently higher number of dikinetids in girdle (87-121 *vs.* 51-77) and ventral kinety (38-49 *vs.* 18-29) (Figs 3C-F) (Lei *et al.* 1999).

Spirostrombidium platum (Song *et* Packroff, 1997) Song, Wilbert *et* Warren, 1999 differs from *S. cinctum* in: (1) larger size (70-100 *vs.* 45-65 μm), (2) absence of thigmotactic membranelles (*vs.* present), (3) fewer anterior (15-18 *vs.* 23-28) and ventral membranelles (8-11 *vs.* 12-15), and (4) absence of a ventral kinety (*vs.* present) (Figs 3G, H) (Song and Packroff 1997).

Spirostrombidium echini Song, Wilbert *et* Warren, 1999 is an endocommensal form which can be clearly separated from *S. cinctum* by its biotope within the digestive tract of the urchins (*vs.* free-living) and the opened girdle kinety, in which the posterior end is divided into two parts giving the impression that this species has two ventral kineties (Figs 3J-L) (Song *et al.* 1999).

Considering the general morphology, comparisons should also be made with three *Strombidium*-species: *Strombidium elegans* Florentin, 1901; *S. clavellinae* von Buddenbrock, 1922 and *S. latum* Kahl, 1932, in which the infraciliature of the latter two remain unknown (Table 2). *Spirostrombidium cinctum* differs from *Strombidium clavellinae* mainly in terms of presence of 3-4 thigmotactic membranelles (*vs.* 4) and different arrangement of extrusomes (covering completely the body portion *vs.* limited in the posterior half when viewed ventrally) (Fig. 3R) (von Buddenbrock 1922).

Strombidium latum Kahl, 1932 is characterized by its giant body size (110-170 *vs.* 45-65 μm), deeply extended buccal field (*ca* 2/3 *vs.* 2/5 of body length), presence of 2 thigmotactic membranelles (*vs.* 3-4) and possibly less dominant extrusomes (Fig. 3Q) (Fauré-Fremiet 1950), thus it can be easily distinguished from *Spirostrombidium cinctum* (Table 2).

Strombidium elegans Florentin, 1901 was redefined very recently (Song *et al.* 2000). It differs from *Spirostrombidium cinctum* in several aspects, i.e. (1) the number of thigmotactic membranelles (2 *vs.* 3-4), (2) the somatic ciliary pattern (girdle kinety closed and inserted by ventral kinety *vs.* girdle kinety dextrally spiraled and its posterior portion parallel to ventral kinety), and (3) less dominant buccal apparatus with fewer ventral membranelles (8-10 *vs.* 12-15), much less number of anterior membranelles (12-15 *vs.* 23-28), dikinetids in girdle kinety (37-34 *vs.* 51-57) and in ventral kinety (9-21 *vs.* 18-29) (Figs 3M-P).

Occurrence and ecology

Kahl (1932) discovered *Spirostrombidium cinctum* in the sand of Kieler Bay at a salinity of 9‰. Raikov (1963) observed this species in sands of the Ussuri Gulf (Japan Sea). Furthermore, it occurred in the sand of North Sea around the Island of Sylt (Hartwig 1973). The Mexican population reported by Aladro-Lubel (1984) was from tropical and calcareous sediments near Enmedio Island, Mexico.

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