

## On Two Marine Oligotrich Ciliates, *Spirostrombidium agathae* n. sp. and *S. schizostomum* (Kahl, 1932) n. comb. from China, with a Key to the Identification of Seven Well-Characterized *Spirostrombidium* spp. (Ciliophora: Oligotrichida)

Dapeng XU<sup>1</sup>, Weibo SONG<sup>1, 2</sup>, Xiaofeng LIN<sup>2</sup> and Alan WARREN<sup>3</sup>

<sup>1</sup>Laboratory of Protozoology, KLM, Ocean University of China, Qingdao, P. R. China; <sup>2</sup>Laboratory of Protozoa, College of Life Science, South China Normal University, Guangzhou, P. R. China; <sup>3</sup>Department of Zoology, Natural History Museum, Cromwell Road, London, UK

**Summary.** Two marine oligotrich ciliates, *Spirostrombidium agathae* n. sp. and *S. schizostomum* (Kahl, 1932) n. comb. (basonym: *Strombidium schizostomum* Kahl, 1932), were isolated from the littoral zone of coastal waters near Qingdao (Tsingtao), northern China. The morphology and infraciliature of each was studied from live and protargol-stained specimens. The new species *S. agathae* is distinguished from its congeners by the following combination of characters: small size, distribution of extrusomes, presence of two prolonged thigmotactic membranelles, number of anterior and ventral membranelles, and number of dikinetids in girdle and ventral kineties. The ciliary pattern of the poorly known *Strombidium schizostomum* Kahl, 1932, which has never been studied using modern methods since it was originally reported, indicates that it should be a member of the genus *Spirostrombidium*. Thus a new combination, *Spirostrombidium schizostomum* (Kahl, 1932) n. comb., is proposed. Based on the present studies and data published previously, a key to the identification of seven well-characterized *Spirostrombidium* spp. is provided.

**Key words:** infraciliature, morphology, *Spirostrombidium agathae* n. sp., *S. schizostomum* n. comb., Strombidiidae, taxonomy.

### INTRODUCTION

The genus *Spirostrombidium* Jankowski, 1978 was established by Jankowski (1978) on the basis of the spiraled "cytoskeleton", which Agatha (2004) considered to be a misinterpretation of the distribution of extrusomes, as the main distinguishing feature. Petz

*et al.* (1995) improved the diagnosis mainly by including the arrangement of the ventral and girdle kineties, which are parallel to one another in the posterior portion of the cell. Agatha (2004), however, noted that this arrangement of somatic kineties can be achieved in one of two ways and that these can be distinguished by the ciliation of the dikinetids that comprise the kineties, i.e. either the anterior or posterior basal bodies can be ciliated (but not both). Based on this character, Agatha (2004) split the genus into two: *Spirostrombidium (sensu stricto)* in which the ventral kinety and posterior portion of the

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Address for correspondences: Weibo Song, Laboratory of Protozoology, KLM, Ocean University of China, Qingdao, 266003, P. R. China; Fax +86-532-82032283; E-mail address: wsong@ouc.edu.cn

girdle kinety are inversely orientated, i.e. anterior basal bodies are ciliated in the ventral kinety vs. posterior basal bodies ciliated in the girdle kinety, and *Parallelostrombidium* in which the ventral kinety and posterior portion of the girdle kinety have the same orientation, i.e. the anterior basal bodies are ciliated in both cases. Consequently Agatha (2004) supplied a further improved diagnosis of *Spirostrombidium*: girdle kinety dextrally spiraled, posterior portion inversely orientated and parallel to longitudinal ventral kinety.

During surveys of the ciliate fauna in the costal regions near Qingdao, China, two oligotrich ciliates were collected. After comparison with similar taxa, one of them is believed to be a new form of *Spirostrombidium*, *S. agathae* n. sp., and the other is conspecific with *Strombidium schizostomum* Kahl, 1932. Based on its pattern of somatic ciliature, the latter is transferred to the genus *Spirostrombidium* as a new combination.

## MATERIALS AND METHODS

*Spirostrombidium agathae*. Organisms were sampled from coastal waters near Qingdao (Tsingtao, 36°08'N; 120°43'E) on 1 March 2005. The upper 10 cm layer of sand was collected with seawater *in situ*. The samples were then transferred to Petri dishes and maintained in the laboratory for several days at room temperature (*ca* 22°C) as a raw culture for further studies.

*Spirostrombidium schizostomum*. Samples were collected on 5 August 2002 from shrimp-farming ponds near Qingdao, China. Glass slides fixed in a slide frame served as artificial substrates and were immersed in the water until a biofilm was formed (~10 days). Then the slides were retrieved out and transferred to Petri dishes with marine water from the sampling site.

The locomotion of the organisms was studied in Petri dishes under a dissecting microscope. The morphology was investigated using bright field and differential interference contrast microscopy. The infraciliature was revealed by protargol impregnation (Wilbert 1975). Drawings of live 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 Agatha *et al.* (2005).

## RESULTS

Order Oligotrichida Bütschli, 1889

Family Strombidiidae Fauré-Fremiet, 1970

Genus *Spirostrombidium* Jankowski, 1978

### *Spirostrombidium agathae* n. sp. (Figs 1, 2; Table 1)

**Diagnosis:** Small marine *Spirostrombidium*, approximately 35 × 25 µm *in vivo*, and 30 × 23 µm after

protargol staining; dorsoventrally flattened *ca* 2:3; body oblong (length: width ratio ~2:1) with apical protrusion at right anterior end; transparent hemitheca which covers posterior half of cell composed of two parts; buccal cavity extends posteriorly to about one-third of cell length; on average 14 anterior and 7 ventral membranelles; two posteriorly directed thigmotactic membranelles; one ellipsoidal macronucleus; extrusomes prominent, composed of three parts: (1) on ventral side, the extrusomes evenly arranged along the girdle kinety, (2) an extra extrusomes group arranged along shoulder area on dorsal side, and (3) the extrusomes arranged along the margin of hemitheca on dorsal side; girdle and ventral kineties consist of approximately 37 and 16 dikinetids respectively.

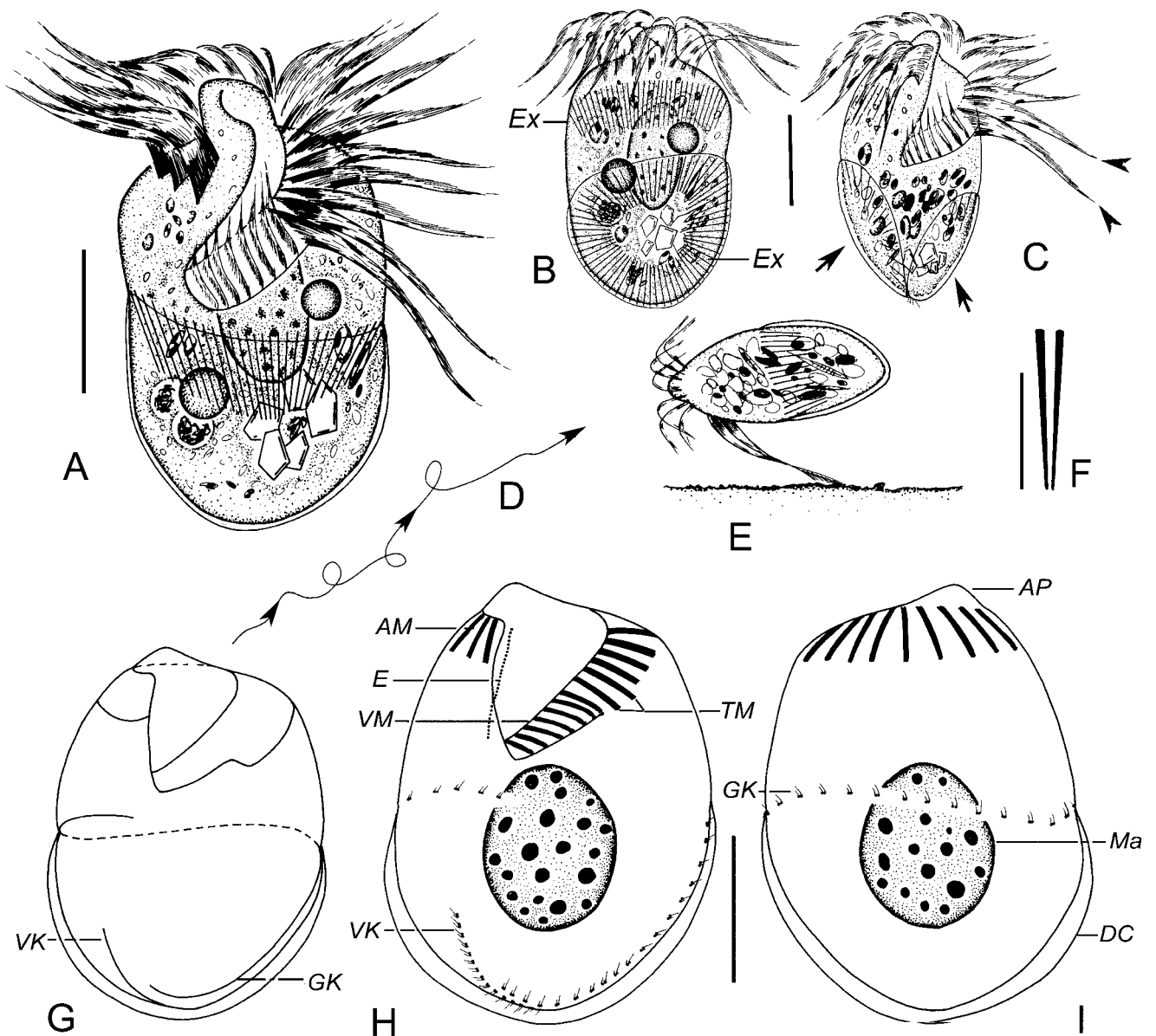
**Type locality and ecological features:** Marine sand in coastal area near Qingdao (Tsingtao, 36°08'N; 120°43'E), China; water temperature *ca* 6°C, salinity *ca* 32 ‰, and pH *ca* 7.9.

**Deposition of slides:** Two permanent slides of protargol-impregnated specimens are deposited as a holotype and a paratype in the Laboratory of Protozoology, OUC, China with registration numbers 2005:03:01:01 and 2005:03:01:02, respectively.

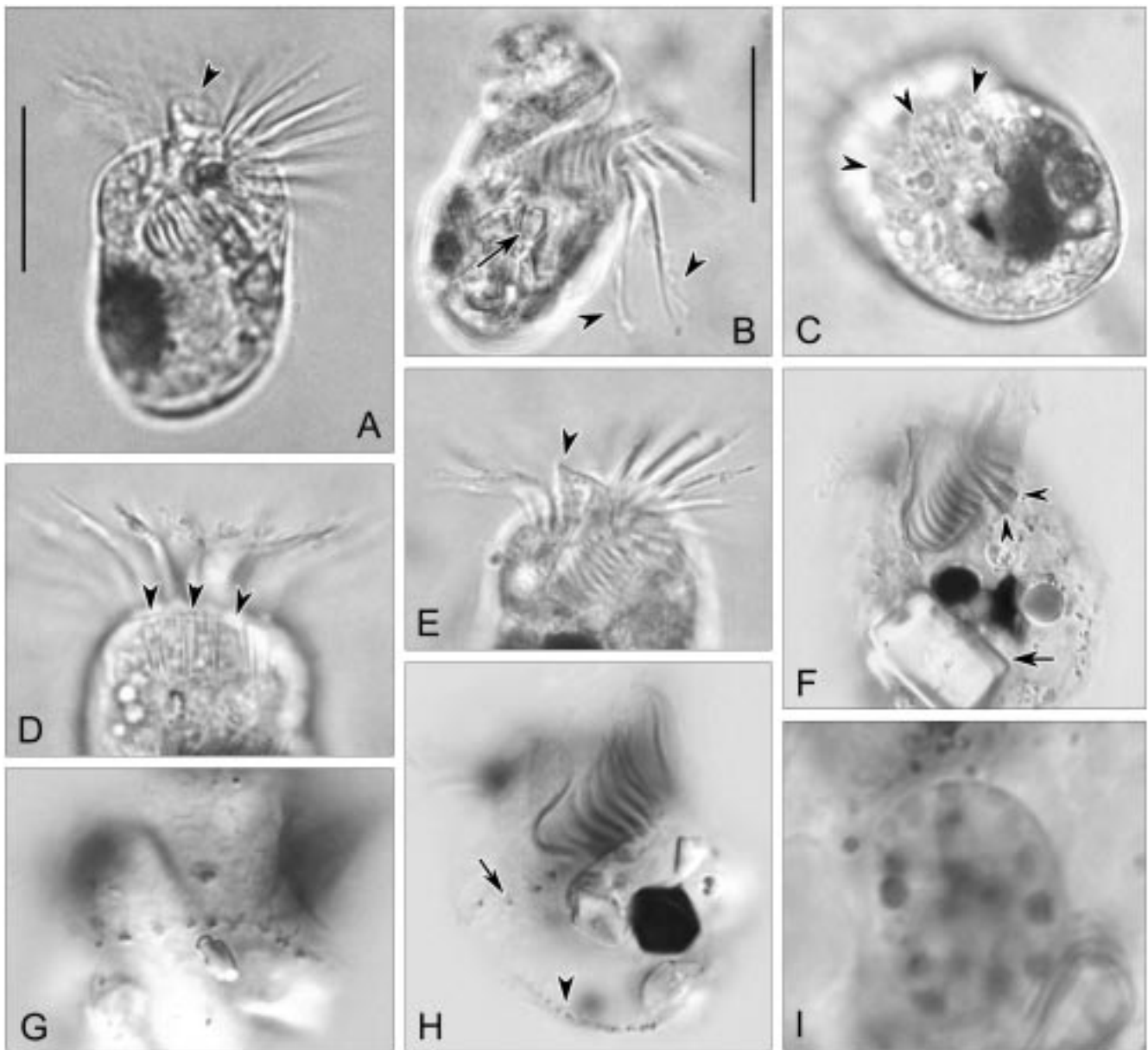
**Dedication:** We dedicate this new species to Dr Sabine Agatha, University of Salzburg, Austria, in acknowledgement of her contribution to the taxonomy of marine planktonic ciliates.

**Description:** Cells *in vivo* 30–35 × 25–30 µm, mostly 35 × 25 µm. Cell shape constant, oblong (length: width ratio ~2:1) with posterior end bluntly rounded; when viewed from ventral side, usually broadest at the “shoulder” region, equatorial area always slightly constricted (Figs 1A; 2A). Anterior end of cell transversely truncated with hyaline, *ca* 5 µm high, apical protrusion (also known as the peristomial collar) at right side of peristome that can be recognized *in vivo* but usually disappears or becomes undetectable after protargol impregnation (Figs 1A; 2A, arrowhead). Cell dorsoventrally flattened with thickness:width ratio approximately 2:3 (Fig. 1C).

Cell fragile, sensitive to presence of coverslip and easily bursts when water temperature increases or on contact with the water surface. Pellicle delicate with thin, transparent hemitheca that covers posterior half of cell (Figs 1A, B, C arrows). Hemitheca obliquely oriented and composed of two parts: one ventral, which is comparatively small, and one dorsal which is larger (Figs 1B, C). No cortical platelets recognizable either *in vivo* or in silvered specimens. Cytoplasm colourless, contains



**Figs 1A-I.** *Spirostrombidium agathae* n. sp. from life (A-F) and after protargol impregnation (G-I). **A** - ventral view of a representative specimen; **B, C** - dorsal (B) and right lateral (C) views, arrows indicate the transparent hemitheca, arrowheads mark the two prolonged membranelles; **D** - swimming trace; **E** - to show the creeping state, note the cell attached to the substrate by its two thigmotactic membranelles; **F** - extrusomes; **G** - pattern of somatic ciliature; **H, I** - ventral (H) and dorsal (I) views of ciliary pattern. AM - anterior membranelles; AP - apical protrusion; DC - distended cell surface; E - endoral membrane; Ex - extrusomes; GK - girde kinety; Ma - macronucleus; TM - thigmotactic membranelles; VK - ventral kinety; VM - ventral membranelles. Scale bars: 10  $\mu$ m (A, H, I); 5  $\mu$ m (F).



**Figs 2A-I.** Photomicrographs of *Spirostrombidium agathae* n. sp. from life (A-E) and after protargol impregnation (F-I). **A** - resting specimen showing typical body shape, arrowhead marks the apical protrusion; **B** - left lateral view, arrow indicates refractive particles in cytoplasm and arrowheads mark two thigmotactic membranelles; **C** - posterior view to show extrusomes (arrowheads); **D** - dorsal view, arrowheads demonstrate extrusomes; **E** - arrowhead to show apical protrusion; **F** - ventral view, arrowheads indicate thigmotactic membranelles and arrow marks the large refractive particle in cytoplasm; **G** - to show dikinetids in girdle kinety; **H** - ventral view, arrow indicates girdle kinety while arrowhead marks ventral kinety; **I** - to demonstrate macronucleus. Scale bars: 20  $\mu$ m (A, B).

green inclusions 1-2  $\mu$ m across and quartz particles 3-10  $\mu$ m in size (Figs 2B, F, arrow). Extrusomes prominent, acicular, 6-8  $\mu$ m long, composed of three parts: (1) on ventral side, the extrusomes evenly arranged along the girdle kinety (Fig. 1A), (2) an extra extrusomes group

arranged along shoulder area on dorsal side (Figs 1B; 2D, arrowheads), and (3) the extrusomes arranged along the margin of hemitheca on dorsal side (Figs 1B; 2C, arrowheads). Neither contractile vacuole nor cytophyge detected. Single macronucleus broadly ellipsoidal in shape

**Table 1.** Morphometric characterization of *Spirostrombidium agathae* n. sp. (upper line) and *S. schizostomum* (Kahl, 1932) n. comb. (lower line). Data based on protargol-impregnated specimens. All measurements in  $\mu\text{m}$ . Abbreviations: M - median, Max - maximum, Mean - arithmetic mean, Min - minimum, n - number of specimens, SD - standard deviation.

Characters	Min	Max	Mean	M	SD	n
Cell length	26	34	29.4	28	2.6	15
	40	60	45.3	45	4.8	20
Cell width	20	28	23.1	24	2.2	15
	24	32	28.2	28	3.6	20
Apex to cytostome, distance*	14	16	14.8	15	0.9	15
	16	26	20.9	20	2.9	20
Apex to the macronucleus	11	13	11.9	12	1.0	15
	8	14	11.0	10	1.5	20
Apex to anterior end of girdle kinety, distance	12	16	14.1	14	1.3	14
	8	13	10.5	10	1.4	16
Posterior cell end to posterior end of girdle kinety, distance	3	6	4.4	4.5	1.0	14
	3	6	4.4	4.0	0.9	16
Anterior membranelles, number	13	16	14.1	14	0.8	11
	16	19	17.3	17	1.1	18
Ventral membranelles, number	7	8	7.4	7	0.5	11
	10	12	10.5	10	0.7	18
Thigmotactic membranelles, number	2	2	2	2	0.0	18
	0	0	0	0	0	20
Macronucleus length	10	14	12.6	13	1.7	16
	14	28	21.6	22	3.8	18
Macronucleus width	9	11	9.8	10	0.6	16
	10	13	11.9	12	1.4	18
Girdle kinety, number of dikinetids	34	42	36.9	36	2.8	14
	46	67	56.3	56	5.9	16
Ventral kinety, number of dikinetids	14	17	15.5	16	0.8	14
	12	18	14.7	14	2.1	16
Ventral kinety, length	10	13	11.2	10.5	1.4	14
	16	20	17.3	18	1.6	16

\* Measured from anteriormost point of cell to the posterior end of buccal zone of membranelles (= ventral membranelles);

and centrally located, containing many small nucleoli each  $\sim 3 \mu\text{m}$  across (Figs 1I, 2I). No micronucleus detected possibly due to its poor uptake of silver.

Locomotion by one of two mechanisms: moderately fast when crawling on debris with its ventral side facing down and two thigmotactic membranelles in contact with substrate (Fig. 1E), or swimming smoothly while rotating about longitudinal body axis (Fig. 1D).

Somatic ciliature composed exclusively of dikinetids (Figs 1H, I; 2G, H). Girdle kinety consists of approximately 37 (34-42) dikinetids, each having a cilium (*ca*  $1.5 \mu\text{m}$  in length) associated with the left basal body and a relatively shorter fibre (?) with the right basal body. Girdle kinety extends from mid-ventral side transversely across ventral and dorsal sides, curves posteriad along left margin, across posterior pole and terminates subterminally on right ventrolateral area (Figs 1G, H, I; 2G, H, arrow). Thus, the girdle kinety spirals approxi-

mately once around cell. Ventral kinety, which is composed of approximately 16 (14-17) densely arranged dikinetids, each having a cilium associated with the anterior basal body (*ca*  $1.5 \mu\text{m}$  in length) and a relatively shorter fibre (?) with the posterior one. Ventral kinety extends anteriorly from posterior pole, parallel to distal end of girdle kinety and terminates in sub-equatorial region (Figs 1G, H; 2H, arrowhead). Girdle and ventral kineties have inverse orientation in terms of their basal body ciliation. No fragment-like "extra" kinety observed.

Oral apparatus occupies anterior end of cell (Figs 1H, I; 2F, H). Buccal cavity extending obliquely to the right and terminating about 1/3 of the way down the cell (Figs 1A, 2A). Adoral zone of membranelles divided into anterior and ventral portions separated by the two thigmotactic membranelles. Anterior portion with about 14 (13-16) membranelles, ventral portion with about

7 (7-8) membranelles, all of which are composed of three rows of basal bodies. Cilia of anterior membranelles *ca* 15  $\mu\text{m}$  in length, stretching laterally or slightly anteriorly when swimming (Fig. 1A). Two thigmotactic membranelles distinct *in vivo* due to their longer cilia (about 20  $\mu\text{m}$  long) and always directed posteriorly like two tails (Figs 1A, C; 2B, arrowheads), but less conspicuous in protargol impregnations where the bases (about 5  $\mu\text{m}$  long) are only slightly longer than those of the anterior membranelles. Bases of ventral membranelles 2-3  $\mu\text{m}$  long and bases of anterior membranelles about 4  $\mu\text{m}$  long. Endoral membrane on inner wall of buccal lip on right side of oral cavity, rarely recognizable either in protargol-stained specimens or *in vivo*, probably composed of a single row of monokinetids. Pharyngeal fibres not observed.

**Remarks:** There are only two congeners with thigmotactic membranelles, i.e., *Spirostrombidium cinctum* and *Spirostrombidium urceolare* (Table 2). Unlike the genus *Strombidium*, the hemitheca of which is composed of polygonal cortical plates (Song *et al.* 2000, Agatha *et al.* 2005, Xu *et al.* 2005), no cortical platelets are recognizable in either of the *Spirostrombidium* spp. species studied here, either *in vivo* or in silvered specimens. Furthermore the hemitheca of both species is obliquely orientated and appears to be composed of two parts whereas the hemitheca of most other strombidiids is usually cup-like. However, it is unknown whether it is a species-specific feature or merely depends on the nutritional state (hungry/well fed) or the nutrition type (mixotrophy/heterotrophy) of the species. According, such a difference should not be overinterpreted.

*Spirostrombidium agathae* can be separated from *S. urceolare* (Stein, 1867) Lei, Xu *et al.* Song, 1999 by its smaller cell size (30-35  $\times$  25-30 *vs.* 70-80  $\times$  35-50  $\mu\text{m}$  *in vivo*), different course of the girdle kinety (originates from mid-ventral cell *vs.* originates from dorsal side of cell), and in having fewer: thigmotactic membranelles (2 *vs.* 3); anterior membranelles (13-16 *vs.* 20-22); ventral membranelles (7-8 *vs.* 13-16); dikinetids in the girdle kinety (34-42 *vs.* 87-21) and ventral kinety (14-17 *vs.* 38-48) (Lei *et al.* 1999).

*Spirostrombidium cinctum* (Kahl, 1932) Petz, Song *et al.* Wilbert, 1995 has a similar cell shape to *S. agathae* and is also a marine species. It can be differentiated from *S. agathae* by presence of extra kinety (*vs.* absence in *S. agathae*) and having more: anterior membranelles (23-28 *vs.* 13-16); ventral membranelles (12-15 *vs.* 7-8); thigmotactic membranelles (3-4 *vs.* 2);

dikinetids in the girdle kinety (51-77 *vs.* 34-42) and ventral kinety (18-29 *vs.* 14-17) (Xu and Song 2006).

*Spirostrombidium sauerbreyae* (Kahl, 1932) Petz, Song *et al.* Wilbert, 1995 is the type species of the genus although its infraciliature remains unknown. It can be separated from *S. agathae* by its much larger cell size (100-55 *vs.* 30-35  $\mu\text{m}$ ) and its lack of thigmotactic membranelles (*vs.* 2 thigmotactic membranelles in *S. agathae*) (Sauerbrey 1928, Kahl 1932).

Considering their morphology of live specimens, three marine *Strombidium* spp. (the infraciliature of which remain unknown), which also have thigmotactic membranelles, should be compared with our new species, namely: *S. fourneleti* (Dragesco, 1960) Maeda *et al.* Carey, 1985, *S. faurei* Dragesco, 1960, and *S. clavellinae* von Buddenbrock, 1922.

*Strombidium faurei* was found in fine sand at Roscoff and also has two thigmotactic membranelles. It can be distinguished from *S. agathae*, however, by its much larger cell size (75 *vs.* 30-35  $\mu\text{m}$ ), position of thigmotactic membranelles (separating from the adoral membranelles *vs.* locating in the adoral membranelles) and absence of a hemitheca (*vs.* present in *S. agathae*) (Dragesco 1960).

Although it has two thigmotactic membranelles, *Strombidium fourneleti* can be separated from our new species by its cell shape (globular *vs.* elongated barrel-shaped), presence of polygonal cortical platelets (*vs.* absent), position of thigmotactic membranelles (separating from the adoral membranelles *vs.* locating in the adoral membranelles), and larger cell size (50-65 *vs.* 30-35  $\mu\text{m}$ ) (Dragesco 1960).

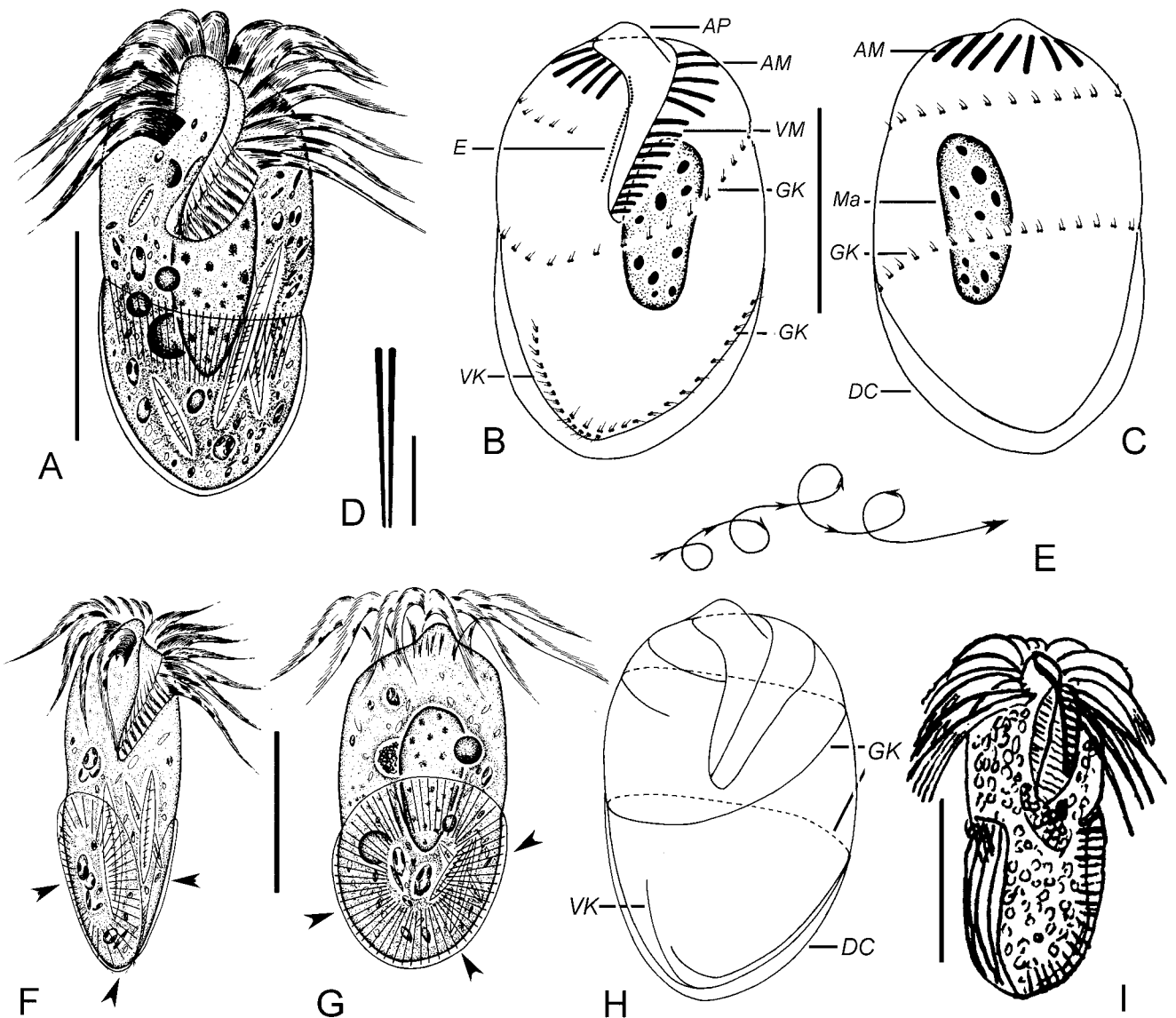
*Strombidium clavellinae* is similar to *Spirostrombidium agathae* in terms of its general appearance (Buddenbrock 1922). It differs from the latter, however, by its much larger cell size (70-80 *vs.* 30-35  $\mu\text{m}$ ) and having 4 thigmotactic membranelles (*vs.* only 2 in *S. agathae*).

***Spirostrombidium schizostomum* (Kahl, 1932) n. comb. (Figs 3, 4; Table 1)**

Basionym: *Strombidium schizostomum* Kahl, 1932

The infraciliature of *Spirostrombidium schizostomum* has never been described, hence we here supply an improved diagnosis based on previous as well as present data.

**Improved diagnosis:** Medium-sized marine *Spirostrombidium*, approximately 50-70  $\times$  25-30  $\mu\text{m}$  *in vivo*, and 45  $\times$  28 after protargol staining; dorsoventrally flattened *ca* 2:3; body asymmetric barrel-shaped with

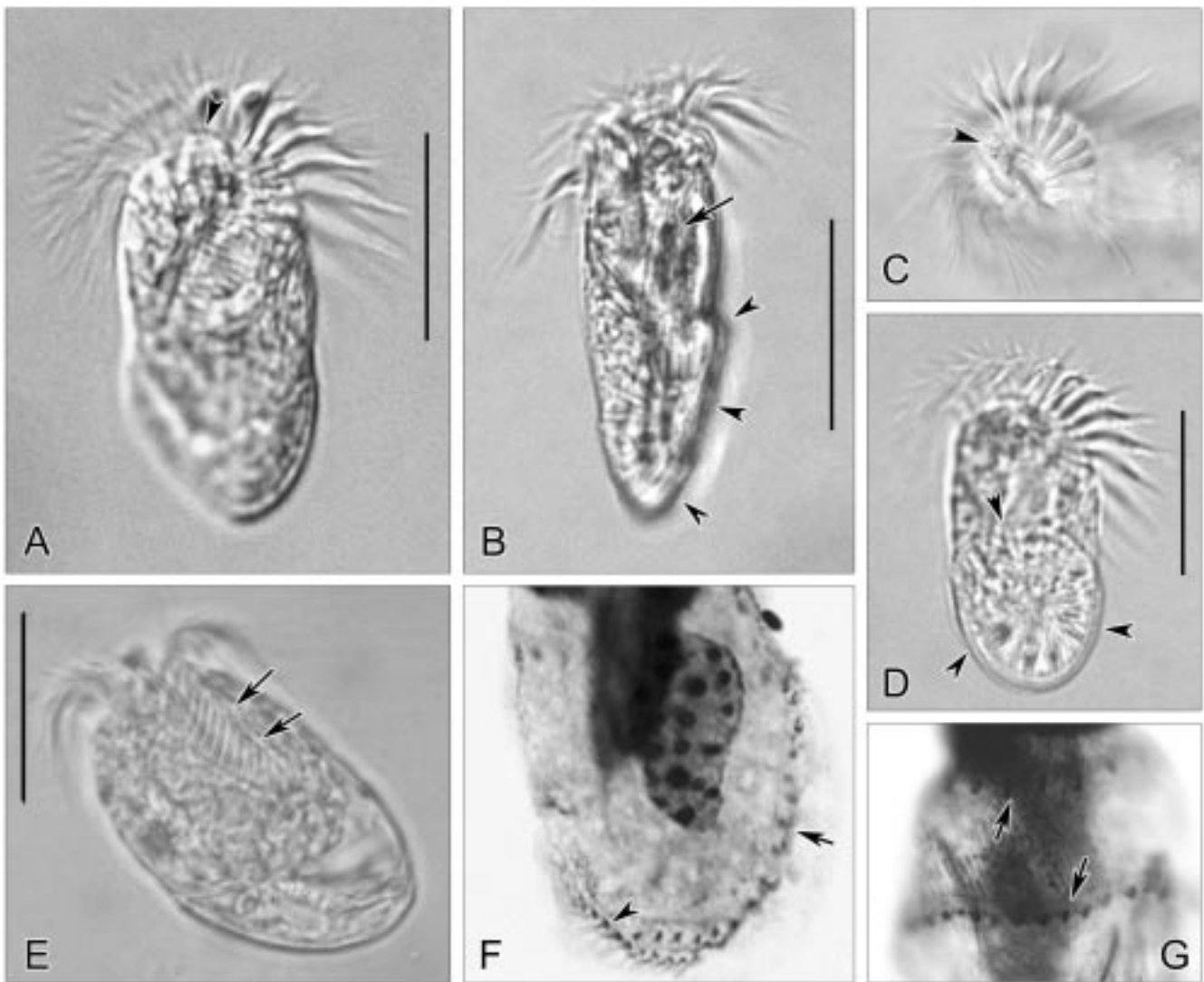


**Figs 3A-I.** *Spirostrombidium schizostomum* from life (A, D, E-G, I) and after protargol impregnation (B, C, H). **A** - ventral view of a typical specimen; **B, C** - ventral (B) and dorsal (C) views of ciliary pattern; **D** - extrusomes; **E** - pattern of locomotion; **F, G** - right lateral (F) and dorsal (G) views, arrowheads mark the hemitheca; **H** - pattern of somatic ciliature; **I** - ventral view of specimen (from Kahl, 1932). AM - anterior membranelles; AP - apical protrusion; DC - distended cell surface; E - endoral membrane; GK - girdle kinety; Ma - macronucleus; VK - ventral kinety; VM - ventral membranelles. Scale bars: 25  $\mu$ m (A, C, G); 5  $\mu$ m (D); 40  $\mu$ m (I).

apical protrusion; on average 17 anterior and 11 ventral membranelles; one ellipsoidal macronucleus; extrusomes prominent, arranged along equatorial area on ventral of cell and along the margin of hemitheca on dorsal side; girdle and ventral kineties consist of about 56 and 14 dikinetids respectively.

**Deposition of slide:** One voucher slide (registration number 2002:08:05) with protargol-impregnated specimens is deposited in the Laboratory of Protozoology, Ocean University of China.

**Description of the Qingdao population:** Cells *in vivo* mostly about 55-65  $\times$  25-30  $\mu$ m. Cell shape gener-



**Figs 4A-G.** Photomicrographs of *Spirostombidium schizostomum* from life (A-E) and after protargol impregnation (F, G). **A** - ventral view of a typical specimen; **B** - right lateral view, to show the hemitheca (arrowheads) and diatoms in cytoplasm (arrow); **C** - top view, to show the apical protrusion (peristomial collar, arrowhead); **D** - dorsal view, arrowheads indicate hemitheca; **E** - ventral view of a slightly compressed individual, arrows mark ventral membranelles; **F** - ventral view, to show the macronucleus, girdle kinety (arrow) and ventral kinety (arrowhead); **G** - dorsal view, arrows mark the girdle kinety. Scale bars: 30 μm (A, B, D).

ally constant, slightly asymmetric and elongated barrel-shaped with posterior end bluntly pointed; when viewed from ventral side equatorial area always slightly narrowed (Figs 3A, 4A). Cell dorsoventrally flattened, thickness: width ratio about 2:3 (Figs 3F, 4B). Collar region domed to form a conspicuous apical protrusion (also known as the peristomial collar), *ca* 5 μm high, which may disappear or become undetectable after protargol impregnation (Figs 3A; 4A, C, arrowhead). No thigmotactic membranelles detected.

Pellicle relatively rigid with thin and transparent hemitheca covering the posterior 1/2 of cell. Hemitheca obliquely oriented and composed of two parts: a ventral part, which is comparatively small, and a dorsal part which is larger (Figs 3F, G). No cortical platelets recognizable either *in vivo* or in silvered specimens. Cell surface distinctly distended posterior to equatorial area in fixed cells. Cytoplasm colourless to grayish, sometimes yellow-green due to ingested algae including large diatoms (Figs 3A, 4B), which often render cell dark or

even opaque when observed at low magnifications. Extrusomes prominent, acicular, *ca* 8–10  $\mu\text{m}$  long, evenly arranged along equatorial area of cell on ventral side and along margin of hemitheca on dorsal side, not in bundles (Figs 3A, F, G). Neither a contractile vacuole nor a cytophyge were detected. Macronucleus ovoid to ellipsoid, centrally located, containing numerous small globular nucleoli about 2  $\mu\text{m}$  across (Fig. 4F). Micronucleus not found. Moving fast in wild spirals and changing direction frequently, never observed to stop swimming (Fig. 3E).

Somatic ciliature as shown in Figs 3B, C, H; 4F, G and composed exclusively of dikinetids. Girdle kinety consists of 56 (range 46–67) dikinetids, each having a cilium (*ca* 1.5  $\mu\text{m}$  in length) associated with the left basal body and a relatively shorter fibre (?) with the right one. Girdle kinety starts in shoulder area on right ventral side, follows an almost complete circle across dorsal and ventral sides, then curves posteriad along left margin, across posterior pole and terminates subterminally in right ventrolateral area (Figs 3B, C, H; 4F, G). Thus, the girdle kinety spirals approximately twice around cell. Ventral kinety composed of approximately 14 (12–18) densely arranged dikinetids, each having a cilium (*ca* 1.5  $\mu\text{m}$  in length) associated with the anterior basal body and a relatively shorter fibre (?) with the posterior one. Ventral kinety located to right of girdle kinety, extends anteriad from posterior pole parallel to distal end of girdle kinety and terminates at subequatorial level (Figs 3B, H; 4F, arrowhead). The girdle kinety and ventral kinety have inverse orientation. No “extra” kinety detected.

Oral apparatus consists of an endoral membrane on inner wall of buccal lip and a membranellar zone (Figs 3B, C). Buccal cavity deep and prominent, extending obliquely to the right and terminates about 1/3 of the way down the body (Figs 3A; 4A, E, arrows). Membranellar zone bipartite with anterior and ventral portions comprising about 17 (16–19) and 10 (10–12) membranelles respectively, all of which are composed of three rows of basal bodies (Figs 3A, B; 4A). Cilia of anterior membranelles about 20–25  $\mu\text{m}$  long, stretching laterally or even slightly posteriorly as shown in Figs 3A, 4A. Endoral membrane (E) on inner wall of buccal lip on right side of oral cavity, probably composed of a single row of monokinetids but rarely recognizable either in protargol preparations or *in vivo*, probably due to being covered by perilemma (Fig. 3B). Pharyngeal fibres not found.

**Remarks:** *Spirostrombidium schizostomum* was originally reported by Kahl (1932) under the name *Strombidium schizostomum*. Its infraciliature, however, remained undescribed until the present study. Consequently we identify our organism mainly on its basic morphology *in vivo* and habitat, *viz.* the cell shape and size, the shape of the macronucleus, the number of anterior membranelles, and its marine biotope. Kahl (1932) mentioned the presence of 4 “pointed” membranelles in his organism. We also found several anterior membranelles that appear somewhat pointed in some, though not all, specimens during live observation. We do not, however, consider this to be a significant character for species circumscription. Furthermore, the buccal cavity of Kahl’s population is narrow, almost entirely covered with the buccal lip, and extends almost longitudinally. We surmise these differences could be population-dependent and should not be exaggerated. Given the strong similarities between the Qingdao population and that described by Kahl (1932), we are confident that the two are conspecific.

*Spirostrombidium pseudocinctum* (Wang, 1934) Petz, Song *et* Wilbert, 1995 resembles *S. schizostomum* in having similar cell size after staining, a similar number of dikinetids in the girdle kinety and in lacking thigmotactic membranelles. The former can nevertheless be clearly separated from *S. schizostomum* by: (1) the girdle kinety spirals only once around the cell (*vs.* girdle kinety spirals almost twice in *S. schizostomum*); (2) the horizontal arrangement of the ventral membranelles (*vs.* ventral membranelles almost longitudinally positioned in *S. schizostomum*); (3) the greater number of anterior membranelles (26–29 *vs.* 16–19 in *S. schizostomum*) and ventral membranelles (14–17 *vs.* 10–12 in *S. schizostomum*); (4) the number of dikinetids in the ventral kinety (*ca* 6 *vs.* 12–18 in *S. schizostomum*) (Petz *et al.* 1995).

*Spirostrombidium cinctum* (Kahl, 1932) Petz, Song *et* Wilbert, 1995 differs from *S. schizostomum* in having extra kinety (*vs.* absence), more anterior membranelles (23–28 *vs.* 16–19), more dikinetids in the ventral kinety (18–29 *vs.* 12–18), and 3–4 thigmotactic membranelles (*vs.* absent) (Xu and Song 2006).

Based on both morphology of live specimens and infraciliature (Petz *et al.* 1995, Song and Packroff 1997, Lei *et al.* 1999, Song *et al.* 1999, Xu and Song 2006, present study), a key to the identification of the seven *Spirostrombidium* spp. that have been examined following silver impregnation is supplied:

1 Thigmotactic membranelles present.....	2
1' Thigmotactic membranelles absent.....	4
2 Extra kinety present.....	3
2' Extra kinety absent.....	<i>S. cinctum</i>
3 Girdle kinety originates from mid-ventral cell.....	<i>S. agathae</i>
3' Girdle kinety originates from dorsal side of cell.....	<i>S. urceolare</i>
4 Girdle kinety spirals approximately twice around cell.....	<i>S. schizostomum</i>
4' Girdle kinety spirals approximately once around cell.....	5
5 Free living.....	6
5' Endocommensal in sea urchin.....	<i>S. echini</i>
6 >25 anterior membranelles.....	<i>S. pseudocinctum</i>
6' <25 anterior membranelles.....	<i>S. platum</i>

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