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## Morphology of Three Aloricate Choreotrich ciliates, Including Description of a New Species *Parastrombidinopsis costalis* sp. n. (Ciliophora, Choreotrichia), and phylogeny of the genus *Parastrombidinopsis*

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**Abstract.** As a follow-up part of our studies of the tropical ciliate fauna carried out in the last decade, we investigated the morphology and phylogeny of three choreotrich ciliates, viz., *Parastrombidinopsis costalis* sp. n., *P. pelagica* (Fauré-Fremiet, 1924) comb. n. and *P. minima* Tsai *et al.*, 2008. The new species is characterized by its unique asymmetrical cell shape, 19–21 collar membranelles, one buccal membrane, and eight unevenly distributed somatic kineties. An improved diagnosis of *P. pelagica* (Fauré-Fremiet, 1924) comb. n. is given based on the original and current studies, the species is characterized by a large cell size, an elongate obconical cell shape, 31–36 collar membranelles and 13–15 somatic kineties. The species *P. minima* is redescribed based on a new population with some new features supplemented. SSU rRNA genes of both the new species and *P. minima* were sequenced and a phylogenetic review of related taxa obtained has been performed in order to reveal their systematic relationships. The monophyly of the genus *Parastrombidinopsis* is highly supported in our phylogenetic analyses.

**Keywords:** choreotrich ciliates; ciliature; new combination; Strombidinopsidae; SSU rRNA gene; taxonomy

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## INTRODUCTION

Aloricate choreotrich ciliates, together with tintinnid and oligotrich ciliates, are often dominant components of the marine microzooplankton, and play important roles in the microbial food web (Liu *et al.* 2017, Santoferrara and McManus 2017, Santoferrara *et al.* 2017, Smith *et al.* 2018, Song *et al.* 2018, Wang *et al.* 2018, Zhang *et al.* 2017). Comprising four families, aloricate choreotrichs have been well investigated in terms of morphology and phylogeny (Lynn and Montagnes 1988; Montagnes and Taylor 1994; Petz *et al.* 1995; Song and Packroff 1997; Song and Bradbury 1998; Song *et al.* 1999, 2000, 2009; Agatha 2003; Agatha *et al.* 2005; Song 2005; Agatha and Strüder-Kypke 2007, 2012; Gao *et al.* 2009, 2016a, b, 2017; Xu *et al.* 2009, 2017; Kim *et al.* 2010; Zhang *et al.* 2010; Li *et al.* 2013; Huang *et al.* 2014, 2016; Chen *et al.* 2016; Yan *et al.* 2016; Zhao *et al.* 2017), and some new taxa have been reported in recent years (Kim *et al.* 2005, Xu *et al.* 2006, 2008, Alekperov *et al.* 2007, Liu *et al.* 2012, 2016, Chen *et al.* 2017).

Members of the choreotrich family Strombidinopsidae Small & Lynn, 1985 are mostly small to medium-size, spheroid to conoid ciliates that are characterized by the possession of a circle of collar membranelles and longitudinal kineties equally distributed around the cell (Agatha 2003, Lynn 2008, Liu *et al.* 2012). There are three genera in the family, namely, *Strombidinopsis* Kent, 1881 (type), *Parastrombidinopsis* Kim *et al.*, 2005, and *Parastrombidium* Fauré-Fremiet, 1924. *Parastrombidinopsis* differs from the others in having an open zone of collar membranelles which are not distally elongated. Up to now, *Parastrombidinopsis* includes only two species (Kim *et al.* 2005, Tsai *et al.* 2008).

During a faunistic study of choreotrich ciliates in China, three *Parastrombidinopsis* species, including one new form, were isolated; here, their morphological information and SSU rRNA gene sequences are reported.

## MATERIALS AND METHODS

### Collection, isolation and morphological studies

Information on sampling sites and ecological features are displayed in Table 1.

Samples of *Parastrombidinopsis costalis* sp. n. (Zhuhai) and *P. minima* were collected directly from surface coastal waters (0–

0.5 m) using 100 ml bottles (Figs 1C, D); samples of *P. costalis* sp. n. (Sanya) were collected directly from mangrove wetland using 100 ml bottles (Fig. 1E); samples of *Parastrombidinopsis pelagica* (Fauré-Fremiet, 1924) comb. n. were collected using a 20 µm mesh plankton net from surface coastal water (0–0.5 m) (Fig. 1B). The samples were then transferred to Petri dishes and the specimens were immediately isolated for further investigation. The morphology of living cells was studied using bright-field and differential interference contrast microscopy. Live observations were performed at ~20 °C. Protargol staining (Wilbert 1975) was used to reveal the infraciliature, and protargol was made according to Pan *et al.* (2013). Drawings of live cells were based on photomicrographs; those of silver-stained cells were made with the help of a camera lucida. Counts and measurements were performed at a magnification of ×1000. Terminology is according to Agatha and Riedel-Lorjé (2006) and taxonomic system is mainly according to Gao *et al.* (2016a).

### Extraction, amplification and sequencing of DNA

Genomic DNA was extracted using a DNeasy Blood & Tissue kit (Qiagen, CA) following the manufacturer's instruction (1–5 cells per 45 µl of ATL solution). Unfortunately, the DNA extraction of Sanya population of *P. costalis* sp. n. and *P. pelagica* failed, and thus only *P. minima* and Zhuhai population of *P. costalis* sp. n. are available for further study. The PCR conditions and the primers used to amplify the SSU rRNA gene followed that of previous studies (Medlin *et al.* 1988). PCR products were sequenced without cloning. Sequencing was conducted bidirectionally on an ABI-PRISM 3730 sequencer (Shanghai Sunny Biotechnology Co. Ltd., Shanghai, China). The sequences were assembled by ContigExpress.

### Phylogenetic analyses

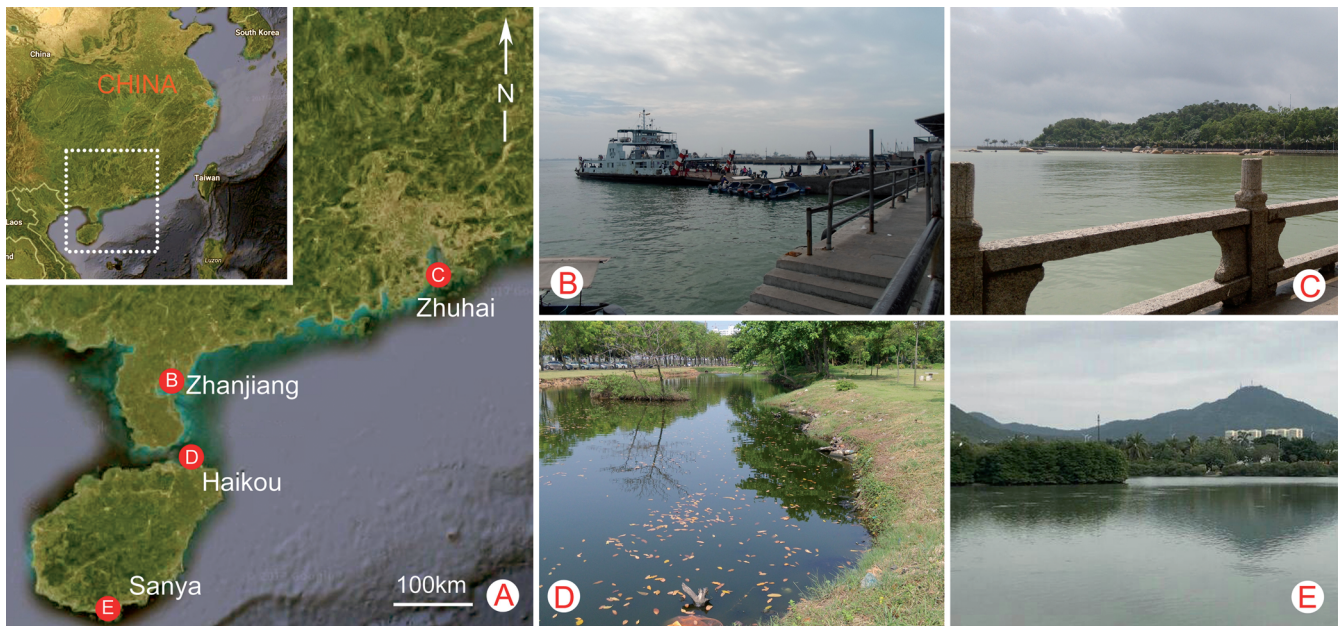
In addition to the two species of *Parastrombidinopsis* in this study, the SSU rRNA gene sequences of 42 species of Choreotrichia and Oligotrichia obtained from GenBank were used to construct the phylogenetic trees; five species (representing five genera) of Halteriidia and Hypotrichia were used as the outgroup taxa.

All 49 sequences were aligned using Bioedit (Hall 1999) with default parameters, and the ends of alignments were trimmed yielding a matrix of 1650 characters. Maximum likelihood (ML) analyses were conducted using RaxML-HPC2 on XSEDE (8.2.10) (Stamatakis 2006, Stamatakis *et al.* 2008) with the default model provided on the online server CIPRES Science Gateway (Miller *et al.* 2010). The reliability of internal branches was assessed using a nonparametric bootstrap method with 10<sup>3</sup> replicates.

Bayesian inference (BI) analysis was performed using MrBayes 3.2.6 on XSEDE v 3.2.6 (Ronquist and Huelsenbeck 2003) provided on the CIPRES Science Gateway with the model GTR+I+G selected by the Akaike Information Criterion (AIC) in MrModeltest v2 (Nylander 2004). Markov chain Monte Carlo analyses were run for 4×10<sup>6</sup> generations with two parallel runs, each with four simultaneous chains, sampling every 100 generations. The first 10000 trees were discarded as burn-in. The remaining trees were used to calculate the posterior probabilities (PP) applying the majority rule consensus.

**Table 1.** Ecological features of the sampling sites for the three *Parastrombidinopsis* species. T, water temperature; S, salinity.

Species	T °C	S ‰	pH	Sampling site	Date
<i>P. costalis</i> sp. n. Zhuhai pop.	29.2	2.5	8.0	Zhujiang estuary in Zhuhai (22°29'N, 113°58'E), China	June, 2014
<i>P. costalis</i> sp. n. Sanya pop.	27.0	30.0	–	coastal waters off Sanya (18°14'N, 109°31'E), China	April, 2018
<i>P. pelagica</i> (Fauré-Fremiet, 1924) comb. n.	25.2	25.0	7.8	coastal waters off Zhanjiang (21°14'N, 110°35'E), China	November, 2013
<i>P. minima</i> Tsai <i>et al.</i> , 2008	29.9	18.2	8.8	brackish waters in Haikou (20°06'N, 110°33'E), China	April, 2014

**Fig. 1.** Satellite photograph of South-East China (A) and pictures of sampling sites (B–E). (A) The position of Zhanjiang, Zhuhai, Haikou and Sanya. (B) Coastal waters off Zhanjiang. (C) Coastal waters off Zhuhai. (D) Brackish waters in Haikou. (E) Mangrove wetland in Sanya.

## RESULTS

Subclass Choreotrichia Small & Lynn, 1985  
 Order Choreotrichida Small & Lynn, 1985  
 Family Strombidinopsidae Small & Lynn, 1985  
 Genus *Parastrombidinopsis* Kim *et al.*, 2005

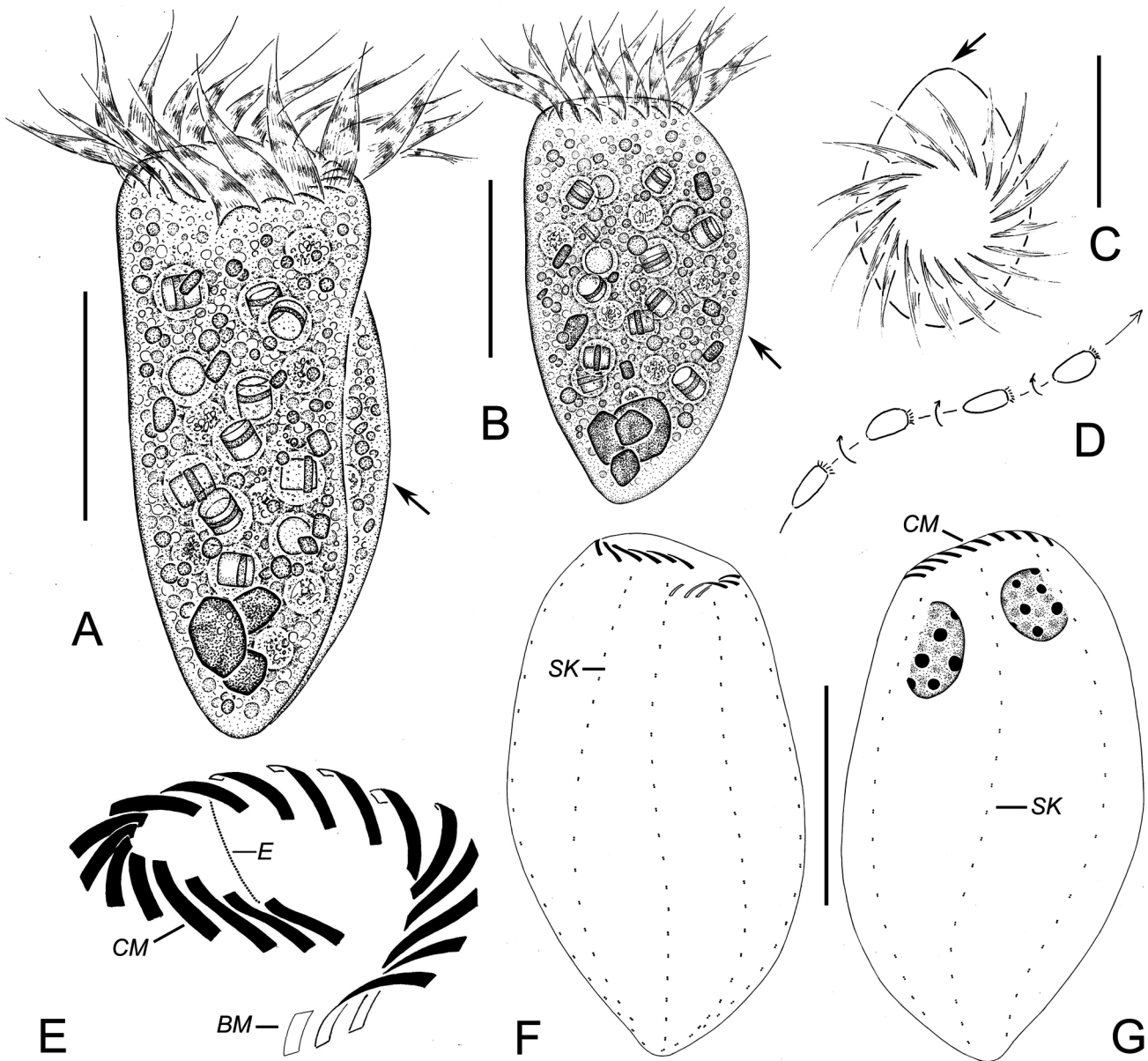
### *Parastrombidinopsis costalis* sp. n. (Fig. 2, 3, Table 2)

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The diagnosis and description are based on two populations, one collected from Zhuhai and one collected

from Sanya. The Zhuhai population is the type population for this new species.

**Diagnosis:** Cell size 30–70 × 15–40 μm *in vivo*, 25–73 × 13–42 μm after protargol staining. Body asymmetrically cylindrical with an obvious longitudinal ridge on dorsal side. One buccal membranelle and 16–21 collar membranelles, with proximal end located below the distal end. Six to eight somatic kineties with three to five kineties extending ventrally and three kineties extending dorsally, each composed of 8–33 diki-netids. Two ellipsoidal macronuclear nodules. Brackish and marine habitat.



**Fig. 2.** *Parastrombidinopsis costalis* sp. n. of Zhuhai population from life (A–D) and after protargol staining (E–G). (A, B) Ventral and left lateral views of representative individuals; arrows mark the dorsal ridges. (C) Apical view, showing collar membranelles and body shape; arrow marks the dorsal ridge. (D) Locomotion. (E) Oral ciliature. (F, G) Ventral and dorsal views of the same specimen. BM, buccal membranelle; CM, collar membranelles; E, endoral membrane; SK, somatic kinety. Scale bars: 25  $\mu$ m.

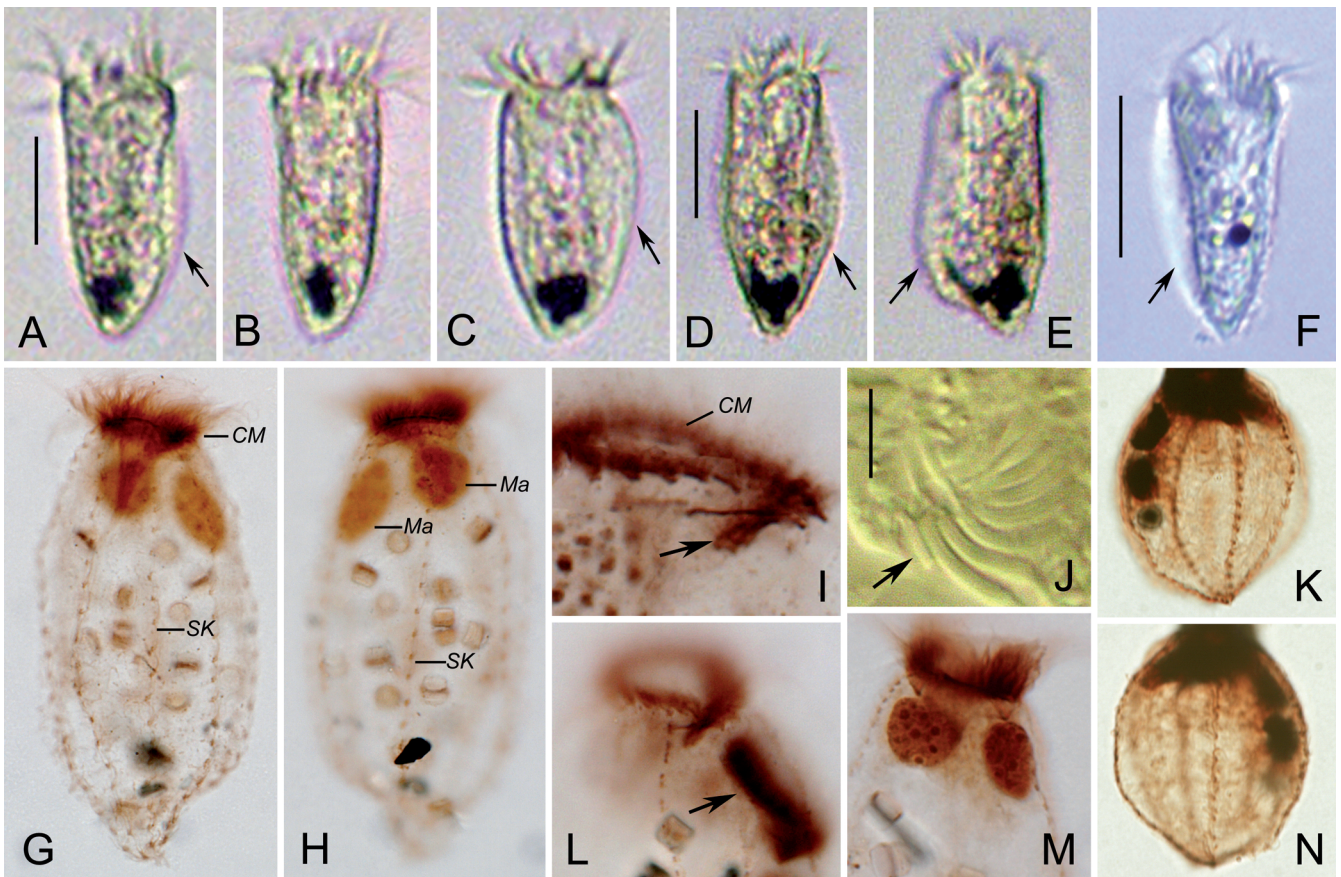
**Type locality:** Zhujiang estuary in Zhuhai (22°29'N, 113°58'E), China. Salinity about 2.5 ‰.

**Type material:** A protargol slide containing the holotype specimen marked with an ink circle and another two protargol slides containing the paratype specimens are deposited in Laboratory of Protozoology,

OUC, China (registration number: SW2014060201-1, SW2014060201-2).

**Etymology:** The Latin word 'costalis' used as species name refers to the longitudinal ridge of cell.

**Gene sequence data:** The small subunit rRNA gene sequence of *Parastrombidinopsis costalis* sp. n. col-



**Fig. 3.** Photomicrographs of *Parastrombidinopsis costalis* sp. n. from life (A–F, J) and after protargol staining (G–I, K–N), (A–E, G–J, L, M) from Zhuhai population, and (F, K, N) from Sanya population. (A–C) Ventral-left, ventral, left-lateral views of one individual, arrows mark the dorsal ridge. (D, E) Ventral-left, ventral-right views of one individual; arrows mark the dorsal ridge. (F) Ventral-right view of one individual, arrow marks the dorsal ridge. (G, H) Ventral and dorsal views of one specimen, showing the somatic ciliature. (I) Ventral view of oral ciliature; arrow marks buccal membranelle. (J) Bases of oral membranelles; arrow marks buccal membranelle. (L) An early divider; arrow marks the oral primordium. (M) Two macronuclei. (K, N) Ventral and dorsal views of one specimen, showing the somatic ciliature. CM, collar membranelles; Ma, macronucleus; SK, somatic kinety. Scale bars: 25  $\mu\text{m}$  (A, D); 20  $\mu\text{m}$  (F); 3  $\mu\text{m}$  (J).

lected from Zhuhai was deposited in GenBank with accession number MH469225.

**Description:** Cell size 60–70  $\times$  25–40  $\mu\text{m}$  *in vivo*, 50–73  $\times$  23–42  $\mu\text{m}$  after protargol staining in Zhuhai population, cell size 30–40  $\times$  15–20  $\mu\text{m}$  *in vivo*, 25–45  $\times$  13–26  $\mu\text{m}$  after protargol staining in Sanya population. Body elongated cylindrical with anterior end transversely truncated and posterior end bluntly pointed; length: width ratio about 2:1 (Figs 2A, 3A–F). An obvious ridge longitudinally extending in the middle of dorsal side in all individuals, making the outline of the cell asymmetrical under lateral and apical views (Figs 2B, C, 3C). The ridge not recognised after protargol staining.

Cytoplasm colourless, with some lipid droplets about 2  $\mu\text{m}$  across and food vacuoles  $\sim$  5  $\mu\text{m}$  across contain-

ing remnants of ingested bacteria and diatoms; in Zhuhai population, several mineral grains usually found in posterior cell portion, rarely in the middle of cell, dark *in vivo* (Figs 2A, B, 3A–E). Cell surface smooth, without mineral envelope. Extrusomes and contractile vacuole not observed. Two macronuclear nodules located in the anterior one-fourth to half of cell, 9–15  $\times$  5–11  $\mu\text{m}$  in size after protargol staining in Zhuhai population, 4–11  $\times$  3–8  $\mu\text{m}$  in size after protargol staining in Sanya population, ellipsoidal, with several nucleoli 2–3  $\mu\text{m}$  across (Figs 2G, 3M). Micronucleus not recognisable in either live or fixed specimens. Locomotion by swimming forward while rotating about the main cell axis (Fig. 2D).

Oral apparatus *in vivo* about 25–30  $\mu\text{m}$  across and 13–20  $\mu\text{m}$  across after protargol impregnation in Zhuhai

**Table 2.** Morphometric data on *Parastrombidinopsis costalis* sp. n. of Zhuhai population (first row), *P. costalis* sp. n. of Sanya population (second row), *P. pelagica* (Fauré-Fremiet, 1924) comb. n. (third row) and *P. minima* (fourth row).

Character	Min	Max	Mean	SD	SE	CV	n
Body length	50	73	60.9	7.23	1.66	11.9	19
	25	45	34.8	5.25	1.36	15.1	15
	95	135	116.4	13.93	5.26	12.0	7
	67	83	77.4	4.57	1.02	5.9	20
Body width	23	42	32.9	4.86	1.15	14.8	18
	13	26	19.6	3.44	0.89	17.5	15
	40	66	54.3	9.55	3.61	17.6	7
	55	75	65.4	5.36	1.23	8.2	19
Anterior cell end to buccal vertex, distance	5	8	5.9	1.09	0.27	18.5	16
	4	7	5.2	0.83	0.19	16.0	20
	13	15	14.0	0.71	0.32	5.1	5
	7	15	10.2	2.32	0.53	22.8	19
Oral diameter	13	20	15.4	1.57	0.36	10.2	19
	8	19	11.9	2.51	0.58	21.1	19
	30	38	34.3	2.55	0.85	7.4	9
	24	31	28.3	1.83	0.39	6.5	22
Number of collar membranelles	19	21	19.8	0.84	0.37	4.2	5
	16	19	17.7	1.53	0.88	8.6	3
	31	36	33.2	1.94	0.79	5.9	6
	16	18	16.6	0.70	0.22	4.2	10
Number of buccal membranelles	1	1	1.0	0.00	0.00	0.0	2
	1	1	1.0	0.00	0.00	0.0	2
	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
Number of somatic kineties	8	8	8.0	0.00	0.00	0.0	19
	6	8	7.1	0.44	0.10	6.2	21
	13	15	14.3	0.76	0.29	5.3	7
	14	15	14.4	0.49	0.12	3.4	17
Number of dikinetids in per somatic kinety	17	33	21.5	3.35	0.60	15.6	31
	8	22	15.2	3.56	0.84	23.4	18
	52	79	61.6	7.54	2.27	12.2	11
	25	39	31.3	5.31	2.01	17.0	7
Number of macronuclei	2	2	2.0	0.00	0.00	0.0	19
	2	2	2.0	0.00	0.00	0.0	18
	2	2	2.0	0.00	0.00	0.0	11
	2	2	2.0	0.00	0.00	0.0	22
Macronucleus length	9	15	11.6	1.70	0.68	14.6	17
	4	11	7.9	1.81	0.43	23.0	18
	10	17	14.3	2.43	0.92	17.0	7
	14	21	16.9	2.34	0.62	13.8	14
Macronucleus width	5	11	8.2	1.70	0.44	16.5	17
	3	8	4.4	1.38	0.33	31.4	18
	8	12	10.1	1.57	0.59	15.5	7
	12	14	13.2	0.70	0.19	5.3	14
Number of micronuclei	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
	3	3	3.0	0.00	0.00	0.0	2
	–	–	–	–	–	–	–

All data are based on randomly selected protargol-stained specimens (Wilbert, 1975). Measurements in  $\mu\text{m}$ . CV, coefficient of variation in %; Max, maximum; Mean, arithmetic mean; Min, minimum; n, number of specimens investigated; SD, standard deviation; SE, standard error of arithmetic mean; '–', the character not observed or detected.

population, for Sanya population, oral apparatus *in vivo* about 15–20  $\mu\text{m}$  across and 8–19  $\mu\text{m}$  across after protargol impregnation; oral cavity extending posteriad to about one-tenth of cell length. Adoral zone of membranelles (AZM) composed of 19–21 collar and one buccal membranelles in Zhuhai population, 16–19 collar and one buccal membranelles in Sanya population, both with a conspicuous gap at the ventral side. The AZM slightly spiralled with proximal end located below the distal end. The cilia of the collar membranelles about 15  $\mu\text{m}$  long in Zhuhai population, about 10  $\mu\text{m}$  long in Sanya population, while the bases of the collar membranelles extend obliquely across the peristomial rim, each composed of three rows of basal bodies; bases about 2  $\mu\text{m}$  long, except for two to three elongate bases at the proximal end of the AZM, about 3  $\mu\text{m}$  long, extending into the oral cavity. One buccal membranelle located parallel to the most proximal collar membranelle on the oral cavity, with a base about 1  $\mu\text{m}$  long (Figs 2E, 3I, J). Endoral membrane inconspicuous, composed of a single row of basal bodies, lying on the inner wall of the buccal cavity (Fig. 2E).

Somatic ciliature arranged in six to eight kineties which generally commence below the membranelar zone and extend longitudinally to the posterior end of cell, without forming a subterminal or subcaudal suture. In stained specimens of Zhuhai population, five somatic kineties arranged on ventral side with distance between adjacent kineties about 8  $\mu\text{m}$ , three somatic kineties arranged on dorsal side, with distance between adjacent kineties about 15  $\mu\text{m}$  (Figs 2F, G, 3G, H). Each somatic kinety consisting of 17–33 dikinetids; in each dikinetid, both basal bodies bear a 3  $\mu\text{m}$  long cilium. In stained specimens of Sanya population, three to five somatic kineties arranged on ventral side with distance between adjacent kineties about 7  $\mu\text{m}$ , three somatic kineties arranged on dorsal side, with distance between adjacent kineties about 10  $\mu\text{m}$  (Figs 3K, N). Each somatic kinety consisting of 8–22 dikinetids; in each dikinetid, both basal bodies bear a 2  $\mu\text{m}$  long cilium. One early divider was observed in Zhuhai population, its oral primordium located anteriorly at the body left (Fig. 3L).

***Parastrombidinopsis pelagica* (Fauré-Fremiet, 1924) comb. n.** (Figs 4, 5, Table 2)

**Synonyms:** *Strobilidium pelagicum* Fauré-Fremiet, 1924

*Strombidinopsis pelagicum* (Fauré-Fremiet, 1924) Jankowski, 2007

This species was simply described by Fauré-Fremiet (1924), here we present an improved diagnosis based on the original description and the present population.

**Improved diagnosis:** Cell size 100–170  $\times$  40–60  $\mu\text{m}$  *in vivo*, 95–135  $\times$  40–66  $\mu\text{m}$  after protargol staining. Body broadly elongated obconical with posterior portion pointed. About 31–36 collar membranelles with proximal end located below the distal end, no elongated membranelles and buccal membranelle. About 13–15 somatic kineties equally spaced around the body, with 52–79 dikinetids each. Three globular micronuclei, located between two ellipsoidal macronuclear nodules. Marine habitat.

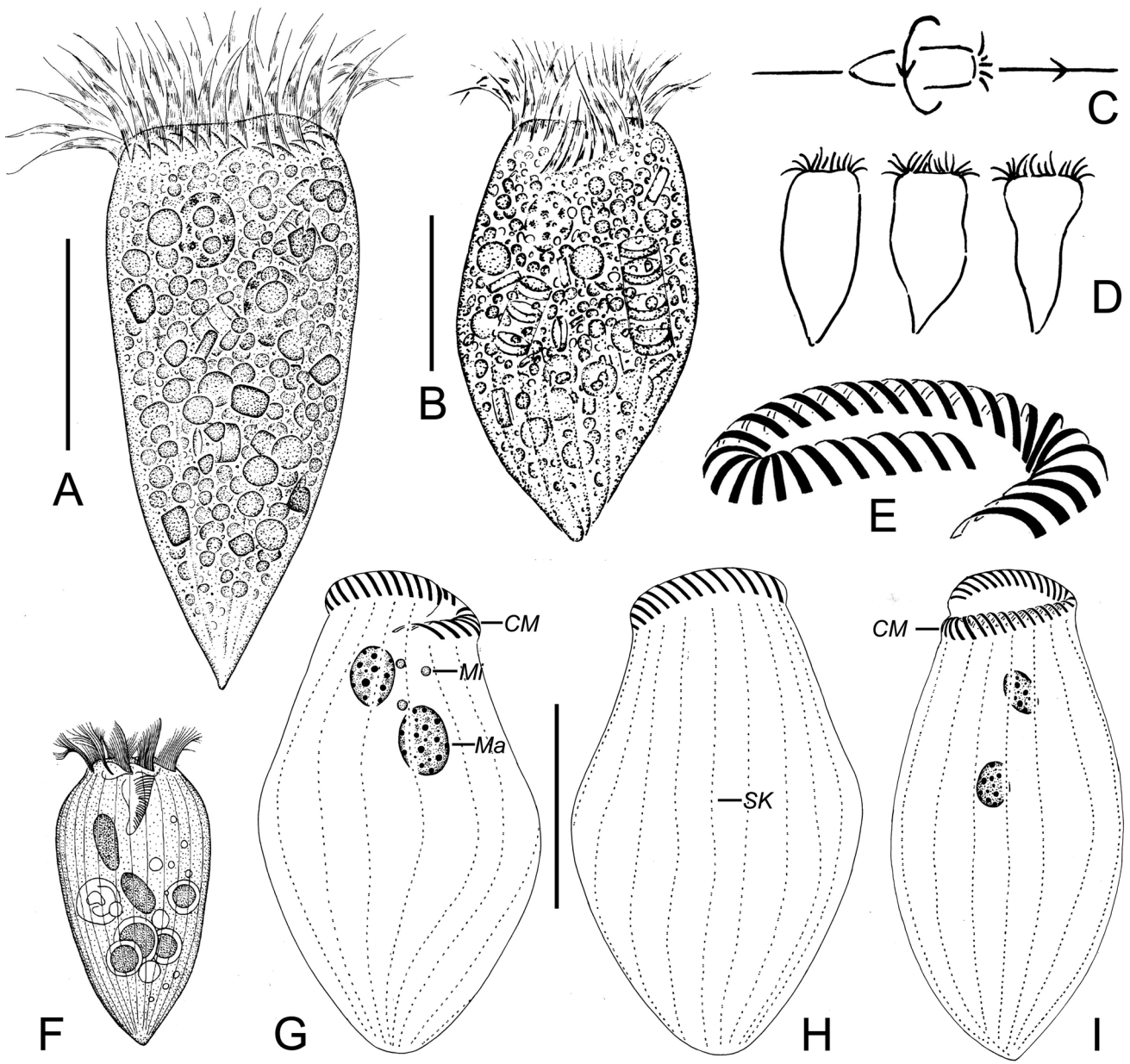
**Locality:** Coastal waters off Zhanjiang (21°14'N, 110°35'E), China. Salinity about 25.0 ‰.

**Voucher slide:** A protargol slide containing voucher specimens is deposited in the Laboratory of Protozoology, OUC, China (registration number: SW2013110202).

**Description:** Cell size 100–130  $\times$  40–60  $\mu\text{m}$  *in vivo*, 95–135  $\times$  40–66  $\mu\text{m}$  after protargol staining. Although body shapes vary from slimly to broadly elongated obconical, in essence, anterior two-thirds of cell cylindrical and posterior one-third of cell pointed; length: width ratio about 2.5:1 *in vivo*. Anterior end of body transversely truncated (Figs 4A, B, D, 5A–D).

Cytoplasm colourless, with some lipid droplets about 2  $\mu\text{m}$  across and food vacuoles  $\sim$  8  $\mu\text{m}$  across containing some yellow algae (Figs 5C, L). Cell surface smooth, without mineral envelope. Extrusomes and contractile vacuole not observed. Two macronuclear nodules arranged obliquely up and down and located in the anterior half of cell, 10–17  $\times$  8–12  $\mu\text{m}$  in size after protargol staining, ellipsoidal, with several nucleoli  $\sim$  2–3  $\mu\text{m}$  across. Three globular micronuclei about 2  $\mu\text{m}$  across, located between two macronuclear nodules (Figs 4G, 5F). Locomotion by swimming forward while rotating about the main cell axis, or by touching the substrate with collar membranelles (Fig. 4C).

Oral apparatus *in vivo* about 45  $\mu\text{m}$  across, but only 30–38  $\mu\text{m}$  across after protargol impregnation; oral cavity extending posteriad to about one-tenth of cell length (Figs 5E, G). About 31–36 collar membranelles formed a slightly spiralled membranelar zone, with a conspicuous gap at the ventral side, and cilia about 25  $\mu\text{m}$  long. Bases of the collar membranelles extend obliquely across the peristomial rim, about 10  $\mu\text{m}$  long; no elongate bases recognized (Figs 4E, I, 5K). Buccal membranelle not observed, nor the endoral membrane, which may be due to poor staining.

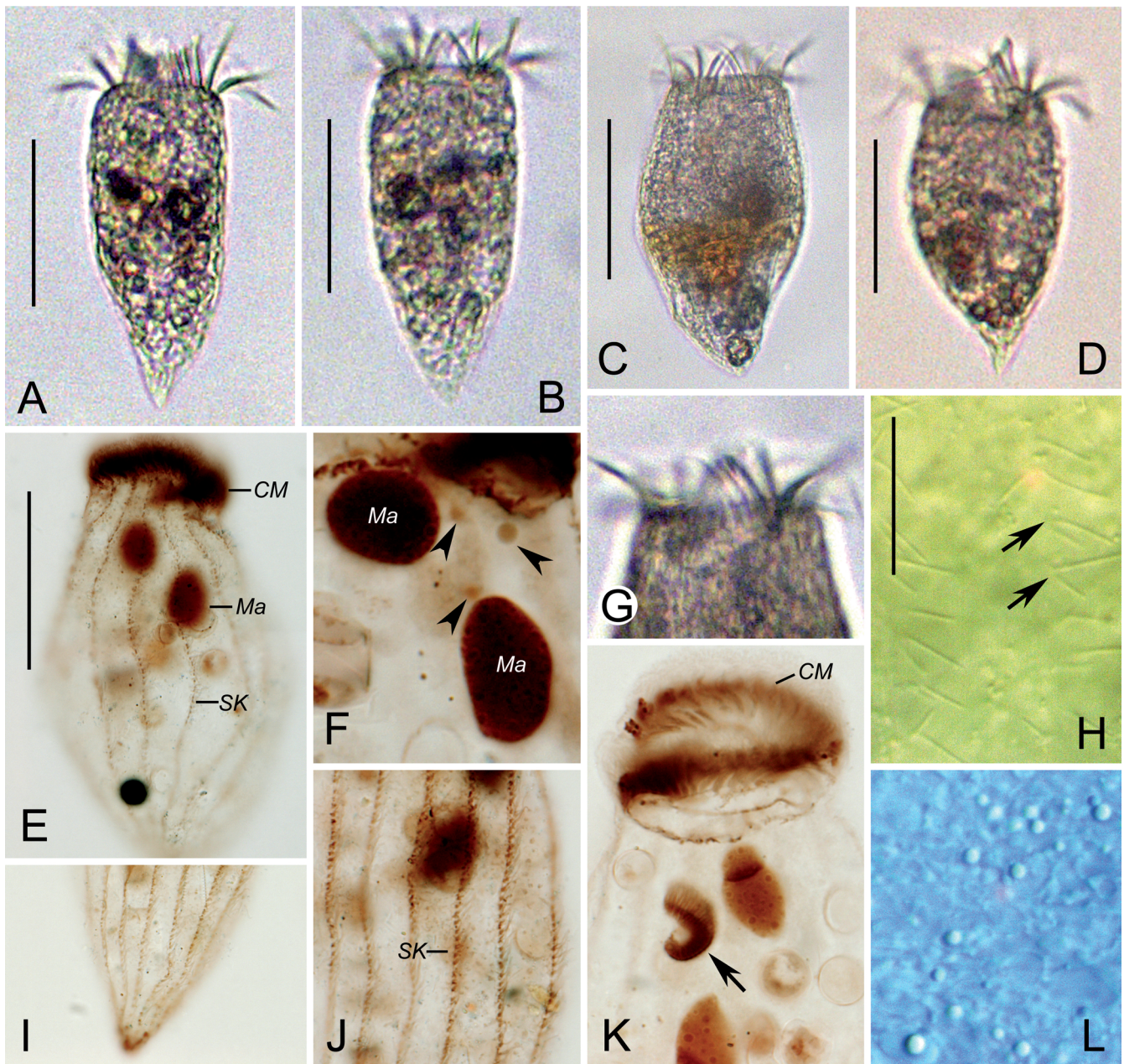


**Fig. 4.** *Parastrombidinopsis pelagica* (Fauré-Fremiet, 1924) comb. n. from life (A–D, F) and after protargol staining (E, G–I). (A, B) Ventral views of two individuals. (C) Swimming pattern. (D) Different individuals to show the variations in body shape. (E) Detail of oral membranelles. (F) From Fauré-Fremiet (1924). (G, H) Ventral and dorsal views of the same specimen showing the ciliature. (I) Lateral view of a specimen. CM, collar membranelles; Ma, macronucleus; Mi, micronucleus; SK, somatic kinety. Scale bars: 50  $\mu$ m.

Somatic ciliature arranged in 13–15 kineties and evenly distributed around body. The somatic kineties generally commence below membranelar zone and extend to posterior end of cell, without forming a subterminal or subcaudal suture (Figs 4H, 5I, J). Each somatic kinety consists of 52–79 dikinetids; in each dikinetid,

both basal bodies bear cilia, with anterior cilium about 4  $\mu$ m long and the posterior cilium about 2  $\mu$ m long (Fig. 5H). One early divider was observed; its oral primordium was located anteriorly at the left of the body (Fig. 5K).





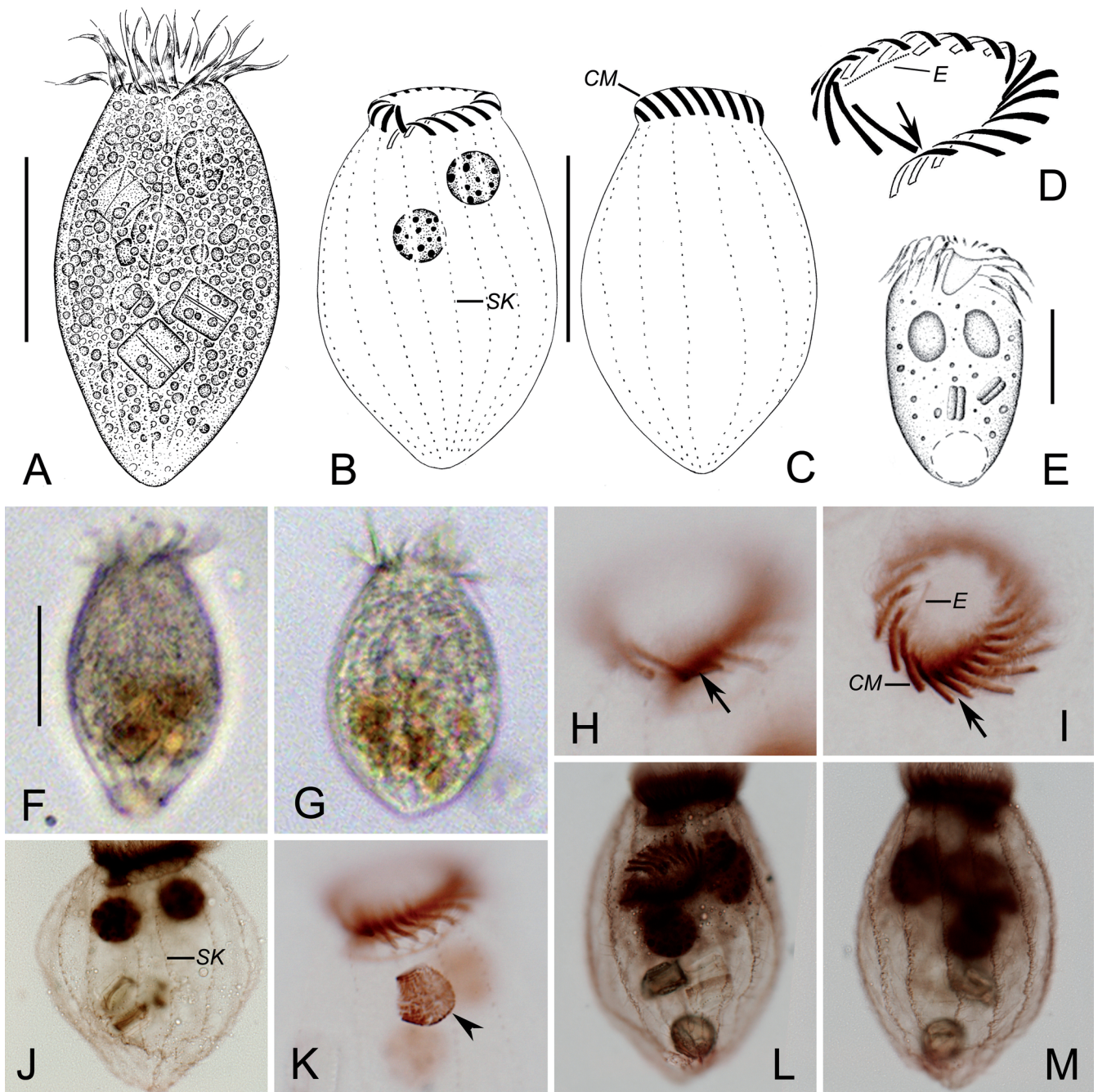
**Fig. 5.** Photomicrographs of *Parastrombidinopsis pelagica* (Fauré-Fremiet, 1924) comb. n. from life (A–D, G, H, L) and after protargol staining (E, F, I–K). (A–D) Four individuals, showing the cell shape. (E) Ventral view, showing ciliature. (F) Detail of macronuclei, arrowheads mark micronuclei. (G) Ventral view of oral membranelles. (H) Arrows mark somatic cilia. (I, J) Showing somatic kineties. (K) An early divider; arrow marks oral primordium. (L) Cytoplasm. CM, collar membranelles; Ma, macronucleus; SK, somatic kinety. Scale bars: 20  $\mu\text{m}$  (A–E); 10  $\mu\text{m}$  (H).

*Parastrombidinopsis minima* Tsai *et al.*, 2008 (Fig. 6, Table 2)

Although the morphology of *Parastrombidinopsis minima* was well studied by Tsai *et al.* (2008), some new features have been found in the Haikou population. Thus, a brief description of the new population is sup-

plied, along with a phylogenetic analysis based on its SSU rRNA gene sequence.

**Locality of the Haikou population:** Brackish water in Haikou (20°06'N, 110°33'E), China, Salinity about 18.2 ‰.



**Fig. 6.** Drawings and photomicrographs of *Parastrombidinopsis minima* from life (A, E–G) and after protargol staining (B–D, H–M). (A, F, G) Views of three individuals showing the body shape. (B, C) Ventral and dorsal views of the same specimen showing the ciliature. (D, H, I) Detail of collar membranellae; the arrows mark the ventral gap. (E) From Tsai *et al.* (2008). (J) Somatic ciliature. (K) An early divider; arrowhead marks the oral primordium. (L, M) Ventral and dorsal views of same specimen showing the ciliature. CM, collar membranellae; E, endoral membrane; SK, somatic kinety. Scale bars: 25  $\mu$ m.

**Voucher slide:** A protargol slide containing voucher specimens is deposited in the Laboratory of Protozoology, OUC, China (registration number: SW2014042501).

**Gene sequence data:** The small subunit rRNA gene sequence of *Parastrombidinopsis minima* Tsai *et al.*, 2008 was deposited in GenBank with accession number MH469226.

**Description:** Cell size 60–70 × 30–40 μm *in vivo*, 67–83 × 55–75 μm after protargol staining. Body broadly ellipsoidal with posterior one-third of cell bluntly pointed; length: width ratio about 1.5:1 *in vivo*. Adoral region narrower than body portion (Figs 6A, F, G).

Cytoplasm colourless, with some lipid droplets about 2 μm across and food vacuoles ~ 8 μm across containing some yellow algae (Figs 6F, G). Cell surface smooth, without mineral envelope. Extrusomes and contractile vacuole not observed. Two macronuclear nodules located in the anterior half of cell, 14–21 × 12–14 μm in size after protargol staining, global to ellipsoidal, with several nucleoli ~ 2–3 μm across (Figs 6B, J). Micronucleus not observed. Locomotion by swimming in slow motion, sometimes touching the substrate with collar membranelles.

Oral apparatus *in vivo* about 20 μm across, 24–31 μm across after protargol impregnation; oral cavity extending posteriad to about one-tenth of cell length. 16–18 collar membranelles arranged horizontally, with an inconspicuous gap at the ventral side, and cilia about 12 μm long. Bases of the collar membranelles extend obliquely across the peristomial rim, each composed of three rows of basal bodies, about 10 μm long; the most proximal three to four bases posteriorly prolonged and extending into the oral cavity. Among the elongated membranelles, the most proximal one slightly shorter than others in terms of the length of the base (Figs 6D, H, I). No buccal membranelle observed. Endoral membrane inconspicuous, composed of a single row of basal bodies and lying within the inner wall of buccal cavity (Figs 6D, I).

Somatic ciliature arranged in 14–15 kineties which generally commence below membranelar zone and extend to posterior end of cell, without forming a subterminal or subcaudal suture. Each somatic kinety consisting of 52–79 dikinetids; in each dikinetid, both basal bodies bear a 4 μm long cilium (Figs 6C, L, M).

#### SSU rRNA gene sequences and phylogenetic analyses

The SSU rRNA gene sequences of species *Parastrombidinopsis costalis* sp. n. (Zhuhai) and *P. minima* studied in this work have been deposited in GenBank. The lengths and GC contents, for *Parastrombidinopsis*

*costalis* sp. n. and *P. minima* SSU rRNA gene sequences are as follows: *Parastrombidinopsis costalis* sp. n. (1676 nt, GC = 46.72%); *P. minima* (1691 nt, GC = 46.48%).

Since the tree topologies from the BI and ML algorithms are similar, only the BI tree is presented, although support values from both methods are indicated at the branch nodes (Fig. 7). In both trees, all *Parastrombidinopsis* species cluster together, which are the sister to two *Strombidinopsis* species. In the clade, two sequences of *P. minima* form a group with full support; then cluster with *P. shimi* with support values of 0.99 BI, 80% ML. *Parastrombidinopsis costalis* sp. n. also clusters with them with support values 1.00 BI, 97% ML. The genus *Strombidinopsis* is polyphyly by forming two clusters, with one being the basal branch to the subclass Choreotrichia, the other one cluster with *Parastrombidinopsis* species.

The divergences (base differences per site) between four *Parastrombidinopsis* SSU rRNA gene sequences (1731 sites) were calculated, ranges from the lowest 0.0035 (two populations of *P. minima*) to the highest 0.0362 (*P. costalis* and *P. shimi*).

## DISCUSSION

### *Parastrombidinopsis costalis* sp. n.: comparison between populations and related species (Tables 2, 3)

The two populations correspond well with each other in terms of cell shape, longitudinal ridge and unevenly spaced somatic kineties, except the Sanya population is smaller than Zhuhai population in cell size (25–45 × 13–26 μm vs 50–73 × 23–42 μm after protargol staining), as well as other size related characters such as number of collar membranelles (16–19 vs 19–21) and number of somatic kineties (6–8 vs 8). We think the cell size difference can be considered as population-dependant, and the two populations are conspecific.

The genus *Parastrombidinopsis* was established by Kim *et al.* (2005). Prior to the present work, two species have been assigned to the genus: *P. shimi* Kim *et al.* 2005 and *P. minima* Tsai *et al.* 2008. *Parastrombidinopsis costalis* sp. n. can be separated from the other congeners and all the poorly described choreotrich species by its unique longitudinal ridge on dorsal side.

Besides, the new species differs from the type species *Parastrombidinopsis shimi* by its: 1) smaller cell size (30–70 × 15–40 μm vs. 100–170 × 40–60 μm *in vivo*); 2) fewer collar membranelles (16–21 vs. 36–48); and 3) fewer somatic kineties (6–8 vs. 36–50).

**Table 3.** Morphological comparison of *Parastrombidinopsis* species. All data are based on descriptions of the original and present populations. Measurements in  $\mu\text{m}$ . CM, collar membranelles; BM, buccal membranelles; SK, somatic kineties.

Character	<i>P. costalis</i> sp. n.	<i>P. shimi</i>	<i>P. minima</i>	<i>P. pelagica</i>
Body size <i>in vivo</i>	30–70 × 15–40	112–221 × 88–176	60–95 × 30–70	100–170 × 40–60
Body size after staining	25–73 × 13–42	88–225 × 55–163	43–83 × 23–75	95–135 × 40–66
Body shape	cylindrical with dorsal ridge	oval	cylindrical	elongated obconical
Oral diameter after staining	8–20	53–98	13–31	30–38
Number of CM	16–21	36–48	12–18	31–36
Number of BM	1	2	0	0
Number of SK	6–8	36–50	11–15	13–15
Number of dikinetids in per somatic kinety	8–33	68–105	20–39	52–79
Number of macronuclei	2	1–4	2	2
Number of micronuclei	–	–	–	3
Sources	present work	Kim <i>et al.</i> , 2005	Tsai <i>et al.</i> , 2008 and present work	Fauré-Fremiet, 1924 and present work

In addition, the new species differs from *P. minima* in the arrangement of the AZM (the proximal end located below the distal end vs. distributed on same horizontal level) and fewer somatic kineties (6–8 vs. 11–15).

#### ***Parastrombidinopsis pelagica* (Fauré-Fremiet, 1924) comb. n. and comparison with related species (Table 3)**

This species was first reported as *Strobilidium pelagicum* by Fauré-Fremiet (1924) with a brief description of the organism *in vivo*. Jankowski (2007) transferred the species to the genus *Strombidinopsis* Kent, 1881 based only on the original description. The Zhanjiang population corresponds well with the original description regarding the cell size, cell shape, macronucleus, oral membranelles, somatic kineties and habitat, thus, we think our population can be conspecific to *Strobilidium pelagicum* Fauré-Fremiet, 1924. Given the ventral gap of collar membranelle bases revealed by protargol staining, however, we think this species should be transferred to the genus *Parastrombidinopsis* Kim *et al.*, 2005 as *P. pelagica* (Fauré-Fremiet, 1924) comb. n.

*Parastrombidinopsis pelagica* (Fauré-Fremiet, 1924) comb. n. most resembles *P. shimi* in its cell size and number of collar membranelles, but can be easily separated from the latter by having fewer somatic kineties (13–15 vs. 36–50). *Parastrombidinopsis pelagica* (Fauré-Fremiet, 1924) comb. n. differs from *P. costalis* sp. n. by having: 1) a larger cell size (100–170 × 40–60  $\mu\text{m}$  vs. 60–70 × 25–40  $\mu\text{m}$  *in vivo*); 2) more collar membranelles (31–36 vs. 19–21); and 3) more somatic

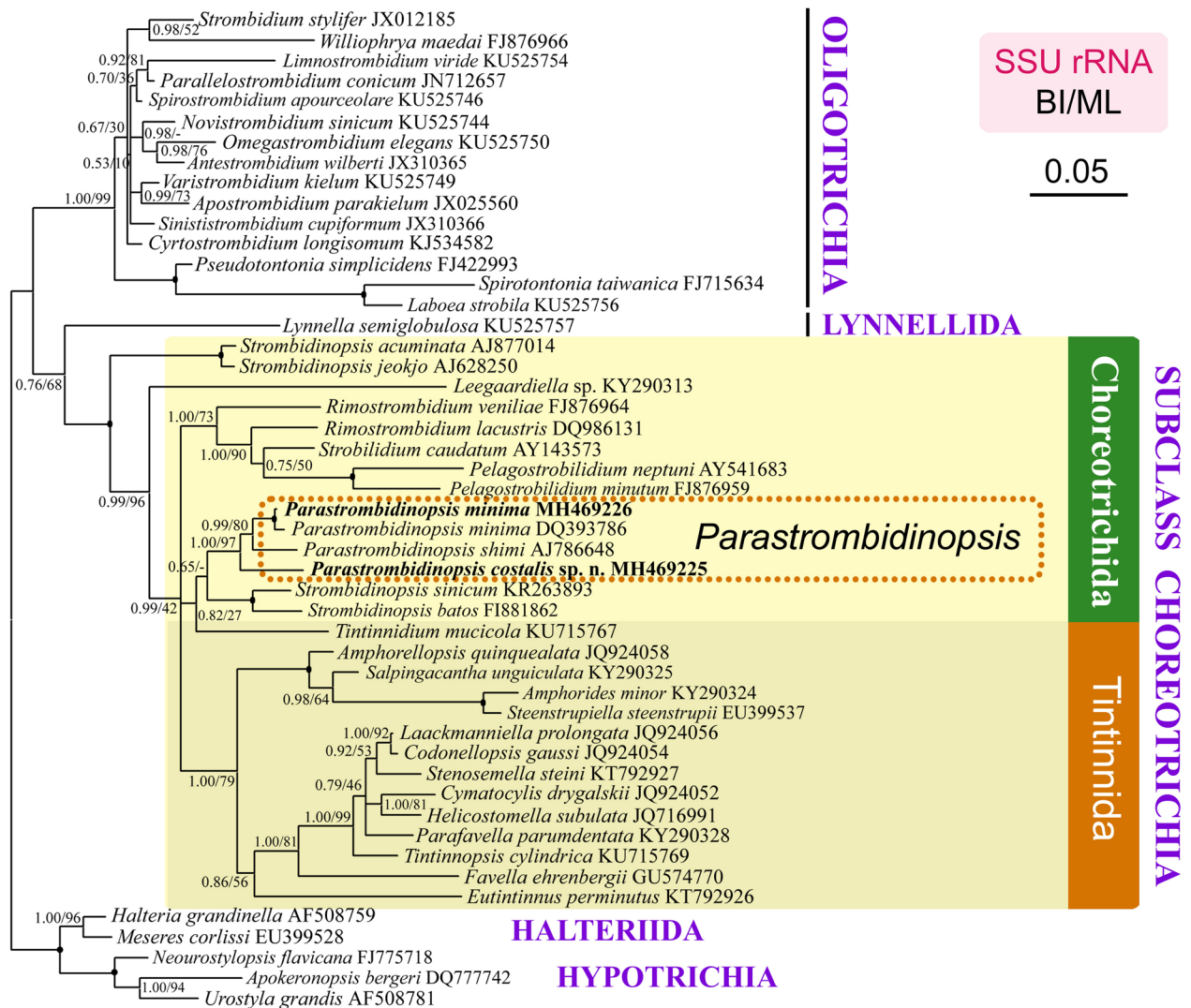
kineties (13–15 vs. 6–8). In addition, *Parastrombidinopsis pelagica* (Fauré-Fremiet, 1924) comb. n. can be separated from *P. minima* by: 1) the arrangement of the AZM (the proximal end located below the distal end vs. distributed on same horizontal level); 2) the larger cell size (100–170 × 40–60  $\mu\text{m}$  vs. 60–95 × 30–70  $\mu\text{m}$  *in vivo*); 3) and the presence of more collar membranelles (31–36 vs. 12–18).

#### ***Parastrombidinopsis minima* Tsai *et al.*, 2008 and comparison with related species (Table 3)**

*Parastrombidinopsis minima* was originally reported by Tsai *et al.* (2008) based on a Taiwan population. The Haikou population matches the original description in all characteristics, except some population-dependent differences: a slightly larger cell size (67–83 × 55–75  $\mu\text{m}$  vs. 43–71 × 23–42  $\mu\text{m}$  in the original population after protargol staining), accompanied by higher numbers of collar membranelles (16–18 vs. 12–16) and dikinetids in each somatic kinety (14–15 vs. 11–13). In addition, the large empty vacuole which was described in the posterior cell end of the original population, was not detected in the present population. We thought those differences should be population-dependent, therefore, the Haikou population is in fact conspecific to *Parastrombidinopsis minima* Tsai *et al.*, 2008.

#### **Phylogenetic analyses**

In both trees, four *Parastrombidinopsis* sequences group together with high support values suggesting the



**Fig. 7.** Bayesian-Inference tree inferred from SSU rRNA gene sequences, indicating the polygenetic positions of species of the genus *Parastrombidinopsis*. Numbers at the nodes represent support values in the following order: BI posterior probabilities and ML bootstrap values. Disagreements in topology between the BI and ML trees are indicated by a hyphen. Nodes that were well supported (1.00 BI; 100% ML) are represented by filled circles. Bar = 5 substitutions per 100 nucleotide positions. Species sequenced in the present study are shown in bold type.

monophyly of the genus as well as the generic affiliation of the new species. The cluster of the two sequences of *Parastrombidinopsis minima*, as well as their lowest divergence value among the four *Parastrombidinopsis* sequences, support their conspecific relationship. The genus *Strombidinopsis* is polyphyly, which is consistent with previous study (Liu *et al.* 2016). The new species shows highest divergences between the other congeners, valuing 0.0362 with *P. shimi*, 0.0321 and 0.0356 with *P. minima*, which is consistent with its high morphological difference with other congeners.

The ventrally opened AZM, which is commonly displayed in hypotrichs and oligotrichs, has been interpreted as an important plesiomorphic character in the class Spirotrichea, while the closed AZM in choreotrichs is regard as a derived state during evolution (Agatha 2004). Phylogenetic analyses based on molecular data, however, suggest that the *Parastrombidinopsis* species, who also have a ventral gap in the AZM, nested within the choreotrich clade rather than clustering with either hypotrichs or oligotrichs, as would be expected. Considering the morphological uniformity between *Parastrom-*

*bidinopsis* and other choreotrichs in terms of the distribution of the somatic kineties, as well as in the arrangement of the macronuclear nodules, *Parastrombidinopsis* surely belongs to the subclass Choreotrichia, as is also suggested by the molecular phylogeny tree. The ventral gap in *Parastrombidinopsis*, as well as *Parastrombidium* and *Lynnella*, probably occurred secondarily during the evolution and represents a synapomorphic retrogression to the plesiomorphic (open) state, as suggested in both Agatha and Strüder-Kypke (2012) and Liu *et al.* (2015).

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