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ORIGINAL ARTICLE

Morphological Characterizations of Four Species of *Parallelostrombidium* (Ciliophora, Oligotrichia), with a Note on the Phylogeny of the Genus

Wen Song[a,1], Lun Wang[a,1], Lifang Li[b], Saleh A. Al-Farraj[c], Abdullah Aleidan[c], Susan Smith[d] & Xiaozhong Hu[a]

a Laboratory of Protozoology, Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao 266003, China
b Marine College, Shandong University, Weihai 264209, China
c Zoology Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
d Department of Marine Science, University of Connecticut, Groton, Connecticut 06340, USA

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New species; oligotrichid ciliates; *Omegastrombidium*; SSU rRNA gene; Strombidiidae.

Correspondence
X. Hu, Institute of Evolution and Marine Biodiversity, OUC, Qingdao 266003, China
Telephone number: +86-0532-82032338;
FAX number: +86-0532-82032338;
e-mail: xiaozhonghu@ouc.edu.cn
and
L. Li, Marine College, Shandong University, Weihai 264209, China
Telephone number: +86-0631-5688303;
FAX number: +86-0631-5688303;
email: qd_liliy@sina.com

[1]These two authors contribute equally.

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ABSTRACT

The morphology and phylogeny of four oligotrichid ciliates, *Parallelostrombidium paraellipticum* sp. n., *P. dragescoi* sp. n., *P. jankowskii* (Xu et al. 2009) comb. n., and *P. kahli* (Xu et al. 2009) comb. n., are described or redescribed based on live observation, protargol stained material, and SSU rRNA gene sequences. The new species *P. paraellipticum* sp. n. is characterized by its obovoidal cell shape, adoral zone composed of 17–21 collar, 9–11 buccal, and two thigmotactic membranelles, and extrusomes attached in one row along the girdle kinety. The new species *P. dragescoi* sp. n. is distinguished from its congeners by its obovoidal cell shape and a lack of thigmotactic membranelles. Based on ciliary patterns recognizable in the original slides, *Omegastrombidium jankowskii* Xu et al. 2009 and *O. kahli* Xu et al. 2009 should be transferred to the genus *Parallelostrombidium* Agatha 2004. Phylogenetic analyses based on SSU rRNA gene sequence data demonstrate that all four new sequences cluster with previously described congeners. The genus *Parallelostrombidium* is separated into two clusters, suggesting its non-monophyly and probably corresponding to the two subgenera proposed by Agatha and Strüder-Kypke (2014), as well as their morphological difference (cell dorsoventrally flattened vs. unflattened).

EXISTING in great abundance among the marine microzooplankton, oligotrichids play a vital role in the planktonic food web (Dolan 2017; Dolan et al. 2016; Grattepanche et al. 2014; Santoferrara et al. 2016, 2017; Xu et al. 2017; Yan et al. 2016). However, the taxonomy and phylogeny of this group have poorly been studied, and only about 100 well described morphospecies have been documented (e.g. Chen et al. 2017; Lei et al. 1999; Liu et al. 2009, 2011, 2013, 2015a, 2016, 2017; Lynn and Montagnes 1988; Martin and Montagnes 1993; Montagnes et al. 1988, 1990; Petz et al. 1995; Song 2005; Song and Packroff 1997; Song et al. 2000, 2009, 2013, 2015a,b; Tsai et al. 2010, 2015; Wilbert and Song 2005; Xu et al. 2008). Among these, approximately 30 species from the subclass Oligotrichia have SSU rRNA gene sequence data available in GenBank (Gao et al. 2009, 2016a, 2017; Santoferrara and McManus 2017; Zhang et al. 2010). With the current progress in molecular technologies, recent studies have discovered large numbers of rare taxa in oligotrichids, indicating that this group is much more diverse than previously supposed (Chen et al. 2016a,b; Gao et al. 2012, 2014, 2016b; Liu et al. 2015a, 2017; Wang et al. 2017a,b; Xiong et al. 2016; Zhao et al. 2017).

Comprising ventral and girdle kineties, the ciliary pattern in strombidiids is highly variable (Agatha 2004; Agatha and Strüder-Kypke 2014; Jankowski 1978; Liu et al. 2011, 2015b; Song and Bradbury 1998; Xu et al. 2009). *Parallelostrombidium* Agatha 2004 was separated from the

genus *Spirostrombidium* Jankowski 1978 by its ventral kinety, which is parallel to the posterior portion of a dextrally spiralled girdle kinety with the same orientation. Simultaneously, *Spirostrombidium* was redefined to possess a posterior girdle kinety portion that is inversely orientated and parallel to the ventral kinety. Agatha and Strüder-Kypke (2014) proposed a division of *Parallelostrombidium* by establishing two subgenera, *Parallelostrombidium* Agatha and Strüder-Kypke 2014 and *Asymptokinetum* Agatha and Strüder-Kypke 2014;. The subgenus *Parallelostrombidium* is characterized by a ventral kinety that is entirely parallel to the girdle kinety, while the subgenus *Asymptokinetum* is characterized by a ventral kinety of which only the posterior portion is parallel to the girdle kinety. Prior to the present work, the subgenus *Parallelostrombidium* included two species, namely, *P. rhyticollare* and *P. siculum*, both of which lack molecular data (Montagnes and Taylor 1994; Petz et al. 1995). The subgenus *Asymptokinetum* included four species, namely, *P. obesum*, *P. paralatum*, *P. ellipticum* and *P. conicum*, for all of which SSU rRNA gene sequences are available (Liu et al. 2013, 2015a; Xu et al. 2006).

During a faunistic study of oligotrichid ciliates in China, four *Parallelostrombidium* species, including two new forms, were isolated; here, their morphologies and SSU rRNA gene sequences are reported.

MATERIALS AND METHODS

Collection, isolation, and morphological studies

Parallelostrombidium paraellipticum sp. n. was collected from the Zhujiang Estuary in Zhuhai (22°29′N, 113°58′E), China, in May 2014; the water temperature was 29.2 °C, the salinity was 2.5‰, and the pH was 8.0. *Parallelostrombidium dragescoi* sp. n. was collected from a brackish water lake in Haikou (20°06′N, 110°33′E), China, in April 2014; the water temperature was 29.9 °C, the salinity was 18.2‰, and the pH was 8.8. *Parallelostrombidium jankowskii* (Xu et al. 2009) comb. n. was isolated from coastal waters off Zhanjiang (21°14′N, 110°35′E), China, in October 2013; the water temperature was 24.6 °C, the salinity was 22.6‰, and the pH was 7.6. *Parallelostrombidium kahli* (Xu et al. 2009) comb. n. was isolated from coastal waters off Qingdao (36°05′N, 120°09′E), China, in October 2012; the water temperature was 21.5 °C, the salinity was 33.2‰, and the pH was 8.0 (Fig. S1).

All samples were collected directly from surface waters (0–0.5 m) in100 ml bottles. The samples were transferred into Petri dishes and immediately isolated for further investigation. The morphology of living cells was studied, using bright-field and differential interference contrast microscopy (OLYMPUS BX53). Live observations were performed at about 20 °C. Protargol staining following Wilbert's (1975), method was used to reveal the ciliary pattern and the nuclear apparatus; the protargol was made according to Pan et al. (2013). Drawings of live cells were based on photomicrographs, and ciliary patterns were drawn using a camera lucida. Counts and measurements were performed at a magnification of ×1,000. Terminology is according to Agatha and Riedel-Lorjé (2006), and the taxonomic system is mainly according to Gao et al. (2016b).

Extraction, amplification and sequencing of DNA

Genomic DNA was extracted, using a DNeasy Tissue kit (Qiagen, CA, USA), following the manufacturer's instructions. The PCR conditions and the primers used to amplify the SSU rRNA gene followed those of previous studies (Huang et al. 2016). Sequencing was conducted bidirectionally on an ABI-PRISM 3730 sequencer (Shanghai Sunny Biotechnology Co., Ltd., Shanghai, China).

Phylogenetic analyses

In addition to the newly obtained sequences of four *Parallelostrombidium* species in this study, the SSU rRNA gene sequences of 54 species of Oligotrichia and Choreotrichia obtained from GenBank were used to construct the phylogenetic trees; five species (representing five genera) of Halteriida and Hypotrichia were used as outgroup taxa.

All 63 sequences were aligned, using Bioedit (Hall 1999) with default parameters, and the ends of the alignments were trimmed, yielding a matrix of 1,624 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 of CIPRES Science Gateway (Miller et al. 2010). The reliability of internal branches was assessed, using a nonparametric bootstrap method with 10^3 replicates.

A 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+Γ selected by the Akaike Information Criterion (AIC) in MrModeltest v2 (Nylander 2004). Markov chain Monte Carlo chains were run for 4×10^6 generations with two parallel runs, each with four simultaneous chains, sampling every 100 generations. The first 10,000 trees were discarded as burn-in prior to consensus tree construction.

An approximately Unbiased (AU) test was used to test the monophyly of the genus *Parallelostrombidium*.

RESULTS

Description of *Parallelostrombidium paraellipticum* sp. n

Cell size 40–60 × 30–45 μm in vivo, 39–57 × 28–43 μm after protargol staining. Body obovoidal and asymmetrical, dorsoventrally flattened, with a width: thickness ratio of about 1.5:1 (Fig. 1A, B, 2A–D). Anterior end domed with a conspicuous apical protrusion about 5 μm high, at right side of peristome (Fig. 1A, B). Posterior end broadly rounded and slightly slanted to the right. Widest at level of horizontal part of girdle kinety. Hemitheca not recognizable.

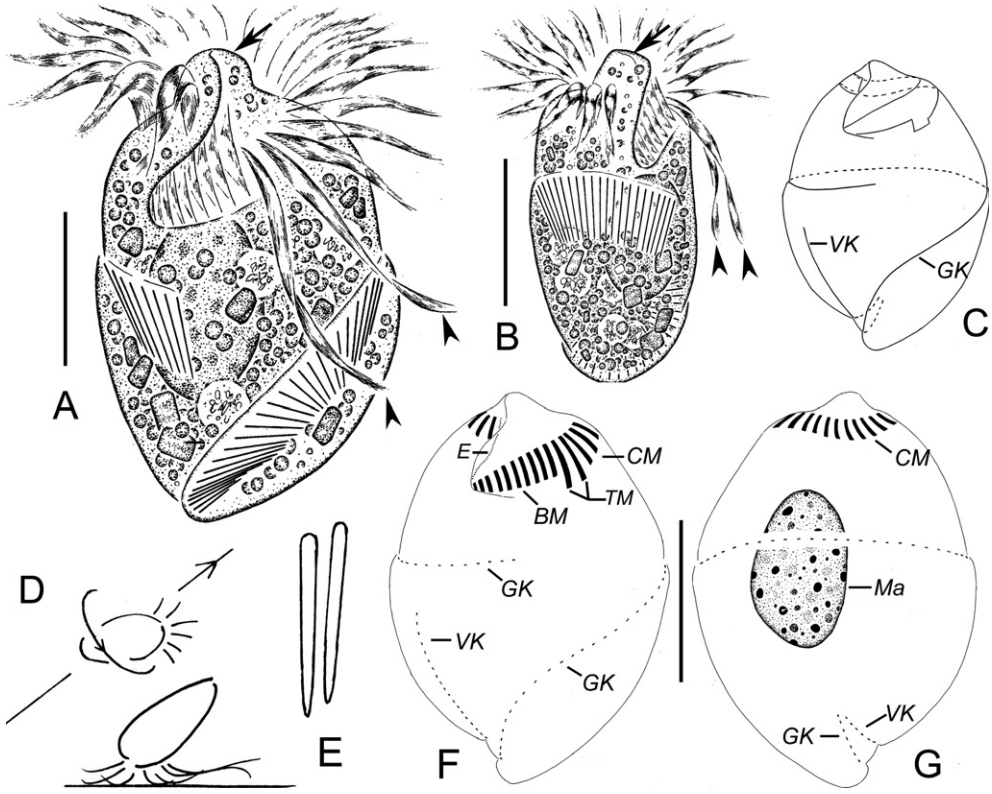

Figure 1 *Parallelostrombidium paraellipticum* sp. n. from life **(A, B, D, E)** and after protargol staining (**C, F, G**). A, B. Ventral and right lateral views of representative individuals, arrows mark apical protrusion, arrowheads mark thigmotactic membranelles. C. Showing pattern of somatic ciliature. D. Locomotion. E. Extrusomes. F, G. Ventral and dorsal views of the same specimen. BM = buccal membranelles; CM = collar membranelles; E = endoral membrane; GK = girdle kinety; Ma = macronucleus; TM = thigmotactic membranelles; VK = ventral kinety. Scale bars = 15 μm.

Cytoplasm colourless, contains numerous lipid droplets (2–5 μm across), food vacuoles (about 8 μm across), and several mineral grains (about 8 × 6 μm in size) gathered in posterior cell portion (Fig. 2A, C, D). Extrusomes needle-shaped, about 12 × 1 μm in size, attached in one row to cortex about 3 μm anteriorly to girdle kinety (Fig. 1A, B, E, 2H). Macronucleus equatorially located, 13–23 × 7–16 μm in size after protargol staining, ellipsoidal with several nucleoli (about 2–3 μm across; Fig. 1G, 2I). Micronucleus and cytopyge not recognizable. Locomotion by crawling over debris, using the thigmotactic membranelles; sometimes swimming in a straight line by rotating about main cell axis (Fig. 1D).

Oral apparatus occupies about 1/3 of cell length in vivo. Adoral zone composed of 17–21 collar, 9–11 buccal, and two thigmotactic membranelles (Fig. 1F, G). Each collar membranelle consists of three rows of basal bodies, with cilia up to about 15 μm long and bases of membranelles about 6 μm wide. Each buccal membranelle with cilia up to about 5 μm long, and bases of membranelles decrease in width from 5 μm at distal end to 3 μm at proximal end of zone portion. Thigmotactic membranelles located between collar and buccal portions, each consists of three rows of basal bodies, with cilia up to about 25 μm long and bases of membranelles about 7 μm wide (Fig. 1A, B, 2F, G, J, arrowheads). Endoral membrane located on inner wall of buccal lip, composed of a single row of densely packed basal bodies (Fig. 1F).

Somatic ciliature composed of a girdle kinety and a ventral kinety (Fig. 1C, F, G, 2E, I). Girdle kinety dextrally spiralled with one and a half whorls, starting ventrally at 2/5 of cell length and about seven dikinetids left to the ventral kinety, extending horizontally across the right and dorsal sides, then extending obliquely across the ventral side, crossing the posterior-right margin, extending anteriorly and terminating at 5/6 of cell length on the dorsal side. Girdle kinety composed of 63–75 dikinetids, each dikinetid bearing a 2 μm long cilium only at the left basal body (Fig. 2E, K, L). Ventral kinety starting at about four dikinetids below the girdle kinety, extending posteriorly and crossing the posterior-right margin, then extending parallel to the girdle kinety on the dorsal side, terminating close to the ending point of the girdle kinety. Ventral kinety composed of 17–26 dikinetids, each dikinetid bearing a 2 μm long cilium only at the anterior basal body (Fig. 2J, L). One early divider was observed, its oral primordium located left of the ventral kinety and the starting point of the girdle kinety, and anterior to the obliquely extending portion of the girdle kinety (Fig. 2J).

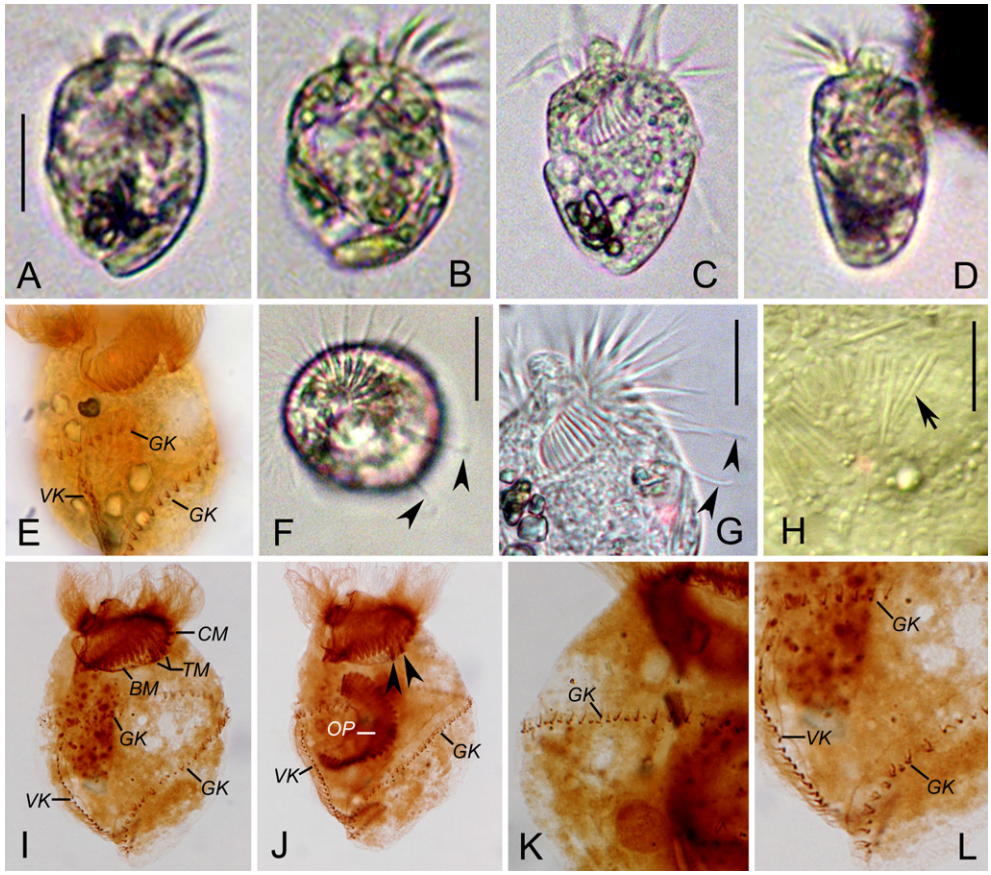

Figure 2 Photomicrographs of *Parallelostrombidium paraellipticum* sp. n. from life (**A–D, F–H**) and after protargol staining (**E, I–L**). A–C. Ventral views of different individuals. D. Lateral view of representative individual. E. Ventral view showing the somatic ciliature. F. Top view, arrowheads mark thigmotactic membranelles. G. Detail of adoral zone, arrowheads mark thigmotactic membranelles. H. Ventral side, arrow marks extrusomes. I, J. Ventral views of a specimen and a middle divider showing ciliary pattern, arrowheads mark thigmotactic membranelles. K. Detail of girdle kinety. L. Detail of somatic kineties. BM = buccal membranelles; CM = collar membranelles; GK = girdle kinety; Ma = macronucleus; PO = oral primordium; TM = thigmotactic membranelles; VK = ventral kinety. Scale bars = 20 μm (A); 15 μm (F); 10 μm (G, H).

Description of *Parallelostrombidium dragescoi* sp. n

Cell size 50–70 × 35–45 μm in vivo, 40–70 × 27–59 μm after protargol staining. Body obovoidal and asymmetrical, dorsoventrally flattened with a width: thickness ratio of about 4:3. Anterior end domed with a conspicuous apical protrusion about 5 μm high, at right side of peristome, posterior end narrowly rounded and slightly slanted to the right. Usually widest at the shoulder portion of cells, sometimes at mid-body, possibly due to presence of food vacuoles (Fig. 3A, C, 4A–D). Hemitheca not recognizable.

Cytoplasm colourless, contains numerous lipid droplets (about 2 μm across) and food vacuoles (about 6 μm across); in some individuals, cytoplasm filled with mineral particles (about 15 × 10 μm in size) (Fig. 4B–D). Extrusomes needle-shaped, about 10 × 0.5 μm in size, attached in one row to cortex anteriorly to the girdle kinety (Fig. 3A, C, 4G, H). Macronucleus centrally located, 22–34 × 14–23 μm in size after protargol staining, broadly ellipsoidal with several nucleoli (about 2 μm across; Fig. 3G, 4I, L). Micronucleus and cytopyge not recognizable. Locomotion by swimming in spirals and rapid changing direction, sometimes remains for 3–20 s (Fig. 3B).

Oral apparatus occupies about 1/3 of cell length in vivo. Adoral zone composed of 18–22 collar and six or seven buccal membranelles; thigmotactic membranelles absent (Fig. 3F, G). Each collar membranelle consists of three rows of basal bodies, with cilia up to about 25 μm long and bases of membranelles 10–12 μm wide. Each buccal membranelle consists of three rows of basal bodies, except for the most proximal buccal membranelle consists of two rows; cilia of buccal membranelles up to 8 μm long, bases of membranelles decrease in width from 6 μm at distal end to 2 μm at proximal end of zone portion (Fig. 3E, 4E, F, J, K). Endoral membrane located on inner wall of buccal lip, composed of a single row of densely packed basal bodies (Fig. 3F).

Somatic ciliature composed of a girdle kinety and a ventral kinety (Fig. 3D). Girdle kinety dextrally spiralled with one and a half whorls, starting ventrally at 1/3 of cell length and below the buccal membranelles, extending

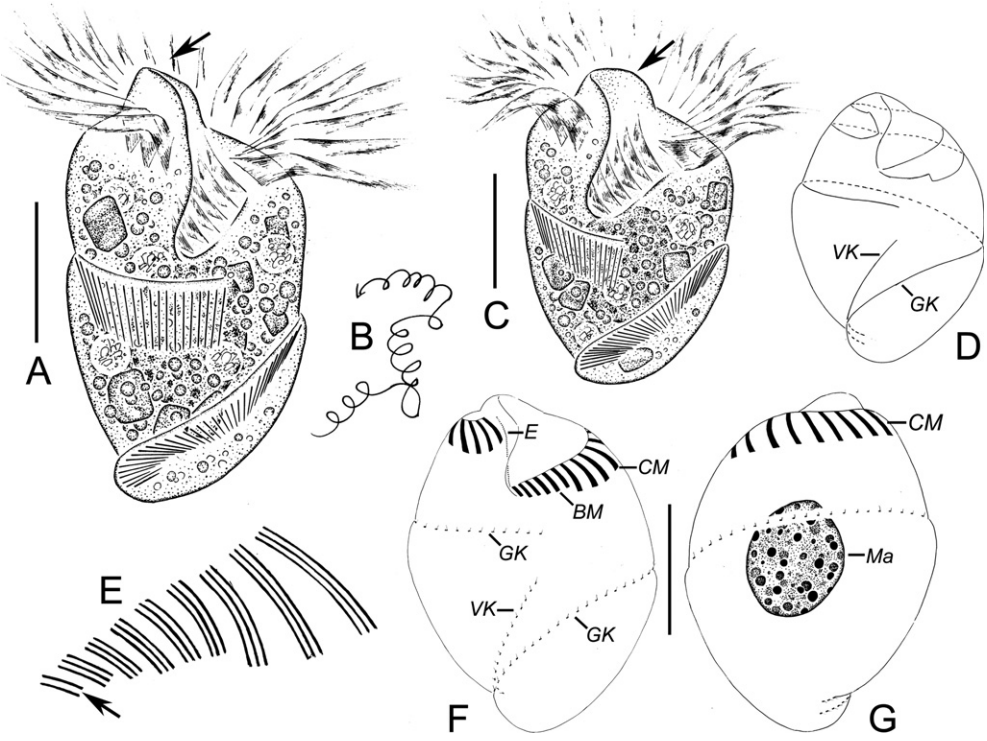

Figure 3 *Parallelostrombidium dragescoi* sp. n. from life (**A–C**) and after protargol staining (**D–G**). A, C. Ventral views of two individuals, arrows mark apical protrusions. B. Swimming trace. D. Scheme showing pattern of somatic ciliature. E. Detail of adoral membranelles, arrow marks the two-rowed membranelle. F, G. Ventral and dorsal views of the same specimen. BM = buccal membranelles; CM = collar membranelles; E = endoral membrane; GK = girdle kinety; Ma = macronucleus; VK = ventral kinety. Scale bars = 20 μm.

horizontally across right and dorsal sides, then extending obliquely across ventral side, crossing the posterior-right margin, extending posteriorly and terminating close to the end of dorsal side. Girdle kinety composed of 50–71 dikinetids, each dikinetid bearing a 2 μm long cilium only at the left basal body. Ventral kinety starting at about 8 μm below the starting point of girdle kinety, extending posteriorly and crossing the posterior-right margin, then extending parallel to the girdle kinety on the dorsal side, terminating close to the ending point of the girdle kinety. Ventral kinety composed of 13–23 dikinetids, each dikinetid bearing a 2 μm-long cilium only at anterior basal body (Fig. 4I, L). One early divider was observed, its oral primordium located left of the ventral kinety and the starting point of the girdle kinety, and anterior to the obliquely extending portion of the girdle kinety (Fig. 3M).

Description of Zhanjiang population of *Parallelostrombidium jankowskii* (Xu et al. 2009) comb. n

Cell size 105–140 × 40–50 μm in vivo, 99–138 × 49–66 μm after protargol staining. Body elongate obconical, with anterior 1/3 cylindrical and posterior 2/3 obconical. Anterior end domed with a conspicuous apical protrusion about 8 μm high, at right side of peristome (Fig. 5A, B, 6A–C). Widest at level of horizontal part of girdle kinety. Hemitheca not recognizable.

Cytoplasm colourless, contains numerous lipid droplets (about 2–4 μm across) and food vacuoles (about 5 μm across) with ingested algae. Extrusomes needle-shaped, about 16 × 1 μm in size, extrusome stripe dextrally spiralled; on dorsal side, extrusomes attached in one row to cortex about 3 μm anteriorly to the girdle kinety (Fig. 6D); on posterior ventral side, extrusomes attached in two rows to cortex extending longitudinally (Fig. 6E). Macronucleus nodules scattered, 24–32 in number, ellipsoidal or irregular with nucleoli (about 2 μm across; Fig. 5F, G, 6F, G, J). Micronucleus and cytopyge not recognizable. Locomotion by rotation about main cell axis while swimming in a straight line, occasionally staying on the spot, and sometimes touching the bottom of the dish (Fig. 5C).

Oral apparatus occupies about 1/3 of cell length in vivo. Adoral zone composed of 24–27 collar, 12–18 buccal, and two thigmotactic membranelles (Fig. 5F, G, 6H). Each collar membranelle with cilia up to about 30 μm long and bases of membranelles about 10 μm wide. Each buccal membranelle with cilia up to about 15 μm long, bases of buccal membranelles decrease in width from 12 μm at distal end to 6 μm at proximal end of zone portion. Thigmotactic membranelles located between collar and buccal portions, with cilia up to about 40 μm long and bases of membranelles about 12 μm wide (Fig. 6H). Endoral membrane located on inner wall of buccal lip, composed of a single row of densely packed basal bodies (Fig. 5F).

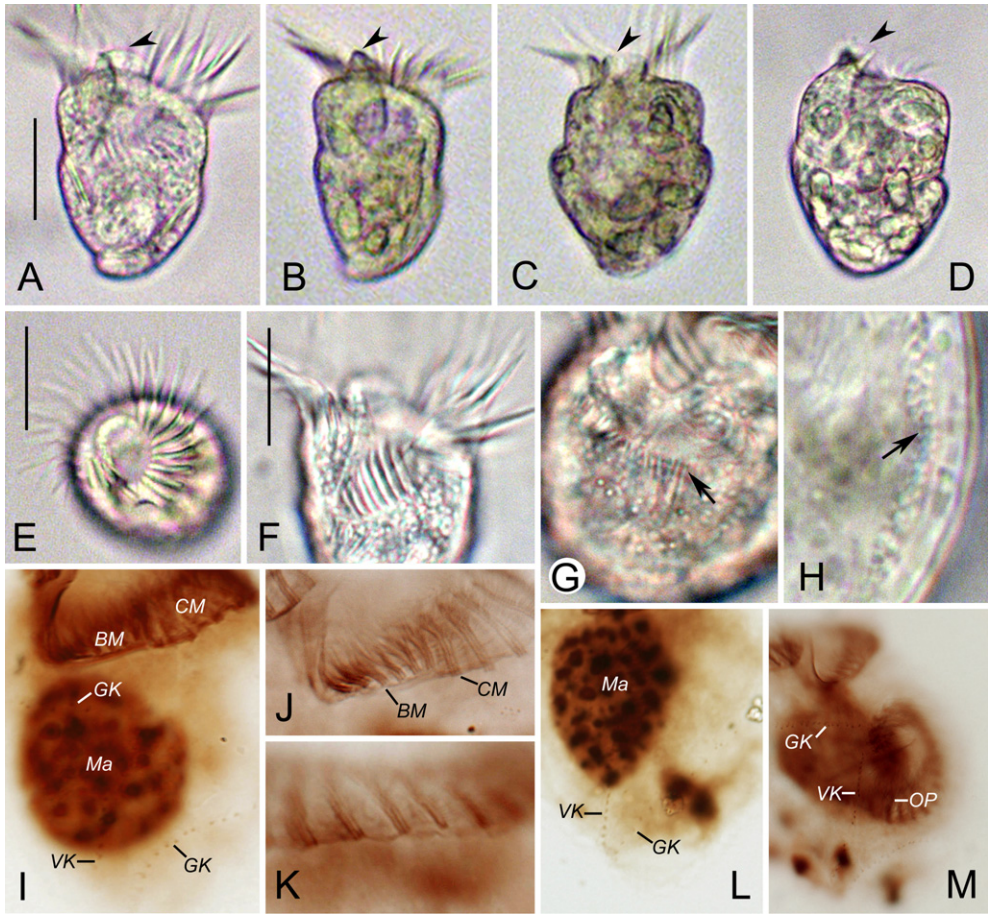

Figure 4 Photomicrographs of *Parallelostrombidium dragescoi* sp. n. from life (**A**–**H**) and after protargol staining (**I**–**M**). A–B. Ventral views of four individuals, arrowheads mark apical protrusions. E, F. Top and ventral views of adoral membranelles. G, H. Arrows mark anterior ends of extrusomes. I. Ventral view showing ciliature. J. Bases of adoral membranelles on ventral side. K. Bases of collar membranelles on dorsal side. L, M. Posterior ventral cell portion showing the somatic ciliature in a specimen and a middle divider. BM = buccal membranelles; CM = collar membranelles; GK = girdle kinety; Ma = macronucleus; OP = oral primordium; VK = ventral kinety. Scale bars = 20 μm.

Somatic ciliature composed of a girdle kinety and a ventral kinety (Fig. 5D, E). Girdle kinety dextrally spiralled with one and a half whorls, starting ventrally at 1/3 of cell length and below the thigmotactic membranelles, extends horizontally across the right and dorsal sides, then extending obliquely across the left and ventral sides, and terminating at posterior cell end (Fig. 5F, G). Girdle kinety composed of 93–126 dikinetids, each dikinetid bearing a 1 μm long cilium only at the left basal body. Ventral kinety starting at about 2–8 μm posteriorly to the starting point of the girdle kinety, extending posteriorly with a few dikinetids, then parallel to the girdle kinety on ventral side, terminating at posterior cell end. Ventral kinety composed of 14–20 dikinetids, each dikinetid bearing a 2 μm long cilium only at the anterior basal body (Fig. 6F, G, I, J).

Description of Qingdao population of *Parallelostrombidium kahli* (Xu et al. 2009) comb. n

Cell size 55–70 × 40–45 μm in vivo, 57–90 × 47–59 μm after protargol staining. Body roughly obconical, anterior end with conspicuous apical protrusion, posterior end pointed. Hemitheca not recognizable.

Cytoplasm colourless, contains food vacuoles (about 10–15 μm across) filled with ovoidal green and yellow algae, which render cells dark under lower magnification. Extrusomes needle-shaped, about 10 μm in length. Macronucleus nodules scattered, 22–34 in number, each about 6 × 4 μm in size after protargol staining, ellipsoidal with nucleoli (about 2 μm across; Fig. 7A, B). Micronucleus and cytopyge not recognizable.

Oral apparatus occupies about 1/3 of cell length in vivo. Adoral zone composed of 47–57 collar, 6–11 buccal, and two thigmotactic membranelles (Fig. 7A, B). Bases of collar and buccal membranelles about 6–8 μm wide. Thigmotactic membranelles located between collar and buccal portions, bases of thigmotactic membranelles about 10 μm wide. Endoral membrane located on the inner wall of buccal lip, composed of a single row of densely packed basal bodies (Fig. 7A).

Somatic ciliature composed of a girdle kinety and a ventral kinety (Fig. 7C, F). Girdle kinety dextrally spiralled,

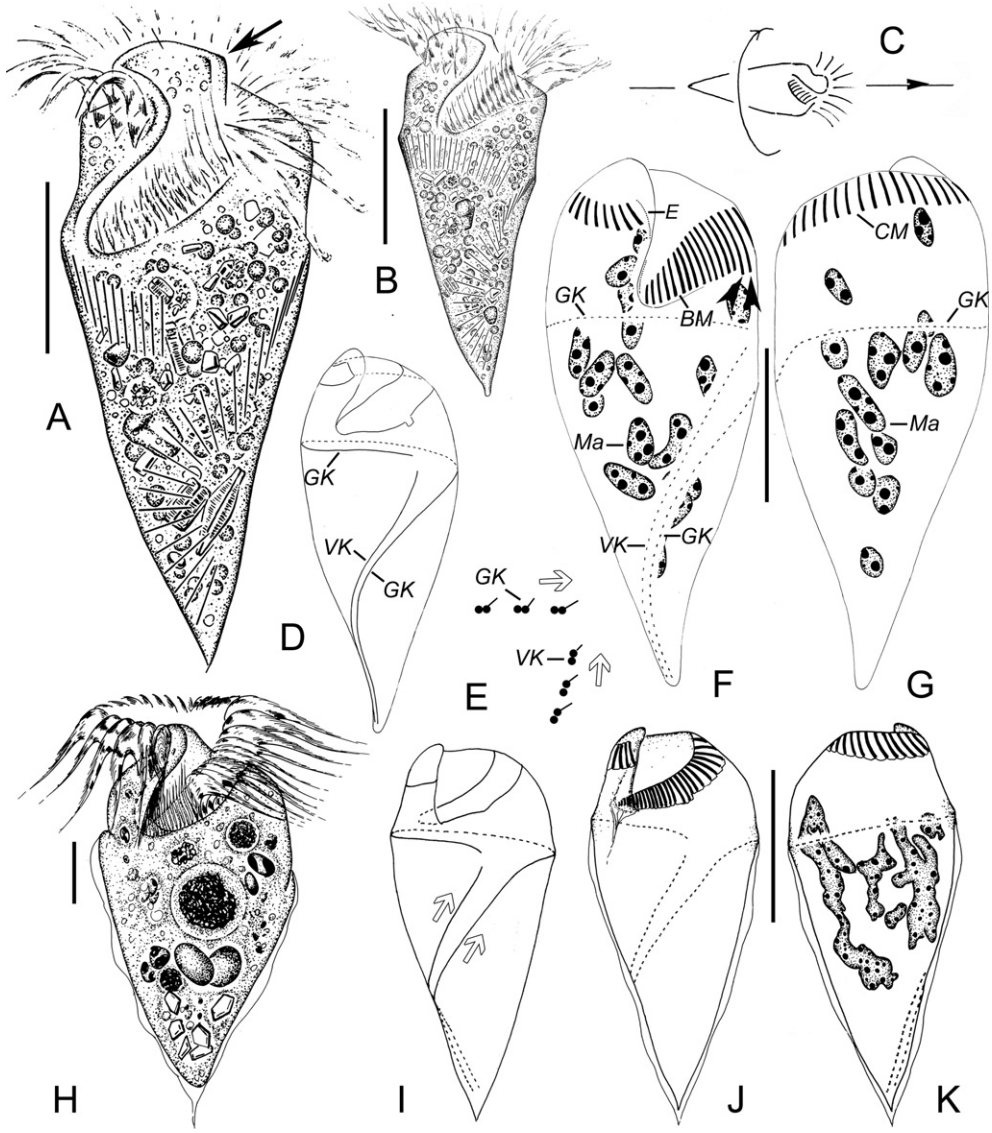

Figure 5 *Parallelostrombidium jankowskii* (Xu et al. 2009) comb. n. from life (**A–C, H**) and after protargol staining (**D–G, I–K**). Figures H-K are from Xu et al. (2009). A, B. Ventral views of representative individuals, arrow marks the transparent apical protrusion. C. Locomotion. D. Scheme showing pattern of somatic ciliature. E. Detail of girdle and ventral kineties, arrows mark orientation. F. G. Ventral and dorsal views of the same specimen, showing ciliature and macronucleus nodules, arrowheads mark thigmotactic membranelles. H. Ventral view of representative individual. I. Scheme showing pattern of somatic ciliature. J, K. Ventral and dorsal views of the same specimen, showing ciliature. BM = buccal membranelles; CM = collar membranelles; E = endoral membrane; GK = girdle kinety; Ma = macronucleus nodules; VK = ventral kinety. Scale bars = 50 μm.

starting below the collar membranelles on the left dorsal side, extending horizontally around cell with nearly one whorl, before performing nearly two whorls, terminating at posterior cell end. Girdle kinety composed of 129–195 dikinetids, each dikinetid bearing a 2 μm long cilium only at the left basal body. Ventral kinety starting at about 3–10 μm posteriorly to the starting point of the girdle kinety on dorsal side, extending posteriorly with a few dikinetids, then parallel to the spiralled portion of girdle kinety for about two whorls, terminating at posterior cell end. Ventral kinety composed of 69–90 dikinetids, each dikinetid bearing a 2 μm-long cilium only at the anterior basal body (Fig. 7A, B, G–J).

SSU rRNA gene sequences of the four species

The SSU rRNA gene sequences of the four species studied in this work have been deposited in GenBank. The lengths, GC contents, and GenBank accession numbers are as follows: *Parallelostrombidium paraellipticum* sp. n. (1,676 nt, GC = 46.72%, GenBank accession number: MF445657); *P. dragescoi* sp. n. (1,691 nt, GC = 46.48%,

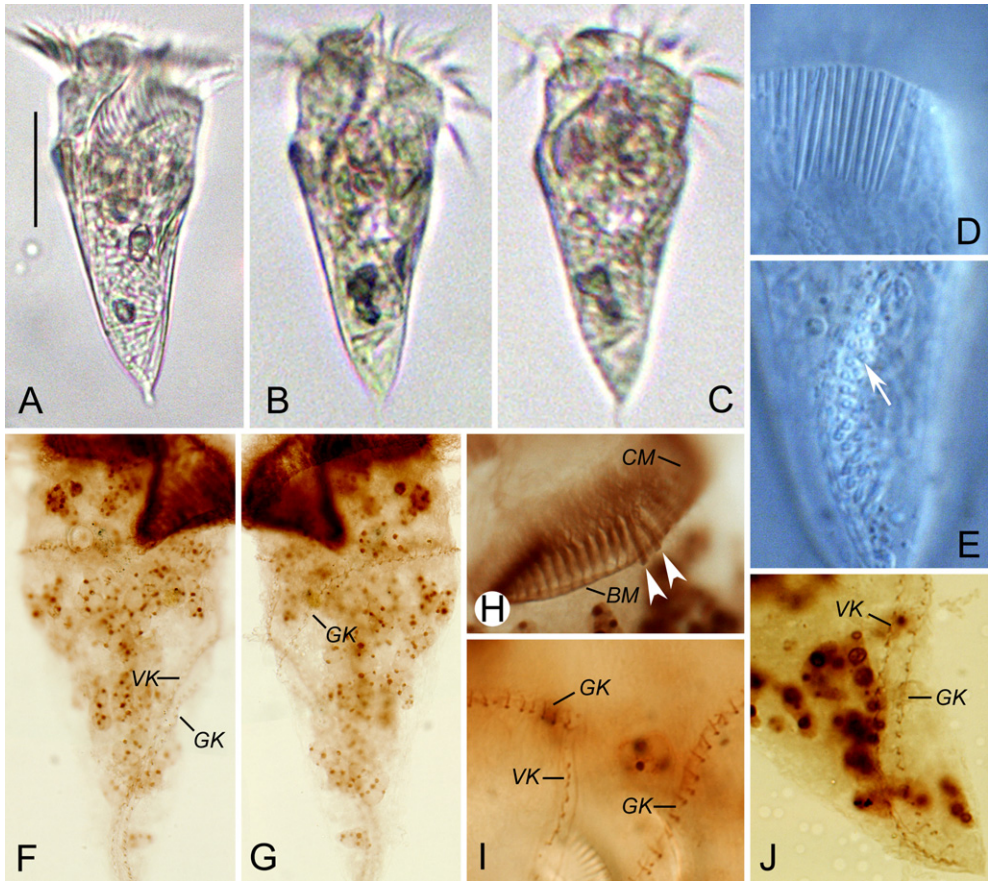

Figure 6 Photomicrographs of *Parallelostrombidium jankowskii* (Xu et al. 2009) comb. n. from life (**A–E**) and after protargol staining (**F–J**). A–C. Ventral views of three individuals. D. Extrusomes on dorsal side. E. Arrow marks the tops of extrusomes on posterior ventral side. F, G. Ventral and dorsal views of one specimen showing ciliature. H. Bases of adoral membranelles, arrowheads mark thigmotactic membranelles. I. Detail of somatic kineties on ventral side. J. Posterior portion of somatic kineties. BM = buccal membranelles; CM = collar membranelles; GK = girdle kinety; VK = ventral kinety. Scale bars = 40 μm.

GenBank accession number: MF445658); *P. jankowskii* (Xu et al. 2009) comb. n. (1,503 nt, GC = 48.57%, GenBank accession number: MF445659); *P. kahli* (Xu et al. 2009) comb. n. (1,724 nt, GC = 48.26%, GenBank accession number: MF445656) (Fig. 8).

Phylogenetic analyses

As the tree topologies from the BI and ML algorithms are similar, only the BI tree is presented with support values from both methods at the branch nodes. In both trees, *P. paraellipticum* sp. n. clusters with *P. obesum* with support values of 0.84 BI and 43% ML; *P. dragescoi* sp. n. groups with *P. ellipticum*, with support values of 0.83 BI and 55% ML. In both trees, *P. jankowskii* (Xu et al. 2009) comb. n. groups with *P. kahli* (Xu et al. 2009) comb. n., with support values of 0.85 BI and 48% ML; this cluster is sister to *P. conicum* with support values of 0.78 BI and 57% ML (Table 1).

A pairwise distance matrix of eight *Parallelostrombidium* species was generated, using the p-distance method (Table 2). Nucleotide pairwise distances ranged from 0.0080 to 0.0376. The highest nucleotide variations were between *P. ellipticum* and *P. jankowskii* (Xu et al. 2009) comb. n.; with a genetic distance value of 0.0376 (3.76%). The lowest genetic distances were found between *P. conicum* and *P. kahli* (Xu et al. 2009) comb. n.; with a nucleotide divergence value of 0.0080 (0.8%).

DISCUSSION

Comparison of *Parallelostrombidium paraellipticum* sp. n. with related species

Until now, excluding the four members in this work, six species had been assigned to *Parallelostrombidium*. *Parallelostrombidium paraellipticum* sp. n. differs from the other congeners and all the poorly described oligotrich species by: obovoidal, asymmetrical, and dorsoventrally flattened cell shape; number of collar, buccal, and thigmotactic membranelles; number of girdle and ventral dikinetids; and extrusomes arranged in one row. In detail,

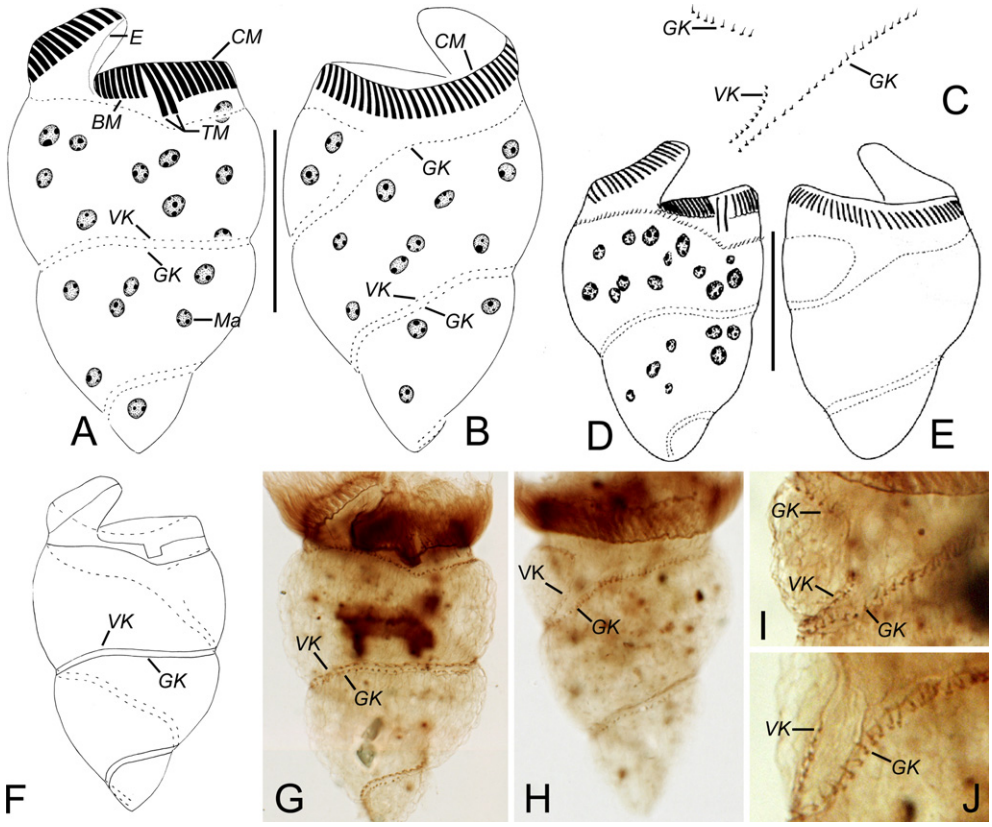

Figure 7 *Parallelostrombidium kahli* (Xu et al. 2009) comb. n. after protargol staining (**A**–**J**). A, B. Ventral and dorsal views of the same specimen showing the numerous macronucleus nodules and the ciliature. C. Detail of somatic kineties on dorsal side. D, E. Ventral and dorsal views from Xu et al. (2009). F. Scheme showing somatic ciliature. G, H. Ventral and dorsal views of same specimen showing the ciliature. I, J. Detail of somatic kineties on dorsal side. BM = buccal membranelles; CM = collar membranelles; E = endoral membrane; GK = girdle kinety; Ma = macronucleus nodules; TM = thigmotactic membranelles; VK = ventral kinety. Scale bars = 40 μm.

Parallelostrombidium ellipticum Liu et al., 2015 mostly resembles *P. paraellipticum* sp. n. in terms of the dorsoventrally flattened cell shape, cell size, number of collar membranelles, buccal membranelles, girdle dikinetids, and ventral dikinetids. However, *P. ellipticum* differs from the latter in the following features: (1) the extrusomes arranged in three rows (vs. one row); (2) the anterior end of the girdle kinety close to the ventral kinety (vs. left of ventral kinety with a distance of about seven dikinetids); and (3) the girdle kinety extends obliquely across the dorsal side (vs. horizontally). Additionally, the SSU rRNA gene sequences differ between these two species by 1.68% (26 bp) (Table 2).

Parallelostrombidium paraellipticum sp. n. resembles two congeners in a dorsoventrally flattened cell and two thigmotactic membranelles (Liu et al. 2015a; Xu et al. 2006). *Parallelostrombidium paralatum* Xu et al. 2006 differs from *P. paraellipticum* sp. n. by having: (1) more collar membranelles (26–30 vs. 17–21); (2) more buccal membranelles (15–19 vs. 9–11); and (3) more dikinetids in the ventral kinety (32–42 vs. 17–26). *Parallelostrombidium obesum* Liu et al., 2015 can be distinguished by having: (1) a doliform cell shape (vs. obovoid); (2) more buccal membranelles (12–20 vs. 9–11); (3) more dikinetids in the girdle kinety (101–164 vs. 63–75); and (4) extrusomes arranged in three rows (vs. one row).

Comparison of *Parallelostrombidium dragescoi* sp. n. with related species

Parallelostrombidium dragescoi sp. n. clearly differs from the other congeners and all the poorly described oligotrich species by its obovoidal, asymmetrical, and dorsoventrally flattened cell shape and a lack of thigmotactic membranelles. In detail, *P. dragescoi* sp. n. differs from *P. conicum*, *P. jankowskii* (Xu et al. 2009) comb. n., *P. kahli* (Xu et al. 2009) comb. n., *P. rhyticollare* and *P. siculum* by its obovoidal cell shape (vs. obconical). It clearly differs from *P. ellipticum*, *P. obesum*, *P. paralatum* and *P. paraellipticum* sp. n. by a lack of thigmotactic membranelles (vs. with thigmotactic membranelles).

Generic affiliation of *Parallelostrombidium jankowskii* (Xu et al. 2009) comb. n

Parallelostrombidium jankowskii (Xu et al. 2009) comb. n. was originally reported by Xu et al. (2009) based on a Qingdao population. Xu and colleagues described the cell

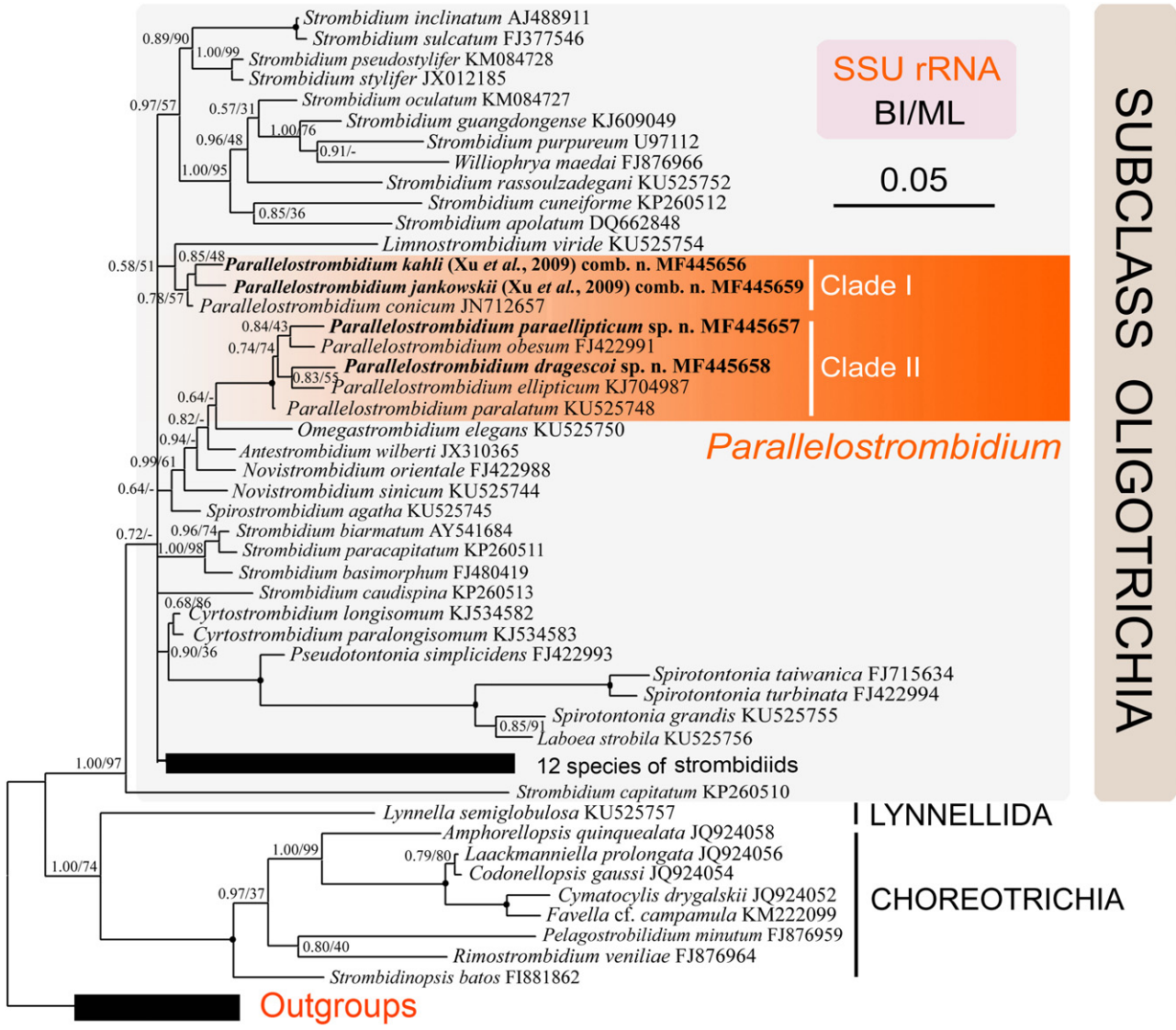

Figure 8 Bayesian-Inference tree inferred from SSU rRNA gene sequences, indicating the polygenetic positions of species of the genus *Parallelostrombidium*. Numbers at the nodes represent support values in the following order: BI posterior probabilities and ML bootstrap values. Disagreements in topology 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.

as having a girdle kinety horizontally orientated in the anterior dorsal cell portion while its two ends extend to the posterior cell end, based on this somatic ciliary pattern, the authors assigned this species to the genus *Omegastrombidium* Agatha 2004;. A re-investigation of the original slides necessitated a redescription of the species owing to a misinterpretation of the somatic ciliary pattern by Xu et al. (2009). Based on the inverse orientation of the dikinetids and the enlarged distance between the horizontal girdle kinety portion and the right longitudinal portion, we conclude that the species has a dextrally spiralled girdle kinety and a ventral kinety that is parallel to the posterior portion of the girdle kinety, except for its anterior most portion. Accordingly, this species should be transferred to the genus *Parallelostrombidium* Agatha 2004. Otherwise, the Zhanjiang population matches the original description in all characteristics, except for the hemitheca described in the original population, which was not observed in the Zhanjiang population. Given that the hemitheca is delicate and thus difficult to observe or might disappear, both populations are considered conspecific.

Parallelostrombidium species, i.e. *P. conicum*, *P. rhyticollare*, *P. siculum*, share a similar posteriorly pointed cell shape with *P. jankowskii* (Xu et al. 2009) comb. n., however, they can be easily distinguished from the latter by: (1) lacking thigmotactic membranelles (present) and (2) a single macronucleus (vs. multiple macronuclei).

Table 1. Morphometric data on *Parallelostrombidium paraellipticum* sp. n. (first row), *P. dragescoi* sp. n. (second row), *P. jankowskii* (Xu et al. 2009) comb. n. (third row) and *P. kahli* (Xu et al. 2009) comb. n. (fourth row)

Character	Min	Max	Mean	SD	SE	CV	n
Body length	39	57	46.5	5.84	1.51	12.5	15
	40	70	55.0	8.01	1.60	14.6	25
	99	138	127.6	12.65	4.22	9.4	9
	57	90	78.3	9.71	3.24	12.4	9
Body width	28	43	34.3	4.79	1.38	14.0	12
	27	59	42.9	8.99	2.12	20.9	18
	49	66	55.8	4.47	1.69	8.0	8
	40	59	48.5	6.10	1.93	12.6	10
Distance from	13	19	14.6	1.75	0.43	11.9	16
anterior cell end	14	20	17.2	1.57	0.31	9.2	25
to buccal vertex	30	34	31.1	1.45	0.51	4.7	9
	16	22	19.4	1.77	0.59	9.1	9
Distance from	11	18	15.1	2.05	0.51	13.6	16
anterior cell end	12	23	–	–	–	–	2
to macronucleus*	–	–	–	–	–	–	–
	–	–	–	–	–	–	–
Distance from	19	27	21.8	2.41	0.69	11.0	12
anterior cell end	17	27	21.1	3.07	0.70	14.6	19
to the anterior end	30	41	35.9	3.00	1.06	8.4	9
of girdle kinety	18	34	24.5	4.36	1.54	17.8	8
Distance from	23	32	28.0	3.00	0.90	10.7	11
anterior cell end	26	43	35.0	5.40	1.71	15.4	10
to the anterior end	33	47	40.7	4.06	1.43	9.8	9
of ventral kinety	28	43	33.3	4.58	1.62	13.8	8
Number of collar	17	21	18.7	1.19	0.36	6.4	11
membranelles	18	22	19.8	1.01	0.25	5.1	17
	24	27	25.4	0.83	0.29	3.3	9
	47	57	51.1	3.18	1.20	6.2	7
Number of buccal	9	11	9.6	0.67	0.20	7.0	11
membranelles	6	7	6.4	0.49	0.12	7.8	17
	12	18	14.4	1.70	0.60	11.8	9
	6	11	8.2	–	–	–	6
Number of	2	2	2.0	0.00	0.00	0.0	9
thigmotactic	0	0	0.0	0.00	0.00	0.0	20
membranelles	2	2	2.0	0.00	0.00	0.0	9
	2	2	2.0	0.00	0.00	0.0	12
Girdle kinety,	63	75	–	–	–	–	2
number of	50	71	56.1	5.03	1.22	9.0	17
dikinetids	86	109	100.4	7.60	2.69	7.6	9
	129	195	162.6	24.0	9.09	14.8	7
Ventral kinety,	17	26	21.5	2.78	0.98	12.9	8
number of	13	23	16.6	3.27	1.03	19.7	10
dikinetids	24	49	38.4	6.98	2.47	18.1	9
	69	90	78.7	–	–	–	6
Macronucleus	13	23	17.6	2.87	0.77	16.3	14
length	22	34	26.0	3.97	0.87	15.2	21
	–	–	–	–	–	–	–
	5	7	6.0	1.00	0.71	16.7	10
Macronucleus	7	16	11.1	2.71	0.73	24.3	14
width	14	23	18.8	3.20	0.70	17.0	21
	–	–	–	–	–	–	–
	4	5	4.5	0.50	0.35	11.1	10
	1	1	1.0	0.00	0.00	0.0	15
Macronucleus	1	1	1.0	0.00	0.00	0.0	25
number	24	32	27.5	–	–	–	6
	22	34	28.6	4.03	1.80	14.1	5

All data are based on randomly selected protargol-stained specimens (Wilbert 1975). Measurements in μm. 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.

*Only in species with single macronucleus.

Generic affiliation of *Parallelostrombidium kahli* (Xu et al. 2009) comb. n

Parallelostrombidium kahli (Xu et al. 2009) comb. n. was originally reported based on a Qingdao population. Xu and colleagues described the species as having an "Ω" shaped girdle kinety in which the parallel course of the ends starts on dorsal side, and the parallel portions perform 1.5 whorls around cell. Accordingly, the authors assigned this species to the genus *Omegastrombidium* Agatha 2004;. Like in the previous species, the pattern was also misinterpreted in the present one. Again, based on the inverse orientation of the dikinetids and the enlarged distance between the horizontal girdle kinety portion and the right longitudinal portion found by the re-investigation of the holotype and paratype slides, we conclude that the species has a dextrally spiralled girdle kinety and a ventral kinety that is parallel to the posterior portion of the girdle kinety, except for its anterior most portion. Accordingly, this species should be transferred to the genus *Parallelostrombidium* Agatha 2004. The present population matches the original description in all characteristics, so both are conspecific.

Parallelostrombidium kahli (Xu et al. 2009) comb. n. differs from other congeners including *P. jankowskii* (Xu et al. 2009) comb. n. by a ventral kinety that is dextrally spiralled with ca. two whorls. *Parallelostrombidium rhyticollare* (Corliss & Snyder 1986) Agatha, 2004 also possesses a dextrally spiralled ventral kinety, but it can be distinguished from this species by having (1) a ventral kinety dextrally spiralled with a half whorl (vs. about 2 whorls) and (2) one macronucleus (vs. 20–34 macronuclear nodules).

Phylogenetic analyses

In the SSU rRNA gene-based trees, all four species clustered with their congeners. Consistent with previous phylogenetic studies, the genus *Parallelostrombidium* is not monophyletic (Liu et al. 2013, 2015a), i. e., the eight *Parallelostrombidium* species form two clusters, and the monophyly of the genus was rejected by the AU test ($p = 1e - 004$). Cluster I includes *P. conicum*, *P. jankowskii* (Xu et al. 2009) comb. n., and *P. kahli* (Xu et al. 2009) comb. n. Cluster II includes the remaining five species. The separation of the eight *Parallelostrombidium* species corresponds to differences in cell shape and

Table 2. Estimates of evolutionary divergence between sequences

		1	2	3	4	5	6	7	8
P. kahli	1								
P. jankowskii	2	0.0134							
P. conicum	3	0.0080	0.0087						
P. paraellipticum	4	0.0342	0.0342	0.0302					
P. dragescoi	5	0.0349	0.0355	0.0308	0.0188				
P. ellipticum	6	0.0369	0.0376	0.0328	0.0168	0.0181			
P. paralatum	7	0.0255	0.0268	0.0214	0.0107	0.0141	0.0121		
P. obesum	8	0.0322	0.0329	0.0282	0.0121	0.0188	0.0181	0.0107	

The pairwise distance between sequences are shown. All ambiguous positions were removed for each sequence pair. There were a total of 1,495 positions in the final dataset. Evolutionary analyses were conducted in MEGA5, using the p-distance model.

somatic ciliary pattern. The species of cluster I share an obconical cell shape with a pointed posterior end and their ventral kinety parallel to the girdle kinety except the anterior most portion. The species of cluster II share a dorsoventrally flattened cell shape with a rounded posterior end, and only the posterior portion of the ventral kinety is parallel to the girdle kinety. The bipartition of the genus in the gene trees is thus supported by morphological features, namely, the cell shape and the pattern of the somatic kineties.

The subgenus assignment of the four species

Agatha and Strüder-Kypke (2014) proposed a division of *Parallelostrombidium* Agatha 2004 based on whether the entire ventral kinety or only its posterior portion is parallel to the girdle kinety. Later, an intermediate pattern of the genus, in which the anterior end of the ventral kinety is slightly separated from the girdle kinety, was found in *P. conicum*; in the present study, two further species showing this pattern are redescribed, namely *P. jankowskii* (Xu et al. 2009) comb. n. and *P. kahli* (Xu et al. 2009) comb. n. Due to the lack of genetic data for the type species of the subgenus *Parallelostrombidium* Agatha and Strüder-Kypke 2014 (*P. rhyticollare*) and small deviations in the somatic ciliary pattern, these species with the intermediate pattern are here tentatively affiliated with the subgenus *Parallelostrombidium* Agatha and Strüder-Kypke 2014 whose members should have the ventral kinety entirely parallel to girdle kinety. The species *P. paraellipticum* sp. n. and *P. dragescoi* sp. n. are assigned to the subgenus *Asymptokinetum* Agatha and Strüder-Kypke 2014. Without genetic data from species showing the three somatic ciliary patterns, their phylogenetic relationships cannot be inferred.

TAXONOMIC SUMMARY

Order Strombidiida Petz & Foissner, 1992
Family Strombidiidae Fauré-Fremiet, 1970
Genus *Parallelostrombidium* Agatha 2004
Subgenus *Asymptokinetum* Agatha and Strüder-Kypke 2014

Parallelostrombidium paraellipticum sp. n.

Diagnosis. Size 40–60 × 30–45 μm in vivo, 39–57 × 28–43 μm in protargol stained specimens; obovoidal and asymmetrical, dorsoventrally flattened, with conspicuous apical protrusion. Oral apparatus occupies about 1/3 of cell length. Extrusomes needle-shaped, about 12 μm long, arranged in one row along girdle kinety. Macronucleus ellipsoidal. Adoral zone composed of 17–21 collar, 9–11 buccal, and two thigmotactic membranelles. Girdle kinety dextrally spiralled with about one and half whorls, with anterior end located ventrally to the left of ventral kinety, composed of 63–75 dikinetids. Ventral kinety parallel with girdle kinety only with posterior portion, composed of 17–26 dikinetids. Saline habitat.

Type locality and habitat. Zhujiang estuary (22°29′N, 113°58′E), Zhuhai, China: salinity 2.5‰, temperature 29 °C, and pH 8.0.

Deposition of slides. A slide with the holotype specimen (registration number: NHMUK 2018.2.15.1) is deposited in the Natural History Museum, London. A slide with paratype specimens (registration number: SW2014053101-2) is deposited in the Laboratory of Protozoology, OUC.

Etymology. Combining the Latin prefix *para-* (meaning close) with the species epiphet *ellipticum* indicates the similarity in cell shape and oral membranelles between this species and *P. ellipticum*.

ZooBank registration. urn:lsid:zoobank.org:act:7DA18C8A-A4F9-43C7-A1F8-698C274D140F

Parallelostrombidium dragescoi sp. n.

Diagnosis. Size 50–70 × 35–45 μm in vivo, 40–70 × 27–59 μm in protargol stained specimens; obovoidal and asymmetrical, dorsoventrally flattened, with conspicuous apical protrusion. Oral apparatus occupies about 1/3 of cell length. Extrusomes needle-shaped, about 10 μm long, arranged in one row along girdle kinety. Macronucleus ovoidal. Adoral zone composed of 18–22 collar and 6 or 7 buccal membranelles. Girdle kinety dextrally spiralled with about one and half whorls, with anterior end located ventrally and anteriorly to ventral kinety, composed of 50–71 dikinetids. Ventral kinety parallel with girdle kinety only with posterior portion, composed of 13–23 dikinetids. Saline habitat.

Type locality and habitat. A brackish water lake in Haikou (20°06′N, 110°33′E), China: temperature 30 °C, salinity 18‰, and pH 8.8.

Deposition of slides. A slide with the holotype specimen (registration number: NHMUK 2018.2.15.2) is deposited in

the Natural History Museum, London. A slide with paratype specimens (registration number: SW2014042201-2) is deposited in the Laboratory of Protozoology, OUC.

Etymology. We dedicate this species to Prof. Dr. Jean Dragesco, the late great French protozoologist, in recognition of his significant contribution to taxonomic study of ciliates.

ZooBank registration. urn:lsid:zoobank.org:act:C558F19D-37D2-4E8B-A99D-058AAEB97538

Subgenus *Parallelostrombidium* Agatha and Strüder-Kypke 2014

Parallelostrombidium jankowskii (Xu et al. 2009) comb. n.

Basionym: *Omegastrombidium jankowskii* Xu et al. 2009

Based on new information from this study, an improved diagnosis is supplied here.

Improved diagnosis. Size 105–150 × 40–75 μm in vivo, 99–138 × 49–66 μm in protargol stained specimens; broadly obconical with a prominent apical protrusion and posterior end pointed. Oral apparatus occupies nearly 1/3 of cell length. Extrusomes needle-shaped, about 16 μm long, arranged in one row along girdle kinety. 24–32 macronuclear nodules scattered. Adoral zone composed of 24–28 collar, 12–19 buccal, and two thigmotactic membranelles. Girdle kinety dextrally spiralled with about one and half whorls, with anterior end located ventrally and anteriorly to ventral kinety, composed of 86–109 dikinetids. Ventral kinety composed of 24–49 dikinetids. Saline habitat.

Deposition of voucher slides. A voucher slide with protargol-impregnated specimens was deposited in the Laboratory of Protozoology, OUC, Qingdao, China (registration number: SW2013110601).

Parallelostrombidium kahli (Xu et al. 2009) comb. n.

Basionym: *Omegastrombidium kahli* Xu et al. 2009

Based on new information from this study, an improved diagnosis is supplied here.

Improved diagnosis. Size 55–80 × 40–50 μm in vivo, 57–90 × 47–59 μm in protargol stained specimens; broadly obconical with posterior end pointed. Oral apparatus occupies about 1/3 of cell. Extrusomes needle-shaped, about 10 μm long. 20–34 ovoidal macronuclear nodules scattered. Adoral zone composed of 47–60 collar, 6–15 buccal, and two thigmotactic membranelles. Girdle kinety dextrally spiralled with nearly three whorls, with anterior end located dorsally and anteriorly to ventral kinety, composed of 129–195 dikinetids. Ventral kinety composed of 69–90 dikinetids. Saline habitat.

Deposition of voucher slides. A voucher slide with protargol-impregnated specimens was deposited in the Laboratory of Protozoology, OUC, Qingdao, China (registration number: SW2012100901).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Satellite photograph of South-East China (**A**) and pictures of sampling sites (**B-F**). **A.** The position of Qingdao, Zhanjiang, Zhuhai and Haikou; **B.** Coastal waters off Qingdao. **C.** Coastal waters off Zhuhai. **D, E.** Coastal waters off Zhanjiang. **F**. Brackish waters in Haikou.