Taxonomy, morphology and molecular systematics of three oligotrich ciliates, including a description of *Apostrombidium parakielum* spec. nov. (Ciliophora, Oligotrichia)

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Three oligotrich ciliates, *Apostrombidium parakielum* spec. nov., *Novistrombidium apsheronicum* (Alekperov & Asadullayeva, 1997) Agatha, 2003 and *Novistrombidium testaceum* (Anigstein, 1914) Song & Bradbury, 1998 were collected from the coastal waters of China and their morphology and small-subunit rRNA (SSU rRNA) gene sequences were studied. The novel species can be recognized by the combination of its obconical body shape, 14–16 anterior and 6–8 ventral membranelles, somatic kinety in three parts and conspicuously long dorsal cilia.

Based on the data obtained for this novel species, an improved diagnosis of the genus *Apostrombidium* is supplied. Descriptions of the population of *N. apsheronicum* and *N. testaceum* collected in this study are also provided and compared with the existing descriptions.

In addition, the phylogenetic positions of these three species are inferred from their SSU rRNA gene sequence data. The results indicate that the genus *Apostrombidium*, the systematics of which has not previously been discussed using molecular information, clusters with *Varistrombidium kielum* and *Omegastrombidium elegans*, whereas *N. testaceum* and *N. apsheronicum* form a single clade.

INTRODUCTION

Oligotrich ciliates are often dominant in microzooplankton communities in both marine and freshwater habitats (Agatha, 2011a; Fenchel, 2008; Jiang *et al.*, 2012; Krainer, 1991; Pierce & Turner, 1992). Being small and fragile, many of these organisms have been rather superficially investigated with descriptions usually only based on observations of living cells or Lugol’s-fixed samples (Fauré-Fremiet, 1924; Kahl, 1932; Maeda & Carey, 1985). The combination of living observation and silver impregnation has come to be regarded as essential for adequate species description and, as a consequence, during the past three decades many oligotrichs have been reported (Agatha, 2003a, 2003b, 2004a, 2004b, 2010a, 2010b, 2011b; Agatha *et al.*, 2004; Kim *et al.*, 2010; Modeo *et al.*, 2003; Song & Bradbury, 1998; Song *et al.*, 2000; Tsai *et al.*, 2010; Xu & Song, 2006; Xu *et al.*, 2005). There is still much to be discovered about the diversity of oligotrichs, however, and many ambiguities concerning the identification of taxa within this species-rich group need to be resolved by combining behavioural, infraciliature and molecular data (Agatha & Strüder-Kypke, 2012; Agatha *et al.*, 2005; Jeong *et al.*, 2004; Kim *et al.*, 2005; Liu *et al.*, 2011a, 2011b, 2012; McManus *et al.*, 2010; Modeo *et al.*, 2003; Xu *et al.*, 2012).

In the present work, the morphology of three oligotrich species, namely *Apostrombidium parakielum* spec. nov., *Novistrombidium apsheronicum* and *Novistrombidium testaceum* was investigated based on living observation and silver impregnation. In addition, the small subunit (SSU) rRNA genes of *A. parakielum* spec. nov. and *N. apsheronicum* were sequenced in order to

†These authors contributed equally to this work.

Abbreviations: BI, Bayesian inference; ML, maximum likelihood; SSU, small subunit.

The GenBank/EMBL/DDBJ accession numbers for the SSU rRNA gene sequences of *Novistrombidium apsheronicum* and *Apostrombidium parakielum* are FJ876958 and JX025560, respectively.
determine their phylogenetic position within the sub-class Oligotrichia.

METHODS

Morphological studies. *A. parakielum* spec. nov. was isolated from a sandy beach at Qingdao (39°10′ N 117°06′ E), Shandong Province, China, on 12 May 2009; the water was 16.5 °C, salinity 30.3% and pH 8.2. *N. aphthericum* was collected from Daya Bay (22°43′ N 114°32′ E), Guangdong Province, China, on 21 August 2007; the water was 31.2 °C, salinity 31.8% and pH 8.2. *N. testaceum* was collected from a mangrove wetland near Shenzhen (22°37′ N 114°04′ E), Guangdong Province, China, on 30 April 2008; the water was 27.0 °C, salinity 17.0%, and pH 8.2.

The morphology of living cells was studied using bright-field and differential interference contrast microscopy. The infraciliature was revealed through protargol impregnation (Wilbert, 1975). Drawings of live cells were based on photomicrographs, while those of silver-impregnated cells were made using a camera lucida. Counts and measurements were performed at a magnification of ×1250. Terminology is mainly according to Agatha (2004a).

SSU rRNA gene sequence and phylogenetic analyses. Genomic DNA of *A. parakielum* spec. nov. and *N. aphthericum* was extracted using the REDExtract-N-Amp Tissue PCR kit (Sangon) with modifications suggested by Gao et al. (2009). The primers used to amplify the nuclear SSU rRNA gene were eukaryotic universal, namely, forward primer Euk A and reverse primer Euk B (Medlin et al., 1988). The PCR protocol followed Huang et al. (2012). The purified PCR product was inserted into the pUCm-T vector (Sangon) and cultured in *E. coli* DH5α cells plated on an agar medium. Overnight, the positive clones were picked out, recultured simultaneously, and detected by PCR amplification with the primers M13F and M13R (Yi & Song, 2011; Zhang et al., 2010). The recultured clones were sequenced in both directions with a 3730 DNA Analyser (Applied Biosystems).

Apart from *A. parakielum* spec. nov. and *N. aphthericum*, the SSU rRNA gene sequences of 62 species were retrieved from the NCBI/GenBank database. All 64 sequences were then aligned using the CLUSTAL W program implemented in BioEdit 7.0.0 with default parameters (Hall, 1999). The ends were trimmed and ambiguous regions including about 95% gaps and few parts which appear four kinds of bases irregularly were eliminated manually. Finally, an alignment consisting of 1760 sites was used in the analyses. The hypotrich species *Oxytricha longa*, *Tetramena pustulata*, *Pseudokeronopsis flava*, *Nathoholosticha fasciola* and *Bergeriella ovata* were used as outgroup.

The Bayesian inference (BI) analysis was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) using the evolutionary model GTR + I (0.5424) + G (0.5537) selected under the AIC criterion by MrModeltest v.2 (Nylander, 2004). Markov chain Monte Carlo simulations were run for 1 200 000 generations, with two parallel runs, each with four simultaneous chains, sampling every 100 generations. Three thousand trees were discarded as a burn-in. All the remaining trees were used in the calculation of posterior probabilities applying the majority rule consensus.

The program Modeltest v.3.7 (Posada & Crandall, 1998) selected GTR + I (0.5424) + G (0.5537) under the AIC criterion as the best fitting model, which was subsequently used for maximum-likelihood (ML) analysis. The ML tree was carried out using the PhyML program (Guindon & Gascuel, 2003; Guindon et al., 2005). The reliability of the internal branches was assessed using a non-parametric bootstrap method with 1000 replications.

RESULTS

Order: Strombidiida Petz & Foissner, 1992
Family: Strombidiidae Fauré-Fremiet, 1970

Genus *Apostrombidium* Xu et al., 2009

*Apostrombidium parakielum* spec. nov. (Figs 1, 2; Table 1)

Diagnosis: body shape obconical to ovoid, about 30–50 × 25–40 μm *in vivo*. Extrusomes grouped in rows and arranged along horizontal part of the girdle kinety and longitudinally in dorsal centre. Adoral zone of membranelles composed of 14–16 anterior and 6–8 ventral membranelles. Somatic ciliation composed of three kinety fragments. K-1 started at the dorsal posterior end and extended obliquely up to the ventral centre. K-2 started above K-1, run horizontally around the left side of the body, extended to dorsal posterior and formed a ‘U-shape’. K-3 started on the right dorsal side, turned to the ventral side, and extended obliquely down to be inversely orientated and parallel to K-1. The cilia on the dorsal side conspicuously longer than others. Single ellipsoidal macro-nucleus.

Type locality: a sandy beach at Qingdao (39°10′ N 117°06′ E), China.

Etymology: the specific epithet ‘parakielum’ refers to the similarity in morphology between this species and *Varistrombidium kielum*. For example, the obconical body shape, the extremely long cilia on the dorsal side and the sandy living habitat.

Slide deposition: a protargol slide with the holotype specimen (marked with a blue circle) is deposited in the Laboratory of Protozoology, OUC, with registration number JMJ09051201-1. Two paratype slides with protargol-impregnated specimens are deposited in the Laboratory of Protozoology, OUC, with registration numbers JMJ09051201-2 and JMJ09051201-3, respectively.

Description: cell size about 30–50 × 25–40 μm *in vivo*. Body broadly obconical to ovoid with posterior end rounded (Figs 1a, b and 2a–d). Apical protrusion conspicuous, about 5 μm high on the right of the peristome, which normally disappears after protargol impregnation (Fig. 2e, arrow). Posterior portion of the cell covered by hemitheca with polygonal platelets about 2 μm across (Fig. 1f).

Cytoplasm colourless, filled with silt particles 2–5 μm across and some food vacuoles containing ingested algae, which often made the posterior portion dark *in vivo* (Figs 1a, b and 2a, c, g). Extrusomes rod-shaped, about 10 μm in length, mostly grouped in short rows which are composed of about five extrusomes and obliquely arranged anterior to
the horizontal part of the girdle kinety (Figs 1a, b, d and 2g, arrowhead). Some further extrusomes, however, grouped in two or three rows, longitudinally located in the dorsal centre (Fig. 1b; 1e, arrowhead). Macronucleus broadly ellipsoidal, about 16 × 12 μm after protargol impregnation, and centrally located (Figs 1h and 2j). Micronucleus, cytopyge and contractile vacuole not observed.

Cells rotated around the main axis when swimming and sometimes drifted in the water when motionless. Cells attached to debris using their anterior membranelles when crawling (Fig. 1c).

Buccal field wide, occupied one third of the body in length in vivo (Figs 1a and 2a). Anterior zone portion composed of 14–16 membranelles whilst ventral zone portion composed of 6–8 membranelles. Each membranelle included three rows of basal bodies. The bases of the ventral membranelles (2–4 μm wide) gradually decreased in width from distal to proximal end of zone portion (Figs 1a, h and 2i); the bases of the anterior membranelles conspicuously long (5 μm wide). Both ventral and anterior membranelles continuously arranged. Cilia of anterior membranelles up to 18 μm long in vivo, compared to about 6–8 μm for the cilia of ventral membranelles. Endoral membrane composed of a row of monokinetids located at right inner wall of buccal cavity (Fig. 1h).

Somatic ciliature composed of three kinety fragments. K-1 started at the dorsal posterior end, turned to the ventral side and extended obliquely up to about two thirds of the body length (Figs 1g, h and 2i, o, p), composed of about 15 dikinetids. K-2 started at the ventral centre and above the anterior end of K-1, run horizontally around the left side of the body to the dorsal side, turned down to the end and
then turned up to about two thirds of the body length to form a ‘U-shape’ on the dorsal side (Figs 1g–i and 2i, m, n, p), composed of about 55 dikinetids. K-3 started on the right dorsal side near the end of the second part, turned to the ventral side, extended obliquely down so that it is inversely orientated and parallel to K-1, then terminated at the dorsal end (Figs 1g, h and 2i, m–p), composed of about 27 dikinetids. All the dikinetids bear a cilium with left basal bodies. Each dikinetid of K-2 in the posterior dorsal portion bore a cilium about 15 μm long in vivo (Fig. 1b, arrowhead; Figs 1e, i and 2h, arrow), which was flexible and conspicuously longer than those in the other portion (which were about 2 μm in vivo) (Fig. 1i, arrowhead). No ventral kinety.

Gene sequence: the SSU rRNA gene sequence of *Apostrombidium parakielum* spec. nov. has been deposited in the GenBank database with accession number JX025560. The length and G+C content of the SSU rRNA gene were 1776 bp and 47.92 mol% respectively.

Fig. 2. Photomicrographs of *Apostrombidium parakielum* spec. nov. from live material (a–h) and after protargol impregnation (i–p). (a–d) Different body shapes. (e) Ventral view of the buccal field, arrow notes the apical protrusion. (f) Apical view of the adoral zone of membranelles. (g) Detail of cell showing the extrusomes (arrowheads) and silt particles (arrow). (h) Detail of cell showing the extremely long cilia (arrows). (i, m) Ventral and dorsal views of the same specimen, to show the infraciliature. (j) Detail of cell to show the macronucleus. (k) Dorsal view to show the kinety fragments and the attachment sites of extrusomes (arrow). (l) Buccal field. (n) Dorsal view to show the K-2 and K-3. (o) Ventral view to show the K-1 and K-3. (p) Ventral view to show the K-1, K-2 and K-3. K-1–3, Kinety 1–3; Ma, macronucleus. Bars: 20 μm (a–d); 5 μm (e, f); 10 μm (g, h).
Table 1. Morphometric characterizations of *Apostrombidium parakielum* spec. nov. (first row), *Novistrombidium apsheronicum* (Alekperov & Asadullayeva, 1997) Agatha, 2003 (second row) and *Novistrombidium testaceum* (Anigstein, 1914) Song & Bradbury, 1998 (third row)

All data are based on randomly selected protargol-impregnated specimens. Measurements in μm. Mean, arithmetic mean; \( n \), number of individuals examined; SD, standard deviation; –, data not available.

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*Ventral kinety in *N. apsheronicum* and *N. testaceum*.
†Girdle kinety in *N. apsheronicum* and *N. testaceum*. 
Genus Novistrombidium Song & Bradbury, 1998

Novistrombidium apsheronicum (Alekperov & Asadullayeva, 1997) Agatha, 2003 (Figs 3, 4; Table 1)

Description of the Guangdong population: cell size 50–60 × 45–55 μm in vivo. Body subcordate, with a slightly sharp posterior end, widest at shoulder. Anterior end slanted, with the right peristomial region slightly protruded, about 2 μm high (Figs 3a and 4a–c). The posterior cell portion, below the stripes of extrusome attachment sites, covered by hemitheca composed of polygonal platelets, about 2–3 μm across (Fig. 4f, g).

Cytoplasm colourless and filled with numerous food particles, probably ingested diatoms about 1 μm across. Extrusomes rod-shaped, about 15 μm in length, mostly grouped in bundles below the anterior adoral membranelles, with anterior ends attached the cell surface forming a fusiform shape; further extrusomes clustered as a row that converges to the ventral kinety (Figs 3a, b and 4e, f, g, arrow). Macronucleus question mark-shaped, the right part transversely positioned and the left part curved posteriorly (Figs 3f and 4k). Micronucleus, cytopyg and contractile vacuole not observed. Usually the cell swam in a zig-zag trace, swinging in a large amplitude (Fig. 3d).

Buccal field broad and extended to about two fifths of the body length. Adoral zone composed of about 13–16 anterior and 9–12 ventral membranelles (Figs 3c and 4c, d). Each membranelle composed of three rows of basal bodies. The cilia of the anterior membranelles up to 30 μm long in vivo, and the bases of the anterior membranelles about 15 μm wide (Figs 3e, f and 4d, h). The cilia of the ventral membranelles up to 15 μm long in vivo, and the bases of the ventral membranelles gradually decrease in width posteriad from 12 μm to 5 μm. Endoral membrane composed of a row of monokinetids.

Somatic ciliature comprised of a girdle kinety and a ventral kinety. Girdle kinety opening ventrally and commencing at the right side of the buccal lip, dextrally spiralling around the body, and terminating at the dorsal posterior end, composed of 36–49 dkinetids. Ventral kinety inserted into the open field of the girdle kinety, beginning just below the
buccal field, obliquely extending to the right, and finally terminating in the posterior dorsal portion, close to the left end of the girdle kinety and composed of about 17–23 dikinetids (Figs 3c, g and 4i, j). The anterior basal body of each ventral dikinetid and the left basal body of each girdle dikinetid possessing a cilium about 2 μm long. The oral primordium of the opisthe originated on the left ventral side, between the adoral zone of membranelles and the girdle kinety (Fig. 4l).

Gene sequence: the SSU rRNA gene sequence of *Novistrombidium apsheronicum* was deposited in the GenBank database with the accession number FJ876958. The length and G+C content of the SSU rRNA gene were 1774 bp and 46.56 mol% respectively.

*Novistrombidium testaceum* (Anigstein, 1914)
*Song & Bradbury, 1998* (Figs 5, 6; Table 1)

Description of the Guangdong population: cell size 35–55 × 45–60 μm in vivo. Body oblate heart shape. Usually, the body with a length-width ratio of up to 1:1.5, widest at shoulder. The anterior cell end with an inconspicuous apical protrusion and the posterior end slanted toward the left. Hemitheca not observed (Figs 5a, b and 6a–c; Fig. 6d, i, arrow). Cytoplasm colourless, containing some lipid droplets and food vacuoles with ingested bacteria. Extrusomes rod-shaped and about 18 μm in length. Some of the extrusomes grouped in bundles located below the anterior membranelles (Figs 5c and 6f, g, arrow). Their anterior ends attached to the cell surface in the shoulder with their attachment sites form a fusiform shape (Figs 5e and 6h, arrow), and their posterior portions inserted obliquely into the cell, to form a groove in the middle of the body (Fig. 6b, c, arrow). Other extrusomes, however, arranged in a single row along with the ventral kinety (Figs 5g and 6l, arrowhead). Macronuclear shape stable C-shaped; no other shapes observed. Micronucleus, cytopyge and contractile vacuole not observed (Figs 5g, 6k). Swimming achieved by slowly rotating in a large amplitude, moving faster when disturbed (Fig. 5d).
Buccal field broad and extended to mid-body (Fig. 5a, b). The anterior part of adoral zone composed of 15–16 membranelles with the cilia up to 30 μm long, the bases of which are about 15 μm wide. The ventral part composed of about 9 membranelles, the cilia of which are about 7–10 μm in length. Ventral membranelles connected with anterior membranelles while the bases of the ventral membranelles gradually shortened from the distal to proximal end of the zone portion (Figs 5f, h and 6d, j; Fig. 6i, arrowhead). All membranelles have three rows of basal bodies. Endoral membrane composed of a row of kinetids (Fig. 5f, h).

Somatic ciliature comprised a girdle kinety and a ventral kinety. Girdle kinety composed of about 63–76 dikinetids, dextrally spiralled, commenced below the right end of anterior membranelles, extended to the dorsal side, terminated at the posterior dorsal end, close to the posterior third of the ventral kinety. Ventral kinety composed of about 19–23 dikinetids, commenced at the posterior dorsal region and extended obliquely to the ventral side, terminated at the ventral midbody (Figs 5g, h and 6k, l). Each ventral dikinetid bearing a 3 μm long anterior cilium, each girdle dikinetid bearing a 2 μm long left cilium.

**Fig. 5. Novistrombidium testaceum** from life (a–e) and after protargol impregnation (f–h). (a) Ventral view of a typical specimen. (b) Small specimen. (c) Detail of the extrusomes below the anterior membranelles (arrow). (d) Pattern of locomotion. (e) Apical view to show the anterior membranelles (arrowheads) and anterior ends of extrusomes (arrows). (f, g) Ventral and dorsal views of the same specimen, to show the infraciliature. (h) Apical view of a specimen showing the adoral zone of membranelles. AM, anterior membranelles; E, endoral membrane; GK, girdle kinety; Ma, macronucleus; VK, ventral kinety; VM, ventral membranelles. Bars: 20 μm (a, b, f–h); 10 μm (c).

**Phylogenetic analyses**

The tree topologies inferred using BI and ML were basically identical and thus were combined here in a single tree (Fig. 7). The monophyly of the order Strombidiida was supported with high node values (1.00 BI, 89 % ML). *Annulostrombidium parakielum* spec. nov. basally clustered with the clade of *Varistrombidium kielum* and *Omegastrombidium elegans*, although this was low supported (0.56 BI, 28 % ML). Four species of *Novistrombidium* split into two clades, one of which, including *N. testaceum* and *N. apsheronicum*, was well-supported, while the other, including *Novistrombidium sinicum* and *Novistrombidium orientale*, grouped with *Parallestrombidium* sp. with high to low support values (1.00 BI, 57 % ML). *Williphyra maedai*, together with most *Strombidium* species, formed another clade. All of these organisms above form the group of the family Strombidiidae (in Fig. 7), which is a sister to the Tontoniidae clade made up of *Laboea strobila*, *Pseudotontonia simplicides*, *Spirotontonia turbinate* and *Spirotontonia taiwanica*. Interestingly, *Lynnella semiglobulosa* represents the basal branch of the subclass Oligotrichia rather than Choreotrichia which was highly or moderately supported (0.98 BI, 80 % ML).
DISCUSSION

Comparison with similar species

The genus *Apostrombidium* was recently established by Xu et al. (2009) based on the monotype *Apostrombidium pseudokielum*. This species is characterized by having two gaps at the posterior parts of the somatic kinety on both the ventral and dorsal sides, thus dividing the kinety into two parts. In our novel species, as described above, however, the somatic kinety is divided into three parts by three gaps, one gap on the posterior end, two gaps on the left and the right turning on the ventral and dorsal sides, respectively. In addition, our species can be differentiated from *A. pseudokielum* described by Xu et al. (2009) in the extremely long cilia present in the posterior dorsal dikinetids in our species [vs their absence in *A. pseudokielum* of Xu et al. (2009)].

Since no molecular data regarding *A. pseudokielum* is available, we tentatively suggest that the numbers of gaps/parts of the kinety in genus *Apostrombidium* could be variable but the general pattern and position of the kinety is stable. We therefore propose an improved diagnosis of *Apostrombidium* here.

Genus *Apostrombidium* Xu et al., 2009

Improved diagnosis: Strombidiidae with a single, strongly curved and discontinuous somatic kinety, which extends to the posterior end of the cell on both the ventral and dorsal sides, ventral kinety absent.

Considering other possible points of general morphological comparison the extremely long cilia on the dorsal side in combination with the sandy living habitat of *Apostrombidium parakielum* resembles *Varistrombidium kielum* (Maeda & Carey, 1985) Xu et al., 2009. However, the former differs from the latter in the pattern of somatic ciliature (somatic kinety divided into 3 parts which are horizontally positioned on lateral side and longitudinally extending to the posterior end of cell on both the ventral and dorsal sides vs somatic kinety divided into 5 parts.

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**Fig. 6.** Photomicrographs of *Novistrombidium testaceum* from live cells (a–i) and after protargol impregnation (j–l). (a) Ventral view of a typical specimen. (b) Small specimen, arrow marks the groove in the middle of the body. (c) Dorsal view, arrow marks the groove. (d) Apical view to show anterior membranelles (arrowheads) and the apical protrusion (arrow). (e) Ventral view to show the distribution of extrusomes (arrow and arrowhead). (f, g) To show the extrusomes below the adoral zone of membranelles (arrows). (h) Apical view showing the anterior ends of extrusomes (arrows). (i) Oral field, to show the ventral membranelles (arrowhead) and the apical protrusion (arrow). (j) Apical-ventral view, to show the adoral zone of membranelles. (k) Aboral views showing the somatic kinety. (l) Ventral views showing the girdle kinety and the ventral kinety. AM, anterior membranelles; GK, girdle kinety; Ma, macronucleus; VK, ventral kinety; VM, ventral membranelles. Bars: 20 μm (a–d); 10 μm (e–i).
which obliquely run across the whole cell) (Xu et al., 2009, 2011).

*N. testaceum* (Anigstein, 1914) Song & Bradbury, 1998 is similar to *N. apsheronicum* (Alekperov & Asadullayeva, 1997) Agatha, 2003 in both its ciliary pattern and the shape of the macronuclear nodule. However, the former is clearly different from the latter in its length : width ratio (1.5 : 1 vs 1 : 1), and the position of the posterior end of the girdle kinety (which is distinctly separated from the ventral kinety vs being close to the ventral kinety).

Two other *Novistrombidium* species, *N. orientale* Liu et al., 2009 and *N. sinicum* Liu et al., 2009, should be compared with *N. apsheronicum* and *N. testaceum*. *N. apsheronicum* and *N. testaceum* differ from *N. orientale* and *N. sinicum* in:

1) the absence of thigmotactic membranelles (vs their presence); 2) a C-shaped macronuclear nodule (vs ovoid); 3) the arrangement of the extrusomes (grouped in bundles below the anterior membranelles vs arranged evenly along with the girdle kinety) (Liu et al., 2009).

**Intraspecific comparison**

*N. apsheronicum* was first reported from the Caspian Sea as *Strombidium apsheronicum* (Alekperov & Asadullayeva, 1997). Based on the re-description of the population from Saudi Arabia (Agatha, 2003a), the species was transferred to the genus *Novistrombidium* as *N. apsheronicum*. This is the first time the species has been reported from Guangdong, China. The Guangdong population corresponds well with previous descriptions in both its general morphology (e.g. subcordinate body shape, extrusomes

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**Fig. 7.** Phylogenetic trees (BI/ML) based on small subunit rRNA genes. Newly sequenced species in the present study are shown in bold. GenBank database accession numbers are given in parentheses following the species names. Numbers near the branch node represent the posterior probability value in BI analyses and the bootstrap values from ML method; solid circles indicate full bootstrap support in both algorithms; -, represents disagreement between BI and ML topologies. Bar, 5 substitutions per 100 nt positions.
grouped in bundles and C-shaped macronucleus) and in the features of its protargol-impregnated infraciliature (e.g. the number of anterior and ventral membranelles, and the posterior ends of the girdle and ventral kineties being close to each other). Although the body length of our population in vivo is slightly shorter than that of the Saudi Arabia population (50–60 μm vs 60–95 μm), and the ends of both the girdle kinety and the ventral kinety extend to the dorsal side (vs terminate at the right ventral side) (Agatha, 2003a), we believe those differences to be population-dependent rather than indicative of a separate species.

*Novistrombidium testaceum* is the type species of the genus *Novistrombidium*, usually seen in various habitats in the sea (Modeo et al., 2003; Song & Bradbury, 1998). It is the first time this species has been reported from Guangdong Province, China. With references to its live features (e.g. oblate heart body shape, extrusomes grouped in bundles) and the features of its protargol-impregnated infraciliature (e.g. the number of anterior and posterior membranelles, spiralled girdle kinety and ventral kinety extending from posterior region to gap of girdle kinety), the current population corresponds very well with the previous description. However, our population is more similar to that of Modeo et al. (2003) than that of Song & Bradbury, (1998), referring to the extrusomes arranged along the ventral kinety (present in former vs absent in latter), and the apical protrusion (present in formers vs absent in latter) (Modeo et al., 2003; Song & Bradbury, 1998). Considering that the structures such as apical protrusion and extrusomes along the ventral kinety are difficult to observe in vivo and easily disappear in impregnated specimens, they were probably not observed by Song & Bradbury (1998). In addition, the macronuclei show different appearances in our population and that of Song & Bradbury (1998) (C-shaped macronucleus in former vs two transversely located band-like macronuclei joined together in latter), but both above forms of macronucleus were found by Modeo et al. (2003). Therefore, we believe the form of macronucleus is variable.

For *N. testaceum*, there are only two populations with available SSU rRNA gene sequences. One was reported by Modeo et al. (2003) with GenBank no. AJ488910; and the other one was submitted by Zhang et al. (2010) based on our population. The identity for these two sequences is 99.02 % (for the co-contained region), which, therefore, further confirms that they are conspecific.

Phylogenetic analyses of *Apostrombidium* and *Novistrombidium*

In the SSU rRNA gene trees of *A. parakielum* spec. nov. is closely related to *Varistrombidium kielum*. This result tends to suggest a close relationship between these two genera in evolution, which is consistent with their morphological similarities, such as the girdle kinety being divided into some fragments, and the lack of a ventral kinety.

In addition, the trees form a well-supported clade composed of *N. testaceum* and *N. apsheronicum* whereas the other *Novistrombidium* species, *N. orientale* and *N. sinicum*, cluster with *Parallelostrombidium* species. This corresponds with previous studies (Li et al., 2013; Liu et al., 2011a; Liu et al., in press) and further suggests the separation of *N. orientale* and *N. sinicum* from *Novistrombidium* considering the morphological data.

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