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LIANG ET AL. – Morphology and Phylogeny of *Vorticella* Species

Morphology and Phylogeny of Four New *Vorticella* Species (Ciliophora: Peritrichia) from Coastal Waters of Southern China

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ABSTRACT

Four new species of *Vorticella*, *V. parachiangi* sp. n., *V. scapiformis* sp. n., *V. sphaeroidalis* sp. n., and *V. paralima* sp. n., were isolated from coastal brackish waters of southern China. Their morphology, infraciliature, and silverline system were investigated based on observations of specimens both in vivo and following silver staining. *Vorticella parachiangi* sp. n. is distinguished by: a J-shaped macronucleus; a single dorsally located contractile vacuole; a two-rowed infundibular polykinetid 3, in which row 1 is shorter than row 2; 21-31 silverlines between peristome and aboral trochal band, 6-11 between aboral trochal band and scopula. *Vorticella scapiformis* sp. n. is characterized by its

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conspicuously thin and irregularly edged peristomial lip; a J-shaped macronucleus; a single, ventrally located contractile vacuole; row 1 of the infundibular polykinetid 3 proximally shortened; 18-25 silverlines between peristome and aboral trochal band, 8-12 between aboral trochal band and scopula. *Vorticella sphaeroidalis* sp. n. can be identified by its small, sub-spherical zooid; a C-shaped macronucleus; a ventrally located contractile vacuole; an aboral trochal band adjacent to the scopula; 16-18 silverlines between peristome and aboral trochal band, two between aboral trochal band and scopula. *Vorticella paralima* sp. n. can be identified by its ovoidal zooid; a J-shaped macronucleus; a dorsally positioned contractile vacuole; rows 1 and 2 of the infundibular polykinetid 3 proximally shortened; 26-35 silverlines from peristome to aboral trochal band, and 7-13 from aboral trochal band to scopula. The SSU rDNA genes of these four species were sequenced and their phylogeny was analyzed.

Keywords: Biodiversity; brackish water; mangrove wetland; Vorticellida; taxonomy.

THE peritrich order Vorticellida which comprises over 100 genera, is a large, distinctive assemblage of ciliates that are commonly found in a wide variety of marine, freshwater, and terrestrial habitats (Foissner et al. 2010; Li et al. 2015; Lynn 2008; Shen et al. 2017; Sun et al. 2015). The sessilids comprise two major sub-clades, i.e., the refined sub-orders Vorticellina de Fromentel. 1875 and Operculariina Jankowski, 1980, as recognised by Jankowski (2007). Based on analyses of their molecular phylogeny, Utz et al. (2010) suggested that the two sessilid sub-clades should be assigned to the rank of order, i.e., Vorticellida and Operculariida. Discussion of peritrich taxonomy above the rank of family is outside the scope of this paper. *Vorticella* Linnaeus, 1767 was the first reported genus of peritrichs. The generic definition was refined by Ehrenberg (1838), who limited it to the solitary Vorticellidae with an unbranched, spirally contractile stalk. Subsequently, *Vorticella* became the largest genus of sessile peritrichs with over 300 species having been described in the 250 years since it was created. Noland and Finley (1931) published the first comprehensive revision of the genus, listing 220 species or named varieties that had been assigned to the genus by 1930, and reduced its membership to 95 species following the elimination of many that obviously belonged to other peritrich genera, to other ciliate taxa, or to animal phyla (e.g., rotifers, cnidarians, crinoids). An additional 113 species, varieties, or forms of *Vorticella* were described in the period between Noland and Finley's paper and publication of the next major revision of the genus by Warren (1986) who excluded over 100 dubious species, reducing the total membership of the genus to 86 species. Many new species of *Vorticella* have since been described, raising the total to more than 100 again (e.g., Song 1997; Sun et al. 2006, 2009, 2017).

The specialized morphology of *Vorticella* reflects its sessile, suspension-feeding lifestyle. The cell body, or zooid, is usually shaped like an inverted bell but may be cylindroid, conical or sub-spherical. The exposed part of the oral region is the peristome, a generally flattened surface with a thickened border or lip and a raised epistomial disc in the center. Two bands of cilia, the haplokinety and polykinety, run counterclockwise around the peristome and beat to create a vortex that carries suspended particles into the oral opening or infundibulum. The shape and size of the zooid, width of

the peristome, height and contour of the epistomial disc and thickness of the peristomial lip have been used as taxonomic characters. Species identification of *Vorticella* is, however, frequently very difficult because of its variable zooid shape and size and its highly contractile nature resulting in the creation of numerous species and varieties of doubtful taxonomic validity (Itabashi et al. 2002; Noland and Finley 1931; Warren 1986).

In the last 50 years, silver staining methods have been used to reveal additional characters for species circumscription and identification. These include the transverse pellicular ridges that encircle the zooid and can be visualized either as silverlines by staining with silver nitrate or directly by scanning electron microscopy, and the structure of the infundibular polykineties within the infundibulum, which can be revealed by staining with protargol. These characters are now routinely used in the determination of species (Sun et al. 2006, 2017). More recently, gene sequence information has also been included in species descriptions and redescriptions (Ji et al. 2015; Sun et al. 2012). Phylogenetic studies based on gene sequence data derived from carefully identified species have shown that *Vorticella* is a paraphyletic group (Gao et al. 2016; Sun et al. 2012, 2013).

In the present study, four new solitary vorticellids were discovered during a survey of ciliate diversity in coastal waters of southern China. Detailed investigations of the specimens, both in vivo and following silver staining (protargol and silver nitrate), and analysis of their small subunit (SSU) rDNA gene sequences revealed them to be new members of the genus *Vorticella*.

MATERIALS AND METHODS

Sample collection

Vorticella parachiangi sp. n. was collected on 14th March 2010 from a mangrove wetland on Techeng Island (21°16'N; 110°44'E) in Zhanjiang, Guangdong Province, China, at a water temperature of 25 °C, a salinity of 25 ‰, and a pH of about 7.

Vorticella scapiformis sp. n. was collected on 21st March 2010 from a sewage discharge outlet on Donghai Island (21°02'N; 110°34'E) in Zhanjiang, Guangdong Province, China, at a water temperature of 25 °C, a salinity of 12 ‰, and a pH of about 9.

Vorticella sphaeroidalis sp. n. was collected on 5th May 2011 from a small ditch near the coast (23°38'N; 116°68'E) of Shantou, Guangdong Province, China, at a water temperature of 23 °C, a salinity of 5 ‰, and a pH of about 7.

Vorticella paralima sp. n. was collected on 11th October 2009 from Gaoqiao mangrove wetland (21°53'N; 110°28'E) in Zhanjiang, Guangdong Province, China, at a water temperature of 23 °C, a salinity of 23 ‰, and a pH of about 7.8.

Morphological studies

Ciliates were observed in vivo using bright field and differential interference contrast microscopy (Nikon 80i, Japan). The infraciliature and silverline system were revealed by the protargol method following Wilbert (1975) and the Chatton-Lwoff silver nitrate-stain method following Song and Wilbert (1995), respectively. Counts and measurements of stained specimens were performed at

1,000× magnification. Drawings of stained specimens were conducted by hand with the help of a camera lucida at 1,000× magnification. Classification and terminology are according to Lynn (2008).

SSU rDNA gene sequencing and phylogenetic analysis

Genomic DNA was extracted, using the RED Extract-N-Amp Tissue PCR Kit. The SSU rDNA sequence was amplified by PCR, using the following primers: EukA (5'-AACCTGGTTGATCCTGCCAGT-3'), EukB (5'-TGATCCTTCTGCAGGTTACCTAC-3') (Medlin et al. 1988). Cycling parameters were as follows: one cycle for initial denaturation (94 °C for 5 min); 35 cycles for amplification (94 °C for 1 min; 56 °C for 2 min; 72 °C for 2 min) and one cycle for the final extension (72 °C for 10 min).

Phylogenetic trees were constructed by two methods: Bayesian inference (BI) and maximum likelihood (ML). Fifty-six taxa were used for phylogenetic analyses, including the four that were newly sequenced and 52 previously sequenced obtained from the NCBI GenBank database. The SSU rDNA of three *Vorticella* species sequenced by Sun et al. (2013), namely, *V. chlorostigma*, *V. striata* and *V. natans*, were removed from the alignment after preliminary analysis because of their short lengths. The hymenostomes *Tetrahymena bergeri* and *T. corlissi* were selected as outgroup taxa. Sequences were aligned with the ClustalW Multiple alignment in BioEdit 7.2.5 (Hall 1999) and then refined by removing ambiguous gaps at both termini of the alignment and in the highly variable V4 and V9 regions. The length of the final alignment was 1,616 bp.

The tree topologies were inferred, using the best-fit model GTR + I + G selected by AIC criterion in MrModeltest 2.2 (Nylander 2004). A 50 % majority-rule Bayesian inference tree was constructed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The chain length for the analysis was 1,000,000 generations with trees sampled every 100 generations; the first 25% of trees were discarded as burn-in (Li et al. 2015). The Maximum likelihood trees inferred from the sequences were constructed with the PhyML 3.0 program (Guindon et al. 2005) with a parametric bootstrap base frequency of 1,000.

ZooBank registration: ZooBank registration number of the present work (see Recommendation 8A of ICZN 2012): urn:lsid:zoobank.org:pub: C1F52FCF-46AB-4444-900E-1CD9B58FA00A.

RESULTS

Description of *Vorticella parachiangi* sp. n.

Zooid inverted campanulate when expanded, size 50-65 µm × 55-70 µm in vivo, ratio of length to width about 1:1, maximum width at peristome. Peristomial lip thin, ca. 4 µm thick. Epistomial disc slightly elevated above peristome (Fig. 1A, C, 2A, C). Length of oral cilia about 12 µm. Pellicle occasionally slightly folded on ventral and dorsal sides (Fig. 1C, 2D). Transverse pellicular striations visible at 1000 × magnification (Fig. 2E, arrows). Telotroch not observed.

Cytoplasm semi-transparent, slightly gray, usually containing a few food vacuoles about 10-15 µm in diameter (Fig. 1A, C). Contractile vacuole near dorsal wall of infundibulum, approximately 10 µm in diameter when fully expanded (Fig. 1A, 2D). Macronucleus J-shaped, apical portion horizontally oriented around infundibulum, hook-shaped posterior portion extends almost longitudinally to level of aboral trochal band. Micronucleus not observed.

Stalk up to 350 µm long and 4 µm in diameter, surface smooth without attached bacteria. Stalk spasmoneme about 2 µm in diameter, with a string of light gray thecoplasmic granules (ca. 0.5 µm in diameter) sparsely distributed along its length (Fig. 2F).

Haplokinety and polykinety describe about 1¼ turns around peristome and continue into infundibulum where they separate and make a further turn. Polykinety transforms into three infundibular polykineties (P1-3) at lower half of infundibulum. P1 and P2 are composed of three distinct rows of kinetosomes, P3 is two-rowed (Fig. 1E, F). Row 1 of P3, i.e. the one nearest to P2, is only half length of row 2 and terminates together with P2, while row 2 of P3 extends to the proximal end of P1 (Fig. 1F, 2H). Germinal kinety lies in distal half of infundibulum, parallel to haplokinety. Epistomial membrane located at entrance of infundibulum (Fig. 1F, 2H). Aboral trochal band dikinetidal, encircles zooid at 80 % distance from peristome to scopula (Fig. 1E, 2G). Silverline system consists of 21-31 silverlines between peristome and aboral trochal band and 6-11 between aboral trochal band and scopula; with sparsely distributed pellicular pores (Fig. 1D, 2I; Table 1).

Description of *Vorticella scapiformis* sp. n.

Zooid elongate obconical in expanded state, size 40-50 µm × 40-55 µm in vivo, maximum width at peristome, with constriction posterior to peristomial lip, which is approximately 2 µm thick. Peristome with irregular protuberances on rim. Epistomial disc slightly convex (Fig. 3A, C, 4A, B). Length of oral cilia about 10 µm. Telotroch not observed.

Cytoplasm semi-transparent and slightly grayish, usually containing a few to several ellipsoidal or spherical granules (3-6 µm in diameter) and food vacuoles (8-10 µm in diameter) (Fig. 3A, 4A, D). Contractile vacuole located near ventral wall of infundibulum at about 25 % of body length, approximately 12 µm in diameter when fully expanded (Fig. 3A, B, 4C). Macronucleus J-shaped, apical portion horizontally oriented around infundibulum, hook-shaped posterior portion extends almost longitudinally to level of aboral trochal band (Fig. 3E, 4G). Micronucleus not observed. Pellicular striations closely spaced, visible at 1,000× magnification (Fig. 4E).

Stalk 500-600 µm in length and 6 µm in diameter with smooth surface. Stalk spasmoneme about 2 µm in diameter with dark gray thecoplasmic granules (ca. 0.5 µm in diameter) sparsely distributed at periphery (Fig. 3D, 4F). Haplokinety and polykinety make approximately 1¼ turns around peristome before entering infundibulum where they separate and make a further half-turn. Polykinety transforms into three infundibular polykineties (P1-3) at lower half of infundibulum. P1-3 composed of three kinetosome rows each. P1 converges with P3 in proximal portion of infundibulum and terminates at same level as the latter. Row 1 of P3 shortened proximally and terminates at curvature of P1. P2 terminates near the curvature of P1, just above row 1 of P3. Germinal kinety lies in distal portion of infundibulum, parallel to haplokinety (Fig. 3E, F, 4H). Epistomial membrane located near entrance of infundibulum. Aboral trochal band dikinetidal, encircles zooid at 85 % distance from peristome to scopula (Fig. 3E). Silverline system consists of 18-25 silverlines between peristome and aboral trochal band and 8-12 between aboral trochal band and scopula (Fig. 4I; Table 1).

Description of *Vorticella sphaeroidalis* sp. n.

Zooid almost globular, size 20-30 µm × 15-25 µm in vivo, maximum width at mid-body. Peristome approximately 15 µm across. Peristomial lip moderately thick. Epistomial disc elevated high above peristome (Fig. 5A, B, 6A, B). Length of oral cilia about 7 µm. Pellicular striations and several grayish granules visible on zooid surface at 400 × magnification (Fig. 5E, 6E, G). Telotroch not observed.

Cytoplasm semi-transparent, slightly grayish and containing a few food vacuoles, 3-5 µm in diameter (Fig. 6D). Contractile vacuole located near ventral wall of infundibulum, approximately 6 µm in diameter when fully expanded (Fig. 5A, B, 6C). Macronucleus C-shaped, transversely oriented, surrounding opening of infundibulum (Fig. 5D, 6D, H). Micronucleus not observed.

Stalk approximately 40-50 μm long, 2 μm across, surface smooth occasionally with some attached bacteria and algae. Spasmoneme about 1 μm in diameter, with several slightly grayish thecoplastic granules (ca. 0.3 μm in diameter) distributed in periphery.

Haplokinety and polykinety make approximately $1\frac{1}{4}$ turns around peristome and extend into infundibulum where they separate and make a further turn. Polykinety transforms into three infundibular polykineties (P1-3) at lower half of infundibulum. P1 and P2 are composed of three distinct rows of kinetostomes. Arrangement of P3 unclear due to limited number of specimens and improper specimen orientation for observation. P3 very likely two-rowed (Fig. 5C, D). Aboral trochal band dikinetid, encircles zooid at 88 % distance from peristome to scopula (Fig. 5D, 6G).

Silverline system consists of 16-18 silverlines between peristome and aboral trochal band and two between aboral trochal band and scopula; with sparsely distributed pellicular pores (Fig. 5E, 6J, K; Table 1).

Description of *Vorticella paralima* sp. n.

Zooid obovoid, with truncated oral end and narrowed aboral end in expanded state, size 35-50 μm $\times$ 20-30 μm in vivo. Cell proper usually widest at mid-body, with constriction posterior to peristomial lip (Fig. 7A, B, 8A, B). Peristomial lip everted and thin, approximately 3 μm thick. Epistomial disc elevated about 4 μm above peristome. Oral cilia about 12 μm long (Fig. 7A, B, 8A, C). Pellicular striations observable at 1,000 x magnification (Fig. 8D, E).

Cytoplasm semi-transparent and slightly grayish, usually containing a few to several food vacuoles 3-5 μm in diameter. Contractile vacuole located at dorsal wall of infundibulum in anterior fourth of zooid, about 7 μm in diameter when fully expanded (Fig. 8A, C). Macronucleus J-shaped, apical portion horizontally oriented around infundibulum, hook-shaped posterior portion terminates slightly below level of aboral trochal band (Fig. 8F). Micronucleus not observed.

Stalk approximately 80-120 μm long and 4 μm in diameter, surface smooth with a few attached bacteria and algae. Spasmoneme about 2 μm in diameter; thecoplastic granules not observed (Fig. 7B).

Haplokinety and polykinety make approximately $1\frac{1}{2}$ turns around peristome and extend into infundibulum where they separate and make another turn. Polykinety transforms into three infundibular polykineties (P1-3) at lower half of infundibulum. P1-3 three-rowed. P1 and P3 converge at curvature of the latter. Row 3 of P3 extends to the end of infundibulum where it terminates near the end of P1. Rows 1 and 2 of P3 are half the length of row 3, terminating together with P2 (Fig. 7D, 8G). Germinal kinety lies in distal portion of infundibulum parallel to haplokinety. Epistomial membrane near entrance of infundibulum (Fig. 7E). Aboral trochal band dikinetid, encircles zooid at 75 % distance from peristome to scopula (Fig. 7D, 8F).

Silverline system consists of 26-35 silverlines from peristome to aboral trochal band and 7-13 from aboral trochal band to scopula; with numerous irregularly distributed pellicular pores (Fig. 7F, 8H, I; Table 1).

Analyses of SSU rDNA sequence

The four new SSU rDNA sequences have been deposited in the GenBank database with length and GC content as follows: *Vorticella parachiangi* sp. n., 1,729 bp, 44.07%; *V. scapiformis* sp. n., 1,727 bp, 43.66%; *V. sphaeroidalis* sp. n., 1,742 bp, 43.51%; *V. paralima* sp. n. 1,651 bp, 43.50%.

Phylogenetic trees constructed with ML and BI analyses had almost identical topologies, therefore only the ML tree is shown here (Fig. 9). In all phylogenetic analyses, the three new species clustered

with species of *Vorticella* and *Pseudovorticella* within the family Vorticellidae. Within the *Vorticella*-*Pseudovorticella* subclade, *V. parachiangi* sp. n. is sister group to *V. scapiformis* sp. n. with maximal support (100% ML, 1.00 BI), *V. sphaeroidalis* sp. n. and *Vorticellides astyliformis* cluster together with high support (95% ML, 1.00 BI) and are sister to the *Vorticellides aquadulcis*-complex with differential support (99% ML, 0.53 BI). *Vorticella paralima* sp. n. and *Pseudovorticella* species form a clade that is sister to the grouping of *V. parachiangi* sp. n. and *V. scapiformis* sp. n.

DISCUSSION

Commonly accepted characters for species circumscription in *Vorticella* include zooid shape and size, number and location of contractile vacuoles, shape and position of the macronucleus, oral morphology (appearance of peristomial lip and epistomial disc), oral infraciliature (especially structure of P3), features of the silverline system, and habitat (Sun et al. 2006, 2017). The four new species could each be separated from their congeners and other similar taxa based on combinations of these characters.

Comparison of *Vorticella parachiangi* sp. n. with related species.

Vorticella parachiangi sp. n. resembles *V. chiangi* Sun et al., 2017 in terms of its zooid shape. Nevertheless, it can be separated from the latter by the zooid size in vivo (50-65 μm $\times$ 50-65 μm vs. 35-50 μm $\times$ 35-50 μm), the number of silverlines (21-31 vs. 33-37 between peristome and aboral ciliary wreath; 6-11 vs. 10-14 between aboral ciliary wreath and scopula), and the structure of P3 (two-rowed vs. three-rowed) (Sun et al. 2017).

Another congener that closely resembles *V. parachiangi* sp. n. is *V. fornicata* (Dons, 1915), which has a similar zooid shape, but these species differ in the zooid size in vivo (50-65 μm $\times$ 50-65 μm vs. 24-32 μm $\times$ 24-37 μm) and the shape of the macronucleus (J-shaped vs. horseshoe-shaped) (Song 1991).

Living cells of three *Pseudovorticella* species, namely, *P. patellina* Müller, 1776, *P. marina* (Greeff, 1870) Ji et al., 2009 and *P. zhengae* Ji et al., 2006, bear some resemblance to *V. parachiangi* sp. n. at low magnifications, although all three can be separated from the latter by having a reticulate (vs. transverse) silverline system. In addition, *P. patellina* has a much larger zooid in vivo than *V. parachiangi* sp. n. (55-110 μm $\times$ 50-100 μm vs. 50-65 μm $\times$ 50-65 μm) and two (vs. one) contractile vacuoles (Song and Warren 2000). *Vorticella parachiangi* sp. n. can be separated from *P. marina* by zooid size in vivo (50-65 μm $\times$ 50-65 μm vs. 40-50 μm $\times$ 35-45 μm) and the structure of P3 (two-rowed vs. three-rowed) (Sun et al. 2009). *Vorticella parachiangi* sp. n. can be separated from *P. zhengae* by the number of transverse silverlines between the aboral trochal band and the scopula (6-11 vs. 11-14), and the structure of P3 (two-rowed vs. three-rowed) (Ji et al. 2006).

Comparison of *Vorticella scapiformis* sp. n. with congeners.

Vorticella similis Stokes, 1887 and *V. spuripicta* Song, 1997 bear some resemblance to *V. scapiformis* sp. n. in terms of zooid shape, macronucleus shape, and habitat (Song 1997). *Vorticella scapiformis* sp. n. can be separated from *V. similis* by the shorter zooid (40-50 μm vs. 52-58 μm), fewer transverse silverlines both between the peristome and the aboral trochal band and between the aboral trochal band and the scopula (18-25 and 8-12 vs. 75-80 and 28-34) and in the position of the contractile vacuole (in the anterior 1/4 of zooid vs. in mid-zooid) (Song 1997).

Vorticella spuripicta differs from *V. scapiformis* sp. n. in respect to the number of silverlines between the aboral trochal band and both the peristome and the scopula (35-39 and 11-15 vs. 18-25 and 8-12, respectively), and in having two (vs. one) contractile vacuoles (Song 1997).

Comparison of *Vorticella sphaeroidalis* sp. n. with congeners.

Although the structure of P3 could not be clearly observed, *V. sphaeroidalis* sp. n. can be easily distinguished from its congeners by the combination of its small and globular zooid, and the location of the aboral trochal band close to the scopula. Three congeners, *V. parapulchella* Sun et al., 2006, *V. pulchella* Sommer, 1951, and *V. sinica* Sun et al., 2009, have to be compared with the new species. *Vorticella sphaeroidalis* sp. n. resembles *V. parapulchella* in zooid size and number of silverlines. Nevertheless, the former differs from the latter in respect to the conspicuously more posterior position of its aboral trochal band, the globular (vs. barrel-shaped) zooid, and its brackish water (vs. marine) habitat (Sun et al. 2006).

Like *V. sphaeroidalis* sp. n., *V. pulchella* has widely spaced pellicular striations. However, the new species can be separated by its much shorter zooid size (20-30 μm vs. 40-46 μm) and more posterior position of the aboral trochal band (Sommer 1951).

Vorticella sinica resembles *V. sphaeroidalis* sp. n. in terms of zooid size and shape and widely spaced pellicular silverlines. However, *V. sphaeroidalis* sp. n. can be separated from the former by its single-layered (vs. double-layered) peristomial lip, very likely two-rowed (vs. three-rowed) P3, and the conspicuously more posterior position of its aboral trochal band (Sun et al. 2009).

Comparison of *Vorticella paralima* sp. n. with congeners.

The congener that most closely resembles *Vorticella paralima* sp. n. is *V. lima* Kahl, 1933 which was transferred to the genus *Pseudovorticella* by Jankowski (1976), and transferred back to the genus *Vorticella* by Warren (1986). *Vorticella paralima* sp. n. differs from *V. lima* by: (1) its shorter zooid in vivo (35-50 μm vs. 60-70 μm); (2) the shape of the macronucleus (very long, with a distinctly horizontal anterior portion and a recurved posterior portion vs. elongate, only slightly curved anteriorly and not recurved posteriorly); (3) habitat (brackish. vs. marine); (4) the dorsal (vs. ventral) position of the contractile vacuole (Kahl 1933).

Vorticella hamata Ehrenberg, 1831 also resembles *V. paralima* sp. n. in terms of its zooid shape and size, habitat and number of silverlines. Nevertheless, the two can be separated by the shape of the macronucleus (C-shaped vs. J-shaped) (Song 1991).

The present form is similar to *V. striata* Dujardin, 1841 in terms of body shape and general appearance in vivo. However, the latter species can be clearly separated from *V. paralima* sp. n. by its shorter zooid in vivo (22-40 μm vs. 35-50 μm), C-shaped (vs. J-shaped) macronucleus, and the number of silverlines between the aboral trochal band and the scopula (5-7 vs. 7-13) (Song 1991).

Living cells of *V. utriculus* Stokes, 1885 and *V. aequilata* Kahl, 1935 also bear some resemblance to *V. paralima* sp. n. in terms of their zooid size and shape and numbers of silverlines. However, *V. utriculus* can be distinguished from *V. paralima* sp. n. by its C-shaped (vs. J-shaped) macronucleus and indistinct (vs. distinct) pellicular striations (Song 1991; Stokes 1885). *Vorticella aequilata* can be distinguished from *V. paralima* sp. n. by its C-shaped (vs. J-shaped) macronucleus (Kahl 1935).

Phylogenetic positions of four *Vorticella* species within the Vorticellidae

In the past decade, several studies have suggested that the family Vorticellidae is a nonmonophyletic assemblage of morphologically diverse groups (Miao et al. 2004; Sun et al. 2012, 2013). Among the genera of Vorticellidae, *Vorticella* Linnaeus, 1767, *Pseudovorticella* Foissner and Schiffmann, 1974, and *Epicarchesium* Jankowski, 1985 were reported to be nonmonophyletic (Sun et al. 2016).

In this study, *Vorticella* species are distributed among two clades: the majority cluster with *Pseudovorticella* species in the family Vorticellidae whereas the remainder, including *V. microstoma*, *V. infusionum*, *V. mayeri*, *V. sphaeroidalis* sp. n. and *Vorticellides* species, group with the Astylozoidae, the sister group of the Vorticellidae. The topology of our tree is consistent with previous studies (Ji et al. 2015; Li et al. 2015; Sun et al. 2012, 2013).

Among the new species, *V. paralima* sp. n. groups with *Pseudovorticella punctata* first with maximal support and both cluster with two *Pseudovorticella* species with a strongly support. This indicates a very close phylogenetic relationship of *V. paralima* sp. n. with these three *Pseudovorticella* species. However, they can be clearly differentiated by the zooid shape and silverline system (Ji et al. 2003, 2004, 2006). *Vorticella parachiangi* sp. n. and *V. scapiformis* sp. n. cluster together with maximal support to form a group that is sister to the subclade of *Pseudovorticella* species and *V. paralima* sp. n.

Vorticella sphaeroidalis sp. n. clusters with *Vorticellides* species to form a strongly supported subclade that is sister to the Astylozoidae. Foissner et al. (2010) transferred a few *Vorticella* species to the newly established genus *Vorticellides* Foissner et al., 2010 based on the possession of two epistomial membranes, a small barrel-shaped zooid (usually < 60 µm long), and a minute two-rowed P3. Molecular data also supported a close phylogenetic relationship between small, barrel-shaped species, such as *V. astyliformis* and *V. microstoma*, and certain stalkless peritrichs such as *Opisthonecta* spp. and *Astylozoon enriquesi* (Foissner et al. 2010). Thus, it is not surprising that *V. sphaeroidalis* sp. n. clusters with members of *Vorticellides* and Astylozoidae based on its small zooid (20-30 µm × 15-25 µm), globular shape, and the structure of P3.

TAXONOMIC SUMMARY

Order Sessilida Kahl, 1933

Family Vorticellidae Ehrenberg, 1838

Genus *Vorticella* Linnaeus, 1767

Vorticella parachiangi sp. n.

Diagnosis. Zooid inverted bell-shaped, 50-65 µm × 50-65 µm in vivo. Macronucleus J-shaped. Single contractile vacuole dorsally located. 21-31 transverse silverlines between peristome and aboral trochal band, 6-11 between aboral trochal band and scopula. P3 two-rowed; row 1 only half the length of row 2, and terminates together with P2; row 2 of P3 extends to proximal end of P1.

Type locality and habitat: Mangrove wetland on Techeng Island in Zhanjiang (21°16'N; 110°44'E), Guangdong Province, China. Water temperature 25 °C, salinity 25 ‰, pH about 7.

Deposition of slides: A protargol slide with the holotype specimen circled by ink is deposited in the Laboratory of Protozoology, Ocean University of China, Qingdao, China with the registration number SZ-2010-0314-01-3. One paratype slide with silver nitrate-stained specimens is deposited in the Natural History Museum, London, UK, with the registration number NHMUK 2018:3:1.1.

Etymology: The Greek word *para* (similar, near) indicates that the type population is similar to *Vorticella chiangi* Sun et al., 2016.

Gene sequence. The SSU rDNA sequence has been deposited in GenBank with the accession number KY594767.

ZooBank registration: ZooBank registration number of the present work (see Recommendation 8A of ICZN 2012): urn:lsid:zoobank.org: act:87851AFC-EB3D-4F02-A909-D78ECF79CAEB

***Vorticella scapiformis* sp. n.**

Diagnosis: Zooid elongate obconical, 40-50 μm $\times$ 40-55 μm in vivo, with conspicuously everted peristomial lip. Macronucleus J-shaped. Single contractile vacuole ventrally located. 18-25 silverlines between peristome and aboral trochal band, 8-12 between aboral trochal band and scopula. P3 three-rowed; rows 2 and 3 terminate together with P1; row 1 of P3 is proximally shortened terminating with P2 at curvature of P1.

Type locality and habitat: Sewage outlet on Donghai Island Zhanjiang (21°02'N; 110°34'E), Guangdong Province, China. Water temperature 25 °C, salinity 12 ‰, pH about 9.

Type specimens: A protargol slide with the holotype specimen circled by ink is deposited in the Laboratory of Protozoology, Ocean University of China with the registration number SZ-2010-0321-01-3. One paratype slide with silver nitrate-stained specimens is deposited in the Natural History Museum, London, UK, with the registration number NHMUK 2018.3.1.2.

Etymology: The Latin adjective *scapiform-is*, *-is*, *-e* ([m, f, n]; bacilliform) refers to the bacilliform body shape.

Gene sequence. The SSU rDNA sequence has been deposited in GenBank with the accession number KY594768.

ZooBank registration: ZooBank registration number of the present work (see Recommendation 8A of ICZN 2012): urn:lsid:zoobank.org: act:06390D5C-670F-4899-87FC-A9D6E05D23A8

***Vorticella sphaeroidalis* sp. n.**

Diagnosis: Zooid globular, 20-30 μm $\times$ 15-25 μm in vivo, not constricted below peristome. Macronucleus C-shaped. Single contractile vacuole ventrally located. 16-18 transverse silverlines between peristome and aboral trochal band, two between aboral trochal band and scopula.

Type locality and habitat: A small ditch near the coast of Shantou (23°38'N; 116°68'E), Guangdong Province, China. Water temperature 23 °C, salinity 5 ‰, pH about 7.

Type specimens: A protargol slide with the holotype specimen circled by ink is deposited in the Laboratory of Protozoology, Ocean University of China, Qingdao, China with the registration number SZ-2011-0505-01-2. One paratype slide with silver nitrate-stained specimens is deposited in the Natural History Museum, London, UK, with the registration number NHMUK 2018.3.1.3.

Etymology: The Latin adjective *sphaeroidalis* refers to the globular zooid shape.

Gene sequence. The SSU rDNA sequence has been deposited in GenBank with the accession number KY594766.

ZooBank registration: ZooBank registration number of the present work (see Recommendation 8A of ICZN 2012): urn:lsid:zoobank.org: act:F91AC656-E89C-4309-9FB8-5F9BE331F530

***Vorticella paralima* sp. n.**

Diagnosis: Zooid inverted bell-shaped, 35-50 μm $\times$ 20-30 μm in vivo. Width of peristomial lip about equal to maximum body width. Macronucleus J-shaped or vermiform. Single contractile vacuole dorsally located. 26-35 silverlines between peristome and aboral trochal band, 7-13 between aboral

trochal band and scopula. Rows 1 and 2 of P3 are half the length of row 3; P2 and P3 terminate at curvature of P1.

Type locality and habitat: Mangrove wetland at Gaoqiao town, Zhanjiang (21°53'N; 110°28'E), Guangdong Province, China. Water temperature 23 °C, salinity 23 ‰, pH 7.8.

Type specimens: A protargol slide with the holotype specimen circled by ink is deposited in the Laboratory of Protozoology, Ocean University of China, Qingdao, China with the registration number SZ-2009-1011-01-2; One paratype slide with silver nitrate-stained specimens is deposited in the Natural History Museum, London, UK, with the registration number NHMUK 2018.3.1.4.

Etymology: Composite of the Greek word *para*-(similar, near) and the specific epithet *lima* indicating that the type population is similar to *Vorticella lima* Kahl, 1933.

Gene sequence. The SSU rDNA sequence has been deposited in GenBank with the accession number KY594765.

ZooBank registration: ZooBank registration number of the present work (see Recommendation 8A of ICZN 2012): urn:lsid:zoobank.org: act:6E42E1AE-8F12-4E53-9920-0C7ACD25BE0D

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Figure legends

Figure 1 *Vorticella parachiangi* sp. n. in vivo (A–C) and after silver nitrate (D) and protargol (E, F) staining. **A.** General view of typical zooid; **B.** Showing proportion of stalk and zooid; **C.** Typical zooid. Arows indicate folds on the surface; **D.** Silverline system. Arrow marks the aboral trochal band; arrowhead indicates pellicular pore; **E.** Infraciliature and macronucleus; **F.** Oral ciliature. Arrowhead marks the epistomial membrane. H, haplokinety; G, germinal kinety; Ma, macronucleus; P1–3, infundibular polykinety1–3; Po, polykinety. Scale bars: 40 µm (A); 200 µm (B); 25 µm (E).

Figure 2 Photomicrographs of *Vorticella parachiangi* sp. n. in vivo (**A-F**) and after protargol (**G-H**) and silver nitrate (**I**) staining. **A.** Lateral view showing the stalk length relative to the zooid; **B.** Apical view. Arrows mark margin of peristomial lip; **C.** Lateral view of zooid showing thickness of peristomial lip (arrow); **D.** Contractile vacuole (arrow) and food vacuole (arrowhead); **E.** Zooid at high magnification and with differential interference contrast optics. Arrows indicate pellicular striations; **F.** Detail of stalk; **G.** Macronucleus (arrows) and aboral trochal band (arrowhead); **H.** Oral ciliature. Arrow marks infundibular polykinety 3; **I.** Lateral view showing silverline system. Scale bars: 135 μm (**A**); 30 μm (**B**); 15 μm (**F**); 15 μm (**G**).

Figure 3 Morphology and infraciliature of *Vorticella scapiformis* sp. n. in vivo (**A-D**) and after protargol staining (**E, F**). **A.** General lateral view of typical zooid; **B.** Lateral view showing the proportion of stalk and zooid; **C.** Showing irregular protuberances on edge of peristomial lip (arrows); **D.** Details of stalk; **E.** Showing general infraciliature and macronucleus (arrow); **F.** Oral ciliature. Arrowhead indicates epistomial membrane. H, haplokinety; G, germinal kinety; P1-3, infundibular polykinety 1-3; Po, polykinety. Scale bars: 40 μm (**A**); 200 μm (**B**); 15 μm (**E**).

Figure 4 Photomicrographs of *Vorticella scapiformis* sp. n. in vivo (**A-F**) and after protargol (**G-H**) and silver nitrate (**I**) staining. **A.** Lateral view, showing the proportion of stalk and zooid; **B.** Showing irregular protuberances on edge of peristomial lip (arrows); **C.** Showing contractile vacuole (arrow); **D.** Showing food vacuole (arrow); **E.** Zooid at high magnification. Arrows show pellicular striations; **F.** Details of stalk. Arrow indicates thecoplasmic granules in the spasmoneme; **G.** Arrows show macronucleus; **H.** Oral ciliature. Arrow indicates infundibular polykinety 3; **I.** Silverlines. Scale bars: 200 μm (**A**); 20 μm (**B, D, G**).

Figure 5 Morphology and infraciliature of *Vorticella sphaeroidalis* sp. n. in vivo (**A-B**), after protargol (**C, D**) and silver nitrate (**E**) staining. **A.** General view of typical zooid; **B.** Lateral view, showing the proportion of stalk and zooid; **C.** Oral ciliature. Arrowhead marks epistomial membrane; **D.** General infraciliature and myoneme system; **E.** Silverline system. Arrow marks the aboral trochal band. H, haplokinety; G, germinal kinety; P1-3, infundibular polykinety 1-3; Po, polykinety. Scale bars: 15 μm (**A**); 40 μm (**B**); 6 μm (**D**).

* In **C**, the initial (i.e. proximal) position of P3 is shown, but the terminal (i.e. distal) position and the number of kinetosomal rows is indeterminate.

Figure 6 Photomicrographs of *Vorticella sphaeroidalis* sp. n. in vivo (**A-E**), after protargol (**F-G**) and silver nitrate (**H-I**) staining. **A.** Typical zooid in vivo; **B.** Showing contractile vacuole (arrow); **C.** Showing food vacuole (arrowhead) and macronucleus (arrowhead); **D.** Granules on the surface (arrow); **E.** Zooid at high magnification. Arrows show pellicular striations; **F.** Arrow shows myoneme system; **G.** Showing macronucleus (arrow), infundibular polykinety 3 (arrowhead) and aboral trochal band (double-arrowheads); **H.** Showing silverline system. Arrow indicates silverline; **I.** Showing pellicular pores (arrowheads). Scale bars: 15 μm (**A**); 10 μm (**H, I**).

Figure 7 Morphology and infraciliature of *Vorticella paralima* sp. n. in vivo (**A-B**), after protargol (**C-D**) and silver nitrate (**E**) staining. **A.** General view of typical zooid; **B.** Showing the proportion of stalk and zooid; **C.** Showing myoneme system and macronucleus; **D.** General infraciliature. Arrow

indicates macronucleus. Arrowhead marks aboral trochal band; **E.** Showing oral ciliature. Arrowhead marks epistomial membrane. H, haplokinety; G, germinal kinety; P1-3, infundibular polykinety 1-3; Po, polykinety. Scale bars: 30 μm (A); 90 μm (B); 20 μm (E).

Figure 8 Photomicrographs of *Vorticella paralima* sp. n. in vivo (**A-C**), after protargol (**D-E**) and silver nitrate (**F-G**) staining. **A.** Lateral view of zooid at low magnification; **B.** Typical zooid in vivo. Arrowhead marks contractile vacuole. Arrow indicates depression on surface; **C.** Showing pellicular striations (arrow); **D.** Showing macronucleus (arrow) and aboral trochal band (arrowhead); **E.** Showing oral infraciliature. Arrow indicates infundibular polykinety 3; **F.** Showing pellicular pores; **G.** Showing silverlines. Scale bars: 60 μm (A); 20 μm (B, E); 15 μm (F, H).

Figure 9 Maximum likelihood tree inferred from small subunit rDNA gene sequences indicating phylogenetic positions of *Vorticella parachiangi* sp. n., *V. scapiformis* sp. n., *V. sphaeroidalis* sp. n. and *Vorticella paralima* sp. n. (bold typeface and arrows). Numbers at the nodes represent support values in the following order: Maximum Likelihood (ML) bootstrap values and Bayesian inference (BI) posterior probabilities. GenBank accession numbers follow each species name. Nodes absent from one of the two phylogenetic analyses are indicated by a hyphen instead of a support value. The scale bar indicates two substitutions per 100 nucleotides.

Table 1. Characterization of zooid size and silverline system of *Vorticella parachiangi* sp. n., *V. scapiformis* sp. n., *V. sphaeroidalis* sp. n. and *V. paralima* sp. n.

Characters ^a	Species	Min	Max	Mean	SD	n
Zooid length in vivo ^b	<i>V. parachiangi</i>	50	65	55.70	4.76	10
	<i>V. scapiformis</i>	40	50	44.31	4.50	7
	<i>V. sphaeroidalis</i>	20	30	23.33	4.08	6
	<i>V. paralima</i>	35	50	42.50	4.63	8
Zooid width in vivo ^b	<i>V. parachiangi</i>	50	65	53.00	3.50	10
	<i>V. scapiformis</i>	40	55	44.30	6.07	7
	<i>V. sphaeroidalis</i>	15	25	18.33	4.08	6
	<i>V. paralima</i>	20	30	23.75	3.54	8
Number of silverlines ^c	<i>V. parachiangi</i>	21	31	25.72	2.66	22
	<i>V. scapiformis</i>	18	25	22.57	2.70	7
	<i>V. sphaeroidalis</i>	16	18	17.50	2	8
	<i>V. paralima</i>	26	35	30.50	2.70	14
Number of silverlines ^d	<i>V. parachiangi</i>	6	11	7.50	1.41	22
	<i>V. scapiformis</i>	8	12	9.86	1.35	7
	<i>V. sphaeroidalis</i>	2	2	2	0	8
	<i>V. paralima</i>	7	13	10.36	1.7	14

^aData of silverlines based on Chatton-Lwoff silver nitrate-stained specimens.

Min, minimum; Max, maximum; Mean, arithmetic mean; SD, standard deviation; n, sample number.

^bMeasurements in μm .

^cNumber of silverlines between peristome and aboral trochal band.

^dNumber of silverlines between aboral trochal band and scopula.