



Morphology of four new solitary sessile peritrich ciliates from the Yellow Sea, China, with description of an unidentified species of *Paravorticella* (Ciliophora, Peritrichia)

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Abstract

Sessile peritrichs are a large assemblage of ciliates that have a wide distribution in soil, freshwater and marine waters. Here, we document four new and one unidentified species of solitary sessile peritrichs from aquaculture ponds and coastal waters of the northern Yellow Sea, China. Based on their living morphology, infraciliature and silverline system, four of the five forms were identified as new members belonging to one of three genera, *Vorticella*, *Pseudovorticella* and *Scyphidia*, representing two families, Vorticellidae and Scyphidiidae. The other isolate was found to be an unidentified species of the poorly known genus *Paravorticella*. *Vorticella chiangi* sp. nov. is characterized by its inverted bell-shaped zooid, short row 3 in infundibular polykinety 3 and marine habitat. *Pseudovorticella liangae* sp. nov. possesses a thin, broad peristomial lip and a granular pellicle. *Pseudovorticella haiboensis* sp. nov. is differentiated from its congeners by having an elongated zooid that is covered by a layer of thin pellicular vesicles, and two rows of kineties in infundibular polykinety 3. *Scyphidia perezuzae* sp. nov. and *Paravorticella* sp. are stalkless ectoparasites or ectocommensals of aquatic animals. The former has a short, plump body, a narrow peristomial lip and a conspicuous, flattened, disc-shaped scopula for adhesion. *Paravorticella* sp. has an extremely elongated clavate body, a broad peristomial lip, and a narrow scopula.

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Introduction

Sessile peritrichs are a large, distinctive group of ciliates comprising over 800 species (Corliss 1979; Foissner et al. 2009; Lynn 2008). The majority of sessile peritrichs attach

to a substrate via a stalk, lorica, or the scopula, although a few never attach and are permanently motile. Stalked peritrichs can be divided into two easily recognized subgroups: solitary, i.e., those with an unbranched stalk, and colonial, i.e., those with a branched stalk (Kahl 1935; Foissner et al. 1992; Ji et al. 2011; Li et al. 2015; Sun et al. 2009, 2015; Warren 1986, 1987). Among the many genera of solitary peritrichs, *Vorticella* Linnaeus, 1767 and *Pseudovorticella* Foissner

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and Schiffmann, 1975 are the most commonly encountered (Foissner 1979; Foissner et al. 1992; Ji et al. 2005; Sun et al. 2006a,b, 2007). The genus *Vorticella* was established by Linnaeus (1767) and later refined by Ehrenberg (1838) to include non-colonial peritrichs with a helically contractile stalk. Over 200 species of *Vorticella* were subsequently described (Noland and Finley 1931; Warren 1986). Foissner and Schiffmann (1975) erected the genus *Pseudovorticella* which is characterized by possessing an unbranched, helically contractile stalk and a reticulate silverline system, thus differentiating it from *Vorticella* which has a transverse silverline system. This resulted in the transfer of all vorticellids with a reticulate silverline system into the genus *Pseudovorticella* leaving only those with a transverse silverline system in the genus *Vorticella* (Warren 1987).

The genera *Paravorticella* Kahl, 1933 and *Scyphidia* Dujardin, 1841 belong to the family Scyphidiidae Kahl, 1933, species of which are usually found inhabiting the body surface or body cavity of zooplankton organisms, e.g., copepods, other crustaceans and fishes (Chakravorty 1937; Irwin and Lynn 2015; Mackenzie 1969; Pane et al. 2014). Species of both genera lack a stalk and attach to their substrate via the scopula which, in *Paravorticella*, is very narrow whereas in *Scyphidia*, is thicker and sometimes forms a conspicuous, flattened disc for adhesion.

During a survey of ciliate biodiversity in marine coastal waters and aquaculture ponds of northern China during the period 2004–2006, five solitary sessilids belonging to the genera *Vorticella*, *Pseudovorticella*, *Paravorticella* and *Scyphidia* were discovered. Investigations of the living morphology, infraciliature and silverline system revealed that four of them were undescribed species. Here, we provide detailed morphological descriptions of these four species. The fifth species belongs to *Paravorticella* but, since we lack sufficient data to fully characterize it, this isolate is preliminarily described as *Paravorticella* sp. Unfortunately, accompanying molecular information of these species cannot be supplied because facilities for molecular analyses were not available in the Laboratory of Protozoology at the Ocean University of China when this research was conducted.

Material and Methods

All species were collected from indoor aquaculture ponds and coastal waters of the Yellow Sea, Shandong, China, either using artificial substrates, or directly from their hosts. For isolation using artificial substrates, glass microscope slides were fixed to a frame and left immersed at a depth of 1 m for 7–10 days to allow colonization by the peritrichs (Small 1973). *Vorticella chiangi* was isolated in June, 2004, from an indoor pond in the Department of Aquaculture, Ocean University of China, Qingdao, that was used for the cultivation of the rockfish *Sebastes fuscescens* (Actinopterygii, Scorpaeniformes). The water temperature was 21 °C and salinity ca. 31‰. *Pseudovorticella haiboensis* and *P. liangae* were

collected from scallop-farming areas near Taiping Bay, Qingdao (water temperature 18 °C, salinity ca. 30‰) in June, 2004, and from mussel-farming areas near the Haibo River, Qingdao (water temperature 14 °C, salinity ca. 31‰) in April, 2005, respectively. *Paravorticella* sp. was found on the body surface of an unidentified metazoan from coastal waters near Zhanqiao pier, Qingdao (water temperature 15 °C, salinity ca. 31‰) in October, 2005. The host was not identified into a specific taxonomic group due to the absence of related knowledge by the authors. *Scyphidia perezuzae* was isolated from the body surface of *Sebastes fuscescens* from indoor fish-farming ponds near Penglai Bay, Shandong (temperature 21 °C, salinity ca. 30‰) in May, 2006. For the waters in the indoor fish-pond, the source of where the waters were originally collected is untraceable.

For observations of specimens in vivo, wet mounts of ciliates were made, viewed under compound microscopes with bright field and Nomarski optics (Olympus, Nikon), and photographed with a digital camera (Pixra, Olympus). The infraciliature was revealed by the protargol staining method of Wilbert (1975). The silver nitrate method of Song and Wilbert (1995) was used to demonstrate the silverline system. Counts and measurements of stained specimens were made under 100–1250× magnification. Drawings of stained specimens were performed at 1000× with the aid of a camera lucida. Terminology follows Lynn (2008).

Results and Discussion

Order: Sessilida Kahl, 1933

Family: Vorticellidae Ehrenberg, 1838

Genus: *Vorticella* Linnaeus, 1767

Vorticella chiangi sp. nov. (Fig. 1A, B, G–M; Table 1)

Diagnosis. Marine *Vorticella*. Zooid inverted bell-shaped, 40 × 40 μm in vivo. Macronucleus J-shaped. Single contractile vacuole ventrally located. 33–37 silverlines from peristome to trochal band, 10–14 from trochal band to scopula. Infundibular polykinety 3 consisting of three rows, row 3 about one-third the length of the other two.

Type locality. Indoor ponds for cultivating *Sebastes fuscescens* in Department of Aquaculture, Ocean University of China, Qingdao, China (36°3′58.85″N, 120°20′19.63″E).

Deposition of slides. A protargol slide (registration number SP-2004-0616-01) containing the holotype specimen (Fig. 1J) is deposited in the Laboratory of Protozoology, Ocean University of China, China. One paratype slide with silver nitrate-stained specimens is deposited in the Natural History Museum, London (NHMUK 2016.27.10.1).

Etymology. This species is named in honor of Prof. Kuoping Chiang, a protozoologist at the Ocean University of Taiwan, China, in recognition of his significant contributions to the field of protozoan ecology.

Description. Zooid inverted bell-shaped, 35–50 × 35–50 μm in vivo, with ratio of length to width about 1:1 on average (Table 1). Peristomial lip approximately

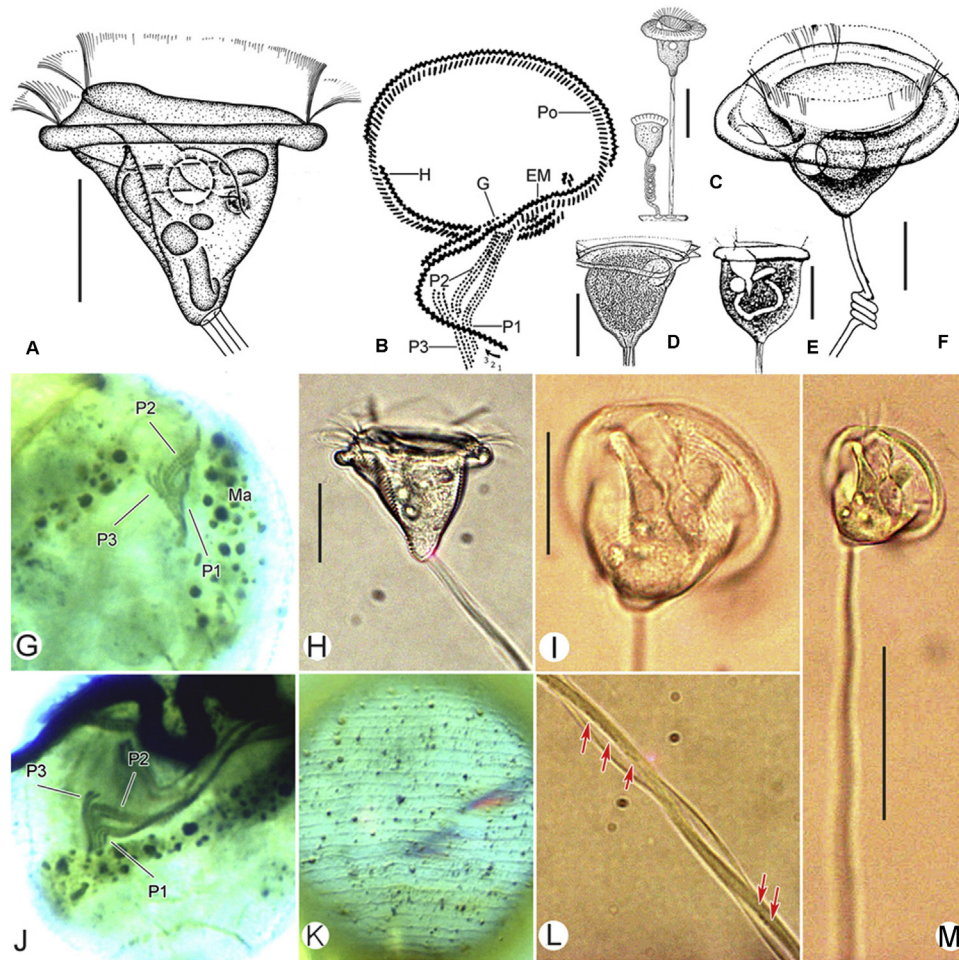


Fig. 1. (A–M) Morphology of *Vorticella chiangi* sp. nov. from life (A, H, I, L, M) and after staining with protargol (B, G, J) and silver nitrate (K); related species (C–F). **A, H, I, M** – typical zooids; **B** – oral infraciliature in bottom view, arrow with numerals indicates numbering convention for infundibular polykineties, and rows of kinetosomes within each polykinety; **C** – a living individual of *Vorticella cratera* (from Kent 1880–1882); **D** – a living individual of *Vorticella campanula* (from Ehrenberg 1831); **E** – A living individual of *Vorticella marina* (from Song 1991); **F** – a living individual of *Vorticella fornicata* (from Dons 1915); **G, J** – detail of infundibular polykineties in a paratype (G) and the holotype (J); **K** – Silverline system; **L** – detail of stalk, arrows mark grayish thecoplasmic granules on spasmoneme. EM, epistomial membrane; G, germinal kinety; H, haplokinety; Ma, macronucleus; P1–3, infundibular polykinety 1–3; Po, polykinety. Scale bars: 25 μm in A; 100 μm in C; 50 μm in D; 20 μm in E, F; 30 μm in H, I; 70 μm in M.

5–6 μm thick and wide, body not constricted beneath lip (Fig. 1A, H). Peristomial disc flattened and slightly elevated when zooid fully extended. Pellicle flexible, usually indented near ventral side of zooid (Fig. 1H, I). Pellicular striations easily detectable above 400 \times magnification, zooid surface appears smooth at low magnifications (Fig. 1H, I, M).

Cytoplasm colorless or slightly grayish, usually containing a few to several food granules or vacuoles (Fig. 1A, H, I, M). Single contractile vacuole located in adoral position beneath peristomial lip and near ventral wall of infundibulum. Macronucleus long, J-shaped, with its semicircular oral arm oriented orthogonal to long axis and located in upper third of zooid (Fig. 1A, G, J). Micronucleus not observed. Stalk approximately 170–200 μm long, 4–5 μm in diameter, with smooth surface (Fig. 1M). Spasmoneme approximately 1.5–2.0 μm in diameter, with numerous, gray-

ish thecoplasmic granules 0.4–0.8 μm in diameter (Fig. 1L, arrows).

Oral infraciliature as shown in Fig. 1B, G and J. Haplokinety and polykinety both making 1.25 turns around peristome and one additional turn within infundibulum. Infundibular polykineties (P1–3) each consisting of three rows of kinetosomes (Fig. 1B). P1 and P2 longer than P3. All rows of P1 almost equal in length, terminating at slightly different levels near cytostome. Rows of P2 terminating adstomally at curvature of P1 (Fig. 1B). Rows of P3 parallel, with anterior end of row 1 slightly displaced relative to the other two. Rows 1 and 2 of P3 almost equal in length, row 3 conspicuously shorter than other two (Fig. 1B, G, J). Epistomial membrane short, located near opening of infundibulum (Fig. 1B, arrow). Germinal kinety lies parallel to haplokinety in upper half of infundibulum (Fig. 1B).

Table 1. Morphometric data of *Vorticella chiangi* sp. nov. (V chi), *Pseudovorticella liangae* sp. nov. (P lia), *Pseudovorticella haiboensis* sp. nov. (P par), *Paravorticella* sp. (P sp) and *Scyphidia perezuae* sp. nov. (S per).^a

Character	Species	Min	Max	Mean	SD	M	CV	N
Body length in vivo	V chi	35.0	50.0	42.0	3.1	41.0	7.3	12
	P lia	75.0	85.0	79.4	3.1	78.0	3.9	10
	P par	65.0	75.0	70.5	2.3	69.0	3.3	11
	P sp	60.0	70.0	68.5	1.5	67.0	2.2	12
	S per	50.0	60.0	57.5	1.5	56.0	2.6	11
Body width in vivo	V chi	35.0	50.0	42.0	2.8	41.0	6.7	12
	P lia	70.0	80.0	75.8	1.5	75.0	2.0	10
	P par	25.0	35.0	30.4	1.7	29.0	5.6	11
	P sp	15.0	25.0	21.5	2.7	21.0	12.6	12
	S per	50.0	70.0	63.1	3.7	61.0	5.9	11
Number of silverlines from peristome to trochal band	V chi	33.0	37.0	34.8	1.6	33.0	4.6	6
	P lia	35.0	39.0	36.6	1.5	36.0	4.1	5
	P par	24.0	28.0	25.6	1.5	25.0	5.9	8
	S per	22.0	30.0	25.5	2.95	26.0	11.6	6
Number of silverlines from trochal band to scopula	V chi	10.0	14.0	12.3	1.2	13.0	9.8	6
	P lia	12.0	16.0	13.7	1.3	13.0	9.5	6
	P par	8.0	10.0	9.2	0.7	9.0	7.6	6
	S per	20.0	24.0	22.0	1.4	21.0	6.4	6

^aAll measurements in μm . Abbreviations: CV, coefficient of variation (%); M, median; Max, maximum; Mean, arithmetic mean; Min, minimum; N, number of specimens; SD, standard deviation.

Silverline system comprises transverse silverlines, approximately 33–37 between peristome and trochal band and 10–14 between trochal band and scopula (Fig. 1K; Table 1).

Remarks and comparisons. Considering its living morphology, four congeners, i.e., *Vorticella cratera* Kent, 1881, *V. fornicata* Dons, 1915, *V. campanula* Ehrenberg, 1831 and *V. marina* Greeff, 1870, should be compared with *V. chiangi* sp. nov. *Vorticella cratera* can be separated from the new species by having larger body size (120–130 μm vs. 35–50 μm in vivo) and a freshwater (vs. marine) habitat (Fig. 1C; Kent 1880–1882). Similarly, *Vorticella campanula* is a freshwater (vs. marine) species which has larger zooid (50–160 μm vs. 35–50 μm in vivo) and more silverlines between the peristome and the trochal band (69–77 vs. 33–37) (Fig. 1D; Foissner et al. 1992). The marine species *V. marina* resembles the new species in having a similar zooid size, an extended peristomial lip, and a flat peristomial disc. But *V. marina* can be distinguished from the new species by the shape of the macronucleus (C-shaped in the former vs. J-shaped in the latter), and the position of contractile vacuole (just beneath peristome in the former vs. near middle of upper one third of zooid in the latter) (Fig. 1E; Song 1991). *Vorticella fornicata* is also a marine species but can be separated from *V. chiangi* sp. nov. by having a smaller zooid size (24–32 \times 24–37 μm vs. 35–50 \times 35–50 μm in vivo) and C-shaped (vs. J-shaped) macronucleus (Fig. 1F; Song 1991).

Genus: *Pseudovorticella* Foissner and Schiffmann, 1975

Pseudovorticella liangae sp. nov. (Fig. 2A–N; Table 1)

Diagnosis. Marine *Pseudovorticella*. Zooid inverted bell-shaped, about 80 \times 75 μm in vivo. Peristomial lip thin and

wide. Single contractile vacuole ventrally located. Macronucleus J-shaped. Transverse silverlines numbering 35–39 from peristome to trochal band, 12–16 from trochal band to scopula. Infundibular polykinety 3 consisting of three rows, with row 1 conspicuously shorter than the other two.

Type locality. Taiping Bay, Qingdao, China (36 05'01"N, 120 35'29"E).

Deposition of slides. The protargol slide (registration number SP-2004-0628-01) containing the holotype specimen (Fig. 2N) is deposited in the Laboratory of Protozoology, Ocean University of China, China. One paratype slide with silver nitrate-stained specimens is deposited in the Natural History Museum, London (NHMUK 2016.10.27.2).

Etymology. This species is named in honor of Prof. Aihua Liang, a molecular ciliatologist at Shanxi University, China, in recognition of her significant contributions to the field of ciliate genetics.

Description. Zooid inverted bell-shaped to subconical, approximately 75–85 \times 70–80 μm in vivo, with maximum width at peristomial area (Fig. 2A, B, E, F). Peristomial lip thin, smooth and wide (ca. 90–100 μm across), zooid not constricted beneath lip (Fig. 2H). Peristomial disc extremely flattened and slightly elevated above peristomial lip when zooid is fully extended (Fig. 2A, F, H). Pellicle smooth under low magnifications, reticulate pellicular striations and pellicular granules only detectable under 1000 \times magnification (Fig. 2G).

Cytoplasm slightly grayish and usually with a few food vacuoles and granules, approximately 4–8 μm in diameter (Fig. 2E, J). Single contractile vacuole located near ven-

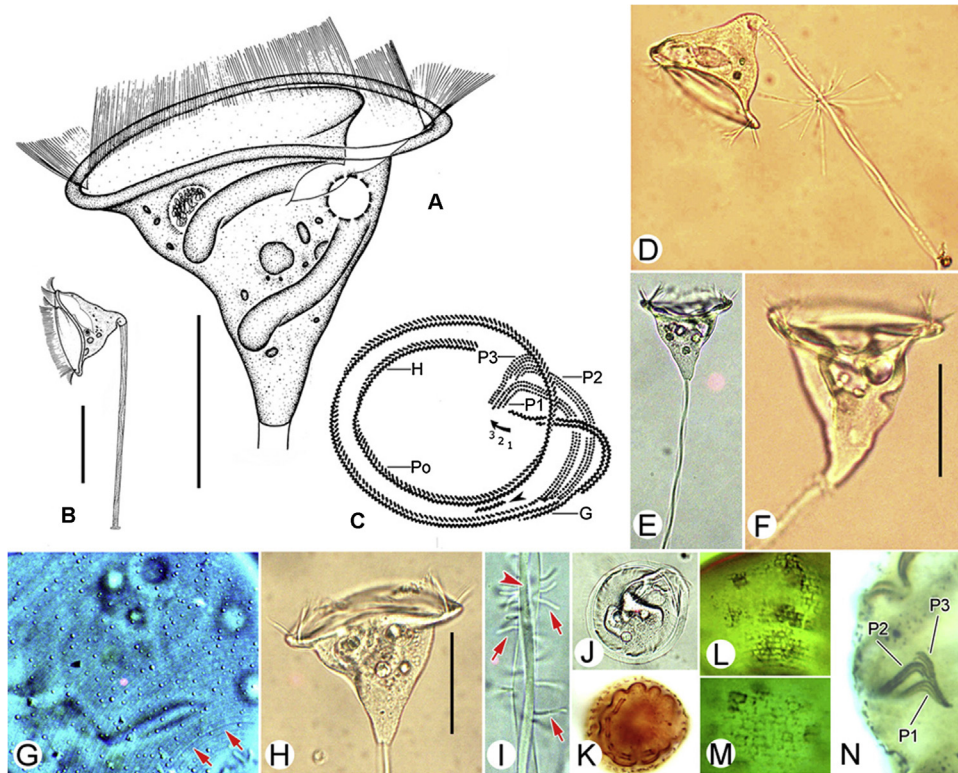


Fig. 2. (A–N) *Pseudovorticella liangae* sp. nov. from life (A, B, D–J) and after staining with protargol (C, K, N) and silver nitrate (L, M). **A, F, H** – living zooids at high magnifications; **B, D, E, J** – living zooids at low magnifications; **C** – top view of entire oral infraciliature, arrowhead marks epistomial membrane, arrow with numerals indicates numbering convention for polykineties and rows of kinetosomes within each polykinety; **G** – living zooid at high magnification focused to show the pellicular striations (arrows); **I** – detail of stalk in vivo, arrows mark rod-like bacteria and arrowhead marks grayish thecoplasmic granules on spasmoneme; **K, N** – oral infraciliature and detailed arrangement of infundibular polykineties in paratype (K) and holotype (N); **L, M** – silverline system. G, germinal kinety; H, haplokinety; P1–3, infundibular polykineties 1–3; Po, polykinety. Scale bars: 40 μm in A; 80 μm in B; 50 μm in F, H.

tral wall of infundibulum (Fig. 2A). Macronucleus slender, roughly J-shaped (Fig. 2A, N). Micronucleus not observed.

Stalk approximately 360–400 μm in length, 5–6 μm in diameter, usually with smooth surface (Fig. 2D). Sometimes filose bacteria, 4–6 μm long, attached to stalk (Fig. 2I, arrows). Stalk spasmoneme 2–3 μm in width, with sparsely distributed grayish granules, 0.4–0.6 μm (Fig. 2I).

Oral infraciliature as shown in Fig. 2C, K and N. Haplokinety and polykinety make one and a half turns around peristome before entering infundibulum where they make a further turn (Fig. 2K). Haplokinety passes around infundibulum on wall opposite to the three infundibular polykineties. Each infundibular polykinety consists of three rows of kinetosomes in lower half of infundibulum. P1 and P2 longer than P3. Three rows of P1 almost equal in length and terminate adstomally at slightly different levels (Fig. 2C). Adstomal ends of P2 terminate between P1 and P3. Rows of P3 parallel, row 1 approximately half length of other two (Fig. 2C, N). Germinal kinety parallel to abstomal half of haplokinety (Fig. 2C). Epistomial membrane located near opening of infundibulum as commonly seen in other peritrichs (Fig. 2C, arrowhead).

Silverline system consists of closely spaced, reticulate silverlines (Fig. 2G, L, M). Approximately 35–39 transverse silverlines from peristome to trochal band and 12–16 between trochal band and scopula (Fig. 2L, M; Table 1).

Remarks and comparisons. *Pseudovorticella patellina* (Müller 1776) Song and Warren, 2000 bears a strong resemblance to the new species in terms of zooid shape, appearance of the peristomial lip, shape of the macronucleus and pattern of P3. However, the former can be differentiated from the latter by possessing a wider range of body size (55–110 \times 50–100 μm vs. 75–85 \times 70–80 μm), fewer silverlines between the peristome and the trochal band (19–22 vs. 35–39) and in having two (vs. one) contractile vacuoles (Fig. 3A; Table 2; Song and Warren 2000).

Pseudovorticella bidulphiae (Stiller, 1939) Ji, Sun, Song and Warren in Sun, Ji, Song and Warren, 2009, *P. pseudocampanula* Foissner, 1979 and *P. monilata* (Tatem, 1870) Foissner and Schiffmann, 1975 are similar to *P. liangae* sp. nov., especially in body shape (Fig. 3B–D). However, *P. bidulphiae* has a different pattern of P3, smaller zooid size (30–40 \times 35–40 μm vs. 75–85 \times 70–80 μm in vivo) and fewer transverse silverlines both above (24–28 vs. 35–39) and

Table 2. Comparisons of *Pseudovorticella liangae* sp. nov. and *P. haiboensis* sp. nov. with morphologically similar species.

Character	<i>P. liangae</i>	<i>P. patellina</i>	<i>P. bidulphiae</i>	<i>P. pseudocampanula</i>	<i>P. haiboensis</i>	<i>P. elongata</i>	<i>P. plicata</i>	<i>P. clampi</i>
Body size in vivo (μm)	50–60 × 50–70	55–110 × 50–100	30–40 × 35–40	32–50 × 28–33	65–75 × 25–35	48–92 × 32–44	80–90 × 40–45	70–106 × 50–75
Body shape	Inverted bell	Inverted bell	Elongate	Conical	Elongate	Elongate	Elongate	Elongate
Number and position of contractile vacuole(s)	1, ventral	2, dorsal	2, ventral	1, ventral	1, ventral	1, ventral	1, ventral	1, ventral
Number of silverlines above and below trochal band	35–39; 12–16	19–22; 13–16	25–31; 9–13	28–33; 16–18	24–28; 8–10	51–66; 10–20	41–46; 14–17	38–42; 16–19
Structure of polykinety 3	Three rows	Three rows	Two rows	–	Two rows	Three rows	Three rows	Three rows
Habitat	Marine	Marine	Marine	Freshwater	Marine	Freshwater	Marine	Marine
Data source	Present study	Song and Warren (2000)	Sun et al. (2009)	Foissner (1979)	Present study	Leitner and Foissner (1997)	Sun et al. (2009)	Ji et al. (2005)

“–”, data is not available.

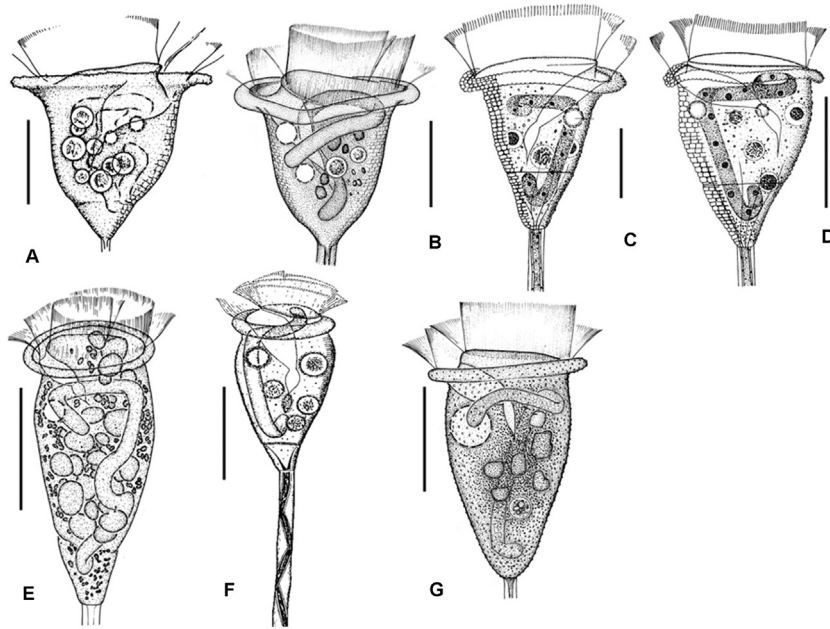


Fig. 3. (A–G) Closely related species of *Pseudovorticella liangae* and *P. haiboensis*. **A** – *Pseudovorticella patellina* (from Song and Warren 2000); **B** – *Pseudovorticella bidulphiae* (from Sun et al., 2009); **C** – *Pseudovorticella pseudocampanula* (from Foissner 1979); **D** – *Pseudovorticella monilata* (from Foissner 1979); **E** – *Pseudovorticella plicata* (from Sun et al., 2009); **F** – *Pseudovorticella elongata* (from Leitner and Foissner 1997); **G** – *Pseudovorticella clampi* (from Ji et al., 2005). Scale bars: 40 μm in A, D–G; 20 μm in B, C.

below (9–11 vs. 12–16) the trochal band (Fig. 3B; Table 2; Ji et al., 2011).

Pseudovorticella pseudocampanula can be differentiated from the new species by its smaller zooid length (32–50 μm vs. 75–85 μm in *P. liangae*), fewer silverlines above the trochal band (28–33 vs. 35–39 in *P. liangae*) and freshwater (vs. marine) habitat (Fig. 3C; Foissner 1979). *Pseudovorticella monilata*, a freshwater species, can be morphologically separated from *P. liangae* sp. nov. by having fewer silverlines above the trochal band (19–23 vs. 35–39 in *P. liangae*) and two (vs. one) contractile vacuoles (Fig. 3D; Table 2; Foissner 1979).

Pseudovorticella haiboensis sp. nov. (Fig. 4A–K; Table 1)

Diagnosis. Marine *Pseudovorticella*. Zooid elongate, inverted bell-shaped, about $70 \times 30 \mu\text{m}$ in vivo with a relatively broad peristomial lip. Single contractile vacuole ventrally located. Macronucleus J-shaped. 24–28 transverse silverlines between peristome and trochal band, 8–10 between trochal band and scopula. P3 having two rows, with row 1 conspicuously shorter than row 2 and diverging from row 2 at its abostomal end.

Type locality. Coastal area near Haibo River, Qingdao, China ($36^{\circ}6'23.85''\text{N}$, $120^{\circ}17'52.15''\text{E}$).

Deposition of slides. The protargol slide (registration number SP-2005-0422-01) containing the holotype specimen (Fig. 4I) is deposited in the Laboratory of Protozoology, Ocean University of China, China. One paratype slide with silver nitrate-stained specimens is deposited in the Natural History Museum, London (NHMUK 2016.10.27.3).

Etymology. The species is named after the area (Haibo River) where the sample was collected.

Description. Zooid slender-campanulate, slightly constricted beneath peristomial lip, $65\text{--}75 \times 25\text{--}35 \mu\text{m}$ in vivo, ratio of length to width 2.3:1 on average (Fig. 4A, D, G; Table 1). Peristomial lip approximately 5–6 μm thick and broad (Fig. 4A, D, G). Peristomial disc flat, slightly elevated above peristomial lip (Fig. 4D, F). Pellicle appears smooth at low magnifications (Fig. 4F) but thin (approximately 1.5–2.0 μm) layer of pellicular vesicles detectable under high magnification (Fig. 4D, G, arrows).

Cytoplasm slightly grayish, usually with several food vacuoles or granules 4–8 μm in diameter, in central part of zooid (Fig. 4A, G). Single contractile vacuole located near ventral wall of infundibulum (Fig. 4A). Macronucleus J-shaped, semicircular oral arm immediately below peristome and oriented orthogonal to long axis, remaining part extends aborally, recurved forming bottom of “J” (Fig. 4A). Micronucleus not observed.

Stalk approximately 5–7 times zooid length, 6–7 μm in diameter, surface usually smooth (Fig. 4B, E, F). Spasmoneme approximately 2–3 μm in diameter with sparsely distributed grayish or black granules (Fig. 4H, arrowheads).

Oral infraciliature as shown in Fig. 4C, I. Haplokinety and polykinety make 1.25 turns around peristome and one additional turn within infundibulum. P1 and P2 each composed of three distinct rows of kinetosomes. Adstomal ends of rows of P1 terminate at slightly different levels (Fig. 4C). P2 terminates adstomally far above adstomal end of P1. P3 consists of two rows, row 1 converging with row 3 of P1 at adstomal

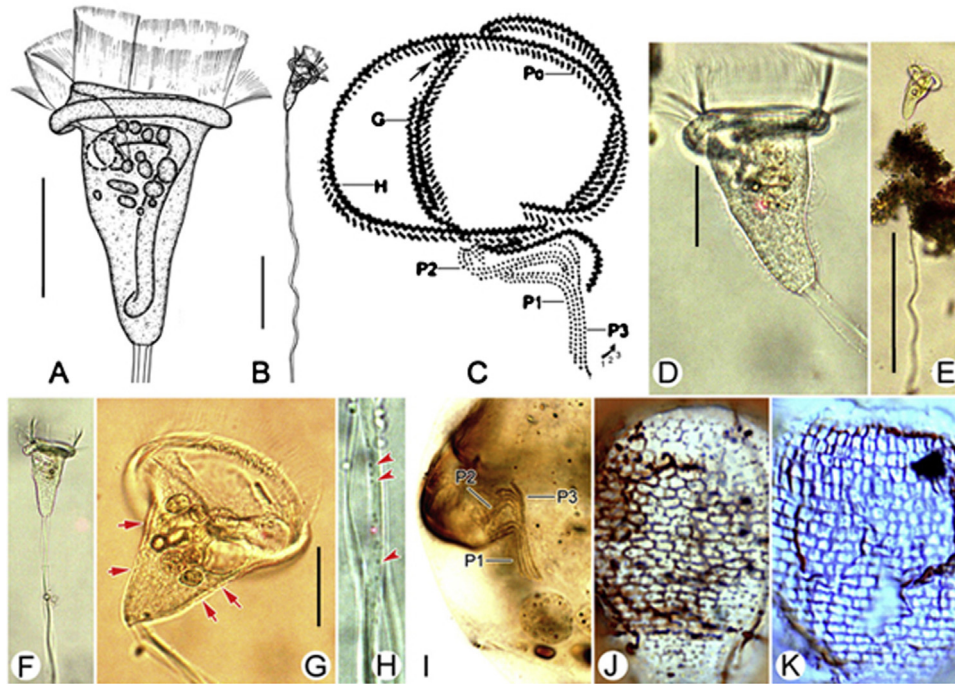


Fig. 4. (A–K) *Pseudovorticella haiboensis* sp. nov. from life (A, B, D–H) and after protargol (C, I) and silver nitrate (J, K) staining. A, D, G – typical zooids at high magnifications, arrows in G mark the thin layer of pellicular vesicles; B, E, F – typical zooids at low magnifications; C – top view of entire oral infraciliature, arrow marks epistomial membrane, arrow with numerals indicates numbering convention for polykineties and rows of kinetosomes within each polykinety; H – detail of stalk, arrowheads mark thecoplasmic granules on spasmoneme; I – detail of infundibular polykineties in holotype specimen; J, K – silverline system. G, germinal kinety; H, haplokinety; P1–3, infundibular polykinety 1–3; Po, polykinety. Scale bar: 30 μm in A, D, G; 150 μm in B, E.

end of infundibulum and slightly separated from row 2 at abostomal end. Epistomial membrane short, located near opening of infundibulum (Fig. 4C, arrow). Germinal kinety lies parallel to haplokinety within upper half of infundibulum (Fig. 4C).

Silverline system reticulate (Fig. 4J, K). Approximately 24–28 transverse silverlines between peristome and trochal band, and 8–10 between trochal band and scopula (Table 1).

Remarks and comparisons. *Pseudovorticella haiboensis* sp. nov. resembles *P. elongata* (Fromentel, 1876) Leitner and Foissner, 1997 in body shape and size and in the position of the contractile vacuole. However, *P. elongata* can be separated from the new species by having a different pattern of P3, more silverlines above the trochal band (51–66 vs. 24–28), and by its freshwater (vs. marine) habitat (Fig. 3F; Table 2; Leitner and Foissner 1997).

Pseudovorticella plicata (Gourett and Roeser, 1886) Sun, 2007 is a marine species that resembles *P. haiboensis* sp. nov. in zooid shape and size, shape of the macronucleus and appearance of the pellicle. However, the former has numerous tubercles on the peristomial lip (vs. absent in *P. haiboensis*), more silverlines both above and below the trochal band (41–46, 14–17 vs. 24–28, 8–10 in *P. haiboensis*) and a different pattern of P3 (Fig. 3E; Table 2; Sun 2007).

Regarding its zooid shape and size, *Pseudovorticella clampi* Ji et al., 2005 should also be compared with *P. haiboensis* sp. nov., however *P. clampi* has numerous tiny

pellicular granules (vs. absent in *P. haiboensis*), more silverlines both above and below the trochal band (41–46, 14–17 vs. 24–28, 8–10 in *P. haiboensis*) and a different pattern of P3 (Fig. 3G; Table 2; Ji et al. 2005).

Family: Scyphidiidae Kahl, 1933

Genus: *Paravorticella* Kahl, 1933

Paravorticella sp. (Fig. 5A–M; Table 1)

Brief characterization. Marine *Paravorticella*. Zooid extremely elongate to slightly clavate, 60–70 \times 15–25 μm in vivo with a relatively broad peristomial lip. Single contractile vacuole apically located. Macronucleus C-shaped, transversely oriented. Silverline system with about 87–95 transverse silverlines based on live observation. P3 with three rows; row 1 diverges from rows 2 and 3 which lie in parallel.

Locality of specimens. Attached to body surface of an unknown metazoan (see Section “Material and Methods”) host collected near Zhanqiao Pier, Qingdao, China (36°03′28.58″N, 120°18′56.26″E).

Deposition of voucher slide. A protargol slide (registration number SP-2005-1011-01) is deposited as a voucher in the Laboratory of Protozoology, Ocean University of China, China.

Description. Zooid extremely elongated, conical to clavate, approximately 70 \times 20 μm on average in vivo (Fig. 5A, D–H). Peristomial lip broad, approximately 22–30 μm in diameter, body not constricted beneath lip (Fig. 5A, F–H). Peristomial disc prominent, obliquely ele-

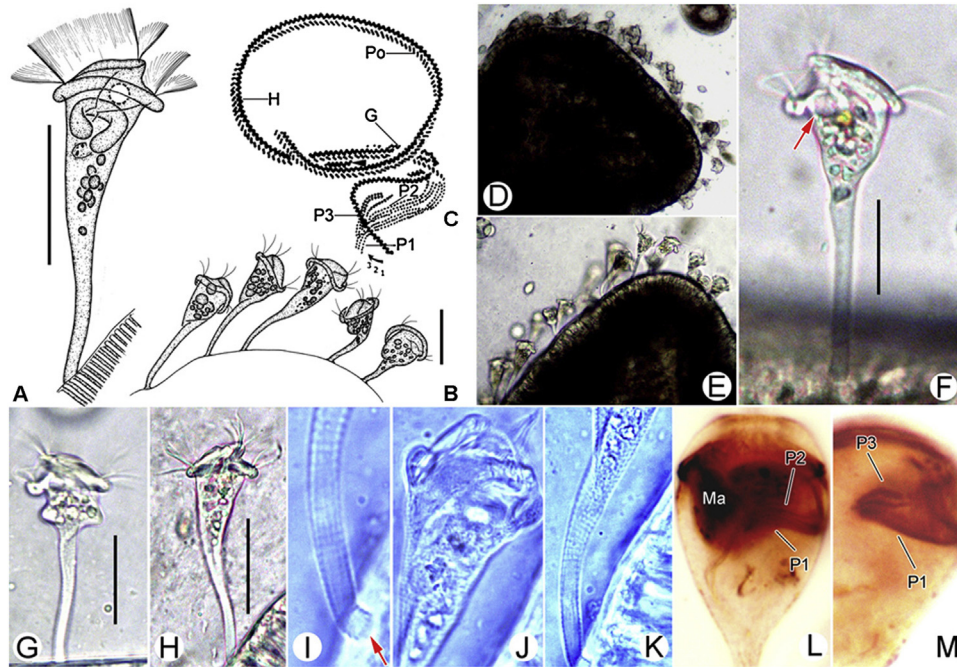


Fig. 5. (A–M) *Paravorticella* sp. from life (A, B, D–K) and after protargol staining (C, L, M). A, F, G, H – typical zooids at high magnifications, arrow in F marks contractile vacuole; B, D, E – typical zooids at low magnifications; C – top view of entire oral infraciliature, arrowhead marks epistomial membrane, arrow with numerals indicates numbering convention for polykineties and rows of kinetosomes within each polykinety; I – detail of aboral end of cell, arrow indicates scopula; J, K – living zooids at high magnification focused to show the pellicular striations; L, M – detail of infundibular polykineties. G, germinal kinety; H, haplokinety; P1–3, infundibular polykinety 1–3; Ma, macronucleus; Po, polykinety. Scale bar: 30 μm in A, B, G, H; 20 μm in F.

vated above peristome (Fig. 5F). Aboral end of zooid slender, sometimes curved (Fig. 5B, K). Pellicle smooth at low magnifications, transverse striations visible at 1000 \times magnification (Fig. 5J, K).

Cytoplasm colorless to grayish, usually containing several light or dark greenish food granules, 4–12 μm in diameter, in center of zooid (Fig. 5A, B, F–H). Single contractile vacuole apically located (Fig. 5A, F, arrow). Macronucleus C-shaped, transversely oriented, lying in the oral fourth of zooid (Fig. 5A, L). Micronucleus not observed. Stalkless, attached directly to substrate via scopula (Fig. 5I, arrow).

Oral apparatus as shown in Fig. 5C, L and M. Haplokinety and polykinety make 1.25 turns around peristome before entering infundibulum. Epistomial membrane short, located at opening of infundibulum (Fig. 5C, arrowhead). Haplokinety passes around infundibulum on wall opposite to the three infundibular polykineties. Germinal kinety lies parallel to haplokinety within upper half of infundibulum (Fig. 5C). P1 accompanied for part of its length by P2 and P3 (Fig. 5C). P1 consisting of three parallel rows of kinetosomes that extend to cytostome. All rows of P2 terminate between, and abostomally to, the adstomal ends of P1 and P3. P3 with three rows, abostomal end of row 1 diverges from the other two (Fig. 5C, M). In total, about 87–95 transverse lines based on live observation of five individuals (Fig. 5I–K).

Remarks and comparisons. *Paravorticella* was erected by Kahl (1933), with *P. terebellae* (Fauré-Fremiet, 1920) as

type species by monotypy, to include species that are solitary, stalkless and adhere to their substrate directly by a narrow scopula. Later, Kahl (1935) added two further species, namely *P. clymenellae* (Shumway, 1926) Kahl, 1935 and *P. crassicaulus* (Kent 1880) Kahl, 1935 which is a member of the genus *Haplocaulus*. With the addition of *P. lycastis* Chakravorty, 1937 and *P. nerillae* Jankowski, 1993, a total of five species have been assigned to *Paravorticella*. Hitherto, no species of *Paravorticella* has been investigated by silver staining methods. However, because very few individuals were available in the present study, it was only possible to investigate specimens in vivo and with protargol staining. The pattern and number of silverlines therefore had to be inferred from observations of specimens in vivo under high magnifications which revealed them as transverse pellicular striations.

Considering its marine habitat and elongated conical to clavate zooid, two congeners, *P. terebellae* and *P. clymenellae*, should be compared with the present species. *Paravorticella terebellae* can be differentiated from *Paravorticella* sp. by having a larger zooid (120 μm vs. 60–70 μm long in vivo), an S-shaped (vs. C-shaped) macronucleus, and a ventrally (vs. apically) located contractile vacuole (Fig. 7A; Kahl 1933). Like *Paravorticella* sp., *P. clymenellae* also has a C-shaped macronucleus. However, unlike *Paravorticella* sp. the macronucleus is oriented longitudinally (vs. transversely) and the contractile vacuole is located ventrally (vs. apically),

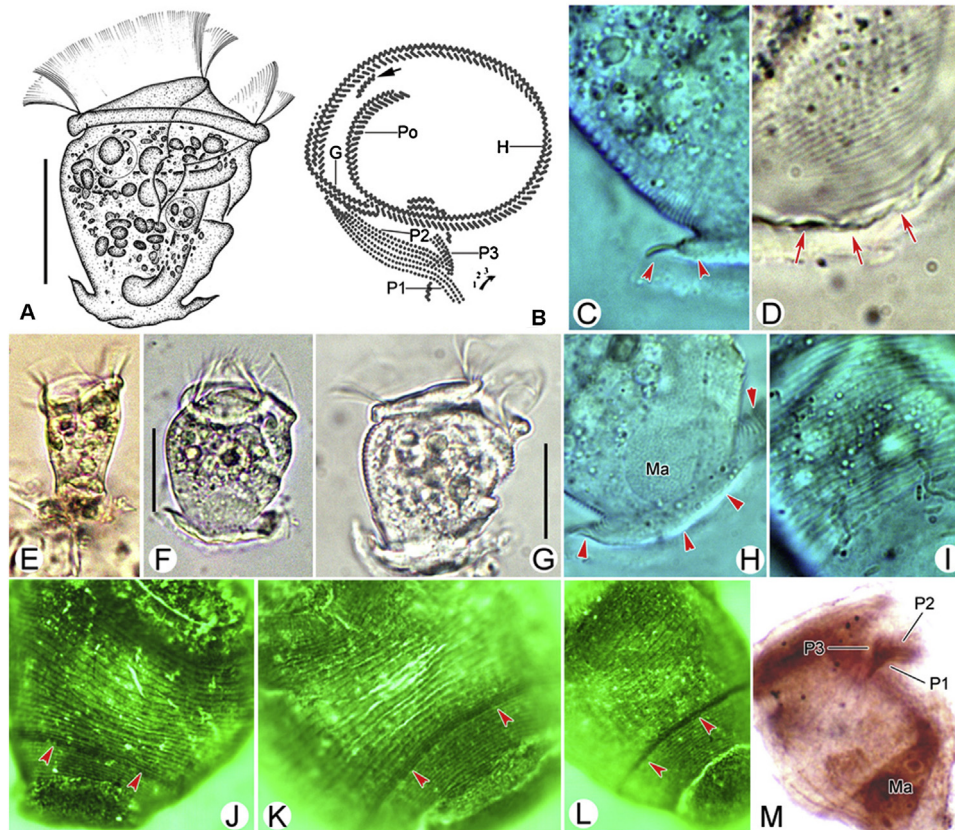


Fig. 6. (A–M) *Scyphidia perezuzae* sp. nov. from life (A, C–I) and after staining with protargol (B, M) and silver nitrate (J–L). A, F, G – living zooids at high magnifications; B – top view of entire oral infraciliature, arrow marks epistomial membrane and arrow with numerals indicates numbering convention for polykineties and rows of kinetosomes within each polykinety; arrow marks epistomial membrane; C, D, H – arrowheads in C, H and arrows in D to show scopula in vivo; E – living zooid at low magnifications; I – living zooid at high magnification focused to show the pellicular striations; J–L – silverline system; M – detailed arrangement of infundibular polykineties in holotype specimen. G, germinal kinety; H, haplokinety; Ma, macronucleus; P1–3, infundibular polykinety 1–3; Po, polykinety. Scale bars: 30 μ m in A, F, G.

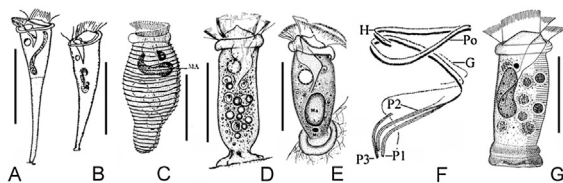


Fig. 7. (A–G) Closely related species of *Paravorticella* sp. and *Scyphidia perezuzae*. A – *Paravorticella terebellae* (from Kahl 1935); B – *Paravorticella clymenellae* (from Kahl 1935); C – *Paravorticella nerillae* (from Magagnini and Verni 1988); D–F – *Scyphidia* sp. (from Song and Wilbert 2002); G – *Scyphidia physarum* (from Foissner and Schiffmann 1979). Scale bars: 60 μ m in A, B, D–F; 30 μ m in C.

thus these two taxa are probably not conspecific (Fig. 7B; Kahl 1935). It is highly likely that the Qingdao isolate is a new species of *Paravorticella*, however further information concerning the identity of its host and the number and pattern of silverlines as revealed by silver nitrate staining is required before it can be established as such.

Another marine *Paravorticella* species, *P. nerillae*, found as epibionts of the polychaete *Nerilla antennata* from the

Livorno coast, also has a similar zooid size to that of *Paravorticella* sp. However, *P. nerillae* can be separated from *Paravorticella* sp. by having significantly fewer silverlines (40 in the former vs. 87–95 in the latter) and a relatively truncated posterior end (vs. posterior region of zooid conspicuously tapered in the latter) (Fig. 7C; Jankowski 1993).

Genus: *Scyphidia* Dujardin, 1841

Scyphidia perezuzae sp. nov. (Fig. 6A–M; Table 1)

Diagnosis. Marine *Scyphidia*. Zooid truncated bell-shaped, 50–60 \times 50–70 μ m in vivo. Peristomial lip about equal to maximum zooid width. Macronucleus J-shaped. Transverse silverlines numbering 22–30 from peristome to trochal band, 20–24 from trochal band to scopula. P3 composed of three parallel, equal-length rows.

Type locality. Body surface of *Sebastodes fuscus* from indoor fish-farming ponds, Penglai Bay, Shandong, China (37°41′46.83″N, 120°52′22.62″E).

Deposition of slides. The protargol slide (registration number SP-2006-0507-01) containing the holotype specimen (Fig. 6M) is deposited in the Laboratory of Protozoology, Ocean University of China, China. One paratype slide with silver nitrate-stained specimens is

deposited in the Natural History Museum, London (NHMUK 2016.10.27.5).

Etymology. This species is named in honor of Dr Blanca Perz-Uz, Universidad Complutense de Madrid, Spain, in recognition of her contributions to the fields of ciliate systematics and ecology.

Description. Zooid truncated bell-shaped, approximately $50\text{--}60 \times 50\text{--}70 \mu\text{m}$ in vivo, conspicuously constricted beneath peristomial lip (Fig. 6A, G). Peristomial lip about equal to maximum body width. Peristomial disc flat, elevated above peristomial lip when zooid fully extended (Fig. 6A, E–G). Pellicle smooth and flexible, sometimes folded, with conspicuous striations clearly visible under $1000\times$ magnification (Fig. 6I). Zooid attached to substratum via scopula which forms a conspicuous, flattened disc (Fig. 6C, D, H, arrowheads and arrows).

Cytoplasm dark gray, usually containing several gray to light-green food granules, $4\text{--}8 \mu\text{m}$ in diameter (Fig. 6A, E–G). Contractile vacuole not observed. Macronucleus J-shaped (Fig. 6M). Micronucleus not observed.

Oral apparatus as shown in Fig. 6B, M. Haplokinety and ploykinety parallel to each other and make about 1.25 turns around peristome before entering infundibulum. Epistomial membrane short, located at opening of infundibulum (Fig. 6B, arrow). After entering infundibulum, haplokinety and polykinety spiral on opposite walls. Infundibular part of haplokinety accompanied by germinal kinety in abostomal half (Fig. 6B). P1–P3 each composed of three rows of kintosomes. P2 terminates adstomally far above adstomal end of P1. Three rows of P3 parallel, approximately equal in length (Fig. 6B).

Silverline system transverse; silverlines widely spaced above trochal band, closely spaced below trochal band (Fig. 6J–L). Trochal band composed of closely arranged kintosomes encircling cell at about third of body length above scopula (Fig. 6J–L, arrowheads). Approximately 22–30 silverlines from peristome to trochal band, and 20–24 from trochal band to scopula (Fig. 6J–L).

Remarks and comparisons. *Scyphidia physarum* Lachmann, 1856 and *Scyphidia* sp. in Song and Wilbert (2002) are similar to the new species and thus should be compared with it. *Scyphidia physarum* can be separated from *S. perezuzae* sp. nov. by its larger size ($80\text{--}110 \mu\text{m}$ vs. $50\text{--}60 \mu\text{m}$ long in vivo), band-shaped (vs. J-shaped) macronucleus, different numbers of silverlines above and below the trochal band ($54\text{--}62$, $8\text{--}10$ vs. $22\text{--}30$, $20\text{--}24$ in *S. perezuzae*) and its freshwater (vs. marine) habitat (Fig. 7G; Foissner and Schiffmann 1979).

Song and Wilbert (2002) described a *Scyphidia* collected from the mantle cavity of clams in Antarctica. This unidentified species can be separated from the new species by its larger size ($70\text{--}120 \mu\text{m}$ vs. $50\text{--}60 \mu\text{m}$ long in vivo) and more silverlines ('numerous' vs. $22\text{--}30$, $20\text{--}24$) (Fig. 7D–F; Song and Wilbert 2002).

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