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Redescription of *Pseudovorticella cylindrica* (Dons,) nov. comb. and *Zoothamnium hiketes* Precht, , two poorly defined marine peritrichs (Ciliophora: Peritrichia) from the north China Sea

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Redescription of *Pseudovorticella cylindrica* (Dons, 1915) nov. comb. and *Zoothamnium hiketes* Precht, 1935, two poorly defined marine peritrichs (Ciliophora: Peritrichia) from the north China Sea

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Abstract

The living morphology, silverline system and infraciliature of two marine peritrichous ciliates, *Pseudovorticella cylindrica* (Dons, 1915) nov. comb. (formerly *Vorticella cylindrica*) and *Zoothamnium hiketes* Precht, 1935, collected in Qingdao, China, were investigated. Based on the Qingdao populations, both species are redefined. *Pseudovorticella cylindrica* is characterized by: cell inverted bell-shaped, about $45 \times 50 \mu\text{m}$ in vivo; macronucleus J-shaped; one apically positioned contractile vacuole; pellicle smooth with inconspicuous, closely spaced striations; the number of transverse silverlines from peristome to aboral ciliary wreath 33–40, from aboral ciliary wreath to scopula 14–19; the inner row of peniculus 3 conspicuously short. An updated and supplementary description of *Zoothamnium hiketes* is also supplied: size of zooid in vivo $70 \times 35 \mu\text{m}$ on average, elongate in shape with inconspicuously double-layered peristomial lip; macronucleus C-formed; one large contractile vacuole apically located; colony dichotomously branched; the number of striations from peristome to aboral ciliary wreath 89–109, from aboral ciliary wreath to scopula 35–43; rows in peniculus 3 parallel to each other.

Keywords: Marine ciliate, Peritrichia, *Pseudovorticella cylindrica* nov. comb., *Zoothamnium hiketes*

Introduction

In recent years, it has been proposed that a minimum requirement for an acceptable species-level description in peritrichous ciliates is that it should be based on observations of the organisms both in vivo and following silver impregnation (Foissner 1984).

Among the solitary peritrichs, members of the genus *Pseudovorticella* and *Zoothamnium*, which have plastic body shape, variable size and highly contractile nature, are difficult to identify to species level. This problem is compounded by the large number of taxa

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described, and a lack of information on the infraciliature and the silverline system based on silver impregnation (Kahl 1935; Stiller 1971; Warren 1986, 1987; Foissner et al. 1992).

During faunistic surveys of marine ciliates in coastal waters of north China, two less common peritrich species were isolated and investigated. After comparison with known congeners, one of them is believed to be a poorly described *Pseudovorticella* which, for many decades, has been known as *Vorticella cylindrica* Dons, 1915, and another one is identified as *Zoothamnium hiketes*.

Materials and methods

Pseudovorticella cylindrica (Dons, 1915) nov. comb. was discovered in a shrimp-breeding pond on the coast of Qingdao (Tsingtao, 36°08'N, 120°43'E), China in April 2004 with salinity ca 30 and pH 8.1. *Zoothamnium hiketes* was collected in June 2003 from an abalone-farming pond near Qingdao with water temperature 22°C and salinity ca 28.

Ciliates were studied in vivo using a differential interference contrast microscope. The infraciliature was revealed with protargol impregnation according to Wilbert (1975), while the silverline system was impregnated by the Chatton-Lwoff silver nitrate method according to Song and Wilbert (1995). Drawings of stained specimens were performed at 1250× with the aid of a camera lucida. Terminology is mainly according to Corliss (1979), Foissner et al. (1985) and Warren (1986, 1987).

Voucher slides

Silver nitrate and protargol-prepared materials are deposited as voucher slides in the collection of the Laboratory of Protozoology, Ocean University of China with the following registration numbers: *Pseudovorticella cylindrica*, 04040801–2; *Zoothamnium hiketes*, 03061901–2.

Results

Subclass PERITRICHIA Stein, 1859

Order SESSILIDA Kahl, 1933

Family VORTICELLIDAE Ehrenberg, 1938

Genus *Pseudovorticella* Foissner and Schiffmann, 1974

Pseudovorticella cylindrica (Dons, 1915) nov. comb.

(Figures 1–18; Table I)

Synonym *Vorticella cylindrica* Dons, 1915.

Since no ciliature information was previously available for this organism, here we supply an improved diagnosis and detailed data on the infraciliature as well as on the morphology of living cells, based on the Qingdao population.

Revised diagnosis

Inverted bell-shaped *Pseudovorticella*, length in vivo 40–50 µm and length: width ratio about 1:1; macronucleus J-formed; one contractile vacuole apically located; reticulate silverline system with 33–40 transverse striations between oral area and aboral ciliary wreath, and

Figures 1–8. Morphology of *Pseudovorticella cylindrica* from life (1–5), after protargol (6, 7) and silver nitrate impregnation (8). (1) A typical zooid. (2) Detail of stalk, arrows to show conspicuous theoplasmic granules in spasmoneme. (3) From Dons (1915) (called *Vorticella cylindrica*). (4) From Song (1991a) (called *Vorticella cylindrica*). (5) Typical zooids at low magnification. (6) Buccal apparatus, arrow indicates epistomial membrane. (7) Variable shape of macronucleus. (8) Silverline system. ACW, aboral ciliary wreath; G, germinal kinety; H, haplokinety; P1–3, peniculi 1–3; PD, peristomial disc; PL, peristomial lip; Po, polykinety; Sc, scopula; Spa, spasmoneme. Scale bars: 30 μ m (1); 20 μ m (4); 50 μ m (5).

14–19 striations between aboral ciliary wreath and scopula. The inner row of peniculus 3 conspicuously shorter than other two. Marine.

Description

Cell constant in size and shape, about $40\text{--}50 \times 45\text{--}50 \mu\text{m}$ in vivo, usually with a slight constriction in the aboral region. Maximum width of cell at peristomial area with thin and relatively rigid peristomial lip (PL; Figures 1, 5, 9). Peristomial disc (PD) flat and only

Figures 9–18. Photomicrographs of *Pseudovorticella cylindrica* from life (9–11, 13), after protargol (12, 15, 17, 18) and silver nitrate impregnation (14, 16). (9) A fully extended zooid at low magnification. (10) Detail of stalk, arrows show theoplasma granules in spasmoneme. (11) Zooids at low magnification. (12) Arrow indicates epistomial membrane. (13) A contracted zooid at low magnification. (14, 16) Silverline system, showing reticulate meshes, double arrowhead marks aboral ciliary wreath. (15) Anterior end of zooid showing peniculi 1–3. (17) Lateral view, showing aboral ciliary wreath (arrowheads). (18) Apical view, showing polykinety and haplokinety. H, haplokinety; P1–3, peniculi 1–3; Po, polykinety. Scale bars: 120 μ m (9); 200 μ m (11); 60 μ m (13).

slightly elevated when cell fully extended (Figures 1, 9). Pellicle generally smooth, while a finely reticulate pattern of striations can be observed only under high magnification (1000 $\times$) (Figure 1).

Cytoplasm colourless or greyish, usually containing several refractive particles, 4–7 μ m in length (Figures 1, 5, 13). Single contractile vacuole large, apically located, contracting every 2–3 min. Macronucleus J-shaped, variable in shape and generally situated transversely (Figure 7). Micronucleus not observed.

Table I. Morphometric data on *Pseudovorticella cylindrica* (P. c.) and *Zoothamnium hiketes* (Z. h.).

Characters	Minimum		Maximum		Mean		SD		SE		CV		N	
	P. c.	Z. h.	P. c.	Z. h.	P. c.	Z. h.	P. c.	Z. h.	P. c.	Z. h.	P. c.	Z. h.	P. c.	Z. h.
Body length in vivo (µm)	40	60	50	80	ca 45	ca 70	2.67	6.03	0.89	1.67	5.9	8.6	9	13
Body width in vivo (µm)	45	30	55	40	ca 50	ca 35	2.67	4.11	0.89	1.14	2.0	11.7	9	13
Body length after fixation (µm)	24	36	36	60	28.9	50.9	3.89	6.22	1.30	1.79	13.4	12.2	9	12
Body width after fixation (µm)	24	24	36	32	31.6	28.4	3.71	2.47	1.24	0.71	11.7	8.7	9	12
No. of silverlines from peristome to aboral ciliary wreath	33	89	40	109	36.0	97.1	2.11	6.29	0.67	1.48	5.8	6.5	10	18
No. of silverlines from aboral ciliary wreath to scopula	14	35	19	43	16.3	38.2	1.80	3.40	0.64	1.52	11.0	8.9	7	5

Stalk usually two to four times zooid length, relatively thick and about 5 µm in diameter (Figure 11). The spasmoneme (Spa) about 2 µm in diameter, with a string of conspicuous, dark grey thecoplasmic granules (ca 0.3 µm in diameter) (Figures 2, 10, arrows).

Individuals often closely grouped together thus forming pseudocolonies of up to 30 zooids. Telotroch (swarmer) was not observed.

Infraciliature as shown in Figures 6, 12, 15, 17, 18. Haplokinety (H) and polykinety (Po) describing about 1.5 turns around peristomial disc before entering vestibulum, where they make a further turn. Similar to that of other congeners, polykinety forms three peniculi in lower half of vestibulum, each consisting of three rows. The posterior ends of peniculus 1 (P1) and P3 terminate at the same level whereas P2 situates between and terminates conspicuously above P1 and P3 (Figures 6, 15). The inner row of P3 is characteristically displaced relative to the other two: very short, about one-third length of the outer two rows which converge with P1. The haplokinety passing around the vestibulum on the opposite wall to the peniculi. Germinal kinety (G) consisting of zig-zag structure of kinetosomes and extending to the upper one-third of the vestibulum, where it passes immediately beyond the haplokinety (Figure 6). The epistomial membrane is short, located near the opening of the infundibulum (Figures 6, 12, arrow). Aboral ciliary wreath encircles cell in posterior region and seems to be composed of a Z-rowed structure (Figure 14, double arrowhead; Figure 17, arrowheads).

Silverline system reticulate (Figure 16). Transverse lines in the anterior region of the cell are more widely spaced than those in the posterior region (Figures 8, 14, 16). The number of transverse silverlines from peristome to aboral ciliary wreath (=aboral trochal band), 33–40; from aboral ciliary wreath to scopula, 14–19 (Table I).

Comparison

Pseudovorticella cylindrica was originally described by Dons (1915) (Figure 3) under the name of *Vorticella cylindrica*. Its infraciliature remained unknown though it was redescribed in the last decade by Song (1991a) (Figure 4). The Qingdao population

Table II. Comparison of *Pseudovorticella cylindrica* with five closely related *Vorticella/Pseudovorticella* species.

Species	Body length in vivo (µm)	Body width in vivo (µm)	No. of silverlines from scopula to aboral ciliary wreath	No. of silverlines from aboral ciliary wreath to peristome	No. of contractile vacuoles	Habitat	Data source
<i>Pseudovorticella cylindrica</i>	40–50	45–50	14–19	33–40	1	Marine	Present work
<i>Pseudovorticella patellina</i>	55–110	50–100	13–16	19–22	1 or 2	Marine	Song and Warren (2000)
<i>Pseudovorticella punctata</i>	40–50	40	?	?	1	Marine	Warren (1987)
<i>Vorticella campanula</i>	50–160	35–100	27–33	69–77	1	Freshwater	Foissner et al. (1992)
<i>Vorticella nebulifera</i>	30–67	17–33	8–12	35–39	1	Marine	Song (1991a)
<i>Vorticella marina</i>	47–57	34–50	?	?	1	Marine	Song (1991a)

?, Data not available.

resembles the previous descriptions in all aspects, e.g. body shape, size, number of contractile vacuoles, as well as marine habitat, hence we are confident that the identification is correct.

A summary of comparisons between *P. cylindrica* and similar *Pseudovorticella* and *Vorticella* spp. is given in Table II.

Morphologically, *Pseudovorticella patellina* (Müller, 1776) Song and Warren, 2000 (Figure 39) should be closely related to *P. cylindrica*. Nevertheless, the former differs from *P. cylindrica* in its larger size (55–110 versus 40–50 µm), the number of transverse silverlines between the aboral ciliary wreath and the anterior end (19–22 versus 33–40), number of contractile vacuoles (two versus one), and the extreme outwards extension of the peristomial lip. In addition, the structure of peniculus 3 is completely different (see Song and Warren 2000).

Pseudovorticella punctata (Dons, 1918) Warren, 1987 (Figure 43) is most similar to *P. cylindrica*, especially in terms of body size and the habitat. However, *P. punctata* can be clearly distinguished from the latter by the appearance of the peristomial lip (considerably thick versus thin), the lower position of the contractile vacuole (below the vestibulum versus apically located), and having significantly fewer transverse lines (about 30 versus 52) (Warren 1987).

Considering the general morphology, further comparisons should be also made with some *Vorticella* species. *Vorticella campanula* Ehrenberg, 1831 (Figures 40, 41) corresponds very well in vivo with *Pseudovorticella cylindrica*. In the past 80 years, *P. cylindrica* was repeatedly considered to be a marine variety of *V. campanula* (Noland and Finley 1931; Warren 1987). In 1992, a thorough redescription of *V. campanula* using modern techniques was given by Foissner et al. (1992). Thus, we have the opportunity to compare these two forms. *Vorticella campanula* can be separated from *P. cylindrica* by size (50–160 versus 40–50 µm), the habitat (freshwater versus marine), *Vorticella*-type silverline system (versus *Pseudovorticella* pattern), and dissimilarities considering the infraciliature (Foissner et al. 1992).

Vorticella nebulifera Müller, 1786 also bears some resemblance to *Pseudovorticella cylindrica*, particularly with respect to the smooth pellicle and cell size. However, the two

Figures 19–26. Morphology of *Zoothamnium hiketes* from life (19–23), after protargol (24, 25) and silver nitrate impregnation (26). (19) General view of a typical zooid. (20) Showing varieties of body shape and macronucleus. (21) Detail of stalk, arrowheads show many rod-shaped bacteria on stalk surface. (22) *Zoothamnium hiketes* (from Precht 1935). (23) Colony form. (24) Detail of the three oral peniculi, arrows mark separated upper end of the outer two rows in P2. (25) Oral infraciliature, arrow notes epistomial membrane, double arrowhead indicates the oral distal fragment. (26) Silverline system. ACW, aboral ciliary wreath; G, germinal kinety; H, haplokinety; P1–3, peniculi 1–3; P0, polykinety. Bars: 30 μ m (19); 20 μ m (21, 22a); 100 μ m (22b); 150 μ m (23).

taxa can be clearly separated by the position of the contractile vacuole (lower ventral versus apically dorsal located) (Figure 42), different pattern of silverline system, and the body shape (slender in *V. nebulifera*) (Song 1991a).

With reference to body shape and the habitat, another similar form, *Vorticella marina* Greeff, 1870 whose silverline system and infraciliature remain unclear, should also be compared with *P. cylindrica*. The former differs from the latter, even at the in vivo level, in the lower position of contractile vacuole (Figure 44) and appearance of pellicle (distinctly striated versus finely striated) (Song 1991a).

Figures 27–38. Photomicrographs of *Zoothamnium hiketes* from life (27–31), after protargol (32–34, 36–38) and silver nitrate impregnation (35). (27) Colony at low magnification. (28) Detail of stalk, arrowheads showing rod-shaped bacteria on stalk surface. (29) Zooids at 400 $\times$ magnification. (30) Varieties of body shape, arrows indicate contractile vacuole. (31) Lateral view of a zooid at 1250 $\times$ magnification, showing the pellicle striations. (32) Arrows mark aboral ciliary wreath. (33) Oral apparatus, indicating three peniculi (P1–3). (34) To show the branching form. (35) General silverline system, arrow marks the aboral ciliary wreath. (36) Arrow showing the distal fragment of oral apparatus. (37) General appearance, to mark aboral ciliary wreath (arrowheads). (38) To show epistomial membrane (arrow) and germinal kinety (double arrowhead). P1–3, peniculi 1–3. Scale bars: 200 μ m (27); 50 μ m (29); 70 μ m (30); 100 μ m (34).

Figures 39–44. Species of *Vorticella* and *Pseudovorticella* that are similar to *Pseudovorticella cylindrica*. (39) *Pseudovorticella patellina* (from Song and Warren 2000). (40) *Vorticella campanula* (from Kahl 1935). (41) *Vorticella campanula* (from Noland and Finley 1931). (42) *Vorticella nebulifera* (from Song 1991a). (43) *Pseudovorticella punctata* (from Warren 1987). (44) *Vorticella marina* (from Song 1991a). Scale bars: 70 μm (39); 30 μm (41, 42); 20 μm (43, 44).

Genus *Zoothamnium* Bory de St. Vincent, 1826

Zoothamnium hiketes Precht, 1935

(Figures 19–38; Table I)

Zoothamnium hiketes was originally described by Precht (1935) from the North Sea, Germany. No further studies have been carried out since then and hence neither the infraciliature nor silverline system is clear. Based on the Qingdao population and previous studies, an updated diagnosis and a detailed redescription are presented here.

New diagnosis

Marine *Zoothamnium* with dichotomously branched stalk. Zooids elongate, measuring about $40\text{--}80 \times 30\text{--}40 \mu\text{m}$, with fine pellicular striations. Peristomial lip double-layered. Single contractile vacuole apically located. Macronucleus generally C-shaped and transversely orientated. Number of transverse lines from oral area to aboral ciliary wreath 89–109, from aboral ciliary wreath to scopula, 35–43.

Description of Qingdao population

Zooids usually elongated vase-shaped, in vivo about $60\text{--}80 \times 30\text{--}40 \mu\text{m}$, and only slightly constricted below the thick peristomial lip, which is inconspicuously double-layered; large peristomial disc highly elevated (Figures 19, 29).

Pellicle smooth when observed at low magnification, fine striations recognizable under high magnification ($\times 400$ or higher) (Figure 31).

Cytoplasm colourless or slightly greyish, usually contains several to many large granules ($4\text{--}8\text{ }\mu\text{m}$ in diameter) (Figures 19, 29). One large contractile vacuole apically located, contracting every 4 min (Figure 19; Figure 30, arrows). Macronucleus band-like, generally C-shaped, transversely orientated (Figures 19, 20); micronucleus not observed.

Colony dichotomously branched with zooids located regularly in pairs (Figures 23, 27), consisting of up to 100 zooids in a large colony (Figure 34). Stalk up to $400\text{ }\mu\text{m}$ long, diameter about $12\text{ }\mu\text{m}$ in main stalk and $6\text{--}8\text{ }\mu\text{m}$ in accessory branches. Surface of stalk smooth and often covered with rod-shaped bacteria (about $1.2\text{--}1.8\text{ }\mu\text{m}$ in length) (Figures 21, 28, arrowheads). Spasmoneme about $2\text{ }\mu\text{m}$ in diameter, without visible mitochondria (thecoplasmic granules).

Oral infraciliature as shown in Figures 24, 25, 33, 36, 38, haplo- and polykinety circling about 1.5 turns around peristomial disc, and making a further turn after entering vestibulum. Near distal end of haplo- and polykinety, always one short kinety fragment recognizable (Figure 25, double arrowhead; Figure 36, arrow).

At lower half of vestibulum, polykinety transforming into three peniculi (P1–3), each of which consists of three rows. P1 longer than other two, extending to cytostome. P2

Figures 45–55. Comparison of some morphotypes closely related to *Zoothamnium hiketes*. (45) *Zoothamnium cienkowskii* (from Kahl 1935). (46, 47) *Zoothamnium affine* (from Song 1991b). (48, 49) *Zoothamnium maximum* (from Ji and Song 2004). (50, 51) *Zoothamnium duplicatum* (from Ji et al. 2005). (52, 53) *Zoothamnopsis mengi* (from Song 1997). (54, 55) *Zoothamnopsis sinica* (from Ji and Song 2004). Scale bars: $50\text{ }\mu\text{m}$ (45, 46, 48, 50, 52, 54); $100\text{ }\mu\text{m}$ (47, 51, 53); $300\text{ }\mu\text{m}$ (49, 55).

interposes between P1 and P3 and ends at curvature of P1. Rows in P3 are parallel to each other (Figure 33). Germinal kinety parallel to haplokinety within upper half of vestibulum (Figure 25; Figure 38, double arrowhead). Epistomial membrane located at opening of oral cavity (Figures 25, 38, arrow). Aboral ciliary wreath formed by probably double-rowed kineties (Figure 26; Figures 32, 35, arrow; Figure 37, arrowheads).

Silverline system genus typical, 89–109 striations between peristomial area and aboral ciliary wreath, 35–43 striations from aboral ciliary wreath to scopula, with many sparsely distributed pellicular pores (Figures 26, 35).

Comparison

Zoothamnium hiketes Precht, 1935 was originally found on the seta of *Gammarus* species as epizoons, and the brief description concerned only the morphology of live cells (Precht 1935; Figure 22a, b). Since then, no redescrptions have been made. In the absence of data concerning the infraciliature and silverline system, we identified our organism mainly on the basis of its body shape, appearance of the thick peristomial lip (inconspicuous double-layered), position of contractile vacuole, branching form, and marine habitat. The only difference is the size of the zooid. According to the original description, the zooid of *Z. hiketes* is smaller, about 40–55 µm in length, while in the Qingdao population the length is about 60–80 µm. Since the size of the organism, to the authors' knowledge, is usually a population-dependent feature in most cases, we consider this difference as an intra-species variation.

Morphologically, *Zoothamnium cienkowskii* Wrzesniowski, 1877 (Figure 45; Table III) is similar to *Z. hiketes* in vivo. Unfortunately, neither the infraciliature nor silverline system of the former has been described. However, *Z. cienkowskii* can be distinguished at least by the

Table III. Morphological comparison between *Zoothamnium hiketes* and other similar morphotypes.

Species	Body length in vivo (µm)	Body width in vivo (µm)	No. of silverlines from scopula to aboral ciliary wreath	No. of silverlines from aboral ciliary wreath to peristome	Appearance of peristomial lip	Appearance of stalk	Data source
<i>Zoothamnium hiketes</i>	60–80	30–40	35–43	89–109	Inconspicuous double layer	Smooth	Present work
<i>Zoothamnium cienkowskii</i>	35–50	?	?	?	Single layer	Transversely wrinkled at accessory branch	Kahl (1935)
<i>Zoothamnium maximum</i>	81–121	47–64	44–60	85–98	Inconspicuous double layer	Smooth	Ji and Song (2004)
<i>Zoothamnium duplicatum</i>	45–84	27–49	25–28	50–54	Double layer	Smooth	Ji et al. (2004)
<i>Zoothamnium affine</i>	55–82	45–60	44–50	75–81	Double layer	Cross-striated	Song (1991b)
<i>Zoothamnopsis mengi</i>	50–70	30–40	30–36	76–87	Single layer	Smooth	Song (1997)
<i>Zoothamnopsis sinica</i>	70–105	45–52	48–55	82–98	Double layer	Smooth	Ji and Song (2004)

?, Data not available.

appearance of the stalk (transversely wrinkled at accessory branch versus smooth) and differentiation of the zooids (having enlarged zooids versus all zooids about equal size) (Kahl 1935).

Zoothamnium affine sensu Song, 1991 (Figures 46, 47) whose infraciliature also remains unknown, matches in some aspects *Z. hiketes*, e.g. in body size, in branching form and in some other morphometric features (Table III). However, the former can be identified by having fewer silverlines between oral area and aboral ciliary wreath (75–81 versus 89–109) and a cross-striated stalk (versus smooth) (Song 1991b).

Another similar form, *Zoothamnium maximum* Song, 1986 (Figures 48, 49; Table III) differs from *Z. hiketes* in: (1) the larger size (80–120 versus 40–80 µm); (2) more silverlines between aboral ciliary wreath and scopula (45–58 versus 35–43); (3) the appearance of P3 (the outer row loosely ciliated, about two-thirds of the other two in length versus inner row slightly longer than the other two) (Song 1986; Ji and Song 2004).

Zoothamnium duplicatum Kahl, 1933 (Figures 50, 51; Table III) also exhibits a similar size and dichotomous branching pattern, but differs from *Z. hiketes* by the following features: (1) significantly fewer silverlines between peristomial area and aboral ciliary wreath (48–53 versus 89–109); (2) the form of P3 (outer two rows close set, with their upper halves separated widely from the inner one versus three rows parallel to each other) (Ji et al., 2005).

Zoothamnopsis mengi Song, 1997 (Figures 52, 53) and *Zoothamnopsis sinica* Ji and Song, 2004 (Figures 54, 55) might also be similar to the present form (Table III). However, they differ from *Z. hiketes* by the different silverline system (*Pseudovorticella*-type versus *Vorticella*-type) and the structure of P3 (Song 1997; Ji and Song 2004).

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