

Taxonomic Characterization of Three Marine Peritrichous Ciliates from China: *Zoothamnium alrasheidi* spec. nov., *Z. marinum* Kahl, 1933 and *Z. vermicola* Precht, 1935 (Ciliophora: Peritrichia)

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Summary. Three marine peritrichous ciliates, *Zoothamnium alrasheidi* spec. nov., *Z. marinum* Kahl, 1933 and *Z. vermicola* Precht, 1935, were isolated from littoral areas near Qingdao, China. The living morphology, infraciliature and silverline system were studied in living and silver-impregnated specimens. *Zoothamnium alrasheidi* is distinguished from its congeners by the giant, leaf-shaped colony, the differentiation of zooids, the structure of the infundibular polykineties and in having 57–75 silverlines between the oral area and the trochal band and 24–42 between the trochal band and the scopula. *Zoothamnium marinum* and *Z. vermicola* are reported for the first time in over 70 years. Each was identified by its zooid shape and size, colony shape, the branching pattern of its stalk and its marine habitat. As a result of the present study, additional features for characterizing these species now include the structure of infundibular polykinety 3 and the number of silverlines between the aboral trochal band and (a) the scopula, and (b) the peristomial lip. Redescriptions and improved diagnoses of both species are supplied based on the Chinese populations.

Key words: Marine ciliate, morphology, new species, Peritrichida, *Zoothamnium*.

INTRODUCTION

Among the peritrichous ciliates, *Zoothamnium* is one of the largest and most taxonomically complex genera, with over 70 nominal species (Kent 1880–1882; Kahl 1935; Song 1986, 1991; Stiller 1971). There has been no revision of this genus since that of Kahl (1935), and few

Zoothamnium species have been studied using modern methods (e.g., silver impregnation) as recommended by Foissner & Schiffmann (1974). Consequently, species separation and identification among congeners of *Zoothamnium* are often very difficult (Kahl 1933; Precht 1935; Sommer 1951; Stiller 1953a, b; Stiller and Stevčić 1967). Information about the silverline system and the infraciliature, especially the details of infundibular polykineties, should therefore be included when a new species is described as these are important characters for species circumscription and identification (Clamp 1992, 1997; Foissner *et al.* 1992; Ji and Song

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2004; Ji *et al.* 2005a, b, c, 2006a; Li *et al.* 2008; Peng *et al.* 2007; Sun *et al.* 2005a, b, 2007).

Three *Zoothamnium* species were collected during surveys of the marine ciliate fauna of the littoral zone near Qingdao in 2002 and 2003. After comparison with known congeners, two were identified as *Z. marimum* and *Z. vermicola* respectively, both of which are reported here for the first time in over 70 years. The third isolate could not be identified with any of its congeners and so represents a new species. In this paper, we supply a detailed description of the new species and re-descriptions of *Z. marimum* and *Z. vermicola* including new information about their morphology.

MATERIALS AND METHODS

Sample collection

Zoothamnium alrasheidi and *Z. marimum* were collected from a shrimp-farming pond in the Qingdao region, P.R. China, (36°28'N, 120°64'E) using glass slides as artificial substrates. The slides were fixed to a frame which was immersed in water and left for 10 days to allow colonization to occur. After this time the slides were retrieved and transported to the laboratory for examination. *Zoothamnium vermicola* was isolated from the mantle cavity of marine clams (*Meretrix* sp.) collected from a tidal flat near Qingdao.

Observations

Ciliates were observed *in vivo* using bright field and differential interference contrast microscopy. The infraciliature was revealed with protargol impregnation according to Wilbert (1975). The “dry” (Foissner 1976) and “wet” (Song and Wilbert 1995) silver nitrate methods were used to demonstrate the silverline system.

Drawings and terminology

Drawings of impregnated specimens were made with the help of a camera lucida at $\times 1250$ magnification. Terminology is mainly according to Warren (1986) and Ji *et al.* (2006b).

RESULTS

Zoothamnium alrasheidi spec. nov. (Figs 1, 2; Table 1)

Diagnosis: Colony planar, leaf-shaped, up to 2 mm high. Stalk alternately branched in small colonies (< 50 zooids) and in accessory branches of large colonies (up to 300 zooids), while secondary stalks of large colonies sometimes irregularly branched from primary stalk, i.e., with two adjacent accessory branches sometimes located on same side of main trunk. Zooids bell-shaped, differentiated into two types: (1) mature zooids, somewhat asymmetric, $80\text{--}120 \times 50\text{--}60 \mu\text{m}$ in size, with wide, double-layered peristomial lip; (2) immature (newly

Table 1. Morphometric data of *Zoothamnium alrasheidi* spec. nov. (1st line), *Z. marimum* (2nd line) and *Z. vermicola* (3rd line). Min – minimum, Max – maximum, Mean – arithmetic mean, SD – standard deviation, n – sample number.

Characteristics	Min	Max	Mean	SD	n
Body length <i>in vivo</i> (μm)	80*	120*	98.8*	12.90*	22*
	96	108	–	–	4
	65	95	81.2	7.75	17
Body width <i>in vivo</i> (μm)	50*	60*	56.2*	4.36*	22*
	52	58	–	–	4
	40	50	43.6	3.10	17
Number of silverlines from aboral trochal band to peristome	50	75	65.4	7.07	21
	60	75	–	–	4
	50	68	60.2	4.74	31
Number of silverline from aboral trochal band to scopula	24	42	34.3	4.96	21
	30	40	–	–	4
	32	53	39.1	5.46	26

* Data from mature zooids.

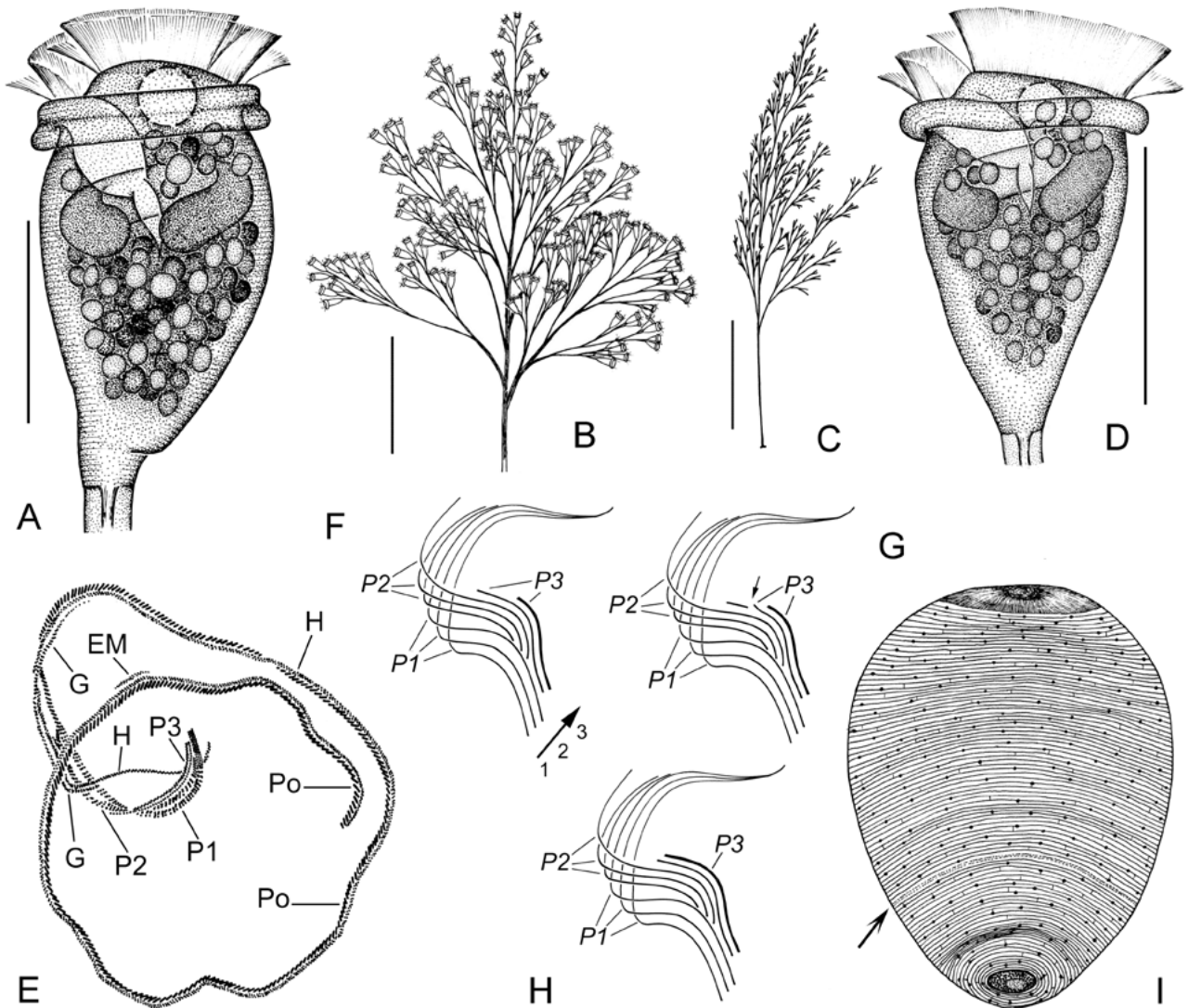


Fig. 1. Morphology of *Zoothamnium alrasheidi* spec. nov. **A** – a mature zooid; **B, C** – colony form; **D** – an immature zooid; **E** – apical view of oral infraciliature; **F–H** – varieties of arrangement of infundibular polykinety 3, arrow with numbers shows convention for numbering rows within each polykinety, arrow in **G** depicts the gap in row 2 of **P3**; **I** – silverline system, arrow marks the aboral trochal band. EM – epistomial membrane; G – germinal kinety, H – haplokinety, P1–3 – infundibular polykinety 1–3, Po – polykinety. Scale bars: 50 μ m (A, D), 300 μ m (B, C).

divided) zooids, uniformly subconical, 60–70 \times 30–40 μ m in size, with narrow, single-layered peristomial lip. Macronucleus C-shaped, transversely oriented. Single contractile vacuole apically located near dorsal wall of infundibulum. Pellicle finely striated, 50–75 striations between aboral trochal band and peristomial lip, 24–42 between aboral trochal band and scopula. Rows 2 and 3

of infundibular polykinety 3 (P3) lying in close proximity to one another and terminating below inner row at abostomal end. Marine habitat.

Type specimens: Two type slides are deposited in the collection of the Laboratory of Protozoology, Ocean University of China, Qingdao, P.R. China as follows: holotype (protargol preparation, No. 0205220301); para-

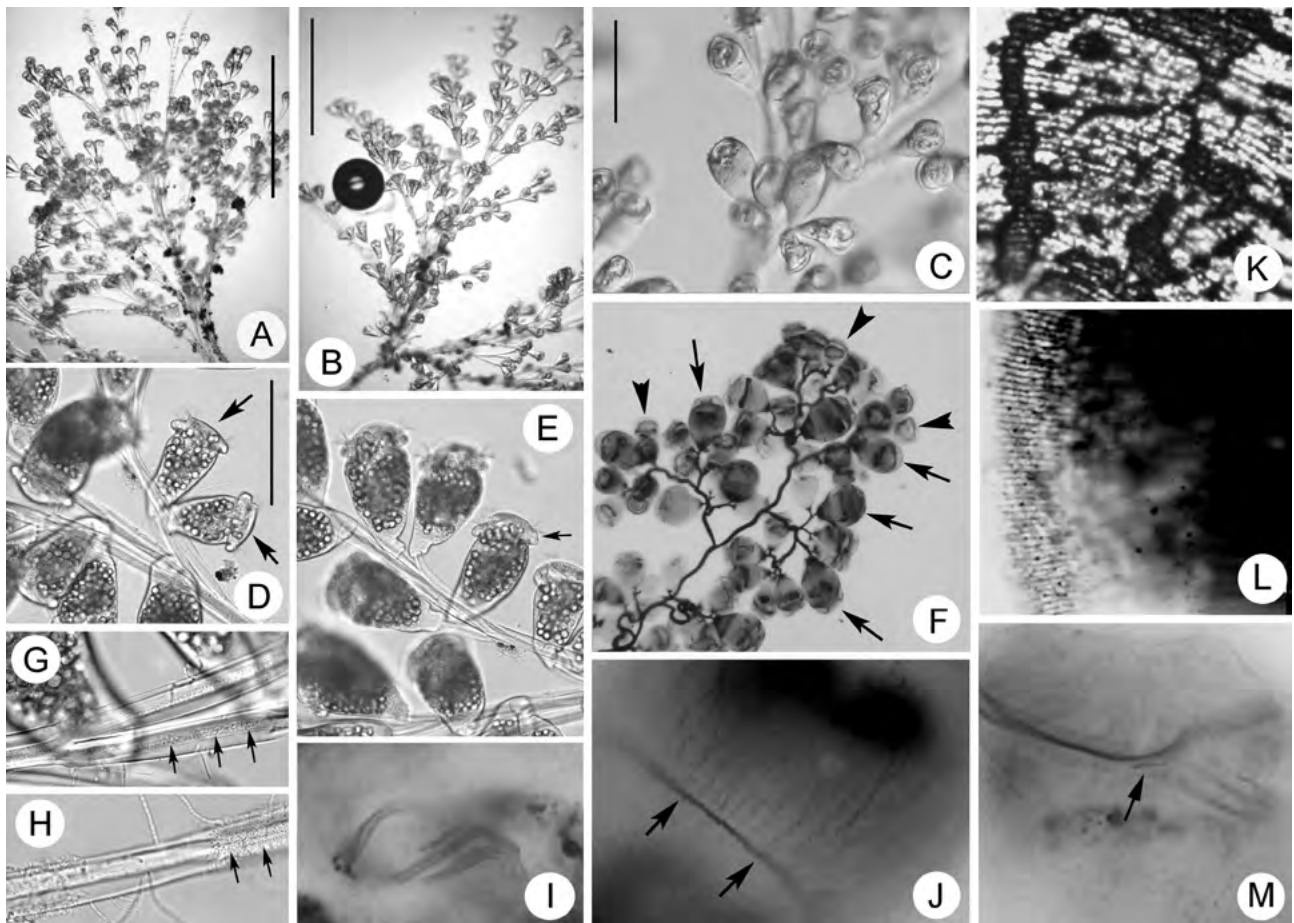


Fig. 2. Photomicrographs of *Zootheramnium alrasheidi* from life (A–E, G, H), after protargol (F, I, J, M) and “dry” silver nitrate (K, L) impregnations. **A, B** – colonies at low magnification; **C, D** – to show variability of zooid size, arrow marks small (immature) zooids just after division; **E** – to show thick peristomial lip of mature zooid (arrow); **F** – contrast between immature (arrowheads) and mature (arrows) zooids; **G** – to show mitochondria in spasmoneme (arrows); **H** – to show bacteria on stalk surface (arrows); **I** – oral infraciliature; **J** – lateral view, arrows mark the aboral ciliary wreath; **K, L** – silverline system; **M** – to show the epistomial membrane (arrow). Scale bars: 400 μm (A, B), 150 μm (C), 100 μm (D).

type, “dry” silver nitrate preparation (No. 0205220303). A second paratype slide is deposited in the Natural History Museum, London, U.K., with registration number 2009:3:31:1.

Type locality: Shrimp-farming pond near Qingdao, China (36°28'N, 120°64'E); water temperature 20–25°C, salinity 25–35‰.

Etymology: This species is named in honour of Prof. Dr. Khaled A. S. Al-Rasheid, Zoology Department, King Saud University, Saudi Arabia, in recognition of his contributions to the taxonomy of ciliates.

Description: Colony planar, leaf-shaped, up to 2 mm high. Secondary stalks branching off primary stalk

(main trunk) alternately in small colonies (< 50 zooids), and this continues in third and fourth level stalks of large colonies (up to 300 zooids). Branching pattern irregular in some large colonies with two adjacent secondary branches located on same side of main trunk (Figs 1B, C, 2A, B). This is caused by partial replacement of main trunk by a highly developed accessory branch from its basal ramification. Consequently the original continuation of main trunk becomes a secondary branch, which is located on same side of colony as last secondary branch, thus disturbing branching order.

Zooids bell-shaped, differentiated into two types: mature and immature (Figs 2C, F). Mature zooids

somewhat asymmetric, 80–120 × 50–60 µm in size, widest at mid-body, with wide (8–12 µm), double-layered peristomial lip and moderately elevated peristomial disc (Figs 1A, 2E); immature (newly divided) zooids uniformly subconical, 60–70 × 30–40 µm in size, with narrow, single-layered peristomial lip (Figs 1D, 2D). Pellicle finely striated, striations only visible at magnifications of ×400 or greater. Proportion of two types of zooids depends on trophic condition of water, colonies in eutrophic water having a higher proportion (> 90%) of mature zooids, whereas in oligotrophic water this proportion is lower (ca. 60%).

Macronucleus C-shaped, transversely oriented, surrounding aboral end of infundibulum (Figs 1A, D). Single contractile vacuole apically located near dorsal wall of infundibulum. Cytoplasm colorless and transparent but usually packed with yellowish or grey food granules, 8–10 µm in diameter, rendering zooid dark at low magnifications (Figs 1A, D, 2D, E).

Stalk surface smooth, sometimes with dense accumulations of attached bacteria (Fig. 2H, arrows). Primary stalk 20 µm in diameter, secondary to quaternary stalks 15 µm in diameter, distal stalks 10 µm in diameter. Myoneme system comprising stalk spasmoneme and connecting somatic myonemes. Spasmoneme 9 µm wide in primary stalk, 4 µm in accessory and distal stalks, and surrounded by a helical band of tiny (0.5–0.8 µm) thecoplastic granules (mitochondria) (Fig. 2G, arrows). Somatic myonemes extending from scopula to oral area (Fig. 2J) where they link to each other forming a transverse strand just beneath peristomial lip.

Oral structure as shown in Figs 1E–H, 2I, M. Haplokinety (H) and polykinety (Po) making one and one-third turns together around peristome before entering infundibulum, where they make a further turn on opposite walls. Epistomial membrane (EM) located near opening of infundibulum as commonly seen in other peritrichs (Figs 1E, 2M, arrow). Germinal kinety (G) running parallel to H in ab stomal half of infundibulum. Infundibular part of Po, namely infundibular polykinety 1 (P1), consisting of three rows of kinetosomes and accompanied by two additional infundibular polykineties (P2, P3) (Figs 1E, 2I). Rows of P1 approximately equal in length, terminating at infundibulum-cytostome boundary. Row 3 of P2 separated from the other two rows at ab stomal end; all three rows of P2 extending ab stomally and terminating at curvature of P1. P3 consisting of three ciliary rows, one inner (row 1) and two outer (rows 2 and 3) that lie in close proximity to one

another, arranged in one of three configurations: 1) usually outer rows continuous and terminate below inner row at ab stomal end (Fig. 1F); 2) sometimes inner row discontinuous with a small gap in ab stomal region (Fig. 1G, arrow); 3) occasionally all three rows about equal length (Fig. 1H).

Aboral trochal band composed of fine, zigzag band of kinetosomes encircling body at 3/4 distance from peristome to scopula (Fig. 2J, arrows).

Silverline system consisting of parallel, transverse rows (Figs 1I, 2K, L), 50–75 between peristomial area and aboral trochal band, 24–42 from aboral trochal band to scopula, with sparsely distributed pellicular pores.

Remarks: *Zoothamnium alrasheidi* is characterized by its large, planar, leaf-shaped colony and the differentiation of zooids into two types: mature and immature. Thus it can be readily distinguished from its congeners by characters visible in the living organism, even at low magnifications.

In terms of its colony shape, *Z. alrasheidi* closely resembles *Z. alternans* Claparède and Lachmann, 1859, *Z. plumula* Kahl, 1933, and *Z. wangi* Ji *et al.*, 2005. *Zoothamnium alrasheidi*, however, can be recognized by its thick double-layered (vs. thin, single-layered) peristomial lip and larger body size (80–120 µm vs. < 80 µm long; Table 2) in the mature zooids (Ji *et al.* 2005c, 2006a; Song *et al.* 2002).

Zoothamnium alrasheidi has an irregular alternately branched stalk and an inconspicuously double-layered peristomial lip, and thus somewhat resembles *Z. maximum* Song, 1986, *Z. duplicatum* Kahl, 1933, *Z. mucedo* Entz, 1886 and *Z. foissneri* Ji *et al.*, 2005. However, the planar leaf-shaped colony of the new species differs distinctly from 3-dimensional umbellate or dendriform colonies of the latter congeners. Furthermore, P3 of *Z. alrasheidi* appears to comprise only two rows due to rows 2 and 3 being very closely spaced (vs. P3 distinctly three-rowed with rows 2 and 3 distinctly separated) (Ji and Song 2004, Ji *et al.* 2005a; Table 2).

Small colonies of *Z. alrasheidi* can easily be confused with *Z. nii* Ji *et al.*, 2005, which also has a leaf-like colony with alternately arranged branches and zooids with a double-layered peristomial lip. However, the former differs from *Z. nii* in the size of the mature zooid (80–120 µm vs. 65–85 µm long) and the appearance of ciliary rows 2 and 3 in P3 (closely spaced vs. distinctly separated). In addition, the zooids of *Z. alrasheidi* are differentiated into two types whereas *Z. nii* has only one zooid type (Ji *et al.* 2005c).

Table 2. Morphological comparison between the species of *Zoothamnium* (*Z. alrasheidii*, *Z. marinum* and *Z. vermicola*) and similar congeners. Measurements in μm .

Species	Body length <i>in vivo</i>	Body width <i>in vivo</i>	No. silverlines from aboral trochal band to peristome	No. silverlines from aboral trochal band to scopula	Appearance of peristomial lip	Branching pattern	Zooid differen- tiation	No. ciliary rows in P3	Data source
<i>Z. alrasheidii</i>	80–120	50–60	50–75	24–42	inconspicuous double layer	irregular	present	3	present study
<i>Z. alternans</i>	40–56	26–32	40–55	20–30	single layer	alternate	present	3	Ji <i>et al.</i> (2006a)
<i>Z. commune</i>	60–104	48–56	59–70	38–43	single layer	alternate	absent	3	Ji <i>et al.</i> (2006a)
<i>Z. cupiferum</i>	82–105	44–63	54–67	20–28	single layer	dichotomous	absent	2	Song (1986)
<i>Z. duplicatum</i>	60–85	35–45	48–53	22–27	double layer	dichotomous	absent	3	Ji <i>et al.</i> (2005a)
<i>Z. hiketes</i>	60–80	30–40	89–109	35–43	inconspicuous double layer	dichotomous	absent	3	Sun <i>et al.</i> (2005a)
<i>Z. intermedium</i>	35–52	27–37	–	–	single layer	dichotomous	absent	–	Song (1991)
<i>Z. marinum</i>	96–108	52–58	60–75	30–40	single layer	dichotomous	absent	3	present study
<i>Z. maximum</i>	81–121	47–64	82–95	45–58	double layer	dichotomous	absent	3	Ji and Song (2004)
<i>Z. mucedo</i>	50–90	30–48	81–94	35–45	double layer	dichotomous	absent	3	Ji <i>et al.</i> (2005a)
<i>Z. nii</i>	70–80	40–50	47–58	22–30	double layer	alternate	absent	3	Ji <i>et al.</i> (2005c)
<i>Z. paraentzii</i>	50–80	25–45	75–83	28–33	single layer	dichotomous	absent	3	Sun <i>et al.</i> (2005b)
<i>Z. paragammani</i>	36–60	21–35	57±	24–28	single layer	dichotomous	absent	2	Song (1991)
<i>Z. penaei</i>	54–95	38–62	115–130*	–	single layer	dichotomous	present	3	Song (1992)
<i>Z. perejaslawzewae</i>	67–74	40–47	–	–	single layer	–	absent	–	Song (1991)
<i>Z. plumula</i>	50–60	30	24–28	70	single layer	alternate	present	3	Song <i>et al.</i> (2002)
<i>Z. vermicola</i>	65–95	40–50	50–68	32–53	single layer	dichotomous	absent	3	present study
<i>Z. wangi</i>	65–90	45–55	70–85	38–50	single layer	alternate	absent	2	Ji <i>et al.</i> (2005c)

* Total number of silverlines from scopula to peristome.

***Zoothmanium marinum* Kahl, 1933 (Figs 3, 4; Table 1)**

Zoothmanium marinum was first reported by Kahl (1933) with only a superficial description. Since no information of the infraciliature and the silverline system is available for this species, we supply an improved diagnosis and a detailed description of its infraciliature, silverline system, and morphology *in vivo*, based on a Chinese population.

Improved diagnosis: Colony three-dimensional, umbellate in outline, up to 1 mm in height, stalk dichotomously branched. Zooids elongate bell-shaped, about $95\text{--}110 \times 50\text{--}60 \mu\text{m}$ in size, with single-layered peristomial lip. Macronucleus C-shaped, transversely oriented. Single contractile vacuole apically located near dorsal wall of infundibulum. Sixty to 75 transverse silverlines between peristomial area and aboral trochal band, about 30–40 between aboral trochal band and scopula. P3 consisting of three ciliary rows, ab stomal half of row 3 with kinetosomes irregularly aligned, separated from row 1 and converging with ab stomal end of row 2 in its mid-region. Marine habitat.

Deposition of voucher slides: Two slides (Nos. 0208250101, 0208250102) with protargol and “dry” silver nitrate impregnated specimens, respectively, are deposited at the Laboratory of Protozoology, OUC, China.

Ecological features: Water temperature 30°C , salinity 28‰.

Redescription: Colony usually containing over 50 zooids, three-dimensional and umbellate in outline, up to 1 mm high and 1 mm in diameter (Figs 3E, 4A). Stalk dichotomously branched, primary stalk $16\text{--}20 \mu\text{m}$ in diameter, accessory branches $14 \mu\text{m}$ in diameter, distal branches $9 \mu\text{m}$ in diameter. Stalk surface smooth with many attached bacteria (Fig. 4D). Spasmoneme in primary stalk $10 \mu\text{m}$ in diameter, $4 \mu\text{m}$ in distal branches, surrounded by a helical band of thecoplasmic granules (mitochondria) (Fig. 4D).

Zooids elongate bell-shaped (subconical), about $95\text{--}110 \times 50\text{--}60 \mu\text{m}$ in size (Figs 3A, D). Body deeply constricted below the single-layered peristomial lip; maximum width of cell usually at peristomial lip and in oral third of body; peristomial disc moderately elevated above peristomial lip (Figs 3A, 4C). Pellicular striations easily detectable above $\times 400$ magnification, but pellicle appears completely smooth when observed at lower magnifications.

Cytoplasm colourless or slightly gray, some well-fed zooids packed with many gray or yellowish food

granules $8\text{--}10 \mu\text{m}$ in diameter (Figs 3A, 4B, C). Single contractile vacuole apically located near dorsal wall of infundibulum. Macronucleus C-shaped, transversely oriented, surrounding micronucleus and lower half of infundibulum (Fig. 3A).

Oral infraciliature as shown in Figs 3B, C, F, 4E–G. Haplo- and polykinety circling one and one-third turns around peristome, and making a further turn after entering infundibulum. Haplokinety parallel to polykinety before entering infundibulum, but located on opposite wall within infundibulum (Fig. 3F). Epistomial membrane short, located at opening of oral cavity (arrows in Figs 3F, 4E). Germinal kinety parallel to haplokinety within upper half of infundibulum (Figs 3F, 4G). Infundibular part of polykinety (P1) accompanied by two other infundibular polykineties, i.e., P2 and P3. P1 and P2 much longer than P3. P2 terminating ad stomally with P3 at curvature of P1. Ab stomal end of row 3 in P2 conspicuously separated from the other two rows as commonly seen in other congeners (Figs 3B, F). Row 2 in P3 about half length of rows 1 and 3, close set and parallel to ad stomal half of row 3. In some zooids row 2 is located very close to row 3 to form a zigzag arrangement of kinetosomes (Fig. 3C). Row 1 in P3 parallel to P2, widely separated from rows 2 and 3 in ab stomal half (Figs 3B, C, 4F). Ab stomal half of row 3 in P3 composed of an irregularly aligned series of kinetosomes, re-converging with row 1 at its ab stomal end (Figs 3B, C).

Aboral trochal band composed of double row of kinetosomes that encircles cell at $2/3$ distance from peristome to scopula (Fig. 4H, arrows).

Silverline system consisting of parallel, transverse rows, 60–75 between peristomial area and aboral trochal band, 30–40 between aboral trochal band and scopula (Fig. 4I).

Remarks: *Zoothamnium marinum* is characterized mainly by its regular dichotomously branched stalk, single-layered peristomial lip, large zooid size and marine habitat, all of which correspond well with the descriptions by Kahl (1933, 1935).

Zoothamnium marinum closely resembles *Z. hiketes* Precht, 1935 in terms of zooid shape and size and the branching style of the stalk. They differ mainly in the number of silverlines between the aboral trochal band and peristome (60–75 in *Z. marinum* vs. 89–109 in *Z. hiketes*) and the arrangement of P3 which comprises three uniformly parallel ciliary rows in *Z. hiketes*, whereas in *Z. marinum* there is a distinct gap between row 1 and rows 2 and 3 (Figs 3B, C) (Sun *et al.* 2005b).

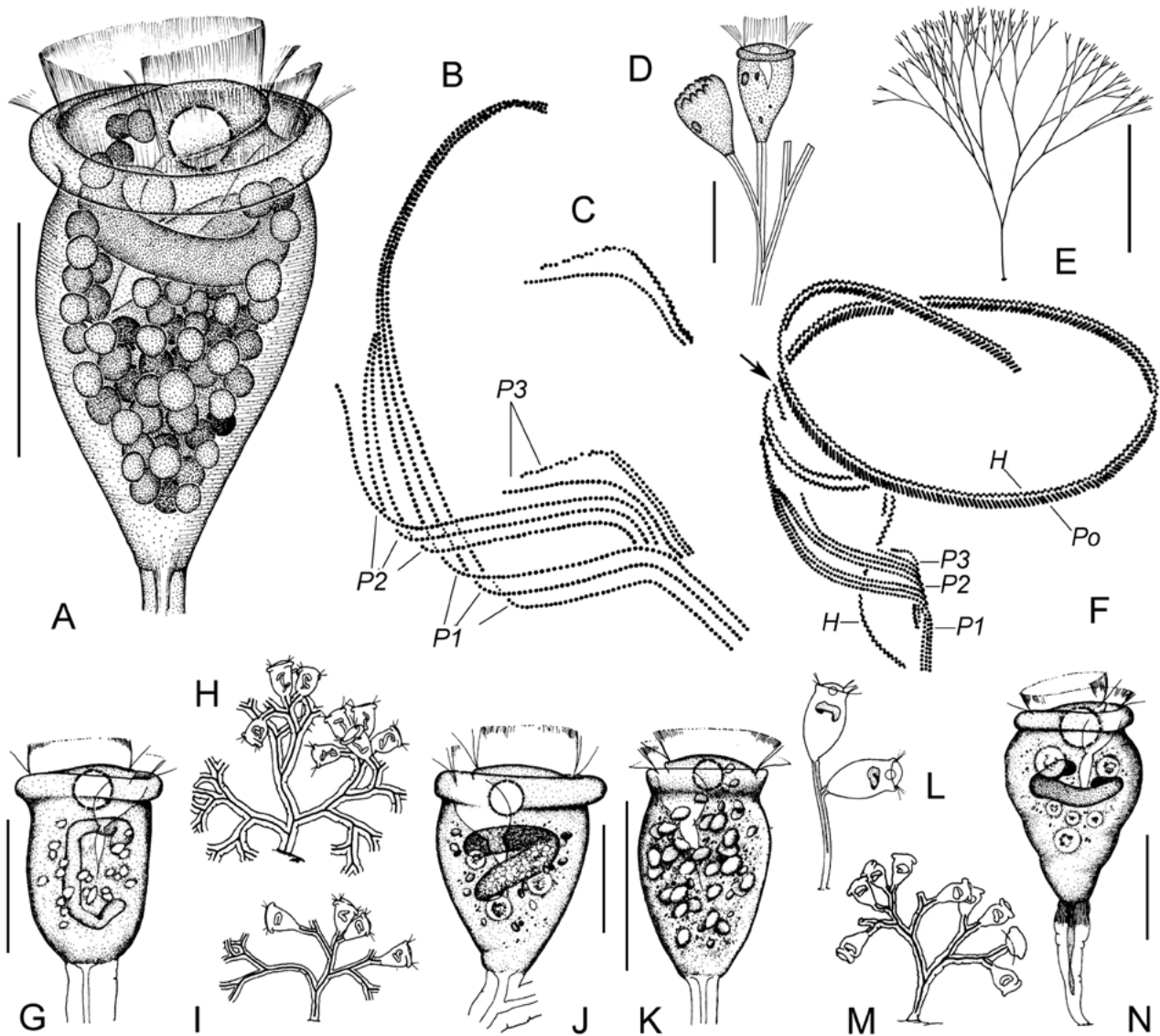


Fig. 3. Morphology of *Zoothamnium marinum* (A–F) and similar congeners (G–N, from Song 1991). **A** – a representative zooid; **B** – detailed arrangement of infundibular polykineties; **C** – variations of infundibular polykinety 3; **D** – after Kahl (1933); **E** – branching style of colony; **F** – oral infraciliature, arrow marks the epistomial membrane; **G**, **H** – *Zoothamnium cupiferum* Song, 1986; **I**, **J** – *Zoothamnium intermedium* Precht, 1935; **K**, **L** – *Zoothamnium perejaslawzewae* sensu Song, 1991; **M**, **N** – *Zoothamnium paragammari* Song, 1991. H – haplokinety, P1–3 – infundibular polykinety 1–3, Po – polykinety. Scale bars: 20 μm (J, N), 50 μm (A, G, K), 100 μm (D), 400 μm (E).

Four other marine congeners with a single-layered peristomial lip and similar stalk branching pattern should also be compared with *Z. marinum*, namely *Z. cupiferum* Song, 1986, *Z. intermedium* Precht, 1935, *Z. paragammari* Song, 1991 and *Z. perejaslawzewae* sensu Song, 1991.

Zoothamnium cupiferum can be separated from *Z. marinum* by its cylindroid (vs. bell-shaped) zooid

and thicker (> 20 μm in diameter vs. 10 μm) distal stalk branches (Song 1986). *Zoothamnium intermedium*, *Z. paragammari* and *Z. perejaslawzewae* have significantly smaller zooids (< 75 μm vs. 95–110 μm in *Z. marinum*). Furthermore, in *Z. paragammari* and *Z. perejaslawzewae* the peristomial lip is narrower than the maximum body width (vs. peristomial lip wider than maximum body width in *Z. marinum*) while the zooid

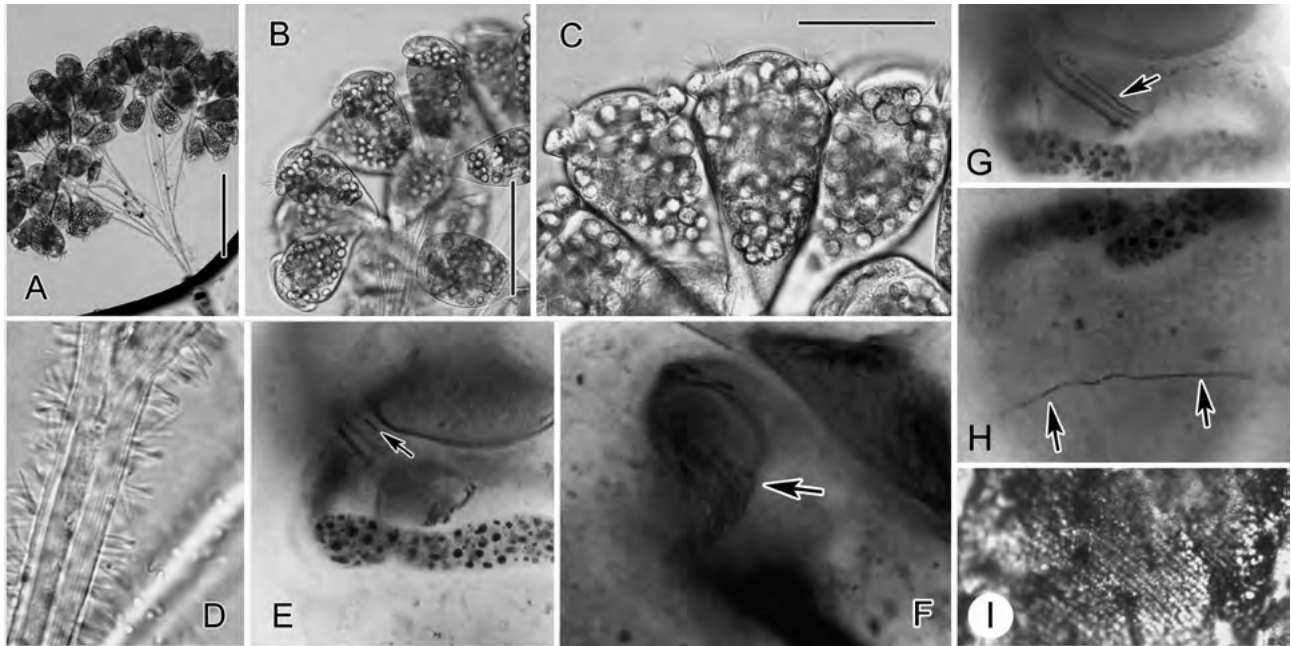


Fig. 4. Photomicrographs of *Zoothamnium marinum* from life (A–D), after protargol (E–H) and “dry” silver nitrate (I) impregnation. A – colony outline; B – zooids at low magnification; C – zooids at $\times 200$ magnification; D – stalk and spasmoneme; E – to show the epistomial membrane (arrow); F – to show infundibular polykineties, arrow marks row 3 of polykinety 3 separated from rows 2 and 3 in the mid-region; G – to show the germinal kinety (arrow); H – to show the aboral trochal band (arrows); I – silverline system. Scale bars: 200 μm (A), 100 μm (B), 50 μm (C).

of *Z. intermedium* is significantly less elongate than that of *Z. marinum* (ratio of length to width ca. 1.3:1 vs. ca. 2:1 in *Z. marinum*) (Song 1991).

In terms of their patterns of branching and the shapes of their colonies, *Z. duplicatum* Kahl, 1933, *Z. maximum* Song, 1986, *Z. mucedo* Entz, 1884 and *Z. rigidum* Precht, 1935 also resemble *Z. marinum*. However, the zooids of these four species have a distinctly double-layered (vs. single-layered) peristomial lip and thus can be easily separated from the latter (Ji *et al.* 2005a, Precht 1935, Song 1991; Table 2).

***Zoothamnium vermicola* Precht, 1935 (Figs 5, 6; Table 1)**

No investigation based on silver impregnated specimens of *Z. vermicola* has previously been carried out; therefore, an improved diagnosis and detailed redescription are supplied here based on the Chinese population.

Improved diagnosis: Primary stalk dichotomously branched but colony asymmetrical owing to differing lengths of secondary stalks; first ramification very close to attachment point of primary stalk. Zooids elongate,

65–95 \times 40–50 μm , with thick, single-layered peristomial lip. Single contractile vacuole apically located near dorsal wall of infundibulum. Macronucleus C-shaped, transversely to obliquely oriented. Pellicle finely striated, 50–68 pellicular striations between aboral trochal band and peristomial lip, about 32–53 between aboral trochal band and scopula. P3 consists of three parallel, closely arranged ciliary rows terminating adstomally above P1. Marine habitat.

Deposition of voucher slides: Two slides (Nos. 0304180101, 0304180102) with protargol and silver nitrate impregnated specimens respectively are deposited at the Laboratory of Protozoology, OUC, China.

Ecological features: Water temperature 15°C, salinity 25‰.

Redescription: Stalk dichotomously branched in asymmetric fashion, first ramification very close to attachment point of primary stalk (Figs 5F, 6A). Zooids of one side growing and dividing faster than the other side, thus forming a colony shaped like a scalene triangle (5F, 6A). Stalk surface smooth, sometimes wrinkled (Fig. 6G). Primary stalk 12–14 μm in diameter, distal

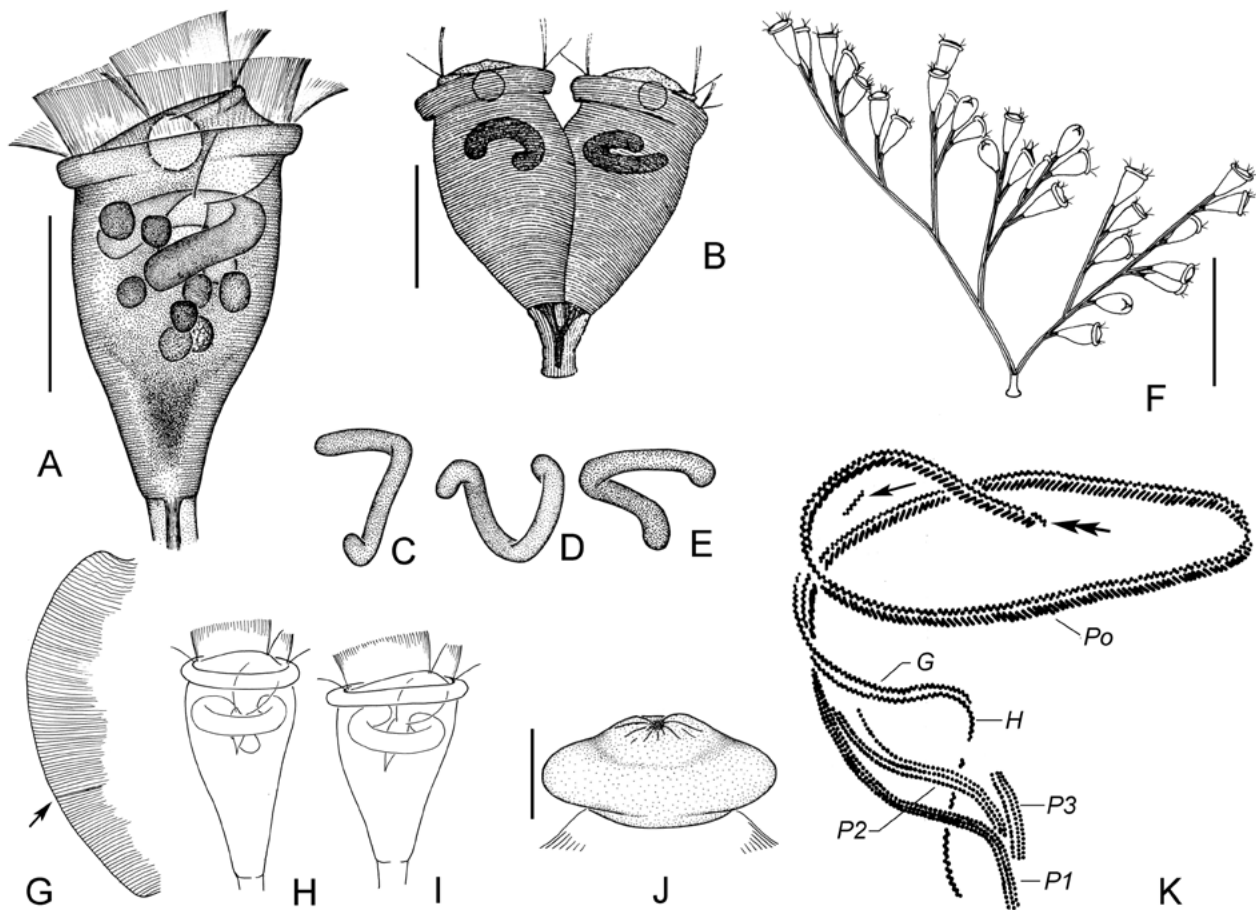


Fig. 5. Morphology of *Zoothamnium vermicola* from life (A–F, H–J), after protargol (K) and silver nitrate impregnation (G). A – general view of a typical zooid; B – from Precht (1935); C–E – shape varieties of macronucleus; F – colony form; G – silverline system, arrow marks the aboral trochal band; H, I – shape variability of zooids; J – telotroch; K – oral infraciliature, arrow marks the epistomial membrane, double-arrow indicates the distal fragment. G – germinal kinety, H – haplokinety, P1–3 – infundibular polykinety 1–3, Po – polykinety. Scale bars: 20 μm (J), 40 μm (A, B), 200 μm (F).

branches 10 μm in diameter. Spasmoneme 4 μm in diameter with black, sparsely distributed thecoplasmic granules (mitochondria) 0.5 μm across (Fig. 6G).

Zooids elongate bell-shaped (subconical), 65–95 \times 40–50 μm *in vivo*, without differentiation of macro- and macrozooids (Figs 5A, H, I, 6C). Body widest at single-layered peristomial lip which is 6 μm wide (Figs 5A, 6C). Peristomial disc flat, elevated obliquely above peristomial lip. Pellicle appears smooth at low magnifications but very fine, densely arranged (0.8 μm intervals) striations visible at $\times 1000$ magnification (Fig. 6J). Zooids insensitive to stimulation, usually contracting separately rather than as a whole colony. Telotroch discoid, 25 μm thick and 60 μm in diameter (Figs 5J, 6D, E).

Cytoplasm opaque, grayish brown in colour. Cell containing a mass of yellowish brown cytoplasm in aboral third of body and several gray or yellow food granules (4–7 μm in diameter) in mid-body region (Figs 5A, 6B, C). Single contractile vacuole apically located near dorsal wall of infundibulum. Macronucleus usually C-shaped and transversely oriented, surrounding the micronucleus and lower half of infundibulum (Figs 5A, B, 6I). Occasionally macronucleus is either obliquely oriented or irregular in shape (Figs 5C–E, 6F).

Peristomial part of oral infraciliature consisting of haplo- and polykinety, which are parallel to one another throughout their length and make one and one-quarter turns of peristome before entering infundibulum (Figs

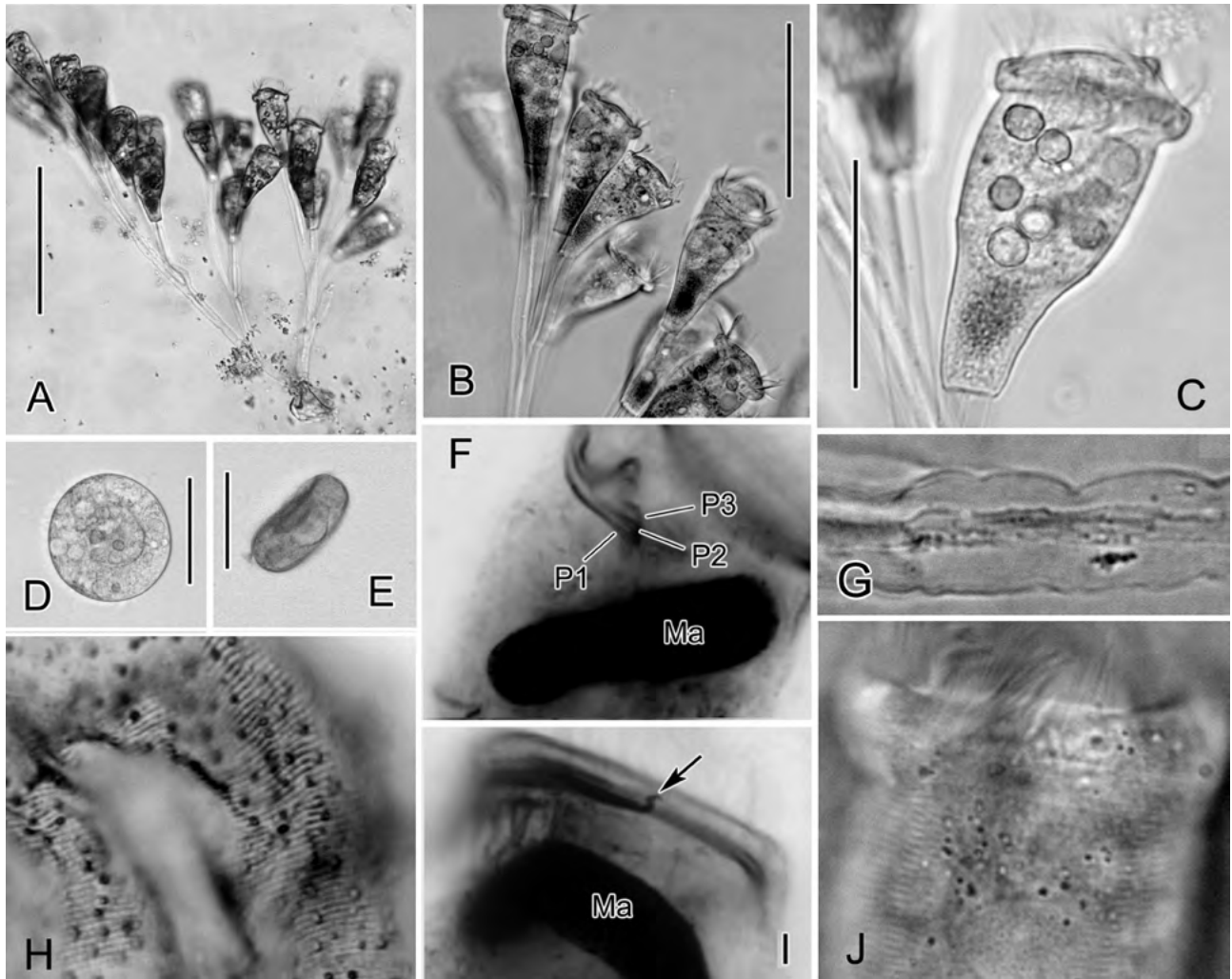


Fig. 6. Photomicrographs of *Zoothamnium vermicola* from life (A–E, G, J), after protargol (F, I) and silver nitrate impregnation (H). A – to show colony outline; B – zooids at low magnification; C – a typical zooid at high magnification; D, E – telotroch; F, I – lateral view, to show the arrangement of infundibular polykineties (P1–3), macronucleus (Ma) and the distal fragment (arrow); G – stalk structure; H – silverline system; J – to show pellicle striations. Scale bars: 200 µm (A), 100 µm (B), 50 µm (C–E).

5K, 6F, I). Short kinety fragment present at peristomial end of haplokinety (Figs 5K, double-arrowhead, 6I, arrow). Epistomial membrane located near opening of infundibulum as commonly seen in other congeners (Fig. 5K, arrow) Within infundibulum haplokinety and polykinety spiral on opposite walls, terminating at border of cytostome. Infundibular part of haplokinety accompanied by germinal kinety in upper half (Fig. 5K). Infundibular polykinety 1 (P1) accompanied by two additional infundibular polykineties (P2, P3) (Figs 5K, 6F). P1 consists of three parallel rows of kinetosomes that extend to cytostome. Three rows of P2 originate

in mid-infundibulum, with row 3 separated from the other two at this point (Fig. 5K); P2 terminating with P3 at curvature of P1. P3 consists of three parallel ciliary rows which are about equal in length, rows 2 and 3 being closer to each other than row 1 (Fig. 5K).

Silverline system consisting of parallel transverse rows, 50–68 between peristomial area and aboral trochal band, 32–53 between aboral trochal band and scopula, and with numerous randomly distributed pellicular pores (Fig. 6H). Aboral trochal band stains heavily with silver, probably composed of two or three rows of closely spaced silverline fragments (Fig. 5G).

Remarks: The original description of *Z. vermicola* was based on an immature colony with only two zooids so comparison with our isolate is difficult (Precht 1935). However, both populations share similar zooid morphologies and both have a very short stalk length between the point of attachment of the primary stalk and the first ramification, a feature that is seldom seen in other congeners. On this basis we identify our isolate as *Z. vermicola*.

In terms of the branching pattern of its stalk, *Z. vermicola* resembles *Z. paraentzii* Song, 1991 and *Z. penaei* Song, 1992. However, *Z. paraentzii* has a slender (vs. bell-shaped) body shape and fewer silverlines between the scopula and the aboral trochal band (28–33 vs. 32–53) while *Z. penaei* has two types of zooid (vs. one type) that are ovoid (vs. bell-shaped). Thus, both of these species can be readily distinguished from *Z. vermicola* (Song 1991, 1992; Table 2).

Zoothamnium hiketes and *Z. marinum* Kahl, 1933 also resemble *Z. vermicola* in terms of their body shape, but they can be easily separated from the latter by their umbellate (vs. scalene triangle-shaped) colonies.

Acknowledgements. This work was supported by the Natural Science Foundation of China (project number: 30700066, 30870264) and the 111 Project (No. B08049). The authors would like to thank Ms. Xiaofeng Lin, South China Normal University, for her help with sampling.

REFERENCES

- Clamp J. C. (1992) Three new species of lagenophryid peritrichs (Ciliophora) ectocommensal on freshwater decapod crustaceans from Madagascar. *J. Protozool.* **39**: 732–740
- Clamp J. C. (1997) Redescription of *Ellobiophrya brevipes* (Laird, 1959) n. comb. (Ciliophora, Peritrichia) and the fine structure of its pellicle and cinctum. *J. Eukaryot. Microbiol.* **44**: 374–382
- Foissner W. (1976) Erfahrungen mit einer trockenen Silberimprägnationsmethode zur Darstellung argyrophiler Strukturen bei Protisten. *Verh. Zool.-bot. Ges. Wien* **115**: 68–79
- Foissner W., Berger H., Kohmann F. (1992) Taxonomische und ökologische Revision der Ciliaten des Saprobien-systems – Band II: Peritrichida, Heterotrichida, Odontostomtida. Informationsberichte der Bayer. Land. Wass. 5, 1–502
- Foissner W., Schiffmann H. (1974) Vergleichende Studien an argyrophilen Strukturen von vierzehn peritrichen Ciliaten. *Protistologica* **10**: 489–508
- Ji D., Song W. (2004) Notes on a new marine peritrichous ciliate (Ciliophora: Peritrichida), *Zoothamnopsis sinica* n. sp. from north China, with reconsideration of *Zoothamnium maximum* Song, 1986. *Acta Protozool.* **43**: 61–71
- Ji D., Song W., Al-Rasheid K. A. S., Sun P. (2005a) Description of three marine peritrichous ciliates, *Zoothamnium foissneri* n. sp., *Z. duplicatum* Kahl, 1933 and *Z. mucedo* Entz, 1884 (Ciliophora: Peritrichia). *Eur. J. Protistol.* **41**: 45–56
- Ji D., Song W., Al-Rasheid K. A. S., Li L. (2005b) Taxonomic characterization of two new marine peritrichous ciliates, *Pseudovorticella clampi* n. sp. and *Zoothamnium parabuscula* n. sp. (Protozoa: Ciliophora: Peritrichida) from north China. *J. Eukaryot. Microbiol.* **52**: 159–169
- Ji D., Song W., Warren A. (2005c) Two new marine sessile peritrichous ciliates (Protozoa, Ciliophora). *Acta Zootax. Sin.* **30**: 702–705
- Ji D., Song W., Warren A. (2006a) Redescriptions of three marine peritrichous ciliates, *Zoothamnium alternans* Claparède et Lachmann, 1859, *Z. sinense* Song, 1991 and *Z. commune* Kahl, 1933 (Ciliophora: Peritrichia), from north China. *Acta Protozool.* **45**: 27–39
- Ji D., Song W., Clamp J. (2006b) *Pseudovorticella zhengae* n. sp., *P. difficilis* (Kahl, 1933) Jankowski, 1976, and *P. punctata* (Dons, 1918) Warren, 1987, three marine peritrichous ciliates from north China. *Eur. J. Protistol.* **42**: 269–279
- Kahl A. (1933) Ciliata libera et ectocommensalia. In: Die Tierwelt der Nordund Ostsee, Lief. 23 (Teil, II, c₃), (Eds. G. Grimpe & E. Wagler), Leipzig, 147–183
- Kahl A. (1935) Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 4. Peritricha und Chonotricha. *Tierwelt Dtl.* **30**: 651–886
- Kent W. S. (1880–1882) A Manual of the Infusoria: including a description of all known flagellate, ciliate, and tentaculiferous protozoa British and foreign, and an account of the organization and affinities of the sponges. David Bogue, London. (Vol. II 1881: 473–913; Vol. III 1882: Plates)
- Li L., Song W., Warren A., Shin M. K., Chen Z., Ji D., Sun P. (2008) Reconsideration of the phylogenetic positions of five peritrich genera – *Vorticella*, *Pseudovorticella*, *Zoothamnopsis*, *Zoothamnium* and *Epicarchesium* (Ciliophora, Peritrichia; Sessilida), based on small subunit rRNA gene sequences. *J. Eukaryot. Microbiol.* **55**: 448–456
- Peng X., Shi X., Warren A. (2008) Establishment of a new peritrich ciliate genus, *Pseudepistylis* n. gen. (Ciliophora: Peritrichia: Epistylididae), with a description of a new freshwater species, *Pseudepistylis songi* n. sp. from Wenzhou, China. *Zootaxa* **1524**: 35–45
- Precht H. (1935) Epizoen der Kieler Bucht. *Nova Acta Leopold.* **3**: 405–474
- Sommer G. (1951) Die peritrichen Ciliaten des großen Plöner Sees. *Arch. Hydrobiol.* **44**: 349–440
- Song W. (1986) Descriptions of seven new species of peritrichs on *Penaeus orientalis* (Peritricha: Zoothamniidae, Epistylididae). *Acta Zootax. Sin.* **11**: 225–235 (in Chinese with English summary)
- Song W. (1991) Contribution to the commensal ciliates on *Penaeus orientalis*. II. (Ciliophora, Peritrichida). *J. Ocean Univ. Qingdao* **21**: 45–55 (in Chinese with English summary)
- Song W. (1992) A new marine ciliate, *Zoothamnium penaei* sp. nov. (Ciliophora, Peritrichida). *Oceanol. Limnol. Sin.*, **23**: 90–94 (in Chinese with English summary)
- Song W., Al-Rasheid K. A. S., Hu X. (2002) Notes on the poorly-known marine peritrichous ciliate, *Zoothamnium plumula* Kahl, 1933 (Protozoa: Ciliophora), an ectocommensal organism from cultured scallops in Qingdao, China. *Acta Protozool.* **41**: 163–168
- Song W., Wilbert N. (1995) Benthische Ciliaten des Süßwassers. In: Praktikum der Protozoologie, (Ed. R. Röttger). Gustav Fischer Verlag, New York, 156–168

- Stiller J. (1953a) Epizoische Peritrichen aus dem Balaton III. *Hydrobiologia*, **5**: 189–221
- Stiller J. (1953b) Die Protozoen des Pécsely-Baches in Ungarn. *Ann. Hist.-Nat. Mus. Nat. Hung.* **44**: 47–70
- Stiller J. (1971) Szájkoszorús Csillósok-Peritricha. *Fauna Hung.* **105**: 1–245
- Stiller J., Stevčić Z. (1967) Epibionten der Meeresspinne *Maja squinado* (Herbst) Nebst Beschreibung Drei Neuer Entokommensaler Peritrichen Arten. *Thal. Jugosl.* **3**: 161–172
- Sun P., Ji D., Song W. (2005a) Notes on a new marine peritrichous ciliate (Ciliophora: Peritrichida), *Zoothamnium xuianum* n. sp., with redescription of *Z. paraentzii* Song, 1991 from northern China. *Zootaxa* **1075**: 41–53
- Sun P., Song W., Ji D., Hu X. (2005b) Redescription of *Pseudovorticella cylindrica* (Dons, 1915) nov. comb. and *Zoothamnium hiketes* Precht, 1935, two marine peritrichs (Ciliophora: Peritrichida) from north China Sea. *J. Nat. Hist.* **39**: 2953–2965
- Sun P., Song W., Xu D. (2007) Two new marine species of *Pseudovorticella* (Ciliophora, Peritrichia) from Qingdao, north China. *Acta Protozool.* **46**: 55–64
- Warren A. (1986) A revision of the genus *Vorticella* (Ciliophora: Peritrichida). *Bull. Br. Mus. nat. Hist. (Zool.)* **50**: 1–57
- Wilbert N. (1975) Eine verbesserte Technik der Protargolimprägation für Ciliaten. *Mikrokosmos* **64**: 171–179

Received on 9th February, 2009; revised version on 8th April, 2009; accepted on 9th April, 2009

