

Notes on a New Marine Peritrichous Ciliate (Ciliophora: Peritrichida), *Zoothamnopsis sinica* sp. n. from North China, with Reconsideration of *Zoothamnium maximum* Song, 1986

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Summary. The morphology, infraciliature and silverline system of a new marine peritrichous ciliate, *Zoothamnopsis sinica* sp. n., isolated from an abalone-farming pond off the coast of Qingdao, China, are investigated. *Z. sinica* is characterized by: colony dichotomously branched, zooids elongated and vase-shaped measuring $70-105 \times 40-55 \mu\text{m}$ *in vivo*, double-layered peristomial lip; contractile vacuole located apically; number of transverse silverlines from peristomial area to aboral ciliary wreath *ca* 82-98, from aboral ciliary wreath to scopula, 48-55. Based on a reexamination on the slides deposited in the Laboratory of Protozoology, Ocean University of China, and on the original records, an updated and supplementary description on the infraciliature and morphology of *Zoothamnium maximum* Song, 1986 is supplied.

Key words: Marine Ciliophora, Peritrichida, *Zoothamnium maximum*, *Zoothamnopsis sinica* sp. n.

INTRODUCTION

Colonial peritrichous ciliates are common and dominant in marine waters, especially in littoral eutrophic biotopes (Kahl 1933, 1935; Precht 1935; Sommer 1951; Stiller 1971; Küsters 1974; Jankowski 1976, 1985). Compared with the forms found in freshwater habitats, however, most of marine species remain extremely less-known or only insufficiently described, which often

render great difficulties in related fields, e.g. ecological and pathogenic studies involved in aquaculture (Song 1986, 1991a, 1992a, b; Hu and Song 2001; Song *et al.* 2002). As accepted by taxonomists, criteria for species identification in this group at least comprise: (1) branching style and features of stalk, (2) zooid shape (including feature of peristomial lip) and size, (3) number of transverse silverlines (i.e. after silver nitrate impregnation), (4) infraciliature (i.e. position of epistomial membrane and detail arrangement of three oral peniculi, protargol impregnation is needed), (5) colony size and number of zooids, (6) habitat and (7) number and position of contractile vacuoles.

Zoothamnopsis is a newly erected genus (Song 1997) to include those peritrichs which are morphologi-

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cally similar to *Zoothamnium* but have reticulate silverline system with both latitudinal and longitudinal lines. Up to date, only two species have been included (Song 1997).

In May 2002, some peritrichs were isolated from an abalone-culturing pond near Qingdao, including an unknown morphotype with reticulate silverline system. After careful comparison, we convinced that it represents a new member in the genus *Zoothamnopsis*, which is described here. As a morphologically similar form, the buccal structure of *Zoothamnium maximum* Song, 1986, was re-checked and some additional descriptions are supplied.

MATERIALS AND METHODS

Zoothamnopsis sinica sp. n. was collected on 18 May, 2002. Ciliates were isolated from the surface of green alga *Ulva* sp., which attached to the stones at the bottom of an abalone-farming pond near Qingdao (Tsingtao), China. Individuals were observed *in vivo* using an oil immersion objective and differential interference contrast microscopy. The infraciliature was revealed with protargol impregnation method according to Wilbert (1975). Silver nitrate method was used to demonstrate the silverline system (Song and Wilbert 1995).

Drawings of impregnated specimens were made with the help of a camera lucida at 1250 \times magnification. Terminology is mainly according to Corliss (1979) and Warren (1986).

RESULTS

Description of *Zoothamnopsis sinica* sp. n. (Figs 1-8, 17-25; Table 1)

Class: Oligohymenophora de Puytorac *et al.* 1974

Order: Peritrichida Stein, 1859

Family: Vorticellidae Ehrenberg, 1838

Genus: *Zoothamnopsis* Song, 1997

Diagnosis for *Zoothamnopsis sinica* sp. n.: Marine *Zoothamnopsis* with colony dichotomously branched; zooids elongated vase-shaped, *in vivo* about 70-105 \times 40-55 μ m, with double-layered, thick peristomial lip; contractile vacuole apically located; macronucleus C-shaped and horizontally oriented; number of transverse lines from oral area to aboral ciliary wreath *ca* 82-98, from aboral ciliary wreath to scopula, 48-55.

Type specimens: One holo- and one paratype slides (registration number: 0205180101, 0205180102) with silver nitrate and protargol impregnated specimens are deposited at the Laboratory of Protozoology, Ocean University of China, China.

Ecological features: Ciliates were found in great abundance in a clean abalone-farming pond, with salinity about 30‰, water temperature 15°C.

Morphology: Cell size and shape consistent, *in vivo* about 70-105 \times 40-55 μ m, generally 80-90 \times 45-50 μ m, and slender vase-shaped. Body constricted slightly below the double-layered, thick peristomial lip; maximum width of cell mostly at oral border; large peristomial disc highly elevated (Figs 1, 3, 18). Pellicle very finely striated, which can be detected only using powerful objectives (\times 400 or higher) (Fig. 19), yet completely smooth when observed at low magnification.

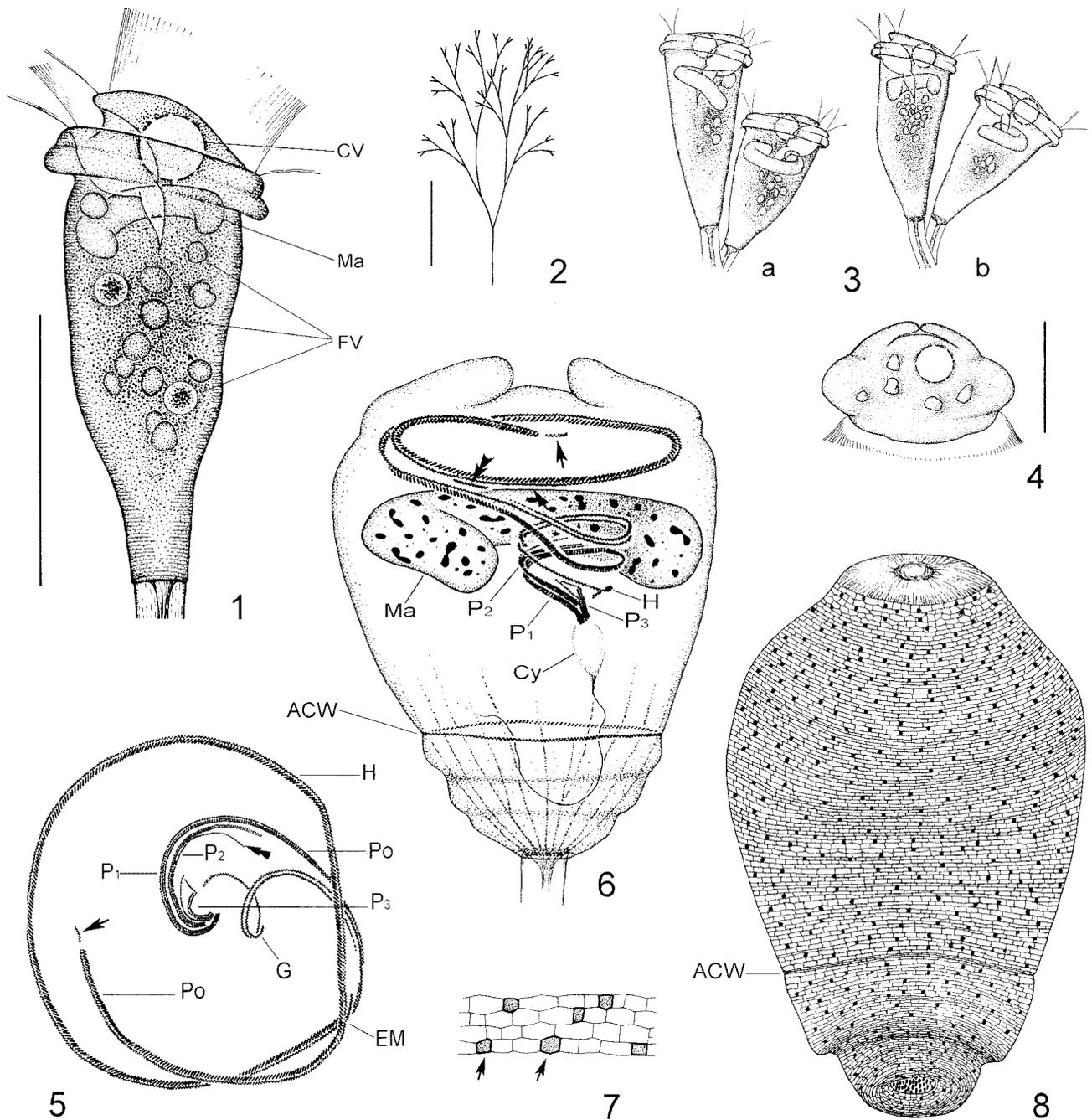
Cytoplasm colorless to slightly greyish, usually containing several large greenish food vacuoles (5-10 μ m across) (Figs 1, 18), and numerous small greyish granules which are 1-2 μ m in diameter. One large, apically located contractile vacuole (Fig. 1) rather inactive, and contracting at a rate of *ca* 3 min. Macronucleus thick and C-shaped, horizontally located (Figs 1, 3, 6); micronucleus not observed.

Small colony (< 30 zooids) regularly dichotomously branched, whereas irregularly branched in large colony, with up to 100 zooids (Figs 2, 17). Stalk up to 1500 μ m long, diameter about 15 μ m in main branch and 10 μ m in distal part, surface smooth under low magnification, with very fine striations (Fig. 1). Myoneme system consisting of thick spasmoneme, which are 6-7 μ m in width in main branch and 3 μ m at distal ends, granules extremely fine (< 0.5 μ m) on spasmoneme. Around scopula, myoneme extending anteriorly to central region of the cell as commonly seen in other peritrichs (Fig. 6).

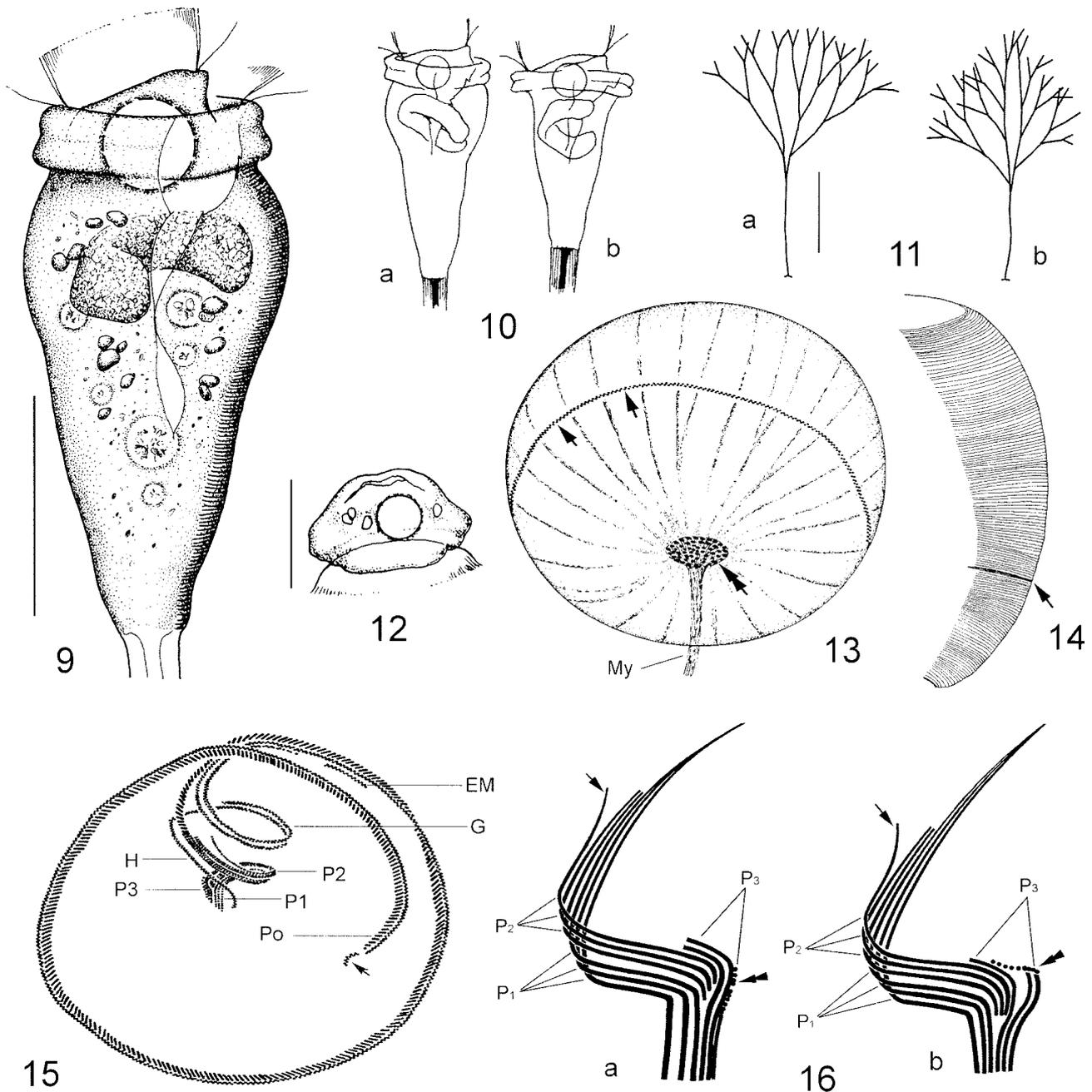
Cells not differentiating to micro- and macrozooid, and insensitive to stimuli. Contracted zooid usually oval-shaped. Telotroch flattened, *ca* 40 \times 60 μ m in size (Figs 4, 18, arrow).

Infraciliature as shown in Figs 5 and 6, which are similar to that of other congeners. Haplokinety (H) and polykinety (Po) describing about 1.5 turns around peristomial disc before entering vestibulum, where they make a further turn (Figs 5, 6). Near distal end of haplo- and polykinety, always one kinety fragment recognizable (Figs 5, 6, 21, arrows).

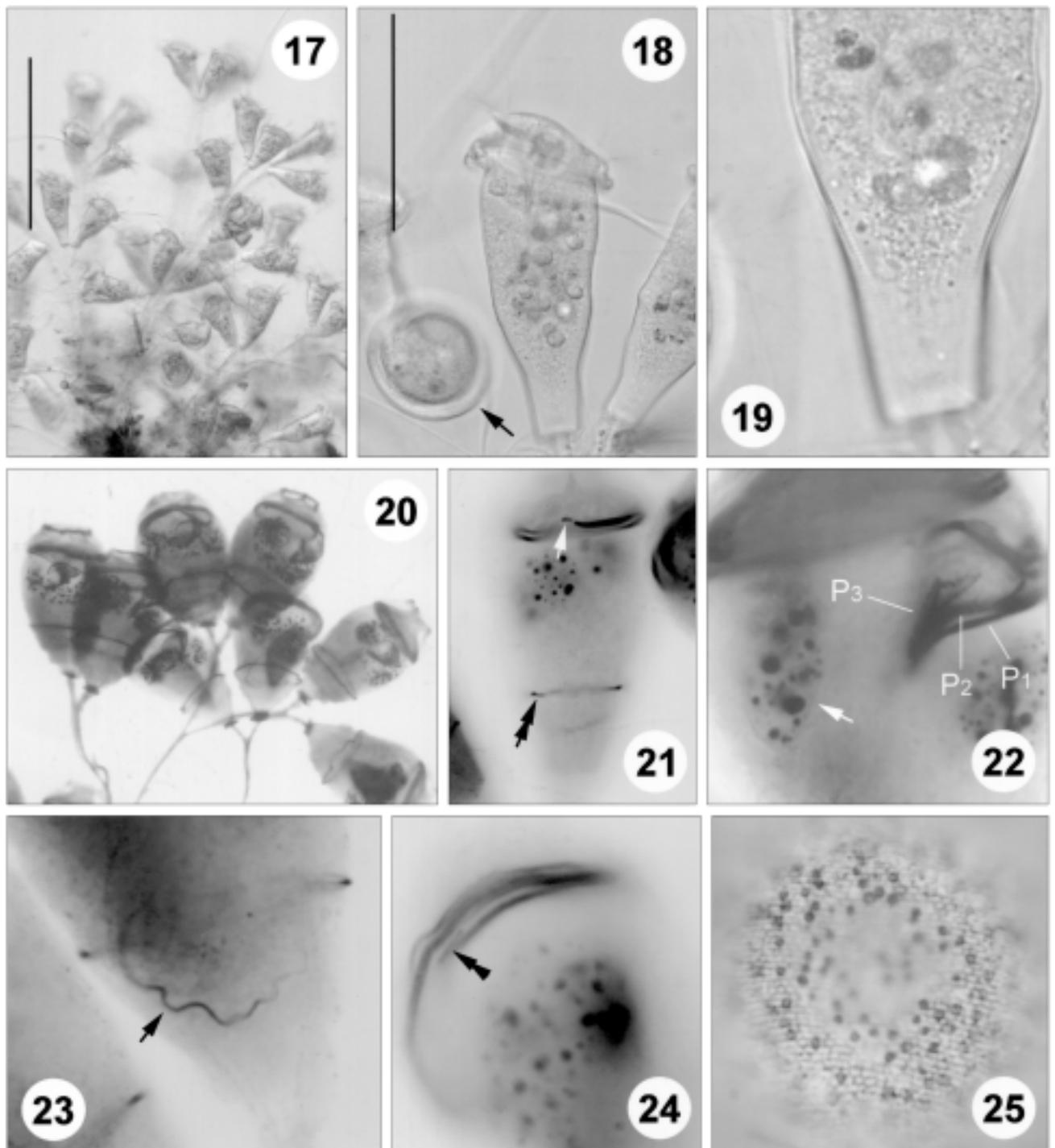
Polykinety forming three peniculi in lower half of vestibulum, each consisting of three kineties. Peniculus 1 (P1) and 2 (P2) much longer than peniculus 3 (P3). At anterior end of P2, the outer kinety apparently separated from the other two (Fig. 5, double arrowheads); P1 and P3 terminating at about same level whereas P2 ending between and above these two; characteristically, two close-set outer kineties in P3 mostly separated from the



Figs 1-8. Morphology of *Zoothamnopsis sinica* sp. n. from life (1-4), after silver nitrate (5-6) and protargol (7-8) impregnations. **1** - general view of a typical zooid; **2** - colony form; **3** - zooids at low magnification, showing varieties of body shape and macronucleus; **4** - a telotroch; **5** - apical view of the oral apparatus, noting the arrangement of three peniculi and the distal fragment (arrow). Double-arrowheads indicate the separated kinety of peniculus 2; **6** - general infraciliature, double-arrowheads mark the epistomial membrane, arrow denotes the distal fragment; **7** - details of silverline system, arrows mark the pellicle pores; **8** - general silverline system. ACW - aboral ciliary wreath, CV - contractile vacuole, Cy - cytopharynx, EM - epistomial membrane, FV - food vacuole, G - germinal kinety, H - haplokinety, Ma - macronucleus, P₁₋₃ - peniculus 1-3, Po - polykinety. Scale bars 50 μm (1); 300 μm (2); 40 μm (4).



Figs 9-16. Morphology of *Zoothamnium maximum* from life (9-12), after protargol (13, 15-16) and silver nitrate (14) impregnations (9-12, after Song 1986; 13-16, original). **9** - general view of a typical zooid; **10** - zooids of different shape; **11** - two colony forms; **12** - a telotroch; **13** - lateral view, showing the myoneme system. Double-arrows indicate the scopula, arrows mark the aboral ciliary wreath; **14** - silverline system, arrow marks aboral ciliary wreath; **15** - apical view of the oral apparatus, arrow marks the distal fragment; **16** - comparison of detailed arrangement of three peniculi between *Z. maximum* (a) and *Zoothamnopsis sinica* (b), showing the different pattern of peniculus 3 (double-arrowheads). EM - epistomial membrane, G - germinal kinety, H - haplokinety, My - myoneme, P1-3 - peniculus 1-3, Po - polykinety. Scale bars 50 μ m (9); 40 μ m (12); 300 μ m (11).



Figs 17-25. Photomicrographs of *Zoothamnopsis sinica* sp. n. from life (17-19), after protargol (Figs 20-24) and silver nitrate (25) impregnations; **17** - colony at low magnification; **18** - zooids at 400 × magnification, arrow marks the telotroch in formation; **19** - lateral part of a zooid at 1250 × magnification, showing the pellicle striations; **20** - to show the branching form; **21** - to show the distal fragment (arrow) and the aboral ciliary wreath (double-arrows); **22** - oral apparatus, indicating three peniculi ($P_{1,2,3}$) and macronucleus (arrow); **23** - the posterior end of the cytopharynx (arrow); **24** - to show the epistomial membrane (double-arrowheads); **25** - silverline system. Scale bars 200 μ m (17); 50 μ m (18).

Table 1. Morphometrical characterizations of *Zoothamnopsis sinica* sp. n. (upper line) and *Zoothamnium maximum* (Song, 1986) (lower line). Measurements in μm . Min - minimum, Max - maximum, Mean - arithmetic mean, SD - standard deviation, SE - standard error of the mean, Vr - coefficient of variation, n - sample number.

Character	Min	Max	Mean	SD	SE	Vr	n
Body length <i>in vivo</i>	70	105	91.4	10.10	2.81	11.1	13
	81	121	-	-	-	-	-
Body width <i>in vivo</i>	40	52	47.3	3.34	0.96	7.1	12
	47	64	-	-	-	-	-
Body length after protargol	43	68	54.6	5.44	1.01	10.0	29
	36	51	44.6	5.00	0.96	11.2	27
Body width after protargol	25	38	31.8	4.27	0.79	13.4	29
	26	42	34.7	5.03	5.20	2.9	27
Number of silverlines from oral area to aboral ciliary wreath	82	98	89.7	4.72	0.67	5.3	7
	82	95	-	-	-	-	4
Number of silverlines from aboral ciliary wreath to scopula	48	55	51.7	2.58	1.05	5.0	6
	45	58	-	-	-	-	4

third one and only converged with it at the end of vestibulum (Figs 5, 6, 16b, double-arrowheads, 22).

Haplokinety passing around vestibulum on opposite wall to peniculi. Cytopharynx highly developed, the distal end hook-shaped and extending below aboral ciliary wreath (Figs 6, 23). Germinal kinety (G) lying parallel to haplokinety within upper half of vestibulum (Fig. 5). Epistomial membrane (EM) short, located near upper level of vestibulum (Figs 5, 24). Aboral ciliary wreath (ACW) formed by double-rowed kineties, which encircle cell in posterior region (Figs 6, 21).

Silverline system, as shown in Figs 7, 8 and 25, genus typical. Transverse lines equably close-set in whole region, mesh rows *ca* 0.5 μm at interval. Pellicle pores distributed irregularly, densely located in some areas but sparsely in others (Figs 7, arrows; 8, 25). ACW represented by two or three parallel lines (Fig. 8). Number of transverse silverlines from peristome to ACW, 82-98; from ACW to scopula, 48-55.

Redescription of *Zoothamnium maximum* Song, 1986 (Figs 9-16, 26-34; Table 1)

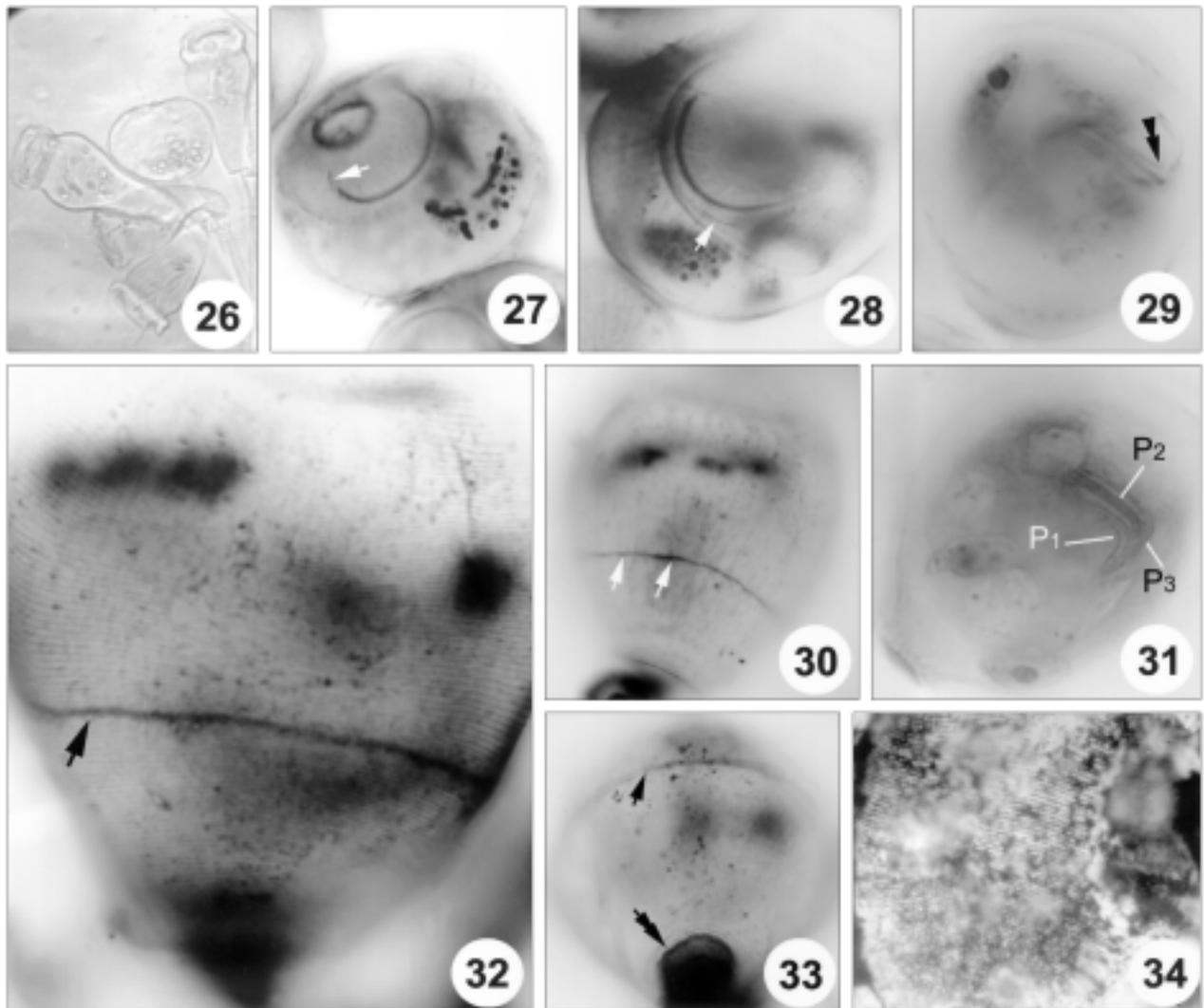
Zoothamnium maximum was originally found from some shrimp-farming waters in the Yellow Sea, China (Song 1986). As the report was made in Chinese and descriptions on some oral structures were not supplied in sufficient details, a complementary redescription is thus added based on re-checking of the slides and the documents reserved by the junior author.

Morphology: Body flexible and slightly variable, but generally elongate and slender vase-shaped (Figs 9, 10,

26), widest at anterior 1/3 of the body, and usually constricted below peristomial lip. Fully extended zooid *ca* 110 (80-120) x 50 (40-55) μm in size, mostly length to width about 2-2.2:1. Peristomial disc strongly elevated (Fig. 9). Peristomial lip conspicuously thick, double-layered (Fig. 9). Pellicle smooth at low magnification, fine striations detected only with more powered objectives (x 400 or higher). Cytoplasm colourless and transparent, several large light-reflecting granules (*ca* 3-5 μm in diameter) often present. Macronucleus relatively short, C-shaped and horizontally oriented. Single large contractile vacuole apically located (Fig. 9).

Stalk strong, about 12 μm thick with smooth surface. Colony typically dichotomously branched and reaching a total length of 1-1.5 mm with often more than 50 homomorphic zooids (Fig. 11). Stalk myoneme conspicuous, theoplasmic granules indetectable. Telotroch flattened, *ca* 60 μm in diameter (Fig. 12).

Oral apparatus typical of genus. Haplokinety (H) and polykinety (Po) circling about one and half turns around peristomial disc and accomplishing a further turn after plunging into vestibulum (Fig. 15). All peniculi consisting of 3 kineties while peniculus 1 (P1) and P2 are much longer than P3. Three kineties in P1 about equally long, converged with P3 at posterior end (Fig. 16a). The outer kinety of P3 loosely ciliated, about 2/3 of the other two in length and closely located to the middle one (Fig. 16a, double arrowheads). P2 interposed between P1 and P3, the anterior end of the outer kinety apart from the other two (Fig. 16a, arrow; 29, double-arrowheads), and all of them terminating at different levels above P1 and P3



Figs 26-34. Photomicrographs of *Zoothamnium maximum* from life (26), after protargol (27-33) and silver nitrate (34) impregnations. 26 - zooids at low magnification; 27 - apical view, showing the distal fragment (arrow) of oral apparatus; 28 - apical view, arrow marks epistomial membrane; 29 - oral apparatus, denoting the outer kinety of peniculus 2 (double-arrowheads); 30 - side view, showing aboral ciliary wreath (arrows) and myoneme system; 31 - detailed arrangement of three peniculi (P_{1-3}); 32, 34 - general silverline system, arrow marks the aboral ciliary wreath; 33 - lateral view, showing the scopula (double-arrows) and the aboral ciliary wreath (arrow).

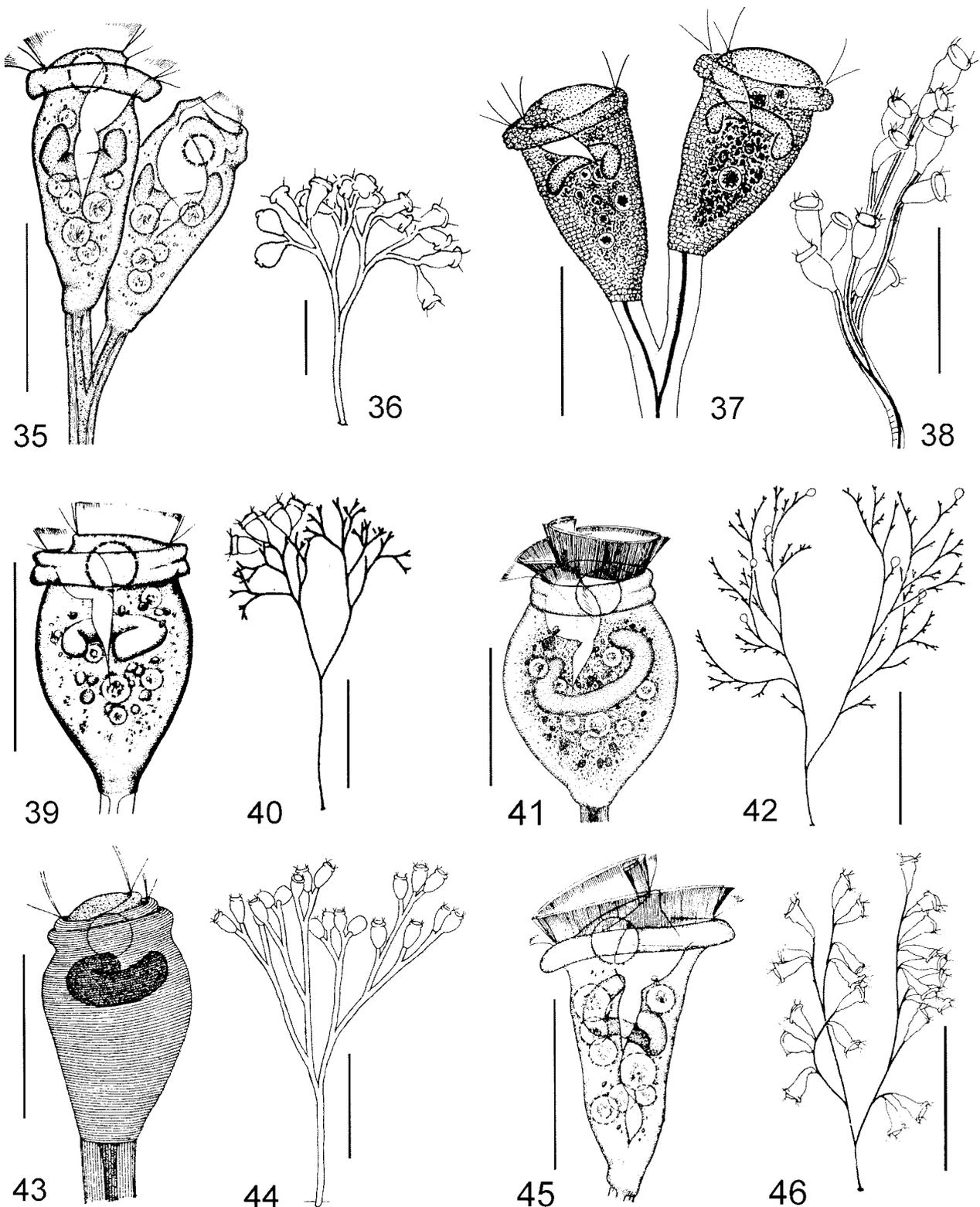
(Fig. 16a). Haplokinety passing around vestibulum on opposite wall to peniculi. Germinal kinety (G) lying parallel to haplokinety within upper half of vestibulum. Epistomial membrane (EM) relatively long, and located near opening of vestibulum (Figs 15, 28). Aboral ciliary wreath composed of zig-zag structure of kinetosomes, which encircles cell at about the level of posterior 1/3 of body length (Figs 13, 30, arrows).

Myoneme system similar to that of *Vorticella* spp., consisting of strong spasmoneme in stalk and thin myonemes (My) around scopula, which extend anteriorly to oral area (Figs 13, 30). Silverline system typical

of *Zoothamnium*-pattern (Figs 14, 32-34), striations close-set and no conspicuous pellicular pores associated with silverlines. Number of transverse silverlines between peristomial lip and aboral ciliary wreath *ca* 82-95, between aboral ciliary wreath and scopula, 45-58.

COMPARISON AND DISCUSSION

The genus *Zoothamnopsis* was established by Song very recently (Song 1997) with two species included, *Z. mengi* Song, 1997 (Figs 35-36) and *Z. perlatum*



Figs 35-42. Comparison of some closely related morphotypes. **35-36** - *Zoothamnopsis mengi* (after Song 1997); **37-38** - *Zoothamnopsis perlatum* (after Stiller 1946); **39-40** - *Zoothamnium duplicatum* (after Song 1991a); **41-42** - *Zoothamnium penaei* (after Song 1992b); **43-44** - *Zoothamnium rigidum* (after Precht 1935); **45-46** - *Zoothamnium paraentzii* (after Song 1991b). Scale bars 50 μ m (35, 37, 39, 41, 43, 45); 100 μ m (36); 200 μ m (38, 40, 44, 46); 400 μ m (42).

Table 2. Morphological comparison between *Zoothamnopsis sinica* sp. n. and other morphologically similar *Zoothamnopsis* and *Zoothamnium* species. Measurements in μm . ? - data not available.

Species	Body length <i>in vivo</i>	Body width <i>in vivo</i>	Number of transverse silverlines from scopula to aboral ciliary wreath	Number of transverse silverlines from aboral ciliary wreath to anterior end	Appearance of peristomial lip	Habitat	Number of zooids	Data source
<i>Zoothamnopsis sinica</i> sp. n.	70-105	45-52	48-55	82-98	double-layer	marine	ca 50	Original
<i>Zoothamnopsis mengi</i>	50-70	30-40	30-36	76-87	single-layer	marine	ca 20	Song 1997
<i>Zoothamnopsis perlatum</i>	60-100	-	-	-	single-layer	freshwater	10-12	Stiller 1946
<i>Zoothamnium duplicatum</i>	45-84 (69)	27-49 (44)	25-28	50-54	double-layer	marine	>100	Kahl 1933, Song 1991a
<i>Zoothamnium maximum</i>	81-121 (109)	47-64 (56)	45-58	82-95	double-layer	marine	-	Song 1986, Original
<i>Zoothamnium paraentzii</i>	50-80 (68)	27-43 (35)	ca 27	ca 60	single-layer	marine	20-30	Song 1991b
<i>Zoothamnium penaei</i>	54-95 (78)	38-62 (51)	-	-	double-layer	marine	>100	Song 1992b
<i>Zoothamnium rigidum</i>	70-95	-	-	-	double-layer	marine	ca 20	Precht 1935

(Stiller, 1946) Song, 1997 (Figs 37-38). *Z. sinica* sp. n. differs from *Z. mengi* by its larger size (70-105 × 40-55 vs. 50-70 × 20-30 μm), double-layered peristomial lip (vs. single-layered) and distinctly more transverse silverlines from the scopula to the aboral ciliary wreath (48-55 vs. 30-36) (Table 2). *Z. perlatum* has similar size to *Z. sinica*, but can be recognized by freshwater habitat (vs. marine) and less transverse striations (*ca* 40, from original illustration vs. over 130).

Considering the living appearances and the marine habitat, some *Zoothamnium* species with dichotomously-branched stalk and double-layered peristomial lip should be compared with the new organism, i.e. *Z. maximum* Song, 1986, *Z. duplicatum* Kahl, 1933, *Z. penaei* Song, 1992 and *Z. rigidum* Precht, 1935 (Table 2).

Morphologically, *Zoothamnium duplicatum* (Figs 39, 40) is possibly most related to *Zoothamnopsis sinica in vivo*, which can be separated, however, besides the different silverline system (*Vorticella*-type vs. *Pseudovorticella*-type), by the conspicuously thicker peristomial lip and less slender body shape (Kahl 1933, Song 1991a).

Different from the giant form, *Zoothamnium maximum*, the new species is relatively smaller (90 vs. 110 μm on average), possesses thinner peristomial lip and more elevated peristomial disc (Figs 1, 9, 18) (Table 2). In addition, they have completely different pattern of silverline system as well.

Zoothamnium penaei (Figs 41, 42) has a conspicuously oval body shape and differentiated zooids (macro- and microzooids?), thus can be clearly identified at the level of living observations.

Besides different silverline pattern (Precht 1935), *Zoothamnopsis sinica* can be distinguished from *Zoothamnium rigidum* (Figs 43, 44) by more elevated peristomial disc, thicker oral border and conspicuously slender body.

Zoothamnium paraentzii (Figs 45, 46), found from the body surface of marine shrimp *Penaeus sinensis* by Song (1991b), exhibits thin, single-layered oral border and less transverse silverlines from scopula to aboral ciliary wreath (*ca* 27 vs. 48-55), thus can be clearly separated from the current organism.

As summarized in Table 2, *Zoothamnium maximum* differs from other related congeners, *Z. duplicatum*, *Z. paraentzii* and *Z. penaei* in the following combination: large size and higher number of silverlines (Table 2).

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