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New discoveries of the genus *Thuricola* Kent, 1881 (Ciliophora, Peritrichia, Vaginicolidae), with descriptions of three poorly known species from China

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Abstract. Members of the genus *Thuricola* are a highly specialized group of peritrichous ciliates possessing a protective barrel-shaped lorica. The genus presents many difficulties in terms of species separation and definition, and in this context the present study investigates three species by protargol staining and analyses of SSU rDNA sequences. Based on their morphologic characteristics and biotope, they were identified as three poorly known forms in *Thuricola*, namely *T. obconica* Kahl, 1933, *T. kellicottiana* (Stokes, 1887) Kahl 1935 and *T. folliculata* Kent, 1881, respectively. *T. obconica* is characterized by possessing curved lorica and a single valve *in vivo*. *T. kellicottiana* is distinguished by two valves with a spine on the main valve, and a generally long internal stalk upon which the zooids sit. *T. folliculata* also has two valves but lacks a spine. The ciliature of the three species are basically similar. The main features are characterized as follows: infundibular polykineties 1–3 (P1–3) are relatively long and composed of three rows each; P1 bends twice and comprises three equally long rows; P2 ends near the second bend of P1 with all rows exhibiting a staggered arrangement; P3 converges with P1 at adstomal end, its row 1 at least twice as long as the other two rows; epistomial membranes 1 and 2 are present. Silverline system in *Vorticella*-pattern. Phylogenetic analyses indicate that the three ciliates in this study cluster together within one of the two major sub-clades within the Vaginicolidae.

Keywords: Ciliate; Loricate sessilids; Molecular phylogeny; Morphology; Silverline

INTRODUCTION

Sessile peritrichs are a large assemblage of ciliates that have a wide distribution in aquatic and terrestrial habitats (Kahl 1935, Foissner *et al.* 1992, Ji *et al.* 2015, Sun *et al.* 2017, Shen *et al.* 2017). Up to now, more than 800 species have been reported (Lynn 2008, Foissner *et al.* 2010), and continuously emerging new taxa suggest that there is a large undiscovered diversity of this group (Peng *et al.* 2007, Foissner *et al.* 2010, Ji *et al.* 2015, Zhuang *et al.* 2016, Liu *et al.* 2017, Shen *et al.* 2017, Sun *et al.* 2017).

Unfortunately, the loricate sessilids have been largely neglected in modern taxonomic studies compared to non-loricate forms owing to their relatively low abundance in nature. As the biggest family among the

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loricate peritrichs, including nearly 200 nominal species, the Vaginicolidae in particular is insufficiently known and is thus in need of thorough research, especially in respect to the ciliature and molecular information.

Thuricola is a relatively small genus of the Vaginicolidae, and was established by Kent (1881). It is distinguished from other vaginicolids by the closure apparatus of the lorica, which is the crucial feature of the genus and is used to enclose the organism (Kahl 1935; Trueba 1978, 1980; Warren 1982, 1991). A series of studies have been carried out on this genus since the late nineteenth century, before Trueba (1980) revised the genus to include 11 species (Wright 1858, Kent 1881; Stokes 1882, 1887; Daday 1910, Kahl 1933, Sommer 1951, Hammann 1952, Biernacka 1963, Bock 1963, Küsters 1974). Thereafter, six new species found in China were added to the genus (Gong 1989a, b; Xu 1990; Sun *et al.* 2009, 2012; Shen and Gu 2016).

Similar to other vaginicolids, ciliature data on *Thuricola* are very scarce (Kahl 1935; Trueba 1978, 1980; Xu 1990, Warren 1991, Zhuang *et al.* 2016), and many descriptions are either outdated or very ambiguous (Fromentel 1874; Penard 1914, 1922; Dons 1922, 1948; Kahl 1933, Bock 1952, Stiller 1971). In addition, although molecular data are playing an increasingly important role in the taxonomy and systematics of ciliates (Li *et al.* 2013, Chen *et al.* 2016; Gao *et al.* 2016a, 2017; Huang *et al.* 2016, Sun *et al.* 2016, Gentekaki *et al.* 2017, Wang *et al.* 2017, Zhuang *et al.* 2018), little such information is so far available for *Thuricola*.

The current work is a new contribution and a part of the ongoing faunistic study of ciliates in China (Qu *et al.* 2015; Liu *et al.* 2016; Lu *et al.* 2017; Pan *et al.* 2017). Three little-known species of *Thuricola* are described based on careful observations of specimens *in vivo* and after silver staining. Their living morphology and ciliature are presented, together with a phylogenetic analysis based on their SSU rDNA sequences.

MATERIALS AND METHODS

Materials and morphological methods: *Thuricola obconica* was isolated from the surface of green algae (*Enteromopha* sp.) collected on February 20th 2017 from an aquaculture pond with brackish water (salinity 11 ‰) on Meishan Island (N29°36′; E121°48′), Ningbo, China. The water temperature was 14.5 °C.

Thuricola kellicottiana was also isolated from the surface of *Enteromopha* algae, which was collected on March 10th 2017 near a floodgate on Meishan Island (N29°36′; E121°48′), Ningbo, China. The water temperature was 13.5 °C and the salinity 6.5 ‰.

Population-I of *Thuricola folliculata* was obtained from samples of the aquatic plant *Ceratophyllum demersum, collected on March 16th 2017 from the wetland of* Hangzhou Bay (N30°22′; E121°12′), Ningbo, China. The water temperature was 9.5 °C and the salinity was 2 ‰. Population-II was isolated on April 24th 2017 from a fresh water lake (N36°04′; E120°21′) in Qingdao, China. The water temperature was 14 °C.

The living morphology was investigated under a compound microscope equipped with a high-power oil immersion objective as well as differential interference contrast optics. The ciliature was revealed using the protargol staining method (Wilbert 1975). The protargol powder was manually synthesized, following the method prescribed by Pan *et al.* (2013). Counts and measurements were performed at 400–1,000 × magnification. Drawings were made with the help of a camera lucida. Terminology is mainly according to Trueba (1980) and Lynn (2008) except for the following:

Junctional membrane (JM): The lorica closure apparatus is a distinctive feature of *Thuricola*. It is composed of valve(s) that can close the lorica through traction of a thin membrane. This membrane is funnel-shaped, surrounds zooid and stalk. View from narrow side of lorica, its anterior portion is normally invisible because it attaches to inner wall tightly. Due to the lack of study of this genus, there is no accepted term for this thin membrane. Moreover, the term ‘membrane’ in ciliates often refers to orderly arranged cilia. Considering that it connects the valve to the bottom of the lorica, we therefore introduce this term here.

Lorica base (LB): A term referring to the bottom of lorica that attaches to the substrate.

DNA extraction, PCR amplification and sequencing: Genomic DNA was extracted from cells using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The small subunit (SSU) rDNA sequence was amplified by PCR using the primers: 82F (5′ -GAA ACT GCG AAT GGC TC-3′) (Jerome *et al.* 1996) and 18s-R (5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′) (Medlin *et al.* 1988). Q5® Hot Start High--Fidelity DNA Polymerase (New England BioLabs, USA) was used to minimize the possibility of PCR amplification errors. Sequencing was performed bidirectionally by the Tsingke Biological Technology Company (Beijing, China).

Phylogenetic analyses: In addition to the three SSU rDNA sequences obtained in this study, 42 sequences of representative peritrichs downloaded from the GenBank database were included in the present phylogenetic analyses. Three hymenostomatian species, *Tetrahymena thermophila*, *Tetrahymena corlissi* and *Glaucoma chattoni* were used as the outgroup taxa. All sequences were first aligned using the GUIDANCE2 algorithm (Landan and Graur 2008; Sela *et al.* 2015). The resulting alignment was then manually refined by trimming both ends using the program BioEdit 7.0 (Hall 1999), resulting in a matrix of 1763 characters. Maximum likelihood (ML) analysis was computed at the CIPRES website (http://www.phylo.org), using the GTR + gamma model performed by RAxML-HPC2 v.8.2.10 on XSEDE (Stamatakis 2014). The highest-scoring ML tree was assessed by 1,000 bootstrap replicates. Bayesian inference (BI) analysis was performed with MrBayes v.3.2.6 on XSEDE (Ronquist *et al.* 2012) using the GTR+ I+ G model selected by MrModeltest 2.2 (Nylander 2004) under the Akaike Information Criterion. Markov chain Monte Carlo simulations were run for 6,000,000 generations with a sample frequency of 100 generations. The first 10% of trees were discarded as burn-in. All the remaining trees were used to calculate the posterior probability using a 50% majority rule consensus. Tree topologies were visualized using MEGA 7.0 (Kumar *et al.* 2016). Systematic classification is according to Sun *et al.* (2012) and Gao *et al.* (2016b).

RESULTS

Subclass Peritrichia Stein, 1859

Order Sessilida Kahl, 1933

Family Vaginicolidae Fromentel, 1874

Genus *Thuricola* Kent, 1881

Hitherto *Thuricola* has never been defined based on the ciliature information and descriptions of most thuricolas are incomplete. Here, three species of *Thuricola* were investigated carefully *in vivo* and their ciliature were revealed for the first time. Thus we provide an improved diagnosis as follows.

Emended diagnosis: One or two zooids within a closeable lorica. Zooid(s) attached to base of lorica directly or via a single stalk. Lorica attached to substrate directly. Valve and junctional membrane present. Infundibular polykinety 3 consisting of three rows of kinetosomes, row 1 of which is distinctly longer than the other two. Silverline system in *Vorticella*-pattern.

Thuricola obconica Kahl, 1933 (Figs 2A–R, 3A–S, Table 1)

1933 ***Thuricola obconica*** n. sp.–Kahl, Tierwelt Nord Ostsee., p. 135, Fig. 24: 7.

1935 ***Thuricola obconica*** Kahl, 1933–Kahl, Tierwelt Dtl., p. 786, Fig. 145: 7–8.

1952 ***Thuricola obconica*** Kahl, 1933–Bock, Kieler Meeresforsch., 8: p. 227, Fig. 2a, b.

1952 ***Thuricola kamptostoma*** n. sp.–Bock, Kieler Meeresforsch., 8: p. 227–228, Fig. 3.

1980 ***Thuricola obconica*** Kahl, 1933–Trueba, Beaufortia, 30: p. 134, Fig. 5g.

Although *T. obconica* had been described several times, morphological data on the species remain somewhat deficient. Furthermore, the inconsistencies in the descriptions provided in the literature suggest that the previous studies may contain some misidentifications. A detailed redescription based on modern criteria is therefore imperative.

Emended diagnosis: Lorica colourless and ca. 120–210 μm long, cross-section sub-circular, nearly symmetrical on wider side and asymmetrical on narrower side, curved before valve base, posterior portion tapering to a base with two annular bulges, base expanded. Valve in upper third of lorica. One or two zooids, 195–310 × 15–32 μm *in vivo*. Stalk about 10 μm long. Peristomial lip single-layered and strongly everted. Contractile vacuole dorsally located. Row 1 of infundibular polykinety 3 about twice as long as the other two. About 105–120 transverse silverlines from peristome to trochal band, 95–110 from trochal band to scopula.

Voucher slides: Six voucher slides with protargol stained specimens (registration number LU20170220-01-01-06) have been deposited in the Laboratory of Protozoology, Ocean University of China.

Description: Cell size about 195–310 × 15–25 μm (n = 9) *in vivo*. Generally, one or two zooids within a lorica. When two zooids co-exist, they are abreast of each other but anisometric. Solitary zooid relatively smaller. When extended, zooid is slender and trumpet-shaped, expanded gradually anteriad, widest at oral opening (Figs 2A–D, 3A–E). Zooid highly flexible and contractile. When stimulated, zooid contracts into lorica and becomes ellipsoid or broadly conical in outline (Fig. 3F–H). Peristomial lip thin and single-layered, about 38–48 μm in diameter when fully expanded. Peristomial disc strongly elevated (Figs 2A, B, 3A–E, I). Pellicle covered with shallow, narrowly spaced traverse striations (Figs 2J, 3N). Striations hardly recognizable at low magnifications, but clearly visible above 400 × magnification. Trochal band, a conspicuously bulged ring-like structure, located near mid-body (Figs 2J, 3N). About 105–120 striations from peristome to trochal band and 95–110 striations from trochal band to scopula (counted from live materials; n = 4).

Cytoplasm colourless and full of granules usually less than 2 μm in diameter. Single contractile vacuole located at the same level as peristomial lip and on the dorsal wall of infundibulum (Figs 2A, B, 3I). Food vacuoles about 5–10 μm across, usually positioned in the middle portion of body (Figs 2A, B, 3A–H). Sometimes, several large irregular-shaped refractive granules scattered in cytoplasm.

Macronucleus rather hyaline and thus difficult to recognize *in vivo*, vermiform in shape, longitudinally oriented, nearly passing through the whole body with a transverse curvature at oral end, attenuating gradually from top to bottom (Figs 2C, 3O–Q). Due to

Table 1. Morphometric data of *Thuricola obconica* (first line), *Thuricola kellicottiana* (second line) and *Thuricola folliculata* (population I, third line; population II, last line).

Characters[a]	Min	Max	Mean	SD	CV	n
Body length	195	310	243.0	38.1	15.7	9
	230	377	287.9	45.5	15.8	9
	165	288	237.1	42.2	17.8	7
	180	313	221.6	43.7	19.7	10
Body width	18	23	20.6	1.6	7.8	9
	21	27	23.9	1.7	7.1	9
	18	23	20.1	1.9	9.5	7
	16	23	19.3	2.1	10.9	10
Peristomial lip width	38	48	43.2	3.7	8.6	9
	40	48	42.7	2.5	5.9	9
	36	42	39.0	2.0	5.1	7
	37	50	40.2	4.0	10.0	10
Lorica length	155	210	178.6	16.2	9.1	10
	190	225	201.5	12.6	6.3	6
	155	186	167.4	10.7	6.4	7
	127	168	139.7	14.6	10.5	6
Lorica width from narrow side	39	50	46.5	3.4	7.3	10
	44	50	46.8	2.0	4.3	6
	45	51	48.9	2.7	5.5	7
	39	46	42.2	2.8	6.6	5
Lorica width from wide side	47	47	47.0	0	0	1
	51	54	52.5	2.1	4.0	2
	50	50	50.0	0	0	1
	48	52	50.0	2.8	5.6	2
Aperture width from narrow side	31	43	37.7	4.4	11.7	7
	32	40	35.5	2.9	8.2	6
	33	39	36.4	2.0	5.5	7
	32	39	35.5	2.6	7.3	6
Aperture width from wide side	47	47	47.0	0	0	1
	49	51	50.0	1.4	2.8	2
	50	50	50.0	0	0	1
	48	52	50.0	2.8	5.6	2
Stalk length	6	14	10.6	2.6	24.5	9
	4	18	14.0	5.1	36.5	6
	5	13	7.3	2.8	38.4	7
	3	6	4.6	1.1	23.9	5
Number of valves	1	1	1.0	0	0	10
	2	2	2.0	0	0	6
	2	2	2.0	0	0	7
	2	2	2.0	0	0	6
Number of striations from peristome to TB[b]	104	120	111.8	7.1	6.4	4
	95	103	99.0	5.7	5.8	2
	110	115	112.5	3.5	3.1	2
	97	111	107.3	6.8	6.3	4
Number of striations from TB to scopula[b]	96	111	101.8	6.5	6.4	4
	100	108	103.6	3.2	3.1	5
	95	108	103.0	2.7	2.6	4
	87	106	93.8	8.7	9.3	4

[a] Data based on living specimens. Measurements in µm.

[b] Rough values.

CV, coefficient of variation in %; Max, maximum; Mean, arithmetic mean; Min, minimum; *n*, number of individuals examined; TB, trochal band.

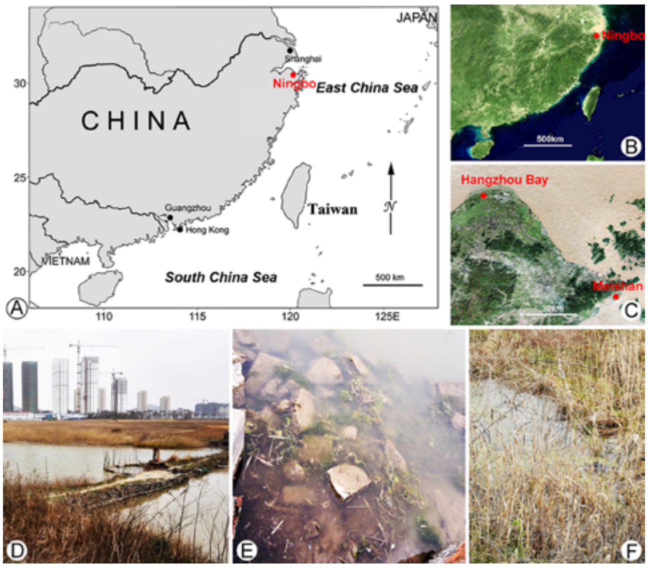

Fig. 1. (A, B) Map of East China Sea and South China Sea, red dot indicates the location of Ningbo. **(C)** Satellite map of North-east Ningbo, red dots indicate the two sampling areas (Wetland of Hangzhou Bay and Meishan Island). **(D)** A brackish aquaculture pond in Meishan Island. **(E)** Close up of a watercourse in Meishan Island. **(F)** Wetland of Hangzhou Bay.

significant shrinkage in the fixation process, macronucleus in protargol stained specimens highly twisted (Figs 2L, 3O–Q).

Zooid attached to lorica via an inner stalk, around 10 µm long (Figs 2A–D, 3A–H). Stalk has longitudinal striations only visible at high magnifications. Both ends of stalk expanded (Figs 2I, 3K).

Lorica of *Thuricola obconica* transparent and colourless, about 155–210 µm long, conical in aboral third and with a laterally bent anterior portion. In lateral view from the narrow side, lorica about 40–50 µm (average 46.5 µm, n = 10) wide, widest about two-thirds of its length from aperture to base (Figs 2A–C, F–H, 3A–H). Lorica usually bent differently at anterior third (Figs 2A–H, 3A, D, E, F, H). In lateral view from the wide side, lorica slightly wider (47 µm, n = 1), and almost rectangular in outline at upper two thirds (Figs 2D, 3C). At posterior third, lorica narrowed with two inconspicuous annular bulges (Figs 2A–H, 3A–H, K, L). Bottom expanded (Figs 2I, 3K, arrow). One valve inserted at anterior third (Figs 2A–H, 3A–H, M). Its base curved and thus conducive to closing and opening (Figs 2A–C, F–H, 3A, B, D–H, M). Valve connected to a junctional membrane (JM). JM thin and hyaline, making it hard to confirm fine three-dimensional structure under light microscope. Viewed from the narrower side of lorica, JM presents two short “V” shaped lines above inner stalk and two short longitudinal lines around stalk (Figs 2A–C, F–H, 3K, arrow), whereas it shows two long lines that nearly symmetrical with smooth curves above inner stalk and two short longitudinal lines around stalk between valve and lorica floor when lorica rotated by 90° (Figs 2E, 3C, L, arrowheads).

Oral cilia about 18 µm long, positioned around peristomial disc and lip. Oral apparatus composed of haplokinety and polykinety; both circling about 1.5 turns around peristomial disc before entering infundibulum where they make a further turn. Infundibular part of polykinety (P1) accompanied by other two kineties (P2–3), composed of three parallel rows of kinetosomes each. Three rows of P1 equal in length and extending to cytostome (Figs 2M, 3R, S). P2 interposed between P1 and P3. Arrangement of three kinetosomal rows of P2 somewhat staggered: row 1 extends abstomally and considerably longer than rows 2 and 3, terminating adstomally ahead of row 2 and 3 at the last curvature of P1; row 2 runs parallel with row 1 and separate from row 3 at abstomal half (Figs 2M, 3R). Row 1 of P3 originates from the middle of P2, while rows 2 and 3 become shortened abstomally. Adstomally P3 converges with P1, both of which terminate at the end of the infundibulum (Figs 2M, 3R, S). Epistomial membrane 1 located near the entrance of infundibulum (Figs 2M, 3R). Epistomial membrane 2 short, located ahead of the beginning of polykinety and haplokinety (Figs 2M, 3R). Trochal band encircling body equatorially. Germinal kinety parallel to haplokinety, ending at last curve of haplokinety (Figs 2M, 3R).

Thuricola kellicottiana (Stokes 1887) Kahl, 1935 (Figs 4A–Q, 5A–T, Table 1)

1884 ***Thuricola innixa* Stokes, 1882**–Kellicott, Proc. Amer. Soc. Microsc., Seventh Annual Meeting., 6: p. 120, Pl.3: Fig. 5.

1887 ***Thuricolopsis kellicottiana* spec. nov.**–Stokes, Proc. Amer. Soc. Microsc., p. 251, Fig. 17.

1888 ***Thuricolopsis kellicottiana* Stokes, 1887**–Stokes, J. Trenton Nat. Hist. Soc., p. 253–254, pl. 9: Fig.12.

1904 ***Cothurnia crystallina* Ehrbrg.**–Entz, Mat. Naturwiss. Ber. Ungarn., 19: p. 140–141, Fig. 10g.

1914 ***Cothurnia castellensis***–Penard, Mém. Soc. Phys. Hist. Nat. Genève, 37: p. 59–60, Pl. 3, Figs 9–11, 13, 14.

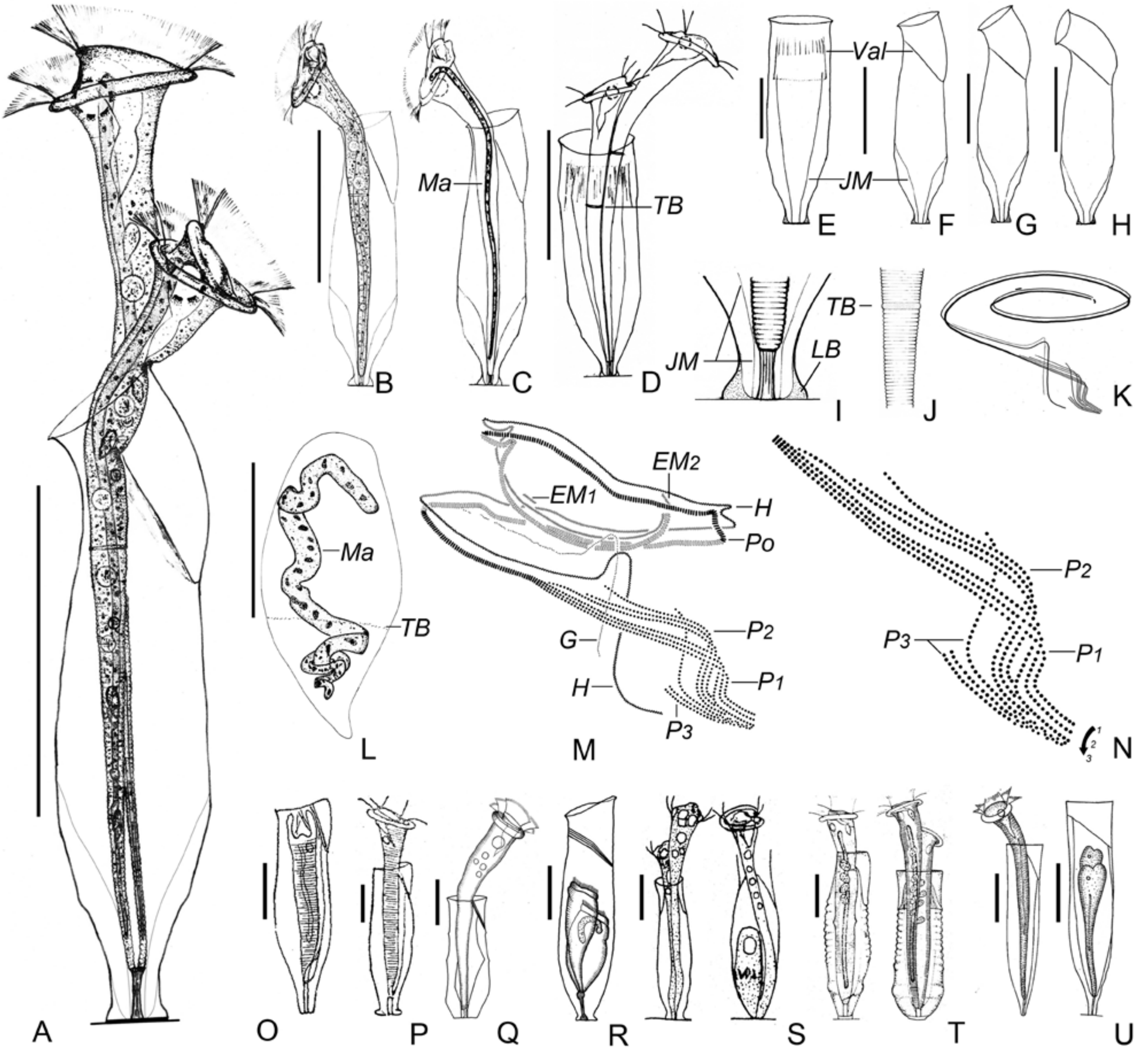

Fig. 2. Morphology and ciliature of *Thuricola obconica*. **(A)** Narrow side view of an individual with two zooids. **(B)** Narrow side view of an individual with single zooid. **(C)** Narrow side view of an individual with single zooid, to show macronucleus. **(D)** Wide side view of an individual with two zooids. **(E)** Wide side view of lorica. **(F–H)** Narrow side view of different curved lorica. **(I)** Base of lorica. **(J)** Detail of pellicle, to show the transverse striations and trochal band. **(K)** Model pattern of oral ciliature. **(L)** Macronucleus after protargol staining. **(M)** Oral ciliature. **(N)** Detail of infundibular polykineties. **(O)** *T. obconica* from Kahl (1933). **(P)** *T. obconica* from Kahl (1935). **(Q)** *T. obconica* from Bock (1952). **(R)** *T. kamptostoma* from Bock (1952) (synonym of *T. obconica*). **(S)** *T. obconica* from Biernacka (1963) (possible misidentification). **(T)** *T. obconica* from Küsters (1974) (probably misidentified). **(U)** *T. obconica* from Shen & Gu (2016). EM1–2, epistomial membrane 1–2; G, germinal kinety; H, haplokinety; JM, junctional membrane; LB, lorica base; Ma, macronucleus; Po, polykinety; P1–3, infundibular polykineties 1–3; TB, trochal band; Val, valve. Scale bars = 100 µm (A, B, D); 50 µm (E–H, L, O–U).

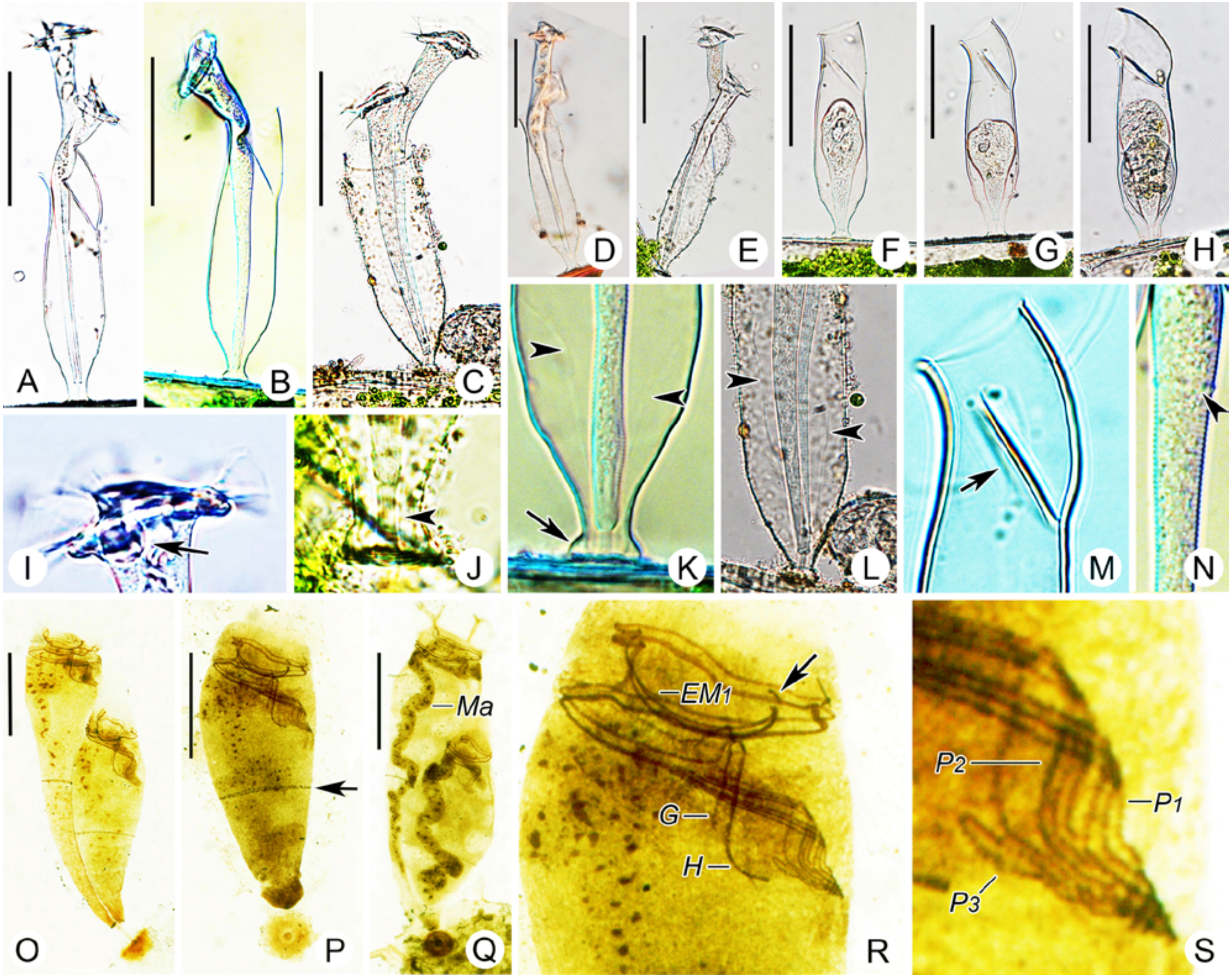

Fig. 3. Photomicrographs of *Thuricola obconica* from life **(A–N)** and after protargol staining **(O–S)**. **(A)** Narrow side view of an individual with two zooids. **(B)** Narrow side view of an individual with single zooid. **(C)** Wide side view of an individual with two zooids. **(D, E)** Narrow side view of different individuals. **(F–H)** Narrow side view of different curved loricas. **(I)** Anterior portion of body, arrow marks contractile vacuole. **(J)** Posterior portion, arrowhead marks junctional membrane around the inner stalk. **(K)** Posterior portion, arrow marks the expanded lorica base, arrowheads mark the junctional membrane. **(L)** Wide side view of lorica, arrowhead marks the junctional membrane. **(M)** Narrow side view of anterior portion of lorica, arrow marks valve. **(N)** Detail of pellicle, arrowhead marks aboral trochal band. **(O–Q)** Ciliature of three specimens, arrow marks trochal band. **(R)** Oral ciliature, arrow marks the epistomial membrane 2. **(S)** Detail of infundibular polykineties 1–3. EM1, epistomial membrane 1; G, germinal kinety; H, haplokinety; Ma, macronucleus; P1–3, infundibular polykineties 1–3. Scale bars = 100 µm (A–H); 50 µm (O–Q).

1922 ***Cothurnia kellicottiana* Stokes**–Penard, Georg & Cie, Genève, p. 290, Fig. 272.

1935 ***Thuricola* (*Thuricolopsis*) *kellicottiana* (Stokes 1887)**–Kahl, Tierwelt Dtl., p. 785, Fig. 145: 5–6.

1951 ***Thuricola amphora* n. sp.**–Sommer, Arch. Hydrobiol., p. 410–411, Pl. 20, Fig. 36.

1970 ***Thuricola kellicottiana* (Stokes 1887) Kahl, 1935**–Nusch, Arch. Hydrobiol., 37: p. 313, Fig. 33.

1971 ***Thuricola amphora* Sommer, 1951**–Stiller, Peritricha. Fauna Hung., 105: p. 214, Fig. 131c.

1980 ***Thuricola kellicottiana* (Stokes 1887) Kahl, 1935**–Trueba, Beaufortia, 30: p. 131–132, Figs 2e, 3c, d.

1982 ***Thuricola kellicottiana* (Stokes 1887) Kahl, 1935**–Bernerth, Cour. Forsch.-Inst. Senckenberg., 57: p. 170.

1988 ***Thuricola kellicottiana* (Stokes 1887) Kahl, 1935**–Foissner, Hydrobiologia, 166: p. 44 (record only).

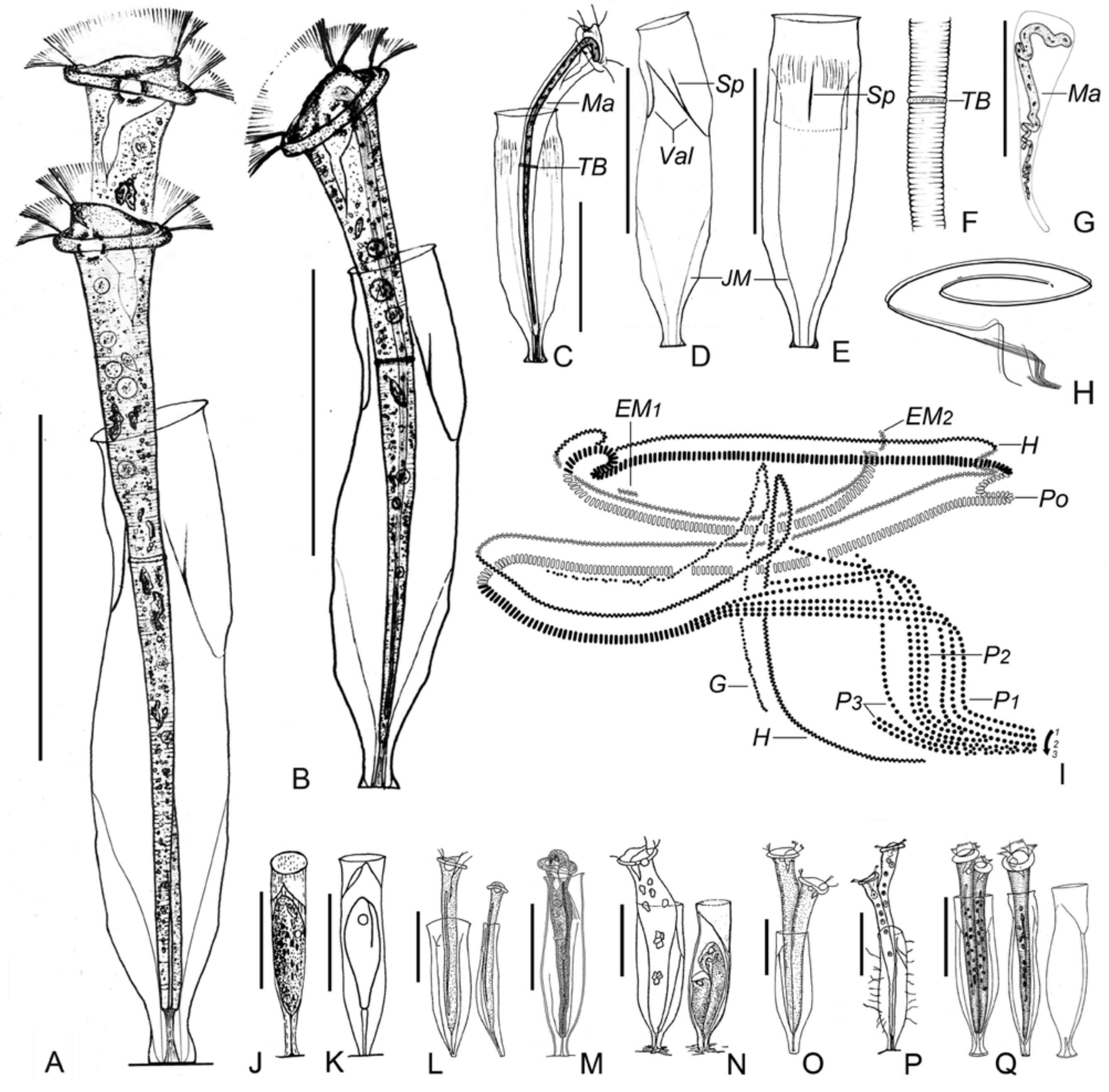

Fig. 4. Morphology and ciliature of *Thuricola kellicottiana*. **(A)** Narrow side view of an individual with two zooids. **(B)** Narrow side view of an individual with single zooid. **(C)** Wide side view of an individual with single zooid, to show macronucleus *in vivo*. **(D)** Narrow side view of lorica. **(E)** Wide side view of lorica. **(F)** Detail of pellicle, to show the transverse striations and aboral trochal band. **(G)** Macronucleus after protargol staining. **(H)** Model pattern of oral ciliature. **(I)** Oral ciliature. **(J)** *T. innixa* from Kellicottiana, 1884. **(K)** *Thuricolopsis kellicottiana* from Stokes, 1887. **(L)** *Cothurnia castellensis* from Penard, 1914. **(M)** *T. amphora* from Sommer, 1951. **(N)** *T. kellicottiana* from Bernerth, 1982. **(O)** *T. kellicottiana* from Nusch, 1970. **(P)** *T. kellicottiana* from Trueba, 1980. **(Q)** *T. kellicottiana* from Shen and Gu (2016). EM1–2, epistomial membrane 1–2; G, germinal kinety; H, haplokinety; JM, junctional membrane; Ma, macronucleus; Po, polykinety; P1–3, infundibular polykineties 1–3; Sp, spine; TB: trochal band; Val, valve. Scale bars = 100 µm.

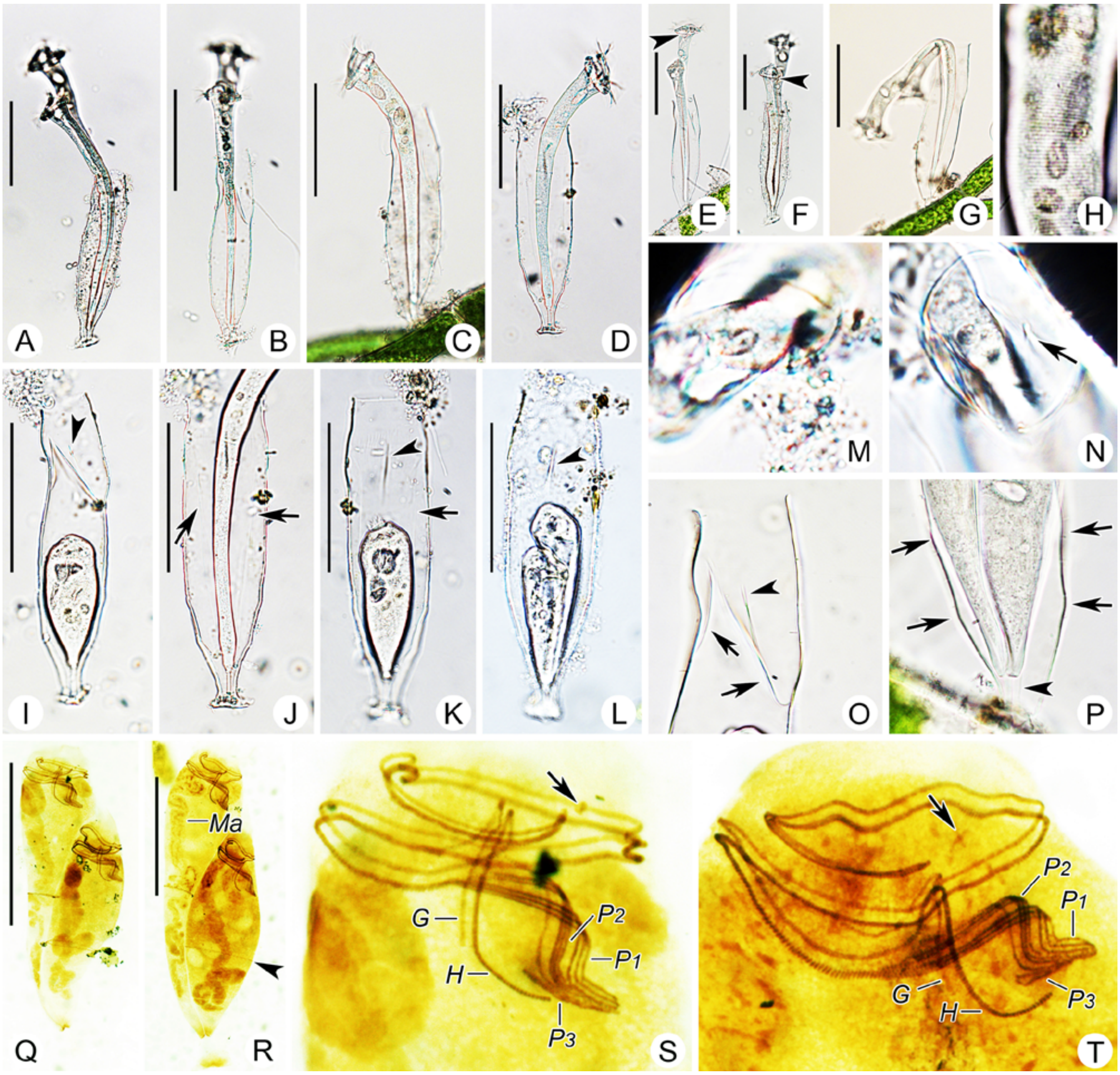

Fig. 5. Photomicrographs of *Thuricola kellicottiana in vivo* **(A–P)** and after protargol staining **(Q–T)**. **(A–C)** Narrow side view of individuals with two zooids **(A, B)** and single zooid **(C)**. **(D)** Wide side view of a single zooid individual. **(E–G)** Narrow side views of different individuals, arrowheads mark contractile vacuoles. **(H)** Detail of pellicle, to show the transverse striations. **(I)** Narrow side view of lorica, arrowhead marks the valve spine. **(J–L)** Wide side view of lorica, arrows mark junctional membrane, arrowheads mark valve spine. **(O)** Narrow side view of anterior portion of lorica, arrows mark valves, arrowheads marks valve spine. **(P)** Posterior portion, arrows mark the bulge in the lorica, arrowhead marks junctional membrane around the inner stalk. **(Q, R)** Ciliature of two specimens, arrowhead marks trochal band. **(S)** Oral ciliature, arrow marks epistomial membrane 2. **(T)** Detail of infundibular polykinety 1–3, arrow marks epistomial membrane 2. G, germinal kinety; H, haplokinety; Ma, macronucleus; P1–3, infundibular polykineties 1–3. Scale bars = 100 μm.

1992 ***Thuricola kellicottiana* (Stokes 1887) Kahl, 1935**–Foissner *et al.*, Landesamtes Wasserwirtsch., 278–280.

2016 ***Thuricola kellicottiana* (Stokes 1887) Kahl, 1935**–Shen & Gu, Fauna Sinica, Invertebrata, 45: p. 333–334. Pl. CLI, Fig. 456a–e.

Although *Thuricola kellicottiana* had been reported several times, studies have not been thorough and its morphology remains vague (Stokes 1887). Thus, a redescription is necessary for the sake of clarification.

Emended diagnosis: Lorica colourless and ca. 160–290 μm long, elliptical to sub-circular in cross-section, nearly symmetrical on wider side and asymmetrical on narrower side. Two anisometric valves in upper third of lorica, larger valve with a spine in the middle; anterior third portion inclined with convex under aperture on secondary valve side, posterior portion tapering to base with two annular bulges to form a long slender rear; aperture clearly elliptical, base expanded. Zooid ca. 180–435 × 20–35 μm *in vivo*. Stalk about 15 μm long. Peristomial lip single-layered and strongly everted. Contractile vacuole dorsally located. Row 1 in infundibular polykinety 3 about twice the length of the other two. Transverse silverlines numbering 95–105 from peristome to trochal band, 100–110 from trochal band to scopula.

Voucher slides: Two voucher slides with protargol stained specimens (registration number: LU20170310-03-01-02) have been deposited in the Laboratory of Protozoology, Ocean University of China.

Description: One or two trumpet-shaped zooids that taper towards the scopula, 230–380 × 20–30 μm (n = 9) *in vivo* (Figs 4A–C, 5A–G). Zooid very flexible and can bend extensively (Fig. 5G). When disturbed, zooid contracts and becomes ellipsoid or broadly conical in outline (Fig. 5I, K, J). Single-layered peristomial lip relatively thin and conspicuously everted, about 40–48 μm across (Figs 4A–C, 5A–G). Peristomial disc obliquely elevated above persitome (Figs 4A–C, 5A–G). Trochal band forming a circular bulge in mid-region of zooid (Fig. 4C, F). About 95–105 striations from peristome to trochal band and 100–110 striations from trochal band to scopula (counted from live cells, n = 5).

One contractile vacuole located at the same level as peristomial lip and on the dorsal wall of infundibulum (Figs 4A, 5E, F). Cytoplasm hyaline and contains numerous small granules and several food vacuoles (Figs 4A, B, 5A–G). Food vacuoles spindle-shaped when freshly detached from cytostome.

Macronucleus vermiform, extends almost entire length of zooid, relatively straight but curved transversely above peristomial lip, widens gradually towards anterior end (Figs 4C, G, 5Q, R). Due to shrinkage during fixation, macronucleus in protargol stained specimens is more twisted than *in vivo* (Figs 4G, 5Q, R).

Stalk about 15 μm long in average, both ends slightly expanded (Figs 4A–C, 5A–G).

Lorica hyaline, about 190–225 μm long. Narrow side about 44–50 μm across and wide side about 51–54 μm across; wall smooth and colourless. Viewed from the narrower side, the upper third portion of lorica inclined (Figs 4A, B, D, 5I, O). Aperture slightly everted and elliptical. Lorica conspicuously convex under aperture on secondary valve side and with a bulge in region above base of main valve (Figs 4A, B, D, 5I, O). Viewed from the wider side, lorica approximately symmetrical. Posterior region tapering and step-like (Figs 4A–E, 5A–G, I–L, P). Lorica widened at base (Figs 4A–E, 5A–G, I–L, P). One main valve and one secondary valve located in the upper third of lorica. Viewed from the wider side, valves include some longitudinal fibril-like elements (Figs 4C, E, 5K, L). Large main valve curved at base, with central spine that is variable in length (Figs 4D, E, 5I, K, L, N). Secondary valve smaller and easily overlooked (Figs 4D, E, 5I, K, L, O). Main valve connected to inconspicuous junctional membrane (Fig. 5I).

Oral cilia approximately 18 μm long. Haplokinety and polykinety commencing together to form a spiral that performs about 1.5 turns around peristomial disc before plunging into infundibulum where they perform a further turn (Figs 4H, I, 5Q–T). Within infundibulum, haplo- and polykineties spiral on opposite walls, terminating at cytostome. The polykinety transforms into three infundibular polykineties (P1–3), each consisting of three parallel rows of kinetosomes (Figs 4L, M, 5S, T). All rows of P1 are equally long (Figs 4I, 5S, T). Three rows of P2 in a staggered arrangement, that is, row 1 commences slightly behind P1 and terminates just before second turn of P1; row 2 starts and ends a little behind row 1; the abstomal end of row 3 starts away from row 2 while the adstomal end terminates beyond row 2 (Figs 4I, 5S, T). Row 1 of P3 commences at the middle of P2 and approximately twice as long as the other two rows; adstomal ends of these three rows disordered (Figs 4I, 5S, T). Epistomial membrane 1 located near the entrance of infundibulum (Fig. 4I). Epistomial membrane 2 ahead of polykinety and haplokinety (Figs 4I, 5S, T). Trochal band in mid-region of zooid (Figs 4C, G, 5Q, R). Germinal kinety parallel to haplokinety and ends at last bend of haplokinety (Figs 4I, 5S, T).

***Thuricola folliculata* Kent, 1881 (Figs 6A–U, 7A–Q, Table 1)**

1881 ***Thuricola folliculata***–Kent, Manual infusoria II., p. 718–719, Pl. XL, Figs 6–8.

1914 ***Cothurnia regalis***–Penard, Mém. Soc. Phys. Hist. Nat. Genève, p. 58–59, Fig. 3: 1–8.

1922 ***Cothurnia crystallina* Ehrenberg, 1838**–Penard, Georg & Cie, Genève, p. 285, Figs 268–271.

1935 ***Thuricola folliculata* (O. F. Müller 1786)**–Kahl, Tierwelt Dtl., 30: p. 785, Fig. 145: 1, 2.

1939 ***Thuricola folliculata* (O. F. Müller, 1786)**–Kudo, Protozoology, second edition, p. 624, Fig. 283e. (Recorded in each editions)

1951 ***Thuricola folliculata* (O. F. Müller 1786) Fromentel-Kent**–Sommer, Arch. Hydrobiol., 44: p. 411, Fig. 37a, b.

1951 ***Thuricola obliqua* n. sp.**–Sommer, Arch. Hydrobiol., 44: p. 413, Fig. 39.

1962 ***Thuricola folliculata* Kent, 1881**–Liebmann, Handbuch der Frischwasser und Abwasser-Biologie I., p. 479, Pl. 14, Fig. 32.

1970 ***Thuricola folliculata* (O. F. Müller 1786)**–Nusch, Arch. Hydrobiol., 37: p. 31–32, Fig. 32.

1971 ***Thuricola folliculata* Kent, 1881**–Stiller, Peritricha. Fauna Hung., 105: p. 214, Fig. 130a, b.

1972 ***Thuricola folliculata* Kent, 1881**–Bick, World Health Organization, Geneva, p. 128–129, Fig. 66.

1980 ***Thuricola folliculata* Kent, 1881**–Eperson, Protistologica, 16: 549–564, Figs 1–26.

1980 ***Thuricola folliculata* Kent, 1881**–Trueba, Beaufortia, 30: 132 (revision).

1983 ***Thuricola folliculata* Kent, 1881**–Jiang, Shen & Gong, Beijing: Science Press., p. 191, Pl. 39, Fig. 335a, b.

1992 ***Thuricola folliculata* Kent, 1881**–Foissner *et al*., Landesamtes Wasserwirtsch. 5/92, 273–277, Figs 1–26

2016 ***Thuricola folliculata* Kent, 1881**–Shen & Gu, Fauna Sinica, Invertebrata., 45: p. 332. Pl. CL, Fig. 453a–c.

Thuricola folliculata is a widespread species first described by Kent (1881) who identified his population as the undefined *Vorticella folliculata* Müller, 1786 from *Cyclops*. Based on a careful reading of Müller's original description, however, and the fact that *Thuricola* has never been detected epizootically, Trueba (1980) thought *Vorticella folliculata* sensu Kent (1881) is in fact a species of *Thuricola* and recognized Kent as the authority for *T. folliculata*. Although *T. folliculata* has been described many times, taxonomic information is still incomplete and past descriptions are often contradictory. Thus, we here supply a redescription based on two populations.

Emended diagnosis: Lorica colourless, about 125–300 μm long, with two valves at upper third; cross-section elliptical to sub-circular, wider side nearly symmetrical, narrower side asymmetrical with a neck at the upper third, aperture elliptical, base expanded. Zooid 165–420 × 15–27 μm *in vivo*. Stalk usually less than 10 μm long. Peristomial lip single-layered and strongly everted. Contractile vacuole dorsally located. Row 1 in infundibular polykinety 3 about two to three times the length of the other two. Transverse silverlines numbering 95–115 from peristome to trochal band, 85–110 from trochal band to scopula.

Voucher slide: A voucher slide with protargol stained specimens of Ningbo population (registration number: LU20170316-01-01) and five voucher slides with protargol stained specimens of Qingdao population (registration numbers: LU20170424-02-01-05) have been deposited in the Laboratory of Protozoology, Ocean University of China.

Description: Morphological data of two populations are in accordance with each other. One or two anisometric zooids about 165–315 × 15–25 μm (n = 13), which live solitarily or coupled with another anisometric zooid in a lorica. If two zooids inhabit the same lorica, when fully extended larger zooid projects nearly one-half its length beyond aperture of lorica, while smaller one usually protrudes almost one-third of its body out of lorica (Figs 6A, C, 7A–C). If solitary, zooid is relatively small (Figs 6B, 7D). Zooid slender, widest at peristomial lip, and flexible, often bending (Fig. 7E–G). Contracted bodies elongate obovate (Fig. 7H). Single-layered, thin peristomial lip protrudes well beyond body, about 36–50 μm in diameter (Figs 6A–C, 7A–E). Peristomial disc obliquely elevated above peristome lip (Figs 6A–C, 7A–E). Pellicular striations inconspicuous at low magnifications but distinct at 400 x magnification and higher (Figs 6G, 7M). About 95–115 striations from peristome to trochal band (n = 6) and 85-110 striations from trochal band to scopula (n = 8). Trochal band forms an inconspicuous bulge near midbody (Figs 6D, G, 7M).

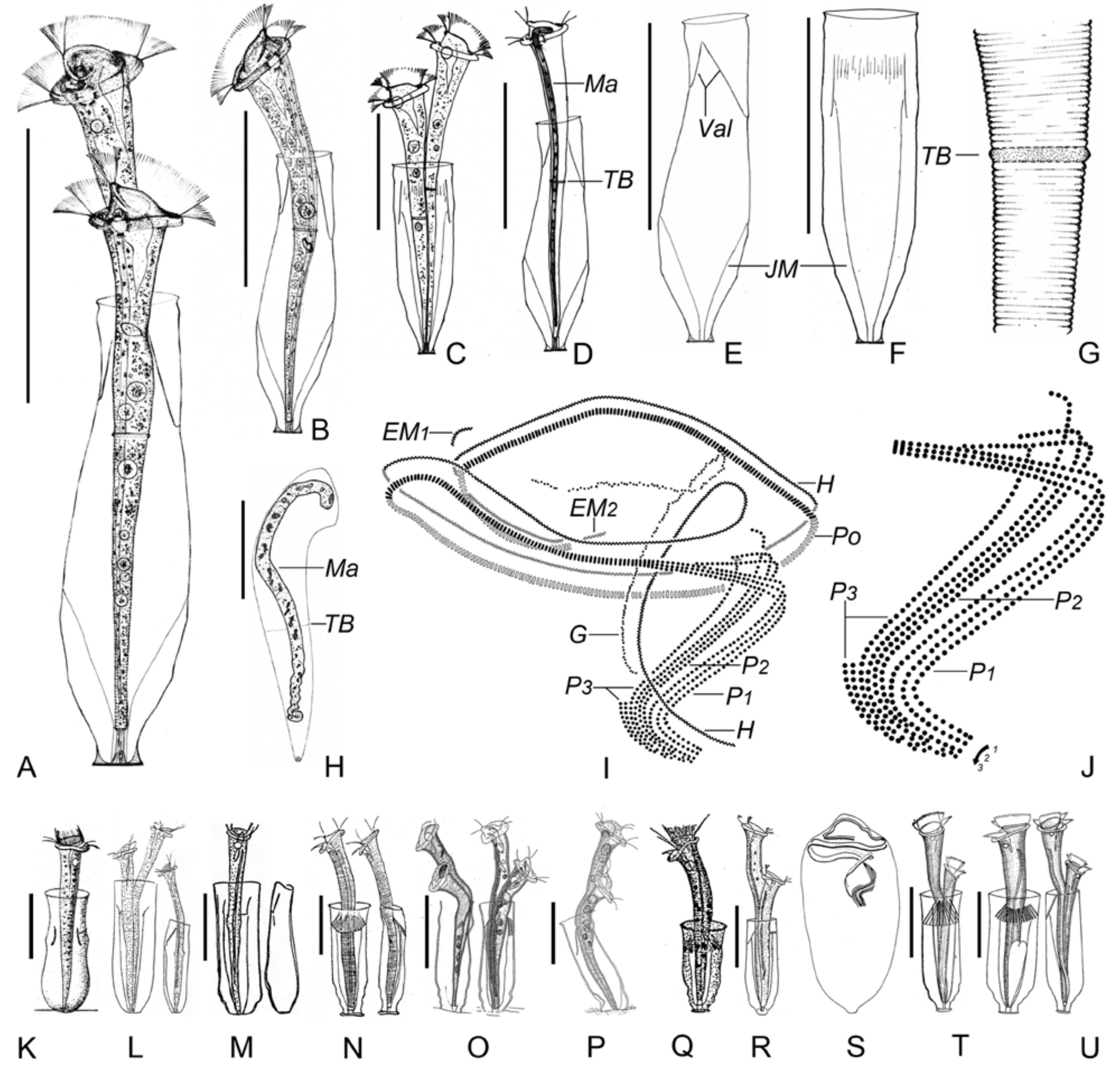

Fig. 6. Morphology and ciliature of *Thuricola folliculata*. **(A)** Narrow side view of an individual with two zooids. **(B)** Narrow side view of an individual with single zooid. **(C)** Wide side view of an individual with two zooids. **(D)** Macronucleus *in vivo*. **(E)** Narrow side view of lorica. **(F)** Wide side view of lorica. **(G)** Detail of pellicle, to show the transverse striations and aboral trochal band. **(H)** Macronucleus after protargol staining. **(I)** Oral ciliature. **(J)** Detail of infundibular polykineties. **(K)** *T. folliculata* from Kent (1881). **(L)** *Cothurnia regalis* from Penard (1914) (synonym of *T. folliculata*). **(M)** *Cothurnia crystallina* from Penard (1922) (synonym of *T. folliculata*). **(N)** *T. folliculata* from Kahl (1935). **(O)** *T. folliculata* from Sommer (1951). **(P)** *T. obliqua* from Sommer (1951). **(Q)** *T. folliculata* from Liebmann (1962). **(R)** *T. folliculata* from Nusch (1970). **(S)** Ciliature of *T. folliculata* from Eperson (1982). **(T)** *T. folliculata* from Jiang *et al.* (1983). **(U)** *T. folliculata* from Shen and Gu (2016). EM1–2, epistomial membrane 1–2; G, germinal kinety; H, haplokinety; JM, junctional membrane; Ma, macronucleus; Po, polykinety; P1–3, infundibular polykineties 1–3; TB, trochal band; Val, valve. Scale bars = 100 μm.

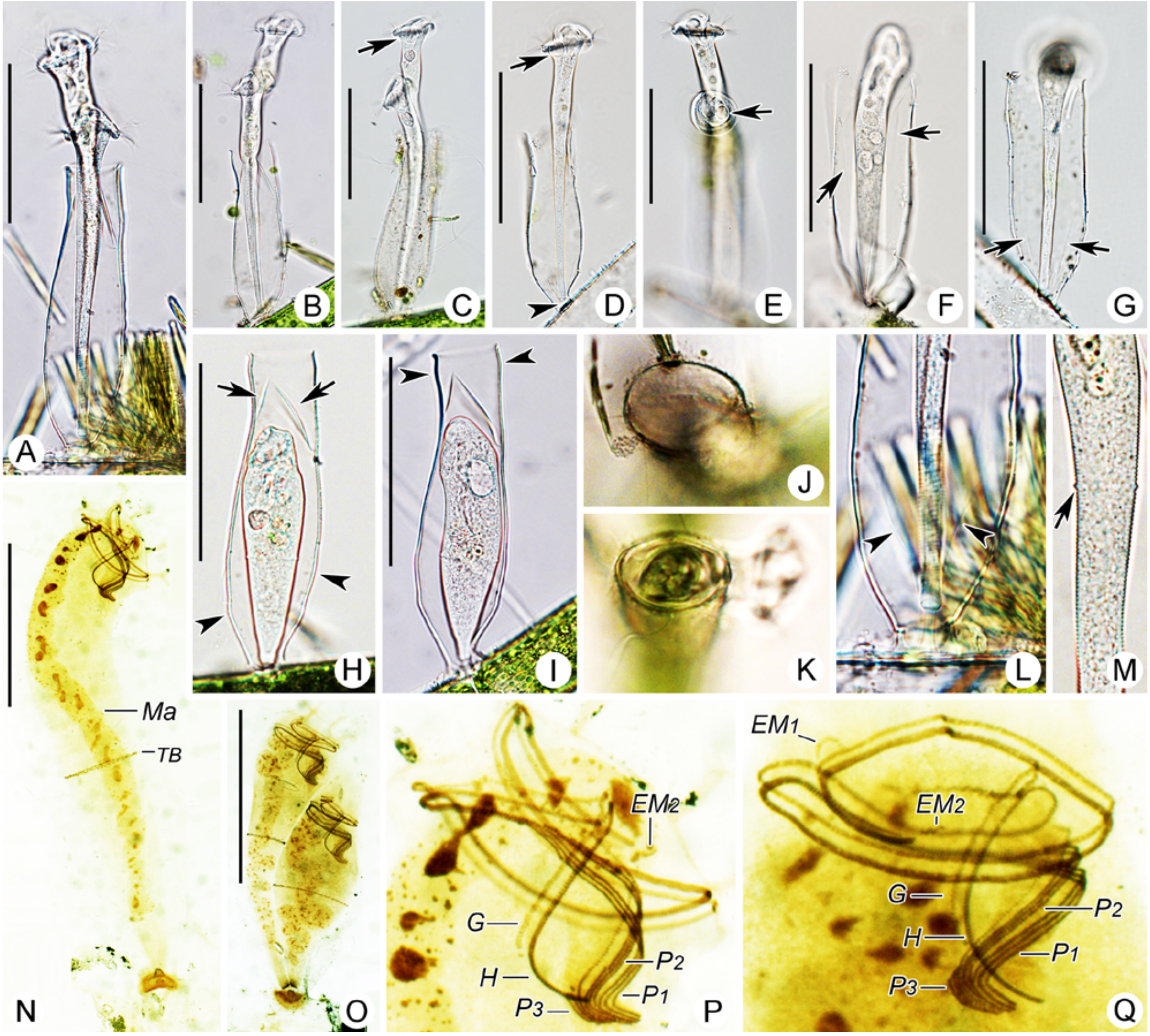

Fig. 7. Photomicrographs of *Thuricola folliculata in vivo* **(A–M)** and after protargol staining **(N–Q)**. **(A–E)** Narrow side views of different individuals with two zooids **(A–C, E)** and single zooid **(D)**, arrows mark contractile vacuole. **(F, G)** Wide side views of lorica, arrows mark the junctional membrane. **(H, I)** Narrow side views of lorica, arrows in H mark valves, arrowheads in H mark posterior bulge of lorica, arrowheads in (I) mark anterior bulge of lorica. **(J, K)** Aperture of lorica. **(L)** Posterior portion of lorica, arrowheads mark junctional membrane. **(M)** Detail of pellicle, to show the transverse striations, arrow marks the aboral trochal band. **(N, O)** Ciliature of two specimens. **(P, Q)** Oral ciliature. EM1–2, epistomial membrane 1–2; G, germinal kinety; H, haplokinety; Ma, macronucleus; P1–3, infundibular polykineties 1–3; TB, trochal band. Scale bars = 100 µm.

Contractile vacuole located at the level of peristomial lip near dorsal wall of infundibulum (Figs 6A, 7C–E). Cytoproct not observed. Endoplasm highly granulated with a few fusiform or globular food vacuoles (Figs 6A–C, 7A–D), usually scattered in the anterior portion of body.

Macronucleus vermiform extending almost entire length of zooid (Figs 6D, H, 7N, O), rather hyaline, and invisible *in vivo*. Due to significant shrinkage in the fixation process, macronucleus highly twisted in most of protargol stained samples (Fig. 7O). One extended individual was obtained in protargol preparations, which revealed that main section of macronucleus to be relatively straight with anterior section curved transversely, gradually widening towards top (Figs 6D, 7N).

In 12 investigated samples, only one individual had a 13 μm long stalk, while stalks of the others being less than 10 μm long: on average, 7.3 μm long in Ningbo population and 4.6 μm long in Qingdao population. Two ends of stalk slightly widened (Figs 6A–E, 7A, D).

Lorica about 125–190 μm tall, with colourless wall. Lorica nearly symmetrical and about 48–52 μm wide on its wider side (Figs 6C, F, 7F, G), asymmetrical and about 39–51 μm wide on its narrower side. Viewed from the narrower side, lorica reaches its greatest width at the posterior third and neck-like at the anterior third (Figs 6E, 7H, I). Aperture slightly everted and elliptical (Figs 6E, 7H, I). Usually with a convex ring immediately under aperture (Figs 6E, 7H, I). Rear third conical tapered with two annular bulges (Figs 6A–F, 7A–D, H, I). Lorica base expanded (Figs 6A–F, 7H, I, L). Two valves in anterior third of lorica, one large (main) and one small (secondary) (Figs 6A, B, D, E, 7A–D, H, I). One larger main valve and one smaller secondary valve. Both valves contain some longitudinal fibriform elements (Figs 6C, F, 7F, G). Base of main valve bent (Figs 6E, 7H, I).

Oral ciliature largely resembles previous two species in this study. Haplokinety and polykinety make approximately a 1.5 turn around peristome and a further circuit on opposite walls inside infundibulum (Figs 6I, 7N–Q). P1–P3 composed of three distinct rows of kinetosomes each (Figs 6I, J, 7N–Q). P1 bends twice and comprises three equally long rows (Figs 6I, J, 7N–Q). P2 ends near second bend of P1 and between P1 and P3. Three rows of P2 also form a staggered pattern, i.e. row 1 commences and terminates before row 2; row 3 starts and ends a little behind row 2, with abstomal end detached from rows 1 and 2 (Figs 6I, J, 7N–Q). P3 converges with P1 at adstomal end. Row 1 of P3 extremely long, about twice to three times as long as two other rows (Figs 6I, J, 7N–Q). Epistomial membrane 1 short, located near opening of infundibulum (Figs 6I, 7Q). Epistomial membrane 2 located before haplo- and polykinety (Figs 6I, 7P, Q). Trochal band in mid-region of zooid. Infundibular part of haplokinety accompanied by germinal kinety until its last curve (Figs 6H, 7N, O).

Molecular data and phylogeny: The new SSU rDNA sequences of these three species have been deposited in the GenBank database with length (bp), GC content and GenBank accession numbers as follows: *T. obconica* – 1,592, 42.46%, MH035973; *T. kellicottiana* – 1,596, 42.11%, MH035974; *T. folliculata* – 1,605, 42.12%, MH035975. There were no differences between the SSU rDNA sequences of the two populations of *T. folliculata* (Ningbo pop I and Qingdao pop II), thus only pop I's (Ningbo) data was deposited in the GenBank database. The sequence of *T. obconica* differs from *T. folliculata* and *T. kellicottiana* in two and nine nucleotides, respectively. *T. folliculata* differs from *T. kellicottiana* in seven nucleotides.

The phylogenetic trees inferred from the SSU rDNA sequences using ML and BI methods resulted in the same topologies, therefore, only the ML tree (with nodal support from both methods) is shown (Fig. 8). The three species of *Thuricola* clustered together with a putative species of *Vaginicola* (KU363269, star) (62% ML, 0.77 BI), and then grouped with other *Vaginicola* species and the genus *Usconophrys* to form a sister clade (40% ML, 0.60 BI) to the clade of cothurnids (63% ML, 0.80 BI), thus falling within the clade of loricate sessilids (Vaginicolidae, Usconophryidae) and clearly distinct from other taxa without lorica.

DISCUSSION

Comments on the genus *Thuricola*: Since it was erected by Kent (1881) dozens of nominal species have been reported. In many earlier studies, however, the information necessary for species circumscription, such as morphometric data, detailed live features, and ciliature, were frequently insufficiently described or unreported (Kahl 1935, Eperson 1980, Trueba 1980, Foissner *et al.* 1992, Shen and Gu 2016).

Hitherto, seventeen species were recognized within *Thuricola*. Prior to this study, *T. folliculata, T. valvata* Wright, 1858 and *T. parafolliculata* Sun *et al.*, 2009 were investigated using silver staining methods. Unfortunately, this staining for *T. folliculata* was unsuccessful (Eperson 1980) and ciliature of *T. valvata* is dubious because the staining is not clearly enough to confirm the details of P3 (Sun *et al.* 2009).

Interestingly, the ciliature data of the three species in our work (*T. obconica*, *T. kellicottiana* and *T. folliculata* based on present study) and *T. parafolliculata* are basically congruent. Due to the lack of ciliature data in respect to other congeners, however, we cannot draw any conclusions as to whether this is a coincidence or a phenomenon shared among the *Thuricola* spp. From our perspective, we would lean towards the latter. The main differences among these three species lie in lorica

Table 2. Morphological comparison of *T. obconica* Kahl, 1933, *T. kellicottiana* Stokes, 1882 and *T. folliculata* Kent, 1881 with their congeners.

Characters^{a}	*T. obconica*	*T. kellicottiana*	*T. folliculata*	*T. obconica*	*T. kellicottiana*	*T. folliculata*	*T. similis*	*T. innixa*	*T. elegans*	*T. constricta*	*T. limbata*
Body length	195–310	230–377	165–313	270	180–435	180–420	220–290, average 250	145–190	100	205–291	125–160
Body width	18–23	21–27	16–23	ca. 32	20–35	20–27	N/A	18–27	N/A	20–30	23
PL width	38–48	40–48	36–50	ca. 45	30–50	35–55	35–42	30–40	N/A	37–42	33
Lorica length	155–210	190–225	127–186	120–220	160–290	125–300	158–248, average 183	110–200	not exceed 65	136–174	115–120
Lorica width	39–50^{N} 47^{W}	44–50^{N} 51–54^{W}	39–51^{N} 48–52^{W}	48–63	40–50^{Nb} 45–55^{b} (74 extremely)^{W}	45–55 (generally) 75 (extremely)	40–57	ca. 30–52	N/A	43–70	40–43
Aperture width	31–43^{N} 47^{W}	32–40^{N} 49–51^{W}	32–39^{N} 48–52^{W}	30–50	27-50^{N} 40-65^{Wb}	45–55 (generally) 75 (extremely)	average 45	30–35	N/A	31–67	36
Stalk length	relatively long (6–14, average 11)	usually long (4–18, average 14)	short (pop I: 5–13, average 7; pop II: 3–6, average 5)	long or relatively long	usually long (7–47, usually less than 25μm)	short or absent (usually less than 10)	long (10–20, average 14)	long or short	short or absent	very short	absent*
Number of striations^{1}	104–120	95–103	97–115	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Number of striations^{2}	96–111	100–108	87–108	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Ma	long, vermiform	long, vermiform	long, vermiform	long, vermiform	long, vermiform	long, vermiform	long, vermiform	long, vermiform	short, horse-shoe–shaped	N/A	long, vermiform
Habitat	BW (S: 11)	BW (S: 6.5)	FW or BW (S: 2)	BW	FW	FW	FW	FW	BW (S: 7)	BW or FW	BW
Data source	Original	Original	Original	List 1	List 2	List 3	Bock 1963	List 4	Biernacka 1963	List 5	Xu 1990

[a] Data based on randomly selected living specimens, measurements in μm; [b] Rough values; [N] Narrow side; [W] Wide side; [1] From peristome to trochal band; [2] From trochal band to scopula; ? possibly incorrect; BW, brackish water; FW, fresh water; N/A, not available; PL, peristomial lip; S, salinity.

List 1: Kahl (1933, 1935); Bock (1952); Trueba (1980).

List 2: Kellicott (1884); Stokes (1887, 1888b); Entz (1904); Penard (1914, 1922); Kahl (1935); Sommer (1951); Nasch (1970); Stiller (1971); Trueba (1980); Bernerth (1982), Foissner (1988); Foissner *et al*. (1992); Shen and Gu (2016).

List 3: Kent (1881); Penard (1914, 1922); Kahl (1935); Kudo (1939); Sommer (1951); Liebmann (1962); Stiller (1971); Bick (1972); Trueba (1980); Jiang *et al* (1983); Foissner *et al*. (1992); Shen and Gu (2016).

List 4: Fromentel (1874); Kent (1881); Stokes (1882, 1887); Kahl (1935); Trueba (1980); Shen and Gu (2016).

List 5: Küsters (1974); Trueba (1980); Shen and Gu (2016).

Table 3. Lorica characteristics of *T. obconica*, *T. kellicottiana* and *T. folliculata* and their congeners (data source same as Table 2).

Characters	Shape quotient	Cross-section (anterior/posterior)	Aperture	Narrow side	Wide side	Posterior constricted portion	Base	Valve (number)		
				Anterior	Middle	Posterior				
T. obconica	3.5–4.2	elliptical/sub-circular	elliptical, everted	curved above valve	characterless	taper to base with two bulges	slightly bulge at valve portion, nearly symmetrical	moderate long	flat, expand	1
T. kellicottiana	4.0–4.5	elliptical/sub-circular	elliptical, everted	oblique, bulge under aperture at secondary valve side, bulge above main valve base	characterless	taper to base with two bulges	slightly bulge at valve portion, nearly symmetrical	long	flat, expand	2, spine
T. folliculata	3.2–4.0	elliptical/sub-circular	elliptical, everted	circular bulge under aperture, neck-like	characterless	taper to base with two bulges	slightly bulge at valve portion, nearly symmetrical	short	flat, expand	2
T. obconica	3.0–5.0	elliptical to sub-circular/ sub-circular	elliptical to sub-circular, everted	slightly curved^{a}	characterless	taper to base	nearly symmetrical	moderate long	flat, expand	1
T. kellicottiana	4–5	elliptical/sub-circular	elliptical, everted	N/A	characterless	taper to base with two bulges	nearly symmetrical	long	flat, expand	2 or 1^{b}
T. folliculata	3	elliptical/sub-circular	elliptical, everted	bulge under aperture, neck-like	characterless	taper to base with two bulges	nearly symmetrical	short	flat, expand	2 or 1^{b}
T. similis	4.0–4.5	sub-circular/ sub-circular	widest, evert	N/A	fluctuant	taper to base with bulges	nearly symmetrical^{a}	long	flat	1
T. innixa	3	circular/circular	circular	N/A	characterless	taper in a curve to base	nearly symmetrical^{a}	inconspicuous	N/A	2
T. elegans	N/A	N/A	N/A	neck-like, with annular beads	characterless	taper to base with bulges	nearly symmetrical^{a}	inconspicuous	N/A	2
T. constricta	3	elliptical/ elliptical^{a}	elliptical, slightly wavy	a clear constriction above middle	characterless	taper to base with bulges^{a}	nearly symmetrical	short	flat, expand	1
T. limbata	3	elliptical/sub-circular	clearly wavy	narrowed slightly	characterless	taper to base in curve^{a}	nearly symmetrical^{a}	inconspicuous	flat^{a}, expand^{a}	1

^{a} Based on drawing and description; ^{b} Likely small secondary valve was overlooked; N/A, not available.

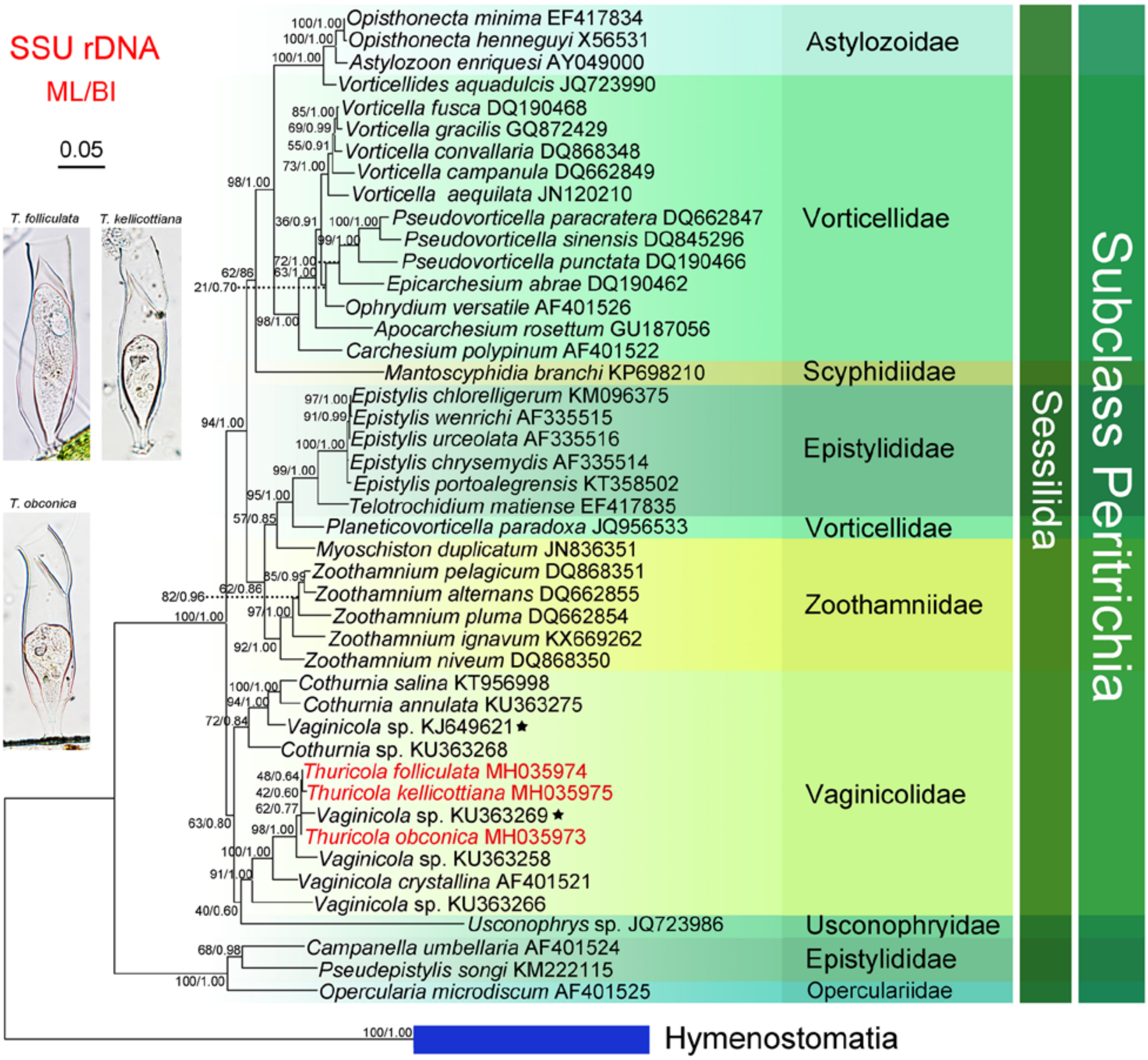

Fig. 8. Phylogenetic tree inferred from SSU rDNA sequences, revealing the phylogenetic positions of *Thuricola folliculata*, *Thuricola kellicottiana* and *Thuricola obconica* (red fonts). The two sequences marked by stars (KJ649621 and KU363269) are probably misidentified. Numbers near nodes denote Maximum likelihood (ML) bootstrap values and Bayesian inference (BI) posterior probability, respectively. The scale bar indicates five substitutions per 100 nucleotides. Systematic classification follows Sun *et al.* (2012) and Gao *et al.* (2016b).

structure. Thus, complete observations of live materials, especially wider and narrower side views of lorica, are extremely important for species separation in vaginicolids.

Some literatures once state the JM is a semi-cylindrical cuticle, which is probably incorrect. Our work demonstrates it cannot be semi-cylindrical, because its posterior portion possesses a circular cross-section definitely. Furthermore, rear-end of JM connects to bottom of lorica rather than the aboral end of zooid as reported by some previous work.

Comments on *Thuricola obconica*: *T. obconica* was originally reported by Kahl (1933) from brackish water in Bottsand near Kiel, Germany. He later added more information on this species (Kahl, 1935). Bock (1952) reported two species from brackish water, namely

T. obconica and *T. kamptostoma*. The lorica of the former is plumper than that of Kahl's form. According to Bock's description, *T. kamptostoma* has a lorica that is slimmer, longer and smoother than that of *T. obconica*; otherwise, however it resembles Kahl's report of *T. obconica*, and we therefore followed Trueba's (1980) proposal to recognize *T. kamptostoma* as a synonym of *T. obconica*.

Biernacka (1963) and Küsters (1974) also described brackish water populations of *T. obconica*. Their figures and descriptions did not accord well with Kahl's report, however. From Biernacka's drawing, his population has two valves rather than the one valve in Kahl's report. Küsters indicated that there are annular ridges in the middle portion of the lorica, which does not occur in the original population. Therefore these two thuricolas cannot be *T. obconica*. Recently, Shen and Gu (2016) reported a freshwater form under the name of *T. obconica*, but the lorica in this form is not curved above the valve (from the drawing). Thus, it is probably distinct species of *Thuricola*.

Our population matches well with Kahl's description in basic morphological characters (cell and lorica size, valve number and inner stalk length) and habitat (brackish water). Although Kahl did not describe the lorica curve above the valve, he did illustrate this character. It is therefore reasonable for us to identify our form as *T. obconica* (Kahl 1933, 1935; Trueba 1980).

Within the genus *Thuricola*, only four species, namely *T. obconica* Kahl, 1933, *T. elegans* Biernacka, 1963, *T. constricta* Küsters, 1974 and *T. limbata* Xu, 1990, have been found in brackish water habitats. *T. obconica* can be easily distinguish from the others by its distinctive feature: i.e. that the lorica is clearly curved above the valve.

Compared to *T. elegans*, *T. obconica* is separated by its longer lorica (155–210 μm vs. less than 65 μm), number of valves (1 vs. 2) and the shape of the macronucleus (long and vermiform vs. short and horseshoe-like) (Biernacka 1963).

T. constricta differs from *T. obconica* mainly by a clear constriction above the middle portion, which is lacking in *T. obconica* (Küsters 1974, Trueba 1980).

T. limbata is distinguished from *T. obconica* by the shape of the lorica: the aperture is circular and clearly wavy and not bent in the front of the valve (Xu 1990).

The population of *T. kellicottiana* reported in this paper was also isolated from a brackish water habitat. It, however, has two valves and a spine in the middle of the main valve, and thus it cannot be conspecific with *T. obconica*.

Comments on *Thuricola kellicottiana*: Based on the revision of Trueba (1980), so far *Thuricola kellicottiana* had been investigated for at least fifteen times, however its taxonomic characteristics are still incomplete. In the literature, the descriptions of this species are often contradictory, especially in respect to whether there are one or two valves. Since the secondary valve is small, we believe that it may have been overlooked in some previous studies. After comparing our population with all valid thuricolas, we identify it with *T. kellicottiana* because it is consistent with the reports in the literature in terms of the basic morphology (zooid size, lorica size, strongly constricted lorica tail, long inner stalk and two valves), even though the inconspicuous spine in the middle of main valve has not been mentioned previously (Kellicott 1884; Stokes 1887, 1888b; Penard 1914, Kahl 1935, Sommer 1951, Nusch 1970, Stiller 1971, Trueba 1980, Bernerth 1982, Foissner *et al*. 1992, Shen and Gu, 2016).

Thuricola kellicottiana has previously been found only in fresh water whereas our population was collected from brackish water (salinity 6.5 ‰). This species is characterized by its closure apparatus: one larger valve with a spine in the middle and one smaller valve. Only three other thuricolas, namely *T. folliculata*, *T. innixa* Stokes, 1882 and *T. elegans* Biernacka, 1963, have previously been reported as having two valves. These valves, however, lack a spine. Considering that this tiny structure is easily overlooked, the presence or absence of a spine is not enough to differentiate *T. kellicottiana* from these other species. It is also noteworthy that since the smaller secondary valve is also easily overlooked, it is not clear whether it is also present in some thuricolas that have previously been reported as only having one valve. *T. kellicottiana* can be distinguished from most of these other species by its long inner stalk except *T. similis*.

The shape of the lorica of *T. folliculata* is different from *T. kellicottiana*, in the former this is usually a convex ring occurring immediately under the aperture, whereas in the latter it is obviously convex at the secondary valve side with a bulge occurring on the base of the main valve.

T. innixa is a poorly known species, but its inner stalk is short or absent compared to the usually long stalk in *T. kellicottiana*. Besides, the base of the lorica is rounded and tapered in a curve while it is strongly constricted and tapered step-like in *T. kellicottiana* (Stokes 1882, 1888a).

T. elegans can be distinguished from *T. kellicottiana* by the much smaller lorica (less than 65 μm long vs.

190–225 µm long), the shape of macronucleus (short and horseshoe-like vs. long and vermiform) (Biernacka 1963).

T. similis is similar to *T. kellicottiana* in its inner stalk length but it only has one valve (vs. two) and is found in activated sludge (vs. fresh or brackish water) (Bock 1963).

Comments on *Thuricola folliculata*: *T. folliculata* is a widespread species that has been reported many times. Our population corresponds well with previous descriptions in the lorica structure and macronucleus shape, stalk length and zooid size. As with *T. kellicottiana*, there is again an inconformity in the number of valves reported in the literature. Consistent with a few previous studies, our population consistently has two valves in the lorica (Penard 1922). Most researchers, however, report only one valve in the lorica (Kent 1881, Penard 1922, Kahl 1935, Kudo 1939, Sommer 1951, Liebmann 1962, Nusch 1970, Bick 1972, Trueba 1980, Jiang *et al.* 1983, Foissner 1992; Shen and Gu 2016). Considering that the secondary valve is small, possibility of neglect cannot be excluded.

Up to now, eleven thuricolas were founded in fresh water. Besides *T. folliculata*, two of these species definitely possess two valves: *T. kellicottiana* and *T. innixa*. As mentioned above, *T. kellicottiana* differs from *T. folliculata* in its lorica shape and stalk length. *T. innixa* can also be distinguished from *T. folliculata* by its lorica shape: tapering to the base in a curve (vs. step-like) and circular cross-section (vs. clearly elliptical in the front third portion) (Fromentel 1874, Kent 1881, Stokes 1887, Penard 1914, Kahl 1935, Trueba 1980).

We followed Trueba (1980) to treat the following forms as synonyms of *Thuricola folliculata*: *Cothurnia regalis* reported by Penard (1914), *Cothurnia crystallina* sensu Penard (1922) and *Thuricola obliqua* Sommer, 1951.

Phylogenetic analysis: As previous studies have shown (Sun *et al.* 2016, Zhuang *et al.* 2016), the loricate sessilids (Vaginicolidae and Usconophryidae) formed a branch separated from the aloricate sessilids even though the confidence value was relatively low (63% ML, 0.80 BI). Considering that the addition of three new sequences of *Thuricola* in this study has reduced the support for this branch, additional new sequences are needed in order to reveal the true phylogenetic relationships within the loricate sessilids.

In terms of the loricate group, the Vaginicolidae is formed of two sub-clades: *Cothurnia* spp. together with *Vaginicola* sp. (KJ649621, star) (possibly misidentified) form one sub-clade, while the other vaginicolids group into the other sub-clade, which it sister to *Usconophrys*. Since *Cothurnia* has a stalk outside the lorica while other vaginicolids and *Usconophrys* lack this stalk, this structure is probably a key character for the evolution of vaginicolids. Strangely, our three thuricolas group with *Vaginicola* sp. (KU363269, star). Considering this sequence (KU363269) was obtained from environmental sequencing without morphological information, it cannot be excluded that this species was misidentified.

To date, about two hundred nominal species have been assigned in the family Vaginicolidae, however, the SSU rDNA sequences are only available for eight taxa. Furthermore, only one species (*Cothurnia salina*) has thorough taxonomic data; most other sequence data were based on ecological samples without species identities, leaving open the possibility that there have been some misidentifications and synonyms. It is also possible that the present topology is biased due to limited taxon sampling. Additional sequences of vaginicolids are required in order to construct a more robust phylogeny for this complex group.

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