



Morphology and molecular analyses of four epibiotic peritrichs on crustacean and polychaete hosts, including descriptions of two new species (Ciliophora, Peritrichia)

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Abstract

Four epibiotic sessilid peritrichs, i.e., *Zoothamnium wilberti* n. sp., *Baikalonis microdiscus* n. sp., *Epistylis anastatica* (Linnaeus, 1767) Ehrenberg, 1830, and *Rhabdostyla commensalis* Möbius, 1888, were isolated from one syllid polychaete and three crustacean hosts in Qingdao, China. For each species, specimens were observed both in vivo and following silver staining. Their SSU rDNA was also sequenced for phylogenetic analyses. *Zoothamnium wilberti* n. sp. is characterized by the appearance of its colony, which is up to 350 μ m high, and usually has fewer than 16 zooids, and the dichotomously branched stalk with transverse wrinkles, the conspicuously conical peristomial disc, and infundibular polykinety 3 comprising three isometric ciliary rows. *Baikalonis microdiscus* n. sp. can be recognized by its barrel-shaped zooid, small peristomial disc, smooth and short stalk, and its unusual infundibular polykinety 3 comprising a long inner row and a short outer row. Two poorly known species, i.e., *Epistylis anastatica* and *Rhabdostyla commensalis*, are redescribed and redefined. Phylogenetic analyses reveal that: (i) *R. commensalis* is closely related to the family Astylozoidae rather than to the morphologically similar Epistylidiidae; (ii) *B. microdiscus* n. sp. is sister to the family Scyphidiidae; (iii) *E. anastatica* groups with vorticellids and ophrydiids, which further supports the polyphyly of the genus *Epistylis*; and (iv) *Z. wilberti* n. sp. is nested within the Zoothamniidae, as expected.

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Keywords: Crustacea; Epibionts; Peritrich ciliates; Polychaete; SSU rDNA; Taxonomy

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Introduction

Peritrich ciliates are adapted to a wide range of ecological conditions and are found worldwide where there is sufficient water for their survival (Entz 1884; Foissner et al. 1992; Hu et al. 2019; Kahl 1935; Kent 1880–1882; Kent, 1880; Kent 1880–1882; Li et al. 2019; Lynn 2008; Penard 1922; Song et al. 2009; Wilbert and Song 2005). Members of the subclass Peritrichia Stein, 1859 are assigned to one of two orders, Sessilida or Mobilida, based on the structure of both the aboral region and the organelles of attachment (Lynn 2008). According to Foissner et al. (2009), the Sessilida contains about 105–140 genera and more than 800 species.

Although sessilids have been investigated for nearly 350 years, many species still lack complete morphological information based on modern criteria. Furthermore, the majority of investigations have focused mainly on sessilids attached to aquatic plants and submerged abiotic substrates (Foissner et al. 1992; Hu et al. 2019; Kahl 1935; Kühner et al. 2016; Liang et al. 2019; Lu et al. 2019; Shen et al. 2017; Sun et al. 2017; Wang and Ji 2019). By contrast, sessilid epibionts on aquatic animals are insufficiently studied despite the fact that they constitute a diverse and speciose assemblage (Clamp 1973, 1987, 1991; Fernandez-Leborans and Tato-Porto 2000; Foissner et al. 1999; Guhl 1979; Keiser 1921; Kitching 2003; Matthes, 1982; Mayen-Estrada and Clamp, 2016; Mikac et al. 2019; Nenninger, 1948; Ramírez-Ballesteros et al. 2018; Scheubel 1973; Schödel 1987, 2004, 2006; Sommer 1951; Stiller 1971; Wang et al. 2016). Moreover, molecular data for epibiotic peritrichs are scant despite the importance of such data in helping to resolve evolutionary relationships within the Peritrichia (Gentekaki et al. 2017; Jiang et al. 2019; Sun et al. 2010; Wang et al., 2019; Zhuang et al. 2018).

In the present work species belonging to each of the following four genera are investigated: *Epistylis* Ehrenberg, 1830, *Rhabdostyla* Kent, 1881, *Baikalonis* Jankowski, 1982 and *Zoothamnium* Bory de St. Vincent, 1824. *Epistylis* contains more than 260 nominal species (H. Berger, pers. comm.) and is characterized by its colonial habit, non-contractile stalk, well-defined peristomial lip, oral ciliary rows completing less than two circuits before entering the infundibulum, and transverse silverline system (Corliss 1979; Kahl 1935; Kühner et al. 2016). *Rhabdostyla* contains more than 60 nominal species (H. Berger, pers. comm.) and is commonly solitary with a very short, non-contractile stalk (Kahl 1935; Kent 1881). This genus is poorly known in the sense that the infraciliature has not been described for any species and only two gene sequences (JF523346 and GU586189), both from unidentified species, are available in GenBank. *Baikalonis* contains three species and is characterized by its solitary habit, short unbranched stalk with a straight spasmoneme that extends its entire length, *Opercularia*-like peristomial disc, and the mode of contraction during which the stalk shortens longitudinally and is enveloped by the zooid (Jankowski 1982; Warren 1988). Hitherto, both ciliature and sequence data were lacking for this genus. *Zoothamnium* is a speciose

genus of colonial sessilids having a transverse silverline system and a continuous spasmoneme that extends throughout the entire colony and causes the stalk to contract in a “zig-zag” fashion (Bory de St. Vincent 1824; Corliss 1979). There are about 140 nominal species of *Zoothamnium* (H. Berger, pers. comm.), most of which lack data concerning the silverline system, infraciliature, or DNA gene sequences (Jankowski 2007; Ji et al. 2015; Schuster and Bright 2016; Shen et al. 2017).

In this study we describe the morphology of four epibiotic sessilid peritrichs representing each of the above four genera, and analyze their SSU rDNA-based phylogeny.

Material and methods

Sampling and morphological methods (Fig. 1A–D)

All species were collected in Qingdao, China. Two populations of *Epistylis anastatica* attached to cyclopoid copepods were collected on 27 March and 3 July 2017 from freshwater ponds at two different parks (population I: N35°55'37"; E120°12'00" and population II: N36°03'58"; E120°20'24", Fig. 1B, respectively). *Baikalonis microdiscus* n. sp. was isolated from a harpacticoid copepod collected on 22 June 2017 from an intertidal pool (N36°03'03"; E120°21'01") (Fig. 1C). *Zoothamnium wilberti* n. sp. was isolated from the crustacean *Gammarus* sp., collected on 13 June 2017 at the same locality as *Baikalonis microdiscus* n. sp. *Rhabdostyla commensalis* was isolated from the polychaete *Salvatoria* sp., which was collected on 4 September 2017 from an intertidal pool near Zhanqiao Pier (N36°03'39"; E120°19'09") (Fig. 1D).

Live cells were observed by differential interference contrast microscopy. They were first observed together with their host at low magnification and then detached from the host using a micropipette for observation at high magnification. The ciliature was revealed using the protargol staining method (Ji and Wang 2018). The protargol powder was manually synthesized following the method prescribed by Pan et al. (2013). Both “wet” and “dry” silver nitrate methods were used to demonstrate the silverline system (Foissner 2014; Song and Wilbert 1995). Counts and measurements were performed at 400–1000× magnifications. Drawings of live organisms were based on living observations and photomicrographs, whereas those of stained specimens were made with the help of a drawing device. Terminology is according to Warren (1986).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted according to the methods described by Luo et al. (2018). The SSU rDNA was amplified using the primers 82F (5'-GAA ACT GCG AAT GGC TC-3')



Fig. 1. A–D. Map and sampling sites. (A) Red dot indicates the location of Qingdao, China. (B) The freshwater pond where *Epistylis anastatica* was collected. (C) The intertidal zone where *Baikalonis microdiscus* n. sp. and *Zoothamnium wilberti* n. sp. were collected. (D) Zhanqiao Pier where *Rhabdostyla commensalis* was collected (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

(Jerome et al. 1996), 18s-F (5'-AAC CTG GTT GAT CCT GCC AGT-3') and 18s-R (5'-TGA TCC TTC TGC AGG TTC ACC TAC-3') (Medlin et al. 1988). The PCR amplification was according to Chen et al. (2018). 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 (Qingdao, China).

Phylogenetic analyses

Four newly characterized sequences, and another 92 sequences obtained from GenBank (85 sessilids, three mobilids and four hymenostomatians), were used in the phylogenetic analyses. The four hymenostomatians, i.e., *Glaucoma chattoni* X56533, *Ichthyophthirius multifiliis* U17354, *Tetrahymena corlissi* U17356 and *T. pyriformis* EF070254, were used as the outgroup taxa. Sequences were aligned using the GUIDANCE2 algorithm online with default parameters (Landan and Graur 2008; Sela et al. 2015). The two ends of the resulting alignment were trimmed manually using the program BioEdit 7.0 (Hall 1999). The final length of the SSU rDNA alignments was 1778 positions.

Maximum likelihood (ML) analysis with 1000 bootstrap replicates was computed at the CIPRES Science Gateway (<http://www.phylo.org>), using the GTR + gamma model performed by RAxML-HPC2 v.8.2.10 on XSEDE (Stamatakis 2014). Bayesian inference (BI) analysis was carried out using

MrBayes v.3.2.6 on XSEDE (Ronquist et al. 2012) with the SYM + G model selected by MrModeltest 2.2 (Nylander 2004) via the Akaike Information Criterion. Markov chain Monte Carlo simulations were run for 6,000,000 generations with a sample frequency of 100 generations and a burn-in of 6000 trees (10%). All the remaining trees were used to calculate posterior probabilities using a 50% majority rule consensus. Tree topologies were visualized using MEGA 7.0 (Kumar et al. 2016). Systematic classification follows Lynn (2008).

Results

Epistylididae Kahl, 1933

Epistylis Ehrenberg, 1830

***Epistylis anastatica* (Linnaeus, 1767) Ehrenberg, 1830 (Figs. 2A–I, 3A–U; Table 1)**

This is a common species found as an epibiont on copepods and has been repeatedly reported in previous studies (Clamp et al. 2016; Foissner et al. 1999). The details of its oral ciliature, however, remain unknown. Therefore, a detailed redescription based on two Qingdao populations, and an improved diagnosis based on previous and present observations, are provided here.

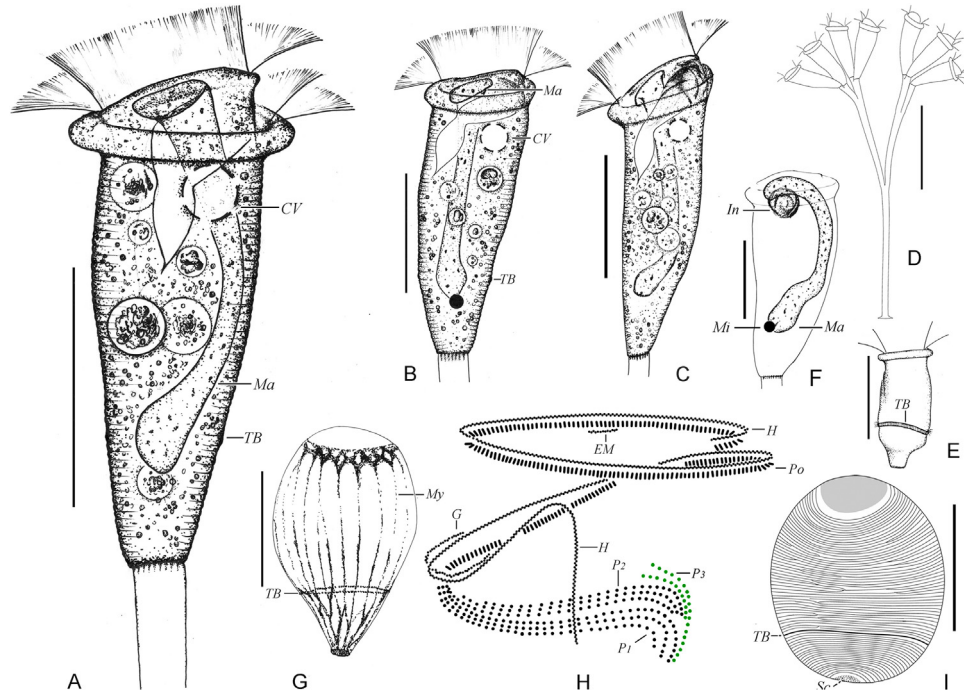


Fig. 2. A–I. Illustrations of *Epistylis anastatica* in vivo (A–F), after protargol (G–H) and dry silver nitrate (I) staining. (A–C) Different zooids, showing the variation of zooid shape. (D) A typical colony. (E) A swarmer freshly detached from stalk. (F) Showing the macronucleus and micronucleus. (G) Myoneme system. (H) Oral ciliature. (I) Silverline system. CV, contractile vacuule; EM, epistomial membrane; G, germinal kinety; H, haplokinety; In, infundibulum; Ma, macronucleus; Mi, micronucleus; My, myoneme; Po, polykinety; P1–3, infundibular polykineties 1–3; Sc, scopula; TB, trochal band. Scale bars: 30 μm in A–C, E–I; 100 μm in D.

Improved diagnosis. Colony usually less than 500 μm high. Stalk dichotomously branched. Zooid elongate bell-shaped, usually $60\text{--}100 \times 20\text{--}35 \mu\text{m}$ in vivo. Peristomial lip slightly everted. Single contractile vacuole located ventrally, below peristomial lip. Macronucleus sausage-shaped, longitudinally oriented. Transverse silverlines numbering 40–61 from peristome to trochal band, 16–26 from trochal band to scopula. Infundibular polykinety 3 consists of one long and one conspicuously shorter row of kinetosomes.

Deposition of voucher slides. Four protargol slides (registration numbers: LBR20170327-01-01, LBR20170327-01-02, LBR20170703-02-01, LBR20170703-02-02) and three “dry” silver nitrate slides (registration numbers: LBR20170327-01-03, LBR20170327-01-04, LBR20170327-01-05) with voucher specimens were deposited in the Laboratory of Protozoology, Ocean University of China, Qingdao, China.

Host and ecological features. Cyclopoid copepods (Fig. 3A, B) from freshwater ponds at two different parks (N35°55′37″; E120°12′00″ and N36°03′58″; E120°20′24″). The water temperature was 12 °C and 26 °C, respectively.

Description based on population II from Qingdao (Figs. 2A, B, H, 3A, B, D–H, J, O–Q, T; Table 1). Zooid about $60\text{--}75 \times 20\text{--}25 \mu\text{m}$ in vivo, usually conical in shape (Figs. 2A, B, 3D, F, H, J). Peristomial lip about 20–30 μm across, about 3 μm thick, and slightly everted (Figs. 2A, B, 3D–J). Peristomial disc slightly convex, obliquely elevated

in fully extended zooids (Figs. 2A, B, 3D–H). Pellicular striations conspicuous when viewed at magnifications above $400\times$. Oral cilia about 15 μm long in live cells.

Cytoplasm colourless, usually contains a few to several yellow or colourless food granules. Contractile vacuole about 5 μm in diameter, situated at ventral wall of infundibulum beneath peristomial lip, pulsating at intervals of about 10 s (Figs. 2A, B, 3D, F–H). Macronucleus elongate sausage-shaped, longitudinally oriented, anterior portion lying transversely in peristomial region, posterior portion slightly curved (Figs. 2A, B, 3J).

Colony up to 350 μm high, usually with 4–8 zooids. Stalk dichotomously branched, with smooth surface and very fine longitudinal striations (Fig. 3B). When solitary, zooid often sits on a relatively short stalk.

Haplokinety and polykinety make approximately 1.25 circuits around peristome before entering infundibulum where they make a further circuit (Figs. 2H, 3O, P). Polykinety transforms into infundibular polykinety 1 (P1), which is accompanied by infundibular polykineties 2 and 3 (P2 and P3) within infundibulum (Figs. 2H, 3O, P). P1 composed of three rows of kinetosomes, adstomal ends of which terminate at slightly different levels: row 1 terminates above row 2 which terminates slightly above row 3 (Figs. 2H, 3T). P2 terminates adstomally at posterior curvature of P1 (Figs. 2H, 3T). Row 1 of P2 converges with P1 abstomally. P3 two-rowed: inner row shorter and terminates at almost same

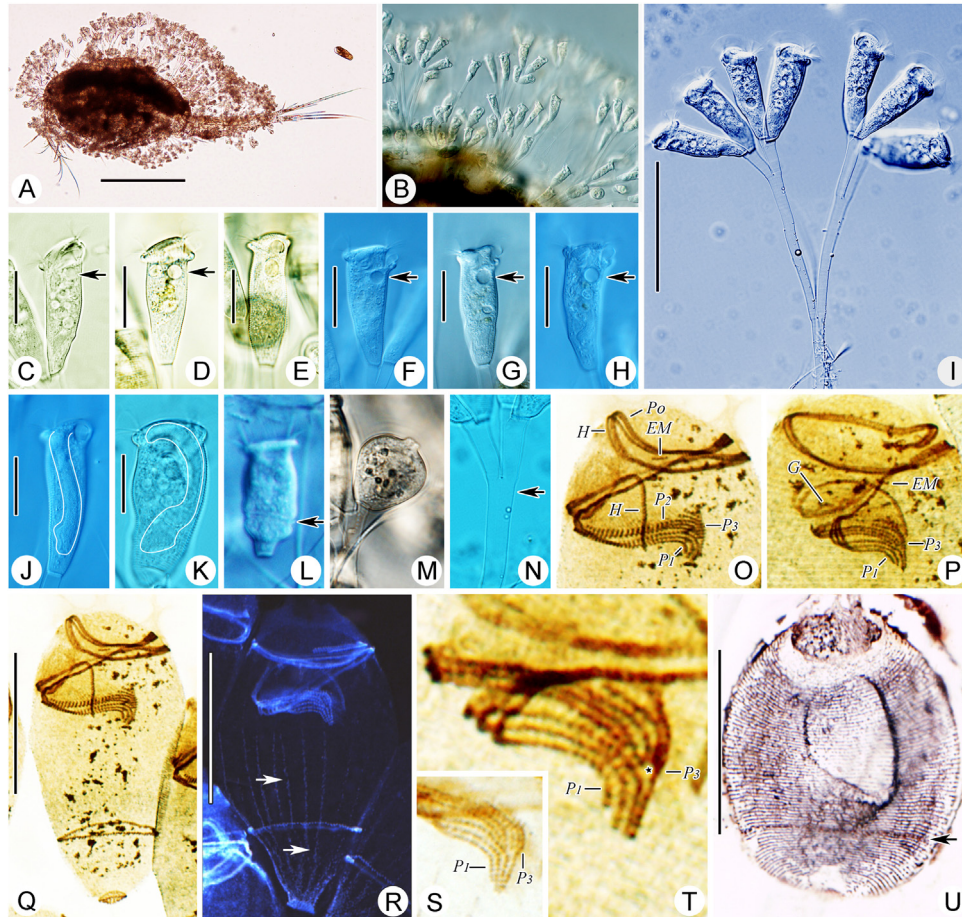


Fig. 3. A–U. Photomicrographs of *Epistylis anastatica* in vivo (A–N), after protargol (O–T) and dry silver nitrate (U) staining. (A) Cyclopid host of *E. anastatica*. (B) Colonies at low magnification, showing the branches. (C–H) Different zooids, showing the variation of shape, arrows mark the contractile vacuole. (I) A typical colony. (J, K) White contours mark the macronucleus. (L) A swarmer freshly detached from stalk, arrow marks trochal band. (M) A contracted zooid. (N) Stalk at high magnification (arrow). (O, P) Oral ciliature. (Q, R) Two stained specimens, showing ciliature (Q) and myoneme system (R, arrows). (S, T) Infundibular polykineties 1–3, asterisk in T marks the adstomal end of inner row in P3; (U) Silverline system, arrow marks trochal band. EM, epistomial membrane; G, germinal kinety; H, haplokinety; Po, polykinety; P1–3, infundibular polykineties 1–3. Scale bars: 500 μm in A; 30 μm in C–H, J, K, R, S, U; 100 μm in I.

level as P2; outer row significantly longer and terminates at same level as P1 (Figs. 2H, 3T). Epistomial membrane short, located at entrance of infundibulum (Figs. 2H, 3O). Germinal kinety lies parallel to haplokinety in upper half of infundibulum (Figs. 2H, 3P). Trochal band dikinetid and encircles zooid at a point about 2/3 of the distance from peristome to scopula (Fig. 3Q).

Silverline system consists of closely spaced, transverse silverlines numbering 43–47 between peristome and trochal band, 16–19 between trochal band and scopula.

Description based on population I from Qingdao (Figs. 2C–G, I, 3C, I, K–N, R, S, U; Table 1). The population I of *Epistylis anastatica* is identical in all features with population II, except some zooids are larger (up to 85 μm long). Its myoneme system is shown in Figs. 2G, 3R, and silverline system is shown in Figs. 2I, 3U.

Epistylidae Kahl, 1933

Rhabdostyla Kent, 1881

Rhabdostyla commensalis Möbius, 1888

(Figs. 4A–E, 5A–R; Table 1)

Rhabdostyla commensalis is a poorly known species. Its superficial description, lack of information on key morphological features and the absence of molecular data necessitate a reinvestigation using modern techniques (Lang 1948; Möbius 1888; Precht 1935). We here provide an improved diagnosis based on previous and present observations.

Improved diagnosis. Zooid pyriform, 35–70 \times 20–30 μm in vivo. Peristomial lip thick and moderately everted. Single contractile vacuole located ventrally, partly below peristomial lip. Stalk length less than zooid length. Macronucleus C-shaped, transversely oriented. Transverse silverlines numbering 33–40 from peristome to trochal band, 13–15 from trochal band to scopula. P3 single-rowed, terminates above P1. Marine habitat. Epizoic.

Deposition of voucher slides. Six protargol slides (registration numbers: LBR20170904-03-01 to LBR20170904-

Table 1. Morphometric data of Chinese populations of *Epistylis anastatica* (population I: *E. ans* I; population II: *E. ans* II), *Rhabdostyla commensalis* (*R. com*), *Baikalonis microdiscus* n. sp. (*B. mic*) and *Zoothamnium wilberti* n. sp. (*Z. wil*).

Characters	Species	Min	Max	Mean	M	SD	CV	N
Body length ^a (μm)	<i>E. ans</i> I	60	85	73.0	75	10.37	14.21	5
	<i>E. ans</i> II	60	75	64.0	60	6.52	10.19	5
	<i>R. com</i>	35	55	45.6	45	7.80	17.11	5
	<i>B. mic</i>	20	30	25.6	26	3.85	15.04	5
	<i>Z. wil</i>	45	65	53.0	50	9.08	17.13	5
Body width ^a (μm)	<i>E. ans</i> I	20	30	25.0	25	3.61	14.44	5
	<i>E. ans</i> II	20	25	21.6	21	2.10	9.72	5
	<i>R. com</i>	20	30	25.4	25	3.65	14.37	5
	<i>B. mic</i>	15	20	17.4	17	2.07	11.90	5
	<i>Z. wil</i>	30	40	33.2	32	4.15	12.50	5
Diameter of peristomial lip ^a (μm)	<i>E. ans</i> I	23	30	27.2	28	2.59	9.52	5
	<i>E. ans</i> II	23	30	25.6	25	2.97	11.60	5
	<i>R. com</i>	20	25	23.8	25	2.17	9.12	5
	<i>B. mic</i>	14	16	14.8	15	0.84	5.68	5
	<i>Z. wil</i>	30	40	33.6	33	3.91	11.64	5
Stalk length ^a (μm)	<i>E. ans</i> I	–	–	–	–	–	–	–
	<i>E. ans</i> II	–	–	–	–	–	–	–
	<i>R. com</i>	5	16	9.8	10	4.02	41.02	5
	<i>B. mic</i>	5	12	8.0	6	2.45	30.63	5
	<i>Z. wil</i>	–	–	–	–	–	–	–
Number of silverlines from peristome to TB ^b	<i>E. ans</i> I	40 ^c	48 ^c	45.4 ^c	46	2.18	4.80	13
	<i>E. ans</i> II	43 ^a	47 ^a	45.5 ^a	46	1.73	3.80	4
	<i>R. com</i>	33 ^d	40 ^d	36.3 ^d	36	3.30	9.10	4
	<i>B. mic</i>	–	–	–	–	–	–	–
	<i>Z. wil</i>	35 ^a	40 ^a	37.7 ^a	38	2.52	6.68	3
Number of silverlines from TB to scopula ^b	<i>E. ans</i> I	17 ^c	20 ^c	18.8 ^c	19	0.93	4.95	13
	<i>E. ans</i> II	16 ^a	19 ^a	17.3 ^a	17	1.26	7.28	4
	<i>R. com</i>	13 ^d	15 ^d	13.6 ^d	13	0.89	6.54	5
	<i>B. mic</i>	–	–	–	–	–	–	–
	<i>Z. wil</i>	28 ^a	32 ^a	30.3 ^a	30.5	2.06	6.80	4
Total number of silverlines ^b	<i>E. ans</i> I	58 ^c	68 ^c	64.2 ^c	65	2.44	3.80	13
	<i>E. ans</i> II	62 ^a	63 ^a	62.8 ^a	63	0.50	0.80	4
	<i>R. com</i>	46 ^d	55 ^d	50.0 ^d	49.5	4.24	8.48	4
	<i>B. mic</i>	40 ^a	40 ^a	40.0 ^a	40	0	0	1
	<i>Z. wil</i>	60 ^a	63 ^a	61.3 ^a	61	1.53	2.50	3

Abbreviations: CV, coefficient of variation in %; Max, maximum; Mean, arithmetic mean; M, Median; Min, minimum; N, number of specimens studied; TB, trochal band; –, data unavailable.

^aData based on live specimens.

^bApproximate values.

^cData based on “dry” silver nitrate stained specimens.

^dData based on “wet” silver nitrate stained specimens.

03-06) and one silver nitrate slide (registration number: LBR20170904-03-07) with voucher specimens were deposited in the Laboratory of Protozoology, Ocean University of China, Qingdao, China.

Host and ecological features. The host polychaete *Salvatoria* sp. (Fig. 5A–C) was isolated from an intertidal pool near Zhanqiao Pier (N36°03′39″; E120°19′09″). The water temperature was 25 °C and the salinity was 30 psu.

Description of Chinese population. Solitary, zooid about 35–55 × 20–30 μm in vivo, pyriform, gradually widens

towards mid-body (Figs. 4A–C, 5D–G). Peristomial lip about 4 μm thick, moderately everted, about 20–25 μm in diameter, nearly as wide as mid-region of zooid (Figs. 4A–C, 5D–G). Peristomial disc obliquely elevated above lip when zooid fully extended, central region sometimes protuberant (Figs. 4A–C, 5D–G). Pellicular striations easily detectable above 400× magnification. Oral cilia about 15 μm long.

Cytoplasm colourless, usually contains numerous small globular particles (0.5–1.0 μm in diameter) and food gran-

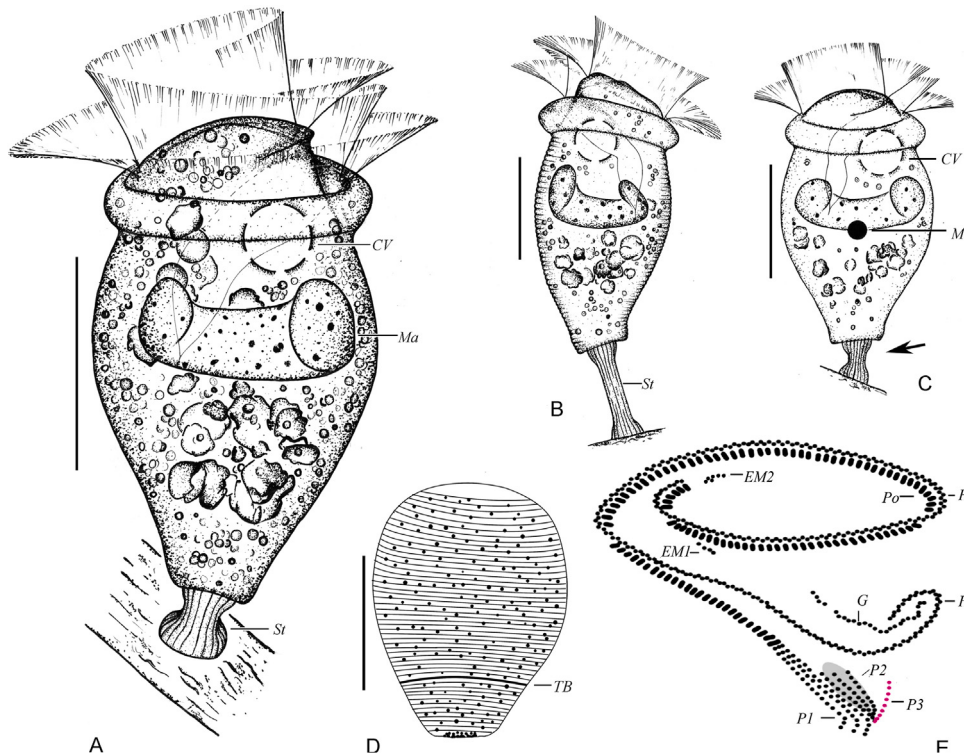


Fig. 4. A–E. Illustrations of *Rhabdostyla commensalis* in vivo (A–C), after wet silver nitrate (D) and protargol (E) staining. (A, B) Different individuals, showing the variation of zooid shape and stalk length. (C) Showing the position of micronucleus. (D) Silverline system and pellicular pores. (E) Oral ciliature. CV, contractile vacuole; EM1,2, epistomial membrane 1,2; G, germinal kinety; H, haplokinety; Ma, macronucleus; Mi, micronucleus; Po, polykinety; P1–3, infundibular polykineties 1–3; St, stalk; TB, trochal band. Scale bars: 20 μm .

ules (2–8 μm in diameter). Contractile vacuole about 8 μm across when fully expanded, located on ventral wall of infundibulum, partly below level of peristomial lip (Figs. 4A–C, 5D–G). Macronucleus C-shaped, transversely oriented, slightly above mid-region of zooid (Figs. 4A–C, 5N, O). Micronucleus globular, lies within curvature of macronucleus (Figs. 4C, 5M).

Stalk stout, 5–16 μm long and 4–5 μm wide, rigid, conspicuously striated longitudinally, sometimes enveloped by debris, both ends somewhat expanded (Figs. 4A–C, 5J).

Haplo- and polykineties make approximately 1.25 turns around peristome before descending into infundibulum where they make a further turn (Figs. 4E, 5N–R). P1 and P2 each three-rowed. Rows of P1 are of equal length, terminating adstomally at the same level (Figs. 4E, 5R). Rows of P2 also of equal length, terminating adstomally at convergence of P1 and P3 (Figs. 4E, 5Q, R). P3 single-rowed, clearly shorter than P2 and terminates adstomally together with P2 (Figs. 4E, 5Q, R). Epistomial membrane 1 located at entrance of infundibulum, epistomial membrane 2 located near distal end of polykinety (Figs. 4E, 5P, R). Germinal kinety lies parallel to haplokinety in upper two-thirds of infundibulum (Figs. 4E, 5Q, R). Trochal band dikinetid, located about 3/4 down length of zooid (Figs. 4D, 5N, O).

Silverline system consists of closely spaced, parallel, transverse silverlines, numbering 33–40 between peristome

and trochal band, 13–15 between trochal band and scopula (Figs. 4D, 5L).

Vorticellidae Ehrenberg, 1838

Baikalonis Jankowski, 1982

***Baikalonis microdiscus* n. sp. (Figs. 6A–E, 7A–S; Table 1)**

Diagnosis. Zooid barrel-shaped, about 20–30 \times 15–20 μm in vivo. Peristomial lip single-layered. Stalk shorter than zooid. Single contractile vacuole located ventrally, below peristomial lip. Macronucleus C-shaped, transversely oriented. P3 consists of one long inner row and one short outer row. Silverline system consists of closely spaced transverse lines. Marine habitat.

Type locality and ecological features. Intertidal pool near Taipingjiao Park (N36°03'03"; E120°21'01"), Qingdao, China. The water temperature was 24 °C and the salinity was 32 psu.

Host. Harpacticoid copepod (Fig. 7A).

Deposition of type slides. The protargol slide (registration number: NHMUK 2019.11.1.1) with the holotype specimen (Fig. 7L) and the silver nitrate slide with paratype specimens (registration number: NHMUK 2019.11.1.2) were deposited in the Natural History Museum, London, UK.

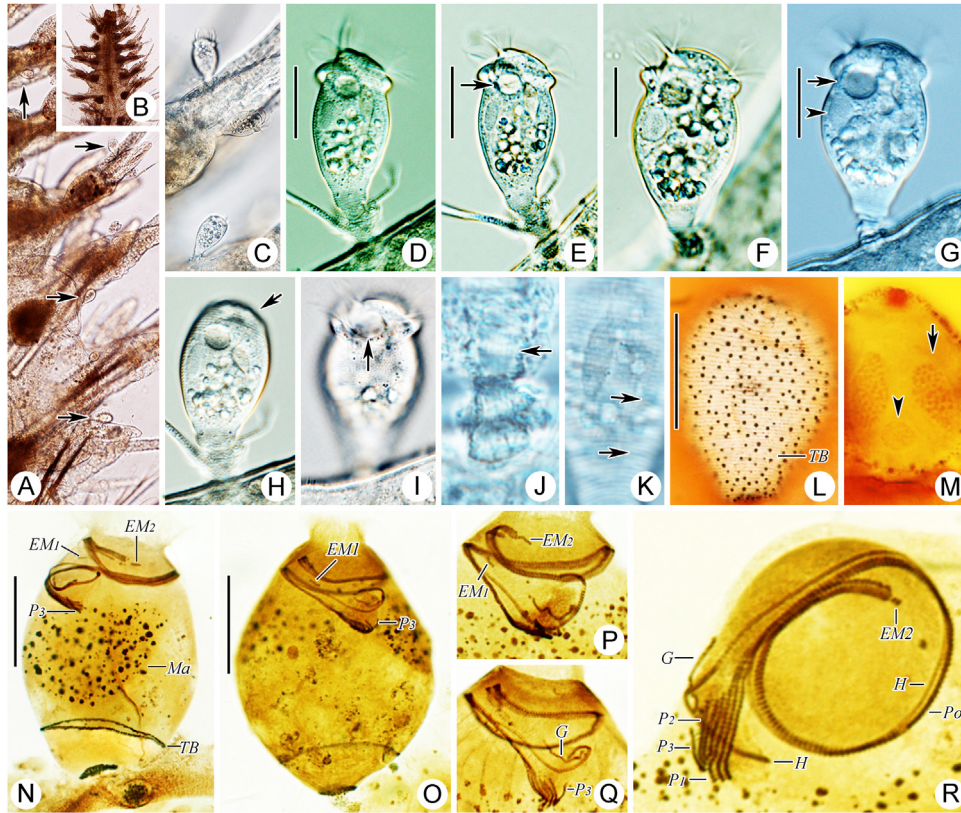


Fig. 5. A–R. Photomicrographs of *Rhabdostyla commensalis* in vivo (A–K), after wet silver nitrate (L, M) and protargol (N–R) staining. (A, B) Part portion of the host (*Salvatoria* sp.), arrows mark individuals of *R. commensalis*. (C) Individual of *R. commensalis* on its host. (D–G) Slender (D, E) and plumper (F, G) individuals, arrows in E and G mark the contractile vacuole, arrowhead in G marks one end of macronucleus. (H) A contracted individual, arrow marks the contracted peristome. (I) Anterior portion, showing the opening of infundibulum (arrow). (J) Stalk (arrow). (K) Pellicular striations (arrows). (L) Silverline system and pellicular pores. (M) Arrow and arrowhead mark the macronucleus and micronucleus, respectively. (N, O) Ciliature. (P–R) Oral ciliature. EM1,2, epistomial membrane 1,2; G, germinal kinety; H, haplokinety; Ma, macronucleus; Po, polykinety; P1–3, infundibular polykineties 1–3; TB, trochal band. Scale bars: 20 μm .

Etymology. The species-group name “*microdiscus*” is a composite of *micro-* (small) and *discus* (disc) and recalls the small peristomial disc of this species.

Description. Solitary, zooid barrel-shaped, about 20–30 \times 15–20 μm in vivo, maximum width in mid-region, tapering slightly posteriorly (Figs. 6A, B, 7B–E). Peristomial lip single-layered, about 14–16 μm in diameter, only slightly everted (Figs. 6A, B, 7B–E, H, I). Peristomial disc conspicuously small, somewhat convex at centre, raised above peristome on a stalk-like extension, base of which is slightly constricted when zooid is fully extended (Figs. 6A, B, 7B–D, I). Pellicular striations very fine, faintly detectable when viewed in vivo at magnifications above 400 \times . Oral cilia about 10 μm long.

Cytoplasm transparent with numerous small globular particles and several food granules. One contractile vacuole about 5 μm across when fully expanded, located on ventral wall of infundibulum below peristomial lip (Figs. 6A, B, 7C–F). Macronucleus C-shaped, transversely oriented, located slightly above mid-region of zooid (Figs. 6C, 7L). Micronucleus globular, lies within curvature of macronucleus (Figs. 6C, 7L).

Stalk about 5–12 μm long and 5 μm wide, enveloped by zooid when contracted (Fig. 7G). Cortex of stalk colourless and transparent (Figs. 6A, B, 7J). Spasmoneme about 2 μm wide. Upon contraction, stalk shortens linearly causing cortex to wrinkle (Figs. 6A, B, 7J).

Haplo- and polykineties make approximately 1.5 turns of peristome before plunging into infundibulum where they make a further turn (Figs. 6D, 7L). P1 and P2 each three-rowed (Figs. 6D, 7O, R). Rows of P1 nearly equal in length (Figs. 6D, 7L, Q). P2 terminates adstomally at convergence of P1 and P3 (Figs. 6D, 7L, Q). Row 3 of P2 slightly longer than inner two rows and is detached from them at abstomal end (Figs. 6D, 7O, Q). P3 two-rowed: outer row very short and composed of a few kinetosomes; inner row much longer, terminating adstomally above P1 (Figs. 6D, 7M–R). Epistomial membrane 1 located at entrance of infundibulum, epistomial membrane 2 located near distal end of polykinety (Figs. 6D, 7L). Germinal kinety lies parallel to haplokinety in upper two-thirds of infundibulum (Figs. 6D, 7L). Trochal band dikinetid and located about 3/4 down length of zooid (Figs. 6C, 7L, M).

Silverline system consists of closely spaced, transverse silverlines, about 40 in total ($n = 1$) (Figs. 6E, 7S).

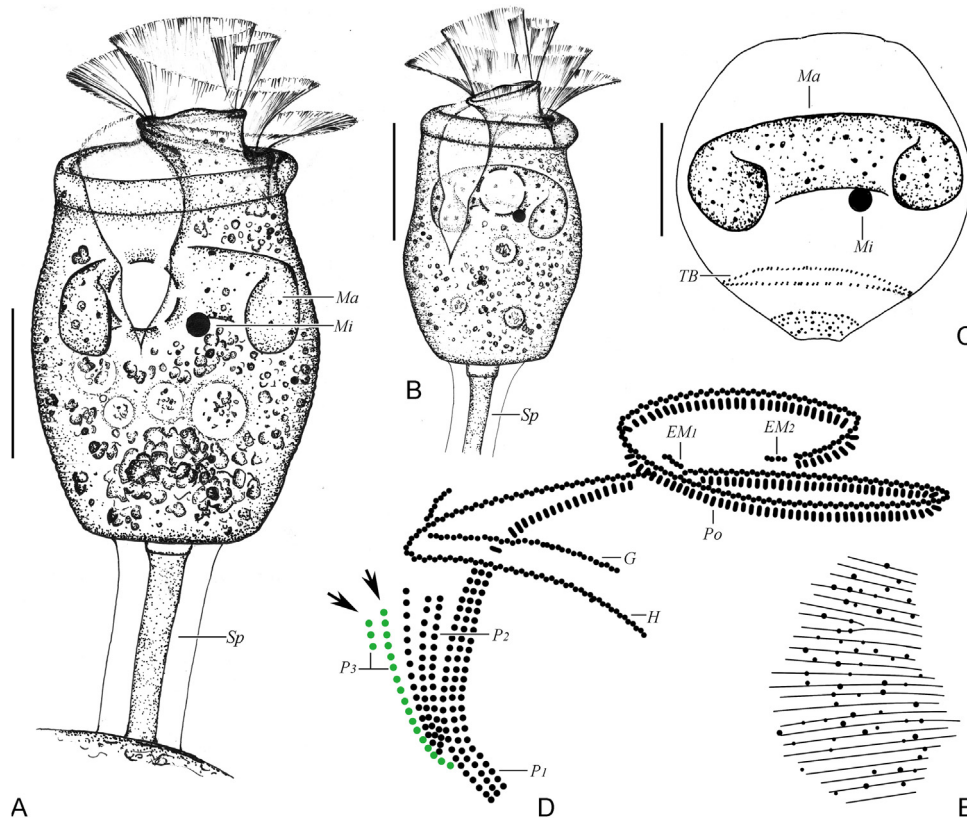


Fig. 6. A–E. Illustrations of *Baikalonis microdiscus* n. sp. in vivo (A, B), after protargol (C, D) and wet silver nitrate (E) staining. (A, B) Different individuals, showing the variation of zooid shape. (C) Showing the nuclear apparatus and trochal band. (D) Oral ciliature, arrows indicate P3. (E) Showing part of silverline system and pellicular pores. EM1,2, epistomial membrane 1,2; G, germal kinety; H, haplokinety; Ma, macronucleus; Mi, micronucleus; Po, polykinety; P1–3, infundibular polykineties 1–3; Sp, spasmoneme; TB, trochal band. Scale bars: 10 μm .

Zoothamniidae Sommer, 1951

Zoothamnium Bory de St. Vincent, 1824

***Zoothamnium wilberti* n. sp. (Figs. 8A–G, 9A–R; Table 1)**

Diagnosis. Colony up to 350 μm high, stalk dichotomously branched. Zooid 45–65 \times 30–40 μm in vivo, widest in mid-region apart from peristomial lip which is thick, strongly everted and 30–40 μm across. Peristomial disc conical, conspicuously elevated above peristome. Single contractile vacuole apically located on dorsal wall of infundibulum. Macronucleus C-shaped, transversely or obliquely oriented. P3 with three equal-length rows, terminates above P1. Transverse silverlines numbering 35–40 from peristome to trochal band, 28–32 from trochal band to scopula. Marine habitat.

Type locality and ecological features. Intertidal pool near Taipingjiao Park (N36°03'03"; E120°21'01"), Qingdao, China. The water temperature was 24 °C and the salinity was 32 psu.

Host. *Gammarus* sp. (Fig. 9A–C).

Deposition of type slides. The protargol slide (registration number LBR-20170613-01-01) with the holotype

specimen (Fig. 9M), nine other protargol slides (registration numbers: LBR-20170613-01-02 to LBR-20170613-01-10) and two silver nitrate slide (registration numbers: LBR-20170613-01-11, LBR-20170613-01-12) were deposited in the Laboratory of Protozoology, Ocean University of China, Qingdao, China. One protargol slide with paratype specimens (registration number: NHMUK 2019.11.1.3) and another silver nitrate slide with paratype specimens (registration number: NHMUK 2019.11.1.4) were deposited in the Natural History Museum, London, UK.

Etymology. This species is named in honor of Prof. Norbert Wilbert, University of Bonn, Germany, in recognition of his significant contributions to the field of ciliatology.

Description. Zooid about 45–65 \times 30–40 μm in vivo, gradually widens towards mid-body, somewhat variable in shape (Figs. 8A–C, 9D–J). Peristomial lip about 30–40 μm across, single-layered, thick and strongly everted (Figs. 8A–C, 9D–J). Peristomial disc conical, conspicuously elevated above peristome in fully extended zooids (Figs. 8A–C, 9D–J). Pellicular striations conspicuous when viewed in vivo at magnifications above 400 \times . Oral cilia about 15 μm long.

Cytoplasm colourless, with numerous yellow or grey food granules. Contractile vacuole about 10 μm across, api-

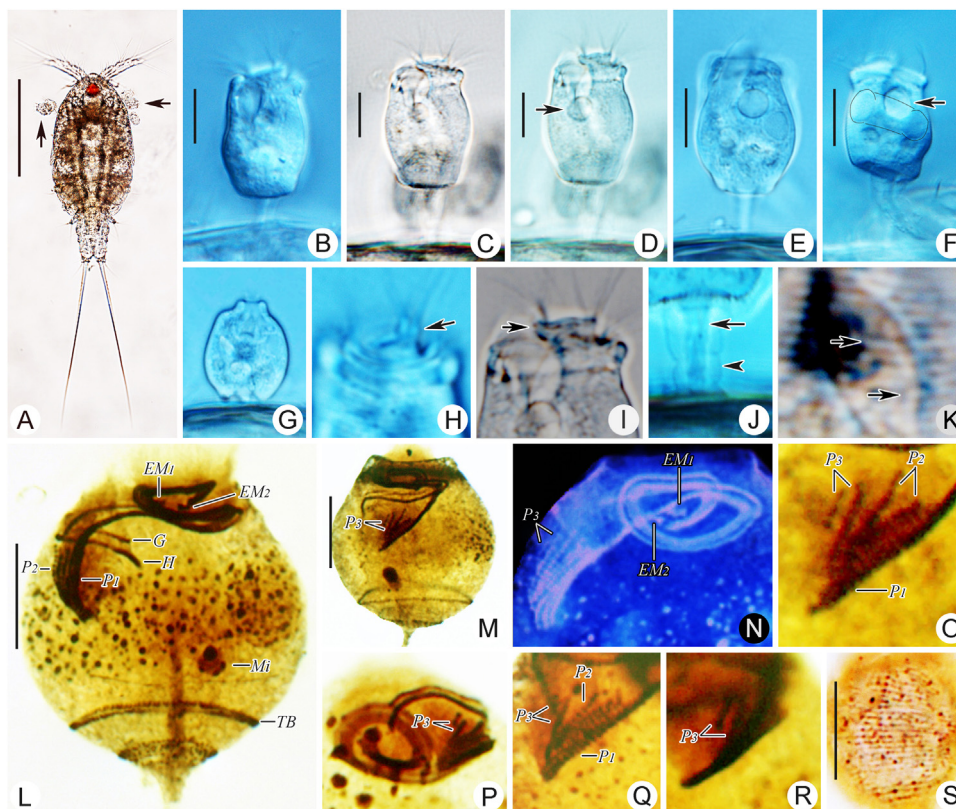


Fig. 7. A–S. Photomicrographs of *Baikalonis microdiscus* n. sp. in vivo (A–K), after protargol (L–R) and wet silver nitrate (S) staining. (A) The host copepod, arrows mark *B. microdiscus* n. sp. (B–F) Several individuals, showing the variation of zooid shape and stalk length, arrows mark the contractile vacuole, black contour in F marks the macronucleus. (G) A contracted individual. (H, I) Anterior portion of zooid, arrows mark peristomial disc. (J) Stalk, arrow marks the spasmoneme and arrowhead indicates the sheath. (K) Pellicular striations (arrows). (L, M) Stained specimens, showing ciliature. (N, P) Oral ciliature. (O, Q, R) Infundibular polykineties. (S) Silverline system and pellicular pores. EM1,2, epistomial membrane 1,2; G, germinal kinety; H, haplokinety; Mi, micronucleus; P1–3, infundibular polykineties 1–3; TB, trochal band. Scale bars: 10 μ m in B–F, L, M, S; 200 μ m in A.

cally located in centre of peristomial disc on dorsal wall of infundibulum (Figs. 8A–C, 9D, E, G). Macronucleus C-shaped, transversely or obliquely oriented (Figs. 8A–C, E, 9N). Micronucleus not observed.

Colony up to 350 μ m high, usually contains fewer than 16 zooids. Stalk dichotomously branched, branches become progressively narrower and shorter from primary stalk to terminal branches (Figs. 8D, 9C). Stalk sheath colourless, with several transverse annular bulges and inconspicuous longitudinal striations (Figs. 8D, 9K). Spasmoneme slender, with numerous small (about 0.5 μ m across), dark granules (Figs. 8D, 9K).

Haplo- and polykineties make approximately 1.25 circuits around peristome before plunging into infundibulum where they make a further circuit (Figs. 8F, 9M). Infundibular polykineties three-rowed (Figs. 8F, 9O–Q). Rows of P1 nearly equal in length. P2 terminates adstomally at convergence of P1 and P3 (Figs. 8F, 9O–Q). Rows 1 and 2 of P2 converge with P1 and are detached from shorter row 3 at abstomal ends (Figs. 8F, 9O–Q). Rows of P3 equally long and terminate adstomally above P1. Row 1 slightly detached from rows 2 and 3 at abstomal end (Figs. 8F, 9O–Q). Epistomial

membrane 1 located at entrance of infundibulum, epistomial membrane 2 adjacent to distal end of polykinety (Figs. 8F, 9M). Germinal kinety lies parallel to haplokinety in upper half of infundibulum (Figs. 8F, 9M). Trochal band dikinetid, located slightly below mid-region of zooid (Figs. 8E, 9M).

Silverline system consists of closely spaced transverse silverlines, 35–40 between peristome and trochal band, 28–32 between trochal band and scopula (Figs. 8G, 9R).

Molecular data and phylogenetic trees (Fig. 10)

The four newly obtained SSU rDNA sequences are deposited in GenBank database with length (bp), GC content and accession numbers as follows: *Epistylis anastatica* 1654, 42.74%, MN543650; *Rhabdostyla commensalis* 2035, 42.56%, MN543651; *Baikalonis microdiscus* n. sp. 1675, 42.27%, MN543652; *Zoothamnium wilberti* n. sp. 1620, 42.41%, MN543653.

Phylogenetic trees constructed with ML and BI analyses had almost identical topologies, therefore only the ML tree is shown here (Fig. 10). The newly sequenced *Epistylis anastatica* did not group with its congeners, rather it clustered with

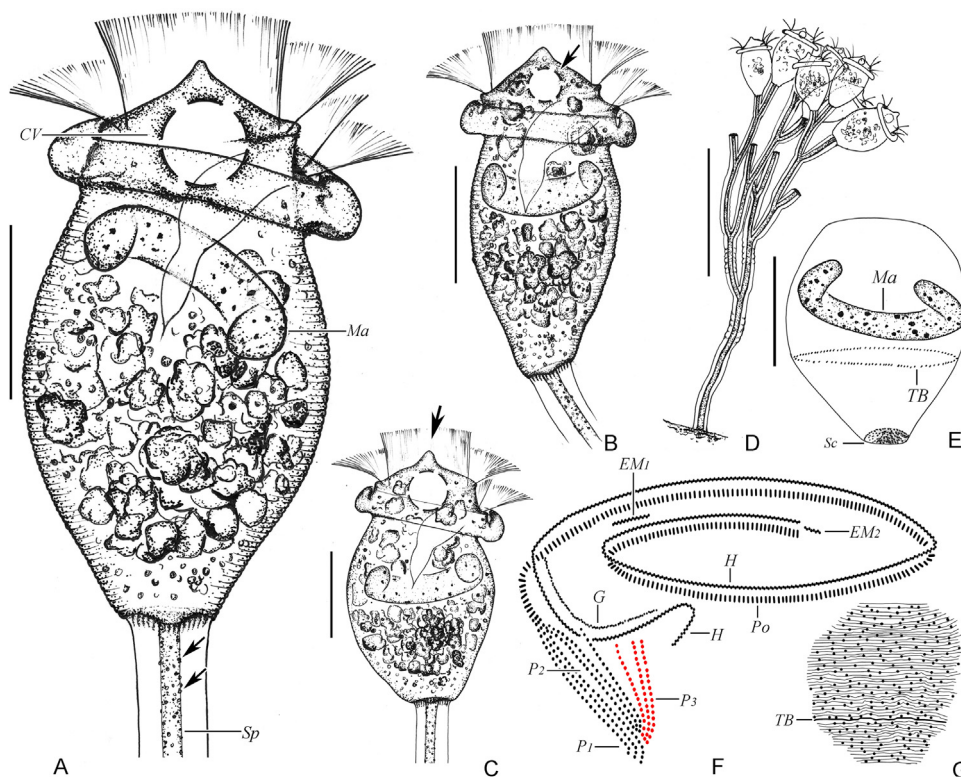


Fig. 8. A–G. Illustrations of *Zoothamnium wilberti* n. sp. in vivo (A–D), after protargol (E, F) and wet silver nitrate (G) staining. (A–C) Different zooids, showing the variation of zooid shape, arrows in A mark the granules on spasmoneme, arrow in B marks the contractile vacuole and arrow in C marks the wart-like protuberance of peristomial disc. (D) A typical colony. (E) Showing the macronucleus, trochal band and scopula. (F) Oral ciliature. (G) Silverline system and pellicular pores. CV, contractile vacuole; EM1,2, epistomial membrane 1,2; G, germinal kinety; H, haplokinety; Ma, macronucleus; Po, polykinety; P1–3, infundibular polykineties 1–3; Sc, scopula; Sp, spasmoneme; TB, trochal band. Scale bars: 20 μm in A–C, E; 100 μm in D.

Carchesium polypinum and *Ophrydium* spp. with low support (28% ML, 0.67 BI), forming a clade within the Vorticellidae. *Rhabdostyla commensalis* occupied the basal position within the Astylozoidae (sensu Sun et al. 2012) clade with maximal support (100% ML, 1.00 BI). *Baikalonis microdiscus* n. sp. grouped with the family Scyphidiidae with moderate to high support (85% ML, 1.00 BI), forming a clade that was sister to the crown group comprising Vorticellidae, Ophrydiidae, Astylozoidae and two epistylidid species (*Epistylis anastatica* and *Rhabdostyla commensalis*). The genus *Zoothamnium* is paraphyletic, its species being distributed among three clades and several other branches within the assemblage comprising the families Zoothamniidae, Opisthonectidae, Epistylididae and the free-swimming vorticellid *Planeticovorticella paradoxa*. *Zoothamnium wilberti* n. sp. was sister to the clade comprising *Z. grossi* and *Epistylis elongata*, with moderate to high support (83% ML, 1.00 BI) within one of the three clades of the family Zoothamniidae.

Discussion

Numerous peritrichs are known to live as epibionts (e.g. Cabral et al. 2016, 2017; Clamp 1991; Fernandez-Leborans

and Tato-Porto 2000; Keiser 1921; Ma and Overstreet 2006; Mayen-Estrada and Clamp 2016; Nenninger 1948; Ramírez-Ballesteros et al. 2018; Schödel 1987, 2004, 2006). Most epibionts, however, are insufficiently described and lack information on their infraciliature and molecular data.

Epistylis anastatica (Linnaeus, 1767) Ehrenberg, 1830

Identification of *Epistylis anastatica*

Linnaeus' (1767) description is widely recognized as the first valid record of *E. anastatica* (original combination *Vorticella anastatica*) although without an illustration. Our population is consistent with this original description.

Foissner et al. (1999) made a revision of this species including details of its historical reports, synonyms, diagnosis, morphology, occurrence and distribution. They proposed the following key characters for the identification of *E. anastatica*: (i) extended zooids about 60–100 μm long, ratio of length to width 2–2.5:1, usually somewhat conical in shape, posterior portion hyaline; (ii) macronucleus longitudinally oriented, slightly curved to form a “C-shaped”; (iii) contractile vacuole located at ventral wall of infundibulum; (iv) stalk smooth; (v) epizooplanktonic, usually on crustaceans. Apart from the slightly slender zooid shape, our population matches

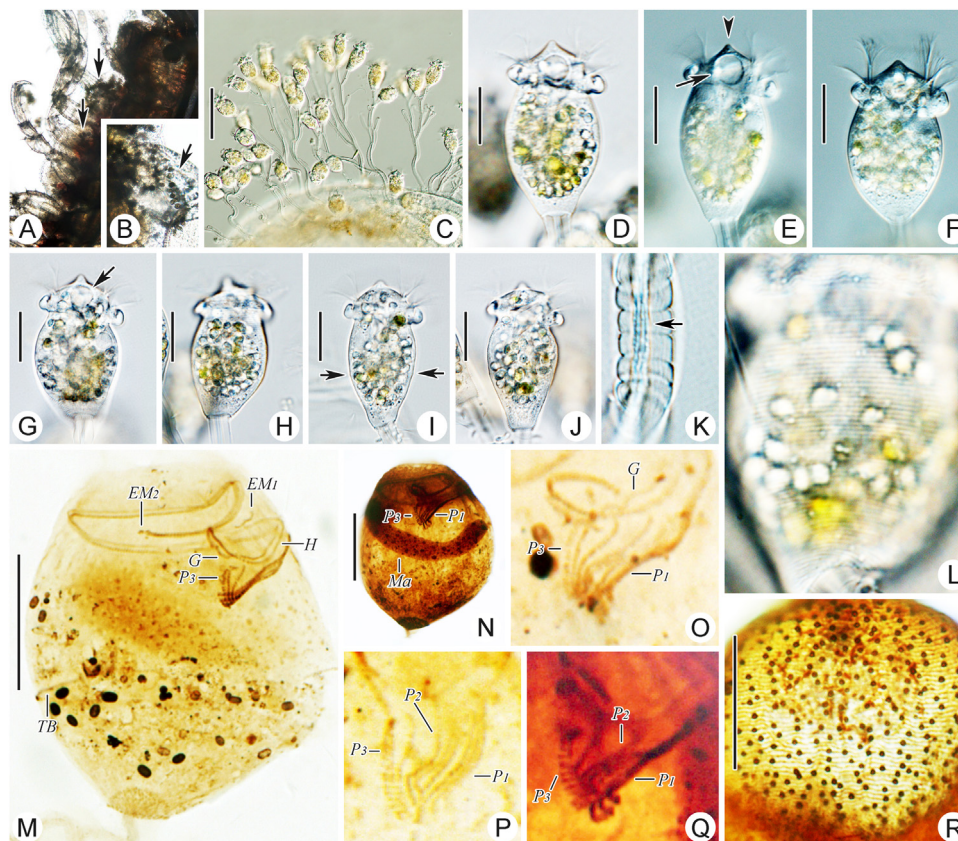


Fig. 9. A–R. Photomicrographs of *Zoothamnium wilberti* n. sp. in vivo (A–L), after protargol (M–Q) and wet silver nitrate (R) staining. (A) Detail of the host *Gammarus* sp., arrows mark *Z. wilberti* n. sp. (B) Site of attachment to host of *Z. wilberti* n. sp. (arrow). (C) Showing colonies of *Z. wilberti* n. sp. attached to its host. (D–J) Different individuals, showing variation in zooid shape, arrowhead in E marks the wart-like protuberance of peristomial disc, arrows in E and G mark the contractile vacuole, arrows in I mark trochal band. (K) Portion of contracted stalk, arrow marks the spasmoneme. (L) Pellicular striations. (M, N) Showing the ciliature and macronucleus. (O–Q) Infundibular polykineties. (R) Silverline system and pellicular pores. EM1,2, epistomial membrane 1,2; G, germinal kinety; H, haplokinety; Ma, macronucleus; P1–3, infundibular polykineties 1–3; TB, trochal band. Scale bars: 20 μm in C–J, M, N, R; 100 μm in C.

these characters sufficiently well for it to be identified with confidence as *E. anastatica*.

Remarks on *Epistylis anastatica* (Fig. 11, Table 2)

Epistylis anastatica is a common species that has been reported on numerous occasions (Clamp et al. 2016; Ehrenberg 1830, 1838; Foissner and Schiffmann 1975; Imhof 1884; Jiang et al. 1983; Kahl 1935; Kent 1881; Lom 1964; Müller 1786; Sommer 1951; Stiller 1940, 1971; Wang et al. 1976; Wang and Nie 1933; Vávra 1963) (Fig. 11A–N). Foissner et al. (1999) synonymized *E. lacustris* Imhof, 1884 with *E. anastatica* and reidentified *Epistylis nympharum* Engelmann, 1862 sensu Foissner and Schiffmann (1975) as a population of *E. anastatica* (Fig. 11H).

Vorticella anastatica sensu Müller (1786) was accepted by Foissner et al. (1999) as a population of *E. anastatica*. According to Müller's (1786) illustration and description, some colonies are, however, indefinable because: (i) they are attached to plants rather than animals; (ii) the stalk surface

is coarse; and (iii) zooids are located at conspicuously different levels within the colony (Fig. 11A). None of these characters is consistent with most reports of *E. anastatica*, in which hosts are aquatic animals, the stalk is smooth and lacks attached particulate matter, and the zooids are located at nearly the same level within the colony. Some colonies described by Müller (1786) might therefore represent one or more different species.

Vávra's (1963) study was not mentioned in the revision by Foissner et al. (1999) even though he provided a detailed description of *E. anastatica* (Fig. 11E). Vávra (1963) also tested the host specificity of *E. anastatica* and found that swimmers supplied with only inanimate substrates died within few hours and those attached to organisms that were not their preferred hosts fail to develop normally. It was concluded that the characteristic movement of the host is necessary for the survival of *E. anastatica* (Vávra 1963). These findings call into question the identity of populations identified as *E. anastatica* attached to motionless substrates such as aquatic plants. Based on reports in the literature (e.g., Clamp

Table 2. Historical reports of *Epistylis anastatica* and its synonymous forms *E. nympharum* sensu Foissner and Schiffmann, 1975 and *E. lacustris* (measurements in μm).

Species/ population	Body length	Body width	Shape quotient	Colony height	Number of zooids	PD	Ma	Stalk	Host	Data source
<i>V. anastatica</i>	–	–	–	–	–	–	–	–	shellfish, plants	Linnaeus (1767)
<i>V. anastatica</i>	–	–	–	–	–	–	–	with attachments	animals, plants	Müller (1786)
<i>E. anastatica</i>	up to 90	–	–	150–1500	–	–	–	D	crustaceans, <i>Ceratophyllum</i> sp.	Ehrenberg (1838)
<i>E. anastatica</i>	90	–	3	up to 1500	–	SC	–	D, S	<i>Entomostraca</i> sp., plants	Kent (1881).
<i>E. anastatica</i>	80 ^a	22 ^a	3–4	–	–	SC	ELC	D, S	<i>Cyclops</i> sp.	Wang and Nie (1933)
<i>E. anastatica</i>	60–115	20–45	3	–	200–300 ^b	SC, P ^c	ELC	D, S, CS ^c , W ^c	copepods	Vávra (1963)
<i>E. anastatica</i>	70–90	–	–	up to 1500	–	flat	SLC	D, S	copepods, cladocerans, plants, snail house	Stiller (1971)
<i>E. anastatica</i>	70–90	–	3	259	32	SC	SLC	D, S	plants	Jiang et al. (1983)
<i>E. nympharum</i>	60–90	–	2–2.5	–	–	SC	ELC	D, S, W ^c	<i>Cyclops</i> sp.	Foissner and Schiffmann (1975); Foissner et al. (1992)
<i>E. anastatica</i>	60–100	–	2–2.5	–	–	SC	ELC	D, S	crustaceans	Foissner et al. (1999)
<i>E. anastatica</i>	–	–	–	75–256	25–30, over 240	–	ELC	D	<i>Mesocyclops isabellae</i>	Clamp et al. (2016)
<i>E. lacustris</i>	60–68	–	–	–	–	flat	ELC	D, S	copepods, <i>Bythotrephes longimanus</i>	Imhof (1884)
<i>E. lacustris</i>	50–70	–	–	–	1, 2, 8–16	SC	ELC	D, S, W ^c	<i>Cyclops</i> sp.	Kahl (1935)
<i>E. lacustris</i>	–	–	–	–	1, 2, many	–	–	D, S, CS ^c , W ^c	<i>Cyclops</i> sp.	Stiller (1940)
<i>E. lacustris</i>	45	–	–	137	up to 30	–	–	D, S	water mite, cyclopoids	Sommer (1951)
<i>E. lacustris</i>	45–75	–	2.3–2.5	30–316	up to 30	SC	ELC	D, S, W ^c	cyclopoids, cladocerans	Wang et al. (1976)

Abbreviations: CS, cross-striation; D, dichotomous; ELC, elongate, longitudinal, somewhat C-shaped, upper part lying in the peristome and lower part curved; Ma, macronucleus; P, protuberance in the center of peristomial disc; PD, peristomial disc; S, smooth; SC, slightly convex; SLC, short, longitudinal, and C-shaped; *V.*, *Vorticella*; W, wrinkled; –, data not available.

^a Average value.

^b Probably a group of colonies.

^c Occasionally.

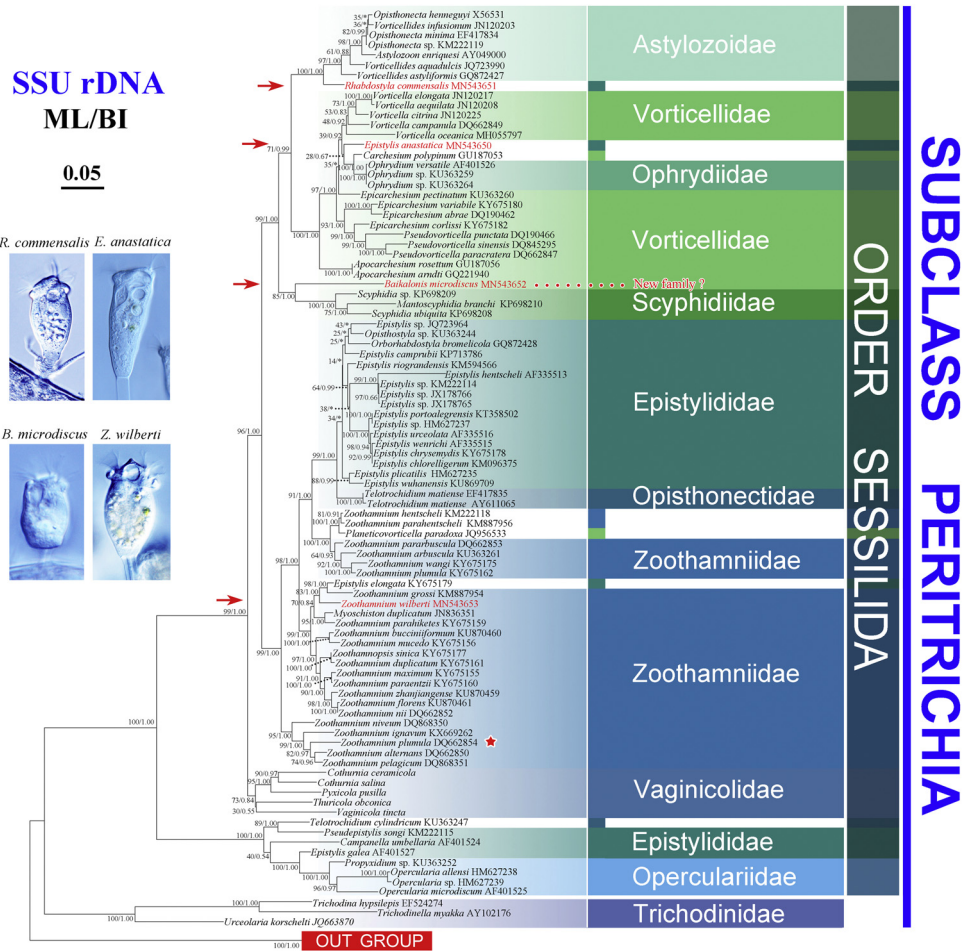


Fig. 10. Phylogenetic tree inferred from SSU rDNA sequences, revealing the phylogenetic positions of *Epistylis anastatica*, *Rhabdostyla commensalis*, *Baikalonis microdiscus* n. sp. and *Zoothamnium wilberti* n. sp. (arrows). Numbers near nodes denote maximum likelihood (ML) bootstrap values and Bayesian inference (BI) posterior probability, respectively. Asterisks indicate disagreements between ML and BI. The sequence of *Zoothamnium plumula* (DQ662854, marked by red star) was deposited in Genbank database under a wrong name *Zoothamnium pluma*, the identification needs to be confirmed. The scale bar indicates five substitutions per 100 nucleotide position.

2016; Kahl 1935; Wang and Nie 1933), we suggest that *E. anastatica* is host-specific for cyclopoid copepods.

Schödel (1986, 1987) reported a population as *E. anastatica* from the gammarids. According to his drawing and photomicrograph, the macronucleus of his population is confined to the anterior half of the zooid (vs. extends longitudinally almost the entire length of the zooid in other populations) and the peristomial disc is conspicuously convex (vs. slightly convex other populations).

Comparison with morphologically similar congeners (Fig. 12A–D, Table 3)

In terms of its colony shape, dichotomously branched stalk, somewhat conical zooid shape, slightly everted peristomial lip, and longitudinally oriented macronucleus, five congeners, viz. *E. digitalis* (Linnaeus, 1758) Ehrenberg, 1830, *E. nympharum* Engelmann, 1862, *E. fugitans* Kellicott,

1884, *E. daphniae* Fauré-Fremiet, 1905, and *E. breviramosa* Stiller, 1931, should be compared with *E. anastatica*.

Epistylis digitalis differs from *E. anastatica* in having an annulated (vs. smooth) stalk (Linnaeus 1758; Ehrenberg 1838; Foissner et al. 1999) (Fig. 12A).

Epistylis nympharum can be separated from *E. anastatica* by its longer zooid (up to 150 µm vs. 60–100 µm long in vivo), barrel-shaped (vs. somewhat conical) zooid outline and the presence (vs. absence) of granules in the posterior region of the zooid (Engelmann 1862; Foissner et al. 1999) (Fig. 12B).

Epistylis fugitans is very similar to *E. anastatica* in all characters except for its shorter, plumper zooid in vivo (50–60 µm long, length: width ratio 1.5–2:1 vs. 60–100 µm long, length:width ratio 2–3:1) and short stalk (usually less than zooid length vs. usually longer than zooid length) (Kellicott 1884) (Fig. 12C).

Table 3. Morphometric comparison of *Epistylis anastatica* with closely related congeners (based on specimens in vivo, measurements in μm).

Species	Body length	Body width	Shape quotient	Infundibulum extends to	PD	Ma	Stalk	Host	Data source
<i>E. anastatica</i>	60–85	20–30	2.5–3	ca. anterior third of body	flat or slightly convex	longitudinal, upper part lying in the peristomial lip and lower part curved	dichotomous, smooth, short to long	<i>Cyclops</i>	Present work
<i>E. anastatica</i>	60–100	–	2–2.5	ca. anterior third of body	slightly convex	longitudinal, both ends slightly curved to C-shaped	dichotomous, smooth, usually long	crustaceans	Foissner et al. (1999)
<i>E. digitalis</i>	60–120	–	3–4	ca. anterior quarter of body	slightly convex	longitudinal	dichotomous ^a , annulated, short to long	<i>Cyclops</i> , <i>Eucyclops</i> , <i>Canthocampus</i> , mites	Ehrenberg (1838); Foissner et al. (1999)
<i>E. nympharum</i>	up to 150	–	ca. 3	ca. mid-body	convex ^a	longitudinal	fairly thick	fly larvae	Engelmann (1862)
<i>E. fugitans</i>	50–60	–	1.5–2	–	narrow	–	short	<i>Sida</i>	Kellicott (1884)
<i>E. daphniae</i>	–	–	–	–	–	–	slender, long and undulating, transverse bands at intervals	<i>Daphnia</i>	Fauré-Fremiet (1905)
<i>E. breviramosa</i>	40–60	25–30	–	ca. anterior third of body	convex or conical	longitudinal, both ends curved	dichotomous ^a , faintly transversely folded ^a , short	<i>Daphnia</i>	Sommer (1951); Stiller (1931, 1971)

Abbreviations: PD, peristomial disc; Ma, macronucleus; –, data not available.

^aInferred from the drawing.

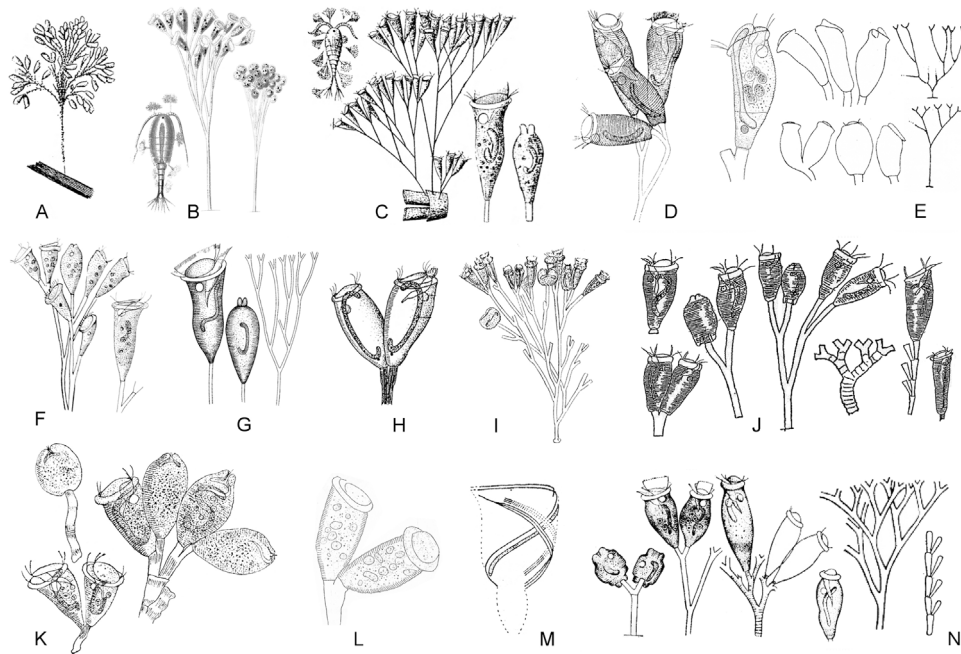


Fig. 11. A–N. Illustrations from previous reports of *Epistylis anastatica* (A–G) and the synonymous forms *E. nympharum* sensu Foissner and Schiffmann (1975) (H) and *Epistylis lacustris* (I–N). (A) *Vorticella anastatica* sensu Müller (1786). (B) from Ehrenberg (1838). (C) from Kent (1881). (D) from Wang and Nie (1933). (E) from Vávra (1963). (F) from Stiller (1971). (G) from Jiang et al. (1983). (H) *Epistylis nympharum* sensu Foissner and Schiffmann (1975). (I) *Epistylis lacustris* from Imhof (1884). (J) from Kahl (1935). (K) from Stiller (1940). (L) from Sommer (1951). (M) incomplete oral ciliature from Lom (1964). (N) from Wang et al. (1976).

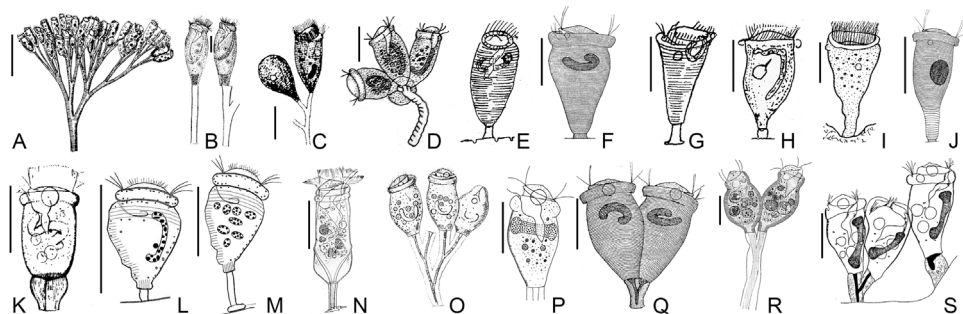


Fig. 12. A–Q. Morphologically similar congeners of *Epistylis anastatica* (A–D), *Rhabdostyla commensalis* (G–M), from *Baikalonis microdiscus* n. sp. (N), and *Zoothamnium willberti* n. sp. (O–S). (A) *E. digitalis* from Ehrenberg (1838). (B) *E. nympharum* from Engelmann (1862). (C) *E. fugitans* from Kellicott (1884). (D) *E. breviramosa* from Stiller (1931). (E) *Rhabdostyla commensalis* from Möbius (1888). (F) *Rhabdostyla commensalis* from Precht (1935). (G) *R. arenicolae* from Fabre-Domergue (1888). (H) *R. arenaria* from Cuénot (1891). (I) *R. variabilis* from Dons (1918). (J) *R. nereicola* from Precht (1935). (K) *R. scyphoides* from Song (1986). (L) *R. mapuche* from Álvarez-Campos et al. (2014). (M) *R. taboadai* from Álvarez-Campos et al. (2014). (N) *B. foissneri* from Jankowski (1982). (O) *Z. affine* from Stein (1854). (P) *Z. affine* from Schödel (2006). (Q) *Z. vermicola* from Precht (1935). (R) *Z. oviforme* from Sommer (1951). (S) *Zoothamnium* sp. from Bierhof and Roos (1977). Scale bars: 200 μ m in A; 30 μ m in B–Q.

Epistylis daphniae differs from *E. anastatica* by its stalk, which is undulating and with transverse bands (vs. straight and smooth) (Fauré-Fremiet 1905).

Compared with *E. breviramosa*, *E. anastatica* has a larger zooid (40–60 μ m vs. 60–100 μ m long in vivo), usually flat or slightly convex peristomial disc (vs. convex or conical) and smooth (vs. transversely folded) stalk (Sommer 1951; Stiller 1931, 1971) (Fig. 12D).

Rhabdostyla commensalis Möbius, 1888

The genus *Rhabdostyla* was established by Kent (1881). It resembles other genera that are solitary such as *Vorticella* Linnaeus, 1767 and its morphological relatives, *Apiosoma* Blanchard, 1885, *Baikalonis* and *Haplocaulus* Warren, 1888. In *Rhabdostyla* the stalk is shorter than the zooid (vs. usually longer than the zooid in *Vorticella* and its morphological

relatives) and lacks a spasmoneme and so is non-contractile (vs. spasmoneme present and stalk contractile in *Vorticella* and its morphological relatives). *Rhabdostyla* differs from *Apiosoma* by always having a stalk (vs. stalk sometimes absent), a sausage-shaped (vs. conical) macronucleus, and being attached to invertebrate (vs. vertebrate) hosts (Curds et al. 1983). Both *Baikalonis* and *Haplocaulus* possess a spasmoneme, plus the former has an “opercularid” peristomial disc, and thus can be separated from *Rhabdostyla* which lacks these features (Jankowski 1982; Warren 1988). Kahl (1935) and Penard (1922) reported that some species of *Rhabdostyla* have a branched stalk, however such species should belong to a different genus as the possession of an unbranched stalk is a diagnostic character for this genus (Curds et al. 1983; Lynn and Small, 2002). There are more than 60 nominal species of *Rhabdostyla*, however, very few have been investigated using modern methods (Álvarez-Campos et al. 2014; Foissner et al. 1999; Kahl 1935; Righi 1973; Song 1986; Stiller 1971). Most are freshwater species and are epibionts, mainly on invertebrates including crustaceans, polychaetes and aquatic insect larvae (Álvarez-Campos et al. 2014; Dias et al. 2007; Fernandez-Leborans and Tato-Porto 2000; Kahl 1935; Stiller 1971).

Identification of *Rhabdostyla commensalis*

This species was originally described by Möbius (1888) as an epibiont of the polychaete *Terebellides stroemi* and later redescribed by Precht (1935) and Lang (1948) from the same host. All populations are very similar except for the body size (35–55 µm of our population vs. 62–70 µm long of Precht’s population vs. 58–65 µm long of Lang’s population) which could be interpreted as population-dependent difference. The present population corresponds perfectly with previous descriptions in the following characters: pyriform body, convex peristomial disc with a protuberance in its centre, short stalk, transversely oriented macronucleus, and epizoic on a marine polychaete (Fig. 12E, F). Thus, we identified our population to *Rhabdostyla commensalis*.

Shen and Gu (2016) recorded a Chinese population of *R. commensalis* in their monograph. Considering their population was founded on the mayfly larvae from freshwater rather than polychaetes from seawater where type population was found, the species identity may be questionable.

Comparison with morphologically similar congeners (Fig. 12G–L, Table 4)

Seven species of *Rhabdostyla* should be compared with *R. commensalis* because they are all epizoic marine forms: *R. arenicolae* Fabre-Domergue, 1888, *R. arenaria* Cuénot, 1891, *R. variabilis* Dons, 1918, *R. nereicola* Precht, 1935, *R. scyphoides* Song, 1986, *R. mapuche* Álvarez-Campos, Fernández-Leborans and Verdes in Álvarez-Campos et al.,

2014, and *R. taboadai* Álvarez-Campos, Fernández-Leborans, Riesgo and Martin in Álvarez-Campos et al., 2014.

Rhabdostyla arenicolae can be distinguished from *R. commensalis* by its funnel-shaped (vs. pyriform) zooid, flat peristomial disc (vs. with a protuberance in the centre) and twisted (vs. C-shaped) macronucleus (Fabre-Domergue 1888) (Fig. 12G).

Rhabdostyla arenaria is similar to *R. commensalis* in respect to its zooid size and shape, but its macronucleus is J-shaped (vs. C-shaped), thus the two can be clearly separated (Cuénot 1891) (Fig. 12H).

Although insufficiently described, *R. variabilis* can be separated from *R. commensalis* by its larger zooid (60–90 µm vs. 35–70 µm long in vivo) and reniform (vs. C-shaped) macronucleus (Dons 1918) (Fig. 12I).

Rhabdostyla nereicola differs from *R. commensalis* by its widened (vs. uniformly narrow) pellicular striations in the posterior portion of body and its concave and discoidal (vs. vermiform C-shaped) macronucleus (Precht 1935) (Fig. 12J).

Rhabdostyla scyphoides was described by Song (1986) as an epibiont of the crustacean *Penaeus orientalis* in China. It differs from *R. commensalis* by its peristomial disc which is not elevated (vs. obliquely elevated) above the peristome, the swollen stalk which is about two-thirds (vs. less than one-third) of the zooid width, the “S-shaped” and nearly longitudinally oriented (vs. C-shaped and transversely oriented) macronucleus, the apically placed contractile vacuole (vs. slightly below the peristomial lip) and two-rowed (vs. single-rowed) P3 (Fig. 12K).

Rhabdostyla mapuche and *R. taboadai* are epibionts of syllid polychaetes from Chile and Spain respectively (Álvarez-Campos et al. 2014). The former can be separated from *R. commensalis* by its smaller zooid (23–31 µm long vs. 35–70 µm long in vivo) and longitudinal (vs. transverse) macronucleus (Fig. 12L). The latter can be separated from *R. commensalis* by its multilobular (vs. vermiform C-shaped) macronucleus (Fig. 12M).

Baikalonis microdiscus n. sp.

The genus *Baikalonis* was erected by Jankowski (1982) based on the type species *Baikalonis foissneri* Jankowski, 1982 which is an epibiont living on the larvae of *Baicalina bellicose*, a caddisfly endemic to Lake Baikal. It can be readily distinguished from other genera by its “opercularid-type” peristomial disc and axially shortened stalk. Warren (1988) transferred two species, *Vorticella undulata* (Dons, 1918) Noland and Finley, 1931 (= *Vorticellopsis undulata* Dons, 1918) and *Haplocaulus* sp. sensu Bierhof and Roos (1977), to *Baikalonis*. However, *V. undulata* was indefinable because of the oversimplified description, and *Haplocaulus* sp. does not correspond with the diagnosis of *Baikalonis* in terms of the “opercularid-type” disc. Consequently, they were not accepted as valid species of *Baikalonis* by Jankowski (2007). Details of the infraciliature and molecular

Table 4. Morphometric comparison of *Rhabdostyla commensalis* with closely related congeners (based on specimens in vivo, measurements in μm).

Species	Body length	Body width	Body shape	Shape quotient	PD	CV	Ma	Stalk	Host	Data source
<i>R. commensalis</i>	35–55	20–30	pyriform	1.5–2	convex, sometime protuberant in centre	slightly below the PL	C-shaped, transverse	5–16 long	<i>Gammarus</i> sp.	Present work
<i>R. commensalis</i>	–	–	pyriform	ca. 2	protuberant in centre	below the PL	C-shaped, transverse	short	<i>Capitella capitata</i> , <i>Terebellides stromii</i>	Möbius (1888)
<i>R. commensalis</i>	62–70	–	pyriform	–	protuberant in centre	partly above PL ^a	C-shaped, transverse	short	<i>Terebellides stromii</i>	Precht (1935)
<i>R. commensalis</i>	58–65	–	–	–	–	below the PL or same level as PL	C-shaped, transverse	short	<i>Terebellides stromii</i>	Lang (1948)
<i>R. arenicolae</i>	45–60	–	conical	ca. 2 ^a	–	below the PL	C-shaped (?)	less than body length	<i>Arenicola piscatorum</i>	Fabre-Domergue (1888)
<i>R. arenaria</i>	average: 56; up to 65	–	pyriform	ca. 2 ^a	convex ^a	above mid-body	J-shaped	short, can up to body length	<i>Synapta inhaerens</i>	Cuénot (1891)
<i>R. variabilis</i>	60–90	ca. 40	irregularly conical	ca. 2 ^a	slightly convex	–	kidney-shaped	very short	<i>Terebellide</i> sp.	Dons (1918)
<i>R. nereicola</i>	55–62	–	elengate pyriform	ca. 2.8 ^a	slightly convex ^a	below the PL ^a	sheet-like and concave	very short	<i>Nereis dumerili</i>	Precht (1935)
<i>R. scyphoides</i>	35–44	24–27	cylindrical	ca. 1.5 ^a	slightly convex ^a	same level as PL ^a	S-shaped, nearly longitudinal	very short, swollen anteriorly	<i>Penaeus orientalis</i>	Song (1986)
<i>R. mapuche</i>	23–31	16–19	pyriform	1.5–2.0 ^b	convex	–	C-shaped, longitudinal	3–6 long	<i>Syllis</i> spp., <i>Salvatoria</i> spp.	Álvarez-Campos et al. (2014)
<i>R. taboadai</i>	30–45	15–23	pyriform	ca. 2 ^b	convex	–	multilobed	14–17 long	<i>Syllis prolifera</i>	Álvarez-Campos et al. (2014)

Abbreviations: CV, contractile vacuole; Ma, macronucleus; PD, peristomial disc; PL, peristomial lip; –, data not available; (?), not sure.

^aInferred from the drawing.

^bInferred from the photomicrographs.

data are lacking for *Baikalonis* and therefore its systematic position remains unknown (Jankowski 2007). Although *Baikalonis* was included in the family Vorticellidae by Lynn (2008), neither its “opercularid-type” peristomial disc nor its mode of contraction, i.e., stalk shortens longitudinally and is enveloped by the zooid, fit the diagnosis of Vorticellidae.

Comparison of *Baikalonis microdiscus* n. sp. with the type species *B. foissneri* (Fig. 12N; Table 5)

Baikalonis microdiscus n. sp. clearly differs from *B. foissneri* by its smaller zooid size (20–30 μm long vs. 56–60 μm long in vivo), the single-layered (vs. double-layered) peristomial lip, the peristomial disc which is narrow and slightly convex in its center (vs. wide and conical), absence (vs. presence) of a conspicuous fibrillar structure in the lower part of the zooid and its marine (vs. freshwater) habitat (Jankowski 1982).

***Zoothamnium wilberti* n. sp.**

Comparison with morphologically similar congeners (Fig. 12O–S; Table 6)

Zoothamnium wilberti n. sp. is mainly characterized by its dichotomously branched stalk, single-layered peristomial lip, conical peristomial disc with wart-like protuberance, and apically positioned contractile vacuole. Considering these characters, three congeners should be compared with *Z. wilberti* n. sp., namely *Z. affine* Stein, 1854, *Z. vermicola* Precht, 1935, and *Z. oviforme* Sommer, 1951.

Zoothamnium affine is a common epibiont on Gammaridae and has been reported many times. Schödel (2006) summarized the previous work of this species and made a revision. According to Schödel’s review, *Z. wilberti* n. sp. can be distinguished from *Z. affine* by having fewer (35–40 vs. 65–79) silverlines between the peristome and the trochal band, and its marine (vs. freshwater or stenohaline) habitat (Schödel 2006; Stein 1854) (Fig. 12O, P).

Zoothamnium vermicola resembles *Z. wilberti* n. sp. in terms of zooid size and shape and marine habitat. However, the primary stalk of *Z. vermicola* is always shorter than the zooid (vs. stalk longer than zooid in *Z. wilberti* n. sp.) (Precht 1935) (Fig. 12Q). Ji et al. (2009) made a redescription of *Z. vermicola* based on a population from China. Besides the shorter primary stalk, their population has more silverlines between the peristome and the trochal band than *Z. wilberti* (50–68 vs. 35–40). According to Precht (1935), *Z. vermicola* has a wart-like protuberance in the centre of peristomial disc which was not observed in the Chinese population (Ji et al. 2009). We therefore believe this is a population-dependent difference.

Zoothamnium oviforme differs from *Z. wilberti* n. sp. by its larger zooid (64–84 μm vs. 45–65 μm long in vivo), the absence (vs. presence) of a wart-like protuberance on the peristomial disc, the peristomial lip being hardly (vs. con-

Table 5. Morphometric comparison of *Baikalonis microdiscus* n. sp. with *Baikalonis foissneri* (based on specimens in vivo, measurements in μm).

Species	Body length	Body width	Pellicle	Diameter of PL	PL	PD	CV	Ma	Stalk length	Host	Habitat	Data source
<i>B. microdiscus</i> n. sp.	20–30	15–20	finely striated	14–16	slightly everted	small, slightly convex	clearly below PL	C-shaped, transverse	5–12	copepods	MW	Present work
<i>B. foissneri</i>	56–60	20	transversely lower part with fibrillar structure	slightly narrower than body ^a	slightly everted	wide, conical	ca. mid-body curved	slightly curved	12–14	<i>Baikalina bellitcosa</i>	FW	Jankowski (1982, 2007); Warren (1988)

Abbreviations: CV, contractile vacuole; FW, freshwater; Ma, macronucleus; MW, marine water; PD, peristomial disc; PL, peristomial lip.

^aInferred from the drawing.

Table 6. Morphometric comparison of *Zoothamnium wilberti* n. sp. with closely related congeners (based on specimens in vivo, measurements in μm).

Species	Body length	Body width	Diameter of PL	PL	PD	CV	Ma	Stalk	Colony	Host	Habitat	Data source
<i>Z. wilberti</i> n. sp.	45–65	30–40	30–40, ca. body width	clearly everted	conical, with wart-like protuberance	apical	C-shaped, transverse	up to 300 long, partly wrinkled	less branched, usually ≤ 16 zooids	<i>Gammarus</i> sp.	MW	Present work
<i>Z. affine</i>	50–100	24–45	ca. body width	everted	conical, with wart-like protuberance	apical	C-shaped, transverse	usually about 200 and can up to 500, partly wrinkled	usually ≤ 12 zooids	Gammaridae	FW, stenohaline	Stein (1854); Schödel (2006)
<i>Z. vermicola</i>	55–65	–	ca. body width	clearly everted	conical, with wart-like protuberance	apical	C-shaped, transverse	less than body length, wrinkled, spasmoneme detached from substrate	less branched, usually ≤ 4 zooids	<i>Castalia punctata</i> , <i>Eteonc longa</i> , <i>Lagis koreni</i> , <i>Nephtys</i> sp., <i>Ophioglypha albida</i>	MW	Precht (1935)
<i>Z. oviforme</i>	64–84	30–44	21–26, less than body width	hardly everted	convex to somewhat conical	same level as PL	C-shaped, transverse	long main stalk (102–195 long), short branches	less branched	<i>Potamogeton</i> sp., <i>Asellus aquaticus</i>	FW	Sommer (1951)
<i>Z. sp.</i>	65	40	ca. body width	clearly everted	conical, with wart-like protuberance	apical	sausage-shaped, longitudinal	–	less branched, 2–4 zooids	<i>Gammarus pulex</i>	FW	Bierhof and Roos (1977)

Abbreviations: CV, contractile vacuole; FW, freshwater; Ma, macronucleus; MW, marine water; PD, peristomial disc; PL, peristomial lip; –, data not available.

spicuously) everted, and the freshwater (vs. marine) habitat (Sommer 1951) (Fig. 12R).

Bierhof and Roos (1977) described an unnamed species of *Zoothamnium* found on *Gammarus pulex*. This species was tentatively identified as *Z. affine* by Schödel (2006) although its longitudinally oriented macronucleus is not consistent with *Z. affine* which has a transversely oriented macronucleus. This population closely resembles *Z. wilberti* n. sp. although it can be separated from the latter by its longitudinally oriented and relatively straight (vs. transversely oriented and C-shaped) macronucleus and its freshwater (vs. marine) habitat (Fig. 12S).

Phylogenetic analyses

The present study provides the first molecular data for *Rhabdostyla* and *Baikalonis*. The SSU rDNA tree, however, challenges the systematic positions of these genera based on morphological characters. Traditionally, *Rhabdostyla* has been classified in the family Epistylididae based on its non-contractile stalk without spasmoneme and its “epistylid”-like peristome (Corliss 1979; Lynn 2008). Morphologically, it most closely resembles *Orborhabdostyla* Foissner et al., 2009 which is also a solitary epistylid but with a discoidal to ellipsoidal macronucleus. However, in the SSU rDNA tree, *Rhabdostyla* (represented by *R. commensalis*) clustered with the family Astylozoidae, distant from both *Orborhabdostyla* and the family Epistylididae. The family Astylozoidae, which was redefined by Sun et al. (2012) based on SSU rDNA information, includes *Opisthonecta* spp., *Astylozoon enriquesi* and *Vorticellides* spp. The morphology and lifestyle of these taxa are, however, distinctly different: *Opisthonecta* spp. and *A. enriquesi* are stalkless and free swimming whereas *Vorticellides* has a stalk with spasmoneme and is sessile for most its life history. *Rhabdostyla* has a non-contractile and unbranched stalk. Consequently, we failed to find morphological or life history apomorphies to support the inclusion of *R. commensalis* within the family Astylozoidae. Data for additional gene markers and increased taxon sampling for *Rhabdostyla* and astylozoid peritrichs are needed in order to verify this relationship (Sun et al. 2013).

Lynn (2008) assigned *Baikalonis* to the family Vorticellidae despite it having a straight spasmoneme and a stalk that shortens longitudinally upon contraction. The SSU rDNA tree indicates a close relationship between *Baikalonis* (represented by *B. microdiscus* n. sp.) and the family Scyphidiidae, rather than with the main clades of Vorticellidae (e.g., *Vorticella* and *Carchesium*). Nevertheless, few morphological similarities could be recognized between *Baikalonis* and scyphidiids which are stalkless and attach to their substrate directly via the scopula. Although the findings of this study support the separation of *Baikalonis* from the Vorticellidae, its affiliation to any other family could not be resolved. It is possible that *Baikalonis* represents a new family.

In the SSU rDNA trees, *Epistylis anastatica* did not group with its congeners but clustered with *Carchesium polyp-*

inum and species of *Ophrydium*, albeit with low support (28% ML, 0.67 BI). A systematic affiliation between these taxa and *E. anastatica* is unexpected because, other than their colonial lifestyle, they are not morphologically similar. *Ophrydium* is nested deep within the Vorticellidae, a family that is characterized by having a helically contractile spasmoneme whereas zooids of *Ophrydium* attach to their substrate via a non-contractile stalk. Sun et al. (2016) suggested that *Ophrydium* represents a lineage that acquired its non-contractile stalk by reduction and loss of the spasmoneme in its ancestor. Similarly, *E. anastatica* may have also derived from an ancestor that lost its spasmoneme. This hypothesis was indirectly supported by Zhuang et al. (2018), who proposed that *Zoothamnium*, a genus with a continuous spasmoneme that contracts in a “zig-zag” fashion, was ancestral to *Epistylis* s. str., the fully developed spasmoneme therefore being plesiomorphic. By contrast, the placement of *E. anastatica* within the Vorticellidae assemblage suggests that the spasmoneme of its ancestor was more likely helically contractile. Thus, *E. anastatica* might represent a different evolutionary lineage to that of its congeners.

The separation of *Zoothamnium wilberti* n. sp. from its congeners is consistent with its distinct morphological features, i.e., it has a conical peristomial disc, whereas all other species of Zoothamniidae for which sequence data are available have a flat or convex peristomial disc. Although there are several species with a conical disc and many epibiotic species of *Zoothamnium*, none of these have been sequenced. Consequently, it is not currently possible to determine their taxonomic affiliations using molecular systematics.

Author contributions

XH conceived and guided the study. BL conducted sampling and performed laboratory work. ZS and BL identified the species. QZ did the phylogenetic analyses and interpreted the results. BL and ZS wrote the manuscript, and AW, XH and WS made further revisions. AW also provided access to the older literature. All authors read and approved the final version of manuscript.

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