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Description of two species of caenomorphid ciliates (Ciliophora, Armophorea): Morphology and molecular phylogeny

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Abstract

Most species in the anaerobic ciliate family Caenomorphidae Poche, 1913 lack detailed descriptions based on modern morphologic and molecular methods. In this report, two species, *Caenomorpha medusula* Perty, 1852 and *Sulfonecta uniserialis* (Levander, 1894) Jankowski, 1978, were isolated from freshwater anaerobic sediments in Qingdao, China. Another population of *C. medusula* was recorded from Boise, Idaho, USA. We reinvestigated live morphology, ciliature, and the small-subunit (SSU) rRNA gene sequence of both species. The current study supports the opinion of Jankowski that, due to the variation in macronuclear nodule number, this character is of limited taxonomic significance in *C. medusula*. Scanning electronic micrographs clearly reveal that this species has one posterior spine. The China population of *Sulfonecta uniserialis* corresponds well with previously described populations in having a single macronucleus, a complex posterior spine, and one bell kinety. Our phylogenetic analyses reveal that the Caenomorphidae is monophyletic. However, the placement of the family is uncertain as it forms a closer relationship with the Litostomatea but with only a medium support value.

Keywords: Anaerobe; Armophorida; Caenomorphidae; Phylogeny; Silver staining; SSU rDNA

Introduction

Anaerobic and micro-aerobic free-living ciliates are important bioindicators in running and stagnant waters, wastewater treatment plants, and soil habitats (Foissner 2016a; Kahl 1932; Wetzel 1928), most of them belonging to the Armophorea and Plagiopylea (Hu 2014; Lynn 2008; Paiva et al. 2017); they also include some members of genera *Cyclidium* and *Loxodes* (Esteban et al. 1993; Xu et al. 2015). Armophorean ciliates, formerly assigned to the Heterotrichia, are typically small to medium-sized cells with highly variable body shapes representing a special assemblage within the Ciliophora Doflein, 1901 (Lynn 2008). The free-living armophoreans are widely distributed, occupying

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hypoxic habitats worldwide (Bourland et al., 2017; Kahl 1932; Mieczan et al. 2013; Silva-Neto et al. 2015).

Caenomorphidae Poche, 1913, which includes the genera *Caenomorpha* Perty, 1852, *Ludio* Penard, 1922, and *Sulfonecta* Jankowski, 1978, is a highly specialized group comprising some hydrogenosome-bearing ciliates inhabiting anaerobic or microaerophilic brackish, freshwater and seawater habitats with a high concentration of hydrogen sulfide (Dragesco 1960; Jankowski 2007). The genus *Cirranter* Jankowski, 1964, formerly included in Caenomorphidae, has recently been transferred to the Apometopidae Foissner, 2016 (Foissner 2016b). The Caenomorphidae are morphologically characterized by a medusoid-shape with somatic ciliature reduced to small kineties, referred to as bell kineties, and several to many oral polykinetids in a small oral cavity (Jankowski 1964; Lynn 2008). *Caenomorpha* is a relatively species-rich genus in the family. For species separation, such features as numbers of macronuclei, posterior spines and the bell kineties are considered to be of high taxonomic value. Several known species in the genus exhibit an increasing complexity of posterior spines, from the simplest ones (*C. simplex*) to such highly organized forms as *C. sapropelica* (Jankowski 1964).

Caenomorpha medusula, type species of the genus, was first described by Perty (1852), and re-described many times since (Dragesco and Dragesco-Kernéis 1986; Kahl 1932; Martin-Gonzalez et al. 1988). Its cortical morphogenesis and conjugation process was investigated only once (Martin-Gonzalez et al. 1988). Jankowski (1978) transferred *Caenomorpha uniserialis* to the genus *Sulfonecta* (*S. uniserialis* was fixed as type species by original designation), and then both Lynn (2008) and Paiva et al. (2013) accepted this classification. Since the original description by Levander (1894), *S. uniserialis* has been investigated by many researchers (Decamp and Warren 1997; Jankowski 1964; Kahl 1932; Sola et al. 1990). However, inconsistencies in the descriptions of these two species persist. During a faunistic survey of free-living anaerobic ciliates in Qingdao, China, and Boise, Idaho, USA, we found two species of caenomorphids. In this paper, we provide a detailed description of their morphology, morphometrics, and 18S rRNA gene sequence. Phylogenetic analyses based on SSU rRNA gene sequence were performed to assess their systematic position in the class Armophorea.

Material and Methods

Sample collection, observation and morphologic study

Caenomorpha medusula and *Sulfoneta uniserialis* were collected from sediments of a freshwater pond with abundant reeds in Qingdao (36° 07′36″N, 120° 35′ 20″E), Shandong Province, China in May 2015 (Fig. 1A, D). The water temperature was 18.3 °C, and pH 6.8. The black sediments had a strong sulfidic odor. Another population of *C. medusula* was sampled from sulfidic sediments of a permanent eutrophic pond in Boise, Idaho, U.S.A. (43°38′ 31.37″N 116°11′12.01″W) during August 2015 through October 2016 (Fig. 1B, C). The water temperature was 19.8 °C, salinity 332 μS/cm, and pH 7.6. The anaerobic ciliates could survive several days in the sampling containers. Attempts to establish clonal cultures were unsuccessful. Scanning electron microscope preparations were made according to Foissner (2014). Preparations were imaged in the Hitachi SU3500 scanning electron microscope.

Living organisms were observed under a light microscope equipped with differential interference contrast illumination. The silver carbonate impregnation method was used to reveal the infraciliature and nuclear apparatus (Fernández-Galiano 1976). Measurements and counts were performed at 1000× magnification. Drawings of specimens were done with the aid of a drawing attachment.

DNA extraction and gene sequencing

Several cells of *Caenomorpha medusula* and *Sulfonecta uniserialis* were picked out with glass micropipettes, washed three to five times using the filtered, sterile habitat water and then used for DNA extraction. Genomic DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. TaKaRa ExTaq polymerase (TaKaRa Biomedicals, Japan) was used to amplify the small-subunit (SSU) rRNA gene with universal primers, EukA and EukB (Medlin et al. 1988) and PCR conditions following Gao et al. (2016). Cloning and sequencing were performed as described by Gao et al. (2016). For the Idaho population, DNA was extracted from single washed cells and the SSU rRNA gene was amplified with primers EukA and EukB. After purification with ExoSAP IT (Affymetrix, Santa Clara, CA) sequencing was done at GenWiz, LLC (South Plainfield, NJ).

Phylogenetic analyses

In total, 42 SSU rDNA sequences of 26 species of armophoreans obtained from the NCBI GenBank database were used in the phylogenetic analyses. Two species, namely, *Protocruzia adherens* and *P. contrax*, were selected as outgroup taxa. Sequences were aligned using the GUIDANCE algorithm with the default parameters via the GUIDANCE web server (Penn et al. 2010). Sequences were manually edited to produce an identity matrix using the program BioEdit 7.0.5.2 (Hall 1999). Ambiguously aligned regions were excluded, resulting in a matrix of 1460 characters. Maximum-likelihood (ML) analyses were carried out on CIPRES Science Gateway using RAxML-HPC2 version 8.2.9 (Miller et al. 2010; Stamatakis et al. 2008) with the GTR + I + G nucleotide substitution model. Support for the

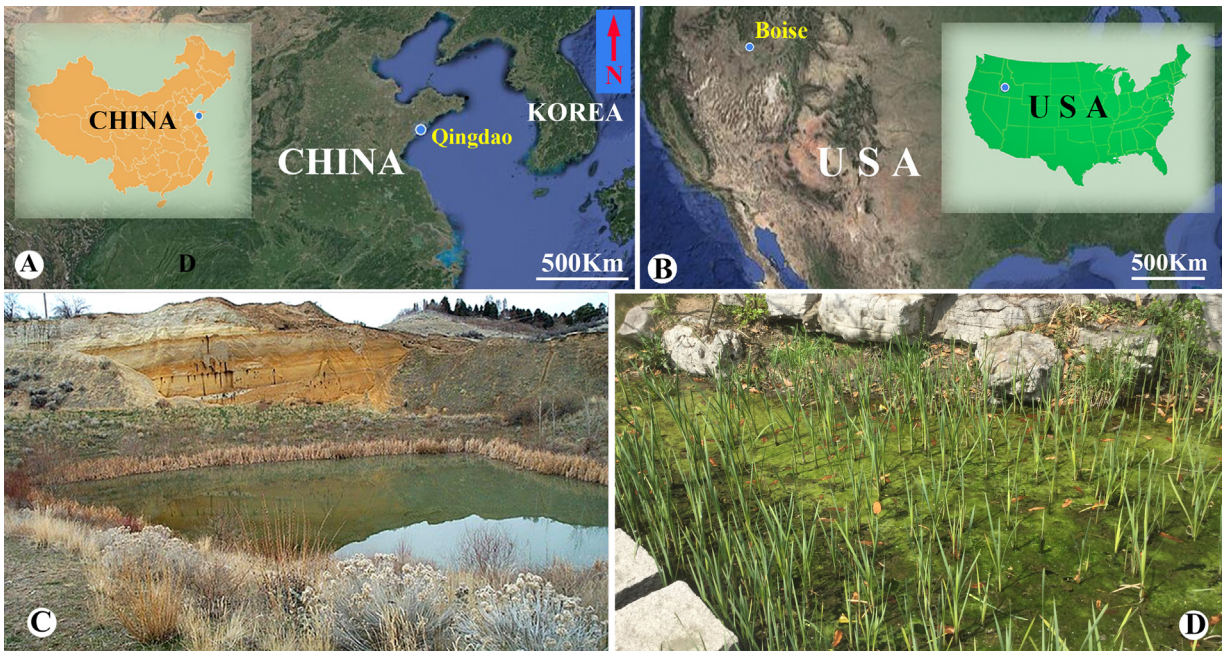

Fig. 1. A–D Sample location. **(A)** Map showing the location of Qingdao, China. **(B)** Map showing the location of Boise, USA. **(C)** The sludge pool in Boise. **(D)** The sludge pool in Qingdao.

best ML tree came from a majority rule consensus tree of 1000 bootstrap replicates. A Bayesian inference (BI) analysis was performed using MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003) using the GTR + I + G as the best model (selected by MrModeltest v.2.0; Nylander 2004) on CIPRES Science Gateway. Markov chain Monte Carlo simulations were run with two sets of four chains for 1,000,000 generations with a sample frequency of 100 generations, and with the first 25% being discarded as burn-in. The remaining trees were used to calculate posterior probabilities using a majority rule consensus.

Topology testing

Constrained trees were created and compared with the unconstrained (i.e. best scoring) ML tree topology, generating a file of per-site log-likelihoods in the RAxML framework for comparison of constrained and unconstrained tree topologies (Stamatakis et al. 2008). To this end, the approximately unbiased (AU) test in CONSEL ver. 0.1k (Shimodaira 2002, 2008; Shimodaira and Hasegawa 2001) was applied. A p-value of <0.05 was chosen for rejection of the null hypothesis.

Results and Discussion

Caenomorpha medusula Perty, 1852 (Figs. 2 A–G, 3 A–L, 4 A–E; Table 1)

Caenomorpha medusula was originally described by Perty (1852). There are numerous descriptions of this species (Jankowski 1964; Martin-Gonzalez et al. 1988; Schmall 1976; Wetzel 1928), but none provided information using scanning electron microscopy and small-subunit rRNA gene sequencing. Thus, based on two populations from China and USA, a complete description is provided here.

Description: Body medusoid, covered with a transparent rigid pellicle, 112–125 × 50–65 μm in vivo with a ratio of length to width about 2:1 (Figs. 2 A, D, 3 A–E, 4 A–C); length of silver carbonate-impregnated specimens variable, ranging from 73 to 122 μm (Table 1). Posterior spine slender, about 45–55 μm long in vivo; ratio of spine length to body length about 0.4. Plump rod-shaped epibiotic bacteria often found in US population (Fig. 4E, F), but not found in China population. Cytoplasm clear and colourless, with some dark globules (1–2 μm across) and an aggregate of transparent granules in anterior body part (Fig. 2A), rod-like bacteria exist in cytoplasm in China population (Fig. 3F), very likely endosymbionts. Edges of preoral bell never adjoin closely to posterior body; peristome narrow, deep funnel-shaped. Cytostome near base of spine (Fig. 2E, F); undulating membrane recognizable after staining, length of membrane about 50 μm long (Figs. 3 J, 4 B, E). Contractile vacuole located near base of spine (Figs. 2 A, 3 H, 4 A), about 15–20 μm in diameter, pulsates at intervals of 3–5 min. Three (41 of 60 cells [Chinese population], 36 of 45 cells [US population]), four (19 of 60 cells [Chinese population], 8 of 45 cells [US population]) or five (1 of 45 cells [US population]) macronuclear nodules, usually ovoid or ellipsoidal, arranged in line, located in center of cell, sometimes incompletely separated, scattered 1–2 μm diameter nucleoli in silver carbonate preparations (Figs. 3 K, L, 4 B, C); one micronucleus, ellipsoidal, near macronuclear nodules. Movement leisurely, spiraling while rotating around the long axis of the body.

Table 1. Morphometric characteristics of *Caenomorpha medusula* (upper lines from Qingdao population, middle lines from Boise population) and *Sulfonecta uniserialis* (lower lines).

Characteristic[a]	Mean	M	SD	CV	Min	Max	*n*
Body, length	94.7	97.0	9.2	9.7	73	108	21
	102.3	105.0	11.36	11.1	81	122	20
	55.8	55.0	3.1	5.6	49	62	17
Body, total width	68.7	70.0	11.1	16.0	41	92	21
	64.9	66.0	9.82	15.1	46	78	20
	50.2	50.0	6.0	12.0	40	59	17
Body length: body width ratio	1.4	1.0	0.2	17.6	1	2	21
	1.6	1.6	0.25	15.9	1.2	2.1	20
	1.4	1.0	0.2	15.8	1.0	2.0	17
Macronuclear nodules, number[b]	3.3	3.0	0.5	13.7	3	4	60
	3.2	3.0	0.39	12.2	3	4	45
	1	1	0	0	1	1	17
Macronucleus nodule, length	14.1	14.0	2.4	16.9	10	18	21
	15.8	16.0	2.58	16.3	11	22	24
	14.7	15.0	3.0	20.4	10	19	17
Macronucleus nodule, width	10.8	12.0	2.4	21.8	6	14	21
	10.1	10.0	2.03	20.0	7	17	24
	13.2	13.0	2.8	22.3	9	17	17
Micronucleus, diameter	3.9	4.0	0.5	13.7	3	5	21
	5.0	4.9	0.73	14.7	4	6	13
	3.1	3.0	0.4	14.3	2	4	17
Bell kinety I, length	68.3	68.0	9.7	14.3	50	95	21
	53.8	54.0	3.44	6.40	48	62	13
	19.1	19.0	2.5	13.3	15	23	17
Bell kinety II, length	34.9	33.0	8.7	25.0	23	50	21
	31.1	32.0	3.71	11.9	23	37	13
	–	–	–	–	–	–	–
Bell kinetosomes I, number	94.0	94.0	13.4	14.2	63	122	21
	108.1	109.0	12.93	12.0	84	136	11
	46.0	43.0	7.6	16.6	36	64	17
Bell kinetosomes II, number	56.0	57.0	6.1	11.1	40	66	21
	59.1	58.0	6.65	11.3	50	74	13
	–	–	–	–	–	–	–
Perizonal ciliary stripe kineties, number	141.0	142.0	12.2	8.7	114	169	21
	155.3	156.0	18.2	11.7	123	180	13
	92.0	92.0	6.0	6.5	82	102	17
Adoral membranelles, number	53.0	54.0	6.2	11.9	41	61	21
	59.4	61.0	7.37	12.4	47	67	7
	38.0	38.0	3.1	8.3	32	44	17
Posterior spine kineties, number	2.0	2.0	0.0	0.0	2	2	21
	2.0	2.0	0.0	0.0	2	2	20
	3.0	3.0	0.0	0.0	3	3	16
Kinetosomes in posterior spine kinety, number	25.0	25.0	3.1	12.3	18	31	21
	27.4	27.0	5.38	19.6	20	38	16
	17.0	17.0	1.3	7.5	14	19	16
Paroral membrane, length	–	–	–	–	–	–	–
	48.7	49.0	2.28	4.7	43	52	11
	–	–	–	–	–	–	–

Measurements in μm. Abbreviations: CV, coefficiency of variation in %; M, median; Max, maximum; Min, minimum; n, number of specimens measured; SD, standard deviation of the arithmetic mean.

[a] Data based on silver carbonate stained specimens.

[b] One of 45 cells had five macronuclear nodules in Boise population of *C. medusula*.

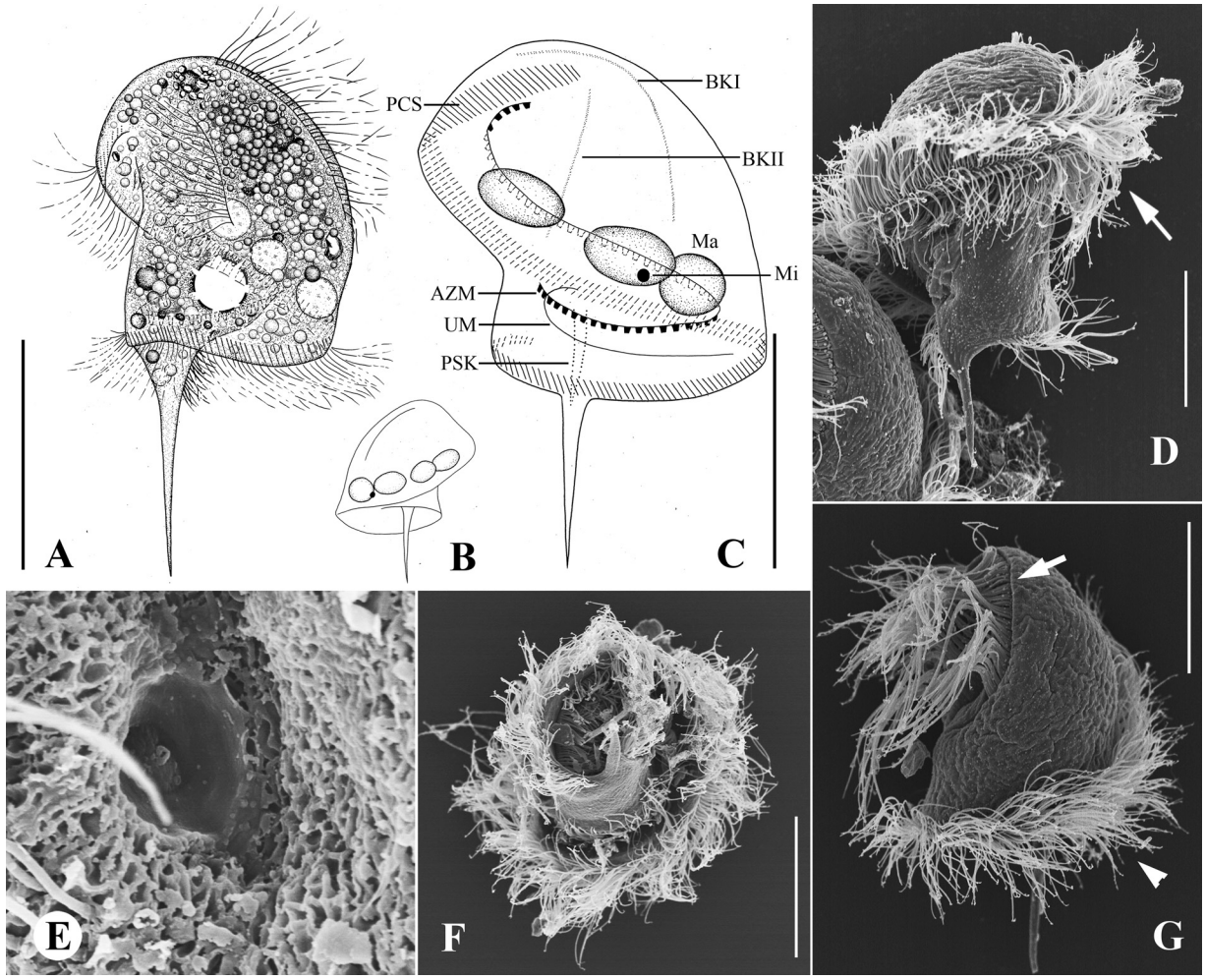

Fig. 2. A–G China population of *Caenomorpha medusula* from life (A), silver carbonate impregnation (B, C), and scanning electron microscopy (D–G). **(A)** Dorsal side of a representative individual. **(B)** An individual with four macronuclear nodules. **(C)** Dorsal view, showing ciliature and nuclear apparatus. **(D)** Left lateral view, showing conspicuous posterior spine and perizonal cilia (arrow). **(E)** Enlarged image showing the cytostome. **(F)** Posterior view. **(G)** Left lateral view, showing bell kinety I (arrow) and PCS (arrowhead). AZM, adoral zone of membranelles; BKI, II, bell kinety I and II; Ma, macronuclear nodule; Mi, micronucleus; PCS, perizonal ciliary stripe; PSK, posterior spine kineties; UM, undulating membrane. Scale bars: Figs (A, C), 50 μm; Figs (D, F, E), 30 μm.

Infraciliature as shown in Figs. 2 C, 3 F, J, 4 B, C. Two strongly thigmotactic bell kineties about 68 μm and 35 μm long, respectively, located in anterior part of dorsal side of cell (Fig. 4A), consist of about 94 and 56 cirri (Chinese population), and 108 and 59 cirri (US population) respectively (n = 21); cirri in each kinety arranged in indistinct zig-zag pattern (Figs. 3 G, 4C).

Perizonal stripe beginning near anterior end of cell, about 6.4 μm wide at middle part, composed of 114–180 kineties (114–169 in China population, 123–180 in US population), spiraling 450° around axis; each kinety inclined about 60° to edge of shield; longest kinety (at middle of stripe) composed of about 15 pairs of kinetosomes in both populations, whereas ones near oral region with only two pairs of kinetosomes (Figs. 2 C, 3 J, 4 B, C). Adoral zone composed of 41-67 membranelles (Chinese population), each with three or four rows of kinetosomes, spiraling 360° around body axis from near the distal perizonal stripe, terminates near cytostome (Figs. 2 C, 3 F, 4 B, C). Undulating membrane on undersurface of preoral bell (i.e. roof of peristomial region), about 50 μm long (Figs. 2 C, 3 F, G). Cilia on base of spine, invariably arranged in two short kineties, each 10–15 μm long, composed of about 25 kinetosomes each. The two spine kineties inclined about 20° to each other, converge posteriorly.

Remarks: Although this species has been studied for more than 150 years, species identification remains problematic because many descriptions are based only on live materials without redescription of the ciliature. Furthermore, some features, e.g. body shape, appear to vary among individuals in different environments, but, nonetheless, have traditionally been used as key characters in the taxonomy of *Caenomorpha*.

The original description of *Caenomorpha medusula* by Perty (1852) was rather superficial and failed to note some

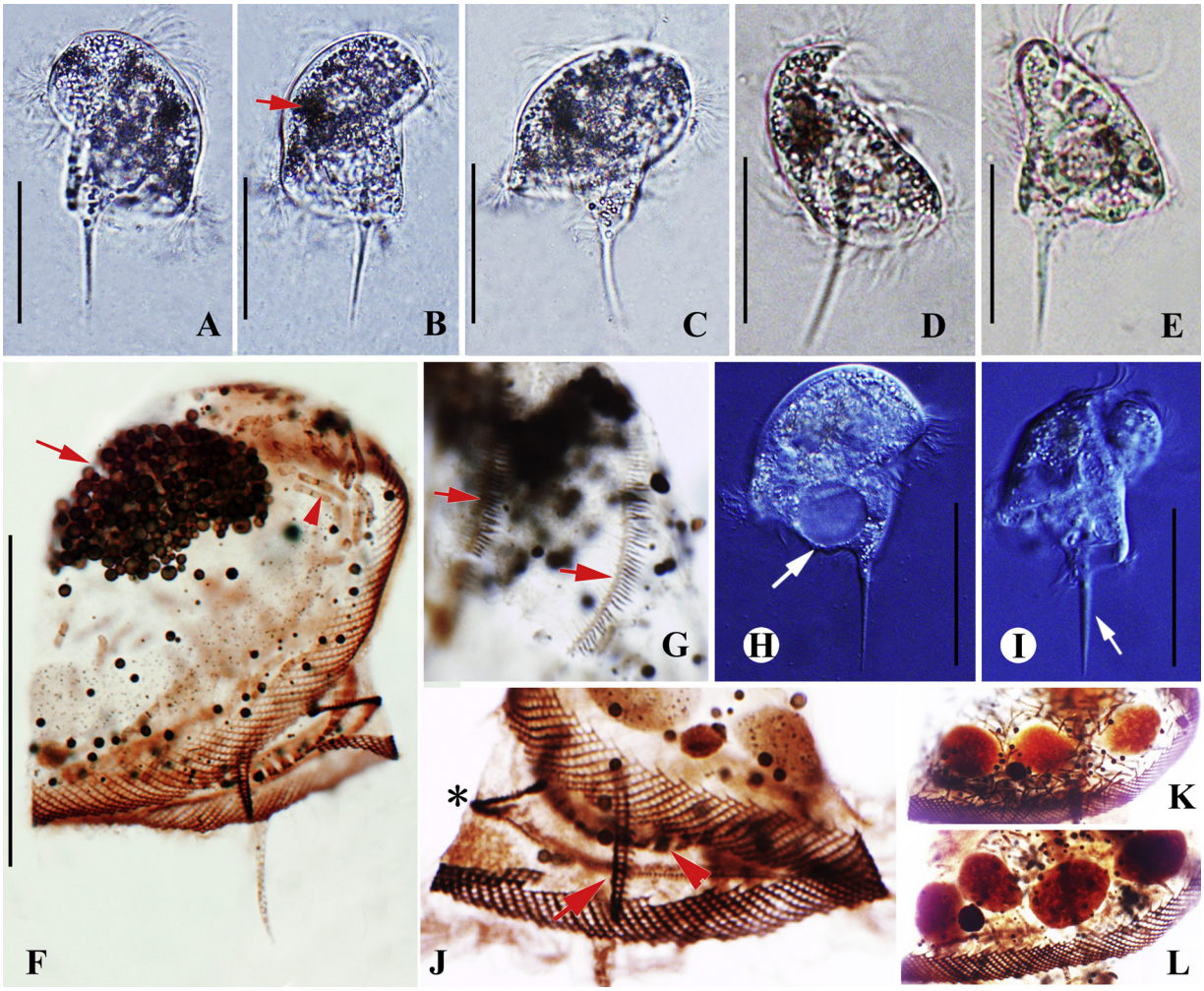

Fig. 3. A–L China population of *Caenomorpha medusula* from life, with bright field (A–E) and differential interference contrast illumination (H, I), and after silver carbonate impregnation (F, G, J–L). **(A–C)** Ventral view of a representative specimen, showing dark granule aggregate (arrow). **(D, E)** ventral view (D) and dorsal view (E), showing the variation of body shape. **(F)** Dorsal view of infraciliature, showing dark granule aggregate in the anterior part (arrow) and the symbiotic rod-like bacteria (arrowhead). **(G)** Details of two bell kineties (arrows). **(H)** Dorsal view, showing the contractile vacuole in diastole (arrow) and one conspicuous posterior spine. **(I)** Right side of the cell, showing posterior spine (arrow). **(J)** Detail of the posterior part showing adoral membranelles (arrowhead), posterior spine kineties (arrow), undulating membrane (asterisk) and the posterior part of the perizonal ciliary stripe. **(K, L)** Two individuals with three or four macronuclear nodules, respectively; one micronucleus adjacent to macronuclear nodule. Scale bars: 50 μm.

important features (e.g. the numbers of adoral membranelles, bell kineties and spines, etc.), which renders the identification of this organism difficult. However, according to the original and subsequent investigations, this species can be recognized by a combination of the following characters: (i) multiple macronuclear nodules; (ii) two unequal-length bell kineties; (iii) one conspicuous posterior spine.

Both the China and US populations resemble the original description well in general body shape and size, multiple macronuclear nodules and two bell kineties, so species identification is without question. Nevertheless, minor differences exist when compared with previous descriptions. Both populations have three or four macronuclei (one cell from the Idaho population had five nodules), agreeing with Jankowski's description but differing slightly from the populations (vs. 2 or 3) of Wetzel (1928) and Kahl (1927). Jankowski (1964) noted that individuals with three or four macronuclear nodules were common in Russian populations of this species. No differences in morphology correlated with the variation in the macronuclear nodule number. Considering the study by Martin-Gonzalez et al. (1988) showing two to five macronuclear nodules could exist during the life cycle, we agree with Jankowski (1964) that the number of macronuclear nodules in this species can be variable, and these populations should be conspecific.

The length of the bell kineties agrees with previous descriptions, but the cirri number in them in our populations were significantly greater than in some former descriptions (40–136 vs. 8–10). The current study revealed that even the shorter kinety has 40 or more cirri, so very likely Perty (1852)

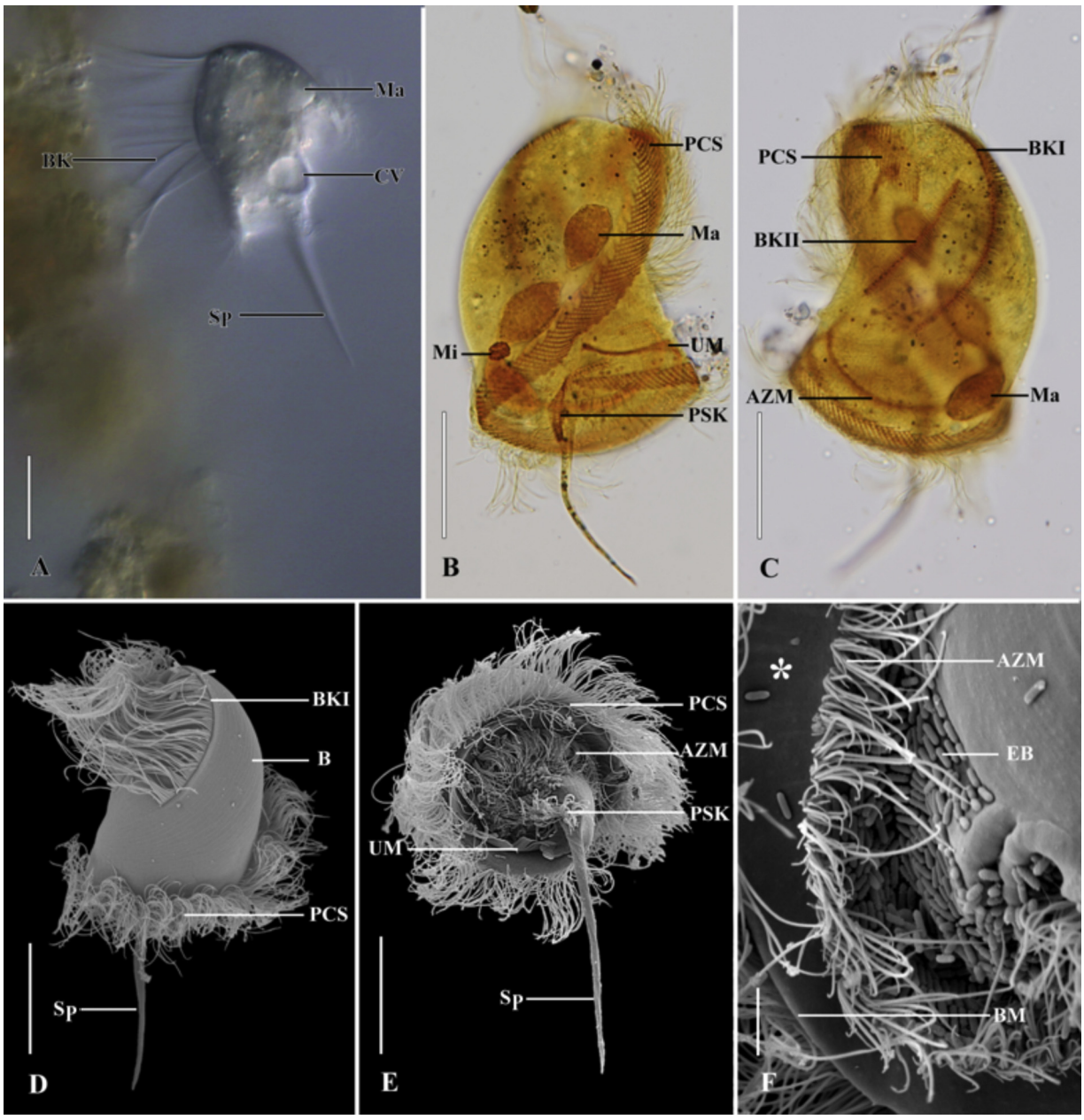

Fig. 4. A–F US population of *Caenomorpha medusula* from life (A), silver carbonate impregnation (B, C, same individual), and scanning electron microscopy (D–F). **(A)** Right lateral view of a representative individual attached to substrate by thigmotactic cilia of bell kineties. **(B)** Right lateral view. **(C)** Left lateral view. **(D)** Left lateral view. **(E)** Posterior view. **(F)** Undersurface of preoral bell (asterisk) showing epibiotic bacteria. AZM, adoral zone of membranelles; B, preoral bell; BKI, II, bell kinety I and II; BM, margin of preoral bell; CV, contractile vacuole; EB, epibiotioc bacteria; Ma, macronuclear nodule; Mi, micronucleus; PCS, perizonal ciliary stripe; PSK, posterior spine kineties; Sp, posterior spine; UM, undulating membrane. Scale bars: (A–E), 25 μm; (F), 5 μm.

and Jankowski (1964) gave an incorrect estimate since counts of individual cirri are difficult in live or simply fixed material. Additionally, in Martin-Gonzalez et al.'s description (1988), the number of spine kineties is higher than in our populations (3 vs. 2), so this variable feature may also be population-specific.

In terms of macronuclear nodules numbers, only *Caenomorpha lata* Kahl, 1927 can be compared with *C. medusula* since others have one macronuclear nodule. The former differs from the latter by the following characters: (i) a very wide (up to 85 μm vs. less than 70 μm) preoral bell; (ii) fewer macronuclear nodules (2 vs. 2–5, usually 3); (iii) an unusually long, thin spine that may exceed the body length (Jankowskii 1964; Kahl 1927). However, these character differences may reflect environment influences. More data, especially molecular information, are required in order to determine if *C. lata* is a distinct species.

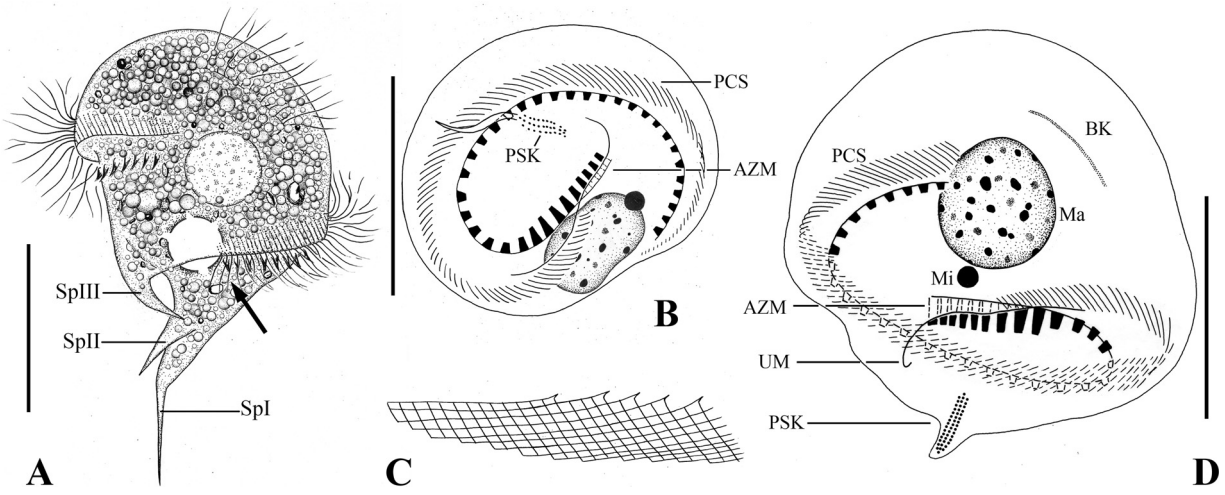

Fig. 5. A–D *Sulfonecta uniserialis* from life (A) and silver carbonate impregnation (B–D). **(A)** Ventral view of a representative individual, showing contractile vacuole (arrow). **(B)** General posterior polar view, showing infraciliature and nuclear apparatus. **(C)** Orientation of perizonal stripe fiber structure. **(D)** General lateral view of infraciliature and nuclear apparatus. AZM, adoral zone of membranelles; BK, bell kinety; Ma, macronucleus; Mi, micronucleus; PCS, perizonal ciliary stripe; PSK, posterior spine kineties; Sp, posterior spines (I, II and III); UM, undulating membrane. Scale bars: 30 μm.

Sulfonecta uniserialis (Levander, 1894) Jankowski, 1978 (Figs. 5 A–D, 6 A–M; Table 1)

Sulfonecta uniserialis was originally described by Levander (1894) and since then it was redescribed in more or less detail several times (Decamp and Warren 1997; Foissner et al. 1992; Kahl 1927; Schmall 1976). We provide a detailed morphological description and could sequence this species for the first time.

Subsequent papers failed to correlate morphologic information with SSU rRNA gene sequence. We provide these data here.

Description: Body medusoid with a ratio of length to width 1.75:1 (Figs. 4 A, 5 A–D), in vivo about 60–80 μm long, on average 56 μm long in silver carbonate preparations. Cells possess transparent rigid pellicle without any keels or ribs. Cytoplasm colourless, filled with numerous colourless granules, spherical, 1–2 μm in diameter in vivo. Aggregate of dark spherical particles (about 3 μm across) always positioned in anterior part of cell, as in *C. medusula*. Some rod-shaped (about 10 μm long) cytoplasmic bacteria recognizable after silver carbonate impregnation (Fig. 6I). Macronucleus globular to ellipsoidal, 9–19 μm across (Fig. 6E, J). Micronucleus ovoid, about 3 μm across, adjacent to macronucleus. A shallow funnel between preoral dome and posterior body surface. Small spine (SpIII) at lower margin of bell, in perizonal area. Main spine (SpI) 20–25 μm long in vivo, bearing another shorter spine (SpII) on its root (Figs. 5 A, 6 F). Contractile vacuole about 20 μm across, posterior, located near sytostome (Fig. 6B), contracts at intervals of >5 min. Movement leisurely, spiraling while rotating around long axis of body.

Infraciliature as shown in Figs. 5 B, D, 6 J, L, M. One bell kinety in anterior part of body, composed out 36–64 dikinetids in zig-zag pattern (Fig. 6G); cilia 10–16 μm long in vivo. Perizonal stripe composed of 82–102 kineties, inclined about 45° to margin of shield, spiraling about 360° around axis; kinety in middle portion consisting of 10–15 pairs of kinetosomes, those near cytostome with three or four pairs (Fig. 6K). Adoral zone composed of 32–44 membranelles, spiraling about 360°; base of membranelles up to about 7 μm wide. Cilia of SpI arranged in three short parallel, unequal length spine kineties; longest one up to 15 μm long, composed of 14–19 kinetosomes (Fig. 6H).

Remarks: Since the original report by Levander (1894), *Sulfonecta uniserialis* has been re-described on several occasions (Foissner et al. 1992; Kahl 1927; Schmall 1976; Sola et al. 1990). The type population is different from others in the number of spines (2 vs. 3), but considering the methodologic limitations of the time, one of the smaller spines was likely overlooked. Our form corresponds well with the original and subsequently described populations in having one bell kinety and one large spherical macronucleus. However, the shape of the spine I shows minor differences among populations. One type of spine I has an obvious expansion in the middle part (Foissner et al. 1992; Jankowski 1964; Schmall 1976), but the other type does not (Decamp and Warren 1997; Levander 1894). The China population belongs to the latter form. Considering that the shape of spine I is unstable among populations, we consider it as a population-dependent phenomenon.

The other difference is the shape of the bell. In the original description and most re-descriptions, it is pyriform, with less of an overhang. In Schmall's population (Schmall 1976), the preoral dome was flatter, and in Kahl's form (1932), it is oblique, with a prominent overhang. But in the China population we find both forms; also, cell shape may vary with exposure to air for a period of time (e.g. about 20 min), so

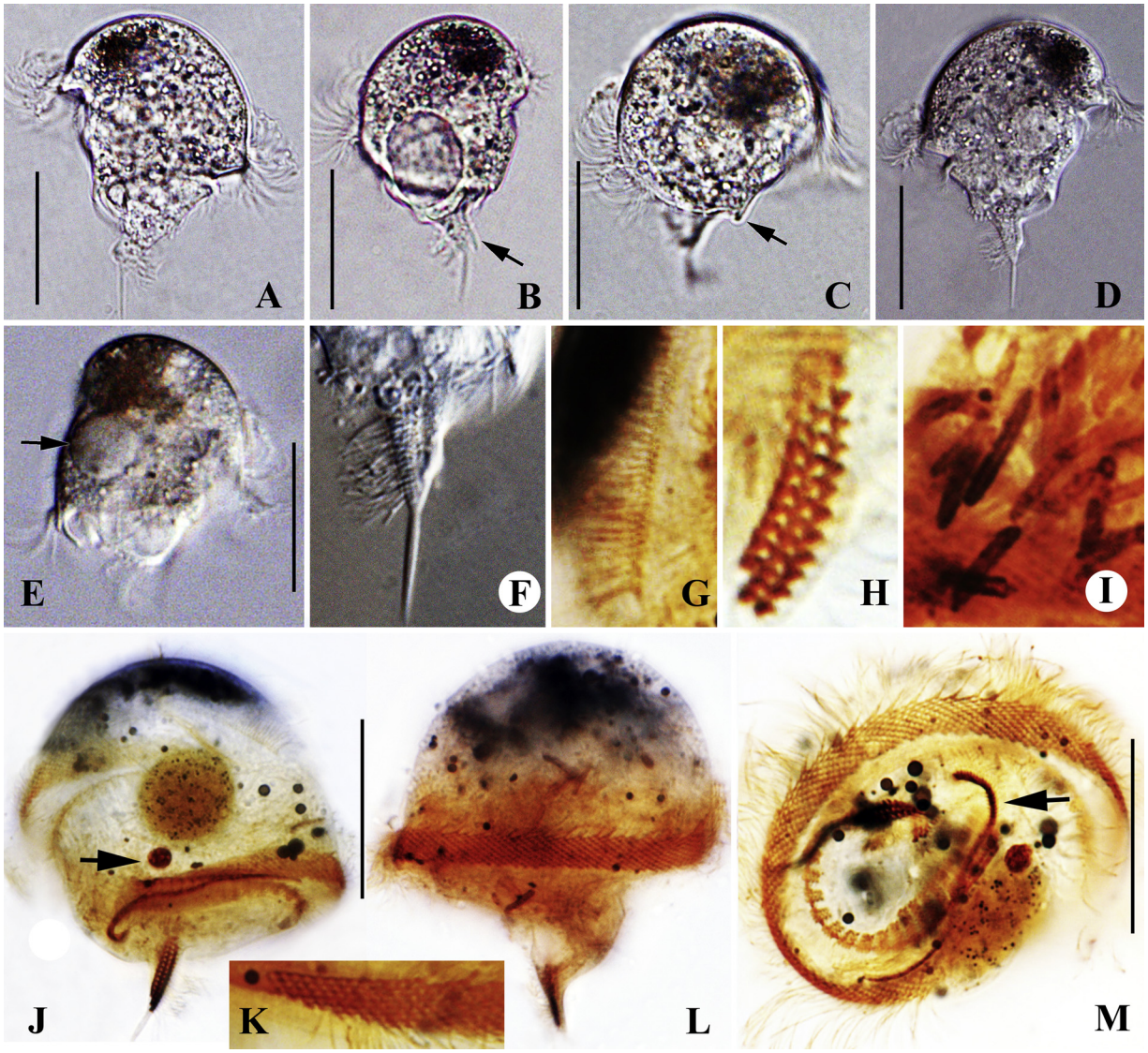

Fig. 6. A–M *Sulfonecta uniserialis* from life, with bright field (A–C), differential interference contrast illumination (D–F) and after silver carbonate impregnation (G–M). **(A)** Dorsal view of a typical specimen. **(B)** Ventral view of the same specimen as in A, showing Spine II (arrow). **(C)** Slightly anterior view of preoral dome, showing Spine III (arrow). **(D)** Outline of the posterior spine I. **(E)** Dorsal view, showing macronucleus (arrow). **(F)** Detail of the posterior end, showing spine I and cilia of posterior spine kineties. **(G)** Orientation of the cirri in the bell kinety. **(H)** Posterior spine kineties. **(I)** Rod-like bacteria in cytoplasm, very likely endosymbionts **(J, L)** Ventral and dorsal view of the same specimen, showing infraciliature, macronucleus and micronucleus (arrow). **(K)** Proximal part of the perizonal stripe. **(M)** Posterior polar view, arrow indicates undulating membrane. Scale bars: 30 μm.

we assume the shape of cell may not be a stable character.

Additionally, Decamp and Warren (1997) recorded a population with a wider range of body length than the China population (36–90 μm vs. 49–62 μm). A possible reason for this difference could be that the former population was obtained from much more varied environments. In the description of Decamp and Warren (1997) and Sola (1990), the number of perizonal ciliary stripe kineties is greater than in China population (100–120 vs. 82–102) and the number of posterior spine kineties (3 or 4 vs. 3) is more variable. Finally, it is notable that the black granule aggregation in anterior portion of cell is very obvious in the China population but was not mentioned by previous authors and may be a special characteristic in this population.

Sulfonecta uniserialis is the only species in the genus (Jankowski 2007). It is similar to *Caenomorpha simplex* Jankowski, 1964 in having one macronucleus and one bell kinety (Jankowski 1964). Nevertheless, the former has a complex compound spine (a long spine with a short affiliated one vs. a short triangular spine), while the latter has a single, simple, short spine, so the two can be easily distinguished.

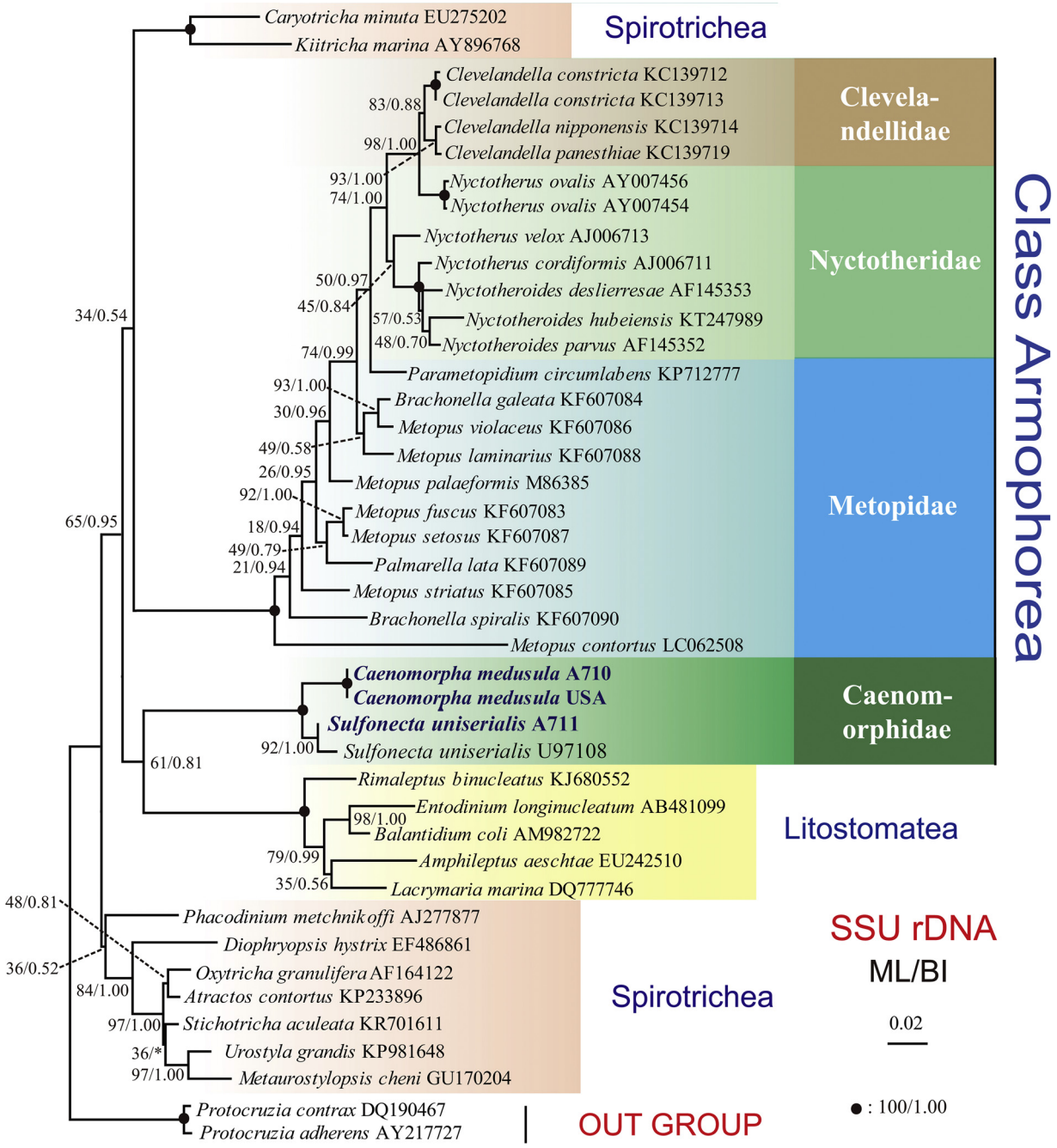

Fig. 7. Maximum likelihood (ML) tree based on SSU rRNA gene sequence data. Sequences from this study are in bold. Support values at the nodes are for BI and ML, respectively (BI/ML). All branches are drawn to scale. The scale bar corresponds to two substitutions per 100 nucleotide positions. GenBank accession numbers are given for each species.

Systematic position of *Caenomorpha* and *Sulfonecta* (Fig. 7; Table 2)

The class Armophorea Lynn, 2004 represents one of the two "riboclasses" the establishment of which is based solely upon similarities of the small subunit rRNA gene sequences shared by its members (Lynn 2008). Although the order Odontostomatida was previously included in Armophorea, it is now assigned to Plagiopylea (Stoeck et al. 2007). Prior to the present study there was only one caenomorphid SSU rRNA gene sequence of *C. uniserialis* (accession number U97108) in the GenBank database. Here we added sequences of three strains of caenomorphids. The lengths, GC contents, and accession numbers of the sequences are as follows: *Caenomorpha medusula* (Qingdao population; 1595 bp, 42.82 mol%, KP010148), *C. medusula* (US population; 1641 bp, 42.98 mol%, KT222269) and *Sulfonecta uniserialis* (1638 bp, 41.76 mol%, KP010149).

Table 2. Approximately Unbiased (AU) test results for trees comparisons considering different topological scenarios.

Topology constraints	-Ln likelihood	AU value (*p*)
0 Unconstrained	9631.30650	0.953
1 Monophyly of family Metopidae	9644.27330	0.066
2 Monophyly of family Nyctotheridae	9665.61001	0.006
3 Monophyly of genus *Brachonella*	9682.94761	0.002
4 Monophyly of genus *Metopus*	9681.11077	0.007
5 Monophyly of genus *Nyctotheroides*	9638.08995	0.155
6 Monophyly of genus *Nyctotherus*	9730.34687	9e−022

$p < 0.05$ refute monophyly, which are shown with shade; $p > 0.05$ do not refute the possibility of monophyly.

The two sequences of China populations of *C. medusula* and *S. uniserialis* differ in 116 nucleotides. The sequences of the Chinese and USA populations of *C. medusula* are identical. While 27 nucleotide differences are found between the China population of *S. uniserialis* and another one (U97108, see GenBank for details), morphological information about the latter was not provided, thus identification can not be certain. This underscores the importance of correlating careful morphologic and morphometric data with ciliate sequences in molecular databases.

As shown in Fig. 7, topologies of the ML and BI trees were basically congruent. In this tree, *Caenomorpha medusula* is sister to *Sulfonecta uniserialis* with full support (100% ML, 1.00 BI), which supports the monophyly of the Caenomorphidae.

Furthermore, our results did not support the monophyly of armophoreans, though it was not completely rejected by the AU-test (p-values = 0.270). Phylogenetic analyses demonstrate the non-monophyly of the two families (Metopidae and Nyctotheridae) and three genera (*Brachonella*, *Metopus* and *Nyctotherus*), which is in accordance with previous works (Bourland et al. 2017, 2014; Lynn and Wright 2013; Paiva et al. 2013). AU tests were also performed to test the phylogenetic associations of those taxa (Table 2). At the 5% significance level, most of the alternative hypotheses were clearly rejected (i.e. the monophyly of Nyctotheridae, $p = 0.006$; *Brachonella*, $p = 0.002$; *Metopus*, $p = 0.007$; *Nyctotherus*, $p =$ 9e-022); by contrast, the putative cluster of the forced grouping of the Metopidae was not rejected ($p = 0.066$). However, all these hypotheses still need to be tested further with more molecular data.

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