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Taxonomy and SSU rDNA-based phylogeny of three new *Euplotes* species (Protozoa, Ciliophora) from China seas

Chunyu Lian [a,b], Yuanyuan Wang [a], Lifang Li [b,*], Khaled A.S. AL-Rasheid [c], Jiamei Jiang [d,*], Weibo Song [a]

[a] Institute of Evolution & Marine Biodiversity, Ocean University of China, Qingdao 266003, China
[b] Marine College, Shandong University, Weihai 264209, China
[c] Zoology Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[d] Shanghai Universities Key Laboratory of Marine Animal Taxonomy and Evolution, Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, National Demonstration Center for Experimental Fisheries Science Education, Shanghai Ocean University, Shanghai 201306, China

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ABSTRACT

As widely accepted, Euplotid ciliates, a unique mono-cellular organisms, play important roles in ecological, environmental, evolutionary and basic biological studies. We investigate the living morphology, infraciliature and silverline system of three new marine *Euplotes* found from China in this study: *Euplotes bergeri* n. sp., *E. weissei* n. sp. and *E. shini* n. sp. The new species *Euplotes bergeri* is characterized by its dominant adoral zone of membranelles, ten frontoventral cirri, three or four caudal cirri, 12–14 dorsal kineties with 14–20 dikinetids in the mid-dorsal row, and a dorsal silverline system of the double-*eurystomus* type. *Euplotes weissei* can be recognized by its tiny size, having two conspicuous dorsal grooves, nine frontoventral cirri, one marginal cirri, six or seven dorsal kineties, ca. 10 basal bodies in the mid-dorsal row and a mix single-double silverline system. *Euplotes shini* is a medium-sized form with the ciliary and silverline pattern of the well-known *E. minuta* but differs from the latter mainly in SSU rDNA gene sequences and usually different-sized marginal cirri. Phylogenetic analyses based on molecular data documented the systematic positions of those new taxa and support the validity of all three organism as distinct species.

1. Introduction

The ciliate genus *Euplotes* is a highly diverse and cosmopolitan genus, with a variety of species distributed in marine, freshwater and terrestrial biotopes (Curds, 1975, Wilbert and Song, 2008). Traditionally, *Euplotes* species have been identified based on the biotope, the living morphology and the ciliary pattern (Kahl, 1932). Silver staining methods have also been used to highlight the infraciliature and silverline system of the *Euplotes* species, with these new specific features being applied to distinguish *Euplotes* species (Petz et al., 1995, Schwarz et al., 2007). Recently, molecular analyses have also been widely referred to when dealing with new or ambiguous species (e.g. Di Giuseppe et al., 2014, Sheng et al., 2018, Zhao et al., 2018). Based on this information, more than ten new species have established in the last two decades (e.g. Chen et al., 2019, Jiang et al., 2019, Lian et al., 2019).

Recent surveys of the ciliate fauna in around China sea have so far revealed 18 species of *Euplotes* including six that are new to science (Song et al., 2009, Liu et al., 2017, Lian et al., 2018, Yan et al., 2018, Hu et al., 2019). In the present paper three forms are documented. SSU rRNA gene sequences data are also supplied and their phylogenetic positions in the SSU rRNA gene tree are determined.

2. Material and methods

Euplotes bergeri n. sp. was found from off-shore water near Chizhou Island (22°28′N; 114°36′E), China on 4 May 2018, when the water temperature was 26 °C and salinity was 32‰ (Fig. S1A, D, E). *Euplotes weissei* n. sp. was isolated on 8 December 2017 from a water tank in the Laboratory of Protozoology (OUC) (36°04′N; 120°20′E) (Fig. S1A, B, C), China, where the water temperature was 19 °C and salinity was 30‰. *Euplotes shini* n. sp. was collected

* Corresponding authors.
E-mail address: jm-jiang@shou.edu.cn (J. Jiang).

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in off-shore water on 1 April 2018 on Baishazhou (22°40′N; 114°38′E), China, when the water temperature was 25 °C and salinity was 35‰ (Fig. S1A, F, G). Cells were observed *in vivo*, after protargol (Wilbert, 1975) and silver nitrate staining (Foissner, 2014). Terminology is according to Curds (1975).

DNA extraction, PCR amplification, sequencing (including other 73 ciliates) and data analyses are according to Luo et al. (2018) and Lu et al. (2018).

3. Results and discussion

3.1. Euplotes bergeri n. sp. (Figs 1A–K, 6A–L; Table 1, S1)

Diagnosis: In vivo about 70–80 × 45–50 μm; buccal field about 80% of body length with approximately 50 adoral membranelles; paroral conspicuously long; ten frontoventral cirri; two marginal, three or four caudal cirri; 12 to 14 dorsal kineties with about 17 dikinetids in mid-dorsal kinety; macronucleus C-shaped; dorsal silverline system double-*eurystomus* type. Marine habitat.

Type specimens: The holotype slide (No. LCY2018050402-1) with protargol stained holotype specimen and one paratype slide (No. LCY2018050402-2) with a protargol stained specimen are deposited in the Laboratory of Protozoology (OUC).

Etymology: We dedicate this new species to Dr. Helmut Berger, Salzburg, Austria, in recognition of his great contributions to the ciliatology studies.

ZooBank registration of Euplotes bergeri sp. n.: urn:lsid:zoobank.org:act:0349ECA6-68D2-4179-A310-21A0254E4021

Morphological description: Cells *in vivo* 70–80 × 45–50 μm. Outline broadly D-shaped, anterior end with a distinct projection on the right side (Fig. 1A). Ventral side with several short ridges between transverse cirri and two longer ridges between frontoventral cirri (Fig. 1A). On dorsal side, some ellipsoid granules clustered around each dorsal cilium, forming rosettes beneath pellicle; on ventral surface, granules packed all around cirri and adoral membranelles (Fig. 1E, F, G). Cytoplasm colourless, with numerous granules and several different-sized food vacuoles, making the cell somewhat opaque *in vivo* (Fig. 1A). Contractile vacuole about 10 μm across, located posteriorly near right body margin (Fig. 1A). Macronucleus mirror-inverted C-shaped; micronucleus not recognizable (Fig. 1C, H). Locomotion typically by moderately fast crawling or incessant jerking on substrate.

Buccal field approximately 80% of cell length with 43–57 adoral membranelles (AZM), breadth of adoral membranelles bases up to 14 μm long (Fig. 1A, B, H). Paroral membrane thin and very long (Fig. 1B, H, J). Consistently ten frontoventral cirri, about 20 μm in length; five transverse cirri, about 25 μm in length; usually three caudal cirri (six out of 25 cells have four such cirri), about 15 μm long; two marginal cirri about 17 μm long (Fig. 1A, B, H, K). Mostly 12 dorsal kineties and mid-dorsal kinety with 14–20 dikinetids (Fig. 1C, I, Table 1). Dorsal silverline system of double-*eurystomus* type (Fig. 1D).

Comparison with congeners: Considering the marine habitat, the double-*eurystomus* type silverline system as well as the general morphological features, especially the conspicuous broad and dominant adoral zone, two similar forms should be compared with our *E. bergeri*, namely *E. acanthodus* Petz et al., 1995 and *E. charon* (Müller, 1773) Ehrenberg, 1830.

Euplotes acanthodus was first reported by Petz et al. (1995) from the sea ice of the Weddell Sea, Antarctica. It differs from *E. bergeri* in having more adoral membranelles (60–76 vs. 43–57), fewer caudal cirri (2 vs. 3 or 4), and the arrangement of marginal cirri: in the former, two marginal cirri (MC) closely together and positioned immediately behind the proximal part of AZM, while in our new species, two MC are far apart and away from the AZM (Fig. S2; Petz et al., 1995).

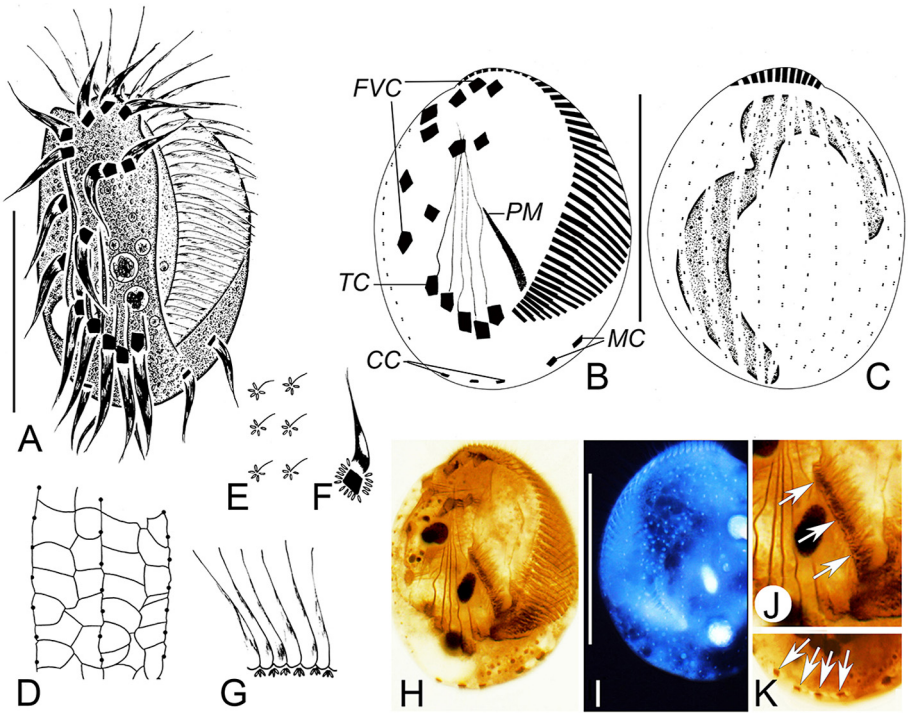

Fig. 1. Morphology and infraciliature of *Euplotes bergeri* n. sp. *in vivo* (A, E–G), after protargol staining (B, C, H–K) and silver nitrate (D) staining. (A) Ventral view. (B, C, H, I) Ventral (B, H) and dorsal (C, I) views, showing the infraciliature. (D) Silverline system on dorsal side. (E) Sub-pellicular rosette-like structures around dorsal cilia. (F, G) Showing the cortical granules around the cirri (F) and adoral membranelles (G). (J) showing paroral membrane (arrows). (K) Individual with four caudal cirri (arrows). CC, caudal cirri; FVC, frontoventral cirri; MC, marginal cirri; PM, paroral membrane; TC, transverse cirri. Scale bars: 50 μm.

Table 1
Morphometric data of *Euplotes bergeri* n. sp., *E. weissei* n. sp. and *E. shini* n. sp.

	species	Min	Max	Mean	Med	SD	CV	n
Body length	*E. bergeri*	49	82	65.6	63	7.3	11.2	25
	E. weissei	34	45	38.9	38	2.7	6.9	25
	E. shini	59	75	66.7	67	3.6	5.4	25
Body width	*E. bergeri*	42	68	52.0	51	6.3	12.1	25
	E. weissei	19	26	22.8	23	1.7	7.3	25
	E. shini	40	54	46.2	46	3.3	7.2	25
No. of adoral membranelles	*E. bergeri*	43	57	48.9	48	4.2	8.7	25
	E. weissei	20	28	23.8	23	1.7	7.1	25
	E. shini	37	46	42.1	43	2.4	5.6	25
No. of frontoventral cirri	*E. bergeri*	10	10	10.0	10	0.0	0.0	25
	E. weissei	9	9	9.0	9	0.0	0.0	25
	E. shini	10	10	10	10	0.0	0.0	25
No. of marginal cirri	*E. bergeri*	2	2	2.0	2	0.0	0.0	25
	E. weissei	1	1	1.0	1	0.0	0.0	50
	E. shini	2	2	2.0	2	0.0	0.0	25
No. of caudal cirri	*E. bergeri*	3	4	3.2	3	0.4	13.5	25
	E. weissei	2	2	2.0	2	0.0	0.0	25
	E. shini	2	3	2.1	2	0.3	13.3	25
No. of dorsal kineties	*E. bergeri*	12	14	12.5	12	0.6	4.7	25
	E. weissei	6	7	6.0	6	0.2	3.3	25
	E. shini	9	9	9.0	9	0.0	0.0	25
No. of dikinetids in middle kinety	*E. bergeri*	14	20	16.5	16	2.0	12.1	25
	E. weissei	7	11	8.9	9	0.9	10.2	25
	E. shini	10	14	12.3	12	1.1	9.0	25

All data are based on protargol-impregnated specimens. Abbreviations: CV = Coefficient of variation in %, Max = Maximum, Mean = Arithmetic mean, Med = Median, Min = Minimum, *n* = Number of cells measured, SD = Standard deviation.

The well-known species *Euplotes charon* was repeatedly reported by numerous followers after its original report (Table S1). Morphologically, *Euplotes bergeri* differs from *E. charon* in smaller cell size (generally smaller than 80 μm vs. about 100 μm), body shape (broadly D-shaped vs. broadly oval) and in the appearance of adoral zone (dominantly long bases of membranelles vs. no special trait), paroral membranelles (extremely long and narrow, length–width ratio about 13:1 vs. common shaped, 3:1) (Fig. S2B–L).

3.2. *Euplotes weissei* n. sp. (Figs 2A–L; Table 1)

Diagnosis: Cell size *in vivo* 35–40 × 20–30 μm; with two deep grooves on dorsal side, extending nearly entire length of dorsal kinetids; buccal field about 2/3 of body length with approximately 24 adoral membranelles; nine frontoventral cirri; one marginal cirrus; six or seven dorsal kineties with about nine dikinetids in mid-dorsal kinety; macronucleus C-shaped; dorsal silverline system mix single-double type. Marine water.

Type specimens: One holotype slide (No. LCY2017120801-1) with a protargol stained holotype specimen and two paratype slides (No. LCY2017120801-2; LCY2017120801-3) with Chatton–Lwoff method impregnation specimens are deposited in the Laboratory of Protozoology (OUC).

Etymology: We dedicate this new species to Prof. Thomas Weisse, University of Innsbruck, Austria, in recognition of his great contributions to the ciliatology studies.

ZooBank registration of Euplotes weissei sp. n.: urn:lsid:zoobank.org:act:2D43B6D5-8056-46F8-B86A-E8F349FC1909

Morphological description: Cells measuring 35–40 × 20–30 μm *in vivo*. Body more or less triangle-shaped, dorsoventrally flattened about 2:1 (Fig. 2A, G, H). Three conspicuous ventral ridges and some shorter ridges between transverse cirri (Fig. 2A). Two dominant and deep grooves on dorsal side (Fig. 2D, H, I, arrows). Dorsally several ellipsoid granules surrounding the dorsal cilium formed a rosette row along the dorsal kineties (Fig. 2B, I). Cytoplasm colourless, several different-sized lipid droplets and food vacuoles scattered (Fig. 2A, G, H, L). Contractile vacuole located posteriorly near right body margin (Fig. 2A). Macronucleus typically shaped in the form of the letter C, micronucleus not recognizable (Fig. 2F). Locomotion by crawling slowly on substrate while, occasionally, remaining stationary for rather long periods.

Adoral zone about 66% of body length with 20–28 membranelles (Fig. 2A, E, G, J). Invariably nine frontoventral cirri, about 15 μm long; five transverse cirri about 15 μm long, two caudal and one marginal cirri, cilia about 10 μm long (Fig. 2A, E, G, J). Six or seven dorsal kineties, with about ten dikinetids in the mid-dorsal kinety (Fig. 2F). The dorsal silverline system typically single-*vannus* type while in groove area the polygons are always folded and seem to be (?) double-*eurystoma* type (Fig. 2C, K).

Comparison and remarks on the silverline system of E. weissei n. sp.: As a tiny form, *E. weissei* can be easily recognized by having two dominant grooves on dorsal, which are, as far as we know, special in this species-rich genus. Regarding this feature as well as other morphological and ecological characters, e.g. presence of the single marginal cirrus, the small shape, the silverline system and the marine habitat, no similar forms could be compared (Curds, 1975; Liu et al., 2017; Song et al., 2009).

According to the pattern of silverlines, Curds (1975) defined five types of dargyrome in *Euplotes*, i.e. single-*vannus*, double-*eurystomus*, double-*patella*, multiple and complex. According to Valbonesi and Luporini (1995), however, the varying dorsal shapes especially the dorsal ridges might considerably affect the appearance of the dargyrome, and hence the typology of the argyrome of *Euplotes* should in fact be restricted to three basic types, i.e. single, double, and multiple. This point of view was partly supported by Foissner et al. (1991) who reported that the structures on the dorsal side might influence the appearance (with more prominent ridges, a double-*eurystomus* shifts to a double-*patella* type).

Euplotes weissei seems to be rather unique, that is, it presents a "mixed mode" (combined single and double types) as mentioned. The pattern is of more or less "double-type" (could be caused by folding of cell surface?) while in other part, the dargyrome is clearly in single mode. So far, no report available demonstrates a

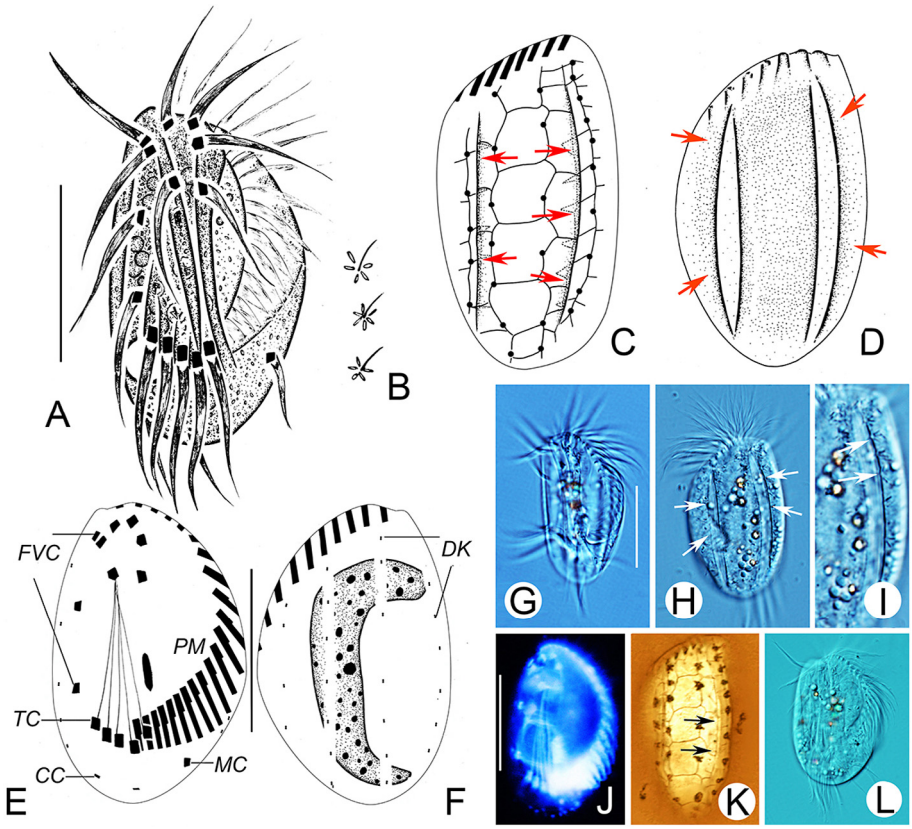

Fig. 2. *Euplotes weissei* n. sp. *in vivo* (A, B, D, G–I, L), after protargol (E, F, J) and silver nitrate (C, K) staining. (A, G, L) Ventral views. (B) Sub-pellicular rosette-like structures around dorsal cilia. (C, K) Silverline system on the dorsal side, arrows indicate the fold (?) of the polygons. (D, H, I) Showing the grooves on dorsal sides (arrows in D, H) and cortical granules (arrow in H) (E, F, J) Ventral (E, J) and dorsal (F) views, showing the infraciliature and nuclear apparatus, J is false-coloured. CC, caudal cirri; DK, dorsal kinety; FVC, frontoventral cirri; MC, marginal cirri; PM, paroral membrane; TC, transverse cirri. Scale bars: 20 μm.

"mixed pattern" of the single and double types in known *Euplotes*. Nevertheless, the silverline pattern of our new species wait for further defining.

Euplotes shini n. sp. (Figs 3fig3A–L, 6 M–Z; Table 1, S2)

Diagnosis: Medium-sized marine *Euplotes*, *in vivo* 65–75 × 35–45 μm; outline generally oval shaped, buccal field about 75% of body length with about 40 adoral membranelles; ten frontoventral cirri; two marginal, two or three caudal cirri; nine dorsal kineties with about 12 dikinetids in mid-dorsal kinety; macronucleus 3-shaped; dorsal silverline system single-*vannus* type. Marine water.

Type specimens: One holotype slide (No. LCY2018040103-1) with a protargol stained holotype specimen, one paratype slide (No. LCY2018040103-3) with Chatton–Lwoff method impregnation specimens are deposited in the Laboratory of Protozoology (OUC).

Etymology: We dedicate this new species to Pro. Mann Kyoon Shin, University of Ulsan, Korea, in recognition of his great contributions to the ciliatology studies.

ZooBank registration of Euplotes shini sp. n.: urn:lsid:zoobank.org:act:77253F72-61E3-463D-B764-D062A22B4228

Morphological description: Cell size *in vivo* 65–75 × 35–45 μm, generally oval in outline. Both left and right margin convex, anterior end narrowly rounded with a distinct projection on right side (Fig. 3A, D). Buccal field approximately 75% of body length (Fig. 3A, B, D, E, I). Cytoplasm colourless, transparent near cell margin, while opaque in central (Fig. 3A, D–F). Contractile vacuole l0 μm across, located posteriorly right (Fig. 3A). Macronucleus shaped in the form of an inverted number 3; micronucleus not observed (Fig. 3C, I, J). Locomotion by fast crawling and jerking movements.

Adoral zone prominent, composed of 37–46 membranelles, bending sharply in the proximal part into the cystostome, bases up to 15 μm long (Fig. 3A, B, I). Paroral membrane very short (Fig. 3B, I). Invariably ten frontoventral cirri, cilia about 17 μm long; five transverse cirri with cilia about 27 μm long; two marginal cirri, cilia about 15 μm long, the upper one usually bearing larger basal plaque than the lower one; usually two caudal cirri about 12 μm in length (Fig. 3A, B, I, K, L). Consistently nine dorsal kineties, with 10–14 dikinetids in mid-dorsal kinety (Fig. 3C, J). Silverline system of regularly single-*vannus* type (Fig. 3G, H).

Comparison with congeners: Morphologically, two small marine species with similar body shape and the single-*vannus* type silverline system should be compared with our new form: *E. cristatus* Kahl, 1932 and *E. minuta* Yocum, 1930.

Euplotes cristatus Kahl, 1932, was first discovered in the Kiel Bay, Germany (Kahl, 1932). Subsequently, this organism has been redescribed by Tuffrau (1960), Kattar (1970), Carter (1972), Carey (1992) and Park et al. (2010). *Euplotes shini* differs from *E. cristatus* in (1) the appearance of the adoral zone (evenly bending vs. sharply curved, nearly 90° bent in the posterior end), (2) the size of the two marginal cirri (upper one usually obviously bigger than lower one vs. approximately the same sized), and (3) the number of dorsal kineties (9 vs. 7–8) (Fig. S2U–Z). With reference to the morphology, *E. shini* appears to resemble *E. minuta* Yocum, 1930 which was "well-studied" after it was reported (Yocom, 1930, Borror, 1962, Kattar, 1970, Agamaliev, 1971, Song and Wilbert, 1997, Al-Rasheid, 1999, Park et al., 2010), and is hard to be distinguished from the latter though it seems to have different-sized marginal

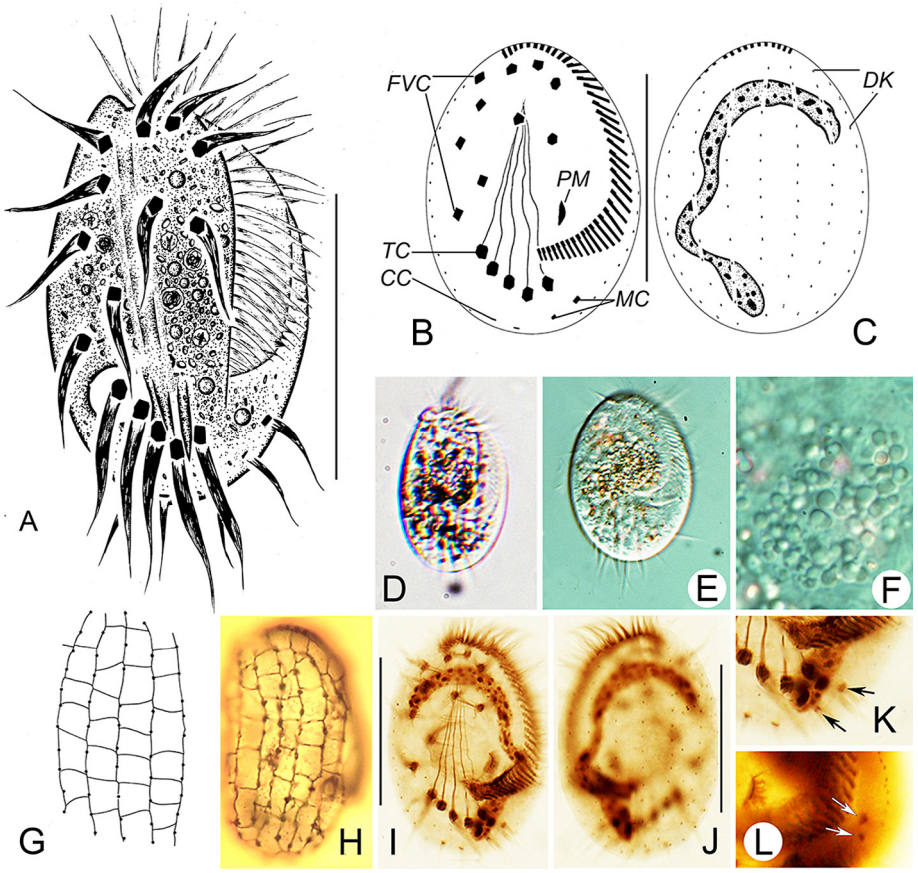

Fig. 3. Morphology and infraciliature of *Euplotes shini* n. sp. from life (A, D–F) and after protargol staining (B, C, I–L) and silver nitrate (G, H) staining. (A, D) Ventral views. (B, C, I, J) Ventral (B, I) and dorsal (C, J) views showing the infraciliature and nuclear apparatus. (E, F) Showing endoplasm. (G, H) Silverline system on dorsal side. (K, L) Ventral views, arrows indicate two marginal cirri. DK, dorsal kinety; FVC, frontoventral cirri; Ma, macronuclear nodules; MC, marginal cirri; PM, paroral membrane; TC, transverse cirri. Scale bars: 50 μm.

cirri (vs. approximately same sized). However, it is distinctly apart from the latter according to the molecular information (Fig. 4), that is, the SSU rDNA sequences have a similarity of 97.1% (compared with AJ310490, isolated from Germany rather than the type locality; Bernhard et al., 2001). Thus, these two species are not conspecific. We also suppose that the historical populations of *E. minuta* is probably a species complex (Table S2).

4. Systematic positions of three new species based on molecular data

All new SSU rDNA sequences have been deposited in the GenBank with accession numbers, length and GC contents as follows: *Euplotes bergeri* (MN593322, 1922 bp, 43.12%), *E. weissei* (MN593323, 1676 bp, 46.6%) and *E. shini* (MN593321, 1681 bp, 44.68%).

The topologies of the ML and BI trees were almost identical except for some minor differences, and therefore only the ML tree is presented here with bootstraps and posterior probabilities from both algorithms. At the same time, we also show the different topology of the BI tree branch including *E. shini*.

Euplotes bergeri did not cluster with the morphologically similar species (i.e. *E. charon* Muller, 1773). In fact, it clustered with *E. qatarensis*, forming a clade that was sister to the clade comprising *E. wuhanensis*, *E. raikovi*, *E. nobilii*, *E. estuarinus*, and *E. curdsi*.

Euplotes weissei clustered with a large-sized species *E. dammamensis*, thereby forming a sister clade with *E. parabalteatus*, together with *E. sinicus* and *E. petzi*, branching at the basal position for all other *Euplotes* species.

In the ML tree, *Euplotes shini* is closely related with a fully-supported assemblage including *E. charon*, *E. parkei*, *E. quinquecarinatus* and *E. focardii*. However, the BI tree exhibits another possible placement for *E. shini*, i.e. it is placed outside a large group comprising *E. minuta*, *E. antarcticus*, *E. cristatus*, *E. vannus*, *E. crassus*, *E. charon*, *E. parkei*, *E. quinquecarinatus* and *E. focardii* (as shown in Fig. 4).

5. Some new consideration on the phylogeny of species related

As most previous analysis showed (Chen et al., 2018, Wang et al., 2019), the phylogenetic analyses demonstrate that the order Euplotida and its five families, i.e. Gastrocirrhidae, Certesiidae, Uronychiidae Aspidiscidae, and Euplotidae, are all monophyletic groups. The monophyly of the order Euplotida is also fully supported (100% ML, 1.00 BI).

According to Syberg-Olsen et al. (2016), Lian et al. (2019) and Yan et al. (2018), *E. curdsi*, *E. nobilii*, *E. raikovi*, *E. elegans*, *E. estuarinus*, *E. qatarensis* and *E. wuhanensis*, which all have a similar dargyrome type, are closely related. Likewise, our new species *E. bergeri*, which shares a double-*eurystomus* type silverline system with the above six species, falls within a fully-supported group including *E. curdsi*, *E. nobilii*, *E. raikovi*, *E. elegans*, *E. qatarensis*, *E. estuarinus* and *E. wuhanensis* in the updated phylogeny of *Euplotes*. This is consistent with their morphological similarities and indicates that dargyrome type could be a very important evolutionary characteristic in this subclade.

The newly obtained *E. weissei* is closely related with *E. dammamensis* in both the ML and BI trees, however, the morphological

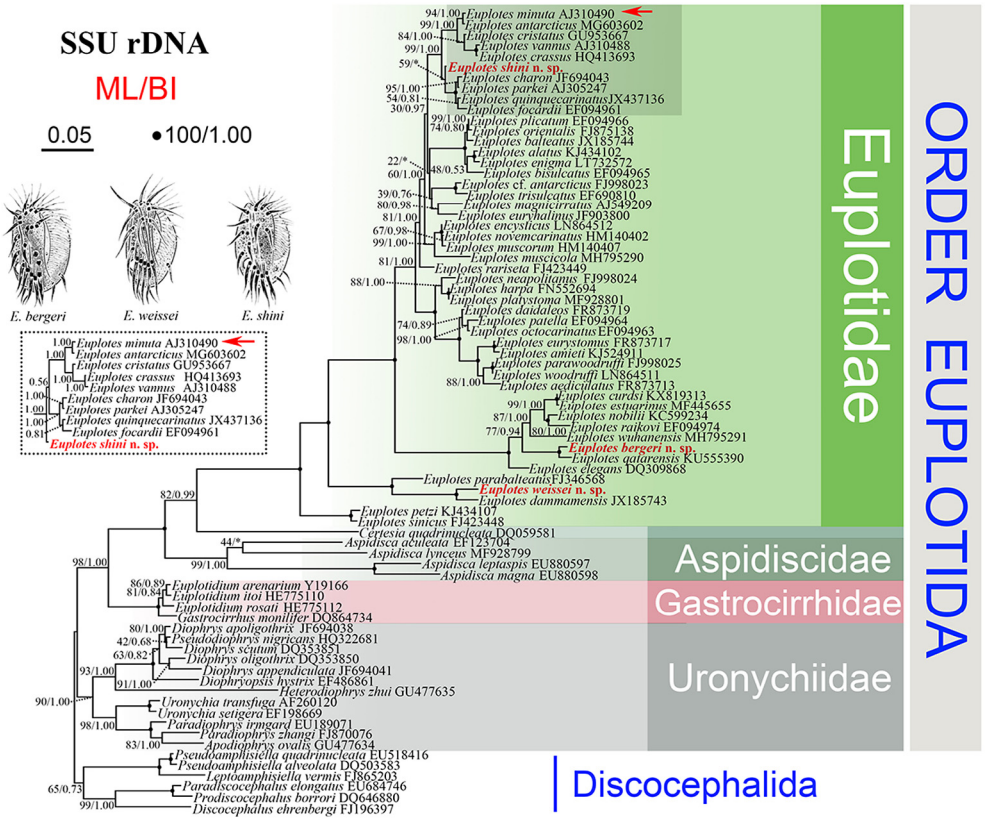

Fig. 4. The Maximum likelihood (ML) tree inferred from 18S rRNA gene sequences, showing the position of the three new *Euplotes* species. Numbers at nodes represent the bootstrap values of the ML analysis and the posterior probability of the Bayesian inference (BI) analysis. Fully supported (1.00/100) branches are marked with solid circles. Asterisk indicates a disagreement between BI tree and the reference ML tree. Arrow indicates a species morphologically similar to *E. shini*. Scale bar corresponds to five substitutions per 100 nucleotide positions.

characters of the two species were different both *in vivo* and after protargol impregnation. Besides, *E. weissei* did not cluster between *Euplotes* species with single type and double type dargyrome but was located outside the main *Euplotes* clade.

Eupotes shini is closely related in both trees with the four mentioned morphospecies. It, however, is clustered outside of the group of four *Euplotes* species with a single-*vannus* type silverline system. Indeed, the phylogenetic position of *E. shini* is not yet stable since it is placed in a different position in both trees.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2019.11.013.

References

Agamaliev, F., 1971. Complements to the fauna of psammophilic ciliates of the western coast of the Caspian Sea. Acta Protozool. 8, 379–404.
Al-Rasheid, K., 1999. Free-living marine interstitial hypotrichid ciliates from Jubail marine wildlife sanctuary in the Arabian Gulf. J. Agric. Mar. Sci. 4, 53–62.
Bernhard, D., Stechmann, A., Foissner, W., Ammermann, D., Hehn, M., Schlegel, M., 2001. Phylogenetic relationships within the class Spirotrichea (Ciliophora) inferred from small subunit rRNA gene sequences. Mol. Phylogen. Evol. 21, 86–92.
Borror, A.C., 1962. *Euplotes minuta* Yocom (Ciliophora, Hypotrichida). J. Protozool. 9, 271–273.
Carey, P.G., 1992. Marine interstitial ciliates. Chapman and Hall, 1–351.
Carter, H.P., 1972. Infraciliature of eleven species of the genus *Euplotes*. Trans. Amer. Micros. Soc. 91, 466–492.
Chen, X., Wang, Y., Sheng, Y., Warren, A., Gao, S., 2018. GPSit: An automated method for evolutionary analysis of nonculturable ciliated microeukaryotes. Mol. Ecol. Resour. 18, 700–713.
Chen, X., Jiang, Y., Gao, F., Zheng, W., Krock, T.J., Stover, N.A., Lu, C., Katz, L.A., Song, W., 2019. Genome analyses of the new model protist Euplotes vannus focusing on genome rearrangement and resistance to environmental stressors. Mol. Ecol. Resour., 10.1111/1755-0998.13023
Curds, C.R., 1975. Guide to the species of the genus *Euplotes* (Hypotrichida, Ciliatea). Bull. Br. Mus. Nat. Hist. (Zool.) 28, 1–61.
Di Giuseppe, G., Erra, F., Frontini, F.P., Dini, F., Vallesi, A., Luporini, P., 2014. Improved description of the bipolar ciliate, *Euplotes petzi*, and definition of its basal position in the *Euplotes* phylogenetic tree. Eur. J. Protistol. 50, 402–411.
Foissner, W., Berger, H., Blatterer, H., Kohmann, F., 1991. Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft, 1–471.
Foissner, W., 2014. An update of 'basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa'. Int. J. Syst. Evol. Microbiol. 64, 271–292.
Hu, X., Lin, X., Song, W., 2019. Ciliate Atlas: Species Found in the South China Sea. Springer, 1–631.
Jiang, Y., Zhang, T., Vallesi, A., Yang, X., Gao, F., 2019. Time-course analysis of nuclear events during conjugation in the marine ciliate *Euplotes vannus* and comparison with other ciliates (Protozoa, Ciliophora). Cell Cycle 18, 288–298.
Kahl, A., 1932. Urtiere oder Protozoa I: Wimpertiere oder Ciliata (Infusoria) 3. Spirotricha. Tierwelt Dtl. 25, 399–650.
Kattar, M., 1970. Estudo dos Protozoarios ciliados psamófilos do litoral Brasileiro. Bol. Zool. Biol. Mar. (São Paulo) N S 27, 123–206.
Lian, C., Luo, X., Fan, X., Huang, J., Yu, Y., Bourland, W., Song, W., 2018. Morphological and molecular redefinition of *Euplotes platystoma* Dragesco & Dragesco Kernéis, 1986 and *Aspidisca lynceus* (Müller,1773) Ehrenberg, 1859, with reconsideration of a "well-known" *Euplotes* ciliate, *Euplotes harpa* Stein, 1859 (Ciliophora, Euplotida). J. Eukaryot. Microbiol. 65, 531–543.
Lian, C., Zhang, T., Al-Rasheid, K.A., Yu, Y., Jiang, J., Huang, J., 2019. Morphology and SSU rDNA-based phylogeny of two *Euplotes* species from China: *E. wuhanensis*

sp. n. and *E. muscicola* Kahl, 1932 (Ciliophora, Euplotida). Eur. J. Protistol. 67, 1–14.
Liu, W., Jiang, J., Xu, Y., Pan, X., Qu, Z., Luo, X., El-Serehy, H.A., Warren, A., Ma, H., Pan, H., 2017. Diversity of free-living marine ciliates (Alveolata, Ciliophora): faunal studies in coastal waters of China during the years 2011–2016. Eur. J. Protistol. 61, 424–438.
Lu, X., Huang, J., Shao, C., Berger, H., 2018. Morphology, cell-division, and phylogeny of *Schmidingerothrix elongata* spec. nov. (Ciliophora, Hypotricha), and brief guide to hypotrichs with *Gonostomum*-like oral apparatus. Eur. J. Protistol. 62, 24–42.
Luo, X., Yan, Y., Shao, C., Al-Farraj, S.A., Bourland, W.A., Song, W., 2018. Morphological, ontogenetic and molecular data support strongylidiids as being closely related to Dorsomarginalia (Protozoa, Ciliophora) and reactivation of the family Strongylidiidae Fauré-Fremiet, 1961. Zool. J. Linn. Soc. 184, 237–254.
Park, M.H., Kim, S.J., Min, G.S., 2010. First record of two *Euplotes* ciliates (Ciliophora: Spirotrichea: Euplotida) from Korea. Koean J. Syst. Zool. 26, 21–27.
Petz, W., Song, W., Wilbert, N., 1995. Taxonomy and ecology of the ciliate fauna (Protozoa, Ciliophora) in the endopagial and pelagial of the Weddell Sea, Antarctica. Stapfia, 1–223.
Schwarz, M.V.J., Zuendorf, A., Stoeck, T., 2007. Redescription of *Euplotes elegans* Kahl 1932 (Hypotrichida; Euplotidae) isolated from the anoxic Mariager Fjord, Denmark. J. Eukaryot. Microbiol. 54, 125–136.
Sheng, Y., He, M., Zhao, F., Shao, C., Miao, M., 2018. Phylogenetic relationship analyses of complicated class Spirotrichea based on transcriptomes from three diverse microbial eukaryotes: *Uroleptopsis citrina*, *Euplotes vannus* and *Protocruzia tuzeti*. Mol. Phylogenet. Evol. 128, 338–345.
Song, W., Wilbert, N., 1997. Morphological investigations on some free living ciliates (Protozoa, Ciliophora) from China sea with description of a new hypotrichous genus. Hemigastrostyla nov. gen. Arch. Protistendk. 148, 413–444.
Song, W., Warren, A., Hu, X., 2009. Free-living ciliates in the Bohai and Yellow Seas, China. Science Press, Beijing, pp. 1–518.
Syberg-Olsen, M.J., Irwin, N.A., Vannini, C., Erra, F., Di Giuseppe, G., Boscaro, V., Keeling, P.J., 2016. Biogeography and character evolution of the ciliate genus Euplotes (Spirotrichea, Euplotia), with description of Euplotes curdsi sp. nov. PloS one 11, e0165442.
Tuffrau, M., 1960. Révision du genre *Euplotes*, fondée sur la comparaison des structures superficielles. Hydrobiologia 15, 1–77.
Valbonesi, A., Luporini, P., 1995. *Euplotes bisulcatus*, a key species for a better understanding of the organization and evolution of *Euplotes* argyrome patterns. Eur. J. Protistol. 31, 32–37.
Wang, Y.R., Wang, C., Jiang, Y., Katz, L.A., Gao, F., Yan, Y., 2019. Further analyses of variation of ribosome DNA copy number and polymorphism in ciliates provide insights relevant to studies of both molecular ecology and phylogeny. Sci. China: Life Sci. 62, 53–64.
Wilbert, N., 1975. Eine verbesserte Technik der Protargol Imprägnation fur Ciliaten. Mikrokosmos 64, 171–179.
Wilbert, N., Song, W., 2008. A further study on littoral ciliates (Protozoa, Ciliophora) near King George Island, Antarctica, with description of a new genus and seven new species. J. Nat. Hist. 42, 979–1012.
Yan, Y., Fan, Y., Luo, X., El-Serehy, H.A., Bourland, W., Chen, X., 2018. New contribution to the species-rich genus *Euplotes*: morphology, ontogeny and systematic position of two species (Ciliophora; Euplotia). Eur. J. Protistol. 64, 20–39.
Yocom, H.B., 1930. Two new species of *Euplotes* from Puget Sound. Pub. Puget Sound Biol. Sta. 7, 241–248.
Zhao, Y., Yi, Z., Warren, A., Song, W., 2018. Species delimitation for the molecular taxonomy and ecology of the widely distributed microbial eukaryote genus *Euplotes* (Alveolata, Ciliophora). Proc. R. Soc. B 285, 20172159.