Two new marine ciliates, *Euplotes sinicus* sp. nov. and *Euplotes parabalteatus* sp. nov., and a new small subunit rRNA gene sequence of *Euplotes rariseta* (Ciliophora, Spirotrichea, Euplotida)

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The morphology, infraciliature and silverline system of two marine *Euplotes*, *Euplotes sinicus* sp. nov. and *Euplotes parabalteatus* sp. nov., isolated from seawater near Qingdao, China, were investigated. *E. sinicus* is characterized by having conspicuous dorsal ridges, a single marginal cirrus and a silverline system of the double-*patella*-I type. *E. parabalteatus* is an extremely small form (only about 35 μm long) with 6–7 dorsal kineties and a silverline system of the double-*eurystomus* type. Small subunit (SSU) rRNA-based phylogenetic trees were constructed with three different methods and these firmly demonstrated that the novel species represent two distinct phylogenetic lineages within the genus *Euplotes*, branching as a sister group to all other sequenced congeners. In addition, the SSU rRNA gene of another rare, morphologically similar form, *Euplotes rariseta*, was sequenced. This revealed the phylogenetic position of *E. rariseta* to be basal to one of the major groups of *Euplotes* rather than close to *Euplotes nobilii.*

INTRODUCTION

Among ciliates, the genus *Euplotes* Ehrenberg, 1830 apparently has no counterpart with regard to the variety of species, worldwide distribution and adaptive plasticity. Tuffrau (1954), Borror (1972) and Carter (1972) revised the genus considering the pattern of the silverline system as an important character for species classification. In the last three decades, about 30 new morphospecies of *Euplotes* have been reported in addition to the 51 species classified by Curds (1975) in his guide to *Euplotes* taxonomy.

In more recent studies on ciliate fauna in northern China seas, several species of *Euplotes* have been identified and redescribed (Song & Packroff, 1997; Song & Wilbert, 1997; Jiang *et al.*, 2008). The present paper describes two novel species based on their morphology, diagnostic characters, small subunit rRNA (SSU rRNA) gene sequence homology and phylogenetic relationship with their congeners.

Abbreviations: BI, Bayesian inference; ML, maximum-likelihood; MP, maximum-parsimony; SSU rRNA, small subunit rRNA.

The GenBank/EMBL/DDBJ accession number for the small subunit rRNA gene sequence of *Euplotes rariseta* is FJ423449.

METHODS

Euplotes sinicus sp. nov. and *Euplotes parabalteatus* sp. nov. were collected in September 2007 from seawater off Qingdao (Tsingtao, 120°18′E; 36°04′N), China. Glass slides were used as artificial substrates to collect ciliates. Briefly, the slides were carefully taken out after being exposed to the seawater for about 7–10 days, and transferred to Petri dishes with seawater from the sampling site. Isolated specimens were maintained in the laboratory for observation and further studies (Hu, 2008).

The specimens were examined *in vivo* at different magnifications before silver impregnation. Live observations were carried out using an oil immersion objective with bright-field and Nomarski differential interference contrast optics (Song *et al.*, 2009). The infraciliature was impregnated by using the protocol of Wilbert (1975). The Chatton–Lwoff method was used for revealing the silverline systems (Wilbert & Song, 2008). Counts and measurements on stained specimens were performed at a magnification of × 1000 with a × 1.25 optovar device. Drawings were made with the help of a camera lucida. Terminology is mainly according to Curds (1975, 1977) and Berger (2006).

The SSU rRNA gene from one population of *E. rariseta* isolated from Qingdao, China, was sequenced. Genomic DNA extraction, PCR amplification and sequencing of the SSU rRNA gene were performed according to Yi *et al.* (2008a). Two primers were used: 18S-F (5′-AACCTGGTTGATCCTGCCAGT-3′) and 18S-R (5′-TGATCCTTCT-GCAGGTTCACCTAC-3′). Sequences of the SSU rRNA gene of *E. sinicus* and *E. parabalteatus* were obtained from the GenBank database (accession numbers FJ346568 and FJ423448, respectively); these were published recently as unidentified (Yi *et al.*, 2009). The other

Table 1. SSU rRNA sequences from the GenBank database used in this study

Species	Accession no.	Species	Accession no.
Amphisiella annulata	DQ832260	*Euplotes patella*	EF094964
Aspidisca steini	AF305625	*Euplotes plicatum*	EF094966
Aspidisca aculeata	EF123704	*Euplotes raikovi*	EF094973
Certesia quadrinucleata	DQ059581	*Euplotes rariseta*	AJ305248
Diophrys appendiculata	AY004773	***Euplotes rariseta* QD-2**	**FJ423449**
Diophrys oligothrix	DQ353850	*Euplotes sinicus*	FJ423448
Diophrys scutum	DQ353851	*Euplotes* sp.	AF492706
Diophrysopsis hystrix	EF486861	*Euplotes trisulcatus*	EF690810
Euplotes aediculatus	M14590	*Euplotes vannus*	AY004772
Euplotes bisulcatus	EF094965	*Euplotes woodruffi*	AF492707
Euplotes charon	AF492705	*Euplotidium arenarium*	Y19166
Euplotes crassus	AY361895	*Gastrocirrhus monilifer*	DQ864734
Euplotes daidaleos	EF690811	*Laboea strobila*	AF399151
Euplotes elegans	DQ309868	*Loxodes striatus*	U24248
Euplotes encysticus	EF535728	*Onychodromopsis flexilis*	AY498652
Euplotes euryhalinus	EF094968	*Phacodinium metchnikoffi*	AJ277877
Euplotes eurystomus	AJ310491	*Prodiscocephalus borrori*	DQ646880
Euplotes focardii	EF094960	*Protocruzia adherens*	AY217727
Euplotes harpa	AJ305252	*Protocruzia contrax*	DQ190467
Euplotes magnicirratus	AJ549210	*Paradiophrys irmgard*	EU189070
Euplotes minuta	AY361900	*Paradiophrys* sp.	EU189071
Euplotes muscicola	AJ305254	*Strombidium apolatum*	DQ662848
Euplotes nobilii	EF094970	*Strombidinopsis jeokjo*	AJ628250
Euplotes octocarinatus	EF094963	*Tintinnidium mucicola*	AY143563
Euplotes parabalteatus	FJ346568	*Uronychia binucleata*	EF198667
Euplotes parawoodruffi	AF452708	*Uronychia setigera*	EF198669
Euplotes parkei	AJ305247	*Uronychia transfuga*	AF260120

nucleotide sequences used in this study are all available in the GenBank database (Table 1).

The structural similarities among sequences of *E. sinicus*, *E. rariseta* and *Euplotes raikovi* were calculated pairwise as described by Elwood *et al.* (1985). Phylogenetic trees based on the SSU rRNA gene sequences for the family Euplotidae were constructed using three different methods: Bayesian inference (BI), maximum-likelihood (ML) and maximum-parsimony (MP). *Loxodes striatus* (U24248) was selected as the out-group species. Phylogenetic analyses were performed according to Yi *et al.* (2008b).

The topologies of the BI, ML and MP trees were almost identical. Therefore, they were merged into a single tree for purposes of illustration. This tree was formatted by using MEGA (Kumar *et al.*, 2004) and exported from the program as a graphics file for construction of the final tree.

RESULTS AND DISCUSSION

Euplotes sinicus sp. nov.

Diagnosis. Marine *Euplotes* with conspicuous dorsal ridges, 60–95 × 35–65 μm *in vivo*. Buccal field about two-thirds of cell length with about 41 membranelles; always 10 frontoventral, 5 transverse, 2 caudal and single fine marginal cirri; 7 dorsal kineties with about 12 dikinetids in mid-dorsal row. Macronucleus curved-bar- or C-shaped. Dorsal silverline system double-*patella*-I type. Morphometric data are summarized in Table 2.

Type location. Isolated from Qingdao, China, on 17 September 2007. Salinity about 27‰ and water temperature about 23 °C.

Type specimens. One holo- and one paratype slide with protargol- and silver nitrate-impregnated specimens, respectively, have been deposited in the Natural History Museum, London, UK (2008:8:4:1 and 2008:8:4:2, respectively), and another set of paratype slides have been deposited in the Laboratory of Protozoology, Ocean University of China (OUC), Qingdao, PR China (JJM2007091703-1 and JJM2007091703-2).

Etymology. The species name *sinicus* (Latin adjective, Chinese) refers to the fact that this species was first discovered in China.

Description. Cells *in vivo* usually 70–80 μm long, generally oval in outline as shown in Figs 1(a–c) and 2(a, c, d). Left and right margins usually less convex in slim individuals (Figs 1c, 2d) than those in well-fed cells (Figs 1b, 2c);

Table 2. Morphometric data for *E. sinicus* sp. nov. (upper rows) and *E. parabalteatus* sp. nov. (lower rows)

Data are based on protargol-impregnated specimens. CV, coefficient of variation (%).

Characteristic	Min	Max	Mean	SD	CV	*n*
Cell length (μm)	65	92	77.5	7.29	9.4	16
	35	44	38.3	2.78	7.3	13
Cell width (μm)	37	62	48.0	7.18	15.0	16
	26	36	26.7	1.70	9.5	13
Adoral membranelles	38	46	40.9	5.38	10.3	16
	19	23	20.4	1.39	6.8	13
Frontoventral cirri	10	10	10	0	0	16
	10	10	10	0	0	13
Transverse cirri	5	5	5	0	0	25
	5	5	5	0	0	25
Marginal cirrus	1	1	1	0	0	25
	2	2	2	0	0	25
Caudal cirri	2	2	2	0	0	25
	2	2	2	0	0	25
Dorsal kineties	7	7	7	0	0	16
	6	7	6.1	0.28	4.6	13
Dikinetids in mid-dorsal kinety	11	16	12.0	1.37	11.4	16
	8	11	9.3	0.75	8.1	13
Dikinetids in leftmost dorsal kinety	3	4	4.1	0.50	12.1	16
	2	3	2.3	0.48	20.1	13

anterior end narrowly rounded with a distinct projection at right side (Fig. 1a, g), while posterior end widely rounded. Cell body dorsoventrally flattened about 2 : 1 with ventral side somewhat convex and dorsal side strongly arched (Fig. 2b). Buccal field approximately two-thirds of cell length. On ventral side, 3 conspicuous ridges extending posteriorly to transverse cirri (Figs 1a, 2e); on dorsal side about 5 dominant dorsal ridges almost extending over the entire cell length (Fig. 1d; Figs 1f, 2b, f, arrows). Dorsal cilia conspicuous, about 5 μm long (Fig. 2g).

Numerous granules (possibly mitochondria) (Fig. 2h, arrows) about 2 μm across, extremely densely packed beneath pellicle (Fig. 1h). Cytoplasm colourless, highly transparent at marginal area, but opaque in central part where several to many different-sized lipid droplets and a few food vacuoles are included. Contractile vacuole adjacent to the rightmost transverse cirrus (Fig. 1a). Macronucleus variable in shape: from typical C-shaped (mostly) to slightly curved (Fig. 1e).

Locomotion typically by moderately fast crawling or slight jerking.

Infraciliature as shown in Figs 1(g, i, j) and 2(j, k). Paroral membrane small, typically composed of many irregularly arranged kinetosomes; positioned below the buccal lip (Fig. 1g). Adoral zone prominent, composed of 38–46 membranelles. Consistently 10 frontoventral cirri arranged in normal pattern, 5 strong transverse cirri and 2 caudal cirri. Single fine marginal cirrus located on left side of the cell posterior to buccal field. Always 7 dorsal kineties almost extending over entire length of the cell except the leftmost one which includes about 4 dikinetids; middle row with about 11–16 dikinetids (Fig. 1j). Silverline system on dorsal side double-*patella*-I type (Fig. 1j).

Comparison and discussion. Hitherto, only five morphotypes possessing the single marginal cirrus and a double-*patella* silverline pattern have been reported: *Euplotes algivora*, *Euplotes zenkewitchi*, *Euplotes raikovi*, *Euplotes rariseta* and *Euplotes strekovi*. Hence, we only compared these species with *E. sinicus*.

Among these species, *E. algivora* is very similar to *E. sinicus* in terms of its infraciliature (Agatha *et al.*, 1990; Fig. 3a–c, Table 3). However, *E. algivora* can be separated from *E. sinicus* by its slender cell shape (vs oval to broadly oval), conspicuous long and strong marginal cirrus (vs short and fine marginal cirrus), and 2 (vs 5) dorsal ridges.

E. zenkewitchi can be clearly distinguished from *E. sinicus* by the number of frontoventral cirri (9 vs 10) and dorsal kineties (8–10 vs 7), as well as the double-*patella*-II type of dorsal silverline system (vs double-*patella*-I) (Burkovsky, 1970; Fig. 3d, e, Table 3).

Both *E. strekovi* and *E. raikovi* resemble *E. sinicus* in cell size and shape; however, *E. strekovi* and *E. raikovi* possess one reduced cirrus (absent in *E. sinicus*), fewer frontoventral cirri (9 and 8, respectively, vs 10), and thus cannot be confused with *E. sinicus*. Additionally, *E. strekovi* has the double-*patella*-II type of dorsal silverline system (vs double-*patella*-I type) and 6 (vs 5) transverse cirri (Agamaliev, 1967; Jiang *et al.*, 2008; Fig. 3f–i, Table 3).

E. rariseta differs from *E. sinicus* in cell size (30–50 × 20–40 vs 65–92 × 37–62 μm), number of membranelles (17–22 vs 38–46) and number of dikinetids in the middle dorsal kinety (5–7 vs 11–16) (Ma *et al.*, 2007; Fig. 3j–m, Table 3).

SSU rRNA gene sequence analysis. The SSU rRNA gene sequence of *E. sinicus* is 1.72 kb in length and has a GC content of 45.0 mol%. The dissimilarity between *E. sinicus*, *E. rariseta* and *E. raikovi* is supported by pairwise comparison of their sequences. Sequences of *E. sinicus* and *E. rariseta* differ in 256 nucleotides and exhibit 89.2 % similarity, whereas *E. sinicus* differs in 309 nucleotides from *E. raikovi* with a similarity of only 86.7 %

Euplotes parabalteatus sp. nov.

Diagnosis. Small-sized marine *Euplotes*, about 35 μm long *in vivo*, slender oval; no conspicuous dorsal or ventral ridges. Buccal field over two-thirds of cell length with about 20 membranelles; consistently 10 frontoventral cirri, 2 marginal cirri positioned posterior to 5 relatively fine transverse cirri and close to 2 caudal cirri; 6–7 dorsal kineties with about 9 dikinetids in mid-dorsal row. Macronucleus slightly curved-bar-shaped. Dorsal silver-

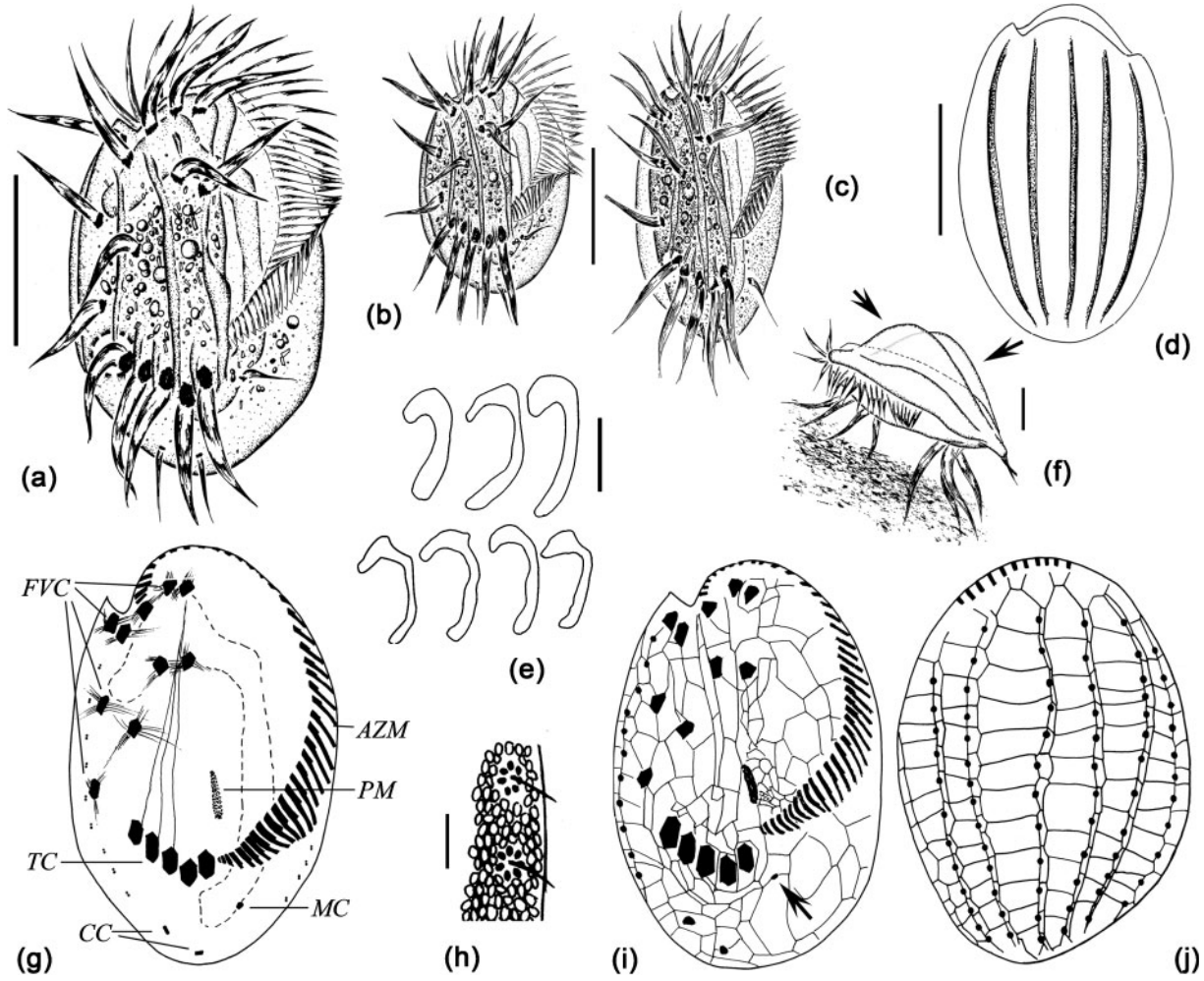

Fig. 1. *E. sinicus* sp. nov. *in vivo* (a–d, f, h), and after protargol (e, g) and silver nitrate (i, j) impregnation. (a–c) Ventral view of different individuals. (d) Dorsal view, showing ridges. (e) Different shapes of macronucleus. (f) Lateral view, showing conspicuous ridges (arrows). (g) Ventral view, showing infraciliature. (h) Portion of dorsal view, showing the granules beneath the pellicle. (i, j) Silverline system on ventral and dorsal sides; note the single fine marginal cirrus (arrow). AZM, adoral zone of membranelles; CC, caudal cirri; FVC, frontoventral cirri; MC, marginal cirrus; PM, paroral membrane; TC, transverse cirri. Bars, 30 μm [a (also applies to g, i and j) and d–f], 40 μm (b and c) and 10 μm (h).

line system double-*eurystomus* type. Morphometric data are summarized in Table 2.

Type location. Occurred in Qingdao, China, on 17 September 2007. Salinity about 27 ‰ and water temperature about 23 °C.

Type specimens. One holo- and one paratype slide with protargol- and silver nitrate-impregnated specimens, respectively, have been deposited in the Laboratory of Protozoology, Ocean University of China (OUC), Qingdao, PR China (JJM2007091701-1 and JJM2007091701-2, respectively), and another paratype slide with protargol-impregnated specimens has been deposited in the Natural History Museum, London, UK (2008 : 8 : 5 : 1).

Etymology. The species name *parabalteatus* is a composite of the prefix *para-* (Greek preposition, beside, like) and the species name *balteatus*, and refers to the similarity of this species to *Euplotes balteatus*.

Description. Cells *in vivo* about 30–35 μm long; cell body shape stable, generally elongate oval as shown in Figs 4(a, b) and 5(a–c); some specimens possibly broadly oval in outline prior to division; dorsoventrally highly flattened with dorsal side little arched, ventral side concave (Fig. 4c). Adoral zone prominent, about two-thirds to three-quarters of cell length (Fig. 4a), and composed of 19–23 adoral membranelles.

One short evident ridge on ventral side located between transverse cirri (Fig. 5e, arrow). On dorsal surface neither

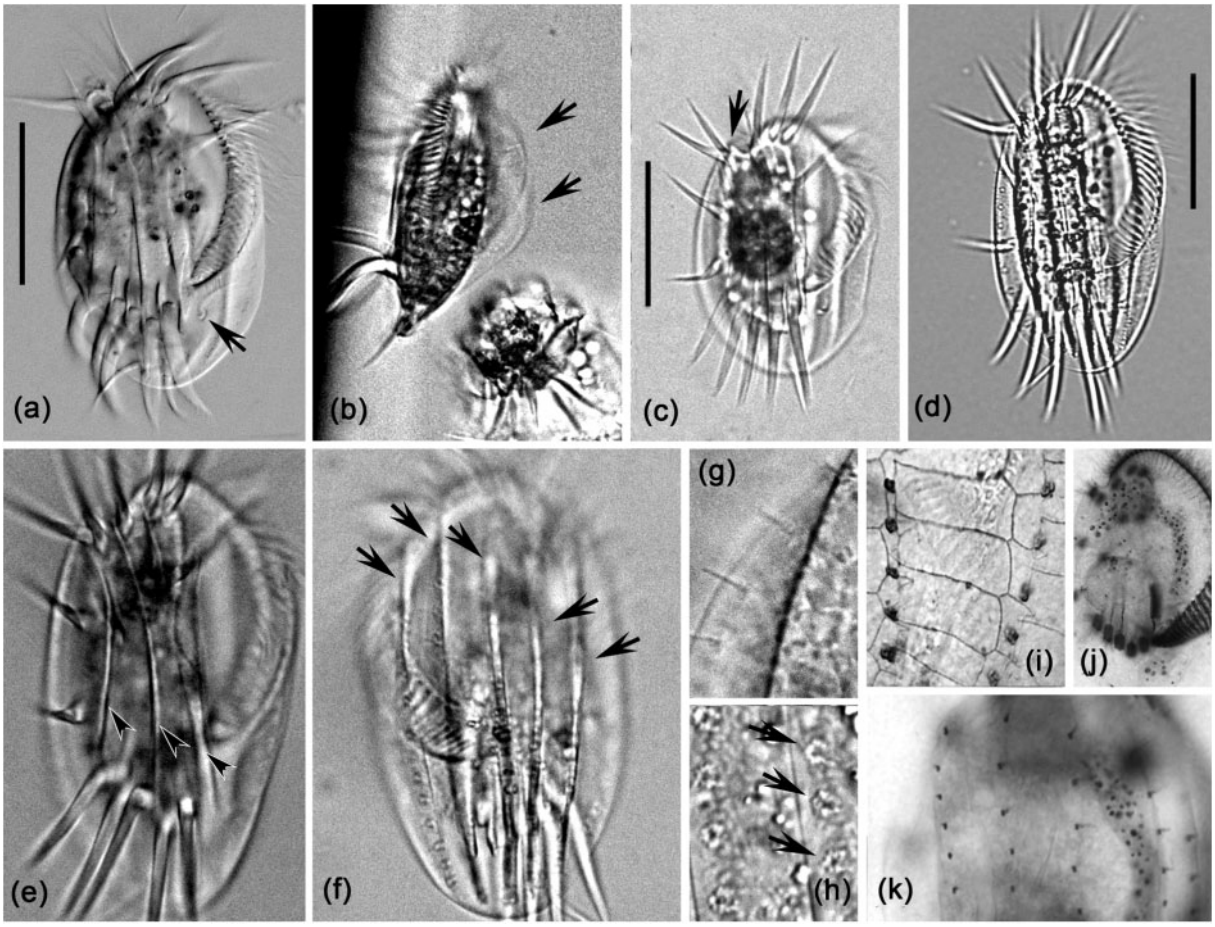

Fig. 2. Photomicrographs of *E. sinicus* sp. nov. *in vivo* (a–h), and after silver nitrate (i) and protargol (j, k) impregnation. (a) Ventral view of a representative specimen. Arrow shows the fine marginal cirrus. (b) Lateral and underside views; arrows point to the dominant dorsal ridges. (c, d) Ventral views of other individuals. Arrow in (c) shows the projection on anterior right of the cell body. (e) Ventral view, showing three long ridges on ventral side (arrowheads). (f) Dorsal view, showing the dorsal ridges (arrows). (g) Detailed view, showing the dorsal cilia. (h) Detailed view; arrows point to the granules around the dorsal cilia. (i) Portion of dorsal silverline system. (j) Ventral view, showing the typical shape of macronucleus. (k) Portion of dorsal view, showing the arrangement of the dorsal kineties. Bars, 40 μm (a, c, d).

ridges nor grooves detectable (Fig. 5f). Ellipsoidal granules (about 1.5×0.8 μm) packed together around the dorsal cilia beneath pellicle in a flower pattern (Figs 4d, 5f, arrows).

Cytoplasm colourless, containing some shining globules and food vacuoles with bacteria (possibly?). Contractile vacuole posterior to the rightmost transverse cirrus (Fig. 5d, arrow). Macronucleus slightly curved-bar-shaped (Fig. 4k). Locomotion typical of the genus.

Ciliary pattern rather stable, always 10 frontoventral and 5 transverse cirri (Fig. 4h, j); cirrus V/2 (Fig. 5g, arrowhead) very close to cirrus VI/2; cirrus II/1 almost at the same level as VI/1 (Fig. 5g, arrows). Two left marginal cirri (Fig. 4j, arrows) positioned posterior to the transverse cirri and close to two caudal cirri. Dorsal kineties number 6–7 (mostly 6), leftmost one of which is remarkably shortened at its anterior end, and consists of only 2–4 dikinetids; all kineties somewhat sparsely ciliated, middle dorsal kinety with about 8–11 dikinetids. Dorsal silverline system double-*eurystomus* type (Figs 4h, i and 5h).

Comparison and discussion. Until now, 9 small marine *Euplotes* with the double-*eurystomus* type dorsal silverline system have been reported. Six of these, *Euplotes alatus* Kahl, 1932, *Euplotes balteatus* (Dujardin, 1841) Kahl, 1932, *Euplotes magnicirratus* Kahl, 1932, *Euplotes quinquecarinatus* Gelei, 1950, *Euplotes plicatum* Valbonesi, 1997 and *Euplotes trisulcatus* Kahl, 1932, have 10 frontoventral and 2 marginal cirri, and thus can be compared with *E. parabalteatus*. However, these six species have conspicuous dorsal ridges which are absent in

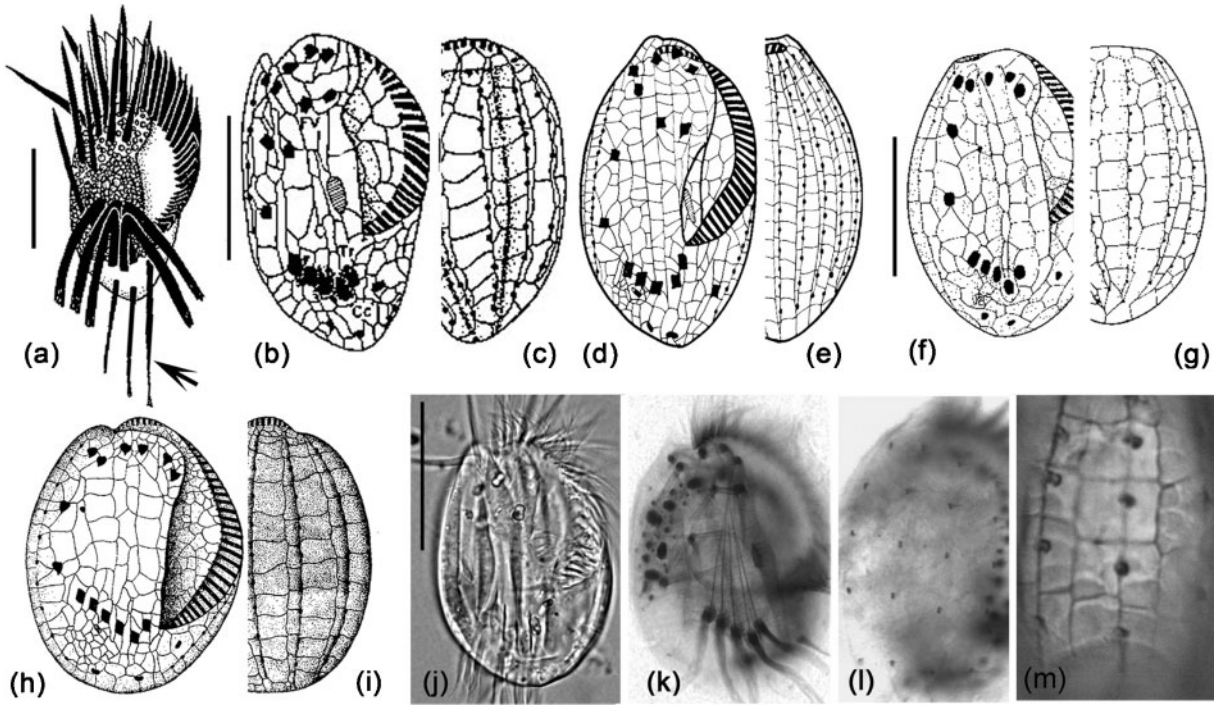

Fig. 3. Morphologically similar marine *Euplotes* species with double-*patella* silverline pattern and single marginal cirrus. (a–c) *E. algivora* Agatha, 1990 (Agatha *et al.*, 1990; arrow marks the single long marginal cirrus); (d, e) *E. zenkewitchi* Burkovsky, 1970 (Burkovsky, 1970); (f, g) *E. raikovi* Agamaliev, 1966 (Washburn & Borror, 1972); (h, i) *E. strekovi* Agamaliev, 1967 (Agamaliev, 1967); (j–m) *E. rariseta* Curds *et al.*, 1974 (Ma *et al.*, 2007). Bars, 30 μm [a (also applies to d and e), b (also applies to c), f (also applies to g–i) and j (also applies to k and l)].

E. parabalteatus. Posterior-located marginal cirri and cirrus V/2 of *E. parabalteatus* also distinguish this novel species from some of its congeners. Other differences used for further species separation are presented below.

According to Borror (1968), *E. alatus* has a relatively shorter adoral zone (half vs over two-thirds of cell length) and a C-shaped macronucleus (vs curved-bar-shaped) (Borror, 1968; Fig. 6a–c, Table 4).

Table 3. Comparison of six morphologically related marine *Euplotes* species with the double-*patella* dorsal silverline pattern and a single marginal cirrus

Species: 1, *E. sinicus*; 2, *E. algivora*; 3, *E. zenkewitchi*; 4, *E. raikovi*; 5, *E. rariseta*; 6, *E. strekovi*. NA, Not available.

Characteristic	1	2	3	4	5	6
Cell size *in vivo* (μm)*	65–92 × 37–62	40–59 × 24–40	70–90 × 45–55	50–64 × 40–56	30–50 × 20–40	45–60 × 38–40
Adoral membranelles	38–46	28–37	50–60	22–-30	17–22	33–38
Frontoventral cirri	10	10	9	8†	10	9†
Dorsal kineties	7	6	8–10	7–8	7	6
Dikinetids in mid-dorsal kinety	11–16	7–12	16–18	10–13	5–7	10
Silverline system	Double-*patella*-I	Double-*patella*-I	Double-*patella*-II	Double-*patella*-I	Double-*patella*-I	Double-*patella*-II
Other special features	Dorsal side highly ridged; fine marginal cirrus	2 Dorsal ridges; marginal cirrus conspicuously long	NA	Dorsal side slightly ridged; single reduced cirrus present	Dorsal side slightly ridged	Single reduced cirrus
Reference	Present work	Agatha *et al.* (1990)	Burkovsky (1970)	Jiang *et al.* (2008)	Ma *et al.* (2007)	Agamaliev (1967)

*Based on impregnated specimens.
†Including the reduced cirrus.

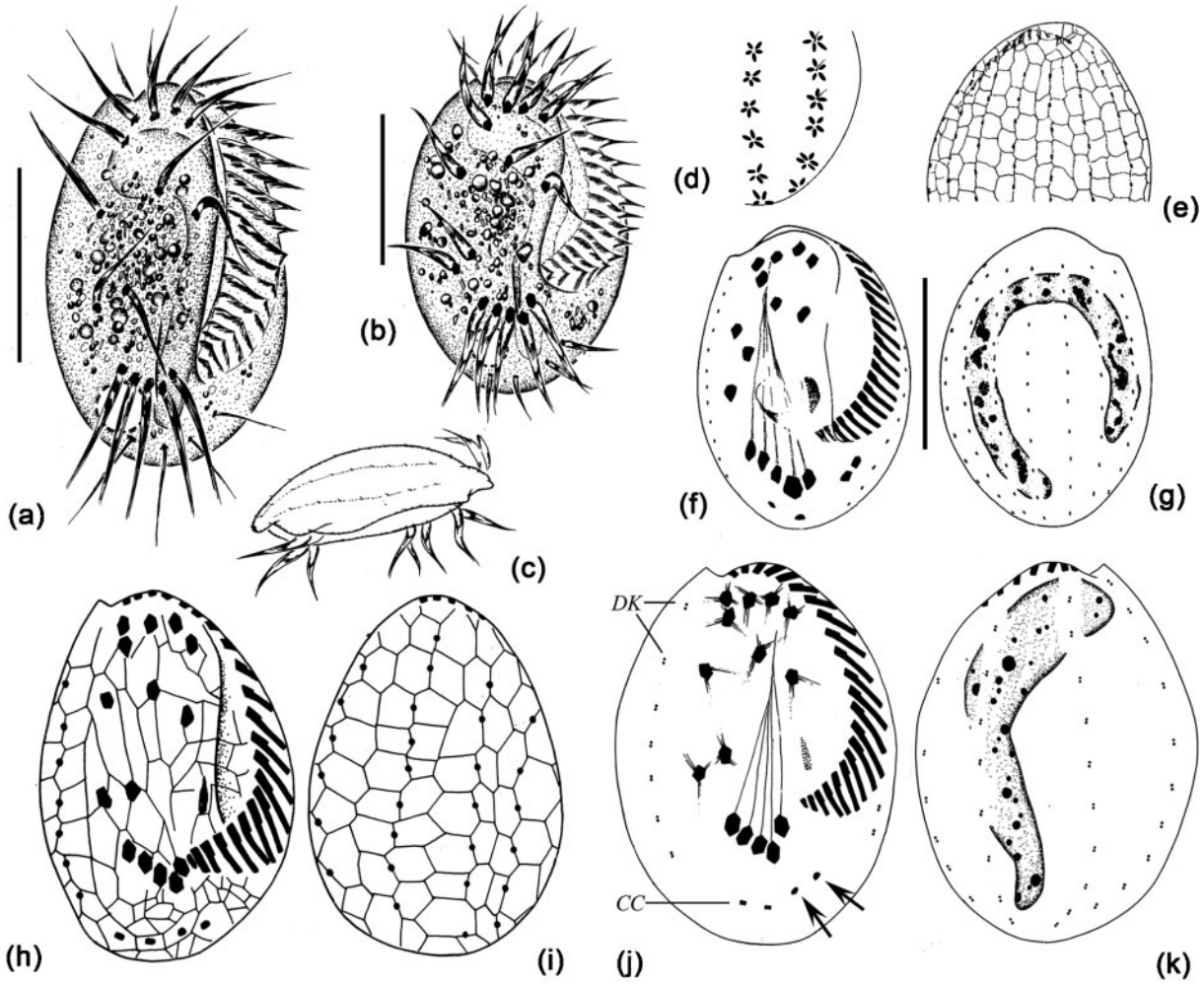

Fig. 4. *E. parabalteatus* sp. nov. *in vivo* (a–d), and after silver nitrate (h, i) and protargol (j, k) impregnation, and *E. balteatus* Kahl, 1932 (e–g) (Song & Wilbert, 2002). (a) Ventral view of a representative specimen. (b) Ventral view of a plumper individual. (c) Lateral view. (d) Portion of dorsal view, showing the granules around dorsal cilia beneath the pellicle. (e) Portion of dorsal silverline system. (f, g) Infraciliature of the same individual on ventral (f) and dorsal (g) sides. (h, i) Silverline system on ventral (h) and dorsal (i) sides. (j, k) Ventral and dorsal views, respectively, of the same specimen, showing infraciliature and nuclear apparatus. Arrows in (j) indicate the marginal cirri. CC, caudal cirri. DK, dorsal kineties. Bars, 20 μm [a (also applies to h–k), b and f (also applies to g)].

E. quinquecarinatus was first briefly described by Gelei (1950). Borror (1968) identified a morphospecies as *E. quinquecarinatus*, and described the silverline system for the first time as 'extremely similar to *E. charon*', but when it came to the comparison, he mistook it as the *patella*-type. Curds (1975) accepted Borror's identification. Compared to *E. parabalteatus*, *E. quinquecarinatus* has more dorsal kineties (9 vs 6–7) and a C-shaped macronucleus (vs curved-bar-shaped) in addition to the differences mentioned above (Borror, 1968; Fig. 6d–f, Table 4).

E. magnicirratus differs from *E. parabalteatus* in its relatively strong cirri (vs normal cirri), more adoral membranelles (49–52 vs 19–23) and the inverted-C-shaped macronucleus (vs curved-bar-shaped) (Carter, 1972; Fig. 6g–i, Table 4).

E. plicatum can be clearly separated from *E. parabalteatus* by more dorsal kineties (10 vs 6–7) and the inverted-C-shaped macronucleus (vs curved-bar-shaped) (Valbonesi *et al.*, 1997; Table 4).

E. trisulcatus resembles *E. parabalteatus* in cell size, infraciliature and macronucleus shape. However, three prominent furrows in *E. trisulcatus* were described by both Tuffrau (1960) and Carter (1972) (Fig. 6j–l, Table 4). In combination with difference in the position of cirrus V/2 as mentioned above, we suggest these should be treated as two distinct species.

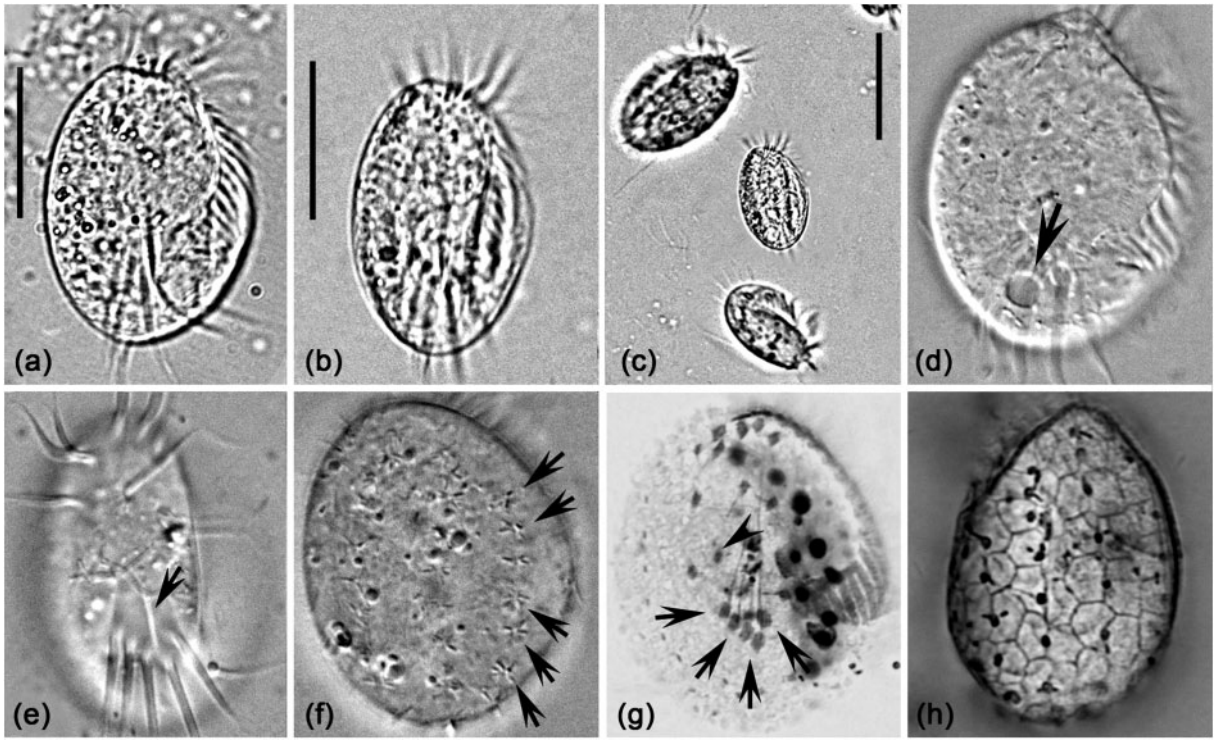

Fig. 5. Photomicrographs of *E. parabalteatus* sp. nov. *in vivo* (a–f), and after protargol (g) and silver nitrate (h) impregnation. (a, b) Ventral views. (c) Differently sized and shaped cells. (d) Ventral view. Arrow points to the contractile vacuole. (e) Ventral view. Arrow shows the only short ridge between transverse cirri on ventral side. (f) Dorsal view. Arrows point to the granules around the dorsal cilia. (g) Ventral view of infraciliature. Arrowhead points to the posterior-located cirrus V/2; arrows indicate the closely arranged transverse cirri. (h) Dorsal view of silverline system. Bars, 20 μm [a, also applies to d–h) and b] and 30 μm (c).

Tuffrau (1959) described a high level of variability in cell size (30–150 μm long) and in the number of adoral membranelles (25–30 to 70–80) of *E. balteatus*, which depend upon its food source. This description by Tuffrau (1959) is a brief description without details of other characters (e.g. the number of dorsal kineties and dikinetids in the mid-dorsal kinety). Here, we can only compare *E. parabalteatus* morphometrically with the population of *E. balteatus* described by Song & Wilbert (2002) (Fig. 4e–g, Table 4). Both are similar in cell shape and size, ciliary pattern and their silverline system; *E. parabalteatus*, however, can be separated from *E. balteatus* by having fewer dorsal kineties (6–7 vs 9) and a less curved macronucleus in addition to the differences mentioned above.

Phylogenetic analyses of the two novel species and *E. rariseta* based on SSU rRNA gene sequences

The phylogenetic trees constructed using three different methods (BI, ML and MP) showed identical topological structure, hence only one tree is presented here (Fig. 7). The topologies are consistent with previous molecular analyses (Yi *et al.*, 2009). As shown in Fig. 7, all *Euplotes* species form a well-supported group with high posterior probability and bootstrap values (BI >0.95, ML and MP >0.90). This group includes five well-supported clades and several species for which relationships remain unresolved. *E. sinicus* and *E. parabalteatus* fall within the family Euplotidae in all three trees (BI 1.00, ML and MP 100 %) and both branch independently at the basal position as a sister group to all other *Euplotes* species. The SSU rRNA genes of the two morphologically closely related species, *E. algivora* and *E. balteatus*, have not been sequenced yet and therefore their genetic separation from their congeners remains unknown.

It is noteworthy that the new sequence of *E. rariseta* reported here did not cluster with the sequence (AF492706) obtained by our group in 2002 (Song *et al.*, 2004). Our isolate of *E. rariseta* clusters strongly with another isolate of this species from Italy (AJ305248). Both sequences branch within the poorly resolved radiation of *Euplotes*, including two monophyletic clades represented by *E. muscicola* and *E. magnicirratus*, respectively.

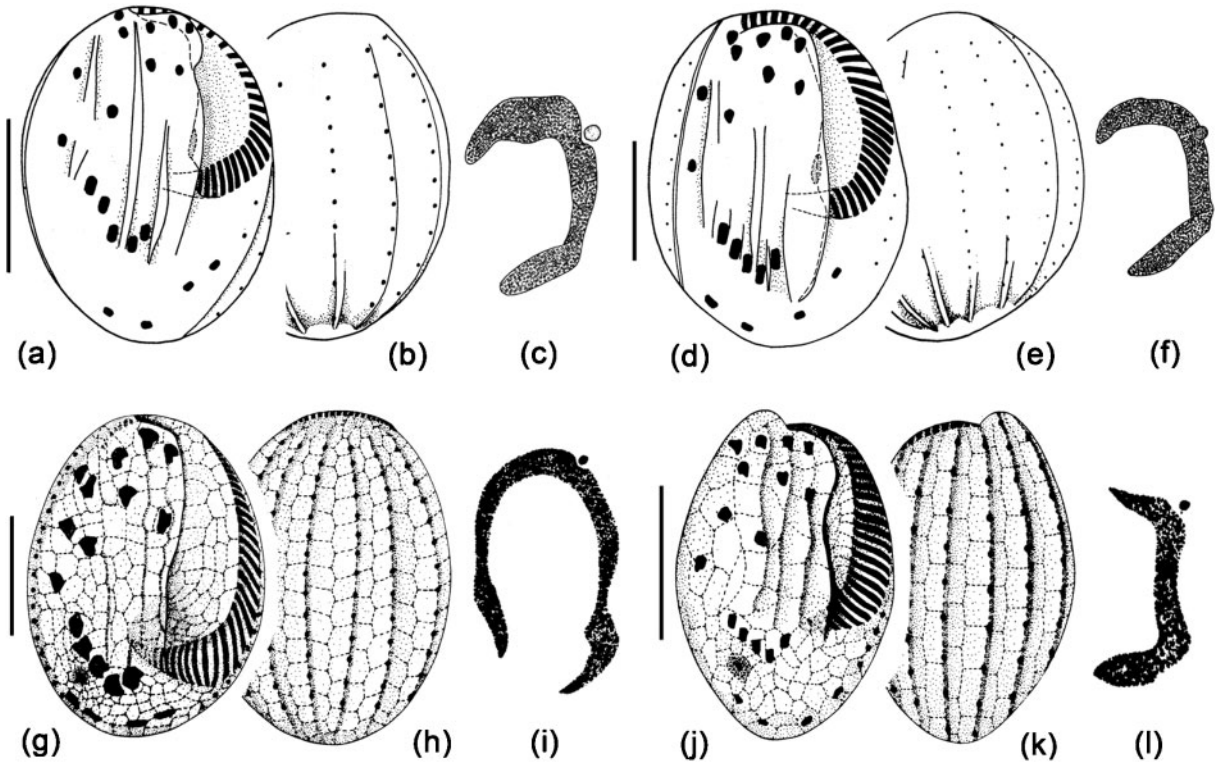

Fig. 6. Morphologically similar small marine *Euplotes* species with a double-*eurystomus* silverline pattern, and 10 frontoventral and 2 marginal cirri. (a–c) *E. alatus* Kahl, 1932 (Borror, 1968), (d–f) *E. quinquecarinatus sensu* Borror, 1968 (Borror, 1968), (g–i) *E. magnicirratus* Carter, 1972 (Carter, 1972), (j–l) *E. trisulcatus* Kahl, 1932 (Carter, 1972). Bars, 20 μm [a (also applies to b and c), d (also applies to e and f), g (also applies to h and i) and j (also applies to k and l)].

However, the so-called *E. rariseta* isolate from Song *et al.*, (2004) (AF492706) clusters with *E. nobilii* as a sister group to this radiation. By rechecking the slides deposited in our lab, we found that the isolate was misidentified. Unfortunately, however, the quality of the specimen is now too poor to allow for accurate identification, but it is clear that the cells are larger and have very obvious ridges on the ventral and dorsal sides, and thus cannot be *E. rariseta* (Song & Packroff, 1997). It was treated as an unidentified *Euplotes* species in this analysis.

Table 4. Comparison of seven morphologically related small marine *Euplotes* species with the double-*eurystomus* dorsal silverline pattern, and 10 frontoventral and 2 marginal cirri

Species: 1, *E. parabalteatus*; 2, *E. alatus*; 3, *E. quinquecarinatus*; 4, *E. magnicirratus*; 5, *E. plicatum*; 6, *E. trisulcatus*; 7, *E. balteatus*.

Characteristic	1	2	3	4	5	6	7
Cell size *in vivo* (μm)	30–35	About 40	About 60	54*	42–55	35–50	40–70
Adoral membranelles	19–23	About 30†	About 30†	49–52	22–25	25–36	27–33
Dorsal kineties	6–7	8	9	8	10	7	8–10
Dikinetids in mid-row	8–11	About 10†	About 13†	13–17	14	About 9?	9–14
Shape of macronucleus	Curved-bar-shaped	C-shaped	C-shaped	Inverted C-shaped	Inverted C-shaped	Curved-bar-shaped	Inverted C-shaped
Reference	Present work	Borror (1968)	Borror (1968)	Carter (1972)	Valbonesi *et al.* (1997)	Carter (1972)	Song & Wilbert (2002)

*Probably from impregnated specimens.
†Counted from illustrations.

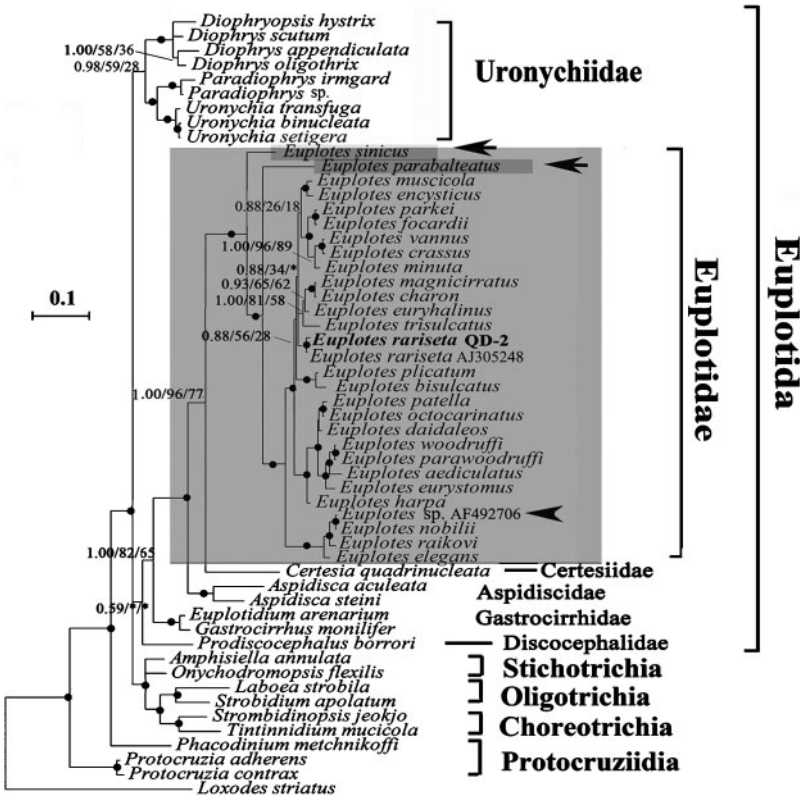

Fig. 7. Phylogenetic tree based on SSU rRNA gene sequences showing the position of *E. sinicus*, *E. parabalteatus* and *E. rariseta* by BI, ML and MP. Numbers near branches are: BI posterior probability value/ML bootstrap value/ MP bootstrap value. An asterisk indicates disagreement among phylogenies. The well-supported (>0.95 BI, >90 % ML, >90 % MP) branches are marked with solid circles. Family Euplotidae is highlighted in grey. The positions of *E. sinicus* and *E. parabalteatus* are denoted with arrows and highlighted in darker grey; the arrowhead refers to the previously misidentified *E. rariseta*. The scale bar corresponds to 10 substitutions per 100 nucleotide positions.

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