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Redescriptions of *Euplotes encysticus* and *E. rariseta* (Protist: Ciliophora: Euplotida)

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Two euplotid ciliates, *Euplotes encysticus* Yonezawa, 1985 and *E. rariseta* Curds *et al*., 1974, were isolated from a freshwater pond called Mulgol in Dokdo of the East Sea and from Masan Bay/Jeju Island, Korea, respectively. Both species are redescribed based on live observations and protargol impregnation. Cells of *Euplotes encysticus* are asymmetrically oval, 63-79 × 41-61 μm in vivo and capable of encystment. The cells have 31-36 adoral zone of membranelles (AZM), 9 fronto-ventral cirri (FVC), 5 transverse cirri (TC), 2-3 caudal cirri (CC), 2 marginal cirri (MC), 7 dorsal kineties (DK), and 19-22 dorsal cilia in middle DK. The cells of *Euplotes rariseta* has a small ovoid form and are 32-44 × 23-35 μm in vivo, 18-22 AZM, 10 FVC, 5 TC, 2 CC, 1 MC and 6 DK.

Keywords: Dokdo, *Euplotes encysticus, Euplotes rariseta*, Euplotid ciliate, Jeju Island, Masan Bay

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Introduction

Euplotes is the species-rich genus, and has a wide distribution and high adaptive potentialities (Curds, 1975; Lobban *et al*., 2005; Schwarz *et al*., 2007; Wilbert and Song, 2008). The identification of *Euplotes* species is difficult because (1) many of the distinguishing characters are known to vary considerably even within clonal cultures, (2) certain common species are morphologically confused, and (3) there is the added difficulty of having to search through a considerable body of literature before an identification can be attempted (Curds, 1975; Yonezawa, 1985; Song *et al*., 1998; Lobban *et al*., 2005; Schwarz and Stoeck, 2007).

About 100 *Euplotes* species have been reported from various habitats (e.g. Tuffrau, 1960; Curd, 1975; Yonezawa, 1985; Song *et al*., 1998; Berger, 2001; Lobban *et al*., 2005; Schwarz and Stoeck, 2007; Wilbert and Song, 2008; Fan *et al*., 2010; Jiang *et al*., 2010a; 2010b), but 18S rRNA sequences of about 34 species have been submitted to GenBank of the NCBI (15 Sep. 2018).

To date, 11 species of *Euplotes* have been reported in Korea: *E. aediculatus, E. charon, E. cristatus, E. eurystomus, E. minuta, E. muscicola, E. muscorum, E. parawoodruffi, E. patella, E. rariseta and E. vannus* (Shin and Kim, 1988; Shin *et al*., 1992; Shin and Kim, 1995; 1996; Jo and Shin, 2003; Kwon and Shin, 2006; Kwon *et al*., 2007; Jung *et al*., 2017; Park *et al*., 2017).

Here we redescribe two euplotid species, *Euplotes encysticus* and *E. rariseta*, based on their living morphology and protargol impregnation.

Materials and Methods

Sample collection, observation and identification

The specimens of *Euplotes encysticus* and *E. rariseta* were collected at a freshwater pond (Mulgol) in Dokdo (island; ~87 km from Ulleungdo) of the East Sea and at marine intertidal sediments of Masan Bay/Jeju Island, Korea, respectively. A single *Euplotes* cell was isolated by micropipetting from a well plate containing sterile seawater or freshwater, supplemented at 1% v/v with lysogeny broth (LB), inoculated into a well plate and maintained at 21°C. For mass and routine cultures, *Euplotes rariseta* inoculated into a well plate containing sterile seawater with a barley grain, and *E. encysticus* inoculated into a well plate containing sterile seawater (15 psu and 30 psu) and freshwater with a barley grain. For routine maintenance, subculturing was performed every two weeks.

Live and protargol impregnated specimens were observed with a Leica DMR microscope (Germany) equipped with a Zeiss Axiocam HR digital camera and its associ-

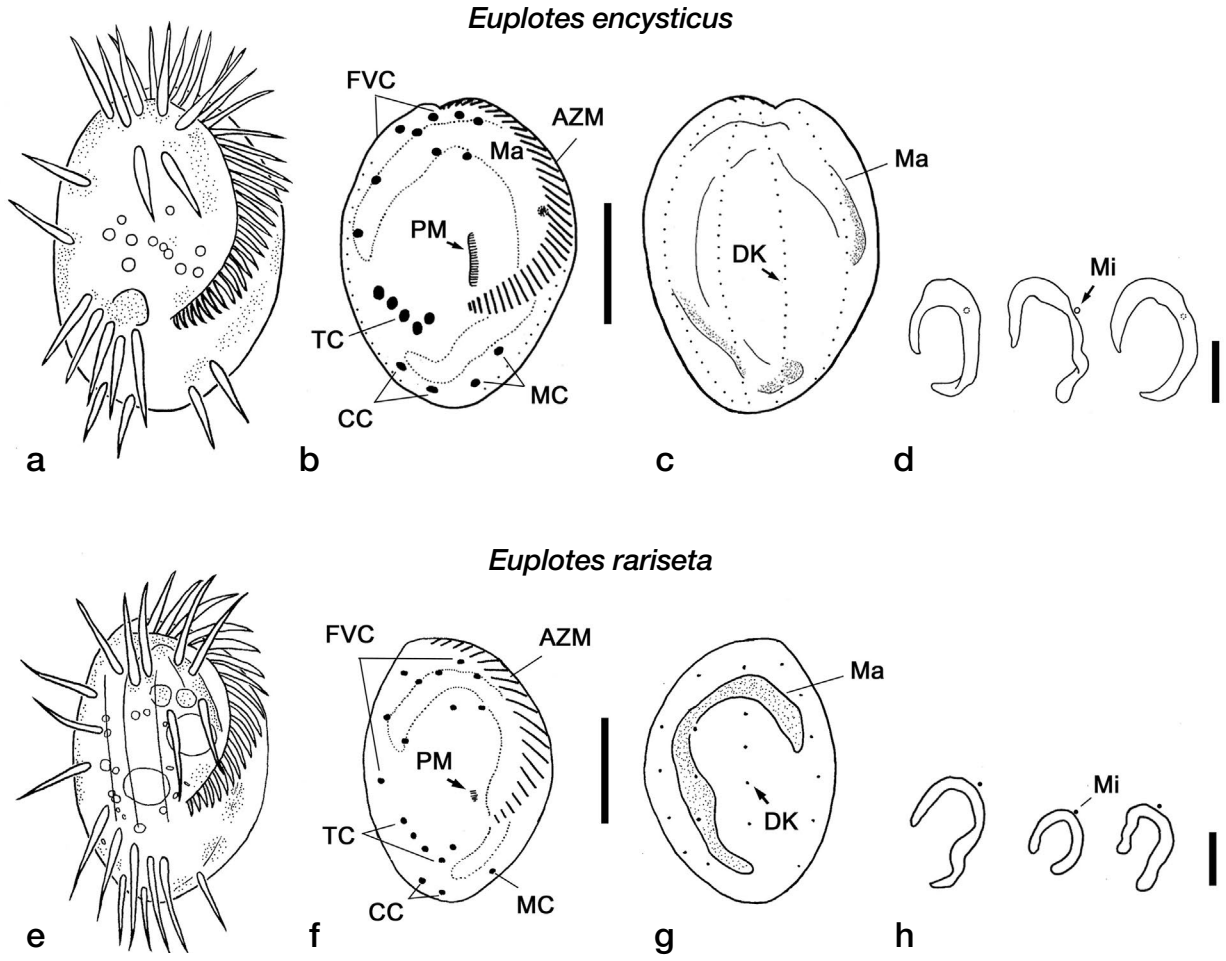

Fig. 1. Drawings of *Euplotes encysticus* (a-d) and *E. rariseta* (e-h). (a) *E. encysticus* and (e) *E. rariseta* in vivo, *E. encysticus* (b-d) and *E. rariseta* (f-h) after protargol impregnation. (b) and (f) ventral views, (c) and (g) dorsal views, (d) and (h) different shapes of macronucleus. AZM, adoral zone of membranelles; PM, paroral membrane; FVC, fronto-ventral cirri; TC, transverse cirri; CC, caudal cirri; MC, marginal cirrus; DK, dorsal kineties; Ma, macronucleus; Mi, micronucleus. Scale bars in (b) for (a-c) and in (d) represents 20 μm, in (f) for (e-g) and in (h) represent 10 μm.

ated software (Axiovision 4.6). The infraciliature was revealed using the protargol method (Wilbert, 1975). Measurements and counts were performed at magnifications of 400-1000. Classification follows Lynn (2008), while terminology follows Curds (1975) and Song *et al.* (2009); Adoral zone of membranelles (AZM, hereafter), adoral membranelles (AM), contractile vacuole (CV), dorsal kineties (DK), macronucleus (Ma), micronucleus (Mi), paroral membrane (PM), fronto-ventral cirri (FVC), transverse cirri (TC), caudal cirri (CC), marginal cirri (MC).

DNA extraction, PCR amplification and sequencing

A 10-mL sample of about one-week-old culture was centrifuged at 5,900 g for 5 min, and then the DNA was extracted using a Qiagen Blood & Cell Culture Extraction Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. 18S ribosomal DNA (18S rDNA) sequences were obtained by PCR amplification using a combination of the eukaryote primers EukA (5′-AACCTGGTTGATCCTGCCAGT-3′) and EukB (5′-TGATCCTTCTGCAGGTTCACCTAC-3′) (Medlin *et al.*, 1988). For the PCR amplification, a reaction volume of about 20 μL was used that included 1.5 μL of 10 μM stocks of the primers EukA and EukB, 2 μL of 0.25 mM dNTP-mix, 1.8 μL of 25 mM $MgCl_2$, 0.7 μL total of 5U/μL *Taq* DNA polymerase (Bioneer, Korea), and 5 μL of DNA template. The cycling conditions started with a denaturing step at 94°C for 5 min, followed by 35 cycles of 30 s at 94°C, 1 min of annealing at 55°C, and extension at 72°C for 2 min (10 min at 72°C for the final cycle only). A PCR product corresponding to the expected size was gel-isolated, directly purified, then sequenced by Sanger sequencing using PCR primers along with internal sequencing primers (514F,

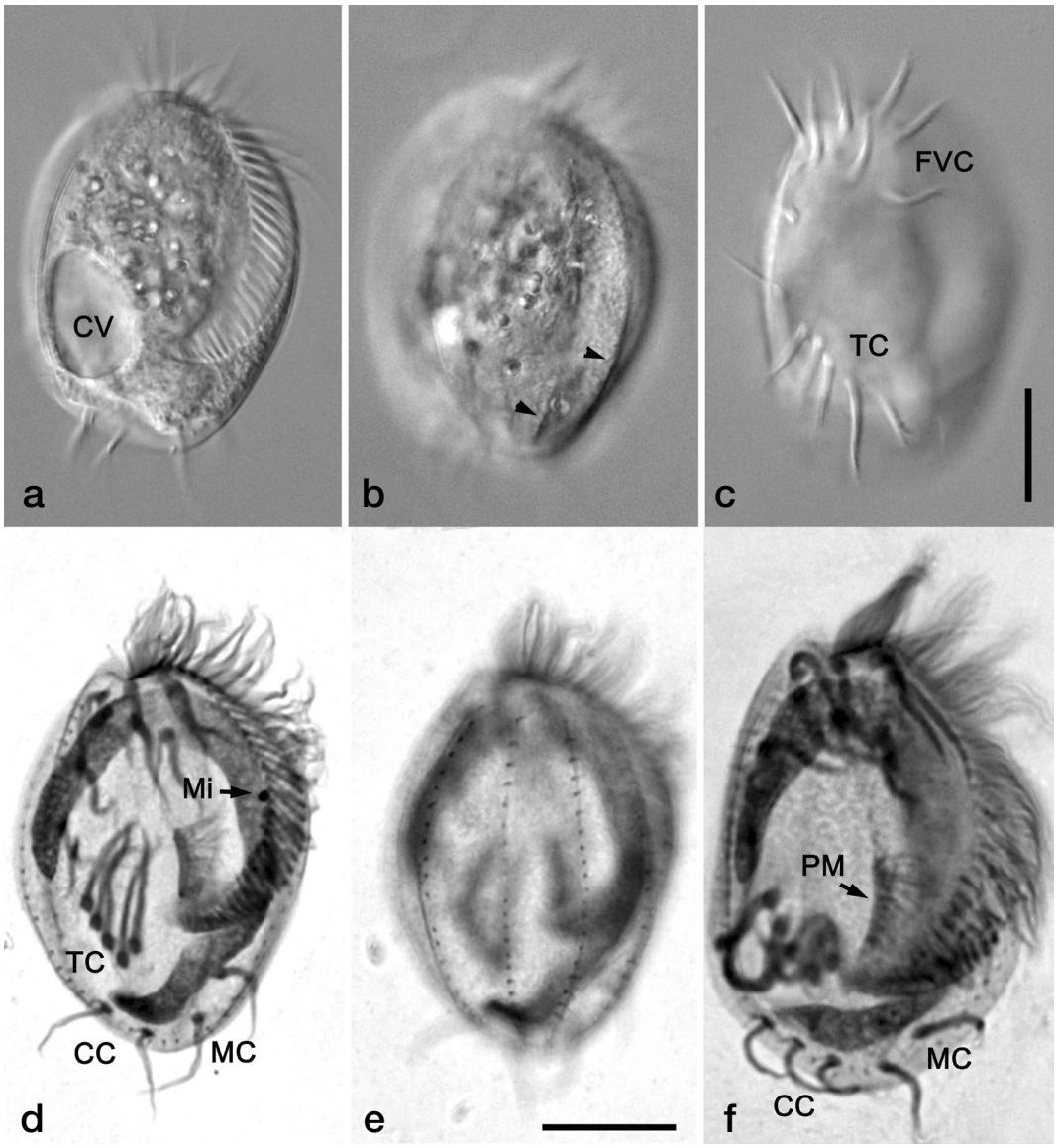

Fig. 2. Micrographs of *Euplotes encysticus* strain KF403 (a-c) in vivo and (d-f) after protargol impregnation. (a) Dorsal views showing contractile vacuole (CV) and adoral membranelles, (b) dorsal ridges (arrowheads), (c) ventral side showing FVC (fronto-ventral cirri) and TC (transvers cirri), (d) ventral view showing AZM (adoral zone of membranelles), PM (paroral membrane), FVC, TC, CC (caudal cirri), MC (marginal cirri), Ma (macronucleus), Mi (micronucleus), (e) dorsal side showing DK (dorsal kineties), (f) note 3 CC. Scale bars in (c) for (a-c) and in (f) for (d-f) represent 20 μm.

5′-GGTGCCAGCASCCGCGGTAA-3′and Euk1209R 5′-GGGCATCACAGACCTG-3′). Individual reads were assembled using Geneious ver. 8.1.5 (Kearse *et al*., 2012).

Results and Discussion

Phylum Ciliophora Doflein, 1901
Class Spirotrichea Bütschli, 1889
Subclass Hypotrichia Stein, 1859
Order Euplotida Small and Lynn, 1985
Family Euplotidae Ehrenberg, 1838
Genus *Euplotes* Müller, 1786

1. *Euplotes encysticus* Yonezawa, 1985 (Figs. 1a-d, 2, 3)

Material examined. Korea: Dokdo (37°14′22″N, 131° 52′08″E), 27 September 2017, from a freshwater pond (Mulgol), collected by Jong Soo Park. Type strain, live cells are kept with the Korean Culture Collection of Protists, Kyungnam University, Korea, reference ‘KF403’.

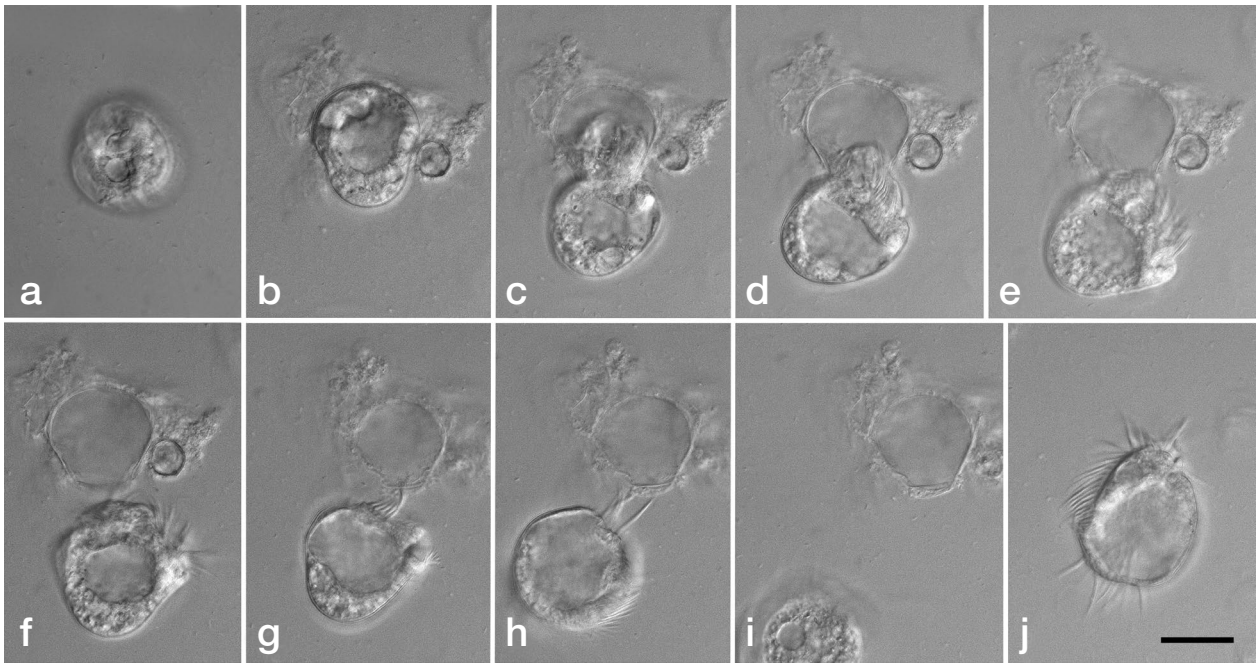

Fig. 3. Micrographs of excystment of *Euplotes encysticus*. (a) Cyst of cell 1, (b-j) time series lasting 6 minutes 52 seconds documenting excystment of cell 2, (i) empty cyst and trophozoit, (j) trophozoit. All images are DIC images. Scale bar in (j) = 25 μm.

Description. Cells are 63-79 × 41-61 μm in vivo and 44-56 × 28-38 μm in protargol, asymmetrically oval with posterior portion slightly wider than anterior, and dorso-ventrally flattened. The peristome is narrow and extremely long, extending approximately 72% of cell length and surrounded by 31-36 adoral membranelles. Adoral zone of membranelles (AZM) curved (Figs. 1a, 1b, 2a, 2d, 2f). Cytoplasm colourless, often filled with many light reflecting granules and few food vacuoles (Fig. 2a). Single contractile vacuole located to right of transverse cirri (TC) (Fig. 2a). Seven dorso-lateral ridges (Figs. 2b, arrowheads; 2e). Dorsal kineties (DK) inserted in concaves down the ridges (Figs. 1c, 2e). The kinetosome number of the mid-dorsal kinety is 19-22. Cirri usually strong; 9 fronto-ventral, 5 transverse, 2-3 (mostly 2) caudal and 2 left marginal cirri. Paroral membrane (PM) about 10 μm long. Macronucleus (Ma) usually C-shaped, with the anterior and posterior ends sometimes curving (Figs. 1d, 2d, 2f). Micronucleus (Mi) small, nearly spherical and located in the upper right half of the macronucleus (Figs. 1b, 1d, 2d). The cells are capable of encystment and excystment (Fig. 3). Move by crawling on crumb or substrate.

Remarks. *Euplotes encysticus* was originally reported by Yonezawa (1985) as a freshwater form. We performed the inoculation of our strain KF403 into well plates containing seawater (15 and 30 psu) with barley grains. The strain grew well in the seawater, thus this species is adaptable to both seawater and freshwater. Our observations are in accord with those of Yonezawa (1985) and Fan *et al*. (2010). *Euplotes encysticus* is very similar to *E. muscorum* in general appearance, but according to several researchers (e.g., Dragesco, 1970; Dragesco and Dragesco Kernéis, 1986; Jo and Shin, 2003) they can be distinguished from each other because (1) no cyst has been observed so far in *E. muscorum*, (2) the obvious dorsal-lateral ridges and the granules surrounding the dorsal cirri have not observed in *E. muscorum*, (3) the AZM of *E. encysticus* outspreads more broadly than that of *E. muscorum*, and (4) the AZM covers 3/4 body length in *E. encysticus*, and 2/3 in *E. muscorum*. Our strain has all of these characters specific to *E. encysticus*. Additionally, these two species are clearly distinguished by the differences in their 18S rRNA gene sequences, of which *E. encysticus* differs from *E. muscorum* by 2.04% (Fan *et al*., 2010). *Euplotes encysticus* is similar to *E. petzi, E. roscoffensis* and *E. sinicus* in cell length, but the numbers of AM, DK and dikinetids in mid-DK in *E. encysticus* are higher than those in *E. petzi*. *Euplotes roscoffensis* and *E. sinicus* have higher number of AM than that of *E. encysticus* (Tables 1, 2). *Euplotes sinicus* has 11-16 dikinetids in mid-DK (vs 5-7) (Tables 1, 2).

NCBI BLASTN search result shows that the closest strains to our strain KF403 are *E. encysticus stains* [GenBank Accession Nos. FJ346569, EF535728, LN864512; Identity 99%]. The 18S rRNA sequence of our stain is 1,663 bp in length.

Habitat. Marine and Freshwater

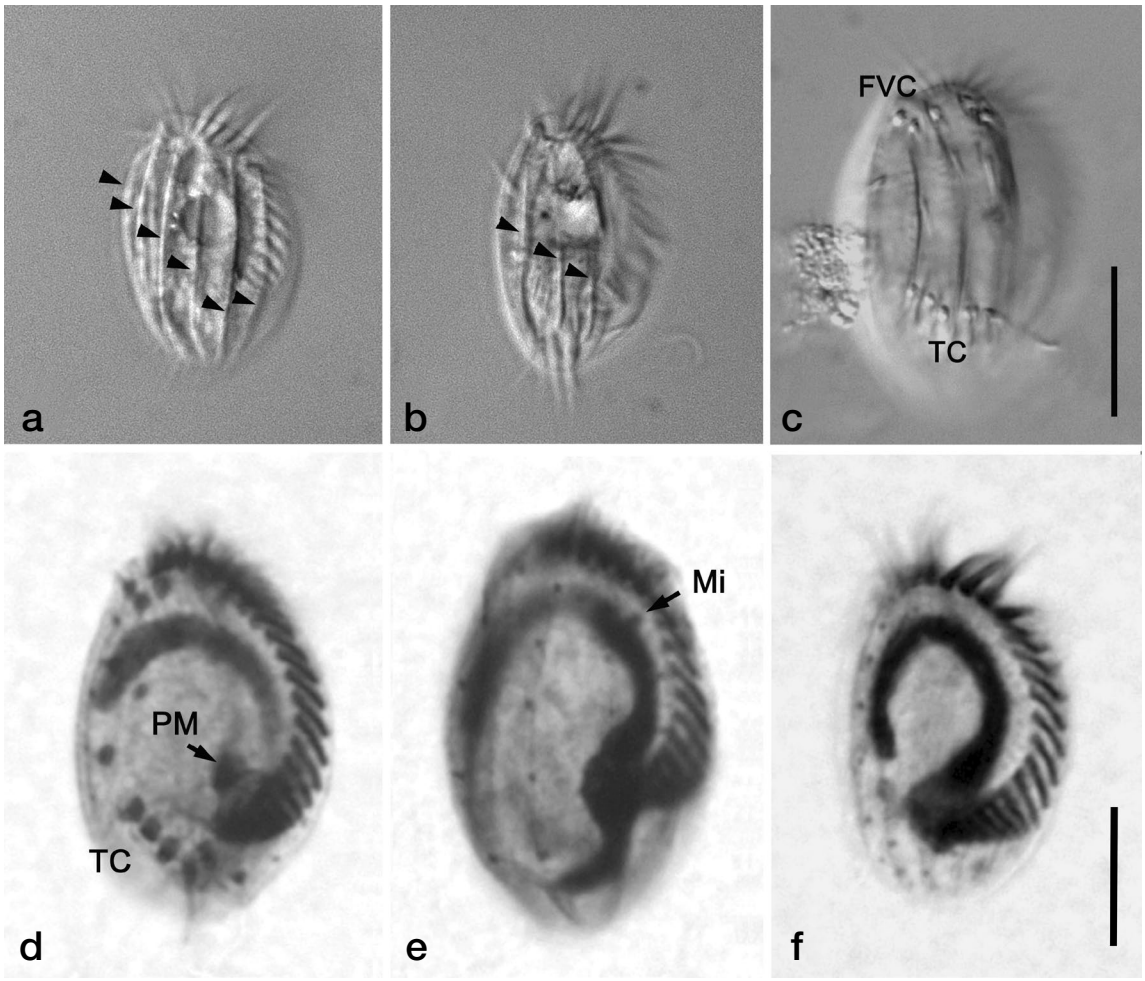

Fig. 4. Micrographs of *Euplotes rariseta* strain KM401 (a-c) in vivo and strain KM444 (d-f) after protargol impregnation. (a) Dorsal ridges (arrowheads) of *E. rariseta*, (b) ventral view showing longitudinal ridges (arrowheads), (c) ventral view showing FVC and TC, (d) ventral view showing AZM (adoral zone of membranelles), FVC (fronto-ventral cirri), TC (transvers cirri), PM (paroral membrane), (e) dorsal view showing dorsal kineties, (f) ventral view showing ventral kineties and Ma (macronucleus). Scale bars in (c) for (a-c) represent 20 μm and in (f) for (d-f) represent 10 μm.

Distribution. Freshwater habitats: Korea (Dokdo, this study), Japan (Yonezawa, 1985) Marine habitats: China (Daya Bay, Fan *et al*., 2010)
Deposition. National Institute of Biological Resources, Korea (NIBRPR0000109440)
NCBI GenBank Accession Number: MK026950

2. *Euplotes rariseta* Curds, West and Dorahy, 1974 (Figs. 1e-h, 4a-f)

Material examined. Korea: Masan Bay (35°10′08″N, 128°35′58″E), 31 May 2016 and Jongdal-ri Beach, Jeju Island (33°30′47″N, 126°53′56″E), 14 July 2018, from marine intertidal sediments, collected by Won Je Lee. Type strains, live cells are kept with the Korean Culture Collection of Protists, Kyungnam University, Korea, reference 'KM401' and 'KM444'.
Description. Cells are 32-44 × 23-35 μm in vivo and 23-30 × 17-20 μm in protargol, ovoid and dorso-ventrally flattened. The peristome is wide, spindle-shaped and surrounded by 18-22 adoral membranelles, which extend for about 72% of the cell length (Figs. 1e, 1f, 4a, 4d-f). Cytoplasm colorless. Few food vacuoles and single contractile vacuole located in the middle of the cell (Fig. 4a, 4b). Cirri usually strong; 10 fronto-ventral, 5 transverse, and 2 caudal cirri (Fig. 4b, 4c). Caudal cirrus below AZM is slightly stout. Ventral surface heavily sculptured with 6 longitudinal ridges (Fig. 4b, 4c, arrowheads). Dorsal surface with 6 double-edged longitudinal ridges (Fig. 4a, arrowheads). Dorsal bristles sparse; 6 kinetics (Figs. 1g, 4e). The macronucleus (Ma) is horseshoe-shaped, and the micronucleus (Mi) is small and situated anteriorly (Figs.

Table 1. Morphometric data of *Euplotes encysticus* and *E. rariseta*. AZM, adoral zone of membranelles; CL, cell length; AM, adoral membranelles; FVC, frontoventral cirri; TC, transverse cirri; MC, marginal cirri; CC, caudal cirri; DK, dorsal kinety; DDK, dikinetids in mid-DK; PM, paroral membrane; n, number.

Characteristics		*Euplotes encysticus*			*Euplotes rariseta*			
Cell length (μm)	in vivo	63-79 (68.5 ± 4.92)	62-74	80-90	32-44 (36.6 ± 5.88)	30-45		30-50
Cell width (μm)	in vivo	41-61 (50.2 ± 5.71)	41-53	50-65	23-35 (26.1 ± 3.73)	20-31		20-40
Cell length (μm)	Protargol	44-56 (50.1 ± 3.30)		67-90	23-30 (26.6 ± 1.88)		22-30	
Cell width (μm)	″	28-38 (33.9 ± 2.58)		44-67	17-20 (17.6 ± 1.14)		17-23	
AZM length (μm)	″	32-39 (36.1 ± 1.87)		50-80	18-21 (19.1 ± 0.83)			
CL: AZM ratio	″	0.66-0.78 (0.72 ± 0.04)		0.6-0.9	0.28-0.77 (0.72 ± 0.04)			
AM (n)	″	31-36 (34.0 ± 1.75)	30-35	35-43	18-22 (20.3 ± 1.23)		17-21	17-22
FVC (n)	″	9	9	9	10	10	10	
TC (n)	″	5	5	5	5			
MC (n)	″	2	2	2	1	1		
CC (n)	″	2-3 (2.1 ± 0.27)	2	2	2	2		
DK (n)	″	7		7	6	6		7
DDK (n)	″	20-22 (20.3 ± 0.65)		21-30	5-7 (6.1 ± 0.80)	6	5-7	5-7
PM (μm)	″	9-14 (10.3 ± 1.25)			2-3 (2.73 ± 0.41)			
Macronuclear shape	″	C-shape	C-shape	C-shape	Horseshoe-shape	S-shape		
Cell numbers, measured		25			21			
References		Present study	Yonezawa (1985)	Fan *et al*., 2010	Present study	Curd *et al*. (1974)	Song and Packoff (1997)	Ma *et al*. (2007)

Table 2. Comparison of morphologically similar *Euplotes* species. AM, adoral membranelles; FVC, frontoventral cirri; TC, transverse cirri; MC, marginal cirri; CC, caudal cirri; DK, dorsal kinety; DDK, dikinetids in mid-DK; n, number.

Characteristics	*E. orientalis*	*E. patabalteatus*	*E. petzi*	*E. roscoffensis*	*E. sinicus*	*E. trisulcatus*
Cell size in vivo (μm)	35-45	30-35	43-50 × 30-37	60-70 × 35-44	65-92 × 37-62	35-50 × 25-40
AM (n)	18-25	19-23	30-33	40-50	38-46	25-36
FVC (n)	8	10	10	–	10	–
TC (n)	5	5	5	–		–
MC (n)	2	2	2	–		–
CC (n)	2	2	2	–		–
DK (n)	6-7	6-7	5-6	?	7	7
DDK (n)	6-8	8-11	9-10	?	11-16	7-10
Macronuclear shape	C-shaped	Curved-bar-shaped	Hook-shaped	C-shaped	C-shaped or curved-bar shaped	C-shaped
References	Jiang *et al*. (2010b)	Jiang *et al*. (2010a)	Giuseppr *et al*. (2014)	Dragesco (1966)	Jiang *et al*. (2010a)	Carter (1972)

1f-h, 4e). The cells are capable of encystment. Move by crawling on substrate. This species was found at Masan Bay (Korea; Strain KM401) and Jongdal-ri Beach (Jeju, Korea; Strain KM444).

Remarks. *Euplotes rariseta* was originally reported by Curds *et al*. (1974) from Aberystwyth, Wales (UK). Our observations are in good agreement with those of Curds *et al*. (1974), Ma *et al*. (2007) and Song and Packoff (1997). *Euplotes rariseta* is similar to *E. parabalteatus* and *E. trisulcatus* in cell length, but can be distinguished because *E. parabalteatus* has 8-11 dikinetids in mid-DK (vs 5-7) and inconspicuous dorsal ridges, and *E. trisulcatus* has 25-36 AM (vs 18-22) and 7-11 dikinetids in mid-DK, and (Tables 1, 2). This species is mostly similar to *E. orientalis* in cell length and in the numbers of AM, DK and dikinetids in mid-DK, but they can be distinguished because *E. rariseta* has 10 FVC (vs 8) and 1 MC (vs 2) (Tables 1, 2). In addition, the 18S rRNA sequences of *E. rariseta* and *E. orientalis* have 93% similarity.

NCBI BLASTN search result shows that the closest strains to our strains (KM401, KM444) are *E. rariseta* stains (GenBank Accession No. AJ305248, FJ423449; Identity 99%). The 18S rRNA sequences of stain KM401 and KM444 are 1,742 bp and 1,738 bp in length, respectively.

Habitat. Marine

Distribution. Korea (Inchon, Park *et al*., 2017; Masan Bay and Jeju Island, this study), Antarctica (Ross Sea, Valbonesi and Luporini, 1990; Albergoni *et al*., 2000), Bellings-hausen Sea (Thompson, 1972), China (Song and Packroff, 1997; Jiang *et al*., 2010b), Indian Ocean (Dallai *et al*., 1987), Red Sea (Solar lake; Wilbert and Kahan, 1981), Somalia (Gesira; Dallai *et al*., 1987), UK (Aberystwyth, Wales, Curds *et al*., 1974), Weddell Sea (Agatha *et al*., 1990; Petz *et al*., 1995).

Deposition. National Institute of Biological Resources, Korea (NIBRPR0000107971) for KM401 strain.

NCBI GenBank Accession Numbers: Strain KM 401: MK028833, Strain KM444: MK050525

Acknowledgements

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