Morphological and molecular characterization of a new species of *Euplotes* (Ciliophora, Hypotrichida, Euplotidae) and suggestion of a new genus

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SUMMARY

A new *Euplotes* ciliate collected from the estuary of the Taehwagang River in Ulsan, Korea is characterized by its morphology and 18S rRNA gene sequence. The new species is unique in size, shape, nuclear apparatus, and infraciliature compared to congeners. The major differences between *Euplotes* n. sp. and its most similar species, *E. raikovi,* are the position and number of fronto-ventral and transverse cirri. The SSU rRNA gene of *Euplotes* n. sp. is sequenced and analyzed phylogenetically. The molecular phylogenetic inferences using BI, ML, NJ, and MP show that *Euplotes* n. sp. is closely related to *E. raikovi* and *E. nobilii*. Its sequence similarities with them are, respectively, 82.35% and 81.83%. The morphological and molecular phylogenetic inferences suggest establishing a new species and a new genus.

INTRODUCTION

To investigate rare and poorly understood ciliate species, we examined one ciliate species from an estuary in Korea. We identified it tentatively as an *Euplotes* n. sp. because the species morphology closely resembles that of the species of genus *Euplotes*, but the exact taxonomic position and phylogenetic relations could not be determined. The species belonging to *Euplotes* are frequently encountered and can be found in diverse habitats. In the literature, over 80 species of *Euplotes*, in a wide interpretation, have been described throughout the world and eight species have been reported from Korea (Kwon and Shin, 2006). Identification of species using DNA sequences is the basis for DNA barcoding and molecular identification. Currently, intensive attention is devoted to using several molecular markers for this purpose. Ribosomal RNA genes are a candidate for this purpose because of their universal occurrence; however, it has been assumed that these genes are highly conserved to delineate the species level. In contrast, it is known that some regions of ribosomal genes harbor highly divergent parts. The phylogenetic reconstructions and sequence similarities of this gene might also be independent criteria to determine the status of species or higher taxa (Petroni et al., 2002; Yi et al., 2009).

MATERIALS AND METHODS

Collection, culture, and morphological observation

Samples were collected from the estuarine littorals of the Taehwagang River in Ulsan, Korea, during April 2008 – September 2008 (N35°32'55", E129°18'27" and N35°33'27", E129°18'24") using a hand net and scoop. Cultures were maintained in petri dishes providing rice grains as a food source to enrich bacterial growth in culture. Living specimens were observed under a light microscope (Axioscope II; Carl Zeiss Inc., Germany) at 40 × to 1,000 × magnifications. Protargol impregnation was conducted to reveal their infraciliatures. The mode of morphological analysis follows that used by Kwon and Shin (2006).

DNA extraction, sequencing and phylogenetic analysis

Genomic DNA of *Euplotes* n. sp., was extracted using a RED-Extract-N-Amp Tissue PCR Kit (Sigma, St. Louis, USA). The PCR amplifications of SSU rRNA gene were performed using primers 16s-like F (5'-AAC CTG GTT GAT CCT GCC AG-3') and 16s-like F (5'-TGA TCC TTC TGC AGG TTC AC-3') (Chen and Song, 2002). Sequencing was conducted using a sequencer (ABI 3700; Applied Biosystems, Foster City, USA). For phylogenetic analyses, sequences of 32 SSU rRNA genes from NCBI/GenBank were used in addition to one that was newly sequenced in this study. The sequence alignments and phylogenetic reconstructions using Bayesian inference (BI), maximum likelihood (ML), neighbor-joining (NJ), and maximum parsimony (MP) were performed mostly according to Yi et al. (2009).

RESULTS

Morphological diagnosis

Body size is small *in vivo* 35–50 × 15–30 mm. It is slightly elongate, with the body axis curved to the left side. The adoral zone of membranelles (AZM) covered 67 –75% of the cell length with 20–22 adoral membranelles (AM). The collar base of AZM is oblique from right to left, with three distinct grooves or ridges on dorsal and ventral surfaces. Contractile vacuole is spherical and positioned at posterior right. Seven fronto-ventral cirri (FVC) are positioned at the anterior part of body. Uniquely, 4 transverse cirri (TC) are respectively positioned at posterior part and separated in two groups consisting of 2 cirri, 2 caudal cirri (CC) and 1 marginal cirrus (MC), 7–8 dorsal kineties (DK), and mid-DK comprising 10–11 bristles. The dargyrome pattern was double-patella type. One macronucleus was completely C-shaped with no micronucleus

or with one micronucleus at the anterior on the left side of macronucleus.

Morphological comparison with related species

Euplotes n. sp. showed high morphological similarity with related species, *E. raikovi* Agamaliev, 1966, *E. rariseta* (Curds et al., 1974) and *E. petzi* (Wilbert and Song, 2008). The most similar morphospecies, *E. raikovi,* differs from the present species in the number (7+1 *vs.* 7) and position (anteriormost *vs.* anterior and mid-part of body) of FVC, the number (6 *vs.* 3) of dorsal ridges, and the number (5 *vs.* 4) of TC. *Euplotes rariseta* differs from the present species in the number (10 *vs*. 7) of FVC, the number (5 *vs*. 4) of TC, and the number (5–9 *vs*. 10–11) of dorsal bristles in DK. *Euplotes petzi* differs from it in the number (10 *vs.* 7) of FVC, the number (25–33 *vs*. 20–22) of AM, the number (2 *vs*. 1) of MC and the number (5 *vs*. 4) of TC (Schwarz et al., 2007; Wilbert and Song, 2008).

Small subunit rRNA gene sequence and phylogenetic analysis

The SSU rRNA gene of *Euplotes* n. sp. was sequenced using 1729 positions. All methods of tree reconstruction independently supported the constant position of *Euplotes* n. sp. The inferred position of this species is between its sister group and the group formed by *Euplotes* sp. JJM07091701 (Acc # FJ346568) including an uncultured marine eukaryote (Acc # EF527100). In the radial/crown tree, *Euplotes* n. sp. displayed a distinct position with a long-branch attraction.

The clade of *Euplotes* n. sp. showed a high bootstrap value (>90%) with neighbor-joining (NJ), maximum likelihood (ML), and high posterior probability (1.00) in Bayesian inference (BI). The sequence similarities of *Euplotes* n. sp. with *E. raikovi* (ACC. # AJ305251) and *E. nobilii (*ACC. # EF094970*)* were, respectively, 82.35% and 81.83%. These two species formed sister groups of *Euplotes* n. sp., exhibiting the closest molecular relation based on reconstructed trees. However, the pairwise sequence differences (>15%) of *Euplotes* n. sp. with its all congeneric species in this study were considerably high and significant to allow the present species to be subordinated to the different new genus.

The relation with the most similar morphospecies *E. raikovi* was proven by having the adjacent phylogenetic position as a sister taxon of *Euplotes* n. sp. through molecular analysis. In addition, *Euplotes rariseta* (ACC. # AJ305248) showed a slightly distant phylogenetic relation with it than *E. raikovi*, which also holds substantial morphological similarities. Although the other two species (*Euplotes* sp. JJM07091701 and uncultured marine eukaryote) were also represented as significantly related species, they are not described morphologically to infer any reliable comparison. Four segments [A, B, C, and D] of SSU rRNA gene sequences were uniquely conserved in *Euplotes* n. sp., *E. raikovi*, and *E. nobilii*, in contrast to other congeners. In addition, one segment [E] has some unique deletions in *Euplotes* n. sp. and three non-defined *Euplotes* species.

DISCUSSION

The present species from *Euplotes* was studied under both morphological and molecular aspects. *Euplotes* n. sp. clearly has unique morphological characteristics by which it differs from its congeners. This species exhibited the highest morphological similarity with *E. raikovi*, which varied significantly by the number and position of fronto-ventral cirri, the number of dorsal ridges, and transverse cirri. These morphologically distinct characteristics indicate this species as a new species in *Euplotes*. Furthermore, the molecular study established a distinct position for *Euplotes* n. sp. with a high level of supporting values from individual phylogenetic analysis. The pairwise sequence comparison analysis for SSU rRNA gene of *Euplotes* n. sp. with its congeneric species showed high divergence, strongly supporting the evaluation of this new species to form a new genus.

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