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Short communication

DNA Barcoding for *Diophrys quadrinucleata* (Ciliophora: Euplotia) from South Korea

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ABSTRACT

One marine ciliate, *Diophrys quadrinucleata* Zhang *et al*., 2020 was newly recorded from South Korea in this study. We provided morphological diagnosis and images of the Korean *D. quadrinucleata* population. We determined the small subunit ribosomal DNA (SSU rDNA) and cytochrome oxidase subunit I (CO1) sequence data of *D. quadrinucleata*, and then the sequences were compared with other *Diophrys* species. Intra-specific variation between the Korean and type (Chinese) populations was identical in the SSU rDNA, while the inter-specific variations between seven *Diophrys* species were 0.3−3.8% in the SSU rDNA and 12.6−18.2% in the CO1. In this study, we obtained 18S and CO1 data from species with identified morphology. As the importance of securing 18S and CO1 based on morphology increases in current studies, this study will contribute to ciliate studies.

Keywords: CO1, Hypotrichia, morphology, seawater, SSU rDNA

INTRODUCTION

The genus *Diophrys* Foissner et al., 1991 currently comprises 11 species, this genus is mainly characterized by the combined features of the oral area with the conspicuous adoral zone of membranelles and separate paroral and endoral membranes, five frontal, two ventral, five transverse and one or two left marginal cirri, and three caudal cirri (Zhang et al., 2020). Recently, three *Diophrys* species have been recorded in South Korea: *D. appendiculata* (Ehrenberg, 1838) Schewiakoff, 1893; *D. oligothrix* Borror, 1965; and *D. scutum* (Dujardin, 1841) Kahl, 1932 (Kwon and Shin, 2006; Kwon et al., 2008; Jung et al., 2017). In this study, we found an unrecorded *Diophrys* species, *D. quadrinucleata* Zhang et al., 2020, in South Korea.

Small subunit ribosomal DNA (SSU rDNA) has been commonly used for phylogenetic studies of ciliates, but the approach based on SSU rDNA could mislead the understanding of ciliate evolution (Dunthorn et al., 2011). Therefore, several barcode studies have been conducted recently, and the suitability of cytochrome c oxidase subunit 1 (CO1) barcodes has been proven in some Oligohymenophorea and Spirotrichea species (Barth et al., 2006; Lynn and Strüder-Kypke, 2006; Chantangsi and Lynn, 2008; Gentekaki and Lynn, 2009; Jung et al., 2011; Kher et al., 2011; Tarcz et al., 2012, 2013, 2014; Zhao et al., 2013, 2016; Song et al., 2014; Park et al., 2018).

Seawater samples were collected in November 2020. For sample collection, depth of 30 cm of sand was dug in Eulwang-ri Beach, and seawater was collected there. The water temperature was approximately 3.5°C, and the salinity was 27.7‰. Specimens were maintained in the laboratory as raw cultures for one to two weeks at 4°C and 20°C, and rice grains were used to promote the growth of bacteria as food for the ciliates. Raw cultures were microscopically observed in vivo (Leica DM2500; Wetzlar, Germany) from ×50 to ×1,000 magnification. Cell staining was performed to use Procedure A method described by Foissner (2014). Classification and terminology are according to Zhang et al. (2020) and Lynn (2008).

DNA extract, polymerase chain reaction (PCR) amplification and sequencing were performed according to the methods of Jung et al. (2012) and Park et al. (2018). Amplification of the nuclear 18S rDNA gene was performed using the forward primer EukA (5′-AAC CTG GTT GAT CCT GCC AGT-3′) and reverse primer EukB (5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′) (Medlin et al., 1988). PCR amplification of CO1 was performed using the ciliate specific CO1 primers, CiCO1 Fv2 (5′-GWT GRG CKA TGA TYA CAC C-3′) and

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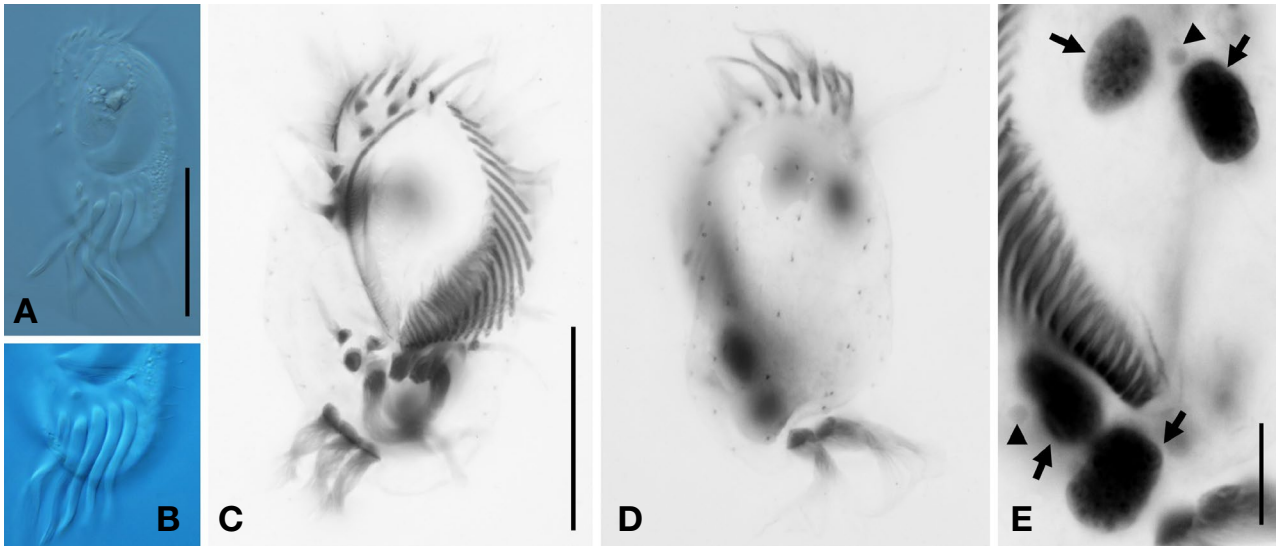

Fig. 1. Photomicrographs of *Diophrys quadrinucleata* from live (A, B), after protargol impregnation (C–E). A, B, Ventral views showing cirral pattern; C, D, Ventral and dorsal view; E, Arrows and arrowheads indicate macronuclear nodules and micronuclei, respectively. Scale bars: A, C=50 μm, E=10 μm.

CiCO1 Rv2 (5′-ACC ATR TAC ATA TGA TGW CC-3′) (Park et al., 2018). The 18S and CO1 sequences of *D. quadrinucleata* and other *Diophrys* species retrieved from GenBank were aligned using BioEdit (Hall, 1999), and the pairwise distances and number of nucleotide differences were calculated using MEGA 11 (Tamura et al., 2021).

RESULTS AND DISCUSSION

Phylum Ciliophora Doflein, 1901
Class Spirotrichea Bütschli, 1889
Subclass Hypotrichia Stein, 1859
Order Euplotida Small & Lynn, 1985
Family Uronychiidae Jankowski, 1975
Genus *Diophrys* Dujardin, 1840

***Diophrys quadrinucleata* Zhang et al., 2020 (Fig. 1)**
Diophrys quadrinucleata Zhang et al., 2020: 979, fig. 2.

Material examined. Seawater sample (27.7‰) from Eurwang-dong, Incheon-si, Korea (37°26′50″N, 126°22′9.5″E), on Nov 2020.
Diagnosis. Size about 80–110 × 50–70 μm in vivo; body elliptical, greyish; 27–39 adoral zone membranelles; 4 macronuclear nodules; 2 or 3 micronuclei; 5 frontal cirri; 2 ventral cirri; 5 transverse cirri; 1 left marginal cirrus; 5 dorsal kineties; 3 caudal cirri.
Distribution. China and Korea.
Remarks. *Diophrys quadrinucleata* differs from other *Diophrys* species by the number of macronuclear nodules (4 vs. 2 or 7–23) (Zhang et al., 2020). The Korean population of *Diophrys quadrinucleata* morphologically corresponds to the type population by the number of frontal cirri, ventral cirri, marginal cirri, transverse cirri, caudal cirri, and dorsal kineties (Zhang et al., 2020).
Voucher slides. One slide with protargol-impregnated specimens was deposited at the National Institute of Biological Resources (NIBRPR0000111058).

The alignment length of the two 18S rDNA sequence (GenBank accession No. OP070164, OP070165) of the Korean *D. quadrinucleata* population and seven *Diophrys* species was 1,613 bp. The intra-specific variation of *D. quadrinucleata* was identical. Inter-specific variation in *D. quadrinucleata* and other congeners was 0.3–3.8% (Table 1).

The alignment length of the four CO1 sequences (GenBank accession No. OP096457–OP096460) the Korean *D. quadrinucleata* population (26.3% GC content) and the three species in the genetic comparison was 476 bp. The intra-specific variation of *D. quadrinucleata* were identical. Inter-specific variation in between *D. quadrinucleata* and *D. scutum* was 11.1–13.9% (Table 2). Inter-specific variation in between *D. quadrinucleata* and *D. appendiculata* was 14.3%. Inter-specific variation in between *D. quadrinucleata* and *D. oligothrix* was 17.5–17.7%. Inter-generic variations within the genus *Diophrys* were in the range of 11.1–17.7%. In a previous study, intra- and inter-specific variations in CO1 sequences of genus *Diophrys* were 0.00–4.54% and 13.49–22.01% (Park et

Table 1. The number of nucleotides differences (above the diagonal) and pairwise distances (below the diagonal) between selected *Diophrys* 18S rDNA sequences

	1.	2.	3.	4.	5.	6.	7.	8.	9.
1. *Diophrys quadrinucleata* OP070164, OP070165		0	4	23	39	42	43	59	59
2. *Diophrys quadrinucleata* MT109370	0.000		4	23	39	42	43	59	59
3. *Diophrys apoligothrix* JF694038	0.003	0.003		25	39	42	43	61	61
4. *Diophrys scutum* MT109372	0.015	0.015	0.016		46	47	51	62	62
5. *Diophrys oligothrix* MG603603	0.025	0.025	0.025	0.029		7	10	56	56
6. *Diophrys blakeneyensis* JN172996	0.027	0.027	0.027	0.030	0.004		15	58	58
7. *Diophrys appendiculata* MG603601	0.027	0.027	0.027	0.033	0.006	0.009		53	53
8. *Diophrys appendiculata* AY004773	0.038	0.038	0.039	0.040	0.036	0.037	0.034		0
9. *Diophrys parappendiculata* EU267928	0.038	0.038	0.039	0.040	0.036	0.037	0.034	0.000	

Table 2. The number of nucleotides differences (above the diagonal) and pairwise distances (below the diagonal) between selected *Diophrys* CO1 sequences

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
1. *Diophrys quadrinucleata* OP096457–OP096460		53	53	54	54	54	59	66	68	83	84	84
2. *Diophrys scutum* MG594861	0.111		1	2	2	2	20	55	81	87	87	87
3. *Diophrys scutum* MG594860	0.111	0.002		1	1	1	20	56	82	88	88	88
4. *Diophrys scutum* MG594858	0.113	0.004	0.002		0	0	21	57	83	89	89	89
5. *Diophrys scutum* MG594859	0.113	0.004	0.002	0.000		0	21	57	83	89	89	89
6. *Diophrys scutum* MG594857	0.113	0.004	0.002	0.000	0.000		21	57	83	89	89	89
7. *Diophrys scutum* MG594862	0.124	0.042	0.042	0.044	0.044	0.044		61	83	85	85	85
8. *Diophrys scutum* MG594863	0.139	0.116	0.118	0.120	0.120	0.120	0.128		75	88	89	89
9. *Diophrys appendiculata* MG594867	0.143	0.170	0.172	0.174	0.174	0.174	0.174	0.158		84	85	85
10. *Diophrys oligothrix* MG594866	0.175	0.183	0.185	0.187	0.187	0.187	0.179	0.185	0.177		1	1
11. *Diophrys oligothrix* MG594864	0.177	0.183	0.185	0.187	0.187	0.187	0.179	0.187	0.179	0.002		0
12. *Diophrys oligothrix* MG594865	0.177	0.183	0.185	0.187	0.187	0.187	0.179	0.187	0.179	0.002	0.000	

al., 2018), respectively. The results of this study overlap with previous studies and show that the CO1 barcode is effective in distinguishing *D. quadrinucleata* from other species in the genus *Diophrys*. Therefore, additional studies involving CO1 barcodes show that they could be effective to resolve the discrepancy between morphology and DNA taxonomy. Currently, most ciliate studies provide only morphology and 18S rDNA, so it is necessary to obtain CO1 to avoid misleading phylogenetic studies. However, CO1 and morphology must be provided together. Therefore, the importance of a study that provides 18S rDNA and CO1 together based on a fully identified individual such as this study will increase in the future, and this study will be helpful.

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CONFLICTS OF INTEREST

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