

COI DNA Barcoding for *Sterkiella multicirrata* (Ciliophora: Oxytrichidae) from South Korea

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ABSTRACT

In the present study, the first mitochondrial cytochrome *c* oxidase subunit I gene (*COI*) sequence of *Sterkiella multicirrata* Li et al., 2018 is presented. To begin with, this species has been also morphologically recorded from South Korea, and this study was performed using genomic DNA of the Korean population. The newly obtained *COI* sequences of *S. multicirrata* were identical. And the inter-specific variation between *S. multicirrata* and *S. histriomuscorum* was noted at 14.3%. These values correspond well with the results of previous studies. However, because there are very few available *COI* sequences of stichotrichian in GenBank, it is concluded that continuous accumulation of data is needed for further study.

Keywords: Ciliophora, *COI*, DNA barcode, Stichotrichia, Styloynchinae

INTRODUCTION

The genus *Sterkiella* Foissner et al., 1991 currently comprises 11 species (Berger, 1999; Chen et al., 2015; Kumar et al., 2015; Foissner, 2016; Chen et al., 2017; Li et al., 2018), four species have been recorded in South Korea: *S. cavicola* (Kahl, 1935) Foissner et al., 1991; *S. histriomuscorum* (Foissner et al., 1991) Foissner et al., 1991; *S. multicirrata* Li et al., 2018; and *S. tricirrata* (Buitkamp, 1977) Berger, 1999 (see Jung et al., 2017; Kim and Min, 2018; Kim et al., 2018). It is characterized by the following features: body broadly elliptical, size *in vivo* 120–165 × 50–90 μm, semi-rigid; four macronuclear nodules; more than 18 frontal-ventral-transverse (FVT) cirri, three frontal, one buccal, five or six frontoventral, five postoral ventral, one or no pretransverse ventral, and four or five transverse cirri; one left and one right marginal cirral row; six dorsal kineties; 3 caudal cirri (Fig. 1).

The sequences of the small and large subunit ribosomal RNA genes have been used most commonly for the phylogenetic studies of ciliates. However, they are considered unsuitable molecular markers at the population and species levels because of their high conservation (Yi et al., 2008; Jung et al., 2014). Recently, it started to evaluate DNA barcode utility of the mitochondrial *COI* gene, which is used generally for DNA

barcoding of metazoan invertebrates, in ciliates (Strüder-Kypke and Lynn, 2010; Zhao et al., 2013; Park et al., 2019).

Soil samples (pH 5.0) were obtained from the sloppy grassland Bangeo-dong, Ulsan, South Korea (35°28'N, 129°25'E), in November 2015. Dried soil samples were transferred into Petri dishes, mixed with tap water, and allowed to stand at room temperature (20°C). *Sterkiella multicirrata* individuals were isolated from raw cultures. Some specimens were deposited at the National Institute of Biological Resources, Korea (NIBR accession No. NIBRPR0000107255, NIBRPR0000107269).

PCR amplification was performed with the ciliate specific *COI* primers, CiCO1 Fv2 (5'-GWT GRG CKA TGA TYA CAC C-3') and CiCO1 Rv2 (5'-ACC ATR TAC ATA TGA TGW CC-3') (Park et al., 2019), under the following conditions: 2 min at 95°C; followed by 35 cycles at 95°C for 15 s, 53°C for 30 s, and 72°C for 60 s; and a final extension at 72°C for 5 min. The newly obtained *COI* sequence for *S. multicirrata* was registered to GenBank (GenBank accession No. MK976655, MN607038–MN607041). The sequences of *S. multicirrata* and other oxytrichids retrieved from GenBank were aligned using BioEdit (Hall, 1999), and then the pairwise distances and the number of nucleotide differences were calculated using MEGA 5.0 (Tamura et al., 2011).

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RESULTS AND DISCUSSION

Five *COI* sequences of the Korean *S. multicirrata* population were identical and 489 bp in length (67.1% AT content). Intra-specific variations of *S. histriomuscorum* and *O. trifallax* were 1.3% and 0.8%, respectively (Table 1). Inter-specific variation between *S. multicirrata* and *S. histriomuscorum*

was 14.3%. Inter-generic variations within the family Oxytrichidae were in the range of 12.8–20.8%. The previous study was determined that intra- and inter-specific variations of *COI* in the subclass Spirotrichea were in the range of 0.0–5.7% and 9.8–47.3%, respectively (Park et al., 2019). In this study, the intra-specific variations of *Sterkiella* species were well reflected in the previous study (Park et al., 2019). However, inter-specific variation between two *Sterkiella* species, *S. multicirrata* and *S. histriomuscorum* (14.3%) overlaps with inter-generic variations within the family Oxytrichidae (12.8–20.8%). To resolve this incongruence between traditional morphological classification and DNA taxonomy, further research should be considered, including morphological revisions, additional analysis of undetermined taxa, and assessment of additional candidate barcodes.

Stichotrichians show a high diversity and is the most confused in ciliates (Schmidt et al., 2007; Lynn, 2008), with over 1,030 species belonging to about 130 genera (Encyclopedia of Life, <http://eol.org>, 24 Oct 2019). However, 61 *COI* sequences of them have been currently registered in GenBank database (24 Oct 2019). In order to evaluate *COI* DNA barcoding in stichotrichians, additional combined morphological and molecular analyses are required for more species.

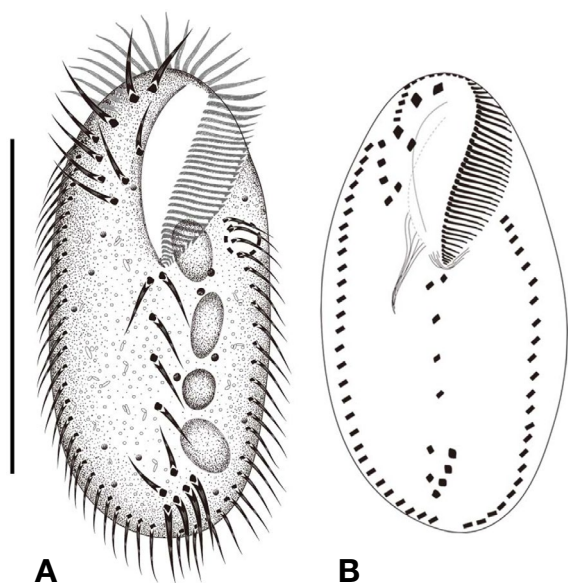


Fig. 1. Morphology of *Sterkiella multicirrata* from live (A) and protargol-impregnated (B) specimens. Scale bar= 100 µm.

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Table 1. The number of nucleotides difference (above the diagonal) and pairwise distance (below the diagonal) between several Oxytrichid ciliates *COI* gene sequence and *Sterkiella multicirrata*

	1	2	3	4	5	6	7	8	9	10
1. <i>Sterkiella multicirrata</i> (MK976655)		68	68	75	76	79	79	81	83	96
2. <i>Sterkiella histriomuscorum</i> (MG594892)	0.143		6	74	72	79	88	83	91	89
3. <i>Sterkiella histriomuscorum</i> (MK976654)	0.143	0.013		75	73	80	86	84	91	89
4. <i>Oxytricha trifallax</i> (MK976656)	0.157	0.155	0.157		4	87	86	97	83	76
5. <i>Oxytricha trifallax</i> (JN383843)	0.159	0.151	0.153	0.008		88	85	99	82	78
6. <i>Rubrioxxytricha</i> sp. (MG594888)	0.166	0.166	0.168	0.182	0.184		76	69	90	84
7. <i>Pseudocyrtohymenidea lacunae</i> (MG594891)	0.166	0.184	0.180	0.180	0.178	0.159		87	61	91
8. <i>Cyrtohymena muscorum</i> (MG594887)	0.170	0.174	0.176	0.203	0.208	0.145	0.182		84	89
9. <i>Pseudocyrtohymena koreana</i> (MG594890)	0.174	0.191	0.191	0.174	0.172	0.189	0.128	0.176		88
10. <i>Pseudouroleptus jejuensis</i> (MG594893)	0.201	0.187	0.187	0.159	0.164	0.176	0.191	0.187	0.184	

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CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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