

DNA Barcoding of a Worldwide Colonial Ascidian, *Diplosoma listerianum* (Asciidiacea: Aplousobranchia: Didemnidae), from East Sea, Korea

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ABSTRACT

Diplosoma listerianum (Milne Edwards, 1841) is a globally distributed species that encompasses the existence of multiple cryptic species. In this study, partial sequences of cytochrome *c* oxidase subunit I (*COI*) from *D. listerianum* in South Korea were determined and compared with *COI* sequences of known *D. listerianum* and other *Diplosoma* species retrieved from GenBank. The results showed that Korean *D. listerianum* matched with clade A of *D. listerianum*, which is a group distributed globally. These new DNA barcodes for Korean *D. listerianum* may be useful in the identification of colonial ascidians, which is difficult to morphological identify.

Keywords: DNA barcoding, *COI*, colonial ascidian, Didemnidae, *Diplosoma listerianum*

INTRODUCTION

Ascidians are among the most important introduced species in the sea (Lambert, 2007). Populations of some introduced ascidians clearly belong to a single species; in other cases, cryptic speciation has been found: *Botryllus schlosseri*, *Ciona intestinalis*, and *Diplosoma listerianum* (Bock et al., 2012; Pérez-Portela et al., 2013; Brunetti et al., 2015). Among these, *D. listerianum* is truly cosmopolitan, recorded in the tropical to temperate waters of the Pacific, Atlantic, and Indian Oceans and the Mediterranean and North Seas (Kott, 2001). Analysis of *D. listerianum* revealed the existence of multiple cryptic species worldwide, and identified a complex geographic structure and multiple clades that occurred in sympatry (clades A–D), based on the dataset of mitochondrial cytochrome *c* oxidase subunit I (*COI*) (Pérez-Portela et al., 2013). The results indicated that *D. listerianum* is a species complex of at least four evolutionarily distinct lineages; however, they were unable to substantiate any clear diagnostic morphological character owing to the scarcity of candidate characters (e.g., the lack of calcareous spicules in the tunic of this genus), and the high variability in the shape and color of the colonies (Pérez-Portela et al., 2013). Among these, clade A of *D. listerianum* appears to have been introduced in different biogeographical areas, as shown by phylogenetic analyses

and population genetic network structure (Pérez-Portela et al., 2013). The *COI* gene has been shown to be extremely useful and accurate for the detection of cryptic speciation and species identification in ascidians (Turon et al., 2003; Tarjuelo et al., 2004; Rius and Teske, 2013). In this study, we obtained partial sequences of *COI* from *D. listerianum* in South Korea and compared it with known *COI* sequences of *D. listerianum* and other *Diplosoma* species.

The colonies were collected from artificial materials in Jukbyeon harbor, South Korea (Fig. 1). All examined colonies were deposited in a 95% ethyl alcohol solution at the Marine Biological Resource Institute of Sahmyook University. Total genomic DNA was extracted from a single zooid in a colony in accordance with the DNeasy Blood & Tissue kit protocol (Qiagen, Hilden, Germany). The partial sequences of *COI* were amplified using a universal primer pair: dinF-Nux1R (Brunetti et al., 2017). Polymerase chain reaction (PCR) was executed in a reaction volume of 20.0 µL AccuPower PCR PreMix & Master Mix (Bioneer, Seoul, Korea) including 1.0 µL of each primer (10 mM) and 1.5 µL of DNA template (> 50 ng/µL) using the following thermocycling profile: 94°C for 3 min; 35 cycles of 94°C for 30 s, 52°C for 60 s, and 72°C for 60 s; and a final extension at 72°C for 5 min. The pairwise distance (p-distance) was calculated using the Kimura 2-parameter model (Kimura, 1980) in MEGA 7.0 (Kumar et al.,

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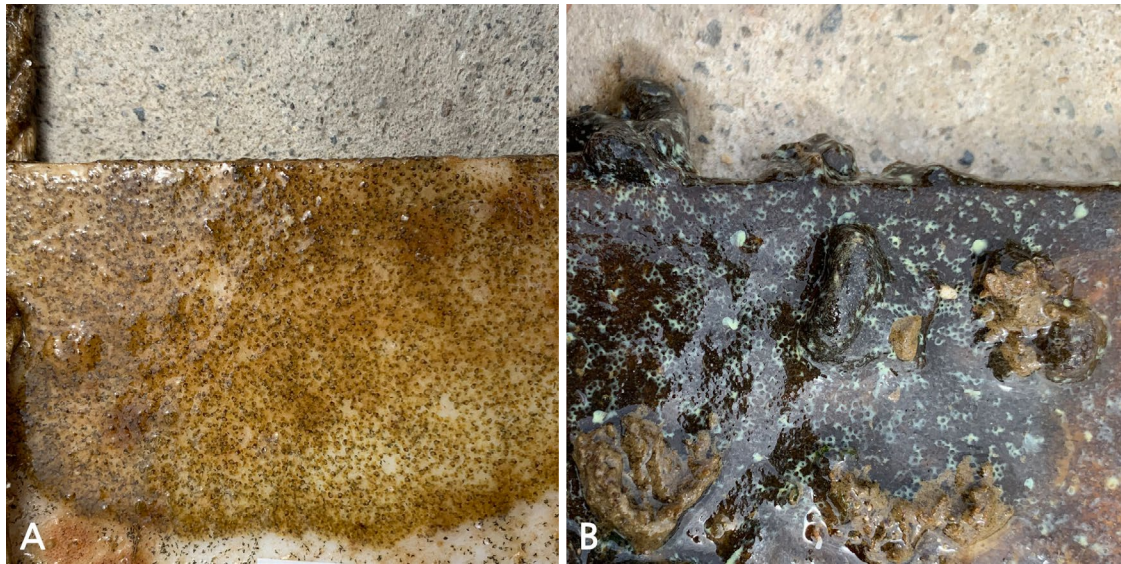


Fig. 1. Various color morph of *Diplosoma listerianum* from South Korea: A, B, Artificial materials (acrylic plate) covered with *D. listerianum* in Jukbyeon harbor.

Table 1. Pairwise distance values (%) based on 516 bp mitochondrial *COI* sequences in this study, calculated using the Kimura 2-parameter method

Species	Clade	n ^a	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 <i>Diplosoma listerianum</i>	Korea	3	0.0													
2	A ^b	28	4.2	5.8												
3	A.1 ^b	8	12.6	12.2	1.3											
4	B ^b	3	18.9	9.4	19.2	5.2										
5	C ^b	4	23.3	23.0	22.3	23.0	4.1									
6	D ^b	2	20.5	20.3	19.8	22.4	19.3	0.2								
7 <i>D. gelatinosa</i>	–	1	18.6	17.8	11.0	24.9	25.5	26.2	0.0							
8 <i>D. multifidum</i>	–	1	25.7	24.4	24.1	32.7	30.8	30.5	27.2	0.0						
9 <i>D. simile</i>	–	1	25.6	25.1	23.3	25.4	23.5	23.3	27.0	31.8	0.0					
10 <i>D. spongiforme</i>	–	1	26.5	26.0	21.5	23.0	26.3	22.9	26.8	32.6	22.7	0.0				
11 <i>D. swamiensis</i>	–	1	19.1	18.2	7.3	25.3	26.9	24.6	16.8	30.0	27.9	25.8	0.0			
12 <i>Diplosoma</i> sp.	–	1	17.7	17.7	16.7	18.1	18.4	18.3	21.7	26.4	20.3	20.2	21.4	0.0		
13 <i>Diplosoma</i> sp.	–	1	17.7	17.7	16.7	18.1	18.4	18.3	21.7	26.4	20.3	20.2	21.4	0.0	0.0	
14 <i>Didemnum vexillum</i>	–	1	33.1	32.6	29.3	32.3	36.3	35.1	31.8	38.9	29.3	38.7	34.8	29.0	29.0	0.0

^aNumber of cytochrome c oxidase subunit I (*COI*) sequences in a clade.

^bThe type of clade is according to Pérez-Portela et al. (2013).

2016). The best-fit model of nucleotide substitution for the *COI* dataset was selected by jModelTest v. 2.1.1 (Darriba et al., 2012) using the Akaike Information Criterion for Maximum Likelihood (ML). The ML tree was constructed using PhyML 3.0 (Guignon et al., 2010) under HKY + I + G model for *COI* dataset, and which included Korean and GenBank of *D. listerianum*, and *D. spongiforme* as an outgroup.

RESULTS AND DISCUSSION

Based on previous morphological studies (Tokioka, 1970;

Rho and Huh, 1984; Kott, 2001; Shenkar et al., 2021), collected colonial ascidians in Jukbyeon harbor were identified as *D. listerianum* (Fig. 1). The newly obtained *COI* sequences (867 bp) of *D. listerianum* were registered in GenBank (accession No. MT983895–MT983897). The type of clade in this study was according to Pérez-Portela et al. (2013). The p-distance values showed that *D. listerianum* could be separated into four groups, with a different species in each group (Table 1). The Korean *D. listerianum* were closer to clade A of *D. listerianum*, than other clades (clade A.1–D) (Table 1). The variation within the Korean and clade A were

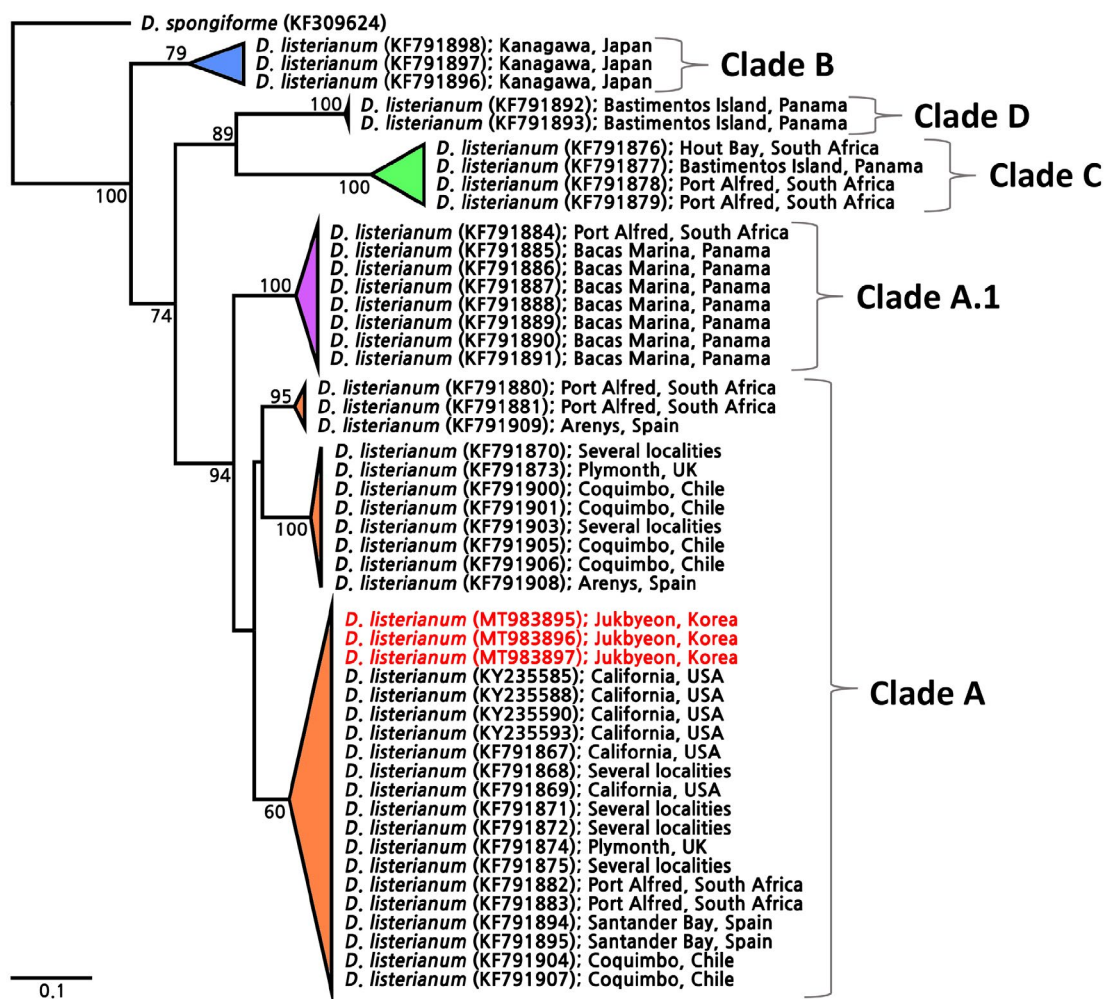


Fig. 2. The maximum likelihood tree of *Diplosoma listerianum* based on the cytochrome *c* oxidase subunit I (COI) dataset (518 bp). The Korean *D. listerianum* indicated with red letters. Bootstrap values (>50) are indicated on each node. The type of clade is according to Pérez-Portela et al. (2013).

4.2% and this value is lower than the intraspecific variation of clade A (5.8%) (Table 1). Among them, the variation within the Korean and closer group in clade A were the mean of 0.8% and the range of 0.0–3.4% (Appendix 1). The variation within Korean and clade A.1–D were 12.6%, 18.9%, 23.3%, and 20.5%, respectively, and which were close to interspecific variations within *D. listerianum* and other *Diplosoma* (Table 1). Pérez-Portela et al. (2013) verified that *D. listerianum* had high intraspecific variations, but they did not find any distinct morphological differences between each clade. The ML tree revealed the same as the p-distances result and Pérez-Portela et al. (2013) (Fig. 2). Among them, Korean *D. listerianum* was distinctly clustered with clade A of *D. listerianum*, and this clade is clearly divided into another clade of *D. listerianum* (Fig. 2). This study contributes in identifying *D. listerianum* and under-

standing taxonomic relationship of *Diplosoma* species.

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CONFLICTS OF INTEREST

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

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