

First Genetic Data of *Nebalia koreana* (Malacostraca, Leptostraca) with DNA Barcode Divergence among *Nebalia* Species

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ABSTRACT

We determined the cytochrome *c* oxidase subunit 1 (*COI*) sequences of *Nebalia koreana* Song, Moreira & Min, 2012 (Leptostraca) collected from five locations in South Korea, and this represents the first genetic data of this species. The maximum intra-species variation was 1.2% within *Nebalia hessleri* Martin, Vetter & Cash-Clark, 1996, while inter-species variation ranged from 9.0% (*N. hessleri* and *Nebalia gerkenae* Haney & Martin, 2000) to 34.8% (*N. hessleri* and *Nebalia pseudotronicosoi* Song, Moreira & Min, 2013). This result is well agreed with the interspecific relationships among *Nebalia* species based on morphological characteristics. In conclusion, this study showed the usefulness of *COI* sequences as a DNA barcode within the genus *Nebalia* Leach, 1814.

Keywords: *COI*, DNA barcode, Leptostraca, *Nebalia*, South Korea

INTRODUCTION

The order Leptostraca Claus, 1880 is the only extant order in the subclass Phyllocarida Packard, 1879, and is considered by many researchers to be the most primitive group in the class Malacostraca Latreille, 1802 (Claus, 1888; Manton, 1934; Hessler and Newman, 1975; Barnes, 1980; Dahl, 1987; Walker-Smith and Poore, 2001). The genus *Nebalia* Leach, 1814 is the largest member of the order Leptostraca (38 of 60 valid species), and so far, the following three species have been reported in South Korea: *Nebalia dolsandoensis* Song & Min, 2017, *Nebalia koreana* Song, Moreira & Min, 2012, and *Nebalia pseudotronicosoi* Song, Moreira & Min, 2013 (see Song et al., 2012, 2013; Song and Min, 2017). *Nebalia koreana* is distributed in Eastern, Western and Southern coastal waters of Korea, while the other two species were found restrictively at their type localities (Song, 2013). *Nebalia koreana* is diagnosed by the following characteristics: antennule fourth article with up to five short distal spines; pleonites 3–7 with rounded denticles on posterior dorsal margins; and pleopod 4 protopod lacking serrations on posterior margin.

Among the mitochondrial genes already examined in most animal phyla, including Crustacea, the cytochrome *c* oxidase subunit 1 (*COI*) gene has proved to be a particularly useful taxonomic marker (Meyran et al., 1997; Wares, 2001; Hajibaei et al., 2006; Clare et al., 2007; Elsasser et al., 2009; Zemlak et al., 2009). However, the usefulness of the *COI* sequences in *Nebalia* species has never been proven. In this study, we firstly determined the *COI* sequence of *N. koreana* (Fig. 1), and attempted to verify its usefulness.

RESULTS AND DISCUSSION

The *COI* sequences of *N. koreana* were obtained from specimens collected from each of the following five locations in South Korea: Bogildo (34°08'10"N, 126°33'48"E), Cheongsando (34°10'54"N, 126°33'59"E), Mokpo (34°48' 19"N, 126°21'43"E), Masan (35°11'11"N, 128°33'53"E), and Sokcho (38°11'50"N, 128°35'00"E) using light traps according to the methods described by Song et al. (2013). The morphological identification, genomic DNA extraction, and PCR conditions were performed according to the methods de-

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Fig. 1. *Nebalia koreana* collected from Sokcho, lateral view.

Table 1. The minimum/maximum values of inter-species pairwise variation (%) between *COI* sequences from *Nebalia* species

	1	2	3	4	5
1. <i>N. koreana</i>	–				
2. <i>N. dolsandoensis</i>	16.4/16.7	–			
3. <i>N. pseudotronicosoi</i>	16.8/17.1	18.7/18.7	–		
4. <i>N. gerkenae</i>	30.8/31.5	29.7/30.4	32.5/32.5	–	
5. <i>N. hessleri</i>	32.2/34.0	31.1/31.9	34.4/34.8	9.0/10.3	–

The GenBank accession numbers used in this study are as follows. *Nebalia koreana*, MK396103–MK396107 (this study); *N. dolsandoensis*, KT229637 and KT229638; *N. pseudotronicosoi*, JX442539–JX442543; *N. gerkenae*, FJ170145–FJ170147; *N. hessleri*, FJ170162–FJ170169

scribed by Song and Min (2017). The sequences were aligned using Geneious 8.1.9 (Biomatters Ltd., Auckland, New Zealand). The *COI* sequences determined from this study and retrieved from GenBank were aligned using MEGA 10 (Kumar et al. 2018). Nucleotide sequence divergence was calculated using the Kimura two-parameter distances.

In our analysis, the maximum intra-species variation was 1.2% within *Nebalia hessleri* Martin, Vetter & Cash-Clark, 1996, while inter-species variation ranged from 9.0% (*N. hessleri* and *Nebalia gerkenae* Haney & Martin, 2000) to 34.8% (*N. hessleri* and *N. pseudotronicosoi*) (Table 1). This result is well agreed with the interspecific relationships among *Nebalia* species based on morphological characteristics (Song and Min, 2017). In conclusion, this study suggests that the *COI* sequences are very useful for the identification of *Nebalia* species, as confirmed by many other crustaceans.

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