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Short communication

DNA Barcoding of the Marine Proteced Species *Pseudohelice subquadrata* (Decapoda, Varunidae, *Pseudohelice*) from the Korean Waters

Ji Min Kim[1,2], Jong-Gwan Kim[1], So Yeon Kim[1,2], Woo Yong Choi[1,2], Hyung Seop Kim[2], Min-Seop Kim[1,*]

[1]National Marine Biodiversity Institute of Korea, Seocheon 33662, Korea
[2]School of Marine Biotechnology, College of Marine Science, Kunsan National University, Gunsan 54150, Korea

ABSTRACT

Pseudohelice subquadrata (Dana, 1851) is endangered due to its restricted habitat; hence, it has been designated as a marine protected species and endangered species by law in Korea. It has been recorded only Jeju-do and Geomun-do, Republic of Korea. The present study, is the first report on a cytochrome *c* oxidase subunit I DNA barcode for *P. subquadrata*. The maximum intra-specific genetic distance among all *P. subquadrata* individuals was found to be 0.5%, whereas inter-genetic distance within the same genus was 17.2−21.5% compared with *Helice tientsinensis* (Rathbun, 1931), *H. tridens* (De Haan, 1835), *H. epicure* (Ng et al., 2018), and *Helicana wuana* (Rathbun, 1931). Our barcoding data can thus be used as reference for restoration and conservation studies on *P. subquadrata*, which are designated as marine protected species.

Keywords: DNA barcode, cytochrome *c* oxidase subunit I, marine protected species, endangered species

INTRODUCTION

The genus *Pseudohelice* K. Sakai, Türkay & Yang, 2006, is one of 36 genera of the family Varunidae, with only one species in the genus reported to date (Ahyoung et al., 2011). *Pseudohelice subquadrata* (Dana, 1851), a monotypic species, usually lives in salt marshes and estuarine environments, especially in the intertidal zone and substrate mangroves (Sakai et al., 2006; Bouchard et al., 2013; Naderloo, 2017; Kim et al., 2018). In Korea, it lives in holes dug in coastal grassland, where stone and sand are mixed (NIBR, 2017). This varunid crab is distributed from the Indian Ocean to the South Pacific, including Japan, China, Thailand, Indonesia, Philippines, Australia, Solomon, and New Caledonia (Shih and Suzuki, 2008; Bouchard et al., 2013; Kim et al., 2018). In Korea, it has been reported to inhabit Jeju-do, which is the northern limit of its natural distribution (Kim, 1973; NIBR, 2017).

Pseudohelice subquadrata is an endangered crab species under the category of wildlife by the Wildlife Protection and Management Act, passed in 2005, and a marine protected species by the Conservation and Management of Marine Ecosystems Act, passed in 2006. Recently, interest in the restoration and conservation of endangered species has been increasing; however, most studies have not achieved much success.

Among the mitochondrial genes already examined in most animal phyla, including Crustacea, cytochrome *c* oxidase subunit I (*COI*) sequence has proven a particularly useful taxonomic marker (Hajibabaei et al., 2006; Elsasser et al., 2009; Zemlak et al., 2009; Song and Min, 2019).

In the present study, we first determined the *COI* sequences of *P. subquadrata* collected from Jeju-do, Republic of Korea, and attempted to verify its application to provide basic data for restoration and conservation studies.

We used four individual crabs collected from two localities in Jeju-do: Yeonpyeong-ri (33°31′--″N, 126°56′--″E) and Wimiri (33°16′--″N, 126°39′--″E). The carapace is deep violet or dark red, while the forehead is short and tilts forward and downward (Fig. 1), making it appear round-shaped compared to the *Helice* group. The morphological identification of this species was based on the description by Kim (1973) and

*To whom correspondence should be addressed
Tel: 82-41-950-0852, Fax: 82-41-950-0851
E-mail: lizard4755@mabik.re.kr

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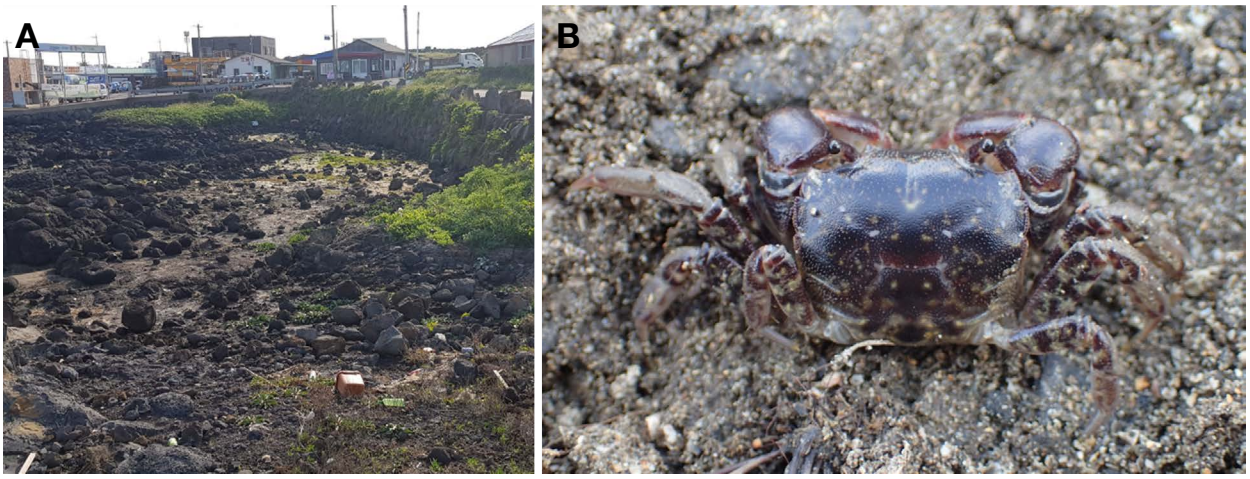

Fig. 1. *Pseudohelice subquadrata* habitat (A), upper view (B).

Table 1. Pairwise genetic distances based on 658 bp size of cytochrome *c* oxidase subunit I (*COI*) genes from *Pseudohelice subquadrata*, *Helice* and *Helicana* species

Species	Location	No.	Accession No.	1	2	3	4	5	6	7	8	9	Data source
P. subquadrata	Yeonpyeong-ri,	1	MN907827										Present study
	South Korea	2	MN907828	0.005									″
	Wimiri,	3	MN907829	0.002	0.003								″
	South Korea	4	MN907830	0.005	0.003	0.003							″
	Japan	5	AB334557	0.003	0.002	0.002	0.002						Shih and Suzuki (2008)
Helice tientsinensis	Ganghwa I., South Korea	6	AB334547	0.184	0.182	0.182	0.180	0.180					″
Helice tridens	Japan	7	AB334548	0.172	0.174	0.174	0.172	0.172	0.049				″
Helicana wuana	Ganghwa I., South Korea	8	AB334551	0.209	0.185	0.211	0.213	0.213	0.189	0.189			″
Helice epicure	Japan	9	LC375189	0.188	0.215	0.185	0.183	0.183	0.032	0.068	0.185		Ng et al. (2018)

Sakai et al. (2006). Genomic DNA was extracted from ambulatory leg muscle tissue using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). All genomic DNA samples were deposited at the National Marine Biodiversity Institute of Korea (MABIK) (Seocheon, Korea) (Voucher Nos. MABIK GR00002588–2591). A portion of the *COI* sequence was amplified by polymerase chain reaction (PCR) using the primers LCO1490 and HCO2198 (Folmer et al., 1994). The PCR assay was performed according to the method described by Sun et al. (2009); the conditions were as follows: an initial 5 min of pre-denaturation at 95°C; 35 cycles of 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 2 min; and a final extension for 5 min at 72°C. Amplified sequences were then aligned using Geneious Primer (Biomatters Ltd., Auckland, New Zealand).

The newly obtained *COI* sequences for *P. subquadrata* were registered in GenBank (GenBank accession Nos. MN907827–907830). We compared and analyzed *COI* gene information (AB334557) with *P. subquadrata* reported from Japan, registered in GenBank.

Pairwise genetic distances among sequences were calculated using the MEGA X program (Kumar et al., 2018) with the Kimura two-parameter model (Kimura, 1980). In order to demonstrate the utility of DNA barcode genes, *COI* sequences of *Helice tientsinensis* (Rathbun, 1931) (AB334547), *H. tridens* (De Haan, 1835) (AB334548), *H. epicure* (Ng et al., 2018) (LC375189), *Helicana wuana* (Rathbun, 1931) (AB 334551) were compared as an outgroup with those of *P. subquadrata* (Shih and Suzuki, 2008).

RESULTS AND DISCUSSION

We obtained four *COI* sequences of 658 bp size from four

P. subquadrata individuals, Intra-specific variation among Korean populations ranged from 0.2−0.5%, while intra-specific variation between the Korean and Japanese populations showed the same 0.2−0.5% (Table 1). In contrast, inter-genetic variation within the Varunidae family was in the range of 17.2−21.5%.

Ranges of 0.15−0.61%, 4.56−10.79%, 16.11−16.57%, respectively, were determined by Shih and Suzuki (2008) as intra-specific, inter-specific, and inter-genetic variations of *COI* in the family Varunidae (*Helice tientsinensis*, *H. tridens*, *Helicana wuana*). These findings were reflected in our results for intra-specific and inter-genetic variations among *P. subquadrata*, 2 *Helice* species, and 1 *Helicana* species.

These results confirmed that *COI* DNA barcodes are useful for the identification of *P. subquadrata*, as shown for many other crustaceans. In addition, our barcoding data can be used as reference data for the restoration and conservation studies on *P. subquadrata*, a marine protected species.

ORCID

Ji Min Kim: https://orcid.org/0000-0002-5065-1974
Jong-Gwan Kim: https://orcid.org/0000-0003-3881-554X
So Yeon Kim: https://orcid.org/0000-0003-0382-9191
Woo Yong Choi: https://orcid.org/0000-0001-6625-1169
Hyung Seop Kim: https://orcid.org/0000-0002-1365-587X
Min-Seop Kim: https://orcid.org/0000-0003-2735-5103

CONFLICTS OF INTEREST

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

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