

## DNA Barcoding of the Marine Protected Species *Chasmagnathus convexus* (Decapoda: Varunidae: *Chasmagnathus*) in Korea

Woo Yong Choi<sup>1</sup>, Chang Ho Yi<sup>1</sup>, Ji Min Kim<sup>1,2</sup>, So Yeon Kim<sup>3</sup>,  
Hyoung Seop Kim<sup>2</sup>, Min-Seop Kim<sup>1,\*</sup>

<sup>1</sup>National Marine Biodiversity Institute of Korea, Seocheon 33662, Korea

<sup>2</sup>School of Marine Biotechnology, College of Marine Science, Kunsan National University, Gunsan 54150, Korea

<sup>3</sup>Korea Fisheries Resources Agency, Gunsan 54021, Korea

### ABSTRACT

*Chasmagnathus convexus* (De Haan, 1835) is a monotypic species belonging to the family Varunidae. *Chasmagnathus convexus* has been designated as a marine protected species and endangered species by the Wildlife Protection and Management Act (2005) of Korea due to its declining population in the wild. This declining population is a result of habitat loss and environmental change. This study is the first to research the mitochondrial cytochrome *c* oxidase subunit I (COI) in Korean *C. convexus*. The maximum intra-specific genetic variation among all *C. convexus* individuals was 1.8%, while the inter-genetic variation among the five varunid species was in the range of 16.0–23.7%. The COI barcodes will be used as a reference for restoration and conservation studies of Korean *C. convexus*.

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

### INTRODUCTION

The common convex crab *Chasmagnathus convexus* (De Haan, 1835) is a monotypic species of the genus *Chasmagnathus* De Haan, 1833.

*Chasmagnathus convexus* distributed in East Asia, specifically Korea, Japan, Taiwan, and China (Shih and Suzuki, 2008). In Korea, this species mainly thrives in burrowed holes in mudflats, marshes, estuaries, and supratidal zones and tends to be more active at night (Kim, 1973) (Fig. 1).

Morphologically, *C. convexus* is characterized by broad teeth on the antero-lateral margin and eight tubercles of different sizes on the suborbital crest from congeners (Kim, 1973) (Fig. 2). While its individuals show two colored forms, purple vs. olive green.

This species was designated as marine protected species by the Conservation and Management of Marine Ecosystems Act in 2007, and endangered by the Wildlife Protection and Management Act in 2005. The reason is the organism's dwindling population in the wild in Korea, mainly attributable to

habitat loss and environmental changes.

DNA barcodes of mitochondrial cytochrome *c* oxidase subunit I (COI) are useful markers for differentiating among several taxonomic groups, including Decapoda (Meyran et al., 1997; Wares, 2001; Hajibaei et al., 2006; Clare et al., 2007; Zemplak et al., 2009; Lee et al., 2019; Song and Min, 2019; Kim et al., 2020). Recently, these markers have been found to be useful in identifying species belonging to the family Varunidae (Kim et al., 2020). In this study, we revealed the COI barcodes of Korean *C. convexus* and assessed their usefulness in providing basic data for restoration and conservation studies of this species.

We used eight individual crabs collected from four localities in Korea: Seocheon-gun (36°08'--"N, 126°34'--"E), Seogwiposi (33°14'--"N, 126°13'--"E), Hadong-gun (35°01'--"N, 127°47'--"E), and Namhae-gun (34°55'--"N, 127°51'--"E). DNA extraction, PCR, and sequencing were performed according to the methods described by Kim et al. (2020). All specimens were deposited at the National Marine Biodiversity Institute of Korea (MABIK) (Seocheon, Chungcheongnam-do,

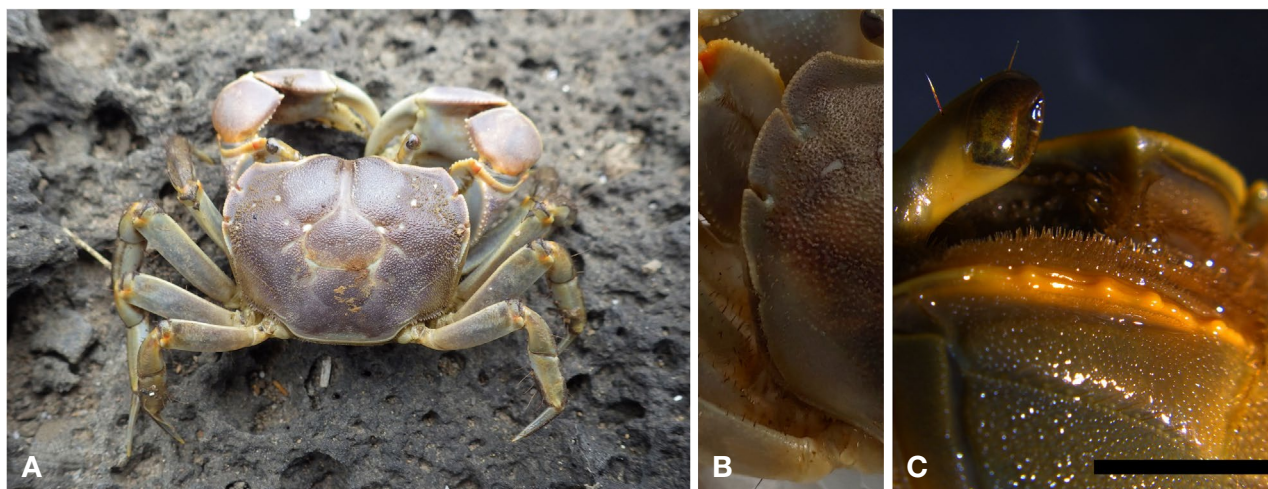
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**\*To whom correspondence should be addressed**

Tel: 82-41-950-0812, Fax: 82-41-950-0811  
E-mail: lizard4755@mabik.re.kr



**Fig. 1.** Photograph of Jeju Island where *Chasmagnathus convexus* is inhabited.



**Fig. 2.** Photographs of *Chasmagnathus convexus* in Jeju island in Korea. A, Top-view; B, Antero-lateral margin; C, Left suborbital crest. Scale bar=5 mm.

Korea) (voucher no. MABIK GR00002607–GR00002614, GR00004007). The newly obtained COI sequences of *C. convexus* were registered in the GenBank nucleotide database (accession no. MT947440–MT947447).

Sequences were aligned using Geneious Pro v.9.1.8 (Biomatters, Auckland, New Zealand). The genetic variations were calculated using MEGA v.6.06 (Tamura et al., 2013). We compared and analyzed the newly obtained COI sequences with that of *C. convexus* (AB334555, AB334556) samples reported in Taiwan and registered with GenBank.

To demonstrate the utility of the DNA barcode, we compared and analyzed the COI sequences of five varunid crabs:

*Helice tientsinensis* Rathbun, 1931 (AB334547), *H. tridens* (De Haan, 1835) (AB334548), *H. epicure* N. K. Ng, Naruse & Shih, 2018 (LC375189), *Helicana wuana* (Rathbun, 1931) (AB334551), and *Pseudohelice subquadrata* (Dana, 1851) (MN907829).

## RESULTS AND DISCUSSION

We obtained newly eight partial COI sequences of 605 bp size from the Korean population of *C. convexus*, showing no intra-specific genetic variations. The intra-specific genetic vari-

**Table 1.** Genetic distance (K2P) based on 605 bp size of COI sequence from *Chasmagnathus convexus* in Korea and Taiwan and within Varunidae family

Species	Location	No.	Accession No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
<i>C. convexus</i>	Seocheon-gun, South Korea	1	MT947440																
	Seogwipo-si, South Korea	2	MT947441	0.000															
	Hadong-gun, South Korea	3	MT947442	0.000	0.000														
	Hadong-gun, South Korea	4	MT947443	0.000	0.000	0.000													
	Namhae-gun, South Korea	5	MT947444	0.000	0.000	0.000	0.000												
	Namhae-gun, South Korea	6	MT947445	0.000	0.000	0.000	0.000	0.000											
	Namhae-gun, South Korea	7	MT947446	0.000	0.000	0.000	0.000	0.000	0.000										
	Taiwan	8	MT947447	0.000	0.000	0.000	0.000	0.000	0.000	0.000									
	Taiwan	9	AB334555	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015							
	Taiwan	10	AB334556	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.003						
<i>Helice tientsinensis</i>	Ganghwa Island, South Korea	11	AB334547	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.160	0.160						
	Japan	12	AB334548	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.052					
<i>H. epicure</i>	Japan	13	LC375189	0.180	0.180	0.180	0.180	0.180	0.180	0.180	0.180	0.173	0.171	0.034	0.069				
	Ganghwa Island, South Korea	14	AB334551	0.225	0.225	0.225	0.225	0.225	0.225	0.225	0.225	0.230	0.227	0.213	0.213	0.203			
<i>Pseudohelice subquadrata</i>	Wimi-ri South Korea	15	MN907829	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.235	0.237	0.200	0.184	0.239	0.200		

K2P, Kimura-2-parameter; COI, cytochrome c oxidase subunit I

ation between the Korean and Taiwanese populations of *C. convexus* showed 1.5–1.8%, while the inter-genetic variations ranged from 16.0% (*C. convexus* vs. *H. tientsinensis*) to 23.7% (*C. convexus* vs. *P. subquadrata*) within three varunid genera (Table 1).

There have been previous studies reporting comparison between COI gene sequences from members of the family Varunidae. Of them, Shih and Suzuki (2008) reported the intra-specific and intergenetic variations as 0.15–0.61% and 13.53–18.39%, respectively, among seven varunid crab species (two *Helice* species, three *Helicana* species, one *Chasmagnathus* species, and one *Pseudohelice* species). Additionally, Kim et al. (2020) reported that the intra-specific and inter-genetic variations were 0.2–0.5% and 17.2–21.5%, respectively, based on (three *Helice* species, one *Helicana* species, and one *Pseudohelice* species). Therefore, the intra-specific and inter-genetic variations within the family Varunidae can be estimated to be 0.15–0.61% and 13.53–21.5%, respectively.

In conclusion, the results of the inter-genetic variation of the family Varunidae in this study and the it of previous studies were similar. In this study, the intra-specific genetic variation of Korean *C. convexus* was within that range of the varunid crab. In contrast, the genetic variation that compared to the Taiwanese population was out of the range. however it was considerably different from the inter-genetic variation.

We reconfirmed the usefulness of COI DNA barcodes for *C. convexus* COI for identifying this species and related taxa.

## ORCID

Woo Yong Choi: <https://orcid.org/0000-0001-6625-1169>

Chang Ho Yi: <https://orcid.org/0000-0002-0934-012X>

Ji Min Kim: <https://orcid.org/0000-0002-5065-1974>

So Yeon Kim: <https://orcid.org/0000-0003-0382-9191>

Hyoung 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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