

DNA Barcoding of the Marine Protected Species *Parasesarma bidens* (Decapoda: Sesarmidea) from the Korean Waters

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ABSTRACT

Parasesarma bidens (De Haan, 1835) has been designated as a marine protected species by the Act on conservation and management of marine ecosystems. This crab has been recorded only from Jeju-do and Geomun-do, Republic of Korea. In this study, we describe for the first time the mitochondrial cytochrome *c* oxidase subunit I (*COI*) sequences of *P. bidens*. The intra-specific genetic distance among the Korean populations and between the Korean and Chinese populations ranged from 0% to 0.9% and 1.9% to 2.7%, respectively. The inter-specific genetic distances among the four *Parasesarma* species ranged from 10.9% to 12.8%. The finding of this study will be helpful to better describe *P. bidens* using *COI* DNA barcodes and can be used as basic data for their restoration and conservation research.

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

INTRODUCTION

The genus *Parasesarma* de Man, 1895, is currently represented by 68 species (Cannicci et al., 2017; Shahdadi and Schubart, 2017; Li et al., 2018; Shahdadi et al., 2018, 2019; Fratini et al., 2019), of which four species have been reported in South Korea: *P. bidens* (De Haan, 1835), *P. pictum* (De Haan, 1835), *P. plicatum* (Latreille, 1803), and *P. tripectin* (Shen, 1940).

Parasesarma bidens (De Haan, 1835) is a mudflat crab typically known to inhabit mangrove swamps (Lee, 1998) and prefers the upper intertidal region of estuaries (Fig. 1). This sesarmid crab is widely distributed in the Indo-West Pacific region, the Bay of Bengal to the Andamans, Malay Archipelago, Hong Kong, Formosa, and Japan (Sakai, 1976; Aiyun and Siliang, 1991). In Korea, it is found restrictively in Jeju-do and Geomun-do, which is the northern limit of its natural distribution. This crab has been designated as a marine protected species by the act on conservation and management of marine ecosystems in 2016 because of the threat to its survival and its high protective value. Recently, studies have been con-

ducted to find ways to increase its population.

The mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene has proven to be a particularly useful taxonomic marker in most animal phyla, including Crustaceans (Meyran et al., 1997; Wares, 2001; Hajibaei et al., 2006; Clare et al., 2007; Elsasser et al., 2009; Zemlak et al., 2009; Song and Min, 2019). In the present study, we first determined the *COI* DNA barcodes of *P. bidens* from Jeju-do, Republic of Korea, and then verified their usefulness for restoration and conservation. We used 15 individuals of *P. bidens*, which were obtained from five localities in Jeju-do, Republic of Korea: Wimi-ri (33°16'--"N, 126°39'--"E), Sehwa-ri (33°18'--"N, 126°48'--"E), Woljeong-ri (33°33'--"N, 126°46'--"E), Gimnyeong-ri (33°33'--"N, 126°44'--"E), Geumseong-ri (33°26'--"N, 126°17'--"E).

Each ambulatory leg was used in October 2017 for genomic DNA extraction. Genomic DNA was isolated from the leg muscle tissue using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). A portion of the *COI* gene was amplified by polymerase chain reaction using the primers LCO1490 and HCO2198 (Folmer et al., 1994) under the following condi-

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Fig. 1. The habitat of *Parasesarma bidens* from Jeju-do.

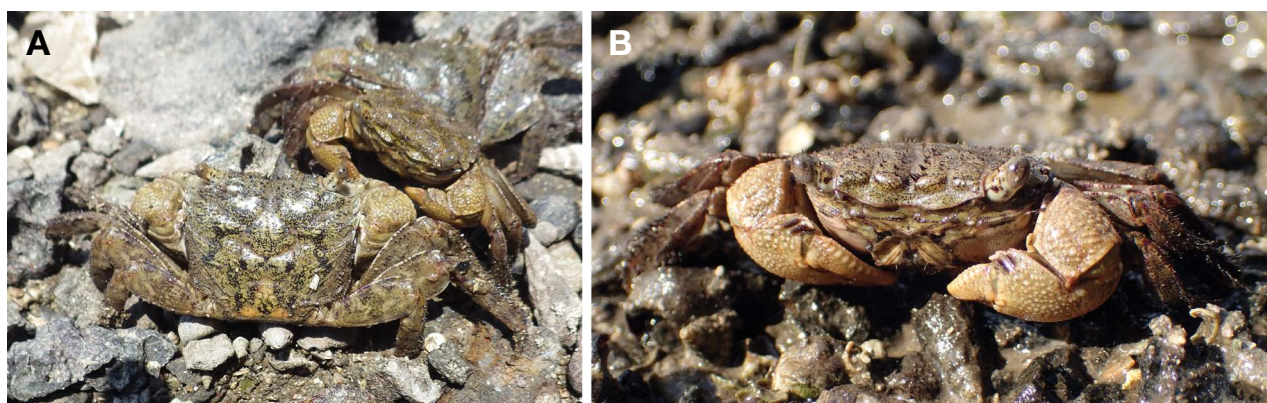


Fig. 2. Photographs of *Parasesarma bidens* from Jeju-do. A, Carapace in dorsal; B, Cheliped.

tions: initial denaturation for 5 min at 95°C, 35 cycles of 30 s at 95°C for denaturation, 30 s at 55°C for annealing, 2 min at 72°C for elongation, and 5 min at 72°C as a final elongation step. The sequences were aligned using Geneious 8.1.9 (Biomatters Ltd., Auckland, New Zealand). We compared and analyzed the *COI* gene information (KM605221–KM605223) with *P. bidens* reported from China, registered with GenBank. The *COI* sequences determined from this study and those retrieved from GenBank were aligned using MEGA v.6.06 (Tamura et al., 2013). To demonstrate the utility of DNA barcode genes, the *COI* sequences of *P. catenatum* (Ortmann, 1897) (MF564013), *P. erythrodictyla* (Hess, 1865) (JX502945), and *P. gazi* (Cannicci, Schubart, Innocenti, Dahdouh-Guebas, Shadadi & Fratini, 2017) (MF564007) were compared as an outgroup with those of *P. bidens*.

RESULTS AND DISCUSSION

Parasesarma bidens is characterized by the existence of

an epibranchial tooth and the structures of the chelipeds distinguish it from the other *Parasesarma* species (Fig. 2). Morphological identification of this species was performed based on the description by Kim (1973). All specimens were deposited at the National Marine Biodiversity Institute of Korea (MABIK) (Seocheon Chungchungnam-do, South Korea) (Voucher No. MABIK GR00002592–00002606).

We obtained 15 partial *COI* sequences of 662 bp from the five Korean populations of *P. bidens* (GenBank accession No. MT117059–73). Intra-specific genetic distances among the Korean populations ranged from 0% to 0.9%, and those between the Korean and Chinese populations ranged from 1.9% to 2.7% (Table 1). The inter-specific genetic distances among the four *Parasesarma* species ranged from 10.9% to 12.8%. We detected no overlap between the intra- and inter-specific genetic variations. A previous study on sesarmid crabs showed similar levels of sequence variation: Cannicci et al. (2017) revealed 6.6% to 10.4% *COI* gene variation among *P. catenatum*, *P. guttatum* (A Milne-Edwards, 1869), *P. leptosoma* (Hilgendorf, 1869), *P. melissa* (de Man, 1888),

Table 1. Pairwise genetic distances of *COI* DNA barcodes among *Parasesarma* species with collecting locations and NCBI accession number

Specific name	Location	NCBI No.	No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
<i>P. bidens</i>	Wimi-ri	MT117059	1																					
		MT117060	2	0																				
		MT117061	3	0	0																			
	Sehwa-ri	MT117062	4	0	0	0																		
		MT117063	5	0	0	0	0																	
		MT117064	6	0.6	0.6	0.6	0.6	0.6																
	Woljeong-ri	MT117065	7	0.8	0.8	0.8	0.8	0.8	0.2															
		MT117066	8	0	0	0	0	0	0.6	0.8														
		MT117067	9	0	0	0	0	0	0.6	0.8	0													
	Gimnyeong-ri	MT117068	10	0.2	0.2	0.2	0.2	0.2	0.8	0.9	0.2	0.2												
		MT117069	11	0	0	0	0	0	0.6	0.8	0	0	0.2											
		MT117070	12	0.6	0.6	0.6	0.6	0.6	0	0.2	0.6	0.6	0.8	0.6										
	Geumseong-ri	MT117071	13	0	0	0	0	0	0.6	0.8	0	0	0.2	0	0.6									
		MT117072	14	0.4	0.4	0.4	0.4	0.4	0.2	0.4	0.4	0.4	0.6	0.4	0.2	0.4								
		MT117073	15	0.6	0.6	0.6	0.6	0.6	0	0.2	0.6	0.6	0.8	0.6	0	0.6	0.2							
<i>P. catenatum</i>	China	KM605221	16	2.3	2.3	2.3	2.3	2.3	2.5	2.7	2.3	2.3	2.5	2.3	2.5	2.3	2.3	2.5						
		KM605222	17	2.3	2.3	2.3	2.3	2.3	2.5	2.7	2.3	2.3	2.5	2.3	2.5	2.3	2.3	2.5	0.8					
		KM605223	18	1.9	1.9	1.9	1.9	1.9	2.1	2.3	1.9	1.9	2.1	1.9	2.1	1.9	1.9	2.1	1.1	1.1				
<i>P. erythrodactyla</i>	South Africa	MF564013	19	12.8	12.8	12.8	12.8	12.8	12.2	12.4	12.8	12.8	12.6	12.8	12.2	12.8	12.4	12.2	12.8	12.8	12.6			
<i>P. gazi</i>	Korea	JX502945	20	11.5	11.5	11.5	11.5	11.5	10.9	11.1	11.5	11.5	11.8	11.5	10.9	11.5	11.1	10.9	11.3	11.3	10.9	12.6		
<i>P. gazi</i>	Kenya	MF564007	21	11.7	11.7	11.7	11.7	11.7	11.1	11.3	11.7	11.7	11.9	11.7	11.1	11.7	11.3	11.1	12.2	12.2	11.9	11.5	9.3	

and *P. samawati* (Gillikin & Schubart, 2004). In contrast, there was no genetic divergence at the intra-specific level. These results confirmed that *COI* DNA barcodes are useful for the identification of *P. bidens*, as shown for many other crustaceans. In conclusion, these barcoding data can provide basic information for the restoration and conservation of *P. bidens*, a marine protected species.

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CONFLICTS OF INTEREST

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

REFERENCES

- Aiyun D, Siliang Y, 1991. Crabs of the China seas. Ocean press, Beijing, pp. 1-682.
- Cannicci S, Schubart CD, Innocenti G, Dahdouh-Guebas F, Shahdadi A, Fratini S, 2017. A new species of the genus *Parasesarma* De Man 1895 from East African mangroves and evidence for mitochondrial introgression in sesarmid crabs. *Zoologischer Anzeiger*, 269:89-99. <https://doi.org/10.1016/j.jcz.2017.08.002>
- Clare EL, Lim BK, Engstrom MD, Eger JL, Hebert PDN, 2007. DNA barcoding of Neotropical bats: species identification and discovery within Guyana. *Molecular Ecology Notes*, 7:184-190. <https://doi.org/10.1111/j.1471-8286.2006.01657.x>
- Elsasser SC, Floyd R, Hebert PDN, Schulte-Hostedde AI, 2009. Species identification of North American guinea worms (Nematoda: Dracunculus) with DNA barcoding. *Molecular Ecology Resources*, 9:707-712. <https://doi.org/10.1111/j.1755-0998.2008.02393.x>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R, 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3:294-299.
- Fratini S, Cannicci S, Porri F, Innocenti G, 2019. Revision of the *Parasesarma guttatum* species complex reveals a new pseudocryptic species in south-east African mangroves. *Invertebrate Systematics*, 33:208-224. <https://doi.org/10.1071/IS18028>
- Hajibaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PDN, 2006. DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America*, 103:968-971. <https://doi.org/10.1073/pnas.0510466103>
- Kim HS, 1973. Illustrated encyclopedia of fauna and flora of Korea. Vol. 14. Anomura: Brachyura. Samwha Publishing Co., Seoul, pp. 1-649.
- Lee SY, 1998. Ecological role of grapsid crabs in mangrove ecosystems: a review. *Marine and Freshwater Research*, 49:335-343. <https://doi.org/10.1071/MF97179>
- Li JJ, Rahayu DL, Ng PKL, 2018. Identity of the tree-spider crab, *Parasesarma leptosoma* (Hilgendorf, 1869) (Decapoda: Brachyura: Sesarmidae), with descriptions of seven new species from the Western Pacific. *Zootaxa*, 4482:451-490. <https://doi.org/10.11646/zootaxa.4482.3.2>
- Meyran JC, Monnerot M, Taberlet P, 1997. Taxonomic status and phylogenetic relationships of some species of the genus *Gammarus* (Crustacea, Amphipoda) deduced from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 8:1-10.
- Sakai T, 1976. Crabs of Japan and adjacent seas. Kodansha Ltd., Tokyo, pp. 1-773.
- Shahdadi A, Davie PJF, Schubart CD, 2019. A new species of *Parasesarma* (Decapoda: Brachyura: Sesarmidae) from northern Australian mangroves and its distinction from morphologically similar species. *Zoologischer Anzeiger*, 279:116-125.
- Shahdadi A, Ng PKL, Schubart CD, 2018. Morphological and phylogenetic evidence for a new species of *Parasesarma* De Man, 1895 (Crustacea: Decapoda: Brachyura: Sesarmidae) from the Malay Peninsula, previously referred to as *Parasesarma indiarum* (Tweedie, 1940). *Raffles Bulletin of Zoology*, 66:739-762.
- Shahdadi A, Schubart CD, 2017. Taxonomic review of *Perisesarma* (Decapoda: Brachyura: Sesarmidae) and closely related genera based on morphology and molecular phylogenetics: new classification, two new genera and the questionable phylogenetic value of the epibranchial tooth. *Zoological Journal of the Linnean Society*, 182:517-548. <https://doi.org/10.1093/zoolinnean/zlx032>
- Song JH, Min GS, 2019. First genetic data of *Nebalia koreana* (Malacostraca, Leptostraca) with DNA barcode divergence among *Nebalia* species. *Animal Systematics, Evolution and Diversity*, 1:37-39. <https://doi.org/10.5635/ASED.2019.35.1.003>
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S, 2013. MEGA 6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30:2725-2729. <https://doi.org/10.1093/molbev/mst197>
- Wares JP, 2001. Patterns of speciation inferred from mitochondrial DNA in North American *Chthamalus* (Cirripedia: Balanomorpha: Chthamaloidea). *Molecular Phylogenetics*

ics and Evolution, 18:104-116. <https://doi.org/10.1006/mpev.2000.0857>

Zemlak TS, Ward RD, Connell AD, Holmes BH, Hebert PDN, 2009. DNA barcoding reveals overlooked marine fishes. *Molecular Ecology Resources*, 9:237-242. <https://doi.org/10.1111/j.1755-0998.2009.02649.x>

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