

Reassessment of the Taxonomic Status of Four *Pagurus* Species (Crustacea: Decapoda: Paguridae) in Korea Using DNA Barcoding

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ABSTRACT

Pagurus is the most diverse hermit crab genus in Korea. In this study, the cytochrome c oxidase subunit I (COI) and 16S rRNA of 24 individuals from four Korean *Pagurus* species (i.e., 7 *Pagurus brachiomastus*, 8 *P. proximus*, 8 *P. simulans*, and 1 *P. rectidactylus*) were sequenced and analyzed. No genetic difference was found between the COI and 16S rRNA sequences of *P. brachiomastus* and *P. simulans*, and the COI sequences of *P. rectidactylus* and *P. quinquelineatus* (comparative species from NCBI). Considering the morphological and ecological characteristics together, we assume that *P. simulans* and *P. rectidactylus* are subspecies of *P. brachiomastus* and *P. quinquelineatus*, respectively. This study should facilitate further research on the taxonomic status of these species.

Keywords: Paguridae, DNA barcoding, COI, 16S rRNA, taxonomic status, East Asia

INTRODUCTION

Pagurus is the most speciose hermit crab genus in Korea, consisting of 30 species (Kim, 1973; Jung and Kim, 2014, 2017; Jung et al., 2018b), and has a large and straight right cheliped, crista dentata with accessory tooth, gonopore without any special character, and twisted and developed abdomen (Kim and Kim, 2014). *Pagurus proximus* and *P. simulans* were separated from *P. brachiomastus* through the morphological reexamination of Komai (2000). In addition, *P. rectidactylus* was reported for the first time based on its morphological and ecological characteristics, such as elongated pereopods, 7 row of spine on the dorsal margin of right chela, and its association with the hydrozoan *Bouillonactinia misakiensis* on its shell (Komai et al., 2015). Recently, numerous *P. simulans* individuals were found to be associated with another hydrozoan, *Hydrissa sodalis* while *P. brachiomastus* and *P. proximus* were not (Jung and Kim, 2017). In this study, we tested the validity of the morphological and ecological characters as evidence of the taxonomic divergence of these four *Pagurus* species via DNA barcoding. Four *Pagurus* species were identified based on morphology, according to Jung et al. (2018a), and whole-body photographs were taken (Fig. 1)

from 11 collecting sites (Fig. 2). For DNA barcoding, the cytochrome c oxidase subunit I (COI) of four Korean *Pagurus* species were sequenced with the universal primers LCO1490 and HCO2198 (Folmer et al., 1994), and sequence variations were determined based on known congener sequences (Jung et al., 2018a). The mitochondrial 16S rRNA of these species were also sequenced, using the primers 16SH2 and 16SL2 (Schubart et al., 2000). All specimens were deposited into the Marine Arthropod Depository Bank of Korea (MADBK).

RESULTS

Twenty-four COI and 16S rRNA sequences of the four Korean *Pagurus* species were registered in GenBank (7 *P. brachiomastus* COI MN549986–MN549992; 16S rRNA MN545761–MN545767, 8 *P. proximus* COI MN549993–MN550000; 16S rRNA MN545768–MN545775, 8 *P. simulans* COI MN550001–MN550008; 16S rRNA MN545776–MN545783, and 1 *P. rectidactylus* COI MN550009; 16S rRNA MN545784). The COI alignment was 564 bp long, and possessed 111 variable sites (19.68%) and 90 parsimony informative sites (15.96%). The 16S rRNA alignment was 498



Fig. 1. Korean *Pagurus* species analyzed in this study. A, *Pagurus brachiomastus*, MADBK160704_019; B, *Pagurus proximus*, MADBK160718_026; C, *Pagurus simulans*, MADBK160719_017; D, *Pagurus rectidactylus*, MADBK160748_001.

bp long, and possessed 32 variable sites (6.43%) and 20 parsimony informative sites (4.02%).

Intraspecific variation among the four Korean *Pagurus* species was low (0–2.66%). The highest intraspecific variation was shown among *P. brachiomastus* individuals. This value was smaller than the minimum interspecific variation of Paguridae (4.79%), which is between *P. lanuginosus* (MG214646) and *P. maculosus* (MG214645) (Jung et al., 2018b).

The interspecific variations from our study are shown in Table 1. Although almost sequences of this study were separated from each other, there were no significant differences between *P. brachiomastus* and *P. simulans* (COI: 1.49, 16S rRNA: 0.22) and the COI sequences of *P. rectidactylus* and *P. quinquelineatus*. These results conflict with the taxonomic status of these species based on the morphological characters of these species (Komai, 2000; Komai et al., 2015; Jung and

Table 1. Sequence pairwise distances (%) of COI and 16S rRNA genes among *Pagurus* species

Species	COI distances (%)						
	Within species	Between species					
		1	2	3	4	5	6
1. <i>Pagurus brachiomastus</i>	0.89–2.66 (1.53)						
2. <i>Pagurus proximus</i>	0–0.71 (0.39)	9.75–11.35 (10.44)					
3. <i>Pagurus simulans</i>	0.53–2.48 (1.37)	0.35–2.48 (1.49)	9.93–10.82 (10.34)				
4. <i>Pagurus rectidactylus</i>	–	9.57–10.82 (10.24)	6.21–6.56 (6.28)	9.75–10.11 (9.82)			
5. <i>Pagurus quinquelineatus</i> (MG214648)	–	9.57–10.82 (10.24)	6.21–6.56 (6.28)	9.75–10.11 (9.82)	0		
6. <i>Pagurus lanuginosus</i> (MG214646)	–	9.40–11.35 (10.19)	8.33–8.87 (8.51)	9.40–10.64 (9.97)	7.98	7.98	
7. <i>Pagurus maculosus</i> (MG214645)	–	10.46–12.06 (10.97)	8.51–8.87 (8.65)	10.46–11.17 (10.79)	7.98	7.98	4.79
16S rRNA distances (%)							
Species	Within species	Between species					
		1	2	3	4		
1. <i>Pagurus brachiomastus</i>	0–0.20 (0.10)						
2. <i>Pagurus proximus</i>	0–0.60 (0.23)	3.61–4.22 (3.90)					
3. <i>Pagurus simulans</i>	0–0.60 (0.30)	0–0.60 (0.22)	3.61–4.41 (3.97)				
4. <i>Pagurus rectidactylus</i>	–	3.82–4.02 (3.90)	2.61–3.21 (2.87)	3.82–4.22 (3.97)			
5. <i>Pagurus maculosus</i> (LC464569)	–	5.12	4.49–5.13 (4.57)	5.13–5.77 (5.21)	5.13		

COI, cytochrome c oxidase subunit I.

Kim, 2017).

Some previous studies have shown conflicts between morphological and molecular identification results (Schubart et al., 2001a, 2001b; Pérez-Barros et al., 2008; Padula et al., 2016). Some suggested recent speciation or continuous gene flow (Schubart et al., 2001a), and others mentioned insufficient morphological identification in the past (Padula et al., 2016). *Pagurus simulans* and *P. rectidactylus* have some morphological characters which is differ to their original species, *P. brachiomastus* and *P. quinquelineatus*, respectively, such as more elongated pereopods and elongated triangle-shaped light chela with seven rows of dorsal spines. In addition, the coloration of *P. simulans* and *P. rectidactylus* is also differ to their original species (Komai, 2000; Komai et al., 2015). However, they are only minor differences so it maybe not enough to separate them as a single species. The relationship

with associated hydrozoa and geographical distribution of *P. simulans* and *P. rectidactylus* are distinguished characteristics to its original species (Fig. 2) (Komai, 2000; Komai et al., 2015; Jung and Kim, 2017). However, no genetic gap between these two species and original species means that these species were geologically separate a recent. Therefore, we suggest that *P. simulans* and *P. rectidactylus* may be subspecies of *P. brachiomastus* and *P. quinquelineatus*, respectively. Further research is needed to rearrange the taxonomic status of these four *Pagurus* species.

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Fig. 2. Collecting sites of five *Pagurus* species specimen in this study.

CONFLICTS OF INTEREST

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

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