

DNA Barcoding of Six Diogenid Species (Crustacea: Decapoda: Paguroidea) in Korea

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ABSTRACT

In this study, cytochrome c oxidase subunit I (*COI*) sequences of 17 individuals from six Korean diogenid species (i.e., 2 *Areopaguristes japonicus*, 4 *A. nigroapiculus*, 3 *Paguristes digitalis*, 4 *P. ortmanni*, 3 *Diogenes edwardsii*, and 1 *Ciliopagurus kempfi*) were determined and analyzed. The DNA barcoding results of this study were consistent with the morphological identification of these six species. Interspecific variations of *COI* sequences within six Korean diogenid species exceeded the minimum interspecific variation of diogenid hermit crabs in previous studies. Little intraspecific variation exists except for *P. digitalis*. This study should facilitate further molecular taxonomy of East Asian diogenids.

Keywords: Diogenidae, DNA barcoding, *COI*, interspecific variation, Korea

INTRODUCTION

Hermit crabs belonging to the family Diogenidae possess a left chela that is similar to or larger than the right one (Kim, 1973), and 20 species have been identified in Korea (Jung and Kim, 2015; Jung et al., 2018b). Among them, *Areopaguristes* and *Paguristes* are distinguished from the other diogenid genera based on the presence of the first pleopods (Kim and Kim, 2014). The identification of these two diogenid genera is important for revealing the marine biodiversity of Korea because they are abundant in intertidal and subtidal environments. However, it is difficult to differentiate between *Areopaguristes* and *Paguristes* because they have similar morphological characteristics except for the number of gills (Komai, 2009; Rahayu and McLaughlin, 2010), which are not visible externally. In addition, because of their similar morphological characteristics, it is difficult to differentiate between *A. japonicus* and *A. nigroapiculus* as well as between *P. digitalis* and *P. ortmanni* (Komai, 2001, 2009), all of which are common species in Korea. Furthermore, their molecular identification is difficult because of the lack of DNA sequences in public databases such as GenBank. In this study, morphological characterization of diogenids was performed according to Jung et al. (2018a), with some modifications and the photographs taken their whole bodies

were presented (Fig. 1). For DNA barcoding approach, the cytochrome c oxidase subunit I (*COI*) sequence of six Korean diogenid species were determined with universal primers LCO1490 and HCO2198 (Folmer et al., 1994) and sequences variations were conducted with known congener sequences (Negri et al., 2014). All specimens are deposited at Laboratory of Systematic and Molecular Evolution (EVOSYS) and Marine Arthropod Depository Bank of Korea (MADBK).

RESULTS

The newly obtained *COI* sequences for the Korean diogenids, which are the length of 558–570 bp, were registered to GenBank (2 *Areopaguristes japonicus* MN114240, MN114241, 4 *A. nigroapiculus* MN114242–MN114245, 3 *Paguristes digitalis* MN114246–MN114248, 4 *P. ortmanni* MN114249–MN114252, 3 *Diogenes edwardsii* MN114253–MN114255, and 1 *Ciliopagurus kempfi* MN114256). The alignment was the length of 558 bp, and possessed 172 variable sites (30.82%) and 157 parsimony informative sites (28.14%).

The intraspecific variation of the six Korean diogenid species showed lower variability (0–2.69%). The highest intraspecific variation was shown within the individuals of *P. digitalis*. This value is smaller than the minimum interspecific

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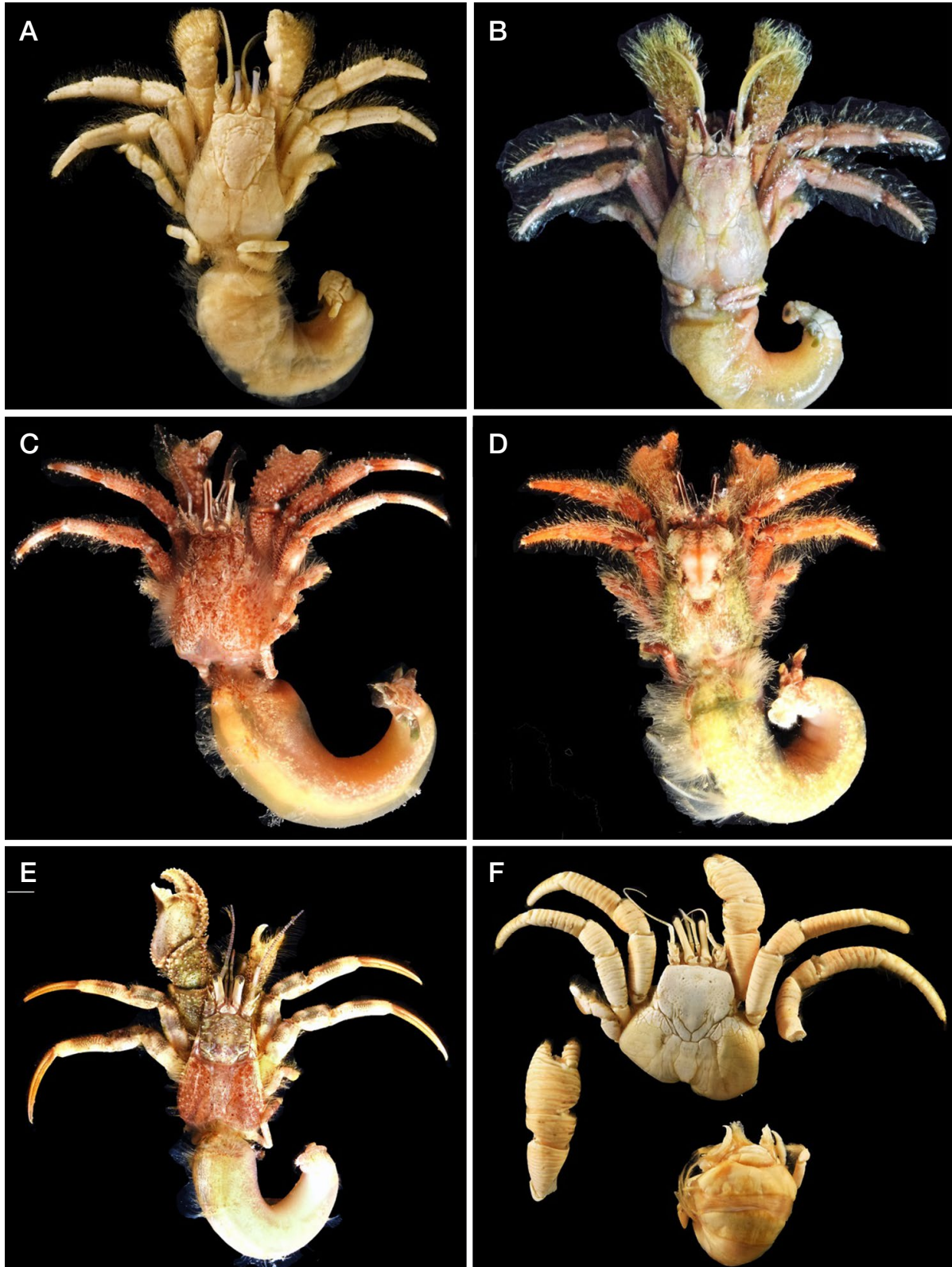


Fig. 1. Korean Diogenidae analyzed by this study. A, *Areopaguristes japonicus*, MADBK160510_001; B, *Areopaguristes nigroapiculus*, MADBK160529_007; C, *Paguristes digitalis*, MADBK160514_001; D, *Paguristes ortmanni*, MADBK160513_041; E, *Diogenes edwardsii*, MADBK160507_008; F, *Ciliopagurus kempfi*, EVOSYS260516_338.

Table 1. Cytochrome c oxidase subunit I (COI) sequences pairwise distances for species of diogenids in this study using pairwise distance (%)

Species	Distances (%)						
	Within species			Between species			
	1	2	3	4	5	6	7
1. <i>Areopaguristes japonicus</i>	1.43						
2. <i>Areopaguristes nigroapicululus</i>	0.54–1.79 (1.16)	12.19–12.90 (12.54)					
3. <i>Paguristes digitalis</i>	2.33–2.69 (2.51)	15.23–16.67 (15.85)					
4. <i>Paguristes ortmanni</i>	0–1.79 (1.02)	14.87–15.77 (15.37)	9.68–11.11 (10.45)				
5. <i>Diogenes edwardsii</i>	0.54–0.72 (0.60)	16.31–17.56 (16.87)	16.30–17.39 (16.73)	18.28–19.00 (18.61)			
6. <i>Ciliopagurus kempfi</i>	0	17.92–18.64 (18.28)	16.49–17.20 (16.79)	17.56–17.74 (17.70)	17.56–17.92 (17.74)		
7. <i>Clibanarius symmetricus</i> ^a	0	21.32–21.86 (21.55)	20.61–21.15 (20.97)	21.33–21.51 (21.42)	19.00–19.35 (19.18)	21.86	
8. <i>Clibanarius vittatus</i> ^a	0	20.07–20.97 (20.56)	19.00–19.35 (19.18)	19.89–20.25 (20.03)	18.82–19.18 (19.00)	20.61	5.56

The parentheses in the value cell represents the mean value of each distance.

^aNCBI sequence of Negri et al. (2014).

variation of Diogenidae (5.56%), which is shown to be between *Clibanarius symmetricus* (JN671551) and *C. vittatus* (JN671534) (Negri et al., 2014) (Table 1).

The interspecific variations in our study are as follow: 12.19–12.90% between *Areopaguristes japonicus* and *A. nigroapicululus*; 9.68–11.11% between *P. digitalis* and *P. ortmanni*, and it is smaller than the minimum value of Negri et al. (2014). The morphological identification result of six diogenid specimens in our study is also consistent with the previous descriptions (Kim, 1973; Oh, 1993; Komai, 2001, 2009). Therefore, our study reaffirms that *COI* could be an efficient marker for DNA barcoding of Korean diogenids.

The barcoding gap of Korean diogenids in our study is slightly narrow. In this study, the minimum interspecific variation is only 3.6 times the maximum intraspecific variation. However, *COI* is used as a common molecular marker in diogenid species (Hirose et al., 2010; Malay et al., 2012; Negri et al., 2014) and lots of data is available in public databases, such as GenBank. From this perspective, *COI* is the best DNA barcoding marker for diogenid species.

The intraspecific variation of *P. digitalis* was slightly larger than that of other species even though the *P. digitalis* specimens were collected from the same locality and at the same time, suggesting the presence of cryptic species in *P. digitalis*, which are difficult to morphologically identify and is considered as a case of demonstrating the value of DNA barcoding as a tool to conduct a more accurate and clear phylogeny study. Further study is needed to examine the genetic variation of *P. digitalis*.

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CONFLICTS OF INTEREST

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

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