

DNA Barcoding of *Koreanohadra kurodana* (Gastropoda: Camaenidae)

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ABSTRACT

The land snail, *Koreanohadra kurodana* (Pilsbry, 1926), is endemic to Korea and was collected from Pocheon, Pyeongchang, and Taebaek in South Korea. This study describes the mitochondrial cytochrome *c* oxidase subunit I gene (*COI*) and 16S ribosomal RNA (16S) sequences of *K. kurodana*, followed by an analysis of the genetic distance between the genus *Koreanohadra* and its congeners. As a result, intra-species variation was 0.2–3.7% in *COI* and 0.3–4.0% in 16S. In the gene sequences of *K. koreana* recently reported by Japanese researchers, both *COI* and 16S sequences were observed to be located within the intra-species variation of *K. kurodana* (1.5–3.5% in *COI*; 0.6–4.0% in 16S). The inter-generic variation between genera *Karaftohelix* and *Koreanohadra*, closely related, was 11.8–26% and 6.2–18.7% in *COI* and 16S, respectively.

Keywords: Camaenidae, *COI*, Korean Peninsula, land snail, 16S

INTRODUCTION

In the order Stylommatophora, 119 species were recorded in South Korea (National Institute of Biological Resources, 2022). Among them, two species, *Koreanohadra kurodana* (Pilsbry, 1926) and *K. koreana* (Pfeiffer, 1850), belong to the genus *Koreanohadra* Kuroda and Habe, 1949 (see Lee, 2013). These two species are endemic to Korea, and the genus *Koreanohadra* is composed of these two species (National Institute of Biological Resources, 2022). The genus *Koreanohadra* is mainly characterized by the following combination of features: medium shell size (diameter of approximately 20 mm); glossy and thin; yellow or light brown with reddish brown color band; and body whorl rounded with narrow umbilicus (Lee, 2013).

Currently, in MolluscaBase and NCBI GenBank, the genus *Koreanohadra* is classified under the genus *Fruticicola* held, 1838. Additionally, in Kimura et al. (2022), *K. kurodana* and *K. koreana* were reclassified under the genus *Karaftohelix* Pilsbry, 1927 and analyzed accordingly. However, the genetic differences between the species belonging to the genus *Fruticicola* and *K. kurodana* are approximately 28% in *COI* (Table 1),

making it difficult to classify them within the same genus. Moreover, since the *K. kurodana* has not yet been registered in the MolluscaBase, it was retained under the genus *Koreanohadra* for the research (MolluscaBase: <https://www.mollusca-base.org/>).

Koreanohadra kurodana is a species endemic to Korea, widely distributed in the central region of South Korea. However, only the Chuncheon population's nucleotide sequence of *K. kurodana* has been reported by Japanese researchers, and no comparative analysis with other populations has been conducted. Therefore, through this study, we aimed to report the nucleotide sequence of a new population of *K. kurodana* and to establish a foundational dataset for genetic diversity research by assessing intra-specific variation. And we performed DNA barcoding using DNA sequences of *K. kurodana* collected from three Korean sites and provided photographs and a brief diagnosis.

Specimens were collected from, *Koreanohadra kurodana*, in Pocheon, Pyeongchang, and Taebaek, South Korea. Morphological verification was performed as described earlier (Lee, 2013). The sequences were aligned and edited using BioEdit (version 7.2.5) (Hall, 1999). Genetic divergence was calculated

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by *p*-distance and neighbor joining (NJ) tree using MEGA 11 (version 11.0.13) (Tamura et al., 2021) with the cytochrome *c* oxidase subunit I (*COI*) and 16S ribosomal RNA (16S) gene sequences of Korean snails, and those of other *Koreanohadra* and *Karaftohelix* species retrieved from GenBank (Table 1). Classification and terminology were based on the methodologies described by Lee (2013).

RESULTS AND DISCUSSION

Phylum Mollusca Linnaeus, 1758
 Class Gastropoda Cuvier, 1795
 Order Stylommatophora A. Schmidt, 1855
 Family Camaenidae Pilsbry, 1895
 Genus *Koreanohadra* Kuroda and Habe, 1949

¹**Koreanohadra kurodana* (Pilsbry, 1926) (Fig. 1)

Eulota (*Euhadra*) *kurodana* Pilsbry, 1926: 455, pl. 33, figs. 3, 4.

Euhadra kurodana Shiba, 1934: 31.

Bradybaena kurodana Kuroda, 1936: 173.

Fruticiola (*Koreanohadra*) *kurodana* Lee, 1956: fig. 33; Kang, 1971: 69; Yamashita and Haba, 1965: 59.

Koreanohadra kurodana Yoo, 1976: 93, pl. 19, figs. 9, 10; Kwon and Habe, 1979: 30; Je, 1989: 34; Kwon, 1990: 365; Kwon et al., 1993: 37, 189, 190, fig. 74; Higo and Goto, 1993: 515; Lee and Min, 2002: 150; Min et al., 2004: 365, figs. 1151-1, 1151-2, 1151-3.

Material examined. Korea: Gyeonggi-do: Pocheon, 37°45' 23"N, 127°10'0"E, Sep 2023; Gangwon-do, Pyeongchang, 37°39'22"N, 128°36'24"E, Oct 2019; Gangwon-do: Taebaek, 37°6'9"N, 129°3'37"E, Jul 2020. The shell specimens have

been deposited at the Endangered Species Research Center of National Institute of Ecology (NIE).

Description. Shell height about 15.9 mm, diameter about 18.3 mm; 5.5 whorls; depressed rather low conical spire and solid though not thick; irregular striae of growth; aperture oblique and broadly lunate shape, width (including peristome) about 10.9 mm; brownish peristome margin, thin, expanded and towards the columellar insertion, partly covering the umbilicus; umbilicus diameter about 1.2 mm; shell yellowish to brown color, gloss; brown band above the periphery.

Remarks on the DNA barcodes of *K. kurodana*. The genus *Koreanohadra* includes only two species, and we were unable to obtain the nucleotide sequence for the valid *K. koreana* from NCBI GenBank. Therefore, instead of analyzing inter-specific variation, inter-generic variation was analyzed by comparing it with species belonging to the closely related genera *Bradybaena*, *Fruticicola*, and *Karaftohelix*.

New *COI* and 16S gene sequences obtained from the four samples of *K. kurodana* were deposited in GenBank (Tables 1, 2). The alignment length was 461 bp (36.9–40.8% of GC content and 157 polymorphic sites) in *COI*, and 341 bp (31.7–37.2% of GC content and 95 polymorphic sites) in 16S. The variation within species was a clear value of 0.4–3.7% in *COI*, and 0–4.0% in 16S, and the variation between species shows a clear value of 11.6–26.3% in *COI*, and 5.2–18.7% in 16S (Tables 1, 2). In both gene regions, *K. kurodana* exhibited an intra-specific variation rate of less than 4%. And comparing the two genetic regions, it was observed that the *COI* has a higher resolution for inter-species variation rates than 16S.

The genus *Koreanohadra* comprises of two species; the *COI* and 16S gene sequences of *K. koreana* were reported in an earlier study (Kimura et al., 2022). However, in this study, *K. koreana* (LC705123, LC705124) showed a variation of 1.5–3.5% in *COI* and 0.6–3.7% in 16S compared to the *K.*

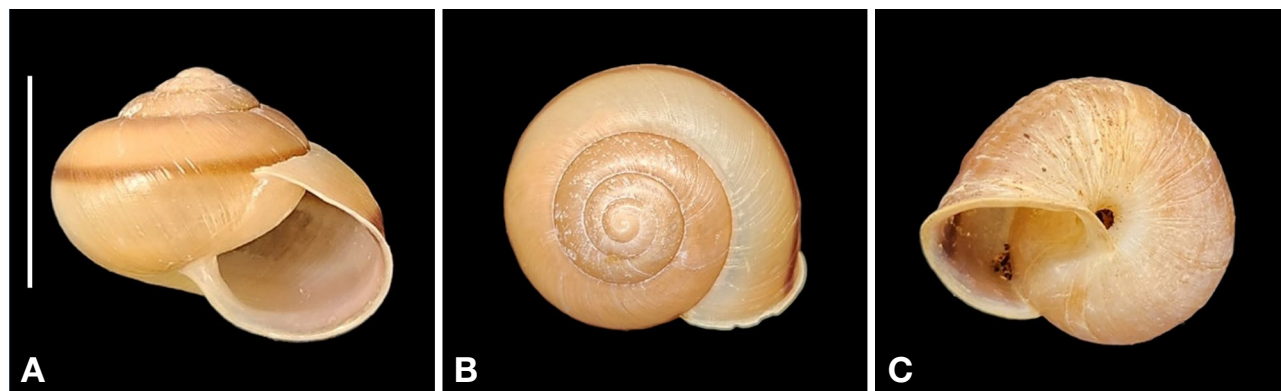


Fig. 1. *Koreanohadra kurodana*. A, Frontal view; B, Dorsal view; C, Ventral view of the shell. Scale bar = 10 mm.

Korean name: ¹*북한산달팽이

Table 1. Genetic distances (*p*-distance) of *COI* gene sequence (461 bp) between *Koreanohadra* species and relatives (%)

No.	Species name	Site	GenBank No.	Reference	1	2	3	4	5	6	7	8	9	10	11
1	<i>K. kurodana</i>	Pocheon	PQ461916	This study											
2	<i>K. kurodana</i>	Pyeongchang	PQ461917	This study	2.4										
3	<i>K. kurodana</i>	Pyeongchang	PQ461918	This study	2.4	0.0									
4	<i>K. kurodana</i>	Taebaek	PQ461919	This study	3.8	1.8	1.8								
5	<i>K. kurodana</i>	Chuncheon	LC705121	Kimura et al. (2022)	2.4	2.2	2.2	3.6							
6	<i>K. kurodana</i>	Chuncheon	LC705122	Kimura et al. (2022)	2.4	2.2	2.2	3.6	0.2						
7	<i>K. koreana</i>	Incheon	LC705123	Kimura et al. (2022)	3.3	1.3	1.3	2.2	3.1	3.1					
8	<i>K. koreana</i>	Incheon	LC705124	Kimura et al. (2022)	3.6	1.5	1.5	2.9	3.3	3.3	0.7				
9	<i>Bradybaena</i> sp.	China	PP723291	Not published	12.8	12.6	12.6	13.1	12.9	12.6	12.8	12.6			
10	<i>Karatohelix twenhuaensis</i>	Russia	LC705114	Kimura et al. (2022)	13.1	13.4	13.4	13.9	13.9	13.9	13.7	12.9	1.1		
11	<i>Karatohelix adamsi</i>	Ulleung	LC705110	Kimura et al. (2022)	24.2	25.5	25.5	26.4	25.3	25.6	25.5	25.5	23.6	23.3	
12	<i>Fruticola fruticum</i>	Russia	KX270742	Hofman et al. (2022)	27.9	28.3	28.3	29.3	27.6	28.0	28.3	28.3	28.3	28.3	26.8

Table 2. Genetic distances (*p*-distance) of 16S gene sequence (347 bp) between *Koreanohadra* species and relatives (%)

No.	Species name	Site	GenBank No.	Reference	1	2	3	4	5	6	7	8	9	10	11
1	<i>K. kurodana</i>	Pocheon	PQ472716	This study											
2	<i>K. kurodana</i>	Pyeongchang	PQ472717	This study	4.0										
3	<i>K. kurodana</i>	Pyeongchang	PQ472718	This study	4.0	0.0									
4	<i>K. kurodana</i>	Taebaek	PQ472719	This study	4.0	1.2	1.2								
5	<i>K. kurodana</i>	Chuncheon	LC705136	Kimura et al. (2022)	1.8	2.7	2.7	2.7							
6	<i>K. kurodana</i>	Chuncheon	LC705137	Kimura et al. (2022)	2.1	2.7	2.7	3.0	0.3						
7	<i>K. koreana</i>	Incheon	LC705138	Kimura et al. (2022)	3.7	0.6	0.6	0.6	2.4	2.1	0.0				
8	<i>K. koreana</i>	Incheon	LC705139	Kimura et al. (2022)	3.7	0.6	0.6	0.9	2.4	2.1	0.0				
9	<i>Bradybaena</i> sp.	China	PP668905	Not published	5.8	5.8	5.8	6.5	5.2	5.5	6.1	6.1			
10	<i>Karatohelix twenhuaensis</i>	Russia	LC705128	Kimura et al. (2022)	5.5	5.5	5.5	6.2	4.9	5.2	5.8	5.8	0.3		
11	<i>Karatohelix adamsi</i>	Ulleung	LC705125	Kimura et al. (2022)	18.7	17.2	17.2	17.9	17.9	17.9	17.9	17.9	18.3	18.7	
12	<i>Fruticola fruticum</i>	Russia	KX270731	Hofman et al. (2022)	21.4	20.6	20.6	21.8	21.4	21.4	21.4	21.4	21.4	21.8	23.1

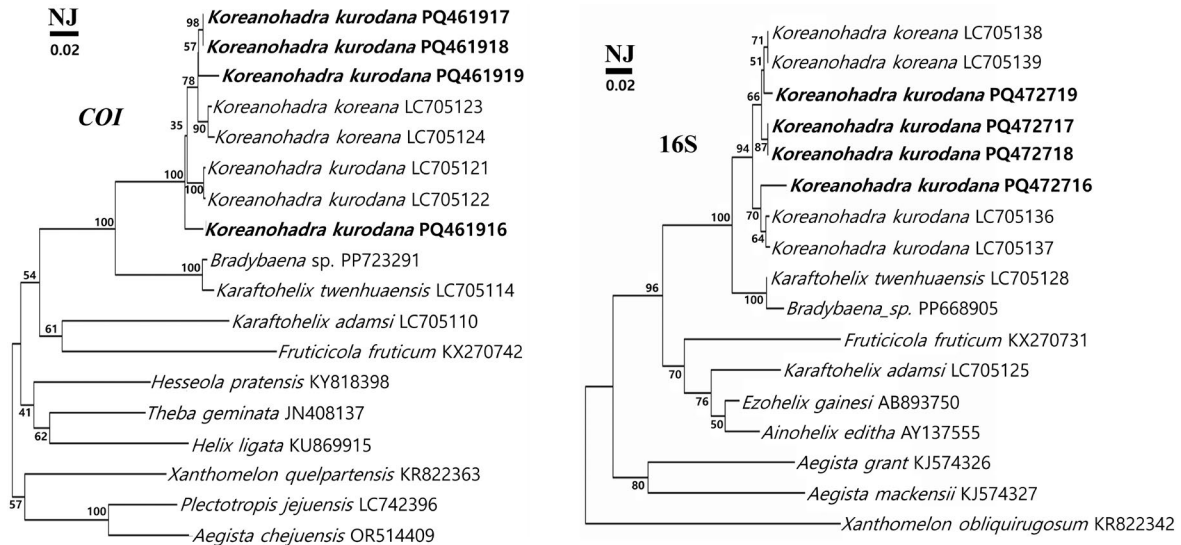


Fig. 2. Neighbor joining (NJ) analyses based on *COI* and *16S* gene sequences. The new sequence provided in the present work is in bold. Numbers at nodes indicate the bootstrap values of NJ out of 1,000 replicates. The scale bar corresponds to one substitution per 10 nucleotide positions.

kurodana, which are included in the intra-specific variation of the *K. kurodana* (*COI*, 0.4–3.7%; *16S*, 0–4.0%) (Tables 1, 2, Fig. 2). In particular, it was confirmed that *K. koreana* (LC705123, LC705124) has a very close genetic relationship with Pyeongchang population of *K. kurodana* (*COI*, 1.5–1.9%; *16S*, 0.6–0.9%) (Tables 1, 2, Fig. 2). And *K. koreana* was confirmed to exist only on two islands, Hongdo and Hateado, in Sinan-gun, Jeollanam-do. Therefore, it was presumed that *K. koreana* (LC705123, LC705124) collected from Uldo, Incheon were misidentified.

Morphological verification is required, however, the morphological description of *K. koreana* in Kimura et al. (2022) was missing, and a morphological comparison with *K. kurodana* in this study could not be performed. The genus *Acusta*, another terrestrial snail, has few shell characteristics and low variation, making morphological comparisons difficult. Species are classified based on their DNA sequences and geographical differences (Hwang et al., 2021; Kim et al., 2023). *Koreanohadra koreana* and *K. kurodana* also have very high morphological similarities; thus, for further studies, morphological data with a large number of samples of these two species, as well as sequence and geographic data, are needed.

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CONFLICTS OF INTEREST

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

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