

# DNA Barcoding of *Aegista chejuensis* and *Plectotropis quelpartensis* (Gastropoda: Stylommatophora: Camaenidae)

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## ABSTRACT

Two land snails, *Aegista chejuensis* (Pilsbry and Hirase, 1908) and *Plectotropis quelpartensis* (Pilsbry and Hirase, 1908), are endemic to Korea and were collected from Hataedo and Jodo Islands in the Yellow Sea of South Korea, respectively. Many terrestrial snail habitats have been confirmed in Korea; however, their genetic sequences have rarely been reported. This study describes the mitochondrial cytochrome *c* oxidase subunit I gene (*COI*) sequences of two species, followed by an analysis of the genetic distance between these two species and their congeners. As a result, there was no intra-species variation in both species *A. chejuensis* or *P. quelpartensis*. However, the inter-species variation was clear (10.3–31.5%). We provide photographs and a brief diagnosis for morphological verification.

**Keywords:** *Aegista*, Camaenidae, *COI*, Korean Peninsula, land snail

## INTRODUCTION

Approximately 77 snail species exist (including subspecies) in the order Stylommatophora recorded in South Korea. Among them, 15 species belong to the genus *Aegista* Albers, 1850 (National Institute of Biological Resources, 2022). The genus *Aegista* is mainly characterized by the following combination of features: small to medium shell size (diameter of approximately 7.5–20 mm); low conical form; whorl rounded or angular periphery; and a wide and deep umbilicus (Lee, 2013). Two subgenera of the genus *Aegista* were recorded in Korea: *Aegista (Aegista)* Albers, 1850, and *Aegista (Plectotropis)* E. von Martens, 1860. However, according to a recent revision of the classification of terrestrial gastropods, these subgenera are now elevated to the generic level, becoming *Aegista* Albers, 1850, and *Plectotropis* E. von Martens, 1860, respectively (Bank, 2017).

Land snails are ideal animals for evolutionary, biogeographical, molecular phylogenetic, and ecological studies be-

cause of their low mobility and limited habitat (Pfenninger et al., 1996; Schweiger et al., 2004). Basic information, such as shell shape, DNA sequence, behavior, and ecological characteristics, is required for these types of studies, and prior research has been conducted to obtain this information (Hirano et al., 2015; Hwang et al., 2021; Kimura et al., 2022). Several snail species have been documented in South Korea by both domestic and international researchers. However, their genetic information, especially their DNA sequences, remains largely unknown.

In this study, we collected two specimens, *Aegista chejuensis* (Pilsbry and Hirase, 1908) and *Plectotropis quelpartensis* (Pilsbry and Hirase, 1908), from the Hataedo and Sangjodo Islands in South Korea, respectively. Morphological verification was performed as previously described (Lee, 2013). Sequences were aligned and edited using BioEdit (version 7.2.5) (Hall, 1999). Genetic divergence was calculated by *p*-distance using MEGA 11 (version 11.0.13) (Tamura et al., 2021) with the cytochrome *c* oxidase subunit I gene (*COI*) sequences of

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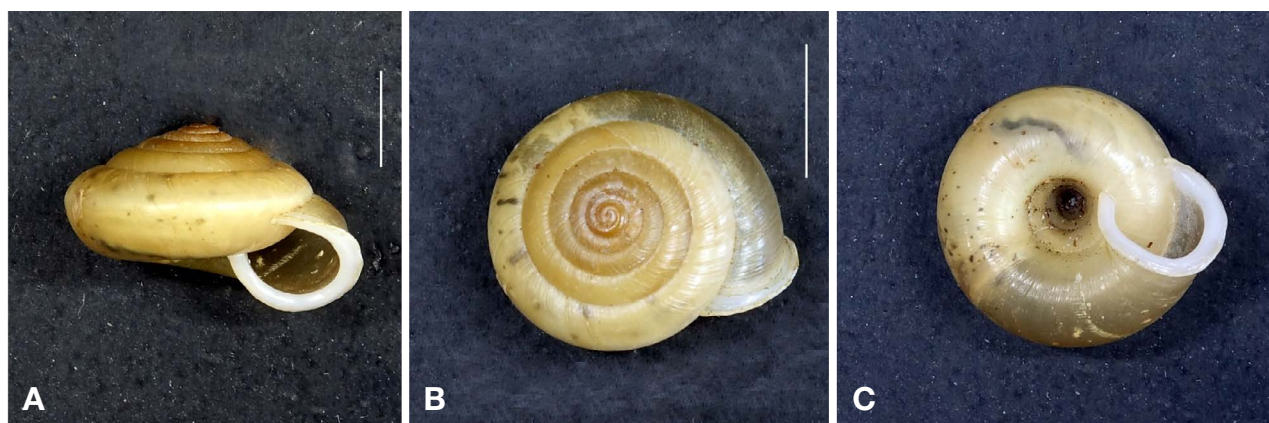
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**Fig. 1.** *Aegista chejuensis*. A, Anterior view; B, Dorsal view; C, Ventral view of the shell. Scale bars: A, B=5 mm.

the two Korean snails and those of the other *Aegista* species retrieved from GenBank (Table 1). Classification and terminology were performed per the methodologies described by MolluscaBase (<https://www.molluscabase.org/>).

## RESULTS AND DISCUSSION

Phylum Mollusca Linnaeus, 1758  
 Class Gastropoda Cuvier, 1795  
 Order Stylommatophora A. Schmidt, 1855  
 Family Camaenidae Pilsbry, 1895  
 Genus *Aegista* Albers, 1850

### <sup>1</sup>\**Aegista chejuensis* (Pilsbry and Hirase, 1908) (Fig. 1)

*Eulota (Aegista) chejuensis* Pilsbry and Hirase, 1908: 61.

*Eulota chejuensis* Pilsbry, 1908: 454.

*Aegista (Aegista) chejuensis* Pilsbry, 1926: 459; Shiba, 1934: 30; Higo and Goto, 1993: 506; Lee and Min, 2002: 149; Min et al., 2004: 361, figs. 1136-1, 1136-2, 1136-3; Lee, 2013: 44.

*Aegista chejuensis* Lee, 1956: 32; Kang, 1971: 69; Kwon, 1979: 21; 1990: 372; Kwon and Habe, 1979: 31; Je, 1989: 34; Kwon et al., 1993: 40, 195, fig. 89 (1-3).

**Material examined.** Terrestrial habitat. Hataedo Island, Sinan-gun, Jeollanam-do, South Korea, 34°23'30"N, 125°17'55"E in July 2023.

**Diagnosis.** Shell height and diameter of approximately 7 and 11 mm, respectively; six and a half whorls; certain depressed low conical and thin shape, solid; light brown color shell, glossy; lacking bristles; smooth periphery of last whorl without angles; large aperture, width (including peristome) of

approximately 6 mm; oblique or round shape; white peristome margin; thick basal lip; round and deep umbilicus with a diameter of approximately 4 mm.

Genus *Plectotropis* E. von Martens, 1860

### <sup>2</sup>\**Plectotropis quelpartensis* (Pilsbry and Hirase, 1908) (Fig. 2)

*Eulota vulgivaga quelpartensis* Pilsbry and Hirase, 1908: 61; Pilsbry, 1908: 454.

*Eulota (Plectotropis) quelpartensis* Pilsbry, 1926: 460, pl. 33, fig. 6.

*Aegista (Aegista) quelpartensis* Shiba, 1934: 31.

*Plectotropis quelpartensis* Lee, 1956: 33; Kang, 1971: 69; Bank, 2017.

*Aegista (Plectotropis) quelpartensis* Shiba 1934: 31; Yoo 1976: 102, pl. 20, figs. 18, 19; Kwon, 1979: 21; 1990: 373; Kwon and Habe, 1979: 31; Je, 1989: 15; Kwon et al., 1993: 40, 195, 196, fig. 91 (1-3); Higo and Goto, 1993: 509; Lee and Min, 2002: 150; Min et al., 2004: 363, figs. 1143-1, 1143-2, 1143-3; Lee, 2013: 58.

**Material examined.** Terrestrial habitat. Sangjodo island, Jindo-gun, Jeollanam-do, South Korea, 34°20'9"N, 126°0'2"E in July 2023.

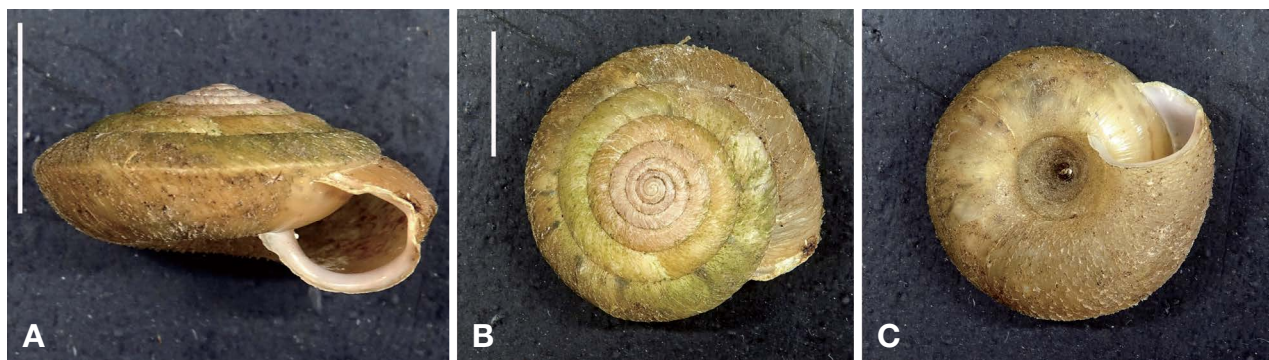
**Diagnosis.** Shell height and diameter of approximately 12 mm and 22 mm, respectively; seven and a half whorls; depressed low conical and thin shape; light brown shell color, low gloss; dense bristles along the growth line on the anterior surface of the shell and on the underside of the body whorl margin; longer bristles on the body whorl margin; a distinct angle at the periphery of the last whorl; aperture width (including peristome) of approximately 10 mm, ovoid and cres-

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**Table 1.** Genetic distances (*p*-distance) of *COI* gene sequence (533 bp) among several *Aegista* species and *Plectotropis quelpartensis* (%)

Species name	GenBank No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1 <i>A. chejuensis</i>	<b>OR514412</b>																					
2 <i>A. chejuensis</i>	<b>OR514413</b>	0																				
3 <i>A. gottschei</i>	AB852630	10.7	10.7																			
4 <i>P. quelpartensis</i>	<b>OR514414</b>	13.0	13.0	10.3																		
5 <i>P. quelpartensis</i>	<b>OR514415</b>	13.0	13.0	10.3	0																	
6 <i>A. vulgivaga</i>	LC514076	15.8	15.8	13.6	12.4	12.4																
7 <i>A. hatakedai</i>	AB852668	16.8	16.8	17.0	16.1	16.1	14.7															
8 <i>A. deflexa</i>	AB852666	16.8	16.8	14.0	13.8	13.8	4.3	14.7														
9 <i>A. aemula</i>	AB852665	17.0	17.0	16.5	16.1	16.1	14.0	3.7	14.9													
10 <i>A. diversifamilia</i>	NC027584	17.2	17.2	17.7	16.5	16.5	17.0	17.5	16.1	17.5												
11 <i>A. horrida</i>	AB852669	17.3	17.3	14.7	14.7	14.7	5.9	14.7	1.9	14.9	16.8											
12 <i>A. kunimiensis</i>	AB852638	18.0	18.0	16.6	16.1	16.1	15.4	18.9	14.9	19.4	18.2	15.6										
13 <i>A. proba</i>	AB852643	18.7	18.7	17.3	17.5	17.5	16.8	18.7	17.7	17.7	19.4	18.2	19.6									
14 <i>A. tumida</i>	AB852659	18.9	18.9	17.3	19.2	19.2	14.9	14.7	17.1	14.9	19.9	17.5	17.1	19.5								
15 <i>A. mikuriyensis</i>	AB852648	19.9	19.9	17.0	17.3	17.3	18.4	18.4	18.7	18.9	19.9	18.9	19.6	19.1	17.5							
16 <i>A. awajjensis</i>	AB852625	21.7	21.7	19.2	18.9	18.9	17.1	15.8	18.3	15.8	18.9	19.0	19.7	20.7	18.8	19.5						
17 <i>A. marginata</i>	AB852678	23.1	23.1	22.9	22.4	22.4	19.1	23.4	20.9	22.9	20.4	21.9	19.9	22.4	20.1	20.4	24.0					
18 <i>A. tokyoensis</i>	AB852656	25.1	25.1	20.0	23.1	23.1	20.8	23.8	21.1	22.9	24.3	21.6	21.8	25.1	23.3	22.5	23.3	25.6				
19 <i>A. cavicollis</i>	AB852661	26.5	26.5	25.1	24.0	24.0	24.0	25.4	24.6	23.8	24.4	25.4	25.1	21.8	23.9	24.0	23.9	27.7	26.2			
20 <i>A. squarrosa</i>	AB852654	26.8	26.8	27.1	29.2	29.2	25.3	26.0	26.4	26.8	26.9	25.6	25.1	28.7	29.6	26.5	27.0	29.5	31.5	29.2		

The species in this study are indicated in bold.  
*COI*, cytochrome c oxidase subunit I.



**Fig. 2.** *Plectotropis quelpartensis*. A, Anterior view of shell; B, Dorsal view of shell; C, Ventral view of shell. Scale bars: A, B=10 mm.

cent-shaped; white peristome margin; thick basal lip; round and deep umbilicus with a diameter of approximately 8.5 mm.

**Remarks on the DNA barcodes of *A. chejuensis* and *P. quelpartensis*.** Previous studies have confirmed that the genus *Aegista* branches into seven clades (A–G) in the gene tree (Hirano et al., 2014, 2015). Two species, *A. chejuensis* and *P. quelpartensis*, were closely related to clade A. Therefore, the 16 *Aegista* species belonging to this clade were selected and included in the analysis (Table 1).

Four new *COI* gene sequences, obtained from two samples each of *A. chejuensis* and *P. quelpartensis*, were registered in GenBank (Table 1). The alignment length was 533 bp (29.1–38.7% GC content, 232 polymorphic sites). Intraspecific distances between *A. chejuensis* and *P. quelpartensis* were both 0% (Table 1). However, the variation between species showed a clear value of 10.3–31.5% (Table 1). As mentioned above, it was compared to phylogenetically close species, and approximately 160 *COI* sequences of the genus *Aegista* have been registered in GenBank (<https://www.ncbi.nlm.nih.gov/>, Aug 2023). A greater difference would be observed if the comparison target were expanded to all species of the genus *Aegista*. *COI* is an effective snail barcode marker for species identification because of the numerous polymorphic sites and large genetic distances between species. However, the *COI* variation rate within the same population for both species was 0%. Thus, comparison with other populations is necessary to analyze intra-species variation.

To date, 15 species of the genus *Aegista* have been reported in Korea. In previous studies, only the nucleotide sequence of *A. gottschei* (Möllendorff, 1887) has been previously reported (Hirano et al., 2015). DNA sequencing is essential to study snails because it is difficult to classify features due to the similarity of shells (Hwang et al., 2021). Moreover, previous geographical studies of snails have not included samples from the Republic of Korea (Hirano et al., 2014; Hwang et al., 2021). Therefore, the continuous discovery of Korean snail DNA sequences is required for further studies.

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## CONFLICTS OF INTEREST

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

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