# Morphological Description of the Newly Discovered Limpet Species Lottia peitaihoensis (Lottiidae: Patellogastropoda) from Korea

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#### ABSTRACT

The family Lottiidae Gray, 1840, belonging to the subclass Patellogastropoda (commonly known as "true limpets"), comprises approximately 200 species worldwide. In Korean waters, 20 species of Lottiidae have been sparsely recorded to date, including eight species from the genus *Lottia* Gray, 1833. *Lottia* species typically exhibit significant ecophenotypic shell variations, which have led to various taxonomic complications, including the proliferation of synonyms due to species misidentification. In this study, we provide detailed information of the shell and radula morphologies using scanning electron microscopy, coupled with mtDNA *cox1* sequences as DNA barcode references for *L. peitaihoensis* (Grabau & S. G. King, 1928), newly discovered in Korean waters. Morphologically, *L. peitaihoensis* can be distinguished from other congeneric species by its shell, characterized by having numerous fine radial riblets with even finer riblets intercalated among them. The radula of this species is characterized by short, pointed first lateral teeth (innermost-lateral teeth), long, slightly rounded second lateral teeth (outermost-lateral teeth), and long, broad, beak-like third lateral teeth (outermost-lateral teeth). In addition, we reconstructed a phylogenetic tree using 25 mtDNA *cox1* haplotype sequences from five *Lottia* species deposited in GenBank, including two newly obtained sequences from Korean *L. peitaihoensis* samples to confirm the species. The detailed morphological data, coupled with molecular information presented in this study, provide valuable information for the accurate delineation and species identification of closely related species within the genus *Lottia*.

Keywords: Patellogastropoda, true limpets, Lottia, radula, SEM, mtDNA cox1, Korea

## INTRODUCTION

Patellogastropoda, commonly known as the "true limpet", is one of the most ancestral gastropod groups of mollusks (Golikov and Starobogatov, 1975; Haszprunar, 1988; Colgan et al., 2000). The family Lottiidae Gray, 1840 belongs to the Patellogastropoda and comprises approximately 200 species worldwide (MolluscaBase, 2024). This family is characterized by a patella-like shell, the presence of a ctenidium in the pallial cavity, and a radula with a formula of 0/1/2-3-0-3-2/1/0. Among these, the type genus *Lottia* Gray, 1833 is distinguished by its conical or slightly convex shell, which often exhibiting prominent radial ridges and a smoothly worn surface (Lindberg, 1998; Okutani, 2017). They are distributed across a broad geographic range, from the tropical to subarctic regions, and are typically found on intertidal rocky shores (Nakano and Ozawa, 2007; Nakano and Sasaki, 2011; Okutani, 2017). The

varied habitats of the intertidal zone contribute to significant variations in shell morphology, even within the same species (Lindberg and Mclean, 1981; Lindberg, 1986; Nakayama et al., 2017; Zhang and Zhang, 2022).

Over the decades, external shell characteristics such as shell shape, size, surface sculpture, color, and pattern have been the most commonly used morphological characters for identifying limpet species. However, species within this group typically exhibit significant ecophenotypic shell variation depending on their local habitats. Accordingly, many previous taxonomic studies have resulted in various complexities, including species misidentification and/or the failure to recognize cryptic species that are morphologically indistinguishable (Nakano and Ozawa, 2005). In addition to shell morphology, a suit of other morphological features such as radula characters, the coloration of foot and pallial tentacles, and gill morphology have been effectively used to resolve these taxonomic com-

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plexities (Teruya et al., 2022). To date, 20 species from the family Lottiidae have been sparsely reported in Korean waters to date, including eight *Lottia* species (National Marine Biodiversity Institute of Korea, 2022): *L. cassis* (Rathke, 1833), *L. dorsuosa* (A. Gould, 1859), *L. kogamogai* Sasaki & Okutani, 1994, *L. langfordi* (Habe, 1944), *L. lindbergi* Sasaki & Okutani, 1994, *L. luchuana* (Pilsbry, 1901), *L. scutum* (Rathke, 1833), and *L. tenuisculpta* Sasaki & Okutani, 1994.

Despite earlier taxonomic efforts that have greatly contributed to the discovery of Korean limpet fauna over several decades, the majority of these studies relied entirely on shell morphology for species identification. This has led to species misidentification and/or the failure to recognize hidden species diversity, including the presence of cryptic species. In this study, we report *L. peitaihoensis* (Grabau & S. G. King, 1928) for the first time in Korea. We provided detailed morphological descriptions of both the shell and radula characteristics using scanning electron microscopy (SEM), along with mtDNA *cox1* sequences as DNA barcode references. The detailed morphological data, coupled with the molecular information presented in this study, will provide valuable insights for the accurate delineation and species identification of closely related species within the genus *Lottia*.

### MATERIALS AND METHODS

Samples were collected from rock surfaces in the intertidal area and preserved in 95% ethyl alcohol. For species identification of the specimens, morphological characters including shell size, height, thickness, aperture shape, apex position, anterior and posterior slopes, shell sculpture, and color were examined using a stereoscopic microscope (Leica M205C; Wetzlar, Germany). The microstructure of radula was examined using an SEM. For SEM preparation, the radula was dissected and incubated in a 10% KOH solution at 50°C for about 5–10 min, then rinsed with distilled water. The specimen was then dried, coated with platinum, and analyzed using



**Fig. 1.** Shell morphology of *Lottia peitaihoensis*. A–C, MABIK voucher specimen No. MO00186626; D–F, MMRBK voucher specimen No. MMRBK 7848; A, D, Dorsal view; B, E, ventral view; C, F, lateral views. Scale bar: A–F=5 mm.

an SEM (Tabletop Microscopes TM4000Plus; Hitachi, Japan). The specimens used for morphological and molecular analysis in this study were deposited in the National Marine Biodiversity Institute of Korea (MABIK voucher specimen No. MO00186626) and the Marine Mollusk Resource Bank of Korea (MMRBK voucher specimen No. MMRBK 7848).

Genomic DNA was extracted from ethanol-fixed tissue (foot muscle) using E.Z.N.A. Mollusc DNA kit (Omega Biotek, Norcross, GA, USA) following the manufacturer's instructions. The mtDNA cox1 sequences were amplified using the universal primers (LCO1490 and HCO2198) (Folmer et al., 1994) in a 50 µL reaction mixture. The mixture contained 33.75  $\mu$ L of distilled water, 5  $\mu$ L of 10 × Ex Tag buffer, 4  $\mu$ L of dNTPs, 2 µL of each primer, 0.25 µL of TaKaRa Ex Taq (TaKaRa Bio, Japan), and 3 µL of template DNA. The PCR was performed with an initial denaturation at 94°C for 3 min, followed by 39 cycles of denaturation at 94°C for 30 s, annealing at 42°C for 30 s, elongation at 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were sequenced using an ABI PRISM 3700 DNA analyzer (Applied Biosystems, USA) and analyzed with Geneious Prime v2024.0.7 (Biomatters, New Zealand). The nucleotide sequences of the partial mtDNA cox1 gene fragment were aligned with homologous sequences from the NCBI database using MAFFT (Katoh and Standley, 2013) in Geneious software, applying default settings. Genetic distances, both between and within species, were calculated as uncorrected p-distances using MEGA v10.2 (Kumar et al., 2018). The Akaike Information Criterion was used via jModelTest (Darriba et al., 2012) to determine the best-fit substitution models for phylogenetic analysis. Phylogenetic relationships were reconstructed using a maximum likelihood approach with RAxML v8.2.9 (Stamatakis, 2014) and branch support was assessed through bootstrap analysis with 1,000 iterations. The mtDNA cox1 sequences of L. peitaihoensis were deposited in GenBank (GenBank accession Nos. PO285411, PO285415).

### SYSTEMATIC ACCOUNTS

Phylum Mollusca Linnaeus, 1758 Class Gastropoda Cuvier, 1795 Subclass Patellogastropoda Lindberg, 1986 Family Lottiidae Gray, 1840 Genus *Lottia* Gray, 1833

<sup>1\*</sup>Lottia peitaihoensis (Grabau & S. G. King, 1928) (Fig. 1) Acmaea peitaihoensis Grabau & S. G. King, 1928a: 39; 1928b: 61; Qi et al., 1989: 21, text-fig. 24; Coan et al., 2015: 204,



**Fig. 2.** Microstructure of radula of *Lottia peitaihoensis* (MABIK voucher specimen No. MO00186626) using scanning electron microscope. Ilt, innermost-lateral teeth; Olt, outermost-lateral teeth; Mt, marginal teeth. Scale  $bar = 50 \ \mu m$ .

fig. 20A-D.

- Acmaea kolarovai Grabau & S. G. King, 1928c: 143, 144, 235, 278, pl. 11, fig. 114.
- Collisella heroldi: Niu et al., 1992: 1406, fig. 2A-C (non Patelloida heroldi (Dunker, 1861)).
- Lottia kogamogai: Sasaki and Okutani, 1994: pl. 2a-c; Fukui and Kashio, 2012: 22, pl. 3, fig. 2 (non *Lottia kogamogai* Sasaki & Okutani, 1994).
- Collisella kolarovai: Qi, 2004: 14, pl. 6F.
- Lottia cf. kogamogai: Kristof et al., 2016: 178, fig. 1a (non Lottia kogamogai Sasaki & Okutani, 1994).
- Lottia goshimai R. Nakayama, Sasaki & T. Nakano, 2017: 239, figs. 2A-H, 3A, 4A-D.

Lottia peitaihoensis: Zhang and Zhang, 2022: 303, figs. 2-11.

**Material examined.** A total of two individuals (MMRBK 7847 [MABIK voucher specimen No. MO00186626], MMRBK 7848) collected from Sinam-ri, Seosaeng-myeon, Ulju-gun, Ulsan (35°20'49.54"N, 129°19'48.38"E) were examined.

**Description.** Shell (Fig. 1) medium-sized (in examined specimen 12 mm [Fig. 1A, B; MMRBK 7847 (MO00186626)] & 10 mm [Fig. 1D, E; MMRBK 7848] in length), relatively high (5 mm in height) (Fig. 1C, F), rather thick; aperture oval; apex curved anteriorly, located one-fifth; anterior slope slightly con-

Korean name: 1\*갈색무늬배말(신칭)

cave; posterior slope slightly convex. Shell sculptured with dense, noticeable, uniformly radial riblets, rough concentric growth lines (Fig. 1A–F). Exterior color white to light brown, featuring 12–13 brown radial stripes, apex white. Interior margin dark brown, intermediate area white, interior stain dark brown, central white.

Radula (Fig. 2; MMRBK 7847 [MO00186626]) first lateral teeth (innermost-lateral teeth; Ilt) short, pointed; second lateral teeth long (outermost-lateral teeth; Olt) slightly rounded; third lateral teeth (outermost-lateral teeth; Olt) broad, beak-like; marginal teeth small, rounded.

**GenBank accession number.** PQ285411, PQ285415. **Distribution.** China, Japan, and Korea.

**Remarks.** This species has undergone many taxonomic revisions due to complications arising from the high morphological variation observed within individuals of the same species. Grabau and King (1928a) initially described this species as *Acmaea peitaihoensis* Grabau & S. G. King, 1928 from Beidaihe, China. In the same year, Grabau and King (1928c) described another species, *Acmaea kolarovai* Grabau & S. G. King, 1928, collected from the same region, named in honor of Dr. F. N. Kolarova. However, Coan et al. (2015) questioned the validity of treating these as separate species due to their

morphological resemblance, such as numerous radial riblets with rough concentric growth lines. Upon re-examination of the syntype specimens of these two species, they confirmed that *A. kolarovai* is a junior synonym of *A. peitaihoensis*.

This species is morphologically similar to the Japanese species Lottia goshimai R. Nakayama, Sasaki & T. Nakano, 2017. However, Zhang and Zhang (2022) suggested that L. goshimai is a junior synonym of L. peitaihoensis (Grabau & S. G. King, 1928) based on shell morphology, radula, and mtDNA cox1 sequences which showed a p-distance ranging from 0.2 to 0.5% between L. peitaihoensis (sampled from China) and L. goshimai (sampled from Japan). In this study, we also performed phylogenetic analysis using mtDNA cox1 sequences to validate our morphology-based species identification. In the phylogenetic tree, the two mtDNA cox1 haplotypes sequenced from Korean L. peitaihoensis specimens were grouped together with other L. peitaihoensis sequences from Japanese and Chinese populations, receiving 99% bootstrap support in the maximum likelihood analysis (Fig. 3). The within-species sequence divergence of L. peitaihoensis populations is very low, ranging from no sequence difference (between Chinese and Japanese samples) to 0.5% (between Chinese and Korean samples). In contrast, the sequence diver-



<sup>0.050</sup> 

**Fig. 3.** Phylogenetic relationships among *Lottia* species using mtDNA *cox1* sequences, inferred from maximum likelihood method. Node supporting values are indicated as bootstrap values ( $\geq$ 50). An asterisk (\*) denotes sequences newly determined in this study.

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L. peitaihoensis (LC137939)																								
L. peitaihoensis (PQ285411)*	0.0																							
L. peitaihoensis (LC137955)	0.0	0.0																						
L. peitaihoensis (LC137921)	0.0	0.0	0.0																					
L. peitaihoensis (LC137928)	0.0	0.0	0.0	0.0																				
L. peitaihoensis (MW812231)	0.0	0.0	0.0	0.0	0.0																			
L. peitaihoensis (MK226734)	0.2	0.2	0.2	0.2	0.2	0.2																		
L. peitaihoensis (MK226733)	0.2	0.2	0.2	0.2	0.2	0.2	0.0																	
L. peitaihoensis (MK226732)	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.0																
L. peitaihoensis (PQ285415)*	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3															
L. peitaihoensis (LC137952)	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3														
L. peitaihoensis (MW812230)	0.3	0.3	0.3	0.3	0.3	0.3	0.5	0.5	0.5	0.5	0.5													
L. luchuana (LC138006)	16.4	16.4	16.4	16.4	16.4	16.4	16.5	16.5	16.5	l6.2 ]	6.4 1	6.4												
L. luchuana (LC138002)	16.1	16.1	16.1	16.1	16.1	16.4	16.5	16.5	16.5	L5.9	6.1 1	6.4 (	0.3											
L. luchuana (LC137990)	16.1	16.1	16.1	16.1	16.1	16.4	16.5	16.5	16.5	[5.9]	6.1 1	6.4 (	0.3	0.0										
L. kogamogai (LC137915)	17.4	17.4	17.4	17.4	17.4	17.5	17.8	17.8	17.8	17.6	7.6 1	7.9 1	7.3 17	.3 17										
L. kogamogai (LC137913)	17.6	17.6	17.6	17.6	17.6	17.7	18.0	18.0	18.0	[7.4]	7.7 1	8.0 1	7.1 17	.1 17	.1 (	.2								
L. kogamogai (LC137909)	17.4	17.4	17.4	17.4	17.4	17.5	17.8	17.8	17.8	17.6	7.6 1	7.9 1	7.6 17	.6 17	.6	0.3	Ŀ.							
L. kogamogai (LC137912)	17.4	17.4	17.4	17.4	17.4	17.5	17.8	17.8	17.8	17.6	7.6 1	7.9 1	7.6 17	.6 17	.6	0.3	.5	0						
L. kogamogai (LC137914)	17.4	17.4	17.4	17.4	17.4	17.5	17.8	17.8	17.8	17.6	7.6 1	7.9 1	7.6 17	.6 17	.6	0.3	.5	0.0	0					
L. lindbergi (LC137971)	26.4	26.4	26.4	26.4	26.4	26.5	26.6	26.6	26.6	26.5	6.4 2	6.9 2!	5.5 25	.5 25	5.5 26	6.7 26	.8 26	4 26.4	4 26.4	4				
L. lindbergi (LC137965)	27.0	27.0	27.0	27.0	27.0	27.0	27.2	27.2	27.2	27.2	7.0 2	7.2 2!	5.4 25	.4 25	6.4 26	6.8 27	.0 26	5 26.	5 26.	50.	0			
L. tenuisculpta (LC137953)	30.3	30.3	30.3	30.3	30.3	30.2	30.5	30.5	30.5	30.5	0.5 3	0.2 28	3.2 28	3.2 28	3.2 29	9.1 29	.2 29	2 29.	2 29.	2 21.	7 22.	50		
L. tenuisculpta (LC137978)	30.2	30.2	30.2	30.2	30.2	30.0	30.3	30.3	30.3	30.3	0.3 3	0.0 2	7.9 27	.9 27	.9 28	3.5 28	.6 28	6 28.	5 28.0	5 21.	5 22.	5 0.8		
L. tenuisculpta (LC137982)	30.3	30.3	30.3	30.3	30.3	30.2	30.5	30.5	30.5	30.5	0.5 3	0.2 28	3.0 28	3.0 28	3.0 28	3.6 28	.8 28	8 28.	8 28.8	8 21.	7 22.	3 0.9	0.2	
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Table 1. Uncorrected *p*-distances (%) of mtDNA cox1 sequences compared among some selected Lottia species

gence between *L. peitaihoensis* and *L. luchuana* ranged from 15.9% to 16.4% (Table 1).

The present study provides detailed information on the shell and radula morphologies, coupled with mtDNA *cox1* sequences as DNA barcode references for *L. peitaihoensis* (Grabau & S. G. King, 1928), newly recorded in Korean waters. The detailed morphological data and mtDNA *cox1* barcode sequence information presented in this study will provide valuable insights for the accurate species identification of closely related species of the genus *Lottia*.

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## **CONFLICTS OF INTEREST**

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

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