# LOTTIA GOSHIMAI NAKAYAMA, SASAKI & NAKANO, 2017, A JUNIOR SYNONYM OF LOTTIA PEITAIHOENSIS (GRABAU & S. G. KING, 1928) (GASTROPODA: LOTTIIDAE)

#### Shuoian Zhang & Suping Zhang

Laboratory of Marine Organism Taxonomy and Phylogeny, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China;

Center for Ocean Mega-Science, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, China

Abstract Examination of the syntypes of Lottia peitaihoensis (Grabau & S. G. King, 1928) showed that this species in shell morphology matches well with Lottia goshimai Nakayama, Sasaki & Nakano, 2017. Phylogenetic analysis of the COI gene based on newly obtained specimens of Lottia peitaihoensis and the sequence data of Lottia goshimai deposited in GenBank provided additional evidence for the conspecificity of these two species. The COI pairwise distance between L. peitaihoensis and L. goshimai ranges from 0-0.5%, a divergence much lower than the known interspecific variation of Lottia spp. (8.6–44.5%). In the present study, we formally synonymise L. goshimai with L. peitaihoensis. A lectotype for L. peitaihoensis is designated from syntypes.

#### Introduction

The genus Lottia Gray, 1833 is a most diverse group of limpets in the family Lottiidae, with more than 60 recognised species (MolluscaBase, 2021). The members of this family, like other patellogastropods, possess high degree of intraspecific variation in shell morphology (phenotype) due to their complex habitat in the intertidal zone (Lindberg & McLean, 1981; Lindberg, 1986; Sasaki & Okutani, 1994). Historical descriptions based on shells only has frequently resulted in ambiguous species delimitation, which has led to many taxonomical confusions and, in turn, synonyms (Sasaki, 1999; Nakano & Spencer, 2007).

Lottia peitaihoensis was first described by Grabau and King in 1928, from Peitaiho (=Beidaihe), Qinhuangdao City, China based on empty shells (Grabau & King, 1928a). Subsequently, this species was recorded from various localities along the coast of Bohai Sea and Yellow Sea, but either placed in different genera (Zhao et al., 1989; Qi, 2014, Zhang et al., 2016) or, sometimes, misidentified (Zhang et al., 2016; Huan et al., 2020). Recently, we examined the type specimens and found that their shell morphology matches well with that of Lottia goshimai Nakayama, Sasaki & Nakano, 2017, a species recently described from northern Japan. In the original description, however, Nakayama et al. (2017) did not compare their new species with L. peitaihoensis. To determine whether the two species are conspecific or not, we collected several fresh specimens in recent years. Based on these materials, the radulae of L. peitaihoensis were closely examined and compared with that of L. goshimai. In addition, we conducted a molecular analysis with mitochondrial COI gene, using the newly obtained materials of *L. peitaihoensis* and the sequence data of the type materials of L. goshimai deposited in GenBank. Morphological and genetic analyses both show that these two species belong to the same species. In the present study, we treat L. goshimai Nakayama, Sasaki & Nakano, 2017 as a junior synonym of *L. peitaihoensis* (Grabau & S. G. King, 1928).

#### MATERIALS AND METHODS

Specimen sampling and preservation Materials studied herein include the syntypes and historical samples of Lottia peitaihoensis deposited in Marine Biological Museum of Chinese Academy of Sciences (MBMCAS), and newly collected specimens from two sites, Beidaihe (type locality) and Qingdao (see Fig. 1). The syntypes and historical samples were examined for shell morphology only, whereas the newly obtained fresh materials were studied not only for shell morphology, but also for radular features and COI sequences. Shells were observed under light microscopy and measured using calipers with accuracy of

Contact author: Shuqian Zhang, zsqtaxon@qdio.ac.cn

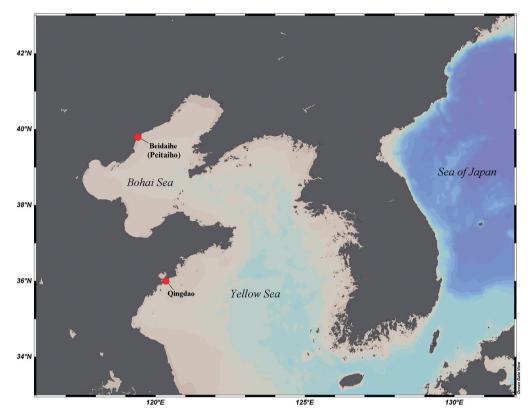


Figure 1 Sampling sites of sequenced specimens of Lottia peitaihoensis used in the present study.

0.1mm. Radulae were examined using Scanning Electron Microscope (SEM). For SEM studies, radulae were cleaned using 10% NaOH for 30 min, rinsed in distilled water, air-dried, coated with gold and examined under SEM at an accelerating voltage of 5kV. All specimens used in this work have been deposited in MBMCAS.

Abbreviations used in present study: GKLNH: Grabau-King Laboratory of Natural History, Peking [Beijing], China; MBM CAS: Maine Biology Museum of Chinese Academy of Sciences, Qingdao, China.

#### Molecular Analysis

Nine newly collected specimens, including three from Qingdao and six from Beidaihe, were genetically analysed. Genomic DNA was extracted with the Column Genomic DNA Isolation Kit (Beijing TIANGEN, China) according to the manufacturer's instructions. DNA was eluted in elution buffer and stored at -20°C until use. The COI region was amplified by polymerase chain reaction (PCR) using the primers LCO1490 (forward: 5′–GGTCAACAAATCATAAAGATATTGG–3′) and HCO2198 (reverse: 5′–TTAACTTCAGGGT GACCAAAAAATCA–3′) (Folmer *et al.*, 1994).

PCR reactions were carried out in a total volume of 50 μL, including 2 μL DNA template, 1.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTPs, 1 μL of both forward and reverse PCR primers, 10× buffer and 2.5 U Taq DNA polymerase. Thermal cycling was performed under the following conditions: 94°C for 3 min (initial denaturation), followed by 35 cycles of 94°C for 30s (denaturation), 45°C for 30s (annealing), 72°C for 60s (extension) and a final extension at 72°C for 10 min. PCR products were verified on a GelRed-stained 1.5% agarose gel and purified with the Column PCR Product Purification Kit (Shanghai Sangon, China).

Sequence were aligned using Clustal W in MEGA X (Kumar *et al.*, 2018) and the MEGA X used to calculate the pairwise distances. Table 1 lists the material analysed and the Genbank numbers of the sequences used. Bayesian Inference phylogenies were inferred using MrBayes 3.2.6 (Ronquist *et al.*, 2012) under HKY+F+I+G4 model (2 parallel runs, 5,000,000 generations), in which the initial 25% of sampled data were discarded as burn-in. ModelFinder (Kalyaanamoorthy *et al.*, 2017) was used to select the best-fit model using AICc criterion. Results were visualised using FigTree v. 1.4.4.

 Table 1
 List of Lottia species and GenBank accession numbers of sequences used in the present study.

Species	Voucher	COI	References
Lottia alveus	11BIOAK-0655	KF643428.1	Layton <i>et al.</i> (2014)
Lottia antillarum	NUGB-L406	AB238462.1	Nakano & Ozawa (2007)
Lottia austrodigitalis	LottiaSobU9	GU443577.1	Kelly & Palumbi (2010)
Lottia austrodigitalis	LottiaSobU8	GU443576.1	Kelly & Palumbi (2010)
Lottia austrodigitalis	LottiaSobU7	GU443575.1	Kelly & Palumbi (2010)
Lottia cassis	QD2	KM221126.1	Lin et al. (2015)
Lottia cassis	QD3	KM221133.1	Lin et al. (2015)
Lottia cassis	QD4	KM221141.1	Lin et al. (2015)
Lottia digitalis	16_STFX_006	MK091869.1	Tabita & Rozycki, unpublished
Lottia digitalis	16_EMSS_001	MK037262.1	Chen & Heine, unpublished
Lottia digitalis	11BIOAK-0657	KF644259.1	Layton <i>et al.</i> (2014)
Lottia emydia	K728	LC416621.1	Nakayama & Nakano, unpublished
Lottia emydia	K518	LC416605.1	Nakayama & Nakano, unpublished
Lottia filosa	NUGB-L507	AB238465.1	Nakano & Ozawa (2007)
Lottia gigantea	KFM-216	JF433982.1	Lipstone et al., unpublished
Lottia gigantea	KFM-217	JF433981.1	Lipstone <i>et al.</i> , unpublished
Lottia gigantea	OGL-E01605	JF433980.1	Lipstone <i>et al.</i> , unpublished
Lottia goshimai	-	MK568805.1	Huan, unpublished
Lottia goshimai	K376	LC416601.1	Nakayama & Nakano, unpublished
Lottia goshimai	K574	LC137999.1	Nakayama et al. (2017)
Lottia goshimai	K522	LC137995.1	Nakayama <i>et al.</i> (2017)
Lottia goshimai	K496	LC137993.1	Nakayama <i>et al.</i> (2017)
Lottia goshimai	K484	LC137989.1	Nakayama <i>et al.</i> (2017)
Lottia goshimai	K483	LC137988.1	Nakayama <i>et al.</i> (2017)
Lottia goshimai	K482	LC137987.1	Nakayama <i>et al.</i> (2017)
Lottia goshimai	K481	LC137986.1	Nakayama <i>et al.</i> (2017)
Lottia goshimai	K480	LC137985.1	Nakayama <i>et al.</i> (2017)
Lottia goshimai	K439	LC137984.1	Nakayama <i>et al.</i> (2017)
Lottia goshimai	K414	LC137976.1	Nakayama <i>et al.</i> (2017)
Lottia goshimai	K404	LC137972.1	Nakayama <i>et al.</i> (2017)
Lottia goshimai	K394	LC137966.1	Nakayama <i>et al.</i> (2017)
Lottia goshimai	K98	LC137920.1	Nakayama <i>et al.</i> (2017)
Lottia kogamogai	NUGB-L143	AB238467.1	Nakano & Ozawa (2007)
Lottia kogamogai	UMUT:RM31933	LC138346.1	Teruya & Sasaki, unpublished
Lottia kogamogai	L1148	LC138008.1	Nakayama <i>et al.</i> (2017)
Lottia kogamogai	L1149	LC138009.1	Nakayama <i>et al.</i> (2017)
Lottia kogamogai	L1147	LC138009.1 LC138007.1	Nakayama <i>et al.</i> (2017)
Lottia kogamogai	L945	LC138007.1 LC138005.1	Nakayama <i>et al.</i> (2017)
	L159	LC138003.1 LC138004.1	Nakayama <i>et al.</i> (2017)
Lottia kogamogai	NUGB-L244	AB238468.1	· · · · · · · · · · · · · · · · · · ·
Lottia langfordi Lottia limatula	NUGB-L244 NUGB-L291	AB238469.1	Nakano & Ozawa (2007)
	NUGB-L291 NUGB-L161	AB238470.1	Nakano & Ozawa (2007)
Lottia lindbergi		LC138348.1	Nakano & Ozawa (2007)
Lottia lindbergi	UMUT:RM31935	LC416602.1	Teruya & Sasaki, unpublished
Lottia lindbergi	K393		Nakayama & Nakano, unpublished
Lottia luchuana	BBW4	KM221049.1	Lin et al. (2015)
Lottia luchuana	BBW3	KM221048.1	Lin et al. (2015)
Lottia luchuana	BBW2	KM221045.1	Lin et al. (2015)
Lottia luchuana	BBW1	KM221044.1	Lin et al. (2015)
Lottia mesoleuca	NUGB-L425	AB238473.1	Nakano & Ozawa (2007)
Lottia onychitis	NUGB-L637	AB238474.1	Nakano & Ozawa (2007)
Lottia orbigny	NUGB-L555	AB238475.1	Nakano & Ozawa (2007)
Lottia paradigitalis	10BCMOL-00081	KF644311.1	Layton et al. (2014)

 Table 1 (Continued)

Species	Voucher	COI	References
Lottia paradigitalis	10BCMOL-00182	KF644279.1	Layton <i>et al.</i> (2014)
Lottia peitaihoensis	MBM286043	MK226732.1	This study
Lottia peitaihoensis	MBM286044	MK226733.1	This study
Lottia peitaihoensis	MBM286045	MK226734.1	This study
Lottia peitaihoensis	LB-001	MW812230	This study
Lottia peitaihoensis	LB-002	MW812231	This study
Lottia peitaihoensis	LB-003	MW812232	This study
Lottia peitaihoensis	LB-004	MW812233	This study
Lottia peitaihoensis	LB-005	MW812234	This study
Lottia peitaihoensis	LB-006	MW812235	This study
Lottia persona	16_TU_011	MK091859.1	Sugio & Šato, unpublished
Lottia pelta	2016_WCHS_009	MK037264.1	Matthew & Tian, unpublished
Lottia pelta	16_EMSS_011	MK037261.1	Bews & Payette, unpublished
Lottia scabra	M0D_13127W_Cho	KJ006004.1	Dawson, et al. (2014)
Lottia scabra	M0D_13125U_Cho	KJ006003.1	Dawson, et al. (2014)
Lottia scabra	M0D_13124T_Cho	KJ006002.1	Dawson, et al. (2014)
Lottia scutum	16_TU_003	MK037252.1	Kikuchi & Matsuda, unpublished
Lottia scutum	11BIOAK-0237	KF644269.1	Layton et al. (2014)
Lottia scutum	17_MOLL_003_073	MK037280.1	Richardson & Froats, unpublished
Lottia smithi	NUGB-L408	AB238480.1	Nakano & Ozawa (2007)
Lottia septiformis	NUGB-L618	AB238479.1	Nakano & Ozawa (2007)
Lottia subrotundata	NUGB-L597	AB238481.1	Nakano & Ozawa (2007)
Lottia tenuisculpta	NUGB-L158	AB238482.1	Nakano & Ozawa (2007)
Lottia tenuisculpta	UMUT:RM31934	LC138347.1	Teruya & Sasaki, unpublished
Perotrochus midas	USNM 888645	AY296820.1	Aktiphis & Giribet (2012)

#### Species delimitation analysis

The Automatic Barcode Gap Discovery (ABGD) method (Puillandre *et al.*, 2012) was used to assess the number of *Lottia* species studied herein. The alignment from the COI gene was uploaded to the online server of ABGD. Analysis was performed with the model of Kimura (K80) TS/TV with the following settings (Pmin=0.001, Pmax=0.1, Steps=10, X=1.0, Nb bins=20).

#### RESULTS

## Morphological comparison

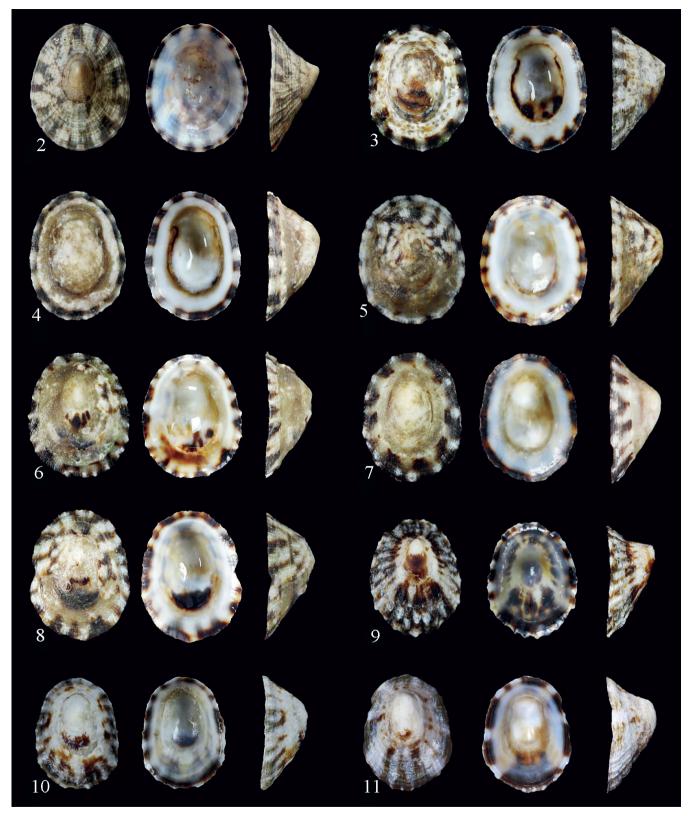
Conchologically, *Lottia peitaihoensis* (Grabau & S. G. King, 1928) matches well with *Lottia goshimai* Nakayama, Sasaki & Nakano, 2017 in general shell shape, presence of regular, prominent radial riblets, and alternating light brown and dark brown bands on shell surface and inner margin. In the original description, however, Nakayama *et al.* (2017) did not compare *L. goshimai* with *L. peitaihoensis*. In that publication, they listed several phenotypes of *L. goshimai*, which are variable

mainly in shell height, strength of spiral sculpture, and colouration. Some of these phenotypical variations can also be found in *L. peitaihoensis* (see Figs 2–11). *L. peitaihoensis* has radula with a formula of 1+3+0+3+1; first lateral tooth relatively short and narrow, second lateral tooth longer and wide, third lateral tooth reduced and wide; marginal tooth small, rounded (see Figs 12–15). This morphology is identical to that of *L. goshimai*.

#### Molecular comparison

A total of nine partial COI sequences were obtained in the present study and have been deposited in GenBank. A phylogenetic tree inferred using BI criteria was reconstructed (Fig. 16). The Bayesian phylogenetic tree shows that *Lottia peitaihoensis* from two sites in China, together with *Lottia goshimai* from Japan form a fully supported clade (PP=1).

The ABGD analysis of the COI sequences resulted in the delimitation of 25 species (see Fig. 16), with values of prior intraspecific divergence



Figures 2-11 Lectotype and sequenced specimens of Lottia peitaihoensis. 2 Lectotype, MBM229002, Beidaihe (=Peitaiho), 11.4×8.4×4.8mm; 3 LB-001, Beidaihe (=Peitaiho), 13.3×10.5×6.0mm; 4 LB-002, Beidaihe (=Peitaiho), 13.2×10.0×5.5mm; **5** LB-003, Beidaihe (=Peitaiho), 15.4×12.7×6.0mm; **6** LB-004, Beidaihe (=Peitaiho), 13.0×10.5×4.9mm; 7 LB-005, Beidaihe (=Peitaiho), 13.7×10.5×5.4mm; 8 LB-006, Beidaihe (=Peitaiho), 13.8×10.6×5.3mm; 9 MBM286043, Qingdao, 11.2×8.7×5.0mm; 10 MBM286044, Qingdao, 9.5×7.2×53.8mm; 11 MBM286045, Qingdao, 11.2×8.5×4.8mm.



**Figures 12–15** Radulae of *Lottia peitaihoensis*. **12, 13** LB-001, Beidaihe (=Peitaiho), China; **14, 15** MBM286042, Qingdao, China. Scale bar=50 µm.

(P) being  $\geq$  0.0017. These groups correspond to clades recovered by BI analysis.

Analysis of the COI gene shows that *Lottia goshimai* and *Lottia peitaihoensis* have almost identical sequences, with pairwise distances ranging from 0–0.5% (see Table 2), a divergence much lower than the known interspecific variation of *Lottia* spp. (8.6–44.5%).

These results provide additional support for the conspecificity of *L. goshimai* and *L. peitaihoensis*.

### **S**YSTEMATICS

Class Gastropoda Cuvier, 1795

Superfamily Lottioidea Gray, 1840

Family Gray, 1840

Genus Lottia Gray, 1833

Type species: *Lottia gigantea* Gray in G. B. Sowerby I, 1834, by subsequent designation).

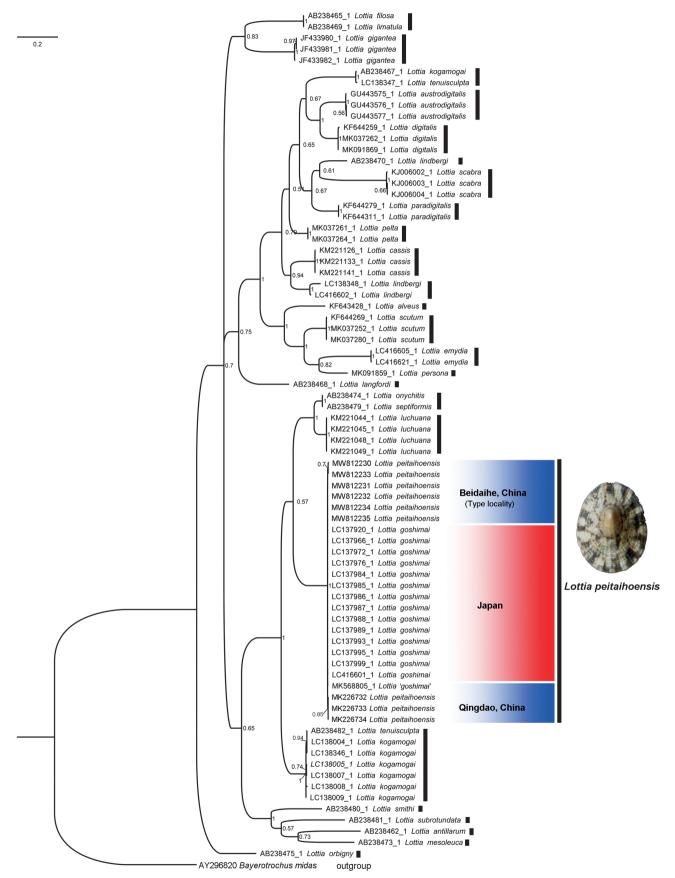


Figure 16 Phylogenetic tree inferred by Bayesian analysis (BI) based on COI gene. Numbers adjacent to nodes refer to BI posterior probability. Black vertical bars indicate the results of ABGD species delimitation.

 Table 2
 Pairwise distance of COI gene sequences among the Lottia peitaihoensis and Lottia goshimai in this study.

Lottia peitaihoensis (Grabau & S. G. King, 1928) (Figs 2–11)

Acmaea peitaihoensis Grabau & King, 1928a: 39; 1928b: 61.; Qi et al., 1989: 21, text-fig. 24.; Coan et al., 2015: 204, figs. 20A-D.

Acmaea kolarovai Grabau & King, 1928c: 143–144, 235, 278, pl. 11, fig. 114.

Collisella kolarovai: Zhang et al., 2016: 17, fig. 17. Collisella peitaihoensis: Qi 2004: 14, pl. 6F.

Patelloida peitaihoensis: Zhao et al., 1989: 20, pl. 2, fig. 2, pl. 3, fig. 3.

Lottia dorsuosa: Zhang et al., 2016: 17, fig. 16. (not Gould, 1859)

Lottia goshimai Nakayama, Sasaki & Nakano, 2017: 239, figs. 2A-H, 3A, 4A-D.

Type material Lectotype (designated herein, MBM286701), and paralectotypes (MBM229002, ex GKLNH, 37 shells), Peitaiho (=Beidaihe), vii 1925; MBM229003, (ex GKLNH, 24 shells), Peitaiho (=Beidaihe), vii 1925.

Othermaterial examined MBM286043-MBM286045, three shells, Qingdao, v 2014; MBM286042, eight specimens, Qingdao, viii 2018; MBM101867, 17 shells, Dalian,×1952; MBM101854, two shells, Rizhao, i 1984; MBM101910, 15 shells, Yantai, iii 1975; MBM103021, four shells, Rongcheng, vii 1952; MBM286837, 20 specimens, Beidaihe, 12 iii 2021.

Type locality Beidaihe (=Peitaiho), Hebei Province, China.

Distribution Intertidal zone along Bohai Sea and Yellow Sea; northern Japan. Live on rocky shore.

Diagnosis Shell medium sized for genus, moderately thick, aperture oval; strongly arched, apex antero-dorsally directed and located at anterior fourth to fifth. Anterior slope straight or slightly concave, posterior slope straight or slightly convex. Teleoconch sculptured with crowded, rough, regularly arranged radial riblets and fine concentric growth lines. Exterior colour white to light brown with 12-16 brown radial bands. Radula has a formula of 1+3+0+3+1. First lateral tooth relatively short and narrow, second lateral tooth longer and wide, third lateral tooth reduced and wide; marginal tooth small, rounded (Figs 12–15).

Remarks Lottia peitaihoensis (Grabau & S. G. King, 1928) was originally described from Beidaihe (=Peitaiho), China as Acmaea peitaihoensis. Later that year, Grabau & King (1928b) proposed a new name Acmaea kolarovai for specimens from the same locality. However, the description of Acmaea kolarovai is identical to that of Acmaea peitaihoensis, and the authors evidently changed their minds about the name. Lottia peitaihoensis (Grabau & S. G. King, 1928), has priority, and Acmaea kolarovai Grabau & King, 1928, is thus a junior synonym (Coan et al., 2015). Grabau & King (1928) did not designate holotype for L. peitaihoensis. Coan et al. (2015) treated all the type materials as syntypes. Herein, we designate a lectotype from the syntypes in order to relate the binomen to a unique voucher (see Fig. 2).

We consider all specimens used in the present study to be L. peitaihoensis without any doubt. Lottia cassis (Eschscholtz, 1833) from the Yellow Sea is the only *Lottia* species known to be sympatric with Lottia peitaihoensis. This species, however, can be easily differentiated from L. peitaihoensis in having smooth shell with solid brown colour, and lacking radial bands. In the type locality of L. peitaihoensis and other region of Bohai Sea, no other Lottia species can be found.

Both morphological and molecular evidence strongly suggest that Lottia goshimai and Lottia peitaihoensis are conspecific. The latter has priority and thus Lottia goshimai should be regarded as a junior synonym.

#### **ACKNOWLEDGMENTS**

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