

Brief Report

# On the Species Identification of Korean Geoduck Clam (*Panopea* sp. 1) Based on the Morphological and Molecular Evidence

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**Abstract:** The geoduck clam is a high-value species in South Korea. However, the molecular and morphological characteristics of geoduck clams from the southern coast of South Korea remain unexamined. The accurate identification of native geoduck clams is crucial for their conservation and management. Therefore, this study used a combination of molecular and morphological analyses to characterize native geoduck clams from the southern coast of South Korea. Based on complete mitochondrial genome and morphological analyses, the native species of geoduck clam from this study area was identified as *Panopea* sp. 1. The complete mitochondrial genome sequencing of *Panopea* sp. 1 revealed a total of 16,225 bp in length with 37 genes (13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes). It was also shown that *Panopea* sp. 1 belongs to the family Hiatellidae based on a phylogenetic analysis tree with 11 bivalve species. In particular, *Panopea* sp. 1 is closely related to three other *Panopea* species (*Panopea* sp., *Panopea abrupta*, and *Panopea japonica*). The phylogenetic analysis correlated with the morphological analysis. Overall, this is the first reliable record of *Panopea* sp. 1 in South Korea. These findings provide a basis for accurate species identification based on morphological characteristics and complete mitochondrial genome sequencing.

**Keywords:** genus *Panopea*; geoduck clam; complete mitochondrial genome; phylogeny; morphology; species identification



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## 1. Introduction

The geoduck clams of the genus *Panopea* de la Groye, 1807, belonging to the family Hiatellidae (Mollusca, Bivalvia), are among the largest deep-burrowing bivalves [1,2]. They are widely distributed in subtidal substrates along shallow coastlines and are buried deeply in muddy sand sediments down to 30–100 cm [3]. To date, approximately 150 fossil species of the genus *Panopea* have been documented, but only 9 extant species have been described, spanning habitats covering from temperate to subtropical regions; 5 of these 9 species are distributed in the Northern Hemisphere [4]. In general, geoduck clams are well suited for aquaculture as a valuable commercial marine fishery resource because of their high protein content and edible proportions, comprising nearly 55% of their bodies [5,6]. Since the 1970s, geoduck clams have been collected and farmed overseas, and their annual production has been reported to be approximately 6000 metric tons; subsequently, their product value is rising owing to increased consumption [7]. The increase in geoduck clam collection has led to the identification and documentation of various species worldwide. Specifically, the Pacific geoduck, *Panopea generosa* A. Gould, 1850, is farmed and collected

in the United States (USA) and Canada [8], the New Zealand geoduck, *Panopea zelandica* Quoy & Gaimard, 1835, is collected in New Zealand, *Panopea abrupta* (Conrad, 1849), is collected on a limited basis in Alaska, USA, and *P. generosa* and *Panopea globose* Dall, 1898, are collected in Mexico [9–11]. Therefore, these newly identified species highlight the importance of the conservation of geoduck clams worldwide and the identification of each region-specific species.

The geoduck clam *Panopea japonica* A. Adams, 1850 is an important bivalve resource and is mainly found in the coastal subtidal regions of Korea, Japan, northern China, and Russia [12]. In South Korea, despite its high commercial value, research on *P. japonica* has been mainly focused on its habitat ecology, growth, reproductive biology, seed production, feeding activity, and environmental tolerance [13–15]. However, specific species identity parameters based on the molecular and morphological characteristics of *P. japonica* remain unclear. Recently, *Panopea* sp. are a newly identified species which are distributed along the east coast of South Korea [16], whereas the geoduck clams inhabiting southern region of South Korea have yet to be examined and characterized. Therefore, it is important to emphasize that species identification is the preliminary step in the appropriate conservation and sustainable management of geoduck clam resources.

To date, mitochondrial DNA markers have been extensively applied in taxonomy and phylogenetics. Furthermore, mitochondrial DNA markers have previously been used for the identification of bivalve species, such as the flat Oyster, *Ostrea denselamellosa* [17], and Geoduck clam, *Panopea* sp. [16], indicating that mitochondrial DNA markers can be successfully used to identify bivalve species, including clams. Therefore, mitochondrial DNA markers were utilized in this study for the accurate species identification of geoduck clams collected from the southern coast of South Korea. Overall, here, we provide the complete mitochondrial genome of the native geoduck clam species from the southern coast of South Korea and compare their mitochondrial DNA genes to those of bivalve species using phylogenetic relationships. Overall, this study contributes to the population stock, conservation, and management of native geoduck clam species, while broadening the knowledge and evolutionary ecology of clams worldwide.

## 2. Materials and Methods

### 2.1. Sample Collection

Adult specimens of harvested native geoduck clams were provided by a local fish market on Geoje Island, South Korea, on 17 February 2023. The collection area is shown in Figure 1. All the protocols used for animal experimentation followed the Guidelines of the Institutional Animal Care and Experimental Committee, approved by the Korea Institute of Ocean Science & Technology (KIOST).

### 2.2. DNA Extraction, PCR Amplification, and DNA Sequencing

To extract genomic DNA, muscle tissue was retrieved from the samples and the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) was used. The quantity and quality of the isolated DNA were analyzed and measured at 230, 260, and 280 nm using a Victor<sup>3</sup> multilabel plate reader (PerkinElmer, Waltham, MA, USA). To construct a genomic library, the TruSeq Nano DNA Kit was used (Macrogen, Seoul, South Korea) and the manufacturer's instructions were followed (Illumina, San Diego, CA, USA). The assembly and annotation of the complete mitochondrial genomes of the geoduck clam species were performed using MITOS [18]. After the completion of the experiment, the voucher material was deposited at the National Marine Biodiversity Institute of Korea (MABIK).

### 2.3. Sequence Alignment and Phylogenetic Analysis

To construct a phylogenetic tree using previously identified species, the complete mitochondrial genomes of 12 bivalve species (five families: Arctiidae, Cardiidae, Hiattellidae, Myidae, and Lucinidae) were collected from the GenBank database (Table 1). The alignment of the concatenated set of 13 protein-coding genes (PCGs) for each mitochondrial

genome was performed using the ClustalW algorithm in the MEGA software (ver. 10.0.1; Centre for Evolutionary Medicine and Informatics, Tempe, AZ, USA). To establish the best-fit substitution model for the phylogenetic analysis, the model with the lowest Bayesian and Akaike information criterion scores was estimated using a maximum likelihood (ML) analysis. Based on the results of the model test, the LG + G + I model was used with 1000 bootstrap replicates.

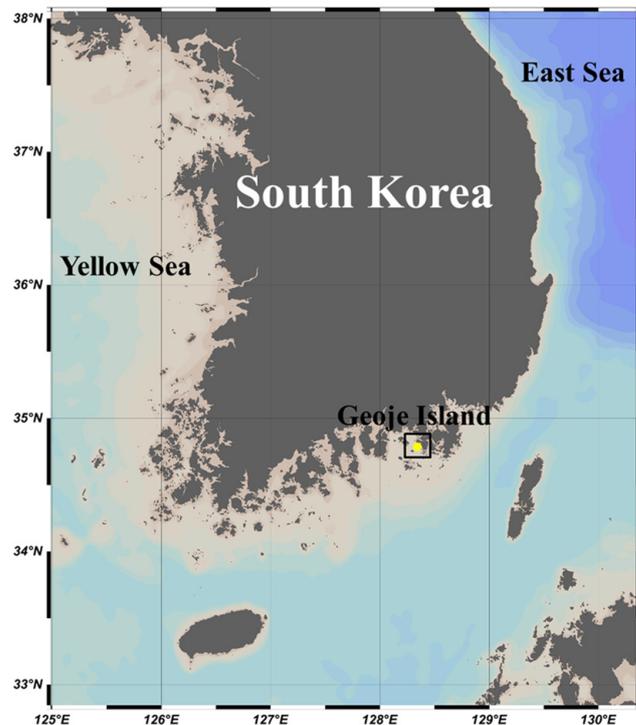


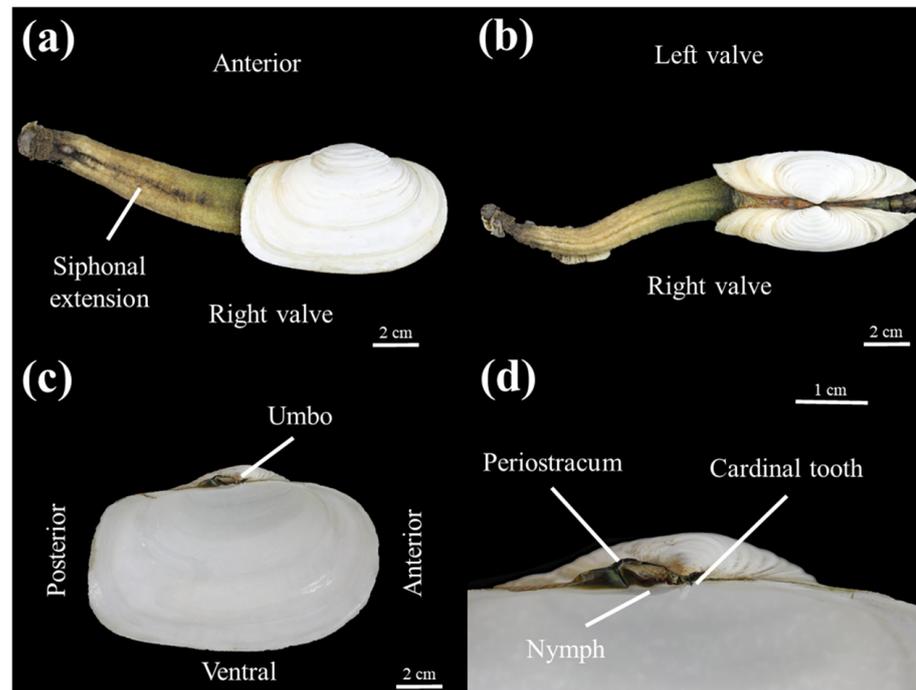
Figure 1. Sampling site of the Geoduck clam, *Panopea* sp. 1, on southern coast of South Korea.

Table 1. List of complete mitochondrial genomes used in this study.

Family	Species	Size (bp)	Accession No.
Arctiidae	<i>Arctica islandica</i>	18,289	NC_022709
Cardiidae	<i>Acanthocardia tuberculata</i>	16,104	NC_008452
Cardiidae	<i>Fulvia mutica</i>	19,110	NC_022194
Cardiidae	<i>Tridacna squamosa</i>	20,930	NC_026558
Hiatellidae	<i>Panopea abrupt</i>	15,381	KX494111
Hiatellidae	<i>Panopea generosa</i>	15,585	NC_025635
Hiatellidae	<i>Panopea globosa</i>	15,469	NC_025636
Hiatellidae	<i>Panopea</i> sp.	16,006	OQ469487
Lucinidae	<i>Loripes lacteus</i>	17,321	NC_013271
Lucinidae	<i>Lucinella divaricata</i>	18,940	NC_013275
Myidae	<i>Mya arenaria</i>	17,947	NC_024738

#### 2.4. Morphological Characteristics

For the morphological identification of the geoduck clam specimen from the southern coast of South Korea, the morphology of its shell was analyzed. Live geoduck clam specimens were fixed in 95% ethanol and their valves were dissected for hinge structure and shell surface sculpture observations. Photographs of the geoduck clams (Figure 2) were taken with a Copy Stand system equipped camera (CS-920, Nikon, Shimadzu, Japan). The voucher material was deposited at the Honam National Institute of Biological Resources (HNIBR). The morphological description was established by referring to Signorelli and Alfaya [19], who carried out a taxonomic revision of *Panopea abbreviata*.

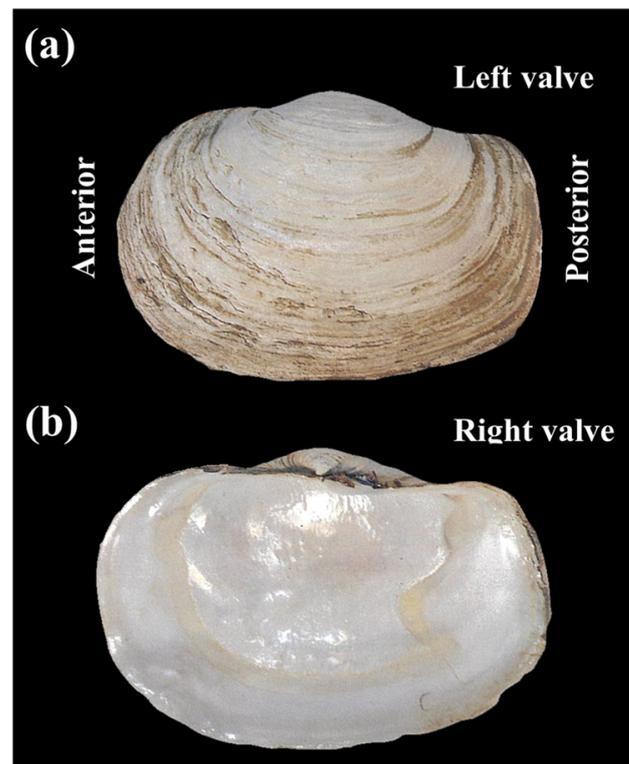


**Figure 2.** Photographs of *Panopea* sp. 1 examined in the present study. (a) Habitus, right side; (b) habitus, dorsal; (c) interior view of left valve; and (d) detail of hinge structure in left valve.

### 3. Results and Discussion

The species identification of geoduck clams in this study was described based on the significant morphological characteristics of the shell, such as shell thick to thin; externally white in color; external surface sculptured by irregular commarginal folds and widely spaced growth lines; outline elongated and subquadrate, with wide gaps at both ends; a length of 10 cm and height of up to 6.4 cm, a length-to-height ratio of 1.56, inequilateral, and slightly longer posteriorly; anterior end rounded, posterior end truncated to slightly produced ventrally, and ventral margin slightly convex; umbo weakly prominent and subcentral anteriorly (length from umbo to anterior end occupying 48% of the total length); a hinge with a dorsally recurved cardinal tooth; nymphal ridge short and covered by a ligament layer; the posterior ligament layer prominent, bilobed, dark brown in color, and with thin periostracum (tan in color); internal surface shear and white in color; pallial line continuous, separated from a ventral margin of approximately 0.9 cm; pallial sinus U-shaped, slightly deeper than high (length-to-height ratio 1.04); adductors positioned dorsally; posterior adductor scar reinforcement; and siphonal extension of 13.2 cm long in preserved specimens (in ethanol).

Based on the identified morphological features, the geoduck clam specimens from the southern coast of South Korea can be placed in the genus *Panopea*: an enlarged siphonal extension that fits into the mantle, elongated and subquadrate shells, a continuous pallial line, a short nymph, and one cardinal tooth in the hinge. Previously, the geoduck clam specimens collected from this region have been identified as *P. japonica*, originally reported from Japan. However, we found significant differences in the shell morphology of the geoduck clam specimens collected in this study and *P. japonica* (cf. Figure 3): (1) the length-to-height ratio of the clam shell was much larger in the study specimens (1.56) compared to that of *P. japonica* (1.39); (2) the postero-dorsal edge was relatively smooth in the study specimens, whereas as it is produced in *P. japonica*; the postero-ventral edge was slightly angular in the studied specimens, whereas it is rounded in *P. japonica*; and (3) the pallial sinus was much deeper in the studied specimens (the length-to-height ratio: 1.04) compared to that of *P. japonica* (1.59). These significant morphological differences made the geoduck clam specimens analyzed in this study readily distinguishable from *P. japonica*.



**Figure 3.** Photographic images of *Panopea japonica* after Okutano (2000) with no scale (a) exterior view of left valve; and (b) interior view of right valve.

Recently, Han et al. [16] reported a novel *Panopea* sp. from the eastern coast of South Korea, including its shell morphology, complete mitochondrial genome sequence, and reproductive information. The native geoduck clam collected for this study from the southern coasts of South Korea was very similar to the eastern *Panopea* sp., however, we found several differences in the conchological features between both species: (1) growth lines on external surface were more widely spaced in the southern population examined in the present study; (2) the umbo was located more anteriorly in the eastern populations (length from umbo to anterior margin occupying 42% in eastern populations, contrary to 48% in the southern population); and (3) the internal surface of the eastern populations had an additional line parallel to the posterior outline that has not been reported from other congeners (Han et al. [16]; Figure 2c). Although the species identification of the genus *Panopea* based on morphological taxonomy is possible, it is difficult to accurately distinguish and identify similar species using morphological data alone due to morphological ambiguity and diversity [20].

In this study, the complete mitochondrial genome of the novel *Panopea* sp. 1 was sequenced and deposited in GenBank (GenBank accession number: OQ469488). The complete mitochondrial genome length of *Panopea* sp. 1 was 15,910 bp. This length is shorter than that of *Panopea* sp. (16,006 bp), but longer than those of *Panopea abrupta* (15,381 bp), *Panopea globosa* (15,469 bp), and *Panopea generosa* (15,585 bp) [16,21]. Furthermore, the mitochondrial genome of *Panopea* sp. 1 encodes a complete set of 37 genes (13 PCGs, 22 transfer RNA genes, and 2 ribosomal RNA genes) and a control region (D-loop) (Table 2 and Figure 4). The nucleotide composition of its mitochondrial genome is: 25.3% A, 39.4% T, 10.6% C, and 24.8% G. This composition is similar, but slightly different from that of other species in the genus *Panopea*, such as *Panopea* sp. (25.8% A, 38.4% T, 11.4% C, and 24.4% G), *P. abrupta* (25.6% A, 38.8% T, 11.3% C, and 24.3% G), *P. generosa* (25.0% A, 38.7% T, 11.2% C, and 25.0% G), and *P. globosa* (23.3% A, 40.4% T, 10.1% C, and 26.1% G). The A + T and G + C contents of the 13 PCGs in the mitochondrial genome of *Panopea* sp. 1 were 64.2% and 35.8%, respectively, whereas those in all sequences of the mitochondrial genome

were 64.7% and 35.3%, respectively. In particular, the A + T content in the mitochondrial genome of *Panopea* sp. 1 was the same as that of *Panopea* sp. (64.2%) and similar to that of *P. abrupta* (64.4%), whereas it was higher than that of *P. generosa* (63.7%) and *P. globosa* (63.7%). Additionally, in *Panopea* sp. 1, all 13 PCGs used ATG as the start codon and TAA/TAG as stop codons. Twelve PCGs in *Panopea* sp. used ATG/GTG as the start codon, whereas *nad4* started with the ATA codon in *Panopea* sp. Collectively, the comparative analysis of the complete mitochondrial genome of *Panopea* sp. 1 revealed intraspecific variations in the mitochondrial genomes of the genus *Panopea*.

**Table 2.** Summary of *Panopea* sp. 1 mitogenome.

Full Gene Name	Location	Size (bp)	Start Codon	Stop Codon	Intergenic Region *
Cytochrome c oxidase subunit I (cox1)	1–1563	1563	GTG	TAG	0
D-lopp (DR)	1564–2308	745	-	-	0
Cytochrome c oxidase subunit II (cox2)	2309–3046	738	ATG	TAA	10
tRNA-Val (trnV)	3057–3121	65	-	-	2
tRNA-Thr (trnT)	3124–3189	66	-	-	68
tRNA-Tyr (trnY)	3258–3319	72	-	-	11
NADH dehydrogenase subunit 4L (nad4l)	3331–3621	291	ATG	TAG	52
ATP synthase F0 subunit 8 (atp8)	3674–3787	114	ATG	TAA	163
NADH dehydrogenase subunit 4 (nad4)	3951–5138	1188	ATG	TAG	0
tRNA-His (trnH)	5139–5202	64	-	-	0
tRNA-Glu (trnE)	5203–5266	64	-	-	−5
tRNA-Ser2 (trnS2)	5262–5324	63	-	-	3
NADH dehydrogenase subunit 3 (nad3)	5328–5693	366	ATG	TAG	2
tRNA-Ile (trnI)	5696–5762	67	-	-	6
tRNA-Asp (trnD)	5769–5832	64	-	-	4
tRNA-Lys (trnK)	5837–5899	63	-	-	2
tRNA-Leu2 (TrnL2)	5902–5966	65	-	-	0
NADH dehydrogenase subunit 1 (nad1)	5967–6890	924	ATG	TAA	1
tRNA-Leu1 (TrnL1)	6892–6956	65	-	-	33
tRNA-Asn (TrnN)	6990–7056	67	-	-	1
NADH dehydrogenase subunit 5 (nad5)	7058–8782	1725	ATG	TAA	−1
NADH dehydrogenase subunit 6 (nad6)	8782–9303	522	ATG	TAG	3
tRNA-Arg (trnR)	9307–9370	64	-	-	2
Cytochrome b (cob)	9373–10,530	1158	GTG	TAA	8
tRNA-Trp (trnW)	10,539–10,605	68	-	-	−52
16S ribosomal RNA (trnL)	10,554–11,838	1285	-	-	0
ATP synthase F0 subunit 6 (atp6)	11,839–12,546	708	ATG	TAA	7
tRNA-Met (trnM)	12,554–12,617	64	-	-	1
12S ribosomal RNA (rrnS)	12,619–13,481	863	-	-	0
Cytochrome c oxidase subunit III (cox3)	13,482–14,267	786	ATG	TAG	4
tRNA-Ser1 (trnS1)	14,272–14,338	67	-	-	0
NADH dehydrogenase subunit 2 (nad2)	14,339–15,385	1047	ATG	TAA	11
tRNA-Gln (trnQ)	15,397–15,462	66	-	-	8
tRNA-Phe (trnF)	15,471–15,533	63	-	-	14
tRNA-Cys (trnC)	15,548–15,612	65	-	-	22
tRNA-Pro (trnP)	15,635–15,701	67	-	-	10
tRNA-Gly (trnG)	15,712–15,777	66	-	-	14
tRNA-Ala (trnA)	15,792–15,856	65	-	-	-

\* Negative numbers indicate overlapping nucleotides between adjacent genes.

The molecular phylogenetic analysis revealed that the phylogenetic placement of *Panopea* sp. 1 was in parallel with previous results [4,16,21]. The molecular phylogenetic tree of the complete mitogenome showed that the *Panopea* sp. 1 identified in this study clustered with the genus *Panopea* (Figure 5). However, within the genus *Panopea*, *Panopea* sp. 1 formed a separate clade from the genus *Panopea*. In particular, *Panopea* sp. 1 constituted three *Panopea* species (*Panopea* sp., *P. abrupta*, and *P. japonica*), which were placed together as sister groups, indicating that they are independent *Panopea* species. Based on these findings, in

In addition to the molecular and morphological results of this study, the native geoduck clam collected from the southern coast of South Korea was identified as *Panopea* species compiles, thus *Panopea* sp. 1. These results corroborate that the complete mitochondrial genome sequence is useful for the accurate species identification in the genera *Panopea* species.

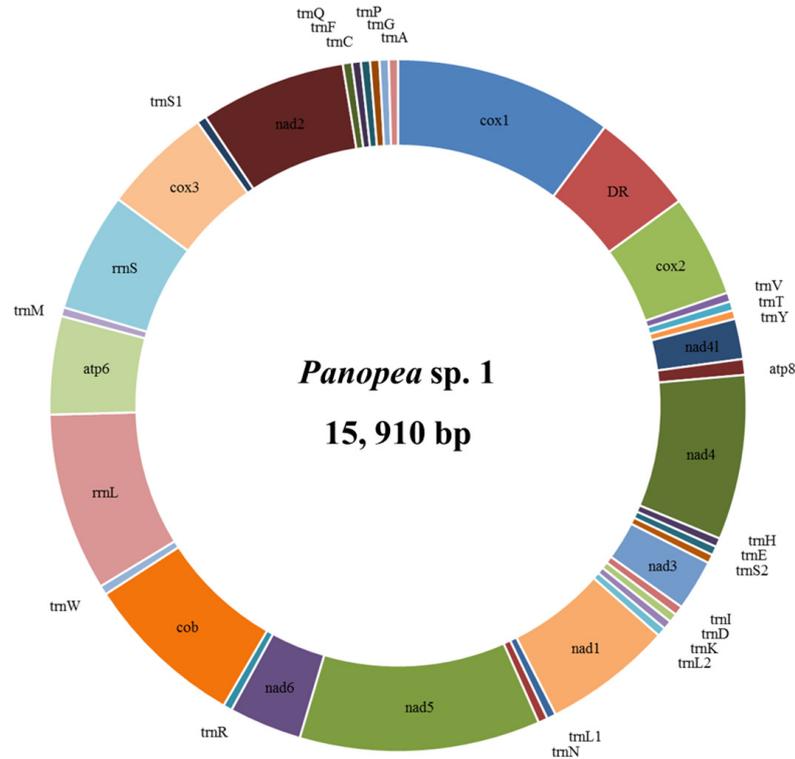


Figure 4. The mitochondrial genome map of *Panopea* sp. 1.

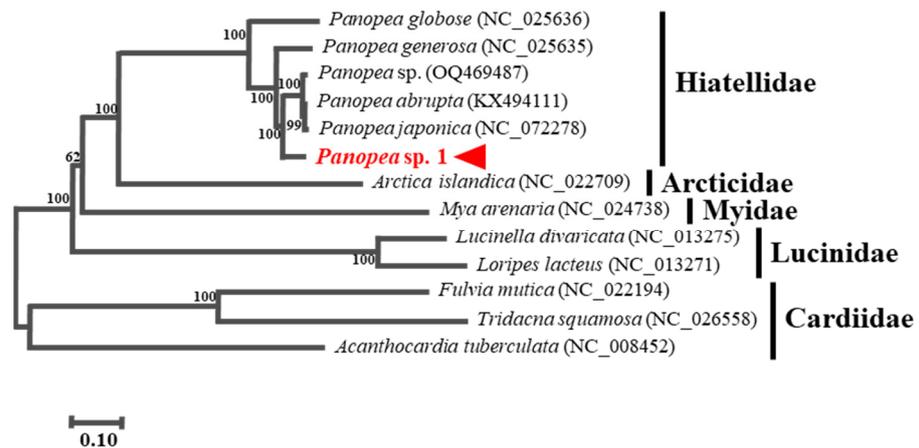


Figure 5. Maximum-likelihood phylogeny of the complete mitogenomes. The red triangle indicates the *Panopea* sp. 1 analyzed in this study.

In summary, based on its mitochondrial DNA sequence and morphological features, *Panopea* sp. 1 is the first-reported species from the southern coast of South Korea. The complete mitochondrial genome sequence was sufficient to identify the *Panopea* species collected from the southern coast of South Korea, supporting the usefulness of mitochondrial DNA-based markers in bivalve species identification. These findings will be useful for substantiating the precise molecular phylogeny for species identification and evolutionary studies in relation to the conservation and management of clam resources.

**Author Contributions:** Data curation, formal analysis, writing—original draft, J.H.; investigation, J.G.K., J.J.C.P. and K.-W.L.; conceptualization, formal analysis, project administration, funding acquisition, O.-N.K. and Y.-U.C. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** All experiments were conducted in compliance with the guidelines of the Institutional Animal Care and Experimental Committee of the Korea Institute of Ocean Science and Technology (KIOST) which approved the experimental protocol.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All data generated or analyzed during this study are available from the KIOST data repository. Materials are available upon request by the corresponding author.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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