

Article

# New Record of Geoduck Clam Collected from the East Coast of South Korea and Its Reproductive Characteristics

Jeonghoon Han <sup>1</sup>, Jong Guk Kim <sup>2</sup>, O-Nam Kwon <sup>3</sup> and Young-Ung Choi <sup>1,\*</sup>

<sup>1</sup> Marine Bio-Resources Research Unit, Korea Institute of Ocean Science & Technology (KIOST), Busan 49111, Republic of Korea

<sup>2</sup> Division of Zoology, Honam National Institute of Biological Resources, Mokpo 58762, Republic of Korea

<sup>3</sup> GABI Co., Ltd., Gangneung 25440, Republic of Korea

\* Correspondence: yuchoi@kiost.ac.kr

**Abstract:** Geoduck clams are important commercial marine resources in Southeast Asia. However, the complete mitochondrial genome of the native geoduck clam in South Korea has not been reported yet. This study reports a geoduck clam collected from the east coast of South Korea. Based on its morphological characteristics and molecular data, the geoduck clam was identified as *Panopea* sp. belonging to the bivalve family Hiatellidae. We determined the complete mitochondrial genome of this species and investigated the seasonal reproductive pattern for monthly changes in sex ratio, gametogenesis, and reproductive cycle from September 2019 to August 2020. The total length of the complete mitochondrial genome sequence is 16,006 bp, containing 13 protein-coding, 22 transfer RNA, 2 ribosomal RNA, and a control region (D-loop). A molecular phylogenetic comparison with 10 bivalve species showed that the geoduck clam specimen belongs to the family Hiatellidae, clustering with *Panopea abrupta* Chinese strain, *P. generosa*, and *P. globosa*. The gametogenesis of the geoduck clam was identified in six stages by histological analysis. The sex ratio was biased towards males from May 2019 to July 2019, the main spawning activity from the frequency of the gonads with ripe and spent stages occurred from October 2018 to April 2019, and there was no clear pattern of spawning peaks from May to August 2019 with gonads developed asynchronously between both sexes. This is the first study to report new records of the genus *Panopea* based on the complete mitochondrial genome and to examine the seasonal reproductive pattern of the native geoduck clam *Panopea* sp. in South Korea, providing valuable information for its sustainable management in South Korea.

**Keywords:** *Panopea*; Hiatellidae; mitochondrial genome; burrowing bivalves



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## 1. Introduction

The genus *Panopea* (geoduck clams) is one of the largest burrowing bivalves in the world and one of the longest-lived animals, with a reported record age of 168 years [1]. These clams were known to be a cosmopolitan species during the Triassic, accommodating 150 fossil species, and 10 living species were distributed in temperate to subtropical waters [2]. In East Asia, two *Panopea* species are commonly found in coastal subtidal regions: *P. abrupta* in China [3] and *P. japonica* in Japan and Korea [2,4,5].

Geoduck clams have been widely cultivated as commercial marine fisheries resources in Southeast Asia and the southeast coastal region of China. Currently, China is one of the largest markets for geoduck clams [6]. However, despite their high commercial importance, natural geoduck clam populations have declined owing to rising market demand and overfishing [7,8]. In Korea, although previous studies on geoduck clams have focused on their ecology, biological characteristics, and seed production [9–12], currently, research on geoduck clams is lacking, and no species identification of geoduck clams has been reported to date. Therefore, understanding species diversity and population density is crucial for

the sustainable management of fishery resources and the conservation of important bivalve species, such as geoduck clams.

Mitochondrial genomes have been widely used as genetic markers for phylogenetic analyses and species identification [13,14]. Recently, complete animal mitochondrial genomes have also been applied to DNA barcoding-based comparative genomics, conservation biology, and evolutionary genomics [15,16]. Previous studies have reported the complete mitochondrial genome of geoduck clams, including *Panopea generosa* [17], *Panopea globosa* [18], and *Panopea abrupta* [3]. However, to date, there has been no report of the complete mitochondrial genome of the native geoduck clam in South Korea.

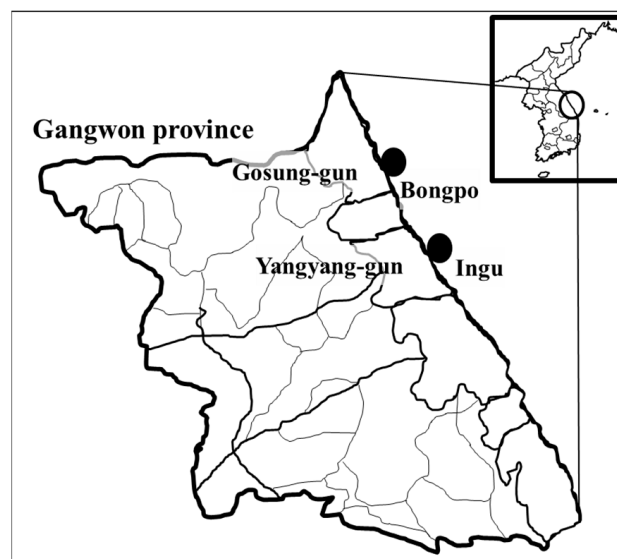
In general, for the conservation, management, and sustainable use of bivalve resources, many environmental factors (e.g., temperature, salinity, and food type) that influence the functioning of bivalve physiological processes must be considered. In particular, temperature is the main environmental factor influencing various physiological processes, including growth and reproduction in marine organisms [19,20]. Temperature influences the reproductive characteristics of marine bivalves, including clams [21–25]. In addition, understanding the reproductive system is essential for the mass production of commercially valuable marine bivalves. However, no information is available on the characteristics of the reproductive biology of the native geoduck clam in South Korea.

In this study, we sequenced the complete mitochondrial genome of native geoduck clam specimens collected from South Korea, providing species identification based on brief morphological information and phylogenetic classification. In addition, we investigated the sex ratio, gametogenesis, and reproductive cycle of native geoduck clams. This study provides important information for future populations and evolutionary studies associated with the conservation of native geoduck clams in South Korea.

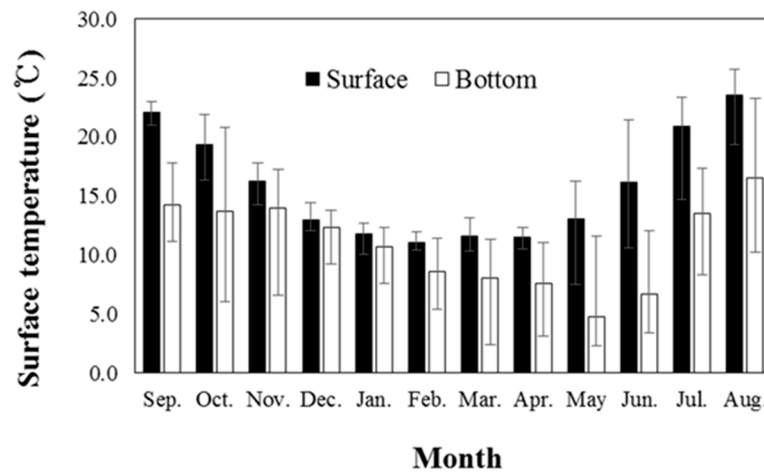
## 2. Materials and Methods

### 2.1. Sampling Collection

Sampling for this study was conducted using a hookah diving system from Gosung's Bongpo and Yangyang's Ingu on the east coast of South Korea. The sampling areas are shown in Figure 1. Live geoduck clams were collected every month (20 bivalves/month) from subtidal medium sand and coarse sand (Mean  $\phi$   $2.1 \pm 0.4$  mm) at 13.0–27.3 m depth in Gangwon province, approximately 40–60 cm deep in soft sediments, from September 2018 to August 2019. At the time of sampling, the surface (the bottom-water temperature at 30 m depth) and the surface (bottom) salinity ranged from 11.0–23.5 (8.0–16.5 °C) and 28.3–34.2 (34.0–35.9 psu), respectively (Figure 2).



**Figure 1.** Sampling sites of the Eastern Geoduck clam, *Panopea* sp. on the east coast of South Korea.



**Figure 2.** Surface (0 m) and bottom (30 m) water temperatures (°C) of the sampling sites of Eastern Geoduck clam, *Panopea* sp. in South Korea.

### 2.2. DNA Extraction and Sequencing

Genomic DNA was extracted from muscle tissues using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer’s protocol. The quantity and quality of the extracted genomic DNA were measured using a Victor<sup>3</sup> multilabel plate reader (PerkinElmer, Waltham, MA, USA). A genomic library was constructed using the TruSeq Nano DNA Kit by a commercial company (Macrogen, Seoul, Republic of Korea) according to the manufacturer’s instructions (Illumina, San Diego, CA, USA). The complete mitochondrial genome of *Panopea* sp. was assembled and annotated using MITOS [26].

### 2.3. Sequence Alignment and Phylogenetic Analysis

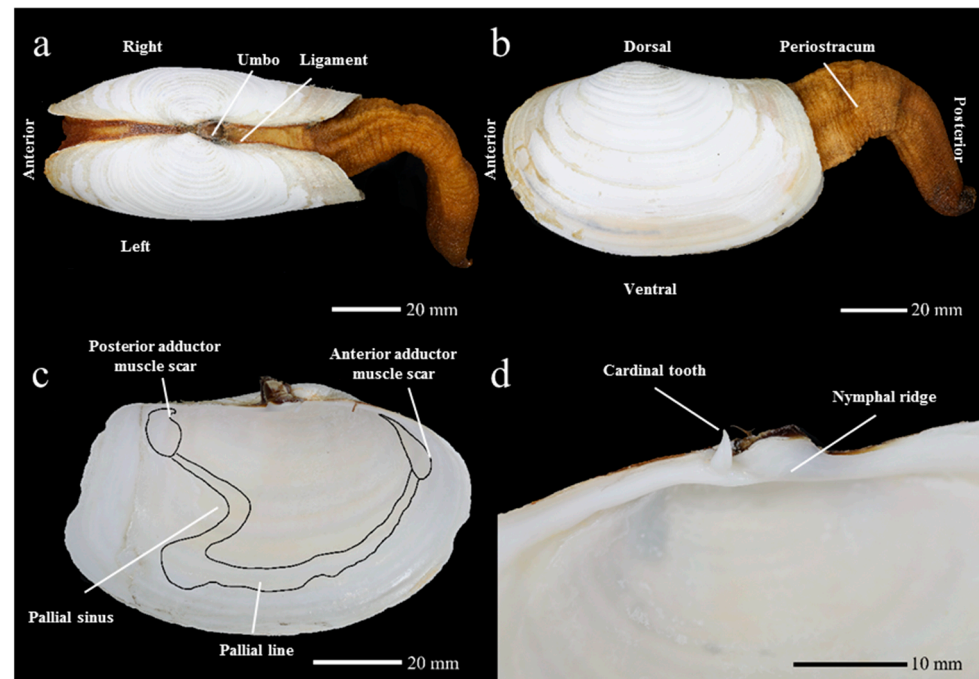
The complete mitochondrial genomes of 10 bivalve species (5 families; Arctiidae, Cardiidae, Hiatellidae, Myidae, and Lucinidae) were downloaded from the GenBank database and used to construct a phylogenetic tree (Table 1). The concatenated set of the 13 protein-coding genes (PCGs) for each mitochondrial genome was aligned using the ClustalW algorithm in MEGA software (ver. 10.0.1; Center for Evolutionary Medicine and Informatics, Tempe, AZ, USA). To establish the best-fit substitution model for phylogenetic analysis, the model with the lowest Bayesian Information Criterion and Akaike Information Criterion scores was estimated using a maximum-likelihood (ML) analysis. According to the results of the model test, ML phylogenetic analyses were performed using the LG + G + I model in the MEGA software. Support for the nodes was calculated using 1000 bootstrap replicates.

**Table 1.** List of complete mt 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 abrupta</i>	15,381	KX494111
Hiatellidae	<i>Panopea generosa</i>	15,585	NC_025635
Hiatellidae	<i>Panopea globosa</i>	15,469	NC_025636
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 species identification of the Korean geoduck clam, a morphological analysis of the shell characters was performed. Living specimens of *Panopea* sp. were fixed in 95% ethanol, and their valves were dissected for observation of hinge structure and shell surface sculpture. Photographic images of *Panopea* sp. (Figure 3) were taken using a camera equipped with a Copy Stand system (CS-920, Nikon, Tokyo, Japan). Voucher material was deposited at the Honam National Institute of Biological Resources (HNIBR). The terminology of morphological description follows a previous study on the taxonomic revision of *Panopea abbreviata* [27].



**Figure 3.** Photographs of *Panopea* sp. examined in the present study. (a) dorsal view of the habitus; (b) lateral view of the habitus (left side); (c) interior of the left valve; and (d) hinge of the right valve.

#### 2.5. Histological Analysis

Every month, 20 samples of geoduck clams were randomly selected for histological examination in the rearing tank. Shell length (right valve) and height were measured using vernier calipers ( $\pm 0.1$  mm), and whole-body weight was weighted to the nearest gram using a digital balance (Toledo, AB-204, error = 0.01 g). The shells were opened, the gonads were collected using scissors and a knife, and two small pieces of each gonad of clam (approximately 0.5 cm) were preserved in a Bouin's solution (5% glacial acetic acid, 9% formaldehyde, and 0.9% picric acid) for 24 h and then stored in 70% ethanol. Gonad samples were preserved for analysis. For analysis, the gonad samples were dehydrated using an ethanol series and soaked in xylene and paraffin. The samples were then embedded in a cassette and sectioned on a microtome. The 5- $\mu$ m sections were placed on a slide and then kept on a hot plate for 24 h. The sectioned sample was stained with Mayer's hematoxylin-eosin and observed under a light microscope (BX 50, X200; Olympus, Tokyo, Japan). Gonadal development was categorized into six stages, as described in a previous study [28]. The sex ratio was estimated for the total sample and analyzed to verify the deviations from a 1:1 proportion using the chi-square test.

### 3. Results and Discussion

The geoduck clam specimens collected from the east coast of South Korea were assigned as members of the genus *Panopea* based on the following generic morphological features: the presence of one cardinal tooth in the hinge and an enlarged siphonal extension

that fit into the mantle [2]. The morphological features of the Korean specimens were as follows: a thick to thin shell, subquadrate, white in color, with gaping anteriorly and posteriorly; a wider posterior gap than the anterior one; length from beak to anterior margin occupying 42% of the total shell length; an external surface with a sculpture composed of commarginal folds and weak growth lines; rounded anterior end, posterior ridge truncated to slightly produced ventrally; slightly concave ventral margin; weakly prominent umbo, subcentral anteriorly; prominent ligament, dark brown in color; thin periostracum, tan in color, dehiscent; short nymph; a hinge with a single cardinal tooth, which slightly curved towards the dorsal margin, in each valve; small anterior adductor scar, dorsally positioned; posterior adductor scar reinforces, positioned dorsally; continuous pallial line; anterior line slightly produced; and posterior line truncate. The pallial sinuses are shallow in both valves. The postvalvular extension was 710 mm long in preserved specimens (in ethanol) with fused siphons distally; a length of up to 960 mm; and a height of up to 590 mm.

In this study, we sequenced the complete mitochondrial genome of *Panopea* sp. The length of the complete mitochondrial genome of *Panopea* sp. was 16,006 bp (GenBank number: OQ469487), which is longer than those of *P. abrupta* (15,381 bp), *P. globosa* (15,469 bp), and *P. generosa* (15,585 bp) [3]. The complete mitochondrial genome of *Panopea* sp. contained 13 PCGs, 22 transfer RNAs, 2 ribosomal RNA genes, and a control region (D-loop) (Table 2 and Figure 4a). The overall base composition of the mitogenome is A 25.8%, T 38.4%, C 11.4%, and G 24.4%, which is similar but slightly different from that of *P. abrupta* (A 25.6%, T 38.8%, C 11.3%, and G 24.3%), *P. generosa* (A 25.0%, T 38.7%, C 11.2%, and G 25.0%), and *P. globosa* (A 23.3%, T 40.4%, C 10.1%, and G 26.1%). Additionally, species-specific differences may be attributed to differences in composition. For example, the A+T and G+C compositions of the 13 PCGs were 63.7% and 36.3% in the mitochondrial genome of *Panopea* sp. and 64.2% and 35.8% in all sequences, respectively. The ratio of A+T nucleotides in the mitochondrial genome of *Panopea* sp. was similar to that of *P. abrupta* (64.4%) but higher than those of congeneric species *P. generosa* (63.7%) and *P. globosa* (63.7%). In addition, in *Panopea* sp., the 12 PCGs initiate with the start codon ATG/GTG whereas nad4 initiates with the start codon ATA. All PCGs were terminated with TAA/TAG. In contrast to *Panopea* sp., 12 PCGs initiate with the start codon ATG/GTG, whereas COXIII has the start codon ATA in *P. abrupta*. All PCGs were terminated with TAA/TAG. Therefore, the comparative analysis of mitogenomes in *Panopea* sp. showed species-specific differences in the mitogenomes of *Panopea* species.

In this study, the molecular phylogenetic tree showed that the *Panopea* species collected from the east coast of South Korea were genetically distinct from each other and belonged to the genus *Panopea* (Figure 4b). In particular, Korean *Panopea* species clustered with the *P. abrupta* Chinese strain with a bootstrap value of 100%. A previous study reported the occurrence of *P. abrupta* on Chinese coasts, determined its complete mitochondrial genome sequence, and found a sister relationship between *P. abrupta* and *P. generosa*. However, the validity of the species identification is uncertain since the species name of *P. abrupta* was previously used for a fossil species [29]. The taxonomy and nomenclature of *P. abrupta* have a complex history. *P. abrupta* was originally introduced from the Miocene deposits of Oregon in the Northwest Pacific region of the USA; however, this species name had been erroneously considered as an extant species *P. generosa* occurring in the Northeastern Pacific coasts in the previous malacological literature published in 1983–2009 [29]. Based on the morphological differences between the holotypes of the two species in the anterior gap, the junior synonym of *P. generosa* from *P. abrupta* was removed. Gould's holotype of *P. generosa* has a distinct anterior gap whereas it is absent in Conard's fossils of *P. abrupta*. Thus, we suspect that the geoduck clam specimens collected from the coast of China might be an undescribed species of *Panopea*. Additionally, in our study, although the molecular phylogenetic results of the complete mitogenomes of *Panopea* sp. from China and South Korea showed a close relationship with *P. generosa* (Figure 4b), they are distinct species based on the following morphological differences: the ratio of shell height/shell length in *P. generosa* (0.66; Figures 5 and 6) is slightly higher than that in *Panopea* sp. (0.61; Figure 3b,c);

the anteroventral margin of the shell in *P. generosa* is weakly angled whereas it is smoothly rounded in our *Panopea* sp.; in dorsal view, the outlines of both shells in *P. generosa* are nearly parallel from the umbo to the posterior end (Figure 4) compared to those of our *Panopea* sp. (Figure 3a); and the pallial sinus of *Panopea* sp. is distinctly deep whereas it is weakly developed in *P. generosa* (Figure 3c) [29]. Taken together, our results suggest that the geoduck clam specimens collected are the first report of the genera *Panopea* on the east coast of South Korea.

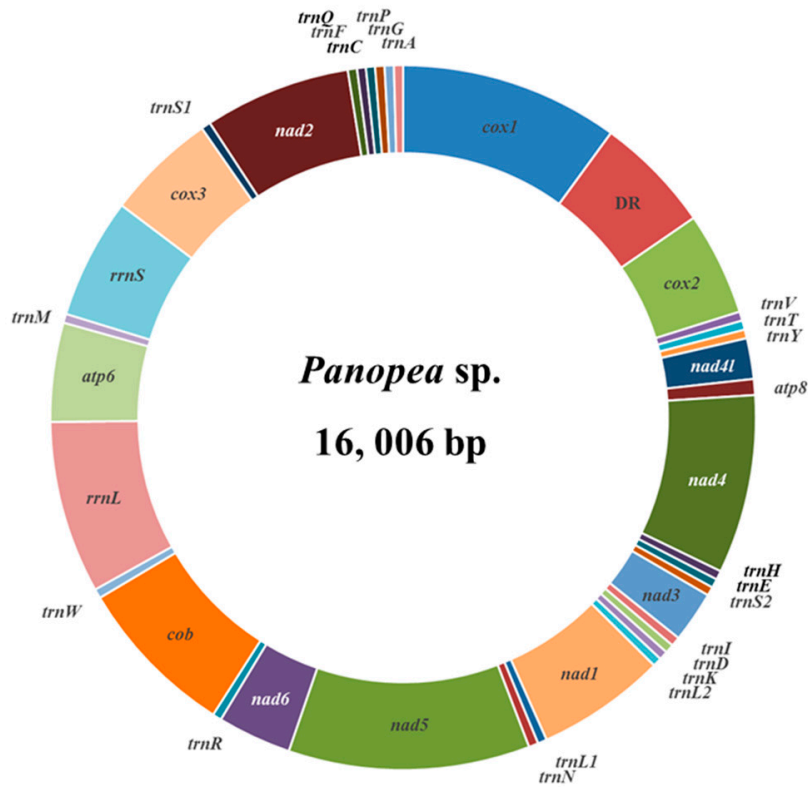
**Table 2.** Summary of *Panopea* sp. mitogenome.

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)	1564–2401	838	-	-	0
Cytochrome c oxidase subunit II (cox2)	2402–3139	738	ATG	TAA	10
tRNA-Val (trnV)	3150–3214	65	-	-	2
tRNA-Thr (trnT)	3217–3282	66	-	-	69
tRNA-Tyr (trnY)	3352–3413	62	-	-	10
NADH dehydrogenase subunit 4L (nad4l)	3424–3714	291	ATG	TAG	52
ATP synthase F0 subunit 8 (atp8)	3767–3880	114	ATG	TAA	163
NADH dehydrogenase subunit 4 (nad4)	4044–5231	1188	ATA	TAG	0
tRNA-His (trnH)	5232–5295	64	-	-	0
tRNA-Glu (trnE)	5296–5359	64	-	-	–5
tRNA-Ser2 (trnS2)	5355–5417	63	-	-	3
NADH dehydrogenase subunit 3 (nad3)	5421–5786	366	ATG	TAG	–1
tRNA-Ile (trnI)	5786–5852	67	-	-	6
tRNA-Asp (trnD)	5859–5922	64	-	-	4
tRNA-Lys (trnK)	5927–5989	63	-	-	2
tRNA-Leu2 (TrnL2)	5992–6056	65	-	-	0
NADH dehydrogenase subunit 1 (nad1)	6057–6980	924	ATG	TAG	1
tRNA-Leu1 (TrnL1)	6982–7046	65	-	-	32
tRNA-Asn (TrnN)	7079–7146	68	-	-	1
NADH dehydrogenase subunit 5 (nad5)	7148–8872	1725	ATG	TAA	–1
NADH dehydrogenase subunit 6 (nad6)	8872–9399	528	ATG	TAA	–1
tRNA-Arg (trnR)	9399–9462	64	-	-	2
Cytochrome b (cob)	9465–10,618	1154	GTG	TAA	13
tRNA-Trp (trnW)	10,632–10,698	67	-	-	0
16S ribosomal RNA (trnL)	10,699–11,931	1233	-	-	0
ATP synthase F0 subunit 6 (atp6)	11,932–12,639	708	ATG	TAA	7
tRNA-Met (trnM)	12,647–12,710	64	-	-	1
12S ribosomal RNA (rrnS)	12,712–13,574	863	-	-	0
Cytochrome c oxidase subunit III (cox3)	13,575–14,360	786	ATG	TAG	4
tRNA-Ser1 (trnS1)	14,365–14,431	67	-	-	0
NADH dehydrogenase subunit 2 (nad2)	14,432–15,478	1047	ATG	TAA	13
tRNA-Gln (trnQ)	15,492–15,557	66	-	-	8
tRNA-Phe (trnF)	15,566–15,628	63	-	-	14
tRNA-Cys (trnC)	15,643–15,707	65	-	-	22
tRNA-Pro (trnP)	15,730–15,796	67	-	-	10
tRNA-Gly (trnG)	15,807–15,872	66	-	-	15
tRNA-Ala (trnA)	15,888–15,952	65	-	-	-

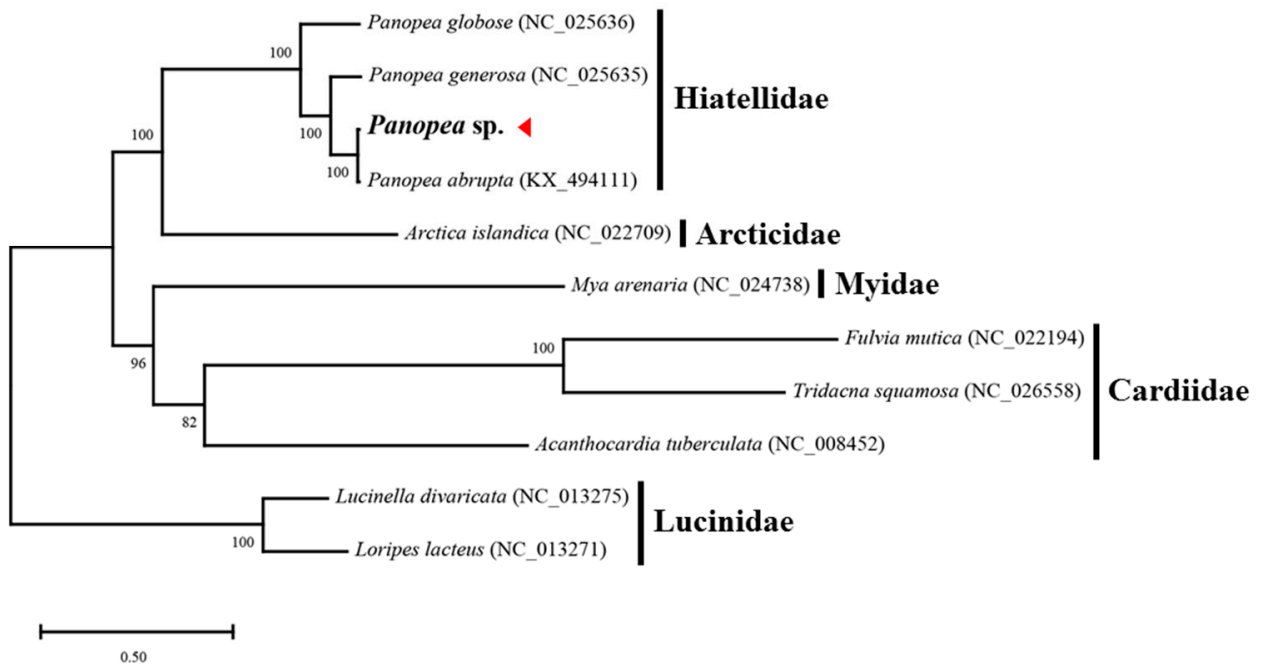
\* Negative numbers indicate overlapping nucleotides between adjacent genes.

Table 3 shows the sex ratio during the study period. Of the total 220 individuals, 77 (35.0%) were females, 137 (62.3%) were males, and 6 (2.7%) were undifferentiated. The sex ratio of the geoduck clams was skewed towards males from May 2019 to July 2019. The overall female-to-male ratio was 0.56:1, which significantly differed from 1:1 (Chi-square test,  $p < 0.05$ ,  $df = 1$ ). A previous study reported that the sex ratio of *P. abbreviata* was biased toward males with a total length of less than 90 mm [28]. Moreover, previous studies have suggested that a disparate sex ratio in *P. abbreviata* related to protandry occurs in the *Panopea* genus [28,30–32]. In the present study, we observed sex ratio bias in males between May 2019 and July 2019, when individuals smaller than females intensively appeared. This also appears to be due to the characteristics of the protandry mechanism of this species. However, the hermaphrodites of this species have not been detected, and the patterns of the protandry mechanism require further investigation.

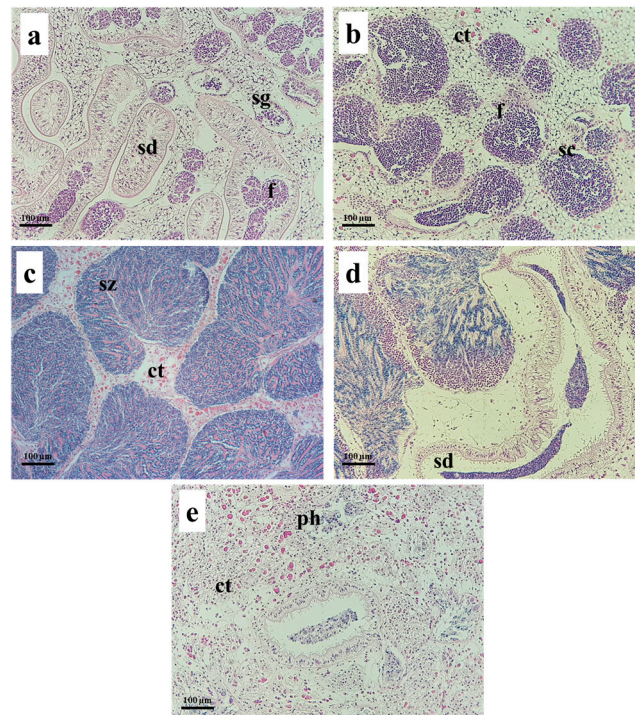
(a)



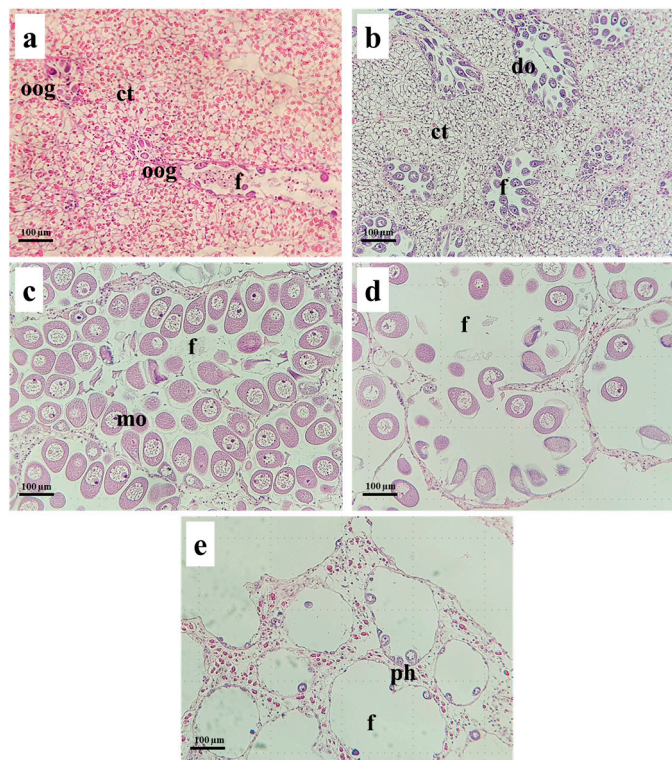
(b)



**Figure 4.** (a) The mitochondrial genome map of geoduck clam, *Panopea* sp. (b) Maximum-likelihood phylogeny of the complete mitogenomes. The red triangle indicates the *Panopea* sp. analyzed in this study.



**Figure 5.** Photomicrographs of histological sections of the geoduck clam, *Panopea* sp. male gonads at gametogenesis. (a) Early active; (b) Late active; (c) Ripe; (d) Spawning, and (e) Spent stages. Scale bar = 100 μm. sg, spermatogonia; sd, sperm duct; ct, connective tissue; f, follicle; sc, spermatopcyte; sz, spermatozoa; ph, phagocytes.



**Figure 6.** Photomicrographs of histological sections of the geoduck clam, *Panopea* sp. female gonad at gametogenesis. (a) Early active; (b) Late active; (c) Ripe; (d) spawning, and (e) Spent stages. Scale bar = 100 μm. oog, oogonia; ct, connective tissue; f, follicle; do, developing oocytes; mo, maturing oocytes; ph, phagocytes.



**Table 3.** Number and size ranges of females and males, and sex ratios for each monthly sample (undifferentiated individuals are omitted).

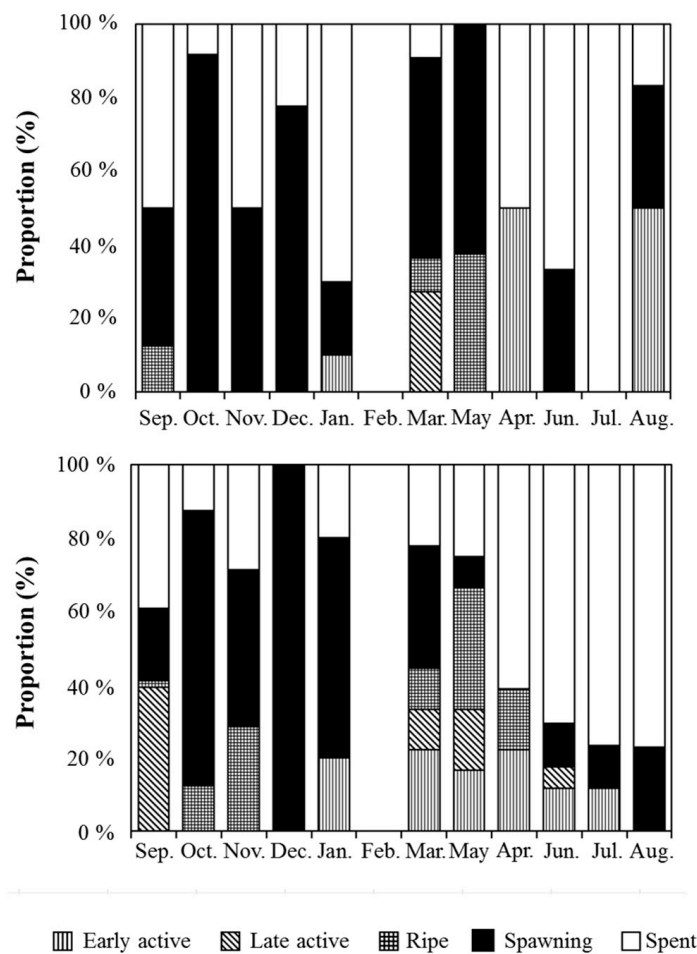
Month	Sex Categories		Sex Ratio
	Female	Male	
September	8 (85.9–111.3)	9 (91.7–113.8)	0.88:1
October	12 (101.5–127.0)	8 (103.5–129.0)	1.50:1
November	6 (82.4–120.7)	14 (80.5–129.0)	0.42:1
January	9 (104.0–117.2)	10 (101.5–134.4)	1:1
February	nd	nd	nd
March	11 (80.5–130.5)	9 (80.5–128.5)	1.22:1
April	8 (80.5–125.1)	12 (82.4–127.0)	0.66:1
May	2 (109.4–117.2)	18 (82.4–122.6)	0.11:1 *
June	3 (105.5–125.1)	17 (78.5–112.8)	0.17:1 *
July	2 (103.3–118.2)	17 (74.6–115.5)	0.11:1 *
August	6 (81.6–135.9)	13 (86.3–112.8)	0.46:1
Total	77	137	0.56:1 *

\*, differs significantly from 1:1 (Chi-square test,  $p < 0.05$ ,  $df = 1$ ); nd, no data available.

The microscopic features of the process of developing gonads in male and female geoduck clams are shown in Figures 5 and 6. The early active stage in males is characterized by small follicles filled with spermatogonia (Figure 5a). Oogonia were attached to the inner follicle wall, and some developing oocytes were observed in females (Figure 6a). In the late active stage, the follicles contained numerous spermatocytes, which are rounded cells that are highly eosinophilic in the male gonads (Figure 5b), and the primary oocytes increased in size and number in the follicles of the female gonads (Figure 6b). In the ripe stage in males, characterized by large, full follicles filled with spermatozoa (Figure 5c), the follicles were filled with mature oocytes in the female gonads (Figure 6c). During the spawning stage of males, the gonad follicles of the spawned males showed an empty area that contained an array of spermatozoa (Figure 5d). Additionally, the female gonad follicles showed a sparse form, indicating that the remaining oocytes were free from the follicle wall (Figure 6d). In the spent stage, the male gonads were atrophic and irregular in follicles and often formed sperm ducts filled with connective tissue (Figure 5e). In females, empty follicles surrounded by connective tissue females and residual oocytes were observed (Figure 6e). In previous studies, the gonadal development of *P. ganerosa*, *P. abrupta*, and *P. abbreviata* was assigned to one of six stages [28,30,32], including undifferentiated stages, which are indistinguishable from immature males and females. In the present study, the gonadal development stage was assigned according to previous studies and classified into six types with slight modifications.

The ripe and spawning stages of gonads were observed from October 2018 to April 2019 with dominant stages in both sexes when the water temperature dropped below 19.3 °C (surface) and 13.7 °C (bottom) and increased to 11.5 °C (surface) and 7.5 °C (bottom) (Figures 2 and 7). Subsequently, the ripe and spawning stages were observed intermittently in both sexes with differences in gonadal development timing or asynchrony from May to August 2019. The frequency of the gonad stage indicated that the main spawning activity occurred from October to April, with no clear spawning pattern from May to August 2019 (Figures 5 and 6). In previous studies, burrowing species such as *Panopea abbreviata*,

*P. japonica*, *Protothaca antiqua*, and *Ensis macha*, these species have a protracted spawning season or two periods of spawning seasons [28,33–36]. Moreover, a previous study reported that *P. abbreviata* populations in northern Argentinean Patagonia show a protected spawning season with no clear pattern of spawning peaks and suggested that *P. abbreviata* live in thermal stability environments, such as burrowing species buried more than 70 cm in soft sediments, which could explain the protracted spawning period [28]. In our study, geoduck clams were sampled at more than 40 cm depth in sediments, and it seems that there was a protracted spawning season with no clear pattern of spawning peaks in thermal stability in sediments. However, the patterns of spawning ratios with the protandry mechanism have not been determined. The occurrence pattern of hermaphrodites at this site should be further investigated to understand their reproductive strategy.



**Figure 7.** Monthly variation in gonad developmental stages of geoduck clam, *Panopea* sp. collected from the east coast of South Korea from September 2019 to August 2020. Upper, females; lower, males.

In summary, we determined, for the first time, the complete mitochondrial genome of the geoduck clam specimen collected from the east coast of South Korea and conducted phylogenetic analyses within the subclass *Heterodonta*. In addition, we investigated the monthly changes in the sex ratio, gametogenesis, and reproductive cycle of *Panopea* sp. from September 2019 to August 2020 in females and males. Overall, this study provides critical information for population and conservation genetics for the management and use of clam resources.

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

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## References

- Bureau, D.; Hajas, W.; Surry, N.W.; Hand, C.M.; Dovey, G.; Campbell, A. *Age, Size Structure, and Growth Parameters of Geoducks (Panopea abrupta Conrad, 1849) from 34 Locations in British Columbia Sampled between 1993 and 2000*; Canadian Technical Report of Fisheries and Aquatic Sciences Nanaimo 2413; Fisheries and Oceans Canada: Nanaimo, BC, Canada, 2002; pp. 1–84.
- Leyva-Valencia, I.; Cruz-Hernández, P.; Álvarez-Castañeda, S.T.; Rojas-Posadas, D.I.; Correa-Ramírez, M.M.; Vadopalas, B.; Lluch-Cota, D.B. Phylogeny and Phylogeography of the Geoduck *Panopea* (Bivalvia: Hiatellidae). *J. Shellfish Res.* **2015**, *34*, 11–20. [[CrossRef](#)]
- Yu, M.; Zhong, S.; Yang, S.; Chen, J.; Saha, T.T. The complete mitochondrial genome of *Panopea abrupta* (Myoida: Hiatellidae). *Mitochondrial DNA Part A* **2016**, *27*, 711–712. [[CrossRef](#)] [[PubMed](#)]
- Habe, T. Fauna of Akkeshi Bay XXI. Pelecypoda and Scaphopoda. *Public Akkeshi Mar. Biol. Stn.* **1955**, *4*, 20–27.
- Min, D.-K.; Lee, J.-S.; Koh, D.-B.; Je, J.-G. *Mollusks in Korea*; Min Molluscan Research Institute: Seoul, Republic of Korea, 2004; p. 566.
- Welch, C. China's Demand for Geoducks Send Prices, Profits Soaring. 2012. The Bellingham Herald Online. Available online: [https://www.heraldandnews.com/china-s-demand-for-geoducks-sends-prices-profits-soaring/image\\_612787da-9bf5-11e1-84c8-0019bb2963f4.html](https://www.heraldandnews.com/china-s-demand-for-geoducks-sends-prices-profits-soaring/image_612787da-9bf5-11e1-84c8-0019bb2963f4.html) (accessed on 15 May 2012).
- Fan, X.B. A model for sustainable fisheries: Canadian geoduck fishery management. *Ocean Fish.* **2012**, *12*, 78–87. (In Chinese)
- Shamshak, G.L.; King, J.R. From cannery to culinary luxury: The evolution of the global geoduck market. *Mar. Policy* **2015**, *55*, 81–89. [[CrossRef](#)]
- Lee, C.S. Studies on feeding activity and environmental tolerance of geoduck clam, *Panopea generosa*. *J. Aquac.* **1997**, *10*, 213–218. (In Korean)
- Lee, C.S.; Rho, S.; Park, Y.J. Studies on the artificial seedling production of geoduck clam, *Panope japonica*. I. Spawning induction and hatching. *J. Aquac.* **1997**, *10*, 113–121. (In Korean)
- Lee, C.S.; Rho, S. Studies on the artificial seedling production of geoduck clam, *Panope japonica*. II. Development of egg and larvae. *J. Aquac.* **1997**, *10*, 25–32. (In Korean)
- Lee, C.S.; Baik, K.K.; Hong, K.E. Ecological studies on the habitat of geoduck clam, *Panope japonica*. *J. Aquac.* **1998**, *11*, 105–111. (In Korean)
- Elson, J.L.; Lightowlers, R.N. Mitochondrial DNA clonality in the dock: Can surveillance swing the case? *Trends Genet.* **2006**, *22*, 603–607. [[CrossRef](#)]
- Botero-Castro, F.; Tilak, M.; Justy, F.; Catzeflis, F.; Delsuc, F.; Douzery, E.J. Next-generation sequencing and phylogenetic signal of complete mitochondrial genomes for resolving the evolutionary history of leaf-nosed bats (*Phyllostomidae*). *Mol. Phylogenet. Evol.* **2013**, *69*, 728–739. [[CrossRef](#)]
- Kurbalija Novicic, Z.; Immonen, E.; Jeli, M.; AnDelkovic, M.; Stamenkovic-Radak, M.; Arnqvist, G. Within-population genetic effects of mtDNA on metabolic rate in *Drosophila subobscura*. *J. Evol. Biol.* **2015**, *28*, 338–346. [[CrossRef](#)]
- Qiu, F.; Kitchen, A.; Beerli, P.; Miyamoto, M.M. A possible explanation for the population size discrepancy in tuna (genus *Thunnus*) estimated from mitochondrial DNA and microsatellite data. *Mol. Phylogenet. Evol.* **2013**, *66*, 463–468. [[CrossRef](#)]
- Bisbal-Pardo, C.I.; Del Río-Portilla, M.A.; Rocha-Olivares, A. The complete mitochondrial DNA of the Pacific Geoduck clam (*Panopea generosa*). *Mitochondrial DNA* **2014**, *27*, 1955–1956. [[CrossRef](#)]
- Bisbal-Pardo, C.I.; Del Río-Portilla, M.A.; Rocha-Olivares, A. Novel gene arrangement in the complete mitochondrial genome of the Cortes Geoduck (*Panopea globosa*). *Mitochondrial DNA Part A* **2016**, *27*, 1957–1958. [[CrossRef](#)]
- Parmesan, C. Ecological and Evolutionary Responses to Recent Climate Change. *Annu. Rev. Ecol. Evol. Syst.* **2006**, *37*, 637–669. [[CrossRef](#)]

20. Paaijmans, K.P.; Heinig, R.L.; Seliga, R.A.; Blanford, J.I.; Blanford, S.; Murdock, C.C.; Thomas, M.B. Temperature variation makes ectotherms more sensitive to climate change. *Glob. Chang. Biol.* **2013**, *19*, 2373–2380. [[CrossRef](#)]
21. Li, Q.; Liu, W.; Shirasu, K.; Chen, W.; Jiang, S. Reproductive cycle and biochemical composition of the Zhe oyster *Crassostrea plicatula* Gmelin in an eastern coastal bay of China. *Aquaculture* **2006**, *261*, 75–759. [[CrossRef](#)]
22. Aragón-Noriega, E.A.; Chávez-Villalba, J.; Gribben, P.E.; Alcántara-Razo, E.; Maeda-Martínez, A.N.; Arambula-Pujol, E.M.; García-Juárez, A.R.; Maldonado-Amparo, R. Morphometric relationships, gametogenic development and spawning of the geoduck clam *Panopea globosa* (bivalvia: Hiatellidae) in the central gulf of California. *J. Shellfish Res.* **2007**, *26*, 423–431. [[CrossRef](#)]
23. Calderon-Aguilera, L.E.; Aragón-Noriega, E.A.; Morales-Bojórquez, E.; Alcántara-Razo, E.; Chávez-Villalba, J. Reproductive cycle of the geoduck clam *Panopea generosa* at its southernmost distribution limit. *Mar. Biol. Res.* **2013**, *10*, 61–72. [[CrossRef](#)]
24. Hamli, H.; Idris, M.H.; Hena, M.K.A.; Rajae, A.H. Fisheries assessment, gametogenesis and culture practice of local bivalve: A review. *Pertanika J. Trop. Agric. Sci.* **2019**, *42*, 103–124.
25. Han, J.; Kim, H.-J.; Oh, S.-Y.; Choi, Y.-U. Reproductive Characteristics of the Flat Oyster *Ostrea denselamellosa* (Bivalvia, Ostreidae) Found on the Southern Coast of South Korea. *J. Mar. Sci. Eng.* **2022**, *10*, 1326. [[CrossRef](#)]
26. Bernt, M.; Donath, A.; Jühling, F.; Externbrink, F.; Florentz, C.; Fritzsche, G.; Pütz, J.; Middendorf, M.; Stadler, P.F. MITOS: Improved de novo metazoan mitochondrial genome annotation. *Mol. Phylogenet. Evol.* **2013**, *69*, 313–319. [[CrossRef](#)] [[PubMed](#)]
27. Signorelli, J.H.; Alfaya, J.E.F. *Panopea abbreviate* (Bivalvia: Hiatellidae) in the Southwestern Atlantic Ocean, taxonomic revision and anatomy. *Malacologia* **2014**, *57*, 279–293. [[CrossRef](#)]
28. Molen, S.V.D.; Kroeck, M.; Ciocco, N. Reproductive cycle of the southern geoduck clam, *Panopea abbreviate* (Bivalvia: Hiatellidae), in north Patagonia, Argentina. *Invertebr. Reprod. Dev.* **2007**, *50*, 75–84. [[CrossRef](#)]
29. Vadopalas, B.; Pietsch, T.W.; Friedman, C.S. The proper name for the geoduck: Resurrection of *Panopea generosa* Gould, 1850, from the synonymy of *Panopea abrupta* (Conrad, 1849) (Bivalvia: Myoida: Hiatellidae). *Malacologia* **2010**, *52*, 169–173. [[CrossRef](#)]
30. Andersen, A.M. Spawning, Growth, and Spatial Distribution of the Geoduck Clam, *Panopea generosa* Gould, in Hood Canal, Washington. Ph.D. Thesis, University of Washington, Seattle, WA, USA, 1971.
31. Campbell, A.; Ming, M.D. Maturity and growth of the Pacific geoduck clam, *Panopea abrupta*, in southern British Columbia, Canada. *J. Shellfish Res.* **2003**, *22*, 85–90.
32. Solan, N.A.; Robinson, S.M.C. Age and gonad development in the geoduck clam *Panopea abrupta* (Conrad) from southern British Columbia. *J. Shellfish Res.* **1984**, *4*, 131–137.
33. Goodwin, L. Observations on spawning and growth of subtidal geoducks (*Panopea generosa*, Gould). *Proc. Natl. Shellfish. Assoc.* **1976**, *65*, 49–58.
34. Borozone, C.C. El ciclo gonadal de *Venus antiqua* King and Brodrip, 1935 (Veneridae, Bivalvia) en el golfo San José. *Physis* **1989**, *47*, 61–72.
35. Barón, P.J.; Real, L.E.; Ciocci, N.; Ré, M.E. Morphometry, growth and reproduction of an Atlantic population of the razor clam *Ensis macha* (Molina, 1782). *Sci. Mar.* **2004**, *68*, 211–217. [[CrossRef](#)]
36. Zhao, Z.J.; Li, F.X.; Ke, C.H. On the sex gonad development and reproductive cycle of clam *Paphia undulata*. *J. Fish. China Shuichan Xuebao* **1991**, *15*, 1–8. [[CrossRef](#)]

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