Phylogeny and Phylogeography of the Geoduck Panopea (Bivalvia: Hiatellidae)

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PHYLOGENY AND PHYLOGEOGRAPHY OF THE GEODUCK *PANOPEA* (BIVALVIA: HIATELLIDAE)

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ABSTRACT  Geoducks (*Panopea* spp.) are recognized as one of the longest-lived and largest burrowing bivalves. Five extant species support commercial fisheries in different countries, yet their phylogenetic relationships are unclear. Phylogenetic analyses using cytochrome oxidase c subunit I, 28S, and 18S partial sequences on five *Panopea* spp. were performed to understand existing biogeography and to unravel taxonomic uncertainties in the genus. The cytochrome oxidase c subunit I sequences revealed two major clades. The first clade included *Panopea zelandica* as a sister taxon of *Panopea globosa*; the second clade included *Panopea abbreviata*, *Panopea generosa*, and *Panopea japonica*. Contrary to expectations, geographically proximate species (*P. generosa* and *P. japonica*) showed lower genetic distance at nuclear loci, suggesting that *P. generosa* could be related to the common ancestor of *P. japonica*. Divergence values for mitochondrial DNA, however, indicated that *P. japonica* might be regarded as a distinct species. Analyses using both nuclear genes suggest that the ancestral species of *P. globosa* may have been broadly distributed through the Pacific coast to South America.

KEY WORDS: *Panopea*, geoduck, phylogeny, phylogeography, evolution, molecular markers

INTRODUCTION

Clams of the genus *Panopea* comprise the largest and longest-lived of all deep-burrowing bivalves; *Panopea generosa* can live up to 168 y (Bureau et al. 2002). Specimens are found in intertidal and subtidal marine and estuarine waters, typically buried ~1 m below the substratum surface in sandy or mud sediments (Feldman et al. 2004). The genus *Panopea* is characterized as having a hinge with one small cardinal tooth in each valve (Cox et al. 1969), and a fully fused siphon and mantle. Other taxonomic traits, such as the shape and depth of the pallial sinus are variable among species (Yonge 1971). The genus *Panopea* was a cosmopolitan genus during the Triassic, and approximately 150 fossil species have been described. Currently only about 10 living species are found in worldwide temperate to subtropical seas and only five species are the subject of commercial fishing activities (Yonge 1971) (Table 1, Fig. 1).

The taxonomy, phylogeny, evolutionary history, and speciation processes of these clams are poorly defined. For example, *Panopea generosa* was incorrectly synonymized with the extinct *Panopea abrupta* for almost 25 y (Vadopalas et al. 2010). The clam *Panopea japonica* from Japan and South Korea has been variously considered as a synonym species of *P. generosa*—one of the 85 bivalve species distributed on the American and Asian sides of the Pacific Ocean, or as closely related species (Coan et al. 2000). Similarly, because of the geographic proximity (~700 km) *Panopea globosa* was described as a variety of *P. generosa*, endemic to the northern Gulf of California (Dall 1898). In addition, the speciation of *P. globosa* was thought to be associated with the formation of the Gulf of California (Hertlein & Emerson 1956). Fossils of the *Latrania* Formation, however, indicate that *P. globosa* lived in the Imperial Sea, California, during the late Miocene (Scott Rugh, Brian F. Smith and Associates, pers. comm., 2011). In addition, geometric morphometric and genetic analyses reveal the presence of *P. globosa* on the western shore of the Baja Peninsula (Bahia Magdalena) in the Pacific Ocean (Leyva-Valencia 2012, Leyva-Valencia et al. 2012, Suárez-Moo et al. 2012). The fossil *Panopea taeniata*, found near Bahia Magdalena and described by Dall (1918), was also long considered a subspecies of *P. generosa*. Recent morphometric analyses, however, revealed that *P. taeniata* is a fossil morphotype of *P. globosa*, changing our understanding of the ancient biogeography of *P. globosa* from the Miocene to the Pleistocene along California and the Baja California Peninsula (Leyva-Valencia et al. 2013).

The biogeographic history of the *Panopea* genus in the southern circum-Pacific is likewise incomplete. Two extant species of *Panopea* occur in New Zealand; *Panopea zelandica* is distinguished from *Panopea smithiae* by morphological differences such as a more shallow pallial sinus and a more squarely truncated posterior end, and inhabiting shallower depths (Beu & Maxwell 1990), and fossils of *Panopea worthingtoni* have been found in Cretaceous sediments in both New Zealand and Antarctica. Fossils of Antarctic *Panopea philippii* and *Panopea andreeae* have a close morphological affinity with the extant South American species *Panopea abbreviata* (Zinsmeister 1984, Studencska 1991). These observations suggest a close relationship among *Panopea* spp. from New Zealand, Antarctica, and South America.

Phylogenetic studies that include the genus *Panopea* are scarce (Adamkewicz et al. 1997, Taylor et al. 2007). A recent

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phylogenetic study of three species of Panopea revealed genetic and morphological variation between Panopea globosa from the Gulf of California and Panopea generosa from the Pacific coast of Baja California (Rocha-Olivares et al. 2010). The authors of these studies concluded that these species do not share a recent ancestor, and proposed trans-Pacific dispersal or vicariance followed by subsequent reproductive isolation between Panopea japonica and P. generosa lineages as possible speciation mechanisms.

<table>
<thead>
<tr>
<th>Species</th>
<th>Authority</th>
<th>Distribution range</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panopea glycimeris</td>
<td>von Born, 1778</td>
<td>Northwestern Spain; Mediterranean Sea to South Africa</td>
<td>Kensley (1974), Rolán (1983), Scotti et al. 2011</td>
</tr>
<tr>
<td>Panopea australis</td>
<td>Sowerby, 1833</td>
<td>Southern and eastern Australia</td>
<td>Grove (2011)</td>
</tr>
<tr>
<td>Panopea zelandica</td>
<td>Quoy and Gaimard, 1835</td>
<td>New Zealand</td>
<td>Breen et al. (1991)</td>
</tr>
<tr>
<td>Panopea smithae</td>
<td>Powell, 1950</td>
<td>New Zealand</td>
<td>Breen et al. (1991)</td>
</tr>
<tr>
<td>Panopea japonica</td>
<td>Adams, 1850</td>
<td>Japan Sea</td>
<td>Coan et al. (2000)</td>
</tr>
<tr>
<td>Panopea bitruncata</td>
<td>Conrad, 1872</td>
<td>North Carolina to the Gulf of Mexico</td>
<td>Robertson (1963), John Slapinskey (FLMNH pers. comm.)</td>
</tr>
<tr>
<td>Panopea globosa</td>
<td>Dall, 1898</td>
<td>Gulf of California, Mexico</td>
<td>Aragón-Noriega et al. (2007), Rocha-Olivares et al. (2010)</td>
</tr>
</tbody>
</table>

FLMNH, Florida Museum of Natural History.

Genes with lower mutational rates such as 18S and 28S are useful for characterizing relationships between distant taxa and old divergence processes in bivalves (Adamkewicz et al. 1997, Winnepenninkx et al. 1998, Taylor et al. 2007), although cytochrome oxidase c subunit I (CO1) is used frequently to distinguish differences between close species. The goals of the current study were to determine the phylogenetic relationships among commercially fished Panopea spp. using molecular markers to infer ancient (18S and 28S) and recent (CO1)
divergences, to propose a hypothesis encompassing both their historical distribution and extant biogeography, and to begin to unravel the taxonomic uncertainties in the genus Panopea.

MATERIALS AND METHODS

Specimens

A total of 52 specimens from five species in the genus Panopea (Fig. 1) were used to obtain individual sequences of the mitochondrial (mtDNA) gene cytochrome oxidase c subunit I (CO1), and the nuclear (nDNA) genes 18S and 28S.

GenBank sequences of Hiattella arctica Linnaeus, 1767 (sister genus to Panopea, accession no. NC008451, AM774511, AM779685) and two species in the subclass Heterodonta (Mya arenaria Lamarck, 1809, accession no. AF120668, AF120560, FM999792; and Thyasira sarsi Philippi, 1845, accession no. AM706509, AM774485, AM779659) were selected as out-groups.

DNA Amplification and Sequencing

Genomic DNA samples were obtained from ethanol-preserved siphon tissues using DNeasy Tissue Kits (Qiagen Inc.). From every specimen, a fragment of each gene was amplified with specific primers (CO1, LCO1490-HCO1498 [Folmer et al. 1994]; 28S, 28MF-28MR [Taylor et al. 2007]; and 18S, 18SF-18SR [Hedin & Maddison 2001]), using polymerase chain reactions (PCR) in a total volume of 50 µL with 2 U Platinum Taq polymerase (Invitrogen Inc.) 100 ng template DNA, 1 µM of each primer, 200 µM of each dNTP, 1× PCR buffer, and 2 mM MgCl2. The PCR cycles were carried out in an iCycler PCR System (Bio-Rad Laboratories, CA) under the following conditions: initial denaturation for 5 min at 94°C, followed by 40 cycles of 45 sec at 94°C, 1 min annealing temperature (45°C for CO1; 53°C for 28S and 18S) and 1 min at 72°C, with a final 10-min extension at 72°C.

The length and quality of PCR products were visualized in 1.5% agarose gels stained with ethidium bromide. Purification and sequencing was performed in both directions using the Macrogen sequencing service (Macrogen, Inc. Korea).

Phylogenetic Analyses

The data were quality filtered by excluding individuals with less than three high-quality gene sequences from downstream analyses. The complementary DNA sequence datasets were edited manually, assembled, and aligned using the software Sequencher 4.10.1 (Gene Codes, Ann Arbor, MI) using default parameters, and were saved in Nexus format for phylogenetic analyses. The program DnaSP (Librado & Rosas 2009) was used to identify the haplotypes for each gene.

To test for saturation, transitions, and transversions, uncorrected p distances were computed in DAMBE 5.2.18 to verify that the sequences had not experienced enough substitution saturation to obscure phylogenetic relationships (Xia & Lemey 2009). To compare the mutation rates among lineages, Tajima’s relative rate test was performed in MEGA 5.03 (Tamura et al. 2011).

The phylogenetic analysis was carried out by using partitioned and complete sequences of each gene (586 bp for CO1, 565 bp for 28S, and 450 bp for 18S), and by using the concatenated set of 1,651 bp. The haplotypes were analyzed with maximum parsimony, Bayesian inference, and maximum likelihood (ML) to estimate tree topology. Maximum parsimony analyses were executed in PAUP 4.10b* (Swofford 2003); node support was assessed via 1,000 bootstrap replicates.

The nucleotide substitution models used in the analyses were chosen for each partition, individual genes, and for the concatenated data set. To determine the best-fit model for Bayesian inference and ML runs, the Akaike information criterion was used as implemented in Modeltest 3.06 (Posada & Crandall 2001, Posada 2009). The ML analysis was performed by a heuristic search with TBR branch swapping and 100 random additions of taxa, performed in PAUP 4.10b*. Node support was obtained by 1,000 bootstrap replicates (Swofford et al. 2001).

Bayesian inference was explored using the program MrBayes 3.1.1 (Ronquist & Huelsenbeck 2003) using four Markov chains and 5,000,000 generations sampled every 100 generations. The ML analyses were carried out using GARLI 0.951 (Zwickl 2006), RAxML GUI v1.1 (Silvestro & Michalk 2011), and Phylogeny.fr (Dereeper et al. 2008) to compare results. Phylogenetic trees were visualized using the program TREEVIEW X (Page 1996).

RESULTS

A total of 120 sequences from five species of Panopea (Table 2) were obtained and 35 haplotypes for all analyzed genes were identified. At CO1, 17 haplotypes with 217 informative sites were found. At 28S, 14 haplotypes with 97 informative sites were found. At 18S, only four haplotypes with 258 informative sites were found. The concatenated data set of the mtDNA and nDNA genes contained 23 haplotypes with 751 informative sites.

The best evolutionary model for the concatenated, CO1, and 28S genes was the generalized time-reversible model plus gamma (Table 2). The Kimura (1980) model was superior for 18S. The parameters for the concatenated data were substitution number = 6; base frequencies of A = 0.2114, C = 0.2382, G = 0.2817, and T = 0.2685; and gamma distribution shape parameter = 0.5808.

Within the genus Panopea, no saturation signal was observed for individual or concatenated sequences. The saturation by substitution index (0.146) was significantly less than the critical value (0.783) for the concatenated analyses (Xia & Lemey 2009).

The greatest genetic divergence at CO1 was between Panopea globosa and Panopea abbreviata (18.2%), whereas the lowest divergence was between Panopea zelandica and P. abbreviata (10%). A divergence of less than 5% was determined between P. zelandica and P. abbreviata with 28S, whereas the lowest divergence (0.3%) was observed between the northern hemisphere geoducks Panopea generosa and Panopea japonica. In contrast, 18S revealed smaller differences (1.3%) between P. zelandica and its congeners. The clams P. globosa and P. abbreviata still had the lowest divergence (0.2%) and even shared one haplotype; P. generosa and P. japonica also shared one haplotype (Table 3).

Phylogenetic Analyses

Maximum parsimony, Bayesian inference, and ML analyses revealed two major clades using both concatenated and individual genes. The concatenated tree (Fig. 2A) showed a polytomy among Panopea generosa–Panopea japonica–Panopea
Haplotypes identified within the five species of the genus *Panopea* from Bahia Magdalena, Puerto Peñasco, Guaymas, San Felipe, Gulf of San Matias, New Zealand, Ensenada, California, Alaska, Washington, and Japan for mitochondrial (CO1) and nuclear genes (28S and 18S).

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Sample</th>
<th>Voucher ID</th>
<th>Haplotype CO1</th>
<th>GenBank accession no.</th>
<th>Haplotype 28S rRNA</th>
<th>GenBank accession no.</th>
<th>Haplotype 18S rRNA</th>
<th>GenBank accession no.</th>
<th>Concatenated haplotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Panopea globosa</em></td>
<td>BM</td>
<td>BM1</td>
<td>1</td>
<td>HCO1</td>
<td>JQ071876</td>
<td>H286</td>
<td>JQ071883</td>
<td>H181</td>
<td>JQ071895</td>
<td>HCN10</td>
</tr>
<tr>
<td><em>P. globosa</em></td>
<td>BM</td>
<td>BM2</td>
<td>2</td>
<td>HCO9</td>
<td>JQ071868</td>
<td>H287</td>
<td>JQ071886</td>
<td>H181</td>
<td>JQ071895</td>
<td>HCN11</td>
</tr>
<tr>
<td><em>P. globosa</em></td>
<td>BM</td>
<td>BM3</td>
<td>3</td>
<td>HCO1</td>
<td>JQ071876</td>
<td>H289</td>
<td>JQ071882</td>
<td>H181</td>
<td>JQ071895</td>
<td>HCN13</td>
</tr>
<tr>
<td><em>P. globosa</em></td>
<td>BM</td>
<td>BM4</td>
<td>4</td>
<td>HCO1</td>
<td>JQ071876</td>
<td>H288</td>
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<td>HCN12</td>
</tr>
<tr>
<td><em>P. globosa</em></td>
<td>BM</td>
<td>BM5</td>
<td>5</td>
<td>HCO1</td>
<td>JQ071876</td>
<td>H286</td>
<td>JQ071883</td>
<td>H181</td>
<td>JQ071895</td>
<td>HCN10</td>
</tr>
<tr>
<td><em>P. japonica</em></td>
<td>JAP</td>
<td>Jap1</td>
<td>33</td>
<td>HCO14</td>
<td>JQ071873</td>
<td>H2812</td>
<td>JQ071881</td>
<td>H184</td>
<td>JQ071894</td>
<td>HCN18</td>
</tr>
<tr>
<td><em>P. japonica</em></td>
<td>JAP</td>
<td>Jap2</td>
<td>34</td>
<td>HCO14</td>
<td>JQ071873</td>
<td>H2812</td>
<td>JQ071881</td>
<td>H184</td>
<td>JQ071894</td>
<td>HCN19</td>
</tr>
<tr>
<td><em>P. japonica</em></td>
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<td>Jap3</td>
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<td>HCO15</td>
<td>JQ071874</td>
<td>H2812</td>
<td>JQ071881</td>
<td>H184</td>
<td>JQ071894</td>
<td>HCN20</td>
</tr>
</tbody>
</table>

continued on next page
Molecular Phylogeny of Five Panopea spp.

Contrary to expectations based on geographic proximity, Panopea genera and Panopea globosa belong to distinct lineages. In addition, the geographically distant species Panopea and Panopea japonica were included in the same clade, and showed lower divergence at both mitochondrial and nuclear loci, suggesting a close evolutionary relationship.

The results from analyzing concatenated and individual genes provide evidence of two principal lineages and reveal surprising phylogenetic relationships within the Panopea genus. Based on the hypothesis of parapatric speciation between Panopea genera and Panopea globosa, nuclear genes were used to provide molecular evidence of ancient phylogeny between them, and included other species of the genus for a broader comparison.

Phylogenetic analyses reveal that the clade containing Panopea abbreviata, Panopea genera, and Panopea japonica is consistent among the concatenated mtDNA and nDNA sequences. The concatenated tree also suggests that Panopea abbreviata and Panopea zelandica may share a common ancestor. The individual genes, however, did not yield sufficient information to resolve the phylogenetic relationships among boreal and austral congeners. The relationships between Panopea zelandica and congeners were dependent on the gene analyzed, whereas Panopea abbreviata appears to have a close phylogenetic relationship with temperate species from the northern hemisphere at both 28S and CO1. Differences among tree topologies may be the result of distinct mutation rates, although other variables such as the evolutionary history of each gene and the phylogenetic algorithms used can influence results.

The species Panopea was a cosmopolitan group during the Triassic period. For example, species such as Panopea glycimeris were widely distributed in the past (Kensley 1974). Extant aggregations of this species now occur from northern Spain to South Africa (Kensley 1976, Rolán 1983, Thomsen et al. 2009, Scotti et al. 2011). Faunal interchange and the speciation process of Panopea zelandica and Panopea abbreviata may have been favored by geological and climatic events. Before the breakup of the Gondwana landmass ~55 million years ago, New Zealand began separating from Antarctica. During this time, Australian species such as Panopea worthingtoni, Panopea andreae, and Panopea philippii occurred in New Zealand, Antarctica, and South America. The progressive movement of the southern continents during the Early Cenozoic resulted in the breakup of the Weddellian Province into smaller, discrete biogeographic units; the distribution of paleoaustral molluscs changed as a result of
the separation and isolation of New Zealand from Antarctica (Zinsmeister 1982).

Past faunal interchange between South America and New Zealand is exemplified by Xymene and Antimelatoma. These genera originated in Patagonia and dispersed to New Zealand three different times: during the Oligocene–Early Miocene, Late Miocene–Pliocene, and Pleistocene–Recent, whereas species of the genera Crosseola, Trichosirius, Ataxocerithium, Penion, Xymenella, Zeacumina, Austromitra, and Eoturris dispersed from New Zealand to Patagonia during the Early Miocene (Del Rio 2004). Before the Tasmanian Seaway and Drake Passage were open and the Isthmus of Panama was closed, ancestors of Panopea zelandica and Panopea abbreviata may have been broadly distributed along the southern Pacific Ocean.

During the Paleogene (23–65 million y ago), global temperatures may have been 10°C warmer than the current temperature (Lyle et al. 2008), making species flow possible across the Arctic. Modeling studies indicate that ocean circulation during the Cenozoic was similar to the modern geographic distribution of circulation gyres and upwelling systems (Thomas et al. 2006, Lyle et al. 2008, Ogasawara et al. 2008). A close relationship between extant species from the northern hemisphere is consistent with the hypothesis of a correlation between the fauna of northern Japan and southern California during the Late Miocene (Otuka 1934), as well as the presence of Panopea generosa fossils in Miocene (Nomura & Niino 1932, Nomura 1935), Pliocene (Yokoyama 1923, Yokoyama 1925), and Pleistocene (Yokoyama 1922) sediments of Japan. Based on the geographic isolation hypothesis, Matsubara (2011) proposed that Panopea japonica has been a distinct species from P. generosa since the Early Miocene, and suggested performing morphology and molecular phylogeny studies to resolve this question. The question of synonymy between P. generosa and P. japonica is a recurrent topic (Coan et al. 2000, Vadopalas et al. 2010).

At CO1, a genetic divergence was observed between Panopea generosa and Panopea japonica of approximately 11%. Divergence values between 10% and 22% at CO1 are considered sufficient to identify separate bivalve species (Therriault et al. 2002, Therriault et al. 2004, Xue et al. 2012), whereas values around of 0.6%–2.0% are typically observed at the intraspecific level (Baldwin et al. 1996, Arnaud et al. 2000, Xue et al. 2012). Thus, the results of the current study are in accord with the hypothesis of Matsubara (2011) that P. generosa and P. japonica are distinct species. However, both nuclear genes revealed low genetic divergence between P. generosa and P. japonica, in accord with the slight 18S gene divergence between P. generosa and P. japonica reported by Rocha-Olivares et al. (2010). Taken together, the results of the current study suggest ancient gene flow between these boreal species. After carefully ruling out contamination or error through repetition of the analyses, the shared 18S haplotype between P. generosa and P. japonica also supports this hypothesis.

The fossil data reveal that despite the close geographic proximity of Panopea globosa and Panopea generosa, they were distinct species prior to the formation of the Gulf of California. The fossil record also indicates that during the Late Miocene to Pleistocene (~10–0.12 million y ago), P. generosa and P. globosa coexisted in the Salton Trough, California (N. Scott-Rugh, SDNHM, pers. comm., 2010) and in the upper Gulf of California (Judith Terry-Smith, USNMH, pers. comm., 2011). The genetic results from the current study indicate that P. generosa is not the ancestral species of P. globosa, and that they are from distinct basal lineages, given the 17% divergence at CO1. Results similar to these were obtained using ITS and 18S rDNA sequences (Rocha-Olivares et al. 2010). Because of the current lack of knowledge of Panopea biogeography, the possibility cannot be excluded that extant aggregations of both species occur in sympatry along the Baja Peninsula.

### TABLE 3.

Divergence percentages within and between Panopea spp. at mitochondrial and nuclear genes using the Kimura two-parameter model.

<table>
<thead>
<tr>
<th>Species</th>
<th>Panopea generosa</th>
<th>Panopea globosa</th>
<th>Panopea abbreviata</th>
<th>Panopea zelandica</th>
<th>Panopea japonica</th>
</tr>
</thead>
<tbody>
<tr>
<td>28S rRNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. generosa</td>
<td>0.16</td>
<td>17.7</td>
<td>12.6</td>
<td>10.9</td>
<td>2.6</td>
</tr>
<tr>
<td>P. globosa</td>
<td>3.1</td>
<td>0.4</td>
<td>2.1</td>
<td>3.8</td>
<td>–</td>
</tr>
<tr>
<td>P. abbreviata</td>
<td>2.6</td>
<td>0.2</td>
<td>1.3</td>
<td>1.3</td>
<td>–</td>
</tr>
<tr>
<td>P. zelandica</td>
<td>3.8</td>
<td>4.0</td>
<td>4.0</td>
<td>2.1</td>
<td>–</td>
</tr>
<tr>
<td>P. japonica</td>
<td>0.3</td>
<td>3.1</td>
<td>1.3</td>
<td>2.1</td>
<td>3.7</td>
</tr>
<tr>
<td>18S rRNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. generosa</td>
<td>–</td>
<td>2.6</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>P. globosa</td>
<td>2.6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>P. abbreviata</td>
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<td>–</td>
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<td>P. zelandica</td>
<td>–</td>
<td>2.6</td>
<td>–</td>
<td>–</td>
<td>1.3</td>
</tr>
<tr>
<td>P. japonica</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

-- No observed genetic divergence.
Knowledge of the historical geographic distribution of *Panopea globosa* is unknown. However, *P. globosa* fossils collected from Miocene (SDNHM 97243) Pliocene (SDNHM 12085, SDNHM 12104, and Pleistocene (USNM118SJ1, USNM865SJ10, and SDNHM2555-108) sediments in southern California, the Gulf of California, and along the Pacific coast of southern Baja California indicate that *P. globosa* had a wide geographic distribution before the last glacial period. Valves of *P. globosa* have also been found in Nayarit, Mexico (SBMNH 135157) and Tumbes, Peru (SBMNH 149357); however, there are no known extant aggregations at these locales. Other bivalves, such as *Atrina maura* and *Argopecten ventricosus*, have a distribution range from the Baja Peninsula to Peru (Keen 1971).

Both the genetic affinity between *Panopea globosa* and *Panopea zelandica* at 28S, and the shared 18S haplotype between *P. globosa* and *Panopea abbreviata* suggest the possibility of a wide-range, warm-water *Panopea* clade distinct from a cold-water clade. As Smith (1991) proposed for several bivalve species, it is speculated that the ancestral species of *P. globosa* dispersed from the western Atlantic to the eastern Pacific by seaways across southern Costa Rica and Panama. Gene flow between eastern Pacific and western Atlantic fauna has been proposed previously (e.g., Rathbun 1918, Marko 2005, Poupin et al. 2005). Before the formation of the Isthmus of Panama, the Atlantic Ocean was a considerably narrower ocean basin than today, and current-mediated larval transport across it may have been feasible during the life span of marine planktonic larvae (Woodring 1982, Schubart et al. 2005). The marine fauna interchange between the Caribbean and the eastern Pacific may have been influenced not only by the closure of the Isthmus of Panama, but also by climate shifts in the Arctic region and the concomitant changes to current systems of the Pacific and Atlantic oceans (Ogasawara et al. 2008).

The only subtropical species known in the genus *Panopea*—*Panopea globosa*—had the greatest number of autapomorphies at CO1 (49), whereas *Panopea generosa* and *Panopea japonica* had only 22 at the same gene. This difference between tropical and temperate species might be related to environmental adaptations and life cycle differences. Studies of reproductive biology indicate that *P. globosa* is well adapted to warm temperatures; their reproductive cycle commences in late summer, when sea surface temperatures reach 28°C, and spawning occurs during winter months, when temperatures are close to 20°C (Aragón-Noriega et al. 2007). Conversely, *P. generosa* spawning peaks in late spring and early summer at temperatures closer to 12°C (Goodwin & Pease 1989, Aragón-Noriega et al. 2007, Arámbula-Pujol et al. 2008). The maximum age recorded...
for *P. globosa* is 47 y (González-Peláez et al. 2013) whereas *P. generosa* can live as long as 168 y (Bureau et al. 2002). Nucleotide substitution rates can be correlated with species body size, metabolic rate, generation time, and environmental temperature (Gillooly et al. 2005, Bromham 2009). Thomas et al. (2010) observed that invertebrate species with shorter generation times exhibited greater substitution rates. Adaptation to warmer temperatures and the shorter generation time for *P. globosa* may likewise be correlated with a greater number of private mutations than its congener.

Both morphology and genetics have been used to elucidate the taxonomy and phylogeny of bivalves (Giribet & Wheeler 2002, Giribet & Distel 2003, Kappner & Bieler 2006, Owada 2007). Although the general *Panopea* morphotype is a successful adaptation given that no significant morphological changes are evident during the past 50,000,000 y, species in the genus *Panopea* can be readily differentiated using shell morphological characteristics (Leyva-Valencia 2012, Leyva-Valencia et al. 2012, Leyva-Valencia et al. 2013). The current results indicate that *Panopea* congeners can also be discriminated via high interspecific genetic variation.

Based on the results of this study, it is hypothesized that the early evolution of *Panopea* occurred in two main lineages. One, associated with colder waters, includes *Panopea abbreviata*, *Panopea generosa*, and *Panopea japonica*. The second lineage, associated with warmer waters, includes the subtropical *Panopea globosa* and the geographically distant species *Panopea zelandica*. It is inferred that the ancestor of *Panopea globosa* was widely distributed during the Middle Miocene, when the Salton Sea was connected with the Pacific Ocean the proto-Gulf of California opened and the Baja California Peninsula began its separation from mainland Mexico (Helenes & Carreñó 1999). This hypothesis will be tested in future studies to help elucidate the evolution of the genus *Panopea*.

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**LITERATURE CITED**


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