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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. globosa*) belong to different lineages, and geographically distant 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 smithae* 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 andreae* have a close morphological affinity with the extant South American species *Panopea abbreviata* (Zinsmeister 1984, Studencka 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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TABLE 1.
Living species of *Panopea* recognized around the world.

Species	Authority	Distribution range	Citation
<i>Panopea glycymeris</i>	von Born, 1778	Northwestern Spain; Mediterranean Sea to South Africa	Kensley (1974), Rolán (1983), Scotti et al. 2011
<i>Panopea australis</i>	Sowerby, 1833	Southern and eastern Australia	Grove (2011)
<i>Panopea zelandica</i>	Quoy and Gaimard, 1835	New Zealand	Breen et al. (1991)
<i>Panopea smithae</i>	Powell, 1950	New Zealand	Breen et al. (1991)
<i>Panopea abbreviata</i>	Valenciennes, 1839	Southwestern Argentina	Morsán & Ciocco (2004)
<i>Panopea japonica</i>	Adams, 1850	Japan Sea	Coan et al. (2000)
<i>Panopea bitruncata</i>	Conrad, 1872	North Carolina to the Gulf of Mexico	Robertson (1963), John Slapcinsky (FLMNH pers. comm.)
<i>Panopea generosa</i>	Gould, 1850	Southern Alaska to Mexico	Goodwin & Pease (1989), Coan et al. (2000), Cadien & Lovell (2008)
<i>Panopea globosa</i>	Dall, 1898	Gulf of California, Mexico	Aragón-Noriega et al. (2007), Rocha-Olivares et al. (2010)

FLMNH, Florida Museum of Natural History.

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.

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, Winnepenninckx 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)

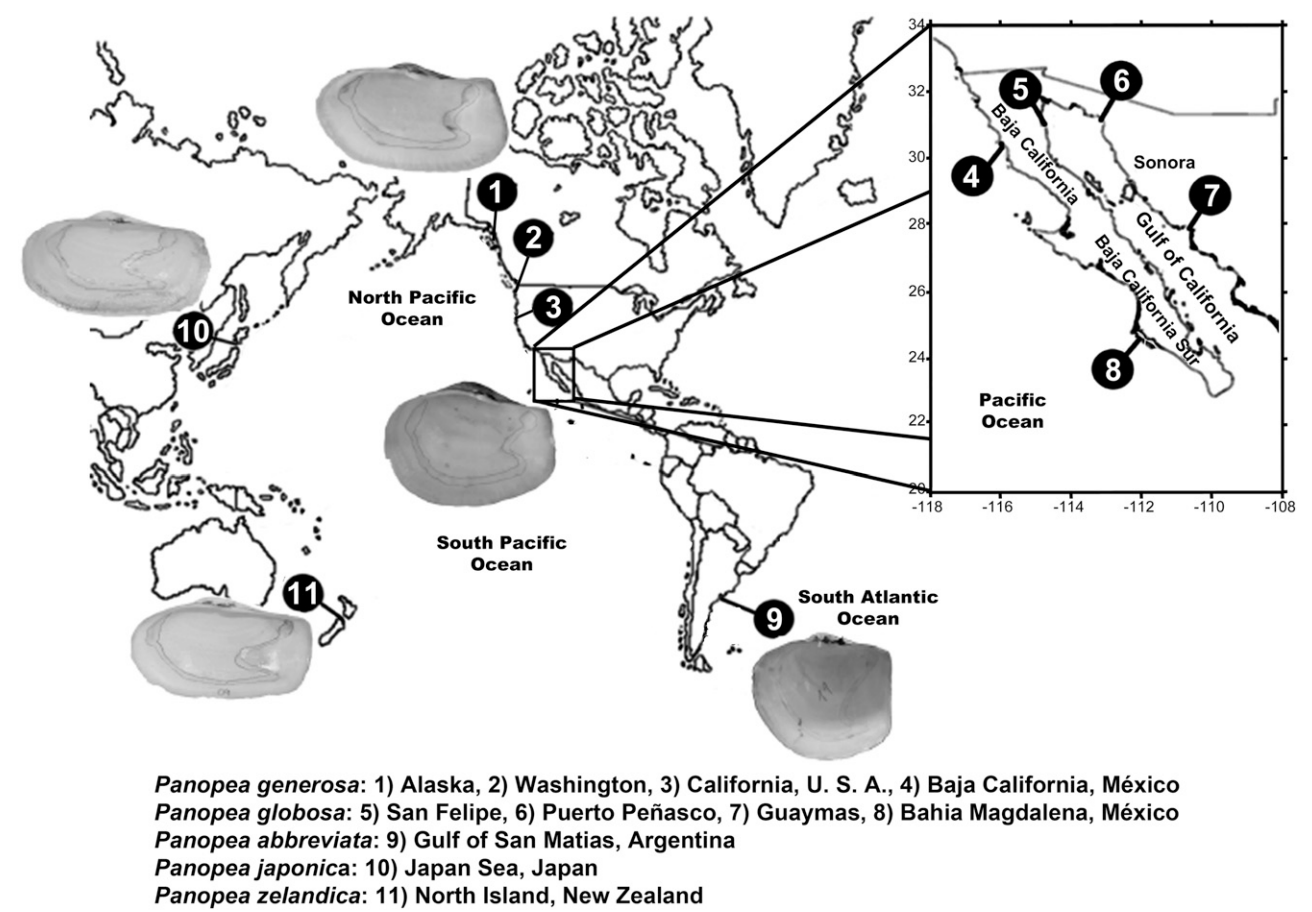


Figure 1. Sampling locales of five geoduck species in the current study.

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 *Hiatella 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 μ L with 2 U Platinum Taq polymerase (Invitrogen Inc.) 100 ng template DNA, 1 μ M of each primer, 200 μ M of each dNTP, 1 \times PCR buffer, and 2 mM $MgCl_2$. 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 strands 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 obtained, whereas 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. 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*

TABLE 2.

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).

Species	Locality	Sample	Voucher ID	Haplotype CO1	GenBank accession no.	Haplotype 28S rRNA	GenBank accession no.	Haplotype 18S rRNA	GenBank accession no.	Concatenated haplotype
<i>Panopea globosa</i>	BM	BM1	1	HCO1	JQ071876	H286	JQ071883	H181	JQ071895	HCN10
<i>P. globosa</i>	BM	BM2	2	HCO9	JQ071868	H287	JQ071886	H181	JQ071895	HCN11
<i>P. globosa</i>	BM	BM3	3	HCO1	JQ071876	H289	JQ071882	H181	JQ071895	HCN13
<i>P. globosa</i>	BM	BM4	4	HCO1	JQ071876	H288	JQ071884	H181	JQ071895	HCN12
<i>P. globosa</i>	BM	BM5	5	HCO1	JQ071876	H286	JQ071883	H181	JQ071895	HCN10
<i>P. globosa</i>	PP	PP1	8	HCO1	JQ071876	H282	JQ071891	H181	JQ071895	HCN5
<i>P. globosa</i>	PP	PP2	9	HCO3	JQ071878	H285	JQ071885	H181	JQ071895	HCN9
<i>P. globosa</i>	PP	PP3	10	HCO5	JQ071872	H284	JQ071889	H181	JQ071895	HCN8
<i>P. globosa</i>	PP	PP4	11	HCO1	JQ071876	H282	JQ071891	H181	JQ071895	HCN5
<i>P. globosa</i>	PP	PP5	12	HCO7	JQ071862	H282	JQ071891	H181	JQ071895	HCN3
<i>P. globosa</i>	GU	GU1	13	HCO6	JQ071870	H281	JQ071892	H181	JQ071895	HCN2
<i>P. globosa</i>	GU	GU2	14	HCO1	JQ071876	H281	JQ071892	H181	JQ071895	HCN1
<i>P. globosa</i>	GU	GU3	15	HCO1	JQ071876	H281	JQ071892	H181	JQ071895	HCN1
<i>P. globosa</i>	GU	GU4	16	HCO1	JQ071876	H281	JQ071892	H181	JQ071895	HCN1
<i>P. globosa</i>	GU	GU5	17	HCO8	JQ071865	H281	JQ071892	H181	JQ071895	HCN4
<i>P. globosa</i>	SF	SF3	6	HCO4	JQ071871	H283	JQ071890	H182	JQ071897	HCN6
<i>P. globosa</i>	SF	SF5	7	HCO2	JQ071877	H284	JQ071889	H182*	JQ071897	HCN7
<i>Panopea abbreviata</i>	GSM	Pabb1	36	HCO16	JQ071864	H2813	JQ071887	H182*	JQ071898	HCN23
<i>P. abbreviata</i>	GSM	Pabb2	37	HCO17	JQ071866	H2813	JQ071887	H182	JQ071898	HCN22
<i>P. abbreviata</i>	GSM	Pabb3	38	HCO17	JQ071866	H2813	JQ071887	H182	JQ071898	HCN22
<i>P. abbreviata</i>	GSM	Pabb4	39	HCO16	JQ071864	H2813	JQ071887	H182	JQ071898	HCN23
<i>P. abbreviata</i>	GSM	Pabb5	40	HCO16	JQ071864	H2813	JQ071887	H182	JQ071898	HCN23
<i>Panopea zelandica</i>	NZ	NZel3	18	HCO10	JQ071875	H2814	JQ071888	H183	JQ071896	HCN14
<i>P. zelandica</i>	NZ	NZel4	19	HCO10	JQ071875	H2814	JQ071888	H183	JQ071896	HCN14
<i>P. zelandica</i>	NZ	NZel5	20	HCO10	JQ071875	H2814	JQ071888	H183	JQ071896	HCN14
<i>Panopea generosa</i>	ENS	Ens1	21	HCO11	JQ071867	H2810	JQ071879	H184	JQ071893	HCN15
<i>P. generosa</i>	ENS	Ens2	22	HCO11	JQ071867	H2810	JQ071879	H184	JQ071893	HCN15
<i>P. generosa</i>	ENS	Ens3	23	HCO11	JQ071867	H2810	JQ071879	H184	JQ071893	HCN15
<i>P. generosa</i>	ENS	Ens4	24	HCO11	JQ071867	H2810	JQ071879	H184	JQ071893	HCN15
<i>P. generosa</i>	CAL	Cal1	25	HCO12	JQ071869	H2810	JQ071879	H184	JQ071893	HCN21
<i>P. generosa</i>	CAL	Cal2	26	HCO11	JQ071867	H2810	JQ071879	H184	JQ071893	HCN15
<i>P. generosa</i>	ALA	Ala77	27	HCO11	JQ071867	H2810	JQ071879	H184	JQ071893	HCN15
<i>P. generosa</i>	ALA	Ala80	28	HCO11	JQ071867	H2811	JQ071880	H184	JQ071893	HCN15
<i>P. generosa</i>	ALA	Ala82	29	HCO13	JQ071863	H2811	JQ071880	H184	JQ071893	HCN15
<i>P. generosa</i>	ALA	Ala83	30	HCO11	JQ071867	H2811	JQ071880	H184	JQ071893	HCN15
<i>P. generosa</i>	ALA	Ala84	31	HCO11	JQ071867	H2811	JQ071880	H184	JQ071893	HCN15
<i>P. generosa</i>	WASH	Wash97	32	HCO12	JQ071869	H2810	JQ071883	H184	JQ071893	HCN17
<i>P. generosa</i>	WASH	Wash101	33	HCO11	JQ071867	H2811	JQ071880	H184	JQ071893	HCN16
<i>Panopea japonica</i>	JAP	Jap1	34	HCO14	JQ071873	H2812	JQ071881	H184†	JQ071894	HCN18
<i>P. japonica</i>	JAP	Jap2	35	HCO14	JQ071873	H2812	JQ071881	H184	JQ071894	HCN19
<i>P. japonica</i>	JAP	Jap3	36	HCO15	JQ071874	H2812	JQ071881	H184	JQ071894	HCN20

continued on next page

TABLE 2.
continued.

Species	Locality	Sample	Voucher ID	Haplotype COI	GenBank accession no.	Haplotype 28S rRNA	GenBank accession no.	Haplotype 18S rRNA	GenBank accession no.	Concatenated haplotype
<i>Hiattella arctica</i>	—	—	—	HCO18	NC008451	H2815	AM779685	H185	AM774511	—
<i>Mya arenaria</i>	—	—	—	HCO19	AF120668	H2816	FM999792	H186	AF120560	—
<i>Thyasira sarsi</i>	—	—	—	HCO20	AM706509	H2817	AM779659	H187	AM774485	—

ALA, Alaska; BM, Bahia Magdalena; CAL, California; ENS, Ensenada; GSM, Gulf of San Matias; GU, Guaymas; JAP, Japan; NZ, New Zealand; PP, Puerto Peñasco; SF, San Felipe; WASH, Washington. Samples in bold type were used to characterize the haplotypes (bold). * One shared haplotype between *Panopea globosa* and *Panopea abbreviata*. † One shared haplotype between *Panopea generosa* and *Panopea japonica*.

abbreviata, placing *Panopea globosa* and *Panopea zelandica* in one major clade (C1), and *P. generosa*, *P. japonica*, and *P. abbreviata* in a second clade (C2). Similar topology was present for the CO1 tree (Fig. 2B) although with this gene, *P. zelandica* was placed as the basal species of the genus. On the 28S tree (Fig. 2C), *P. zelandica* was included with *P. globosa* and a polytomy is shown among *P. generosa*, *P. japonica*, and *P. abbreviata*, whereas the 18S tree (Fig. 2D) showed a basal polytomy among *P. globosa*, *P. zelandica*, and the others. As expected, we found that the relative rates of evolution of the *Panopea* genes were 18S rRNA < 28S rRNA < CO1.

DISCUSSION

Contrary to expectations based on geographic proximity, *Panopea generosa* and *Panopea globosa* belong to distinct lineages. In addition, the geographically distant species *P. generosa* 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 generosa* 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 generosa*, and *Panopea japonica* is consistent among the concatenated mtDNA and nDNA sequences. The concatenated tree also suggests that *P. 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 *P. 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 glycymeris* 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 y 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 paleoaustal molluscs changed as a result of

TABLE 3.

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

Species	Cytochrome oxidase c subunit I				
	<i>Panopea generosa</i>	<i>Panopea globosa</i>	<i>Panopea abbreviata</i>	<i>Panopea zelandica</i>	<i>Panopea japonica</i>
<i>P. generosa</i>	0.16				
<i>P. globosa</i>	17.7	0.4			
<i>P. abbreviata</i>	12.6	18.2	0.1		
<i>P. zelandica</i>	12.6	15.1	10.0	–	
<i>P. japonica</i>	10.9	17.0	11.6	10.0	0.3
28S rRNA					
<i>P. generosa</i>	0.3				
<i>P. globosa</i>	3.1	0.4			
<i>P. abbreviata</i>	2.1	3.8	–		
<i>P. zelandica</i>	3.8	4.0	4.6	–	
<i>P. japonica</i>	0.3	3.1	2.1	3.7	–
18S rRNA					
<i>P. generosa</i>	–				
<i>P. globosa</i>	2.6	–			
<i>P. abbreviata</i>	2.6	0.2	–		
<i>P. zelandica</i>	1.3	1.3	1.3	–	
<i>P. japonica</i>	–	2.6	2.6	1.3	–

–, No observed genetic divergence.

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*, *Zeacuminia*, *Austromitra*, and *Eoturris* dispersed from New Zealand to Patagonia during the Early Miocene (Del Río 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 northwestern 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 COI, a genetic divergence was observed between *Panopea generosa* and *Panopea japonica* of approximately 11%. Divergence values between 10% and 22% at COI 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 COI. 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.

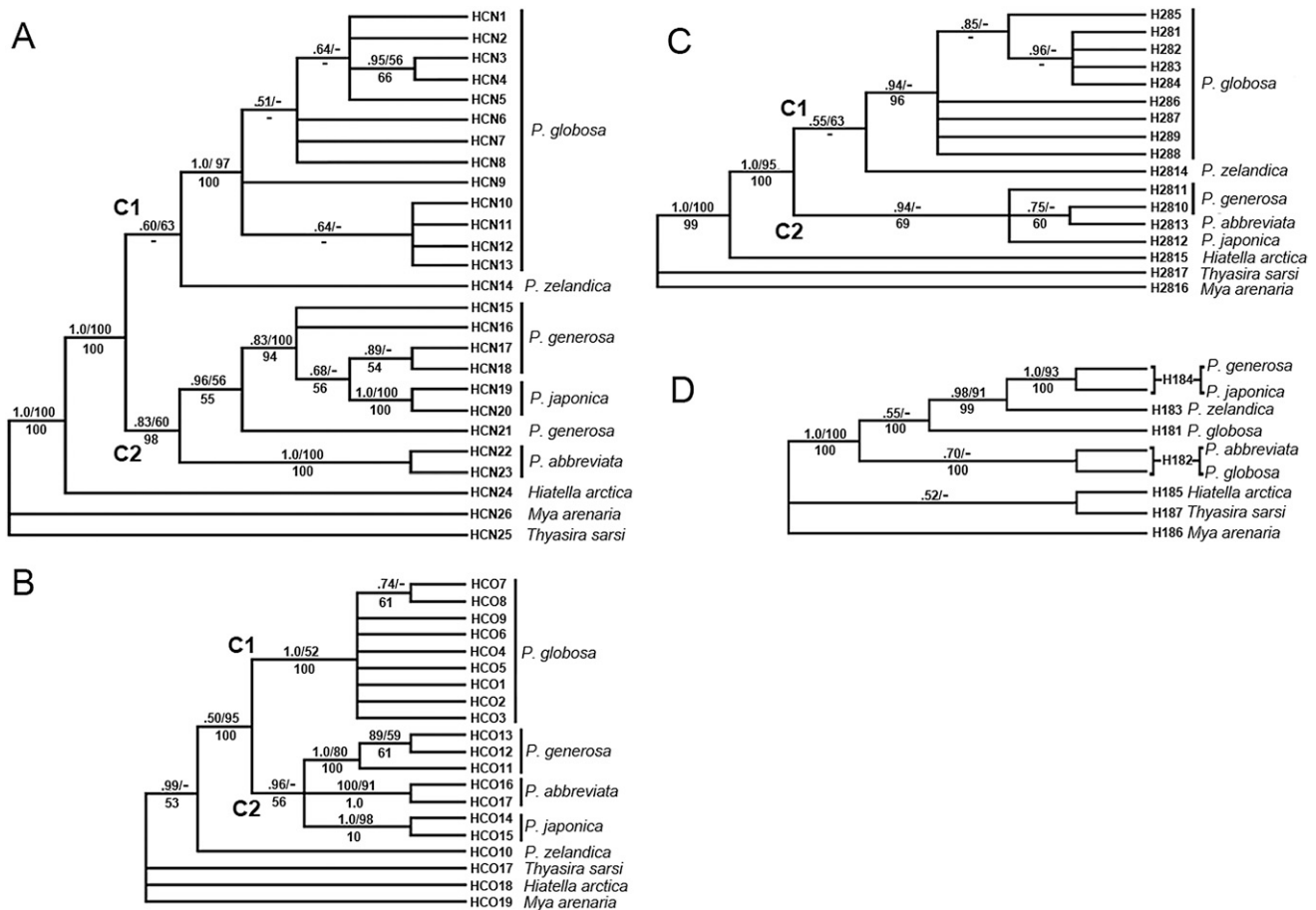


Figure 2. Phylogenetic reconstruction of mitochondrial and nuclear genes between five *Panopea* species. (A) Concatenated sequences. (B) CO1. (C) 28S. (D) 18S. Values over nodes indicate Bayesian interference/maximum likelihood branch support; under nodes, maximum parsimony bootstrap values. Names refer to the haplotype number.

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 (USNM11SJ1, USNM86SJ10, 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 congeners.

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 lineage, associated with colder waters, includes *Panopea abbreviata*, *Panopea generosa*, and *Panopea japonica*. The second lineage, associated with warmer waters, includes the subtropical species *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 & Carreño 1999). This hypothesis will be tested in future studies to help elucidate the evolution of the genus *Panopea*.

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