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A new *Lamellibrachia* species and confirmed range extension for *Lamellibrachia barhami* (Siboglinidae, Annelida) from Costa Rica methane seeps

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Abstract

Lamellibrachia Webb, 1969 has eight currently recognized species reported from chemosynthetic environments in the Pacific, Atlantic, and Mediterranean. Of these, *Lamellibrachia barhami* Webb, 1969 has been reported in the eastern Pacific from Canada to Costa Rica. In this study, phylogenetic analyses of *Lamellibrachia* tubeworms sampled from the Costa Rica margin confirm the large geographic range of *L. barhami* and reveal a new *Lamellibrachia* species from a single methane seep between 999 and 1,040 meters. *Lamellibrachia donwalshi* **sp. nov.** differs genetically and morphologically from all congeneric species. Despite its geographic proximity to the eastern Pacific *L. barhami*, *L. donwalshi* **sp. nov.** formed a clade with Atlantic and Mediterranean *Lamellibrachia* species. This suggests a vicariant event may have occurred after an Atlantic radiation of *Lamellibrachia.*

Key words: Vestimentifera, cold seeps, new species, deep sea, East Pacific

Introduction

The first Vestimentifera described, *Lamellibrachia barhami* Webb, 1969, was from southern California and placed in the phylum Pogonophora. Webb (1969) also erected the Order Vestimentifera in the same paper owing to the unusual morphology of *L. barhami*. Pogonophora (and Vestimentifera) is now referred to as the family Siboglinidae Caullery, 1914, within Annelida, though *Lamellibrachia* Webb, 1969 and its relatives are still often placed in the rank-free taxon Vestimentifera, within Siboglinidae; see Pleijel *et al.* (2009) for details of this complex story. Members of Vestimentifera lack a mouth and gut as adults, instead relying on organic compounds supplied by endosymbiotic chemoautotrophic bacteria for nutrition (Bright & Lallier 2010). Their generally large size (up to 2.4 meters in *Riftia pachyptila* Jones, 1981), high densities in some chemosynthetic environments (Bergquist *et al.* 2003; Levin *et al.* 2012; Shank *et al.* 1998), and their unusual features make them compelling organisms to study.

Of the ten vestimentiferan genera, the majority are known from hydrothermal vents in the Pacific: *Alaysia* Southward, 1991*, Arcovestia* Southward & Galkin, 1997, *Oasisia* Jones, 1985*, Paraescarpia* Southward, Schulze & Tunnicliffe, 2002*, Ridgeia* Jones, 1985*, Riftia* Jones, 1981 and *Tevnia* Jones, 1985. *Escarpia* Jones, 1985 and *Lamellibrachia* are also known from sedimented vents in the Pacific, and *Escarpia* has been reported from whale bones (Feldman *et al.* 1998), but they are primarily seep-associated genera (Bright & Lallier 2010; Kobayashi *et al.* 2015; Nishijima *et al.* 2010; Watanabe *et al.* 2010). There are five seep-associated genera, *Alaysia*, *Escarpia, Lamellibrachia*, *Paraescarpia* and *Seepiophila* Gardiner, McMullin & Fisher, 2001. *Seepiophila* is only known from the Gulf of Mexico, while *Lamellibrachia* and *Escarpia* contain species present in the Pacific or Atlantic/Caribbean/Mediterranean. *Paraescarpia* has been reported in the West Pacific and the very eastern margin of the Indian Ocean (Southward *et al.* 2002; McMullin *et al.* 2003).

Eight *Lamellibrachia* species have been described to date, making the genus the most speciose and one of the most widely spread vestimentiferan clades (distribution shown in Fig. 1). The majority of the diversity of *Lamellibrachia* lies in the Pacific with five of the eight currently accepted species (*L. barhami*, *L. columna*

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Southward, 1991, *L. satsuma* Miura, 1997, *L. juni* Miura & Kojima, 2006, and *L. sagami* Kobayashi, Miura & Kojima, 2015), four of which occur in the West Pacific (Fig. 1). *Lamellibrachia anaximandri* Southward, Andersen & Hourdez, 2011 was described from the Mediterranean, while in the West Atlantic and Caribbean there are two described species: *L. luymesi* van der Land & Nørrevang, 1975, off Guyana, and *L. victori* Mañé-Garzón & Montero, 1985, off Uruguay (Fig. 1, Table 1). Despite being a moderate distance from either type locality, specimens sampled from the Gulf of Mexico have been identified morphologically as *L. luymesi* (Jones 1985; Gardiner & Hourdez 2003; McMullin *et al.* 2003), and *L. victori* was considered "questionably distinct" by Jones (1985) and Gardiner & Hourdez (2003). However, Miglietta *et al.* (2010) and Cowart *et al.* (2014) showed through DNA sequencing that there were several distinct species in the northern Gulf of Mexico, and it remains unclear whether these are *L. luymesi*, *L. victori*, or other species altogether, and the validity of *L. victori* remains in question.

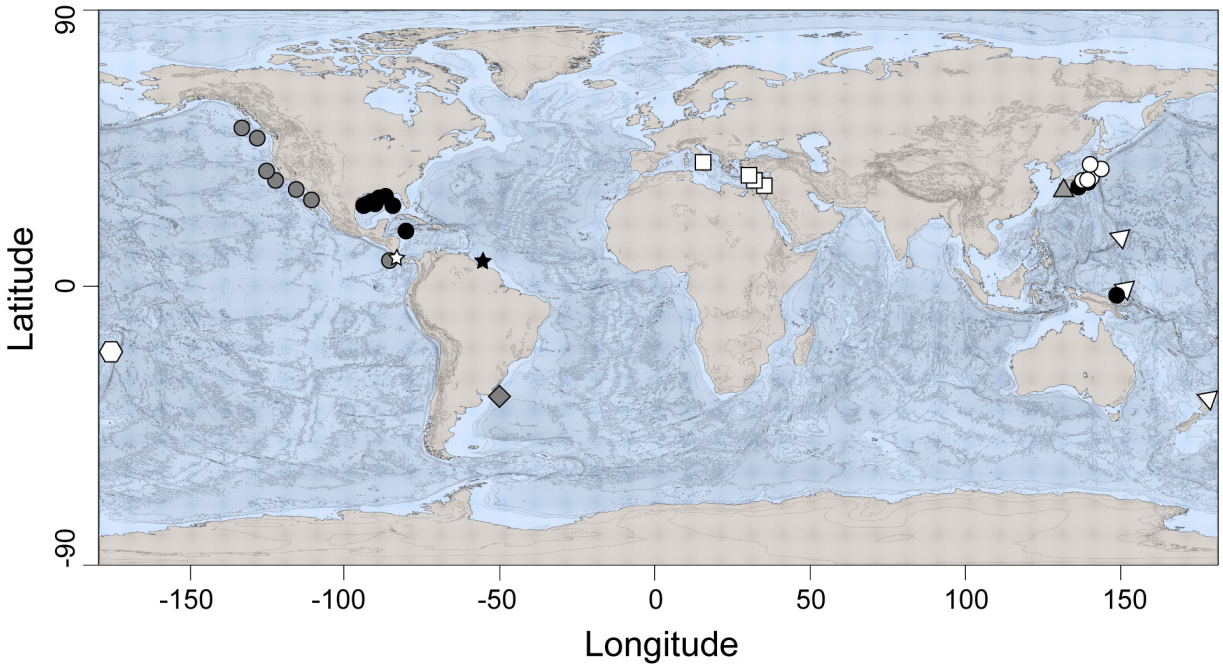

FIGURE 1. Distribution of *Lamellibrachia*. *Lamellibrachia anaximandri* (white square), *Lamellibrachia barhami* (grey circle), *Lamellibrachia columna* (white hexagon), *Lamellibrachia donwalshi* **sp. nov.** (white star), *Lamellibrachia juni* (white triangle), *Lamellibrachia luymesi* (black star), *Lamellibrachia sagami* (grey triangle), *Lamellibrachia satsuma* (white circle), *Lamellibrachia victori* (grey diamond), unresolved *Lamellibrachia* species (black circles): *L.* sp. 1/cf. *luymesi*, *L.* sp. 2, *L.* sp. L4, *L.* sp. L5, *L.* sp. L6.

TABLE 1. Type localities for the eight currently accepted *Lamellibrachia* species.

Name	Region	Locality	Year	Depth (m)	Citation
L. barhami	East Pacific	California coast	1969	1125	Webb, 1969
L. luymesi	South Atlantic	Guyana coast	1975	500	van der Land & Nørrevang, 1975
L. victori	South Atlantic	Uruguay coast	1985	300	Mañé-Garzón & Montero, 1985
L. columna	West Pacific	Lau Basin	1991	1859	Southward, 1991
L. satsuma	Northwest Pacific	Kagoshima Bay	1997	98	Miura, 1997
L. juni	South Pacific	Brothers Caldera	2006	1604	Miura & Kojima, 2006
L. anaximandri	Eastern Mediterranean	Anaximander Mountains	2011	672	Southward, Andersen & Hourdez, 2011
L. sagami	Northwest Pacific	Sagami Bay	2015	853	Kobayashi, Miura & Kojima, 2015

This paper focuses on deep-sea collections of *Lamellibrachia* from the Pacific Ocean off the Costa Rican coast, where there are cold seeps at various depths (Levin *et al.* 2012, 2015; Sahling *et al.* 2008). At these seeps, two species of *Lamellibrachia* have been noted, *L. barhami* at 1,800 meters (Levin *et al.* 2012) and an unidentified *Lamellibrachia* at 1,000 meters (Levin *et al.* 2015). We combine newly generated DNA data for *Lamellibrachia* samples from these sites with previously published DNA data (Braby *et al.* 2007; Cowart *et al.* 2014; Kobayashi *et al.* 2015; Kojima *et al.* 2001, 2006; Li *et al.* 2015, 2017; McMullin *et al.* 2003; Miglietta *et al.* 2010; Sun *et al.*, 2018) and confirm that there is a previously undescribed species of *Lamellibrachia*, which we describe here. This new species has a sister group in the Atlantic (Gulf of Mexico) and the biogeography of *Lamellibrachia* is discussed.

Materials and methods

Sampling and morphological analyses. Sampling was conducted over several years at multiple localities (Fig. 2, Table 2). *Lamellibrachia donwalshi* **sp. nov.** was collected on several dives by the HOV *Alvin* between 2009 and 2017 near Costa Rica at the Mound 12 dive site (1,000 meters) and Mound 11 dive site (1,040 meters). *Lamellibrachia barhami* was also collected on several dives by *Alvin* near Costa Rica at the Quepos Seep (1,400 meters), Jaco Scar (1,800 meters), and Parrita Scar (2,200 meters) dive sites. One specimen of *L. barhami* was collected from a Guaymas Basin seep, referred to as Pinkie's Vent (1,565 meters, see Paull *et al.* 2007), in 2012 via the ROV *Doc Ricketts*.

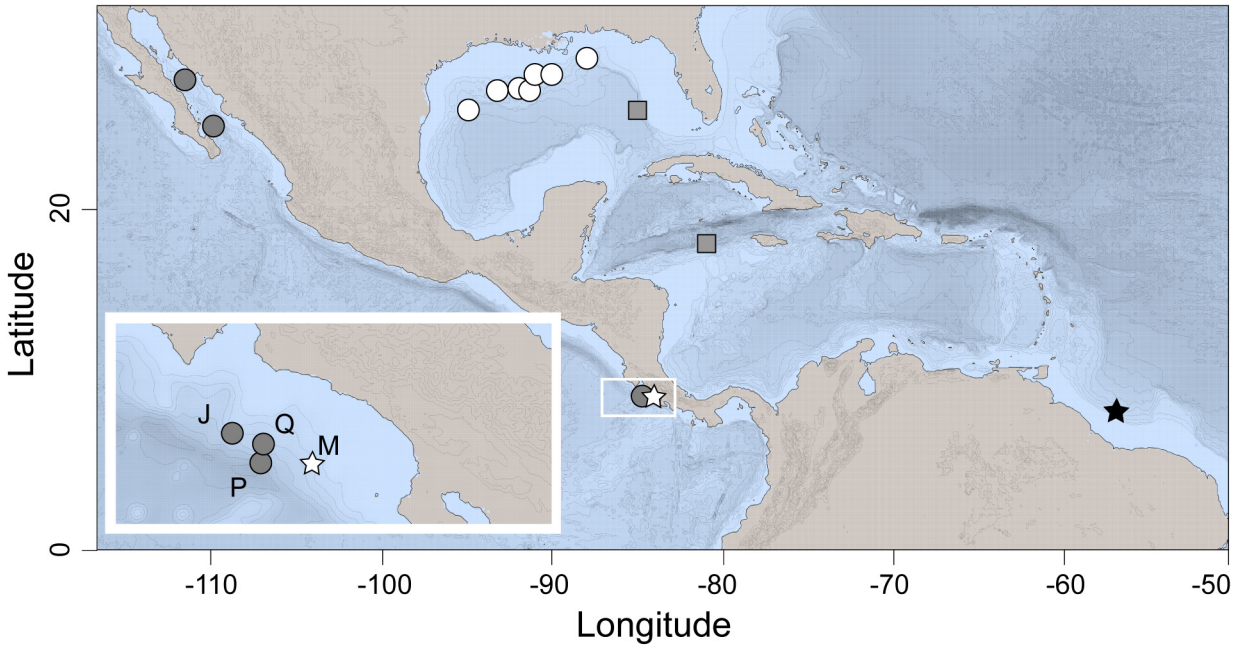

FIGURE 2. *Lamellibrachia* distribution in the Gulf of Mexico, Caribbean, and Costa Rica margin. *Lamellibrachia barhami* (grey circle), *Lamellibrachia donwalshi* **sp. nov.** (white star), *Lamellibrachia luymesi* (black star) *Lamellibrachia* sp. 1/cf. *luymesi* (white circle), *Lamellibrachia* sp. 2 (grey square). Detailed map of sampling at Costa Rica margin within white border: Jaco Scar (J), Quepos Seep (Q), Parrita Scar (P), Mounds 11/12 (M).

For DNA analysis, a portion of the vestimentum and/or trophosome was cut off and preserved in 95% ethanol (details of samples are noted in Material Examined section). One paratype (MZUCR 402-01) is deposited at El Museo de Zoología, Universidad de Costa Rica, San Jose, Costa Rica; the remaining specimens are deposited in the Scripps Institution of Oceanography Benthic Invertebrate Collection (SIO-BIC), La Jolla, California, USA. Whole specimens were photographed prior to preservation using Leica MZ8 or MZ9.5 stereomicroscopes. Post-preservation, specimens were examined and photographed using Leica S8 APO and DMR HC microscopes. Thin pieces of epidermis were cut out from the vestimentum surface near the trunk in the holotype and ten paratypes,

and on the trunk surface near the vestimentum in the holotype and seven paratypes, and placed on a glass slide for observation and measurement of cuticular plaques. A solution of 5% sodium hydroxide in water was added to dissolve tissue and improve observations. Ten plaques from the vestimentum and ten plaques from the trunk were measured from each specimen. Small pieces of the crown (paratype, SIO-BIC A1341), vestimentum (male and putative female paratypes, SIO-BIC A1341), and trunk (male and putative female paratypes, SIO-BIC A1341; holotype SIO-BIC A8382) were embedded in paraffin wax and sectioned on a Spencer '820' microtome. Sections 10µm thick were stained with Azure A for light microscopy. All morphological measurements were made post-preservation. Spearman rank correlations were conducted to test whether certain morphological characteristics (number of sheath lamellae, number of branchial lamellae, vestimental and trunk plaques) were correlated to body size.

DNA extraction, amplification, and sequencing. DNA was extracted from 30 specimens with the Zymo Research DNA-Tissue Miniprep kit, following the protocol supplied by the manufacturer. Approximately 1,050 base pairs (bp) of mitochondrial cytochrome subunit I (COI) were amplified using the polychaete *mtCOI* primer set COIf and COIr (Nelson & Fisher 2000) for multiple specimens in Table 2, and up to 550 bp of 16S rRNA (16S) were amplified using the primer set 16SbrH and 16SarL (Palumbi 1996). The Nelson and Fisher (2000) COIf/COIr primer set did not amplify the COI sequences of a few older specimens, thus these were amplified with the HCO2198 and LCO1490 primer set (Folmer *et al.* 1994) instead. Amplification was carried out with 12.5µl Apex 2.0x Taq RED DNA Polymerase Master Mix (Genesee Scientific), 1µl each of the appropriate forward and reverse primers (10µM), 8.5µl of ddH_2O, and 2µl eluted DNA. The PCR reactions were carried out in a thermal cycler (Eppendorf). The polychaete COI temperature profile was as follows: 95ºC/300s – (94ºC/60s – 55ºC/60s – 72ºC/120s) * 35 cycles – 72ºC/420s. The 16S temperature profile was as follows: 95ºC/180s – (95ºC/40s – 50ºC/40s – 72ºC/50s) * 35 cycles – 72ºC/300s. The universal COI temperature profile was as follows: 94ºC/180s – (94ºC/30s – 47ºC/45s – 72ºC/60s) * 5 cycles – (94ºC/30s – 52ºC/45s – 72ºC/60s) * 30 cycles – 72ºC/300s. The PCR products were purified with the ExoSAP-IT protocol (USB, Affymetrix) and sequencing was performed by Eurofins Genomics (Louisville, KY). The only nuclear gene (18S rRNA [18S]) that has been sequenced for multiple *Lamellibrachia* species has been shown to be uninformative among vestimentiferan species (Halanych *et al.* 2001) and thus was not amplified in this study.

Molecular analyses. Alignments of the newly generated sequences and available sequence data from GenBank for the two genes presented in Table 1 (published in the most recent siboglinid phylogenies [Braby *et al.* 2007; Cowart *et al.* 2014; Kobayashi *et al.* 2015; Kojima *et al.* 2001, 2006; Li *et al.* 2015, 2017; McMullin *et al.* 2003; Miglietta *et al.* 2010; Sun *et al.* 2018]) were performed using MAFFT with default settings (Katoh & Standley 2013) and concatenated with SequenceMatrix v.1.6.7 (Gaurav *et al.* 2011). For those specimens with mitochondrial genomes available on GenBank, 16S and COI genes only were downloaded prior to alignment and concatenation. For species that showed very little variation in COI (*L. anaximandri*, *L.* cf. *luymesi, L. satsuma, L. barhami*), a single individual was chosen to represent that lineage in the phylogenetic analyses. A few terminals included lacked 16S data (Table 1). Maximum likelihood (ML) analyses were conducted on the concatenated dataset using RAxML v.8.2.10 (Stamatakis 2014) with each partition assigned the GTR+G+I model. Node support was assessed via a thorough bootstrapping (1,000 replicates). Bayesian Inference (BI) analyses were also conducted using MrBayes v.3.2.6 (Ronquist *et al.* 2012). Best-fit models for these partitions were selected using the Akaike information criterion (AIC) in jModelTest 2 (Darriba *et al.* 2012; Guindon & Gascuel 2003): GTR+G+I was the best-fit model for both partitions. Maximum parsimony (MP) analyses were conducted using PAUP* v.4.0a161 (Swofford 2002), using heuristic searches with the tree-bisection-reconnection branch-swapping algorithm and 100 random addition replicates. Support values were determined using 100 bootstrap replicates. To check whether different proximate outgroups influenced the topology within *Lamellibrachia*, the previously described analyses were conducted with each of the following outgroups separately: *Escarpia* sp., *Ridgeia piscesae*, and *Riftia pachyptila*. Uncorrected pairwise distances were calculated for the COI dataset (~1275 bp) with PAUP* v.4.0a161 (Swofford 2002). A model-corrected distance analysis for a reduced COI dataset containing *L. donwalshi* **sp. nov.** and its sister clades (supported by the ML analysis) was also conducted with the best-fit model, HKY (Hasegawa *et al.* 1985) selected via AIC in jModelTest 2 (Darriba *et al.* 2012; Guindon & Gascuel 2003). This reduced dataset (n=46) was also analyzed with Automatic Barcoding Gap Discovery (ABGD) (Puillandre *et al.* 2012) with the following settings: p_{min}=0.001, p_{max}=0.1, Steps=20, X=1.5, Nb bins=30. The ABGD analysis was conducted with both Jukes-Cantor and Kimura distances. Haplotype networks of *L. donwalshi* **sp.**

nov., *L. barhami*, *L. anaximandri*, and *L.* sp. 2 were created with PopART v.1.7 (Bandelt *et al.* 1999) using the median-joining option with epsilon set at 0 using the COI dataset (1,177 bp).

Note on some 'Lamellibrachia' sequences on GenBank. Our phylogenetic analyses, which included many sequences from past studies, illuminated a few misidentifications of sequences on GenBank. Though these sequences were identified as *Lamellibrachia*, we found no evidence of their presence in the phylogenetic trees presented in Miglietta *et al.* (2010) (no terminals with matching names or information). We confirmed that the proper identification of these sequences was not *Lamellibrachia* via NCBI BLAST (Altschul *et al.* 1990). These specimens were misidentified as *Lamellibrachia*, and Miglietta *et al.* (2010) did not include them in their *Lamellibrachia* analyses thus we also excluded them from our analyses. Their GenBank accession numbers and approximate identifications are as follows: GU068171 (*Escarpia* sp.), GU059230 (*Seepiophila jonesi*), GU059239 (*Escarpia* sp.), GU059172 (*Seepiophila jonesi*), GU059250 (*Seepiophila jonesi*).

We would also like to note that *Escarpia spicata* (KJ789161) may be a misidentification on GenBank as well*: Escarpia spicata* is known only from the eastern Pacific, while the specimen (KJ789161) identified as *Escarpia spicata* (Li *et al.* 2015) was sampled from the Gulf of Mexico, where only *Escarpia laminata* has been morphologically identified. However, the molecular phylogeny of *Escarpia* lacks the resolution necessary to confirm this with molecular data (Cowart *et al.* 2013) thus we refer to this sequence as *Escarpia* sp.

Results

The ML, BI, and MP analyses of the three different rooting options (*Escarpia* sp., *Riftia pachyptila*, or *Ridgeia piscesae*) were congruent (topology represented in Fig. 3 with the *Ridgeia piscesae* rooting, chosen due to its proximity to *Lamellibrachia* in the recent siboglinid phylogeny by Li *et al.* 2017). *Lamellibrachia juni* was recovered as sister to the remaining *Lamellibrachia* species (ML bootstrap support of 59%) and *L. donwalshi* **sp. nov.** was recovered inside an Atlantic radiation (86%) (Fig. 3). However, the majority of phylogenetic relationships in Fig. 3 were poorly supported, with the exception of the respective sister relationships between *L. columna* and *L. sagami* (100%), and *L.* cf. *luymesi* and *L.* sp. 1 (100%). All analyses (Fig. 3) recovered a grade of Pacific species with respect to the Atlantic species plus *L. donwalshi* **sp. nov.** All analyses also showed *L. donwalshi* **sp. nov.** as sister to a *L.* sp. 2 and *L. anaximandri* (Mediterranean) clade (44%).

Uncorrected pairwise distances for the COI dataset revealed that *L. donwalshi* **sp. nov.** was 2.45% and 2.50% divergent from *L.* sp. 2 (Table 3) and *L. anaximandri* (Table 3), respectively. *Lamellibrachia donwalshi* **sp. nov**. was 5.42% divergent from its geographically nearest relative, *L. barhami* (Table 3). The minimum HKY-corrected distance between *L. donwalshi* **sp. nov.** and *L.* sp. 2 was 1.92%, and the minimum HKY-corrected distance between *L. donwalshi* **sp. nov.** and *L. anaximandri* was 1.81%. Results of the ABGD analyses of COI for the reduced (*L.* sp. 2, *L. anaximandri,* and *L. donwalshi* **sp. nov.**) dataset were identical using either Jukes-Cantor or Kimura distances and showed three distinct clusters, or hypothetical species. Haplotype networks generated for the COI data from *L. donwalshi* **sp. nov.**, *L.* sp. 2, and *L. anaximandri* also showed three distinct species, with some minor variation within each species (Fig. 4). *Lamellibrachia barhami* samples from a variety of depths and localities showed one dominant haplotype in Costa Rica, which was also found in the Gulf of California, Monterey Canyon, and Oregon (Fig. 5).

Taxonomy

Siboglinidae Caullery, 1914

Lamellibrachia Webb, 1969

Lamellibrachia donwalshi sp. nov.

(Figs. 6–11)

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Lamellibrachia sp. (Levin *et al.* 2015)

TABLE 2. Origin of sequenced terminals, vouchers, and GenBank accession numbers. New sequences are set in bold. Sampling sites of new sequences are as follows: Guaymas Basin, 27.59 N, 111.47 W (1,565m); Jaco Scar, 9.12 N, 84.85 W (1,800–1,891m); Quepos Seep, 9.03 N, 84.62 W (1,409m); Parrita Scar, 8.84 N, 84.65W (2,209m); Mound 12, 8.93 N, 84.31 W (999–1,005m); Mound 11, 8.92 N, 84.31 W (1,040m). GM=Gulf of Mexico, CR=Costa Rica, GC=Gulf of California, SP=South Pacific.

Scientific Name	Origin	COI	16S	Voucher or Reference
Escarpia sp.	Mississippi Canyon, GM	KJ789161	KJ789161	Li *et al.* 2015
Lamellibrachia anaximandri	Eastern Mediterranean	EU046616	HM746782	SMH-2007a
Lamellibrachia barhami	Oregon Margin	U74054	-	Black *et al.* 1997
Lamellibrachia barhami	Middle Valley	U74055	-	Black *et al.* 1997
Lamellibrachia barhami	Pescadero Basin, GC	KY581526	-	Goffredi *et al.* 2017
Lamellibrachia barhami	Monterey Canyon	AY129137–38	-	McMullin *et al.* 2003
Lamellibrachia barhami	Oregon	AY129141, AY129145	-	McMullin *et al.* 2003
Lamellibrachia barhami	Vancouver Island Margin	AY129146–47	-	McMullin *et al.* 2003
Lamellibrachia barhami	**Parrita Scar, CR**	**MH670765**	**MH660398**	**SIO-BIC A1564**
Lamellibrachia barhami	**Jaco Scar, CR**	**MH670766–92**	**MH660399**	**SIO-BIC A1824, A1837/A2133, A8305, A8309, A8313–15, A8317–18, A8322–23, A8343–45, A8351, A8375, A8377, A8403–05**
Lamellibrachia barhami	**Guaymas Basin, GC**	**MH670793**	**-**	**SIO-BIC A3433**
Lamellibrachia barhami	**Quepos Seep, CR**	**MH670794–806**	**-**	**SIO-BIC A8432, A8435–40, A8443–45, A8449–50**
Lamellibrachia columna	Fiji-Lau Back Arc Basin	DQ996645	FJ347646	Braby *et al.* 2007
***Lamellibrachia donwalshi* sp. nov.**	**Mound 12, CR**	**MH670826, MH670807–10, MH670813–25, MH670827–34**	**MH664896–MH664918**	**SIO-BIC A1504/A1338, A8266–70, A8272, A8274–77, A8382, A8412, A8416, A1506/A1341**
***Lamellibrachia donwalshi* sp. nov.**	**Mound 11, CR**	**MH670811–12**	**MH664919**	**SIO-BIC A1531**
Lamellibrachia juni	Manus Basin, SP	AB264603	-	Kojima *et al.* 2006
Lamellibrachia luymesi	Green Canyon, GM	GU059225	GU068209	Miglietta *et al.* 2010
Lamellibrachia luymesi	Mississippi Canyon, GM	KJ789163	KJ789163	Li *et al.* 2015
Lamellibrachia sagami	Sagami Bay, Japan	LC064365	-	JAMSTEC 1140043315
Lamellibrachia satsuma	Kagoshima Bay, Japan	KP987801	KP987801	Patra *et al.* 2016
Lamellibrachia sp. 1	GM	GU059165–66, GU059169, GU059227, GU059237	GU068253–54, GU068257, GU068212, GU068227	Miglietta *et al.* 2010
Lamellibrachia sp. 2	GM	GU059173, GU059175–77	GU068265, GU068269	Miglietta *et al.* 2010
Lamellibrachia sp. 2	Mid-Cayman Spreading Center, Caribbean	KM979545	KJ566961	Plouviez *et al.* 2014
Ridgeia piscesae	Hulk, Canada	KJ789165	KJ789165	Li *et al.* 2015
Riftia pachyptila	East Pacific Rise	KJ789166	KJ789166	Li *et al.* 2015

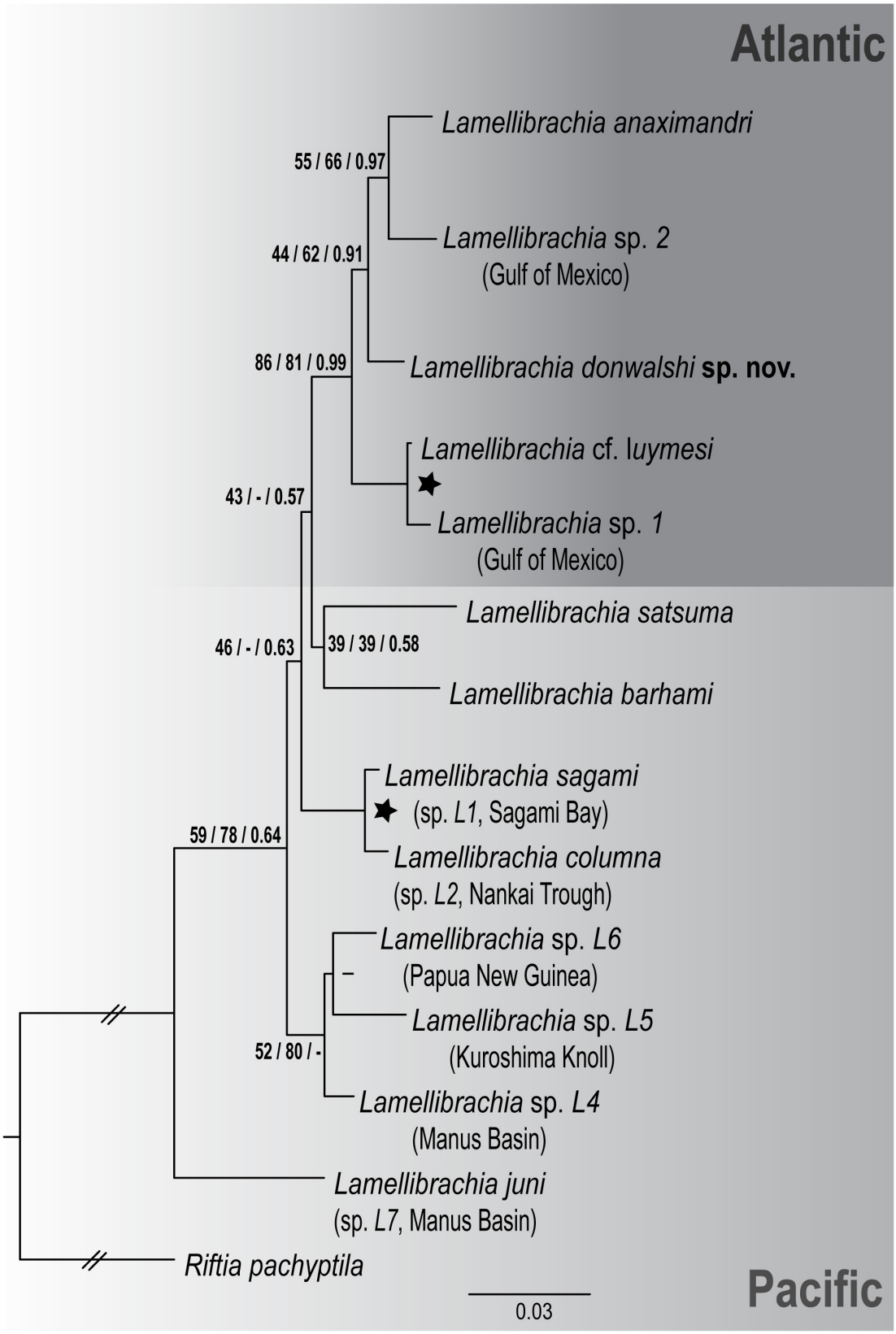

FIGURE 3. Maximum likelihood trees of the combined analysis from two mitochondrial genes (16S, COI) aligned with MAFFT and then concatenated, with three different rooting options: **A** *Escarpia* sp., **B** *Ridgeia piscesae*, **C** *Riftia pachyptila*. Bootstrap support percentages from Maximum Likelihood and Maximum Parsimony analyses (separated by slashes) are followed by Bayesian posterior probabilities. Support values of 95%/0.95 or greater for all analyses are indicated by stars. Nodes with support values less than 50%/0.5 or not recovered in one of the analyses are indicated by a hyphen.

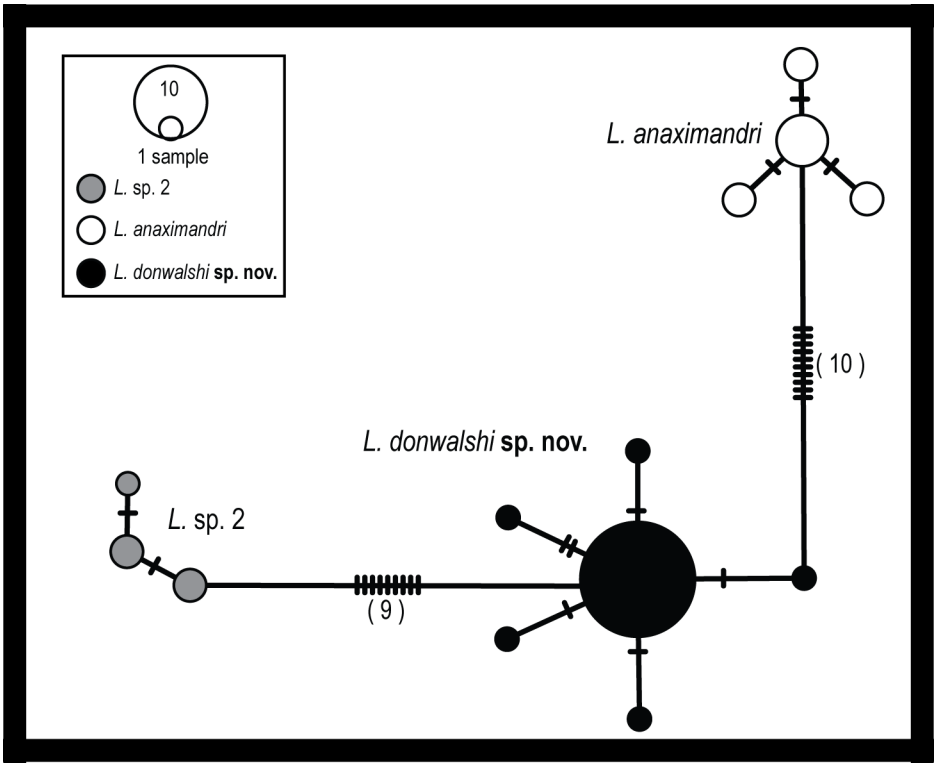

FIGURE 4. Haplotype networks from COI data of *Lamellibrachia* sp. 2 (grey), *Lamellibrachia anaximandri* (white), and *Lamellibrachia donwalshi* **sp. nov.** (black).

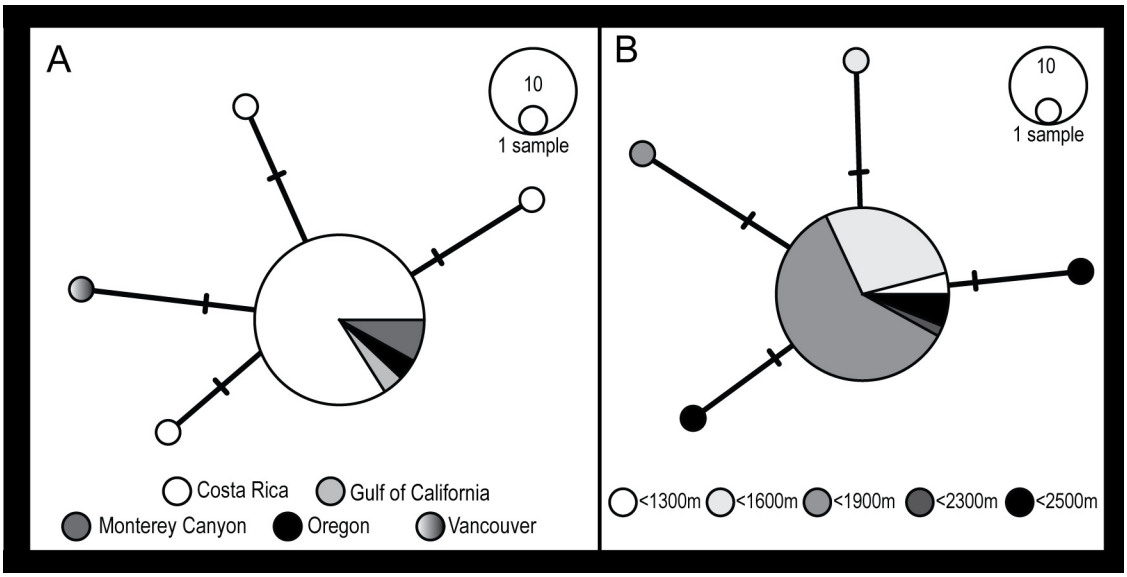

FIGURE 5. Haplotype networks from COI data for *Lamellibrachia barhami* sampled from Costa Rica margin to Vancouver: **A** Color-coded according to sampling locality. **B** Color-coded according to depth of sampling.

Type-locality: Costa Rica, Eastern Pacific, methane seep known as Mound 12, ~1,000 meters depth; 8.93°N, 84.32°W.

Material Examined. Holotype: (SIO-BIC A8382) from type locality, collected by HOV *Alvin,* Dive 4917, 1 June 2016; fixed in 10% SW formalin, preserved in 50% ethanol, putative male.

Paratypes: (SIO-BIC A1341) from type locality, collected by HOV *Alvin* Dive 4503, 24 February 2009; fixed in 10% SW formalin, preserved in 50% ethanol, two males, seven putative females, (see Table 2). One specimen (MZUCR 402-01) from type locality, collected by HOV *Alvin*; fixed in 10% SW formalin, preserved in 50% ethanol.

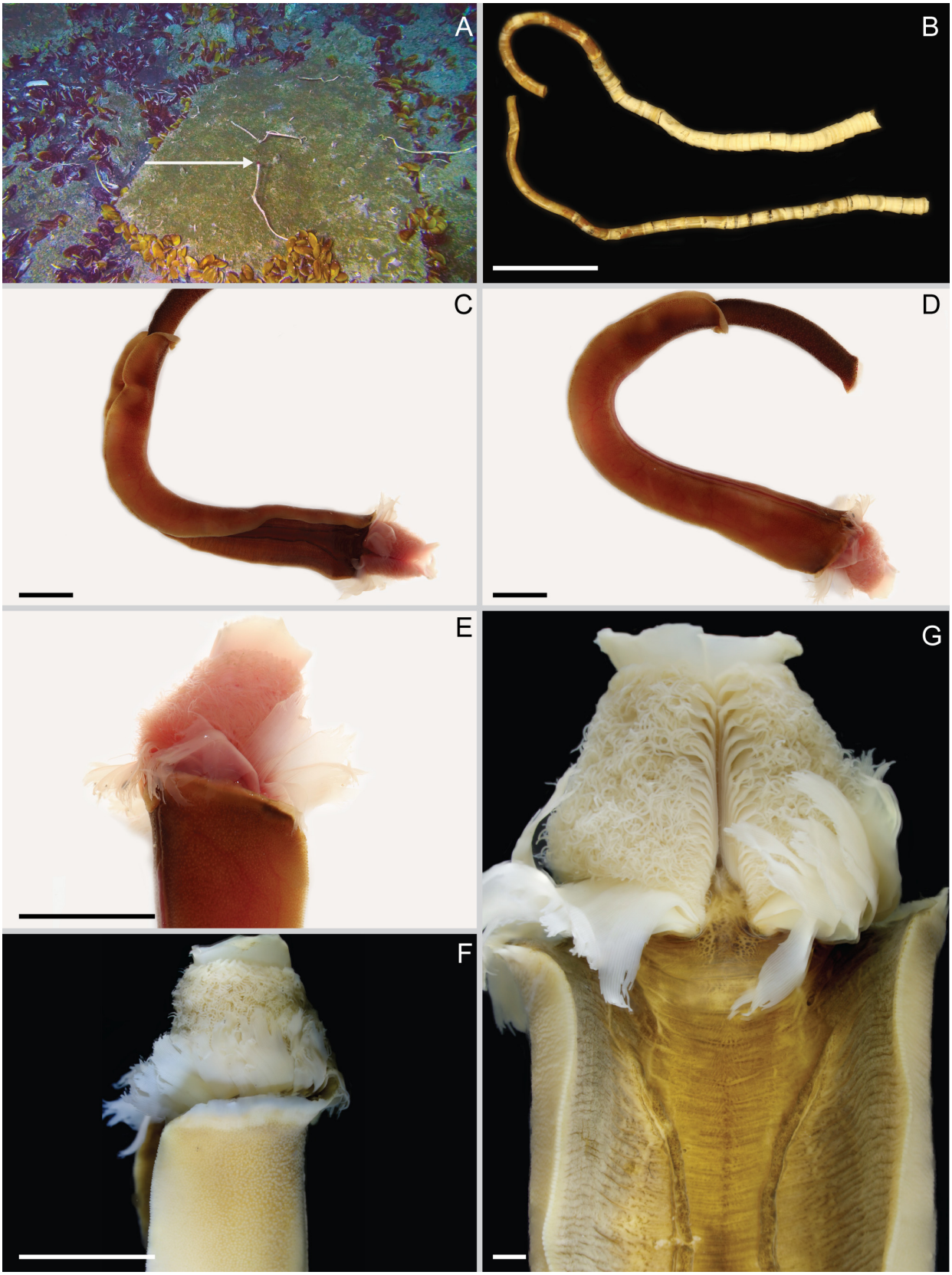

FIGURE 6. *In situ* photograph and micrographs of live *Lamellibrachia donwalshi* **sp. nov. A** In situ photo of *Lamellibrachia donwalshi* **sp. nov.** holotype (SIO-BIC A8382) taken from the HOV *Alvin*, indicated by arrow. **B** Tubes (incomplete) of *Lamellibrachia donwalshi* **sp. nov.** paratypes (SIO-BIC A1341). **C** Holotype, ventro-lateral to dorsal, live. **D** Holotype, ventro-lateral, live. **E** Holotype, left side, live. **F** Holotype, right side, post-preservation. **G** Holotype, dorsal, post-preservation. **G** Holotype, dorsal, post-preservation. Scale bars, B=5cm; C–F=10mm; G=1mm.

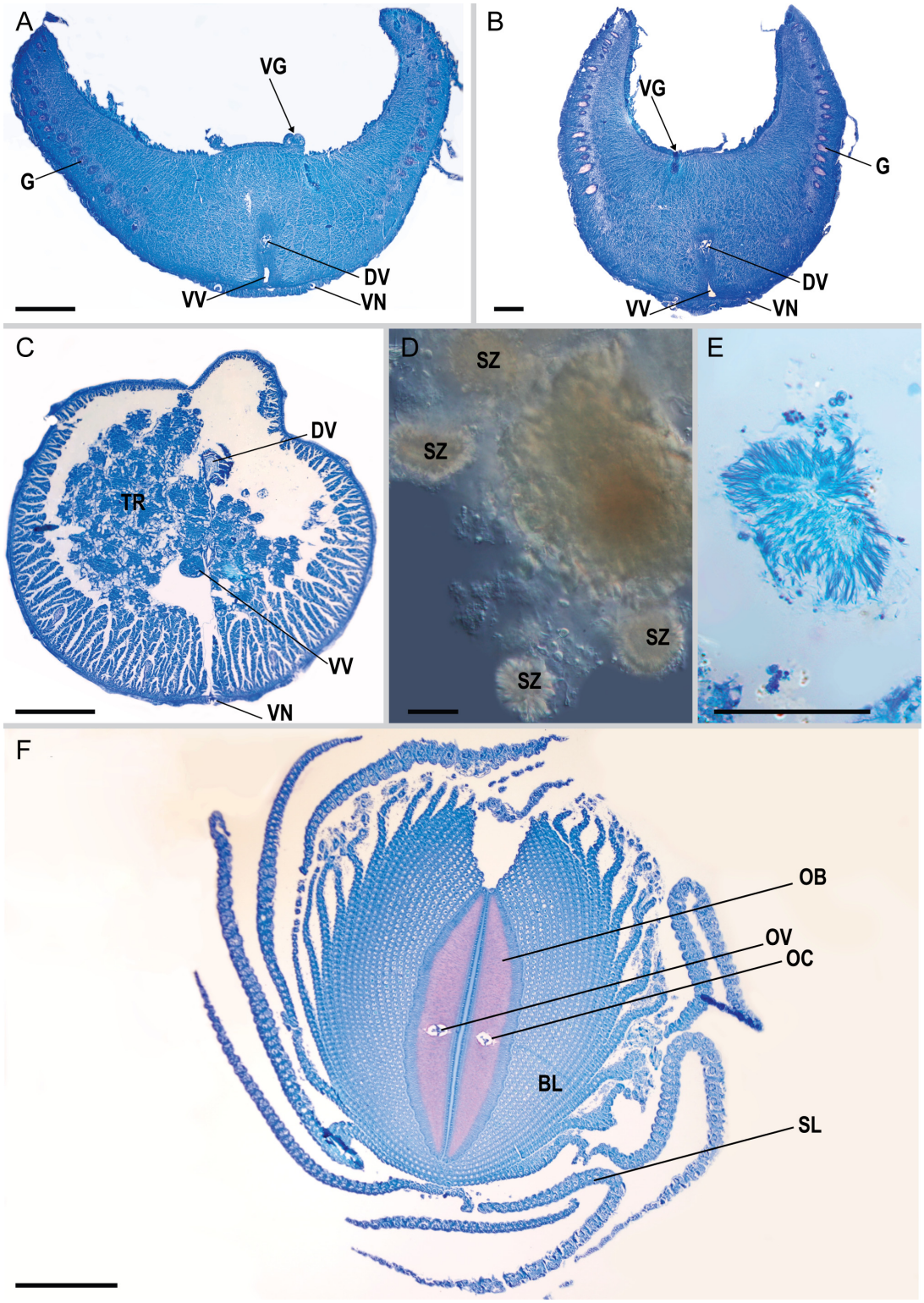

FIGURE 7. Micrographs of vestimental and trunk regions of *Lamellibrachia donwalshi* holotype (SIO-BIC A8382), male paratype (SIO-BIC A1341), and putative female paratype (SIO-BIC A1341). **A** 10 µm transverse section through anterior-middle part of vestimentum, male paratype. **B** 10µm transverse section through anterior-middle part of vestimentum, female paratype. **C** 10µm transverse section through trunk, male paratype. **D** Light micrograph of additional spermatozeugmata dissected from male paratype. **E** Close-up of spermatozeugmata in 10µm transverse section through trunk, male paratype. **F** 10µm transverse section through crown, male paratype. VG, vestimental groove; DV, dorsal blood vessel; VV, ventral blood vessel; VN, ventral nerve cord (Worsaae *et al.* 2016); G, gland; SZ, spermatozeugmata; OB, obturaculum; OV, obturacular blood vessel; OC, coelom of obturacular blood vessel; BL, branchial lamellae; SL, sheath lamellae. Scale bars, A–C=1mm; D=100µm; E–F=1mm.

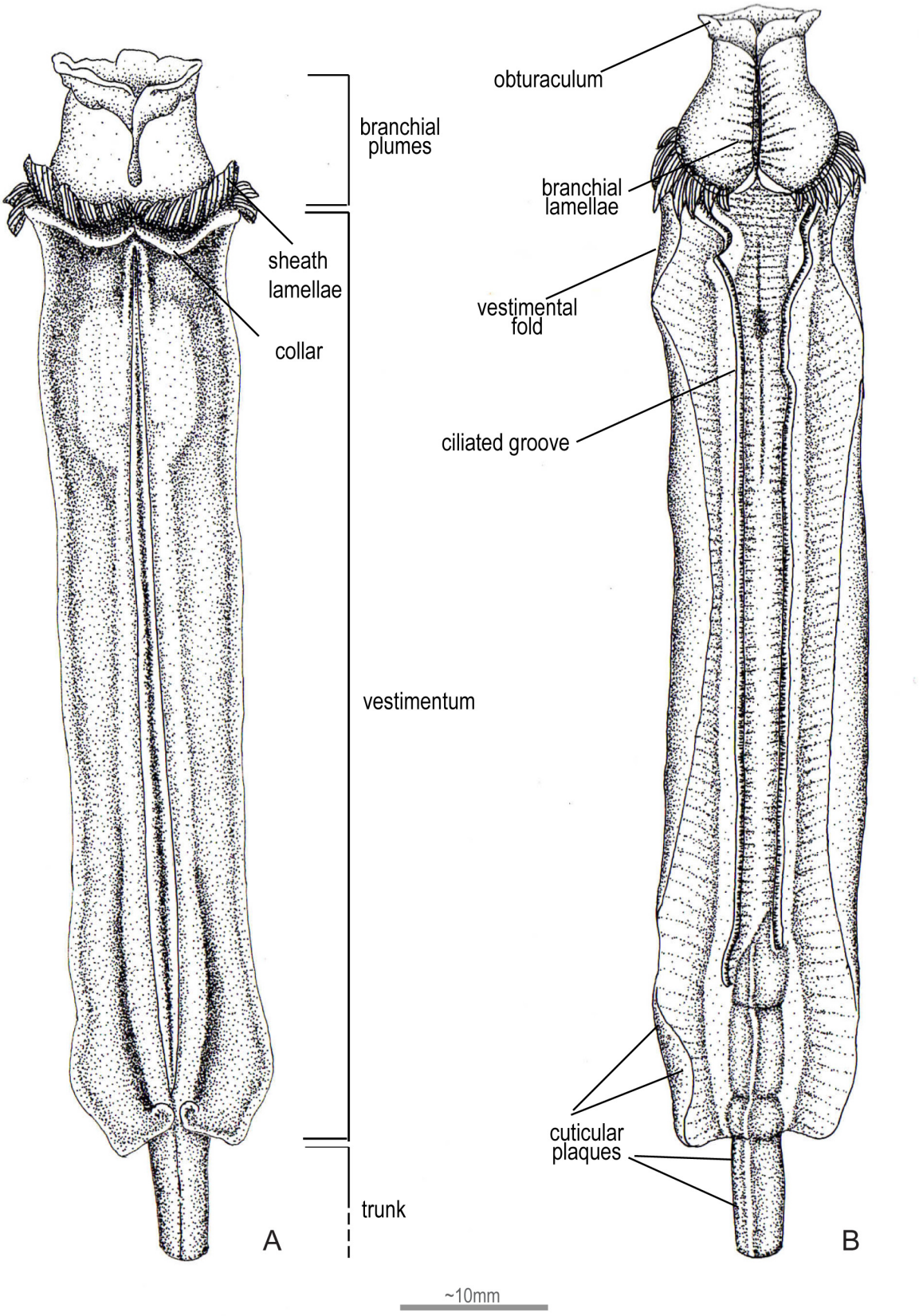

FIGURE 8. Illustration of *Lamellibrachia donwalshi* **sp. nov.** (male), holotype (SIO-BIC A8382). **A** Ventral. **B** Dorsal. Scale bar represents ~10mm.

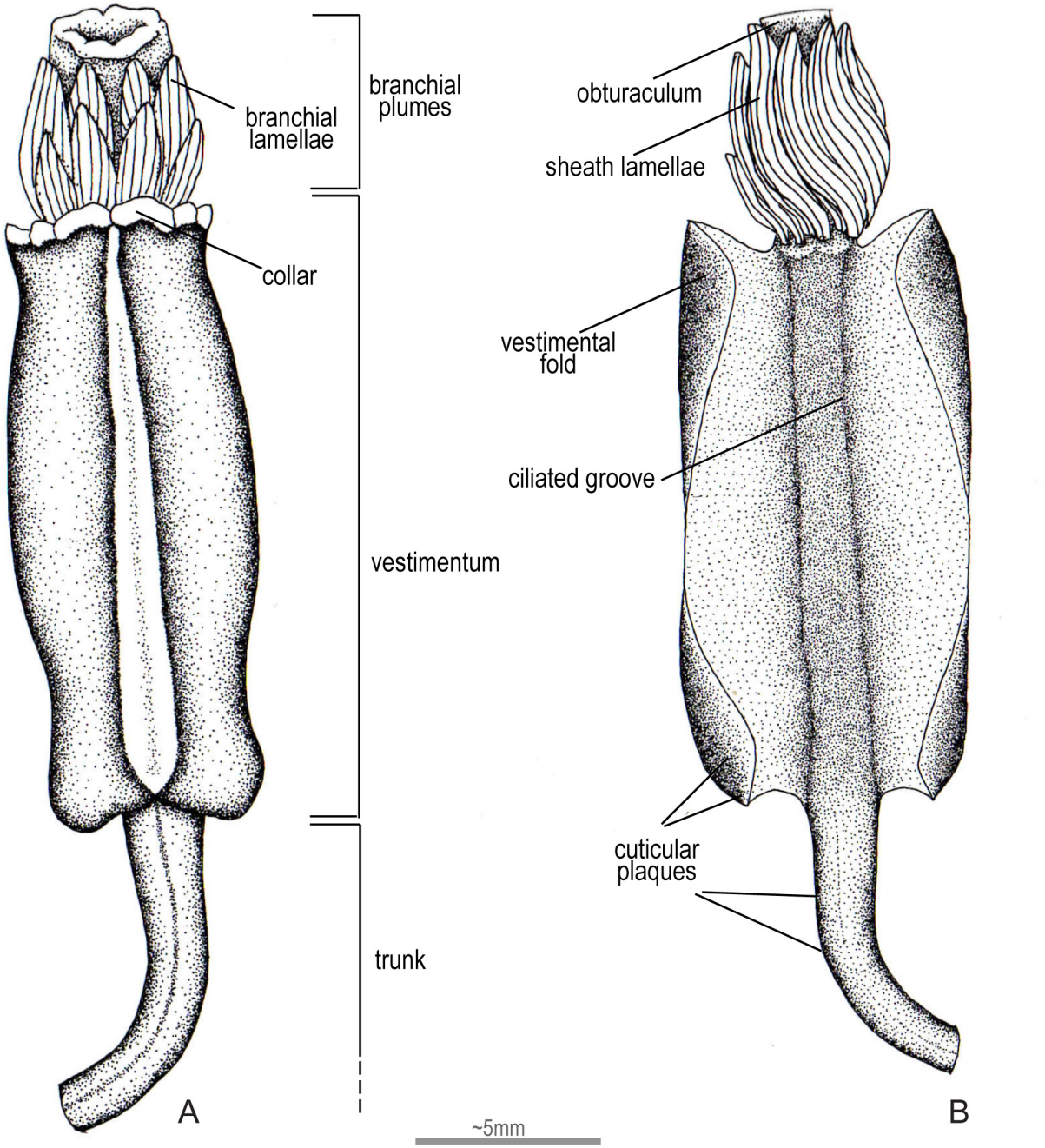

FIGURE 9. Illustration of *Lamellibrachia donwalshi* **sp. nov.** (female), paratype (SIO-BIC A1341). **A** Ventral. **B** Dorsal. Scale bar represents ~5mm.

Description. Tubes incomplete (broken in sampling), 24–26.5cm long, 9–10mm diameter anteriorly (n = 2; photo of tubes in-situ Fig. 6A). Anterior end of tube slightly curved with mostly long tube collars, occasionally interrupted by two or three short tube collars, but varying among specimens (Fig. 6B). Posterior of tubes smooth, curled, without obvious tube collars (Fig. 6B).

Obturaculum length 2.5–9mm (n = 11; holotype 7mm); width 2–8mm (n = 11; holotype 6mm), with bare anterior face, lacking any secreted structures (Figs. 6C–G). Lateral surface of obturaculum surrounded by branchial plumes (Fig. 6E–G). 5–11 pairs sheath lamellae (holotype 11 pairs; Figs 6E–G, 7–9) enclose 10–23 pairs branchial lamellae (holotype 23 pairs; Figs 6G, 8–10) with ciliated pinnules. Ratio of number of branchial lamellae pairs to obturaculum width varied from 1–3.3.

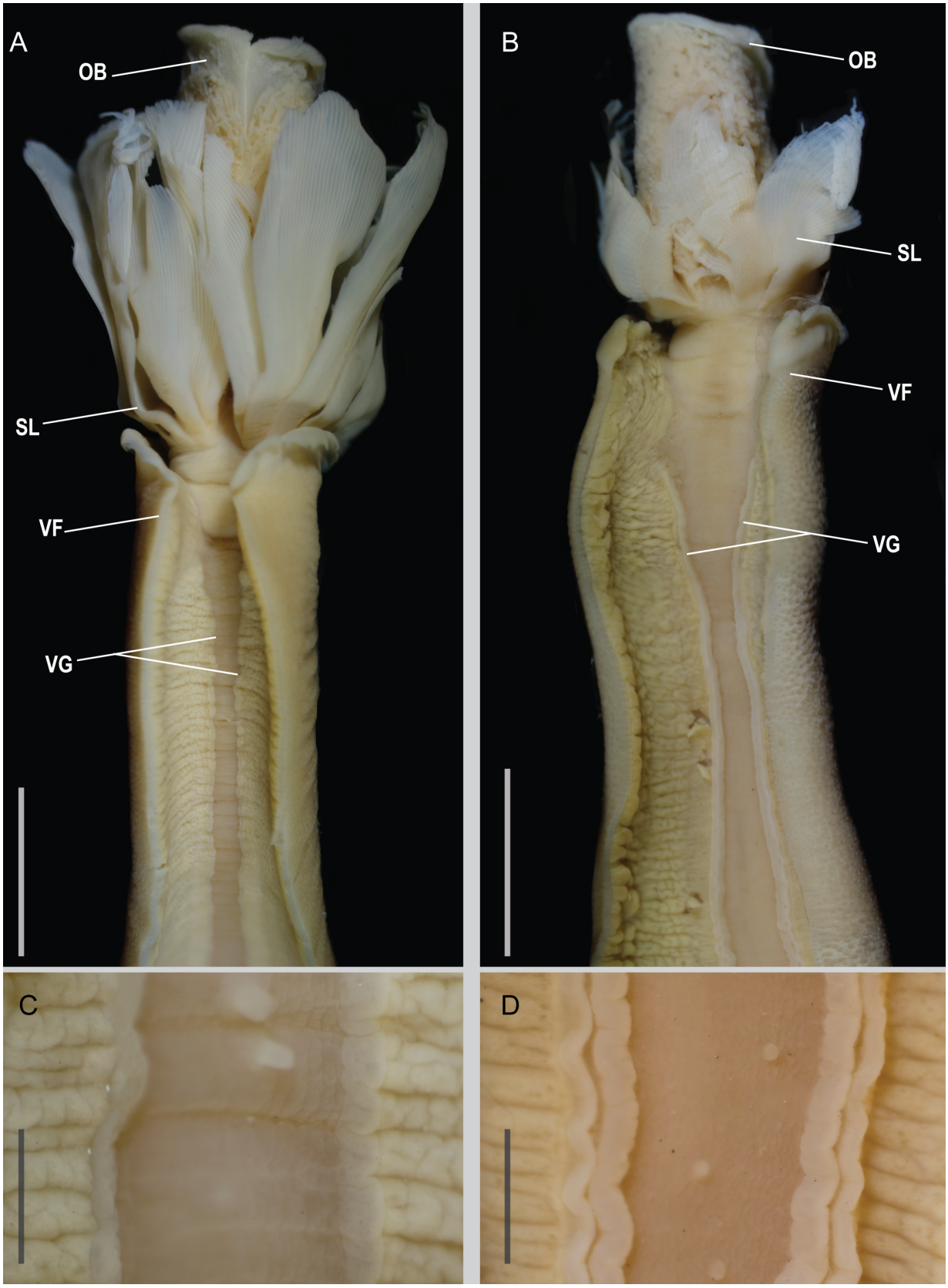

FIGURE 10. Micrographs of *Lamellibrachia donwalshi* **sp. nov.** male and female paratypes (SIO-BIC A1341). **A** Female dorsal anterior and vestimentum. **B** Male dorsal anterior and vestimentum. **C** Female vestimental grooves. **D** Male vestimental grooves. OB, obturaculum; SL, sheath lamellae; VF, vestimental fold; VG, vestimental groove. Scale bars, A–B=5mm; C–D=0.5mm.

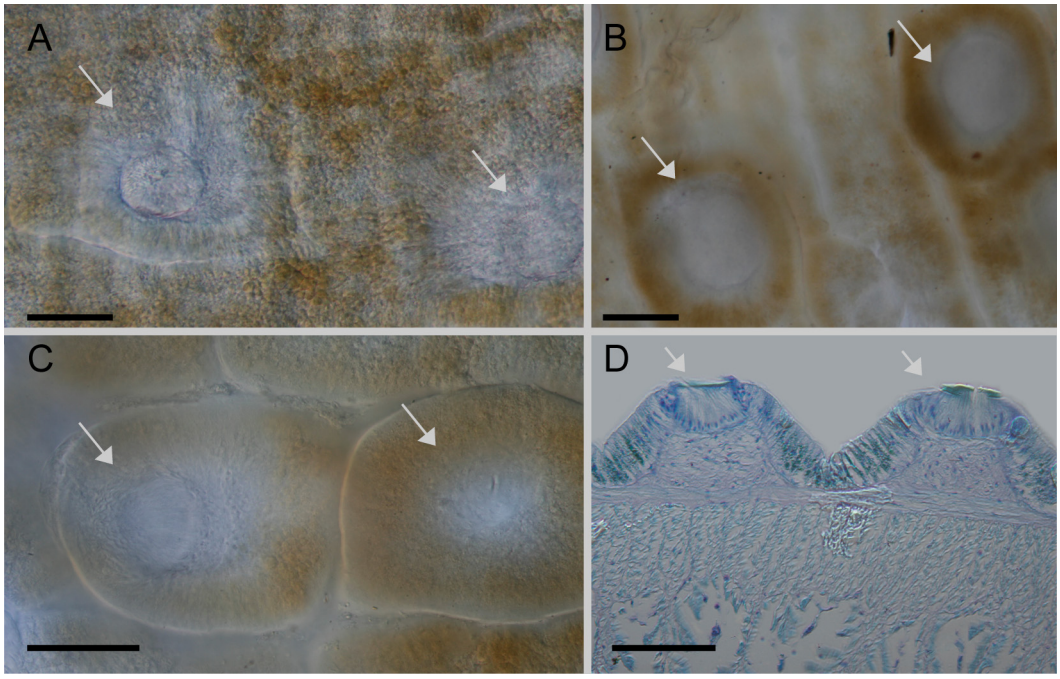

FIGURE 11. Interference contrast micrographs of plaques from *Lamellibrachia donwalshi* **sp. nov.** holotype (SIO-BIC A8382). **A**–**B** vestimental plaques, indicated by arrows. **C** Trunk plaques, indicated by arrows. **D** 10µm transverse section through epidermis showing trunk plaques, indicated by arrows. Scale bars 35µm.

Vestimentum length 22–70mm (holotype 70mm), width 3–12mm with vestimental folds curled (Figs 6C–G, 8A–B, 9B). Anterior vestimentum edge slightly curled forming collar (Figs 8A, 9A); posterior ends of vestimental folds rounded with slight separation at center (Figs 6, 7A–B, 8A, 9A). Dorsal paired vestimental ciliated grooves run down length of vestimentum (Figs 8B, 9B). In males, grooves flanked by ridge-like, conspicuous epidermal folds, spermatozeugmata observed in trunk (Figs 7D–E, 8B); conspicuous epidermal folds not present in putative females (Figs 7A–B, 9B, 10A–D). Both males and females have a few scattered epidermal processes on the internal epidermis of the vestimental cavity (Fig. 10C–D).

All specimens lacking posterior trunks. Anterior portion of trunk (Figs 6C–D, 7C) filled with fragile trophosome tissue (Fig. 7C). Ventral surface of vestimentum covered in cuticular plaques (Figs 11A–B), noticeably smaller than those on trunk (Figs 11C–D). Vestimental plaques measure 33.2–74.7µm in diameter (holotype 41.5–49.8 µm, Fig. 11B). Surface of trunk covered entirely by cuticular plaques, measuring 51.5–83µm in diameter (holotype 41.5–83µm, Fig. 11D). No plaques on middorsal and midventral lines of trunk. Opisthosoma not recovered.

Etymology. Don Walsh was one of the first people to descend to the bottom of the Challenger Deep aboard the bathyscaphe *Trieste* in 1960. He went on to a distinguished career in oceanography and marine policy. We name *Lamellibrachia donwalshi* **sp. nov.** in honor of his contributions to deep sea research and exploration.

Distribution. *Lamellibrachia donwalshi* **sp. nov.** has only been recovered from a single small area (varies by 0.01 N) and depth range of 999 to 1,040 meters. It was previously noted by Levin *et al.* (2015) as *Lamellibrachia* sp.

Remarks. *Lamellibrachia donwalshi* **sp. nov.** differs morphologically from other *Lamellibrachia* species in that it has 5–11 sheath lamellae, 10–23 branchial lamellae, and vestimental plaque diameters of 33.2–74.7µm (Table 4). It is not uncommon for ranges of sheath lamellae, branchial lamellae, and plaque diameters to overlap among *Lamellibrachia* species (Table 4), but no previously described species encompasses the entire range of these morphological traits in *L. donwalshi* **sp. nov.** We found no significant correlation between the body size (length and width of obturaculum and vestimentum) and the number of sheath lamellae, branchial lamellae, or plaque diameters (Spearman rank correlation, 11 specimens, $P > 0.05$). This supports the findings of Kobayashi *et al.* (2015) that the number of lamellae and the diameters of plaques are independent of growth in adults and can be used for morphological comparison across species. Due to a lack of morphological data for *L.* sp. 2, we cannot say

TABLE 3. Uncorrected pairwise distances for COI data (unique taxa only; identical sequences omitted), generated with PAUP*.

	***L.* sp. 1**	***L.* sp. 2**	***L. anaximandri***	***L. barhami***	***L. columna***	***L. juni***	***L.* cf. *luymesi***	***L. donwalshi* sp. nov.**	***L. sagami***	***L.* sp. L4**	***L.* sp. L5**	***L.* sp. L6**
***L.* sp. 2**	3.38	-	-	-	-	-	-	-	-	-	-	-
L. anaximandri	2.50	2.71	-	-	-	-	-	-	-	-	-	-
L. barhami	5.93	4.55	5.52	-	-	-	-	-	-	-	-	-
L. columna	4.92	4.60	5.23	5.68	-	-	-	-	-	-	-	-
L. juni	7.86	7.42	7.79	7.54	7.67	-	-	-	-	-	-	-
L.* cf. *luymesi	1.01	3.36	2.87	6.27	4.28	7.46	-	-	-	-	-	-
***L. donwalshi* sp. nov.**	3.59	2.37	2.50	5.42	4.68	7.25	2.85	-	-	-	-	-
L. sagami	4.49	4.45	4.56	4.99	1.00	6.81	4.14	4.34	-	-	-	-
***L.* sp. L4**	5.16	5.05	5.18	5.09	4.66	6.68	4.09	4.42	4.13	-		-
***L.* sp. L5**	4.71	5.21	4.28	5.30	4.95	6.98	4.47	4.72	4.31	2.62	-	-
***L.* sp. L6**	5.71	5.07	4.74	5.52	4.75	6.70	4.28	4.53	4.00	1.85	2.83	-
L. satsuma	5.94	6.12	6.18	6.06	6.15	8.48	5.12	6.25	5.51	5.69	5.87	5.69

TABLE 4. Morphological characters of *Lamellibrachia donwalshi* **sp. nov.** and congeneric species (1=Kobayashi *et al.* 2015; 2=Southward *et al.* 2011; 3=Miura & Kojima 2006; 4=Gardiner & Hourdez 2003; 5= Miura *et al.* 1997; 6=Southward 1991; 7=Jones 1985; 8=Mañe-Garzón & Montero 1985; 9=van der Land & Nørrevang 1975; 10=Webb 1969; no superscript=this study). OL: obturaculum length, OW: obturaculum width, BL: number of branchial lamellae, SL: number of sheath lamellae, VP: diameter of vestimental plaques, TP: diameter of trunk plaques.

Taxon	**OL (mm)**	**OW (mm)**	**BL**	**SL**	**VP (µm)**	**TP (µm)**
Lamellibrachia anaximandri	5.5–17[2]	1.8–6[2]	8–19[2]	3–9[2]	55–70[2]	60–95[2]
Lamellibrachia barhami	4.5–16[2,7,10]	4.5–12[2,7,10]	?–25[7]/19–25[2,10]	-4[7]/2–5[2,10]	60–150[2]	115–160[2]
Lamellibrachia columna	15–42[2,6]	8–13[2,6]	21[2,6]	8–16[2,6]	65–90[2,6]	70–120[2,6]
***Lamellibrachia donwalshi* sp. nov.**	**2.5–9**	**2–6**	**10–23**	**5–11**	**33.2–74.7**	**53.2–83**
Lamellibrachia juni	6.6–12.9[3]	5.2–8.3[3]	22–35[3]	2–3[3]/-4[2]	87–99[3]	80–98[3]
Lamellibrachia luymesi	13[9]/6.6–16[32,4]	9[9]/3.4–9.7[2,4]	19[9]/15–22[2,4]	6[9]/4–8[2,4]	55–60[2,4]	75–85[2,4]
Lamellibrachia sagami	5.8–22.5[1]	4.4–10.8[1]	19–26[1]	3–6[1]	59–101[1]	67–130[1]
Lamellibrachia satsuma	1.8–9.8[5]	1–5.6[5]	?–19[5]	0–4[5]/4–5[2]	35–63[5]	51–82[5]
Lamellibrachia victori	13[8]	13[2]	7[8]	18[2]	n/a	n/a

at this time whether *L. donwalshi* **sp. nov.** differs morphologically from this close genetic relative (Fig. 3). However, it clearly differs morphologically from its other close relative, *L. anaximandri* (Fig. 3), in having greater numbers of sheath lamellae and branchial lamellae and a shorter obturaculum length (Table 4). *Lamellibrachia donwalshi* **sp. nov.** also demonstrates some of the smallest vestimental plaque diameters reported for the genus (lower bound of 33.2µm, Table 4), though this range is very close to that of *L. sagami* and falls partially within the range of plaque diameters for *L. anaximandri* (also shown in Table 4). *Lamellibrachia donwalshi* **sp. nov.** also closely resembles *L. sagami* in the range of trunk plaque diameters, but numbers of lamellae more closely resemble those of *L. columna* (Table 4).

Discussion

Phylogenetic Support. *Lamellibrachia* shows low levels of variation in the mitochondrial genes 16S and COI and the nuclear gene 18S (Cowart *et al*. 2013, 2014). This is reflected in a number of poorly supported nodes in the phylogenetic analyses conducted with these loci (16S and COI; Fig. 3). The average distance among *Lamellibrachia* species calculated from the uncorrected pairwise distances for taxa in this study was approximately 5%, but several established *Lamellibrachia* species differ by as little as 1–3% (e.g. *L. columna/L. sagami* and *L. anaximandri/L.* sp. 2, respectively). This suggests that the 1.81% and 1.92% minimum distances between *L. donwalshi* **sp. nov.** and its closest relatives *L. anaximandri* and *L.* sp. 2, respectively, is not unusual. The validity of *L. donwalshi* **sp. nov.** is also supported by its geographic separation from its closest relatives, *L. anaximandri* and *L.* sp. 2 by the Panama Isthmus. *Lamellibrachia donwalshi* **sp. nov.** is 5.4% divergent from its sympatric relative, *L. barhami*. While in close proximity, these two species were found at different depths: *L. donwalshi* **sp. nov.** was present only at relatively shallow depths (~1,000m) and at a single location (Mound 12), while *L. barhami* was present at depths of 1,800 meters or greater and multiple sites (Jaco Scar, Parrita Scar, and Quepos Seep).

Gulf of Mexico/Caribbean Taxa. Some *Lamellibrachia* specimens in the upper Gulf of Mexico have often been identified as *L. luymesi*, which was described from much further south off Venezuela at about 500 meters depth. The even more remote Uruguayan species, *L. victori,* trawled from 300 meters depth, has been regarded as "questionably distinct" (Gardiner & Hourdez 2003; Jones 1985). Gardiner & Hourdez (2003) pointed out that the original descriptions of *L. luymesi* and *L. victori* by van der Land & Nørrevang (1975) and Mañé-Garzón & Montero (1985) were based on only one or two specimens. The number of sheath lamellae and the ratio of vestimentum diameter to length for the *L. luymesi* type specimen falls within the ranges reported for the specimens sampled from the Gulf of Mexico. Gardiner & Hourdez (2003) did a detailed morphological study on material collected from less than 1,000 m on the upper Louisiana slope of the Gulf of Mexico and extended the range for *L. luymesi* to that region. Most features of the *L. victori* type specimen (except for vestimentum length and the aperture diameter of the specimen tube) also fall within the range of the sampled Gulf of Mexico specimens, so it was suggested by Gardiner & Hourdez (2003) that *L. victori* may not be distinct from *L. luymesi*. However, the type specimen for *L. victori* differs from the type specimen of *L. luymesi* for most of the features analyzed (numbers of lamellae, obturaculum and vestimentum lengths, etc.) used in Gardiner & Hourdez (2003) and it may still be a valid species. In any case, McMullin *et al.* (2003) reported the *Lamellibrachia* specimens collected from the Louisiana Slope for DNA sequencing as *L.* cf. *luymesi*. The molecular results of Miglietta *et al*. (2010), which included samples from deeper waters in the Gulf of Mexico, then revealed at least two genetically distinct species of *Lamellibrachia*. One included samples studied by McMullin *et al.* (2003), and they referred to this as *L. luymesi*/ sp. 1. There was a second species only found in deeper water, which they called *L.* sp. 2. (Fig. 2). A microsatellite study by Cowart *et al.* (2014) later showed that *L. luymesi/L.* sp. 1 may indeed be distinct species, but we treat them as a single taxon presently based on the mitochondrial data, which does not differentiate the two. The presence of up to three *Lamellibrachia* species in the northern Gulf of Mexico has been corroborated by further sequencing of mitochondrial and nuclear DNA as well as a microsatellite study of Gulf of Mexico *Lamellibrachia* (Cowart *et al.* 2014). No molecular data exists for specimens from either of the type localities of *L. luymesi* or *L. victori* thus until further sampling and DNA sequencing is conducted, there is no way to confirm what the specimens identified as *L.* cf. *luymesi*, *L.* sp. 1, and *L.* sp. 2 from the Gulf of Mexico actually are. Interestingly, *L.* sp. 2 has been recorded south of Cuba at hydrothermal vents at ~2,500 meters in the Mid-Cayman Spreading Center (Plouviez *et al.* 2015).

Biogeographic Implications of Lamellibrachia donwalshi **sp. nov.**. Fig. 3 shows a grade of Pacific species with

respect to Atlantic *Lamellibrachia* species, with the exception of *L. donwalshi* **sp. nov.** (Pacific), which shows a closest relationship with the Atlantic *L.* sp. 2 (Gulf of Mexico) and *L. anaximandri* (Mediterranean) (Fig. 2). As in Southward *et al.* 2011, within the Pacific grade, a West Pacific species (*L. juni*) was recovered as sister to all other *Lamellibrachia*. This suggests a Pacific ancestor for *Lamellibrachia* and may align with the Moalic *et al.* (2012) hypothesis that proposes Atlantic deep-sea chemosynthetic environments were colonized by Pacific deep-sea fauna. The recovery of *L. donwalshi* **sp. nov.** inside the Atlantic clade suggests that a vicariant event may have occurred after an Atlantic radiation of *Lamellibrachia*. The shoaling of deep water as the Panama isthmus began to form (approximately 9–12 Ma [O'Dea *et al.* 2016]) could have shut off the deep-water connection between populations of the *L. donwalshi* **sp. nov.**/*L.* sp. 2/*L. anaximandri* common ancestor. Similar phylogenetic topologies and biogeographic hypotheses have been reported for other deep-sea fauna in chemosynthetic environments in this region, such as the vesicomyids *Pliocardia* Woodring, 1925*, Calyptogena* Dall, 1891, and *Abyssogena* Krylova, Sahling & Janssen 2010 (LaBella *et al.* 2017), and in the annelid *Amphisamytha* Hessle, 1917, which also shows a clear eastern Pacific to Atlantic sister relationship (Stiller *et al.* 2013).

The expanded range of *L. barhami* reported here is also notable. Though originally described from off southern California by Webb in 1969 (sequences not published as yet from the type locality), *L. barhami* has been noted along the Pacific coast of North America from central California to Oregon (Black *et al.* 1997; Suess *et al.* 1985), and as far north as Vancouver Island, Canada (Barry *et al.* 1996; McMullin *et al.* 2003), with DNA sequence data to support this (Black *et al.* 1997; McMullin *et al.* 2003). Sequence data also shows that it occurs in the southern and central Gulf of California (Goffredi *et al*. 2017, present study) and it has been noted as far south as Costa Rica (Han *et al.* 2004; Mau *et al.* 2006; Sahling *et al.* 2008; Southward *et al.* 1996), though until now there been no sequence data to support these southernmost reports. Haplotype networks (Fig. 5) show minimal genetic divergence between *L. barhami* samples from different depths and different localities: Costa Rica (Jaco Scar, Parrita Scar, Quepos Seep), Gulf of California (Guaymas and Pescadero Basins), Monterey Canyon, Oregon, Middle Valley, and Vancouver Island from depths of 1,000–2,416 meters. Though a large proportion of the COI sequences of *L. barhami* were from Costa Rica, even those from as far north as Vancouver Island were identical or differed by at most a single base pair from the most common haplotype, regardless of locality (Fig. 5).

The *Lamellibrachia* phylogeny should also be considered in the context of the phylogeny of Siboglinidae (Rouse 2001; Sun *et al.* 2018). In the Sun *et al.* analysis (2018), generated with 13 mitochondrial genes and two ribosomal RNA genes, all sequenced seep-dwelling Vestimentifera (*Lamellibrachia*, *Escarpia*, *Seepiophila*, and *Paraescarpia*) formed a grade with respect to a vent-dwelling clade (*Riftia*, *Ridgeia, Oasisia*, *Tevnia*). Rouse (2001) showed that the two genera known at that time from seeps (*Lamellibrachia*, *Escarpia*) formed the sister group to the vent clade (*Arcovestia, Alaysia, Riftia*, *Ridgeia, Oasisia*, *Tevnia*) and that, in the context of the overall phylogeny of Siboglinidae, the vent-dwelling clade was derived from seep ancestors. The vent-seep separation is somewhat recurrent geographically: vent Vestimentifera are known only from the Pacific (with the exception of a known occurrence at a submarine volcano north of Sicily [Southward et al. 2011]), with none present at the Mid-Atlantic Ridge (Gebruk *et al.* 1997), Antarctic Ridge (Karaseva *et al.* 2016), or the South-West and Central Indian Ridges (Van Dover *et al.* 2001). The Southern Ocean may act as a barrier to some vent animals (Rogers *et al.* 2012), and at the Central Indian Ridges there is no molecular evidence of a connection between Pacific and Atlantic deep-sea fauna (there are known topographic barriers that could limit dispersal there [Van Dover *et al*. 2001]). However, some non-vestimentiferan taxa inhabiting chemosynthetic environments such as the annelid *Archinome* Kudenov, 1991 and the mussel *Bathymodiolus* Kenk & Wilson, 1985 and two seep vestimentiferan genera (*Lamellibrachia* and *Escarpia*) have distributions in both the Pacific and Atlantic Oceans (Borda *et al.* 2013; Copley *et al.* 2016). The phylogeny of *Escarpia* is not well-resolved and needs further study, but the present results suggest that *Lamellibrachia* originated in the Pacific (Fig. 3). Further molecular data is needed to more clearly elucidate the evolutionary relationships of Pacific and Atlantic seep Vestimentifera.

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