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At least six morphospecies of vestimentiferan tubeworms are associated with cold seeps in the Gulf of Mexico (GOM). The physiology and ecology of the two best-studied species from depths above 1000 m in the upper Louisiana slope (*Lamellibrachia luymesi* and *Seepiophila jonesi*) are relatively well understood. The biology of one rare species from the upper slope (escarpiid sp. nov.) and three morphospecies found at greater depths in the GOM (*Lamellibrachia* sp. 1, *L.* sp. 2, and *Escarpia laminata*) are not as well understood. Here we address species distributions and boundaries of cold-seep tubeworms using phylogenetic hypotheses based on two mitochondrial genes. Fragments of the mitochondrial large ribosomal subunit rDNA (16S) and cytochrome oxidase subunit I (COI) genes were sequenced for 167 vestimentiferans collected from the GOM and analyzed in the context of other seep vestimentiferans for which sequence data were available. The analysis supported five monophyletic clades of vestimentiferans in the GOM. Intra-clade variation in both genes was very low, and there was no apparent correlation between the within-clade diversity and collection depth or location. Two of the morphospecies of *Lamellibrachia* from different depths in the GOM could not be distinguished by either mitochondrial gene. Similarly, *E. laminata* could not be distinguished from other described species of *Escarpia* from either the west coast of Africa or the eastern Pacific using COI. We suggest that the mitochondrial COI and 16S genes have little utility as barcoding markers for seep vestimentiferan tubeworms.

1. Introduction

For the better part of the last century, marine biologists assumed oceans were largely interconnected by currents that enabled larvae and propagules to reach distant shores and assure gene flow even over great distances. More recently, the use of molecular tools has challenged assumptions regarding population structure and speciation in the ocean and demonstrated that marine animals often have genetically distinct populations despite geographic proximity (Palumbi and Warner, 2003). Although sharp genetic breaks between close populations have been recorded throughout the ocean, most of what is known about speciation patterns and phylogeography has been inferred from shallow-water and coastal systems, which represent only about 15% of the aquatic environment. Thus, our knowledge of processes that lead to population divergence and speciation in the open ocean is relatively limited (Thornhill et al., 2008, and references therein; Zardus et al., 2006).

Vestimentiferan tubeworms, which include 10 genera in the polychaete family Siboglinidae (Halanych et al., 2001; Kojima et al., 2002; McMullin et al., 2003; Rouse, 2001), are abundant at deep-sea hydrothermal vents and cold seeps at depths ranging from 80 to 9345 m (Cordes et al., 2007b; Mironov, 2000; Miura et al., 2002). In the deep Gulf of Mexico, six morphospecies have been reported (Cordes et al., 2009). Two described species, *Lamellibrachia luymesi* (van der Land and Narrevang, 1975) and *Seepiophila jonesi* (Gardiner et al., 2001), are relatively well studied, and their ecology and physiology are well understood (Bergquist et al., 2002; Cordes et al., 2007a, b). They occur on the upper Louisiana slope at between ~500 and 950 m depth and occasionally co-occur with a rare undescribed species, escarpiid sp. nov. The three other morphological species are found on the lower Louisiana slope at depths greater than about 950 m (*Lamellibrachia* sp. 1, *L.* sp. 2, and *Escarpia laminata*).

In this paper, we present phylogenetic hypotheses based on the mitochondrial large ribosomal subunit rDNA gene (16S) and mitochondrial cytochrome oxidase 1 gene (COI) of over 200

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vestimentiferans (sequenced for either or both genes) including 180 individuals from the six morphospecies that occur in the Gulf of Mexico. Phylogenetic trees are used to examine the distribution of vestimentiferans in the Gulf of Mexico and their relations to other vestimentiferans around the world. We examined the concordance between the morphological and phylogenetic data to identify differences between the genealogical and morphological species analyzed. Finally, we compared between- and within-species 16S and COI genetic distances and show that these two mitochondrial genes have little utility as "barcoding molecules" for vestimentiferans.

2. Material and methods

2.1. Collection of material

Vestimentiferans were collected in the deep Gulf of Mexico from 12 sites on two cruises in 2006 and 2007, using the DSV ALVIN and R.V. *Atlantis* in 2006 and ROV JASON II and the NOAA ship *Ronald Brown* in 2007 (see Fig. 1). Vestimentiferans were collected using either the Bushmaster Jr. collection device (for samples destined also for community ecology analyses, see Cordes et al., 2010) or the submersible manipulators and placed directly into a collection box. Aboadship, all vestimentiferans were identified using morphological criteria, and subsamples of vestimentum tissue were frozen for subsequent analyses at the Pennsylvania State University. Additional frozen vestimentiferan tissue samples collected previously from shallower sites on the upper Louisiana slope using the DSV JOHNSON SEA LINK were also analyzed for this study (see Table 1 for a complete list of specimens).

2.2. DNA sequencing

DNA was extracted either by boiling a small amount of frozen tissue in 600 μL of 10% Chelex solution (Bio-Rad) or using a CTAB+PVP method modified from Doyle and Doyle (1987), followed by a standard ethanol precipitation.

A 524 bp fragment of the mitochondrial 16S gene was amplified using primers 16Sar and 16Sbr (Kojima et al., 1995). A 689 bp fragment of the mitochondrial gene COI was amplified using the primers HCO and LCO (Folmer et al., 1994). Amplification was performed under the following PCR conditions: 94 °C (1 min); 50 °C (2 min); and 72 °C (2.5 min) for 30 cycles. All PCR reactions were performed using 0.5 μl of each primer, 2.5 μl of 10XBuffer, 2 μl of 10 μM dNTPs, 0.2 μl of taq, 16.5 μl of water, and 3 μl of template. The PCR product was first purified with the ExoSap-it protocol (USB, Affimetrix) and then run on a 2% agarose gel stained with ethidium bromide to enable us to check the quantity and quality of the product. The purified PCR product was used as a template for double-stranded sequencing that was carried out at the Pennsylvania State University Sequencing Core Facility, University Park, Pennsylvania, using ABI 3730 sequencer machines.

2.3. Phylogenetic analysis

Sequences were first assembled and edited using Geneious Pro 4.0.4 (Biomatters Ltd.), and then aligned using ClustalX (Thompson et al., 2002). All alignments were confirmed and edited visually in MacClade 4.06 OS X (Maddison and Maddison, 2000) to insure that indel variation was aligned consistently among all sequenced genes.

Phylogenetic analyses of the aligned sequences were conducted using the maximum parsimony (MP) optimality criterion and neighbor joining (Saitou and Nei, 1987) (NJ) in PAUP* version 4.0b10 for Macintosh (Wilgenbusch and Swofford, 2003), and the maximum likelihood (ML) optimality criterion in GARLI v0.951.OsX-GUI (Zwickl, 2006) and PhylML (Guindon and Gascuel, 2003). The best-fit model used in PhyML and PAUP* was assessed using the akaike information criterion as implemented in modeltest (Posada, 2003; Posada and Crandall, 1998). The best-fit model was (HKY+I+G) for the COI dataset and (GTR+G) for the 16S dataset. Clade stability was assessed by ML bootstrap analysis (Felsenstein, 1985) in GARLI (100 bootstrap replicates) and NJ (1000 replicates) in PAUP*. The ML analyses in GARLI were performed using random starting trees and default termination conditions. Within- and between-species distances were estimated in MEGA 4 (Tamura et al., 2007).

3. Results

The complete COI dataset includes 146 sequences (Table 1) of the six Gulf of Mexico (GOM) cold-seep morphospecies, the available GenBank sequences of *E. southwardae, E. spicata,* and assorted *Lamellibrachia* species from around the world. Sequences from the hydrothermal vent-dwelling genera *Riftia*, *Oasisia*, *Tevnia*, and *Arcovestia* were used as outgroups. We restricted our analyses to

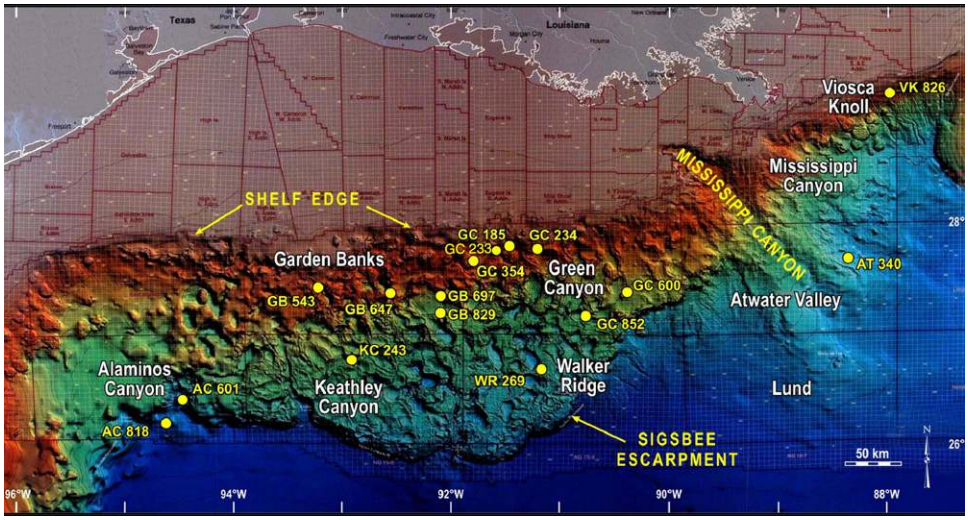

Fig. 1. Map of new deep-water collection sites in the Gulf of Mexico.

Table 1
Genbank accension numbers and genes analyzed.

Sample[a]	Clade	Location[b]	GenBank Accession #	Genes
1.AC818	*Escarpia laminate*	GOM AC818	16S: GU068165 COI: GU059163	16S/COI
2.AC818	*Escarpia laminata*	GOM AC818	COI: GU059196	COI
3.AC818	*Escarpia laminata*	GOM AC818	16S: GU068166 COI: GU059205	16S/COI
4.AC818	*Escarpia laminata*	GOM AC818	16S: GU068167 COI: GU059214	16S/COI
5.AC818	*Escarpia laminata*	GOM AC818	16S: GU068168 COI: GU059222	16S/COI
6.AC818	*Escarpia laminata*	GOM AC818	16S: GU068169 COI: GU059228	16S/COI
7.AC818	*Escarpia laminata*	GOM AC818	16S: GU068170 COI: GU059234	16S/COI
8.AC818	*Lamellibrachia luymesi*/sp. 1	GOM AC818	16S: GU068171	16S
10.GB697	*Escarpia laminata*	GOM GB697	16S: GU068172 COI: GU059164	16S/COI
11.GB829	*Escarpia laminata*	GOM GB829	16S: GU068173 COI: GU059170	16S/COI
12.GB829	*Escarpia laminata*	GOM GB829	16S: GU068174 COI: GU059174	16S/COI
13.GC600	*Escarpia laminata*	GOM GC600	16S: GU068175	16S
14.GC852	*Escarpia laminata*	GOM GC852	16S: GU068176 COI: GU059185	16S/COI
17.GC852	*Escarpia laminata*	GOM GC852	16S: GU068177 COI: GU059192	16S/COI
18.GC852	*Escarpia laminata*	GOM GC852	COI: GU059193	COI
19.GC852	*Escarpia laminata*	GOM GC852	16S: GU068178 COI: GU059194	16S/COI
19B.AC818	*Escarpia laminata*	GOM AC 818	COI: GU059195	COI
20.WR269	*Escarpia laminata*	GOM WR269	16S: GU068179 COI: GU059197	16S/COI
21.WR269	*Escarpia laminata*	GOM WR269	16S: GU068180 COI: GU059198	16S/COI
22.WR269	*Escarpia laminata*	GOM WR269	16S: GU068181 COI: GU059199	16S/COI
23.WR269	*Escarpia laminata*	GOM WR269	COI: GU059200	COI
24.WR269	*Escarpia laminata*	GOM WR269	COI: GU059201	COI
26.AT340	*Escarpia laminata*	GOM AT340	16S: GU068182	16S
27.AT340	*Escarpia laminata*	GOM AT340	16S: GU068183 COI: GU059202	16S/COI
28.AT340	*Escarpia laminata*	GOM AT340	16S: GU068184 COI: GU059203	16S/COI
29.AT340	*Escarpia laminata*	GOM AT340	16S: GU068185 COI: GU059204	16S/COI
30.AT340	*Escarpia laminata*	GOM AT340	16S: GU068186 COI: GU059206	16S/COI
31.AT340	*Escarpia laminata*	GOM AT340	16S: GU068187 COI: GU059207	16S/COI
32.AT340	*Escarpia laminata*	GOM AT340	16S: GU068188 COI: GU059208	16S/COI
33.AT340	*Escarpia laminata*	GOM AT340	16S: GU068189 COI: GU059209	16S/COI
34.WR264	*Escarpia laminata*	GOM WR269	16S: GU068190	16S
35.WR269	*Escarpia laminata*	GOM WR269	16S: GU068191 COI: GU059210	16S/COI
37.AC601	*Escarpia laminata*	GOM AC601	16S: GU068192 COI: GU059211	16S/COI
38.AC601	*Escarpia laminata*	GOM AC601	16S: GU068193 COI: GU059212	16S/COI
39.AC601	*Escarpia laminata*	GOM AC601	16S: GU068194 COI: GU059213	16S/COI
40.AC601	*Escarpia laminata*	GOM AC601	16S: GU068195 COI: GU059215	16S/COI
41.AC601	*Escarpia laminata*	GOM AC601	16S: GU068196	16S
42.AC601	*Escarpia laminata*	GOM AC602	16S: GU068197 COI: GU059216	16S/COI
43.AC601	*Escarpia laminata*	GOM AC601	16S: GU068198 COI: GU059217	16S/COI
44.AC601	*Escarpia laminata*	GOM AC602	16S: GU068199 COI: GU059218	16S/COI
45.AC601	*Escarpia laminata*	GOM AC601	16S: GU068200 COI: GU059219	16S/COI
46.AT340	*Escarpia laminata*	GOM AT340	16S: GU068201	16S
47.AC601	*Escarpia laminata*	GOM AC601	16S: GU068202 COI: GU059220	16S/COI

Table 1 (*continued*)

Sample[a]	Clade	Location[b]	GenBank Accession #	Genes
48.AC601	*Escarpia laminata*	GOM AC601	16S: GU068203	16S
49.AC601	*Escarpia laminata*	GOM AC601	16S: GU068204 COI: GU059221	16S/COI
50.AC601	*Escarpia laminata*	GOM AC601	16S: GU068205 COI: GU059223	16S/COI
51.AT340	*Escarpia laminata*	GOM AT340	16S: GU068206	16S
52.AT340	*Escarpia laminata*	GOM AT340	16S: GU068207	16S
54.AC601	*Escarpia laminata*	GOM AC601	16S: GU068208 COI: GU059224	16S/COI
55.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068209 COI: GU059225	16S/COI
56.*L.* sp. 1 GB697	*Lamellibrachia luymesi*/sp. 1	GOM GB697	16S: GU068210	16S
57.*L. luymesi* GC234	*Lamellibrachia luymesi*/sp. 1	GOM GC234	16S: GU068211 COI: GU059226	16S/COI
58.*L.* sp. 1 GC852	*Lamellibrachia luymesi*/sp. 1	GOM GC852	16S: GU068212 COI: GU059227	16S/COI
59.*L.* sp. 1 AC601	*Lamellibrachia luymesi*/sp. 1	GOM AC601	16S: GU068213	16S
60.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068214	16S
61.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068215	16S
62.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068216 COI: GU059229	16S/COI
63.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068217	16S
64.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068218 COI: GU059230	16S/COI
65.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068219	16S
66.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068220 COI: GU059231	16S/COI
67.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068221	16S
68.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068222 COI: GU059232	16S/COI
69.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068223 COI: GU059233	16S/COI
70.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068224 COI: GU059235	16S/COI
71.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068225	16S
72.*L. luymesi* BP	*Lamellibrachia luymesi*/sp. 1	GOM GC233	16S: GU068226 COI: GU059236	16S/COI
73.*L.* sp. 1 GB697	*Lamellibrachia luymesi*/sp. 1	GOM GB697	16S: GU068227 COI: GU059237	16S/COI
74.*L.* sp. 1 GB697	*Lamellibrachia luymesi*/sp. 1	GOM GB697	16S: GU068228	16S
75.*L.* sp. 1 GB697	*Lamellibrachia luymesi*/sp. 1	GOM GB697	16S: GU068229	16S
76.*L.* sp. 1 GB829	*Lamellibrachia luymesi*/sp. 1	GOM GB829	16S: GU068230 COI: GU059238	16S/COI
77.*L.* sp. 1 GB829	*Lamellibrachia luymesi*/sp. 1	GOM GB829	16S: GU068231	16S
78.*L.* sp. 1 GB829	*Lamellibrachia luymesi*/sp. 1	GOM GB829	16S: GU068232 COI: GU059239	16S
79.*L.* sp. 1 GB829	*Lamellibrachia luymesi*/sp. 1	GOM GB829	16S: GU068233	16S
80.*L.* sp. 1 GB829	*Lamellibrachia luymesi*/sp. 1	GOM GB829	16S: GU068234	16S
81.*L. luymesi* GC234	*Lamellibrachia luymesi*/sp. 1	GOM GC234	16S: GU068235	16S
83.*L. luymesi* GC234	*Lamellibrachia luymesi*/sp. 1	GOM GC234	16S: GU068236 COI: GU059240	16S/COI
84.*L. luymesi* GC234	*Lamellibrachia luymesi*/sp. 1	GOM GC234	16S: GU068237	16S
85.*L. luymesi* GC234	*Lamellibrachia luymesi*/sp. 1	GOM GC234	16S: GU068238	16S
86.*L. luymesi* GC234	*Lamellibrachia luymesi*/sp. 1	GOM GC234	16S: GU068239 COI: GU059241	16S/COI
88.*L.* sp. 1 GC600	*Lamellibrachia luymesi*/sp. 1	GOM GC600	16S: GU068240 COI: GU059242	16S/COI
89.*L.* sp. 1 GC600	*Lamellibrachia luymesi*/sp. 1	GOM GC600	16S: GU068241 COI: GU059243	16S/COI
90.*L.* sp. 1 GC852	*Lamellibrachia luymesi*/sp. 1	GOM GC852	16S: GU068242 COI: GU059244	16S/COI
91.*L.* sp. 1 GC852	*Lamellibrachia luymesi*/sp. 1	GOM GC852	16S: GU068243	16S
92.*L.* sp. 1 GC852	*Lamellibrachia luymesi*/sp. 1	GOM GC852	16S: GU068244 COI: GU059245	16S
93.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068245 COI: GU059246	16S/COI
94.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068246	16S
95.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068247	16S
96.*L. luymesi* BH	*Lamellibrachia luymesi*/sp. 1	GOM GC185	16S: GU068248	16S
97.*L. luymesi* GC234	*Lamellibrachia luymesi*/sp. 1	GOM GC234	16S: GU068249	16S
98.*L. luymesi* GC234	*Lamellibrachia luymesi*/sp. 1	GOM GC234	16S: GU068250	16S
99.*L. luymesi* GC234	*Lamellibrachia luymesi*/sp. 1	GOM GC234	16S: GU068251 COI: GU059247	16S/COI
100.*L.* sp. 1. WR269	*Lamellibrachia luymesi*/sp. 1	GOM WR269	16S: GU068252	16S
102.*L.* sp. 1 WR269	*Lamellibrachia luymesi*/sp. 1	GOM WR269	16S: GU068253 COI: GU059165	16S/COI

Table 1 (*continued*)

Sample[a]	Clade	Location[b]	GenBank Accession #	Genes
103.*L.* sp. 1 AT340	*Lamellibrachia luymesi*/sp. 1	GOM AT340	16S: GU068254 COI: GU059166	16S/COI
104.*L.* sp. 1 WR269	*Lamellibrachia luymesi*/sp. 1	GOM WR269	16S: GU068255 COI: GU059167	16S/COI
105.*L.* sp. 1 WR269	*Lamellibrachia luymesi*/sp. 1	GOM WR269	16S: GU068256 COI: GU059168	16S/COI
107.*L.* sp. 1 AC601	*Lamellibrachia luymesi*/sp. 1	GOM AC601	16S: GU068257 COI: GU059169	16S/COI
110.*L.* sp. 1 AC601	*Lamellibrachia luymesi*/sp. 1	GOM AC601	16S: GU068258 COI: GU059171	16S/COI
112.GB697	*Lamellibrachia* sp. 2	GOM GB697	16S: GU068259	16S
113.GB697	*Lamellibrachia* sp. 2	GOM GB697	16S: GU068260 COI: GU059172	16S/COI
114.GB697	*Lamellibrachia* sp. 2	GOM GB297	16S: GU068261	16S
115.GB697	*Lamellibrachia* sp. 2	GOM GB297	16S: GU068262	16S
116.GB829	*Lamellibrachia* sp. 2	GOM GB829	16S: GU068263	16S
117.GC600	*Lamellibrachia* sp. 2	GOM GC600	16S: GU068264	16S
118.GC852	*Lamellibrachia* sp. 2	GOM GC852	16S: GU068265 COI: GU059173	16S/COI
119.GC852	*Lamellibrachia* sp. 2	GOM GC852	16S: GU068266	16S
120.GC852	*Lamellibrachia* sp. 2	GOM GC852	16S: GU068267	16S
121.WR269	*Lamellibrachia* sp. 2	GOM WR269	16S: GU068268	16S
122.AT340	*Lamellibrachia* sp. 2	GOM AT340	16S: GU068269 COI: GU059175	16S/COI
123.WR2695	*Lamellibrachia* sp. 2	GOM WR269	16S: GU068270 COI: GU059176	16S/COI
124.AC601	*Lamellibrachia* sp. 2	GOM AC601	COI: GU059177	COI
126.AC601	*Lamellibrachia* sp. 2	GOM AC601	COI: GU059178	COI
128.*L.* sp. 1 AT340	*Lamellibrachia luymesi*/sp. 1	GOM AT340	16S: GU068271 COI: GU059179	16S/COI
130.GB697	*Seepiophila jonesi*	GOM GB697	16S: GU068272 COI: GU059180	16S/COI
131.GB647	*Seepiophila jonesi*	GOM GB647	16S: GU068273 COI: GU05981	16S/COI
132.GC234	*Seepiophila jonesi*	GOM GC234	16S: GU068274 COI: GU059182	16S/COI
133.GC234	*Seepiophila jonesi*	GOM GC234	16S: GU068275	16S
134.GC234	*Seepiophila jonesi*	GOM GC234	16S: GU068276 COI: GU059183	16S/COI
134b.GC234	*Seepiophila jonesi*	GOM GC234	16S: GU068277 COI: GU059184	16S/COI
135.GC234	*Seepiophila jonesi*	GOM GC234	16S: GU068278	16S
136.GC234	*Seepiophila jonesi*	GOM GC234	16S: GU068279	16S
137.BH	*Seepiophila jonesi*	GOM GC185	16S: GU068280	16S
138.BH	*Seepiophila jonesi*	GOM GC185	16S: GU068281	16S
139.GC234	*Seepiophila jonesi*	GOM GC234	16S: GU068282	16S
140. GC234	*Seepiophila jonesi*	GOM GC234	16S: GU068283	16S
141.GB647	*Seepiophila jonesi*	GOM GB647	16S: GU068284 COI: GU059186	16S/COI
142.GB647	*Seepiophila jonesi*	GOM GB647	16S: GU068285	16S
143.GB647	*Seepiophila jonesi*	GOM GB647	16S: GU068286 COI: GU059187	16S/COI
144.GB647	*Seepiophila jonesi*	GOM GB647	16S: GU068287 COI: GU059188	16S/COI
145.AC818	*Escarpia laminata*	GOM AC818	16S: GU068288 COI: GU059189	16S/COI
146.BH	*Seepiophila jonesi*	GOM GC185	16S: GU068289	16S
147.GB647	*Seepiophila jonesi*	GOM GB647	COI: GU059190	COI
148.GB647	*Seepiophila jonesi*	GOM GB647	COI: GU059191	COI
149.AC601	*Escarpia laminata*	GOM AC601	COI: GU059248	COI
151.GB697	*Lamellibrachia luymesi*	GOM GB697	COI: GU059250	COI
152.GC234	*Lamellibrachia luymesi*/sp. 1	GOM GC234	COI: GU059253	COI
153.GC600	*Lamellibrachia luymesi*/sp. 1	GOM GC600	COI: GU059254	COI
154.NewEscarpidGB485	Escarpiid sp. nov.	GOM GB425	16S: GU068290 COI: GU059255	16S/COI
155.NewEscarpidGC234	Escarpiid sp. nov.	GOM GC234	16S: GU068291 COI: GU059256	16S/COI
157.*L.* sp. 1 GB697	*Lamellibrachia luymesi*/sp. 1	GOM GB697	COI: GU059229	COI
159.GB697	*Seepiophila jonesi*	GOM GB697	COI: GU059251	COI
160.GB697	*Seepiophila jonesi*	GOM GB697	COI: GU059252	COI
161.GC234	*Lamellibrachia luymesi*/sp. 1	GOM GC234	xxx-xxx	COI
162.GC600	*Lamellibrachia luymesi*/sp. 1	GOM GC600	xxx-xxx	COI
165.GC852	*Lamellibrachia* sp. 2	GOM GC852	xxx-xxx	COI
166.*L.*sp1 AT340	*Lamellibrachia luymesi*/sp. 1	GOM AT340	xxx-xxx	COI
S. jonesi BH	*Seepiophila jonesi*	GOM GC185	AF317287	COI
S. jonesi GB425	*Seepiophila jonesi*	GOM GB425	AF317288	COI

Table 1 (*continued*)

Sample[a]	Clade	Location[b]	GenBank Accession #	Genes
Lamluymesi GC234	*Lamellibrachia luymesi*	GOM GC234	AY129136	COI
Basibranchia mariana 1	*Basibranchia mariana*	West Pacific	U74078	COI
Arcovestia	*Arcovestia ivanovi*	West Pacific	AB073491	COI
E. laminata	*Escarpia laminata*	West Atlantic	U74063	COI
E. southwardae 1	*Escarpia southwardae*	West Africa	AY326304	COI
E. southwardae 2	*Escarpia southwardae*	West Africa	AY326303	COI
E. spicata	*Escarpia spicata*	East Pacific	U84262	COI
L. sp.1_b	*Lamellibrachia luymesi*/sp. 1	GOM AT340	U74061	COI
*Oasisia*HaploA	*Oasisia alvinae*	East Pacific	AY646001	COI
*Oasisia*HaploP	*Oasisia alvinae*	East Pacific	AY646016	COI
*Lam.*2000Nanaki	*Lamellibrachia* sp.	West Pacific	D50592	COI
*Lam.*300Sagami	*Lamellibrachia* sp.	West Pacific	AB088674	COI
*Lam.*300Sagami 1	*Lamellibrachia* sp.	West Pacific	D38029	COI
*Lam.barhami*10b	*Lamellibrachia barhami*	East Pacific	AY129137	COI
*Lam.barhami*11b	*Lamellibrachia barhami*	East Pacific	AY129138	COI
*Lam.barhami*4b	*Lamellibrachia barhami*	East Pacific	AY129147	COI
*Lam.barhami*7	*Lamellibrachia barhami*	East Pacific	AY129146	COI
*Lam.barhami*8b	*Lamellibrachia barhami*	East Pacific	AY129145	COI
*Lam.barhami*9	*Lamellibrachia barhami*	East Pacific	AY129141	COI
*Lam.barhami*b	*Lamellibrachia barhami*	East Pacific	U74054	COI
*L. barhami*2	*Lamellibrachia barhami*	East Pacific	AF315045	16S
*L. barhami*3	*Lamellibrachia barhami*	East Pacific	AF315045	16S
Lam.columna	*Lamellibrachia columna*	West Pacific	U74061	COI
Lam.columna 1	*Lamellibrachia columna*	West Pacific	AB055210	COI
Lam.juni	*Lamellibrachia juni*	West Pacific	AB242858	COI
*Lam.juni*Haplo1	*Lamellibrachia juni*	West Pacific	AB264601	COI
*Lam.juni*Haplo2	*Lamellibrachia juni*	West Pacific	AB264602	COI
*Lam.juni*Haplo3	*Lamellibrachia juni*	West Pacific	AB264603	COI
*Lam.juni*Haplo4	*Lamellibrachia juni*	West Pacific	AB264604	COI
*Lam.juni*Haplo5	*Lamellibrachia juni*	West Pacific	AB264605	COI
*Lam*L4	*Lamellibrachia* sp.	West Pacific	AB055209	COI
*Lam*L5	*Lamellibrachia* sp.	West Pacific	AB055210	COI
*Lam*L6	*Lamellibrachia* sp.	West Pacific	AB088674	COI
*Lam*L7	*Lamellibrachia* sp.	West Pacific	AB088675	COI
*Lamluymesi*BH 2	*Lamellibrachia luymesi*	GOM GC185	AY129133	COI
*Lam.luymesi*BHb	*Lamellibrachia luymesi*	GOM GC185	AY129132	COI
*Lam.luymesi*BP	*Lamellibrachia luymesi*	GOM GC233	AY129139	COI
*Lam.luymesi*GB4252	*Lamellibrachia luymesi*	GOM GB425	AY129135	COI
*Lam.luymesi*GC354	*Lamellibrachia luymesi*	GOM GC354	AY129126	COI
Lam.luymesi VK	*Lamellibrachia luymesi*	GOM VK826	AY129124	COI
*Lam.*Med	*Lamellibrachia* sp. from Med.	Mediterranean	EU046616	COI
*Lam.satsuma*b	*Lamellibrachia satsuma*	West Pacific	AF342671	COI
NewEscarpiidGB425	Escarpiid sp. nov.	GOM GB425	AY129134	COI
Oaisisia fujikurai	*Oasisia fujikurai*	South/West Pacific	AB242857	COI
Paraescarpia	*Paraescarpia* cf. *echinospica*	West Pacific	D50594	COI
Ridgeia	*Ridgeia piscesae*	Juan de Fuca Ridge	AF022233	COI
Ridgeia	*Ridgeia piscesae*	Juan de Fuca Ridge	AF315054	16S
Ridgeia 2	*Ridgeia piscesae*	Juan de Fuca Ridge	AF315051	16S
*Ridgeia*3	*Ridgeia piscesae*	Juan de Fuca Ridge	AF315054	16S
Riftia	*Riftia pachyptila*	East Pacific	AY645989	COI
Tevnia jerichonana	*Tevnia jerichonana*	East Pacific	16S: AF315042 COI: AY645995	16S/COI
S. jonesi BH	*Seepiophila jonesi*	GOM GC185	AF317287	COI
S. jonesi GB425	*Seepiophila jonesi*	GOM GB425	AF317288	COI

[a] Samples analyzed for this study are numbered and labeled as for Figs. 2 and 3. Sequences from Genbank are listed by names assigned in Genbank.

[b] Samples from the Gulf of Mexico are indicated by GOM followed by the abbreviation of their collection sites. VK826, GC185, GC233, GB425, GC234, and GC354 are all on the upper Louisiana slope at depths < 800 m. The other GOM sites are at depths > 900 m and are indicated on Fig. 1.

the species' boundaries for *Lamellibrachia*, *Escarpia,* and *Seepiophila,* and we do not infer higher level phylogenetic relationships among genera because neither 16S nor COI offers sufficient resolution at deeper nodes. The complete and aligned COI dataset included 690 bp, of which 460 were invariant sites, 207 were phylogenetically informative sites, and 23 were autapomorphies.

The complete 16S dataset consisted of 133 sequences (see Table 1 for the complete list of samples), 127 of which were from the Gulf of Mexico. Sequences from the vent-dwelling genera *Tevnia* and *Ridgeia* were used as outgroups. The aligned 16S dataset consisted of 524 bp, of which 433 were invariant sites, 72 were phylogenetically informative, and 19 were autapomorphies.

MP, ML, and NJ analyses produced congruent trees, and the GARLI ML phylogeny is presented in Fig. 2 A and B and 3A and B.

Both 16S and COI phylogenies identify five distinct monophyletic clades of vestimentiferans in the Gulf of Mexico. Four of the clades represent single morphospecies, *S. jonesi*, *E. laminata*, *Lamellibrachia* sp. 2, and escarpiid sp. nov., from the upper slope. However, the fifth clade includes both *Lamellibrachia* sp. 1 from the collections in the deeper GOM and *L. luymesi* from the upper Louisiana slope sites. They were, therefore, considered a single species when within- and between-species distances for the 16S and COI datasets were estimated. Additionally, COI sequences of *E. laminata* did not differ from those of *E. spicata* and

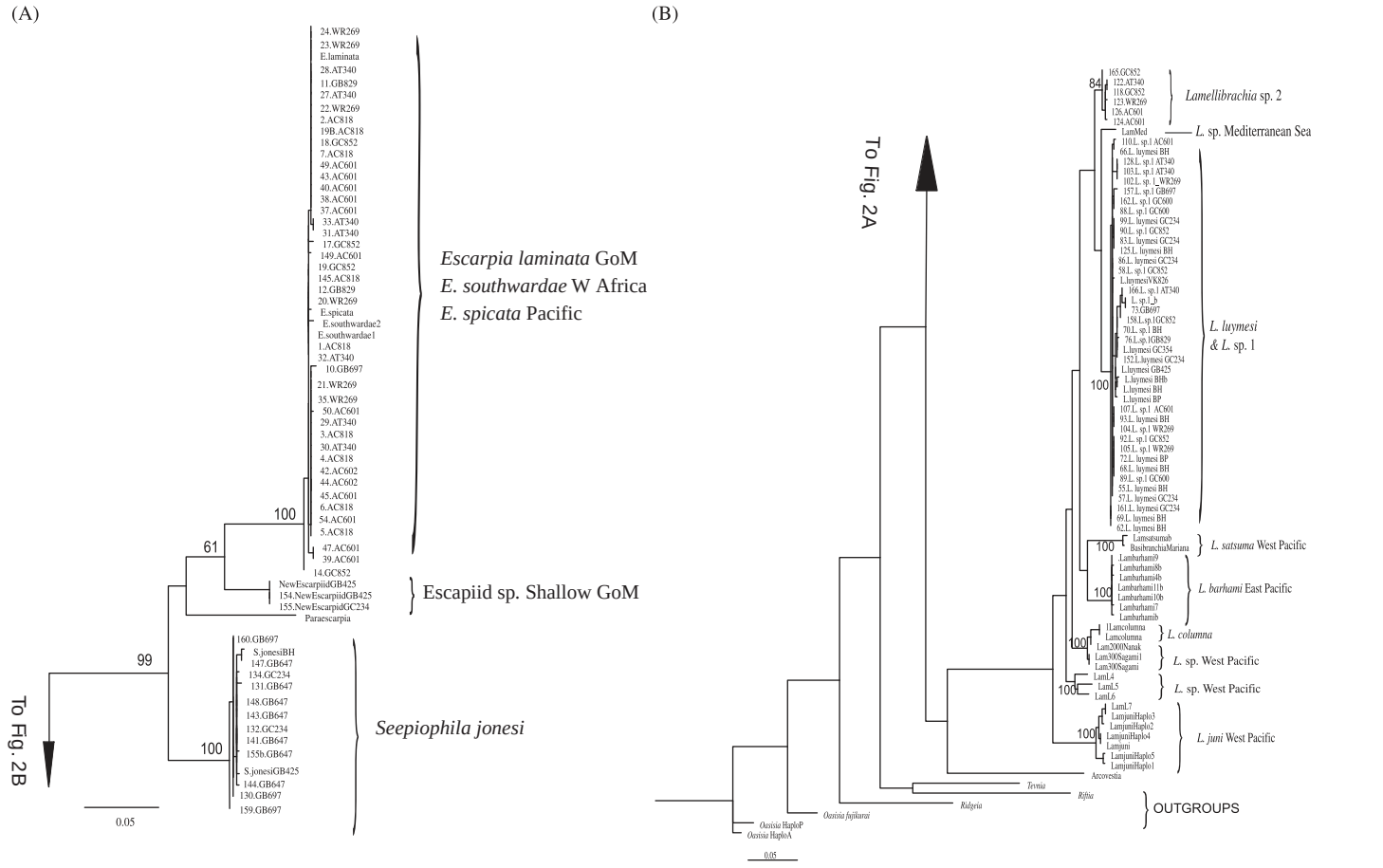

Fig. 2. COI maximum likelihood (ML) tree. Outgroups are shown in italics, and bootstrap support above 50% (NJ 1000 replicates) is indicated below each node. All new sequences are preceded by a number, followed by the abbreviation for the seep site or lease block from which they were collected. VK=Viosca Knoll, BH=Bush Hill, and BP=Brine Pool. Those sites, together with GB425, GC234, and GC354, are from the upper Louisiana slope of the GOM ($<$800 m depth). All other lease blocks are on the lower slope (Fig. 1).

Table 2
16 S Between- and within-species (in bold, on diagonal) *p* distances for the 16 S gene of the GOM species.

	[1] (%)	[2] (%)	[3] (%)	[4] (%)	[5] (%)
[1] *E. laminata*	**0.10**				
[2] *L. luymesi*/ sp. 1	9.60	**0.00**			
[3] *L.* sp. 2	9.00	2.20	**0.10**		
[4] *S. jonesi*	2.00	8.40	8.10	**0.00**	
[5] Escarpid sp. new	3.50	8.30	8.90	3.70	**0.00**

Table 3
Between- and within-species (in bold, on diagonal) *p* distances for the COI gene.

	[1] (%)	[2] (%)	[3] (%)	[4] (%)	[5] (%)
[1] *E. laminata/southwardae/spicata*	**0.9**				
[2] *L. luymesi*/ sp. 1	13.7	**0.4**			
[3] *L.* sp. 2	13.8	2.8	**0.3**		
[4] *S. jonesi*	9.7	14.2	14.4	**0.3**	
[5] Escarpid sp. new	7.1	14.8	14.6	7.1	**0.0**

E. southwardae from the East Pacific and East Atlantic, respectively. We were unable to obtain 16S sequences for *E. spicata* or *E. southwardae*.

Estimates of within- and between-species diversity (*p*) for both genes are shown in Tables 2 and 3. Within a species, *p* distances range from 0% to 0.1% for 16S and 0% to 0.9% for the more variable COI. The very low values for the undescribed escarpiid may reflect the small number of individuals of this species analyzed (n=3 for COI and n=2 for 16S).

4. Discussion

4.1. Distribution of vestimentiferan species in the Gulf of Mexico and relation to other seep species

Vestimentiferans have been collected from both hydrothermal vent and cold-seep sites. The vent and seep species fall into two different clades. However, it should be noted that "seep species" are sometimes found in sedimented hydrothermal vent areas with low levels of diffuse flow, and that "cold-seep" fluids may have temperatures elevated over background (Black et al., 1998; Kojima et al., 1997; MacDonald et al., 2000; Joye et al., 2005); so this separation really reflects more aspects of their habitat than temperature alone. Vestimentiferans found at cold seeps worldwide can be further divided into two clades. One clade includes at least five named and three unnamed species in the genus *Lamellibrachia*. The other clade includes three named species in the genus *Escarpia*, *S. jonesi*, *Paraescarpia echinospica*, and a rarely collected species (escarpiid sp. nov.) from the shallow GOM. Although *Arcovestia* seems basal to the *Lamellibrachia* clade (Fig. 2B), this position is not well supported.

Three species in the escarpiid clade of seep vestimentiferans are found in the GOM: *S. jonesi* has been collected from numerous sites,

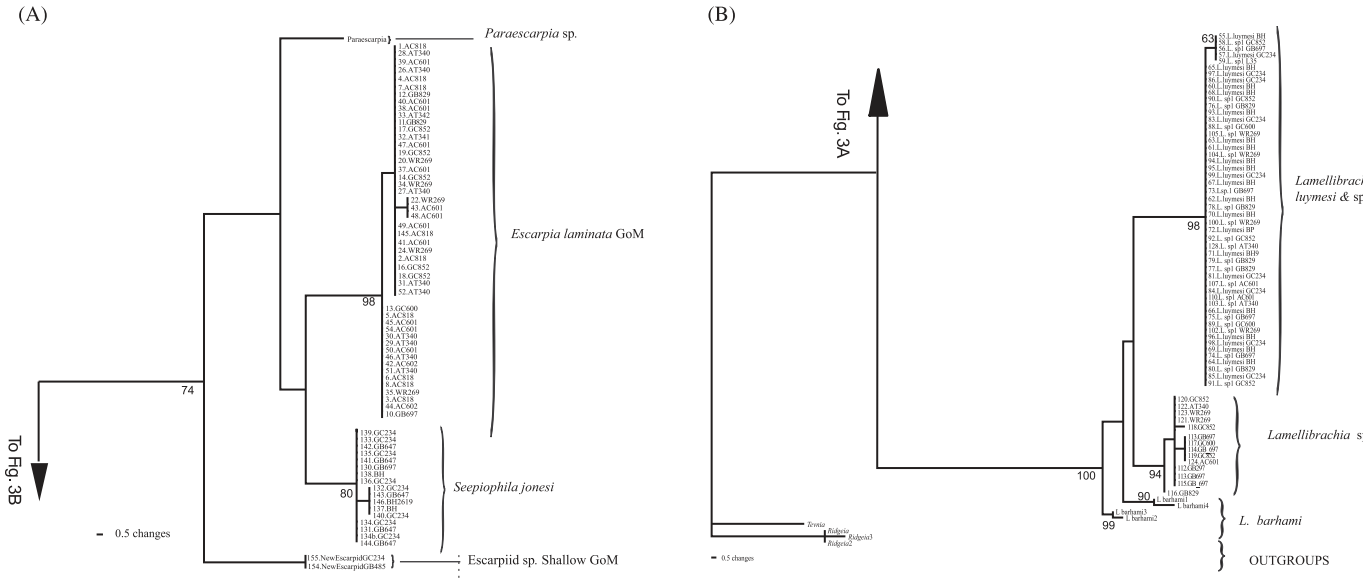

Fig. 3. 16S maximum likelihood (ML) tree. Outgroups are shown in italics and bootstrap support above 50% (NJ 1000 replicates) is indicated below each node. Sample identifications and abbreviations are as in Fig. 2.

ranging in depth from 500 to 950 m; escarpiid sp. nov. from two sites ranging in depth from 600 to 640 m, where it co-occurs with *S. jonesi* (although it has been reported also from GC234 at 525 m; see Cordes et al., 2003); and *E. laminata* from 950 to 3200 m depth. *S. jonesi* and *E. laminata* co-occurred at only one site, GB647, at a depth of 950 m. The undescribed escarpiid differs morphologically from *S. jonesi,* as it lacks the curl of the ventral vestimental fold that is a defining character of the genus *Seepiophila* (Gardiner et al., 2001). Additionally, the obturacular process of the undescribed escarpiid forms a spike, whereas it is flat in *S. jonesi* and barely protrudes from the top of the obturaculum.

Both the COI and 16S phylogenetic trees distinguish these three species and place them within the escarpiid clade of seep vestimentiferans (Figs. 2 and 3). Both the 16S tree and the 16S *p* distance matrix suggest *E. laminata* is more closely related to *S. jonesi* (between-species uncorrected $p=2\%$) than to the undescribed escarpiid (between-species uncorrected $p=3.50\%$). However, the COI tree groups the undescribed escarpiid with the described *Escarpia* spp. The bootstrap value based on COI data supporting this clade is low (61%), and the grouping observed for the 16S dataset has a bootstrap below 50%. Neither tree allows us to state clearly whether this new escapiid is more closely related to *Escarpia*, *Paraescarpia*, or *Seepiophila*.

As previously noted by other authors, COI does not separate *Escarpia southwardae, E. spicata,* and *E. laminata,* respectively, from cold seeps on the west coast of Africa in the eastern Atlantic, Guaymas Basin, off the coast of Mexico, and the GOM (Black et al., 1998). Also, there is very little to no intra-clade diversity within this group (Table 3). This result may indicate that those three nominal species represent a single genealogical species with a surprisingly wide geographic distribution and variable morphology. However, this assumption would require a high level of gene flow between quite distant localities, especially since the closing of the Isthmus of Panama 3.5 million years ago, followed the closing of the deep sea exchange 10 million years ago (Burton et al., 1997). This level of genetic exchange over these distances seems quite unlikely, considering what is known about larval development times for vestimentiferans (Marsh et al. 2001, Young et al., 1996). Although the life span of *Escarpia* larvae has not been determined, the larval life span of the vent species *Riftia pachyptila* is estimated at about three weeks (Marsh et al., 2001) and the larval life span of the seep vestimentiferan *L. luymesi* is estimated to be about one month (Young et al., 1996). Tyler and Young (1999) estimate that the maximal dispersal distances for these species are on the order of 60 km per generation, which is unlikely to support the level of genetic mixing necessary to maintain genetic homogeneity among the three described species of *Escarpia* from such widely separated geographic locations. It is possible, however, that undiscovered seeps around South America could connect all of these species.

The lack of fixed COI differences within *Escarpia spicata*, *E. laminata*, and *E. southwardae* could also be due to different rates of evolution of the COI gene in different taxa. COI has been used for higher level phylogenetic reconstructions in other groups of annelids (Halanych and Janosik, 2006) and has been adopted as an appropriate gene for the "barcode of life" for animals in general by the barcode of life initiative (BOLI; http://www.dnabarcodes.org/). However, the fact that COI fails to identify morphologically distinct populations of *Escarpia* from such widely separated areas implies that in this clade the mutation rate may be considerably slower than in other lineages. Slower rates of evolution in the mitochondrial DNA have been recognized in some other groups, such as the cnidarian class Anthozoa, where this phenomenon has been linked to an especially efficient repair system of their mitochondrial DNA (France and Hoover, 2002; Pont-Kingdon et al., 1998); however, no evidence of a similar system has been found in vestimentiferan mitochondrial DNA. Seep vestimentiferans can also be extremely long-lived (Bergquist et al., 2000; Cordes et al., 2007a), which may contribute to a slower rate of change of mitochondrial DNA (see for example Nabholz et al. (2008) for a consideration of longevity effects on mitochondrial rates of evolution in vertebrates).

In the COI dataset, the *Lamellibrachia* clade is divided into eight distinct groups that represent presumptive species, including five basal species (*L. juni, L. barhami*, *L. satsuma*, *L.* sp. Japan, and *L.* sp. West Pacific), all of which are from the Pacific Ocean and four of which are from the western Pacific. This observation is consistent with the hypothesis that the genus *Lamellibrachia* originated in the Pacific, likely the western Pacific, and subsequently radiated to the eastern Pacific, the Atlantic, and the GOM.

Three morphological species of *Lamellibrachia* were identified in collections from the GOM: *L. luymesi,* from the upper slope at

between about 400 m and 800 m; *L.* sp. 1, from 950 to 2320 m; and *L.* sp. 2, from 1175 to 2320 m. *L. luymesi* and *L.* sp. 1 have a similar number of sheath lamellae, but the deep-water *L.* sp. 1 generally has more gill lamellae, ranging between 21 and 27 in the 28 individuals examined, whereas the shallow-water *L. luymesi* has between 15 and 22 gill lamellae in the 20 individuals examined for the species description. The morphological character that allowed rapid identification of animals aboardship was the relatively short and fat vestimentum of *L.* sp. 1. The ratio of length to width of the vestimentum of *L.* sp. 1 ranges from 2.4 to 4.7 and from 6.2 to 16.4 in *L. luymesi*. *L.* sp. 2 has a similar number of sheath and gill lamellae as *L.* sp. 1, and the vestimentum length to width ratio tends to be shorter (1.9 to 3). The most distinct field character for *L.* sp. 2 is the lack of a ventral vestimental fold, which is present on *L.* sp. 1.

Despite morphological characters that distinguish the three GOM *Lamellibrachia* presumptive species, only either the COI or the 16S phylogenetic trees resolved two of them. Specifically, both genes failed to separate *L. luymesi* from the shallow GOM and *L.* sp. 1 from the deeper GOM sites. This lack of genetic differences between individuals that span such a wide depth range is unusual (Chase et al., 1998; Zardus et al., 2006) and surprising, given the morphological differences. Both 16S and COI genes consistently identify *Lamellibrachia* sp. 2 as a separate clade, sister to the *L. luymesi*/*L.* sp. 1 clade.

There were no apparent geographic distributional patterns that were independent of depth for the seep vestimentiferans in the Gulf of Mexico. The common species present on the upper Louisiana slope (*L. luymesi* and *S. jonesi*) have been found at both the eastern-most and western-most sites where we have collected vestimentiferans. *E. laminata* from the lower slope ranges from the Alaminos Canyon sites, our most westerly collection sites for this study, to the Florida Escarpment in the eastern GOM (Cordes et al., 2009; McMullin et al., 2003). Both of the *Lamellibrachia* spp. found at the deeper sites occurred over the entire E–W range of sites within their depth range (from the Alaminos Canyon sites in the west to AT340 in the east).

4.2. Within-species diversity of the GOM vestimentiferans

Tables 2 and 3 report within- and between-species *p* distance calculated for the GOM genetic species. In most cases, within-species diversity for both 16S and in the COI genes is strikingly low, a finding that is in contrast to previous studies on deep-sea mollusks and echinoderms, where large amounts of genetic variation were observed over small distances (Chase et al., 1998; Howell et al., 2004; Quattro et al., 2001). However, large-scale studies indicate that low within-species genetic variation may be typical of deep-sea organisms (Bisol et al., 1984) and even suggest that it may decrease with increase in depth (France and Kocher, 1996). Genetic variation has been suggested to be an important feature of the genome of an organism that allows it to adapt to a changing environment (Powers et al., 1991). Organisms that live in the deep sea may experience a long-term stable environment, resulting in low levels of within-species genetic diversity. Alternatively, low within-species genetic diversity may be the result of fewer replication errors, more efficient repair in the germ line, or repeated population bottlenecks.

E. laminata, *E. spicata*, and *E. southwardae clade* and *L. luymesi* sp. 1 and *L.* sp. 2 have a moderate degree of intra-specific diversity (Figs. 2 and 3). However, as with all of the GOM vestimentiferans analyzed, none of the within-species clades grouped by specific geographic locations or depth. A similar pattern was found in the seep mussel *Bathymodiolus childressi*, which, based on markers ranging from microsatellites to mitochondrial genes, has a panmictic population in the GOM ranging across 550 km east to west and from 540 to 2200 m depth (Carney et al., 2006; Cordes et al., 2007b). In contrast, genetic breaks and barriers that restrict gene flow were identified in both hydrothermal vent vestimentiferans and mussels along the East Pacific Rise (EPR). Specifically, Won et al. (2003) used COI sequences to identify two highly divergent clades on the EPR on the two sides of the Easter Island Microplate. Similarly, Hurtado et al. (2004) used COI sequences to identify several geographic breaks and barriers that restrict gene flow in three genera of annelids along the EPR, including two species of vestimentiferan (*Riftia pachypitla* and *Tevnia jerichonana*).

5. Summary

In this study, our primary goals were to identify and characterize the distributions of vestimentiferans at seep sites covering a wide geographic and depth range in the Gulf of Mexico and to investigate their relationship to other seep vestimentiferan species, using phylogenetic analysis of mitochondrial gene sequences. Although the genetic analyses confirmed identification of most of the morphological species during collections, we also identified an unexpected discrepancy between the morphospecies identified during the collections and genealogical species identified using the mitochondrial genes COI and 16S. Using morphological characters, we identified two new species of *Lamellibrachia* (spp. 1 and 2). However, neither COI nor 16S distinguished the deeper occurring morphospecies *L.* sp. 1 from *L. luymesi*, the common *Lamellibrachia* species on the upper Louisiana slope. Our molecular genetic analyses confirm the presence of three vestimentiferan species within the escarpiid clade in the Gulf of Mexico. However, since COI also does not differentiate between *E. laminata* found in the Gulf of Mexico and the other described *Escarpia* species off the coast of Africa or in the eastern Pacific Ocean, we suggest that COI or 16S genes may not reliably distinguish closely related species of long-lived seep vestimentiferans. We are currently evaluating the usefulness of several nuclear genes to clarify the relationships among the named species of *Escarpia* and the *Lamellibrachia* species in the Gulf of Mexico.

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