

## Supplementary Material

### A tale of two tubeworms: taxonomy of vestimentiferans (Annelida: Siboglinidae) from the Mid-Cayman Spreading Centre

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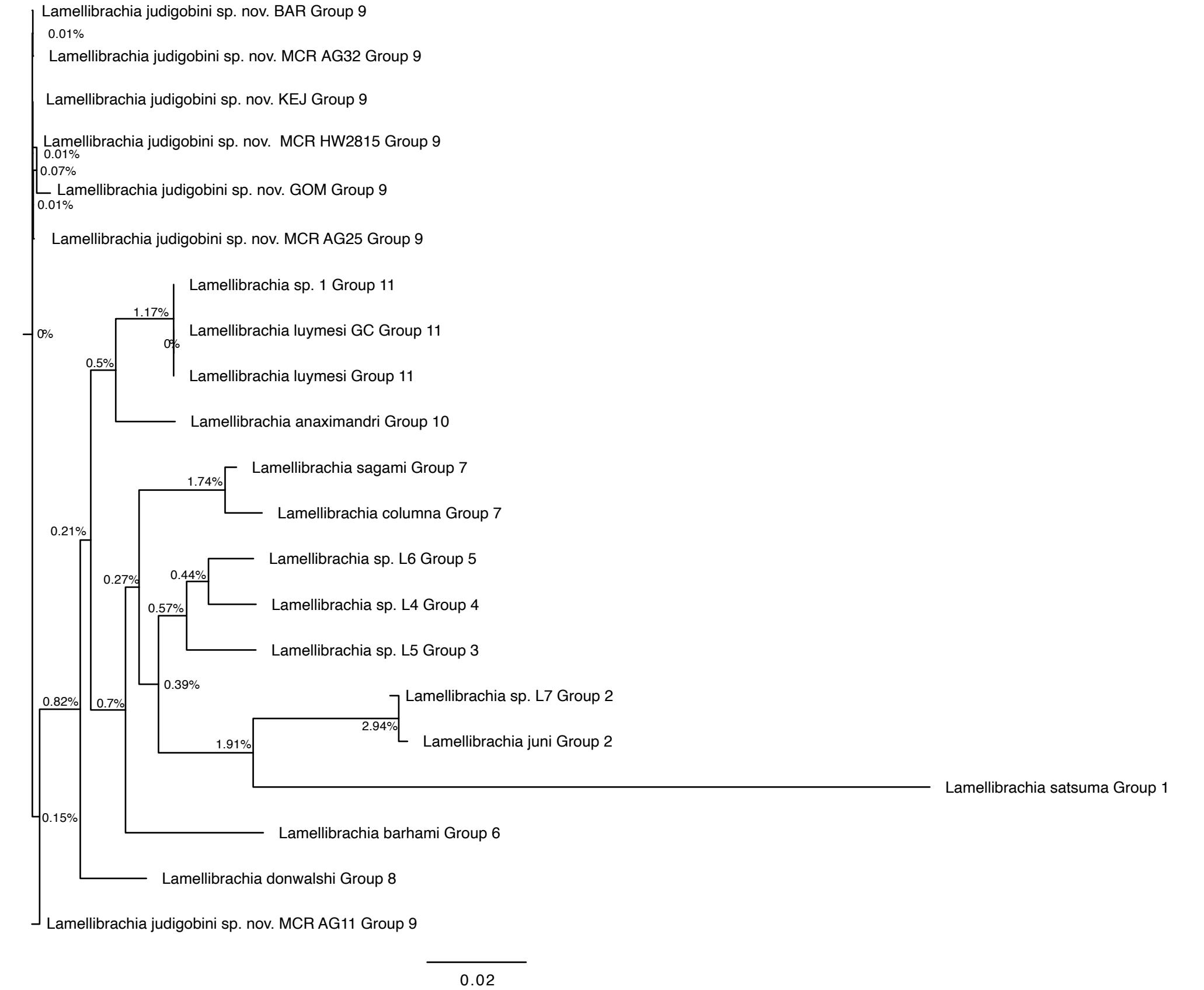
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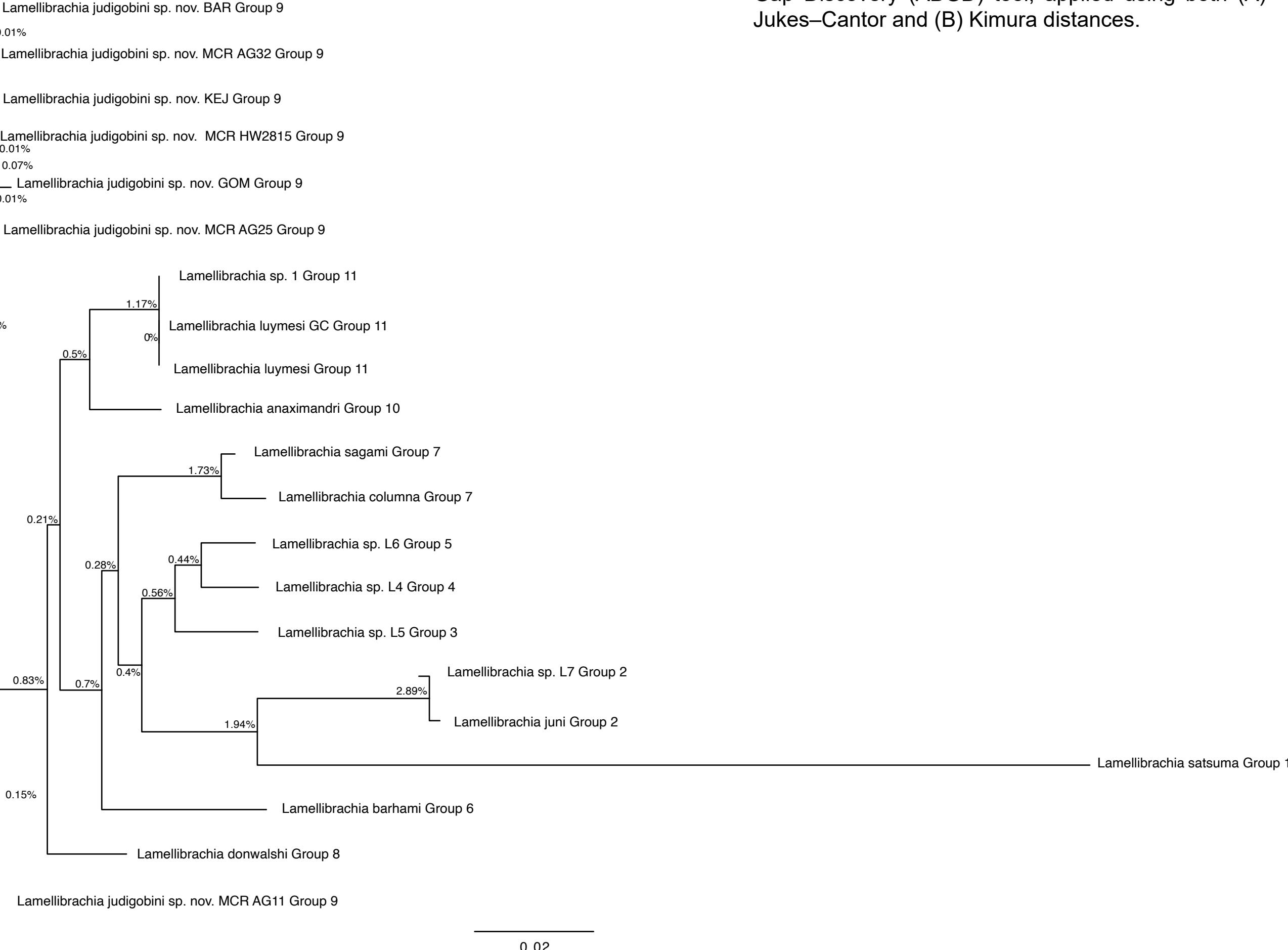
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(A) ABGD result: Jukes Cantor



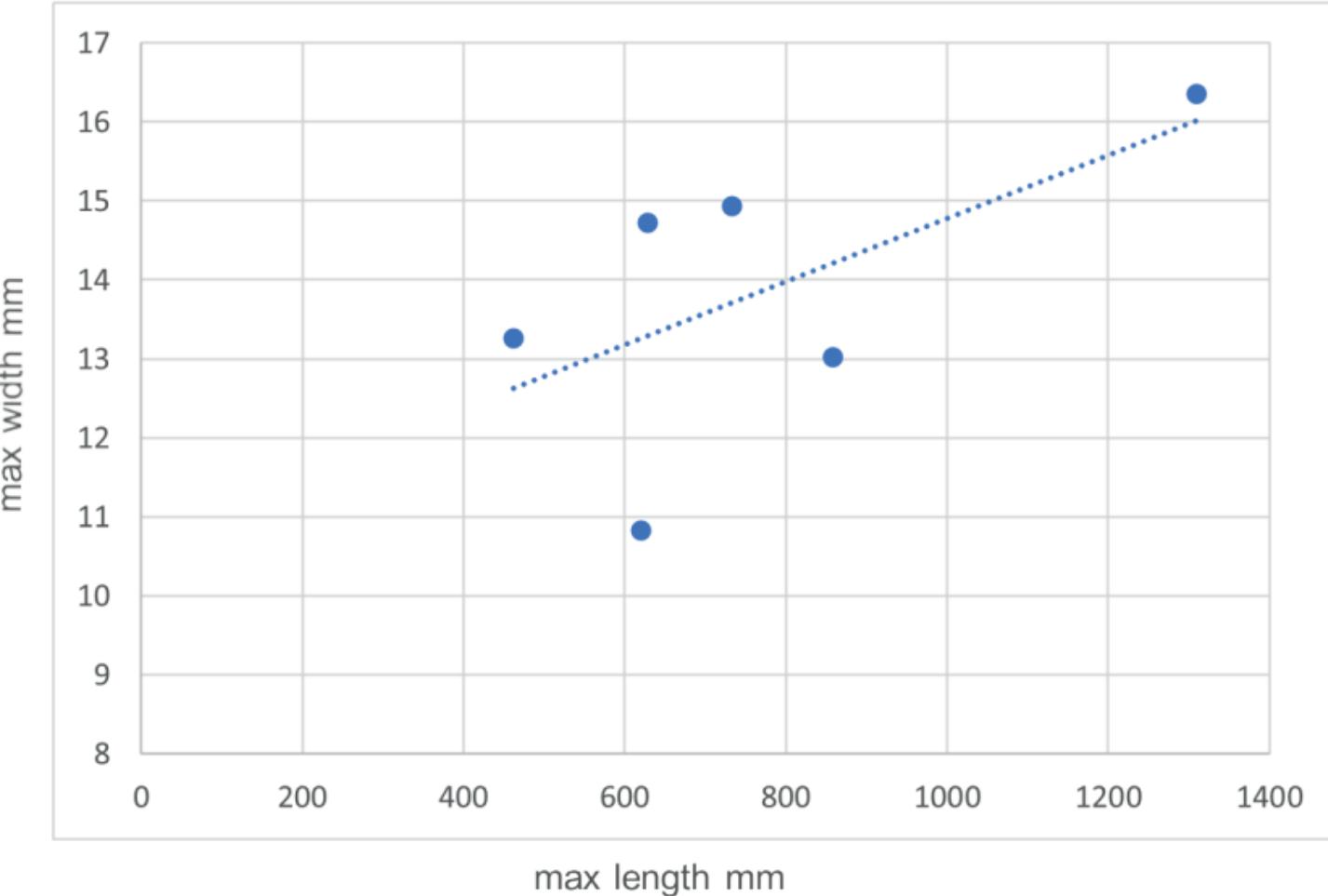
(B) ABGD result: Kimura



**Fig. S1.** Tube results of the Automated Barcode Gap Discovery (ABGD) tool, applied using both (A) Jukes–Cantor and (B) Kimura distances.

**A**

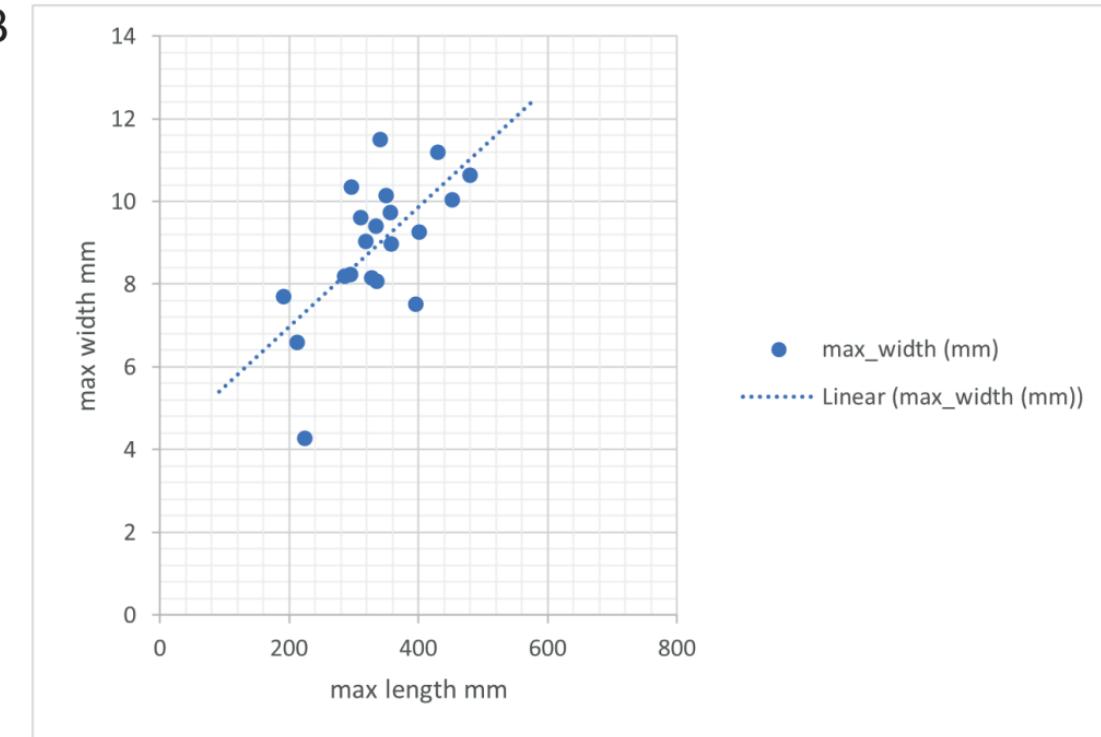
<i>Lamellibrachia</i>	length (mm)	max_width (mm)	min_width (mm)
1	620.263	10.828	2.042
2	628.43	14.719	1.917
3	461.3	13.263	0.943
4	858.418	13.017	0.99
5	733.447	14.94	1.136
6	1309.287	16.362	2.007

**B**

**Fig. S2.** Tube sizes of *L. judigobini* sp. nov. (A) Length and width parameters of the studied tubes. (B) Trend line of the tube growth.

**A** *Escarpia* specimen length (mm) max\_width (mm) min\_width (mm)

1	191.88	7.704	2.374
2	350.18	10.144	1.738
3	334.531	9.406	1.422
4	335.158	8.081	1.077
5	318.279	9.038	0.938
6	286.099	8.196	1.14
7	327.601	8.148	1.093
8	310.06	9.611	0.823
9	357.131	8.979	1.117
10	295.053	8.225	0.678
11	400.704	9.261	0.858
12	296.191	10.358	1.328
13	340.292	11.508	0.797
14	211.641	6.586	1.033
15	224.161	4.265	0.964
16	356.203	9.742	1.11
17	451.888	10.045	0.939
18	430.278	11.19	1.276
19	480.095	10.641	1.09
20	395.536	7.525	0.708



**Fig. S3.** Tube sizes of *E. tridentaculata* sp. nov. (A) Length and width parameters of the studied tubes. (B) Trend line of the tube growth.

**Table S1.** Morphological characters of studied specimens of *L. judigobini* sp. nov.

	Sex	OD (mm)	OL (mm)	NBL left/right	NSL left/right	VP (μm)	TPA (μm)	TPP (μm)	VD (mm)	VL (mm)	VL/ OL	CFW (mm)	TL (mm)	TD (mm)
JC082 912 HOLOTYPE NHMUK ANEA 2023.599	Male	5–7	13	22/23	5/5	74–97	98–140	29–37	10	25	1.9	3	45+	06–10
AT18-16 MCR691 PARATYPE UWIZM 2022.5	Male	9	16	21/21	6/5	73–104	n/a	n/a	14	n/a	n/a	3	n/a	n/a
AT18-16 MCR234 PARATYPE WSBS MSU ZMMU WS16820	Female	5–7	12	18/19	3/3	68–102	76–135	29–50	10	25	2.1	3.5	130+	1.5–9
AT18-16 MCR667	Female	3.5–4	12	12/11	4/3	54–73	86–108	16–23	6	20	1.7	1.5	125+	4–0.5
AT18-16 MCR498 PARATYPE PMJ Ann 289	Male	5	9	17/22	5/3	56–75	55–71	39–46	9	16	1.8	2.5	95+	2–5.5

CFW, width of the ciliary field; CT, thickness of the cuticular crust; CW×SW, cross width × sagittal width; GGL, genital groove length; NHM, The Natural History Museum of London; NL, number of lamellae; OD, obturaculum width; OL, obturaculum length; TD, trunk diameter; TDA, tube anterior diameter; TDP, tube posterior diameter; TL, tube length; TPA, diameter of the papillae cuticular plaque in the anterior trunk; TPP; diameter of the papillae plaque in the posterior trunk; VD, vestimentum diameter; VL, vestimentum length; VP, vestimental papillae; VPM, posterior margin of the wings; NHMUK, Natural History Museum UK; WSBS MSU, the White Sea Biological station of Moscow State University, Russia; UWIZM, University of the West Indies Zoology Museum, Trinidad and Tobago; PMJ Ann, Phyletic Museum Jena, Germany.

**Table S2.** Morphological characters of studied specimens of *E. tridentaculata* sp. nov.

	OL (mm)	OD (mm)	VL (mm)	VD (mm)	NL	VP (μm)	TPA (μm)	TPP (μm)	CFW (mm)	TD (mm)	Cuticular crust	CT (mm)	spike, CWxSW (mm)	GGL (mm)
JC082 919 HOLOTYPE NHMUK ANEA 2023.601	11	7.5–9	16	8	43 & 40	70–106	97–157	54–74	5	5–5	Lamellar	3	1x1.5	12
JC082 915 PARATYPE WSBS MSU ZMMU WS16819		09–10	15	10	38 & 43	53–78	79–127	45–67	7	8–2	Lamellar	0.5	0.5x2.5	14
MCR 507	6	5	>10	6	37	48–70	n/a	n/a	2	n/a	Lamellar	0.5	0,1x1	n/a
MCR010 PARATYPE PMJ Ann 290	6	6	16	7	37 & 34	n/a	n/a	n/a	3	n/a	Lamellar	1	0,7x2	11
MCR017 PARATYPE PMJ Ann 291	4	5.5	10	5	28	65–108	61–98	n/a	3	5	Columnar & lamellar	0.5	0,1x1	8
MCR 1333	n/a	n/a	n/a	n/a	n/a	n/a	100–164	57–106	n/a	2	n/a	n/a	n/a	n/a

CFW, width of the ciliary field; CT, thickness of the cuticular crust; CWxSW, cross width × sagittal width; GGL, genital groove length; NHM, The Natural History Museum of London; NL, number of lamellae; OD, obturaculum width; OL, obturaculum length; TD, trunk diameter; TDA, tube anterior diameter; TDP, tube posterior diameter; TL, tube length; TPA, diameter of the papillae cuticular plaque in the anterior trunk; TPP, diameter of the papillae plaque in the posterior trunk; VD, vestimentum diameter; VL, vestimentum length; VP, vestimental papillae; VPM, posterior margin of the wings; NHMUK, Natural History Museum UK; WSBS MSU, the White Sea Biological station of Moscow State University, Russia; PMJ Ann, Phyletic Museum Jena, Germany.

**Table S3.** PCR primers used in this study.

Gene	Primer	Sequence 5'-3'	Reference
COI	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer <i>et al.</i> (1994)
	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer <i>et al.</i> (1994)
16S	Ann16SF	GCGGTATCCTGACCGTRCWAAAGGT	Sjölin <i>et al.</i> (2005)
	Ann16SR	TCCTAACGCCAACATCGAGGTGCCAA	Sjölin <i>et al.</i> (2005)
18S	18SA	AYCTGGTTGATCCTGCCAGT	Medlin <i>et al.</i> (1988)
	18SB	ACCTTGTACGACTTTACTTCCTC	Nygren and Sundberg (2003)
	620F	TAAAGYTGYTCAGTTAAA	Nygren and Sundberg (2003)
	1324R	CGGCCATGCACCACC	Cohen <i>et al.</i> (1998)
hbB2i	hbB2i_F	TCCATGCCCCAGGCTGTCTTC	Cowart <i>et al.</i> (2013)
	hbB2i_R	GCCTTGAATTGCTTGCTGTT	Cowart <i>et al.</i> (2013)

## References

- Cohen BL, Gawthrop A, Cavalier-Smith T (1998) Molecular phylogeny of brachiopods and phoronids based on nuclear-encoded small subunit ribosomal RNA gene sequences. *Philosophical Transactions of the Royal Society of London – B. Biological Sciences* **353**, 2039–2061. <https://doi.org/10.1098/rstb.1998.0351>
- Cowart DA, Huang C, Arnaud-Haond S, Carney SL, Fisher CR, Schaeffer SW (2013) Restriction to large-scale gene flow vs. regional panmixia among cold seep *Escarapia* spp. (Polychaeta, Siboglinidae). *Molecular Ecology* **22**, 4147–4162. <https://doi.org/10.1111/mec.12379>
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**, 294–299.
- Medlin L, Elwood HJ, Stickel S, Sogin ML (1988) The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* **71**, 491–499. [https://doi.org/10.1016/0378-1119\(88\)90066-2](https://doi.org/10.1016/0378-1119(88)90066-2)
- Nygren A, Sundberg P (2003) Phylogeny and evolution of reproductive modes in Autolytinae (Syllidae, Annelida). *Molecular Phylogenetics and Evolution* **29**, 235–249. [https://doi.org/10.1016/S1055-7903\(03\)00095-2](https://doi.org/10.1016/S1055-7903(03)00095-2)
- Sjölin E, Erséus C, Källersjö M (2005) Phylogeny of Tubificidae (Annelida, Clitellata) based on mitochondrial and nuclear sequence data. *Molecular Phylogenetics and Evolution* **35**, 431–41. <https://doi.org/10.1016/j.ympev.2004.12.018>

**Table S4.** GenBank accession numbers for sequences used in the phylogenetic analysis.

Taxon	COI	16S	18S
<b>Vestimentiferans</b>			
<i>Lamellibrachia anaximandri</i>	EU046616	HM746782	-
<i>Lamellibrachia barhami</i>	AY129137	AF315043	AF168742
<i>Lamellibrachia columna</i>	DQ996645	FJ347646	FJ347679
<i>Lamellibrachia donwalshi</i>	MH670827	MH664910	-
<i>Lamellibrachia juni</i>	AB264603	-	-
<i>Lamellibrachia luymesii</i>	GU059225	GU068209	-
<i>Lamellibrachia luymesii</i>	GU059226	GU068211	-
<i>Lamellibrachia</i> sp. 1	GU059227	GU068212	-
<i>Lamellibrachia sagami</i>	LC064365	-	-
<i>Lamellibrachia satsuma</i>	KP987801	KP987801	FM995543
<i>Lamellibrachia judigobini</i> JC82_912	OQ596968	OQ599463	OQ599465
<i>Lamellibrachia judigobini</i> JC82_913	*OQ596969		
<i>Lamellibrachia judigobini</i> JC82_920	*OQ596970		
<i>Lamellibrachia judigobini</i> JC82_925	*OQ596971		
<i>Lamellibrachia judigobini</i> GOM	GU059176	GU068270	-
<i>Lamellibrachia judigobini</i> BAR	KX901972	-	-
<i>Lamellibrachia judigobini</i> T&T	KX902047	-	-
<i>Lamellibrachia</i> sp. L4	AB055209	-	-
<i>Lamellibrachia</i> sp. L5	AB055210	-	-
<i>Lamellibrachia</i> sp. L6	AB088674	-	-
<i>Lamellibrachia</i> sp. L7	AB088675	-	-
<i>Escarapia laminata</i>	GU059163	GU068165	-
<i>Escarapia spicata</i>	KC870959	KC357364	AF168741
<i>Escarapia southwardae</i>	KC870957	KC357356	-
<i>Escarapia tridentaculata</i> WSBS_MSU_ZMMU_WS16819 (COI, 16S), JC82_918 (18S)	OQ596972	OQ599464	OQ599466
<i>Seepiophila jonesi</i>	GU059186	GU068284	-
<i>Ridgeia piscesae</i>	RPU74057	AF315054	AF168744
<i>Riftia pachyptila</i>	KJ789166	KJ789166	KP119591
<i>Tevnia jerichonana</i>	FJ667531	---	AF168746
<i>Paraescarpia echinospica</i>	D50594	---	FM995546
<i>Oasisia alvinae</i>	AY646020	AF315052	AF168743
<i>Alaysia</i> sp.	FJ667536	---	FM995545
<b>Other siboglinids</b>			
<i>Sclerolinum contortum</i>	KU214832	KU214830	KU214829
<i>Sclerolinum brattstromi</i>	FJ347644	FJ347645	FJ347680
<i>Siboglinum ekmani</i>	KF444429	AF315038	AF315062
<i>Siboglinum fiordicum</i>	---	AF315039	AF315060
<i>Spirobranchia</i> sp.	FJ480372	AF315036	AF168740
<b>Outgroups</b>			
<i>Malacobertos fuliginosus</i>	EF432015	EF431961	EF446961
<i>Sabella pavonina</i>	KF369181	AY340482	U67144

Sequences marked with an asterisk (\*) were only used in genetic distance calculations, and those highlighted in blue are newly provided by this study.

**Table S5.** Sequences used in *Lamellibrachia* sp. 2 *COI* haplotype network.

Haplotype	Collection locality	GenBank ID	Haplotype	Collection locality	GenBank ID
Haplotype1	Gulf of Mexico	KX902153	Haplotype8	Barbados	KX901976
Haplotype1	Gulf of Mexico	KX902162	Haplotype8	Barbados	KX901977
Haplotype1	Gulf of Mexico	KX902165	Haplotype8	Barbados	KX901978
Haplotype1	Gulf of Mexico	KX902175	Haplotype8	Barbados	KX901979
Haplotype2	Gulf of Mexico	KX902138	Haplotype8	Barbados	KX901981
Haplotype2	Gulf of Mexico	KX902140	Haplotype8	Barbados	KX901982
Haplotype2	Gulf of Mexico	KX902141	Haplotype8	Barbados	KX901983
Haplotype2	Gulf of Mexico	KX902142	Haplotype8	Barbados	KX901985
Haplotype2	Gulf of Mexico	KX902143	Haplotype8	Barbados	KX901986
Haplotype2	Gulf of Mexico	KX902144	Haplotype8	Barbados	KX901988
Haplotype2	Gulf of Mexico	KX902145	Haplotype8	Barbados	KX901989
Haplotype2	Gulf of Mexico	KX902147	Haplotype8	Barbados	KX901990
Haplotype2	Gulf of Mexico	KX902149	Haplotype8	Barbados	KX901993
Haplotype2	Gulf of Mexico	KX902150	Haplotype8	Barbados	KX901994
Haplotype2	Gulf of Mexico	KX902151	Haplotype8	Barbados	KX901996
Haplotype2	Gulf of Mexico	KX902152	Haplotype8	Barbados	KX901997
Haplotype2	Gulf of Mexico	KX902154	Haplotype8	Barbados	KX901998
Haplotype2	Gulf of Mexico	KX902155	Haplotype8	Barbados	KX901999
Haplotype2	Gulf of Mexico	KX902156	Haplotype8	Barbados	KX902000
Haplotype2	Gulf of Mexico	KX902157	Haplotype8	Barbados	KX902001
Haplotype2	Gulf of Mexico	KX902158	Haplotype8	Trinidad & Tobago	KX902002
Haplotype2	Gulf of Mexico	KX902159	Haplotype8	Trinidad & Tobago	KX902003
Haplotype2	Gulf of Mexico	KX902160	Haplotype8	Trinidad & Tobago	KX902004
Haplotype2	Gulf of Mexico	KX902161	Haplotype8	Trinidad & Tobago	KX902005
Haplotype2	Gulf of Mexico	KX902163	Haplotype8	Trinidad & Tobago	KX902006
Haplotype2	Gulf of Mexico	KX902164	Haplotype8	Trinidad & Tobago	KX902007
Haplotype2	Gulf of Mexico	KX902167	Haplotype8	Trinidad & Tobago	KX902008
Haplotype2	Gulf of Mexico	KX902168	Haplotype8	Trinidad & Tobago	KX902009
Haplotype2	Gulf of Mexico	KX902169	Haplotype8	Trinidad & Tobago	KX902010
Haplotype2	Gulf of Mexico	KX902170	Haplotype8	Trinidad & Tobago	KX902011
Haplotype2	Gulf of Mexico	KX902171	Haplotype8	Trinidad & Tobago	KX902012
Haplotype2	Gulf of Mexico	KX902173	Haplotype8	Trinidad & Tobago	KX902013
Haplotype2	Gulf of Mexico	KX902174	Haplotype8	Trinidad & Tobago	KX902014
Haplotype2	Gulf of Mexico	KX902176	Haplotype8	Trinidad & Tobago	KX902015
Haplotype2	Gulf of Mexico	KX902177	Haplotype8	Trinidad & Tobago	KX902016
Haplotype2	Gulf of Mexico	KX902178	Haplotype8	Trinidad & Tobago	KX902017
Haplotype2	Gulf of Mexico	KX902180	Haplotype8	Trinidad & Tobago	KX902020
Haplotype2	Gulf of Mexico	KX902181	Haplotype8	Trinidad & Tobago	KX902021
Haplotype2	Gulf of Mexico	KX902182	Haplotype8	Trinidad & Tobago	KX902022
Haplotype2	Gulf of Mexico	KX902184	Haplotype8	Trinidad & Tobago	KX902023
Haplotype2	Gulf of Mexico	KX902185	Haplotype8	Trinidad & Tobago	KX902024
Haplotype2	Gulf of Mexico	KX902186	Haplotype8	Trinidad & Tobago	KX902025
Haplotype2	Gulf of Mexico	KX902187	Haplotype8	Trinidad & Tobago	KX902026
Haplotype2	Gulf of Mexico	KX902188	Haplotype8	Trinidad & Tobago	KX902027
Haplotype2	Gulf of Mexico	KX902189	Haplotype8	Trinidad & Tobago	KX902028
Haplotype2	Gulf of Mexico	KX902190	Haplotype8	Trinidad & Tobago	KX902030
Haplotype3	Barbados	KX901980	Haplotype8	Trinidad & Tobago	KX902031
Haplotype3	Barbados	KX901987	Haplotype8	Trinidad & Tobago	KX902032
Haplotype4	Barbados	KX901975	Haplotype8	Trinidad & Tobago	KX902033
Haplotype4	Trinidad & Tobago	KX902029	Haplotype8	Trinidad & Tobago	KX902034
Haplotype5	Barbados	KX901991	Haplotype8	Trinidad & Tobago	KX902036
Haplotype5	Barbados	KX901992	Haplotype8	Trinidad & Tobago	KX902037
Haplotype6	Gulf of Mexico	GU059177	Haplotype8	Trinidad & Tobago	KX902038
Haplotype6	Gulf of Mexico	KX902109	Haplotype8	Trinidad & Tobago	KX902039
Haplotype6	Gulf of Mexico	KX902118	Haplotype8	Trinidad & Tobago	KX902040
Haplotype6	Gulf of Mexico	KX902183	Haplotype8	Trinidad & Tobago	KX902041
Haplotype7	Barbados	KX901995	Haplotype8	Trinidad & Tobago	KX902043
Haplotype7	Trinidad & Tobago	KX902018	Haplotype8	Trinidad & Tobago	KX902045
Haplotype7	Trinidad & Tobago	KX902019	Haplotype8	Trinidad & Tobago	KX902046
Haplotype7	Trinidad & Tobago	KX902042	Haplotype8	Trinidad & Tobago	KX902047
Haplotype7	Trinidad & Tobago	KX902044	Haplotype8	Mid-Cayman Rise	OQ596968
Haplotype8	Barbados	KX901972	Haplotype8	Mid-Cayman Rise	OQ596969
Haplotype8	Barbados	KX901973	Haplotype8	Mid-Cayman Rise	OQ596970
Haplotype8	Barbados	KX901974	Haplotype8	Mid-Cayman Rise	OQ596971

Haplotype	Collection locality	GenBank ID
Haplotype8	Mid-Cayman Rise	KX902048
Haplotype8	Mid-Cayman Rise	KX902049
Haplotype8	Mid-Cayman Rise	KX902050
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Haplotype8	Mid-Cayman Rise	KX902053
Haplotype8	Mid-Cayman Rise	KX902054
Haplotype8	Mid-Cayman Rise	KX902055
Haplotype8	Mid-Cayman Rise	KX902056
Haplotype8	Mid-Cayman Rise	KX902057
Haplotype8	Mid-Cayman Rise	KX902058
Haplotype8	Mid-Cayman Rise	KX902059
Haplotype8	Mid-Cayman Rise	KX902060
Haplotype8	Mid-Cayman Rise	KX902061
Haplotype8	Mid-Cayman Rise	KX902062
Haplotype8	Mid-Cayman Rise	KX902063
Haplotype8	Mid-Cayman Rise	KX902064
Haplotype8	Mid-Cayman Rise	KX902065
Haplotype8	Mid-Cayman Rise	KX902066
Haplotype8	Mid-Cayman Rise	KX902067
Haplotype8	Mid-Cayman Rise	KX902068
Haplotype8	Mid-Cayman Rise	KX902069
Haplotype8	Mid-Cayman Rise	KX902070
Haplotype8	Mid-Cayman Rise	KX902071
Haplotype8	Mid-Cayman Rise	KX902072
Haplotype8	Mid-Cayman Rise	KX902073
Haplotype8	Mid-Cayman Rise	KX902074
Haplotype8	Mid-Cayman Rise	KX902075
Haplotype8	Mid-Cayman Rise	KX902076
Haplotype8	Mid-Cayman Rise	KX902077
Haplotype8	Mid-Cayman Rise	KX902078
Haplotype8	Mid-Cayman Rise	KX902079
Haplotype8	Mid-Cayman Rise	KX902080
Haplotype8	Mid-Cayman Rise	KX902081
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Haplotype8	Mid-Cayman Rise	KX902085
Haplotype8	Mid-Cayman Rise	KX902086
Haplotype8	Mid-Cayman Rise	KJ566961
Haplotype8	Gulf of Mexico	GU059173
Haplotype8	Gulf of Mexico	GU059175
Haplotype8	Gulf of Mexico	GU059176
Haplotype8	Gulf of Mexico	GU059178
Haplotype8	Gulf of Mexico	KX902087
Haplotype8	Gulf of Mexico	KX902088
Haplotype8	Gulf of Mexico	KX902089
Haplotype8	Gulf of Mexico	KX902090
Haplotype8	Gulf of Mexico	KX902091
Haplotype8	Gulf of Mexico	KX902092
Haplotype8	Gulf of Mexico	KX902093
Haplotype8	Gulf of Mexico	KX902094

Haplotype	Collection locality	GenBank ID
Haplotype8	Gulf of Mexico	KX902095
Haplotype8	Gulf of Mexico	KX902096
Haplotype8	Gulf of Mexico	KX902097
Haplotype8	Gulf of Mexico	KX902098
Haplotype8	Gulf of Mexico	KX902099
Haplotype8	Gulf of Mexico	KX902100
Haplotype8	Gulf of Mexico	KX902101
Haplotype8	Gulf of Mexico	KX902102
Haplotype8	Gulf of Mexico	KX902103
Haplotype8	Gulf of Mexico	KX902104
Haplotype8	Gulf of Mexico	KX902105
Haplotype8	Gulf of Mexico	KX902106
Haplotype8	Gulf of Mexico	KX902107
Haplotype8	Gulf of Mexico	KX902108
Haplotype8	Gulf of Mexico	KX902110
Haplotype8	Gulf of Mexico	KX902111
Haplotype8	Gulf of Mexico	KX902112
Haplotype8	Gulf of Mexico	KX902113
Haplotype8	Gulf of Mexico	KX902114
Haplotype8	Gulf of Mexico	KX902115
Haplotype8	Gulf of Mexico	KX902116
Haplotype8	Gulf of Mexico	KX902117
Haplotype8	Gulf of Mexico	KX902119
Haplotype8	Gulf of Mexico	KX902120
Haplotype8	Gulf of Mexico	KX902121
Haplotype8	Gulf of Mexico	KX902122
Haplotype8	Gulf of Mexico	KX902123
Haplotype8	Gulf of Mexico	KX902124
Haplotype8	Gulf of Mexico	KX902125
Haplotype8	Gulf of Mexico	KX902126
Haplotype8	Gulf of Mexico	KX902127
Haplotype8	Gulf of Mexico	KX902128
Haplotype8	Gulf of Mexico	KX902129
Haplotype8	Gulf of Mexico	KX902130
Haplotype8	Gulf of Mexico	KX902131
Haplotype8	Gulf of Mexico	KX902132
Haplotype8	Gulf of Mexico	KX902133
Haplotype8	Gulf of Mexico	KX902134
Haplotype8	Gulf of Mexico	KX902135
Haplotype8	Gulf of Mexico	KX902136
Haplotype8	Gulf of Mexico	KX902137
Haplotype8	Gulf of Mexico	KX902139
Haplotype8	Gulf of Mexico	KX902166
Haplotype8	Gulf of Mexico	KX902172
Haplotype8	Gulf of Mexico	KX902179
Haplotype 9	Barbados	KX901984
Haplotype 10	Trinidad & Tobago	KX902035
Haplotype 11	Mid-Cayman Rise	KX902052
Haplotype 12	Gulf of Mexico	KX902146
Haplotype 13	Gulf of Mexico	KX902148

Sequences highlighted in blue are newly provided by this study.

**Table S6.** Sequences used in *Escarapia COI* haplotype network.

Haplotype	Species	Collection locality	GenBank ID
Haplotype 1	<i>E. laminata</i>	Gulf of Mexico	GU059213
Haplotype 1	<i>E. laminata</i>	Gulf of Mexico	GU059220
Haplotype 2	<i>E. southwardae</i>	West Africa	KC357341
Haplotype 2	<i>E. southwardae</i>	West Africa	KC870958
Haplotype 3	<i>E. laminata</i>	Gulf of Mexico	GU059207
Haplotype 3	<i>E. laminata</i>	Gulf of Mexico	GU059209
Haplotype 4	<i>Escarapia</i> sp.	Gulf of Mexico	JN021269
Haplotype 4	<i>E. laminata</i>	Alaminos Canyon, Gulf of Mexico	AY129129
Haplotype 4	<i>E. laminata</i>	Florida Escarpment	ELU74063
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	GU059170
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	GU059193
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	GU059195
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	GU059196
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	GU059199
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	GU059200
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	GU059201
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	GU059202
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	GU059203
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	GU059211
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	GU059212
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	GU059215
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	GU059217
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	GU059221
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	GU059234
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC357323
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC357324
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC357325
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC357326
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC357327
Haplotype 5	<i>Escarapia</i> sp.	Mid-Cayman Rise	OQ596972
Haplotype 5	<i>Escarapia</i> sp.	Mid-Cayman Rise	OQ596973
Haplotype 5	<i>Escarapia</i> sp.	Mid-Cayman Rise	KJ566960
Haplotype 5	<i>Escarapia</i> sp.	Gulf of Mexico	JN021268
Haplotype 5	<i>E. spicata</i>	Gulf of California	ESU74064
Haplotype 5	<i>E. spicata</i>	Gulf of California	FJ667537
Haplotype 5	<i>E. spicata</i>	Gulf of California	KC357329
Haplotype 5	<i>E. spicata</i>	Gulf of California	KC357330
Haplotype 5	<i>E. spicata</i>	Gulf of California	KC357331
Haplotype 5	<i>E. spicata</i>	Gulf of California	KC357332
Haplotype 5	<i>E. spicata</i>	Gulf of California	KC357333
Haplotype 5	<i>E. laminata</i>	Alaminos Canyon, Gulf of Mexico	AY129128
Haplotype 5	<i>E. laminata</i>	Florida Escarpment	AY129131
Haplotype 5	<i>E. laminata</i>	Gulf of Mexico	GU059163
Haplotype 5	<i>E. laminata</i>	Gulf of Mexico	GU059174
Haplotype 5	<i>E. laminata</i>	Gulf of Mexico	GU059189
Haplotype 5	<i>E. laminata</i>	Gulf of Mexico	GU059194
Haplotype 5	<i>E. laminata</i>	Gulf of Mexico	GU059197
Haplotype 5	<i>E. laminata</i>	Gulf of Mexico	GU059208
Haplotype 5	<i>E. laminata</i>	Gulf of Mexico	KC357321
Haplotype 5	<i>E. laminata</i>	Gulf of Mexico	KC357322
Haplotype 5	<i>E. laminata</i>	Gulf of Mexico	KC357328
Haplotype 5	<i>E. southwardae</i>	West Africa	KC357334
Haplotype 5	<i>E. southwardae</i>	West Africa	KC357335
Haplotype 5	<i>E. southwardae</i>	West Africa	KC357337
Haplotype 5	<i>E. southwardae</i>	West Africa	KC357338
Haplotype 5	<i>E. southwardae</i>	West Africa	KC357342
Haplotype 5	<i>E. southwardae</i>	West Africa	KC357343
Haplotype 5	<i>E. southwardae</i>	West Africa	KC357344
Haplotype 5	<i>E. southwardae</i>	West Africa	KC870953
Haplotype 5	<i>E. southwardae</i>	West Africa	KC870954
Haplotype 5	<i>E. southwardae</i>	West Africa	KC870955
Haplotype 5	<i>E. southwardae</i>	West Africa	KC870956
Haplotype 5	<i>E. southwardae</i>	West Africa	KC870957
Haplotype 5	<i>E. southwardae</i>	West Africa	AY326304

Haplotype	Species	Collection locality	GenBank ID
Haplotype 6	<i>E. southwardae</i>	West Africa	KC357339
Haplotype 6	<i>E. southwardae</i>	West Africa	KC357340
Haplotype 7	<i>E. spicata</i>	Gulf of California	ESU74065
Haplotype 7	<i>E. spicata</i>	Gulf of California	KC870959
Haplotype 7	<i>E. spicata</i>	Gulf of California	KC870961
Haplotype 8	<i>Escarapia</i> sp.	Gulf of Mexico	JN021270
Haplotype 8	<i>E. laminata</i>	Gulf of Mexico	GU059198
Haplotype 8	<i>E. laminata</i>	Gulf of Mexico	GU059204
Haplotype 8	<i>E. laminata</i>	Gulf of Mexico	GU059205
Haplotype 8	<i>E. laminata</i>	Gulf of Mexico	GU059206
Haplotype 8	<i>E. laminata</i>	Gulf of Mexico	GU059210
Haplotype 8	<i>E. laminata</i>	Gulf of Mexico	GU059214
Haplotype 8	<i>E. laminata</i>	Gulf of Mexico	GU059216
Haplotype 8	<i>E. laminata</i>	Gulf of Mexico	GU059218
Haplotype 8	<i>E. laminata</i>	Gulf of Mexico	GU059219
Haplotype 8	<i>E. laminata</i>	Gulf of Mexico	GU059222
Haplotype 8	<i>E. laminata</i>	Gulf of Mexico	GU059224
Haplotype 8	<i>E. laminata</i>	Gulf of Mexico	GU059228
Haplotype 9	<i>E. laminata</i>	Gulf of Mexico	GU059185
Haplotype 10	<i>Escarapia</i> sp.	Mid-Cayman Rise	OQ596974
Haplotype 11	<i>Escarapia</i> sp.	Gulf of Mexico	JN021267
Haplotype 12	<i>E. southwardae</i>	West Africa	KC357336
Haplotype 13	<i>E. spicata</i>	Gulf of California	KC870960
Haplotype 14	<i>E. laminata</i>	Gulf of Mexico	GU059192
Haplotype 15	<i>E. southwardae</i>	West Africa	AY326303
Haplotype 16	<i>E. laminata</i>	Alaminos Canyon, Gulf of Mexico	AY129130
Haplotype 17	<i>E. spicata</i>	Santa Catalina whalefall	ESU84262
Haplotype 18	<i>E. laminata</i>	Gulf of Mexico	GU059223
Haplotype 19	<i>E. laminata</i>	Gulf of Mexico	GU059164
Haplotype 20	<i>E. laminata</i>	Gulf of Mexico	GU059248
Haplotype 21	<i>E. spicata</i>	off Chile	LC413848

Sequences highlighted in blue are newly provided by this study.

**Table S7.** Sequences used in Escarpia HbB2i haplotype network.

Haplotype	Species	Collection locality	GenBank ID
Haplotype 1	<i>Escarapia</i> sp.	Mid-Cayman Rise	OQ595232
Haplotype 1	<i>Escarapia</i> sp.	Mid-Cayman Rise	OQ595231
Haplotype 1	<i>E. southwardae</i>	West Africa	KC870996
Haplotype 1	<i>E. southwardae</i>	West Africa	KC870995
Haplotype 1	<i>E. southwardae</i>	West Africa	KC870994
Haplotype 1	<i>E. southwardae</i>	West Africa	KC870993
Haplotype 1	<i>E. southwardae</i>	West Africa	KC870992
Haplotype 1	<i>E. southwardae</i>	West Africa	KC870991
Haplotype 1	<i>E. southwardae</i>	West Africa	KC357412
Haplotype 1	<i>E. southwardae</i>	West Africa	KC357411
Haplotype 1	<i>E. southwardae</i>	West Africa	KC357410
Haplotype 1	<i>E. southwardae</i>	West Africa	KC357409
Haplotype 1	<i>E. southwardae</i>	West Africa	KC357408
Haplotype 1	<i>E. southwardae</i>	West Africa	KC357407
Haplotype 1	<i>E. southwardae</i>	West Africa	KC357406
Haplotype 1	<i>E. southwardae</i>	West Africa	KC357405
Haplotype 1	<i>E. southwardae</i>	West Africa	KC357404
Haplotype 1	<i>E. southwardae</i>	West Africa	KC357403
Haplotype 2	<i>E. spicata</i>	Gulf of California	KC871000
Haplotype 2	<i>E. spicata</i>	Gulf of California	KC870999
Haplotype 2	<i>E. spicata</i>	Gulf of California	KC870998
Haplotype 2	<i>E. spicata</i>	Gulf of California	KC357401
Haplotype 2	<i>E. spicata</i>	Gulf of California	KC357400
Haplotype 2	<i>E. spicata</i>	Gulf of California	KC357399
Haplotype 3	<i>E. spicata</i>	Gulf of California	KC871001
Haplotype 3	<i>E. spicata</i>	Gulf of California	KC357402
Haplotype 3	<i>E. spicata</i>	Gulf of California	KC357398
Haplotype 4	<i>E. spicata</i>	off Chile	LC413850
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC870990
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC870989
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC870988
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC870987
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC870986
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC870985
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC870984
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC870983
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC870982
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC357420
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC357419
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC357418
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC357417
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC357416
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC357415
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC357414
Haplotype 4	<i>E. laminata</i>	Gulf of Mexico	KC357413
Haplotype 5	<i>E. spicata</i>	Gulf of California	KC870997

Sequences highlighted in blue are newly provided by this study.

**Table S8.** Confirmed occurrences of Lamellibrachia sp. 2 from DNA sequencing.

Collection region	Locality	Basis	GenBank ID	Latitude	Longitude	Depth (m)	Reference
Gulf of Mexico		GenBank COI	KX902149	26.355217	-94.497700	2214	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902150	26.355217	-94.497700	2214	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902151	26.355217	-94.497700	2214	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902152	26.355217	-94.497700	2214	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902153	26.355217	-94.497700	2214	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902154	26.355217	-94.497700	2214	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902155	26.355217	-94.497700	2214	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902156	26.355217	-94.497700	2214	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902157	26.355217	-94.497700	2214	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902158	26.355217	-94.497700	2214	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902159	26.355217	-94.497700	2214	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902160	26.355217	-94.497700	2214	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902161	26.355217	-94.497700	2214	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902162	26.355217	-94.497700	2214	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902137	26.355484	-94.497899	2206	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902138	26.355484	-94.497899	2206	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902139	26.388330	-94.513330	2323	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	AC601	GenBank COI	GU059177	26.392000	-94.514000	2335	Miglietta <i>et al.</i> (2010)
Gulf of Mexico	AC601	GenBank COI	GU059178	26.392000	-94.514000	2335	Miglietta <i>et al.</i> (2010)
Gulf of Mexico	AC601	GenBank 16S	HE974470	26.392000	-94.514000	2335	Thiel <i>et al.</i> (2012)
Gulf of Mexico	WR269	Microsats	-	26.677000	-91.665000	1975	Cowart <i>et al.</i> (2014)
Gulf of Mexico	WR269	GenBank COI	GU059176	26.677000	-91.665000	1975	Miglietta <i>et al.</i> (2010)
Gulf of Mexico		GenBank COI	KX902094	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902095	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902096	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902097	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico		GenBank COI	KX902098	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	AT340	GenBank COI	GU059175	27.090783	-92.617700	2230	Miglietta <i>et al.</i> (2010)
Gulf of Mexico	AT340	GenBank COI	KX902163	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	AT340	GenBank COI	KX902164	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	AT340	GenBank COI	KX902165	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	AT340	GenBank COI	KX902166	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	AT340	GenBank COI	KX902167	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	AT340	GenBank COI	KX902168	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	AT340	GenBank COI	KX902169	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	AT340	GenBank COI	KX902170	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	AT340	GenBank COI	KX902171	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	AT340	GenBank COI	KX902172	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	AT340	GenBank COI	KX902173	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	AT340	GenBank COI	KX902174	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	AT340	GenBank COI	KX902175	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	AT340	GenBank COI	KX902176	27.090783	-92.617700	1263	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	GC852	Microsats	-	27.095000	-91.265000	1437	Cowart <i>et al.</i> (2014)
Gulf of Mexico	GC852	GenBank COI	GU059173	27.095000	-91.265000	1437	Miglietta <i>et al.</i> (2010)
Gulf of Mexico	GC852	GenBank 16S	HE974469	27.095000	-91.265000	1437	Thiel <i>et al.</i> (2012)
Gulf of Mexico	GB829	GenBank 16S	GU068263	27.184740	-92.123837	1291	Miglietta <i>et al.</i> (2010)
Gulf of Mexico	GB697	GenBank COI	GU059172	27.312000	-92.638000	1281	Miglietta <i>et al.</i> (2010)
Gulf of Mexico		GenBank COI	KX902099	27.338550	-92.360983	964	Plouviez, Ball and Van Dover, unpubl. data
Gulf of Mexico	GC600	GenBank 16S	GU068264	27.374000	-90.573000	1193	Miglietta <i>et al.</i> (2010)







## References

- Cowart DA, Halanych KM, Schaeffer SW, Fisher CR (2014) Depth-dependent gene flow in Gulf of Mexico cold seep Lamellibrachia tubeworms (Annelida, Siboglinidae). *Hydrobiologia* **736**, 139–154. <https://doi.org/10.1007/s10750-014-1900-y>
- Miglietta MP, Hourdez S, Cowart DA, Schaeffer SW, Fisher C (2010) Species boundaries of Gulf of Mexico vestimentiferans (Polychaeta, Siboglinidae) inferred from mitochondrial genes. *Deep-Sea Research – II. Topical Studies in Oceanography* **57**, 1916–1925. <https://doi.org/10.1016/j.dsr2.2010.05.007>
- Thiel V, Hügler M, Blümel M, Baumann HI, Gärtner A, Schmaljohann R, Strauss H, Garbe-Schönberg D, Petersen S, Cowart DA, Fisher CR, Imhoff JF (2012) Widespread occurrence of two carbon fixation pathways in tubeworm endosymbionts: lessons from hydrothermal vent associated tubeworms from the Mediterranean Sea. *Frontiers in Microbiology* **3**, 423. <https://doi.org/10.3389/fmicb.2012.00423>