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10   **Title:** Shared phylogeographic breaks in a Caribbean coral reef sponge and its invertebrate

11   commensals

12

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Abstract

32

Aim: To test whether phylogeographic barriers in the brooding sponge *Callyspongia vaginalis* match breaks previously identified in the Caribbean. We also compared patterns of subdivision in the sponge to those of three of its commensals, the broadcast spawning brittle star *Ophiothrix suensonii* and the brooding amphipods *Leucothoe ashleyae* and *L. kensleyi*, and tested whether any shared breaks arose simultaneously.

38

Location: Florida, Bahamas, and the Caribbean

40

Methods Subdivision of *C. vaginalis* populations was inferred from one mitochondrial (*COI*) and six nuclear loci using clustering analyses. We identified phylogeographic breaks in the sponge and its invertebrate commensals by determining geographic patterns of genetic variation and tested simultaneous population divergence across barriers shared among taxa using hierarchical approximate Bayesian computation.

46

Results: Sponge populations were partitioned into western and eastern groups across the Caribbean, with hierarchical subdivision within regions. The sponge and its commensals shared barriers across their ranges despite differences in dispersal strategy: *C. vaginalis, L. ashleyae*, and *O. suensonii* populations in Central America were isolated from the remainder of the Caribbean and all four taxa shared a break between Florida and the Bahamas, although simultaneous population divergence could not be inferred with statistical certainty. Our results also suggest cryptic speciation within *C. vaginalis*.

54

Main conclusions: Phylogeographic patterns in *C. vaginalis* largely matched barriers previously identified at the Florida Straits, Mona Passage, and Bay of Honduras in other Caribbean taxa. Oceanographic features such as deep water between locations, strong currents, and eddies are likely mechanisms responsible for these breaks.

59

60   **Key words:** amphipod, brittle star, *Callyspongia*, comparative phylogeography, cryptic

61   species, *Leucothoe*, *Ophiothrix*

62

63

64   **Introduction**

65

66   Identifying the mechanisms responsible for the geographic distribution of genetic variation

67   within species advances our understanding of the formation of biodiversity. In terrestrial

68   systems, barriers separating phylogenetic lineages within species, such as mountains or

69   rivers, are often apparent. Although barriers in the ocean are usually less obvious and

70   extended larval duration should in theory reduce differentiation, populations of marine taxa

71   are frequently subdivided. Population subdivision in marine systems is influenced by the

72   relative strength and interaction of several factors, including life history (Shulman &

73   Bermingham, 1995), selection (Prada & Hellberg, 2013), demographic change (DeBiasse *et*

74   *al.*, 2014), oceanography (Alberto *et al.*, 2011), ecology (Selkoe *et al.*, 2010). Associations

75   between interacting species can also dictate phylogeographic structure and lead to shared

76   patterns, especially when a host species' distribution limits where its commensals occur

77   (Duffy, 1992).

78

79   Sponges promote species richness by providing habitat for vertebrate and invertebrate

80   commensals (Fiore & Jutte, 2010). The intimate connection among these species might

81   lead to more tightly correlated patterns of subdivision than for species whose ranges

82   overlap but do not rely on each other for shelter, food, or reproduction. For example,

83   congruent phylogeographic patterns have been documented for shrimp/goby (Thompson *et*

84   *al.*, 2005) and coral/zooxanthellae (Prada *et al.*, 2014) symbiotic pairs. However,

85   symbioses do not guarantee shared phylogeographic structure (Parker *et al.*, 2004; Crandall

86   *et al.*, 2008) and differences in the closeness of commensal relationships or in dispersal

87   strategy might create discordant phylogeographic patterns among species (Hellberg, 2009).

88

89   *Callyspongia vaginalis* (Demospongiae, Haplosclerida, Lamarck, 1813), an abundant

90   western Atlantic sponge, hosts the facultatively commensal brittle star *Ophiothrix*

91   *suensonii* (Echinodermata, Ophiuroidea, Lütken, 1856) (for which *C. vaginalis* is the

92   preferred host; Henkel & Pawlik, 2005) and the obligate commensal amphipods *Leucothoe*

93   *ashleyae* and *L. kensleyi* (Arthropoda, Amphipoda, Thomas & Klebba, 2006). *Ophiothrix*

94   *suensonii* spawns year round and, with a 49 day planktotrophic larval development in

95   culture (Mladenov, 1983), has the potential for long distance dispersal. *Leucothoe ashleyae*

96   and *L. kensleyi* live within the canals of *C. vaginalis*. Females brood eggs and the young

97   disperse as crawl-away juveniles (Thomas & Klebba, 2006). *Callyspongia vaginalis* is a

98   spermcasting hermaphrodite that broods larvae competent to settle immediately upon

99   release (Lindquist *et al.*, 1997).

100

101   Despite a compact size that might be expected to promote homogenization and reduce

102   opportunities for geographic isolation and differentiation, the Caribbean basin contains

103   over 12,000 marine species (Miloslavich *et al.*, 2010), including several endemic radiations

104   (Morrison *et al.*, 2004; Taylor & Hellberg, 2005; Thornhill *et al.*, 2009) and cryptic species

105   (Victor, 2010; Prada & Hellberg, 2013). Several well-documented phylogeographic breaks

106   fall within the distribution of *C. vaginalis* and its commensals in the Caribbean. For

107   example, the Florida Straits between Florida and the Bahamas has been shown to be

108   phylogeographic break for a range of taxa, including fish (Carlin *et al.*, 2003), mussels (Lee

109   & Ó Foighil, 2004), sponges (Chaves-Fonnegra *et al.*, 2015), and corals (Andras *et al.*,

110   2013) and their zooxanthellae (Andras *et al.*, 2011). Another well known break at the Mona

111   Passage between Hispaniola and Puerto Rico demarcates a barrier for *Acropora palmata*

112   and *Montastrea annularis* corals (Baums *et al.*, 2006; Foster *et al.*, 2012). Taylor and

113   Hellberg (2003, 2006) documented genetic breaks at the Mona Passage and across the

114   Central Bahamas in the goby genus *Elactinus*. Central America is a particularly interesting

115   region for phylogeographic barriers. *Montastrea annularis* corals and *L. ashleyae*

116   amphipods both show deep genetic divides over small distances within the Bay of

117   Honduras (Foster *et al.*, 2012; Richards *et al.*, 2012) while other studies showed taxa in

118   Central America to be isolated from the rest of the Caribbean (Colin, 2002; Roberts *et al.*,

119   2002; Taylor & Hellberg, 2006; Andras *et al.*, 2011, 2013).

120

121   Identifying phylogeographic breaks in the sea is important because it provides information

122   about the geographic distribution of genetic variation in marine taxa, which in turn helps

123   researchers determine the mechanisms facilitating population divergence, speciation, and

124   ultimately, biodiversity creation. In this study, we test whether phylogeographic barriers in

125   *C. vaginalis* match previously defined breaks at the Florida Straits, the Mona Passage, and

126   the Bay of Honduras. The ubiquity of these breaks across a range of coral reef taxa is

127   evidence of their wide influence in population subdivision in the Caribbean and we

128   therefore expect them to be present in *C. vaginalis* as well. We also compare population

129   subdivision in the sponge to subdivision in the commensal brittle star *O. suensonii* and the

130   *Leucothoe* amphipods and test the hypothesis that any shared barriers arose simultaneously.

131

132

133   **Materials and methods**

134

135   *Genetic data*

136   We collected 275 *Callyspongia vaginalis* samples from 10 Caribbean locations (Fig. 1,

137   Table 1, Table S1 in Appendix S1). We sequenced cytochrome oxidase I (*COI*) in each

138   individual using protocols in DeBiasse *et al.* (2010) and six nuclear protein-coding genes

139   (catalase, *cata*; cathepsin, *cps*; cirhin, *cir*; elongation factor 1 alpha, *ef*; filamin, *fil*;

140   macrophage expressed protein, *mep*) in a subset of individuals in each location (n =100)

141   using protocols in DeBiasse *et al.* (2014). Sequences were GENEIOUS 4.5.5 (Drummond *et*

142   *al.*, 2012). We resolved alleles for nuclear genes in heterozygous individuals using PHASE

143   v2.1 (Stephens *et al.*, 2001) with a 90% probability limit. Individuals heterozygous for a

144   single insertion/deletion were resolved using CHAMPURU1.0 (Flot, 2007). Individuals with

145   alleles that could not be phased to a probability >90% were cloned using the Invitrogen

146   TOPO kit. At least 8 clones per reaction were sequenced. Individuals not resolved after

147   several rounds of cloning were removed from the dataset (*cata*=1; *cir*=2; *cps*=2; *ef*=4;

148   *fil*=1; *mep*=3). We detected no intra-locus recombination using GARD (Pond *et al.*, 2006).

149   The sponge dataset analysed here contains i) new *COI* and nuclear sequences from

150   Caribbean-wide *C. vaginalis* samples and ii) previously published *COI* and nuclear

151   sequences for 122 *C. vaginalis* individuals sampled in Florida (Table 1, Table S1 in

152   Appendix S1).

153

154   *Genetic diversity indices for* Callyspongia vaginalis

155   We calculated basic summary statistics in DNASP v5.0 (Librado & Rozas, 2009). Because

156   previous research suggested demographic change in *C. vaginalis* at small spatial scales

157   (DeBiasse *et al.*, 2014), we calculated Tajima's *D* (Tajima, 1989) and R_2 (Ramos-Onsins &

158   Rozas, 2002) in DNASP to test for population bottlenecks and expansions.

159

160   *Distribution of genetic variation in* Callyspongia vaginalis

161   We constructed a haplotype network using TCS 1.21 (Clement *et al.*, 2000) with default

162   settings. We used STRUCTURE 2.3.2 (Pritchard *et al.*, 2000) to determine how genetic

163   variation was distributed geographically. We ran clustering analyses for nuclear DNA

164   (nucDNA) alone and for mitochondrial DNA (mtDNA) and nucDNA combined. Sequences

165   were recoded into frequency data and individuals were considered homozygous for their

166   mitochondrial haplotype. In STRUCTURE, we applied the admixture model with correlated

167   allele frequencies, performing 20 iterations, each consisting of 1 million steps and a 10%

168   burnin. For each run, *K* was set to 1 through the number of geographic locations included

169   in that run. The Evanno *et al.* (2005) method, implemented in STRUCTURE HARVESTOR

170   (Earl & vonHoldt, 2011), determined the most likely number of clusters.

171

172   *Testing for shared phylogeographic breaks among taxa*

173   We used the program BARRIER 2.2 (Manni *et al.*, 2004) to identify shared genetic barriers

174   in the co-distributed taxa. The program uses geographic coordinates from each sampling

175   location and a distance matrix for each pair of locations. We obtained geographic

176   coordinates from Google maps and used pairwise ϕ_{ST} values estimated in ARLEQUIN 3.5

177   (Excoffier & Lischer, 2010) to construct distance matrices for all taxa (available on Dryad

178   at www.dx.doi.org/dryadXXX). We determined the strongest barrier in each locus for *C.*

179   *vaginalis*. We used previously published *COI* sequences (Table 1 and Table S1 in

180   Appendix S1) to infer the two strongest barriers in the amphipods and brittle star.

181

182   We tested for simultaneous divergence of the sponge and commensals using hierarchical

183   approximate Bayesian computation implemented in MTML-MSBAYES 20140305 (Huang *et*

184   *al.*, 2011). This method estimates population specific sub-parameters for each taxon and

185   three hyper-parameters that describe the mean, variance, and number of divergence events

186   across population pairs. Using *COI* sequences (Table 1 and Table S1 in Appendix S1), we

187   estimated summary statistics for all taxa. We simulated 3,000,000 datasets drawing

188   population sub-parameters and hyper-parameters from a prior distribution. The upper

189   bound for the number of divergence events (ψ) was set to the number of taxa (4). We

190   approximated the posterior distribution for the hyper-parameters by retaining 900 simulated

191   models whose summary statistic vectors had the shortest Euclidian distances from the

192   summary statistic vector in the empirical data. We used Ω, which measures the

193   incongruence among population divergence times, and ψ to evaluate the relative support of

194   each model (Stone *et al.*, 2012). Ω values of 0 (e.g. no variation in divergence times among

195   taxa) and ψ values of 1 (e.g. a single divergence event for all taxa) indicate simultaneous

196   divergence.

197

198   **Results**

199

200   *Genetic diversity and variation in* C. vaginalis

201   Nuclear loci had higher allelic richness than *COI* (Table S2 in Appendix S1). *Cata* and *fil*

202   had the highest number of alleles (36 and 35, respectively) and *fil* had the highest

203   nucleotide diversity (0.0239). For *COI*, Tajima's *D* values were significantly positive for

204   Key Largo, Marquesas Keys, and Curaçao, significantly negative for Glover's Reef, and

205   nonsignificant for all locations combined. For the nuclear loci, Tajima's *D* was

206   nonsignificant for each sampling location and for all locations combined, with the

207   exception of *cata* in Bimini, *cps* in Key Largo and Long Key, and *fil* in Long Key. The R_2

208   statistic was nonsignificant for all loci except *cps* (Table S2 in Appendix S1).

209

210   *COI* haplotypes in *C. vaginalis* were geographically restricted (Fig. 1). The 'green'

211   haplotype was most frequent (27%) with the widest distribution, ranging from Bimini to

212   Bocas del Toro. It was the only haplotype in Vieques and St. Croix. The 'yellow' haplotype

213   was restricted to Central America (Glover's Reef, Utila, and Roatan). The 'red' haplotype

214   was restricted to Veracruz, Florida, and Bimini. The 'blue' haplotype was common in

215   Florida and Bimini and rare in Central America. The 'brown' haplotype occurred in Florida

216   and Central America and half the individuals in Curaçao. The 'purple' haplotypes, found in

217   Bocas del Toro and Utila, connected to each other but not to the main network at the 95%

218   confidence level, indicating their genetic divergence from the other haplotypes and

219   suggesting they represent a cryptic species found in sympatry with *C. vaginalis*. The K2P

220   distance between the cryptic species and the other *C. vaginalis* individuals was 4% for

221   *COI*. The cryptic species had four private nuclear alleles in three nuclear genes and shared

222   16 alleles with *C. vaginalis* (*cata*=1; *cir*=3; *cps*=4; *ef1a*=3; *fil*=2; *mep*=3).

223

224   The STRUCTURE analyses based on the nucDNA alone showed the best-supported number

225   of genetic clusters was two, with a west-to-east split among the sampling locations. Florida,

226   Glover's Reef, Utila, Roatan, and Bocas del Toro were assigned to the western cluster and

227   Bimini, Crooked Island, St. Croix, Vieques, and Curaçao were assigned to the eastern

228   cluster. A small number of individuals from Florida and Bimini were also assigned to the

229   cluster opposite their geographic position. The cryptic species from Utila and Bocas del

230   Toro were assigned to the eastern cluster (Fig. 2a).

231

232   When the nucDNA and mtDNA were combined, the best *K* was 2 and the west-to-east

233   pattern was recovered (Fig. 2b), however Veracruz clustered with the west and Bocas del

234   Toro clustered with the east, opposite their positions in the nucDNA-only analysis (Fig 2a).

235   In the combined mtDNA and nucDNA analysis, the cryptic species from Bocas del Toro

236   and Utila clustered in the west and east, respectively, and a small number of individuals

237   from Florida and Bimini were assigned to the cluster opposite their geographic location

238   (Fig. 2). The plot of delta *K* produced in the Evanno *et al*. (2005) method showed

239   secondary peaks at *K*=7 and *K*=10 (Fig. 2c and Fig. S1 in Appendix S1), indicating

240   hierarchical subdivision exists among sponge populations within the western and eastern

241   regions. As *K* increased in the STRUCTURE analyses, the cryptic species were assigned to

242   their own cluster (Fig. 2b, Fig. S1 in Appendix S1). When we repeated the STRUCTURE

243 analyses excluding the cryptic species, the same west-to-east pattern was recovered and the

244 sampling locations were assigned to the same clusters.

245

246 *Shared barriers and simultaneous divergence in* C. vaginalis *and its commensals*

247 The most frequent barrier in *C. vaginalis* (observed in four loci) occurred in the centre of

248 the Caribbean, running northwest to southeast below Jamaica (Fig. 3a). The location of this

249 barrier corresponds with the STRUCTURE results, which also divided sponge populations

250 west-to-east. *Leucothoe kensleyi* and *C. vaginalis* shared a barrier separating Vieques and

251 St. Croix from Curaçao (Figure 3a, c) and *L. ashleyae*, *O. suensonii*, and *C. vaginalis* all

252 had barriers isolating Central American locations from the rest of the Caribbean (Fig. 3a, b,

253 c). All four taxa showed a break between Florida and the Bahamas across the Florida

254 Straits (Fig. 3a-d).

255

256 Results from our analyses indicated that gene flow across the Florida Straits was restricted

257 to varying degrees with isolation strongest in the amphipods and weakest in the brittle star.

258 We tested for simultaneous divergence across this region but the results were ambiguous.

259 The mode and mean for Ω were 0.0 and 0.190, respectively, however 95% of the values in

260 the approximated posterior distribution (95% quantile) were between 0.0 and 0.658. While

261 a value of zero indicates simultaneous divergence, values above zero in the 95% quantile

262 suggest variation around the divergence of each of the four population pairs. For ψ, the

263 mode and mean were 1.0 and 1.7, respectively, and the 95% quantile contained all possible

264 numbers of divergence events (1 through 4). The posterior probabilities for models with 1,

265 2, 3, and 4 divergence events were 0.518, 0.308, 0.126, and 0.048, respectively. Although

266 the range of Ω values contained zero, values greater than zero, which indicate variation

267 around the divergence times of co-distributed taxa, were contained within the distribution.

268 For ψ, 80% of the posterior support was divided between models with one and two

269 divergence events, preventing us from distinguishing the best model to describe divergence

270 history for the sponge and its commensals.

271

272 **Discussion**

273 We determined population subdivision in *C. vaginalis* and tested whether phylogeographic

274 patterns in this sponge corresponded to previously documented breaks at the Florida

275 Straits, Mona Passage, and Bay of Honduras. We also compared its genetic structure to

276 structure in three of its invertebrate commensals. We found sponge populations were

277 divided west-to-east across the Caribbean, with hierarchical structure within regions that

278 matched known barriers. The sponge, amphipods, and brittle star shared a well-defined

279 split across the Florida Straits but we were unable to determine whether this break occurred

280 simultaneously across all four taxa.

281

282 Data from *COI* and six nuclear markers showed significant population subdivision in *C.*

283 *vaginalis*. These patterns are consistent with previous sponge studies employing a variety

284 of markers, which often attribute genetic differentiation to limited larval dispersal

285 (Blanquer *et al.*, 2009; López-Legentil & Pawlik, 2009; Blanquer & Uriz, 2010; Dailianis

286 *et al.*, 2011; Pérez-Portela *et al.*, 2014; Chaves-Fonnegra *et al.*, 2015). Our previous work

287 on *C. vaginalis* in Florida at a smaller spatial scale showed genomically discordant patterns

288 of structure: *COI* haplotypes were geographically subdivided while nuclear alleles were

289 panmictic (DeBiasse *et al.*, 2010, 2014). Coalescent simulations and neutrality tests

290 supported population bottlenecks and sperm-biased dispersal as possible drivers of mito-

291 nuclear discordance in *C. vaginalis* (DeBiasse *et al.*, 2014). Across the Caribbean,

292 mitochondrial and nuclear loci had similar patterns of subdivision, suggesting spatial scale

293 may influence concordance among loci.

294

295 We tested the hypothesis that phylogeographic barriers in *C. vaginalis* matched previously

296 defined breaks for other coral reef taxa across the Caribbean basin. The BARRIER and

297 STRUCTURE analyses (Fig. 2, Fig. 3) indicated significant population subdivision between

298 Florida and the Bahamas in *C. vaginalis* and its invertebrate commensals across the Florida

299 Straits, a major barrier to gene flow for many marine species. For example, Andras *et al.*

300 (2013) found allele frequency differences in the sea fan *Gorgonia ventalina* between

301 Florida and the Bahamas, while its algal symbionts shared no alleles across this gap

302 (Andras *et al.*, 2011). Two species of coral, a mussel, and a sponge also have

303 phylogeographic breaks across the Florida Straits (Lee & Ó Foighil, 2004; Brazeau *et al.*,

304   2005; Baums *et al.*, 2010; Chaves-Fonnegra *et al.*, 2015). The geographic proximity of

305   Florida and Bahamian coral reefs (~100 km) makes it unlikely that distance alone is

306   responsible for restricting gene flow between populations in this region. Indeed, previous

307   results showed connectivity in *C. vaginalis* (based on nucDNA) and both amphipod species

308   (based on *COI*) along ~400 km of the shallow Florida reef tract (Richards *et al.*, 2007;

309   DeBiasse *et al.*, 2014), four times the distance of the Florida Straits.

310

311   This dichotomy, connectivity within Florida but structure across the Florida Straits, likely

312   results from a complex interaction of factors including geography, life history, and

313   oceanography. For example, the continuous reef habitat along the Florida coastline might

314   facilitate population connectivity via stepping stone dispersal of gametes, larvae, and/or

315   adults despite the brooding strategy of the sponge and amphipods. Amphipods have been

316   found in detached *C. vaginalis* tubes drifting along the reef substrate (VPR, MBD personal

317   observations) and sponge fragments can support viable sponge larvae (Maldonado & Uriz,

318   1999), suggesting dispersal of amphipods and sponge larvae could occur via drifting in

319   sponge fragments (Highsmith, 1985). While the continuity of the shallow Florida reef tract

320   likely facilitates such dispersal, deep water (~800 m), lack of intervening reef habitat, and

321   rapid transport (3.0×10^7 m^{3}s^{-1}) of the Florida Current, which flows northward between

322   Florida and the Bahamas (Baringer & Larsen, 2001), likely restrict dispersal across the

323   Florida Straits. The pattern of connectivity along a continuous reef tract and isolation

324   between proximal locations separated by deep water was also observed for *L. ashleyae* and

325   the reef fish *Elacatinus lori* in the Belizean barrier reef system (Richards *et al.*, 2012;

326   D'Aloia *et al.*, 2014). Isolation across the Florida Straits was weakest here in the brittle star,

327   perhaps due to its broadcast spawning life history strategy, which might allow dispersal

328   regardless of depth and currents (Sherman *et al.*, 2008). We tested whether the shared

329   Florida Straits barrier produced simultaneous divergence in *C. vaginalis* and its

330   commensals, but the distribution of posterior support among alternative models prevented

331   us from confidently accepting the simultaneous divergence model. Growing evidence

332   shows robust population parameter estimation requires multi-locus genetic data (Heled &

333   Drummond, 2010) and our power to test simultaneous divergence here was likely limited

334   by the use of a single marker.

335

336   The Mona Passage between Hispaniola and Puerto Rico is a well-studied phylogeographic

337   break for many marine taxa (Taylor & Hellberg, 2003; Baums *et al.*, 2005; Dennis *et al.*,

338   2005; Baums *et al.*, 2006; Taylor & Hellberg, 2006), but the broad scale pattern of

339   population subdivision inferred from STRUCTURE did not show a phylogeographic barrier at

340   the Mona Passage for *C. vaginalis*. Locations on either side of the Passage (Bimini,

341   Crooked Island, Vieques, and St. Croix) grouped together in the same cluster (Fig. 2a,b).

342   However, at a finer scale, locations to the east of the Mona Passage (Vieques and St. Croix)

343   grouped in a different cluster than locations to the west of the Passage (Crooked Island),

344   suggesting these sites are isolated from each other across the Mona Passage (Fig. 2c). The

345   BARRIER analyses identified a break in *C. vaginalis* across Hispaniola, separating Vieques

346   and St. Croix from Crooked Island. In contrast to the sponge, the BARRIER analyses did not

347   identify a break between locations on either side of the Mona Passage for the invertebrate

348   commensals. Biophysical oceanographic models suggest deep depths, complex bottom

349   topography, and unique oceanographic features, such as small scale eddies, restrict

350   dispersal across this region (Baums *et al.*, 2006). Robustly identifying the precise location

351   of the break on either side of the passage in *C. vaginalis* will require finer scale sampling

352   across this region.

353

354   Previous research points to middle Central America (Belize and Honduras) as a region of

355   genetic isolation and endemism in terrestrial and marine species (Briggs, 1984; Roberts *et*

356   *al.*, 2002). For example, Andras *et al.* (2011; 2013) reported populations of the sea fan

357   *Gorgonia ventalina* in Belize and Panama were strongly differentiated from the wider

358   Caribbean, as were populations of the sea fan's zooxanthellae. The goby *Elacatinus*

359   *oceanops* was monophyletic for mitochondrial and nuclear markers between Florida and

360   Belize (Taylor & Hellberg, 2006) and Colin (2002) described *E. lori* as endemic to

361   Honduras and Belize. Previous results and analyses we performed here (Fig. 2, Fig. 3)

362   showed Central American populations of *C. vaginalis*, *L. ashleyae*, and *O. suensonii* were

363   genetically isolated from the Caribbean despite differences in dispersal strategy (Richards

364   *et al.*, 2012, 2015). Isolation of marine taxa in Central America is likely due in part to

365   oceanographic currents. Gyres in the Gulf of Honduras (Heyman & Kjerfve, 2000) and off

Multiple analyses showed that some individuals from Central American populations were genetically divergent from sympatric *C. vaginalis* and might represent a cryptic species (Hart & Sunday, 2007). Phylogeographic studies on the Porifera frequently uncover cryptic species (Blanquer & Uriz, 2007; Xavier *et al.*, 2010; Andreakis *et al.*, 2012; de Paula *et al.*, 2012), likely because the morphological characters used to define sponges are simple, plastic, and can vary geographically (Barnes & Bell, 2002; Loh & Pawlik, 2009; DeBiasse & Hellberg, 2015). Although the cryptic species sampled in Bocas del Toro and Utila had private mitochondrial haplotypes and nuclear alleles, it also shared a few nuclear alleles with *C. vaginalis*. Genetic data from additional loci and individuals are needed to determine whether these shared alleles are the result of hybridization, incomplete lineage sorting, or another mechanism.

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Supporting Information

658   Additional Supporting Information may be found in the online version of this article:

659   **Appendix S1** Supplementary tables and one figure.

660

Data Accessibility

662   Sequences for *C. vaginalis* generated for this study are available from the European

663   Nucleotide Archive under accession numbers LK026325-LK026602 (*COI*) and LK026931-

664   LK028492 (nuclear loci). **this excludes *mep* sequences because due to their length, can

665   only be assigned accession numbers once the manuscript is accepted**

666

667 **Biosketch**

668 **MBD's research examines the mechanisms responsible for the distribution of genetic**

669 **and phenotypic variation within and among species of marine invertebrates,**

670 **particularly the Porifera.**

671 Author Contributions: MBD, VPR, MSS, and MEH designed the project; VPR and MBD

672 collected the tissue samples; MBD generated and analysed the genetic data; MBD and

673 MEH wrote the manuscript; all authors commented on and approved the final version of

674 the manuscript.

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676 **Editor: Michael Dawson**

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717 **Table 1** Collection locations and locus sample sizes (number of alleles) for the sponge

718 *Callyspongia vaginalis* and its invertebrate commensals. Sequences from previously

719 published studies are indicated with a superscript letter. All other sequences were generated

720 for this study. Accession numbers for all new and previously published sequences are

721 available in Table S1 in Appendix S1. Abbreviations: *COI*, cytochrome oxidase subunit I;

722 *cata*, *catalase*; *cir*, *cirhin*; *cps*, *cathepsin*; *ef*, *elongation factor 1 alpha*; *fil*, *filamin*; *mep*,

723 *macrophage expressed protein*

724 _____

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726 *Callyspongia vaginalis*

727 Location   *COI*   *cata*   *cir*   *cps*   *ef*   *fil*   *mep*

728 _____

729	Key Largo	30[a]	30[b]	34[b]	34[b]	28[b]	34[b]	34[b]
730	Long Key	34[a]	26[b]	28[b]	26[b]	26[b]	28[b]	28[b]
731	Marquesas Keys	29[a]	20[b]	28[b]	28[b]	26[b]	29[b]	28[b]
732	Dry Tortugas	29[a]	30[b]	32[b]	28[b]	28[b]	32[b]	32[b]
733	Veracruz	16	10	14	14	12	14	12
734	Glover's Reef	33	20	22	20	22	22	22
735	Utila	31	6	20	22	22	22	20
736	Roatan	32	4	20	20	20	20	20
737	Bocas del Toro	24	18	18	18	14	18	28
738	Curaçao	30	26	28	28	26	26	26
739	St. Croix	28	16	18	16	18	18	16
740	Vieques	30	14	12	14	12	12	14
741	Crooked Island	23	8	8	8	8	8	8
742	Bimini	29	16	34	34	32	30	30

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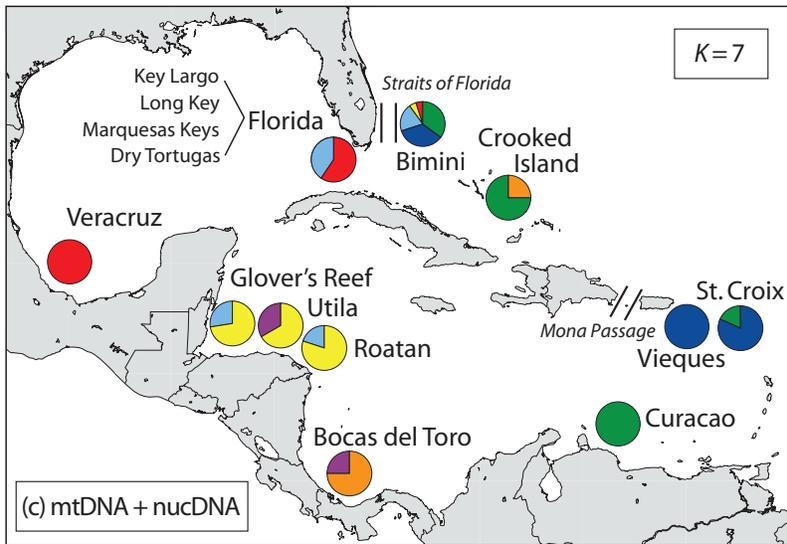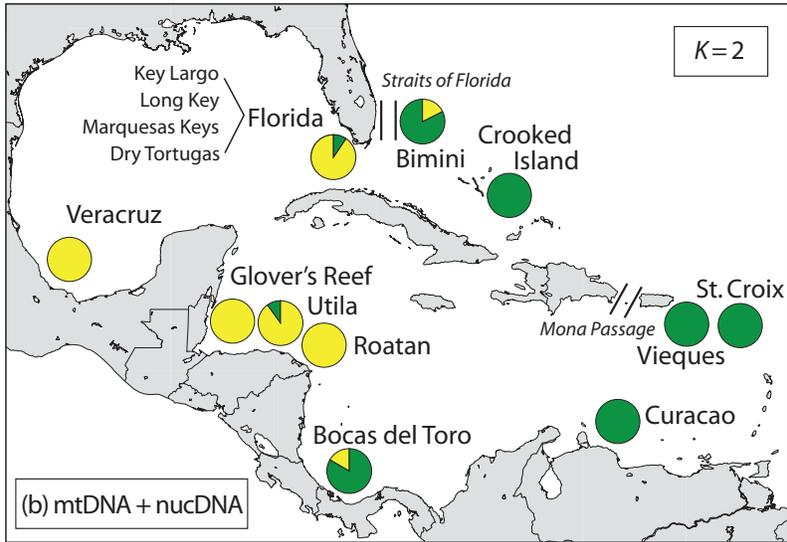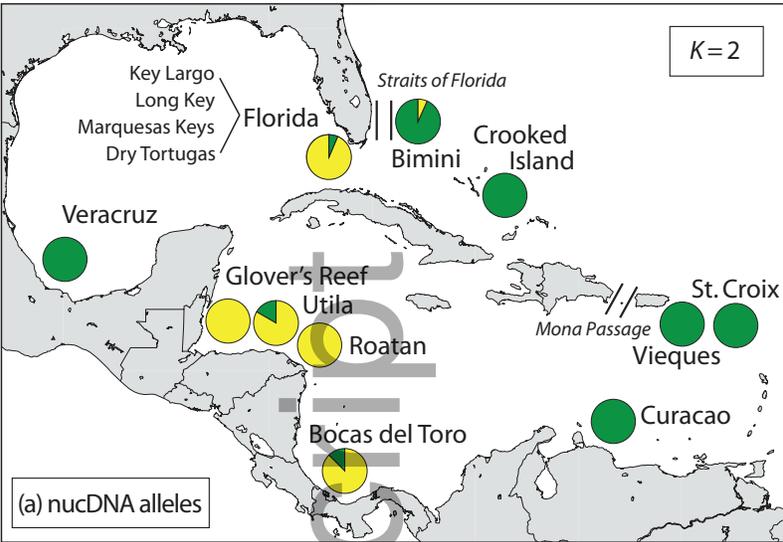|	*Leucothoe ashleyae*		*Leucothoe kensleyi*		*Ophiothrix suensonii*	
746	Location	*COI*	Location	*COI*	Location	*COI*
748	Palm Beach	30[c]	Pam Beach	36[c]	Key Largo	18[e]
749	Ft. Lauderdale	37[c]	Ft. Lauderdale	82[c]	Long Key	27[e]
750	Long Key	23[c]	Long Key	31[c]	Key West	24[e]
751	Key West	29[c]	Key West	33[c]	Marquesas Keys	28[e]
752	Glover's Reef	17[c]	Curaçao	26[d]	Cayman Isl.	31[e]
753	Carrie Bow Cay	21[d]	Vieques	20[d]	Utila	32[e]
754	Roatan	49[d]	Bimini	14[d]	Curaçao	32[e]
755	Curaçao	27[d]			St. Croix	31[e]
756	Vieques	31[d]			Crooked Isl.	33[e]
757	Bimini	30[d]				

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759 [a]DeBiasse *et al.* (2010), [b]DeBiasse *et al.* (2014), [c]Richards *et al.* (2007), [d]Richards *et al.*
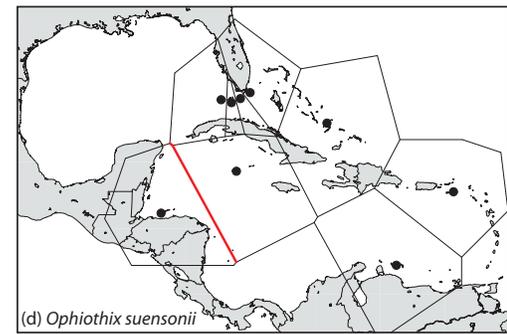
760 (2010), [e]Richards *et al.* (2015).
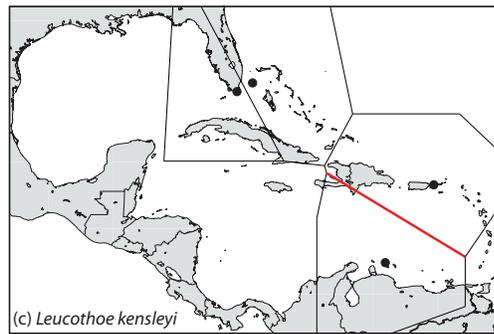
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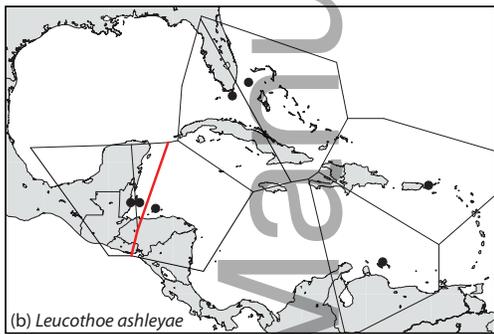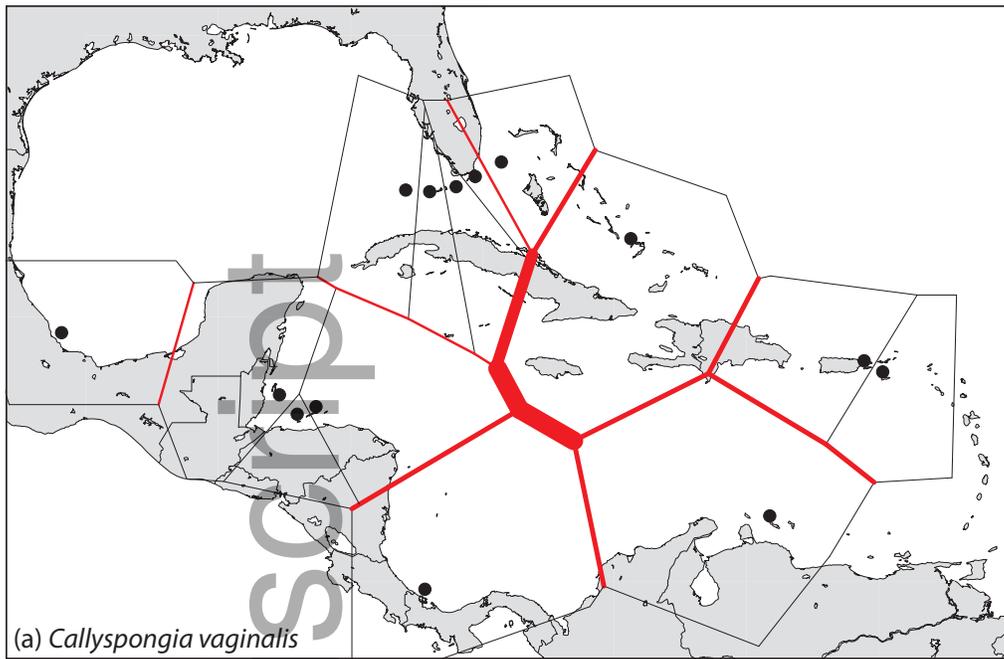Long Key
Marquesas Keys
Dry Tortugas

Straits of Florida

Florida

Bimini

Crooked
Island

Veracruz

Glover's Reef

Utila

Roatan

St. Croix

Mona Passage

Vieques

Curacao

Bocas del Toro

mtDNA haplotypes

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(a) nucDNA alleles

(b) mtDNA + nucDNA

(c) mtDNA + nucDNA

$K = 2$

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Key Largo
Long Key
Marquesas Keys
Dry Tortugas

Florida

Straits of Florida

Bimini

Crooked Island

Veracruz

Glover's Reef
Utila

Roatan

St. Croix

Mona Passage

Vieques

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(a) *Callyspongia vaginalis*

(b) *Leucothoe ashleyae*

(c) *Leucothoe kensleyi*

(d) *Ophiothix suensonii*

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