ORIGINAL ARTICLE

Shared phylogeographical breaks in a Caribbean coral reef sponge and its invertebrate commensals

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ABSTRACT

Aim To test whether phylogeographical 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.

Location Florida, Bahamas and the Caribbean.

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

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*.

Main conclusions Phylogeographical 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.

Keywords
amphipod, brittle star, *Callyspongia*, comparative phylogeography, cryptic species, *Leucothoe*, *Ophiothrix*

INTRODUCTION

Identifying the mechanisms responsible for the geographical distribution of genetic variation within species advances our understanding of the formation of biodiversity. In terrestrial systems, barriers separating phylogenetic lineages within species, such as mountains or rivers, are often apparent. Although barriers in the ocean are usually less obvious and extended larval duration should in theory reduce differentiation, populations of marine taxa are frequently subdivided. Population subdivision in marine systems is influenced by the relative strength and interaction of several factors, including life history (Shulman & Bermingham, 1995), selection (Prada & Hellberg, 2013), demographic change (DeBiasse *et al.*, 2014), oceanography (Alberto *et al.*, 2011) and ecology (Selkoe *et al.*, 2010). Associations between interacting species can also dictate phylogeographical structure and lead to shared patterns, especially when a host

species' distribution limits where its commensals occur (Duffy, 1992).

Sponges promote species richness by providing habitat for vertebrate and invertebrate commensals (Fiore & Jutte, 2010). The intimate connection among these species might lead to more tightly correlated patterns of subdivision than for species whose ranges overlap but do not rely on each other for shelter, food or reproduction. For example, congruent phylogeographical patterns have been documented for shrimp/goby (Thompson *et al.*, 2005) and coral/zooxanthellae (Prada *et al.*, 2014) symbiotic pairs. However, symbioses do not guarantee shared phylogeographical structure (Parker *et al.*, 2004; Crandall *et al.*, 2008), and differences in the closeness of commensal relationships or in dispersal strategy might create discordant phylogeographical patterns among species (Hellberg, 2009).

Callyspongia vaginalis (Demospongiae, Haplosclerida, Lamarck, 1813), an abundant western Atlantic sponge, hosts the facultatively commensal brittle star *Ophiothrix suensonii* (Echinodermata, Ophiuroidea, Lütken, 1856) (for which *C. vaginalis* is the preferred host; Henkel & Pawlik, 2005) and the obligate commensal amphipods *Leucothoe ashleyae* and *L. kensleyi* (Arthropoda, Amphipoda, Thomas & Klebba, 2006). *Ophiothrix suensonii* spawns year round and, with a 49-day planktotrophic larval development in culture (Mladenov, 1983), has the potential for long-distance dispersal. *Leucothoe ashleyae* and *L. kensleyi* live within the canals of *C. vaginalis*. Females brood eggs and the young disperse as crawl-away juveniles (Thomas & Klebba, 2006). *Callyspongia vaginalis* is a spermcasting hermaphrodite that broods larvae competent to settle immediately upon release (Lindquist *et al.*, 1997).

Despite a compact size that might be expected to promote homogenization and reduce opportunities for geographical isolation and differentiation, the Caribbean basin contains over 12,000 marine species (Miloslavich *et al.*, 2010), including several endemic radiations (Morrison *et al.*, 2004; Taylor & Hellberg, 2005; Thornhill *et al.*, 2009) and cryptic species (Victor, 2010; Prada & Hellberg, 2013). Several well-documented phylogeographical breaks fall within the distribution of *C. vaginalis* and its commensals in the Caribbean. For example, the Florida Straits between Florida and the Bahamas has been shown to be phylogeographical break for a range of taxa, including fish (Carlin *et al.*, 2003), mussels (Lee & Ó Foighil, 2004), sponges (Chaves-Fonnegra *et al.*, 2015), and corals (Andras *et al.*, 2013) and their zooxanthellae (Andras *et al.*, 2011). Another well-known break at the Mona Passage between Hispaniola and Puerto Rico demarcates a barrier for *Acropora palmata* and *Montastrea annularis* corals (Baums *et al.*, 2006; Foster *et al.*, 2012). Taylor & Hellberg (2003, 2006) documented genetic breaks at the Mona Passage and across the Central Bahamas in the goby genus *Elactinus*. Central America is a particularly interesting region for phylogeographical barriers. *Montastrea annularis* corals and *L. ashleyae* amphipods both show deep genetic divides over small distances within the Bay of Honduras (Foster *et al.*, 2012; Richards *et al.*, 2012), while other studies showed taxa in Central America to be isolated from the rest of the Caribbean (Colin, 2002; Roberts *et al.*, 2002; Taylor & Hellberg, 2006; Andras *et al.*, 2011, 2013).

Identifying phylogeographical breaks in the sea is important because it provides information about the geographical distribution of genetic variation in marine taxa, which in turn helps researchers determine the mechanisms facilitating population divergence, speciation and ultimately, biodiversity creation. In this study, we test whether phylogeographical barriers in *C. vaginalis* match previously defined breaks at the Florida Straits, the Mona Passage, and the Bay of Honduras. The frequency of these breaks across a range of coral reef taxa suggests their wide influence in population subdivision in the Caribbean, and we, therefore, expect them to be present in *C. vaginalis* as well. We also compare population subdivision in the sponge to subdivision in the commensal brittle star *O. suensonii* and the *Leucothoe* amphipods and test the hypothesis that any shared barriers arose simultaneously.

MATERIALS AND METHODS

Genetic data

We collected 275 *Callyspongia vaginalis* samples from 10 Caribbean locations (Fig. 1, Table 1, Table S1 in Appendix S1). We sequenced cytochrome oxidase I (*COI*) in each individual using protocols in DeBiasse *et al.* (2010) and six nuclear protein-coding genes (catalase, *cata*; cathepsin, *cps*; cirhin, *cir*; elongation factor 1 alpha, *ef*; filamin, *fil*; macrophage expressed protein, *mep*) in a subset of individuals in each location ($n = 100$) using protocols in DeBiasse *et al.* (2014). Sequences were aligned in Geneious 4.5.5 (Drummond *et al.*, 2012). We resolved alleles for nuclear genes in heterozygous individuals using Phase 2.1 (Stephens *et al.*, 2001) with a 90% probability limit. Individuals heterozygous for a single insertion/deletion were resolved using Champuru 1.0 (Flot, 2007). Individuals with alleles that could not be phased to a probability > 90% were cloned using the Invitrogen TOPO kit. At least eight clones per reaction were sequenced. Individuals not resolved after several rounds of cloning were removed from the dataset (*cata*=1; *cir*=2; *cps*=2; *ef*=4; *fil*=1; *mep*=3). We detected no intra-locus recombination using Gard (Pond *et al.*, 2006). The sponge dataset analysed here contains (1) new *COI* and nuclear sequences from Caribbean-wide *C. vaginalis* samples and (2) previously published *COI* and nuclear sequences for 122 *C. vaginalis* individuals sampled in Florida (Table 1, Table S1 in Appendix S1).

Genetic diversity indices for *Callyspongia vaginalis*

We calculated basic summary statistics in DnaSP 5.0 (Librado & Rozas, 2009). Because previous research suggested demographic change in *C. vaginalis* at small spatial

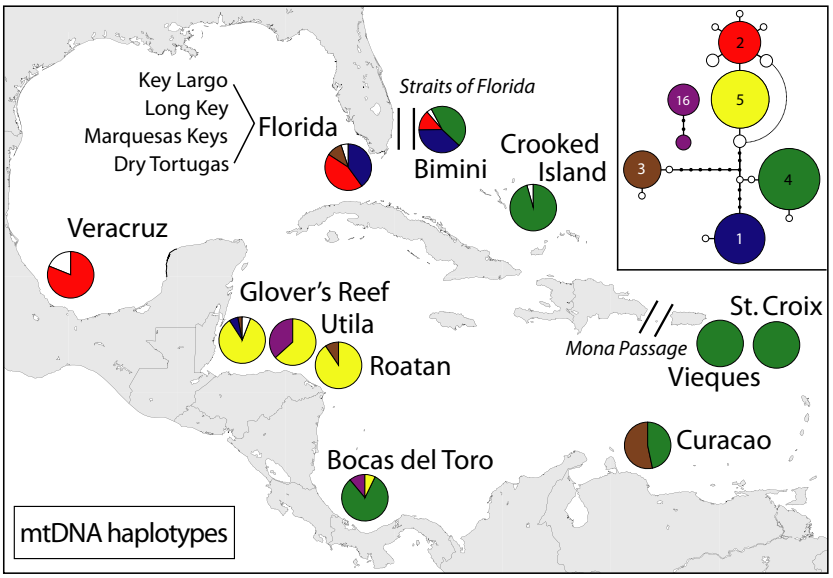

Figure 1 The distribution of mitochondrial DNA (mtDNA) variation in *Callyspongia vaginalis*. The inset shows a parsimony network where coloured circles represent mtDNA haplotypes and circle size is proportional to the frequency of each haplotype. Less frequent haplotypes are in white. Small black dots represent hypothetical haplotypes that were not sampled and lines separating each haplotype represent one mutational change. The dotted line represents an alternative connection among haplotypes. Two haplotypes (purple) connect to each other but not the main network at the 95% confidence interval. The map shows haplotype distributions among sampling locations. Each pie chart shows which haplotypes occur at each sampling location with sector size proportional to haplotype frequency.

scales (DeBiasse *et al.*, 2014), we calculated Tajima's *D* (Tajima, 1989) and R_2 (Ramos-Onsins & Rozas, 2002) in DnaSP to test for population bottlenecks and expansions.

Distribution of genetic variation in *Callyspongia vaginalis*

We constructed a haplotype network using TCS 1.21 (Clement *et al.*, 2000) with default settings. We used Structure 2.3.2 (Pritchard *et al.*, 2000) to determine how genetic variation was distributed geographically. We ran clustering analyses for nuclear DNA (nucDNA) alone and for mitochondrial DNA (mtDNA) and nucDNA combined. Sequences were recoded into frequency data, and individuals were considered homozygous for their mitochondrial haplotype. In Structure, we applied the admixture model with correlated allele frequencies, performing 20 iterations, each consisting of 1 million steps and a 10% burnin. For each run, *K* was set to 1 through the number of geographical locations included in that run. The method of Evanno *et al.* (2005), implemented in Structure Harvestor (Earl & vonHoldt, 2011), determined the most likely number of clusters.

Testing for shared phylogeographical breaks among taxa

We used the program Barrier 2.2 (Manni *et al.*, 2004) to identify shared genetic barriers in the co-distributed taxa. The program uses geographical coordinates from each sampling location and a distance matrix for each pair of locations. We obtained geographical coordinates from Google maps and used pairwise ϕ_{ST} values estimated in Arlequin 3.5 (Excoffier & Lischer, 2010) to construct distance matrices for all taxa (data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.bj546). We determined the strongest barrier in each locus for *C. vaginalis*. We used previously published *COI* sequences (Table 1 and Table S1 in Appendix S1) to infer the two strongest barriers in the amphipods and brittle star.

We tested for simultaneous divergence of the sponge and commensals using hierarchical approximate Bayesian computation implemented in MTML-msBayes 20140305 (Huang *et al.*, 2011). This method estimates population specific subparameters for each taxon and three hyper-parameters that describe the mean, variance and number of divergence events across population pairs. Using *COI* sequences (Table 1 and Table S1 in Appendix S1), we estimated summary statistics for all taxa. We simulated 3,000,000 datasets drawing population subparameters and hyper-parameters from a prior distribution. The upper bound for the number of divergence events (ψ) was set to the number of taxa (4). We approximated the posterior distribution for the hyper-parameters by retaining 900 simulated models whose summary statistic vectors had the shortest Euclidian distances from the summary statistic vector in the empirical data. We used Ω, which measures the incongruence among population divergence times, and ψ to evaluate the relative support of each model (Stone *et al.*, 2012). Ω values of 0 (e.g. no variation in divergence times among taxa) and ψ values of 1 (e.g. a single divergence event for all taxa) indicate simultaneous divergence.

Table 1 Collection locations and locus sample sizes (number of alleles) for the sponge *Callyspongia vaginalis* and its invertebrate commensals. Sequences from previously published studies are indicated with a superscript letter. All other sequences were generated for this study. Accession numbers for all new and previously published sequences are available in Table S1 in Appendix S1. Abbreviations: *COI*, cytochrome oxidase subunit I; *cata*, catalase; *cir*, cirhin; *cps*, cathepsin; *ef*, elongation factor 1 alpha; *fil*, filamin; *mep*, macrophage expressed protein

Callyspongia vaginalis Location	*COI*	*cata*	*cir*	*cps*	*ef*	*fil*	*mep*
Key Largo	30[a]	30[b]	34[b]	34[b]	28[b]	34[b]	34[b]
Long Key	34[a]	26[b]	28[b]	26[b]	26[b]	28[b]	28[b]
Marquesas Keys	29[a]	20[b]	28[b]	28[b]	26[b]	29[b]	28[b]
Dry Tortugas	29[a]	30[b]	32[b]	28[b]	28[b]	32[b]	32[b]
Veracruz	16	10	14	14	12	14	12
Glover's Reef	33	20	22	20	22	22	22
Utila	31	6	20	22	22	22	20
Roatan	32	4	20	20	20	20	20
Bocas del Toro	24	18	18	18	14	18	28
Curaçao	30	26	28	28	26	26	26
St. Croix	28	16	18	16	18	18	16
Vieques	30	14	12	14	12	12	14
Crooked Island	23	8	8	8	8	8	8
Bimini	29	16	34	34	32	30	30

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

[a]DeBiasse *et al.* (2010), [b]DeBiasse *et al.* (2014), [c]Richards *et al.* (2007), [d]Richards *et al.* (2012), [e]Richards *et al.* (2015).

RESULTS

Genetic diversity and variation in *C. vaginalis*

Nuclear loci had higher allelic richness than *COI* (Table S2 in Appendix S1). *Cata* and *fil* had the highest number of alleles (36 and 35, respectively), and *fil* had the highest nucleotide diversity (0.0239). For *COI*, Tajima's *D* values were significantly positive for Key Largo, Marquesas Keys and Curaçao, significantly negative for Glover's Reef, and non-significant for all locations combined. For the nuclear loci, Tajima's *D* was non-significant for each sampling location and for all locations combined, with the exception of *cata* in Bimini, *cps* in Key Largo and Long Key, and *fil* in Long Key. The R_2 statistic was non-significant for all loci except *cps* (Table S2 in Appendix S1).

COI haplotypes in *C. vaginalis* were geographically restricted (Fig. 1). The 'green' haplotype was most frequent (27%) with the widest distribution, ranging from Bimini to Bocas del Toro. It was the only haplotype in Vieques and St. Croix. The 'yellow' haplotype was restricted to Central America (Glover's Reef, Utila and Roatan). The 'red' haplotype was restricted to Veracruz, Florida and Bimini. The 'blue' haplotype was common in Florida and Bimini and rare in Central America. The 'brown' haplotype occurred in Florida and Central America and half the individuals in Curaçao. The 'purple' haplotypes, found in Bocas del Toro and Utila, connected to each other but not to the main network at the 95% confidence level, indicating their genetic divergence from the other haplotypes and suggesting they represent a cryptic species found in sympatry with *C. vaginalis*. The K2P distance between the cryptic species and the other *C. vaginalis* individuals was 4% for *COI*. The cryptic species had four private nuclear alleles in three nuclear genes and shared 16 alleles with *C. vaginalis* (*cata*=1; *cir*=3; *cps*=4; *ef1a*=3; *fil*=2; *mep*=3).

The Structure analyses based on the nucDNA alone showed the best-supported number of genetic clusters was two, with a west-to-east split among the sampling locations. Florida, Glover's Reef, Utila, Roatan, and Bocas del Toro were assigned to the western cluster, and Bimini, Crooked Island, St. Croix, Vieques, and Curaçao were assigned to the eastern cluster. A small number of individuals from Florida and Bimini were also assigned to the cluster opposite their geographical position. The cryptic species from Utila and Bocas del Toro were assigned to the eastern cluster (Fig. 2a).

When the nucDNA and mtDNA were combined, the best *K* was 2 and the west-to-east pattern was recovered (Fig. 2b); however, Veracruz clustered with the west and Bocas del Toro clustered with the east, opposite their positions in the nucDNA-only analysis (Fig 2a). In the combined mtDNA and nucDNA analysis, the cryptic species from Bocas del Toro and Utila clustered in the west and east, respectively, and a small number of individuals from Florida and Bimini were assigned to the cluster opposite their geographical location (Fig. 2). The plot of delta *K* produced in the Evanno *et al.* (2005) method showed secondary peaks at $K = 7$ and $K = 10$ (Fig. 2c and Fig. S1 in Appendix S1), indicating hierarchical subdivision exists among sponge populations within the western and eastern regions. As *K* increased in the Structure analyses, the cryptic species were assigned to their own cluster (Fig. 2b, Fig. S1 in Appendix S1). When we repeated the Structure analyses excluding the cryptic species, the same west-to-east pattern was recovered and the sampling locations were assigned to the same clusters.

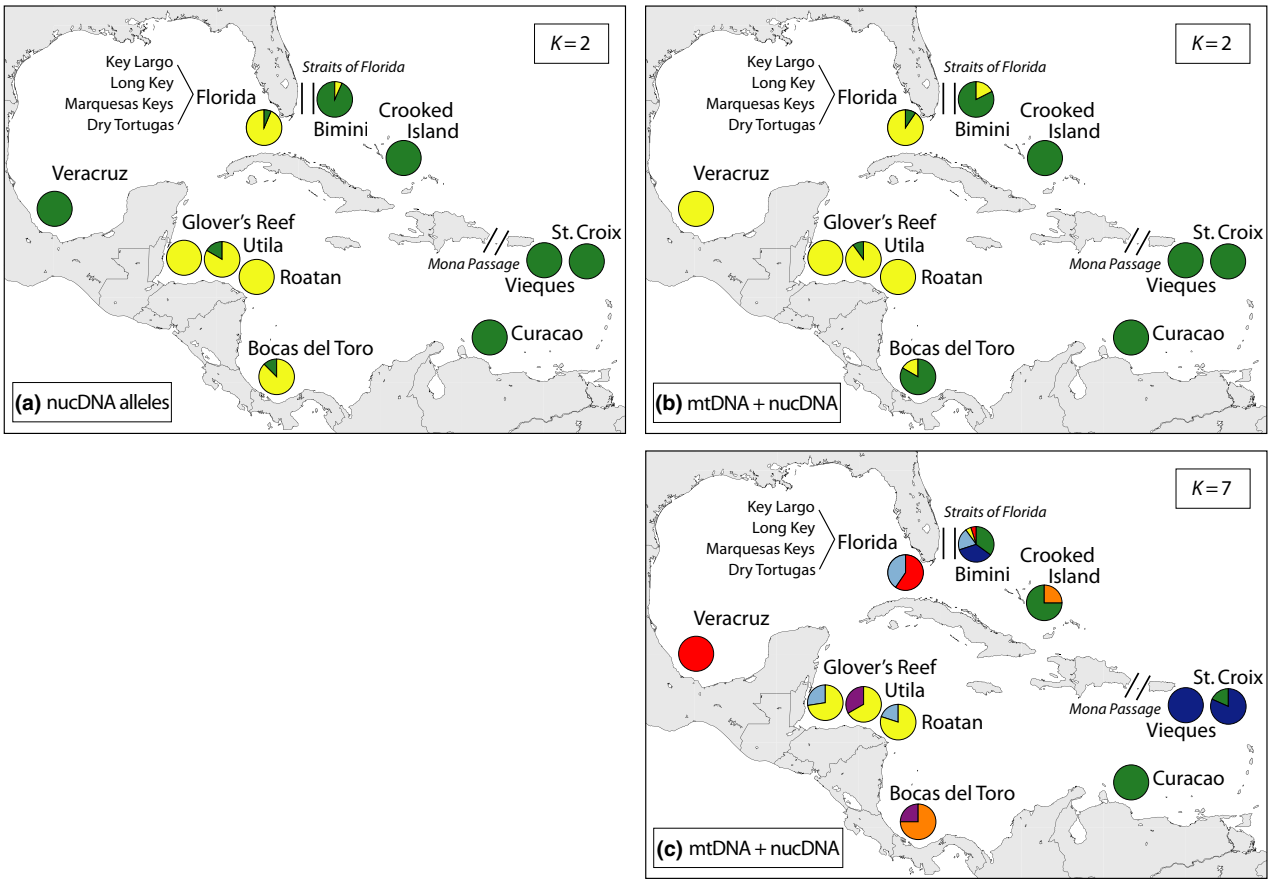

Figure 2 Population assignments of *Callyspongia vaginalis* individuals inferred from the STRUCTURE analyses and the method of Evanno *et al.* (2005). Colours represent different genetic clusters. Pie charts show which clusters occur at each sampling location with sector size proportional to the number of individuals assigned to that cluster. (a) Based on the nuclear DNA (nucDNA) only, the most likely number of genetic clusters, '*K*', was 2 with clusters divided west-to-east across the Caribbean. (b) When the mitochondrial DNA (mtDNA) and nucDNA are combined, the best *K* is again 2. (c) When the mtDNA and nucDNA were combined, the plot of delta *K* (Fig. S1 in Appendix 1) showed a secondary peak at $K = 7$, suggesting hierarchical structure within the western and eastern clusters.

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

The most frequent barrier in *C. vaginalis* (observed in four loci) occurred in the centre of the Caribbean, running north-west to south-east below Jamaica (Fig. 3a). The location of this barrier corresponds with the STRUCTURE results, which also divided sponge populations west-to-east. *Leucothoe kensleyi* and *C. vaginalis* shared a barrier separating Vieques and St. Croix from Curaçao (Fig. 3a,c), and *L. ashleyae*, *O. suensonii* and *C. vaginalis* all had barriers isolating Central American locations from the rest of the Caribbean (Fig. 3a–c). All four taxa showed a break between Florida and the Bahamas across the Florida Straits (Fig. 3a–d).

Results from our analyses indicated that gene flow across the Florida Straits was restricted to varying degrees with isolation strongest in the amphipods and weakest in the brittle star. We tested for simultaneous divergence across this region, but the results were ambiguous. The mode and mean for Ω were 0.0 and 0.190, respectively; however, 95% of the values in the approximated posterior distribution (95% quantile) were between 0.0 and 0.658. While a value of zero indicates simultaneous divergence, values above zero in the 95% quantile suggest variation around the divergence of each of the four population pairs. For ψ, the mode and mean were 1.0 and 1.7, respectively, and the 95% quantile contained all possible numbers of divergence events (1 through 4). The posterior probabilities for models with 1, 2, 3 and 4 divergence events were 0.518, 0.308, 0.126 and 0.048, respectively. Although the range of Ω values contained zero, values greater than zero, which indicate variation around the divergence times of co-distributed taxa, were contained within the distribution. For ψ, 80% of the posterior support was divided between models with one and two divergence events, preventing us from distinguishing the best model to describe divergence history for the sponge and its commensals.

DISCUSSION

We determined population subdivision in *C. vaginalis* and tested whether phylogeographical patterns in this sponge corresponded to previously documented breaks at the Florida

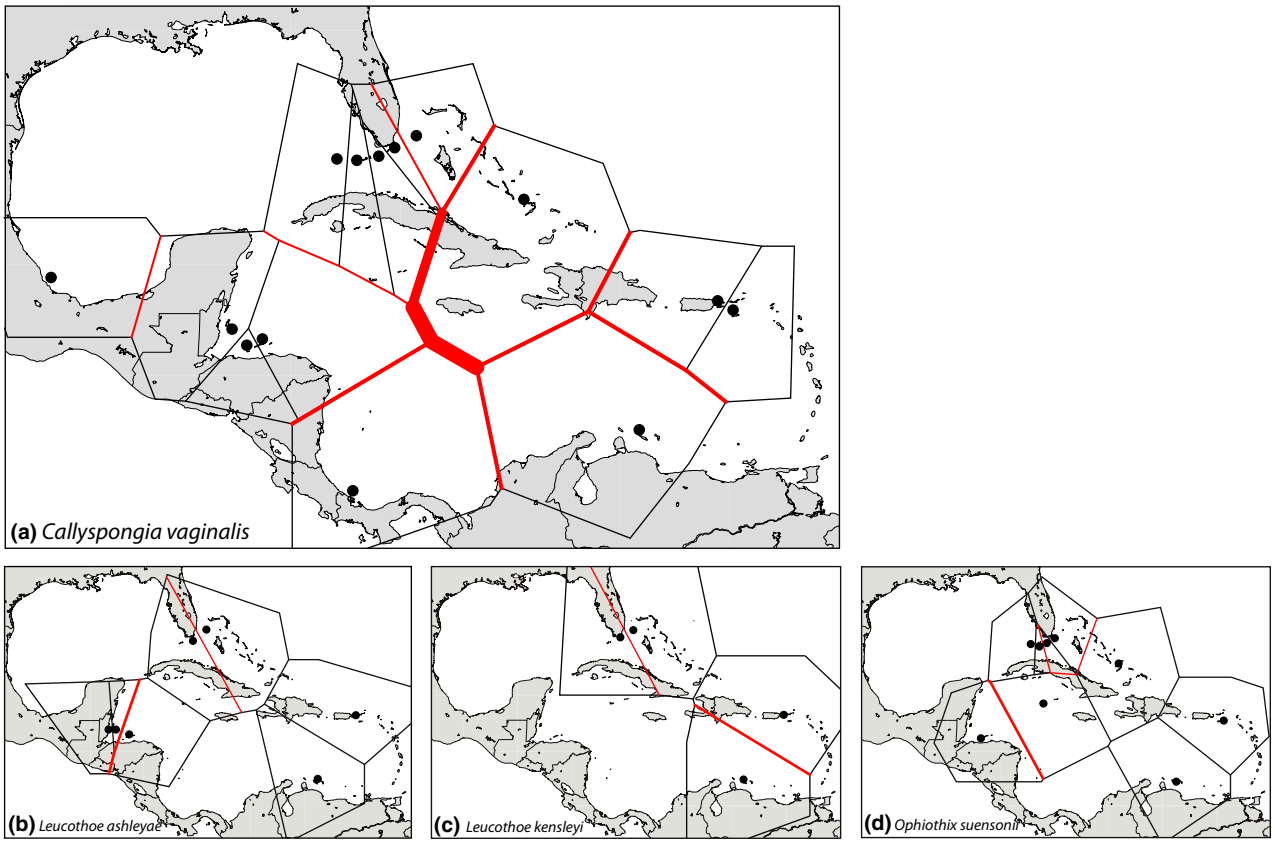

Figure 3 Barriers inferred for (a) *Callyspongia vaginalis*, (b) *Leucothoe ashleyae*, (c) *Leucothoe kensleyi* and (d) *Ophiothrix suensonii*. Circles represent sampled localities and lines represent the Voronoi diagrams. Red lines indicate the inferred barriers. (a) A composite of barriers inferred from seven loci for *C. vaginalis* where the thickness of the lines represents the number of loci that shared a particular barrier. The thickest lines represent four loci, and the thinnest lines represent one locus. (b–d) The thick and thin red lines represent the first and second strongest barrier inferred for *L. ashleyae*, *L. kensleyi* and *O. suensonii*, respectively.

Straits, Mona Passage, and Bay of Honduras. We also compared its genetic structure to structure in three of its invertebrate commensals. We found sponge populations were divided west-to-east across the Caribbean, with hierarchical structure within regions that matched known barriers. The sponge, amphipods, and brittle star shared a well-defined split across the Florida Straits, but we were unable to determine whether this break occurred simultaneously across all four taxa.

Data from *COI* and six nuclear markers showed significant population subdivision in *C. vaginalis*. These patterns are consistent with previous sponge studies employing a variety of markers, which often attribute genetic differentiation to limited larval dispersal (Blanquer *et al.*, 2009; López-Legentil & Pawlik, 2009; Blanquer & Uriz, 2010; Dailianis *et al.*, 2011; Pérez-Portela *et al.*, 2014; Chaves-Fonnegra *et al.*, 2015). Our previous work on *C. vaginalis* in Florida at a smaller spatial scale showed genomically discordant patterns of structure: *COI* haplotypes were geographically subdivided, while nuclear alleles were panmictic (DeBiasse *et al.*, 2010, 2014). Coalescent simulations and neutrality tests supported population bottlenecks and sperm-biased dispersal as possible drivers of mitonuclear discordance in *C. vaginalis* (DeBiasse *et al.*, 2014). Across the Caribbean, mitochondrial and nuclear loci had similar patterns of subdivision, suggesting spatial scale may influence concordance among loci.

We tested the hypothesis that phylogeographical barriers in *C. vaginalis* matched previously defined breaks for other coral reef taxa across the Caribbean basin. The Barrier and Structure analyses (Fig. 2, Fig. 3) indicated significant population subdivision between Florida and the Bahamas in *C. vaginalis* and its invertebrate commensals across the Florida Straits, a major barrier to gene flow for many marine species. For example, Andras *et al.* (2013) found allele frequency differences in the sea fan *Gorgonia ventalina* between Florida and the Bahamas, while its algal symbionts shared no alleles across this gap (Andras *et al.*, 2011). Two species of coral, a mussel, and a sponge also have phylogeographical breaks across the Florida Straits (Lee & Ó Foighil, 2004; Brazeau *et al.*, 2005; Baums *et al.*, 2010; Chaves-Fonnegra *et al.*, 2015). The geographical proximity of Florida and Bahamian coral reefs (~100 km) makes it unlikely that distance alone is responsible for restricting gene flow between populations in this region. Indeed, previous results showed connectivity in *C. vaginalis* (based on nucDNA) and both amphipod species (based on *COI*) along ~400 km of the shallow Florida reef

tract (Richards *et al.*, 2007; DeBiasse *et al.*, 2014), four times the distance of the Florida Straits.

This dichotomy, connectivity within Florida but structure across the Florida Straits, likely results from a complex interaction of factors, including geography, life history, and oceanography. For example, the continuous reef habitat along the Florida coastline might facilitate population connectivity via stepping stone dispersal of gametes, larvae and/or adults despite the brooding strategy of the sponge and amphipods. Amphipods have been found in detached *C. vaginalis* tubes drifting along the reef substrate (VPR, MBD personal observations) and sponge fragments can support viable sponge larvae (Maldonado & Uriz, 1999), suggesting dispersal of amphipods and sponge larvae could occur via drifting in sponge fragments (Highsmith, 1985). While the continuity of the shallow Florida reef tract likely facilitates such dispersal, deep water (~800 m), lack of intervening reef habitat, and rapid transport (3.0×10^7 m^3s^{-1}) of the Florida Current, which flows northward between Florida and the Bahamas (Baringer & Larsen, 2001), likely restrict dispersal across the Florida Straits. The pattern of connectivity along a continuous reef tract and isolation between proximal locations separated by deep water was also observed for *L. ashleyae* and the reef fish *Elacatinus lori* in the Belizean barrier reef system (Richards *et al.*, 2012; D'Aloia *et al.*, 2014). Isolation across the Florida Straits was weakest here in the brittle star, perhaps due to its broadcast spawning life history strategy, which might allow dispersal regardless of depth and currents (Sherman *et al.*, 2008). We tested whether the shared Florida Straits barrier produced simultaneous divergence in *C. vaginalis* and its commensals, but the distribution of posterior support among alternative models prevented us from confidently accepting the simultaneous divergence model. Growing evidence shows that robust population parameter estimation requires multi-locus genetic data (Heled & Drummond, 2010) and our power to test simultaneous divergence here was likely limited by the use of a single marker.

The Mona Passage between Hispaniola and Puerto Rico is a well-studied phylogeographical break for many marine taxa (Taylor & Hellberg, 2003, 2006; Baums *et al.*, 2005, 2006; Dennis *et al.*, 2005), but the broad scale pattern of population subdivision inferred from Structure did not show a phylogeographical barrier at the Mona Passage for *C. vaginalis*. Locations on either side of the Passage (Bimini, Crooked Island, Vieques and St. Croix) grouped together in the same cluster (Fig. 2a,b). However, at a finer scale, locations to the east of the Mona Passage (Vieques and St. Croix) grouped in a different cluster than locations to the west of the Passage (Crooked Island), suggesting these sites are isolated from each other across the Mona Passage (Fig. 2c). The Barrier analyses identified a break in *C. vaginalis* across Hispaniola, separating Vieques and St. Croix from Crooked Island. In contrast to the sponge, the Barrier analyses did not identify a break between locations on either side of the Mona Passage for the invertebrate commensals. Biophysical oceanographic models suggest that deep depths, complex bottom topography, and unique oceanographic features, such as small-scale eddies, restrict dispersal across this region (Baums *et al.*, 2006). Robustly identifying the precise location of the break on either side of the passage in *C. vaginalis* will require finer scale sampling across this region.

Previous research points to middle Central America (Belize and Honduras) as a region of genetic isolation and endemism in terrestrial and marine species (Briggs, 1984; Roberts *et al.*, 2002). For example, Andras *et al.* (2011, 2013) reported populations of the sea fan *Gorgonia ventalina* in Belize and Panama were strongly differentiated from the wider Caribbean, as were populations of the sea fan's zooxanthellae. The goby *Elacatinus oceanops* was monophyletic for mitochondrial and nuclear markers between Florida and Belize (Taylor & Hellberg, 2006), and Colin (2002) described *E. lori* as endemic to Honduras and Belize. Previous results and analyses we performed here (Fig. 2, Fig. 3) showed that Central American populations of *C. vaginalis*, *L. ashleyae* and *O. suensonii* were genetically isolated from the Caribbean despite differences in dispersal strategy (Richards *et al.*, 2012, 2015). Isolation of marine taxa in Central America is likely due in part to oceanographic currents. Gyres in the Gulf of Honduras (Heyman & Kjerfve, 2000) and off the coast of Panama and Colombia (Richardson, 2005) may retain dispersers and prevent migrants from other locations. Indeed, a biophysical model predicting larval fish dispersal found self-recruitment was higher in Central America than elsewhere in the Caribbean (Cowen *et al.*, 2006). Potential mechanism that may isolate populations *within* the Bay of Honduras include freshwater outflow from rivers (Chérubin *et al.*, 2008) and deep water between reefs. For example, geographically proximal populations of *L. ashleyae* and *E. lori* separated by deep water in Belize were shown to be genetically divergent (Richards *et al.*, 2012; D'Aloia *et al.*, 2014).

Although not identified *a priori* in this study, our data suggested a break between Bocas del Toro and Curaçao in *C. vaginalis*. Other studies have found differentiation between Bocas del Toro and Curaçao for coral reef taxa (Baums *et al.*, 2005; Vollmer & Palumbi, 2007; Hemond & Vollmer, 2010; Andras *et al.*, 2011, 2013). The formation of Santa Marta Massif of Colombia, a mountainous feature whose tectonic displacement northward into the Caribbean in the early Pleistocene disrupted habitat along the continental margin, might have contributed to breaks across this region. For example, Betancur-R *et al.* (2010) found that lineages of the marine catfish *Cathorops* were reciprocally monophyletic to either side of the Santa Marta Massif over only 150 km. Additionally, the narrow coastal shelf, cold water upwelling, and strong offshore currents (Cowen *et al.*, 2006) in this region, combined with freshwater outflow from the Magdalena River, likely reduce connectivity between Bocas del Toro and Curaçao.

We found that the cluster affiliation of Bocas del Toro changed depending on whether the mtDNA was included in the Structure analyses (Fig 2). Mitochondrial introgression

would explain such a pattern (Nydam & Harrison, 2011). The introgression of an eastern mtDNA haplotype into a western nucDNA genetic background in sponges from Bocas del Toro is consistent with the alternative cluster assignments of this population and suggests some genetic mixing along the northern South American coastline at the break between Bocas del Toro and Curaçao, but more data are needed to test these hypotheses explicitly.

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). Phylogeographical 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 and 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.

ACKNOWLEDGEMENTS

We thank Michael Dawson and three anonymous reviewers whose constructive comments improved the article. This research was supported by a Louisiana Board of Regents fellowship and a Sigma Xi grant to MBD, the NOAA Center for Sponsored Coastal Ocean Science award #NA04NOS4260065 via the National Coral Reef Institute to MSS, the Guy Harvey Research Institute operational funds to MSS, NSF grant OCE-0550270 to MEH and Iliana Baums and funds from the LSU Department of Biological Sciences.

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SUPPORTING INFORMATION

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

Appendix S1 Supplementary tables and one figure.

DATA ACCESSIBILITY

Sequences for *C. vaginalis* generated for this study are available from the European Nucleotide Archive under accession numbers LK026325-LK026602 (*COI*) and LK026931-LK028492, LT556086 - LT556285 (nuclear loci).

BIOSKETCH

MBD's research examines the mechanisms responsible for the distribution of genetic and phenotypic variation within and among species of marine invertebrates, particularly the Porifera.

Author contributions: M.B.D., V.P.R., M.S.S. and M.E.H. designed the project; V.P.R. and M.B.D. collected the tissue samples; M.B.D. generated and analysed the genetic data; M.B.D. and M.E.H. wrote the manuscript; all authors commented on and approved the final version of the manuscript.

Editor: Michael Dawson