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REPORT

Genetic assessment of connectivity in the common reef sponge, *Callyspongia vaginalis* (Demospongiae: Haplosclerida) reveals high population structure along the Florida reef tract

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Abstract The genetic population structure of the common branching vase sponge, *Callyspongia vaginalis*, was determined along the entire length (465 km) of the Florida reef system from Palm Beach to the Dry Tortugas based on sequences of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene. Populations of *C. vaginalis* were highly structured (overall $\Phi_{ST} = 0.33$), in some cases over distances as small as tens of kilometers. However, non-significant pairwise Φ_{ST} values were also found between a few relatively distant sampling sites suggesting that some long distance larval dispersal may occur via ocean currents or transport in sponge fragments along continuous, shallow coastlines. Indeed, sufficient gene flow appears to occur along the Florida reef tract to obscure a signal of isolation by distance, but not to homogenize COI haplotype frequencies. The strong genetic differentiation among most of the sampling locations suggests that recruitment in this species is largely local source-driven, pointing to the importance of further elucidating general connectivity patterns along the Florida reef tract to guide the spatial scale of management efforts.

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Introduction

An often recommended and implemented strategy for reducing and reversing coral reef degradation is the establishment of marine protected/reserve areas (MPAs). An important criterion to inform the design and assess the effectiveness of MPAs is the level of demographic connection within and among coral reef sections (Palumbi 2003; Almany et al. 2009; Planes et al. 2009). The east coast of Florida, USA, is a densely populated and developed region that also contains the majority of continental US coral reefs, much of it in an advanced state of impairment (Causey et al. 2002; Pandolfi et al. 2005). Less than 5% of Florida's reefs are currently protected under a no-take MPA designation, and there are increasing calls to strategically expand these areas to reduce continued threats to reef health (Pandolfi et al. 2005).

Despite the considerable ecosystem value and importance of the Florida coral reef tract as a socioeconomic resource (Causey 2008), there has been surprisingly little assessment of the detailed dynamics of connectivity within this degrading ecosystem. Since many biological and physical factors influence connectivity patterns among coral reefs (Galindo et al. 2006; Taylor and Hellberg 2006; Underwood et al. 2007), deriving a general picture of connectivity to inform conservation and management efforts will require information from diverse species displaying various life history strategies. Previous work examining detailed connectivity patterns among multiple sampling sites within the Florida reef tract has

characterized the genetic population structure of three invertebrate species (a passively dispersing brittle star and two brooding amphipods) living commensally within the branching vase sponge *Callyspongia vaginalis* (Richards et al. 2007). Despite their different reproductive strategies, all three species showed extensive connectivity along the 355 km of reef tract from Palm Beach to Key West, Florida (Fig. 1 illustrates this stretch of coastline).

To further elucidate patterns of genetic connectivity and biodiversity within the Florida reef tract, genetic differentiation patterns were examined in the sponge *C. vaginalis*, which is common in Florida and Caribbean reefs and serves as host to the commensal species studied by Richards et al. (2007). Sponges are among the most diverse organisms on coral reefs (Diaz and Rützler 2001) with some data indicating sponge biomass may surpass that of corals and algae (Rützler 1978). Sponges also play major ecological roles in promoting reef species richness by providing refugia for many commensal invertebrates, particularly during critical juvenile or reproductive life history phases (Ribeiro et al. 2003), and harbor a substantial biomass of diverse microbial endosymbionts (Corredor et al. 1988; Diaz and Ward 1997; Lopez et al. 1999), many of which produce secondary metabolites of ecological importance (Taylor et al. 2007). Although an essential and conspicuous component of coral reef communities, relatively few studies exist on sponge connectivity and population genetic structure generally (Duran et al. 2004a, b, c; Bentlage and Wörheide 2007; Wörheide et al. 2008; López-Legentil and Pawlik 2009), and the authors are unaware of studies using sponges as models to examine connectivity within the Florida reef tract system.

Callyspongia vaginalis is believed to brood parenchymellar larvae that are released in an advanced stage of development (Lindquist et al. 1997; Maldonado 2006). As such, *C. vaginalis* larvae are assumed to be competent to settle a short time after release and therefore have low dispersal capabilities (Lindquist et al. 1997; Maldonado 2006). Based on this presumed low dispersal, it was hypothesized that *C. vaginalis* samples collected along the entire Florida reef tract (465 km from Palm Beach to the Dry Tortugas; Fig. 1) would be genetically structured. However, increasing evidence has suggested that reproductive strategy is not always a reliable predictor of connectivity (Barber et al. 2000; Sponer and Roy 2002; Levin 2006; Richards et al. 2007).

A handful of studies utilizing the DNA sequence of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene for sponge population genetic studies have suggested this gene may be insufficiently variable to be useful as a population marker (Duran et al. 2004b; Wörheide 2006; Park et al. 2007; however, see López-Legentil and Pawlik 2009). Preliminary studies conducted by us on *C. vaginalis* indicated otherwise, however, and given the very few studies

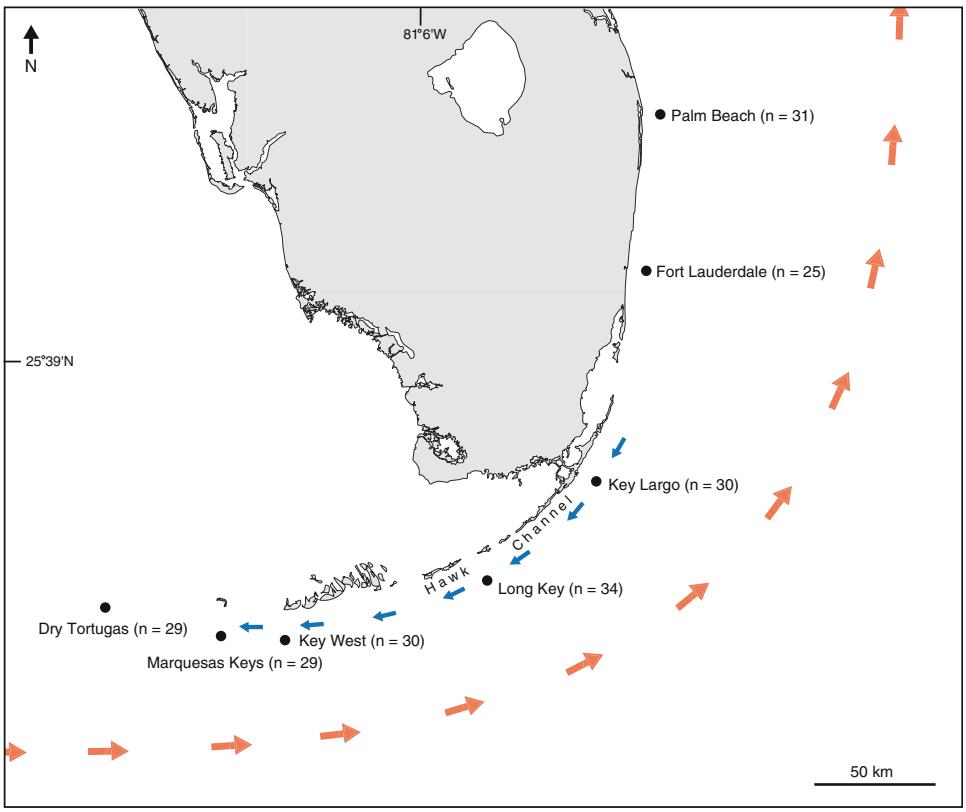

Fig. 1 Map showing sampling sites of *Callyspongia vaginalis* along the Florida reef tract and the location of Hawk Channel. *Red arrows* represent the approximate location and direction of Florida current while *blue arrows* represent the counter current that runs along Hawk Channel. Sponge sample sizes for each location are listed in parentheses. Map created with OMC (http://www.aquarius.geomar.de/omc)

on this issue, the utility of this gene for revealing population differentiation was further explored.

Materials and methods

Sampling sites and collections

A total of 208 *C. vaginalis* samples from seven geographic locations along the southeast coast of Florida and the Florida Keys (Fig. 1) were collected. An ~2-cm section of tissue was collected from one tube in each sponge colony sampled. Samples were stored in 95% ethanol at room temperature before DNA extraction.

Polymerase chain reaction (PCR) and sequencing

All macroinvertebrate commensals were removed from each sponge sample under a dissecting microscope prior to DNA extraction. Genomic DNA was isolated from approximately 25 mg of clean sponge tissue using the DNeasy Tissue Kit (QIAGEN Inc.) and stored at −20°C until needed. The universal primer pair LCO1490 and HCO2198 (Folmer et al. 1994) was used to initially amplify and sequence approximately 670 base pairs at the 5′ end of the COI gene. To avoid possible nonspecific amplification of DNA from microsymbionts living on and within the sponge, the following internal *C. vaginalis* specific primers were designed for subsequent COI gene PCR amplification and sequencing: CvaCOIF11 (5′-GGCATTTAGTATGTTAATCAGATTGGA-3′) and CvaCOIR7 (5′-GGGTGACCAAAAAATCAAAATAAATGTTG-3′). The poriferan origin of the sequences obtained using the *C. vaginalis* primers was confirmed by using the BLAST search engine in GenBank.

Amplifications were conducted in 50-μl reactions consisting of 1 μl of extracted genomic DNA (unquantified) template diluted in 99 μl of water to reduce the effect of secondary metabolites that can act as PCR inhibitors, 5 μl of 10× PCR buffer, 50 μM of each dNTP, 0.25 μM of each primer, and 0.75–1.75 U of HotStar Taq™ DNA polymerase (QIAGEN Inc.). The remaining volume was made up with water. Amplification reactions were performed in a Mastercycler Gradient (Eppendorf Inc.) thermal cycler under the following conditions: an initial denaturation step of 95°C for 15 min, followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, and a final extension step at 72°C for 5 min. PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN Inc.) and sequenced in the forward and reverse directions using standard protocols on an ABI 3130 automated genetic analyzer. Forward and reverse sequences were aligned and edited using the program GENEDOC v. 2.6.02 (Nicholas and Nicholas 1997). Sequences were translated in GENEDOC to check for correct coding of invertebrate mtDNA amino acids, aberrant start/stop codons, and possible nuclear pseudogene amplification.

Data analysis

The program DnaSP v. 4.10 (Rozas et al. 2003) was used to calculate molecular diversity indices within and among all sampling locations. Genetic population structure was estimated using an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) implemented in ARLEQUIN v. 2.0 (Schneider et al. 2000). Pairwise Φ_{ST} values were calculated to estimate population differentiation between each pair of sampling sites and associated *P* values were adjusted using sequential Bonferroni techniques (Rice 1989). In order to further investigate the partitioning of genetic variation within the Florida reef tract, sampling sites were grouped into two regions: the northern region consisted of the continuous coastline connecting Palm Beach and Fort Lauderdale sites, and the southern region consisted of the island sites of the Florida Keys (Key Largo to Key West), the Marquesas Keys, and the Dry Tortugas. This northern vs. southern region comparison was undertaken based on the fact that a haplotype common in southern sites was absent in the northern sites (see Fig. 3, haplotype II), and the recognition by Jaap and Hallock (1990) of the southern portion of the Florida reef tract (Florida Keys and islands south) as more biodiverse than the northern parts of the reef tract. A hierarchical AMOVA was then used to estimate the variance among haplotypes (1) within sampling sites (Φ_{ST}), (2) among sampling sites within regions (Φ_{SC}), and (3) between the northern and southern regions (Φ_{CT}).

To determine if sponges from adjacent locations were more genetically connected to each other than they were to sponges from more distant sampling sites, the isolation by distance program IBD v.1.52 (Bohonak 2002) was used to test for a correlation between pairwise Φ_{ST} values and geographic distances among sampling locations. The geographic distance between any two sampling locations was calculated using Google Earth v. 4.0.2416 as the shortest distance by sea. A network displaying the genetic relationships among different *C. vaginalis* haplotypes, and geography was constructed using the statistical parsimony algorithm of Templeton et al. (1992) as implemented by the software package TCS v.1.21 (Clement et al. 2000). The analysis was conducted using the default settings and provided the most parsimonious connections among haplotypes at the 95% confidence level.

An initial test of the feasibility of estimating migration rates and direction was conducted between a subset of paired sampling locations using the Bayesian framework in the program MIGRATE version 2.1.3 (Beerli 2004, 2006;

Beerli and Felsenstein 2001); this approach allows the setting of prior distributions which generally promotes run convergence in data sets with low sequence variation. For each pairwise comparison, the parameters Θ and M from six preliminary runs with uniform prior distributions (three long chains, 1,000,000 steps sampled, with a burn in of 10,000) were averaged and used to set the boundaries for the exponential prior distributions for the final run. The final run consisted of one long chain, 20,000,000 steps sampled, a burn in of 10,000, and used an adaptive heating scheme with start temperatures of 1.0, 1.2, 1.5, and 3.0.

Results

Sequencing 511 base pairs of the COI gene in 208 *C. vaginalis* individuals from seven sampling locations revealed 20 polymorphic sites and 11 haplotypes (GenBank accession numbers GQ304527-GQ304734). No insertions, deletions, or stop codons were encountered in the sequences. Haplotype diversity (h) within sampling locations ranged from 0.181 (Palm Beach) to 0.584 (Marquesas Keys) with an overall value of 0.650. Nucleotide diversity (π) within sampling locations ranged from 0.0042 (Palm Beach) to 0.0132 (Fort Lauderdale) with an overall value of 0.0132 (Table 1). Haplotype and nucleotide diversity in specimens from Palm Beach were approximately half that found at other sampling sites.

The AMOVA resulted in an overall Φ_{ST} value of 0.332 ($P < 0.0001$) (Table 2). Of 21 total pairwise comparisons, 12 were significantly structured. The highest pairwise Φ_{ST} occurred between Palm Beach and Key West (0.704, $P < 0.0001$), while the lowest value was nonsignificant, occurring between the Marquesas Keys and Fort Lauderdale (−0.021, $P = 0.607$). High pairwise Φ_{ST} values over short geographic distances (35 and 52 km) were observed between the Marquesas Keys and Key West, and Long Key and Key Largo, respectively. Conversely, nonsignificant pairwise Φ_{ST} values over large geographic distances (218 and 393 km) were also observed between Long Key and the Dry Tortugas, and Palm Beach and the Marquesas Keys, respectively. While the hierarchical AMOVA indicated there was significant genetic differentiation within sampling sites ($\Phi_{ST} = 0.395$, $P < 0.0001$) and among sampling sites within regions ($\Phi_{SC} = 0.267$, $P < 0.0001$), there was no significant structure between the northern and southern regions of the reef tract as defined in this study ($\Phi_{CT} = 0.174$, $P = 0.185$) (Table 3). Results of the test for isolation by distance were nonsignificant ($r^2 = 0.035$, $P = 0.164$, 10,000 randomizations).

The TCS analysis generated a nine step statistical parsimony network connecting all 11 haplotypes (Fig. 2). There was one ambiguous loop involving haplotypes II, IV, IX, and X. Three major haplotypes dominated the data set of 208 sequences with frequencies of 90 (haplotype I), 78 (haplotype II), and 30 (haplotype III). Haplotypes I and III were distributed throughout the length of the Florida reef tract, but haplotype II only occurred in the southern portion of the reef tract (Florida Keys, Marquesas Keys, and the Dry Tortugas) (Figs. 2 and 3). Palm Beach, Fort Lauderdale, and Key Largo were dominated by haplotype I, while Long Key, Key West, and the Dry Tortugas were dominated by haplotype II. Interestingly, in contrast to its neighboring sampling sites (i.e., the Dry Tortugas and Key West), the Marquesas Keys were dominated by haplotype I and had a very similar overall haplotype composition to Key Largo (Figs. 2 and 3).

The MIGRATE analysis conducted on the subset of sampling sites resulted in large confidence intervals for estimates of the number of migrants per generation and inconsistent inferences of gene flow direction, likely due to the relatively low sequence variability in the data set.

Table 1 Genetic diversity indices for *Callyspongia vaginalis* in Florida

Location	n	H	S	h	π
Palm Beach	31	2	12	0.181	0.0042
Fort Lauderdale	25	5	16	0.603	0.0132
Key Largo	30	4	16	0.552	0.0107
Long Key	34	4	16	0.446	0.0079
Key West	30	2	12	0.331	0.0078
Marquesas Keys	29	6	20	0.584	0.0120
Dry Tortugas	29	3	16	0.478	0.0099
All Populations	208	11	20	0.650	0.0132

n sample size, H number of haplotypes, S number of segregating sites, h haplotype diversity, π nucleotide diversity

Table 2 *Callyspongia vaginalis* pairwise Φ_{ST} values among Florida sampling locations

	PLB	FTL	KLG	LNK	KWT	MRQ
FTL	0.170					
KLG	0.105	0.046				
LNK	**0.641**	**0.390**	**0.350**			
KWT	**0.704**	**0.428**	**0.439**	0.035		
MRQ	0.089	−0.021	0.017	**0.420**	**0.474**	
DRT	**0.556**	**0.298**	**0.249**	−0.018	0.066	**0.327**

PLB Palm Beach, *FTL* Fort Lauderdale, *KLG* Key Largo, *LNK* Long Key, *KWT* Key West, *MRQ* Marquesas Keys, *DRT* Dry Tortugas

Values significant after Bonferroni correction are indicated in bold

Overall Φ_{ST}= 0.33, $P < 0.0001$

Table 3 Hierarchical analysis of molecular variance (AMOVA) based on cytochrome *c* oxidase I (COI) sequences of *Callyspongia vaginalis*

Source of variation	% Variance	Φ Statistic	*P* value
Between northern and southern regions	17.44	$\Phi_{CT} = 0.174$	0.143
Among sampling sites within regions	22.08	$\Phi_{SC} = \mathbf{0.267}$	<0.00001
Within sampling sites	60.47	$\Phi_{ST} = \mathbf{0.395}$	<0.00001

Northern region includes Palm Beach and Fort Lauderdale. Southern region includes Key Largo, Long Key, Key West, the Marquesas Keys, and the Dry Tortugas

Significant values ($P < 0.05$) are indicated in bold

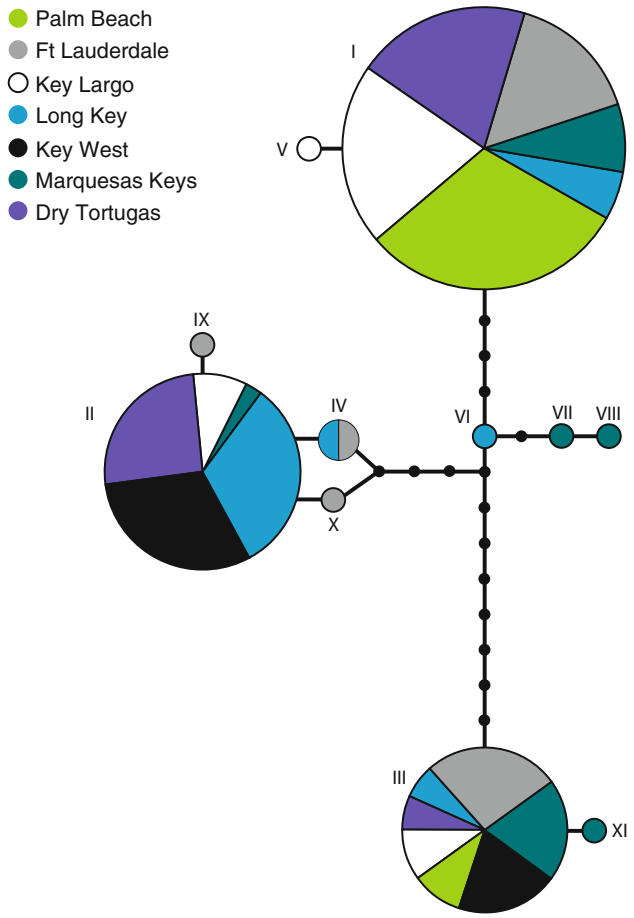

Fig. 2 Unrooted statistical parsimony network for *Callyspongia vaginalis*. *Circles* represent individual haplotypes with circle size proportional to total frequency of occurrence. *Colors* represent sampling sites and the area of each sector or circle is proportional to haplotype frequency at that site. Haplotypes are labeled with *Roman numerals*

Discussion

Genetic diversity in the *Callyspongia vaginalis* COI gene

Recent sponge genetic studies have suggested low levels of intraspecific sequence variation in the COI gene (Duran et al. 2004c; Wörheide 2006; Park et al. 2007). These results have raised questions about the utility of the COI gene (and possibly other mitochondrial genes) for assessing population differentiation in sponges. However, discounting the suitability of mitochondrial genes as population markers for all sponges based on these few studies may be premature, as our results showed low but sufficient genetic variation in the *C. vaginalis* COI gene to reveal population differentiation over relatively short geographic distances. Duran and Rützler (2006) also used COI to determine intraspecific relationships for the sponge C*hondrilla nucula* and reported a total haplotype number and nucleotide diversity ($H = 12$, $\pi = 0.0025$, respectively) similar to those observed in *C. vaginalis*. López-Legentil and Pawlik (2009) have also demonstrated the utility of a different section (I3-M11) of the COI gene (Erpenbeck et al. 2006) for detecting population structure in the giant barrel sponge, *Xestospongia muta*. Although the COI gene may show relatively low intra-specific variation overall, these findings and its ease of amplification keep it a potentially useful locus to investigate as part of the marker repertoire for sponge population studies.

Statistical parsimony analysis

The TCS network indicated that frequencies of the 11 haplotypes found in the 208 *C. vaginalis* individuals were highly variable: three haplotypes dominated the data set, while the remaining eight haplotypes were infrequent. Population dynamics and the reproductive strategy of *C. vaginalis* may have contributed to this pattern. For example, sponge communities can experience rapid declines in diversity and abundance due to predation, storms, and disease (Wulff 2006). However, sponges are also able to quickly recolonize bare areas after disturbance events (Wulff 1991). Furthermore, Maldonado (2006) suggested it is difficult to reconcile the abundance of *C. vaginalis* throughout its range from its modest larval output (see Lindquist et al. 1997) and that population growth may also be accomplished by fragmentation, an important reproductive strategy in many species of branching sponges (Wulff 1991). Although little is known about the prevalence and role of predation and disease as demographic

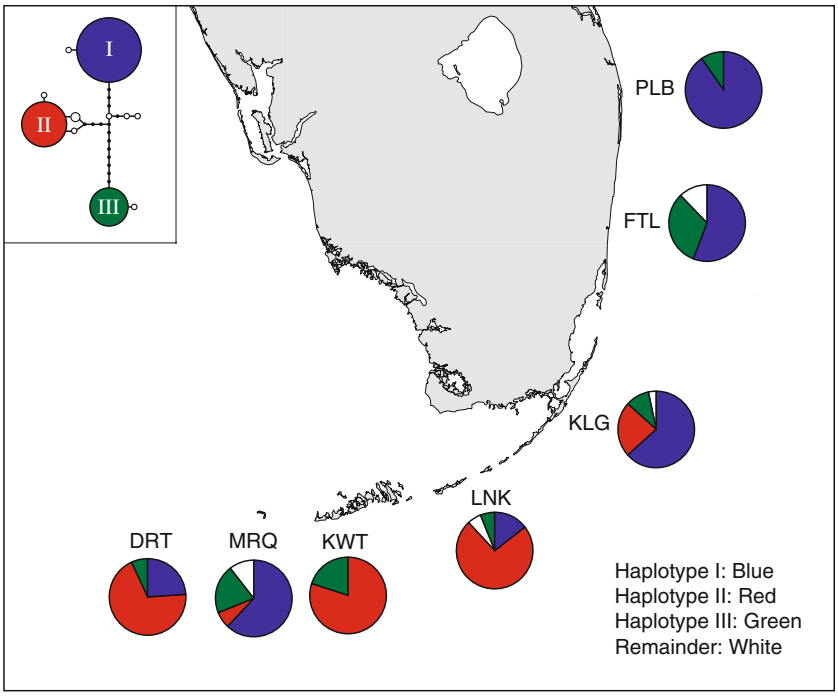

Fig. 3 Map showing the distribution of the *Callyspongia vaginalis* cytochrome *c* oxidase I haplotypes along the Florida reef tract. The three dominant haplotypes, I, II, and III, are represented by the colors *blue*, *red*, and *green*, respectively. Less frequent haplotypes IV–XI are represented in *white*. The area of each sector is proportional to haplotype frequency at each site. *Inset* shows TCS haplotype network with dominant haplotypes I, II, and III represented by the colors *blue*, *red*, and *green*, respectively

influences on *C. vaginalis*, storms are relatively frequent off the Florida coast. The numerical dominance of haplotypes I, II, and III in our data set may be the result of storm-induced declines followed by rapid re-colonization of the Florida reef tract by asexual reproduction (mediated by fragmentation) after such events.

Connectivity in *Callyspongia vaginalis*

Many studies have investigated the phylogeography of continuously distributed marine species along the Florida coast, and there is good evidence for phylogeographic breaks at Cape Canaveral on the mid-Florida Atlantic coast (Saunders et al. 1986; Reeb and Avise 1990; Collin 2001) and between the Gulf and Atlantic coasts of Florida (Felder and Staton 1994; Young et al. 2002; Lee and Foighil 2004; Matthews 2006). A few studies have examined population structure of marine species within the Florida Keys (Lacson et al. 1989, Lacson and Morizot 1991; Kirk et al. 2009); however, with the exception of Richards et al. (2007), there are no published studies elucidating genetic connectivity within the entire Florida coral reef ecosystem at a fine spatial scale.

In contrast to the extensive connectivity found by Richards et al. (2007) using the commensal brooding amphipods and broadcast spawning brittle star as models, populations of their host sponge *C. vaginalis* were highly structured along the Florida reef tract, in some cases over small geographic distances (tens of kilometers). This finding for *C. vaginalis* is consistent with several other studies that have found low connectivity in sponges. For example, Duran et al. (2004a, b, c) observed significant structure over similarly short distances in the Mediterranean sponge *Crambe crambe* using several genetic markers. Similarly, Blanquer et al. (2009) also found strong genetic structure over small scales ($\sim$100 m) in the encrusting sponge, *Scopalina lophyropoda*. López-Legentil and Pawlik (2009) reported significant genetic structuring among populations of *X. muta* among Florida, Bahamas, and Belize sampling sites. Deep genetic divergences in sponge populations (albeit over longer distances) in the Indo-Pacific have also been recently reported (Whalen et al. 2005; Wörheide et al. 2002, 2008). These studies suggest that sponge populations in general may prove to be quite structured, possibly a function of their assumed limited larval dispersal capabilities (Wörheide et al. 2005). However, since very few studies have directly examined the dispersal ability of sponge larvae, variability in dispersal distances is likely to emerge (López-Legentil and Pawlik 2009). In this context, the number of studies investigating sponge connectivity with DNA markers remains far too limited to draw broad conclusions about their genetic structuring patterns (Bentlage and Wörheide 2007).

It is expected for organisms with restricted dispersal capabilities that genetic differentiation will increase as geographic distance among populations increases (Wright 1943). Although the dispersal ability of *C. vaginalis* via larvae is presumably limited, no significant pattern of isolation by distance was detected. Slatkin (1993)

suggested the absence of isolation by distance may be caused by a recent colonization event or by long distance dispersal. Additional work characterizing the population structure of *C. vaginalis* throughout its range which includes the Bahamas and Caribbean is needed to confirm or refute the possibility that this sponge has recently colonized Florida reefs. An alternative explanation for the lack of isolation by distance is that *C. vaginalis* is capable of some long distance movements via its larvae and/or asexual fragmentation. This possibility is consistent with the results of the AMOVA indicating that a few pairwise Φ_{ST} values between distant sampling locations (e.g., Palm Beach and Marquesas Keys; Table 2) were nonsignificant. Long distance dispersal has been shown to negate a pattern of isolation by distance in other marine organisms (Hellberg 1996; Kyle and Boulding 2000; Teske et al. 2005).

The processes that allow long distance dispersal to obscure a pattern of isolation by distance while retaining strong genetic differentiation among populations along the Florida reef tract are likely complex. The majority of *C. vaginalis* larvae presumably settle within hours (precompetency periods after expulsion from the parental sponge in *C. vaginalis* are unknown), as is believed to occur in many sponges (Maldonado 2006). However, it is not unreasonable to expect that some larvae may be transported longer distances by the complex currents around the Florida peninsula (Lee and Williams 1999; Yeung and Lee 2002). Indeed, in a larval tracking experiment on another common, sympatric, haplosclerid Florida reef sponge (*Niphates digitalis*), 24 percent of the mobile larvae released underwater moved up into the water column (Lindquist et al. 1997), suggesting that some larval transport via currents is possible. Transport by Florida reef tract currents was also proposed to explain the complex gene flow patterns observed in the passively dispersing brittle star *Ophiothrix lineata* (Richards et al. 2007), a commensal in *C. vaginalis*. The fact that *C. vaginalis* larvae are chemically defended and unpalatable to common Caribbean reef fish (Lindquist and Hay 1996) should make them less susceptible to predation, facilitating their survival during extended transport periods in the water column.

Fragmentation is also an important method of reproduction and dispersal in many branching sponge species (Wulff 1991; Maldonado 2006), and strong storms can detach and transport individual tubes or sponge fragments from branching colonies (Wulff 1985, 1995). Moreover, even very small sponge fragments can support viable larvae (Maldonado and Uriz 1999). Lindquist et al. (1997) reported observing larvae in *C. vaginalis'* brooding chambers in the Florida Keys, thus making it likely that some long distance larval dispersal is facilitated by their transport in sponge fragments before the larvae are released (Maldonado and Uriz 1999). Transport inside sponge fragments was also suggested by Richards et al. (2007) as a mechanism for long distance dispersal of the brooding amphipods living commensally within *C. vaginalis*.

Statistically robust estimates of gene flow direction could not be obtained using MIGRATE due to lack of run convergence, presumably due low variability in the data set. However, the frequency and distribution of haplotypes along the Florida reef tract, particularly the shared dominance of haplotype I in the Marquesas Keys and the northern reef locations (Palm Beach. Ft. Lauderdale and Key Largo), may be illustrative on this issue. If it is reasonably assumed that the more extensive geographic distribution and much higher frequency of haplotype I in the northern reaches of the reef tract reflect its "ancestral" range, its more sporadic occurrence in southern portion of the reef tract is suggestive of gene flow in *C. vaginalis* occurring in the north to south direction. The strong and complex currents surrounding the Florida peninsula include a well-studied system of counter currents that runs north to south through Hawk Channel (Fig. 1) west of the dominant Florida Current (Lee and Williams 1999; Yeung and Lee 2002). These counter currents may promote southerly biased gene flow among coral reef taxa in the Florida Keys, and were proposed as an explanation for the strong north to south gene flow pattern documented for the *C. vaginalis* commensal brittle star *O. lineata* (Richards et al. 2007).

The findings presented here expand the currently very limited baseline information on connectivity patterns in the Florida reef tract, an ecosystem in urgent need of additional conservation and management intervention. The *C. vaginalis* populations along the Florida reef tract are mostly genetically differentiated, displaying low overall connectivity. The significant Φ_{ST} values for most pairwise sampling locations along the Florida reef tract and overall for *C. vaginalis* suggests that the majority of larval movements are short distance, but with long distance dispersal occurring frequently enough to obscure a pattern of isolation by distance, but not to homogenize haplotype frequencies among populations. The evidence for multiple management units with largely local source-driven recruitment in *C. vaginalis* points to the importance of further elucidating general connectivity patterns along the Florida reef tract. This information will be essential to gauge whether more local-scale management efforts are necessary to prevent further declines of a unique, high-latitude reef ecosystem.

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