Coral Reefs
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REPORT

Patterns of population structure and dispersal in the long-lived "redwood" of the coral reef, the giant barrel sponge (*Xestospongia muta*)

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Abstract Sponges are one of the dominant fauna on Florida and Caribbean coral reefs, with species diversity often exceeding that of scleractinian corals. Despite the key role of sponges as structural components, habitat providers, and nutrient recyclers in reef ecosystems, their dispersal dynamics are little understood. We used ten microsatellite markers to study the population structure and dispersal patterns of a prominent reef species, the giant barrel sponge (*Xestospongia muta*), the long-lived "redwood" of the reef, throughout Florida and the Caribbean. *F*-statistics, exact tests of population differentiation, and Bayesian multi-locus genotype analyses revealed high levels of overall genetic partitioning ($F_{ST} = 0.12$, $P = 0.001$) and grouped 363 individuals collected from the Bahamas, Honduras, US Virgin Islands, Key Largo (Florida), and the remainder of the Florida reef tract into at minimum five genetic clusters ($K = 5$). Exact tests, however, revealed further differentiation, grouping sponges sampled from five locations across the Florida reef tract (~250 km) into three populations, suggesting a total of six genetic populations across the eight locations sampled. Assignment tests showed dispersal over ecological timescales to be limited to relatively short distances, as the only migration detected among populations was within the Florida reef tract. Consequently, populations of this major coral reef benthic constituent appear largely self-recruiting. A combination of levels of genetic differentiation, genetic distance, and assignment tests support the important role of the Caribbean and Florida currents in shaping patterns of contemporary and historical gene flow in this widespread coral reef species.

Keywords Marine sponge · Microsatellite · Genetic connectivity · *Xestospongia muta* · Caribbean

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Introduction

An important component of understanding coral reef ecosystem dynamics is the extent to which populations are genetically connected by larval dispersal, as this information enables the delineation of population boundaries and the identification of small, genetically isolated populations (Hellberg 2007; Jones et al. 2007). Predominately self-seeding, these populations should be a conservation priority as they receive little genetic input from more distant populations, rendering them less able to adapt to rapid environmental impacts or recover from anthropogenic physical damage. Consequently, data on reef connectivity are essential for determining the appropriate scale of reef management and its incorporation into conservation

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strategies should assist their long-term success (Thorrold et al. 2002; Palumbi 2003; Jones et al. 2009).

Sponges remain among the least studied animals despite representing one of the five major animal clades (Porifera), occupying a key position in early animal evolution, and harboring what is likely to be vastly underappreciated biodiversity (Dunn et al. 2015). Sponges are among the most prominent of coral reef taxa and consequently represent an important component of reef ecosystems (Diaz and Rützler 2001). This is particularly evident on Florida and Caribbean reefs where they are one of the dominant fauna, with estimates of species diversity and biomass exceeding that of reef-building corals (Rützler 1978; Diaz and Rützler 2001; Moyer et al. 2003). Sponges also play key ecological roles by providing habitat for a diverse array of invertebrate and microbial taxa, with microbial symbioses likely involved in primary production and the nitrogen cycle (Rützler 1978; Bell 2008; Webster and Taylor 2012). Other important ecological interactions include the impact sponges have on the carbonate reef framework and the effect of sponge water filtering, which, given the large biomass, likely plays a significant role in nutrient and carbon cycling (Diaz and Rützler 2001; Bell 2008; de Goeij et al. 2013).

Few studies have examined genetic connectivity among sponge populations, particularly within Florida and Caribbean regions (but see López-Legentil and Pawlik 2009: *Xestospongia muta*; DeBiasse et al. 2010, 2014: *Callyspongia vaginalis*; Chaves-Fonnegra et al. 2015: *Cliona delitrix*). To date, the handful of population genetic studies conducted on sponges have largely, although not exclusively (see López-Legentil and Pawlik 2009), confirmed the expectation of limited larval dispersal as a result of their short pelagic larval durations (PLDs) (e.g., Bergquist and Sinclair 1973; Ilan and Loya 1990; Meroz and Ilan 1995; Lindquist et al. 1997; and see Maldonado 2006 for a review) and have demonstrated a general pattern of high genetic differentiation among populations (e.g., Wörheide et al. 2002; Duran et al. 2004; Bell et al. 2014; Chaves-Fonnegra et al. 2015; Pérez-Portela et al. 2015).

The giant barrel sponge, *Xestospongia muta*, is a large (often exceeding 1 m in diameter) and dominant member of reef communities throughout Florida and the Caribbean (McMurray et al. 2008; Pawlik et al. 2008). It can cover more than 9% of available substrate (Zea 1993) and may be over 1000 yr old, making *X. muta* one of the longest-lived of all animals, leading to its moniker "the redwood of the reef" (McMurray et al. 2008). Given the giant barrel sponge's ecological and functional importance to coral reef habitat, understanding its genetic connectivity and dispersal capacity is extremely important, especially in the context of widespread reef habitat degradation. Within the Indo-Pacific, recent work on *X. muta*'s congener taxa has found that barrel sponge populations demonstrate high levels of self-recruitment, limited larval dispersal distances, and have numerous genetic lineages suggesting that a diverse species complex may exist (Swierts et al. 2013; Bell et al. 2014). Though the exact PLD of *X. muta* is unknown, it is likely similar to that of its Pacific congener, *X. bergquista*, which settles within ~3 d (Fromont and Bergquist 1994), and while such a short PLD suggests limited dispersal and high local recruitment, the strong currents that flow through the Caribbean, in particular along the eastern Florida coastline (Roberts 1997; Yeung and Lee 2002), may allow for connectivity across relatively broad spatial scales.

To complement previous genetic connectivity work on *X. muta* which used sequences of the mitochondrial cytochrome c oxidase subunit I (López-Legentil and Pawlik 2009), we developed and adopted high-resolution microsatellite markers to assess connectivity over broad and fine spatial scales. In addition, we used an extensive sampling regime that included samples from multiple locations across the Caribbean, and from multiple locations within the Florida reef tract, allowing for a detailed, fine-scale analysis of population differentiation and migration over both evolutionary and ecological timescales within the Florida reef tract and throughout the Florida–Caribbean region.

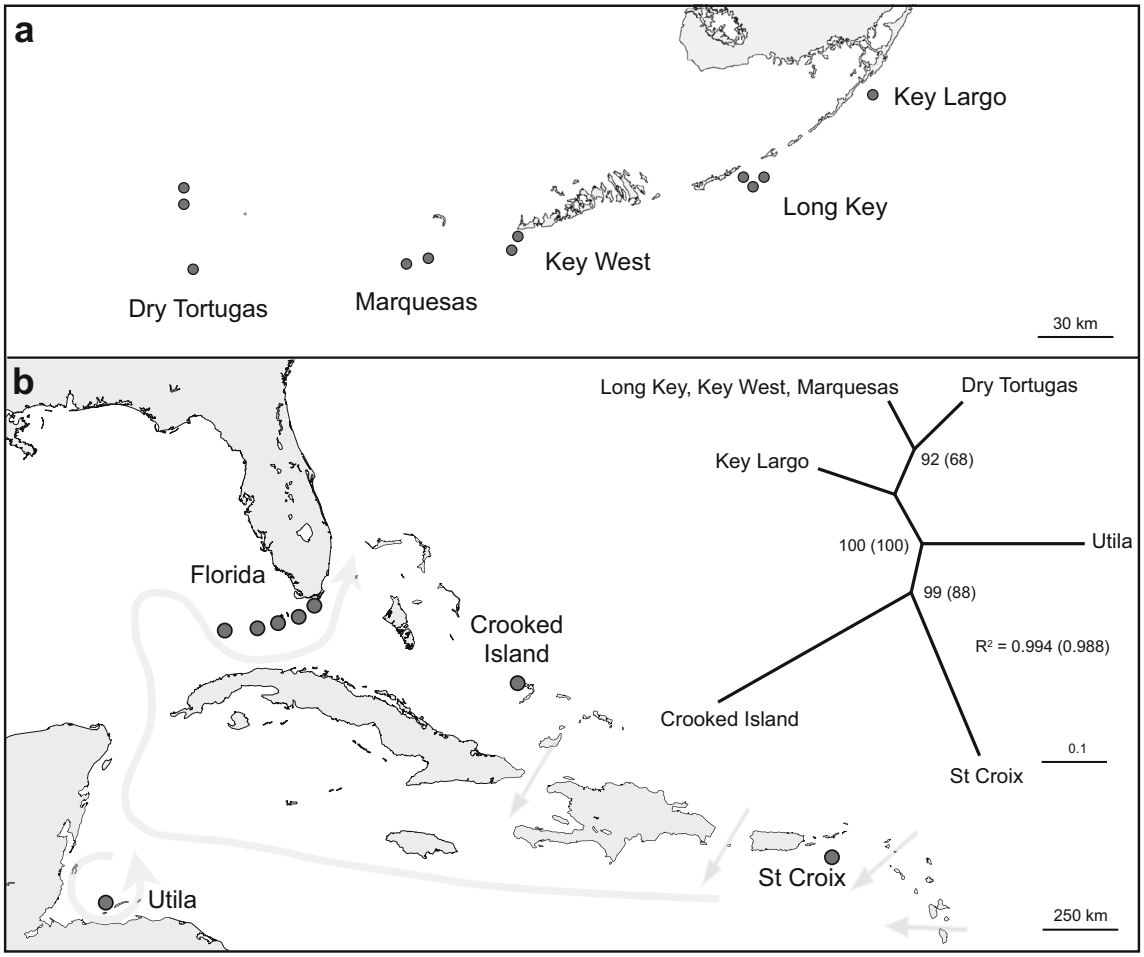
Materials and methods

All laboratory work on these samples was performed in accordance with Nova Southeastern University guidelines.

Sample collection, genomic DNA extraction, and microsatellite marker development

A total of 374 *X. muta* individuals were sampled from 22 reefs from eight locations within the Florida reef tract and the Caribbean (Fig. 1). At each site, specimen collection was opportunistic due to the patchy nature of *X. muta* along the surveyed reef tracts. Where possible, collections from the same reef were performed evenly and the sampling of proximate specimens was avoided. Of the 374 genotyped individuals, five sets of matching genotypes were identified. Duplicate genotypes were discarded from all subsequent analyses, and only unique samples genotyped at a minimum of five of ten surveyed loci (see below) were analyzed ($n = 363$; Fig. 1). All individuals were preserved in 95% ethanol at 4 °C until genomic DNA (gDNA) extraction using the DNeasy Tissue Kit (Qiagen Inc., Valencia, CA).

To develop a suite of ten species-specific *X. muta* microsatellite loci, the enrichment protocol of Glenn and



Map a (Florida Reef Tract)
The Dry Tortugas = 49
The Marquesas Keys = 58
Key West = 31
Long Key = 40
Key Largo = 35

Map b (Florida & the Caribbean)
Florida, USA = 213
Utila, Honduras Bay Islands = 33
Crooked Island, Bahamas = 50
St Croix, USVI = 67

Fig. 1 Sampling locations in **a** Florida reef tract and **b** Florida and the Caribbean. *Gray arrows* describe the general path of major surface currents and gyres. Sample sizes for each location are listed under the maps. **b** also contains the neighbor-joining phylogeny built using Cavalli-Sforza and Edwards chord distance (D_c). Bootstrap support values are shown (1000 replicates). First value is for a phylogeny built using seven loci and the second value in parentheses is for a phylogeny built using all ten loci. The topology of both phylogenies was identical

Schable (2005) was adopted with a few modifications and all polymerase chain reactions (PCRs) were performed in 25 μL volumes (see Electronic Supplementary Material, ESM Methods).

Population genetic structure

Summary and F-statistics

GENEPOP v4.1 (Raymond and Rousset 1995; Rousset 2008) was used to calculate Weir and Cockerham *F*-statistics (Weir and Cockerham 1984), to perform exact tests to assess population differentiation, to estimate observed and expected frequencies of heterozygotes, and to test for Hardy–Weinberg (HWE) and linkage equilibrium (LE) between all locus pairs. Significance of overall population differentiation across all loci in all populations was calculated using FSTAT 2.9.3.2 (Goudet 1995). Statistical significance was adjusted using the sequential Bonferroni correction ($\alpha = 0.05$). FREENA (Chapuis and Estoup 2007) was used to estimate the frequency of null alleles and to calculate F_{ST} values corrected for any positive bias introduced by the presence of null alleles (1000 iterations).

Cluster analyses

The number of genetic clusters (K) was estimated using three separate approaches: (1) STRUCTURE 2.3.4 (Pritchard et al. 2000); (2) GENELAND 4.0.5 (Guillot et al. 2005); and (3) BAPS 5.2 (Corander et al. 2003).

For STRUCTURE, K was estimated by first performing a Bayesian evaluation of genetic partitioning ($K = 1$–10) and then by calculating the ad hoc statistic ΔK based on the second order rate of change of the probability of the data [$L(K)$] (Evanno et al. 2005). STRUCTURE runs were performed using five Markov chains for each value of K, and consisted of 200,000 Markov chain Monte Carlo (MCMC) iterations (including 100,000 MCMC steps burn-in). The ancestry model LOCPRIOR with admixture (Hubisz et al. 2009) was used in combination with the correlated allele frequency model (Falush et al. 2003). STRUCTURE HARVESTER (Earl and vonHoldt 2012) was used to estimate ΔK. Visualization of clustering outcomes was performed using the program DISTRUCT 1.1 (Rosenberg 2004).

GENELAND analyses were conducted incorporating spatial coordinates of sampled individuals, and the null allele model was used to correct for any upward bias on the estimation of K that may occur due to null alleles (Guillot et al. 2008). Using the uncorrelated allele frequency, spatial, and null allele models, five independent Markov chains were run for 1,000,000 MCMC iterations, with a sampling increment of 100. Finally, groups of individuals (i.e., populations defined a priori) were clustered using the software BAPS, which incorporates a stochastic optimization method to determine the optimum partitioning among the sampling sites (K).

Genetic relationships among populations were determined by neighbor-joining (NJ) analysis incorporating Cavalli-Sforza and Edwards' (1967) chord distances. NJ tree construction and statistical support for branch nodes (nonparametric bootstrap; 1000 replicates) were carried out in TreeFit 1.2 (Kalinowski 2009).

Contemporary gene flow

To estimate the extent of contemporary (i.e., ecological timescale) dispersal between *X. muta* populations, the number of first-generation immigrants into each population was inferred using GENECLASS2 (Piry et al. 2004). First-generation migrants were identified using the likelihood criteria of Rannala and Mountain (1997) and the simulated likelihood distribution was generated using the Monte Carlo re-sampling procedure of Paetkau et al. (2004) (10,000 simulated individuals). The test statistic L_h, in combination with an α of 0.01 and 0.05, was used in all analyses as not all source populations were sampled (Paetkau et al. 2004).

Individuals with missing data (nonamplification of alleles) were excluded; reduced sample sizes were St. Croix = 44, Utila = 25, and Crooked Island = 38. For the Florida reef tract, individuals were divided into two groups: group 1 = Key Largo (n = 31) and group 2 = remaining Florida individuals (n = 137). This grouping follows the findings of our population structure analyses (see "Results" section).

Results

Population genetic structure

Summary and F-statistics

All ten of the surveyed microsatellite markers showed high levels of polymorphisms, as overall the number of alleles ranged from five (Xm94) to 70 (Xm202) and expected heterozygosity (H_E) ranged between 0.25 (Xm94) and 0.96 (Xm202) (ESM Tables S1, S2).

When all eight sampling locations were defined a priori as populations, the overall population differentiation was significant ($F_{ST} = 0.119$, $P = 0.001$); however, evidence of a significant heterozygote deficiency was also observed ($F_{IS} = 0.221$, $P = 0.0000$).

Out of 80 exact tests performed to detect the presence of heterozygote deficiencies at each locus within each population, 37 were significant (46.3%) (ESM Table S2). The majority of these significant values were due to deviations found at three loci: Xm24 (eight significant values), Xm43 (seven significant values), and Xm144 (eight values significant). Removal of these three loci reduced the number of significant values to 14 (17.5%) for all populations. Notably, the St. Croix population showed a high level of heterozygote deficiencies as all but one locus deviated significantly within this population. Exact tests performed to ensure LE among loci identified four significant locus comparisons; however, all of the significant comparisons involved the loci Xm43 or Xm144.

The average frequency of null alleles for each locus in all populations ranged from 0.0 to 36.3% (8.5% overall) (ESM Table S2), and the three loci that showed the highest levels of heterozygote deficiency (Xm24, Xm43, and Xm144) also demonstrated the highest frequencies of null alleles: 20.1, 13.9, and 26.0%, respectively. As null alleles have the potential to inflate F_{ST} values (Chapuis and Estoup 2007), post hoc analyses were performed to investigate the potential for over-estimation of population genetic structure for *X. muta* across the surveyed distribution (ESM Results).

The first approach to estimate the number of genetically discrete populations was to follow the ad hoc procedure proposed by Waples and Gaggiotti (2006). Pairwise exact tests of population differentiation were examined and those sampling sites that were connected through a chain of nonsignificant results were considered as belonging to the same population. To accommodate the presence of null alleles, an evaluation of their effect on the level of pairwise differentiation found among a priori populations was performed. For all pairwise tests of differentiation, the locus with the highest frequency of null alleles was systematically removed and then the pairwise test was repeated. Using ten, nine, and eight loci, seven populations were consistently delineated: St. Croix, Utila, Crooked Island, and four populations within the Florida reef tract. Samples from the Marquesas and Key West were consistently grouped into the same population, while samples from Long Key, Dry Tortugas, and Key Largo were significantly differentiated from this grouping and each other (Table 1; Fig. 2). Using seven or fewer loci, broad geographic differences remained; however, Long Key was no longer differentiated from the Marquesas and Key West population grouping (Table 1; Fig. 2). Using four or fewer loci, the Dry Tortugas was nonsignificantly differentiated from the Marquesas, Key West, and Long Key. Key Largo, however, remained significantly differentiated from the remainder of the reef tract until only two loci remained.

Pairwise F_{ST} values (for both ten and seven loci) among the four major locations (Florida, Utila, Crooked Island, and St. Croix) were an order of magnitude higher than the values within the Florida reef tract, with Crooked Island showing the highest differentiation (approximately double that seen among the remaining three locations) (Fig. 2). Of the three Caribbean populations, Utila showed the lowest level of differentiation when compared to Florida.

Cluster analyses

Due to the presence of null alleles, STRUCTURE analyses were run using (1) ten loci and (2) seven loci (with Xm24, Xm43, and Xm144 omitted). Interestingly, the use of ten loci did not inflate ΔK, despite a high frequency of null alleles as both data sets produced a ΔK of 5, which is largely concordant with the results from the exact tests using seven (or fewer) loci, which delineated six populations (Table 1); however, exact tests revealed more fine-scale differences within the Florida reef tract compared to the individual-based clustering method of STRUCTURE.

Membership coefficients (Q) for a K of five showed very strong and consistent membership of individuals from St. Croix, Utila, and Crooked Island to clusters three, four, and five, respectively (Fig. 3). Within the Florida reef tract, analysis of the two data sets showed slightly different patterns. Using seven loci, the majority of the membership of cluster one was from Key Largo, and to a lesser degree, Long Key. Individuals from Long Key, in particular Key West and the Marquesas, had strong membership to cluster two. The Dry Tortugas were largely assigned to cluster two; however, individuals also shared a small amount of co-ancestry with clusters one and four as well. Using ten loci, the pattern was similar for Long Key, Key West, and the Marquesas, but the majority of the membership of cluster one was shared between Key Largo and the Dry Tortugas. Long Key again had a small proportion of the membership of cluster one. This pattern is similar to the results obtained from the exact tests, which showed that the Dry Tortugas was significantly differentiated from the remainder of the reef tract when ten loci were used, but not differentiated when some combinations of fewer loci were used (Fig. 3).

As the model implemented in the clustering program GENELAND corrects for the presence of null alleles, a single data set comprising all ten microsatellite loci was analyzed. GENELAND results suggested the presence of four distinct genetic clusters comprising Florida, Utila, Crooked Island, and St. Croix (Table 1). Similarly, analyses of the two data sets (seven and ten loci) using the program BAPS also identified these same four genetic clusters (Table 1).

Because of the very low levels of differentiation among samples from Long Key, Key West, and the Marquesas, these samples were grouped together for the calculation of chord genetic distances. Genetic distances were calculated for both the seven locus data set (Xm24, Xm43, and Xm144 removed), and the ten locus set.

Table 1 Estimates of the number of genetic populations (K) of *Xestospongia muta* sampled from eight sites within the Florida reef tract and across the Caribbean

Number of loci used	1	2	3	4	5	6	7	8	9	10
Method	Number of populations (K)									
Exact tests	4	4	5	5	7	6	6	7	7	7
STRUCTURE (correlated allele frequencies)							5			5
GENELAND										4
BAPS							4			4

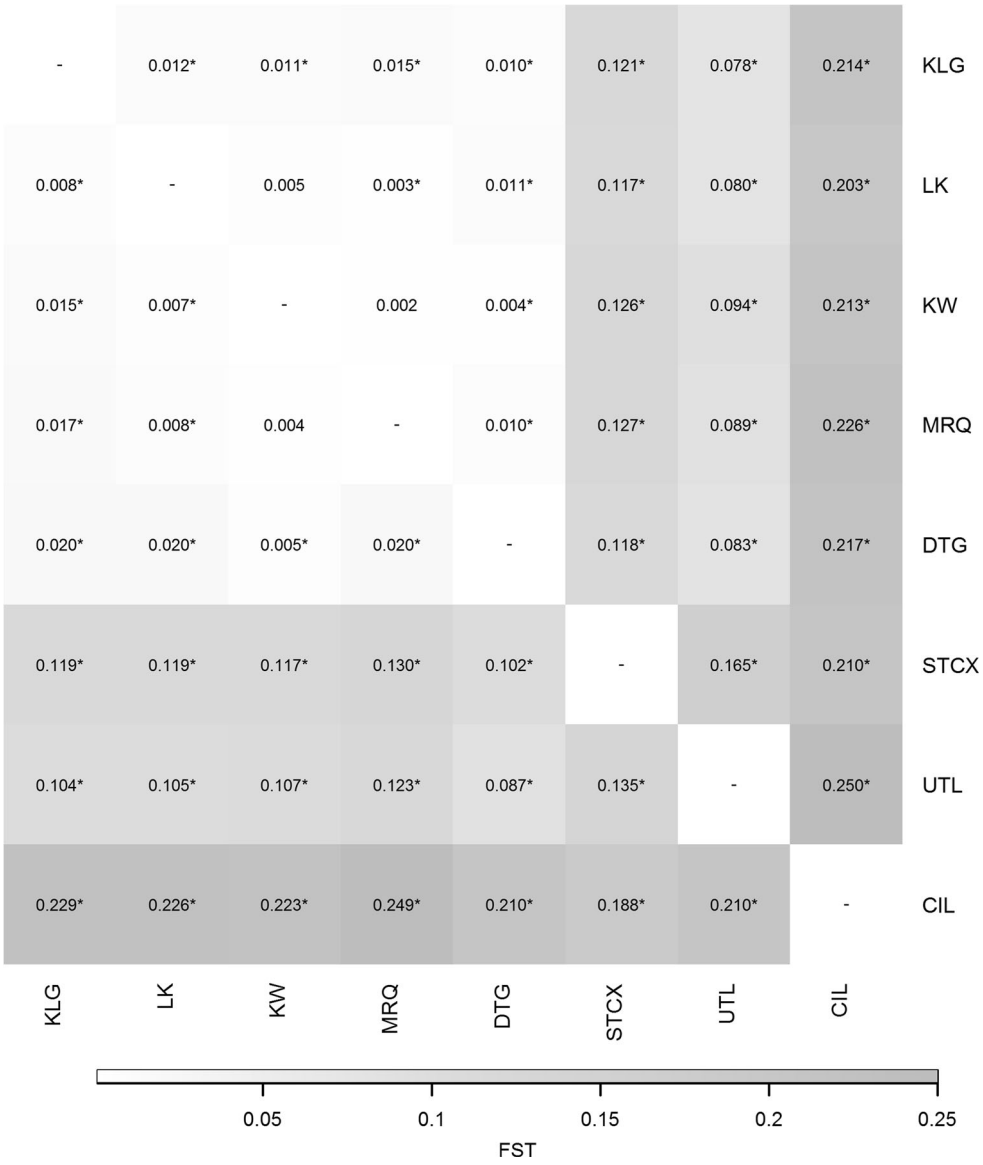


Fig. 2 Heatmap and matrix of pairwise population-level F_{ST} values estimated for seven (*upper triangular*) and ten (*lower triangular*) microsatellite markers, respectively. *Asterisk* indicates a significant exact test after sequential Bonferroni correction: $\alpha = 0.05$. *KLG* Key Largo, *LK* Long Key, *KW* Key West, *MRQ* Marquesas, *DTG* Dry Tortugas, *STCX* St. Croix, *UTL* Utila, *CIL* Crooked Island

The NJ phylogenies produced by the two sets of distances were nearly identical (the Dry Tortugas branch length was slightly longer via analysis of the ten locus data set) and both showed good fit to their respective distance matrices (seven loci: $R^2 = 0.994$, ten loci: $R^2 = 0.988$) (Fig. 1). Phylogenies showed that Utila was more closely related to Florida than Crooked Island or St. Croix. Interestingly, the Key Largo population, which is geographically the most distant from Utila, was the most closely related and Crooked Island was the most distantly related to the Florida populations (the branch connecting Crooked Island to the node connecting Crooked Island to St. Croix was the longest in the phylogeny). Overall, the phylogenies showed a chain of relatedness as follows: Florida, Utila, St. Croix, and Crooked Island.

Contemporary gene flow

With α set to 0.05, and using the seven locus data set, 13% of individuals sampled at Caribbean locations were identified as first-generation immigrants (Utila = 3, Crooked Island = 5, St. Croix = 12). However (with one exception; see below), the origin of these immigrants could not be determined as the highest likelihood values were for the location where each immigrant was sampled. These results suggest that the origin of the identified first-generation immigrants may be un-sampled neighboring reefs. A single immigrant sampled within Utila was identified as having originated from Key Largo.

Within the Florida reef tract, with α set to 0.05, six individuals (17%) sampled within group 1 (Key Largo) and

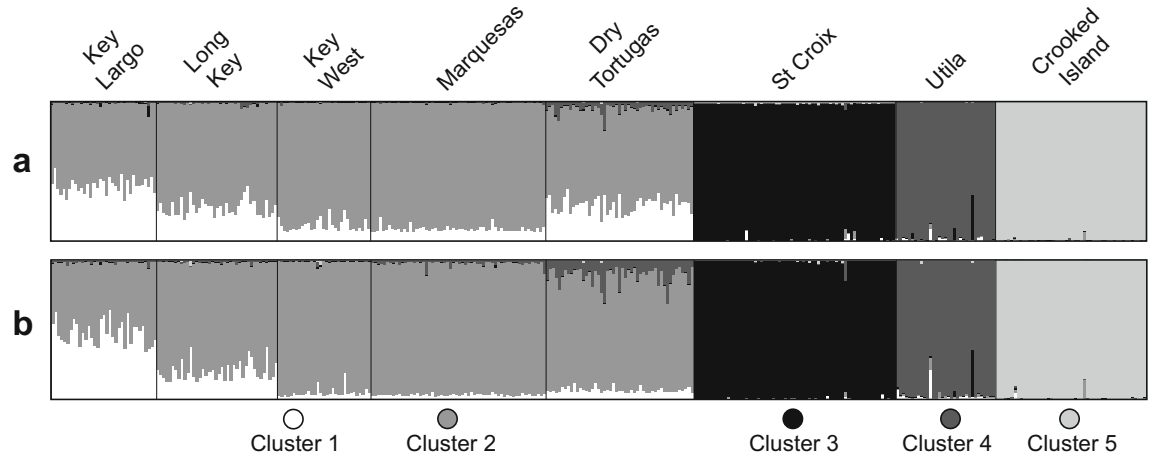


Fig. 3 Plots of membership coefficients (Q) for $K = 5$ clusters generated using the LOCPRIOR ancestry and correlated allele frequency models in STRUCTURE using **a** ten loci, and **b** seven loci. *Different shading* for each of the five clusters is shown under the plots. Individual membership coefficients are divided into eight sections representing sampling sites. *Thin columns* within the sections represent individuals and show the proportion of membership coefficient for different genetic clusters

12 individuals (7%) sampled in group 2 (remaining Florida individuals) were identified as first-generation immigrants. The origin of seven individuals could be identified: three immigrants into group 1 (9%) were from group 2 and four immigrants into group 2 (2%) were from group 1. There were no immigrants into the Florida populations from the Caribbean. GENECLASS2 results describing first-generation migration with α set to 0.01 demonstrated largely similar trends, yet detected fewer migration events overall (ESM Results).

Discussion

Population differentiation and larval dispersal ability

Population genetic results showed a high level of genetic differentiation among *X. muta* populations throughout Florida and the Caribbean. Such findings are in keeping with the growing body of literature showing high genetic partitioning among sponge populations (e.g., Wörheide et al. 2002; Duran et al. 2004; Blanquer et al. 2009; DeBiasse et al. 2010; Bell et al. 2014; Chaves-Fonnegra et al. 2015) and confirm the mitochondrial COI sequence-based findings of López-Legentil and Pawlik (2009) who also reported high genetic partitioning among *X. muta* populations from Florida, Belize, and the Bahamas.

Our genetic survey of Florida and Caribbean populations of *X. muta* suggested that individuals could be delineated into likely six distinct genetic groups, comprising Utila, Crooked Island, St. Croix, and northern (Key Largo), mid (Marquesas, Key West, and Long Key) and southern Florida Keys (Dry Tortugas). Such findings are largely consistent with patterns of regional differentiation within the Caribbean (Cowen et al. 2006). Exact tests differentiated the Key Largo population from the remainder of the southern Florida reef tract (save the two locus comparison), and the Dry Tortugas also showed a degree of distinctiveness as well. However, this site could be eventually connected to the remainder of the reef tract via a nonsignificant comparison with individuals sampled from Key West (using four or fewer loci), suggesting likely genetic connectivity throughout the southern portion of the reef tract. The delineation of sample sites into five rather than six genetic populations was suggested using STRUCTURE; however, the clustering programs GENELAND and BAPS were more conservative and were unable to differentiate the Key Largo population from the other Florida populations.

With one exception, no larval exchange was detected among the four major study regions (Utila, Crooked Island, St. Croix, and Florida), which suggested limited (if any) connectivity among these regions over ecologically relevant timescales; however, we acknowledge the caveat that large geographic distances separated the surveyed areas and that the absence of intermediate sampling sites makes it difficult for us to fully assess connectivity over broader geographic scales. Interestingly, GENECLASS2, however, identified a single individual from within the Utila population as a first-generation migrant and indicated its most likely origin as Key Largo. However, given that the distance between Key Largo and Utila is over 1300 km, it seems highly unlikely that an individual could migrate this distance within a single generation, suggesting that this individual originated from a population much closer to

Utila whose genotypes were more closely related to the Key Largo population.

Null alleles

While the suite of ten polymorphic species-specific microsatellite loci for *X. muta* developed herein was highly effective at differentiating among regions, the high levels of heterozygote deficiency detected for loci Xm24, Xm43, and Xm144 within virtually all populations strongly suggested the occurrence of null alleles at these loci.

The likely presence of null alleles at these three surveyed loci has important implications for our interpretation of the high levels of spatial genetic population structure we found. Since differentiation among populations tends to increase as diversity within a population decreases (Slatkin 1995), by lowering the number of heterozygotes (and the diversity) within populations, null alleles have the potential to artificially inflate measures of differentiation among populations (Chapuis and Estoup 2007). However, while results for *X. muta* showed a general increase in population differentiation with increased frequency of null alleles, this trend was mild when compared to F_{IS} (ESM Fig. S1) which was far more sensitive to the presence of null alleles.

Nevertheless, when the microsatellite markers Xm24, Xm43, and Xm144 were removed from the data analyses, some discrepancies were found with respect to the detected levels of genetic differentiation between data sets (ten vs. seven loci). Exact tests have been shown to be one the most sensitive approaches to infer differentiation but high sensitivity renders such tests susceptible to artifacts such as data error (Waples and Gaggiotti 2006). STRUCTURE analyses also showed mixed susceptibility to the presence of null alleles, as estimated membership coefficients (Q values) of the Dry Tortugas population were variable for the ten and seven locus data sets.

To date, few sets of microsatellite markers have been developed to genotype and survey sponge species and populations, and among these surveys, the level of reported heterozygote deficiencies (and null alleles) across markers and studies has been highly variable. For instance, Blanquer et al. (2009) reported few if any significant heterozygote deficiencies, whereas Duran et al. (2004), Chaves-Fonnegra et al. (2015), and Giles et al. (2015) reported higher levels of either null alleles or heterozygote deficits across markers. Bell et al. (2014) found high levels of heterozygote deficiencies in their survey of Indonesian populations of *Xestospongia* species; however, the authors attributed their results to inbreeding resulting from high levels of self-recruitment, rather than null alleles. With so few studies, generalizations regarding the presence of null alleles in sponges remain difficult. However, certain taxa with large effective population sizes such as insects and mollusks do appear to have high frequencies of null alleles (Chapuis and Estoup 2007), and sponges likely also fall into this category.

Large-scale dispersal dynamics among Florida and Caribbean populations

Larval dispersal dynamics of marine species are complex and may be affected to varying degrees by numerous factors such as ocean currents (Palumbi 1994; Roberts 1997; White et al. 2010), larval behavior (Paris et al. 2007), mortality and diffusion (Cowen et al. 2000, 2006), availability of suitable settlement habitat (García-Machado et al. 2001), and timing of larval release (Baums et al. 2006). Despite the apparent importance of ocean currents in the dispersal of larvae, many studies have shown no correlation between patterns of population genetic connectivity and the present-day direction of current flow (Benzie and Williams 1997; Palumbi et al. 1997; Barber et al. 2002; Wörheide et al. 2002). However, many such studies employed mtDNA sequence data which provides information on migration averaged over thousands of generations (evolutionary timescales). Consequently, DNA sequence data will likely only reflect the direction of current flow if currents have remained constant for many generations. In contrast, due to their rapid evolution, microsatellites provide a means to separate the signals of past and present connectivity (ecological timescales) by examining migration over more intermediate (allele frequency) and contemporary (multi-locus genotype assignment) timescales.

Both mtDNA sequence data (López-Legentil and Pawlik 2009) and microsatellite allele frequency data (this study) suggest that ocean currents throughout Florida and the Caribbean have indeed been important factors affecting the dispersal of *X. muta* larvae for thousands of generations (the contemporary migration data, however, shows little to no first-generation migration over this geographic scale). Both mtDNA and microsatellite data sets show relatively low population differentiation between individuals collected from Utila and the Florida reef tract, despite separation of over 1000 km; in contrast, considerably higher levels of differentiation were found over a much shorter distance between the Florida reef tract and the Bahamas (Fig. 2). While the Caribbean and Loop Currents may bring larvae (for *X. muta* probably over several generations) into the Florida Keys from upstream western Caribbean sources (i.e., Honduras–Belize), facilitating gene flow, significant differentiation between the Florida reef tract and the Bahamas (López-Legentil and Pawlik 2009; this study) may be maintained by the strong flow of the Florida Current serving to restrict gene flow between these two regions. Simulation modeling efforts support this hypothesis, as results have

suggested that larvae entrained in the Florida Current from upstream sources could become derailed in the upper Keys as the current flows close to shore (Yeung and Lee 2002) and that the first point of contact within the Florida reef tract for larvae being transported from upstream sources is the upper Keys. This is consistent with measures of F_{ST} and NJ phylogenies for *X. muta*, which show Key Largo (upper Keys) to have the lowest level of differentiation and closest genetic relationship to Utila compared to all other Florida sampling sites (Figs. 1, 2).

Fine-scale dispersal dynamics within the Florida reef tract

Water circulation within the Florida reef tract is dynamic and strongly influenced by seasonal wind patterns and the onshore meanders of the Florida Current. The interaction of these processes combined with the changing orientation of the reef tract northwards as the current moves away from the Florida mainland generates a complex pattern of counter and eddy currents (Lee and Williams 1999; Lee et al. 1992; Yeung and Lee 2002). Consequently, currents can flow in either direction through the reef tract and may be responsible for the complex patterns of resolved connectivity. For instance, within the upper Keys, the north–south orientation of the reef tract and the close proximity of the flow of the Florida Current often result in a northeasterly current direction, while the west–east orientation of the middle–lower Keys often results in a southwesterly current direction. The GENECLASS2 migration results appear to reflect this dynamic flow pattern within the Florida reef tract. While only low amounts of migration between Key Largo and the remainder of the reef tract were noted, migration was bidirectional and was approximately in equal proportions in either direction. Thus, as previously suggested, Key Largo's genetic distinctiveness may be attributed to the northerly transport of larvae carried by the Florida Current from upstream locations outside of the Florida reef tract. If only a small proportion of these novel genotypes are subsequently transported southwest into the remainder of the reef tract, Key Largo could accumulate a distinctive allelic distribution when compared to the remainder of the Florida Keys.

The Tortugas Gyre, another dominant water circulation feature within the Florida reef tract, may also be serving to isolate Key Largo populations of *X. muta*. This large gyre, which is formed from offshoots of the Florida Current off of the Dry Tortugas, often moves through the lower and middle Keys (Lee et al. 1994). Current recirculation has the potential to retain marine larvae (Shearer and Coffroth 2006; Paris et al. 2007) and genetic isolation of populations due to localized oceanographic circulation has been reported for numerous species (García-Machado et al. 2001; Perrin et al. 2004; Waters and Roy 2004; Taylor and Hellberg 2006). Thus, entrapment of larvae within the Tortugas Gyre may also be contributing to the distinctiveness of the middle/lower Keys from the upper Keys.

The finding of genetic connectivity through a large section of the Florida reef tract (Long Key, Key West, and the Marquesas) for *X. muta*, coupled with significant differentiation among the upper and lower Keys, contrasts with other sponge and coral surveys to date (see Baums et al. 2010; Andras et al. 2013), including those examining Indo-Pacific *Xestospongia* species (Bell et al. 2014). Within the Caribbean, the sponges *Callyspongia vaginalis* (Debiasse et al. 2010) and *Cliona delitrix* (Chaves-Fonnegra et al. 2015) have shown mixed patterns of population genetic structure. *Callyspongia vaginalis* showed significant differentiation among individual sampling sites separated by distances as small as tens of kilometers (Debiasse et al. 2010), whereas *Cliona delitrix* demonstrated higher levels of connectivity, albeit with some differentiation among sites (Chaves-Fonnegra et al. 2015). Assuming a similar reproductive biology and PLD to *Xestospongia* sister taxa (see Fromont and Bergquist 1994), variation in genetic connectivity between *Callyspongia vaginalis* and *X. muta* over similar spatial scales and geographic distributions may be due to life history differences between species. *Callyspongia vaginalis* brood larvae to an advanced stage of development (Lindquist et al. 1997), suggesting rapid larval settlement and limited dispersal via currents, which would promote the evolution of genetic isolation over small spatial scales relative to *X. muta*. Within the Indo-Pacific, a survey of *X. testudinaria* reported larval dispersal distances of <150 m and significant genetic differentiation between sampling sites separated by as little as only 2 km (Bell et al. 2014). Assuming similar PLDs, the high genetic connectivity of *X. muta* throughout the lower and mid Keys contrasts these Indo-Pacific findings, suggesting that the dynamics of these two systems may be quite different and may be greatly influencing genetic connectivity. Interestingly, while *X. testudinaria* populations were found to be largely dependent on local recruitment (as were *X. muta* over somewhat larger spatial scales), akin to the present study, some evidence for long distance dispersal was found.

Extending the previous mtDNA-based Caribbean survey of *X. muta* (López-Legentil and Pawlik 2009), our study revealed a high degree of genetic partitioning among populations found throughout Florida and the Caribbean. Fine-scale population structure within the Florida coral reef tract was detected, showing that reefs in Key Largo and the Dry Tortugas were genetically differentiated from the remainder of the Florida reef tract. Furthermore, this study demonstrates that migration over ecologically relevant timescales in this iconic structural species is likely

restricted to relatively short distances and that populations are likely largely self-recruiting. Genetic connectivity over both ecological and evolutionary timescales is likely driven by oceanographic patterns and currents, both connecting and isolating individuals.

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