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Population structure and connectivity in the Atlantic scleractinian coral *Montastraea cavernosa* (Linnaeus, 1767)

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Abstract

Coral reefs are increasingly threatened worldwide by a variety of biological and physical factors, including disease, bleaching and ocean acidification. Understanding levels of connectivity among widespread populations can assist in conservation efforts and the design of marine protected areas, as larval dispersal scales affect population demography. This study examined genetic connectivity and morphological variation of the broadcast spawning coral *Montastraea cavernosa* (L., 1767) among five locations in the Caribbean and Western Atlantic. Analysis of mtDNA and nuclear rRNA internal transcribed spacers, at both the local and regional scale, revealed that the majority of variation existed within locations rather than among them. Likewise, the majority of pairwise comparisons were non-significant between sites and locations. These results suggest that moderate to high gene flow occurs within and among populations of *M. cavernosa* in the Western Atlantic. The phylogeographic signature and significant pairwise comparisons among several locations, however, indicate that populations are also partially maintained through self-seeding and that gene flow may be restricted over large geographic distances. Additionally, while some anatomical variation is likely attributable to phenotypic plasticity, variations in skeletal morphology between Jamaica and other locations correspond with significant pairwise genetic distances and the presence of private sequence types (limited to a single location), suggesting selection to local environmental conditions.

Introduction

The ability of a coral reef to recover from disturbance events is contingent on the continued recruitment of coral larvae from local and distant populations. Understanding patterns of population connectivity, therefore, increases our understanding of reef resilience. Previous estimates of population connectivity were based on potential larval dispersal capabilities inferred from basic life-history information and ocean current variables (Thorson 1950; Veron 1995; Bohonak 1999; White *et al.* 2010). The incorporation of genetic information, however, has shown high amounts of genetic structure among locations for several species of corals regardless of the duration of their larval phase (Ayre & Hughes 2000; Baums *et al.* 2005; Underwood *et al.* 2007; Vollmer & Palumbi 2007; Miller & Ayre 2008; Goodbody-Gringley *et al.* 2010). For example, in the Caribbean, restricted gene flow has been reported in the broadcasting acroporid corals *Acropora palmata* and *Acropora cervicornis* (Baums *et al.* 2005; Vollmer & Palumbi 2007) and in the brooding faviid coral *Favia fragum* (Goodbody-Gringley *et al.* 2010). Alternatively, Neves *et al.* (2008) found continuous gene flow along the Brazilian coast in the brooding siderastreid corals *Siderastrea stellata* and *Siderastrea radians*, and evidence also exists for panmictic populations of some broadcasting corals spanning over hundreds of kilometers (Ayre *et al.* 1997; Ayre & Hughes 2000; Ridgway *et al.* 2001; Marquez *et al.* 2002; Ng & Morton 2003; Takabayashi *et al.* 2003). Such differences imply that levels of population connectivity are not only a function of the duration of the larval pre-competent phase

but likely involve other physical, biological and behavioral factors as well.

Montastraea cavernosa (Linnaeus, 1767) is a common reef-building coral on fore-reef slopes throughout the Caribbean and Western Atlantic, extending from Bermuda to Brazil and the West African coast (Szmant 1986; Veron 2000; Nunes *et al.* 2009). Colonies of *M. cavernosa* can form massive boulders or flat plates that typically occur in green, brown, gray or orange (Veron 2000). Like its congeneric species in the *Montastraea annularis* complex, *M. cavernosa* is a broadcast spawner, releasing sperm and eggs into the water column where fertilization and development subsequently take place (Szmant 1986). The minimum development period for broadcasting corals is thought to be 4–6 days; however, larvae of broadcast-spawning corals are documented to survive in the laboratory for up to 105 days and still remain competent to settle (Wilson & Harrison 1998). The potentially long larval duration of broadcasting corals could facilitate wide-scale dispersal, as evidenced by the long-distance dispersal documented for this species (Nunes *et al.* 2009). However, the high degrees of endemism and genetic structure documented for populations of endosymbiotic zooxanthellae (*Symbiodinium* spp.) associated with broadcasting corals, may be indicative of similar structure in host populations, as host-symbiont pairings are highly specific (Santos *et al.* 2003; Kirk *et al.* 2005, 2009; Howells *et al.* 2009; Thornhill *et al.* 2009).

This study examines the population genetic structure of *M. cavernosa* across five locations in the Western Atlantic: Barbados, Bermuda, the Flower Garden Banks (USA), Jamaica and the Caribbean coast of Panama. Nunes *et al.* (2009) recently looked at the structure of this species throughout its distribution range using two fragments of the nuclear β-tubulin gene. These authors found high levels of gene flow among the majority of populations, but restricted connectivity across the Atlantic Ocean to geographically isolated populations (Nunes *et al.* 2009). Here we focus on Caribbean and Western Atlantic populations to explore population genetic structure among distantly related locations as well as within each region to examine local recruitment at neighboring sites just a few kilometers apart. We incorporated one of the two β-tubulin fragments used by Nunes *et al.* (2009), a fragment of the nuclear ribosomal internal spacer region, and a non-coding mitochondrial marker to compare diversity among markers with varying evolutionary histories. Using standard population genetic analyses in concert with haplotype networks, we explore spatial patterns of diversity and the potential for population expansion based on low frequency sequence types. In addition, we analyzed variations in skeletal morphology at a regional scale to compare with genetic variation. Four morphological features were examined, corallite diameter, columella diameter, length of longest septum, and distance to nearest neighboring polyp, as these features are documented to be taxonomically informative for this species (Ruiz Torres 2004). Given the relatively long planktonic life stage of broadcast spawning corals, *M. cavernosa* is expected to have a broad dispersal capability resulting in high levels of genetic diversity and gene flow among and within populations. We expect, therefore, to find little evidence of local recruitment and a broad distribution of high-frequency haplotypes. Furthermore, patterns of genetic structure and diversity are hypothesized to vary among markers based on their different evolutionary rates.

Material and Methods

Collection

Samples from a total of 290 *Montastraea cavernosa* colonies were collected from five locations within the Caribbean and Western Atlantic: Barbados, Bermuda, Flower Garden Banks, Jamaica, and Panama (Fig. 1). Within each location, small fragments were collected from 10 *in situ* colonies along fore-reef zones at five sites (nine sites in Bermuda) for a total of 50 sampled colonies per location (90 in Bermuda). Collection sites in Barbados, Bermuda, Jamaica, and Panama ranged in depth from 5 to 10 m, were separated by a minimum of 100 m, and were spread across five linear km in Jamaica, 15 linear km in Barbados, 100 km^2 in Panama and 450 km^2 in Bermuda (Fig. 1). Collection sites at the deep reefs of the Flower Garden Banks ranged in depth from 20 to 30 m, were separated by a minimum of 100 m, and spread across 30 km^2. In the lab a small portion (one to two polyps) of each sample was removed and placed in a 1.5-ml microcentrifuge tube filled with 96% ethanol. Remaining fragments were bleached with a 10% solution of sodium hypochlorite (NaOCl) to expose the bare skeletons for morphological analysis. Collecting expeditions took place in summer 2005 to Bermuda, and spring/summer 2006 to Barbados, Flower Garden Banks, Jamaica, and Panama. Appendix 1 lists all localities and sites included in this study and detailed collecting and permitting information. All skeletons, tissue samples, and DNA extractions were deposited in the collection of the Department of Invertebrate Zoology, Museum of Comparative Zoology, Harvard University.

Morphological analysis

Bleached skeletons (50 per location, 90 in Bermuda) were photographed with a Nikon D70S camera attached to a dissecting microscope outfitted with an optical micrometer

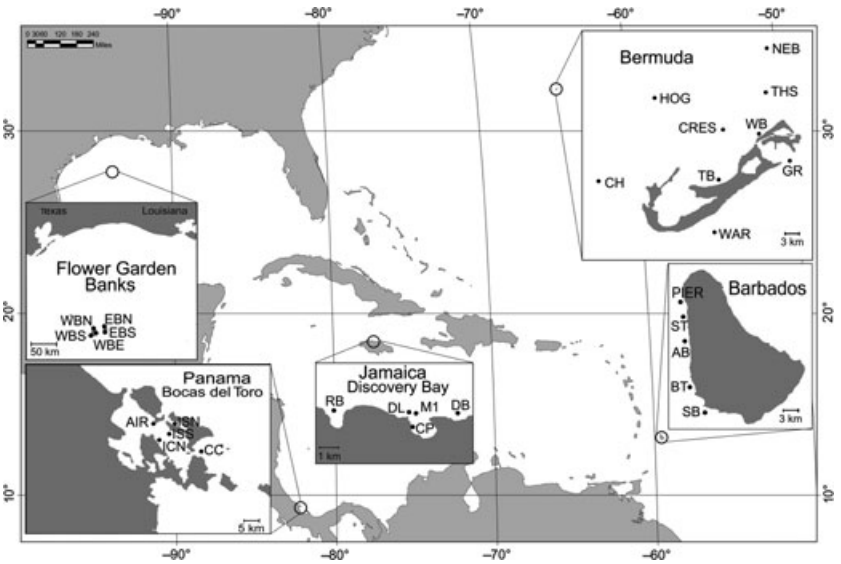

Fig. 1. Map of the Caribbean and Western Atlantic indicating sampling locations in Barbados, Bermuda, Flower Garden Banks, Jamaica, and Panama.

for scale, and analyzed using NIH IMAGE J version 1.34s software (http://rsb.info.nih.gov/ij/). Corallite diameter, columella diameter, length of longest septum and distance to nearest neighbor were measured on four central corallites per colony (Fig. 2). These characters were chosen based on their utility in intraspecific comparisons for *Montastraea cavernosa* (Ruiz Torres 2004), and are easily analyzed using macro-photography. The maximum number of corallites on the smallest bleached fragment determined the number of corallites examined. Data for corallite and columella diameter and septa length met the assumptions of equal variance and normality and were analyzed with a single factor analysis of variance (ANOVA) for the effects of location. Data for distances between neighboring polyps did not meet the assumptions of homoscedasticity and were therefore analyzed for differences in parameters by location using a Wilcoxon–Mann–Whitney test. *Post hoc* analysis for each parameter between locations was done using pairwise Wilcoxon rank sum *W* tests (Dytham 2003).

DNA extraction and sequencing

Genomic DNA was extracted from all samples for each location with an AutoGenprep 965 automated extraction robot following the manufacturer's protocol at the Bauer Center for Genomic Research at Harvard University. A 896-bp region of the ribosomal internal spacer region including internal transcribed spacer 1–5.8S ribosomal RNA – internal transcribed spacer 2 (*ITS* hereafter) was amplified using the 'anthozoan-universal' primer pairs 1S (5′-GGT ACC CTT TGT ACA CAC CGA CCG TCG CT-3′) and 2SS (5′-GCT TTG GGC GGC AGT CCC AAG CAA CCC GAC TC-3′) (Odorico & Miller 1997) and a 576-bp region of β-tubulin (β-*tub* hereafter) previously used for coral phylogenetics using the primers Tub F (5′-GCA TGG GAA CGC TCC TTA TTT-3′) and Tub R (5′-ACA TCT GTT GAG TCA GTT CTG-3′) (Fukami *et al.* 2004; Nunes *et al.* 2008, 2009). A 634-bp region of the mitochondrial inter-genomic spacer between cytochrome *c* oxidase subunit I (cox1) and the formylmethionine

Fig. 2. Images of *Montastraea cavernosa:* colony *in situ* (left), colony close-up (center) and bleached skeleton (right) indicating corallite diameter (A), columella diameter (B), length of longest septum (C), and distance to nearest neighbor (D).

transfer RNA gene (tRNA-Met) (*IGR* hereafter) was amplified using primers IGR F (5′-TGT GTG ACA TAT AGG TTA TGA ACT TG-3′) and IGR R (5′-GTT CTT GGG TTG CAT GGT TT-3′). Primers for *IGR* were developed for this study with PRIMER3 (Rozen & Skaletsky 2000) using pre-existing complete coral mitochondrial genome sequences available on GenBank. Polymerase chain reaction (PCR) amplifications were done in a 25-μl reaction volume containing a bottom mix of 3 μl 3.3× PCR buffer, 1.5 μl MgOAc, 2 μl dNTPs (10 mM), 1.25 μl of each primer (100 mM), and 1 μl of DNA template and a top mix of 4.5 μl 3.3× PCR buffer, 10.4 μl H_2O and 0.1 μl of rTth polymerase XL (Applied Biosystems) following the manufacturer's protocol with thermocycling parameters of 35 cycles of 94 °C/30 s, 49 °C/30 s, 72 °C/150 s.

The double-stranded PCR products were visualized by 1% agarose gel electrophoresis and vacuum-purified using 96-well Millipore Multiscreen® plates. When double bands were visualized (*β-tub* only) amplifications were repeated at an annealing temperature of 50 °C to increase stringency. The purified PCR products were sequenced directly using ABI BigDye Terminator version 3.0 (Applied Biosystems) following standard protocols described by the manufacturer. The BigDye-labeled PCR products were then analyzed using an ABI PRISM 3730 Genetic Analyzer. Sequences were edited and aligned in SEQUENCHER 4.7 (Gene Codes Corporation 1991–2007). Sequenced individuals and accession numbers are listed in Appendix 2; all sequences are available on GenBank (http://www.ncbi.nlm.nih.gov/Genbank).

Population genetic and phylogeographic analyses

The *ITS* region is part of the ribosomal nuclear array and thus multiple copies occur per nuclear genome (Long & Dawid 1980). However, no more than a single polymorphic site was found within any individual through direct sequencing of amplified products. As such, each sequence that contained a polymorphic site was separated into two sequence types to account for each represented nucleotide. The *β-tub* sequences, on the other hand, contained several heterozygous sites. Haplotypes for *β-tub* were therefore estimated using PHASE 2.1 (Stephens *et al.* 2001) on DNASP v.5 (Librado & Rozas 2009), which implements a coalescent-based Bayesian method to infer haplotypes. Population genetic analyses were performed using ARLEQUIN version 3.01 (Excoffier *et al.* 2005). To assess diversity among and within each location, standard diversity indices, including number of haplotypes (N*h*), number of polymorphic sites (Np), haplotypic diversity (*h*) (Nei 1987), nucleotide diversity (pn) (Tajima 1983; Nei 1987), and mean number of pairwise differences (pd) between haplotypes (Tajima 1983) were calculated for each gene. An unweighted analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) was performed to test hierarchical models of genetic variance using pairwise differences among haplotypes as a measure of divergence. Analyses were run with regional subdivisions of Barbados (n = 14 for *ITS*, 28 for *β-tub*, 7 for *IGR*), Bermuda (n = 30 for *ITS*, 16 for *β-tub*, 43 for *IGR*), Flower Garden Banks (n = 18 for *ITS*, 24 for *β-tub*, 22 for *IGR*), Jamaica (n = 18 for *ITS*, 20 for *β-tub*, 25 for *IGR*), and Panama (n = 21 for *ITS*, 18 for *β-tub*, 28 for *IGR*), as well as local subdivisions of sites within each location (see Appendix 1 for additional site details). Population (location) pairwise F_{ST} (Hudson *et al.* 1992) and N_{ST} (Lynch & Crease 1990) values, whose significances were assessed through 10,000 permutation tests, were used to calculate differentiation between locations and between sites within each location using DNASP v.5 (Librado & Rozas 2009).

To test for the possibility of recent population expansion in *Montastraea cavernosa*, Tajima's *D* (Tajima 1989) and Fu's F_S (Fu 1997) were calculated for all specimens considered as one group and also separately for each location ($\alpha = 0.05$). Although Tajima's *D* was originally designed to test for selection, a negative value suggests the presence of an excess of low-frequency haplotypes, which would be expected under an expansion scenario (Aris-Brosous & Excoffier 1996).

Haplotype networks were estimated for each gene with TCS version 1.18 (Clement *et al.* 2000), which implements the statistical parsimony procedure (Templeton *et al.* 1992; Crandall 1994). This method estimates an unrooted tree and provides a 95% plausible set for the relationships among haplotypes.

Concatenated sequences were created with Phyutility (Smith & Dunn 2008) and used to construct strict consensus trees on POY version 4 (Varón *et al.* 2010) with local and regional subdivisions (Appendix 3). Bootstrap values indicating branch support were calculated based on 1000 pseudoreplications.

Results

Three genomic regions totaling almost 3 kb of DNA information were amplified. Alignments of all three genes were trivial and required no insertion/deletion events. The complete *ITS* region was sequenced for 101 individual colonies (Table 1). Over 892 aligned positions, 43 sequence types were recovered with 31 polymorphic sites (3.48% variable). A total of 106 individual sequences were estimated for the *β-tub* region, which contained 25 sequence types with 24 polymorphic sites over 575 aligned positions (4.17% variable) (Table 1). The *IGR* region was sequenced for 125 individual colonies

Table 1. Standard diversity measures for populations of *Montastraea cavernosa* including number of colonies sampled *(n),* number of haplotypes (Nh), number of polymorphic sites (Np), haplotype diversity (*h*), nucleotide diversity (pn), the mean number of pairwise differences (pd), and Tajima's *D* and Fu's F_s statistics.

Location	Gene	*n*	Nh	Np	*h*	pn	pd	Tajima's D	Fu's F_s
ALL	*ITS*	101	43	31	0.9600 ± 0.0080	0.005451 ± 0.002969	4.862574 ± 2.391205	−0.2403	**−25.3866**
Barbados		14	9	12	0.9011 ± 0.0624	0.004780 ± 0.002829	4.263736 ± 2.248018	−0.30909	−1.90789
Bermuda		30	15	24	0.9287 ± 0.0247	0.004904 ± 0.002773	4.374712 ± 2.223050	−0.54521	**−4.62676**
Flower Gardens		18	13	16	0.9608 ± 0.0301	0.005517 ± 0.003151	4.921568 ± 2.513760	0.22144	**−4.68244**
Jamaica		18	13	18	0.9477 ± 0.0392	0.005114 ± 0.002948	4.562091 ± 2.351357	0.224	**−5.84276**
Panama		21	15	20	0.9667 ± 0.0236	0.005392 ± 0.003061	4.809524 ± 2.445947	−0.15755	**−5.23433**
ALL	*β-tub*	106	25	24	0.8246 ± 0.0293	0.007002 ± 0.003904	4.025876 ± 2.026876	−0.33861	**−7.75442**
Barbados		28	10	15	0.7778 ± 0.0655	0.006524 ± 0.003776	3.751323 ± 1.950182	0.01518	−0.94405
Bermuda		16	11	13	0.9417 ± 0.0406	0.008797 ± 0.005048	5.058333 ± 2.591521	0.99498	**−3.26727**
Flower Gardens		24	10	11	0.8841 ± 0.0432	0.007202 ± 0.004140	4.134058 ± 2.131579	1.36638	−0.90792
Jamaica		20	7	10	0.5842 ± 0.1270	0.003961 ± 0.002535	2.273684 ± 1.302596	−0.67615	−0.81599
Panama		18	4	9	0.6275 ± 0.0733	0.006809 ± 0.004005	3.908497 ± 2.055473	1.74955	4.00616
ALL	*IGR*	125	5	21	0.1085 ± 0.0379	0.002467 ± 0.001641	1.564129 ± 0.939638	**−1.69633**	2.2727
Barbados		7	1	0	0	0	0	0	0
Bermuda		43	2	20	0.0465 ± 0.0439	0.001467 ± 0.001145	0.930233 ± 0.653484	**−2.60992**	3.49608
Flower Gardens		22	2	19	0.0909 ± 0.0809	0.002724 ± 0.001840	1.727273 ± 1.045633	**−2.47287**	4.90299
Jamaica		25	4	21	0.2300 ± 0.1095	0.002766 ± 0.001851	1.753333 ± 1.052873	**−2.48684**	1.73017
Panama		28	2	20	0.1376 ± 0.0837	0.004340 ± 0.002639	2.751323 ± 1.502545	**−1.6411**	7.76304

Significant comparisons are given in bold ($\alpha = 0.05$).

(Table 1). Over 634 aligned positions, five haplotypes were recovered with 21 polymorphic sites (3.31% variable).

Haplotype diversity, nucleotide diversity, and the mean number of pairwise differences were higher for *ITS* and *β-tub* than for *IGR* based on non-overlapping standard errors (Table 1). Higher diversity measure for nuclear markers compared to mitochondrial markers is expected given the known rates of evolution for nrRNA *versus* mtDNA in basal metazoans (Hellberg 2007). Diversity measures did not differ among locations in *ITS* and *β-tub*; however, diversity in Barbados was lower than that in all other sites for *IGR* (Table 1).

Tajima's *D* values were negative for all three markers but results were only significant in *IGR* (Table 1). Although Tajima's *D* values varied for each marker within each location, all were negative and significant for *IGR*. Significance for a negative value explains the presence of an excess of low-frequency sequence types and may also provide evidence for recent population growth (*e.g.* Baker *et al.* 2007). Fu's F_S values, however, were non-significant in *IGR,* but were significant in *ITS* and *β-tub*, indicating that there is not enough evidence for accepting or rejecting an expansion scenario (Table 1).

Haplotype diversity, nucleotide diversity, and the number of pairwise differences varied considerably in *ITS* and *β-tub* among sites ranging across a linear distance of 15 km in Barbados (Appendix 4). However, no variation was detected in *IGR*. These parameters also differed among sites sampled across 450 km^2 in Bermuda for *ITS* and *IGR*, with the lowest diversity for *ITS* found at the North East Breakers (NEB), and highest diversity for *IGR* at Hog Breakers (HOG). Diversity measures did not differ among locations for *β-tub*. At the Flower Garden Banks, diversity varied among sites across roughly 30 km^2 for all three markers. Diversity was lowest for *ITS* at the West Bank South (WBS) and at the West Bank East (WBE) for *β-tub*, while diversity in *IGR* was highest at the East Bank North site (EBN). In Jamaica, haplotype diversity, nucleotide diversity, and pairwise differences varied across a 5-km linear distance for *ITS* and *IGR*; however, there were no differences among sites in *β-tub*. Haplotype diversity, nucleotide diversity and mean number of pairwise differences did not vary significantly among sites within a 100-km^2 region of Panama for *ITS*. These parameters did vary among sites in *β-tub,* however, and in *IGR* site Crawl Caye (CC) had higher diversity than any other location.

Phylogeographic distribution of alleles

Networks were constructed separately for each marker analyzed (Fig. 3). Within *ITS,* several high-frequency sequences observed are shared among multiple locations: Barbados, Bermuda, Flower Garden Banks, Jamaica, and Panama, one of which was shared among all five locations (Fig. 3). Likewise, the *β-tub* network contained a single high-frequency sequence shared among all five locations, with several additional sequences shared among two or

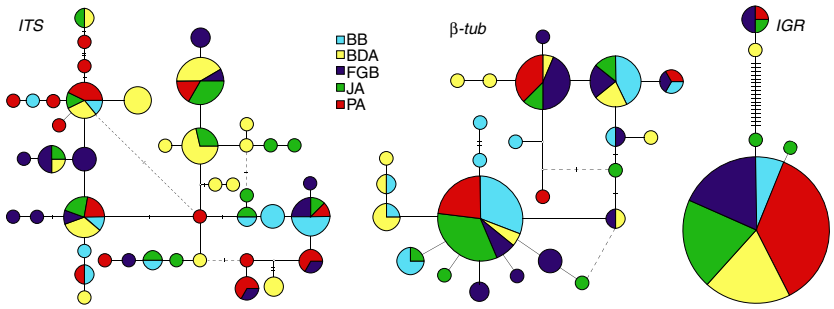

Fig. 3. Haplotype networks for *ITS*, *β-tubulin*, and *IGR* where each line or hatch mark indicates a single nucleotidic change. Area of each circle corresponds to the number of sequence types it represents, and color of circle represents the location where it was found. BB = Barbados; BDA = Bermuda; FGB = Flower Garden Banks; JA = Jamaica; PA = Panama.

Table 2. Number of private sequence types found within each location (Barbados, Bermuda, Flower Garden Banks, Jamaica, and Panama) for each genetic marker (*ITS, β-tub*, and *IGR*).

Location	Gene	Private haplotypes
Barbados (BB)	*ITS*	3
	β-tub	3
	IGR	0
Bermuda (BDA)	*ITS*	9
	β-tub	4
	IGR	1
Flower Garden Banks (FGB)	*ITS*	7
	β-tub	4
	IGR	0
Jamaica (JA)	*ITS*	4
	β-tub	3
	IGR	2
Panama (PA)	*ITS*	8
	β-tub	1
	IGR	0

more sites (Fig. 3). *IGR* contained a single high-frequency sequence shared among all five locations and one sequence shared between Bermuda, Jamaica, and Panama (Fig. 3). Private sequence types (found only at a single location) were also present in all three markers (Table 2). Barbados contained three private sequence types for *ITS*, Bermuda contained nine, the Flower Garden Banks contained seven, Jamaica contained four, and Panama contained eight. In *β-tub*, Barbados contained three private sequence types, Bermuda and the Flower Garden Banks each contained four private sequence types, Jamaica had three private sequence types, and Panama contained a single private sequence type. Jamaica also contained two private haplotypes and Bermuda a single private haplotype in *IGR*.

Population genetic structure

An analysis of molecular variance was used to test for hierarchical population structure with each location expressed as a separate group (Table 3). AMOVA results detected significant genetic structure within populations for *ITS* and *β-tub*, where 92.79% (*ITS*) and 92.51% (*β-tub*) of variation is explained within locations, respectively, as well as a small degree of genetic structure among locations (7.21% in *ITS*; 7.49% in *β-tub*) (Table 3). Furthermore, although significant genetic structure was not identified in *IGR*, all of the variation is explained within localities.

Pairwise F_{ST} and N_{ST} values varied by marker due to their different evolutionary rates (Table 4). Comparisons were significant between samples from Barbados and all other locations in *ITS* for both analyses. In *β-tub*, pairwise comparisons using both analyses were significant for samples from Jamaica compared with those from Bermuda, Flower Garden Banks and Panama, and between samples from Barbados and the Flower Garden Banks (Table 4). Significant pairwise N_{ST} comparisons were also found between samples from Barbados and Bermuda in *β-tub*; however, F_{ST} comparisons for these locations were non-significant. All other comparisons in *ITS* and *β-tub*, as well as all comparisons in *IGR* were non-significant.

Within Barbados, Bermuda, the Flower Garden Banks, Jamaica, and Panama, AMOVA analyses indicate that no genetic structure exists among sample sites at each location ($\alpha = 0.05$), with the majority of variation explained within sites. Significant F_{ST} comparisons were found between sites SB and AB for *ITS* in Barbados, between site WB and sites CH and WAR for *ITS* in Bermuda, between site WBE and sites WBN and WBS for *β-tub* in the Flower Garden Banks, and between sites DB and DL for *β-tub* in Jamaica; however, pairwise F_{ST} values were non-significant for the majority of comparisons between sites at each location across all three markers ($\alpha = 0.05$).

Morphology

Skeletal analysis revealed significant differences among populations in columella diameter ($P < 0.0001$), corallite diameter ($P < 0.0001$), distance to nearest neighbor

Table 3. AMOVA. Each population was considered as a separate group.

Gene	Source of Variation	df	Sum of squares	Variance components	Percent of total variation	Fixation indices
ITS	AMOVA grouping by population					
	Among populations	4	23.28	**0.17784** Va	7.21	F_{ST}: **0.07206**
	Within populations	96	219.85	2.29015 Vb	92.79	
	Total	100	243.13	2.46798		
β-tub	AMOVA grouping by population					
	Among populations	4	20.41	**0.15314** Va	7.49	F_{ST}: **0.07493**
	Within populations	101	190.94	1.89054 Vb	92.51	
	Total	105	211.36	2.04368		
IGR	AMOVA grouping by population					
	Among populations	4	1.12	−0.02190 Va	−2.82	F_{ST}: −0.02819
	Within populations	120	95.85	0.79878 Vb	102.82	
	Total	124	96.98	0.77689		

Significance at the $P < 0.05$ level is indicated in bold.

Table 4. Pairwise F_{ST} (below diagonal lines) and N_{ST} (above diagonal lines) values among locations for each gene.

	Barbados	Bermuda	Flower Gardens	Jamaica	Panama
ITS					
Barbados		**0.24289**	**0.08946**	**0.15223**	**0.10179**
Bermuda	**0.24269**		0.05128	0.00053	0.07391
Flower Gardens	**0.08946**	0.05128		0.01466	−0.00298
Jamaica	**0.15211**	0.00049	0.01475		0.04553
Panama	**0.10169**	0.0738	−0.00291	0.04545	
β-tub					
Barbados		**0.06897**	**0.09589**	−0.00642	0.0462
Bermuda	0.05739		−0.01995	**0.17096**	−0.06093
Flower Gardens	**0.09104**	−0.01271		**0.22061**	−0.02545
Jamaica	0.00213	**0.16683**	**0.21857**		**0.16144**
Panama	0.03874	−0.04386	−0.02068	**0.14924**	
IGR					
Barbados		0	0	0.00371	0.03704
Bermuda	0		−0.0312	−0.02742	−0.00741
Flower Gardens	0	−0.03117		−0.94159	−0.0325
Jamaica	0.00379	0.02736	−0.04153		−0.02913
Panama	0.03704	−0.00741	−0.03249	−0.02913	

Significant comparisons at the $P < 0.05$ level are indicated in bold.

($P < 0.0001$), and septum length ($P < 0.0001$) between locations (Fig. 4). Pairwise comparisons showed that specimens from Jamaica have significantly larger columella diameters and significantly shorter septa than all other locations. Specimens from Flower Garden Banks have significantly larger corallite diameters and significantly longer septa than specimens from any other location, as well as significantly smaller columella diameter than specimens from Barbados, Bermuda or Jamaica. Distance to nearest neighboring polyps was significantly higher in Panama than in any other location and significantly lower in Barbados and Bermuda than at other locations. Columella and corallite diameter differed significantly by site within Barbados, Bermuda, and Panama ($\alpha < 0.05$), length of longest septum differed significantly by site within Bermuda, the Flower Garden Banks, and Panama ($\alpha < 0.05$), and polyp distance to nearest neighbor differed significantly between sites in Barbados, Bermuda, Jamaica, and Panama ($\alpha < 0.05$). Given the few variables available, formal correlations with genetic variance were not attempted.

Discussion

Samples of *Montastraea cavernosa* collected in the Caribbean and nearby Atlantic for this study show that the majority of variation occurs within locations, rather than among them. This indicates that gene flow among

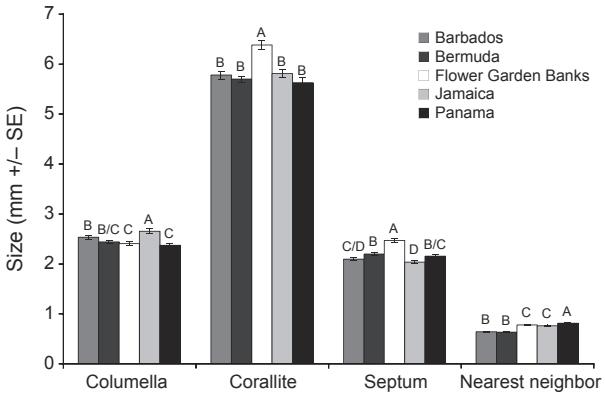

Fig. 4. Comparisons of colony skeletal morphology by location, including columella diameter, corallite diameter, length of longest septum, and distance to nearest neighboring polyp. Statistically similar locations are grouped by letter; locations not represented by the same letter are significantly different ($P < 0.05$).

locations is not restricted, possibly due to the presence of a long-lived larva in the life cycle of the coral. Likewise, no genetic structure was found locally among reefs in each location and pairwise comparisons among reefs in each location revealed non-significant F_{ST} values for the majority of comparisons, indicating high degrees of local genetic connectivity. Although significant differences in skeletal morphology were found between some sites within each location, the lack of genetic structure in such locations indicates that differences in morphology are likely due to phenotypic plasticity. This is in contrast to the brooding coral species *Favia fragum*, where a correlation between genetic and morphometric structure has been shown (Goodbody-Gringley *et al.* 2010). Additionally, non-significant and inconsistent results for Tajima's *D* and Fu's F_S imply that *M. cavernosa* has a stable demographic history throughout the geographic range studied here. These data, as those of Nunes *et al.* (2009), suggest that populations of *M. cavernosa* throughout the Caribbean and Western Atlantic are connected and readily share genetic information. Significant F_{ST} and N_{ST} values for several comparisons in *ITS* and *β-tub* coupled with the presence of extensive private sequence types, however, indicate that local recruitment is also important in determining the genetic structure of this species.

Local recruitment is particularly evident in Barbados, where all comparisons with other locations in *ITS* revealed significant pairwise F_{ST} and N_{ST} values. Additionally, several private sequence types were found in Barbados for both *ITS* and *β-tub*. Populations in Barbados may, therefore, experience decreased gene flow from populations within the Caribbean. Barbados is the southernmost location sampled in this study and lies on the border of the Caribbean and South American plates. Topographical features associated with plate boundaries may restrict dispersal across these regions, resulting in reduced genetic exchange.

Evidence of local recruitment was also found in Jamaica, which contained private haplotypes for all three markers. Furthermore, significant pairwise F_{ST} and N_{ST} values were found in *β-tub* for the majority of comparisons between Jamaica and other locations. Samples from Jamaica differed anatomically as well, having significantly larger columella diameters and significantly shorter septa than those from all other locations. Significant pairwise comparisons coupled with significant anatomical differences imply that despite the potential for long-distance dispersal and the central location of Jamaica within the Caribbean, populations of *M. cavernosa* in Jamaica are partially maintained through self-seeding and may have undergone selective adaptation in response to local environmental conditions. However, we cannot discard the possibility of this being an expression of phenotypic plasticity. These findings contrast with those of Nunes *et al.* (2009), who document a high degree of genetic exchange among several Caribbean locations. Differences between results found in the latter study and ours, however, may be due to variation in mutational rates among the genes used for analyses.

Although the Flower Garden Banks is often considered an isolated reef system due to its location in the Northern Gulf of Mexico and deeper depth, non-significant pairwise comparisons indicate that high degrees of genetic exchange occur among Caribbean populations. Samples from the Flower Garden Banks varied significantly, however, in three of the four morphological characters analyzed. These anatomical differences do not appear to be linked to genetic differences and variation in skeletal morphology may therefore be due to depth-specific phenotypic plasticity, a feature that is known to be high in scleractinian corals (Bruno & Edmunds 1997). The depth of reefs at the Flower Garden Banks required collection at 20–30 m, compared with collections at 5–10 m from all other locations. Depth differences between the Flower Garden Banks and other tested locations are likely responsible for the variation found in skeletal morphology.

Although *M. cavernosa* in the Caribbean likely experiences moderate to high gene flow among locations, genetic exchange may be limited over wider geographic distances (>1500 km) and local populations may also be partially maintained through self-seeding. At the local scale, no genetic structure was found among sites within each location, indicating that reefs separated by tens of kilometers readily exchange genetic information. These results correspond with the findings of Nunes *et al.*

(2009) who found that *M. cavernosa* populations within the Caribbean and Western Atlantic experience high genetic connectivity, whereas peripheral populations have low genetic diversity and are genetically isolated from the Caribbean populations. Likewise, Ayre & Hughes (2000) found several brooding and broadcasting corals on the Great Barrier Reef which rely on self-seeding for population maintenance but also have sufficient gene flow among reefs to prevent accumulation of fixed genetic differences. Additionally, several phylogeographic studies of broadcasting corals in the Pacific have found high levels of gene flow among populations at the local scale, but restricted gene flow over greater distances (Yu *et al.* 1999; Rodriguez-Lanetty & Hoegh-Guldberg 2002; Magalon *et al.* 2005). For example, Magalon *et al.* (2005) found evidence of panmixia among *Pocillopora meandrina* populations in the South Pacific at scales below 10 km, but restricted gene flow at scales over 2000 km. This observation is further supported by the findings of Underwood *et al.* (2009), which suggest that although many coral reefs are genetically differentiated, panmixia can occur at scales of tens of kilometers or less.

The use of ribosomal RNAs (rRNA) in phylogeographic studies is often contested due to the faster rate of speciation in corals relative to the concerted evolution of rRNA resulting in shared ancient rRNA lineages, obscuring processes such as introgressive hybridization (Vollmer & Palumbi 2004). The rate of homogenization and the rate of new mutation, however, vary from species to species (Ohta & Dover 1983) and thus problems associated with concerted evolution and introgressive hybridization may only exist in certain coral genera such as *Acropora* (Chen *et al.* 2004; see Harris & Crandall 2000). While this study incorporated multiple genetic markers and morphology to examine population structure in order to avoid the putative pitfalls associated with the *ITS* marker, the use of *ITS* sequences as a molecular marker relies on the assumption that the rRNA array evolves as a single molecule and results should be interpreted cautiously (Avise 2004; Hellberg 2007; Baums 2008). It is possible, therefore, that concerted evolution may have resulted in a biased assessment of variation, giving us either an over- or under-estimate of diversity and connectivity. However, inclusion of *ITS* sequences in this study revealed patterns of diversity that were not detected by *β-tub* or *IGR*, and thus may be useful for future intraspecific population studies. Additional examination of these samples should include higher resolution markers, such as microsatellites, as well as greater coverage of locations throughout the species distribution.

Gene flow among coral reef populations becomes particularly relevant in light of recent declines in scleractinian abundance on reefs throughout the Caribbean. According to Hughes *et al.* (2003), human impacts on coral reefs such as overfishing, eutrophication, and climate change are threatening coral reefs around the globe. The resilience of coral reefs depends in large part on their ability to re-colonize disturbed areas. Whether planulae that settle at such sites come from local populations or distant locations is critical to our understanding of the corals' ability to recover from environmental perturbations (Brazeau *et al.* 2005; Magalon *et al.* 2005). The present study indicates that while gene flow occurs among populations of *M. cavernosa* within the Caribbean and near Atlantic, populations also rely on local recruitment, and dispersal may be limited across wider geographic distances. Benefits of conservation efforts at any scale will likely extend beyond the target population; however, successful management should address local populations independently, as the degree of self-seeding may vary among locations.

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Appendix 1: Collection locations and permit information.

Location	Site	Abbrev.	Coordinates	CITES permit	Collecting permit
Bermuda	Chub head	CH	32°21′25.69″N, 64°55′08.43″W		
	Crescent reef	CRES	32°22′48.13″N, 64°45′15.66″W		
	Gurnet rock	GR	32°20′21.33″N, 64°39′48.67″W		
	Hog breaker	HOG	32°28′14.98″N, 64°49′21.70″W		
	Tynes Bay	TB	32°18′45.84″N, 64°46′58.25″W	BDA 05-17	SP060801
	Three Hills Shoals	THS	32°25′26.39″N, 64°42′07.93″W		
	Whalebone Bay	WB	32°21′58.11″N, 64°42′53.41″W		
	Warwick	WAR	32°15′00.43″N, 64°48′09.48″W		
	North east breakers	NEB	32°28′58.76″N, 64°41′48.03″W		
Barbados	St James Pier	PIER	13°16′49.84″N, 59°39′02.20″W		
	Bridgetown	BT	13°07′11.12″N, 59°38′11.58″W	1236	No #, approved by coastal zone 4/20/2006
	Allynes Bay	AB	13°11′31.09″N, 59°38′29.56″W		
	Speightstown	ST	13°13′15.75″N, 59°38′45.34″W		
	South Bridgetown	SB	13°04′34.17″N, 59°36′43.57″W		
Flower Garden Banks	West Bank – East	WBE	27°52′31.30″N, 93°48′51.30″W		
	West Bank – North	WBN	27°52′35.10″N, 93°48′54.10″W	N/Awithin USA	
	West Bank – South	WBS	27°52′27.50″N, 93°49′00.40″W		FGBNMS-2006-009
	East Bank – South	EBS	27°54′27.00″N, 93°35′57.40″W		
	East Bank – North	EBN	27°54′39.90″N, 93°35′55.60″W		
Panama	Isla Solarte North	ISN	9°19′50.67″N, 82°12′36.50″W		
	Isla Solarte South	ISS	9°19′19.73″N, 82°13′06.04″W		
	Isla Cristobal	ICN	9°16′54.05″N, 82°15′17.15″W	SEX/A-84-06	SE/A-17-06
	Crawl Caye	CC	9°15′48.14″N, 82°07′19.40″W		
	Bocas Airport	AIR	9°20′27.42″N, 82°15′36.68″W		
Jamaica	Dancing lady	DL	18°28′28.20″N, 77°25′05.18″W	JM 1646	18/27
	Mooring 1	M1	18°28′24.02″N, 77°24′37.19″W		
	Rio Bueno	RB	18°28′58.75″N, 77°27′24.86″W		
	Dairy Bull	DB	18°28′02.10″N, 77°23′16.00″W		
	Columbus Park	CP	18°28′02.89″N, 77°24′52.52″W		

Appendix 2: Individual colony locations, identity and accession numbers. All sequences with a corresponding accession number are available in GenBank.

Location	ID	*ITS*	*β-tub*	*IGR*	Location	ID	*ITS*	*β-tub*	*IGR*
Barbados	AB1a	HM447251	HM447539		Jamaica	CP1a	HM447313	HM447479	
	AB1b		HM447540			CP1b	HM447314	HM447480	
	AB2a		HM447551			CP2a		HM447502	HM447428
	AB2b		HM447552			CP2b		HM447503	
	AB3a		HM447541			CP3	HM447315		
	AB3b		HM447542			CP4a		HM447525	HM447424
	AB4a		HM447549			CP4b		HM447526	
	AB4b		HM447550			CP5	HM447316		HM447425
	AB5a		HM447545			CP6			HM447426
	AB5b		HM447546			CP7			HM447427
	BT1a	HM447252	HM447543			DB1a	HM447317	HM447487	HM447433
	BT1b		HM447544			DB1b	HM447318	HM447488	
	BT2a		HM447559			DB4a	HM447319	HM447530	HM447434
	BT2b		HM447560			DB4b		HM447531	

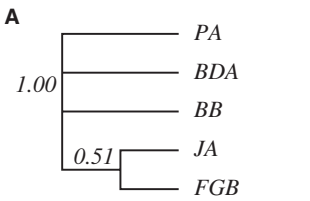
Appendix 2: (Continued)

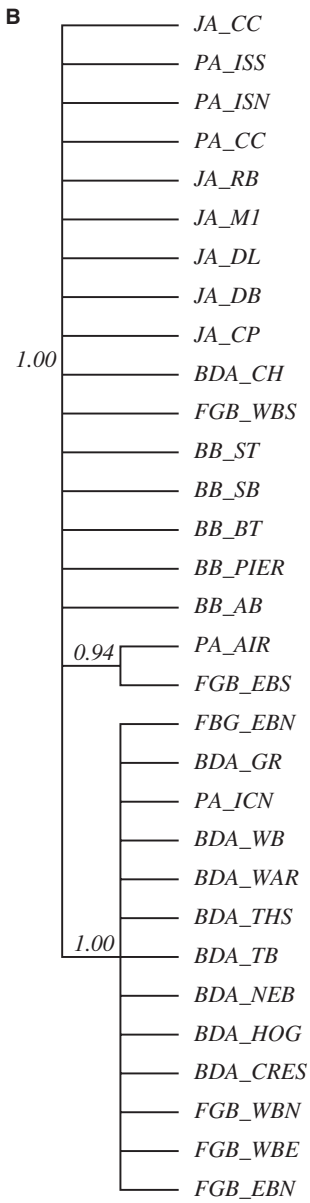
Location	ID	*ITS*	*β-tub*	*IGR*	Location	ID	*ITS*	*β-tub*	*IGR*
	BT3a		HM447555			DB5a		HM447514	
	BT3b		HM447556			DB5b		HM447515	
	BT4a	HM447253	HM447557			DB6a	HM447320		HM447429
	BT4b	HM447254	HM447558			DB6b	HM447321		
Barbados	BT5a		HM447553		Jamaica	DB7	HM447322		HM447430
	BT5b		HM447554			DB8	HM447323		HM447433
	PIERIa		HM447498	HM447352		DL1	HM447324		
	PIERIb		HM447499			DL3	HM447325		HM447434
	PIER2a		HM447516	HM447353		DL4	HM447326		HM447435
	PIER4			HM447354		DL5a		HM447535	HM447436
	PIER5			HM447355		DL5b		HM447536	
	PIER9	HM447256				DL6	HM447327		HM447437
	PIER10	HM447255				M11a	HM447328	HM447489	HM447438
	SB1A	HM447257				M11b		HM447490	
	SB1B	HM447258				M12			HM447439
	SB2	HM447259				M13			HM447440
	SB4	HM447260				M14			HM447441
	SB5a		HM447547			M16			HM447442
	SB5b		HM447548			RB2a		HM447517	HM447445
	ST1b	HM447261	HM447506	HM447356		RB2b		HM447518	
	ST1a		HM447505			RB4	HM447329		HM447446
	ST2			HM447357		RB5a	HM447330	HM447500	
	ST3			HM447358		RB5b		HM447501	
	ST5	HM447262				RB6			HM447443
	ST7	HM447263				RB7			HM447444
	ST8	HM447264							
Bermuda	CH2			HM447359	Panama	AIR1			HM447447
	CH3a	HM447265	HM447494			AIR2			HM447448
	CH3b		HM447495			AIR3	HM447331		HM447449
	CH5a	HM447266	HM447527	HM447360		AIR4a		HM447481	HM447450
	CH5b		HM447528			AIR4b		HM447482	
	CH6			HM447361		AIR5			HM447451
	CH7a	HM447267	HM447509	HM447362		AIR6	HM447332		
	CH7b		HM447510			AIR7	HM447333		
	CH9			HM447363		AIR8a	HM447334	HM447511	HM447452
	CRESIa	HM447268	HM447522			AIR8b		HM447512	
	CRES2	HM447269		HM447364		AIR9a		HM447475	HM447453
	CRES3			HM447365		AIR9b		HM447476	
	CRES4	HM447270				ICN2	HM447341		HM447461
	CRES7			HM447366		ICN3	HM447342		HM447462
	CRES9			HM447367		ICN7	HM447343		HM447463
	GR1			HM447369		ICN8	HM447344		HM447464
	GR2			HM447368		ICN10	HM447340		HM447460
	GR3			HM447370		ISN2a	HM447346	HM447533	HM447466
	HOG3	HM447271				ISN2b		HM447534	
	HOG4	HM447272				ISN4	HM447347		HM447467
	HOG5	HM447273		HM447373		ISN8	HM447348		HM447468
	HOG6			HM447372					
	ISN9			HM447469					
	HOG7			HM447374		ISN10	HM447345		HM447465
	HOG8			HM447375		ISS1	HM447349		
	HOG9			HM447376		ISS2a		HM447507	HM447470
	HOG 10			HM447371		ISS2b		HM447508	

Appendix 2: (Continued)

Location	ID	*ITS*	*β-tub*	*IGR*	Location	ID	*ITS*	*β-tub*	*IGR*
	NEB4			HM447378		ISS4			HM447471
	NEB8	HM447274				ISS7a	HM447350	HM447496	HM447472
	NEB9			HM447379		ISS7b		HM447497	
	NEB10			HM447377		ISS8			HM447473
Bermuda	TB1	HM447275		HM447380	Panama	ISS9	HM447351		HM447474
	TB2			HM447381		CC1	HM447335		
	TB6			HM447382		CC2	HM447336		
	TB7	HM447277		HM447383		CC3	HM447337		HM447455
	TB9	HM447278		HM447385		CC4	HM447338		HM447456
	TB10	HM447276		HM447384		CC5a	HM447339	HM447483	HM447457
	THS4	HM447279		HM447386		CC5b		HM447484	
	THS5a	HM447280		HM447392		CC8a		HM447485	HM447458
	THS5b	HM447281				CC8b		HM447486	
	THS6	HM447282		HM447387		CC9			HM447459
	THS7			HM447388		CC10a		HM447477	HM447454
	THS8a		HM447504	HM447389		CC10b		HM447478	
	THS9a		HM447505	HM447390	Flower Garden Banks	EBN1			HM447403
	THS 10			HM447391		EBN2	HM447295	HM447492	HM447404
	WAR1a	HM447283	HM447513			EBN4	HM447296		HM447402
	WAR2a	HM447284				EBN5	HM447297		HM447405
	WAR2b	HM447285				EBS1			HM447406
	WAR3	HM447286				EBS2a		HM447519	HM447407
	WAR4	HM447287				EBS3			HM447408
	WAR5a	HM447288	HM447524			EBS5a		HM447520	HM447409
	WAR5b	HM447289				EBS5b		HM447521	
	WAR6			HM447394		EBS6a	HM447299		HM447410
	WAR8			HM447395		EBS6a	HM447300		
	WAR9			HM447396		EBS7a		HM447521	
	WAR10			HM447393		EBS8	HM447301		
	WB1			HM447397		WBE1	HM447303		HM447411
	WB4	HM447290		HM447398		WBE2			HM447412
	WB5	HM447291				WBE4			HM447413
	WB6			HM447399		WBE5a	HM447302	HM447538	HM447414
	WB8	HM447292		HM447400		WBE7			
	WB9a	HM447293		HM447401		WBE10			
	WB9b	HM447294				WBN1			HM447415
						WBN2			HM447416

Appendix 3: Strict consensus trees with regional (A) and local (B) subdivisions. Bootstrap values based on 1000 pseudoreplications indicate branch support.





Appendix 4: Jamaica and Panama for *ITS*, *β-tub* and *IGR*, including: number of individual colonies sequenced (*N*), number of haplotypes (Nh), number of polymorphic sites (Np), haplotype diversity (*h*), nucleotide diversity (pn) and the mean number of pairwise differences (pd)

Barbados	Site	*N*	Nh	Np	*h*	pn	pd
ITS	BT	3	3	6	1.0 ± 0.2722	0.004484 ± 0.003811	4.000000 ± 2.725541
	AB	1	1	0	0	0	0
	PIER	2	2	7	1.0 ± 0.5	0.007848 ± 0.008389	7.000000 ± 5.291502
	SB	4	2	1	0.5 ± 0.2652	0.000561 ± 0.000695	0.500000 ± 0.519115
	ST	4	4	9	1.0 ± 0.1768	0.005792 ± 0.004231	5.166667 ± 3.160166
β-tub	AB	10	7	10	0.9111 ± 0.0733	0.007459 ± 0.004562	4.288889 ± 2.319238
	BT	10	4	8	0.7778 ± 0.0907	0.006117 ± 0.003845	3.511111 ± 1.951332
	PIER	4	3	9	0.8333 ± 0.2224	0.009872 ± 0.007146	5.666667 ± 3.435029
	SB	2	2	7	1.00 ± 0.50	0.012195 ± 0.013037	7.00 ± 5.291502
	ST	2	1	0	0	0	0
IGR	PIER	4	1	0	0	0	0
	ST	3	1	0	0	0	0
Bermuda	Site	*N*	Nh	Np	*h*	*p*	np
ITS	CH	3	3	10	1.0000 ± 0.2722	0.007474 ± 0.006051	6.666667 ± 4.327835
	CRES	3	2	4	0.6667 ± 0.3143	0.002990 ± 0.002683	2.666667 ± 1.918994
	HOG	3	2	7	0.6667 ± 0.3143	0.005232 ± 0.004372	4.666667 ± 3.126944
	NEB	1	1	0	0	0	0
	TB	4	4	9	1.0000 ± 0.1768	0.005605 ± 0.004108	5.000000 ± 3.068482
	THS	4	3	4	0.8333 ± 0.2224	0.004297 ± 0.003247	3.833333 ± 2.425439
	WAR	7	6	7	0.9524 ± 0.0955	0.003577 ± 0.002403	3.190476 ± 1.871442
	WB	5	5	9	1.0000 ± 0.1265	0.004933 ± 0.003419	4.400000 ± 2.608429
β-tub	CH	6	5	9	0.9333 ± 0.1217	0.007085 ± 0.004743	4.066667 ± 2.357494
	CRES	2	2	6	1.00 ± 0.50	0.010453 ± 0.011290	6.00 ± 4.582576
	THS	4	3	9	0.8333 ± 0.2224	0.009855 ± 0.007134	5.666667 ± 3.435029
	WAR	4	4	7	1.00 ± 0.1768	0.006377 ± 0.004846	3.666667 ± 2.333333
IGR	CH	5	1	0	0	0	0
	CRES	4	1	0	0	0	0
	GR	3	1	0	0	0	0
	HOG	6		20	0.333 ± 0.2152	0.010515 ± 0.006678	6.666667 ± 3.666667
	NEB	3	1	0	0	0	0
	TB	6	1	0	0	0	0
	THS	7	1	0	0	0	0
	WAR	4	1	0	0	0	0
	WB	5	1	0	0	0	0
Flower Garden Banks	Site	*N*	Nh	Np	*h*	*p*	np
ITS	EBN	4	4	9	1.0000 ± 0.1768	0.005419 ± 0.003985	4.833333 ± 2.976762
	EBS	3	2	7	0.6667 ± 0.3143	0.005232 ± 0.004372	4.666667 ± 3.1269
	WBE	4	4	10	1.0000 ± 0.1768	0.006353 ± 0.004598	5.666667 ± 3.435029
	WBN	6	5	10	0.9333 ± 0.1217	0.005157 ± 0.003400	4.600000 ± 2.626785
	WBS	1	1	0	0	0	0
β-tub	EBN	2	2	7	1.00 ± 0.50	0.012195 ± 0.013037	7.00 ± 5.291502
	EBS	6	4	9	0.8667 ± 0.1291	0.009175 ± 0.005960	5.266667 ± 2.962732
	WBE	2	1	0	0	0	0
	WBN	6	4	9	0.800 ± 0.1721	0.006272 ± 0.004267	3.60 ± 2.121320
	WBS	8	6	9	0.9286 ± 0.0844	0.006035 ± 0.003918	3.464286 ± 1.974020
IGR	EBN	4	2	19	0.500 ± 0.2652	0.014984 ± 0.010429	9.500000 ± 5.537065
	EBS	5	1	0	0	0	0
	WBE	4	1	0	0	0	0
	WBN	6	1	0	0	0	0
	WBS	3	1	0	0	0	0

Appendix 4: (Continued)

Jamaica	Site	*N*	Nh	Np	*h*	pn	pd
ITS	CP	4	4	12	1.0000 ± 0.1768	0.007287 ± 0.005211	6.500000 ± 3.892634
	DB	7	7	8	1.0000 ± 0.0764	0.004324 ± 0.002827	3.857143 ± 2.201334
	DL	4	3	5	0.8333 ± 0.2224	0.002990 ± 0.002381	2.666667 ± 1.778499
	M1	1	1	0	0	0	0
	RB	2	2	7	1.0000 ± 0.5000	0.007848 ± 0.008389	7.000000 ± 5.291502
β-tub	CP	6	2	7	0.3333 ± 0.2152	0.004065 ± 0.002969	2.333333 ± 1.475730
	DB	6	3	7	0.600 ± 0.2152	0.004065 ± 0.002969	2.333333 ± 1.475730
	DL	2	2	6	1.00 ± 0.50	0.010453 ± 0.011290	6.00 ± 4.582576
	M1	2	2	1	1.00 ± 0.50	0.001742 ± 0.002464	1.00 ± 1.00
	RB	4	2	3	0.50 ± 0.2652	0.002613 ± 0.002332	1.50 ± 1.120934
IGR	CP	6	1	0	0	0	0
	DB	4	3	2	0.8333 ± 0.2224	0.001577 ± 0.001563	1.000000 ± 0.829646
	DL	5	1	0			
	M1	5	2	20	0.400 ± 0.2373	0.012618 ± 0.008266	8.000000 ± 4.483030
	RB	5	1	0	0	0	0
Panama	Site	*N*	Nh	Np	*h*	*p*	np
ITS	AIR	4	4	9	1.0000 ± 0.1768	0.005792 ± 0.004231	5.166667 ± 3.160166
	CC	5	4	10	0.9000 ± 0.1610	0.005157 ± 0.003556	4.600000 ± 2.712932
	ICN	5	5	12	1.0000 ± 0.1265	0.006278 ± 0.004239	5.600000 ± 3.234570
	ISN	4	4	7	1.0000 ± 0.1768	0.004297 ± 0.003247	3.833333 ± 2.425439
	ISS	3	2	5	0.6667 ± 0.3143	0.003737 ± 0.0032	3.333333 ± 2.323107
β-tub	AIR	6	2	7	0.5333 ± 0.1721	0.006504 ± 0.004403	3.733333 ± 2.188861
	CC	6	2	7	0.3333 ± 0.2152	0.004065 ± 0.002969	2.333333 ± 1.475730
	ISN	2	2	6	1.00 ± 0.50	0.010453 ± 0.011290	6.00 ± 4.582576
	ISS	4	3	8	0.8333 ± 0.2224	0.007259 ± 0.005429	4.166667 ± 2.609437
IGR	AIR	7	1	0	0	0	0
	CC	6	2	20	0.5333 ± 0.1721	0.016824 ± 0.010331	10.666667 ± 5.672546
	ICN	5	1	0	0	0	0
	ISN	5	1	0	0	0	0
	ISS	5	1	0	0	0	0