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No two reefs are created equal: fine-scale population structure in the threatened coral species *Acropora palmata* and *A. cervicornis*

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ABSTRACT: The population structures of the zooxanthellate corals *Acropora palmata* and *A. cervicornis* were used as a proxy to estimate genetic connectivity between reefs of Puerto Rico. Sequences of the mitochondrial control region were recovered from geographically adjacent and distant reefs inhabited by *A. palmata* (n = 220) and *A. cervicornis* (n = 124). Both species exhibited low levels of mitochondrial nucleotide diversity, a common observation for scleractinian corals. Analysis of molecular variance based on coral colonies collected from 26 reefs of 6 localities suggested that significant population structure exists even between neighboring reefs (*A. palmata*, Φ_{ST} = 0.0863, $p < 0.00098$; *A. cervicornis*, Φ_{ST} = 0.1237, $p < 0.00587$). When all samples from Puerto Rico were combined and compared to samples from Lee Stocking Island, Bahamas, the pairwise genetic distances were also significant. Evidence for population structure in *A. cervicornis* was much stronger when introgressed and native alleles were used in combination rather than alone: *A. cervicornis* from Mona Island was significantly different compared to La Parguera and the Bahamas, but not to Desecheo Island. Recovery of *Acropora* in ecological time might rely on the survival and sexual reproduction of local populations rather than replenishment from distant reefs because of the population subdivision observed at fine geographic scales.

KEY WORDS: Population structure · *Acropora* · Endangered species · Puerto Rico

INTRODUCTION

There has been an unprecedented decline in coral reef cover worldwide over the last 3 decades (Gardner et al. 2003, Bruno & Selig 2007). About 20% of coral reefs have vanished and 16% are severely damaged, while an additional 26% are under threat of a long-term decline (Gardner et al. 2003, Wilkinson 2006). Among the most impacted areas is the Caribbean region (Gardner et al. 2003, Aronson & Precht 2006, Carpenter et al. 2008, Wilkinson & Souter 2008), where the dominant reef-building coral species of the last 500 000 yr have been rapidly disappearing (Gilmore & Hall 1976, Miller et al. 2002). Some of the most rapidly declining corals belong to the genus *Acropora*, which includes 115 species worldwide, the vast majority being distributed in the Pacific (Wallace 1999, Bruno et al. 2007). In the Caribbean, the genus is represented solely by *Acropora palmata* and *A. cervicornis* and the hybrid *A. prolifera*.

Acropora cervicornis, also known as staghorn coral, is a branching hermatypic coral found mostly in patch and barrier reefs around the Caribbean, normally at depths ranging from 3 to 30 m in high-energy areas where fragmentation, due to the coral's thin fragile branches, plays an important role in asexual spreading (Bottjer 1980, Tunnicliffe 1981, Neigel & Avise 1983). *A. cervicornis* is a fast-growing (12 cm yr^{-1}) reef-building species, which provides habitat for a wealth of marine diversity (Tunnicliffe 1981, Bruckner 2002, Precht et al. 2004). *A. palmata* is also a fast-growing coral (5 to 9.5 cm yr^{-1}), but unlike *A. cervicornis*, it is

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thicker and stronger with branches greater than 0.5 m in length and a light tan to brown coloration (Schuhmacher & Plewka 1981). *A. palmata* is normally found at shallow depths ranging from 0 to 15 m (Schuhmacher & Plewka 1981) along the reef crest where wave energy is high, but is also found deeper than 20 m (Zimmer & Precht 2006).

These 2 acroporid species are important not only because they have been the most ecologically and geologically dominant reef-builders, but also because they provide habitat for many reef fishes and invertebrates (Rogers et al. 1982, Vega-Zepeda et al. 2007). However, the deterioration of natural populations of the 2 species is so alarming that both species were listed under the US Endangered Species Act in 2006 (Miller et al. 2002, Precht et al. 2004). The dramatic decline of Caribbean acroporids over the past 3 decades has been attributed to disease, storms, corallivory, hyperthermic stress and pollution (Bruckner 2002, Gardner et al. 2003, Weil 2004, Bruno et al. 2007, Lesser 2007). Despite the Caribbean-wide collapse of acroporids, there is evidence of some recent recovery of *Acropora palmata* at the local scale (Macintyre & Toscano 2007, Zubillaga et al. 2008).

The threatened status of acroporids has generated several studies focusing on the genetic diversity harbored by these species. Van Oppen et al. (2000) examined the species boundaries among *Acropora cervicornis*, *A. palmata* and *A. prolifera* by sequencing portions of the *ITS-1*, *5.8S*, and *PaxC* gene regions. Van Oppen et al. (2001) and Vollmer & Palumbi (2002) studied the genetic footprint of hybridization between these species using nuclear intronic regions and the mitochondrial control region. Baums et al. (2005a) reported high levels of clonality in *A. palmata* in 3 reefs of Key Largo, Florida, whereas in Caribbean-wide studies, *A. palmata* (Baums et al. 2005b, 2006a) and *A. cervicornis* (Galindo et al. 2006, Vollmer & Palumbi 2007) exhibited little or no recent genetic exchange between the western and eastern Caribbean.

The purpose of the present study was to estimate the genetic variability and the population structure of *Acropora palmata* and *A. cervicornis* in the coastal reefs of Puerto Rico. We examined the patterns of variation in the mitochondrial control region as a neutral genetic marker. Our sampling regime emphasized southwest and west Puerto Rico, because the targeted area includes the highest number and largest total area of Marine Protected Areas in Puerto Rico (Aguilar-Perera et al. 2006, their Fig. 1). Information on the levels of population connectivity can be particularly useful in determining the chances of recolonization of reefs by larvae from other populations (Cowen et al. 2006, Hellberg et al. 2002, Zubillaga et al. 2008) and improving the design of marine reserves (Palumbi 2003). Inclusion of geographically separated populations that are connected genetically should be favored in a marine park network such as the one present in Puerto Rico instead of populations with restricted gene flow relying exclusively on self-replenishment. Because of the geographic proximity of sampling areas, we hypothesized that high levels of connectivity would be uncovered. An additional reason for the dense sampling of the area was Puerto Rico's proximity to Mona Passage, a suggested biogeographic barrier to marine fauna (Colin 2003). We hypothesized that the islands of Mona and Desecheo will harbor genetically disconnected populations from the main island of Puerto Rico.

MATERIALS AND METHODS

Study area and sampling locations. A total of 220 *Acropora palmata* and 124 *A. cervicornis* colonies were sampled from 26 reefs at 6 localities in Puerto Rico during 2005 and 2006 and 3 reefs from Lee Stocking Island, Bahamas during 2005 (Figs. 1 & 2, Table 1). Sample locations included Enrique, Laurel, San Cristóbal, Media Luna, Turrumote, Atravesado, El Palo and Margarita reefs in La Parguera Natural Reserve on the southwest coast of Puerto Rico (Table 1). For reference

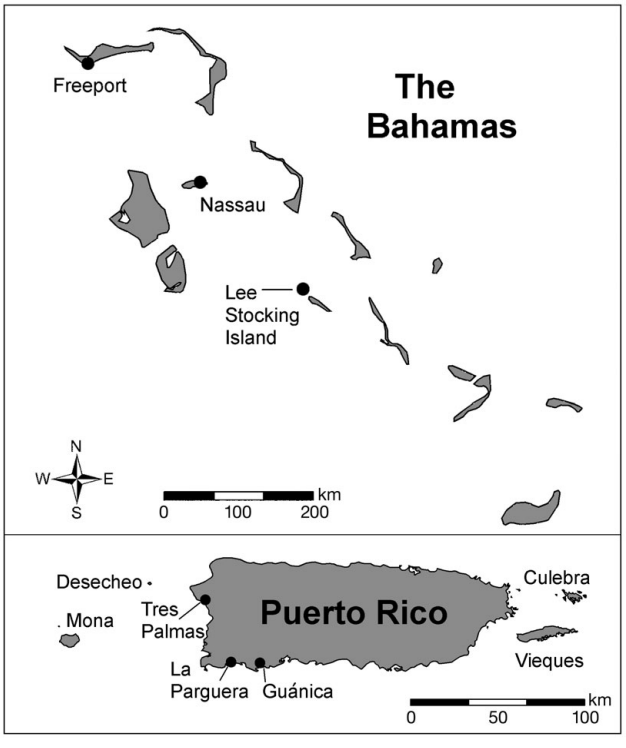

Fig. 1. Sampling localities including La Parguera, Guánica, Tres Palmas, Desecheo Island, Mona Island, Culebra and Vieques from Puerto Rico, and Lee Stocking Island, Bahamas

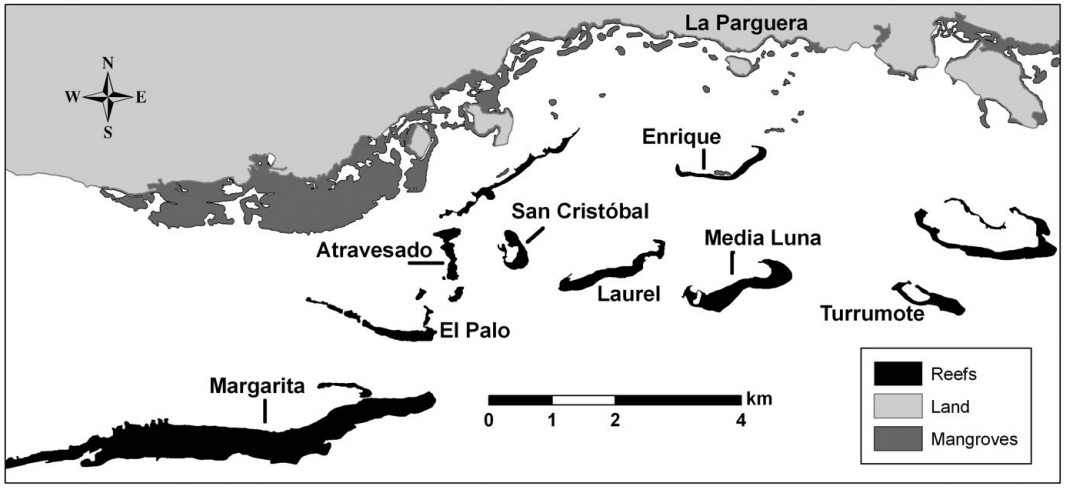

Fig. 2. La Parguera, southwestern Puerto Rico, showing the sampled reefs

Table 1. Sample localities of *Acropora* spp. PR: Puerto Rico; BH: Bahamas. 'Circle' and 'Random' represent the 2 sampling methods. na: no samples were processed

Locality	Reef	Latitude	Longitude	No. of samples	
				Circle	Random
A. palmata					
La Parguera, PR	Laurel	17° 56′ 39″ N	67° 03′ 22″ W	12	6
	Turrumote	17° 56′ 05″ N	67° 01′ 03″ W	11	7
	Media Luna	17° 56′ 29″ N	67° 02′ 25″ W	16	8
	Margarita	17° 55′ 12″ N	67° 05′ 59″ W	13	13
	El Palo	17° 56′ 02″ N	67° 06′ 02″ W	na	12
	Enrique	17° 57′ 18″ N	67° 02′ 37″ W	17	7
Mona Island, PR	Reef 1	18° 4′ 29″ N	67° 51′ 10″ W	na	5
	Reef 2	18° 4′ 11″ N	67° 51′ 21″ W	na	5
	Reef 3	18° 3′ 57″ N	67° 51′ 23″ W	na	2
	Reef 4	18° 5′ 20″ N	67° 56′ 21″ W	na	3
Desecheo Island, PR	Reef 1	18° 22′ 52″ N	67° 29′ 10″ W	na	4
Culebra, PR	Reserva Canal Luis Pena	18° 19′ 14″ N	65° 19′ 23″ W	na	2
Vieques, PR		18° 06′ 27″ N	65° 34′ 20″ W	na	2
Tres Palmas, PR		18° 21′ 01″ N	67° 15′ 56″ W	32	9
Guánica, PR		17° 56′ 26″ N	66° 52′ 07″ W	9	na
Lee Stocking Island, BH	Reef 1	23° 46′ 51″ N	76° 06′ 13″ W	na	4
	Reef 2	23° 47′ 23″ N	76° 08′ 16″ W	na	4
	Reef 3	23° 53′ 53″ N	76° 15′ 37″ W	na	5
A. cervicornis					
La Parguera, PR	Media Luna	17° 56′ 19″ N	67° 03′ 03″ W	23	15
	San Cristóbal	17° 56′ 39″ N	67° 04′ 38″ W	22	8
	Atravesado	17° 56′ 21″ N	67° 05′ 12″ W	12	1
	Laurel	17° 56′ 39″ N	67° 03′ 20″ W	2	8
Mona Island, PR	Reef 1	18° 04′ 15″ N	67° 51′ 11″ W	na	1
	Reef 2	18° 03′ 57″ N	67° 51′ 22″ W	na	2
	Reef 3	18° 02′ 56″ N	67° 52′ 21″ W	na	5
	Reef 4	18° 04′ 37″ N	67° 56′ 31″ W	na	2
	Reef 5	18° 06′ 12″ N	67° 56′ 47″ W	na	2
	Reef 6	18° 06′ 12″ N	67° 56′ 47″ W	na	3
	Reef 7	18° 06′ 12″ N	67° 56′ 47″ W	na	1
	Reef 8	18° 03′ 18″ N	67° 51′ 38″ W	na	1
Desecheo Island, PR	Reef 1	18° 22′ 52″ N	67° 29′ 10″ W	na	4
	Reef 2	18° 22′ 42″ N	67° 29′ 04″ W	na	2
	Reef 3	18° 22′ 52″ N	67° 29′ 10″ W	na	1
Culebra, PR	Reserva Canal Luis Pena	18° 19′ 14″ N	65° 19′ 23″ W	na	1
Lee Stocking Island, BH	Reef 1	23° 46′ 51″ N	76° 06′ 13″ W	na	2
	Reef 2	23° 47′ 23″ N	76° 08′ 16″ W	na	2
	Reef 3	23° 53′ 53″ N	76° 15′ 37″ W	na	2

samples we used *Acropora* tissue from 6 other Puerto Rico locations: Guánica, Tres Palmas Marine Reserve in Rincón, Mona and Desecheo Islands on the west coast and Culebra and Vieques Islands on the east coast. No samples of *A. cervicornis* were collected from Tres Palmas Marine Reserve in Rincón, Guánica or Vieques.

Two sampling methods were used. (1) Circle method. We sampled each available colony using the concentric circle design with a 5 m radius for *Acropora palmata* (Baums et al. 2005a) and a ~10 m radius for *A. cervicornis*, since patches of the latter species were more distantly separated. We restricted our circles to 5 and 10 m because circles of a larger radius do not further contribute to the genotypic variation (Baums et al. 2005a). At least 2 circles were sampled at most reefs (Table 2). (2) Random method. From each reef, we randomly collected tissue from at least 15 colonies of each species at least 5 m apart in order to reduce the probability of collecting clones. Each circle was marked with a GPS coordinate, and the randomly collected colonies were collected far from where the circle method was implemented to avoid sampling colonies twice.

Tissue collection and DNA extraction. Tissue was collected by divers using either snorkels or SCUBA. Using a pair of tweezers, a small piece with 3 or 4 individual polyps, along with the epitheca, was collected from each *Acropora cervicornis* and *A. palmata* colony. The polyps were then suctioned up by a 5 ml plastic pipette and secured with a rubber band, or put directly into a 1.5 ml centrifuge tube whenever feasible. DNA was extracted from freshly collected and ethanol-preserved specimens using a Puregene DNA Purification Kit (Gentra Systems), following the protocol for DNA purification from 5 to 10 mg of solid tissue fixed in ethanol or formalin.

PCR and sequencing conditions. The PCR mix for the control region was identical for *Acropora cervicornis* and *A. palmata*, and contained 1.0 µl of each primer (10 µM μl^{-1}), 5.0 µl of $MgCl_2$ (Promega 25 mM), 0.5 µl of deoxynucleoside triphosphates (dNTPs, 25 mM), 5.0 µl of 10× PCR Buffer (Promega), 1 unit of *Taq* and 36.2 µl of double distilled H_2O (ddH_2O) in each tube. Reactions were run using 1.0 to 2.0 µl of DNA template, which was balanced out by adding or subtracting ddH_2O in order to reach a final volume of 50 µl in each PCR tube. The PCR amplicons were subjected to electrophoresis in a 1% agarose gel and catalogued digitally. PCR reactions were then cleaned from excess dNTPs, primers and other impurities by the enzymatic treatment ExoSap.

Table 2. *Acropora* spp. Summary of the concentric circle collection method. Number of colonies (n) and number of haplotypes (*h*) per circle for each species

Species	Location	Circle 1		Circle 2		Circle 3	
		n	*h*	n	*h*	n	*h*
A. palmata	Tres Palmas	14	5	11	4	7	3
	Laurel	7	3	5	2		
	Margarita	9	5	4	4		
	Turrumote	4	1	7	2		
	Enrique	6	5	11	3		
	Media Luna	8	4	8	2		
	Guánica	5	2	4	1		
A. cervicornis	San Cristóbal	11	2[a]	10	1		
	Media Luna	9	3	14	3		

[a]Two haplotypes were recovered from the same colony

The PCR conditions were identical for *Acropora cervicornis* and *A. palmata*: initial denaturation at 94°C for 3 min, then 35 cycles at 94°C for 15 s, annealing at 46°C for 30 s, extension at 72°C for 45 s and the final extension at 72°C for 5 min. Sequencing reactions were prepared using a DYEnamic ET Terminator Cycle Sequencing Kit (GE) and loaded into a MEGABase 96 lane sequencer for capillary electrophoresis. DNA sequencing trace files were processed using the Phrap/Phred/Consed programs (Ewing & Green 1998, Ewing et al. 1998, Gordon 2003) for base calling, quality assessment, contig assembly and visualization. Edited DNA sequences were imported into MacClade (Maddison & Maddison 2000) to derive a homologous alignment.

Genetic analyses. The genetic divergences within and among reefs and within and among localities were calculated using Arlequin v. 3.11 (Excoffier et al. 2005). Analysis of molecular variance (AMOVA) was used to examine the partition of variance within and between samples (Excoffier et al. 1992). AMOVAs were carried out separately for samples collected using the concentric circle and random methods to compare the effects of the different sampling methods. In some cases, some reefs were sampled by only one collection method and, thus, excluded from analysis. For AMOVA, we did not include sequences from other studies. *Acropora cervicornis* samples from Culebra were left out of the AMOVA analysis because only 1 colony was successfully sequenced. Nuclear diversity indices (π and θ) and haplotype diversity (h_d) were estimated using the program DnaSP v. 4.10 (Rozas et al. 2003). In order to evaluate the differences in genetic diversity between the 2 collection methods, the diversity indices were calculated separately for each method. However, to capture all of the variation in a population, diversity indices were also calculated combining the 2 collection methods, since in some instances the circle method displayed higher diversity than the random method.

Haplotypes from each species were imported into PAUP* v. 4.0b10 (Swofford 2002) to construct maximum likelihood genealogies with estimated model parameters and 500 bootstrap replicates using the fast

step-wise search. The most suitable model of sequence evolution for each species was derived using the hierarchical likelihood ratio tests in ModelTest v. 3.06 (Posada & Crandall 1998). Haplotype networks for each species were constructed using the statistical parsimony function in TCS v. 1.21 (Templeton et al. 1992, Clement et al. 2000). For the construction of haplotype networks, alignment gaps were considered as a fifth state.

RESULTS

Haplotype diversity

The amplified control region after quality verification and trimming was approximately 959bp in length for *Acropora palmata* and 1062 bp for *A. cervicornis*. There were 7 and 12 transitions and 3 and 8 transversions in *A. palmata* and *A. cervicornis*, respectively.

We identified 25 haplotypes for *Acropora palmata* in Puerto Rico and 2 in the Bahamas (GenBank accession nos. GQ421854–GQ421864; GQ421867–GQ421880). The highest number of haplotypes (n = 10) were found at Margarita reef followed by Enrique, Laurel, and Tres Palmas (n = 9) (Table S1 in the Supplement at www.int-res.com/articles/suppl/b010p069_supp.pdf). The smallest number of haplotypes was recovered at reefs with the smallest number of samples (e.g. Guánica, Vieques). Out of 117 colonies of *A. cervicornis*, we identified 24 haplotypes for Puerto Rico and 4 for the Bahamas (GenBank accession nos. GQ421881–GQ421893, GQ421896–GQ421908), twice as many as previously identified for Puerto Rico (Vollmer & Palumbi 2007). In addition to the present data, DNA sequences from Puerto Rico specimens published in Vollmer & Palumbi (2007) (AF507194–AF507196, AF507202–AF507207 and AF507290–AF507309 for *A. cervicornis*; and AF507220–AF507238, and AF507253–AF507255 for *A. palmata*) were included in the construction of the haplotype networks and maximum likelihood genealogies.

One-way gene flow between *Acropora cervicornis* and *A. palmata* in the past has caused the introgression of partial *A. palmata* sequences in *A. cervicornis* (Vollmer & Palumbi 2007). Alleles that did not incorporate *A. palmata* sequences were referred to as native alleles and those that did were referred to as introgressed alleles. The highest number of haplotypes (n = 8) were found at Media Luna reef followed by Atravesado (n = 7) and Mona Island (n = 6; Table S2 in the Supplement). The smallest number of *Acropora cervicornis* haplotypes was found at San Cristóbal (n = 3), despite the large number of surveyed colonies (n = 30). In La Parguera, one additional haplotype of *A. palmata* was observed at Media Luna and Guánica and 6 more haplotypes of *A. cervicornis* were observed at Media Luna and 2 less at San Cristóbal compared to those reported by Vollmer & Palumbi (2002). The circle collection method resulted in highly variable numbers of haplotypes; the lowest numbers of haplotypes were recorded at San Cristóbal (same *A. cervicornis* haplotype in all colonies) (Table 2). In *A. palmata*, there were instances where almost all colonies had different haplotypes (e.g. Margarita, Enrique). In contrast, the circle method at Turrumote yielded few haplotypes (Table 2).

mtDNA diversity indices

The haplotypic diversity of *Acropora palmata* from Puerto Rico was relatively high (h_d = 0.333) and the nucleotide diversity low (π = 0.00075). The overall genetic diversity for *A. palmata* in La Parguera was low (π = 0.00071). Samples from 117 colonies of *A. cervicornis* around Puerto Rico resulted in a slightly higher haplotype diversity (h_d = 0.853) and slightly lower nucleotide diversity (π = 0.0050) than those reported previously from a Caribbean-wide study (276 colonies, h_d = 0.847 and π = 0.0057; Vollmer & Palumbi 2007). Similarly low values were detected in *A. cervicornis* (introgressed and native alleles, π = 0.00512; native alleles, π = 0.0012). When reefs in La Parguera were compared, the highest values of π and θ in *A. palmata* were from samples collected at Enrique, and the lowest were from El Palo (Table 3). For comparison, the present values of nucleotide diversity at Media Luna (π = 0.00055) are higher than those previously reported (π = 0.00026; Vollmer & Palumbi 2002); however, 3 times more colonies were sampled in the present study (Table 3). When all regions were compared for *A. palmata*, the Bahamas showed the highest nucleotide diversity, followed by Mona Island and La Parguera (Table 3). The highest values of π and θ in *A. cervicornis* were found at Laurel and the lowest at San Cristóbal (Table 3). Even though twice as many colonies were sampled from San Cristóbal compared to those sampled from the same reef in Vollmer & Palumbi (2002), much lower values of nucleotide diversity were recovered in the present study, regardless of whether the calculations were based on introgressed and native alleles combined or solely on native alleles (Table 3). The lowest number of colonies with introgressed alleles was observed at San Cristóbal (0 of 30) and the highest at Atravesado (12 of 13) and Media Luna (31 of 38). More than half of the *A. cervicornis* in La Parguera (48 of 90) carried the introgressed alleles. We found 2 colonies with introgressed alleles at Mona and Desecheo Islands. *A. cervicornis* showed the highest nucleotide diversity in La Parguera followed by

Table 3. *Acropora* spp. DNA summary statistics of the control region. Gaps were included in the determination of haplotypes. V&P: control region sequences from Vollmer & Palumbi (2002). *S*: segregating sites; *h*: number of haplotypes; π: average no., and θ: expected no., of differences between pairs of sequences in the sample

Location		No. colonies	*S*	*h*	π	θ
A. palmata						
Tres Palmas	Circle	32	3	8	0.00082	0.00079
	Random	9	1	6	0.00047	0.00041
	All	41	3	9	0.00075	0.00075
Laurel	Circle	12	2	4	0.00027	0.00078
	Random	6	1	5	0.00035	0.00046
	All	18	3	9	0.00048	0.00096
Margarita	Circle	13	3	8	0.00102	0.00105
	Random	13	2	7	0.00045	0.00071
	All	26	3	10	0.0006	0.00085
Turrumote	Circle	11	0	2	0	0
	Random	7	3	6	0.00160	0.00143
	All	18	3	6	0.00061	0.00093
Enrique	Circle	17	3	6	0.00083	0.00096
	Random	7	1	4	0.0002	0.00057
	All	24	3	9	0.00105	0.00086
Media Luna	Circle	16	2	5	0.00069	0.00063
	Random	8	1	3	0.0003	0.00046
	All	24	2	6	0.00055	0.00057
El Palo	Random	12	1	5	0.0004	0.00036
La Parguera	Random	53	4	12	0.0005	0.00094
	Circle	69	5	16	0.00085	0.0011
	All	121	6	20	0.00071	0.00118
Guánica	Circle	9	1	3	0.00012	0.0005
Mona Island		15	2	6	0.00096	0.00064
Desecheo Island		4	1	2	0.00053	0.00057
Bahamas		13	4	6	0.00124	0.00136
V&P	All	22	1	2	0.00018	0.00029
	Media Luna	8	1	2	0.00026	0.00041
	Guánica	7	0	1	0	0
	San Cristóbal	4	0	1	0	0

Location		No. colonies	*S*	*h*	π	θ
***A. cervicornis* (introgressed and native alleles combined)**						
San Cristóbal	Circle	22	0	2	0	0
	Random	8	1	2	0.00024	0.00037
	All	30	1	3	0.00006	0.00024
Media Luna	Circle	23	15	6	0.00486	0.00386
	Random	15	5	3	0.00074	0.00146
	All	38	15	8	0.00468	0.00339
Laurel	All	10	11	4	0.00574	0.00369
Atravesado	All	13	16	76	0.00485	0.00491
La Parguera	Random[a]	31	16	8	0.00461	0.00381
	Random[b]	44	18	14	0.00525	0.00394
	Circle	59	18	13	0.005	0.00366
	All	90	18	19	0.00512	0.00337
Mona Island		17	14	6	0.00281	0.00393
Desecheo Island		7	8	5	0.0038	0.0031
Bahamas		6	10	4	0.00373	0.00415
V&P	All	19	13	6	0.0029	0.00353
	Media Luna	4	2	2	0.00095	0.00104
	San Cristóbal	15	12	4	0.00303	0.0035
***A. cervicornis* (native alleles only)[c]**						
San Cristóbal	Circle	22	0	2	0	0
	Random	8	1	2	0.00024	0.00037
	All	30	1	3	0.00006	0.00024
Media Luna	Circle	7	4	2	0.00181	0.00155
Laurel	Random	5	0	1	0	0
Atravesado		1	0	1	0	0
La Parguera	Random[b]	13	2	3	0.00063	0.00061
	Circle	29	6	4	0.00145	0.00145
	All	42	6	6	0.00121	0.00132
Mona Island		15	6	4	0.00128	0.00175
Desecheo Island		5	3	4	0.00133	0.00137
Bahamas		1	0	1	0	0
V&P	All	17	3	4	0.0013	0.00084
	Media Luna	4	2	2	0.00095	0.00104
	San Cristóbal	13	2	2	0.00097	0.00061

[a]Without Atravesado; [b]with Atravesado; [c]no. of colonies includes only those colonies with native alleles

Desecheo Island, the Bahamas and Mona Island (Table 3). When using native mtDNA, Desecheo Island displayed the highest genetic diversity, followed by Mona Island and La Parguera (Table 3). None of the neutrality tests in *A. palmata* and *A. cervicornis* were significantly different from equilibrium, except at Laurel for *A. cervicornis* (Tajima's $D = -2.48296$, $p < 0.05$), indicative of a past population expansion.

AMOVA for both collection methods, combined and separately

Both collection methods displayed significant population differentiation, but the random method was more robust in detecting differentiation between more than 2 reefs. Sampling colonies at least 5 m apart decreased the chances of sampling genetic clones, ensuring a better estimate of the genetic variability present in a reef. In *Acropora palmata*, the overall Φ_{ST} (0.0863) was significant ($p < 0.00098$) for Puerto Rico (Mona Island, Desecheo Island, La Parguera, Tres Palmas and Guánica) when samples from both collection methods were combined (Appendix 1). Significant population structure was also observed with either of the methods (random, $\Phi_{ST} = 0.1156$, $p < 0.002$; circle, $\Phi_{ST} = 0.0957$, $p < 0.0001$) (Appendix 1). A comparison among reefs in La Parguera showed significant population differentiation with both the random ($\Phi_{ST} = 0.1558$, $p < 0.0225$) and circle method ($\Phi_{ST} = 0.3806$, $p < 0.0001$) (Appendix 1). Pairwise comparisons among all reefs in La Parguera for *A. palmata* showed significant differentiation, except those colonies of Laurel vs. Media Luna and vs. Margarita collected with the circle method (Table 4). The smallest distance between reefs was 1.10 km (Margarita and

Table 4. *Acropora palmata*. Pairwise comparisons (a) between reefs in La Parguera, Puerto Rico (concentric circle collection method) and (b) between regions (random collection method). Values were generated with the Kimura 2-P model of substitution. na: location not sampled with that particular collection method; *p < 0.05, **p < 0.001

(a) Between reefs in La Parguera, PR	Laurel	Turrumote	Enrique	Media Luna	Margarita	El Palo
Laurel	–	0.7717**	0.3285**	−0.1331	0.1202	na
Turrumote	0.3749*	–	0.6795**	0.4272**	0.2774*	na
Enrique	0.365*	0.2867*	–	0.3530**	0.425**	na
Media Luna	0.1828	0.2792**	−0.1139	–	−0.0146	na
Margarita	0.0677	0.3027**	−0.0549	−0.0902	–	na
El Palo	0.3437*	0.2432	−0.1965	−0.0302	0.027	–
(b) Between regions	Mona Island	Desecheo Island	La Parguera	Tres Palmas	Guánica	Bahamas
Mona Island	–	na	na	na	na	na
Desecheo Island	0.1404	–	na	na	na	na
La Parguera	0.1056*	0.5529**	–	0.02926	0.0038	na
Tres Palmas	0.0508	0.581*	−0.0494	–	−0.0599	na
Guánica	na	na	na	na	–	na
Bahamas	−0.04371	0.03254	0.10593*	0.02709	na	–

Table 5. *Acropora palmata.* Corrected (Kimura 2-P model) pairwise comparisons between reefs around Puerto Rico. La Parguera All includes samples collected using both collection methods. *p < 0.05, **p < 0.001

	Mona Island	Desecheo Island	La Parguera All	Tres Palmas
Mona Island				
Desecheo Island	0.1404			
La Parguera All	0.1166*	0.4832**		
Tres Palmas	0.0976*	0.4519**	0.0007	
Guánica	0.0108	0.7234*	−0.0254	−0.0751

El Palo) and the greatest distance was 8.5 km (Margarita and Turrumote). The most genetically disconnected reefs were Turrumote and Laurel. Pairwise comparisons among regions showed significant differentiation in *A. palmata* between Mona Island and most other Puerto Rico locations and between La Parguera and the Bahamas (Table 4). When both collection methods were combined for *A. palmata* in La Parguera, significant pairwise differences were detected between western/southwestern Puerto Rico (Tres Palmas and La Parguera) and Desecheo and Mona Islands in western Puerto Rico (Table 5). When all samples from Puerto Rico were combined and compared to samples from Lee Stocking Island, Bahamas, for *A. palmata*, the overall Φ_{ST} was also significant (0.0726, p < 0.04492) (Appendix 1). The significantly high Φ_{ST} values indicate that there is restriction of gene flow both among reefs within La Parguera, a small natural reserve in southwestern Puerto Rico (Fig. 2), and among sampled regions separated by several hundred kilometers (Fig. 1), agreeing with results from previous studies at some of the same reefs (Vollmer & Palumbi 2002, 2007).

The AMOVA results for *Acropora cervicornis* suggested population trends similar to those of *A. palmata*.

Table 6. *Acropora cervicornis.* Pairwise comparisons (a) between reefs in La Parguera, Puerto Rico (concentric circle collection method) and (b) between regions (random collection method), using introgressed and native alleles combined/native alleles alone. Values were generated with the Kimura 2-P model of substitution. na: location not sampled with that particular collection method. *p < 0.05, **p < 0.001

(a) Between reefs in La Parguera, PR	Media Luna	San Cristóbal	Atravesado	Laurel
Media Luna	–	0.5302**/0.8438**	0.0950/0.6083**	na
San Cristóbal	0.9278**/na	–	0.8055**/1.000*	na
Atravesado	0.5114**/na	0.6831**/0.9395	–	na
Laurel	0.7381**/na	0.3076**/0.8615**	0.4580**/1.0000	–
(b) Between regions	Mona Island	Desecheo Island	Bahamas	La Parguera
Mona Island	–	na	na	na
Desecheo Island	0.0067/−0.051	–	na	na
Bahamas	0.5375**/0.417	0.3899*/0.417	–	na
La Parguera	0.2688**/0.0353	0.1422*/0.1899*	0.0671/0.5440*	–

Population structure was detected among Puerto Rico locations (Mona Island, Desecheo Island, La Parguera; Φ_{ST} = 0.1237, p < 0.0059) (Appendix 1). A comparison in the mtDNA (combined introgressed and native alleles) of *A. cervicornis* among reefs in La Parguera (Media Luna, San Cristóbal, Atravesado and Laurel) displayed significant population structure (random, Φ_{ST} = 0.6665, p < 0.0001; circle, Φ_{ST} = 0.5098, p < 0.0001) (Appendix 1). Genetic differentiation was also observed in La Parguera when using the native alleles (Appendix 1). Almost all pairwise comparisons of randomly collected *A. cervicornis* in La Parguera were significant, using the introgressed and native alleles combined (Table 6). When using only the samples collected by the circle method, differences were observed between all sampled La Parguera reefs (except Media Luna vs. Atravesado) with both the introgressed and native alleles combined and native alleles alone (Table 6). Pairwise comparisons in *A. cervicornis* showed significant differentiation between reefs separated by a few kilometers in La Parguera (Table 6). The smallest geographic distance between reefs was 1.21 km (Laurel and San Cristóbal) and the greatest distance was 4.37 km (Atravesado and Media Luna). As expected, the random collection method was more efficient in revealing significant population structure.

When *Acropora cervicornis* samples from both collection methods were pooled in La Parguera, significant pairwise population differences were detected between Mona Island and La Parguera (introgressed and native alleles combined, Φ_{ST} = 0.1598, p < 0.05) and between Desecheo and La Parguera (native alleles only, Φ_{ST} = 0.148, p < 0.05). The comparison among regions (La Parguera, Mona Island, Desecheo Island and the Bahamas) in the mtDNA also reflected significant population structure (random, Φ_{ST} = 0.2284, p < 0.0001; circle, Φ_{ST} = 0.1678, p < 0.00098) (Appendix 1). Differentiation was detected between populations of *A. cervicornis* in pooled Puerto Rico samples versus Lee Stocking Island, Bahamas (Φ_{ST} = 0.1840, p < 0.0244) (Appendix 1). Additional pairwise population comparisons revealed significant differentiation between Mona Island and the Bahamas, Mona Island and La Parguera, and Desecheo Island and the Bahamas (Table 6).

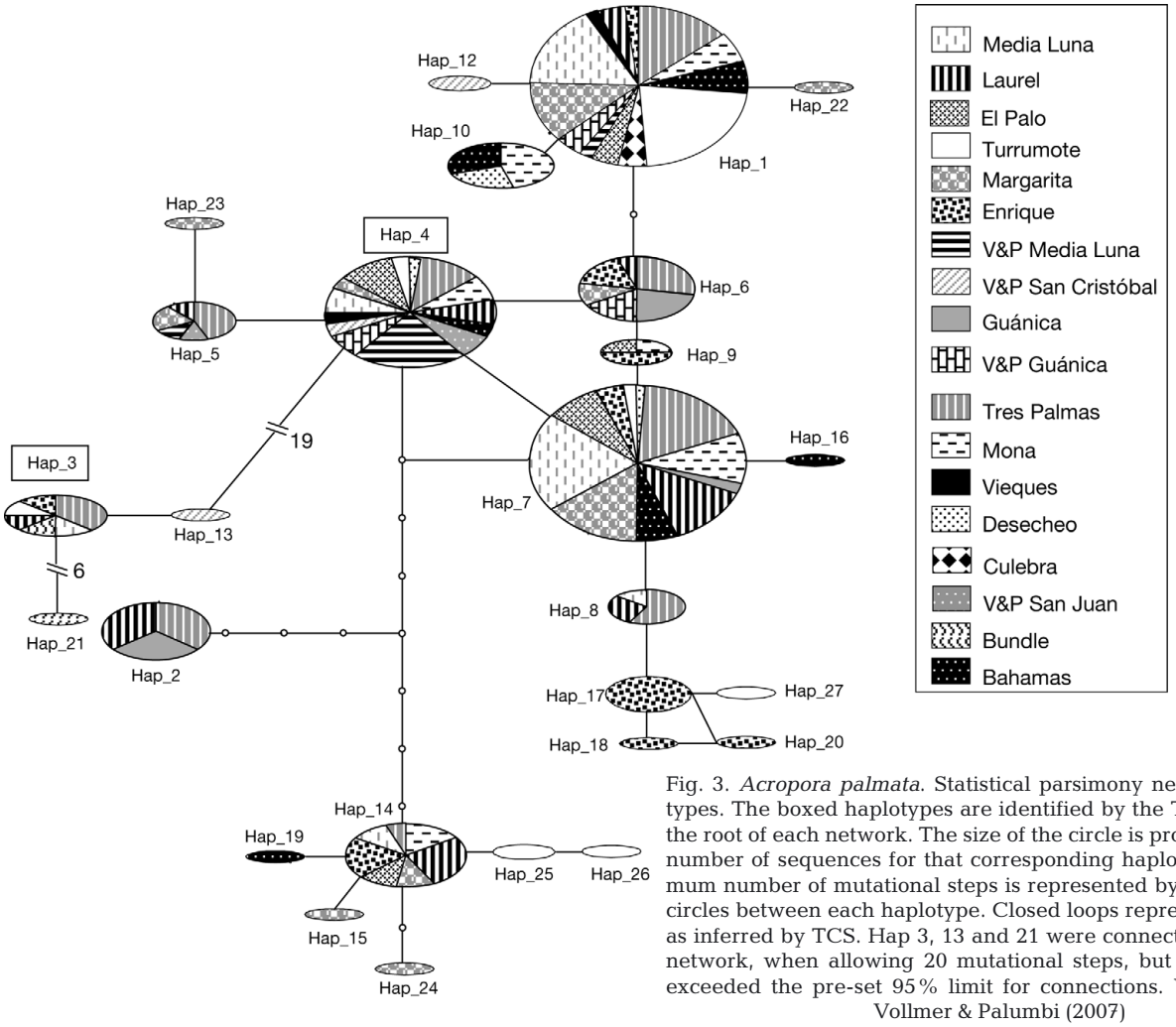

Fig. 3. *Acropora palmata*. Statistical parsimony network of haplotypes. The boxed haplotypes are identified by the TCS program as the root of each network. The size of the circle is proportional to the number of sequences for that corresponding haplotype. The minimum number of mutational steps is represented by the small open circles between each haplotype. Closed loops represent homoplasy as inferred by TCS. Hap 3, 13 and 21 were connected to the main-network, when allowing 20 mutational steps, but the divergence exceeded the pre-set 95% limit for connections. V&P: data from Vollmer & Palumbi (2007)

Meanwhile, the native mtDNA in *Acropora cervicornis* displayed significant population structure among adjacent reefs but not among regions (Appendix 1), which is counterintuitive. Comparisons among reefs (Laurel, San Cristóbal and Atravesado) demonstrated significant population structure (random, Φ_{ST} = 0.919, $p < 0.0001$; circle, Φ_{ST} = 0.8564, $p < 0.0001$). Native mtDNA showed significant differentiation in La Parguera versus Desecheo Island and La Parguera versus the Bahamas only when the colonies were randomly sampled (Table 6).

Gene genealogies

Gene genealogies were constructed in PAUP using maximum likelihood with models Hasegawa-Kishino-Yano (HKY) and K81uf+I+Γ (Kimuras' 1981 model with unequal base frequencies + proportion of invariable sites + Gamma distribution) as the most suitable models of substitution for *Acropora palmata* and *A. cervicornis*, respectively. The HKY model was implemented with unequal base frequencies (0.2411, 0.1618, 0.2701, 0.3270) for *A. palmata* and the K81uf+I+Γ model was implemented with unequal base frequencies (0.3328, 0.2651, 0.1749, 0.2272, I = 0.9730, Γ = 41.6402) for *A. cervicornis*. The resolution of the maximum likelihood tree for *A. palmata* was low and the topology was not informative (data not shown). Parsimony networks were constructed for *A. palmata* (Fig. 3) and *A. cervicornis* (Fig. 4). Hap_1 (20%), Hap_7 (20%) and Hap_4 (11%) are the most widespread and numerous of all haplotypes found in *A. palmata*. These 3 haplotypes were found in almost every reef and region sampled (Fig. 3). The maximum likelihood tree (data not shown) and parsimony network analysis reflected the presence

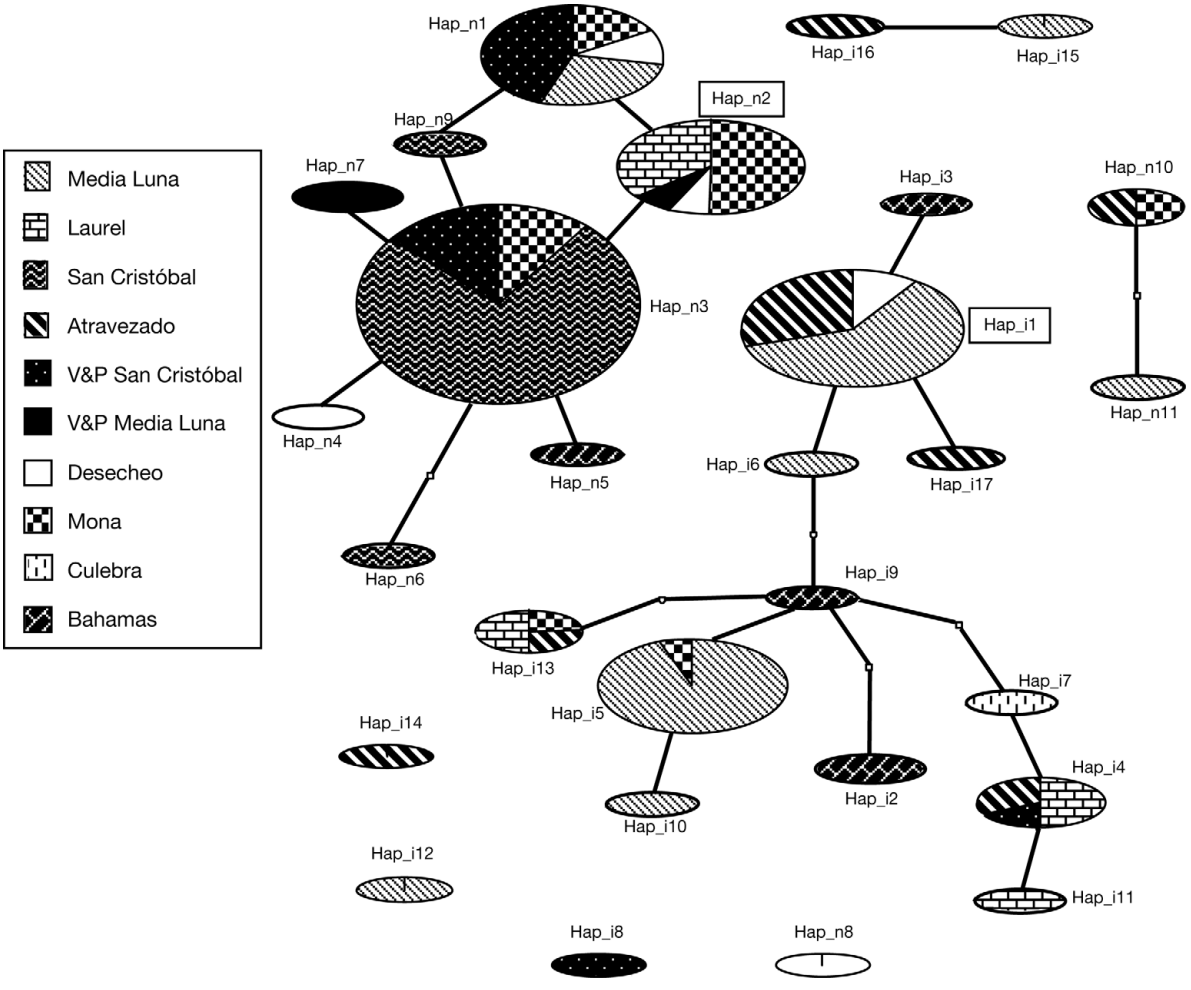

Fig. 4. *Acropora cervicornis*. Statistical parsimony network of haplotypes. The boxed haplotypes are identified by the TCS program as the root of each network. n: native alleles; i: introgressed alleles. The size of the circle is proportional to the number of sequences for that corresponding haplotype. The minimum number of mutational steps is represented by the small open circles between each haplotype. All haplotypes were joined by using the 95% connection limit. V&P: data from Vollmer & Palumbi (2007)

of native and introgressed alleles in *A. cervicornis* (Fig. 4). The native and introgressed alleles formed 2 well-supported monophyletic clades (Fig. 4). Hap_n3 (26%), Hap_n1 (13%) and Hap_n2 (10%) were the most common native haplotypes, while Hap_i1 (14%) and Hap_i5 (12%) were the most common introgressed haplotypes in the sampling area.

DISCUSSION

Structure among reefs in La Parguera, Puerto Rico

Tests for the presence of population structure demonstrated that there is significant differentiation, both between regions and reefs in *Acropora palmata* and between reefs in *A. cervicornis*, regardless of which collection method was used (Appendix 1). Population structure was detected in La Parguera for both species, suggesting restriction of gene flow between some reefs in close proximity. At small geographic scales comparable to those found in the La Parguera reef system, larval dispersal may be greatly influenced by the local oceanographic conditions and the shape and topology of the reef. The island mass effect, described by Hamner & Hauri (1981), could explain the unexpected patterns of genetic connectivity observed locally since it takes into consideration many variables that cause the water flow to vary around islands and reefs. Current speed, tidal flow, size of islands or reefs, depth and type of substratum are some variables which may affect the distribution and abundance of organisms (Hamner & Hauri 1981, Sammarco & Andrews 1989, McGehee 1994, Hohenlohe 2004, Baums et al. 2006a). Coral larvae seem to get trapped in eddies that form around islands, and studies have shown a noticeable decline in recruits farther from the center of the eddy in the Great Barrier Reef (Sammarco & Andrews 1989) and eddies around the Mona Passage (Baums et al. 2006a). Hydrodynamic forces caused by turbulent waters can affect recruitment even during the early phases of larval settlement (Reidenbach et al. 2009). Water motion varies within and between reefs and between depths in both the fore- and back-reef in La Parguera, adding to the complexity of larval transport (Appeldoorn et al. 1994, Lugo-Fernández et al. 1994, McGehee 1994, Mercado-Molina 2008, Williams et al. 2009) and, therefore, influencing patterns and rates of gene flow. Differences in water speed have been reported between the back-reef and fore-reef at Media Luna (0.23 and 1.1 km h^{-1}, respectively) (Williams et al. 2009). The substrata of back-reefs in La Parguera vary from seagrass beds to sand to rubble substrate (Irizarry-Soto 2006), none of which are optimal for larval settlement (Irizarry-Soto 2006, Szmant & Miller 2005). The genetic population structure of the acroporid species observed between some reefs could have resulted from differential larval mortality due to the deflection and entrapment of water in the back-reef. Meanwhile, the fore-reef, because of the high water motion, gets flushed faster, which could aid in the transport of larvae from one reef to another (Hamner & Hauri 1981, Sammarco & Andrews 1989), explaining the lack of genetic differentiation among some reefs in La Parguera.

Twice as many *Acropora cervicornis* haplotypes were detected in the present study compared to those previously reported (Vollmer & Palumbi 2002), except at San Cristóbal reef. While it is possible that we might have missed other haplotypes in that reef, the differences could be also attributed to colony losses from environmental and biological stresses. Sampling in Vollmer & Palumbi (2002) took place in 2001, and since then, Puerto Rico reefs have been devastated by the Caribbean-wide bleaching event of 2005 (NOAA 2005, Donner et al. 2007, Lesser 2007, Wilkinson & Souter 2008) and suffered minor damages by the passing of Hurricane Dean in September 2007 (J. Garcia Reyes & N. V. Schizas pers. obs.). Even though Hurricane Dean crossed the Caribbean hundreds of kilometers south of Puerto Rico, the associated surge and waves caused destruction in coastal reefs of southern Puerto Rico and several of our localities are presently characterized by a barren substrate. For example, San Cristóbal reef was once heavily populated with *A. cervicornis* in the back-reef, but after the white band disease epizootic event, predation and these 2 destructive events, an unprecedented decrease in this species has occurred (J. Garcia Reyes & N. V. Schizas pers. obs.). There are alternate explanations for the fine-scale population structure in the 2 acroporid species. A highly variable recruitment from exogenous populations could drive the patterns of genetic connectivity observed at the local scale. The local population structure of the brooding coral *Seriatopora hystrix* was significantly influenced by occasional recruits from distant sources (Underwood et al. 2007). In the same manner, Puerto Rico represents the mixing zone between western and eastern Caribbean populations of some coral species and this process may lead to genetically heterogeneous reefs locally.

Structure among populations around Puerto Rico

We detected significant population subdivision for *Acropora cervicornis* in southwestern Puerto Rico (Φ_{ST} = 0.1237, $p < 0.0059$) when using both native and introgressed alleles combined. Our values were similar to those of Vollmer & Palumbi (2007), who found signifi-

cant population structure in the mtDNA (Φ_{ST} = 0.130) and in the native mtDNA (Φ_{ST} = 0.235) of *A. cervicornis*. However, contrary to Vollmer & Palumbi (2007), the present analysis of native alleles did not result in significant population structure (Φ_{ST} = 0.0964, p < 0.08211), suggesting high rates of gene flow between reefs. The genetic structure observed in *A. cervicornis* at local and regional scales was mostly due to the introgressed alleles (Vollmer & Palumbi 2007, present study). Sample to sample variability and random senescence of acroporid colonies in La Parguera reefs may have caused the differences between the studies when employing the native mtDNA. We also detected significant population structure (Φ_{ST} = 0.0863, p < 0.00098) for *A. palmata* (which has not experienced introgression like *A. cervicornis*), reflecting diminished gene flow around Puerto Rico at a local spatial scale.

There was significant population differentiation in *Acropora cervicornis* between Puerto Rico and the Bahamas and between Mona Island and west Puerto Rico (Table S2 in the Supplement & Table 3), agreeing with previous reports on the western Caribbean genetic affinities of the Puerto Rican acroporids. The presence of genetic population differences between Puerto Rico and the Bahamas indicates that historically there has been reduced gene flow between these 2 locations. Restricted gene flow between populations of both species may have been caused by the Mona Passage, which separates Puerto Rico from Hispaniola (Baums et al. 2005b, 2006a, Galindo et al. 2006) and has been considered a marine phylogeographic barrier between the western and eastern Caribbean (Colin 2003, Taylor & Hellberg 2003, Baums et al. 2006a,b, Cowen et al. 2006, Galindo et al. 2006). Geographic models proposed by Baums et al. (2006a) and Galindo et al. (2006) suggested that reproductive timing, larval traits and oceanographic features together could inhibit the dispersal of *A. palmata* and *A. cervicornis* larvae between the western and eastern Caribbean. However, larvae do cross sporadically into the western Caribbean, creating a mixing of genetic lineages (Hohenlohe 2004, Baums et al. 2006a,b, Cowen et al. 2006, Galindo et al. 2006).

Conservation genetics of acroporids

Contrary to expectations, in 4 out of the 6 reefs, *Acropora palmata* displayed higher diversity indices with the circle collection method than with the random method. A similar discrepancy was reported by Baums et al. (2005a), who found that sometimes 2 different collecting circles within the same reef can yield completely different results. These observations highlight the genetic heterogeneity that can be observed in a single reef and suggest that samples from the fore-reef and the back-reef may be significantly different. Both species exhibited low genetic diversity, with *A. cervicornis* exhibiting higher values than *A. palmata*. In previous studies, the amount of genetic diversity in the ITS-1 region of *A. cervicornis* ranged from 0 to 13% (van Oppen et al. 2000) and averaged about 7.6% in *A. palmata* (Vollmer & Palumbi 2004). The low genetic diversity of *A. cervicornis* in the present study is not atypical compared to some other coral species (e.g. *A. cervicornis* π = 0.0057, *Siderastrea* sp. π = 0.0034, *Pavona cactus* π = 0.0069 and *P. decussata* π = 0.0079; Forsman et al. 2005, Pillay et al. 2006, Vollmer & Palumbi 2007). However, the genetic diversity of *A. palmata* was amongst the lowest reported values for marine invertebrates.

Low levels of mitochondrial diversity in the acroporids may represent past organelle bottleneck events. Over the last 30 yr, the scleractinian Caribbean acroporids have declined dramatically because of multiple stressors. The first massive die-off of the acroporids was observed in the 1980s during the epizootic event of white band disease (Gladfelter 1982). Another factor responsible for the decline of the 2 acroporids was physical damage inflicted by ship groundings (Bruckner & Bruckner 2001). In Fajardo, Puerto Rico, *Acropora palmata* colonies were severely affected by Hurricane David in 1979, and almost totally destroyed by Hurricane Hugo in 1989 (Weil et al. 2002). The northern inshore localities of Puerto Rico have exhibited a 68.4% decline of *A. palmata* in the last 20 yr, while a decrease of 53.3% has been recorded from the eastern inshore regions (Weil et al. 2002). *A. cervicornis* has suffered a 100% decline in several transects, at both northern inshore and eastern inshore localities (Weil et al. 2002).

The presence of high numbers of *Acropora cervicornis* colonies carrying introgressed alleles in the La Parguera reefs of Media Luna and Atravesado emphasize the importance of hybridization in the evolutionary ecology of both acroporids and the implications of management decisions concerning these 2 endangered species. The hybrid colonies are manifested in several intermediate morphs which may represent new ecotypes, potentially enriching the evolutionary potential of the group (Vollmer & Palumbi 2002). The present study also underscores the spatial variability of the hybridization frequency, since no introgressed alleles were recovered in 30 colonies of *A. cervicornis* from San Cristóbal, a reef located between Media Luna and Atravesado (Fig. 2).

The population and genetic history of both acroporid species before the recent die-offs is largely unknown; therefore, inferences on the long-term survival of the declining populations should be conservative (Grober-Dunsmore et al. 2007). Recovery of reefs in southwest-

ern Puerto Rico and other similar reefs of close spatial proximity might rely upon the survival and sexual reproduction of local populations and less on replenishment from distant reefs (Roberts 1997, Vollmer & Palumbi 2007). Preserving the genetic diversity of declining scleractinian species will increase the probability of the long-term survival of the species, thus underlying the important use of genetic studies. When genetic methods are coupled with ecological and oceanographic studies (Baums et al. 2006a, Galindo et al. 2006, Hellberg 2007, Grober-Dunsmore et al. 2007, Zubillaga et al. 2008), a more comprehensive management plan can be implemented. Management decisions can be further fine-tuned by the inclusion of genetic information in restoration projects (Baums 2008, Shearer et al. 2009) and by the identification of disease-resistant genotypes (Vollmer & Kline 2008).

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LITERATURE CITED

Aguilar-Perera A, Schärer M, Valdés-Pizzini M (2006) Marine protected areas in Puerto Rico: historical and current perspectives. Ocean Coast Manag 49:961–975

Appeldoorn RS, Hensley DA, Shapiro DY, Kioroglou S, Sanderson BG (1994) Egg dispersal in a Caribbean coral reef fish, *Thalassoma bifasciatum*. II. Dispersal off the reef platform. Bull Mar Sci 54:271–280

Aronson RB, Precht WF (2006) Conservation, precaution, and Caribbean reefs. Coral Reefs 25:441–450

Baums IB (2008) A restoration genetics guide for coral reef conservation. Mol Ecol 17:2796–2811

Baums IB, Hughes CR, Hellberg ME (2005a) Mendelian microsatellite loci for the Caribbean coral *Acropora palmata*. Mar Ecol Prog Ser 288:115–127

Baums IB, Miller MW, Hellberg ME (2005b) Regionally isolated populations of an imperiled Caribbean coral, *Acropora palmata*. Mol Ecol 14:1377–1390

Baums IB, Paris CB, Cherubin LM (2006a) A bio-oceanographic filter to larval dispersal in a reef-building coral. Limnol Oceanogr 51:1969–1981

Baums IB, Miller MW, Hellberg ME (2006b) Geographic variation in clonal structure in a reef-building Caribbean coral, *Acropora palmata*. Ecol Monogr 76:503–519

Bottjer DJ (1980) Branching morphology of the reef coral *Acropora cervicornis* in different hydraulic regimes. J Paleontol 54:1102–1107

Bruckner AW (2002) Proceedings of the Caribbean *Acropora* workshop: potential application of the U.S. Endangered Species Act as a conservation strategy. NOAA Tech Memo NMFS-OPR-24, Silver Spring, MD

Bruckner AW, Bruckner RJ (2001) Condition of restored *Acropora palmata* fragments off Mona Island, 2 years after the Fortuna Reefer ship grounding. Coral Reefs 20:235–243

Bruno JF, Selig ER (2007) Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. PLoS ONE 2:e711

Bruno JF, Selig ER, Casey KS, Page CA and others (2007) Thermal stress and coral cover as drivers of coral disease outbreaks. PLoS Biol 5:e124

Carpenter KE, Abrar M, Aeby G, Aronson RB and others (2008) One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science 321:560–563

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9: 1657–1659

Colin PL (2003) Larvae retention: Genes or oceanography? Science 300:1657

Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. Science 311:522–527

Donner SD, Knutson TR, Oppenheimer M (2007) Model-based assessment of the role of human-induced climate change in the 2005 Caribbean coral bleaching event. Proc Natl Acad Sci USA 104:5483–5488

Ewing B, Green P (1998) Base calling of automated sequencer traces using phred. II. Error probabilities. Genome Res 8: 186–194

Ewing B, Hillier L, Wendl M, Green P (1998) Base calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res 8:175–185

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Forsman ZH, Guzman HM, Chen CA, Fox GE, Wellington GM (2005) An ITS region phylogeny of *Siderastrea* (Cnidaria: Anthozoa): Is *S. glynni* endangered or introduced? Coral Reefs 24:343–347

Galindo HM, Olson DB, Palumbi SR (2006) Seascape genetics: a coupled oceanographic–genetic model predicts population structure of Caribbean corals. Curr Biol 16: 1622–1626

Gardner TA, Cote IM, Gill JA, Grant A, Watkinson AR (2003) Long-term region-wide declines in Caribbean corals. Science 301:958–960

Gilmore MD, Hall BR (1976) Life history, growth habits, and constructional roles of *Acropora cervicornis* in the patch reef environment. J Sediment Petrol 46:519–522

Gladfelter WB (1982) White-band disease in *Acropora palmata*: implications for the structure and growth of shallow reefs. Bull Mar Sci 32:639–643

Gordon D (2003) Viewing and editing assembled sequences

using consed. In: Baxevanis AD, Davison DB (eds) Current protocols in bioinformatics. John Wiley, New York, p 11.12.11–11.12.43

Grober-Dunsmore R, Bonito V, Frazer TK (2007) Discernment of sexual recruits is not critical for assessing population recovery of *Acropora palmata*. Mar Ecol Prog Ser 335: 233–236

Hamner WM, Hauri IR (1981) Effects of island mass: water flow and plankton pattern around a reef in the Great Barrier Reef lagoon, Australia. Limnol Oceanogr 26: 1084–1102

Hellberg ME (2007) Footprints on water: the genetic wake of dispersal among reefs. Coral Reefs 26:463–473

Hellberg ME, Burton RS, Neigel JE, Palumbi SR (2002) Genetic assessment of connectivity among marine populations. Bull Mar Sci 70:273–290

Hohenlohe PA (2004) Limits to gene flow in marine animals with planktonic larvae: models of *Littorina* species around Point Conception, California. Biol J Linn Soc 82:169–187

Irizarry-Soto E (2006) Abundance, composition and survivorship of juvenile corals in the southwestern Puerto Rico, La Parguera. MS thesis, University of Puerto Rico, Mayagüez

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Lesser MP (2007) Coral reef bleaching and global climate change: Can corals survive the next century? Proc Natl Acad Sci USA 104:5259–5260

Lugo-Fernández A, Hernández-Avila ML, Roberts HH (1994) Wave-energy distribution and hurricane effects on Margarita Reef, southwestern Puerto Rico. Coral Reefs 13: 21–32

Macintyre IG, Toscano MA (2007) The elkhorn coral *Acropora palmata* is coming back to the Belize Barrier Reef. Coral Reefs 26:757

Maddison DR, Maddison WP (2000) MacClade 4.0. Sinauer Associates, Sunderland, MA

McGehee MA (1994) Correspondence between assemblages of coral reef fishes and gradients of water motion, depth, and substrate size off Puerto Rico. Mar Ecol Prog Ser 105: 243–255

Mercado-Molina AE (2008) Population ecology of the demosponge *Amphimedon compressa*. MS thesis, University of Puerto Rico, Mayagüez

Miller MW, Bourque AS, Bohnsack JA (2002) An analysis of the loss of acroporid corals at Looe Key, Florida, USA: 1983–2000. Coral Reefs 21:179–182

Neigel JE, Avise JC (1983) Clonal diversity and population structure in a reef-building coral, *Acropora cervicornis*: self-recognition analysis and demographic interpretation. Evolution 37:437–453

NOAA (2005) Caribbean basin bleaching event. NOAA, Silver Spring, MD, available at http://coralreefwatch.noaa.gov/caribbean2005/

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. Ecol Appl 13(Suppl):146–158

Pillay KRM, Asahida T, Chen CA, Terashima H, Ida H (2006) ITS ribosomal DNA distinctions and the genetic structures of populations of two sympatric species of *Pavona* (Cnidaria: Scleractinia) from Mauritius. Zool Stud 45: 132–144

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818

Precht WJ, Robbart ML, Aronson RB (2004) The potential listing of *Acropora* species under the US Endangered Species Act. Mar Pollut Bull 49:534–536

Reidenbach MA, Koseff JR, Koehl MAR (2009) Hydrodynamic forces on larvae affect their settlement on coral reefs in turbulent, wave-driven flow. Limnol Oceanogr 54: 318–330

Roberts CM (1997) Connectivity and management of Caribbean coral reefs. Science 278:1454–1457

Rogers CS, Suchanek TH, Pecora FA (1982) Effects of Hurricanes David and Frederic (1979) on shallow *Acropora palmata* reef communities: St. Croix, U.S. Virgin Islands. Bull Mar Sci 32:532–548

Rozas J, Sánchez-DelBarrio JC, Messenger X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497

Sammarco PW, Andrews JC (1989) The Helix experiment: differential localized dispersal and recruitment patters in Great Barrier Reef corals. Limnol Oceanogr 34:896–912

Schuhmacher H, Plewka M (1981) The adaptive significance of mechanical properties versus morphological adjustments in skeletons of *Acropora palmata* and *Acropora cervicornis* (Cnidaria, Scleractinia). Proc 4th Int Coral Reef Symp 2:121–128

Shearer TL, Porto I, Zubillaga AL (2009) Restoration of coral populations in light of genetic diversity estimates. Coral Reefs 28:727–733

Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony*, version 4.0b10. Sinauer Associates, Sunderland, MA

Szmant AM, Miller MW (2005) Settlement preferences and post-settlement mortality of laboratory cultured and settled larvae of the Caribbean hermatypic corals *Montastraea faveolata* and *Acropora palmata* in the Florida Keys, USA. Proc 10th Int Coral Reef Symp 1:43–49

Taylor MS, Hellberg ME (2003) Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299:107–109

Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619–633

Tunnicliffe V (1981) Breakage and propagation of the stony coral *Acropora cervicornis*. Proc Natl Acad Sci USA 78: 2427–2431

Underwood JN, Smith LD, van Oppen MJH, Gilmour JP (2007) Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. Mol Ecol 16:771–784

van Oppen MJH, Willis BL, van Vugt HWJA, Miller DJ (2000) Examination of species boundaries in the *Acropora cervicornis* group (Scleractinia, Cnidaria) using nuclear DNA sequence analyses. Mol Ecol 9:1363–1373

van Oppen MJH, McDonald BJ, Willis B, Miller DJ (2001) The evolutionary history of the coral genus *Acropora* (Scleractinia, Cnidaria) based on a mitochondrial and a nuclear marker: Reticulation, incomplete lineage sorting, or morphological convergence? Mol Biol Evol 18:1315–1329

van Oppen MJH, Koolmees EM, Veron JEN (2004) Patterns of evolution in the scleractinian coral genus *Montipora* (Acroporidae). Mar Biol 144:9–18

Vega-Zepeda A, Hernández-Arana H, Carricart-Ganivet JP (2007) Spatial and size-frequency distribution of *Acropora* (Cnidaria: Scleractinia) species in Chinchorro Bank, Mexican Caribbean: implications for management. Coral Reefs 26:671–676

Vollmer SV, Kline DI (2008) Natural disease resistance in threatened staghorn corals. PLoS ONE 3:e3718

Vollmer SV, Palumbi SR (2002) Hybridization and the evolution of reef coral diversity. Science 296:2023–2025

Vollmer SV, Palumbi SR (2004) Testing the utility of internally transcribed spacer sequences in coral phylogenetics. Mol Ecol 13:2763–2772

Vollmer SV, Palumbi SR (2007) Restricted gene flow in the Caribbean staghorn coral *Acropora cervicornis*: implications for the recovery of endangered reefs. J Hered 98: 40–50

Wallace C (1999) Staghorn corals of the world: a revision of the coral genus *Acropora*. CSIRO Publishing, Collingwood

Weil E (2004) Coral reef diseases in the wider Caribbean. In: Rosenberg E, Loya Y (eds) Coral health and disease. Springer-Verlag, Berlin

Weil E, Hernández-Delgado EA, Bruckner AW, Ortiz AL, Nemeth M, Ruiz H (2002) Distribution and status of Acroporid coral (Scleractinia) populations in Puerto Rico. In: Bruckner AW (ed) Proceedings of the Caribbean *Acropora* workshop: potential application of the US Endangered Species Act as a conservation strategy. NOAA Tech Memo NMFS-OPR-24:71–98

Wilkinson C (2006) Status of coral reefs of the world: summary of threats and remedial action. In: Cote IM, Reynolds JD (eds) Coral reef conservation. Cambridge University Press, Cambridge

Wilkinson C, Souter D (2008) Status of Caribbean coral reefs after bleaching and hurricanes in 2005. Global Coral Reef Monitoring Network and Reef and Rainforest Research Centre, Townsville

Williams SM, Garcia-Sais JR, Capella J (2009) Seasonal variation and spatial distribution of early larval stages of the long-spined sea urchin *Diadema antillarum* in La Parguera, Puerto Rico. Caribb J Sci 45:110–117

Zimmer B, Precht W (2006) Discovery of *Acropora palmata* at the Flower Garden Banks National Marine Sanctuary, northwestern Gulf of Mexico. Coral Reefs 25:192

Zubillaga AL, Marquez LM, Croquer A, Bastidas C (2008) Ecological and genetic data indicate recovery of the endangered coral *Acropora palmata* in Los Roques, Southern Caribbean. Coral Reefs 27:63–72

Appendix 1. *Acropora* spp. Hierarchical analysis of molecular variance (AMOVA). Values were generated with 10 000 permutations using the Kimura 2-P model of nucleotide substitution (Kimura 1980). $^{*}p < 0.05$; $^{**}p < 0.001$

	df	SS	Variance components	% variation	Φ_{ST}
A. palmata					
Between regions					
La Parguera Random vs. Desecheo Island vs. Mona Island vs. Bahamas vs. Tres Palmas					
Among populations	4	4.768	0.05265 Va	11.55	0.1156*
Within populations	90	36.271	0.40302 Vb	88.45	
Total	94	41.039	0.45567		
La Parguera Circle vs. Desecheo Island vs. Mona Island vs. Bahamas vs. Guánica vs. Tres Palmas					
Among populations	5	7.466	0.05109 Va	9.57	0.0957**
Within populations	137	66.142	0.48279 Vb	90.43	
Total	142	73.609	0.53388		
Between reefs					
La Parguera Random (Margarita vs. Enrique vs. Turrumote vs. Laurel vs. Media Luna vs. El Palo)					
Among populations	5	3.095	0.04407 Va	15.58	0.1558*
Within populations	47	11.226	0.23885 Vb	84.42	
Total	52	14.321	0.28292		
La Parguera Circle (Margarita vs. Enrique vs. Turrumote vs. Laurel vs. Media Luna)					
Among populations	4	11.681	0.19276 Va	38.06	0.3806**
Within populations	63	19.766	0.31375 Vb	61.94	
Total	67	31.447	0.5065		
Puerto Rico (La Parguera All vs. Mona Island vs. Desecheo Island vs. Guánica vs. Tres Palmas)					
Among populations	4	5.332	0.03650 Va	8.63	0.0863**
Within populations	186	71.906	0.38659 Vb	91.37	
Total	190	77.238	0.4231		
Puerto Rico All vs. Lee Stocking Island, Bahamas					
Among populations	1	1.311	0.03526 Va	7.26	0.0726*
Within populations	211	95.025	0.45036 Vb	92.74	
Total	212	96.336	0.48561		

Appendix 1 (continued)

	df	SS	Variance components	% variation	Φ_{ST}
A. cervicornis **(introgressed and native alleles combined)**					
Between La Parguera reefs					
Random (Media Luna vs. San Cristóbal vs. Laurel vs. Atravesado)					
Among populations	3	68.962	2.15340 Va	66.65	0.6665**
Within populations	38	40.941	1.07740 Vb	33.35	
Total	41	109.903	3.2308		
Circle (Media Luna vs. San Cristóbal vs. Atravesado)					
Among populations	2	59.509	1.54071 Va	50.98	0.5098**
Within populations	54	79.99	1.48130 Vb	49.02	
Total	56	139.5	3.02202		
Between regions					
La Parguera Random vs. Mona Island vs. Desecheo Island vs. Bahamas					
Among populations	3	37.096	0.71073 Va	22.84	0.2284**
Within populations	67	160.915	2.40172 Vb	77.16	
Total	70	198.011	3.11245		
La Parguera Circle vs. Mona Island vs. Desecheo Island vs. Bahamas					
Among populations	3	28.987	0.47737 Va	16.78	0.1678**
Within populations	86	203.607	2.36752 Vb	83.22	
Total	89	232.594	2.84489		
Between reefs					
Puerto Rico (La Parguera All vs. Mona Island vs. Desecheo Island)					
Among populations	2	19.179	0.35461 Va	12.37	0.1237*
Within populations	110	276.42	2.51291 Vb	87.63	
Total	112	295.599	2.86752		
Puerto Rico All vs. Lee Stocking Island, Bahamas					
Among populations	1	9.483	0.59835 Va	18.40	0.1840*
Within populations	121	321.000	2.65290 Vb	81.60	
Total	122	330.483	3.25124		
A. cervicornis **(native alleles only)**					
Between La Parguera reefs					
Random (Laurel vs. San Cristóbal vs. Atravesado)					
Among populations	2	7.037	0.90822 Va	90.9	0.919**
Within populations	11	0.881	0.08006 Vb	8.1	
Total	13	7.917	0.98827		
Circle (Media Luna vs. San Cristóbal vs. Atravesado)					
Among populations	2	15.953	1.27267 Va	85.64	0.8564**
Within populations	27	5.762	0.21339 Vb	14.36	
Total	29	21.715	1.48606		
Between regions					
La Parguera Random vs. Mona Island vs. Desecheo Island vs. Bahamas					
Among populations	3	2.900	0.05828 Va	9.64	0.0964
Within populations	30	16.384	0.54613 Vb	90.36	
Total	33	19.284	0.60441		
La Parguera Circle vs. Mona Island vs. Desecheo Island vs. Bahamas					
Among populations	3	4.360	0.07627 Va	9.37	0.0937
Within populations	46	33.919	0.73737 Vb	90.63	
Total	49	38.279	0.81363		
Between reefs in Puerto Rico					
La Parguera All[a] vs. Mona Island vs. Desecheo Island					
Among populations	2	2.881	0.05309 Va	7.48	0.0748
Within populations	59	38.762	0.65698 Vb	92.52	
Total	61	41.643	0.71007		

[a]La Paguera All includes samples collected using both the concentric circle and random collection methods

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