Genetic variability and fine-scale population structure in the threatened species *Acropora palmata* and *Acropora cervicornis* around Puerto Rico

by

Joselyd García Reyes

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
in
MARINE SCIENCES

UNIVERSITY OF PUERTO RICO
MAYAGÜEZ CAMPUS
2008

Approved by:

_____	_____
Ernesto Weil, Ph.D. Member, Graduate Committee	Date
_____	_____
Paul M. Yoshioka, Ph.D. Member, Graduate Committee	Date
_____	_____
Nikolaos V. Schizas, Ph.D. President, Graduate Committee	Date
_____	_____
Representative of Graduate Studies	Date
_____	_____
Nilda E. Aponte, Ph.D. Chairperson of the Department	Date

ABSTRACT

The population genetic structure of the zooxanthellate corals *Acropora palmata* and *A. cervicornis* were used as a proxy to understand connectivity between reefs. Sequences of the mitochondrial control region were recovered from geographically adjacent and distant populations of *A. palmata* and *A. cervicornis* around Puerto Rico. AMOVA results from 220 *A. palmata* and 124 *A. cervicornis* colonies collected from 26 reefs of six localities suggest that significant population structure exists (Φ_{ST}=0.0863, P<0.00098; Φ_{ST}=0.1237, P<0.00587, for *A. palmata* and *A. cervicornis*, respectively). Significant Φ_{ST}'s between reefs of Puerto Rico suggest that there is fine scale population structure. Although these species displayed significant population structure, both species exhibited low levels of nucleotide diversity which is common for scleractinian corals. Recovery of reefs in southwestern Puerto Rico might rely on the survival and sexual reproduction of local populations rather than replenishment from distant reefs because of the high levels of population subdivision.

RESUMEN

La estructura genética poblacional de los corales zooxantelados *Acropora palmata* y A. *cervicornis* fue usada como indicador para estudiar la conectividad entre arrecifes. Secuencias de la región control mitocondrial fueron recuperadas de poblaciones geográficamente adyacentes y distantes de *A. palmata* y *A. cervicornis* alrededor de Puerto Rico. Resultados de AMOVA para 220 colonias de *A. palmata* y 124 colonias de *A. cervicornis* muestreadas en 26 arrecifes en seis localidades sugieren que existe una estructura poblacional significativa (Φ_{ST}=0.0863, P<0.00098; Φ_{ST}=0.1237, P<0.00587, para *A. palmata* y *A. cervicornis*, respectivamente). Valores de Φ_{ST}'s significativos entre arrecifes en Puerto Rico sugiere que existe estructura poblacional a menor escala. Aunque estas especies revelan estructura poblacional significativa, las dos especies exhibieron bajos niveles de variabilidad genética lo cual es común entre corales escleractínios. La recuperación de los arrecifes en el suroeste de Puerto Rico podría depender de la sobrevivencia y reproducción sexual de poblaciones locales en lugar del abastecimiento por arrecifes distantes debido a los altos niveles de subdivisión poblacional.

Signed:

Date:

I dedicate my thesis to Jose, Sr., Lydia, and Jose Jr. as thanks for all the support they have given me not only throughout my years here at the University of Puerto Rico-Mayagüez, but in all aspects of my life. Without them, I would not be where I am today.

I love you!!

ACKNOWLEDGEMENTS

I am grateful to the many people who lent me a hand during my field collection: Dannise Ruiz Ramos, Brandi Todd, Stacey Williams, Alex Mercado Molina, Helena Antoun, Carlos Prada, Hernan Torrés-Pratts, Hector Ruiz, Michelle Scharer, Aldo Croquer, Emmanuel Irrizary, and Godoberto Lopez. I would like to thank Dr. Nikolaos Schizas for all his guidance, support, and patience throughout the years and I would like to thank Dr. Paul Yoshioka and Dr. Ernesto Weil for their advice and support. I would like to thank my graduate school representative Dr. Edgardo Ojeda for his comments and suggestions. I would also like to thank Aldo Croquer, Katie Flynn, Anthony Marshak, Alex Mercado, and Carlos Prada for their comments on an earlier version of this manuscript. I acknowledge the Department of Marine Sciences from the University of Puerto Rico-Mayagüez and Sea Grant Puerto Rico for the use of the SCUBA diving and boat facilities, assistantships, and funding for travel to several conferences for presenting my thesis work. I would like to thank all the wonderful friends, colleagues, and workers from the Department of Marine Sciences. These years would not have been the same without your friendship and support. Thanks to the administrative staff: Zulma Martinez, Lillivette Valles, Monserrate (Taty) Casiano, Nilda Ramirez, Nereida Santiago. Many special thanks to Helena, Brandi, Stacey, Katie, Alex, Dihalia, Dannise, Brenda, Maria, Sara, Carlos Prada, Diana, Hernan, Yaritza, Rosalina, Aldo, Emmanuel and more recently Carmen, Chino, Suhey, Orian, Panky, and Daniel for all the unforgettable moments spent together throughout the years. I thank Dr. Owen McMillan and Jenny Acevedo at the University of Puerto Rico-Rio Piedras for all their help and training in molecular techniques. Most of all I would like to thank my family, who has been there every step of the way.

Funds for this project came from a Caribbean Coral Reef Institute grant and a Program Development grant from the Caribbean Marine Research Center (Project #05-PRKS-01-05A) awarded to Schizas, a Sigma Xi Grant-in-Aid of Research (ID Number: G20073121644256671), the José M. Berrocal Scholarship, La Unidad Latina Foundation Leadership Award to Garcia, the UPRM-Department of Marine Sciences and a Sea Grant Seed Money grand awarded to Garcia, Prada and Schizas. Collection permits for acroporids were issued by the Puerto Rico Department of Natural and Environmental Resources. DNA sequencing was performed in the Sequencing and Genotyping facility of the University of Puerto Rico-Río Piedras which is supported in part by NCRR AABRE Grant #P20 RR16470, NIH-SCORE Grant #S06GM08102, University of Puerto Rico Biology Department, NSFCREST Grant #0206200.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

INTRODUCTION

There has been an unprecedented decline of coral reef cover worldwide over the last three decades (Gardner et al. 2003, Bruno and Selig 2007). About 20% of coral reefs have vanished and 16% are severely damaged while 26% more are in 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 and Precht 2006, Wilkinson and Souter 2008), where the dominant reef-building coral species of the last 500,000 years have been rapidly disappearing (Gilmore and 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 only represented by *Acropora palmata* and *A. cervicornis* and a 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. The species is normally found at depths ranging from 3-30 meters in high-energy areas where fragmentation, due to its thin fragile branches, plays an important role in asexual spreading (Bottjer 1980, Tunnicliffe 1981, Neigel and Avise 1983). *Acropora cervicornis* is a fast growing (12 cm $\cdot$ yr^{-1}) reef building species, which provides habitat for a wealth of marine diversity (Bruckner 2003, Precht et al. 2004, Tunnicliffe 1981). *Acropora palmata* is also a fast growing coral (5-9.5 cm $\cdot$ yr^{-1}) (Schuhmaker and Plewka 1981), but unlike *A. cervicornis* it is thicker and stronger and normally found along the reef crest where wave energy is high. The branches of *A. palmata* are greater than 0.5 m in length with a light tan to brown coloration (Schuhmaker and Plewka 1981). Unlike *A. cervicornis*, *A. palmata* is found at depths ranging from 0-15 m (Schuhmaker and Plewka 1981) and more recently at depths >20m (Zimmer and Precht 2006).

These two scleractinian species are ecologically important, not only because they have been the most 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 two species is so remarkable 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 three decades has been attributed to disease, storms, corallivory, hyperthermic stress, and pollution (Rinkevich 2000, Bruckner 2003, Gardner et al. 2003, Vargas-Angel et al. 2003, Weil 2004, Lesser 2007, Bruno et al. 2007).

Both, *A. cervicornis* and *A. palmata* reproduce sexually and asexually. There is an annual sexual cycle culminating in one or two spawning events per year. In Puerto Rico, the acroporids spawn three to six days after the full moon of August and/or September (Szmant-Froelich 1986). Bundles containing eggs and sperm are released from the colonies and within minutes they reach the water surface (Szmant-Foelich 1986, pers. obs.). Once in the surface, the bundles break and fertilization occurs during the mixing of eggs and sperm. Acroporid larvae settle out of the water column after three to five days but can remain viable up to 20 days in aquaria (Baums et al 2006b). Mass spawnings of corals provide overwhelming opportunities for hybridization to occur, which has contributed to the evolution of scleractinian corals (McMillan et al. 1991, van Oppen et al. 2001, Vollmer and Palumbi 2002, van Oppen et al. 2004). Hybridization between the two Caribbean acroporids results in the formation of *A. prolifera* which is sterile (Vollmer and Palumbi 2002). Genetically, *A. prolifera* contains alleles from both *A. cervicornis* and *A. palmata* (Vollmer and Palumbi 2002) and morphologically *A. prolifera* resembles the species which donated the egg. The most prevalent mode of reproduction in both *A. cervicornis* and *A. palmata* is through fragmentation (asexual reproduction) (Bak and Engel

1979, Tunnicliffe 1981, Highsmith 1982, Lirman 2000, Bruckner 2003, Baums et al. 2005a, Baums et al. 2005b). However, more recent reports have noted that sexual reproduction might play a more important role than previously thought. Reefs throughout Los Roques National Park in Venezuela exhibited high allozyme variation (118 different genotypes in 120 colonies) that could be indicative of a high degree of sexual reproduction (Zubillaga 2008). Congruently, a Florida study revealed relatively high survivorship (29%) of *A. palmata* recruits over seven months in field experiments (Szmant and Miller 2006). Therefore, sexual reproduction is probably a fundamental process in the long-term recovery of the acroporids (Szmant and Miller 2006, Zubillaga 2008). On the other hand, asexual reproduction can play a significant role in the short-term clonal replication and short distance dispersion of genets of these species (Tunnicliffe 1981, Smith and Hughes 1999). Although fragmentation can produce many colonies or replace dead ones, it can also lead to extensive reef areas with populations consisting of one genotype (Baums et al. 2005a), thereby decreasing the available allelic combinations. The numerical dominance of one genotype in a reef may indicate its high fitness in the prevailing environmental conditions (Robson et al. 1999). However, if substantial environmental changes occur, the prevalent genotype may exhibit lower fitness than other genotypes.

The threatened status of acroporids has generated several studies focusing on genetic diversity at the population level. van Oppen et al. (2000) examined the species boundaries within *A. cervicornis*, *A. palmata* and *A. prolifera* by sequencing portions of ITS-1, 5.8S, and PaxC gene regions. Vollmer and Palumbi (2002) also studied the hybridization between these coral species using nuclear intronic regions and the mitochondrial putative control region. Both of these studies determined that there is significant divergence between the Caribbean acroporids. Although neither of these studies focused on the intraspecific variation, they did

report that there was some variation within each species. In *A. cervicornis*, the amount of genetic diversity in the ITS-1 region ranged from 0-13% (van Oppen et al. 2000) and 7.6% in *A. palmata* (Vollmer and Palumbi 2004). Baums et al. (2005a) used eight microsatellite markers to report variation of *A. palmata* colonies among three reefs in Key Largo, Florida. Out of the 93 colonies sampled, only 15 genets were represented at those three reefs. The reefs were located 3.3 m to 15.4 km apart, raising concerns that these populations could be extremely vulnerable to disturbances caused by climate change (Baums et al. 2005a). In a Caribbean-wide study conducted by Baums et al. (2006a), *A. palmata* has had little or no recent genetic exchange between the western and eastern Caribbean, with Puerto Rico as the mixing point between these two regions. There is evidence that the Mona Passage restricts genetic mixing of the Western and Eastern Caribbean lineages of *A. palmata* (Baums et al. 2006a, Baums et al. 2006b) and *A. cervicornis* (Galindo et al. 2006). The Mona Passage which separates Puerto Rico from Hispaniola has been considered as a marine phylogeographic barrier between these two regions (Colin 2003, Taylor and Hellberg 2003, Cowen et al. 2006, Baums et al. 2006a, Baums et al. 2006b). Vollmer and Palumbi (2007) reported significant population subdivision in *A. cervicornis* across the Caribbean using multiple genes, suggesting that there is restriction of gene flow. The mtDNA data showed the highest population structure (Φ_{ST} = 0.235) within *A. cervicornis*, when only native alleles were considered. One-way gene flow between *A. cervicornis* and *A. palmata* in the past has caused the introgression of partial *A. palmata* sequences in *A. cervicornis* (Vollmer and 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. Fine-scale population structure was discovered in reefs of southwestern Puerto Rico separated by a distance of only 2 km (Vollmer and Palumbi 2007).

Spatial scales of connectivity for wide dispersal species might be smaller than previously assumed (Palumbi 2003, Cowen et al. 2006). Presumably, a species with large dispersal capabilities could disperse over longer distances (Bohonak 1999, Riginos and Victor 2001). This might be true for some species but dispersal capabilities might not be directly correlated with gene flow (Cowen et al. 2006, Hellberg 2007). Many studies have focused on marine organisms that have large dispersal capabilities over large-spatial scales, yet significant population structure was discovered at smaller-than-expected spatial scales (Taylor and Hellberg 2003, Sotka et al. 2004, Jose and Solferini 2007, Zardi et al. 2007).

Fine-scale corals studies, ranging from hundreds of meters to few km, have been scarce because large geographic separation of localities is presumably needed for detection of population structure. The more recent studies of Baums et al. (2005a,b) and Vollmer and Palumbi (2007) have focused on Caribbean-wide spatial scales although they have both reported fine-scale population structure within their study. The purpose of the present study is to estimate the genetic variability and the genetic population structure of *A. palmata* and *A. cervicornis* in the coastal reefs of Puerto Rico. Information on the level of connectivity (rate of gene flow) around Puerto Rico can aid in determining the chances of recolonization of populations by immigrants from other populations (Cowen et al. 2000, Hellberg et al. 2002, Zubillaga 2008). Elucidation of gene flow patterns provides estimates of reef connectivity, which can better help to design marine reserves (Palumbi 2003). Geographically separated populations that are connected genetically should be preferentially included in a marine park network, instead of populations with restricted gene flow relying exclusively on self-replenishment. The detection of source and sink populations of larvae by genetic methods and local oceanographic models, can fine-tune the design of marine protected areas.

MATERIALS AND METHODS

Study area and sampling locations

A total of 220 *Acropora palmata* and 124 *A. cervicornis* colonies were sampled from 26 reefs of six localities in Puerto Rico and three reefs from Lee Stocking Island, Bahamas (Figures 1 and 2, Tables 1 and 2). Sample locations included Enrique, Laurel, San Cristobal, Media Luna, Turrumote, Atravesado, El Palo, and Margarita keys from the La Parguera Natural Reserve on the southwest coast of Puerto Rico (Tables 1 and 2). For reference samples we used *Acropora* tissue from six other Puerto Rico locations: Guánica, Tres Palmas Marine Reserve in Rincón, Mona Island, and Desecheo Island on the west coast, Culebra and Vieques islands on the east coast. Additionally, one location was sampled near Lee Stocking Island, Bahamas to serve as an outgroup (Tables 1 & 2). No samples of *A. cervicornis* were collected in the Tres Palmas Marine Reserve in Rincón, Guánica, and Vieques.

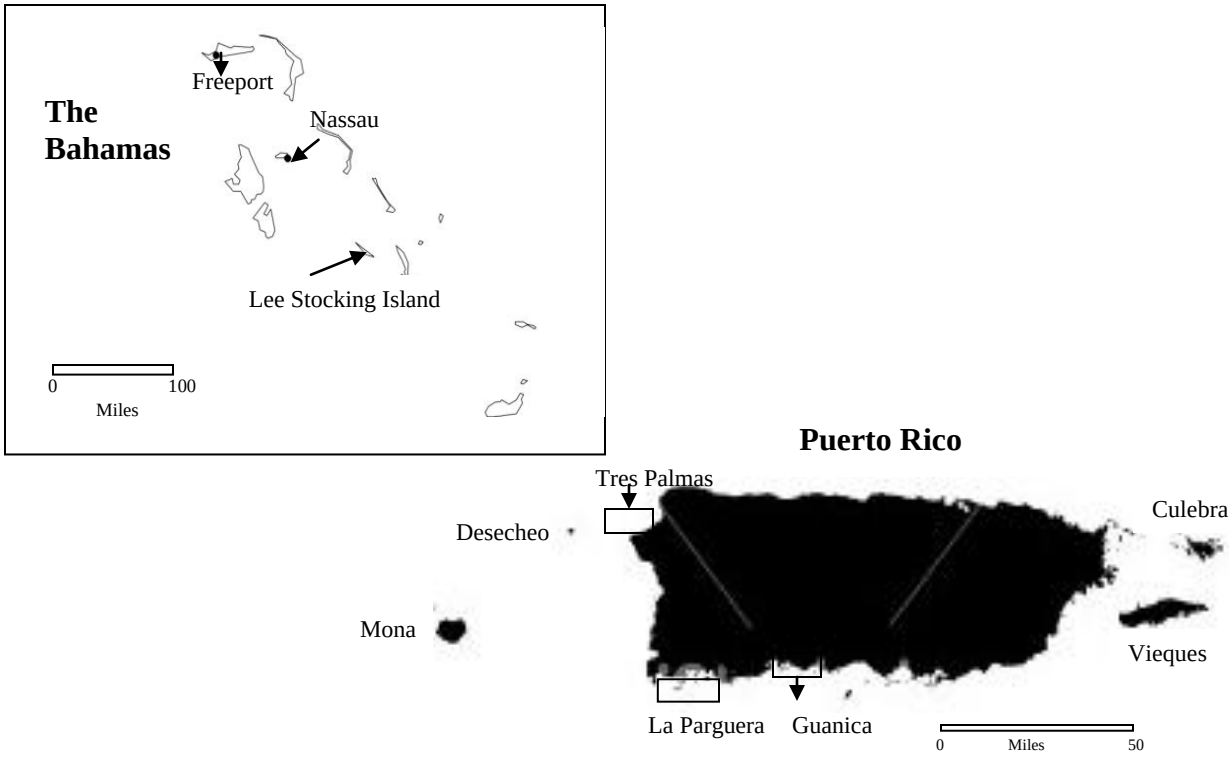

Figure 1. Map of the sampling localities including La Parguera, Guanica, Tres Palmas, Desecheo Island, Mona Island, Culebra, and Vieques from Puerto Rico and Lee Stocking Island from Bahamas.

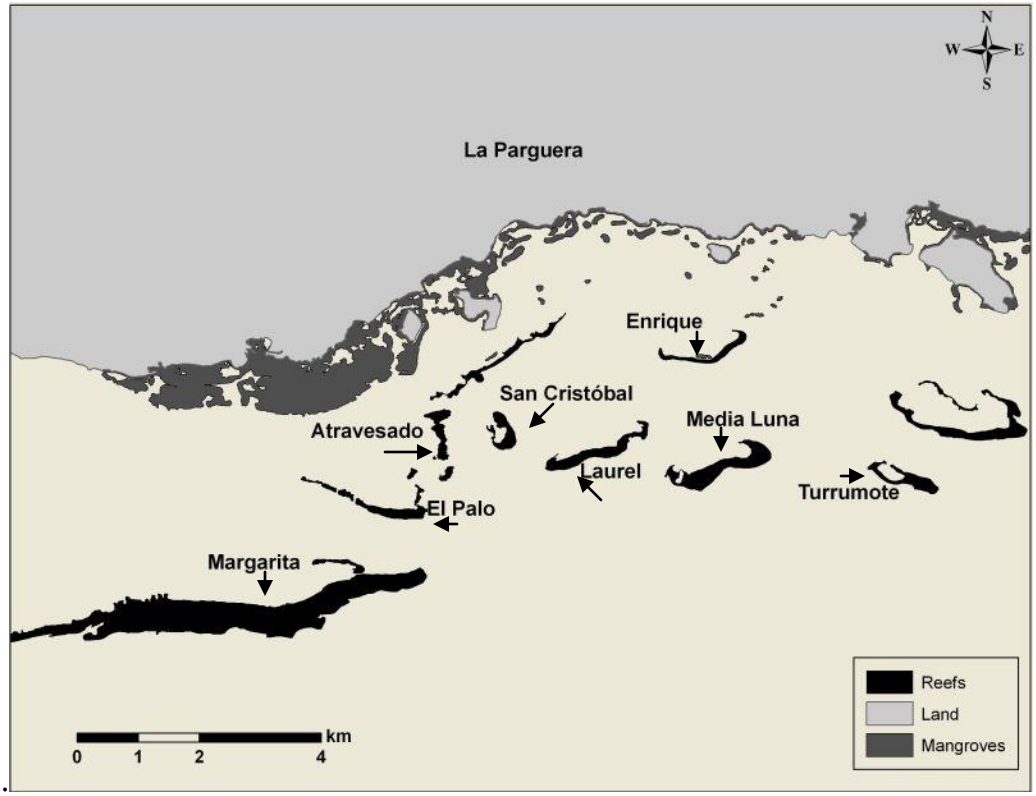

Figure 2. Map of La Parguera, Puerto Rico showing the sampled reefs.

Table 1. Samples localities of *Acropora palmata*.

Localities	Reefs	Latitude	Longitude	# samples from circles	# samples from haphazard
A. palmata					
La Parguera, PR					
	Laurel	N 17°56.649'	W 67°03.36'	12	6
	Turrumote	N 17°56.075’	W 67°01.047’	11	6
	Media Luna	N 17° 56.484'	W 67° 02.422'	16	8
	Margarita	N 18° 27.353'	W 65° 59.387'	13	13
	El Palo	N 17° 56.031’	W 67°06.031’	N/A	13
	Enrique	N 17° 57.298'	W 67° 02.618'	16	7
Mona Island, PR					
	Reef 1	N 18°07.468'	W 67°85.276'	N/A	5
	Reef 2	N 18°06.966'	W 67°85.575'	N/A	5
	Reef 3	N 18°06.594'	W 67°85.629'	N/A	2
	Reef 4	Sardinera		N/A	3
Desecheo Island, PR					
	Reef 1	N 18°38.124'	W 67°48.606'	N/A	4
Culebra, PR	Reserva Canal Luis Pena	N 18°19.238’	W 65°19.385’	N/A	2
Vieques, PR		N 18°06.449’	W 65°34.330’	N/A	2
Tres Palmas, PR		N 18°21.018'	W 67°15.938'	32	9
Guanica, PR		N 17°56.429'	W 66°52.115'	9	N/A
Lee Stocking Island, Bahamas					
	Reef 1	N 23°46.85’	W 76°06.21’	N/A	4
	Reef 2	N 23°47.383’	W 76°08.264’	N/A	4
	Reef 3	N/A	N/A	N/A	5

Table 2. Sample localities of *Acropora cervicornis.*

Localities	Reefs	Latitude	Longitude	# samples from circles	# samples from haphazard
A. cervicornis					
La Parguera, PR					
	Media Luna	N 17°56.312'	W 67°03.044'	23	15
	San Cristobal	N 17°56.645'	W 67°04.63'	22	8
	Atravesado	N 17° 56.354'	W 67° 05.206'	12	N/A
	Laurel	N 17°56.655'	W 67°03.339'	N/A	11
Mona Island, PR					
	Reef 1			N/A	1
	Reef 2	N 18°06.594'	W 67°85.629'	N/A	2
	Reef 3	N 18°04.899'	W 67°87.249'	N/A	5
	Reef 4	N 18°07.688'	W 67°94.200'	N/A	2
	Reef 5	N 18.065911	W 67.856297	N/A	1
	Reef 6	N 18.048994	W 67.872486	N/A	2
	Reef 7	N 18.076877	W 67.942003	N/A	3
	Reef 8	N/A	N/A	N/A	1
Desecheo Island, PR					
	Reef 1	N 18°38.124'	W 67°48.606'	N/A	4
	Reef 2	N 18°3783.441'	W 67°48.431'	N/A	2
	Reef 3	N/A	N/A	N/A	1
Culebra, PR	Reserva Canal Luis Pena	N 18°19.238’	W 65°19.385’	N/A	1
Lee Stocking Island, Bahamas					
	Reef 1	N 23°46.85’	W 76°06.21’	N/A	2
	Reef 2	N 23°47.383’	W 76°08.264’	N/A	2
	Reef 3	N/A	N/A	N/A	2

Two sampling methods were implemented. First, to exhaustively sample a specific location, we sampled each species using a concentric circle design with a five meter radius for *A. palmata* (Figure 3) as implemented by Baums et al. (2005a) and about a 10 m radius for *A. cervicornis* (Figure 4), since patches were seen more distantly separated. We restricted our circles to 5 m and 10 m for the two acroporids because circles of a larger radius did not further contribute to the genotypic variation as suggested by Baums et al. (2005b). The distances of the

colonies from the center point, angle measurements, and samples were taken for each colony. Secondly to assess the genetic variability in the reef as a whole, we haphazardly collected at least 15 colonies of each species at least 5 m apart from each reef in order to reduce the probability of collecting clones. Each circle was marked with a GPS coordinate, and the haphazardly collected colonies were collected far from where the circle method was implemented. Therefore, colonies were not sampled twice.

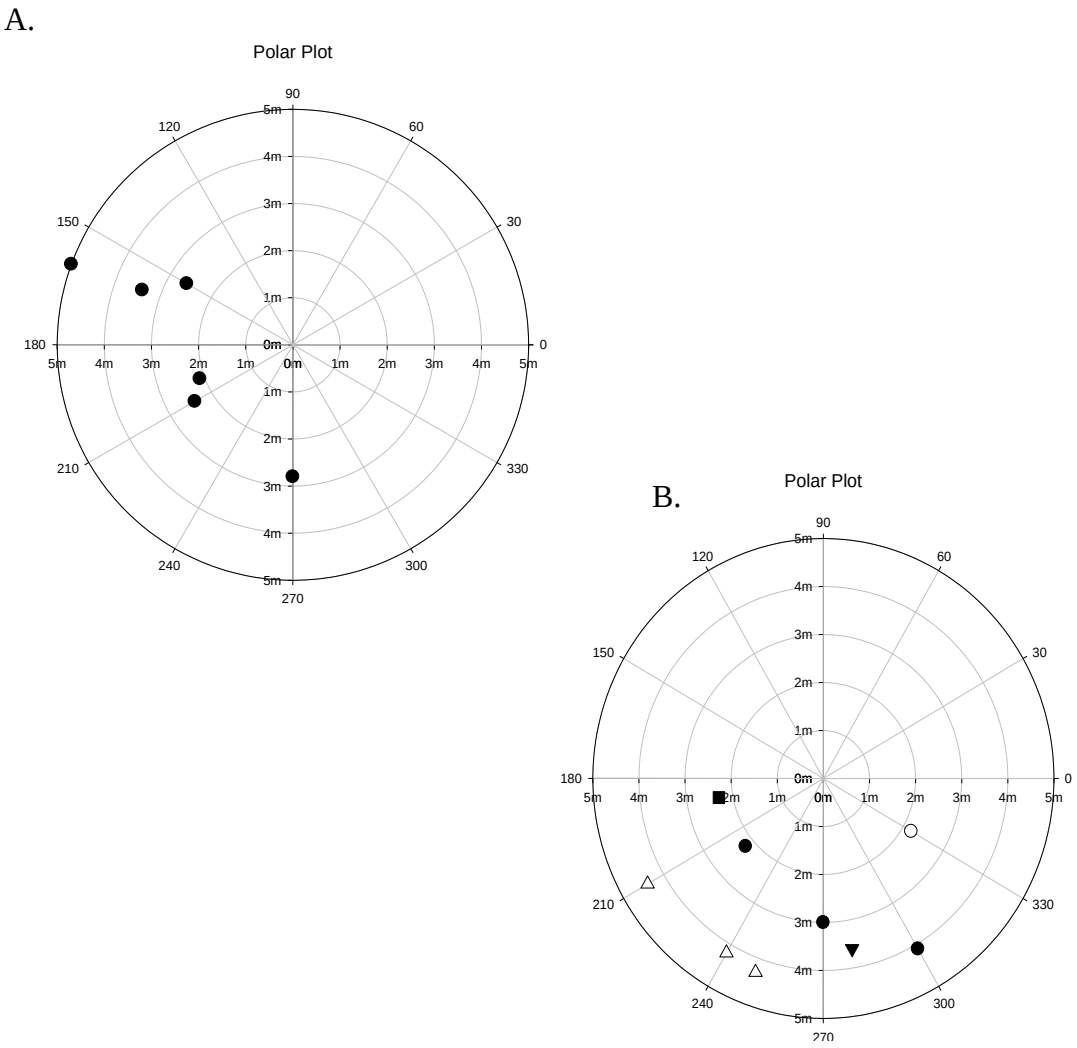

Figure 3. Polar plot of the concentric circle method for *A. palmata*. (A)Reflects one circle from the Turrumote reef where there is no haplotypic variation and (B) reflects one circle from the Margarita reef where there are five different haplotypes. Each unique symbol represents a distinct haplotype.

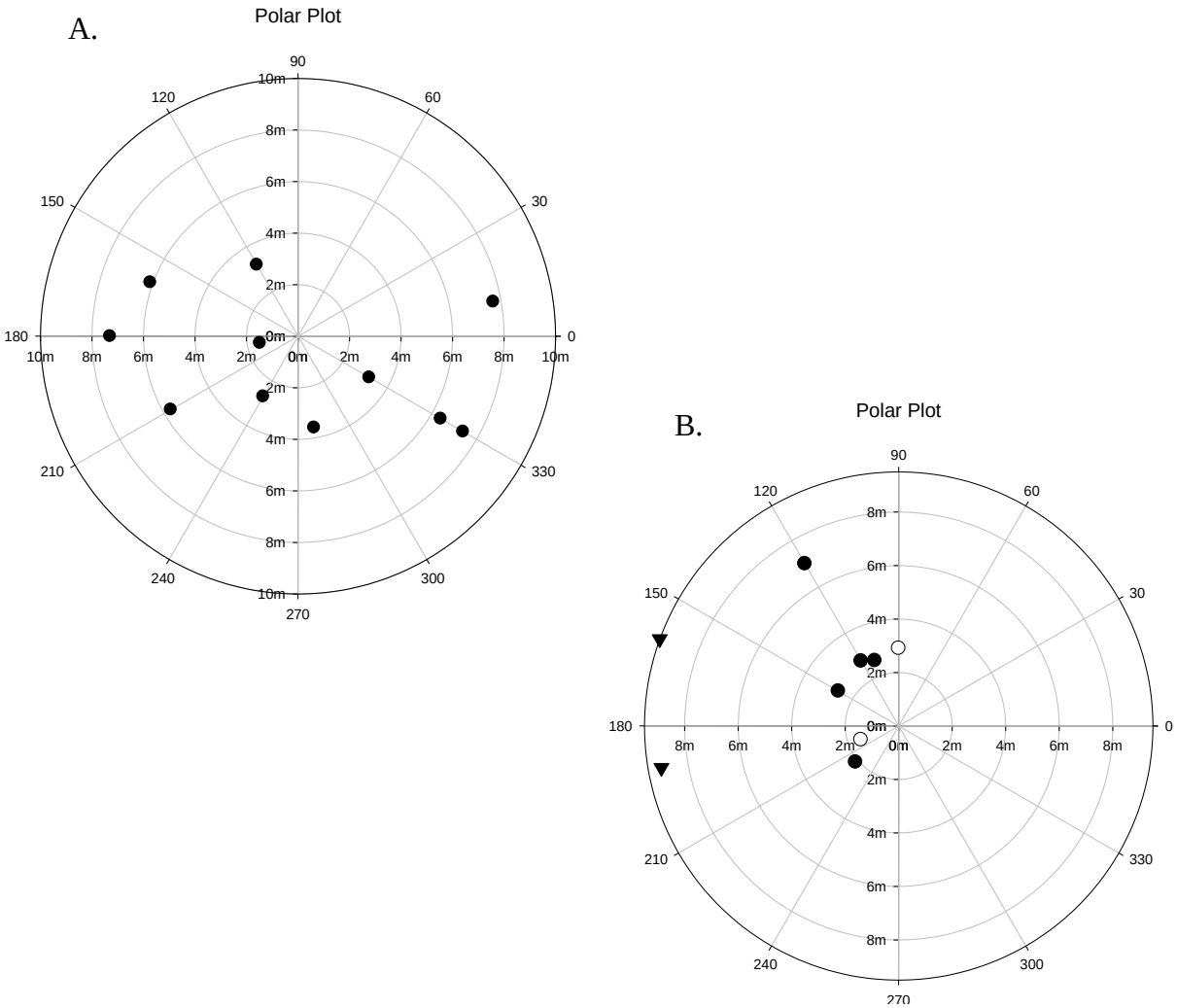

Figure 4. Polar plot of the concentric circle method for *A. cervicornis.* (A) Reflects one circle for the San Cristobal reef where there is no haplotypic variation and (B) reflects one circle for the Media Luna reef where there are three different haplotypes. Each unique symbol represents a distinct haplotype.

Collection of tissue and DNA extraction

Tissue was collected during either snorkeling or SCUBA diving. Using a pair of tweezers, a small piece with three or four individual polyps, along with the epitheca was collected per *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 in a 1.5 ml centrifuge tube whenever feasible. Tissue was stored in 70-100% ethanol and placed at -20° C until extraction.

DNA was extracted from freshly collected and/or stored specimens using a PUREGENE DNA Purification Kit (Gentra Systems) following the protocol for DNA purification from 5-10 mg of solid tissue fixed in ethanol or formalin. Samples were hydrated with 50 µl of the Hydration Solution. DNA samples were quantified and stored at 4° C or –20° C after concluding the extraction.

PCR and Sequencing Conditions

Standard PCR amplifications were performed in an Eppendorf mastercycler. After screening of several nuclear and a mitochondrial marker for genetic variation, the only gene that proved to be polymorphic enough for the geographic scale of our study was the mitochondrial control region (1062 bp). The PCR mix for the control region was identical for *A. cervicorni*s and *A. palmata*, and contained 1.0 µl of each primer (10 µM/µl), 5.0 µl of $MgCl_2$ (Promega 25 mM), 0.5 µl of dNTPs (25 mM), 5.0 µl of 10xPCR Buffer (Promega), 1 unit of Taq, and 36.2 µl of ddH2O 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 evaluated using a 1% agarose gel electrophoresis and catalogued digitally. PCR reactions were then cleaned from excess dNTPs, primers and other impurities by the enzymatic treatment ExoSap. The quality and quantity of the ExoSap product was evaluated in a 1% agarose gel.

The PCR conditions were identical for *A. cervicorni*s and *A. palmata*: initial denaturation at 94°C for 3 minutes, then at 94°C for 15 seconds, annealing at 46°C for 30 seconds, extension at 72°C for 45 seconds, and the final extension at 72°C for 5 minutes. The denaturing, annealing, and extension steps were repeated 35 times, before the final extension step took place.

Sequencing reactions were prepared with a DYEnamic ET Terminator Cycle Sequencing Kit (GE) and loaded in a MEGABase 96 lane Sequencer for capillary electrophoresis. DNA sequencing trace files were processed with the Phrap/Phred/Consed programs (Ewing and Green 1998, Ewing et al. 1998, Gordon 2003) for base calling, quality assessment, contig assembly, and visualization. Edited DNA sequences were imported in MacClade (Maddison and Maddison 2000) to derive a homologous alignment.

Genetic Analysis

The genetic divergences (Φ_{ST}) within and among reefs and within and among localities were calculated in Arlequin ver 3.11 (Excoffier et al. 2005). The AMOVA method was implemented 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 method, and the haphazardly collected colonies, to compare the effects of the different sampling methods. In some cases, some reefs were sampled by one collection method and excluded from analysis. For AMOVA, we did not include sequences from other studies. *A. cervicornis* samples from Culebra were left out of the AMOVA analysis due to the isolation of only one individual. The Bonferroni correction was used for multiple testing errors.

Nuclear diversity indices (π and θ) and haplotype diversity were estimated using the program DNAsp 4.0 (Rozas et al. 2003). In order to evaluate the differences in genetic diversity between the two 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 two collection methods since in some instances the circle method displayed higher diversity than the haphazard method.

Haplotypes from each species were imported into PAUP* 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 by the hierarchical likelihood ratio tests implemented in MODELTEST 3.06. Haplotype networks for each species were constructed by statistical parsimony in TCS 1.21 (Templeton et al. 1992, Clement et al. 2000). For the construction of haplotype networks, gaps were considered as a 5^{th} state. The Puerto Rico sequences from the Vollmer and Palumbi (2007) study were obtained from GeneBank (AF507194-AF507196; AF507202-AF507207; AF507290-AF507309 for *A. cervicornis*) and (AF507220-AF507238; AF507253-AF507255 for *A. palmata*) and were included in the construction of the haplotypes networks and maximum likelihood genealogies.

RESULTS

The amplified control region after quality verification and trimming was approximately 959bp long for *A. palmata* and 1062bp for *A. cervicornis.* We identified 25 haplotypes for *A. palmata* in Puerto Rico and two in the Bahamas (Appendix I). DNA analysis from 200 *A. palmata* colonies from Puerto Rico resulted in a relatively high haplotype diversity (h_d= 0.333) and low nucleotide diversity (π= 0.00075). We also identified 24 haplotypes for *A. cervicornis* around Puerto Rico and four in the Bahamas, twice as many as previously identified for Puerto Rico in a previous study (Vollmer and Palumbi 2007) (Appendix II). 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) (Table 4), than those reported previously from a Caribbean wide study (276 colonies, h_d= 0.847 and π= 0.0057) (Vollmer and Palumbi

2007). In La Parguera, one additional haplotype of *A. palmata* was observed in Media Luna and Guánica) and six more haplotypes of *A. cervicornis* were observed in Media Luna and two less in San Cristobal compared to those reported by Vollmer and Palumbi (2002). There were seven and 12 transitions and three and eight transversions in *A. palmata* and *A. cervicornis*, respectively.

AMOVA results including both collection methods

For *A. palmata*, the overall Φ_{ST} was significant (0.0863, P< 0.00098) for the Puerto Rico locations (Mona Island, Desecheo Island, La Parguera, Tres Palmas, and Guánica; collection methods combined) (Table 3). The Φ_{ST} between all of Puerto Rico vs. Lee Stocking Island, Bahamas was also significant (0.0726, P< 0.0401) (Table 3). A significant Φ_{ST} indicates that there is restriction in gene flow both among reefs within a region separated by a few km and among regions separated by several hundred km, agreeing with results from previous studies in some of the same reefs (Vollmer and Palumbi 2002, 2007). These results show that both collection methods were sensitive enough to detect significant population structure. In *A. cervicornis*, the overall Φ_{ST} for all Puerto Rico locations (Mona Island, Desecheo Island, La Parguera) was significant (0.1237, P<0.0059) (Table 4). La Parguera included samples collected with both methods. The Φ_{ST} between all of Puerto Rico vs. Lee Stocking Island, Bahamas was also significant in *A. cervicornis* (0.1840, P< 0.0244) (Table 4).

Table 3. Hierarchical analysis of molecular variance (AMOVA) for *A. palmata*. Mar=Margarita, Enr=Enrique, Turr=Turrumote, Lau=Laurel, and ML=Media Luna. Values were generated with 1000 permutations, using the Kimura 2-P model. * significant at p<0.05; **significant at p<0.001.

A. palmata	**d.f.**	**Sum of squares**	**Variance components**	**% of variation**	Φ_{ST}
Between regions (La Parguera haphazard 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		
Between regions (La Parguera circles vs. Desecheo Island vs Mona Island vs. Bahamas vs. Guanica 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 in La Parguera haphazard method (Mar vs. Enr vs. Turr vs. Lau vs. ML vs. EP)					
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		
Between reefs in La Parguera circle method (Mar vs. Enr vs. Turr vs. Lau vs. ML)					
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		
Between reefs in Puerto Rico (All La Parguera vs. Mona Island vs. Desecheo Island vs. Guanica 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		
Between reefs in All Puerto Rico 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		

Table 4. Hierarchical analysis of molecular variance (AMOVA) for *A. cervicornis* using the introgressed and native alleles, combined). ML= Media Luna, SC=San Cristobal, Lau=Laurel, and Atra=Atravesado. Values were generated with 1000 permutations, using the Kimura 2-P model. * significant at p<0.05; **significant at p<0.001.

***A. cervicornis* mtDNA**	**d.f.**	**Sum of squares**	**Variance components**	**% of variation**	Φ_{ST}
Between reefs in La Parguera using haphazard collection method (ML vs. SC vs. Lau vs. Atr)					
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		
Between reefs in La Parguera using circle collection method (ML vs. SC vs. Atr)					
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 haphazard 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		
Between regions (La Parguera circles 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 in Puerto Rico (All La Parguera 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		
Between reefs in All Puerto Rico 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		

Both methods display significant differentiation but the haphazard method was more successful in detecting differentiation between more than two reefs. Sampling colonies at least every 5m (haphazard in this study) decreases the chances of sampling clones, expecting to capture more differentiation between colonies. However, when both collection methods were combined for *A. palmata* in La Parguera, there were significant pairwise differences between Mona Island vs. La Parguera, Mona Island vs. Tres Palmas, Desecheo Island vs. La Parguera, Desecheo Island vs. Tres Palmas, and Desecheo Island vs. Guánica (Table 5). When both collection methods were combined for *A. cervicornis* in La Parguera, significant pairwise

differences were detected between Mona Island vs. La Parguera (including both introgressed and native alleles) and between Desecheo Island vs. La Parguera (including native alleles) (Table 6). When all samples from Puerto Rico were combined and compared to samples from Lee Stocking Island, Bahamas for *A. palmata* and *A. cervicornis*, the pairwise comparisons were also significant (0.07260, P< 0.04492 and 0.18404, P< 0.01953, respectively) (data not shown). Significant pairwise comparisons demonstrate that there is significant population differentiation, both, between regions and between reefs in *A. palmata* and between reefs in *A. cervicornis*, regardless of which collection method was used.

Table 5. Pairwise differences between reefs in *A. palmata* around Puerto Rico. All La Parguera includes samples collected using both collection methods. Values were generated with 1000 permutations, using the Kimura 2-P model. * significant at p<0.05 before correction; **significant at p<0.001. Numbers in bold are significant after Bonferroni correction.

A. palmata				
	Mona Is.	Desecheo Is.	All La Parguera	Tres Palmas
Mona Is.				
Desecheo Is.	0.1404			
La Parguera	0.1166*	**0.4832****		
Tres Palmas	0.0976*	**0.4519****	0.0007	
Guanica	0.0108	0.7234*	-0.0254	-0.0751

Table 6. Pairwise differences of *A. cervicornis* between reefs around Puerto Rico including all colonies, regardless the sampling method. Values were generated with 1000 permutations, using the Kimura 2-P model. The upper values reflect the native mtDNA and the bottom reflect the introgressed and native alleles combined. * significant at the p<0.05.

A. cervicornis			
	Mona is	Desecheo Is.	La Parguera
Mona Is.		-0.051	0.0624
Desecheo Is.	0.0067		0.148*
La Parguera	0.1598*	0.0389	

AMOVA results including only the haphazard method

AMOVA tests showed significant differentiation among populations (reefs) and/or regions in both species (Tables 3 and 4). In *A. palmata* a comparison among reefs in La Parguera (Margarita, Enrique, Turrumote, Laurel, Media Luna, and El Palo) showed significant

population structure (Φ_{ST} = 0.1558, P< 0.0225) (Table 3). The smallest distance between reefs was 1.10 km (Margarita and El Palo) and the greatest distance was 8.5 km (Margarita vs. Turrumote). When a comparison among regions (La Parguera, Mona Island, Bahamas, Desecheo Island, Tres Palmas, and Guánica) was performed, significant population structure was also observed (Φ_{ST} = 0.1156, P< 0.002). Guánica was excluded since the haphazard method was not carried out there.

AMOVAs for *A. cervicornis* suggested similar population trends. A comparison in the mtDNA (including introgressed and native alleles) of *A. cervicornis* among reefs in La Parguera (Media Luna, San Cristobal, Atravesado, and Laurel) displayed significant population structure (Φ_{ST} = 0.6665, P< 0.0001) (Table 4). The smallest distance between reefs was 1.21 km (Laurel vs. San Cristobal) and the greatest distance was 4.37 km (Atravesado vs. Media Luna). The comparison among regions (La Parguera, Mona Island, Desecheo Island, and Bahamas) in the mtDNA also reflected significant population structure (Φ_{ST} = 0.2284, P< 0.0001). Meanwhile, the native mtDNA in *A. cervicornis* displayed significant population structure among reefs but not among regions (Table 7). Comparisons among reefs (Laurel, San Cristobal, and Atravesado) demonstrated significant population structure (Φ_{ST} =0.919, P< 0.0001). However, comparisons among regions (La Parguera, Mona Island, Desecheo Island, and Bahamas) suggested no population structure. No population structure indicates that there is enough gene flow between reefs so that they are genetically homogeneous. As mentioned earlier significant population structure suggests restrictions on gene flow even at a finer scale which can be observed when you compare populations within La Parguera. The population structure is not seen at larger scales (between regions) since the regions potentially share most of the haplotypes.

Table 7. Hierarchical analysis of molecular variance (AMOVA) for *A. cervicornis* using only the native mtDNA. ML= Media Luna, SC=San Cristobal, Lau=Laurel, and Atra=Atravesado. All La Parguera includes samples collected using both collection methods. Values were generated with 1000 permutations, using the Kimura 2-P model. * significant at $p<0.05$; **significant at $p<0.001$.

***A. cervicornis* native mtDNA**	**d.f.**	**Sum of squares**	**Variance components**	**% of variation**	Φ_{ST}
Between reefs in La Parguera using haphazard collection method (Lau vs. SC vs. Atra)					
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		
Between reefs in La Parguera using circle collection method (ML vs. SC vs. Atra)					
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 haphazard 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		
Between regions (La Parguera circles 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 (All La Parguera 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		

Pairwise comparisons among reefs in La Parguera for *A. palmata* showed significant differentiation. Significant differentiation was detected between Turrumote vs. Laurel, Enrique vs. Laurel, and Enrique vs. Turrumote (Table 8). Pairwise comparisons among regions showed significant differentiation in *A. palmata* between Mona Island vs. La Parguera, Desecheo Island vs. Tres Palmas, Desecheo Island vs. La Parguera, and Bahamas vs. La Parguera (Table 9).

Table 8. Pairwise differences of *A. palmata* between reefs in La Parguera, PR. The upper values reflect the concentric circle collection method while the bottom values reflect the haphazard collection method. Values were generated with 1000 permutations, using the Kimura 2-P model. * significant at p<0.05; **significant at p<0.001, N/A = location not sampled with that particular collection method. Numbers in bold are significant after Bonferroni correction.

A. palmata						
	Laurel	Turrumote	Enrique	Media Luna	Margarita	El Palo
Laurel		**0.7717****	**0.3285****	-0.1331	0.1202	N/A
Turrumote	0.3749*		**0.6795****	**0.4272****	0.2774*	N/A
Enrique	0.365*	0.2867*		**0.3530****	**0.425****	N/A
Media Luna	0.1828	**0.2792****	-0.1139		-0.0146	N/A
Margarita	0.0677	**0.3027****	-0.0549	-0.0902		N/A
El Palo	0.3437*	0.2432	-0.1965	-0.0302	0.027	

Table 9. Pairwise differences between regions in *A. palmata*. The upper values reflect the concentric circle collection method and the bottom reflect the haphazard collection method. Values were generated with 1000 permutations, using the Kimura 2-P model. * significant at p<0.05; **significant at p<0.001. Numbers in bold are significant after Bonferroni correction.

A. palmata						
	Mona Is.	Desecheo Is.	La Parguera	Tres Palmas	Guanica	Bahamas
Mona Is.		0.1404	0.1243*	0.0999*	0.0108	-0.0437
Desecheo Is.	0.1404		0.455*	**0.4261****	0.7234*	0.0325
La Parguera	0.1056*	**0.5529****		0.02926	0.0038	0.1061*
Tres Palmas	0.0508	0.581*	-0.0494		-0.0599	0.0709
Guanica	N/A	N/A	N/A	N/A		-0.0106
Bahamas	-0.04371	0.03254	0.10593*	0.02709	N/A	

Pairwise comparisons in *A. cervicornis* showed significant differentiation between reefs separated by a few km in La Parguera (Tables 10 and 11). As expected, the haphazard collection method was more efficient in revealing significant population structure in the control region. Pairwise comparisons among regions (separated by hundreds of km) also showed significant differentiation in *A. cervicornis* (Tables 12 and 13). Significant population differentiation was detected between Mona Island vs. Bahamas, La Parguera vs. Mona Island, Desecheo Island vs. Bahamas, and La Parguera vs. Desecheo Island (includes both introgressed and native alleles). The native mtDNA showed significant reef subdivision in La Parguera vs. Desecheo Island and La Parguera vs. Bahamas.

Table 10. Pairwise differences of *A. cervicornis* between reefs in La Parguera, Puerto Rico, using introgressed and native alleles combined. Values were generated with 1000 permutations, using the Kimura 2-P model. The upper values reflect the concentric circle collection method and the bottom reflect the haphazard collection method. * significant at the p<0.05; ** significant at p<0.001. Numbers in bold are significant after Bonferroni correction.

***A. cervicornis* mtDNA**				
	Media Luna	San Cristobal	Atravesado	Laurel
Media Luna		**0.5302****	0.0950	N/A
San Cristobal	**0.9278****		**0.8055****	N/A
Atravesado	**0.5114****	**0.6831****		N/A
Laurel	**0.7381****	**0.3076****	**0.4580****	

Table 11. Pairwise differences of *A. cervicornis* between reefs in La Parguera, Puerto Rico, using native alleles only. Values were generated with 1000 permutations, using the Kimura 2-P model. The upper values reflect the concentric circle collection method and the bottom reflect the haphazard collection method. * significant at the p<0.05; ** significant at p<0.001. Numbers in bold are significant after Bonferroni correction.

***A. cervicornis* native mtDNA**				
	Media Luna	San Cristobal	Atravesado	Laurel
Media Luna		**0.8438****	**0.6083****	N/A
San Cristobal	N/A		1*	N/A
Atravesado	N/A	0.9395		N/A
Laurel	N/A	**0.8615****	1.0000	

Table 12. Pairwise differences of *A. cervicornis* between regions in Puerto Rico using introgressed and native alleles. Values were generated with 1000 permutations, using the Kimura 2-P model. The upper values reflect the concentric circle collection method and the bottom reflect the haphazard collection method. * significant at the p<0.05; ** significant at p<0.001. Numbers in bold are significant after Bonferroni correction.

***A. cervicornis* mtDNA**				
	Mona Is.	Desecheo Is.	Bahamas	La Parguera
Mona Is.		0.0067	**0.5375****	**0.1643****
Desecheo Is.	0.0067		0.3899*	0.0177
Bahamas	**0.5375****	0.3899*		0.1992*
La Parguera	**0.2688****	0.1422*	0.0671	

Table 13. Pairwise differences of *A. cervicornis* between regions in Puerto Rico using the native mtDNA alleles. Values were generated with 1000 permutations, using the Kimura 2-P model. The upper values reflect the concentric circle collection method and the bottom reflect the haphazard collection method. * significant at the p<0.05; ** significant at p<0.001.

***A. cervicornis* native mtDNA**				
	Mona Island	Desecheo Is.	Bahamas	La Parguera
Mona Is.		-0.051	0.3659	0.0715
Desecheo is	-0.051		0.417	0.1379
Bahamas	0.417	0.417		0.1944
La Parguera	0.0353	0.1899*	0.5440*	

AMOVA results including only the circle collection method

In *A. palmata* a comparison among reefs in La Parguera (Margarita, Enrique, Turrumote, Laurel, Media Luna, and El Palo) showed significant population structure (Φ_{ST} = 0.3806, P<

0.0001) (Table 3). El Palo was excluded since no circle method was implemented there. The smallest distance between reefs was 1.10 km (Margarita and El Palo) and the greatest distance was 8.5 km (Margarita vs. Turrumote). When a comparison among regions (La Parguera, Mona Island, Bahamas, Desecheo Island, Tres Palmas, and Guánica) was performed, significant population structure was also observed (Φ_{ST} = 0.0957, P< 0.0001) (Table 3).

AMOVAs for *A. cervicornis* indicated similar population subdivisions. A comparison in the mtDNA (including introgressed and native alleles) of *A. cervicornis* among reefs in La Parguera (Media Luna, San Cristobal, Atravesado) displayed significant population structure (Φ_{ST} = 0.5098, P< 0.0001) (Table 4). The smallest distance between reefs was 1.21 km (Laurel vs. San Cristobal) and the greatest distance was 4.37 km (Atravesado vs. Media Luna). The comparison among regions (La Parguera, Mona Island, Desecheo Island, and Bahamas) in the mtDNA also reflected significant population structure (Φ_{ST} = 0.1678, P< 0.00098) (Table 4).

Meanwhile, the native mtDNA in *A. cervicornis* displayed significant population structure among reefs but not among regions. The comparison among reefs (Media Luna, San Cristobal, and Atravesado) resulted in significant population structure within La Parguera (Φ_{ST} = 0.8564, P< 0.0001) (Table 7). However, comparisons among regions (La Parguera, Mona Island, Desecheo Island, and Bahamas) suggested lack of population structure indicating that these regions are genetically homogeneous.

Pairwise comparisons among reefs in La Parguera for *A. palmata* showed significant differentiation between Margarita and Turrumote, Margarita vs. Enrique, Media Luna vs. Turrumote, Media Luna vs. Enrique, Enrique vs. Turrumote, and Turrumote vs. Laurel (Table 8). Pairwise comparisons among regions detected significant differentiation in *A. palmata* between Mona Island vs. Tres Palmas, Desecheo Island vs. Tres Palmas, Mona Island vs. La Parguera,

Desecheo Island vs. La Parguera, Guánica vs. Desecheo Island, and Bahamas vs. La Parguera (Table 9).

Pairwise comparisons among regions (separated by hundreds of km) also showed significant differentiation in mtDNA of *A. cervicornis* between Mona Island vs. Bahamas, La Parguera vs. Mona Island, Desecheo Island vs. Bahamas, and La Parguera vs. Bahamas (Table 10). The native alleles did not show significant population structure when the circle collection method was used.

mtDNA diversity indices

The overall genetic diversity for *A. palmata* in La Parguera was low (π = 0.0007). Similarly low values were detected in *A. cervicornis*, mtDNA (π = 0.00512, including both introgressed and native alleles) and (π = 0.0012) for the native mtDNA. When reefs in La Parguera were compared, the highest values of π and θ of *A. palmata* were from samples collected in Enrique (Table 14). The highest values of π and θ of *A. cervicornis* were found in the Laurel population (Table 15). The lowest values of π and θ of *A. palmata* were found in the samples from El Palo (Table 14). The lowest values of π and θ of *A. cervicornis* were found in San Cristobal (Table 15). When all the regions were compared for *A. palmata*, Bahamas showed the highest nucleotide diversity followed by Mona Island and La Parguera. *A. cervicornis* showed the highest nucleotide diversity in La Parguera followed by Desecheo Island, Bahamas, and Mona Island (Table 15). When using the native mtDNA, Desecheo Island displayed the highest genetic diversity followed by Mona Island and La Parguera (Table 16). None of the neutrality tests in *A. palmata* and *A. cervicornis* (except Laurel) were significantly different from equilibrium.

Table 14. DNA summary statistics of the mtDNA for *Acropora palmata*. Gaps were included in the analysis. The V&P sequences refer to the control region sequences from Vollmer and Palumbi (2002). S= segregating sites and *h*= number of haplotypes. **significance at p<0.01. N/A = test not performed

A. palmata						
Location	**# of colonies**	***S***	***h***	***π***	***θ***	**Tajima's *D***
Tres Palmas circles	32	3	8	0.00082	0.00079	-0.1821
Tres Palmas haphazard	9	1	6	0.00047	0.00041	0
All Tres Palmas	41	3	9	0.00075	0.00075	-0.42728
Laurel circles	12	2	4	0.00027	0.00078	-1.14053
Laurel haphazard	6	1	5	0.00035	0.00046	-0.93302
All Laurel	18	3	9	0.00048	0.00096	-1.50776
Margarita circles	13	3	8	0.00102	0.00105	-0.27429
Margarita haphazard	13	2	7	0.00045	0.00071	-1.14915
All Margarita	26	3	10	0.0006	0.00085	-0.31053
Turrumote circles	11	0	2	0	0	0
Turrumote haphazard	6	3	5	0.00113	0.00162	0.31063
All Turrumote	17	3	6	0.00064	0.00095	-0.69681
Enrique circles	16	3	6	0.00083	0.00096	1.03439
Enrique haphazard	7	1	4	0.0002	0.00057	0
All Enrique	23	3	9	0.00089	0.00087	1.58255
Media Luna circles	16	2	5	0.00069	0.00063	-1.16221
Media Luna haphazard	8	1	3	0.0003	0.00046	0
All Media Luna	24	2	6	0.00055	0.00057	-1.15933
El Palo haphazard	13	1	5	0.0004	0.00036	0
La Parguera haphazard	53	4	12	0.0005	0.00094	-1.3586
La Parguera circles	69	5	16	0.00085	0.0011	-0.19459
All La Parguera	121	6	20	0.00071	0.00118	-0.76515
Guánica	9	1	3	0.00012	0.0005	0
Mona Island	16	2	6	0.00096	0.00064	1.03439
Desecheo Island	4	1	2	0.00053	0.00057	-0.61237
Bahamas	13	4	6	0.00124	0.00136	-0.64598
All Palumbi	22	1	2	0.00018	0.00029	-0.64112
Palumbi_Media Luna	8	1	2	0.00026	0.00041	-1.05482
Palumbi Guanica	7	0	1	0	0	N/A
Palumbi San Cristobal	4	0	1	0	0	N/A

Table 15. DNA summary statistics of the mtDNA for *Acropora cervicornis.* Gaps were included in the analysis. The V&P sequences refer to the control region sequences from Vollmer and Palumbi (2002). S= segregating sites and h= number of haplotypes. **significance at p<0.01.

***A. cervicornis* mtDNA**						
Location	**# of colonies**	**S**	**h**	**π**	**θ**	**Tajima's *D***
San Cristobal circles	22	0	2	0	0	0
San Cristobal haphazard	8	1	2	0.00024	0.00037	-1.05482
All San Cristobal	30	1	3	0.00006	0.00024	-1.147
Media Luna circles	23	15	6	0.00486	0.00386	0.92229
Media Luna haphazard	15	5	3	0.00074	0.00146	-1.66013
All Media Luna	38	15	8	0.00468	0.00339	1.22462
Laurel haphazard	7	8	2	0.00361	0.0031	0.87554
All Laurel	10	11	4	0.00574	0.00369	2.48296**
Atravesado circles	12	11	6	0.00391	0.00346	0.54678
La Parguera haphazard w/o Atravesado	29	16	8	0.00469	0.00387	0.72332
La Parguera haphazard w/ Atravesado	41	18	14	0.00538	0.00399	1.12767
La Parguera circles	60	18	13	0.005	0.00366	1.11663
All La Parguera	89	18	19	0.00512	0.00337	1.48976
Mona Island	17	14	6	0.00281	0.00393	-1.09397
Desecheo Island	7	8	5	0.0038	0.0031	1.18198
Bahamas	6	10	4	0.00373	0.00415	-0.61195
All Palumbi	19	13	6	0.0029	0.00353	-0.67065
Palumbi Media Luna	4	2	2	0.00095	0.00104	-0.7099
Palumbi San Cristobal	15	12	4	0.00303	0.0035	-0.53793

Table 16. DNA summary statistics of the native mtDNA for *Acropora cervicornis.* Gaps were included in the analysis. The number of colonies only include those colonies with native alleles. The V&P sequences refer to the control region sequences from Vollmer and Palumbi (2002). S= segregating sites and h= number of haplotypes.

***A. cervicornis* native alleles**						
Location	**# of colonies**	**S**	**h**	**π**	**θ**	**Tajima's *D***
San Cristobal circles	22	0	1	0	0	0
San Cristobal haphazard	8	1	2	0.00024	0.00037	-1.05482
All San Cristobal	30	1	3	0.00006	0.00024	-1.147
Media Luna circles	7	4	2	0.00181	0.00155	0.79674
Laurel haphazard	5	0	1	0	0	0
Atravesado	1	0	1	0	0	0
La Parguera haphazard w/ Atravesado	13	2	3	0.00063	0.00061	0.09664
La Parguera circles	29	6	4	0.00145	0.00145	-0.00136
All La Parguera	42	6	6	0.00121	0.00132	-0.22119
Mona Island	15	6	4	0.00128	0.00175	-0.93509
Desecheo Island	5	3	4	0.00133	0.00137	-0.17475
Bahamas	1	0	1	0	0	0
All Palumbi	17	3	4	0.0013	0.00084	1.53282
Palumbi Media Luna	4	2	2	0.00095	0.00104	-0.7099
Palumbi San Cristobal	13	2	2	0.00097	0.00061	1.66129

Gene genealogies

Gene genealogies were constructed in PAUP using maximum likelihood with the HKY and K81uf+I+G models as the most suitable models of substitution for *A. palmata* and *A. cervicornis* as suggested by ModelTest. The HKY model was implemented with unequal base frequencies (0.2411, 0.1618, 0.2701, 0.3270) for *A. palmata* and the K81uf+I+G model was implemented with unequal base frequencies (0.3328, 0.2651, 0.1749, 0.2272, I = 0.9730, G = 41.6402) for *A. cervicornis* (Figure 5). The resolution of the maximum likelihood tree for *A. palmata* was not informative (data not shown). Maximum parsimony networks were constructed for *A. palmata* and *A. cervicornis* (Figures 6 and 7). Hap_1 (20%), Hap_7 (20%), and Hap_4 (11%) are the most wide-spread and numerous of all haplotypes found for *A. palmata*. These three haplotypes were found in almost every reef and region sampled (Appendix III, Figure 6).

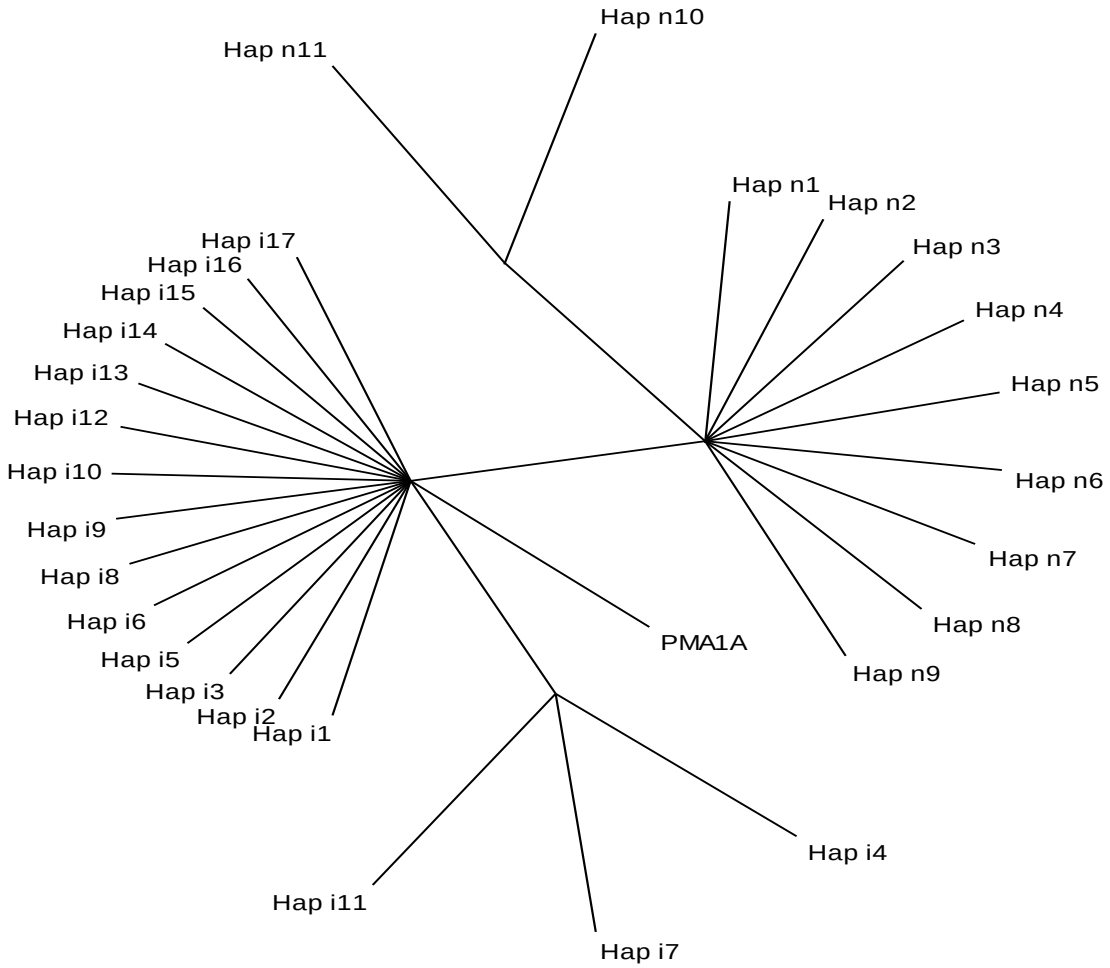

Figure 5. Maximum likelihood tree of *A. cervicornis* under the K81uf+I+G model with unequal base frequencies (0.3328, 0.2651, 0.1749, 0.2272, I = 0.9730,G = 41.6402). The haplotypes with an "i" in front of them indicate introgressed haplotypes, while those with "n" indicate native haplotypes. PMA1A is an *A. palmata* sequence as an outgroup. Hap n7 (AF507294) and Hap i8 (AF507297) are from Vollmer and Palumbi (2002).

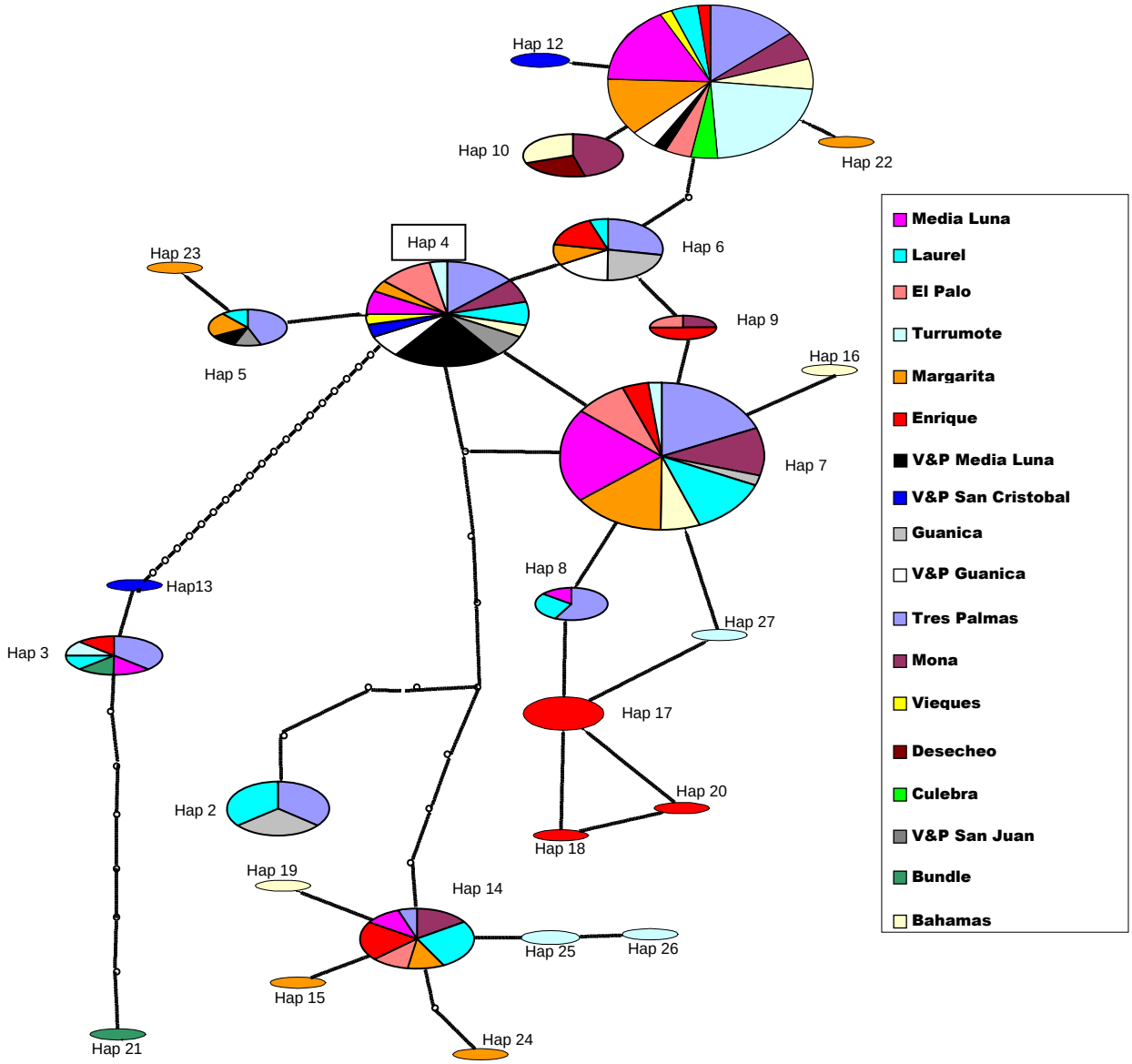

Figure 6. Statistical parsimony network of *A. palmata* haplotypes. The boxed haplotype is identified by TCS as the root of the network. The size of the circle is proportional to the number of sequences for that corresponding haplotype. The minimum number of steps is represented by the small empty circles between each haplotype. Hap 12 (AF507256) and Hap 13 (AF507220) are from Vollmer and Palumbi (2002). Closed loops represent homoplasy as inferred by the TCS program. Hap3, 13, and 21 were connected to the main network, when allowing 20 mutational steps, but the divergence exceeded the 95% limit for connections.

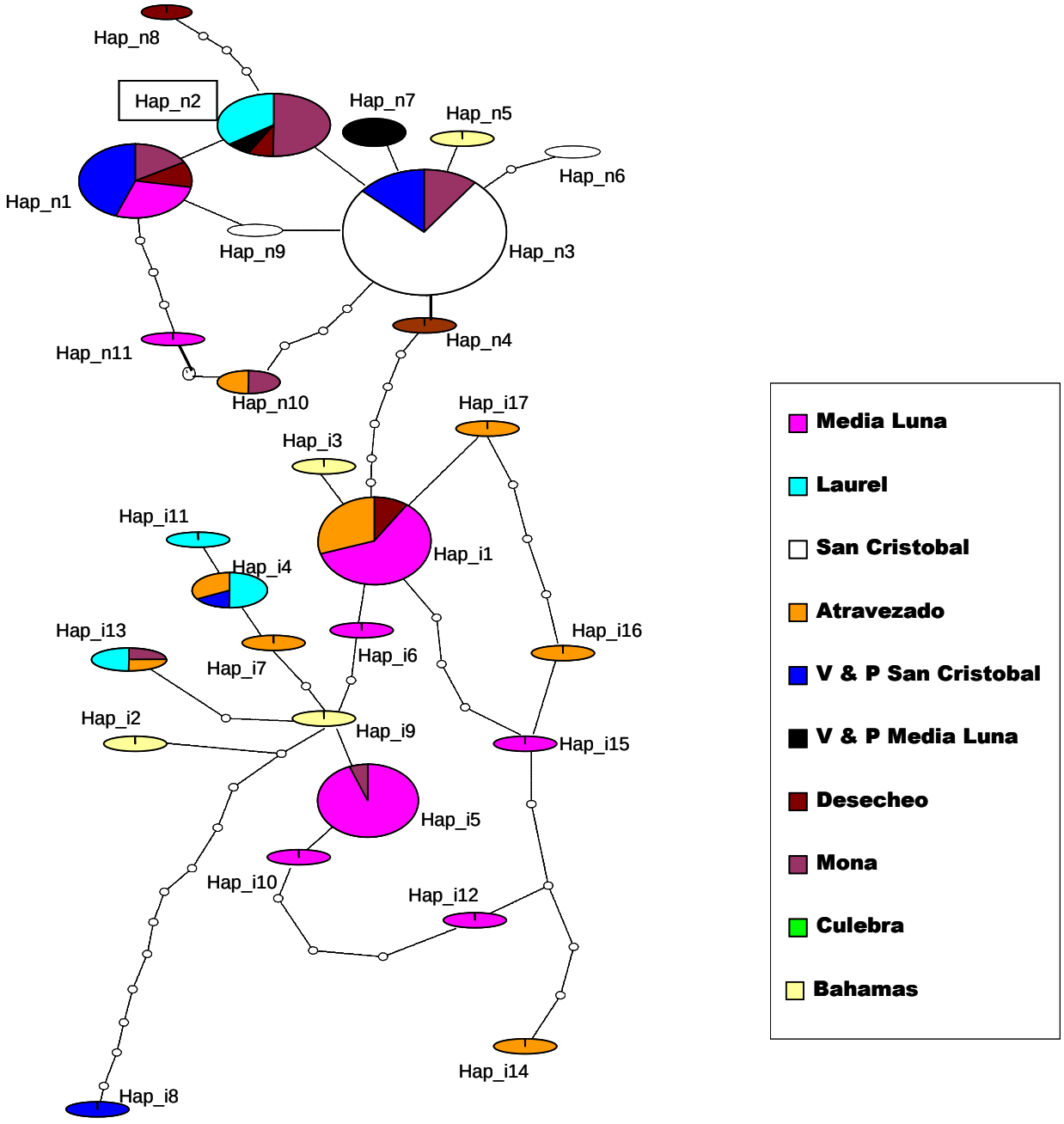

Figure 7. Statistical parsimony network of *A. cervicornis* haplotypes. The boxed haplotypes are identified by TCS as the root of each network. Haplotypes with an "n" in front of them describe the native alleles while those with an "i" in front of them describe the introgressed alleles. The size of the circle is proportional to the number of sequences for that corresponding haplotype. The minimum number of steps is represented by the small empty circles between each haplotype. Hap n7 (AF507294) and Hap i8 (AF507297) are from Vollmer and Palumbi. All haplotypes were jointed by using the 95% limit for connections for the control region.

The maximum likelihood tree and maximum parsimony network reflected similar topologies for *A. cervicornis.* Both maximum likelihood and maximum parsimony displayed two clades, one containing the native alleles (n#) and the other clade the introgressed alleles (i#) for *A. cervicornis* (Figures 5 and 7). 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 (Appendix IV, Figures 5 and 7) in the sampling area.

DISCUSSION

Structure among reefs in La Parguera, Puerto Rico

Significant genetic population structure was detected in La Parguera for *Acropora palmata* and *A. cervicornis* suggesting that there has been restriction of gene flow between reefs in both species. Pairwise comparions suggest that exchange of genetic material between the coral colonies has been occurring between some reefs and not in others. The Island mass effect, described by Hamner and Hauri (1981), could influence patterns of genetic connectivity observed in La Parguera. This effect takes into consideration many variables that cause the water flow to vary around islands and reefs. Variations in current speed, tidal flow, size of islands or reefs, depth, and substrate are some of these variables which affect the distribution and abundance of organisms (Hamner and Hauri 1981, Sammarco and Andrews 1989, McGehee 1994, Hohenlohe 2004, Baums et al. 2006b). Through the deployment of multiple drogues in the Great Barrier Reef, Hamner and Hauri (1981) noticed that there were differences in water motion not only around different sized reefs but also during different tidal levels. Plankton biomass varied depending on tidal time; during ebb tide there were higher copepod densities while larvaceans were in higher concentrations during floodtide (Hamner and Hauri 1981). Coral

larvae also seemed to get trapped in eddies that formed around islands with a noticeable decline of recruits farther from the center of the eddy in the Great Barrier Reef (Sammarco and Andrews 1989) and eddies around the Mona Passage (Baums et al. 2006b). 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 (Appledoorn et al. 1994, Lugo-Fernández et al. 1994, McGehee 1994, Mercado-Molina 2008, Williams et al. in review, S. Williams pers. comm.), influencing patterns and rates of gene flow. A comparison of different reefs determined that water motion was significantly different between back reefs, with the largest movement found in San Cristobal followed by Margarita, and Laurel (McGehee 1994). Also water motion was significantly different in the fore reef, with the largest movement observed in Margarita followed by Laurel, San Cristobal, and Enrique (McGehee 1994). There have been differences in water speed reported between the back reef and fore reef at Media Luna (0.23 km $\cdot$ hr^{-1} and 1.1 km $\cdot$ hr^{-1}, respectively) (Williams et al. in review, S. Williams pers. comm.).

Reefs vary in size, shape and substrate adding to the habitat heterogeneity observed in La Parguera. For instance, Margarita reef is one of the largest reefs and is oriented in an east to west position, San Cristobal is one of the smallest reefs also oriented in an east to west position, however, Atravesado reef is oriented in a north to south orientation (Almy and Carrión-Torres 1963, pers. obs.). The substrate of back reefs in La Parguera varies from seagrass beds, sand to rubble substrate (Irizarry-Soto 2006); none of these habitats are optimal for larval settlement (Szmant and Miller 2006, Irizarry-Soto 2006).

The genetic population structure observed between some reefs in the acroporid species 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 and Hauri 1981, Sammarco and Andrews 1989) resulting in the observed population connectivity. Settlement suitability, differences in water motion and the difference in size and orientation of reefs also could play a significant role in larval dispersal in La Parguera therefore influencing the patterns of gene flow from reef to reef in *A. palmata* and *A. cervicornis.* Current patterns in conjunction with direct observations of newly settled planulae and indirect methods of population connectivity (through genetic data) might provide a better indication of the various natural processes governing the coral distribution around La Parguera.

In smaller geographic scales like 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. San Cristobal reef in the fall, experiences reduced current speed and frequent nighttime current reversals which limited dispersal of fish larvae and aid in the settlement within 2 km of the natal reef (Appledoorn et al. 1994). Recovery of reefs in southwestern Puerto Rico and other similar reefs of close spatial proximity, might rely upon the survival and sexual reproduction of local populations rather than replenishment from distant reefs because of the high levels of population subdivision (Roberts 1997, Vollmer and Palumbi 2007).

The differentiation seen in La Parguera could also be due to historical declines of the acroporid species. Core data from the US Virgin Islands, indicated the dominant presence of *A. palmata* in reefs about 11,000 years ago (Hubbard et al. 2005). Since then, there have been at least two periods where *A. palmata* were not observed in core samples in some Caribbean locations; approximately 5,600 years and 2,600 years ago (Hubbard et al. 2005, Toscano and Macintyre 2003). These declines were not attributed to sea level rise therefore climatic or environmental stress conditions could have induced their decline. *A. palmata* declined around

the southwestern part of Barbados during the Mid-Holocene era during severe storm activity (Macintyre 2007). Similarly, Cobler reef in Barbados was destroyed 3,000 to 4,000 years ago. Due to the strong trade wind activity, sedimentation, and the decrease of herbivory on algae, *A. palmata* has not been able to recover to previous densities in Cobler reef (Macintyre 2007). In La Parguera, 10,000 years ago, the reefs were distributed along the edge of the continental shelf and were dominated by *A. palmata* (Hubbard et al. 1997). Reef accretion ceased at the edge of the continental shelf 7,000 years ago and newly formed reefs landwards were dominated by the sediment tolerant species *Montastrea annularis* (Hubbard et al. 1997). The apparent shift from a *A. palmata*- to *M. annularis*-dominant reefs could be considered as past population bottleneck event. More recently, Hurricane Edith in 1963 devastated areas in the windward outer reef zones in La Parguera (Glynn et al 1964). Partial to complete destruction was observed in Turrumote and Media Luna reefs while a portion of Enrique reef was destroyed (Glynn 1964).

Structure among populations around Puerto Rico

We detected significant population subdivision for *A. cervicornis* in southwestern Puerto Rico (Φ_{ST} = 0.1237, P<0.0059) when using both native and introgressed alleles combined (mtDNA). Our values were similar to those of Vollmer and Palumbi (2007), who found significant 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 and Palumbi (2007), the native alleles only in the present study did not show significant population structure, (Φ_{ST} =0.0964, P<0.08211), suggesting high rates of gene flow between reefs in native alleles. The genetic structure observed in *A. cervicornis* at local and regional scales was mostly due to the introgressed alleles (Vollmer and Palumbi 2007, this study). Sample to sample variability and random senescence of acroporid colonies in La Parguera reefs may have caused the differences

in results 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.

Significant genetic population structure (Φ_{ST} = 0.0410) has been reported for *A. palmata* (Baums et al. 2006a) and for *A. cervicornis* (Vollmer and Palumbi 2007) in a wider geographic scale around the Caribbean. Even though the focus of our study was at a smaller geographic scale, we did detect significant population structure for both acroporids between Puerto Rico and Bahamas (Tables 6, 7). Significant genetic differences were also detected in *A. cervicornis* between Curaçao and the rest of the Caribbean localities sampled by Vollmer and Palumbi (2007). Curaçao was classified as part of the Eastern Caribbean while the other sampled localities (Panama, Bahamas, Belize, and Turks and Caicos) fell in the Western Caribbean. Puerto Rico was genetically distinct compared to all localities except Panama. However, the significant differences between Puerto Rico and Curaçao were not as high when comparing Curaçao to other localities. For example, the pairwise Φ_{ST} between Curaçao and Belize was 0.364 (P<0.001) while the pairwise Φ_{ST} between Curaçao and Puerto Rico was 0.213, (P<0.05), suggesting that Puerto Rico could be a mixing area for *A. cervicornis* (Vollmer and Palumbi 2007). In our study, we found significant pairwise differences in both acroporid species between Puerto Rico and Lee Stocking Island, Bahamas 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 Bahamas indicates that historically there has been a cessation of gene flow between these geographically distant locations.

Restricted gene flow between populations of both species may have been caused by the Mona Passage which flows between Puerto Rico and Hispaniola (Galindo et al. 2006, Baums et al. 2006a, Baums et al. 2006b). A geographic model proposed by Baums et al (2006b) and Galindo et al. (2006) shows that with 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. Currents flowing around Mona Island induce the formation of eddies trapping larvae from both the Western and Eastern Caribbean. However, there could have been instances where the larvae crossed into the Western Caribbean creating a mixing of genetic lineages (Hohenlohe 2004, Cowen et al. 2006, Galindo et al. 2006, Baums et al. 2006a, Baums et al. 2006b).

Our sampling regime intentionally included large numbers of samples around Puerto Rico resulting in higher number of reported haplotypes, because of the Island's proximity to Mona Passage, a suggested biogeographic barrier separating genetically some of the Western from the Eastern Caribbean fauna (Baums et al. 2006, Galindo et al. 2006). Even though our intensive sampling found twice as many haplotypes than previously reported, our study found two less in *A. cervicornis* from San Cristobal, La Parguera. While it is possible that our sampling could have missed other haplotypes, the difference could also be due to environmental and biological stresses. Vollmer and Palumbi sampled in 2001, and since then, Puerto Rico reefs were devastated by the Caribbean wide bleaching event of 2005, (http://coralreefwatch.noaa.gov/caribbean2005/, Donner et al. 2007, Lesser 2007, Wilkinson and Souter 2008) and suffered minor damages by the passing of Hurricane Dean in September 2007 (pers. obs.). Even though Hurricane Dean crossed the Caribbean hundreds of kilometers south of Puerto Rico, the associated surge and waves caused major destruction in coastal reefs of southern

Puerto Rico. For example, several of our localities are presently characterized by a barren substrate. San Cristobal reef was once heavily populated with *A. cervicornis* in the back reef, but after the white band disease epizootic event, predation, and these two destructive events, an unprecedented decrease of this species has occurred (Weil pers. comm., Garcia and Schizas, unpub. data). Longer term field observations are needed to assess the population dynamics of *A. cervicornis*, since the colony densities fluctuate widely throughout years in some La Parguera reefs (Yoshioka pers. comm.).

Conservation genetics of acroporids

Genetic comparisons between reefs and regions might have been influenced by the sampling method. The majority of the reefs in this study were sampled with both, the concentric circle and the haphazard method, however, in *A. cervicornis*, some reefs displayed higher molecular diversity with the circle method, while in other reefs sampling with the haphazard method resulted in higher molecular diversity values. We expected to find more genetic variation from the haphazard method which sampled colonies that were farther apart to avoid the potential of collecting clones than colonies that were closely spaced (circle method). However, contrary to expectations, in four out of the six reefs, *A. palmata* displayed higher diversity indices with the circle method than with the haphazard method. This could be due to the small sample sizes of the haphazard method. However, a similar discrepancy was reported by Baums et al. (2005b) who found that sometimes two circles within the same reef can be genetically distinct. 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 and perhaps sampling methods may need to be more comprehensive. Increasing the sampling size by collecting samples closer together (e.g. 2 m apart rather than 5 m apart) and increasing

the sample area by tens of meters (up to 60 m or more when possible) in both the back and fore reef could provide a more comprehensive look at the genetic connectivity around La Parguera.

Haplotype diversity was relatively high around Puerto Rico even though the nucleotide diversity was low. Both species exhibited low genetic diversity, with *A. cervicornis* having higher values than *A. palmata*. Even though the levels of genetic diversity of *A. cervicornis* were low, they were not atypical compared to some other coral species (*A. cervicornis* π=0.0057, *Siderastrea* sp. π=0.0034, *Pavona cactus* π=0.0069, *Pavona decussata* π=0.0079) (Stephan and Langley 1992, Forsman et al. 2005, Pillay et al. 2006, Vollmer and Palumbi 2007). However, *A. palmata*'s genetic diversity was amongst the lowest reported values for marine invertebrates.

The low genetic variation of acroporids at the population level is concordant with previous reports on the mitochondrial genetic diversity from some scleractinian corals (van Oppen et al. 1999, Shearer et al. 2002, van Oppen et al. 2004, Fukami and Knowlton 2005, Hellberg 2006). Levels of nucleotide substitution at the mitochondrial level are generally low in scleractinians, rendering comparisons even between conspecific taxa unattainable (Neigel et al. 2007). Low levels of mitochondrial diversity may also represent past organelle bottleneck events. Over the last 30 years 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 two acroporids was physical damage inflicted by hurricanes (Bruckner and Bruckner 2001). In Fajardo, Puerto Rico *A. palmata* colonies were severely affected during the 1979 Hurricane David, and almost totally destroyed during 1989 because of Hurricane Hugo (Weil et al. 2003). The northern inshore localities of Puerto Rico have exhibited a 68.4% decline of *Acropora palmata* in the last 20 years, while a decrease of 53.3% has been

recorded from the eastern inshore (Weil et al. 2003). *Acropora cervicornis* has suffered a 100% decline in several sampled transects, in both northern inshore localities and eastern inshore localities (Weil et al. 2003).

As the effective population size (N_e) of a species is determined by the harmonic mean of population sizes across generations, the effective population size of acroporids has been largely determined by the most severe bottleneck events. Additional environmental and biological disturbances (e.g. recurring white band disease outbreaks, snail predation, Hurricane Dean and the 2005 Bleaching event) might have caused further decreases in N_e. Successive population bottlenecks in acroporids might have reduced heterozygosity and depleted the haplotypic diversity, limiting the spectrum of organismal responses to future stresses. The population decline of *Acropora* poses a question about the ecological and evolutionary survival of these important coral reef builders. Reduction of genetic variability and accumulation of deleterious mutations in clonal organisms may eventually decrease species diversity by leading to genetically monomorphic populations which are more susceptible to extinction (Miller and van Oppen 2003). The reduction of genetic variability may be more exacerbated in organisms that rely, wholly or partly upon asexual reproduction such as some groups of scleractinian corals (e.g. acroporids). Since mutations are the raw material of evolution, genetic clones have limited or no potential for withstanding new biotic or abiotic challenges. If the majority of the surviving *Acropora* patches are composed of undifferentiated clonal individuals, then no new genetic variation can be introduced to the gene pool except from the slow processes of mutation, insertion, deletion and gene duplications, immigration of sexual planulae from other localities, genomic disturbances caused by transposable elements, and hybridization with other closely related scleractinian corals. Sexual reproduction generates new gene combinations as a result of

recombination, which purge deleterious mutations (Bachtrog 2003) and adds more genetic variation to the population and increases the probability that one of the variants will be able to survive to changing environmental conditions and defend successfully against parasites and other diseases, increasing the chances for long term survival (Otto and Michalakis 1998, Robson et al. 1999, Bachtrog 2003). The low genetic diversity and the presence of one or two haplotypes of *Acropora* in some reefs may be indicative of limited evolutionary solutions to future environmental changes. Reefs that harbor few haplotypes could be in danger of being decimated by chance (e.g. severe storm) or during another event (e.g. epizootic, hyperthermic). However, by not knowing the comprehensive population and genetic history of either acroporid species before the die-offs, inferences on the long-term survival of the declining populations should be conservative (Grober-Dunsmore et al. 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, a more comprehensive management plan can be implemented (Zubillaga et al. 2008, Hellberg 2006, Grober-Dunsmore et al. 2007, Galindo et al. 2006).

CONCLUSIONS

Significant population structure found among different reefs in Puerto Rico suggests that gene flow is restricted between closely spaced reefs. Pairwise genetic differences showed that some reefs in La Parguera were more connected than others. Reef heterogeneity found in La Parguera and past bottleneck events could have affected the patterns and rates of genetic connectivity between reefs. Significant genetic population structure and pairwise differences

were observed around Puerto Rico as well, suggesting restriction on gene flow in larger geographic scales. Even though our focus was not Caribbean-wide, we found significant pairwise differences between the Bahamas and Puerto Rico supporting previous reports of Puerto Rico having an affinity to the Western Caribbean. In accordance, the Mona Passage could be a potential barrier for both species decreasing the connectivity between localities of the Western and Eastern Caribbean. Therefore, larval dispersal could be limited over long distances restricting larval replenishment of reef systems from other regions. Chances of recovery of impacted reefs may increase when interconnected, genetically diverse reefs are protected.

LITERATURE CITED

Almy, C.C. and C. Carrión-Torres. 1963. Shallow-water stony corals of Puerto Rico. Carib. J. Sci. 3: 133-162.

Appeldoorn, R.S., D.A. Hensley, D.Y. Shapiro, S. Kioroglou and B.G. Sanderson. 1994. Egg dispersal in a Caribbean coral reef fish, *Thalassoma bifasciatum*. II. Dispersal off the reef platform. Bull. Mar. Sci. 54: 271-280.

Aronson, R.B. and W.F. Precht. 2006. Conservation, precaution, and Caribbean reefs. Coral Reefs 25: 441-450.

Bachtrog, D. 2003. Adaptation shapes patterns of genome evolution on sexual and asexual chromosomes in *Drosophila*. Nature Genetics 34: 215-219.

Bak, R.P.M. and M.S. Engel. 1979. Distribution, abundance and survival of juvenile hermatypic corals (Scleractinia) and the importance of life history strategies in the parent coral community. Mar. Biol. 54: 341-352.

Baums, I.B., C.R. Hughes and M.E. Hellberg. 2005b. Mendelian microsatellite loci for the Caribbean coral *Acropora palmata*. Mar. Ecol. Prog. Ser. 288: 115-127.

Baums, I.B., M.W. Miller and M.E. Hellberg. 2005a. Regionally isolated populations of an imperiled Caribbean coral, *Acropora palmata*. Mol. Ecol. 14: 1377-1390.

Baums, I.B., M.W. Miller and M.E. Hellberg. 2006a. Geographic variation in clonal structure in a reef-building Caribbean coral, *Acropora palmata*. Ecol. Monogr. 76: 503-519.

Baums, I.B., C.B. Paris and L.M. Cherubin. 2006b. A bio-oceanographic filter to larval dispersal in a reef-building coral. Limnology and Oceanography 51: 1969-1981.

Bjorndal, K.A., A.B. Bolten and S. Troeng. 2005. Population structure and genetic diversity in green turtles nesting at Tortuguero, Costa Rica, based on mitochondrial DNA control region sequences. Mar. Biol. 147: 1449-1457.

Bohonak, A.J. 1999 Dispersal, gene flow, and population structure. The Quarterly Review of Biology 74: 21-45.

Bottjer, D.J. 1980. Branching morphology of the reef coral *Acropora cervicornis* in different hydraulic regimes. Journal of Paleontology 54: 1102-1107.

Bruckner, A.W. 2003. Proceedings of the Caribbean *Acropora* Workshop: potential application of the U.S. Endangered Species Act as a Conservation Strategy. NOAA Technical Memorandum NMFS-OPR-24, Silver Spring, MD, Miami, FL, p 1-206.

Bruckner, A.W. and R.J. Bruckner. 2001. Condition of restored *Acropora palmata* fragments off Mona Island, 2 years after the *Fortuna Reefer* ship grounding. Coral Reefs 20: 235-243.

Bruno, J.F. and E.R. Selig. 2007. Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. PLoS Biology 5: 1-8.

Bruno, J.F., E.R. Selig, K.S.Casey, C.A. Page, B.L. Willis, C.D. Harvell, H. Sweatman and A.M. Melendy. 2007. Thermal stress and coral cover as drivers of coral disease outbreaks. PLoS ONE 5: 1220-1227.

Clement, M., D. Posada and K.A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9: 1657-1659.

Colin, P.L. 2003. Larvae retention: genes or oceanography? Science 300: 1657-1659.

Cowen, R.K., C.B. Paris and A. Srinivasan. 2006. Scaling of connectivity in marine populations. Science 311: 522-527.

Donner, S.D., T.R. Knutson, and M. Oppenheimer. 2007. Model-based assessment of the role of human-induced climate change in the 2005 Caribbean coral bleaching event. Proc. Nat. Acad. Sci. 104: 5483-5488.

Ewing, B. and P. Green. 1998. Base calling of automated sequencer traces using phred. II. Error probabilities. Genome Research 8: 186-194.

Ewing, B., L. Hillier, M. Wendl and P. Green. 1998. Base calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Research 8: 175-185.

Excoffier, L., G. Laval and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online.

Excoffier, L., P.E. Smouse and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application. Genetics 131: 479-491.

Forsman, Z.H., H.M. Guzman, C.A. Chen, G.E. Fox and G.M. Wellington. 2005. An ITS region phylogeny of *Siderastrea* (Cnidaria: Anthozoa): is *S. glynni* endangered or introduced? Coral Reefs 24: 343-347.

Fukami, H. and N. Knowlton. 2005. Analysis of complete mitochondrial DNA sequences of three members of the *Montastraea annularis* coral species complex (Cnidaria, Anthozoa, Scleractinia). Coral Reefs 24: 410-417.

Galindo, H.M., D.B. Olson and S.R. Palumbi. 2006. Seascape Genetics: A coupled oceanographic-genetic model predicts population structure of Caribbean corals. Current Biology 16: 1622-1626.

Gardner, T.A., I.M. Cote, J.A. Gill, A. Grant and A.R. Watkinson. 2003. Long-term region-wide declines in Caribbean corals. Science 301: 958-960.

Gil-Agudelo, D.L., G.W. Smith and E. Weil. 2006. The white band disease type II pathogen in Puerto Rico. Rev. Biol. Trop. 54: 59-67.

Gilmore, M.D. and B.R. Hall. 1976. Life history, growth habits, and constructional roles of *Acropora cervicornis* in the patch reef environment. Journal of Sedimentary Petrology 46: 519-522.

Gladfelter, W.B. 1982. White-Band Disease in *Acropora palmata*: Implications for the Structure and Growth of Shallow Reefs. Bull. Mar. Sci. 32: 639-643.

Glynn, P.W., L.R. Almodóvar and J.G. Gonzalez. 1964. Effects of Hurricane Edith on marine life in La Parguera, Puerto Rico. Carib. J. Sci. 4: 335-345.

Gordon, D. 2003. Viewing and editing assembled sequences using consed. In: Baxevanis AD, Davison DB (eds.) Current Protocols in Bioinformatics. John Wiley & Co., New York, p 11.12.11-11.12.43.

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

Hamner, W.M. and I.R. Hauri. 1981. Effects of Island mass: Water flow and plankton pattern around a reef in the Great Barrier Reef lagoon, Australia. Limnology and Oceanography 26: 1084-1102.

Hellberg, M.E., R.S. Burton, J.E. Neigel, and S.R. Palumbi. 2002. Genetic assessment of connectivity among marine populations. Bull. Mar. Sci. 70: 273-290.

Hellberg, M.E. 2006. No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. BMC Evolutionary Biology 6: 24.

Hellberg, M.E. 2007. Footprints on water: the genetic wake of dispersal among reefs. Coral Reefs 26: 463-473.

Highsmith, R.C. 1982. Reproduction by fragmentation in corals. Mar. Ecol. Prog. Ser. 7: 207-226.

Hohenlohe, P.A. 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.

Hubbard, D.K., I.P. Gill, R.B. Burke and J. Morelock. 1997. Holocene reef backstepping - southeastern Puerto Rico shelf. Proc. 8th Int. Coral Reef Symp. Panama 2: 1779-1784.

Hubbard, D.K., H. Zankl, I. Van Heerden and I.P. Gill. 2005. Holocene reef development along the northeastern St. Croix shelf, Buck Island, U.S. Virgin Islands. J. Sediment. Res. 75: 97-113.

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, P.R., 74 pp.

Jose, J. and V.N. Solferini. 2007. Population genetics of *Collisella subrugosa* (Patellogastropoda: Acmaeidae): evidence of two scales of population structure. Genetica 130: 73-82.

Lesser, M.P. 2007. Coral reef bleaching and global climate change: Can corals survive the next century? Proc. Nat. Acad. Sci. 104: 5259-5260.

Lirman, D. 2000. Fragmentation in the branching coral *Acropora palmata* (Lamarck): growth, survivorship, and reproduction of colonies and fragments. J. Exp. Mar. Biol. Ecol. 251: 41-57.

Lugo-Fernández, A., M.L. Hernández-Avila and H.H. Roberts. 1994. Wave-energy distribution and hurricane effects on Margarita Reef, southwestern Puerto Rico. Coral Reefs 13: 21-32.

Macintyre, I.G., P.W. Glynn and M.A. Toscano. 2007. The demise of a major *Acropora palmata* bank-barrier reef off the southeast coast of Barbados, West Indies. Coral Reefs 26: 765-773.

Maddison, D.R. and W.P. Maddison. 2000. MacClade 4.0, Vol. Sinauer Associates, Inc., Sunderland, Massachusetts.

Mayor, P.A., C.S. Rogers and Z.M. Hillis-Star. 2006. Distribution and abundance of elkhorn coral, *Acropora palmata*, and prevalence of white-band disease at Buck Island Reef National Monument, St. Croix, US Virgin Islands. Coral Reefs 25: 239-242.

McGehee, M.A. 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.

McMillan, F., T. Mahony, J.E.N. Veron and D.J. Miller. 1991. Nucleotide sequencing of highly repetitive DNA from seven species in the coral genus *Acropora* (Cnidaria: Scleractinia) implies a division contrary to morphological criteria. Mar. Biol. 110: 323-327.

Mercado-Molina, A.E. 2008. Population ecology of the demosponge *Amphimedon compressa*. MS thesis University of Puerto Rico- Mayagüez, P.R., 72 pp.

Miller, D.J. and M.J.H. van Oppen. 2003. A 'fair go' for coral hybridization. Mol. Ecol. 12: 805-807.

Miller, M.W., A.S. Bourque and J.A. Bohnsack. 2002. An analysis of the loss of acroporid corals at Looe Key, Florida, USA: 1983-2000. Coral Reefs 21: 179-182.

NOAA. 2005 Caribbean Basin Bleaching Event. http://coralreefwatch.noaa.gov/caribbean2005/

Neigel, J.E. and J.C. Avise. 1983. Clonal diversity and population structure in a reef-building coral, *Acropora cervicornis*: Self-recognition analysis and demographic interpretation. Evolution 37: 437-453.

Neigel, J., A. Domingo and J. Stake. 2007. DNA barcoding as a tool for coral reef conservation. Coral Reefs 26: 487-499.

Ortiz-Prosper, A.L. 2005. Population dynamics of hurricane-generated fragments of elkhorn coral *Acropora palmata* (Lamarck, 1816). Doctorate dissertation, University of Puerto Rico-Mayagüez, P.R., 89 pp.

Otto, S.P. and Y. Michalakis. 1998. The evolution of recombination in changing environments. Trends Ecol.Evol. 13: 145-151.

Palumbi, S.R. 2003. Population genetics, demographic connectivity, and the design of marine reserves. Ecol. Appl. 13: S146-S158.

Pillay, K.R.M., T. Asahida, C.A.Chen, H. Terashima and H. Ida. 2006. ITS ribosomal DNA distinctions and the genetic structures of populations of two sympatric species of *Pavona* (Cnidaria: Scleractinia) from Mauritius. Zoological Studies 45: 132-144.

Precht, J.W., M.L. Robbart and R.B. Aronson. 2004. The potential listing of *Acropora* species under the US Endangered Species Act. Mar. Poll. Bull. 49: 534-536.

Riginos, C. and B.C. Victor. 2001. Larval spatial distributions and other early life-history characteristics predict genetic differentiation in eastern Pacific blennioid fishes. Proc. R. Soc. B. 268: 1931-1936.

Rinkevich, B. 2000. Steps towards the evaluation of coral reef restoration by using small branch fragments. Mar. Biol. 136: 807-812.

Roberts, C.M. 1997. Connectivity and management of Caribbean coral reefs. Science 278: 1454-1457.

Robson, A.J., C.T. Bergstrom and J.K. Pritchard. 1999. Risky business: sexual and asexual reproduction in variable environments. J. Theor. Biol. 197: 541-556.

Rogers, C.S., T.H. Suchanek and F.A. Pecora. 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., J.C. Sánchez-DelBarrio JC, X. Messenger and R. Rozas. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19: 2496-2497.

Sammarco, P.W. and J.C. Andrews. 1989. The Helix experiment: Differential localized dispersal and recruitment patters in Great Barrier Reef corals. Limn. Oceanogr. 34: 896-912.

Schuhmacher, H. and M. Plewka. 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. Canada 2: 121-128.

Shearer, T.L., M.J.H. van Oppen, S.L. Romano and G. Worheide. 2002. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Mol. Ecol. 11: 2475-2487.

Smith, L.D. and T.P. Hughes. 1999. An experimental assessment of survival, re-attachment and fecundity of coral fragments. J. Exp. Mar. Biol. Ecol. 235: 147-164.

Sotka, E.E., J.P. Wares, J.A. Barth, R.K. Grosberg and S.R. Palumbi. 2004. Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula*. Mol. Ecol. 13: 2143-2156.

Stephan, W. and C.H. Langley. 1992. Evolutionary consequences of DNA mismatch inhibited repair opportunity. Genetics 132: 567-574.

Swofford, D.L. 2002. PAUP*: Phylogenetic analysis using parsimony*, version 4.0b10, Vol, Sunderland, MA, Sinauer.

Szmant, A.M. and M.W. Miller. 2006. Settlement preferences and post-settlement mortality of laboratory cultured and settled larvae of the Caribbean hermatypic corals *Montastrea faveolata* and *Acropora palmata* in the Florida Keys, USA. Proc. 10th Int. Coral Reef Symp. Japan. p. 43-49.

Szmant-Froelich, A.M. 1986. Reproductive ecology of Caribbean reef corals. Coral Reefs 5: 43-54.

Taylor, M.S. and M.E. Hellberg. 2003. Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299: 107-109.

Templeton, A.R., K.A.Crandall and C.F. Sing. 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.

Toscano, M.A. and I.G. Macintyre. 2003. Corrected western Atlantic sea-level curve for the last 11,000 years based on calibrated ^{14}C dates from *Acropora palmata* framework and intertidal mangrove peat. Coral Reefs 22: 257-270.

Tunnicliffe, V. 1981. Breakage and propagation of the stony coral *Acropora cervicornis.* Proc. Nat. Acad. Sci. 78: 2427-2431.

van Oppen, M.J.H, B.L. Willis and D.J. Miller. 1999. Atypically low rate of cytochrome *b* evolution in the scleractinian coral genus *Acropora*. Proc. R. Soc. B. 266: 179-183.

van Oppen, M.J.H., E.M. Koolmees and J.E.N. Veron. 2004. Patterns of evolution in the scleractinian coral genus *Montipora* (Acroporidae). Mar. Biol. 144: 9-18.

van Oppen, M.J.H., B.J. McDonald, B. Willis and D.J. Miller. 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, M.J.H., B.L. Willis, H.W.J.A. Van Vugt and D.J. Miller. 2000. Examination of species boundaries in the *Acropora cervicornis* group (Scleractinia, Cnidaria) using nuclear DNA sequence analyses. Mol. Ecol. 9: 1363-1373.

Vargas-Angel, B., J.D. Thomas and S.M. Hoke. 2003. High-latitude *Acropora cervicornis* thickets off Fort Lauderdale, Florida, USA. Coral Reefs 22: 465-473.

Vega-Zepeda, A., H. Hernández-Arana and J.P. Carricart-Ganivet. 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, S.V. and S.R. Palumbi. 2002. Hybridization and the evolution of reef coral diversity. Science 296: 2023-2025.

Vollmer, S.V. and S.R. Palumbi. 2004. Testing the utility of internally transcribed spacer sequences in coral phylogenetics. Mol. Ecol. 13: 2763-2772.

Vollmer, S.V. and S.R. Palumbi. 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, Queensland, Australia.

Weil, E., E.A. Hernández-Delgado, A.W. Bruckner, A. L. Ortiz, M. Nemeth and H. Ruiz. 2003. Distribution and status of Acroporid coral (Scleractinia) populations in Puerto Rico. In Bruckner, compiler, Proceedings of the Caribbean *Acropora* Workshop: Potential

application of the US Endangered Species Act as a conservation strategy, 2002 NOAA technical memorandum NMFS-OPR-24, Miami, Florida pp 71-98.

Weil, E. 2004. Coral Reef Diseases in the Wider Caribbean. In: Rosenberg E and Loya Y (ed.) Coral Health and Disease. Springer-Verlag, Berlin.

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.

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

Zardi, G.I., C.D. McQuaid, P.R. Teske and N.P. Barker. 2007. Unexpected genetic structure of mussel populations in South Africa: indigenous *Perna perna* and invasive *Mytilus galloprovincialis.* Mar. Ecol. Prog. Ser. 227: 135-144.

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

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

Appendix I. Segregating sites in the control region haplotypes of *Acropora palmata*. The numbers indicate the original alignment position. Hap_12 (AF507256) and Hap_13 (AF507220) are from Vollmer and Palumbi (2002).

```
                 22236667777777778888888888888888888888889
          1111111845880351366789999113555556666666666777775
         90123456017250995334431234891567890123456789012345 8
Hap_1    TAATAAAA--TGTTATCACGCG----GT-AATGGGGG-AAATTTATTTGA-
Hap_2    ----------TGTTATCACGCGGGG-GT-AATGGGGG-AAATTTATTTGA-
Hap_3    TAATAAAA--TGTTATCACGCGGGG-GT----------------------
Hap_4    TAATAAAA--TGTTATCACGCGGGG-GT-AATGGGGG-AAATTTATTTGA-
Hap_5    TAATAAAA--TGTCATCACGCGGGG-GT-AATGGGGG-AAATTTATTTGA-
Hap_6    TAATAAAA--TGTTATCACGCGGG--GT-AATGGGGG-AAATTTATTTGA-
Hap_7    CAATAAAA--TGTTATCACGCGGGG-GT-AATGGGGG-AAATTTATTTGA-
Hap_8    CAATAAAA--GGTTATCACGCGGGG-GT-AATGGGGG-AAATTTATTTGA-
Hap_9    CAATAAAA--TGTTATCACGCGGG--GT-AATGGGGG-AAATTTATTTGA-
Hap_10   TAATAAAA--TGTTGTCACGCG----GT-AATGGGGG-AAATTTATTTGA-
Hap_11   ?AATAAAA--TGTTATCACGCGGGG-GT-AATGGGGG-AAATTTATTTGA-
Hap_12   TAATAAAA--TGTTATCACGCG----GT-AATGGGGG-AAATTTATTTGAT
Hap_13   TAATAAAA--TGTTATCACGCGGGG-GT----------------------T
Hap_14   ----------TGTTATCACGCGGGG-GT-AATGGGGG-AAATTTATTTGA-
Hap_15   ----------TGTTATTACGCGGGG-GT-AATGGGGG-AAATTTATTTGA-
Hap_16   CAATAAAA--TGTTACCACGCGGGG-GT-AATGGGGG-AAATTTATTTGA-
Hap_17   CAATAAAA--GGTTATCACGCGGGGGGT-AATGGGGG-AAATTTATTTGA-
Hap_18   CAATAAAA--GGTTATCACGCAGGGGGT-AATGGGGG-AAATTTATTTGA-
Hap_19   ----------TGCTATCACGCGGGG-GT-AATGGGGG-AAATTTATTTGA-
Hap_20   CAATAAAA--GGTTATCACGCGGGG-GT-AATGGGGG-AAATTTATTTGA-
Hap_21   TAATAAAA--TGTTAT----AGGGG-------------------------
Hap_22   TAATAAAA-ATGTTATCACGCG----GT-AATGGGGG-AAATTTATTTGA-
Hap_23   TAATAAAAG-TGTCATCACGCGGGG-GT-AATGGGGG-AAATTTATTTGA-
Hap_24   ----------TGTTATCACGCGGGG-GTGAATGGGGGGAAATTTATTTGA-
Hap_25   ----------GGTTATCACGCGGGG-GT-AATGGGGG-AAATTTATTTGA-
Hap_26   ----------GCTTATCACGCGGGG-GT-AATGGGGG-AAATTTATTTGA-
Hap_27   CAATAAAA--TGTTATCACGCGGGGGGT-AATGGGGG-AAATTTATTTGA-
```

Appendix II. Segregating sites in the control region haplotypes of *Acropora cervicornis*. The numbers indicate the original alignment position. Hap_n7 (AF507294) and Hap_i8 (AF507297) are from Vollmer and Palumbi (2002)

```
                                                                  1
            222222222222333335566666666677888890
          233336627788888888802992791236788885800000 5
          934564575612345678983786046321945672767896 0
Hap_n1    TAAAATA-GGTCCTGCGGGGG--GGGGTAAT----CGA-GG-T
Hap_n2    TAAAATA-GGTCCTGCGGGGT--GGGGTAAT----CGA-GG-T
Hap_n3    TAAAATA-TGTCCTGCGGGGT--GGGGTAAT----CGA-GG-T
Hap_n4    TAAAATA-TGTCCTGCGGGGT--GGTGTAAT----CGA-GG-T
Hap_n5    CAAAATA-TGTCCTGCGGGGT--GGGGTAAT----CGA-GG-T
Hap_n6    TAAAATA-TGTCCTGCGGGGTCCGGGGTAAT----CGA-GG-T
Hap_n7    TAAAATA-TGTCCTGCGGGGT--GGGGCAAT----CGA-GG-T
Hap_n8    TAAAATA-GGTCCTGCGGGGT--GGGGTAATTCCGCGA-GG-T
Hap_n9    TAAAATA-TGTCCTGCGGGGG--GGGGTAAT----CGA-GG-T
Hap_n10   TGCCCTA-TGTCCTGCGGGGT--GGGGTAAT----CGA-GG-T
Hap_n11   TGCCCTA-GGTCCTGCGGGGG--GGGGTAAT----CGA-GG-T
Hap_i1    TAAAATATTGTCCTGCGGGAT--AATATAAC----CGA-GG-T
Hap_i2    CAAAATATTGTCCTGCGGGAT--AATATAGT----CGAGGG-C
Hap_i3    TAAAATATTTTCCTGCGGGAT--AATATAAC----CGA-GG-T
Hap_i4    TAAAACATTGTCCTGCGGGAG--AATATGGT----CGA-GG-C
Hap_i5    TAAAATATTGTCCTGCGGGAT--AATATAGT----TGA-GG-C
Hap_i6    TAAAATATTGTCCTGCGGGAT--AATATAAC----CGA-GG-C
Hap_i7    TAAAACATTGTCCTGCGGGAT--AATATGGT----CGA-GG-C
Hap_i8    TAAAATATTG---------AT--AATATAGT----CA-GGG-C
Hap_i9    TAAAATATTGTCCTGCGGGAT--AATATAGT----CGA-GG-C
Hap_i10   TAAAATATTGTCCTGCGGGAT--AATATAGT----TGA-GG-T
Hap_i11   TAAAACATTGTCCTGCGGGAG--AATATGGT----CGA-GGGC
Hap_i12   TGCCCTATTGTCCTGCGGGAT--AATATAGT----TGA-GG-T
Hap_i13   TAAAATATTGTCCTGCGGGAT--AATATAGT----CGA----C
Hap_i14   TGCCCCATTGTCCTGCGGGAG--AATATGGT----CGA-GG-T
Hap_i15   TGCCCTATTGTCCTGCGGGAT--AATATAAC----CGA-GG-T
Hap_i16   TGCCCTCTTGTCCTGCGGGAT--AATATAAC----CGA-GG-T
Hap_i17   TAAAATCTTGTCCTGCGGGAT--AATATAAC----CGA-GG-T
```

Appendix III. Distribution of *Acropora palmata* haplotypes by region and populations. Hap_12 (AF507256) and Hap_13 (AF507220) are from Vollmer and Palumbi (V&P) (2002). *Haplotype was recovered from a gamete bundle from Rincon Tres Palmas.

	La Parguera								Guanica						Culebra		Bahamas	
Locus/ alleles	Media Luna	Laurel	El Palo	Turrumote	Margarita	Enrique	V&P Media Luna	V&P San Cristobal	Guanica	V&P Guanica	Tres Palmas	Mona Island	Desecheo Island	Vieques	Reserva Luis Pena	V&P San Juan	Lee Stocking Island	Total
Hap 1	8	2	2	11	6	1	1			2	7	3		1	2		3	49
Hap 2		5							4		5							14
Hap 3	1	1		1		1					3							7
Hap 4	2	2	3	1	1		6	1		2	4	2		1		2	1	28
Hap 5		1			2		1				4					1		9
Hap 6		1			2	3			4	3	5							18
Hap 7	10	6	4	1	7	2			1		9	5					3	48
Hap 8	1	2									4							7
Hap 9			1			2						1						4
Hap 10												6	3				4	13
Hap 11													1					1
Hap 12								2										2
Hap 13								1										1
Hap 14	2	5	2		2	4					1	3						19
Hap 15					1													1
Hap 16																	1	1
Hap 17						9												9
Hap 18						1												1
Hap 19																	1	1
Hap 20						1												1
*Hap 21																		
Hap 22					1													1
Hap 23					1													1
Hap 24					1													1
Hap 25				2														2
Hap 26				1														1
Hap 27				1														1
Total	24	25	12	18	24	24	8	4	9	7	42	20	4	2	2	3	13	241

Appendix IV. Distribution of *Acropora cervicornis* haplotypes by region and population. Introgressed haplotypes (i) are in gray and native haplotypes (n) are in white. Hap_n7 (AF507294) and Hap_i8 (AF507297) are from Vollmer and Palumbi (V&P) (2002)

	La Parguera								Culebra	Bahamas	
Locus/ alleles	Media Luna	Laurel	San Cristobal	Atravezado	V&P San Cristobal	V&P Media Luna	Desecheo	Mona	Reser. Luis Pena	Lee Stocking Island	Total
Hap_n1	5				8		2	3			18
Hap_n2		5				1	1	7			14
Hap_n3			28		5			4			37
Hap_n4							1				1
Hap_n5										1	1
Hap_n6			1								1
Hap_n7						3					3
Hap_n8							1				1
Hap_n9			1								1
Hap_n10				1				1			2
Hap_n11	2										2
Hap_i1	12			6			2				20
Hap_i2										3	3
Hap_i3										1	1
Hap_i4		3		2	1						6
Hap_i5	16							1			17
Hap_i6	1										1
Hap_i7									1		1
Hap_i8					1						1
Hap_i9										1	1
Hap_i10	1										1
Hap_i11		1									1
Hap_i12	1										1
Hap_i13		2		1				1			4
Hap_i14				1							1
Hap_i15	1										1
Hap_i16				1							1
Hap_i17				1							1
Total	39	11	30	13	15	4	7	17	1	6	143