UC San Diego
UC San Diego Electronic Theses and Dissertations

Title

Biodiversity and connectivity in peripheral populations of corals of the South and Eastern Atlantic

Permalink

Author

Nunes, Flávia

Publication Date

2009

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA, SAN DIEGO

Biodiversity and connectivity in peripheral populations of corals

of the South and Eastern Atlantic

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in

Oceanography

by

Flávia Nunes

Committee in charge:

    Professor Nancy Knowlton, Co-Chair
    Professor Richard D. Norris, Co-Chair
    Professor Ronald Burton
    Professor Greg Rouse
    Professor Steven Vollmer
    Professor Christopher Wills

2009

The Dissertation of Flávia Nunes is approved, and it is acceptable

in quality and form for publication on microfilm and electronically:

_____

_____

_____

_____

_____

Co-Chair

_____

Co-Chair

University of California, San Diego

2009

To Nicolas Le Dantec,

whose perseverance and dedication I admire

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I arrived at Scripps Institution of Oceanography as a geologist, and my move towards the biological sciences was a challenge that could only be faced with the help of numerous friends and colleagues. First and foremost I would like to thank Dick Norris who always encouraged me to do research on topics that I found exciting, even if it wasn't easy. His enthusiasm for science and discovery are inspiring and I owe him a great deal for supporting and encouraging me through a Masters and a PhD. I am also greatly indebted to Nancy Knowlton, who on a leap of faith, welcomed me into her lab and encouraged my interest in Brazilian corals. Without her it would have been impossible to get this project off the ground.

I would also like to thank my committee members; Ron Burton, Greg Rouse, Steve Vollmer and Chris Wills for comments and advice that helped shape my doctoral work. Members of the Burton and Rouse labs received regular visits from me, and for their help and patience, I thank them. I would also like to thank Peter Andolfatto who served on my committee until his move to Princeton. Thank you, Peter, for the discussions and ideas.

Hironobu Fukami and Steve Vollmer are two people who were instrumental in getting me up to speed with molecular methods and concepts in evolutionary biology. Hiro mentored me in the early days of my PhD and I will always smile when I think about how much I learned from hand-drawn sketches of molecules – a picture is really worth a thousand words.

先生ありがとうございました

Steve Vollmer mentored me when I was a visiting scholar at the Smithsonian in Panamá and his continued support has really made a difference in my work. I had a great time in Panamá, thanks to the wonderful people who helped me on a daily basis in Naos – Carla Hurt, Eyda Gomez, Javier Jara and Kip Gupta – and those that made field work in Bocas del Toro memorable – Steve, Steph and Miles Vollmer, Davey Kline, Armando Castillo, and Carmen Schloeder. It was a blast, but I hope I never have to share a dorm room with 5 guys again. I also want to thank the friends from La Jaula and Friday night soccer games – Panamá would not have been the same without you.

During my PhD I made several visits to the lab of Dr. Antonio Solé-Cava at the Federal University of Rio de Janeiro, to process and sequence Brazilian coral samples. I love interacting with Brazilian researchers – you are all so much fun! A special thanks goes out to Antonio Solé-Cava, Cristiano Lazoski and Anderson Vilasboa (who healed my Arlequin handicap) and the rest of the Laboratório de Biodiversidade Molecular. Vocês têm uma energia e dinâmica maravilhosa. Obrigada por tudo! Sampling in Brazil went smoothly thanks to the organization and experience of Bertran Feitoza, Eduardo Freitas and the folks at Projeto Netuno.

Another unforgettable group of colleagues are the members of the São Tomé & Príncipe expedition: João Gasparini (ó o moshquito), Luiz Rocha (Sshhgente), Cadu Ferreira (Tiburão Trige), Sergio Floeter , Buia Sampaio, Kerry Nichols (kerryophillia), Arthur Anker, Denishka Poddoubtchencko, Peter Wirtz and Nancy Knowlton. When are we going to Cabo Verde? A big thanks also goes to the crew of Club Maxel.

Phil, Matt and Sandy. Our lab meetings were so diverse, it opened up my mind to a range of fascinating (and on occasion, random?) topics. Special mention goes to Jessica Carilli and Celli Hull who have shared many insightful comments and opinions on my work and on various topics in evolution and marine ecology, and to Adam Young, who made the template for this thesis and saved me countless hours of mindless formatting.

Finally I would like to thank my family and friends for the support and encouragement they have given me through many years of graduate work. To my mom and dad who have motivated me to find a profession which brings me enjoyment and satisfaction despite the hard work. To my big brother who has chased after his dreams and made them happen – I want to have the guts to take risks like you have. To my talented sister, who knows the trials and tribulations of a PhD as well as I do, and whose laughter reminds me of what's important in life. Thank you to the wonderful cohort of SIO graduate students (2002 – 2009) who made life in California so much fun. I am so fortunate to have known you! As we go off in our separate ways, I can only imagine – where will our next adventure be?

Nicolas Le Dantec has been there for me through it all. He has helped me through so many challenges and I'm happy to have shared with him my victories and accomplishments. I dragged him around the tropics with hammer, chisel and very heavy luggage to sample corals in Panamá and Brazil. We worked long hours in the field, in the lab, at home – even writing our dissertations side by side. I'm happy we

are together in this journey. Wherever we go next, it will always be home because you will be there with me.

MATERIAL SUBMITTED FOR PUBLICATION IN THE DISSERTATION

Chapter I, in full, is a reprint of the material as it appears in Coral Reefs, 2008, Flavia Nunes, Hironobu Fukami, Steven V. Vollmer, Richard D. Norris and Nancy Knowlton, Springer Publishing Company, 2008. The dissertation author was the primary investigator and author of this paper.

Chapter II, in full, has been submitted and accepted for the publication of the material as it may appear in Molecular Ecology, 2009, Nunes, Flavia; Norris, Richard D.; Knowlton, Nancy, Wiley-Blackwell Publishing, Inc. 2009. The dissertation author was the primary investigator and author of this paper.

Chapter III is currently being prepared for submission for publication of the material. The dissertation author was the primary investigator and author of this material.

Chapter IV is currently being prepared for submission for publication of the material. The dissertation author was the primary investigator and author of this material.

VITA

2009   Doctor of Philosophy, University of California, San Diego, USA

2004   Master of Science, University of California, San Diego, USA

2002   Honours Bachelor of Science, University of Toronto, Canada

PUBLICATIONS

Nunes F, Norris RD and Knowlton N. (accepted) Implications of low genetic diversity in peripheral isolates of an amphi-Atlantic coral, *Molecular Ecology*

Knowlton, N and **Nunes F**. (2008) Atlantic corals – Least of our concerns? *Science*, E-letter, http://www.sciencemag.org/cgi/eletters/321/5888/560

Nunes F, Fukami H, Vollmer S, Norris RD, and Knowlton N. (2008) Re-evaluation of the systematics of the endemic corals of Brazil by molecular data, *Coral Reefs* 27, 423-432

Nunes F, and Norris RD. (2006) Abrupt reversal in ocean overturning during the Palaeocene/Eocene warm period. *Nature*, 439, 60-63.

Nunes F, and Norris RD. (2004) Data report: high-resolution stable isotope records across the Paleocene/Eocene boundary, ODP Sites 1220 and 1221, in Wilson, P. A., Lyle, M. W., and Firth, J. V., eds., *Proceedings of the Ocean Drilling Program, Scientific Results*, 199, 1-12, doi:10.2973/odp.proc.sr.199.206.2005

Nunes F (2004) Abrupt change in thermohaline overturning across the Paleocene-Eocene boundary: University of California, 50 p. (thesis)

ABSTRACT OF THE DISSERTATION

Biodiversity and connectivity in peripheral populations of corals

of the South and Eastern Atlantic

by

Flávia Nunes

Doctor of Philosophy in Oceanography

University of California, San Diego, 2009

Professor Nancy Knowlton, Co-Chair

Professor Richard D. Norris, Co-Chair

The coral reefs of the South and Eastern Atlantic are impoverished in species diversity and abundance relative to the Caribbean because they occupy areas of suboptimal conditions for coral growth. Biodiversity and connectivity are important for the resilience and persistence of reefs, but corals inhabiting the periphery, or limits of the species range, lack the buffering qualities of ecological redundancy that accompanies biodiversity and may be isolated from sources of migrants that could replenish populations in the event of disturbance. Conversely, peripheral populations may be the site of divergence, local adaptation and speciation as a result of isolation. I

explore the contributions of peripheral coral populations in Brazil and West Africa to preserving and generating biodiversity and their role in maintaining genetic connectivity across long distances in the Atlantic. Current estimates of coral biodiversity are confounded by pervasive morphological convergence, potentially underrating regional endemism. Molecular data presented here suggest that biodiversity has been underestimated for *Mussismilia*, a genus endemic to Brazil, and that the South Atlantic can be the site of species origination, such as for *Favia gravida*. Over ecological time scales, high connectivity provides a means for populations to persist in impacted locations by the influx of migrants from distant healthy reefs, while over longer time scales, isolation can be a creative mechanism that promotes diversification. I present the first molecular datasets that examine basin-scale dispersal in seven species of amphi-Atlantic corals. Isolation among regions is inferred for all but one species, indicating that coral populations must rely on regional recruitment to persist, but that local adaptation and future diversification may also take place. Within regions, however, differences in connectivity appear to be associated with reproductive mode. Among coral populations of Brazil, connectivity was greatest for broadcasting species and more restricted for brooding species, suggesting that aspects of reproduction may aid in dispersal at local scales. Peripheral coral reefs in the Atlantic are important sanctuaries for a host of endemic corals and other marine species and should be recognized for their distinctiveness, their role as sites of diversification, but also protected because of their isolation and vulnerability.

CHAPTER I

Re-evaluation of the systematics of the endemic corals of Brazil by

molecular data

ABSTRACT

Recent genetic work on various coral genera has shown that morphological convergence between Atlantic and Pacific corals obscures evolutionary relationships and inferred levels of endemicity between the regions. Based on DNA sequences from nuclear and mitochondrial loci that provide higher resolution than those previously presented, this study shows that relationships within parts of the Atlantic coral fauna are also in need of substantial revision. The data presented here indicate that (1) the endemic Brazilian genus *Mussismilia* is a monophyletic clade, (2) *Mussismilia* is more closely related to the Caribbean Faviidae than Mussidae, the family in which it is currently placed, (3) the Brazilian endemic coral *Favia leptophylla* is much more closely related to *Mussismilia* than other species of *Favia* and has most likely been incorrectly placed in the genus *Favia* and (4) the other endemic *Favia* species found in Brazil, *Favia gravida*, is genetically distinct from *Favia fragum*, a Caribbean congener with which it is frequently synonymized. The nuclear data also suggest the possible presence of a cryptic species within *Mussismilia*, but additional sampling and morphological information is required to confirm this finding.

INTRODUCTION

An accurate classification scheme is essential for testing evolutionary hypotheses about corals, but recent molecular work finds many conflicts with traditional morphological systematics at the levels of order, suborder, family, genus and species (Knowlton et al. 1992; Weil and Knowlton 1994; Romano and Palumbi 1996, 1997; Lopez et al. 1999; Romano and Cairns 2000; van Oppen et al. 2001, 2004; Chen et al. 2002; Fukami et al. 2004a, b; Kerr 2005; Watanabe et al. 2005; Medina et al. 2006). The reasons for the lack of molecular support for many aspects of morphologically based taxonomy include morphological convergence, the failure to use truly homologous morphological characters and phenotypic plasticity (Foster 1979, 1980, 1985; Lasker 1981; Budd et al. 1994; Bruno and Edmunds 1997; Budd and Klaus 2001; Todd et al. 2001; Gittenberger and Hoeksema 2006; Klaus et al. 2007). It has thus become increasingly evident that current classification schemes are in need of significant revision. In this study, molecular data were used to improve our understanding of the phylogenetic standing and relationships of the endemic Brazilian corals conventionally placed in the families Mussidae (*Mussismilia*) and Faviidae (*Favia leptophylla* and *Favia gravida*).

Brazilian coral reefs

In the Atlantic Ocean, the highest diversity of corals is found in the Caribbean, where clear, warm waters provide an ideal habitat for coral growth, but corals are also

found in other parts of the Atlantic such as along the coasts of Brazil, West Africa and on various Mid-Atlantic islands (Laborel 1969a, b, 1974). In these regions, corals commonly grow as isolated colonies on what are primarily rocky reefs. Despite less favorable conditions, corals persist in these regions in low abundance and diversity. True biogenic coral reefs (those formed by the biological accumulation of organisms with calcareous skeletons) in the South Atlantic are found only in Brazil, but these reefs are very different both in species composition and in growth form from Caribbean reefs. Brazilian reefs are comprised of a low diversity fauna of 15 hermatypic coral species, five of which are endemic to Brazilian waters (*F. leptophylla*, *Mussismilia braziliensis*, *Mussismilia harttii*, *Mussismilia hispida* and *Siderastrea stellata*) and one which is endemic to the South Atlantic (*F. gravida*) (Maida and Ferreira 1997; Castro and Pires 2001). This modest number of species is likely a result of (1) a narrow continental shelf, which limits the habitat space for corals and (2) high sedimentation from rivers, which decreases water clarity needed for photosynthesis (via symbionts) and can potentially smother corals (Leão and Ginsburg 1997). Corals are found along ~2,400 km of the coast, ranging from as far north as Maranhão State (00°53'S, 044°16'W) to Santa Catarina State in the south (27°34'S, 048°37'W) (Castro and Pires 2001). Their distribution is bound by the Amazon River to the north and cool waters to the south, with interruptions in coral occurrence near large river outflows such as the São Francisco and Doce Rivers, where high sedimentation rates and low salinity hinder coral growth (Laborel 1969a). The only true biogenic reefs are found on the Abrolhos Bank, a volcanic platform that

extends 200 km offshore (Leão and Kikuchi 2000). On the north shore of Brazil, reefs nearly as diverse those of Abrolhos are found in Parcel Manuel Luiz, but it is not clear whether these are biogenic reefs because a substantial part of the reef is composed of rock formations (Rocha and Rosa 2001). The primary reef builders of Brazil are corals in the endemic genus *Mussismilia* (Leão 1996). Extant members of this genus are restricted to Brazil, with some species such as *M. braziliensis* being restricted to the Abrolhos Bank (Laborel 1969b). Reefs built by these species of coral display a unique growth morphology not seen on other reefs, such as the mushroom-shaped "chapeirões", coral banks formed by the fusion of multiple colonies. *Mussismilia* is thought to have a Miocene origin, with fossil specimens of *Mussismilia provincialis* being found in the Aquitainian and Burdigalian of France (~23–16 Ma) (Chevalier 1959, 1961) and *Mussismilia vindobonensis* being found in the Middle Miocene (Vindobonian) of Spain and France (~16– 11.6 Ma) (Chevalier 1961; age estimates from Gradstein et al. 2004). Until recently, the fossil record for extant species was limited to *M. hispida* from the Pliocene Tamiami Formation of Florida (3.5–3.0 Ma) (Meeder 1987) and *M. harttii* from the Pliocene Mao Formation of the Dominican Republic (4.0–3.4 Ma) (Budd et al. 2001; age estimates from Budd 2000). New paleontological collections (see NMITA database, Budd et al. 2001, updated 2007) have revealed occurrences of *M. hispida* in the Pliocene of Panamá (2.2–1.8 Ma, age estimate from Budd and Klaus 2001) and *Mussismilia aff. harttii* from deposits in the Dominican Republic, Curaçao and Costa Rica, ranging from the Late Miocene (~8.3 Ma, age estimate from Saunders et al. 1986) to Middle Pleistocene (~400 Ka, age

estimate from Schellmann et al. 2004). The Caribbean fossil occurrences suggest that the genus *Mussismilia* was once widely distributed, and only recently has been restricted to Brazil. The rarity of this genus in earlier fossil collections from Caribbean deposits may be due in part to the morphological similarity of *Mussismilia* to other Caribbean members of the family Mussidae, to which *Mussismilia* has been traditionally assigned. However, recent molecular work suggests that its closest relatives may be members of a new Caribbean clade that contains taxa from both the Mussidae and Faviidae (Fukami et al. 2004b).

MATERIALS AND METHODS

Sampling and DNA extraction

Coral tissue samples were collected from Brazil and two additional locations in the Atlantic Ocean for biogeographic comparisons: Panamá and the Gulf of Guinea. The Brazilian endemic corals were collected from the Abrolhos Banks in Bahia State in 2002. Caribbean and Atlantic coral species were collected in Panamá at the Bocas del Toro Field Station in September of 2005, and in São Tomé Island in the Gulf of Guinea during February 2006. Fresh tissue was scraped off the surface of a small piece (~2–5 cm2) of living coral and put into vials containing CHAOS solution (4 M guanidine thiocyanate, 0.1% sodium N-lauroyl sarcosine, 10 mM Tris-HCl pH8, 0.1 M 2-mercaptoethanol) (Fukami et al. 2004b). Samples were stored at room temperature, and can be kept in these conditions for upwards of several months.

Skeletal material was labeled, bleached and dried for morphological identification. For DNA extraction, equal volumes of coral tissue digested in CHAOS solution and phenol extraction buffer (100 mM Tris-HCl pH8, 10 mM EDTA, 0.1% sodium dodecyl sulfate (SDS) were mixed, then added to twice the volume of phenol:chloroform:isoamyl alcohol (25:24:1). This mixture was vortexed, centrifuged at high speed and the supernatant recovered. This step was repeated 1–2 more times, until a clear solution remained. DNA was precipitated with isopropanol and resuspended in 10 mM Tris-HCl pH 8.0.

Mitochondrial marker

The mitochondrial marker used in this study was the intergenic region (IGR) between cytochrome oxidase I (cox1) and the formylmethionine transfer RNA gene (trnM) (referred to in the rest of the text as cox1-trnM IGR). The region was amplified using a degenerate primer pair, designed based on sequences of the same region in *Montastraea annularis* (Fukami et al. 2004a). The primer sequences were as follows: NcodF 5'-RAG YTG GGC TTC TTT AGA GTG-3' and NcodR 5'-GCT ACT TAC GGA ATC TCG TTT GA-3'. Internal sequencing primers designed for *F. fragum*/*F. gravida* (FfrNCF) were 5'-CTA CGC GTG GAA ATT GGT TT-3' and (FfrNCR) 5'-AAC CTT TTT CCC TCT TTT CAA-3'. Amplification of the region was performed by polymerase chain reaction (PCR) consisting of 2.5 μl of 10X Buffer, 0.5 μl of dNTP (200 mM), 1.5 μl of primers (10 mM), 1.25 U Taq (Sigma-Aldrich), 1 μl of DNA and water to 25 μl. The thermal cycler profile used had an initial denaturation

step at 94°C for 2 min followed by 35 cycles at 94°C/30 s, 48°C/30 s (52°C/30 s for *Favia* internal primers), 72°C/2 min, with a final extension step at 72°C for 5 min. A 10–15 μl aliquot of the PCR product was purified for sequencing by adding 0.5 U of exonuclease 1, 0.5 U of shrimp alkaline phosphatase (SAP) and 1 μl of 10X SAP buffer, and incubating at 37°C for 60 min. The enzyme was then deactivated by heating to 80°C for 15 min. A cycle sequencing reaction was performed directly on PCR products using ET terminators from MegaBase, following the manufacturer's protocol.

Nuclear marker

Most sequences of the β-tubulin gene were obtained during a previous study for which only exon sequences were used in the phylogenetic analysis (the β-tubulin intron was too variable for alignment over a broad range of coral species) (Fukami et al. 2004b). Since this study focused on a small, closely related group of species, the more variable β-tubulin intron could be used in addition to the exon thus obtaining good phylogenetic resolution among closely related taxa. β-tubulin was amplified, cloned and sequenced according to the published protocol of Fukami et al. (2004b). Additional samples of *F. fragum*, *F. gravida*, *F. leptophylla* and *Montastraea cavernosa* were sequenced directly from PCR products, following the same amplification protocol of Fukami et al. (2004b) and the same sequencing protocol described above for the mitochondrial locus. Sequences for forward and reverse

primers were: TubF (5'-GCA TGG GAA CGC TCC TTA TTT-3') and TubR (5'-ACA TCT GTT GAG TGA GTT CTG-3').

Molecular phylogenetic analysis

Sequence chromatographs were viewed and edited using Sequencher 4.5. Exon and intron regions of β-tubulin (1,000 bp), and 709 bp of the mitochondrial IGR were used for phylogenetic analysis. Sequences are available online through GenBank (accession numbers EU303010– EU303116). The sequences were aligned using the ClustalX v1.83.1 algorithm (Thompson et al. 1997), with *Mussa angulosa* and *Scolymia* spp. as outgroup taxa. For β-tubulin, a second analysis used *M. cavernosa* as the outgroup, in order to resolve the relationship of *Mussismilia* relative to other Caribbean faviids and mussids. Although *M. cavernosa* has conventionally been assigned to the Faviidae, molecular work has shown that this species is quite divergent from other Atlantic mussids and faviids (Fukami et al. 2004b), suggesting that M. cavernosa is not closely related to either family and would thus make a suitable outgroup. The cox1-trnM IGR sequence for *M. cavernosa*, however, was too divergent for alignment with ingroup sequences, and could not be used for phylogenetic analysis. Ten alignments were performed using varying gap opening and extension penalties to monitor the effect of gaps on phylogenetic interpretation for β-tubulin. Low gap opening penalties produced a greater number of short gaps in the 3' end of the intron, whereas default and high gap penalties produced fewer gaps that were longer in length. Neighbor-joining trees were constructed for all ten alignments, and

no change in topology was observed except for variations in length of some branches, particularly the outgroup branch. Because no large gaps were observed in the mitochondrial marker, this type of analysis was not performed for this locus. Default gap penalties were employed in the alignment used for further phylogenetic analyses for both nuclear and mitochondrial markers. Base composition, the number of transitions (Ti) and transversions (Tv), and pairwise sequence divergence were calculated using DAMBE (Xia and Xie 2001). Phylogenetic inference was made on the basis of maximum parsimony (MP), maximum likelihood (ML) and Bayesian methods. MP analyses used a heuristic search and the tree-bisection-reconnection branch swapping algorithm, with 1,000 bootstrap replicates in PAUP*v4.0beta10 (Swofford 2002). For ML and Bayesian analyses, the model of sequence evolution was determined by hierarchical likelihood ratio tests using Modeltest 3.7 (Posada and Crandall 1998) and MrModeltest 2.2 (Nylander 2002) respectively. ML analyses conducted using PhyML (Guindon and Gascuel 2003) used the Hasegawa-Kishono-Yano model (HKY85) (Hasegawa et al. 1985) for both β-tubulin (-lnL of best tree = 3536.44) and cox1-trnM IGR (-lnL = 2265.65). Transition/transversion (Ti/Tv) ratio, proportion of invariable sites and gamma distribution parameter (Γ) were estimated by PhyML for both loci. The Bayesian analysis used the HKY + Γ model for both β-tubulin (-lnL = 3896.63) and cox1-trnM IGR (-lnL = 2330.68) in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Four parallel chains of 2 million generations were run for β- tubulin and cox1-trnM IGR. Trees were sampled every 100 generations, and 5,000 "burn-in" trees were excluded from the consensus tree (both markers). The

average standard deviation of split frequencies after two million generations was 0.005579 for β-tubulin and 0.010630 for cox1-trnM IGR.

RESULTS

Data properties

Base frequencies for β-tubulin and cox1-trnM IGR are shown in Table 1.1.

Table 1.1 Nucleotide frequencies per species for the nuclear gene β-tubulin and the mitochondrial cox1-trnM intergenic region.

Nucleotide Frequencies

	Mitochondrial, *cox1-trnM* IGR				Nuclear, *β-tubulin*			
	p(A)	p(C)	p(G)	p(T)	p(A)	p(C)	p(G)	p(T)
Favia leptophylla	0.2527	0.1843	0.2260	0.3371	0.3075	0.2149	0.1772	0.3004
Mussismilia braziliensis	0.2525	0.1844	0.2241	0.3390	0.3030	0.2162	0.1807	0.3001
Mussismilia harttii	0.2522	0.1837	0.2241	0.3401	0.3064	0.2188	0.1826	0.2924
Mussismilia hispida	0.2511	0.1844	0.2241	0.3404	0.3118	0.2140	0.1760	0.2983
Favia fragum	0.2634	0.1690	0.2321	0.3356	0.3038	0.2181	0.1835	0.2948
Favia gravida	0.2632	0.1696	0.2330	0.3342	0.3040	0.2178	0.1830	0.2953
Diploria clivosa	0.2525	0.1719	0.2405	0.3352	0.3054	0.2177	0.1782	0.2986
Diploria strigosa	0.2532	0.1704	0.2412	0.3352	0.3034	0.2147	0.1795	0.3024
Diploria labyrinthiformis	0.2588	0.1711	0.2348	0.3352	0.3076	0.2200	0.1800	0.2925
Manicina areolata	0.2518	0.1740	0.2348	0.3395	0.3022	0.2199	0.1816	0.2963
Colpophyllia natans	0.2650	0.1816	0.2220	0.3315	0.2940	0.2156	0.1858	0.3046
Mussa angulosa	0.2475	0.1884	0.2361	0.3280	0.2967	0.2188	0.1740	0.3106
Scolymia	0.2438	0.1846	0.2427	0.3290	0.2922	0.2284	0.1797	0.2997
Montastraea cavernosa					0.3070	0.2154	0.1727	0.3049
AVERAGE	0.2544	0.1783	0.2320	0.3354	0.3032	0.2179	0.1796	0.2994
STANDARD DEVIATION	0.0064	0.0072	0.0072	0.0040	0.0055	0.0036	0.0037	0.0051

Although both loci show higher frequencies of A and T ($60.3 \pm 0.6\%$ in β-tubulin and $59.0 \pm 0.8\%$ cox1-trnM IGR; mean $\pm$ SD) relative to C and G, these frequencies are similar between the two loci and vary within a narrow range from taxon to taxon (see Table 1.1 for standard deviations), indicating that base frequency heterogeneity should

not bias phylogenetic interpretation. The number of transitions and transversions between sequence pairs was plotted against genetic distance to test for substitution saturation (Fig. 1.1).
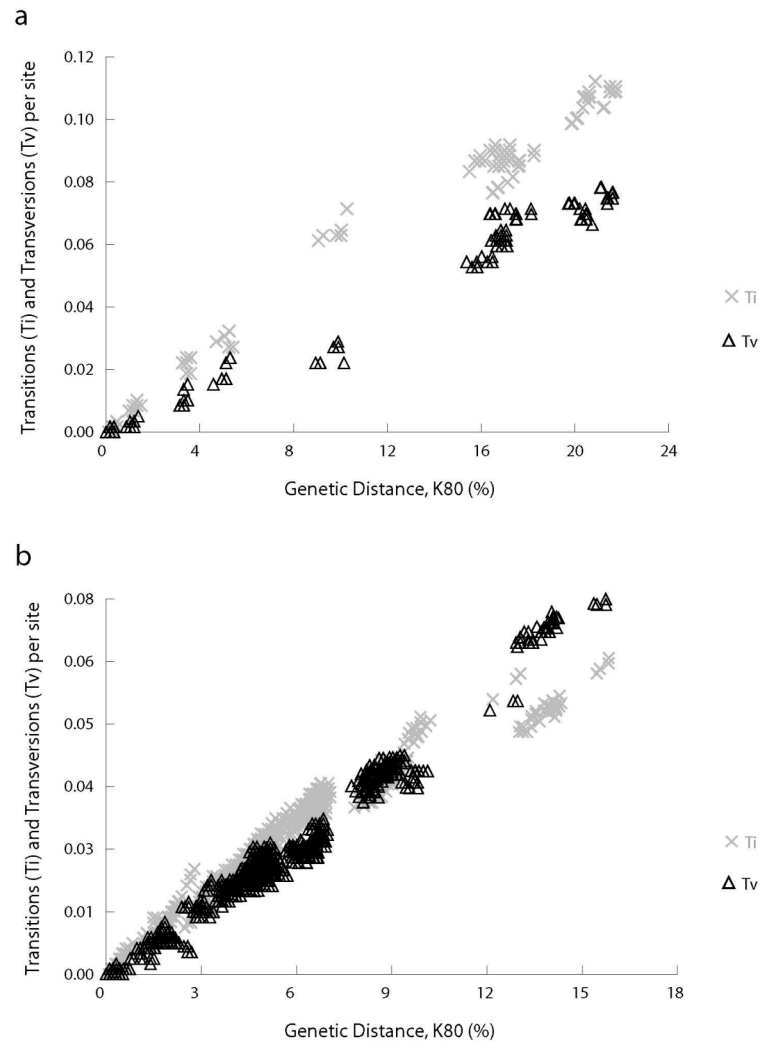
Figure 1.1 Test for substitution saturation for a the mitochondrial cox1-trnM intergenic region, and b the nuclear β-tubulin gene, based on number of transitions (Ti) and transversions (Tv) plotted against Kimura 2-parameter distance (K80)

There is no indication of substitution saturation for cox1-trnM IGR, but in β-tubulin, the number of transversions is approximately equal to transitions when genetic distance is between 8 and 10%, and transitions outnumber transversions at genetic distances greater than 14%. When the outgroup sequence (*M. cavernosa*) was excluded from the test, transitions were always more frequent than or equal to transversions (not shown). This observation indicates that the phylogenetic signal between closely related taxa is reliable, but that some phylogenetic signal may have been lost between the ingroup and outgroup taxa. These data quality tests suggest that the loci used do not contain any biases that may interfere with the phylogenetic signal, and that they should be suitable for inferring relationships among the species studied.

Phylogenetic analysis

The three methods of inferring phylogeny used in this study yielded similar topologies for each locus. Consensus trees built with the Bayesian method are shown in Fig. 1.2 for β-tubulin and cox1-trnM IGR, with posterior probability and bootstrap support for ML and MP based on 1,000 replicates listed for each branch node respectively. Both gene trees are largely congruent in their topologies, but β-tubulin had greater phylogenetic resolution than cox1-trnM IGR for the relationships among the Mussismilia species. For β-tubulin, relationships among the ingroup taxa remain the same whether the phylogeny was rooted with *M. angulosa* + *Scolymia* or *M. cavernosa*. Both markers suggest that *Mussismilia* + *F. leptophylla* is a monophyletic

clade with strong node support (100/100/100 in cox1-trnM IGR and 100/89/76 for β-tubulin).
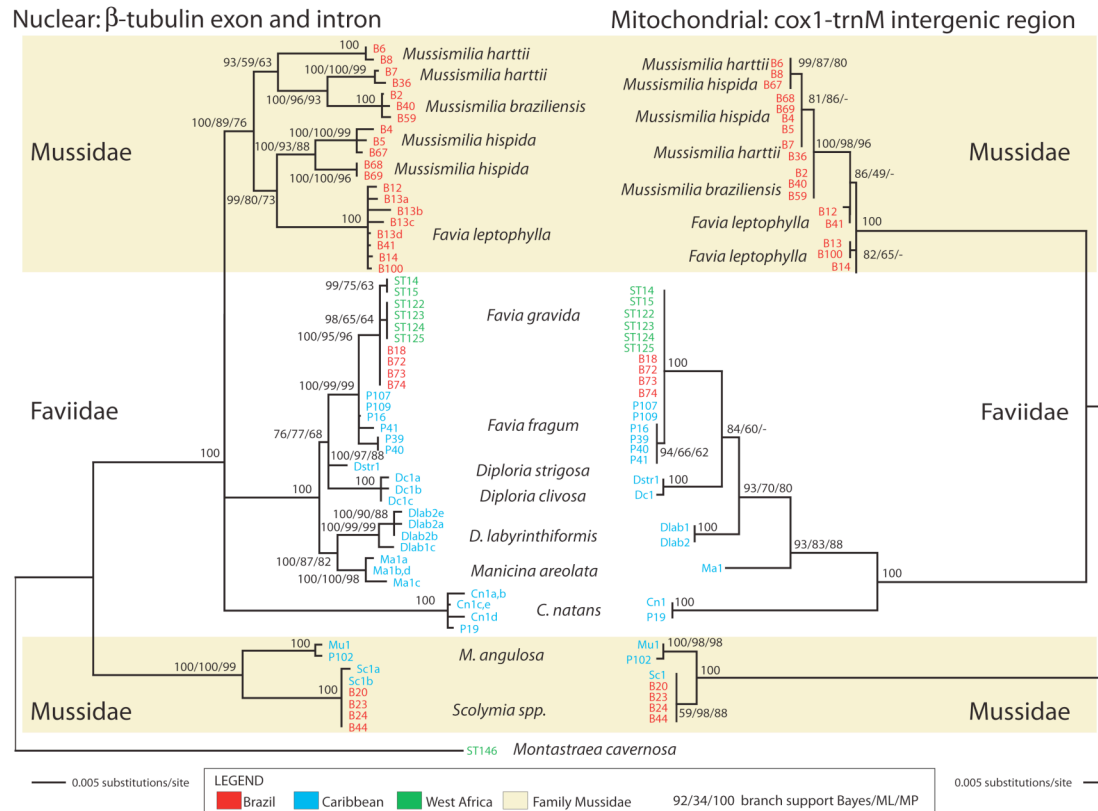
Figure 1.2 Phylogenetic trees of selected Brazilian and Caribbean corals, based on the nuclear *β-tubulin* gene and the mitochondrial *cox1-trnM* intergenic region. Trees shown were inferred by Bayesian methods. Maximum likelihood (ML) and maximum parsimony (MP) yielded similar topologies. Posterior probability and bootstrap support (Bayesian/ML/MP) is reported for each node. Unsupported nodes in the ML or MP analyses are represented by a *dash*. Nodes with the same support value for all three methods of phylogenetic inference are represented by a *single number*. Species are color-coded by region: Caribbean (*blue*), West Africa (*green*) and Brazil (*red*)

This clade of Brazilian corals appears to be more closely related to Atlantic faviids (i.e., *F. fragum* and *Diploria*) than Atlantic mussids (*M. angulosa* and *Scolymia*) (Fig. 1.2) in β-tubulin. Within the *Mussismilia + F. leptophylla* clade, the mitochondrial

locus exhibits 12 polymorphic sites which define seven haplotypes in 19 individuals. All individuals of *M. braziliensis*, *M. harttii* and *M. hispida* share one of three haplotypes, none of which is specific to any one species. The remaining four haplotypes in this clade are found in individuals of *F. leptophylla*, which is basal to the group. In contrast, the β-tubulin locus exhibits 84 polymorphic sites (104 if indels are considered) that define 15 haplotypes in 17 individuals. This marker suggests that *F. leptophylla* and *M. hispida* are sister taxa, each forming a monophyletic clade, and that *M. braziliensis* is paraphyletic with respect to *M. harttii*. Little divergence is observed between *F. fragum* and *F. gravida* at the mitochondrial locus. Only two haplotypes were found in cox1-trnM IGR for both species combined, with one haplotype being shared by both *F. fragum* and *F. gravida* and the other haplotype being found only in *F. fragum*. In the more variable nuclear marker, however, there are seven haplotypes in 16 individuals, none of which are shared between the two species. In addition, each of the three regions where samples were taken (Caribbean, Brazil and West Africa) is represented by unique haplotypes not seen in any of the other regions. Haplotypes from Brazil and West Africa (conventionally named *F. gravida*) fall within a well-supported clade (100/95/96) represented by three haplotypes in ten individuals, while four haplotypes found in six individuals of *F. fragum* from the Caribbean are basal to the *F. fragum*/*F. gravida* clade. Average genetic distances (HKY-corrected) were calculated among sequences of the same individual (intra-individual) and among sequences of individuals of the same species (intra-specific) for the β-tubulin gene (Fig. 1.3).
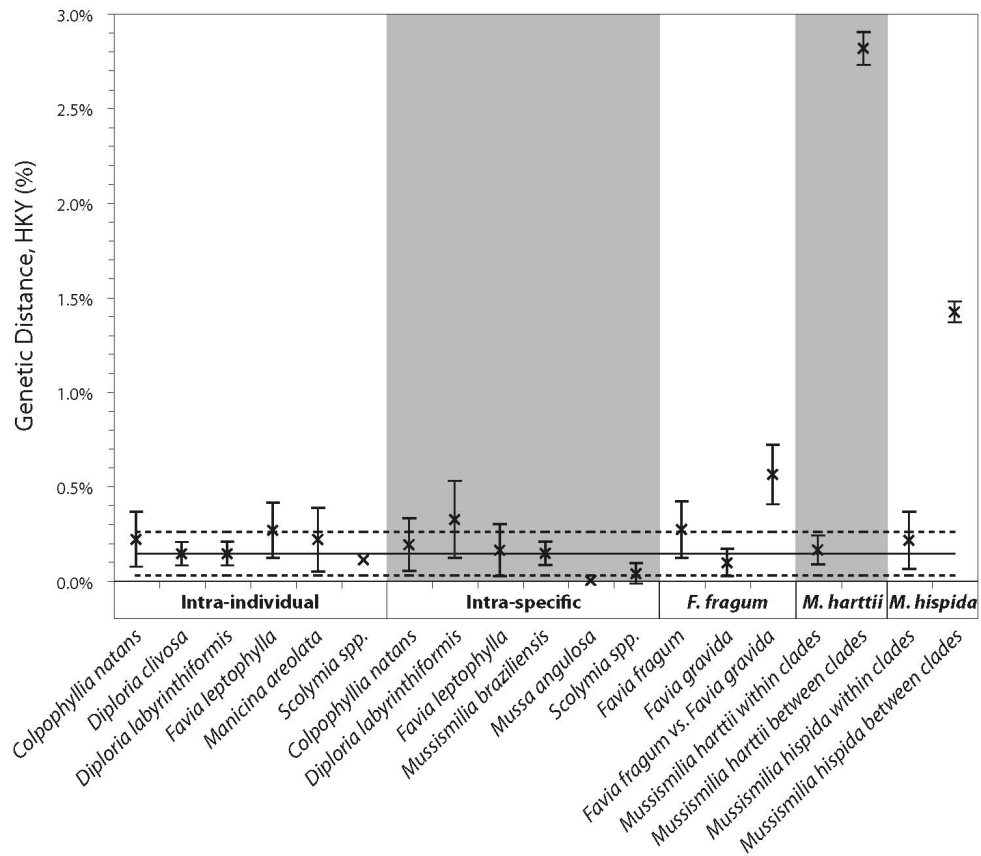
Figure 1.3 Average genetic distance within individuals (intra-individual) and within species (intra-specific) for the β-tubulin gene. Solid and dashed horizontal lines represent the overall intra-specific average and standard deviation of the average, respectively

One individual from the following six species were sequenced for three to five clones: *Colpophyllia natans* (sample Cn1 a-e), *Diploria clivosa* (sample Dc1 a-c), *Diploria labyrinthiformis* (sample Dlab2 a-c), *F. leptophylla* (B13 a-d), *Manicina areolata* (sample Ma1 a-d) and *Scolymia* spp. (Sc1 a, b). Average intra-specific genetic distances were calculated for species where sequences of multiple individuals were available: *C. natans*, *D. labyrinthiformis*, *F. leptophylla*, *M. braziliensis*, *M. angulosa* and *Scolymia* spp. An overall intra-specific average was calculated over these six

species. Intra-specific averages were also calculated for *F. fragum*, *F. gravida*, *M. harttii* and *M. hispida*, but these were not included in the overall average because of the uncertainty regarding these groupings as actual species (see Sect. "Discussion"), and so that comparisons could be made between these groupings and the other recognized species. *M. harttii* and *M. hispida* contain haplotypes that are much more divergent (1.9 ± 1.4% and 0.9 ± 0.6% respectively) (mean ± SD) than the overall intra-specific average (0.14 ± 0.1%). Divergent haplotypes define two clades for each of the two species, with the average divergence between clades being of 2.8 ± 0.1% and 1.4 ± 0.1% for *M. harttii* and *M. hispida* respectively. When genetic distance is calculated for each clade of haplotypes separately, the within-clade divergences fall within the expected intra-specific variation (Fig. 1.3). The average intra-specific genetic distance for *F. fragum* and for *F. gravida* also falls within the overall intra-specific average, but the average genetic distance between *F. fragum* and *F. gravida* (0.56 ± 0.2%) is greater than the overall intra-specific average (0.14 ± 0.1%) (see also Fig. 1.3). In contrast, limited data suggest that *Scolymia* does not exhibit any marked divergence between Caribbean and Brazilian populations.

DISCUSSION

The data presented here show strong support for the monophyly of *Mussismilia* at both nuclear and mitochondrial loci (Fig. 1.2). The results of this study also clearly indicate that *F. leptophylla* is more closely related to *Mussismilia* than other members of *Favia*. In addition, the nuclear marker used in this study suggests that the

Mussismilia + *F. leptophylla* clade is more closely related to Atlantic faviids (*Colpophyllia*, *Diploria*, *Manicina* and *F. fragum*) than Atlantic mussids (*Mussa* and *Scolymia*). A published mitochondrial dataset of combined sequences of the cytochrome b (cytb) and cytochrome oxidase 1 (cox1) genes for this group (Fukami et al. 2004b) as well as phylogenetic analysis based on morphologic characters (Budd and Smith 2005) confirm this finding. The analysis with cox1-trnM IGR is ambiguous regarding the phylogenetic position of *Mussismilia* + *F. leptophylla* relative to other Atlantic mussids because an appropriate outgroup beyond Mussa + Scolymia was not found. The β-tubulin data provide the first dataset from a nuclear locus to confirm these patterns of relationships. Both loci presented here have greater resolution than the previously examined loci of Fukami et al. (2004b).

These molecular findings disagree with traditional morphological taxonomy for these taxa (Vaughan and Wells 1943; Wells 1956). Although there is some congruence between the data presented here and new morphological phylogenies for Atlantic corals (Johnson 1998; Budd and Smith 2005), significant differences are still observed in phylogenies inferred by molecular versus traditional morphologic data. The consistency of molecular results from both nuclear and mitochondrial loci suggests that traditional morphological characters are subject to convergence, and that phylogenetically informative morphological characters remain to be identified. Skeletal microstructural features may prove to be an invaluable source of new morphological characters for coral systematics (Cuif et al. 2003).

In the case of the Mussidae, it has already been shown that Pacific and Atlantic mussids are not closely related, but that Atlantic mussids and faviids are (Fukami et al. 2004b). Mussids are primarily distinguished from faviids by the size of corallites and septal teeth and the number of fan systems of trabeculae (Vaughan and Wells 1943), with members of both families falling along a spectrum of corallite and teeth dimensions (for example, corallites are 1.5 – 10 mm in diameter for faviids and 10 – 15 mm or greater for mussids). The dimensions of these characters, however, may not be useful for discriminating between the Atlantic representatives of these two families. Thus the finding that *Mussismilia* is more closely related to Atlantic faviids suggests that the Family definitions need to be re-evaluated.

In the case of *F. leptophylla*, its distinctiveness with respect to other *Favia* has been noted by earlier researchers, e.g., Laborel (1969b), who noted that "*F. leptophylla* semble nettement separée des autres espèces du genre". However, due to its similarity to the Caribbean fossil species *F. macdonaldi* and the presence of features considered characteristic of *Favia* (Laborel 1969b), *F. leptophylla* was placed in this genus. Several morphological characters are shared by *F. leptophylla* and *Mussismilia* but not by *F. leptophylla* and *F. fragum*: parathecal wall structure, absence of paliform lobes, paddle-shaped septal teeth (Budd and Smith 2005). These characters support the close relationship between *F. leptophylla* and *Mussismilia* inferred by molecular data. The genus *Favia* clearly needs major taxonomic revision. The results of this study show that the two Brazilian species are not closely related to each other, a finding that is supported by morphological phylogenetic data (Johnson

1998; Budd and Smith 2005) and other work suggests that neither are closely related to Pacific Favia (Fukami et al. 2004b).

Within the *Mussismilia + F. leptophylla* clade, only *F. leptophylla* had distinct mitochondrial haplotypes (four in total). The three *Mussismilia* species combined are represented by only three haplotypes, and all of the three species share haplotypes with at least one other species. There is significant morphological disparity defining the three taxa, suggesting that they are likely separate species, but this relationship is not clearly observed at the mitochondrial locus. This may reflect ancestral polymorphisms (Avise et al. 1990) shared among the *Mussismilia* species and the fact that mitochondrial genes show little genetic variation in corals (Shearer et al. 2002). β-tubulin, on the other hand, contains more polymorphic sites within this clade and as a result provides greater phylogenetic resolution than the mitochondrial marker. The data support the genetic distinctiveness of the four described taxa. Moreover, the data suggest the possibility of at least one unrecognized cryptic species. This is supported by the distinctiveness of the two *M. hartii* clades in β-tubulin ($2.8 \pm 0.1\%$ divergence, mean $\pm$ SD, greater than the divergence between species of different genera in several examples, for the same locus), and the fact that the same two individuals (B6 and B8) are distinctive at both loci. *M. hispida* also contains two subclades in the β-tubulin tree, but different individuals define nuclear and mitochondrial clades. Additional data supporting these clades are needed to confirm the presence of cryptic species, however.

The results of this study support recognition of the South Atlantic endemic coral, *F. gravida* as a discrete species. Overlap in morphological characters has led some researchers to synonymize this species with *F. fragum* (Veron and Stafford-Smith 2000). The main morphological feature used to separate the two species is the level of meandrinization in the former species relative to the latter (Laborel 1969b). Studies of skeletal variation in *F. gravida* indicate that the species exhibits significant phenotypic plasticity in several morphological characters used for taxonomy: (1) meandrinization of the exotheca, (2) the size of the calyx (or valleys, for the meandoid forms) (Laborel 1969b) and (3) number of septa per cm (Santos et al. 2004) The variability in morphology of *F. gravida* has been correlated with environmental conditions, with higher meandrinization occurring in areas of greater sedimentation (Laborel 1969b). These data raise the possibility that *F. gravida* and *F. fragum* could be merely ecomorphs, with the less meandroid forms being characteristic of less turbid waters found in the Caribbean and more meandroid forms inhabiting the more turbid waters of Brazil and West Africa. The molecular data presented here, however, argue against this possibility. Data from the variable β-tubulin marker show that the divergence between *F. gravida* and *F. fragum* is greater than the intra-specific variation seen in individuals of other coral species in this study, and no haplotypes are shared between the two species, suggesting that these two morphological species are distinct. Although the mitochondrial marker used in this study suggests very little divergence between *F. fragum* and *F. gravida*, there is growing evidence that the mitochondrial genome in highly conserved in many coral species (van Oppen et al.

1999; Shearer et al. 2002; Hellberg 2006). Although the β-tubulin data provide convincing evidence for the separation of these two species, this conclusion must be taken with caution as it is based on only one molecular marker. Further molecular studies of additional variable loci may help to elucidate the relationship between these two closely related taxa. The data presented here further suggest population subdivision within *F. gravida*. Although divergences between haplotypes are small, individuals of *F. gravida* from Brazil do not share haplotypes with individuals of *F. gravida* from West Africa. Larger sample sizes are needed to fully assess the level of gene flow between the two regions, but this preliminary dataset suggests restricted gene flow across the Atlantic for this coral species.

Re-assessment of the systematics of Brazilian endemic corals has revealed that while some endemic species may represent relicts from the Caribbean (such as *M. hispida* and *M. harttii*, which have fossil occurrences in the Caribbean), other endemics may have originated in the South Atlantic. The data presented here suggest that *F. fragum* and *F. gravida* are closely related, but sufficiently distinct to warrant differentiation. The absence of *F. fragum* in the South Atlantic (and reciprocally, the absence of *F. gravida* in the Caribbean) and the position of *F. gravida* relative to *F. fragum* in the β-tubulin phylogeny suggest allopatric speciation of *F. gravida* from *F. fragum*. It is possible that geographic isolation may have played a role in the speciation of the remaining Brazilian endemic not studied here, *S. stellata*, or the diversiffication of the genus *Mussismilia*. Additional studies are needed to resolve

whether allopatric speciation or range reductions and local extinctions are responsible for the high proportion of endemic species in Brazil.

Atlantic species with Pacific congeners have most likely been incorrectly classified at the genus level hindering evaluation of their evolution and conservation. For example, the results of this study suggest that *F. leptophylla* belongs to the genus *Mussismilia*, a genus that is consequently more diverse than previously thought (four species instead of three). Conversely, the genus *Favia* has only two representatives in the Atlantic (*F. fragum* and *F. gravida*) and Pacific *Favia* (19 species) will not ultimately be classified in this genus, leading to a drop in its diversity. Previous work suggests that Atlantic mussids and faviids form a distinct clade that most likely represents a single Atlantic coral family (Fukami et al. 2004b; Budd and Smith 2005). Inconsistencies between morphological and molecular classification schemes impede our ability to make accurate estimates of diversity and endemism at the species, genus and family level. The resulting difficulty in assessing taxonomic diversity of clades may have important repercussions for conservation when high priority is given to areas with the greatest number of species or evolutionary distinctness.

Chapter I, in full, is a reprint of the material as it appears in Coral Reefs, 2008, Flavia Nunes, Hironobu Fukami, Steven V. Vollmer, Richard D. Norris and Nancy Knowlton, Springer Publishing Company, 2008. The dissertation author was the primary investigator and author of this paper.

REFERENCES

Avise JC, Ankney CD, Nelson WS (1990) Mitochondrial gene trees and the evolutionary relationship of Mallard and Black ducks. *Evolution* **44**, 1109–1119.

Bruno JF, Edmunds PJ (1997) Clonal variation for phenotypic plasticity in the coral <u>Madracis mirabilis</u>. *Ecology* 78, 2177–2190.

Budd AF (2000) Diversity and extinction in the Cenozoic history of Caribbean reefs. *Coral Reefs* **19**, 25–35.

Budd AF, Klaus JS (2001) The origin and early evolution of the <u>Montastraea "annularis"</u> species complex (Anthozoa: Scleractinia). *J Paleontol* **7**, 527–545.

Budd AF, Smith ND (2005) Diversification of a new Atlantic clade of scleractinian reef corals: insights from phylogenetic analysis of morphologic and molecular data. *Paleontological Society Papers* **11**, 103–128.

Budd AF, Johnson KG, Potts DC (1994) Recognizing morphospecies in colonial reef corals.1. Landmark-based methods. *Paleobiology* **20**, 484–505.

Budd AF, Foster CT, Dawson JP, Johnson KG (2001) The Neogene marine biota of Tropical America ("NMITA") database: accounting for biodiversity in paleontology. *J Paleontol* **75**, 743–751.

Castro CB, Pires DO (2001) Brazilian coral reefs: what we already know and what is still missing. *Bull Mar Sci* **69**, 357–371.

Chen CA, Wallace CC, Wolstenholme J (2002) Analysis of the mitochondrial 12S rRNA gene supports a two-clade hypothesis of the evolutionary history of scleractinian corals. *Mol Phylogenet Evol* **23**, 137–149.

Chevalier JP (1959) Quelques considerations sur la vie recifale au Miocène dans le bassin Méditerranéen. *Bull Soc Geol Fr* **4**, 369–374.

Chevalier JP (1961) Recherche sur les madréporaires et les formations récifales Miocènes de la Méditerranée Occidentale. *Mem Soc Geol Fr, Nouv Ser* **40**, 1–562.

Cuif JP, Lecointre G, Perri C, Tillier A, Tillier S (2003) Patterns of septal biomineralization in Scleractinia compared with their 28S rRNA phylogeny: a dual approach for a new taxonomic framework. *Zool Scr* **32**, 459–473.

Foster AB (1979) Phenotypic plasticity in the reef corals Montastraea annularis (Ellis and Solander) and Siderastrea siderea (Ellis and Solander). *J Exp Mar Biol Ecol* **39**, 25–54.

Foster AB (1980) Environmental variation in skeletal morphology within the Caribbean reef corals Montastraea annularis and Siderastrea siderea. *Bull Mar Sci* **30,** 678–709.

Foster AB (1985) Variation within coral colonies and its importance for interpreting fossil species. *J Paleontol* **59**, 359–1381.

Fukami H, Budd AF, Levitan DR, Jara J, Kersanach R, Knowlton N (2004a) Geographic differences in species boundaries among members of the Montastraea annularis complex based on molecular and morphological markers. *Evolution* **58**, 324–337.

Fukami H, Budd AF, Paulay G, Sole-Cava A, Chen CLA, Iwao K, Knowlton N (2004b) Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. *Nature* **427**, 832–835.

Gittenberger A, Hoeksema BW (2006) Phenotypic plasticity revealed by molecular studies on reef corals of <u>Fungia</u> (Cycloseris) spp. (Scleractinia: Fungiidae) near river outlets. *Contrib Zool* **75**, 195–201.

Gradstein F, Ogg J, Amith A (eds) (2004) A geologic time scale 2004. Cambridge University Press, Cambridge.

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* **52**, 696–704.

Hasegawa M, Kishino H, Yano TA (1985) Dating of the human ape splitting by a molecular clock of mitochondrial DNA. *J Mol Evol* **22**, 160–174.

Hellberg ME (2006) No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. *BMC Evol Biol* **6**, 24. doi: 10.1186/1471-2148-6-24

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–755.

Johnson KG (1998) A phylogenetic test of accelerated turnover in Neogene Caribbean brain corals (Scleractinia: Faviidae). *Paleontology* **41**, 1247–1268.

Kerr AM (2005) Molecular and morphological supertree of stony corals (Anthozoa: Scleractinia) using matrix representation parsimony. *Biol Rev* **80**, 543–558.

Klaus JS, Budd AF, Heikoop JM, Fouke BW (2007) Environmental controls on corallite morphology in the reef coral <u>Montastraea annularis</u>. *Bull Mar Sci* **80**, 233–260.

Knowlton N, Weil E, Weigt LA, Guzman HM (1992) Sibling species in <u>Montastraea annularis</u>, coral bleaching, and the coral climate record. *Science* **255**, 330–333.

Laborel J (1969a) Les peuplements de madréporaires des côtes tropicales du Brésil. Ph.D. thesis, Université d'Abidjan, p 260

Laborel J (1969b) Madréporaires et hydrocoralliaires récifaux des côtes brésiliennes. Systématique, écologie, repartition verticale et géographique. *Ann Inst Océanogr Paris* **47**, 171–229.

Laborel J (1974) West African reefs corals: an hypothesis on their origin. *Proc 2nd Int Coral Reef Symp* **1**, 425–443.

Lasker HR (1981) Phenotypic variation in the coral <u>Montastrea cavernosa</u> and its effects on colony energetics. *Biol Bull* **160**, 292–302.

Leão ZMAN (1996) The coral reefs of Bahia; morphology, distribution and the major environmental impacts. *An Acad Bras Ciênc* **68**, 439–452.

Leão ZMAN, Ginsburg RN (1997) Living reefs surrounded by siliciclastics sediments: the Abrolhos coastal reefs, Bahia, Brazil. *Proc 8th Int Coral Reef Symp* **8**, 1767–1772.

Leão ZMAN, Kikuchi RKP (2000) The Abrolhos reefs of Brazil. *Ecol Stud* **144**, 83–96.

Lopez JV, Kersanach R, Rehner SA, Knowlton N (1999) Molecular determination of species boundaries in corals: genetic analysis of the <u>Montastraea annularis</u> complex using amplified fragment length polymorphisms and a microsatellite marker. *Biol Bull* **196**, 80–93.

Maida M, Ferreira BP (1997) Coral reefs of Brazil, an overview. *Proc 8th Int Coral Reef Symp* **1**, 263–274.

Medina M, Collins AG, Takaoka TL, Kuehl JV, Boore JL (2006) Naked corals: skeleton loss in Scleractinia. *Proc Natl Acad Sci USA* **103**, 9096–9100.

Meeder JF (1987) The paleoecology, petrology and depositional model of the Pliocene Tamiami Formation, southwest Florida (with special reference to corals and reef evolution). Ph.D. thesis, University of Miami, p 748

Nylander JAA (2002) MrModeltest, v2.2. Program distributed by the author. Evolutionary Biology Center, Uppsala University, Uppsala Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.

Rocha LA, Rosa IL (2001) Baseline assessment of reef fish assemblages of Parcel Manuel Luiz Marine State Park, Maranhão, north-east Brazil. *J Fish Biol* **58**, 985–998.

Romano SL, Cairns SD (2000) Molecular phylogenetic hypotheses for the evolution of scleractinian corals. *Bull Mar Sci* **67**, 1043–1068.

Romano SL, Palumbi SR (1996) Evolution of scleractinian corals inferred from molecular systematics. *Science* **271**, 640–642.

Romano SL, Palumbi SR (1997) Molecular evolution of a portion of the mitochondrial 16S ribosomal gene region in scleractinian corals. *J Mol Evol* **45**, 397–411.

Santos MG, Amaral FD, Hernández MIM, Knowlton N, Jara J (2004) Variação morfológica de <u>Favia gravida</u> Verrill, 1868 e <u>Siderastrea stellata</u> Verrill, 1868 (Cnidaria, Scleractinia): aspectos esqueléticos. *Bol Mus Nac Rio J Zool* **517**:1–9.

Saunders JB, Jung P, Biju-Duval B (1986) Neogene paleontology in the northern Dominican Republic. Part 1: Weld surveys, lithology environment and age. *Bull Am Paleontol* **89**, 1–79.

Schellmann G, Radtke U, ScheVers A, Whelan F, Kelletat D (2004) ESR dating of corals reef terraces on Curaçao (Netherlands Antilles) with estimates of younger Pleistocene sea level elevations. *J Coast Res* **20**, 947–957.

Shearer TL, van Oppen MJH, Romano SL, Worheide G (2002) Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). *Mol Ecol* **11**, 2475–2487.

Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony (*and other methods), v4.0b10. Sinauer, Sunderland

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Todd PA, Sanderson PG, Chou LM (2001) Morphological variation in the polyps of the scleractinian coral <u>Favia speciosa</u> (Dana) around Singapore. *Hydrobiologia* **444**, 227–235.

van Oppen MJH, Willis BL, Miller DJ (1999) Atypically low rate of cytochrome b evolution in the scleractinian coral genus <u>Acropora</u>. *Proc R Soc London, B* **266,** 179–183.

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

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

Vaughan TW, Wells JW (1943) Revision of the suborders, families and genera of the Scleractinia. *Spec Pap Geol Soc Am* **44**, 1–345.

Veron JEN, StaVord-Smith M (2000) *Corals of the world*. Australian Institute of Marine Science, Townsville

Watanabe T, Nishida M, Watanabe K, Wewengkang DS, Hidaka M (2005) Polymorphism in nucleotide sequence of mitochondrial intergenic region in scleractinian coral (Galaxea fascicularis). *Mar Biotechnol* **7**, 33–39.

Weil E, Knowlton N (1994) A multi-character analysis of the Caribbean coral Montastraea annularis (Ellis and Solander, 1786) and its 2 sibling species, M. faveolata (Ellis and Solander, 1786) and M. franksi (Gregory, 1895). *Bull Mar Sci* **55**, 151–175.

Wells JW (1956) Scleractinia. In: Moore RD (ed) *Treatise on invertebrate paleontology*, vol F. Geological Society of America and University of Kansas Press, New York, pp 328–444

Xia X, Xie Z (2001) DAMBE: software package for data analysis in molecular biology and evolution. *J Hered* **92**, 371–373.

CHAPTER II

Implications of isolation and low genetic diversity in peripheral populations of an amphi-Atlantic coral

ABSTRACT

Limited dispersal and connectivity in marine organisms can have negative fitness effects in populations that are small and isolated, but reduced genetic exchange may also promote the potential for local adaptation. Here, we compare the levels of genetic diversity and connectivity in the coral *Montastraea cavernosa* among both central and peripheral populations throughout its range in the Atlantic. Genetic data from one mitochondrial and two nuclear loci in 191 individuals show that *M. cavernosa* is subdivided into three genetically distinct regions in the Atlantic: Caribbean-North Atlantic, Western South Atlantic (Brazil) and Eastern Tropical Atlantic (West Africa). Within each region, populations have similar allele frequencies and levels of genetic diversity; indeed, no significant differentiation was found between populations separated by as much as 3,000 km, suggesting that this coral species has the ability to disperse over large distances. Gene flow within regions does not, however, translate into connectivity across the entire Atlantic. Instead, substantial differences in allele frequencies across regions suggest that genetic exchange is infrequent between the Caribbean, Brazil and West Africa. Furthermore, markedly lower levels of genetic diversity are observed in the Brazilian and West African populations. Genetic diversity and connectivity may contribute to the resilience of a coral population to disturbance. Isolated peripheral populations may be more vulnerable to human impacts, disease or climate change relative to those in the genetically diverse Caribbean-North Atlantic region.

INTRODUCTION

The Caribbean has extensive reef development, but most Atlantic reefs outside of the Caribbean are not biogenic in origin. Rather, these are rocky reefs sparsely colonized by corals and other benthic invertebrates. These peripheral rocky reefs also have lower coral abundance and fewer coral species than the Caribbean, but host a rich endemic fauna of marine organisms (Floeter *et al.* 2008; Jones 1994; Rios 1994; Rosenberg 1993), including two endemic genera of corals (Laborel 1969b, 1974). Several coral species have a wide distribution across the Atlantic, extending well beyond the Caribbean to the peripheral reefs of the southern and eastern Atlantic. It is unclear, however, whether these broad distributions reflect continued genetic exchange between geographically distant populations. Understanding how peripheral populations are connected to one another and to more central populations can shed light on how distantly corals are capable of dispersing and what types of barriers limit dispersal in the Atlantic. Furthermore, peripheral populations may exhibit lower genetic diversity as a consequence of smaller effective populations sizes and isolation (although exceptions exist, see the review by Eckert *et al.* 2008). Because genetic variation is the raw material that allows populations to evolve in response to changing environments, reduced genetic diversity may increase a population's vulnerability to disturbances. Here we provide the first molecular dataset from a coral species spanning Caribbean and several peripheral reefs in the Atlantic in order to determine the level of genetic diversity and connectivity among these populations.

Genetic exchange in sessile organisms like corals is achieved primarily by larval dispersal. The extent of transport varies depending on larval longevity and behavior, and ocean circulation. Some coral species are able to maintain competent larvae for 50-100 days after fertilization (Graham *et al.* 2008; Richmond 1987), but average larval lifetime can be quite variable across different species (Nishikawa& Sakai 2005), being as short as 10 days for some (Goffredo& Zaccanti 2004). Nutritional reserves and the presence of zooxanthellae can affect potential larval lifetime (Harii *et al.* 2002; Isomura& Nishihira 2001), and substantial mortality as a result of predation by reef fish during coral spawning (Pratchett *et al.* 2001) can reduce the number of dispersing larvae. Ocean currents aid dispersal by carrying larvae downstream and limit dispersal upstream or across strong currents, but making predictions about larval dispersal patterns based solely on currents can be difficult. Large-scale patterns of ocean circulation are averages that do not represent all possible paths for dispersal. Swimming behavior allows larvae to orient and follow settlement cues (Raimondi& Morse 2000), and vertical migration can lead larvae to leave one current and enter another. Low water temperatures or low salinity may pose impermeable barriers to dispersal; the latter can shorten the planktonic period by decreasing the larvae's selectivity for suitable substrate (Vermeij *et al.* 2006). Complex meso-scale circulation and local eddies also play a role, and simulation studies have shown that local retention of larvae can be very high (Cowen *et al.* 2000; Cowen *et al.* 2006; James *et al.* 2002).

Because of the logistic challenges in tracking or simulating larval movement, molecular methods are often used to infer connectivity on coral reefs. However, studies of connectivity in corals are relatively scarce relative to other reef inhabitants, despite their importance as foundation species on reefs. This is in part due to difficulty in finding molecular markers that contain sufficient intraspecific variation. Mitochondrial DNA in corals and other anthozoans, unlike most other organisms, evolves at a much slower rate and is usually invariant at the level of species (Shearer *et al.* 2002). Despite the low levels of variation in the mitochondrial genome, nuclear variation has been found in corals (Hellberg 2006). Allozymes have been employed successfully in studies of population differentiation in Indo-Pacific corals (Ayre& Dufty 1994; Stoddart 1984), and more recently, efforts have been made to develop microsatellite markers for Caribbean coral species (Baums *et al.* 2005a; Severance *et al.* 2004; Shearer& Coffroth 2004). For this study, sequence data from one mitochondrial and two nuclear loci were used to estimate levels of genetic diversity and population differentiation in a common and widespread Caribbean coral.

The coral *Montastraea cavernosa* is an abundant reef builder in the Caribbean, being found throughout the region, from Panamá to Florida, east to the islands of the Lesser Antilles, and as far north as Bermuda (32º18'N, 64º46' W), in the north Atlantic. This species is also widespread in the South Atlantic, being common along the coast of Brazil from Cabedelo, Paraíba (6º 57'S, 34º 49'W) to Vitoria, Espírito Santo (20º 21'S, 40º 16'W) (Laborel 1969a). *M. cavernosa* has also been reported in the offshore island of Fernando de Noronha (Laborel 1969a) as well as Parcel Manuel

Luiz (Moura *et al.* 1999), an offshore reef located 500 km east of the Amazon outflow. Although this species was previously unknown off the north coast of Brazil, we discovered abundant colonies on the offshore reef of Pedra da Risca do Meio (3º 34'S, 38º 24'W) at depths of ~25m. *M. cavernosa* is also one of the most common coral species in the islands of São Tomé, Príncipe and Annobon in the Gulf of Guinea, West Africa, but it has not been reported along the West African mainland nor in the Cape Verde islands further north (Laborel 1974).

M. cavernosa is a gonochoric broadcast spawning species with an annual cycle of gametogenesis (Szmant 1991). Spawning takes place approximately one week after the full moon in August and September, and in some locations in October as well (Acosta& Zea 1997). Oogenesis takes place annually except for 1-2 months after spawning, whereas spermatogenesis occurs from April to August. Zooxanthellae are not present in the eggs and must be acquired from the water column *de novo*. *M. cavernosa* produces larger eggs than *Montastraea annularis*, another abundant reef-builder in the Caribbean, although recent phylogenetic analyses indicate that they are not closely related (Fukami *et al.* 2004b; Fukami *et al.* 2008). The larger eggs of *M. cavernosa* may increase larval survival time and dispersal capability (Acosta& Zea 1997), as well as increase survivorship after settlement.

MATERIALS AND METHODS

Study sites and sample collection

Tissue samples of *M. cavernosa* were collected from eight sites spanning the Atlantic Ocean: three sites within the Caribbean (Belize, Panamá and Puerto Rico), one site in the North Atlantic (Bermuda), three sites in the Western South Atlantic (Abrolhos, João Pessoa and Fortaleza, Brazil) and one site in the Eastern Tropical Atlantic (São Tomé & Príncipe, Gulf of Guinea). A hammer and chisel were used to break a small piece ($\sim 2 - 5$ cm^2) off the coral, taking care to minimize damage to the overall colony. Fresh tissue was scraped off the living surface and put into vials containing guanidine thiocyanate solution (4M guanidine thiocyanate, 0.1% N-lauroyl sarcosin sodium, 10 mM Tris pH8, 0.1M 2-mercaptoethanol). Samples were stored at room temperature, and can be kept in these conditions for upwards of several months. Skeletal vouchers for each sample were labeled, bleached and dried.

DNA extraction, amplification and sequencing

For DNA extraction, equal volumes of coral tissue digested in guanidine thiocyanate solution and phenol extraction buffer (PEB; 100 mM Tris-HCl pH 8.0, 10 mM EDTA, 0.1% SDS) were mixed, then added to twice the volume of phenol:chloroform:isoamyl alcohol (25:24:1). This mixture was vortexed, centrifuged at high speed, and the supernatant recovered. DNA was precipitated with 100% isopropanol and resuspended in 10mM Tris-HCl pH 8.0.

The mitochondrial marker used in this study was the intergenic region (IGR) between cytochrome oxidase I (*cox1*) and the methionine transfer RNA gene (referred to as *cox1-trnM* IGR hereafter). This region was chosen because it was found to be one of the longest and most variable intergenic regions in the mitochondrial genome of the *M. annularis* species complex (Fukami& Knowlton 2005). The region was amplified using primers published in Fukami *et al.* (2004a).

Two nuclear markers were used in this study. *β-tubulin* has previously been used for coral phylogenetics (Fukami *et al.* 2004b; Nunes *et al.* 2008) and to differentiate cryptic species of corals (Lopez& Knowlton 1997). In *M. cavernosa*, amplification of the *β-tubulin* gene yields two bands, one at ~1000 bp *(β-tub1*) and another at 750 bp (*β-tub2*). Both bands were purified and sequenced, revealing two gene products with the same partial amino acid sequence but different intron lengths, most likely the result of a duplication. *β-tub1* has an intron of 611 bp, whereas *β-tub2* has an intron of 284 bp. The two markers were tested for linkage disequilibrium using Arlequin v. 3.11 (Excoffier *et al.* 2005), and although linkage was detected among a subset of nucleotide sites within each locus, no significant linkage was found across the two markers (data not shown). Therefore, these two markers were assumed to be independent loci. *β-tub1* and *β-tub2* were amplified simultaneously, using published primers (Lopez& Knowlton 1997).

Amplification of all three markers was performed by polymerase chain reaction (PCR) consisting of 5 μL of 10X Buffer (containing 100 mM Tris-HCl, pH 8.3, 500 mM KCl,15 mM $MgCl_2$ and 0.01% gelatin), 2 μL of primers (10μM), 1 μL of

dNTP (200mM), 1 U Taq polymerase (Sigma-Aldrich), 1-2 µL of template and water to 50 µL. The thermal cycler profile used had an initial denaturation step at 94°C for 2 min, followed by 35 cycles of 94°C/45sec, 54°C/45sec for *cox1-trnM* IGR or 58°C/45sec for *β-tub1* and *β-tub2*, 72°C/1min 30sec, with a final extension step at 72°C for 5 min.

For the mitochondrial locus, a 10-15µL aliquot of the PCR product was purified for sequencing by adding 0.5 U of exonuclease 1, 0.5 U of shrimp alkaline phosphatase (SAP, New England Biolabs) and 1 µL of 10x SAP buffer, and incubating at 37°C for 60 min. The enzymes were deactivated by heating to 80°C for 15 min. In order to purify the PCR products of the two nuclear markers, *β-tub1* and *β-tub2*, a 40µL aliquot was run through a 1% agarose gel prepared with Tris-acetate EDTA (TAE). Each fragment was excised from the gel and purified using the Qiaquick Gel Extraction Kit (Qiagen) according the manufacturer's protocol.

Sequencing of all loci was performed directly on purified PCR products for both forward and reverse directions on an ABI 3130xl genetic analyzer with the ABI BigDye Terminator v3.1 chemistry (Applied Biosystems). Cycle sequencing reactions were purified with Sephadex G-50 (Sigma). Sequence chromatographs were viewed and edited using Sequencher v4.5 software (Gene Codes Corp). For *β-tub1*, indels were observed at sites 812, 881 and three adjacent indels at sites 450, 451 and 452. In the case of individuals heterozygous for two or more indels, an internal sequencing primer was designed to obtain sequence data between indels (btubintR: 5'- TCA GCT GGT ACT CGT TGT CG -3'). For *β-tub2*, only one indel was found, at site 451.

Heterozygous alleles were identified by double peaks observed in sequences from both directions.

Three of heterozygous individuals from each nuclear locus was cloned using the pGEM-T Vector system (Promega) *(β-tub1* = samples E05, P24, and P570; *β-tub2* = samples E08, E24 and M01). Sequences from 6-8 clones per individual were used as input data along with homozygous direct sequences (n = 64 for *β-tub1* and n = 73 for *β-tub2*) to reconstruct haplotypes for heterozygous individuals using PHASE v 2.1.1 (Stephens& Scheet 2005; Stephens *et al.* 2001). For *β-tub1*, the five indel sites and an additional four sites (sites 301, 438, 524 and 881) containing multiple mutations (>2 phases) were excluded from the analysis. No sites were excluded for *β-tub2*. The algorithm was run three times for 100 iterations with ten thinning interval steps and 100 burn in steps. The best pairs of haplotypes for each individual resulting from the run with the highest average value for the goodness of fit were used for each nuclear locus. Posterior probabilities of >0.90 were observed for 97.7% and 95.0% of base calls for *β-tub1* and *β-tub2* respectively.

Data analysis

A statistical parsimony network was constructed for each locus using TCS v1.21 (Clement *et al.* 2000) after sequences had been aligned using Clustal X v1.83.1 (Thompson *et al.* 1997). Recombination in the two nuclear loci was inferred as reticulations in the haplotype network and tested by the four-gamete test in DnaSP (Librado & Rozas 2009). Six loops (1-3 mutations in length) were observed in *β-tub1*

and five loops (1-2 mutations in length) in *β-tub2*. For clarity, loops were omitted from the haplotype network and only internal branches are shown. For *β-tub1* and *β-tub2*, 10.4% and 5.0% of pairwise comparisons contained all four gametic types, respectively. As a result, analyses using a coalescent framework (that assume no recombination) were not attempted.

A pie chart of the haplotype frequencies for each population was plotted on a map of the Atlantic Ocean for each locus. Due to the high number of haplotypes encountered in the two nuclear loci, pie segments represented the haplotype frequencies of the three most common haplotypes and the following haplotype frequency classes: (1) haplotypes belonging to the most common haplotype clade (Clade A for *βtub1* or Clade C *βtub2*, see results section), (2) haplotypes belonging to the less common haplotype clade (Clade B for *βtub1* or Clade D for *β-tub2*) and (3) private alleles (haplotypes that were restricted to a geographical region). Haplotypes were labeled by numbers preceded by the letters J (representing haplotype clades) for *β-tub1*, by the letter L for *βtub2*, and by the letter M for *cox1-trnM IGR*.

The number of haplotypes (*H*) and segregating sites (*s*) were tallied per population and overall for each locus. Genetic diversity for each population was measured by the gene diversity (*h*), nucleotide diversity (*π*), and average nucleotide changes (*k*). Gene diversity is defined as the probability that two randomly chosen haplotypes are different in the sample, whereas nucleotide diversity is the probability that two randomly chosen homologous sites are different. Population genetic structure was inferred by analysis of molecular variance (AMOVA) in Arlequin v. 3.11

(Excoffier *et al.* 2005). Hierarchical AMOVA was used to estimate levels of genetic differentiation among populations (Φ_{st}), between groups of populations or regions (Φ_{ct}), and between populations within regions (Φ_{sc}). Regions used in the analysis were Brazil (Abrolhos, João Pessoa and Fortaleza), the Gulf of Guinea (São Tomé Island), the Caribbean (Panamá, Belize and Puerto Rico) and the North Atlantic (Bermuda). A second scenario was tested where the Caribbean and North Atlantic groups were merged to form a single region (greater Caribbean). AMOVA was conducted independently for all three loci. Pairwise ϕ_{ST} values were calculated to estimate differentiation between populations. Both AMOVA and ϕ_{ST} calculations used pairwise distances among sequences. Deviations from Hardy-Weinberg equilibrium (HWE), in the form of heterozygote excesses or deficits, were calculated using exact tests in Genepop (Raymond& Rousset 1995). Statistics for neutral sequence evolution (Tajima's D and Fu's Fs) and linkage disequilibrium among and within loci were tested using Arlequin v3.11.

Divergent mitochondrial haplotypes

Three highly divergent mitochondrial haplotypes were observed in a small number of individuals from Belize and Panamá. In order to determine the origin of these divergent mitochondrial haplotypes, the following tests were performed: (1) To determine if the divergent mitochondrial haplotypes represented a cryptic species within *M. cavernosa*, nuclear sequence data (*βtub1* and *βtub2*) were obtained for these individuals to test whether divergence was observed in the nuclear genome of these

individuals as well. (2) To assess whether the divergent mitochondrial haplotypes were introgressed from another species, sequences of the flanking and more conserved gene, cytochrome oxidase 1 (*cox1*), were obtained from four individuals carrying the divergent mitochondrial haplotypes and four individuals carrying the typical *M. cavernosa* haplotype for *cox1-trnM IGR*. Because *cox1* has been used to infer phylogenetic relationships in corals, the sequences obtained from our samples could be compared against the Genbank database and used for species identification in the case of introgression from another species. (3) To determine if the divergent mitochondrial haplotypes resulted from a pseudogene that was amplified simultaneously with *cox1-trnM IGR*, four individuals carrying the divergent allele and four individuals carrying the typical alleles were cloned using the pGEM-T cloning vector system. Ten clones were sequenced per individual in order to detect if multiple regions were being amplified following our protocol.

RESULTS

A total of 191 individuals from eight populations were genotyped for this study (Sequences have been deposited in Genbank, accession numbers FJ854573-FJ854692). Sequence lengths for the three loci were 834 bp for *cox1-trnM IGR* , 961 bp for *βtub1* and 612 bp for *βtub2*. Overall, populations did not show a significant departure from neutrality (p > 0.05), but negative Tajima's D values were observed in *βtub1* for Abrolhos (D = -1.85, p = 0.015) and Fortaleza (D = -1.43, p = 0.048), and negative Fu's Fs values were observed in Belize for both *βtub1* (Fs = -14.82, p=

0.002) and *βtub2* (Fs = -5.455, p=0.053). Significant heterozygote deficits were observed in the João Pessoa population for the *βtub1* locus (p = 0.014) and in the Gulf of Guinea population for *βtub2* (p = 0.013). For all other populations, no significant heterozygote excesses or deficits were observed for either locus. Results for HWE and neutrality tests for the three loci are shown in Table 1.

Divergent mitochondrial haplotypes

A total of nine individuals from two Caribbean populations were found to have highly divergent mitochondrial haplotypes (Belize, n=7 and Panamá, n=2), which differed from the common haplotypes by 40 base substitutions. These polymorphisms were distributed throughout the sequence and no indels were observed. All individuals carrying a divergent mitochondrial haplotype were genotyped as having typical *M. cavernosa* alleles at both nuclear loci. Because there does not appear to be divergence in the nuclear genome separating these individuals from the rest of the population, it is unlikely that they represent a cryptic species.

Sequences of the *cox1* gene were obtained for eight individuals from Panamá and Belize. All eight individuals had identical *cox1* sequences, typical of *M. cavernosa* as identified by BLAST (Altschul *et al.* 1997), irrespective of whether they carried the typical *cox1-trnM IGR* haplotype or the rare divergent *cox1-trnM IGR* haplotype. Since all individuals can be identified as *M. cavernosa* based on *cox1*, and given that the mitochondrial genome does not recombine, it is unlikely that the divergent mitochondrial haplotypes were introgressed from another species.

Ten clones were sequenced per individual out of a sample of eight individuals to check for the presence of a pseudogene that could be amplified simultaneously with *cox1-trnM IGR*. Of the eight cloned individuals, every individual had only one sequence for all ten clones, suggesting that these divergent haplotypes are not derived from a pseudogene.

Although none of the above tests have conclusively determined the origin of the divergent sequences, the results make it unlikely that these sequences represent a cryptic species, an introgressed allele or a pseudogene. Therefore, the divergent sequences were kept in the analyses and treated as regular alleles.

Haplotype distribution

Statistical parsimony networks for haplotypes from each locus are shown in Fig. 2.1 a-c. Over all sampling locations, 74 haplotypes were observed in *β-tub1*, 40 haplotypes were observed in *β-tub2* and six haplotypes were observed in the mitochondrial *cox1-trnM IGR*.

The *β-tub1* network was divided into two clades of haplotypes (Clades A and B), which were separated by at least nine mutations (Fig. 2.1.a). Individuals heterozygous for alleles belonging to each of the two clades were observed in all populations, except for the Gulf of Guinea population, where all observed haplotypes were from Clade A. Six haplotypes (J1, J3, J7, J9, J12 and J13) were shared between Caribbean and Brazilian populations, whereas only two haplotypes (J2 and J5) were shared between populations in Brazil and the Gulf of Guinea. No shared haplotypes

A. β-*tubulin 1* Haplotype Network

B. β-*tubulin 2* Haplotype Network

C. *cox1-trnM IGR* Haplotype Network
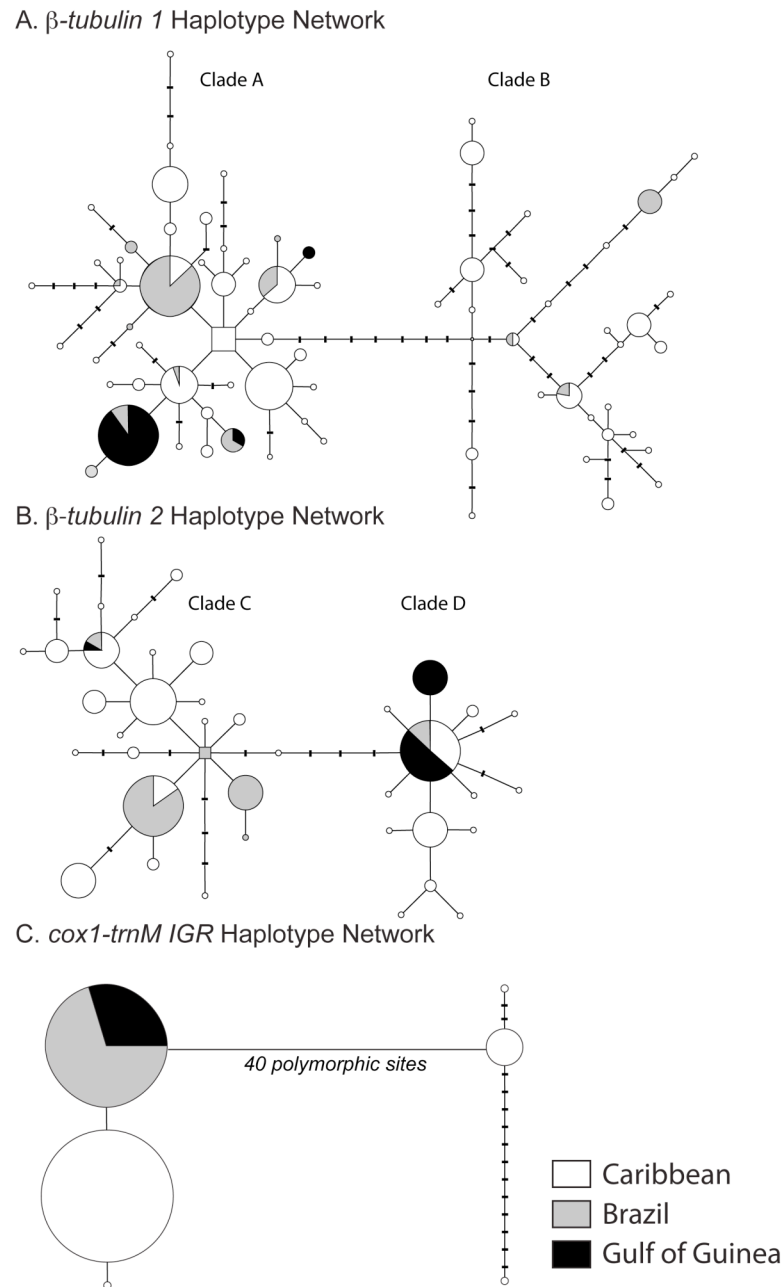
Figure 2.1 **Parsimony haplotype network for A. *β-tubulin 1* B. *β-tubulin 2* and C. mitochondrial *cox1-trnM IGR.* Haplotypes observed in the Caribbean, Brazil and Gulf of Guinea are shown as white, grey and black circles, respectively. The size of each circle reflects the frequency that a haplotype is observed. Notches symbolize intermediate haplotypes not sampled. For the mitochondrial locus, the number of steps between common and divergent haplotype clades could not be estimated by TCS. Instead, the number of polymorphic sites between haplotypes is indicated.**

were found between the Gulf of Guinea and the Caribbean. Sixty-six of 74 haplotypes were endemic to one of the three major regions (Caribbean, Brazil or Gulf of Guinea) and differed by only a few mutations from the more common haplotypes. Forty-four haplotypes (59%) were observed only once.

The haplotype network for *β-tub2* was also separated into two haplotypes clades (Clades C and D) that were at least four mutations apart (Fig. 2.1.b). Individuals heterozygous for the two clades were found in all populations. Two common haplotypes (L1 and L4) were found in all populations across the Atlantic, whereas another haplotype (L2) was shared between the Caribbean and Brazil. All other haplotypes were regionally restricted, although these differed by at most three mutations from haplotypes found in an adjacent region, and 22 haplotypes (55%) were observed only once.

In the mitochondrial marker (Fig. 2.1.c), most Caribbean individuals shared one common haplotype (M2) whereas all South Atlantic individuals shared another common haplotype (M1), which differed from the common Caribbean haplotype by one mutation. The other mitochondrial haplotypes (M3-M6) were observed only in Belize and Panamá and three (M4-M6) differed from the common haplotypes at 40 nucleotide sites. Three haplotypes (50%) were observed only once. The results of additional tests that were performed to identify the origin of the divergent haplotypes are summarized in the preceding section.

Haplotype frequencies

A map of haplotype frequencies for each population and for each locus is shown in Fig. 2.2. For both nuclear loci, Caribbean and North Atlantic populations (Belize, Panamá, Puerto Rico and Bermuda) had similar haplotype frequencies, as did populations along the coast of Brazil (Abrolhos, João Pessoa and Fortaleza). Populations in different regions (Caribbean-North Atlantic vs. Brazil vs. Gulf of Guinea), however, differed in their haplotype frequencies. For example, the frequency of the most common β-*tub1* allele (J1) in the Brazilian populations ranged from 0.71 – 0.75, but in the Caribbean, the frequency of this allele ranged only from 0.08 – 0.10, and was absent in Puerto Rico. The most common β*tub1* haplotype in the Gulf of Guinea (J5, $p = 0.86$) was present in the Brazilian populations, but at a much lower frequency ($p = 0.04 – 0.05$), and was absent from all Caribbean populations. A similar pattern in haplotype frequencies was observed for β-*tub2*. For the mitochondrial locus, South Atlantic populations all shared the same haplotype (M1), whereas Caribbean populations, including Bermuda, shared another common haplotype (M2). Haplotype M3 was observed only once in Bermuda. In the Caribbean, three divergent haplotypes were present at low frequencies in Belize (M4 and M6 $p = 0.026$ each; M5, $p = 0.103$) and Panamá (M5, $p = 0.105$).
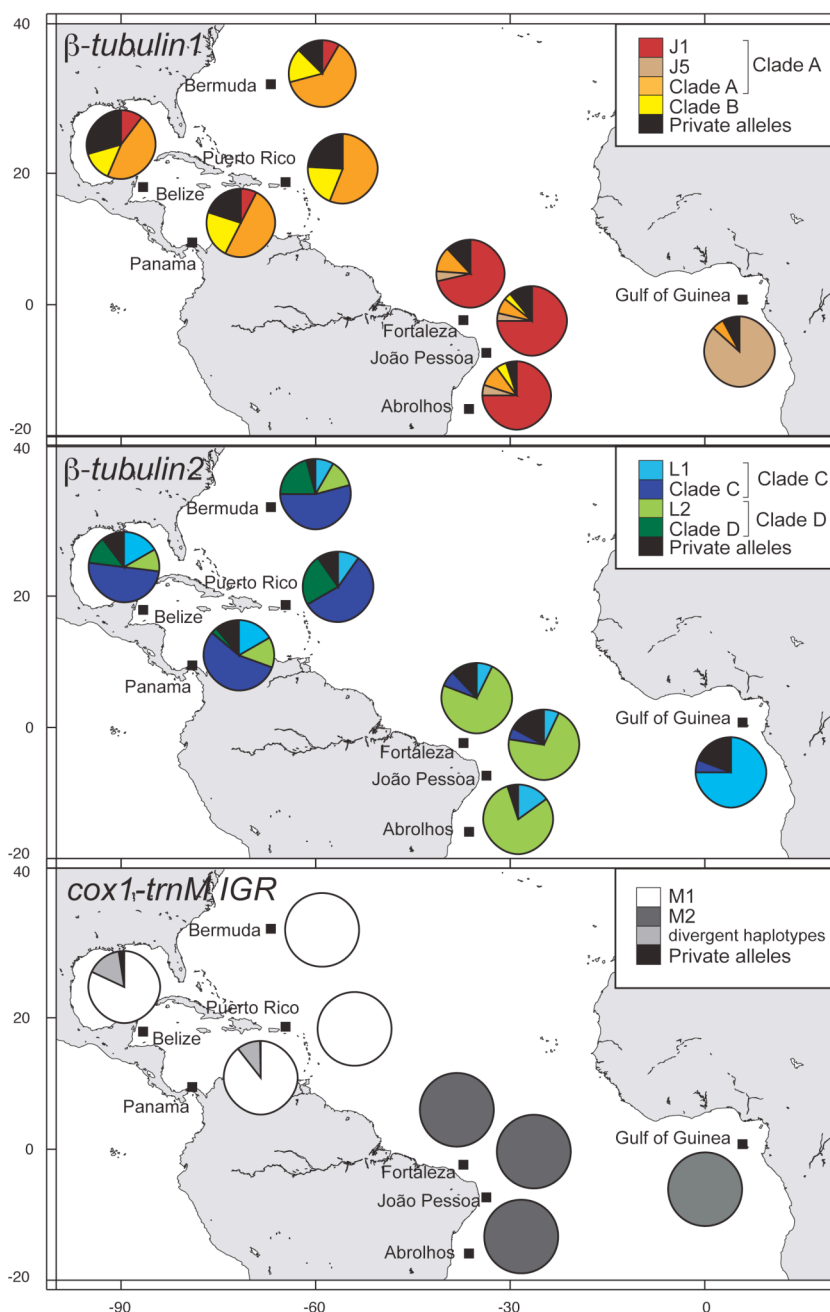
Figure 2.2 **Haplotype frequency pie charts for each of the eight sampled populations. Data for each locus is presented in a separate panel: A. *β-tub1* B. *β-tub2* and C. mitochondrial *cox1-trnM IGR*. Because of the high number of alleles in *β-tub1* and *β-tub2*, haplotype frequencies were binned by haplotype Clade (Clades A and B for *β-tub1* and Clades C and D for *β-tub2*); private alleles for each population are shown as a single category, regardless of Clade. The frequencies of selected common haplotypes are also shown to emphasize the differences among populations. Haplotype frequencies are similar within regions, but are strikingly different from region to region.**

Genetic diversity patterns

Molecular diversity indices for each marker and for each of the populations are shown in Table 1. For all loci, diversity indices were similar between the Caribbean and North Atlantic populations. The Caribbean-North Atlantic sites showed greater levels of molecular diversity than the populations from Brazil and Gulf of Guinea. For example, nucleotide diversity (π) in *β-tub1* ranged from 0.008 ± 0.004 in Bermuda to 0.010 ± 0.005 in Panamá, but in Brazil, π ranged from 0.002 ± 0.002 to 0.004 ± 0.002 and in the Gulf of Guinea, $\pi = 0.001 \pm 0.001$. Nucleotide diversity was an order of magnitude greater in the most diverse site (Belize) relative to the least diverse site (Gulf of Guinea). A similar pattern was observed for gene diversity (h), average nucleotide changes (k), and number of haplotypes and segregating sites for both nuclear loci, with greater genetic diversity in the Caribbean relative to the South Atlantic populations, and with the Gulf of Guinea populations consistently being the least diverse of all (Table 1).

Much lower molecular diversity was observed in the mitochondrial locus relative to the nuclear loci. Most sampled individuals had one of two most common haplotypes, with one haplotype being fixed or nearly so in the South Atlantic (M1) and Caribbean and North Atlantic (M2) populations. As a result, diversity indices were 0 for most locations, except for Belize and Panamá, where three divergent haplotypes occurred at low frequencies.

Table 2.1. **Molecular diversity indices, tests of neutrality and exact tests of heterozygote deficit (HD) and heterozygote excess (HE) for 3 loci used in the study. N_i is the number of sampled individuals, N_a is the number of sampled alleles (N_a = $2N_i$ for diploid loci, N_a = N_i for the haploid locus), H is the number of unique haplotypes observed, s is the number of segregating sites, h is the gene diversity, π is the average nucleotide diversity and k is the average number of nucleotide differences. Statistically significant values (α = 0.05) are highlighted in bold. Values of h, π and k are consistently greater for Caribbean and North Atlantic populations and lower in peripheral populations in Brazil and Gulf of Guinea. For the mitochondrial locus, several diversity indices and tests of neutrality have a value of zero because only one haplotype was observed among all sampled individuals for those populations.**

								Neutrality		HWE	
					b-tubulin 1 = 961 bp						
	N_i	N_a	H	s	h	π	k	Tajima's D	Fu's Fs	HD p-value	HE p-value
CARIBBEAN											
Belize	39	78	39	49	0.959 ± 0.010	0.009 ± 0.005	8.43 ± 3.94	-0.498	**-14.822**	0.121	0.984
Panama	20	40	24	42	0.963 ± 0.014	0.010 ± 0.005	9.33 ± 4.38	-0.194	-5.475	0.357	0.726
Puerto Rico	33	66	29	43	0.918 ± 0.025	0.009 ± 0.005	8.83 ± 4.13	-0.073	-5.565	0.174	0.854
NORTH ATLANTIC											
Bermuda	12	24	13	29	0.928 ± 0.032	0.008 ± 0.004	7.41 ± 3.59	-0.174	-1.041	1	0.412
BRAZIL											
Abrolhos	10	20	5	16	0.442 ± 0.133	0.002 ± 0.002	2.27 ± 1.30	**-1.842**	1.054	1	0.530
Joao Pessoa	28	56	8	25	0.433 ± 0.081	0.004 ± 0.002	4.04 ± 2.05	-0.832	2.655	**0.014**	0.992
Fortaleza	21	42	9	23	0.489 ± 0.094	0.003 ± 0.002	3.05 ± 1.62	**-1.432**	0.078	0.234	0.878
GULF OF GUINEA											
Sao Tome Is.	26	52	4	8	0.248 ± 0.077	0.001 ± 0.001	0.98 ± 0.67	-1.214	0.846	0.132	0.959

								Neutrality		HWE	
					b-tubulin 2 = 612 bp						
	N_i	N_a	H	s	h	π	k	Tajima's D	Fu's Fs	HD p-value	HE p-value
CARIBBEAN											
Belize	39	78	23	29	0.910 ± 0.017	0.008 ± 0.004	4.78 ± 2.36	-0.659	**-5.454**	0.153	0.811
Panama	18	36	14	19	0.924 ± 0.019	0.008 ± 0.004	4.84 ± 2.42	0.087	-1.871	0.164	0.878
Puerto Rico	36	72	14	24	0.843 ± 0.022	0.008 ± 0.004	4.74 ± 2.35	-0.147	0.053	0.758	0.276
NORTH ATLANTIC											
Bermuda	12	24	12	18	0.917 ± 0.032	0.008 ± 0.005	5.01 ± 2.52	-0.004	-1.786	0.314	0.736
BRAZIL											
Abrolhos	10	20	3	8	0.353 ± 0.123	0.003 ± 0.002	2.05 ± 1.20	-0.438	3.300	1	0.692
Joao Pessoa	29	58	6	11	0.488 ± 0.076	0.003 ± 0.002	1.66 ± 0.99	-1.194	0.687	0.230	0.820
Fortaleza	21	42	5	12	0.446 ± 0.090	0.003 ± 0.002	2.00 ± 1.15	-1.121	1.827	0.098	0.952
GULF OF GUINEA											
Sao Tome Is.	26	52	3	9	0.405 ± 0.072	0.002 ± 0.001	1.20 ± 0.78	-1.100	2.734	**0.013**	0.997

								Neutrality	
					cox1-trnM IGR = 834 bp				
	N_i	N_a	H	s	h	π	k	Tajima's D	Fu's Fs
CARIBBEAN									
Belize	39	39	5	47	0.323 ± 0.093	0.013 ± 0.007	10.8 ± 5.04	-0.087	14.381
Panama	19	19	2	40	0.199 ± 0.112	0.010 ± 0.005	7.95 ± 3.87	-1.229	14.208
Puerto Rico	36	36	1	0	0	0	0	0	0
NORTH ATLANTIC									
Bermuda	12	12	1	0	0	0	0	0	0
BRAZIL									
Abrolhos	10	10	1	0	0	0	0	0	0
Joao Pessoa	29	29	1	0	0	0	0	0	0
Fortaleza	20	20	1	0	0	0	0	0	0
GULF OF GUINEA							0		
Sao Tome Is.	26	26	1	0	0	0	0	0	0

Genetic structure

M. cavernosa exhibited significant structure in populations across the Atlantic Ocean in both nuclear and mitochondrial loci. Significant genetic structure was observed between populations (Φst = 0.196 – 0.371) and between regions (Φct = 0.187 – 0.367) for all three loci (see Table 2). However, no significant genetic structure was found among populations within regions for the two nuclear loci (Φsc = 0 – 0.06), and only a small amount of differentiation was present among populations within regions for the mitochondrial locus (Φsc = 0.070). AMOVA values were similar whether Bermuda was treated as a separate group, or was included in the Caribbean group (Table 2). Variance components and the percentage of variation attributed to each hierarchical level for each locus are also shown in Table 2. Variation within populations accounts for 62.9 – 81.9% of the total observed variation, while variation among populations accounts for 17.04 – 36.72%. Less than 1% of the variation is attributed to variation among populations within regions for every locus, suggesting that populations within regions are similar.

Table 2.2. **Hierarchical analysis of molecular variance (AMOVA) was used to estimate levels of genetic differentiation among populations (F_{st}), between groups of populations or regions (F_{ct}), and between populations within regions (F_{sc}). The variance components and percentage of variance attributed to each hierarchical level considered are shown for each locus. Regions used in the analysis were Brazil (Abrolhos, João Pessoa and Fortaleza), the Gulf of Guinea (São Tomé Island), the Caribbean (Panamá, Belize and Puerto Rico) and the North Atlantic (Bermuda). A second scenario was tested where the Caribbean and North Atlantic groups were merged to form a single region (greater Caribbean).**

A. AMOVA results, Bermuda is treated as separate group

| | Mitochondrial DNA | | Nuclear DNA | | | |
| **Locus** | cox1-trnM IGR | | b-tubulin 1 | | b-tubulin 2 | |
	Variance components	% of variation	Variance components	% of variation	Variance components	% of variation
Within populations	1.517	77.06	2.969	81.90	1.69	65.18
Among populations	0.335	17.04	0.658	18.16	0.90	34.52
Among populations within regions	0.116	5.90	-0.002	-0.06	0.01	0.30

Fixation indices

Φ_{st}	**0.229**	**0.181**	**0.348**
Φ_{ct}	**0.170**	**0.182**	**0.345**
Φ_{sc}	**0.071**	-0.001	0.005

B. AMOVA results, Bermuda included in Caribbean group

| | Mitochondrial DNA | | Nuclear DNA | | | |
| **Locus** | cox1-trnM IGR | | b-tubulin 1 | | b-tubulin 2 | |
	Variance components	% of variation	Variance components	% of variation	Variance components	% of variation
Within populations	1.517	75.64	2.969	80.38	1.692	62.92
Among populations	0.375	18.68	0.724	19.59	0.987	36.72
Among populations within regions	0.114	5.68	0.001	0.03	0.010	0.37

Fixation indices

Φ_{st}	**0.244**	**0.196**	**0.371**
Φ_{ct}	**0.187**	**0.196**	**0.367**
Φ_{sc}	**0.070**	0.000	0.006

Pairwise ϕst values for the three markers are shown in Table 3. Over all three markers, there was no significant differentiation among populations from Belize, Panamá, Puerto Rico and Bermuda. Likewise, no significant differentiation was found among populations along the coast of Brazil (Abrolhos, João Pessoa and Fortaleza). In contrast, populations from Brazil and the Gulf of Guinea showed significant differentiation against all other sites, except in the mitochondrial marker, where there was no differentiation between Brazil and the Gulf of Guinea, because the same haplotype (M1) was found in all individuals for the two regions. For the two nuclear loci, differentiation between Caribbean and Brazilian populations was moderate, ranging from 0.060 to 0.164 in *βtub1* and from 0.127 to 0.294 in *βtub2*. Strong differentiation was observed between the Gulf of Guinea and all others populations. In particular, ϕst values were greatest between the Gulf of Guinea and the Brazilian populations (0.458 – 0.595 in *βtub1* and 0.755 – 0.780 in *βtub2*).

DISCUSSION

Divergent mitochondrial haplotypes

The presence of three rare and divergent haplotypes in the *cox1-trnM* IGR locus is unexpected given the low intraspecific variation observed in the mitochondrial genome of corals (Shearer *et al.* 2002). Several tests were conducted to determine the origins of these haplotypes, but a conclusive source was not found.

Although the levels of divergence found in the mitochondrial locus may suggest the presence of a cryptic species, little or no divergence compared to typical

Table 2.3. **Pairwise Φst for each of the three sampled loci. Statistically significant values (α = 0.05) are highlighted in bold.**

Population	Nuclear DNA *b-tubulin 1*							
	1	2	3	4	5	6	7	8
1 Abrolhos	0.000							
2 Joao Pessoa	0.000	0.000						
3 Fortaleza	-0.017	-0.010	0.000					
4 Belize	**0.104**	**0.084**	**0.109**	0.000				
5 Bermuda	**0.083**	**0.060**	**0.089**	-0.010	0.000			
6 Panama	**0.113**	**0.102**	**0.129**	-0.004	-0.005	0.000		
7 Puerto Rico	**0.156**	**0.137**	**0.164**	0.000	0.007	0.008	0.000	
8 Gulf of Guinea	**0.595**	**0.458**	**0.514**	**0.303**	**0.423**	**0.339**	**0.343**	0.000

Population	Nuclear DNA *b-tubulin 2*							
	1	2	3	4	5	6	7	8
1 Abrolhos	0.000							
2 Joao Pessoa	0.000	0.000						
3 Fortaleza	-0.020	-0.016	0.000					
4 Belize	**0.165**	**0.221**	**0.189**	0.000				
5 Bermuda	**0.127**	**0.218**	**0.170**	-0.011	0.000			
6 Panama	**0.129**	**0.182**	**0.147**	-0.001	-0.007	0.000		
7 Puerto Rico	**0.236**	**0.294**	**0.257**	0.008	0.021	0.020	0.000	
8 Gulf of Guinea	**0.755**	**0.780**	**0.757**	**0.427**	**0.514**	**0.547**	**0.444**	0.000

Population	Mitochondrial DNA *cox1-trnM IGR*							
	1	2	3	4	5	6	7	8
1 Abrolhos	0							
2 Joao Pessoa	0	0						
3 Fortaleza	0	0	0					
4 Belize	0.106	**0.183**	**0.154**	0				
5 Bermuda	**1**	**1**	**1**	0.049	0			
6 Panama	0.135	**0.261**	**0.211**	-0.031	0.021	0		
7 Puerto Rico	**1**	**1**	**1**	**0.117**	0	0.110	0	
8 Gulf of Guinea	0	0	0	**0.174**	**1**	**0.245**	**1**	0

M. cavernosa alleles is observed in these individuals at the nuclear loci (0 – 0.15% divergence for both nuclear loci). Lineage sorting in the mitochondrial genome is expected to be 4 times greater than at nuclear loci because of its haploid state and maternal mode of inheritance. Therefore, the 4.8% divergence observed in *cox1-trnM IGR* should be accompanied by ~1.2% divergence at the nuclear loci, but this is not observed in *β-tub1* or *β-tub2*. The nuclear data presented here thus do not support the presence of a cryptic species.

Introgression from another species also appears unlikely. Recent phylogenetic revisions suggest that *M. cavernosa* does not appear to have any close relatives in the Caribbean, its most similar association being *Diploastrea heliopora* (Fukami *et al.* 2008; Fukami *et al.* 2004b), a species found exclusively in the Pacific. *M. cavernosa* and *Montastraea annularis* are divergent at various loci, including the exon of *β-tubulin* (Fukami *et al.* 2008), and most likely do not represent congeneric species. Nevertheless, sequences of *cox1-trnM IGR* from *M. annularis* were compared to typical and divergent haplotypes of *M. cavernosa* to determine if this species could be the source of introgressed alleles. The sequence alignment was very poor owing to multiple indels and numerous substitutions, indicating that the divergent haplotypes in *M. cavernosa* are more similar to typical *M. cavernosa* haplotypes than to *M. annularis*. Sequencing of the adjacent *cox1* gene revealed that individuals with divergent *cox1-trnM IGR* had identical *cox1* to typical individuals of *M. cavernosa.* Given there is no recombination in the mitochondrial genome, the absence of *cox1*

from another species in individuals containing the divergent haplotypes conflicts with an introgression hypothesis.

Amplification of a gene duplication or transfer of a mitochondrial gene to the nuclear genome (pseudogenes) can in some cases be detected by sequencing multiple clones of an amplicon. This test did not reveal any evidence of a pseudogene or duplication of this locus (see Results). Because this is a non-coding region, tests for differences in selective constraints between a gene and its pseudogene (for example, comparing synonymous to non-synonymous substitution rates) could not be conducted. Finally, it is unlikely that these divergent haplotypes have been amplified from contaminants. The primers used for amplification were developed using coral mitochondrial genome sequences (Fukami *et al.* 2004a) and should be specific to corals. Indeed, the most similar sequences resulting from a BLAST search belong to other coral species (although genetic differences are so great from any other sequence found on Genbank, query coverage was only <7%). In addition, independent samples from Belize and Panamá taken on separate sampling expeditions and by different scientists yielded identical haplotypes in six individuals (M5). Although the possibility of a contaminant cannot be eliminated, it appears to be unlikely.

The inclusion of divergent mitochondrial haplotypes in the analysis does not greatly alter the interpretation of the patterns of genetic diversity and connectivity. Differentiation between Caribbean and the South Atlantic is supported whether or not the divergent haplotypes are included. In terms of genetic diversity, however, exclusion of the divergent haplotypes would reduce molecular diversity indices in all

populations to zero. Such low levels of variation render this locus effectively uninformative, and patterns of genetic diversity would then primarily rely on the nuclear loci.

Gene flow between regions of the Atlantic

This is the first study to look at differentiation among coral populations spanning both sides of the Atlantic. The nuclear data reveal significant structure among populations of *M. cavernosa* spanning three regions separated by 4,000 - 10,000 km in the Atlantic Ocean – the Caribbean, Brazil and Eastern Atlantic. The mitochondrial locus supports a Caribbean and South Atlantic split, but the locus is invariant in Brazilian and African populations (all individuals carry a M1 haplotype). As a result, the mitochondrial locus is not informative regarding gene flow among populations of Brazil and West Africa. These data suggest that there are significant barriers to gene flow for this species across its distributional range, perhaps due to the freshwater plume of the Amazon and Orinoco Rivers and the vast expanses of open-ocean separating areas with suitable habitat. Given that *M. cavernosa* appears to be able to maintain gene flow over greater distances within the Caribbean than other corals of this region (see following section), these barriers may be effective for Atlantic corals in general, although further studies are needed to test this prediction.

Restricted gene flow between broad regions in the Atlantic has also been observed in other marine species. Strong differentiation was found between Caribbean and Brazilian populations of the sea urchin *Tripneustes ventricosus* (Lessios *et al.*

2003) ($F_{ST} = 0.73 - 0.82$), with multiple diagnostic mutations separating the eastern and western Atlantic populations. In other cases, differentiation among regions is so strong that species that were once thought to be amphi-Atlantic, such as the sponge *Chondrilla nucula* (Klautau *et al.* 1999) and the blenny *Ophioblennius atlanticus* (Muss *et al.* 2001), are currently being reconsidered as allopatric cryptic species.

Nevertheless, barriers of dispersal are not equally effective for all Atlantic reef organisms, even within related groups. In the sea urchins *Eucidaris tribuloides* and *Diadema antillarium*, no significant differentiation was found between populations in the Caribbean and Brazil, but moderate to strong differentiation was observed between East and West Atlantic populations (Lessios *et al.* 2001; Lessios *et al.* 1999). The opposite trend, however, was found for *Echinometra lucunter* (McCartney *et al.* 2000). Likewise, levels of connectivity in sponges across the Atlantic are variable (Worheide *et al.* 2005). Reef-associated fish can be either panmictic, highly differentiated across their range, or show some intermediate level of connectivity. In certain species, the Amazon freshwater outflow appears to be the strongest barrier to gene flow (*Acanthurus bahianus* (Rocha *et al.* 2002)), whereas the large distances separating the Eastern and Western Atlantic appear to play a more critical role for other species, such as the goldspot goby, (Rocha *et al.* 2005). Overall, pelagic larval duration does not appear to be a good predictor for patterns of differentiation in reef fish (Bowen *et al.* 2006). Rather, it appears that ecology, such as adult habitat preferences (Rocha *et al.* 2002), and historical events play a more important role in determining patterns of connectivity in Atlantic reef fish. The incongruent patterns

observed among sea urchin species also do not correlate with dispersal potential, since four of the studied species have similar larval duration but different levels of genetic differentiation across the Atlantic.

Gene flow within regions of the Atlantic

In contrast to high levels of differentiation observed in *M. cavernosa* between regions of the Atlantic, high levels of gene flow are observed **within** those regions, even at large spatial scales. Notably, the oceanographic isolation of Bermuda from the Caribbean does not appear to have resulted in significant differentiation in populations of *M. cavernosa*. Similarly, there do not appear to be significant barriers to gene flow among three *M. cavernosa* populations spanning 2,000 km of coast in Brazil.

Our findings of low population differentiation within the Caribbean are in agreement with results for *Montastraea faveolata* (Severance& Karl 2006), where little differentiation was observed between populations spanning as much as 2,000 km, but differ from several other studies that have found significant structure among Caribbean coral populations. Differentiation between eastern and western Caribbean corals has been found in *Acropora palmata* (Baums *et al.* 2005b) and *Acropora cervicornis* (Vollmer& Palumbi 2007), and fine scale intraspecific differentiation has also been observed in *Agaricia agaricites* (Brazeau *et al.* 2005) and in some members of the *Montastraea annularis* complex (Severance& Karl 2006). The discrepancy between our findings and previous work on Caribbean corals may in part be a result of limited sampling of populations of *M. cavernosa* within the Caribbean. Our only

sampling location in the eastern Caribbean was Puerto Rico. In the case of *Acropora palmata*, the Puerto Rico population was an intermediate between the eastern and western Caribbean (Baums *et al.* 2005b). However, the finding that Bermuda was not differentiated with respect to the three Caribbean populations suggests that *M. cavernosa* may be able to disperse more widely than other Caribbean corals.

Much like the Caribbean, there appears to be continued gene flow between populations of *M. cavernosa* along the coast of Brazil. This suggests that although the Amazon and Orinoco Rivers pose a major barrier to gene flow between Brazil and the Caribbean, other large rivers within Brazil, such as the São Francisco River, are not impassable for larvae of *M. cavernosa*. High levels of gene flow have also been inferred for populations of other marine invertebrates in Brazil such as the urchin *Diadema antillarium* (Lessios *et al.* 2001) and the shrimps *Farfanteperanaeus brasiliensis* and *Litopenaeus schmitti* (Gusmão *et al.* 2005). However, not all marine invertebrates are capable of dispersing as widely along the Brazilian coast. Populations of both *Siderastrea stellata* and *Siderastrea radians* were found to have high levels of gene flow along the northeast coast (spanning 350 km), but displayed distinct differences between several northeast and southeast populations (separated by a minimum of 1,200 km) (Neves *et al.* 2008). Greater levels of differentiation in these two corals species compared to *M. cavernosa* may result from their different reproductive modes; *M. cavernosa* is a broadcast spawner, whereas *S. stellata* and *S. radians* brood larvae that are typically ready to settle within 48 hours (Neves *et al.* 2008). Lower dispersal capability has also been observed in several species of shrimp

(Gusmão *et al.* 2006; Maggioni *et al.* 2003), oysters (Lazoski 2004) and reef fish (Freitas *et al.* 2003), with significant structure being found along the coast of Brazil. In sum, *M. cavernosa* appears to be able to disperse more widely than several other marine invertebrates along the same stretch of Brazilian coastline, in agreement with our findings for the Caribbean-North American populations.

Levels of genetic diversity

Genetic diversity, measured by haplotype diversity (h), nucleotide diversity (π) and average nucleotide changes (k), was found to be greater in the Panamá, Belize, Puerto Rico and Bermuda, relative to the peripheral populations in Brazil and the eastern Atlantic (see Table 1). Reduced genetic diversity in a peripheral population can result from a small founding population or from small effective size due to genetic drift or inbreeding depression. A combination of these factors is likely at work in the isolated coral populations of *M. cavernosa* in Brazil and the Gulf of Guinea (which has no known neighboring populations along the African mainland and consistently showed the lowest levels of genetic diversity). Evidence for low genetic diversity in isolated, peripheral populations of corals occurs elsewhere. For example, on Lord Howe Island, the southernmost reef in the Pacific located 700 km from the Great Barrier Reef, four of five studied species, which included both brooders and broadcasters with widely varying geographic ranges, showed reduced genetic diversity (Ayre& Hughes 2004). Nevertheless, the high genetic diversity observed for corals in

Bermuda shows that geographic distance alone does not automatically result in isolation and loss of variability.

Depressed levels of genetic diversity can have significant fitness effects resulting in loss of resilience for marine populations. For example, grass beds consisting of single genotypes of the eelgrass *Zostera marina* experienced greater mortality and slower growth rates during an episode of intense heat and supported a less diverse associated invertebrate fauna compared to those composed of multiple genotypes (Reusch *et al.* 2005). These findings suggest that genetic diversity may contribute to a species' ability to cope with disturbances, and that the genetic diversity of foundation species, like the eelgrass, may have broad reaching impacts that effect the entire ecosystem, not just the species itself.

The effects of reduced genetic diversity on the resilience of coral populations remain untested, but other evidence suggests that there may be benefits to genetic diversity. A recent study has found that 3 out of 49 surveyed genotypes of the coral *Acropora cervicornis* were resistant to white band disease in Panamaá (Vollmer & Kline 2008). Similarly, *Montastraea annularis* is sometimes able to sustain only partial bleaching because it harbors a diversity of zooxanthellae strains (Rowan *et al.* 1997), some of which have increased thermal tolerance (Baker 2004; Tchernov *et al.* 2004; van Oppen *et al.* 2009), improving its ability to survive under stressful conditions. Thus the lack of diversity in peripheral coral populations may suggest that these populations will be less resilient to future episodes of environmental or

anthropogenic stress, because they lack the full spectrum of genotypes, some of which may fare better under disturbance.

Although isolated coral populations in the southern and eastern Atlantic demonstrate lower levels of genetic diversity, this does not mean they are evolutionary dead ends that do not merit the attention of conservation efforts. On the contrary, reduced gene flow combined with a set of different selective pressures in peripheral populations may play a role in maintaining and generating biological diversity, via local adaptation or allopatric speciation (Carson & Templeton 1984; Templeton 1980). In Brazil, coral populations are exposed to a different set of stressors than in the Caribbean. High rates of sedimentation from the numerous rivers that discharge on the coast, compounded with high winds that fall upon the nearshore year-round, means that the clear calm waters which are typical of the Caribbean Sea are rarely found in Brazil. Different environmental conditions, combined with restricted gene flow may lead to divergence between regions that can induce local adaptation and given sufficient time, allopatric speciation. Divergence between the Caribbean coral *Favia fragum* and the South Atlantic endemic *Favia gravida* (Nunes *et al.* 2008) suggests that peripheral coral populations can be the site of origination of new species. Therefore, conservation efforts in these regions should not be ignored.

Different environmental regimes in the South Atlantic may favor the selection of resistant genotypes to the stressful environments of the peripheral populations. However, a genotype resistant to a particular set of environmental conditions may no longer be favored following erratic changes in climate or environment. In a diverse

population, new genotypes may be favored and increase in frequency, but in peripheral populations where only a handful of genotypes exist, drastic environmental change could have devastating results.

The vulnerability of isolated peripheral populations highlights the need for increased awareness and immediate action for conservation in these regions. Both in Brazil and on the islands of the São Tomé and Príncipe, a large proportion of the population depends on reef resources (Pikitch& Doukakis 2005). Reefs in Brazil are also highly threatened by coral bleaching (Castro & Pires 1999; Migotto 1997), coral disease (Francini *et al.* 2008), nutrification of coastal environments (Costa 2007; Costa *et al.* 2000; Costa *et al.* 2008), competition with aggressive exotic species (Creed 2006; Paula& Creed 2004, 2005) and other human impacts (Leão& Kikuchi 2005; Leão *et al.* 1994). But due to difficult access and lower research effort in these areas, conservation in these regions often fall by the wayside. Funding for continued research and conservation in these areas are urgently needed.

Chapter II, in full, has been submitted and accepted for the publication of the material as it may appear in *Molecular Ecology*, 2009, Nunes, Flavia; Norris, Richard D.; Knowlton, Nancy, Wiley-Blackwell Publishing, Inc. 2009. The dissertation author was the primary investigator and author of this paper.

REFERENCES

Acosta A, Zea S (1997) Sexual reproduction of the reef coral <u>Montastraea cavernosa</u> (Scleractinia: Faviidae) in the Santa Marta area, Caribbean coast of Colombia. *Marine Biology* **128**, 141-148.

Altschul SF, Madden TL, Schaffer AA*, et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**, 3389-3402.

Ayre DJ, Dufty S (1994) Evidence for restricted gene flow in the viviparous coral <u>Seriatopora hystrix</u> on Australia's Great Barrier Reef. *Evolution* **48**, 1183-1201.

Ayre DJ, Hughes TP (2004) Climate change, genotypic diversity and gene flow in reef-building corals. *Ecology Letters* **7**, 273-278.

Baker AC (2004) Symbiont diversity on coral reefs and its relationship to bleaching resistance and resilience. In: *Coral health and disease.* (eds. Rosenberg E, Loya Y), pp. 177-194.

Baums IB, Hughes CR, Hellberg ME (2005a) Mendelian microsatellite loci for the Caribbean coral <u>Acropora palmata</u>. *Marine Ecology Progress Series* **288**, 115-127.

Baums IB, Miller MW, Hellberg ME (2005b) Regionally isolated populations of an imperiled Caribbean coral, <u>Acropora palmata</u>. *Molecular Ecology* **14**, 1377-1390.

Bowen BW, Bass AL, Muss A, Carlin JL, Robertson DR (2006) Phylogeography of two Atlantic squirrelfishes (Family Holocentridae): exploring links between pelagic larval duration and population connectivity. *Marine Biology* **149**, 899-913.

Brazeau DA, Sammarco PW, Gleason DF (2005) A multi-locus genetic assignment technique to assess sources of <u>Agaricia agaricites</u> larvae on coral reefs. *Marine Biology* **147**, 1141-1148.

Carson HL, Templeton AR (1984) Genetic revolutions in relation to speciation phenomena the founding of new populations. *Johnston, R. F. (Ed.). Annual Review of Ecology and Systematics, Vol. 15. Xiv+601p. Annual Reviews, Inc.: Palo Alto, Calif., USA. Illus*, 97-132.

Castro CB, Pires DO (1999) A bleaching event on a Brazilian coral reef. *Revista brasileira de oceanografia* **47**, 97-90.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**, 1657-1659.

Costa OS (2007) Anthropogenic nutrient pollution of coral reefs in Southern Bahia, Brazil. *Brazilian Journal of Oceanography* **55**, 265-279.

Costa OS, Leão ZMAN, Nimmo M, Attrill MJ (2000) Nutrification impacts on coral reefs from northern Bahia, Brazil. *Hydrobiologia* **440**, 307-315.

Costa OS, Nimmo M, Attrill MJ (2008) Coastal nutritication in Brazil: A review of the role of nutrient excess on coral reef demise. *Journal of South American Earth Sciences* **25**, 257-270.

Cowen RK, Lwiza KMM, Sponaugle S, Paris CB, Olson DB (2000) Connectivity of marine populations: Open or closed? *Science* **287**, 857-859.

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

Creed JC (2006) Two invasive alien azooxanthellate corals, <u>Tubastraea coccinea</u> and <u>Tubastraea tagusensis</u>, dominate the native zooxanthellate <u>Mussismilia hispida</u> in Brazil. *Coral Reefs* **25**, 350-350.

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Molecular Ecology* **17**, 1170-1188

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**.

Floeter SR, Rocha LA, Robertson DR*, et al.* (2008) Atlantic reef fish biogeography and evolution. *Journal of Biogeography* **35**, 22-47.

Francini RB, Moura RL, Thompson FL*, et al.* (2008) Diseases leading to accelerated decline of reef corals in the largest South Atlantic reef complex (Abrolhos Bank, eastern Brazil). *Marine Pollution Bulletin* **56**, 1008-1014.

Freitas JEP, Araújo ME, Solé-Cava AM (2003) Estruturação genética das populações de duas espécies de peixes recifais do Atol das Rocas e da costa do Ceará. *Tropical Oceanography, Recife* **31**, 193-201.

Fukami H, Budd AF, Levitan DR*, et al.* (2004a) Geographic differences in species boundaries among members of the Montastraea annularis complex based on molecular and morphological markers. *Evolution* **58**, 324-337.

Fukami H, Budd AF, Paulay G*, et al.* (2004b) Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. *Nature* **427**, 832-835.

Fukami H, Chen CA, Budd AF*, et al.* (2008) Mitchondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (Order Scleractinia, Class Anthozoa, Phylum Cnidaria). *PLoS One* **3**, e3222.

Fukami H, Knowlton N (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.

Goffredo S, Zaccanti F (2004) Laboratory observations of larval behavior and metamorphosis in the mediterranean solitary coral Balanophyllia europaea (Scleractinia, Dendrophylliidae). *Bulletin of Marine Science* **74**, 449-457.

Graham EM, Baird AH, Connolly SR (2008) Survival dynamics of scleractinian coral larvae and implications for dispersal. *Coral Reefs* **27**, 529-539.

Gusmão J, Lazoski C, Monteiro FA, Sole-Cava AM (2006) Cryptic species and population structuring of the Atlantic and Pacific seabob shrimp species, Xiphopenaeus kroyeri and Xiphopenaeus riveti. *Marine Biology* **149**, 491-502.

Gusmão J, Lazoski C, Sole-Cava AM (2005) Population genetic structure of Brazilian shrimp species (Farfantepenaeus sp., F. brasiliensis, F. paulensis and

<u>Litopenaeus schmitti</u>: Decapoda : Penaeidae). *Genetics and Molecular Biology* **28**, 165-171.

Harii S, Kayanne H, Takigawa H, Hayashibara T, Yamamoto M (2002) Larval survivorship, competency periods and settlement of two brooding corals, <u>Heliopora coerulea</u> and <u>Pocillopora damicornis</u>. *Marine Biology* **141**, 39-46.

Hellberg ME (2006) No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. *Bmc Evolutionary Biology* **6**, 24.

Isomura N, Nishihira M (2001) Size variation of planulae and its effect on the lifetime of planulae in three pocilloporid corals. *Coral Reefs* **20**, 309-315.

James MK, Armsworth PR, Mason LB, Bode L (2002) The structure of reef fish metapopulations: modeling larval dispersal and retention patterns. *Proceedings of the Royal Society of London Series B-Biological Sciences* **269**, 2079-2086.

Jones PJ (1994) Biodiversity in the Gulf of Guinea - an Overview. *Biodiversity and Conservation* **3**, 772-784.

Klautau M, Russo CAM, Lazoski C*, et al.* (1999) Does cosmopolitanism result from overconservative systematics? A case study using the marine sponge <u>Chondrilla nucula</u>. *Evolution* **53**, 1414-1422.

Laborel J (1969a) *Les peuplements de madreporaires des cotes tropicales du Bresil*, Universite d'Abidjan.

Laborel J (1969b) Madreporaires et hydrocoralliaires recifaux des cotes bresiliennes. Systematique, ecologie, repartition verticale et geographique. *Annales De L Institut Oceanographique de Paris* **47**, 171-229.

Laborel J (1974) West african reefs corals: an hypothesis on their origin. *Proceedings of the 2nd International Coral Reef Symposium* **1**, 425-443.

Lazoski CVdS (2004) Sistemática molecular e genética populacional de ostras brasileiras (<u>Crassostrea</u> spp.). *Universidade Federal do Rio de Janeiro*.

Leão ZMAN, Kikuchi RKP (2005) A relic coral fauna threatened by global changes and human activities, Eastern Brazil. *Marine Pollution Bulletin* **51**, 599-611.

Leão ZMAN, Telles MD, Sforza R, Bulhoes HA, Kikuchi RKP (1994) Impact of tourism development on the coral reefs of the Abrolhos area, Brazil. In: *Proceedings of the Colloquium on Global Aspects of Coral Reefs: Health, Hazards and History* (ed. Ginsburg RN), pp. 254-260.

Lessios HA, Kane J, Robertson DR (2003) Phylogeography of the pantropical sea urchin Tripneustes: Contrasting patterns of population structure between oceans. *Evolution* **57**, 2026-2036.

Lessios HA, Kessing BD, Pearse JS (2001) Population structure and speciation in tropical seas: Global phylogeography of the sea urchin Diadema. *Evolution* **55**, 955-975.

Lessios HA, Kessing BD, Robertson DR, Paulay G (1999) Phylogeography of the pantropical sea urchin Eucidaris in relation to land barriers and ocean currents. *Evolution* **53**, 806-817.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451-1452.

Lopez JV, Knowlton N (1997) Discrimination of species in the Montastraea annularis complex using multiple genetic loci. *Proceedings of the 8th International Coral Reef Symposium* **2**, 1613-1618.

Maggioni R, Rogers AD, Maclean N (2003) Population structure of Litopenaeus schmitti (Decapoda : Penaeidae) from the Brazilian coast identified using six polymorphic microsatellite loci. *Molecular Ecology* **12**, 3213-3217.

McCartney MA, Keller G, Lessios HA (2000) Dispersal barriers in tropical oceans and speciation in Atlantic and eastern Pacific sea urchins of the genus Echinometra. *Molecular Ecology* **9**, 1391-1400.

Migotto AE (1997) Anthozoan bleaching on the southeastern coast of Brazil in the summer of 1994. *Proceedings of the 6th International Conference on Coelenterate Biology, 1995*, 329-335.

Moura RL, Rodrigues MCM, Francini-Filho RB, Sazima I (1999) Unexpected richness of reef corals near the southern Amazon River mouth. *Coral Reefs* **18**, 170.

Muss A, Robertson DR, Stepien CA, Wirtz P, Bowen BW (2001) Phylogeography of Ophioblennius: the role of ocean currents and geography in reef fish evolution. *Evolution* **55**, 561-572.

Neves EG, Andrade SCS, da Silveira FL, Solferini VN (2008) Genetic variation and population structuring in two brooding coral species (Siderastrea stellata and Siderastrea radians) from Brazil. *Genetica* **132**, 243-254.

Nishikawa A, Sakai K (2005) Settlement-competency period of planulae and genetic differentiation of the scleractinian coral Acropora digitifera. *Zoological Science* **22**, 391-399.

Nunes F, Fukami H, Vollmer SV, Norris RD, Knowlton N (2008) Re-evaluation of the systematics of the endemic corals of Brazil by molecular data. *Coral Reefs* **27**, 423-432.

Paula AF, Creed JC (2004) Two species of the coral Tubastraea (cnidaria, Scleractinia) in Brazil: A case of accidental introduction. *Bulletin of Marine Science* **74**, 175-183.

Paula AF, Creed JC (2005) Spatial distribution and abundance of nonindigenous coral genus Tubastraea (Cnidaria, Scleractinia) around Ilha Grande, Brazil. *Brazilian Journal of Biology* **65**, 661-675.

Pikitch EK, Doukakis P (2005) Recommendations for fisheries reform for São Tomé and Príncipe. *Commissioned by The Earth Institute at Columbia University for the São Tomé e Príncipe Advisory Project on Sustainable Development Strategies.*

Pratchett MS, Gust N, Goby G, Klanten SO (2001) Consumption of coral propagules represents a significant trophic link between corals and reef fish. *Coral Reefs* **20**, 13-17.

Raimondi PT, Morse ANC (2000) The consequences of complex larval behavior in a coral. *Ecology* **81**, 3193-3211.

Raymond M, Rousset F (1995) Genepop (Version-1.2) – Population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248-249.

Reusch TBH, Ehlers A, Hammerli A, Worm B (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 2826-2831.

Richmond RH (1987) Energetics, competence, and long-distance dispersal of planula larvae of the coral Pocillopora damicornis. *Marine Biology* **93**, 527-533.

Rios EC (1994) *Seashells of Brazil*. Rio Grande, Brazil, 368 p.

Rocha LA, Bass AL, Robertson DR, Bowen BW (2002) Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei : Acanthuridae). *Molecular Ecology* **11**, 243-252.

Rocha LA, Robertson DR, Rocha CR*, et al.* (2005) Recent invasion of the tropical Atlantic by an Indo-Pacific coral reef fish. *Molecular Ecology* **14**, 3921-3928.

Rosenberg G (1993) A database approach to studies of molluscan taxonomy, biogeography and diversity, with examples from Western Atlantic marine gastropods. *American Malacological Bulletin* **10**, 257-266.

Rowan R, Knowlton N, Baker A, Jara J (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. *Nature* **388**, 265-269

Severance EG, Karl SA (2006) Contrasting population genetic structures of sympatric, mass-spawning Caribbean corals. *Marine Biology* **150**, 57-68.

Severance EG, Szmant AM, Karl SA (2004) Microsatellite loci isolated from the Caribbean coral, Montastraea annularis. *Molecular Ecology Notes* **4**, 74-76.

Shearer TL, Coffroth MA (2004) Isolation of microsatellite loci from the scleractinian corals, Montastraea cavemosa and Porites astreoides. *Molecular Ecology Notes* **4**, 435-437.

Shearer TL, Van Oppen MJH, Romano SL, Worheide G (2002) Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology* **11**, 2475-2487.

Stephens M, Scheet P (2005) Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. *American Journal of Human Genetics* **76**, 449-462.

Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics* **68**, 978-989.

Stoddart JA (1984) Genetic differentiation amongst populations of the coral Pocillopora damicornis off Southwestern Australia. *Coral Reefs* **3**, 149-156.

Szmant AM (1991) Sexual reproduction by the Caribbean reef corals Montastrea annularis and M. cavernosa. *Marine Ecology-Progress Series* **74**, 13-25.

Tchernov D, Gorbunov MY, de Vargas C*, et al.* (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 13531-13535.

Templeton AR (1980) The theory of speciation via the founder principle. *Genetics* **94**, 1011-1038.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**, 4876-4882.

van Oppen MJH, Baker AC, Coffroth MA, Willis BL (2009) Bleaching resistance and the role of algal endosymbionts. In: *Coral Bleaching: patterns, processes, causes and consequences* (eds. van Oppen MJH, Lough JM), pp. 83-102. Springer-Verlag Berlin Heidelberg, New York, Berlin.

Vermeij MJA, Fogarty ND, Miller MW (2006) Pelagic conditions affect larval behavior, survival, and settlement patterns in the Caribbean coral Montastraea faveolata. *Marine Ecology-Progress Series* **310**, 119-128.

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

Vollmer SV, Palumbi SR (2007) Restricted gene flow in the Caribbean staghorn coral Acropora cervicornis: Implications for the recovery of endangered reefs. *Journal of Heredity* **98**, 40-50.

Worheide G, Sole-Cava AM, Hooper JNA (2005) Biodiversity, molecular ecology and phylogeography of marine sponges: patterns, implications and outlooks. *Integrative and Comparative Biology* **45**, 377-385.

SUPPLEMENTARY TABLES

Supplementary Table 2.1. Haplotype frequencies per locus for each population.

	BRAZIL			CARIBBEAN			NORTH ATLANTIC	WEST AFRICA
Haplotype	Abrolhos	João Pessoa	Fortaleza	Belize	Panama	Puerto Rico	Bermuda	São Tomé
b-tubulin 1								
J01	15	42	30	8	3	0	2	0
J02	2	1	1	0	0	0	0	2
J03	1	0	0	1	0	0	0	0
J04	1	0	0	0	0	0	0	0
J05	1	2	2	0	0	0	0	45
J06	0	5	3	0	0	0	0	0
J07	0	2	2	0	1	5	1	0
J08	0	1	1	0	0	0	0	0
J09	0	2	0	2	0	3	2	0
J10	0	1	0	0	0	0	0	1
J11	0	0	1	0	0	0	0	0
J12	0	0	1	9	4	2	2	0
J13	0	0	1	1	1	0	1	0
J14	0	0	0	5	1	1	0	0
J15	0	0	0	8	0	17	5	0
J16	0	0	0	1	2	2	0	0
J17	0	0	0	1	0	0	0	0
J18	0	0	0	1	0	0	0	0
J19	0	0	0	1	0	0	0	0
J20	0	0	0	1	0	0	0	0
J21	0	0	0	3	2	2	0	0
J22	0	0	0	1	0	0	0	0
J23	0	0	0	1	0	0	0	0
J24	0	0	0	1	1	2	1	0
J25	0	0	0	2	2	1	2	0
J26	0	0	0	3	1	1	0	0
J27	0	0	0	1	0	0	0	0
J28	0	0	0	5	5	5	4	0
J29	0	0	0	1	0	0	0	0
J30	0	0	0	2	1	0	0	0
J31	0	0	0	1	0	0	0	0
J32	0	0	0	2	4	2	0	0
J33	0	0	0	1	0	0	0	0
J34	0	0	0	1	0	0	0	0
J35	0	0	0	1	0	0	0	0
J36	0	0	0	1	0	0	0	0
J37	0	0	0	1	0	0	0	0
J38	0	0	0	1	0	0	0	0

Supplementary Table 2.1 (continued)

	BRAZIL			CARIBBEAN			NORTH ATLANTIC	WEST AFRICA
Haplotype	Abrolhos	João Pessoa	Fortaleza	Belize	Panama	Puerto Rico	Bermuda	São Tomé
J39	0	0	0	1	0	0	0	0
J40	0	0	0	1	0	0	0	0
J41	0	0	0	1	0	0	0	0
J42	0	0	0	1	1	1	0	0
J43	0	0	0	2	0	0	0	0
J44	0	0	0	1	0	0	0	0
J45	0	0	0	1	0	0	0	0
J46	0	0	0	1	0	0	0	0
J47	0	0	0	1	1	5	0	0
J48	0	0	0	0	1	0	1	0
J49	0	0	0	0	0	0	1	0
J50	0	0	0	0	0	0	1	0
J51	0	0	0	0	0	0	1	0
J52	0	0	0	0	1	1	0	0
J53	0	0	0	0	1	0	0	0
J54	0	0	0	0	1	0	0	0
J55	0	0	0	0	2	0	0	0
J56	0	0	0	0	1	0	0	0
J57	0	0	0	0	1	0	0	0
J58	0	0	0	0	1	0	0	0
J59	0	0	0	0	1	0	0	0
J60	0	0	0	0	0	1	0	0
J61	0	0	0	0	0	2	0	0
J62	0	0	0	0	0	1	0	0
J63	0	0	0	0	0	1	0	0
J64	0	0	0	0	0	2	0	0
J65	0	0	0	0	0	1	0	0
J66	0	0	0	0	0	1	0	0
J67	0	0	0	0	0	1	0	0
J68	0	0	0	0	0	1	0	0
J69	0	0	0	0	0	1	0	0
J70	0	0	0	0	0	1	0	0
J71	0	0	0	0	0	1	0	0
J72	0	0	0	0	0	1	0	0
J73	0	0	0	0	0	1	0	0
J74	0	0	0	0	0	0	0	4
Total	20	56	42	78	40	66	24	52

Supplementary Table 2.1 (continued)

	BRAZIL			CARIBBEAN			NORTH ATLANTIC	WEST AFRICA
Haplotype	Abrolhos	João Pessoa	Fortaleza	Belize	Panama	Puerto Rico	Bermuda	São Tomé
b-tubulin 2								
L01	3	4	3	13	6	7	2	39
L02	16	41	31	8	5	0	3	0
L03	1	6	4	0	0	0	0	0
L04	0	3	3	4	3	19	1	3
L05	0	1	0	0	0	0	0	0
L06	0	3	0	0	0	0	0	0
L07	0	0	1	0	0	0	0	0
L08	0	0	0	1	0	0	0	0
L09	0	0	0	16	4	9	5	0
L10	0	0	0	5	4	9	4	0
L11	0	0	0	1	0	0	0	0
L12	0	0	0	3	2	2	0	0
L13	0	0	0	1	0	0	0	0
L14	0	0	0	2	0	0	1	0
L15	0	0	0	4	4	1	0	0
L16	0	0	0	6	0	17	3	0
L17	0	0	0	3	2	0	1	0
L18	0	0	0	2	1	0	1	0
L19	0	0	0	1	0	0	0	0
L20	0	0	0	1	0	0	0	0
L21	0	0	0	1	0	0	0	0
L22	0	0	0	1	0	0	1	0
L23	0	0	0	1	0	0	0	0
L24	0	0	0	1	0	0	0	0
L25	0	0	0	1	1	0	0	0
L26	0	0	0	1	0	0	0	0
L27	0	0	0	1	0	0	0	0
L28	0	0	0	0	0	0	1	0
L29	0	0	0	0	0	1	1	0
L30	0	0	0	0	1	0	0	0
L31	0	0	0	0	1	0	0	0
L32	0	0	0	0	1	0	0	0
L33	0	0	0	0	1	0	0	0
L34	0	0	0	0	0	1	0	0
L35	0	0	0	0	0	1	0	0
L36	0	0	0	0	0	2	0	0
L37	0	0	0	0	0	1	0	0
L38	0	0	0	0	0	1	0	0
L39	0	0	0	0	0	1	0	0
L40	0	0	0	0	0	0	0	10
Total	20	58	42	78	36	72	24	52

Supplementary Table 2.1 (continued)

	BRAZIL			CARIBBEAN			NORTH ATLANTIC	WEST AFRICA
Haplotype	Abrolhos	João Pessoa	Fortaleza	Belize	Panama	Puerto Rico	Bermuda	São Tomé
cox1-trnM IGR								
M1	10	29	20	0	0	0	0	26
M2	0	0	0	32	17	36	12	0
M3	0	0	0	1	0	0	0	0
M4	0	0	0	1	0	0	0	0
M5	0	0	0	4	2	0	0	0
M6	0	0	0	1	0	0	0	0
Total	10	29	20	39	19	36	12	26

Supplementary Table 2.2. Genotype over all loci for each sampled individual. Haplotype sequences can be found on Genbank (accession numbers FJ854573-FJ854692).

Abrolhos	*β-tub1*		*β-tub2*		*cox1-trnM*
B009	J01	J02	L01	L02	M1
B010	J01	J01	L02	L02	M1
B011	J01	J01	L02	L02	M1
B034	J01	J01	L02	L02	M1
B035	J01	J02	L01	L02	M1
B065	J01	J03	L02	L03	M1
B066	J01	J01	L02	L02	M1
B076	J01	J01	L02	L02	M1
B077	J01	J04	L02	L02	M1
B078	J01	J05	L01	L02	M1

João Pessoa	*β-tub1*		*β-tub2*		*cox1-trnM*
B154	J01	J01	L02	L02	M1
B158	J01	J01	L02	L02	M1
B166	J01	J01	L02	L02	M1
B168	J01	J01	L02	L02	M1
B171	J01	J01	L02	L02	M1
B195	J01	J01	L02	L02	M1
B201	J01	J01	L02	L02	M1
B204	J01	J01	L02	L02	M1
B212	J06	J07	L03	L04	M1
B213	J06	J02	L01	L02	M1
B214	J08	J06	L02	L03	M1
B215	J01	J01	L02	L02	M1
B216	J01	J06	L02	L05	M1

Supplementary Table 2.2 (continued)

João Pessoa	β-tub1		β-tub2		cox1-trnM
B217	J01	J01	L02	L02	M1
B218	J01	J01	L02	L02	M1
B219	J01	J01	L02	L02	M1
B222	J01	J01	L02	L02	M1
B234	J09	J09	L03	L03	M1
B235	J01	J05	L01	L02	M1
B236	J01	J07	L02	L04	M1
B237			L03	L04	M1
B279	J01	J01	L02	L06	M1
B290	J01	J01	L02	L06	M1
B292	J01	J01	L02	L06	M1
B303	J01	J01	L02	L02	M1
B312	J01	J05	L01	L02	M1
B314	J01	J01	L02	L02	M1
B316	J10	J06	L01	L03	M1
B320	J01	J01	L02	L02	M1

Fortaleza	β-tub1		β-tub2		cox1-trnM
B322	J05	J08	L01	L02	M1
B323	J07	J11	L04	L04	M1
B324	J01	J01	L02	L02	M1
B328	J01	J01	L02	L02	M1
B329	J01	J01	L02	L02	M1
B331	J01	J01	L02	L02	M1
B335	J01	J12	L07	L02	M1
B337	J01	J01	L02	L02	M1
B339	J01	J01	L02	L02	M1
B340	J01	J01	L02	L02	M1
B341	J01	J01	L02	L02	M1
B342	J01	J05	L01	L02	M1
B344	J06	J07	L03	L04	M1
B346	J01	J01	L02	L02	M1
B348	J01	J02	L01	L02	M1
B351	J01	J01	L02	L02	M1
B352	J01	J01	L02	L02	M1
B353	J01	J13	L02	L03	
B354	J01	J01	L02	L02	M1
B356	J01	J06	L02	L03	M1
B357	J01	J06	L02	L03	M1

Belize	b-tub1		b-tub2		cox1-trnM
E01	J12	J12	L01	L08	M2
E02	J14	J15	L01	L09	M2
E03	J16	J17	L01	L09	M2
E04	J09	J18	L09	L09	M3
E05	J19	J20	L01	L10	M2
E06	J21	J12	L01	L01	M2

Supplementary Table 2.2 (continued)

Belize	*β-tub1*		*β-tub2*		*cox1-trnM*
E07	J21	J14	L11	L12	M4
E08	J22	J23	L13	L14	M2
E09	J24	J25	L01	L15	M2
E10	J15	J26	L16	L09	M5
E11	J27	J28	L01	L09	M2
E12	J15	J03	L16	L09	M2
E13	J12	J29	L01	L17	M2
E14	J30	J31	L18	L15	M2
E15	J01	J30	L18	L02	M2
E16	J09	J32	L04	L19	M2
E17	J01	J33	L16	L15	M2
E18	J28	J26	L10	L09	M2
E19	J34	J35	L09	L12	M5
E20	J12	J12	L01	L17	M2
E21	J01	J01	L01	L02	M2
E22	J01	J12	L02	L20	M2
E23	J12	J36	L01	L21	M2
E24	J13	J15	L22	L16	M2
E25	J01	J37	L02	L02	M2
E26	J01	J01	L02	L02	M2
E27	J14	J38	L09	L04	M2
E28	J12	J39	L02	L23	M2
E29	J40	J41	L09	L09	M5
E30	J28	J28	L10	L09	M2
E31	J28	J26	L10	L24	M2
E32	J14	J42	L10	L04	M2
E33	J15	J25	L09	L25	M2
E35	J21	J14	L01	L04	M6
E36	J15	J15	L16	L16	M2
E37	J43	J44	L26	L15	M2
E38	J32	J45	L09	L12	M2
E39	J43	J46	L09	L14	M2
E40	J15	J47	L17	L27	M5

Bermuda	*b-tub1*		*b-tub2*		*cox1-trnM*
M01	J48	J49	L01	L02	M2
M02	J28	J50	L18	L10	M2
M03	J12	J28	L10	L17	M2
M04	J15	J51	L16	L09	M2
M05	J13	J15	L22	L14	M2
M06	J24	J15	L01	L16	M2
M07	J15	J28	L10	L16	M2
M08	J09	J25	L09	L09	M2
M09	J09	J25	L09	L09	M2
M10	J01	J28	L02	L10	M2
M11	J01	J12	L28	L02	M2
M13	J07	J15	L29	L04	M2

Supplementary Table 2.2 (continued)

Panamá	b-tub1		b-tub2		cox1-trnM
P09	J25	J52	L15	L12	M2
P14	J25	J53	L15	L12	M2
P24	J07	J54	L04	L17	M2
P31	J01	J55	L01	L02	M2
P32	J21	J55	L01	L02	M2
P33	J01	J56	L09	L25	M2
P34	J28	J57	L10	L15	M2
P35	J12	J28			M2
P36	J47	J30	L18	L09	M2
P37	J28	J32	L10	L30	M5
P561	J12	J32	L01	L31	M2
P562	J21	J42	L32	L10	M2
P563	J28	J58			M2
P564	J01	J13	L02	L02	M2
P565	J14	J26	L09	L09	M5
P566	J12	J16	L01	L01	M2
P567	J48	J16	L01	L02	M2
P568	J32	J32	L15	L04	M2
P569	J12	J28	L33	L04	
P570	J24	J59	L10	L17	M2

Puerto Rico	b-tub1		b-tub2		cox1-trnM
R01	J47	J28	L10	L04	M2
R02	J60	J61	L01	L16	M2
R03			L16	L04	M2
R04	J62	J63	L10	L04	M2
R05	J15	J15	L16	L34	M2
R06	J12	J15	L01	L35	M2
R07	J15	J15	L16	L16	M2
R08	J64	J07	L09	L04	M2
R09	J65	J47	L04	L04	M2
R10	J16	J15	L01	L16	M2
R11	J21	J66	L16	L04	M2
R12	J67	J32	L10	L04	M2
R13	J07	J15	L29	L04	M2
R14	J15	J15	L16	L16	M2
R15	J28	J68	L10	L09	M2
R16	J16	J47	L01	L04	M2
R17	J15	J28	L10	L16	M2
R18	J14	J69	L36	L04	M2
R19	J70	J64	L09	L37	M2
R20	J15	J42	L10	L16	M2
R21	J47	J28	L10	L04	M2
R22	J32	J52	L04	L38	M2
R23	J09	J09	L09	L04	M2
R24	J09	J25	L09	L15	M2
R25	J15	J15	L16	L16	M2

Supplementary Table 2.2 (continued)

Puerto Rico	β-tub1		β-tub2		cox1-trnM
R26	J21	J47	L04	L12	M2
R27	J24	J71	L01	L04	M2
R28			L09	L09	M2
R29			L16	L09	M2
R31	J07	J28	L36	L10	M2
R32	J07	J15	L16	L04	M2
R33	J12	J15	L01	L39	M2
R34	J15	J61	L10	L09	M2
R35	J07	J72	L04	L12	M2
R37	J26	J73	L16	L04	M2
R39	J24	J15	L01	L16	M2

São Tomé	b-tub1		b-tub2		cox1-trnM
ST001	J05	J05	L40	L40	M1
ST002	J05	J05	L01	L40	M1
ST003	J05	J05	L01	L01	M1
ST004	J05	J02	L01	L01	M1
ST028	J05	J05	L01	L40	M1
ST068	J74	J74	L04	L04	M1
ST069	J05	J05	L01	L40	M1
ST109	J05	J10	L01	L40	M1
ST110	J05	J05	L01	L01	M1
ST111	J05	J05	L01	L01	M1
ST112	J05	J02	L01	L01	M1
ST146	J05	J05	L01	L01	M1
ST147	J05	J05	L01	L01	M1
ST180	J05	J05	L01	L01	M1
ST181	J05	J05	L01	L40	M1
ST250	J05	J05	L01	L01	M1
ST252	J05	J05	L01	L01	M1
ST253	J05	J74	L01	L04	M1
ST254	J05	J05	L01	L01	M1
ST255	J05	J05	L01	L01	M1
ST287	J05	J05	L01	L01	M1
ST326	J05	J05	L01	L01	M1
ST333	J05	J74	L01	L01	M1
ST339	J05	J05	L01	L01	M1
ST353	J05	J05	L01	L40	M1
ST358	J05	J05	L40	L40	M1

CHAPTER III

Long-distance dispersal and connectivity in amphi-Atlantic corals at regional and basin scales

ABSTRACT

Among Atlantic scleractinian corals, species diversity is highest in the Caribbean, but low diversity and high endemism is observed in various peripheral populations in central and eastern Atlantic islands and along the coasts of Brazil and West Africa. The degree of connectivity between distantly separated populations is of interest because it provides insight into processes at both evolutionary and ecological scales, such as speciation, recruitment dynamics and the persistence of coral populations. To assess dispersal ability in broadly distributed coral species of the Atlantic, DNA sequence data from two nuclear markers were obtained in six coral species spanning their distributional range. At basin-wide scales, significant differentiation was generally observed among populations in the Caribbean, Brazil and West Africa. Concordance of patterns in connectivity among co-distributed taxa suggests that extrinsic barriers, such as the Amazon freshwater plume or long stretches of open ocean, restrict dispersal of coral larvae from region to region. Within regions, dispersal ability appears to be influenced by aspects of reproduction and life history. Two broadcasting species, *S. siderea* and *M. cavernosa*, were able to maintain gene flow among populations separated by as much as 1,200 km along the coast of Brazil. In contrast, brooding species, such as *F. gravida* and *S. radians* had more restricted gene flow along the Brazilian margin.

INTRODUCTION

The ability to disperse over long distances and maintain connectivity over large geographical areas has important repercussions for the population dynamics of a species. Over ecological time scales, connectivity contributes to the persistence of populations, as the influx of migrants from neighboring healthy ecosystems can buffer mortality due to local disturbances. Large populations are expected to preserve more genetic variety than smaller populations which are susceptible to loss of genetic diversity through the effects of genetic drift. Populations interconnected by regular dispersal and gene flow can behave as large populations and bypass the negative effects of drift. On the other hand, population fragmentation can promote local adaptation and the origination of new species over evolutionary time scales, contributing to the overall diversity of a region. Peripheral populations, those found towards the edges of a species range, are more likely to have reduced number of individuals and lower genetic diversity because they are isolated from the central core and because they are found at the environmental limits of the species range. Understanding connectivity with respect to peripheral populations can provide insight into (1) how these populations are able to persist despite withstanding suboptimal conditions and (2) whether they can contribute evolutionary novelty and distinctiveness to the overall population.

Many marine organisms have broad geographical distributions, but it is unclear whether these distributions reflect the ability to maintain frequent long-distance dispersal or if populations are fragmented across their range. In the Atlantic, the most

extensive and diverse tropical reef ecosystems are found in the Caribbean Sea, but reefs with low overall species richness yet high endemism are also found along the coast of Brazil, on mid-Atlantic islands and in West Africa. Species whose distribution spans the Atlantic provide an interesting natural experiment to explore basin-scale dispersal in benthic marine organisms and the role of peripheral populations in the evolution of biodiversity.

Dispersal in benthic marine organisms can be achieved in various ways. Long-lived pelagic larvae are capable of traversing up to thousands of kilometers either by feeding in the water column or by relying on nutritional resources imparted by the parents. Rafting also provides the means of long distance dispersal, since larvae that settle and survive on floating debris can colonize distant locations through transport by oceanic currents. Habitat preferences also play a role, and species able to survive in a wide range of environments or whose habitat is large and continuous may maintain dispersal over long distances despite shorter larval durations, because dispersal can be achieved as a series of smaller steps. Oceanographic currents can either aid or prevent dispersal of larvae. Physical properties of water masses affect larval survival, and depending on the species, larval dispersal may be limited by temperature, salinity or nutrient content. Dispersal can also be limited by the absence of suitable substrate for settlement because successful recruitment is dependent not only on the larva's ability to reach a destination, but also its ability to settle and become established. Comparative studies across species with different reproductive strategies and life histories can be important in helping to clarify which characteristics

contribute to successful long-distance dispersal, and whether dispersal is limited by intrinsic characteristics of a single species or extrinsic barriers affecting multiple species. An example of such a barrier is observed as concordant breaks in gene flow observed for co-distributed organisms between the Gulf of Mexico and Atlantic Coast of the United States suggests that a major geographic barrier for dispersal exists for a number of marine, coastal and terrestrial species (reviewed in (Avise 2000).

Corals support biodiversity in the oceans by providing the substrate and architecture for a host of other marine species. Understanding the processes that limit or promote dispersal in coral species can provide insight into how and why populations persist and evolve. Although connectivity and dispersal have been explored for a number of co-distributed Pacific corals (Ayres and Hughes, 2000; Ayres and Hughes 2004), basin-scale connectivity among Atlantic corals has only been explored among populations of one species, *Montastraea cavernosa*. For this coral, significant differentiation is found between populations in the Caribbean, Brazil and West Africa, despite this species' ability to maintain connectivity across 3,000 km within the Greater Caribbean, including Bermuda in the North Atlantic (Nunes *et al.*, in press). This suggests that important barriers to dispersal may separate coral populations from different biogeographic regions of the Atlantic, but it is unclear whether these findings can be generalized to all corals, or whether they are specific to the dispersal ability of *M. cavernosa*. Comparative studies across multiple coral species over similar spatial scales can help elucidate this problem.

Corals exhibit a variety of reproductive strategies, making them an interesting group for evaluating the role of reproductive traits in determining dispersal potential. Corals reproduce sexually either by internal fertilization and brooding of larvae or by external fertilization of broadcast gametes followed by development in the water column, and they may also raft and reproduce by fragmentation, although fragments are not expected to travel far and so are unlikely to be important to long-distance dispersal. Various traits aid long-distance dispersal, such as larval longevity, delayed time to competency, and reproductive output. Prolonged longevity allows larvae to be transported greater distances, and the nutritional resources available to the larva will affect its ability to remain alive. Large eggs, such as those observed for many broadcasting species (Soong 1991; Szmant 1986), or larvae harboring zooxanthellae at the time of release, such as for many brooded species, may have improved chances of survival, as the nutrients provided by the egg or the symbionts can enhance survival. Coral larval longevity can reach upwards of two to three months for some species (Graham *et al.* 2008; Richmond 1987), but many larvae will settle in less time, upon encountering suitable substrate. Brooded larvae are more advanced in their development when released into the water column, and are competent for settling in 24 – 48 hours (Atoda 1947a, b, 1951). In contrast, broadcast gametes usually require 5 – 7 days to develop in the water column before being capable of settling (Baird 1998; Harrison& Wallace 1990), increasing the chances of broadcast larvae of being transported to greater distances.

Over evolutionary time scales, even a small number of successful long-distance dispersal events may be enough to maintain gene flow between populations. Because long-distance dispersal can be a stochastic process, the probability of success increases with the number of trials, or in the case of coral dispersal, the greater the number of propagules generated. Estimating the reproductive output of corals is not straightforward, because the number of larvae produced by a colony varies according to several factors, including colony size, the number of eggs produced per polyp, number of reproductive cycles per year, and fertilization success. In particular, the number of reproductive cycles per year may be an important difference between brooding versus broadcasting species, since the former are able to produce larvae through most of the year, while the latter typically have only one to two spawning events annually. Estimates of the annual volume of eggs produced indicate that the annual output of larvae may be greater for brooders (Harrison& Wallace 1990). It also appears that brooders allocate more energy resources to reproduction relative to broadcasters. Broadcasters typically build massive colonies that can withstand storms and disturbances, but to build robust skeletons they must allocate more resources towards growth by spawning once a year. Brooders on the other hand tend to be small in size and inhabit more disturbed or marginal reef environments, which may make them more vulnerable to higher rates of mortality. Brooders may offset their high mortality rates by increasing the amount of energy they allocate to reproduction (Szmant 1986). Finally, sexuality and sex ratio can also play a role in fertilization success. Equal sex ratios, self-compatibility and hermaphrodism may improve

chances of fertilization by maximizing the possibility of successful matings. These adaptations can be advantageous in locations were colonies are sparse or where environmental conditions may hinder outcrossing, such as may be expected in peripheral populations.

Species richness and regional endemism varies widely across the Atlantic. Among the 81 species of reef-building corals found in the Atlantic, 68 species are found in the Caribbean, 23 in Brazil and 18 in the Eastern Atlantic (Supplementary Table 3.1). There are 12 species endemic to either Brazil or the Eastern Atlantic (5 in Brazil and 5 in West Africa, and 2 species found in both regions) whereas 49 species are endemic to the Caribbean. Twenty species have wide distributions that span two or more biogeographic regions of the Atlantic, but only 9 are found across all three regions: *Madracis asperula, M. decactis, M. pharensis, Montastraea cavernosa, Porites astreoides, Siderastrea radians, S. siderea, S. stellata* and *Tubastraea coccinea*. In addition, *Favia fragum* (Caribbean and West Africa) and *F. gravida* (Brazil and West Africa) are closely related and it is debated whether they should be synonymized (Veron& Stafford-Smith 2000), although they can be distinguished on the basis of morphology (Laborel 1969) and genetics (Nunes *et al.* 2008).

It is important to note that species lists and shared occurrences are a work in progress, and can change as more information becomes available. The shared occurrence of some species across biogeographic regions, such as for *Meandrina braziliensis, Siderastrea stellata,* or *Scolymia cubensis* is debated among taxonomists and biogeographers (for example, Neves *et al.* 2006). Future work that uses

morphological and molecular data from specimens collected in both regions are required to confirm occurrences and refine estimates of endemism for each region.

Here we show new genetic data from six coral species and an existing dataset for an additional species that have a broad geographic distribution across the Atlantic and span a range of reproductive strategies to determine patterns of connectivity across corals. Among the sampled species, *Favia fragum*, *Favia gravida*, *Siderastrea radians*, *Siderastrea stellata* and *Porites astreoides* are brooders, while *Siderastrea siderea* and *Montastraea cavernosa* are broadcasters. Most coral species are hermaphroditic, but *M. cavernosa*, *S. radians* and *S. stellata* are gonochoric, with *S. radians* having a sex bias of 1:20 towards females. Colonies of *P. astreoides* have been observed to be either hermaphroditic or separate sexes. Genetic data from random amplified polymorphic DNAs (RAPDs) suggest that *F. fragum* and *P. astreoides* are able to self-fertilize (Brazeau *et al.* 1998). By sampling corals with a variety of biological traits and spanning a wide taxonomic range, we hope to eliminate biases so that our conclusions regarding basin-wide dispersal and connectivity can be representative for Atlantic corals.

METHODS AND MATERIALS

Coral tissue samples were collected from colonies of six coral species: *F. fragum*, *F. gravida*, *S. siderea*, *S. radians*, *S. stellata*, and *P. astreoides*. Sampling took place in Panama in 2005, at various locations on the island of São Tomé in West Africa in 2006 and in three populations separated by >500 km in Brazil (Abrolhos,

João Pessoa and Fortaleza) in 2002 and in 2007. Genomic DNA was preserved and extracted following a protocol described previously (Nunes *et al.* 2008). Due to the unusually low levels of genetic variation found in the mitochondrial DNA of corals (Shearer *et al.* 2002), only nuclear loci were used. The intron and exon of *β-tubulin* and the intron of the *Pax-C* gene, which have previously been shown to contain sufficient intraspecific variation in various corals for population studies, were amplified for all species studied, except for *P. astreoides*, where only *β-tubulin* could be amplified. For *P. astreoides*, the mitochondrial control region (thought to be one of the most variable regions of the coral mitochondrial genome) was amplified and sequenced for 32 individuals from Panama and Brazil, but because all individuals contained identical nucleotide sequences at this locus, this marker was not used for further analysis.

Amplification of the two nuclear loci was performed by polymerase chain reaction using published primer sequences for *β-tubulin* (Lopez& Knowlton 1997) and for *Pax-C* (Van Oppen *et al.* 2000). The thermal cycler profile had an initial denaturation step at 94°C for 2min, followed by 38 cycles of 94°C/45sec, 50-58°C/45sec for *β-tubulin* or 50-54°C/45sec for *Pax-C*, 72°C/90sec, with a final extension step at 72°C for 5 min. An additional forward primer was designed for the amplification and sequencing of *Pax-C* for *F. fragum* (PaxFsh1: 5'- GGA GGA GCT TGC GAA TAA GA -3'). Multiple bands were amplified in *P. astreoides* and the *Siderastrea* species in *β-tubulin*, and for *S. siderea* in *Pax-C*. Bands at ~750bp and ~600 bp were extracted and purified for products of *β-tubulin* and *Pax-C* respectively,

using the Qiaquick Gel Extraction kit. The remaining PCR products were purified either by gel extraction or by elution through a silica-membrane column using the appropriate Qiaquick purification system.

Sequencing of all loci was performed directly on purified PCR products for both forward and reverse directions on an ABI 3130xl genetic analyzer with the ABI BigDye Terminator v3.1 chemistry. Sequence chromatographs were viewed and edited using the Sequencher v4.5 software (Gene Codes Corp). Heterozygous alleles were identified by double peaks observed in sequences from both directions. Indels were observed in the introns of *β-tubulin* for *S. radians* (site 549) and *S. siderea* (site 549), and in sequences of *Pax-C* for *S. radians* (sites 134), *F. fragum* (site 198) and *F. gravida* (site 198). Because only a small number of individuals were heterozygous for indels within any species and indel positions were straightforward to identify, indel sites were kept and considered in the analysis. Since the divergence among haplotypes was small for the two *Favia* species, both species were analyzed as a single taxonomic unit. Interestingly, individuals identified in the field as *S. stellata* contained a combination of haplotypes typical of *S. siderea* and *S. radians* at both loci – suggesting that *S. stellata* is the result of hybridization between these two species. Details on the evidence supporting hybridization between these two species will be outlined elsewhere. For the purposes of this study, only populations of *S. radians* and *S. siderea* were considered. Haplotypes obtained from *S. stellata* were included as part of either a *S. radians* or *S. siderea* population, depending on similarity/identity with haplotypes from those species.

Haplotypes for heterozygous individuals were reconstructed using PHASE v 2.1.1 (Stephens& Scheet 2005; Stephens *et al.* 2001). The algorithm was run three times for 100 iterations with ten thinning interval steps and 100 burn in steps. The best pairs of haplotypes for each individual resulting from the run with the highest average value for the goodness of fit were used for each nuclear locus.

Haplotype frequencies per population, molecular diversity indices, tests of neutrality, analysis of molecular variance (AMOVA) and estimates of population differentiation were calculated using Arlequin v.3.11 (Excoffier *et al.* 2005). Analysis of molecular variance (AMOVA) was used to estimate levels of genetic differentiation among populations of each coral species. Three separate AMOVAS were used to estimate differentiation among (1) all populations ($\Phi_{ST\ ALL}$), (2) among the following regions: Caribbean, Brazil and West Africa ($\Phi_{ST\ REG}$) and (3) among populations within Brazil ($\Phi_{ST\ BR}$), the only region for which multiple populations were sampled. Estimates of ϕst used pairwise distances between haplotypes. Parsimony haplotype networks were constructed with TCS (Clement *et al.* 2000). Recombination in the two nuclear loci was inferred as reticulations in the haplotype network and tested by the four-gamete test in DnaSP (Librado & Rozas 2009). No loops were observed in the haplotype networks of *F. fragum/F gravida* for both loci, or in the network of *P. astreoides* for *β-tubulin*. One loop was observed in the networks of *S. radians* for both loci. For *S. siderea*, no loops were observed in *Pax-C*, however, numerous loops were observed in *β-tubulin*, suggesting that in this locus, several recombination events have occurred. The four gamete test indicates that only a few recombination events

have occurred at for each locus for most species (Rm = 0 – 4), except for *β-tubulin* of *S. siderea*, where a minimum of 12 recombination events are inferred. Estimates of recombination for *β-tubulin1* of *M. cavernosa* have been published elsewhere (Nunes et al., in press). For clarity, loops were omitted from the haplotype networks and only internal branches are shown. Due to the evidence of recombination, analyses using a coalescent framework (which assume no recombination) were not attempted.

RESULTS

A total of 238 individuals across all species were sequenced for *β-tubulin* and 191 for *Pax-C*. Sequences have been deposited in Genbank. The number of individuals, alleles, unique haplotypes and segregating sites per population are shown in Table 3.1, as are standard molecular diversity indices, tests of neutrality and deviations from Hardy-Weinberg equilibrium for each species and each locus. Gene diversity (*h*), which estimates the probability that two randomly sampled haplotypes in a population are different, ranged from 0 – 0.862 in *Pax-C* and 0.235 – 0.986 in *β-tubulin*, indicating that while some populations are dominated by a common haplotype, others have a large diversity of haplotypes. Nucleotide diversity (*π*) ranged from 0.0009 – 0.009 in *Pax-C* and 0.0003 – 0.0158 in *β-tubulin*, and the average number of differences between haplotypes of a population (*k*) ranged from 0.32 – 3.29 in *Pax-C* and 0.23 – 10.5 in *β-tubulin*. The mean and standard deviation for each molecular diversity index is shown graphically in Figures 3.1 – 3.3. Populations

Table 3.1. **Molecular diversity indices and tests of neutrality for *β-tubulin* and *Pax-C* for each species. N_a is the number of sampled alleles, *H* is the number of unique haplotypes observed, *s* is the number of segregating sites, *h* is the gene diversity, π is the average nucleotide diversity and *k* is the average number of nucleotide differences. Statistically significant values (α = 0.05) are highlighted in bold.**

		N_a	H	s	h		p		k		Tajima's D	Fu's Fs
Pax-C	***Favia fragum+Favia gravida* 454bp**											
	BR1 - Abrolhos	26	2	1	0.492	± 0.051	0.0011	± 0.0011	0.4923	± 0.436	1.4372	1.52295
	BR2 - João Pessoa	46	2	1	0.394	± 0.063	0.0009	± 0.0009	0.3942	± 0.375	1.00608	1.40683
	CA1 - Panama	32	3	2	0.619	± 0.044	0.0016	± 0.0014	0.7238	± 0.555	1.53512	0.88595
	WA1 - São Tome	40	1	0	0		0		0		0	0
	***S. radians* 367 bp**											
	BR1 - Abrolhos	14	2	4	0.528	0.064	0.006	0.004	2.110	1.250	2.232	4.844
	BR2 - João Pessoa	27	5	8	0.735	0.059	0.009	0.005	3.293	1.748	1.610	2.968
	BR3 - Fortaleza	20	3	5	0.653	0.065	0.006	0.004	2.105	1.225	1.511	3.402
	CA1 - Panama	21	5	5	0.491	0.127	0.003	0.002	1.210	0.803	-0.213	-0.565
	WA1 - São Tome	44	5	8	0.571	0.052	0.006	0.004	2.229	1.253	0.896	2.316
	***S. siderea* 366 bp**											
	BR2 - João Pessoa	29	3	4	0.493	0.093	0.003	0.002	1.034	0.710	0.041	1.676
	BR3 - Fortaleza	20	5	9	0.600	0.101	0.004	0.003	1.542	0.963	-1.348	-0.007
	CA1 - Panama	27	8	13	0.863	0.035	0.009	0.005	3.288	1.745	-0.085	0.156
	WA1 - São Tome	36	2	3	0.108	0.068	0.001	0.001	0.324	0.335	-1.237	0.939
β-tubulin	***Favia fragum+Favia gravida* 939 bp**											
	BR1 - Abrolhos	38	2	1	0.235	0.081	0.0003	0.0003	0.2347	0.278	-0.0201	0.45459
	BR2 - João Pessoa	52	3	3	0.604	0.034	0.0013	0.0009	1.19	0.775	1.62298	2.69459
	CA1 - Panama	36	4	5	0.533	0.088	0.0014	0.001	1.3206	0.842	0.24847	1.34034
	WA1 - São Tome	40	3	2	0.65	0.038	0.0008	0.0007	0.7885	0.585	1.27808	1.26004
	***S. radians* 665 bp**											
	BR1 - Abrolhos	6	4	6	0.867	0.129	0.005	0.003	3.200	1.918	1.246	0.352
	BR2 - João Pessoa	14	4	6	0.495	0.151	0.002	0.001	1.099	0.765	**-1.499**	-0.214
	BR3 - Fortaleza	14	3	2	0.703	0.062	0.001	0.001	0.879	0.654	1.080	0.586
	CA1 - Panama	20	8	22	0.837	0.051	0.016	0.008	10.505	4.999	2.631	3.722
	WA1 - São Tome	40	5	6	0.677	0.042	0.002	0.001	1.363	0.860	-0.090	0.531
	***S. siderea* 665 bp**											
	BR1 - Abrolhos	10	7	15	0.933	0.062	0.009	0.005	5.778	3.020	0.413	-0.394
	BR2 - João Pessoa	38	19	21	0.926	0.028	0.008	0.004	5.266	2.602	0.180	**-5.479**
	BR3 - Fortaleza	24	8	16	0.844	0.044	0.006	0.004	4.152	2.140	-0.110	0.657
	CA1 - Panama	38	32	34	0.986	0.011	0.012	0.006	7.933	3.771	-0.130	**-21.883**
	WA1 - São Tome	40	5	5	0.236	0.088	0.001	0.001	0.342	0.346	**-1.804**	**-3.315**
	***P. astreoides* 596 bp**											
	BR2 - João Pessoa	42	4	12	0.628	0.041	0.009	0.005	5.087	2.518	2.515	8.829
	CA1 - Panama	24	4	10	0.728	0.045	0.008	0.004	4.649	2.361	2.455	5.884

of *S. siderea* in São Tomé (West Africa) had lower average gene diversity than populations in the Caribbean and Brazil. Reduced gene diversity was also observed for *F. fragum/F. gravida* in West Africa in the *Pax-C* locus; however, this trend was not observed in the *β-tubulin* locus. *S. radians* and *P. astreoides* showed overlap in mean values of gene diversity at both loci (Fig 3.1). Mean values of nucleotide diversity (π) and average number of differences (*k*) overlap for nearly all species in both loci (Fig 3.2 and Fig 3.3), with the exception of populations of *S. radians* in Panama, which appear to have greater values of π and *k* in the *β-tubulin* locus.

Tajima's D and Fu's Fs for each population suggest that the loci used are evolving according to neutral expectations for most populations. Two populations (*S. radians* in João Pessoa and *S. siderea* in São Tomé) had significantly negative values of Tajima's D, suggesting deviations from neutral expectations. Negative values of

Figure 3.1 Mean gene diversity (*h*) per locus for each coral population. Error bars indicate standard deviations.
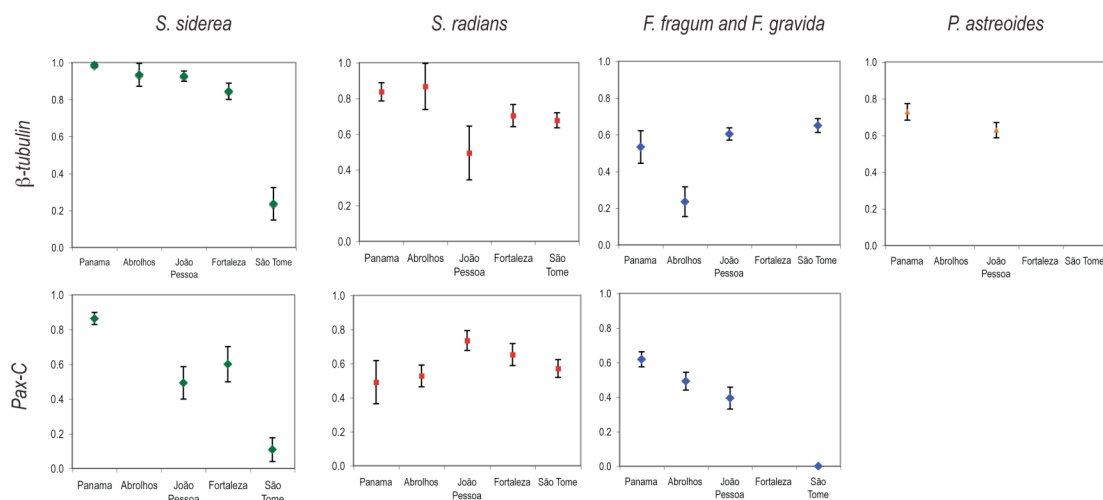
Figure 3.2 Mean nucleotide diversity (π) per locus for each coral population. Error bars indicate standard deviations.
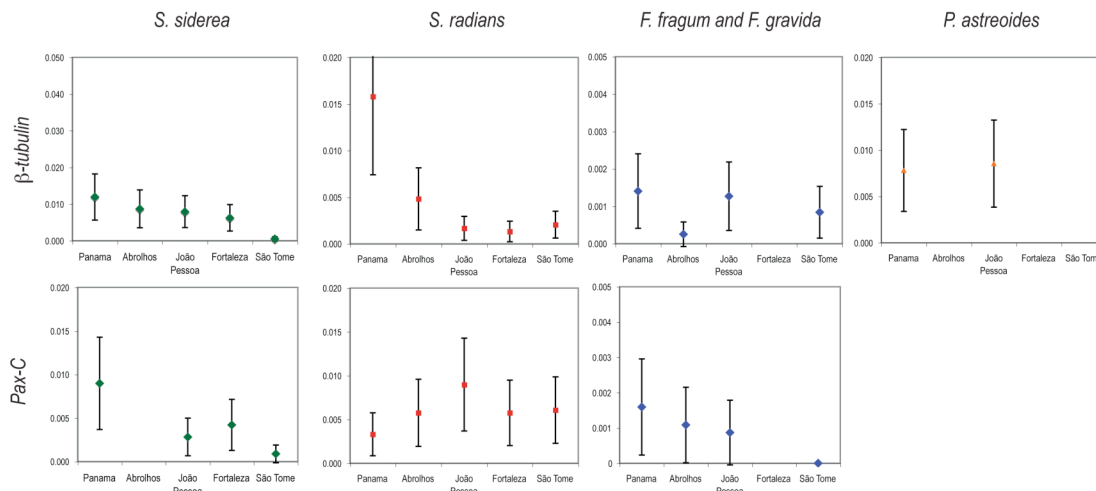
Figure 3.3 Mean average number of differences (k) per locus for each coral population. Error bars indicate standard deviations.
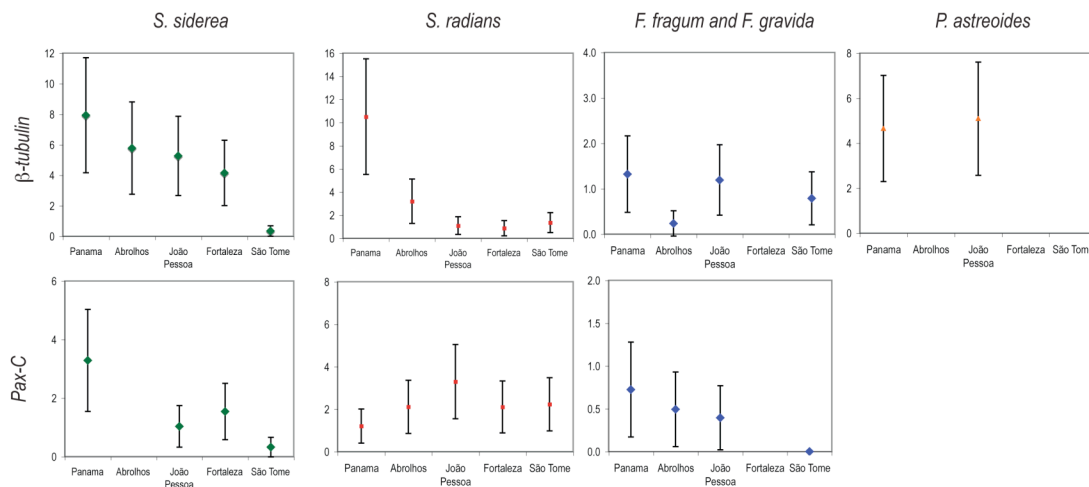
Fu's Fs were observed for three populations of *S. siderea* for the *β-tubulin* locus, but only the population of São Tomé also had a significant negative value of Tajima's D. Fu's Fs is sensitive to changes in demography, and large negative values in *S. siderea* are indicative of population expansion. Aside from the exceptions mentioned above, it

appears that both loci are evolving according to neutral expectations for the remaining populations of the six species.

Significant differentiation among populations was observed for nearly all of the study species at both loci ($\Phi_{ST\ ALL}$ = 0.126 – 0.781, p < 0.05), except for *P. astreoides* ($\Phi_{ST\ ALL}$ = 0.026, p > 0.05). Differentiation among regions was also found to be significant across every sampled species ($\Phi_{ST\ REG}$ = 0.067 – 0.767, p < 0.05) except of *P. astreoides* ($\Phi_{ST\ REG}$ = 0.026, p > 0.05). Furthermore, there was significant intra-regional differentiation between populations of *F. gravida* and *S. radians* of Brazil ($\Phi_{ST\ BR}$ = 0.148 – 0.582), but not among populations of *S. siderea* or *M. cavernosa* within Brazil ($\Phi_{ST\ BR}$ = -0.026 – 0.018, p>0.05) (see Table 3.2). Significant population differentiation was observed among most pairwise comparisons (Table 3.3), with only a few exceptions: (1) populations of *S. siderea* within Brazil were not differentiated at both loci, (2) no significant differentiation was observed between populations of *P. astreoides* in Brazil and the Caribbean and (3) populations of Abrolhos and Fortaleza (both in Brazil) and São Tomé in *S. radians* were not significantly different at *Pax-C*, most likely because similar allele frequencies were observed for two common haplotypes (SRP1 and SRP2). Haplotype frequency pie charts were plotted for each locus and each species on a map to illustrate differences across populations (Fig. 3.4).

Statistical parsimony haplotypes for each species and locus are shown in Fig. 3.5. Haplotype networks were typically composed of a small number of haplotypes (H < 15), although the network for *β-tubulin* in *S. siderea* had 60 haplotypes. Every

Table 3.2. **Analysis of molecular variance (AMOVA) was used to estimate levels of genetic differentiation among all populations ($\Phi_{ST\ ALL}$); among the following regions: Caribbean, Brazil and West Africa ($\Phi_{ST\ REG}$); and among populations within Brazil ($\Phi_{ST\ BR}$), the only region for which multiple populations was sampled. Statistically significant values (α = 0.05) are highlighted in bold.**

	Species	Locus	$\Phi_{ST\ ALL}$	$\Phi_{ST\ REG}$	$\Phi_{ST\ BR}$
Brooders	*F. fragum+F. gravida*	*Pax-C*	**0.781**	**0.767**	**0.208**
	F. fragum+F. gravida	*β-tubulin1*	**0.739**	**0.642**	**0.479**
	S. radians	*Pax-C*	**0.126**	**0.067**	**0.148**
	S. radians	*β-tubulin1*	**0.371**	**0.296**	**0.582**
	P. astreoides	*β-tubulin1*	0.026	0.026	N/A
Broadcasters	*S. siderea*	*Pax-C*	**0.252**	**0.256**	-0.026
	S. siderea	*β-tubulin1*	**0.369**	**0.365**	0.018
	M. cavernosa	*β-tubulin1*	**0.196**	**0.196**	-0.009
	M. cavernosa	*β-tubulin2*	**0.371**	**0.369**	-0.013

haplotype network had 1-5 haplotypes that were shared between one or more regions. Differences between haplotypes from different regions were typically small, on the order of one to a few mutations. There were a relatively high proportion of private alleles (those restricted to only a single population) for each locus and species, ranging from 44% - 87% of the observed haplotypes for a population. The proportion of

alleles that were observed only once was low at each locus for most species (0 – 27%),

except for *β-tubulin* in *S.siderea*, where 72% of haplotypes were observed only once.

Table 3.3. **Pairwise Φst for each of the sampled nuclear loci, for each species. Statistically significant values (α = 0.05) are highlighted in bold.**

β-tubulin							*Pax-C*					
F. fragum+ F. gravida							F. fragum+ F. gravida					
	1	2	3	4	5			1	2	3	4	5
1 BR1-Abrolhos	0						1 BR1-Abrolhos	0				
2 BR2-Joao Pessoa	**0.479**	0					2 BR2-Joao Pessoa	**0.208**	0			
3 Panama	**0.804**	**0.755**	0				3 Panama	**0.804**	**0.809**	0		
4 STP1-Mainland	**0.253**	**0.309**	**0.773**	0			4 STP1-Mainland	**0.610**	**0.198**	**0.849**	0	
5 STP2-Rolas	**0.848**	**0.631**	**0.802**	**0.771**	0		5 STP2-Rolas	**0.511**	0.140	**0.801**	0	0
S. radians							S. radians					
	1	2	3	4	5			1	2	3	4	5
1 Abrolhos	0						1 Abrolhos	0				
2 Joao Pessoa	**0.338**	0					2 Joao Pessoa	**0.193**	0			
3 Fortaleza	**0.463**	**0.72**	0				3 Fortaleza	-0.010	**0.209**	0		
4 Panama	0.175	**0.358**	**0.323**	0			4 Panama	**0.192**	**0.271**	**0.095**	0	
5 Sao Tome	**0.277**	**0.521**	**0.167**	**0.393**	0		5 Sao Tome	-0.040	**0.196**	-0.013	**0.125**	0
S. siderea							S. siderea					
	1	2	3	4	5			1	2	3	4	
1 Abrolhos	0						1 Joao Pessoa	0				
2 Joao Pessoa	-0.042	0					2 Fortaleza	-0.026	0			
3 Fortaleza	0.037	0.039	0				3 Panama	**0.253**	**0.214**	0		
4 Panama	**0.065**	**0.122**	**0.160**	0			4 Sao Tome	**0.087**	**0.097**	**0.370**	0	
5 Sao Tome	**0.735**	**0.597**	**0.748**	**0.359**	0							
P. astreoides												
	1	2										
1 Joao Pessoa	0											
2 Panama	0.026	0										

DISCUSSION

Genetic differentiation across regions of the Atlantic

The data collected for six coral species plus an existing dataset for *Montastraea cavernosa* (Nunes *et al.* in press) suggest that significant differentiation is present between the Caribbean, Brazil and West Africa for most sampled coral species. Concordance in the pattern of regional isolation occurs at the scale of genes and species. Both nuclear markers show consistent patterns in differentiation,

suggesting that this is not the result of regional adaptation of particular genes, but rather reflects a genome-wide pattern. Regional differentiation across multiple species also suggests that barriers to gene flow between biogeographic regions in the Atlantic are effective for most corals.

The most likely biogeographic barrier separating Caribbean and Brazilian coral populations are the deltas and low salinity waters of the Amazon, Orinoco and numerous rivers along the coast of northern South America. The Amazon is the largest among them, and accounts for 16% of the annual freshwater discharge into the world's oceans (Muller-Karger *et al.* 1988). Its plume extends 200 – 500km in width (Lentz 1995) and is recognizable 24-32 m below the surface (Nikiema *et al.* 2007). The Amazon and Orinoco Rivers attained their current drainage configuration around the Late Miocene (Hoorn *et al.* 1995), suggesting that low salinity and high sedimentation may have been a feature of this coastal region for ~10 Ma. Approximately 2,300 km of coastline rich in soft sediment bottoms and low salinity coastal waters between Caribbean and Brazilian reefs may pose substantial barriers to dispersal, because the substrate near river deltas is inadequate for coral settlement and coral larvae are very sensitive to the changes in salinity (Vermeij *et al.* 2006) that these large river plumes create.

The only species for which no significant differentiation was observed between Caribbean and Brazilian populations was *P. astreoides*. For *β-tubulin* (the only marker amplified for this species, see Methods), *P. astreoides* has one common haplotype that is observed both in Brazil and Panama at similar frequencies, with all

Figure 3.4 Haplotype frequency pie charts for coral populations across the Atlantic for A. brooders and B. broadcasters. The three most commonly occurring haplotypes for each species and locus have been coded with the same color (see legend). Private alleles for each geographic region have been binned into one category for clarity.
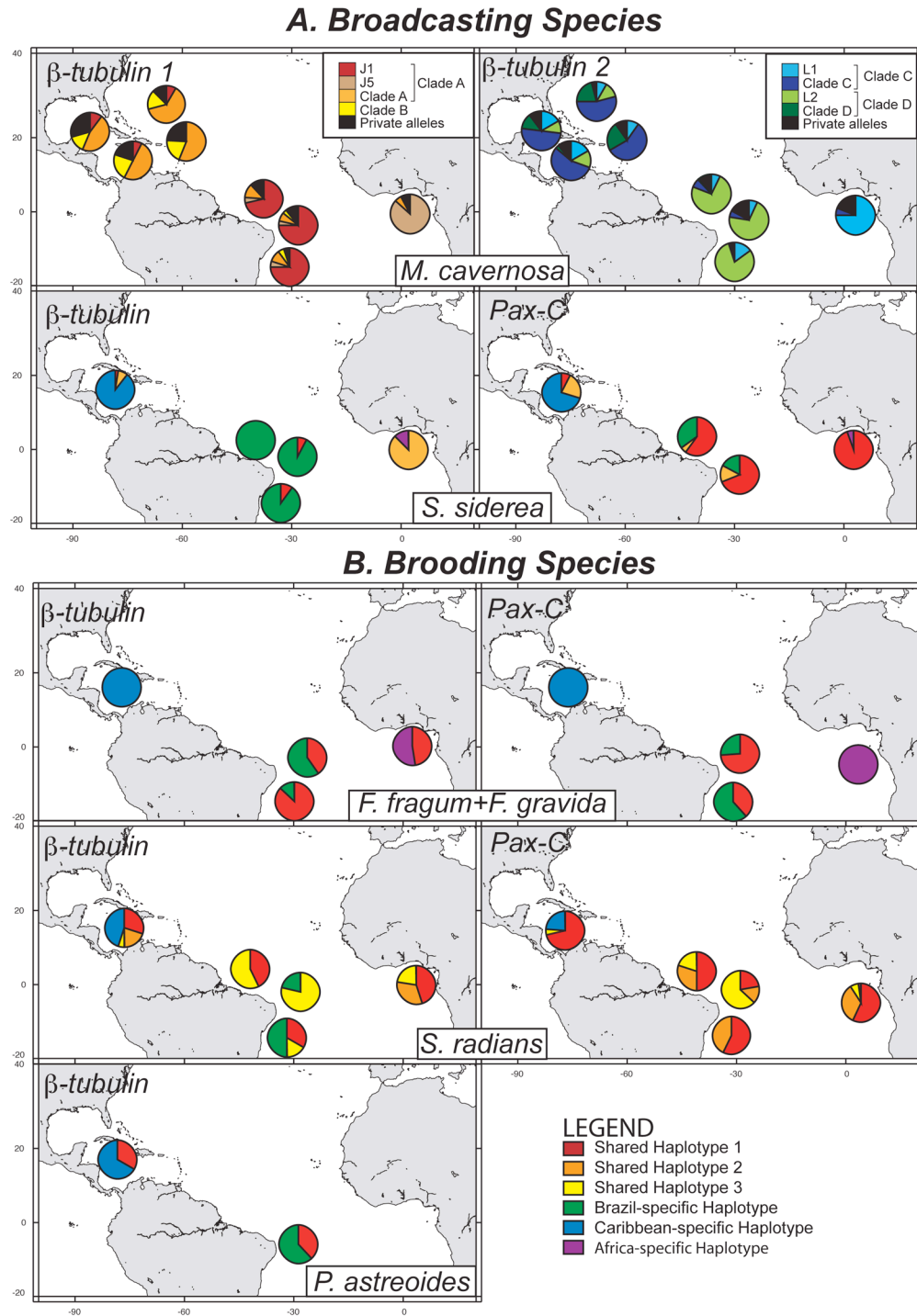
other haplotypes being unique to each respective population (see Fig 3.5). Sequences of *β-tubulin* for *P. astreoides* have lower variation compared to the other species, and this level of variation may be insufficient to detect differences among populations. A common haplotype present in populations of Panamá and Brazil may be an ancestral haplotype that has been maintained over time in the two populations without continued gene flow. Additional support from faster-evolving markers such as microsatellites are required to determine whether continued gene flow is maintained between the Caribbean and Brazil in this species. Alternatively, *P. astreoides*, a brooder, may be able to raft or be more tolerant to freshwater.

Significant regional differentiation was also observed with respect to West Africa (Table 3.3), suggesting that long distances of open ocean may be impassable for coral larvae. The easternmost point of Brazil and São Tomé Island are separated by 4,800 km. Although some mid-Atlantic islands could serve as stepping-stones for dispersal and could shorten dispersal distances by about one half, it appears that even these distances are too great to maintain gene flow across the two regions or that the populations on those islands (such as the St. Peter and Paul rocks) are too small or ephemeral to be significant in trans-Atlantic dispersal. Interestingly, the population of *M. cavernosa* on the island of Bermuda is able to maintain connectivity with Caribbean populations despite being separated by at least 1,000 km (Nunes *et al.* in press). Presumably the fast-moving currents of the Gulf Stream are able to maintain the influx of larvae to Bermuda. This suggests that distance may not be the only important isolating factor for West African coral populations, but that physical

Figure 3.5 **Parsimony haplotype network for A. *F. fragum+F. gravida*, B. *S. radians*, C. *P. astreoides* and D. *S. siderea*. Haplotypes observed in the Caribbean, Brazil and Gulf of Guinea are shown as white, grey and black circles, respectively. The size of each circle reflects the frequency that a haplotype is observed. Notches symbolize intermediate haplotypes not observed.**
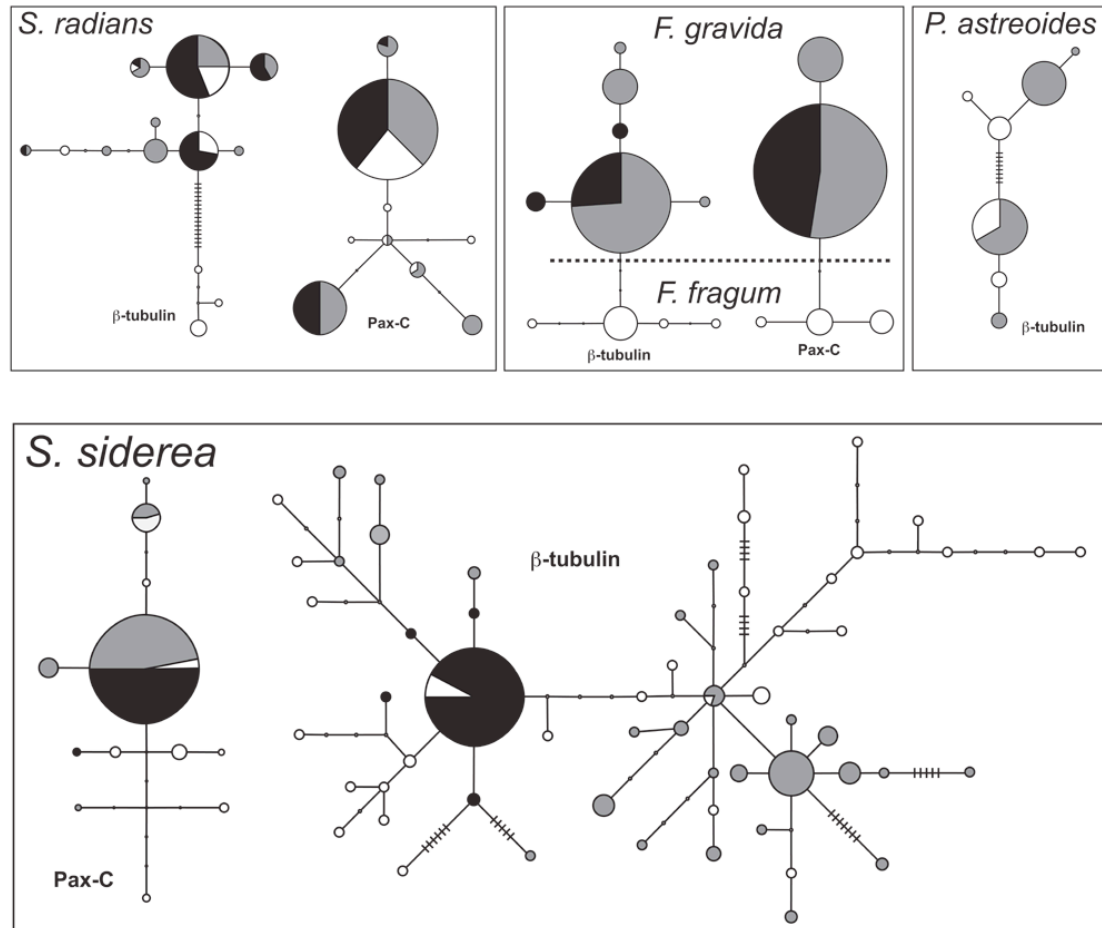
oceanography also plays an important role in the delivery of larvae to or from this region.

Concordance in patterns of regional connectivity in the Atlantic is not observed in most other species of marine organisms. Sea urchins, although sharing similar life histories and pelagic larval duration, show different patterns of differentiation across the Atlantic. While Caribbean, Brazilian and West African *Euciradis tribuloides* form a single panmictic population (Lessios *et al.* 1999), gene flow is interrupted in other

species of sea urchins. While *E. tribuloides* is able to maintain gene flow across the Amazon outflow, it appears to be a significant barrier to gene flow between populations of *Echinometra lucunter* (McCartney *et al.* 2000), *Tripneustes ventricosus* (Lessios *et al.* 2003) and *Diadema antillarum* (Lessios *et al.* 2001). The long distances that separate the East and West Atlantic also appear to be significant barriers to dispersal in some urchin species but not in others. Both *E. lucunter* and *E. tribuloides* populations appear to maintain continued exchange between Brazil, the mid-Atlantic islands and the Gulf of Guinea, while multiple diagnostic mutations separate eastern and western Atlantic populations of *T. ventricosus*, (Lessios *et al.* 2003) and *D. antillarum* (Lessios *et al.* 2001). The discordant patterns observed among sea urchins with similar reproductive strategies and larval durations, suggests that barriers to gene flow in the Atlantic may be permeable depending on aspects other than reproduction and pelagic larval duration.

Among reef fish, similar discordance has been observed across species. Some species achieve high gene flow throughout the Atlantic (*Myripristis jacobus*; (Bowen *et al.* 2006), or throughout their range (*Halichoeres garnoti*; (Rocha 2004), others are highly differentiated (Muss *et al.* 2001; Taylor& Hellberg 2003), while some display intermediate levels of gene flow between these two extremes. Interestingly, broad adult habitat preferences among surgeonfish of the genus *Aulostosomus* correlates well with dispersal ability across the Amazon, suggesting that among reef fish, ecology as well as reproduction play important roles in dispersal potential (Rocha *et al.* 2002).

Figure 3.6 **Reproductive traits of studied species: *Favia fragum, Favia gravida, Porites astreoides, Siderastrea radians, Siderastrea stellata, Siderastrea siderea* and *Montastraea cavernosa*.** (data from Harrison& Wallace 1990; Neves et al. 2008; Soong 1991; Szmant 1986)

	F. gravida	F. fragum	P. astreoides	S. radians	S. stellata	S. siderea	M. cavernosa
Mode of development							
Egg size	0.3 x 0.3 um	0.3 x 0.3* um	0.2 x 0.1 cm	0.7 x 0.3 um	0.7 x 0.3* um	0.8 x 0.3 um	0.6 x 0.6 cm
Sexuality							
Sex ratio				1:20	female biased	1:1	1:1
Annual Egg Volume produced (mm3cm-2)		27.4	0.2			116.6	12.9
Mean Annual Fecundity (cm-2)		3240	2592			2187	576

* inferred from sister species

The similarities in patterns of connectivity observed among various coral species suggests that barriers such as the Amazon or stretches of open ocean are likely impassible for most corals, leading to concordance of patterns. These barriers may be more permeable, however, for other organisms whose ecology and life history permit dispersal at greater distances than corals, resulting in more variable patterns of connectivity observed in other amphi-Atlantic marine organisms.

Genetic differentiation within regions

Although patterns in inter-regional differentiation are concordant among most coral species, different estimates of gene flow are observed <u>within</u> regions across coral species, and the extent of gene flow is correlated with reproductive traits. In this study, multiple populations within a region were sampled only in Brazil. For each species, 2-3 populations each separated by >600 km across a total of 2,000 km along the coast of Brazil were sampled for all species except *P. astreoides*, for which specimens were only taken in João Pessoa (see Fig 3.4). Strong differentiation observed at both loci between populations of the brooder, *F. gravida* in Abrolhos and João Pessoa (Table 3.2) suggests that little exchange is occurring between populations of this species along the coast of Brazil. Intermediate levels of differentiation were observed for the brooder *S. radians*, where AMOVA suggests that differentiation is significant between populations within regions for both loci (Table 3.2), but not all pairwise population comparisons showed significant differentiation (Table 3.3). In contrast, gene flow appears to be maintained between three populations spanning

2,000 km of Brazilian coast of the broadcasting corals, *S. siderea* and *M. cavernosa* (Table 3.2).

The two species that are able to maintain gene flow along the coast of Brazil are both gonochoric broadcasting species, with one reproductive cycle per year. Egg sizes for broadcasting *M. cavernosa* and *S. siderea* are larger than for the brooders *F. gravida* and *P. astreoides*, but only marginally larger than brooding *S. radians* (Fig 3.6). Estimated mean annual fecundity for the two broadcasters, however, is lower than for the brooding species (Harrison& Wallace 1990) because, although broadcasters tend to have large colonies and produce more eggs in a given spawning event, they do so only once a year, while small colonies of brooders reproduce throughout the year. The results of this study indicate that broadcasting species that produce fewer but larger eggs are able to disperse more widely than brooding species that produce a larger number of small eggs.

The species with the greatest population differentiation*, F. gravida* is a hermaphroditic brooder, possibly capable of self-fertilization as is its sibling species *F. fragum* (Brazeau *et al*. 1998). Self-fertilization may ensure reproductive success when population densities are low and sperm limitation reduces chances of fertilization, but with the disadvantage that populations may become inbred and dominated by clonemates. Self-fertilization will also result in apparent reduced gene flow so at least part of the high differentiation among populations of *F. gravida* could reflect inbreeding.

Some Caribbean coral species are able to maintain gene flow throughout the Caribbean and Bermuda, such as *M. cavernosa* (Nunes *et al.* in review) and *M. faveolata* (Severance& Karl 2006), but dispersal within the Caribbean is more restricted for other species. Among the seven Caribbean corals studied to date, gene flow is restricted to < 500 km for most species (Vollmer& Palumbi 2007). *Acropora palmata* (Baums *et al.* 2005) and *Acropora cervicornis* (Vollmer& Palumbi 2007) are able to disperse widely, but their populations are subdivided between the eastern and western Caribbean. Gene flow appears to be restricted to even shorter distances for species such as *Agaricia agaricites* (Brazeau *et al.* 2005) and some members of the *M. annularis* species complex (Fukami *et al.* 2004; Severance& Karl 2006). Significant genetic differentiation was observed among all sampled populations of *A. agaricites*, including sites in the Florida Keys separated by only 12 km of open water (Brazeau *et al.* 2005). Populations of *M. annularis* showed significant genetic divergence across multiple populations (Severance& Karl 2006), including between the Lower Florida Keys and the Dry Tortugas, which are separated by only ~130 km. All of the aforementioned Caribbean corals are broadcasters with the exception of *A. agaricites*. In agreement with findings for Brazilian brooders, *A. agaricites* in the Caribbean has a more fragmented population than broadcasting species, although studies on additional Caribbean brooders are required to confirm the generality of this trend. Among Caribbean broadcasters, there are no clear explanations for differences in dispersal ability. Dispersal ability in *A. palmata* and *A. cervicornis* appears to be more restricted than for *M. cavernosa* and *M. faveolata*, even though Caribbean *Acropora* eggs are at

least 1.4 times greater in size (Soong 1991). Egg size may not be a good predictor of dispersal ability, however, as nutritional reserves may be allocated for settlement rather than survival in the water column. Most striking is the difference in dispersal ability among closely related species with similar reproductive traits, such as short-ranging *M. annularis* and Caribbean-wide *M. faveolata* (Severance& Karl 2006).

In the Indo-Pacific, trends in dispersal ability show even less association with reproductive mode. A survey of nine co-distributed species along 1200km of the Great Barrier Reef showed conflicting patterns between gene flow and reproductive mode. Among brooders, three species were able to maintain high levels of gene flow, while two had fragmented populations, and among the broadcasters, two species were able to maintain gene flow across the region while the other two were not (Ayre& Hughes 2000). All species showed some level of inbreeding inferred by heterozygote deficits in all populations. Likewise, reproductive mode was not a good predictor of connectivity among several coral populations along the GBR and on Lord Howe Island. Among 5 co-distributed coral species, the brooder *Styllophora pistillata*, showed the highest estimate of gene flow while *Seriotopora hystrix*, another brooder, had the lowest (Ayre& Hughes 2004).

Another unexpected pattern in connectivity has been observed between two broadcasting species with very different life history characteristics (Miller& Ayre 2008). *Platygyra danalae,* which is self-incompatible and has positively buoyant gametes with planktonic development of larvae and *Goniastrea favulus*, which produces negatively buoyant eggs and larvae (Miller& Ayre 2008). Estimates of

larval longevity are also different for the two species, with planulae of *P. danalae* living 124 days and *G. favulus* 60+ days (Graham *et al.* 2008). Surprisingly, both species have similar levels of fine to meso-scale population subdivision, and *G. favulus* has higher genetic diversity than the presumably more outcrossed *P. danalae*. Determining which life history traits impart greater success in long-distance dispersal is not straightforward. In Atlantic corals, reproductive mode, and to some extent egg size, appear to provide an advantage for long-distance dispersal, but this trend is not observed for corals in the Indo-Pacific, where both brooding and broadcasting species can either maintain connectivity across long distances or have restricted dispersal. In the Atlantic, brooders tend to build small colonies that inhabit marginal and disturbed environments, but in the Indo-Pacific, some brooding colonies can be large and may be expected to have higher fecundity than Atlantic brooders. These results indicate that single aspects of coral life history, such as reproductive mode, egg size or larval longevity, cannot alone predict dispersal ability and that more likely the complex interactions of multiple factors play a role in determining connectivity. In other organisms, aspects of reproduction and life history, when taken alone, have at times also not been good predictors of dispersal ability and connectivity. In reef fish of the Atlantic, for example, pelagic larval duration is not a good predictor of population connectivity (Bowen *et al.* 2006).

While dispersal ability is an important factor for the maintenance of connectivity, environmental factors may also play an important role in whether migrants can become established and contribute to gene flow in distant reefs. The

transport of larvae is the first step to successful long distance migration, but recruits must also be able to adapt to the environment at a distant location. Interactions with other organisms can also affect successful recruitment. Areas where there is high species diversity and abundance have high competition for benthic space, and incoming migrants must overcome competition. The GBR is oriented along a latitudinal gradient, which creates a large environmental gradient in seasonality and other physical factors. Some of the differences in gene flow across species in this region may be related not only to reproductive mode or dispersal ability, but also to differences in their ability to adapt to environments at different latitudes, such as a greater range in seasonality. The Caribbean, on the other hand, has an east-west orientation within the same band of latitude. Species capable of dispersing widely within this biogeographic region experience more modest differences in environment and seasonality, making successful gene flow more dependent on aspects of reproduction and less affected by environment. Differences in geographic configuration and the resulting environmental gradients between the Caribbean and Indo-Pacific may therefore be reflected on patterns of connectivity in the two basins. Competition for space with macroalgae may be important in Brazil, as algae are abundant in coastal environments (Castro& Pires 2001), but in West Africa, much of the benthic space is open (personal observation), and competition with algae may be less important. Environmental factors that limit recruitment, however, may be strong in areas where corals are already stressed by suboptimal environmental conditions, resulting in reduced connectivity with respect to these areas. In sum, patterns in

connectivity are difficult to predict based on reproductive traits alone and are likely influenced by multiple biotic and abiotic factors.

Patterns of genetic diversity

Mean values of molecular diversity indices overlap among regions for most coral species, although for some comparisons, diversity appears to be lower in the peripheral populations, such as gene diversity in *S. siderea* of West Africa for both loci and *F.gravida* in West Africa for Pax-C (Fig 3.1 – 3.3). On average, however, there does not appear to be a strong trend in decreasing genetic diversity towards the edges of the species range for most species.

This finding is at odds with patterns observed in populations of the coral *M. cavernosa*, where genetic diversity was found to be lower in Brazil and West Africa compared to the Caribbean (Nunes *et al.* in press). Similarly, reduced allozyme allelic diversity and heterozygosity was also found for several coral species on the isolated Lord Howe Island relative to populations on the Great Barrier Reef (Ayre& Hughes 2004) as would be predicted by small population size and founder effects. Peripheral populations that are isolated from the center of species range or center of diversity may have smaller effective population size (N_e) and can suffer loss of diversity via genetic drift. In addition, peripheral populations colonized by a small group of founders may only contain a subset of the full diversity of alleles observed in the main population. However, loss of genetic diversity in Brazil and West Africa does not appear to be a consistent trend among the coral species studied here, or if differences

in genetic diversity exist among regions, they have not been captured with the markers used here.

Overall, levels of polymorphism and the number of haplotypes for the brooding species *F. fragum*, *F. gravida*, *P. astreoides* and *S. radians* were much lower than for the broadcasters *S. siderea* and *M. cavernosa*. For example, 42 – 70 haplotypes were observed for *M. cavernosa* depending on the nuclear marker used and 40 haplotypes were observed for *S. radians*, while the number of haplotypes observed for the four brooding species ranged from 6 – 12. High levels of polymorphism in *M. cavernosa* may have made the patterns of reduced variation in the peripheral populations more pronounced and readily detected. In *S. siderea*, decreasing trends in gene diversity (h) are observed, but there is overlap in mean values of nucleotide diversity (π) and average number of differences (k) among Caribbean, Brazilian and West African populations (Fig 3.1 – 3.3).

Because of the small number of sampled populations (only one population sampled in the Caribbean and in West Africa), only a few measurements were available to estimate regional differences in genetic diversity. This may have reduced the ability to detect differences in genetic diversity among regions. Sampling which includes a greater number of populations in the future may provide more robust results on whether genetic diversity is similar across regions are whether small but significant differences in diversity exist between central and peripheral populations across multiple species.

Regional differences in genetic diversity may have gone undetected, but another reasonable alternative is that the available data reflect true patterns, and that genetic diversity is consistent across the Atlantic for all species but *M. cavernosa*. One possibility is that loss of variation has occurred in peripheral populations of *M. cavernosa*, but not in other species. Although regional isolation is observed for nearly all species, the mechanisms that lead to reduced variation may be less effective in some species relative to others. Loss of variation resulting from genetic drift is most effective in populations with small effective size (N_e). Differences in N_e between the species may result in different rates in the loss of genetic diversity. Founder effects may also be less pronounced if peripheral populations have received multiple founding events. Some of the brooding species studied have multiple mating cycles per year and reproductive strategies which may allow them to establish larger populations over shorter time periods, thereby increasing N_e and buffering the loss of genetic diversity. Another possible scenario is that historically some of the other species were able to maintain gene flow up until more recently, while gene flow for *M. cavernosa* ended a longer time in the past – subjecting the species to a longer period of time for which drift could act upon.

CONCLUSIONS

Patterns of connectivity across broad regions of the Atlantic are congruent across multiple species of corals, suggesting that barriers to dispersal such as the Amazon freshwater plume and the long distances that separate the east and west South

Atlantic are effective for most coral species. At shorter, regional scales, it appears that some aspects of reproduction such as mode of larval development and egg size can influence gene flow, although this pattern cannot be generalized to all scenarios. Broadcasters have greater dispersal ability in the Caribbean, but several exceptions exist in the Indo-Pacific, suggesting that complex interactions between biotic and abiotic factors can limit connectivity rather than single traits of a species. Loss of genetic diversity in peripheral populations of corals is also not a general trend observed among all amphi-Atlantic corals, likely due to differences in population size and timing of isolation across species. Regional isolation may mean that the persistence of peripheral populations relies primarily on local to regional recruitment since migration from other regions must occur rarely. These populations may be more vulnerable to disturbance on ecological time scales as a result of their isolation. On the other hand, isolated peripheral populations with small effective population size may become the sites for local adaptation and allopatric speciation. In both scenarios, these regions require special attention for conservation as a result of their potential vulnerability to environmental change, but also their importance in generating diversity.

ACKNOWLEDGEMENTS

Chapter III, in part, is currently being prepared for submission for publication of the material. Nunes, Flavia; Norris, Richard D.; Knowlton, Nancy. The dissertation author was the primary investigator and author of this paper.

REFERENCES

Atoda K (1947a) The larva and postlarval development of some reef-building corals. I. Pocillopora damicornis cespitosa (Dana). *Science Reports of the Tohoku Imperial University, 4th Series (Biology)* **18**, 24-47.

Atoda K (1947b) The larva and postlarval development of some reef-building corals. II. Stylophora pistillata (Esper). *Science Reports of the Tohoku Imperial University, 4th Series (Biology)* **18**, 48-64.

Atoda K (1951) The larva and postlarval development of some reef-building corals. V. Seriatopora hystrix Dana. *Reports of the Tohoku University, 4th Ser.(Biology)* **19**, 33-39.

Avise JC (2000) *Phylogeography: the history and formation of species* Harvard University Press, Cambridge, MA.

Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. *Evolution* **54**, 1590-1605.

Ayre DJ, Hughes TP (2004) Climate change, genotypic diversity and gene flow in reef-building corals. *Ecology Letters* **7**, 273-278.

Baird A (1998) The length of the larval phase in corals: new insights into patterns of reef connectivity. *Australian Coral Reef Society Newsletter* **27**, 6-8.

Baums IB, Miller MW, Hellberg ME (2005) Regionally isolated populations of an imperiled Caribbean coral, Acropora palmata. *Molecular Ecology* **14**, 1377-1390.

Bowen BW, Bass AL, Muss A, Carlin JL, Robertson DR (2006) Phylogeography of two Atlantic squirrelfishes (Family Holocentridae): exploring links between pelagic larval duration and population connectivity. *Marine Biology* **149**, 899-913.

Brazeau DA, Gleason DF, Morgan ME (1998) Self-fertilization in brooding hermaphroditic Caribbean corals: Evidence from molecular markers. *Journal of Experimental Marine Biology and Ecology* **231**, 225-238.

Brazeau DA, Sammarco PW, Gleason DF (2005) A multi-locus genetic assignment technique to assess sources of Agaricia agaricites larvae on coral reefs. *Marine Biology* **147**, 1141-1148.

Castro CB, Pires DO (2001) Brazilian coral reefs: What we already know and what is still missing. *Bulletin of Marine Science* **69**, 357-371.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**, 1657-1659.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**, 47-50.

Fukami H, Budd AF, Levitan DR*, et al.* (2004) Geographic differences in species boundaries among members of the Montastraea annularis complex based on molecular and morphological markers. *Evolution* **58**, 324-337.

Graham EM, Baird AH, Connolly SR (2008) Survival dynamics of scleractinian coral larvae and implications for dispersal. *Coral Reefs* **27**, 529-539.

Harrison PL, Wallace C (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: *Ecosystems of the World vol. 25, Coral Reefs* (ed. Dubinsky Z), pp. 133-208. Elsevier, Amsterdam.

Hoorn C, Guerrero J, Sarmiento GA, Lorente MA (1995) Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. *Geology* **23**, 237-240.

Laborel J (1969) Madreporaires et hydrocoralliaires recifaux des côtes brésiliennes. Systematique, ecologie, répartition verticale et geographique. *Annales De L' Institut Oceanographique de Paris* **47**, 171-229.

Lentz SJ (1995) Seasonal variations in the horizontal structure of the Amazon plume inferred from historical hydrographic data. *Journal of Geophysical Research-Oceans* **100**, 2391-2400.

Lessios HA, Kane J, Robertson DR (2003) Phylogeography of the pantropical sea urchin Tripneustes: Contrasting patterns of population structure between oceans. *Evolution* **57**, 2026-2036.

Lessios HA, Kessing BD, Pearse JS (2001) Population structure and speciation in tropical seas: Global phylogeography of the sea urchin Diadema. *Evolution* **55**, 955-975.

Lessios HA, Kessing BD, Robertson DR, Paulay G (1999) Phylogeography of the pantropical sea urchin Eucidaris in relation to land barriers and ocean currents. *Evolution* **53**, 806-817.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451-1452.

Lopez JV, Knowlton N (1997) Discrimination of species in the Montastraea annularis complex using multiple genetic loci. *Proceedings of the 8th International Coral Reef Symposium* **2**, 1613-1618.

McCartney MA, Keller G, Lessios HA (2000) Dispersal barriers in tropical oceans and speciation in Atlantic and eastern Pacific sea urchins of the genus Echinometra. *Molecular Ecology* **9**, 1391-1400.

Miller KJ, Ayre DJ (2008) Population structure is not a simple function of reproductive mode and larval type: insights from tropical corals. *Journal of Animal Ecology* **77**, 713-724.

Muller-Karger FE, McClain CR, Richardson PL (1988) The dispersal of the Amazons water. *Nature* **333**, 56-59.

Muss A, Robertson DR, Stepien CA, Wirtz P, Bowen BW (2001) Phylogeography of Ophioblennius: The role of ocean currents and geography in reef fish evolution. *Evolution* **55**, 561-572.

Neves E, Johnsson R, Sampaio C, Pichon M (2006) The occurrence of <u>Scolymia cubensis</u> in Brazil: revising the problem of the Caribbean solitary mussids. *Zootaxa*, 45-54.

Neves EG, Andrade SCS, da Silveira FL, Solferini VN (2008) Genetic variation and population structuring in two brooding coral species (<u>Siderastrea stellata</u> and <u>Siderastrea radians</u>) from Brazil. *Genetica* **132**, 243-254.

Nikiema O, Devenon JL, Baklouti M (2007) Numerical modeling of the Amazon River plume. *Continental Shelf Research* **27**, 873-899.

Nunes F, Fukami H, Vollmer SV, Norris RD, Knowlton N (2008) Re-evaluation of the systematics of the endemic corals of Brazil by molecular data. *Coral Reefs* **27**, 423-432.

Nunes F, Norris RD, Knowlton N (accepted) Implications of low genetic diversity in peripheral isolates of an amphi-Atlantic coral. *Molecular Ecology*

Richmond RH (1987) Energetics, competence, and long-distance dispersal of planula larvae of the coral <u>Pocillopora damicornis</u>. *Marine Biology* **93**, 527-533.

Rocha LA (2004) Mitochondrial DNA and color pattern variation in three western Atlantic Halichoeres (labridae), with the revalidation of two species. *Copeia*, 770-782.

Rocha LA, Bass AL, Robertson DR, Bowen BW (2002) Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonfishes (Teleostei : Acanthuridae). *Molecular Ecology* **11**, 243-252.

Severance EG, Karl SA (2006) Contrasting population genetic structures of sympatric, mass-spawning Caribbean corals. *Marine Biology* **150**, 57-68.

Shearer TL, Van Oppen MJH, Romano SL, Worheide G (2002) Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). *Molecular Ecology* **11**, 2475-2487.

Soong K (1991) Sexual reproductive [atterns of shallow-water reef corals in Panama. *Bulletin of Marine Science* **49**, 832-846.

Stephens M, Scheet P (2005) Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. *American Journal of Human Genetics* **76**, 449-462.

Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics* **68**, 978-989.

Szmant AM (1986) Reproductive Ecology of Caribbean Reef Corals. *Coral Reefs* **5**, 43-53.

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

Van Oppen MJH, Willis BL, Van Vugt H, Miller DJ (2000) Examination of species boundaries in the Acropora cervicornis group (Scleractinia, Cnidaria) using nuclear DNA sequence analyses. *Molecular Ecology* **9**, 1363-1373.

Vermeij MJA, Fogarty ND, Miller MW (2006) Pelagic conditions affect larval behavior, survival, and settlement patterns in the Caribbean coral Montastraea faveolata. *Marine Ecology-Progress Series* **310**, 119-128.

Veron JEN, Stafford-Smith M (2000) *Corals of the World* Australian Institute of Marine Science.

Vollmer SV, Palumbi SR (2007) Restricted gene flow in the Caribbean staghorn coral Acropora cervicornis: Implications for the recovery of endangered reefs. *Journal of Heredity* **98**, 40-50.

SUPPLEMENTARY TABLES

Supplementary Table 3.1. **Coral species list for the Atlantic. Species occurrence in each biogeographic region is indicated by an X. Total species count for each region is found at the bottom of each column.**

Species		Caribbean	Brazil	W. Africa
Acropora	*cervicornis*	X		
Acropora	*palmata*	X		
Agaricia	*fragilis*	X	X	
Agaricia	*undata*	X		
Agaricia	*agaricites*	X	X	
Agaricia	*grahamae*	X		
Agaricia	*humilis*	X		
Agaricia	*tenuifolia*	X		
Agaricia	*lamarcki*	X		
Cladocora	*arbuscula*	X		
Cladocora	*debilis*	X		
Colpophyllia	*natans*	X		
Dendrogyra	*cylindrus*	X		
Dichocoenia	*stellaris*	X		
Dichocoenia	*stokesii*	X		
Diploria	*clivosa*	X		
Diploria	*labyrinthiformis*	X		
Diploria	*strigosa*	X		
Eusmilia	*fastigiata*	X		
Favia	*leptophylla*		X	
Favia	*fragum*	X		X
Favia	*gravida*		X	X
Helioseris	*cucullata*	X		
Isophyllastrea	*rigida*	X		
Isophyllia	*sinuosa*	X		
Leptoseris	*cailleti*	X		
Madracis	*asperula*	X	X	X
Madracis	*brueggemanni*	X	X	
Madracis	*carmabi*	X		
Madracis	*auretenra*	X		
Madracis	*decactis*	X	X	X
Madracis	*formosa*	X		
Madracis	*myriaster*	X		

Supplementary Table 1 (continued)

Species		Caribbean	Brazil	W. Africa
Madracis	*pharensis*	X	X	X
Madracis	*profunda*			X
Madracis	*senaria*	X		
Manicina	*areolata*	X		
Meandrina	*braziliensis*		X	
Meandrina	*danae*	X		
Meandrina	*meandrites*	X		
Montastraea	*annularis*	X		
Montastraea	*faveolata*	X		
Montastraea	*cavernosa*	X	X	X
Montastraea	*franksi*	X		
Mussa	*angulosa*	X		
Mussismilia	*braziliensis*		X	
Mussismilia	*harttii*		X	
Mussismilia	*hispida*		X	
Mycetophyllia	*aliciae*	X		
Mycetophyllia	*danaana*	X		
Mycetophyllia	*ferox*	X		
Mycetophyllia	*lamarckiana*	X		
Mycetophyllia	*reesi*	X		
Oculina	*arbuscula*	X		
Oculina	*diffusa*	X		
Oculina	*patagonica*	X		
Oculina	*robusta*	X		
Oculina	*tenella*	X		
Oculina	*valenciennesi*	X		
Oculina	*varicosa*	X		
Porites	*astreoides*	X	X	X
Porites	*bernardi*			X
Porites	*branneri*	X	X	
Porites	*colonensis*	X		
Porites	*divaricata*	X		
Porites	*furcata*	X		
Porites	*hentscheli*			X
Porites	*porites*	X		X
Schizoculina	*africana*			X
Schizoculina	*fissipara*			X

Supplementary Table 1 (continued)

Species		Caribbean	Brazil	W. Africa
Scolymia	*cubensis*	X	X	
Scolymia	*lacera*	X		
Scolymia	*wellsi*	X	X	
Siderastrea	*stellata*	X	X	X
Siderastrea	*radians*	X	X	X
Siderastrea	*siderea*	X	X	X
Solenastrea	*bournoni*	X		
Solenastrea	*hyades*	X		
Stephanocoenia	*intersepta*	X	X	
Tubastraea	*coccinea*	X	X	X
Tubastraea	*tagusensis*		X	X
TOTAL	81	69	23	18

Supplementary Table 3.2. **Haplotype count for each species, locus and population.**

Favia gravida & Favia fragum, Pax-C

Haplotype	BR1 Abrolhos	BR2 João Pessoa	CA1 Panamá	WA1 Mainland
FFP1	16	12	0	0
FFP2	10	34	0	40
FFP3	0	0	4	0
FFP4	0	0	15	0
FFP5	0	0	13	0
Total	26	46	32	40

Favia gravida & Favia fragum, b-tubulin

Haplotype	BR1 Abrolhos	BR2 João Pessoa	CA1 Panamá	WA1 Mainland
FFB1	33	21	0	19
FFB2	5	0	0	0
FFB3	0	6	0	0
FFB4	0	25	0	0
FFB5	0	0	24	0
FFB6	0	0	4	0
FFB7	0	0	4	0
FFB8	0	0	4	0
FFB9	0	0	0	9
FFB10	0	0	0	12
Total	38	52	36	40

Porites astreoides - b-tubulin

Haplotype	BR2 João Pessoa	CA1 Panamá
PAB1	20	0
PAB2	16	8
PAB3	5	0
PAB4	1	0
PAB5	0	9
PAB6	0	5
PAB7	0	2
Total	42	24

Supplementary Table 2 (continued)

S. radians, Pax-C

Haplotype	BR1 Abrolhos	BR2 João Pessoa	BR3 Fortaleza	CA1 Panamá	WA1 Mainland
SRP1	8	6	10	15	25
SRP2	6	4	6	0	15
SRP3	0	12	0	0	1
SRP4	0	4	4	0	2
SRP5	0	1	0	1	0
SRP6	0	0	0	2	0
SRP7	0	0	0	2	0
SRP8	0	0	0	1	0
SRP9	0	0	0	0	1
Total	14	27	20	21	44

S. radians, b-tubulin

Haplotype	BR1 Abrolhos	BR2 João Pessoa	BR3 Fortaleza	CA1 Panamá	WA1 Mainland
SRB1	2	0	0	0	0
SRB2	2	0	6	6	18
SRB3	1	0	0	0	1
SRB4	1	1	0	0	0
SRB5	0	10	0	0	0
SRB6	0	1	4	0	7
SRB7	0	2	0	0	0
SRB8	0	0	4	1	1
SRB9	0	0	0	5	0
SRB10	0	0	0	4	13
SRB11	0	0	0	1	0
SRB12	0	0	0	1	0
SRB13	0	0	0	1	0
SRB14	0	0	0	1	0
Total	6	14	14	20	40

Supplementary Table 2 (continued)

S. siderea, Pax-C

Haplotype	BR2 João Pessoa	BR3 Fortaleza	CA1 Panamá	WA1 Mainland
SSP1	20	12	2	34
SSP2	5	5	0	0
SSP3	4	1	6	0
SSP4	0	1	0	0
SSP5	0	1	0	0
SSP6	0	0	3	0
SSP7	0	0	4	0
SSP8	0	0	7	0
SSP9	0	0	2	0
SSP10	0	0	1	0
SSP11	0	0	2	0
SSP12	0	0	0	2
Total	29	20	27	36

S. siderea, b-tubulin

Haplotype	BR1 Abrolhos	BR2 João Pessoa	BR3 Fortaleza	CA1 Panamá	WA1 Mainland
SSB1	2	1	1	0	0
SSB2	1	3	0	1	0
SSB3	1	2	0	0	0
SSB4	2	3	0	0	0
SSB5	1	0	0	0	0
SSB6	2	1	3	0	0
SSB7	1	0	0	0	0
SSB8	0	9	7	0	0
SSB9	0	2	0	0	0
SSB10	0	2	0	0	0
SSB11	0	1	0	0	0
SSB12	0	1	0	0	0
SSB13	0	1	0	0	0
SSB14	0	4	1	0	0

Supplementary Table 2 (continued)

S. siderea, b-tubulin (continued)

Haplotype	BR1 Abrolhos	BR2 João Pessoa	BR3 Fortaleza	CA1 Panamá	WA1 Mainland
SSB15	0	1	0	0	0
SSB16	0	2	0	0	0
SSB17	0	1	0	0	0
SSB18	0	1	0	0	0
SSB19	0	1	0	0	0
SSB20	0	1	0	0	0
SSB21	0	1	0	0	0
SSB22	0	0	3	0	0
SSB23	0	0	2	0	0
SSB24	0	0	1	0	0
SSB25	0	0	6	0	0
SSB26	0	0	0	1	0
SSB27	0	0	0	1	0
SSB28	0	0	0	1	0
SSB29	0	0	0	1	0
SSB30	0	0	0	1	0
SSB31	0	0	0	1	0
SSB32	0	0	0	1	0
SSB33	0	0	0	1	0
SSB34	0	0	0	1	0
SSB35	0	0	0	1	0
SSB36	0	0	0	3	35
SSB37	0	0	0	2	0
SSB38	0	0	0	1	0
SSB39	0	0	0	1	0
SSB40	0	0	0	1	0
SSB41	0	0	0	1	0
SSB42	0	0	0	1	0
SSB43	0	0	0	1	0
SSB44	0	0	0	1	0
SSB45	0	0	0	1	0
SSB46	0	0	0	1	0

Supplementary Table 2 (continued)

S. siderea, b-tubulin (continued)

Haplotype	BR1 Abrolhos	BR2 João Pessoa	BR3 Fortaleza	CA1 Panamá	WA1 Mainland
SSB47	0	0	0	1	0
SSB48	0	0	0	1	0
SSB49	0	0	0	1	0
SSB50	0	0	0	4	0
SSB51	0	0	0	1	0
SSB52	0	0	0	1	0
SSB53	0	0	0	1	0
SSB54	0	0	0	1	0
SSB55	0	0	0	1	0
SSB56	0	0	0	1	0
SSB57	0	0	0	0	2
SSB58	0	0	0	0	1
SSB59	0	0	0	0	1
SSB60	0	0	0	0	1
TOTAL	10	38	24	38	40

Supplementary Table 3.3. **Genotype over all loci for each sampled individual. Haplotype sequences can be found on Genbank.**

Sample	β-tubulin				Pax-C			
Favia fragum								
Bocas del Toro, Panamá								
P016	FFB	06	FFB	06				
P039	FFB	08	FFB	08	FFP	03	FFP	03
P040	FFB	08	FFB	08	FFP	03	FFP	03
P041	FFB	07	FFB	07	FFP	04	FFP	04
P106	FFB	05	FFB	05	FFP	05	FFP	05
P107	FFB	05	FFB	05	FFP	05	FFP	04
P108	FFB	05	FFB	05	FFP	04	FFP	04
P109	FFB	05	FFB	05	FFP	05	FFP	05
P110	FFB	05	FFB	05	FFP	05	FFP	05
P111	FFB	05	FFB	05				
P112	FFB	07	FFB	07	FFP	04	FFP	04
P113	FFB	05	FFB	05	FFP	05	FFP	05
P114	FFB	06	FFB	06	FFP	04	FFP	04
P115	FFB	05	FFB	05	FFP	04	FFP	04
P116	FFB	05	FFB	05	FFP	05	FFP	05
P396	FFB	05	FFB	05	FFP	05	FFP	05
P457	FFB	05	FFB	05	FFP	04	FFP	04
P458	FFB	05	FFB	05	FFP	04	FFP	04
Favia gravida								
Abrolhos, Brazil								
B015	FFB	01	FFB	01	FFP	01	FFP	01
B016	FFB	01	FFB	01	FFP	01	FFP	01
B017	FFB	02	FFB	02	FFP	01	FFP	01
B018	FFB	01	FFB	01				
B042	FFB	01	FFB	01	FFP	01	FFP	01
B043	FFB	01	FFB	01	FFP	01	FFP	01
B072	FFB	01	FFB	01				
B073	FFB	01	FFB	01	FFP	02	FFP	02
B074	FFB	01	FFB	01				
B075	FFB	01	FFB	01	FFP	02	FFP	02

Supplementary Table 3.3 (continued)

Sample	β-tubulin				Pax-C			
B079	FFB	01	FFB	01	FFP	02	FFP	02
B080	FFB	01	FFB	01	FFP	01	FFP	01
B081	FFB	01	FFB	01				
B082	FFB	01	FFB	01	FFP	01	FFP	01
B083	FFB	01	FFB	01				
B084	FFB	01	FFB	01				
B085	FFB	02	FFB	02	FFP	02	FFP	02
B086	FFB	01	FFB	01	FFP	01	FFP	01
B087	FFB	01	FFB	02	FFP	02	FFP	02

João Pessoa, Brazil

Sample	β-tubulin				Pax-C			
B150	FFB	03	FFB	03	FFP	02	FFP	02
B162	FFB	04	FFB	04	FFP	02	FFP	02
B164	FFB	04	FFB	04	FFP	02	FFP	02
B170	FFB	04	FFB	04	FFP	02	FFP	02
B178	FFB	04	FFB	04	FFP	02	FFP	02
B209	FFB	01	FFB	01	FFP	01	FFP	01
B210	FFB	01	FFB	01	FFP	01	FFP	01
B211	FFB	01	FFB	01	FFP	01	FFP	01
B242	FFB	03	FFB	03				
B253	FFB	01	FFB	01	FFP	02	FFP	02
B254	FFB	01	FFB	01	FFP	02	FFP	02
B255	FFB	04	FFB	01	FFP	01	FFP	01
B256	FFB	03	FFB	03	FFP	02	FFP	02
B257	FFB	04	FFB	04	FFP	02	FFP	02
B258	FFB	01	FFB	01	FFP	02	FFP	02
B259	FFB	01	FFB	01	FFP	02	FFP	02
B261	FFB	01	FFB	01	FFP	01	FFP	01
B262	FFB	04	FFB	04	FFP	02	FFP	02
B263	FFB	04	FFB	04	FFP	02	FFP	02
B264	FFB	01	FFB	01	FFP	01	FFP	01
B265	FFB	01	FFB	01				
B266	FFB	04	FFB	04				
B300	FFB	04	FFB	04	FFP	02	FFP	02
B309	FFB	04	FFB	04	FFP	02	FFP	02
B313	FFB	04	FFB	04	FFP	02	FFP	02
B315	FFB	04	FFB	04	FFP	02	FFP	02

Supplementary Table 3.3 (continued)

Sample	β-tubulin				Pax-C			
São Tomé, West Africa								
ST014	FFB	09	FFB	09	FFP	02	FFP	02
ST015	FFB	09	FFB	09	FFP	02	FFP	02
ST016	FFB	01	FFB	01	FFP	02	FFP	02
ST017	FFB	01	FFB	01	FFP	02	FFP	02
ST018	FFB	09	FFB	09	FFP	02	FFP	02
ST019	FFB	01	FFB	01	FFP	02	FFP	02
ST122	FFB	10	FFB	10	FFP	02	FFP	02
ST123	FFB	10	FFB	10	FFP	02	FFP	02
ST124	FFB	10	FFB	10	FFP	02	FFP	02
ST125	FFB	10	FFB	10	FFP	02	FFP	02
ST126	FFB	10	FFB	10	FFP	02	FFP	02
ST127	FFB	10	FFB	10	FFP	02	FFP	02
ST370	FFB	01	FFB	01	FFP	02	FFP	02
ST371	FFB	01	FFB	01	FFP	02	FFP	02
ST372	FFB	09	FFB	09	FFP	02	FFP	02
ST373	FFB	09	FFB	01	FFP	02	FFP	02
ST374	FFB	01	FFB	01	FFP	02	FFP	02
ST375	FFB	01	FFB	01	FFP	02	FFP	02
ST376	FFB	01	FFB	01	FFP	02	FFP	02
ST377	FFB	01	FFB	01	FFP	02	FFP	02

Porites astreoides

Sample	β-tubulin			
João Pessoa, Brazil				
B140	PAB	01	PAB	02
B142	PAB	01	PAB	02
B143	PAB	01	PAB	03
B148	PAB	04	PAB	02
B223	PAB	01	PAB	03
B224	PAB	01	PAB	02
B225	PAB	01	PAB	03
B226	PAB	01	PAB	02
B231	PAB	01	PAB	02
B232	PAB	01	PAB	02
B271	PAB	01	PAB	03
B272	PAB	01	PAB	02

Supplementary Table 3.3 (continued)

Sample	β-tubulin				Pax-C			
B273	PAB	01	PAB	02				
B274	PAB	01	PAB	02				
B275	PAB	01	PAB	02				
B280	PAB	01	PAB	02				
B285	PAB	01	PAB	02				
B295	PAB	01	PAB	03				
B296	PAB	01	PAB	02				
B297	PAB	01	PAB	02				
B298	PAB	01	PAB	02				

Bocas del Toro, Panamá

Sample	β-tubulin				Pax-C			
P004	PAB	05	PAB	02				
P018	PAB	02	PAB	06				
P058	PAB	05	PAB	02				
P059	PAB	02	PAB	06				
P136	PAB	05	PAB	06				
P137	PAB	05	PAB	07				
P138	PAB	05	PAB	07				
P139	PAB	02	PAB	06				
P140	PAB	05	PAB	02				
P141	PAB	05	PAB	02				
P142	PAB	05	PAB	05				
P143	PAB	02	PAB	06				

Siderastrea radians

Abrolhos, Brazil

Sample	β-tubulin				Pax-C			
B092	SB	201	SB	202	SP	201	SP	201
B093	SB	201	SB	202	SP	201	SP	201

João Pessoa, Brazil

Sample	β-tubulin				Pax-C			
B133					SP	203	SP	203
B134					SP	203	SP	203
B146	SB	205	SB	205	SP	202	SP	202
B194	SB	205	SB	205	SP	201	SP	202
B283	SB	205	SB	205	SP	201	SP	201

Supplementary Table 3.3 (continued)

Sample	β-tubulin				Pax-C			
Fortaleza, Brazil								
B358	SB	202	SB	208	SP	201	SP	204
B360	SB	202	SB	208	SP	201	SP	204
B361	SB	202	SB	208	SP	201	SP	204
B367	SB	202	SB	208	SP	201	SP	204
Bocas del Toro, Panamá								
P042	SB	202	SB	209	SP	206	SP	207
P043	SB	202	SB	209	SP	206	SP	207
P195	SB	210	SB	209	SP	201	SP	201
P196	SB	210	SB	211	SP	201	SP	201
P197	SB	202	SB	202	SP	201	SP	201
P198	SB	212	SB	213	SP	201	SP	201
P199	SB	210	SB	209	SP	201	SP	201
P200	SB	208	SB	214	SP	201	SP	208
P201	SB	202	SB	202	SP	201	SP	201
P202	SB	210	SB	209	SP	201	SP	201
São Tomé, West Africa								
ST010	SB	210	SB	202	SP	201	SP	202
ST024	SB	210	SB	202	SP	201	SP	204
ST034	SB	210	SB	202	SP	201	SP	202
ST075	SB	210	SB	202	SP	201	SP	202
ST076	SB	210	SB	202	SP	201	SP	202
ST077	SB	210	SB	202	SP	201	SP	202
ST078	SB	210	SB	202	SP	201	SP	202
ST133	SB	210	SB	202	SP	202	SP	202
ST223	SB	202	SB	206	SP	201	SP	202
ST224	SB	210	SB	202	SP	201	SP	202
ST225	SB	210	SB	202	SP	201	SP	202
ST226	SB	202	SB	206	SP	201	SP	202
ST311	SB	203	SB	202	SP	201	SP	201
ST335	SB	210	SB	206	SP	201	SP	209
ST351	SB	202	SB	206	SP	201	SP	202
ST354	SB	210	SB	206	SP	201	SP	202
ST355	SB	202	SB	208	SP	201	SP	204
ST362	SB	210	SB	202	SP	201	SP	202

Supplementary Table 3.3 (continued)

Sample	β-tubulin				Pax-C			

Siderastrea siderea

Abrolhos, Brazil

Sample								
B096	SB	103	SB	104				

João Pessoa, Brazil

Sample								
B131	SB	105	SB	102	SP	101	SP	102
B135	SB	108	SB	109	SP	101	SP	101
B141	SB	111	SB	111	SP	101	SP	101
B149	SB	101	SB	114	SP	103	SP	103
B161	SB	103	SB	117	SP	103	SP	101
B185	SB	105	SB	105	SP	101	SP	101
B186	SB	118	SB	103	SP	103	SP	101
B192	SB	119	SB	119	SP	101	SP	102
B199	SB	104	SB	104	SP	101	SP	101
B240	SB	120	SB	108	SP	101	SP	102
B241	SB	108	SB	108	SP	101	SP	101
B245	SB	108	SB	108	SP	101	SP	102
B252	SB	108	SB	103	SP	101	SP	101
B277	SB	119	SB	119	SP	101	SP	102

Fortaleza, Brazil

Sample								
B325	SB	108	SB	108	SP	101	SP	102
B326	SB	108	SB	102	SP	101	SP	101
B327	SB	101	SB	108	SP	104	SP	105
B330	SB	122	SB	122	SP	102	SP	102
B332	SB	122	SB	119	SP	101	SP	101
B333	SB	108	SB	123	SP	101	SP	102
B345	SB	108	SB	123	SP	103	SP	101
B349						101	SP	102
B350	SB	108	SB	124	SP	101	SP	101
B355	SB	102	SB	102	SP	101	SP	101

Bocas del Toro, Panamá

Sample								
P007	SB	126	SB	127	SP	107	SP	103
P053	SB	128	SB	129	SP	108	SP	108
P054	SB	130	SB	131	SP	107	SP	101

Supplementary Table 3.3 (continued)

Sample	β-tubulin				Pax-C			
P055	SB	132	SB	133	SP	106	SP	103
P088	SB	134	SB	135	SP	106	SP	103
P089	SB	103	SB	136	SP	103	SP	103
P090	SB	137	SB	138	SP	109	SP	109
P091	SB	139	SB	140	SP	107	SP	107
P093	SB	136	SB	143	SP	108	SP	108
P094	SB	144	SB	145	SP	103	SP	110
P095	SB	146	SB	147	SP	108	SP	101
P219	SB	148	SB	149				
P220	SB	150	SB	150				
P222	SB	151	SB	150				
P223	SB	150	SB	152	SP	111	SP	111
P224	SB	137	SB	153				
P502	SB	154	SB	155	SP	108	SP	108
P504	SB	156	SB	136				
São Tomé, West Africa								
ST011	SB	136	SB	136	SP	101	SP	101
ST012	SB	136	SB	136	SP	101	SP	101
ST025	SB	136	SB	136	SP	101	SP	101
ST031	SB	136	SB	136	SP	101	SP	101
ST079	SB	136	SB	136	SP	101	SP	101
ST119	SB	136	SB	136	SP	101	SP	101
ST120	SB	136	SB	136	SP	101	SP	101
ST121	SB	136	SB	136	SP	101	SP	101
ST134	SB	136	SB	136	SP	101	SP	101
ST137	SB	136	SB	136	SP	101	SP	101
ST144	SB	136	SB	136	SP	101	SP	101
ST145	SB	136	SB	136	SP	101	SP	101
ST190	SB	158	SB	136	SP	101	SP	101
ST191	SB	136	SB	136	SP	101	SP	101
ST198	SB	136	SB	136	SP	112	SP	112
ST292	SB	136	SB	136	SP	101	SP	101
ST314	SB	136	SB	136	SP	101	SP	101
ST349	SB	136	SB	160	SP	101	SP	101

Supplementary Table 3.3 (continued)

Sample	β-tubulin				Pax-C			

Siderastrea stellata (*S. radians* x *S. siderea* hybrids)

Abrolhos, Brazil

Sample								
B094	SB	101	SB	101	SP	202	SP	202
B095	SB	102	SB	203	SP	201	SP	201
B097	SB	105	SB	106	SP	202	SP	202
B098	SB	105	SB	102	SP	202	SP	202
B099	SB	107	SB	204	SP	201	SP	201

João Pessoa, Brazil

Sample								
B136	SB	110	SB	205	SP	201	SP	203
B144	SB	205	SB	112	SP	203	SP	203
B145	SB	204	SB	113	SP	203	SP	203
B152	SB	115	SB	205	SP	203	SP	202
B156	SB	109	SB	205	SP	203	SP	203
B159	SB	116	SB	206	SP	201	SP	201
B198	SB	108	SB	207	SP	204	SP	204
B249	SB	121	SB	121	SP	205	SP	101
B284	SB	108	SB	207	SP	204	SP	204

Fortaleza, Brazil

Sample								
B359	SB	125	SB	206	SP	201	SP	202
B362	SB	125	SB	202	SP	201	SP	202
B363	SB	125	SB	202	SP	201	SP	202
B364	SB	125	SB	206	SP	201	SP	202
B365	SB	125	SB	206	SP	201	SP	202
B366	SB	125	SB	206	SP	201	SP	202

Bocas del Toro, Panamá

Sample								
P092	SB	141	SB	142	SP	106	SP	205

São Tomé, West Africa

Sample								
ST132	SB	157	SB	202	SP	201	SP	203
ST222	SB	158	SB	206	SP	201	SP	201
ST265	SB	136	SB	202	SP	201	SP	201
ST338	SB	159	SB	206	SP	201	SP	201

CHAPTER IV

Unforeseen coral diversity and abundance in unexplored mesophotic reefs of Northern Brazil

The north coast of Brazil is considered unexplored in terms of its coral fauna. This 1,000 km stretch of coast represents one of the biggest gaps in current knowledge regarding the distribution of corals in the South Atlantic (Castro and Pires 2001). A recent expedition to the Pedra da Risca do Meio (PRM) Marine State Park (Fig. 4.1), in the heart of the unexplored region, revealed greater coral species diversity and abundance than previously supposed. This is the first report listing coral species found in the Marine State Park.
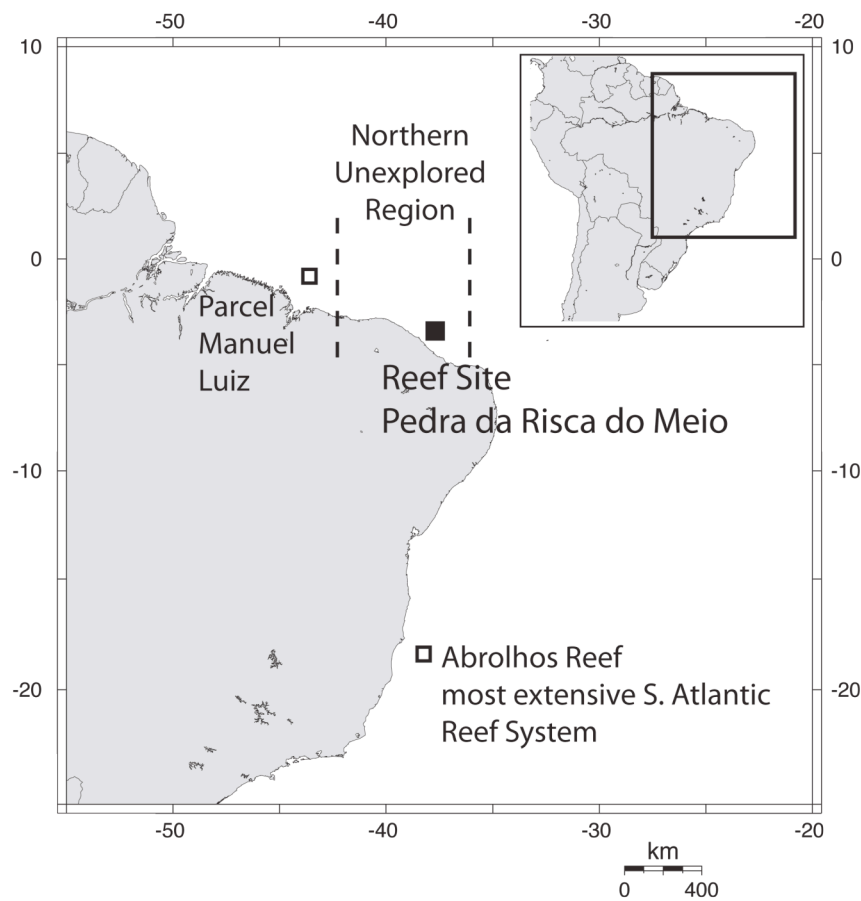

Figure 4.1 Map indicating location of the study site (black square) and other major reefs along the coast of Brazil (open squares). Limits of the Northern Unexplored Region according to Castro and Pires (2001) are indicated by dotted lines.

Of the 15 coral species that occur in Brazil, five were observed in the marine park (*Montastraea cavernosa, Siderastrea stellata, Mussismilia hispida, Stephanocoenia intersepta and Scolymia* sp.) of which two are considered uncommon and have limited ranges along the coast of Brazil (*S.intersepta* and *Scolymia* sp.) (Fig. 4.1). Because only a few dives were made in the area, the total number of species present may be higher. Colonies of *M. cavernosa* and *S. stellata* were the most abundant, followed by *M. hispida*. Coral colonies tended to have a low profile and were laterally extensive; platy colonies of *M. cavernosa* were particularly common. The reef was primarily carbonate in composition with no exposed bedrock. Large sponges, coralline and fleshy algae and carbonate hardground make up much of the remaining benthic cover.

PRM Marine State Park is located 18 km from the coast and covers an area of $32km^2$ within a depth range of 18-30 m (Freitas et al. 2003). Research activities remain infrequent in this area due to difficult access and commonly unfavorable oceanic conditions. Wind speeds of 15-20 km/hr are observed year-round, increasing the water turbidity. Nearshore environments are particularly affected, with visibility in the order of 1 m, resulting in the low coral diversity and abundance previously reported for this area (*S.stellata, Favia gravida* and *Millepora alcicornis*) (Laborel, 1969). The offshore site visited for this study had much greater species diversity and abundance, presumably because as turbidity decreases with increasing depth, an environment more suitable for coral growth can be found. Visibility was ~10 m during our visit in January 2008.

Figure 4.2 This large coral specimen has been visually identified as a colony of *Stephanocoenia michelini* (AF Budd, personal communication), a species currently known from only a few locations in Brazil.

Coral cover at a visual estimate of 20-30% was also greater than expected, raising the question of coral abundance at similar sites along this coast. Corals were believed rare in this area, but the occurrence of even uncommon coral species in the offshore reefs suggests that perhaps they are only rare in nearshore environments, and more abundant offshore, such as in PRM. The fact that Parcel Manuel Luiz, the closest coral reef to the Amazon freshwater outflow located ~800km west of PRM (see Fig 4.1), has some of the highest coral diversity and coral cover in all of Brazil (Amaral et al 1998, Moura et al, 1999) also points to the importance of mesophotic reefs in this

region. Future exploratory work in the northern region of Brazil will help determine biodiversity trends in offshore reefs such as Parcel Manuel Luiz and PRM, and what role these reefs play in maintaining connectivity among corals and other reef inhabitants along the coast of Brazil and with respect to the Caribbean (Rocha et al 2002).

ACKNOWLEDGMENTS

We would like to thank the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) and the Superintendência Estadual do Meio Ambiente do Ceará (SEMACE) for granting permission to sample in PRM Marine State Park. Funding for this research expedition was provided by an anonymous donation to the Scripps Institution of Oceanography (SIO) Graduate Department and by an SIO grant to RDN.

Chapter IV, in part is currently being prepared for submission for publication of the material. Nunes, Flavia; Freitas, J. Eduardo P.; Le Dantec, Nicolas; Norris, Richard D.; Knowlton, Nancy. The dissertation author was the primary investigator and author of this paper.

REFERENCES

Amaral FD, Hudson MM and Coura, MF (1998) Levantamento prelimiar dos corais e hidrocorais do Parque Estadual Marinho do Parcel do Manuel Luiz (MA). *Resumos do XIII Simposio de Biologia Marinha* USO, CEBIMar., 13

Castro CB, Pires DO (2001) Brazilian coral reefs: what we already know and what is still missing. *Bull Mar Sci* **69**, 357-371.

Freitas JEP, Araújo ME, Campos AA and Feitosa RD (2003) Projeto Conservação de Peixes Recifais. *VI Congresso de Ecologia do Brasil, Fortaleza* **1**, 158-160.

Laborel J (1969) Les peuplements de madréporaires des côtes tropicales du Brésil, Ph.D. thesis, Université d'Abidjan, 260p

Moura RL, Rodrigues MCM, Francini-Filho RB and Sazima I. (1999) Unexpected richness of reef corals near the southern Amazon River, *Coral Reefs* **18**, 170

Rocha LA, Bass AL, Robertson DR and Bowen BW (2002) Adult habitat preferences, larval dispersal, and the comparative phylogeography ofthree Atlantic surgeonfishes (Teleostei : Acanthuridae), *Molec Ecol* **11**, 243-252.