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ENDEMISMO E CONECTIVIDADE DE CORAIS-DE-FOGO
(*MILLEPORA* SPP.) NO OCEANO ATLÂNTICO

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“There is grandeur in this view of life... having been originally breathed into a few forms or into one; and that... from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved.”

(Charles Darwin, 1859)

RESUMO

Conhecer o grau de conectividade e de diversidade genética pode auxiliar a elucidar quais são as populações em vias evolutivas de especiação ou que estão mais vulneráveis às mudanças ambientais. Tendo em vista a importância ecológica de milleporídeos no Oceano Atlântico, este estudo objetivou investigar os padrões de conectividade e de diversidade genética de corais-de-fogo do Atlântico tropical, e combinou dados moleculares e morfológicos para melhor distinguir as espécies endêmicas simpátricas. A análise filogenética, baseada na sequência de DNA mitocondrial (DNAm_t) 16S DNAr, corroborou a existência de quatro clados monofiléticos no Atlântico Sul: *Millepora alcicornis*, *M. braziliensis*, *M. nitida* e *M. laboreli*. A morfometria revelou o diâmetro dos gastróporos e dactilóporos como sendo as principais variáveis que distinguiram o morfotipo *M. nitida* incrustante dos outros dois morfotipos, *M. nitida* ramificada e *M. braziliensis*. Entre as regiões do Caribe, Brasil e Atlântico Oriental observou-se alta estruturação genética das populações de *M. alcicornis* ($\Phi_{st} = 0,596$ — $0,680$, $P < 0,05$). No Brasil, as populações das espécies endêmicas *M. braziliensis* ($\Phi_{st} = 0,689$, $P < 0,05$) e *M. nitida* ($\Phi_{st} = 0,828$, $P < 0,05$) mostraram-se altamente estruturadas, ao passo que alta conectividade predominou nas populações de *M. alcicornis* ($\Phi_{st} < 0,106$), com exceção particularmente do Arquipélago de Fernando de Noronha. A diversidade genética decresceu em direção às margens da distribuição de *M. alcicornis* ($h = 0$ — $0,982$), *M. braziliensis* ($h = 0,286$ — $0,702$) e *M. nitida* ($h = 0,255$ — $0,667$). Os resultados de análises de estruturação genética sugerem que a pluma dos rios Amazonas-Orinoco (do inglês “Amazon-Orinoco Plume”, AOP) e a extensão de oceano aberto dividindo o Atlântico Oriental e Ocidental, também conhecida como Barreira do Atlântico Central (do inglês “Mid-Atlantic Barrier”, MAB) são as principais barreiras ao fluxo gênico em *M. alcicornis* ao longo do Caribe, Brasil e Atlântico Oriental. O deságue do rio São Francisco parece restringir a dispersão das espécies endêmicas de forma a evitar a sobreposição de suas áreas, mas ao mesmo tempo é permeável a *M. alcicornis*, espécie de ampla distribuição. A perda de diversidade em direção às margens da distribuição pode ser responsável pela perda da capacidade de resiliência das populações periféricas frente a distúrbios ambientais. Sendo assim, as populações periféricas da espécie de mais ampla distribuição (*M. alcicornis*) e as populações mais centrais das espécies endêmicas (*M. braziliensis* e *M. nitida*) merecem atenção especial dos esforços conservacionistas.

Palavras-chave: fluxo gênico, diversidade genética, barreiras biogeográficas, populações periféricas, morfometria.

ABSTRACT

Knowledge on the degree of connectivity and genetic diversity of corals may help to elucidate which populations are under evolutionary trajectories of speciation or are more vulnerable to environmental changes. Given the ecological importance of milleporids in the Atlantic Ocean, this study aimed to investigate patterns of connectivity and genetic diversity in fire corals from the tropical Atlantic, and combined molecular and morphological data to better distinguish the endemic species. Phylogenetic analyses, based on mitochondrial DNA (mtDNA) 16S rDNA, corroborated the existence of four reciprocally monophyletic clades in the South Atlantic: *Millepora alcicornis*, *M. braziliensis*, *M. nitida* and *M. laboreli*. Morphologically, gastropore's and dactylopor's diameters were the main variables that distinguished encrusting morph from the other two morphs, the ramified colonies of *M. nitida* and *M. braziliensis*. Among Caribbean, Brazil and Eastern Atlantic high levels of genetic structure are observed ($\Phi_{st} = 0.596\text{--}0.680$, $P < 0.05$). Within Brazil, populations of the endemic species *M. braziliensis* ($\Phi_{st} = 0.689$, $P < 0.05$) and *M. nitida* ($\Phi_{st} = 0.828$, $P < 0.05$) are highly structured, while high connectivity predominates in populations of *M. alcicornis* ($\Phi_{st} < 0.106$), with the exception of Fernando de Noronha Archipelago. Genetic diversity decreases towards the edges of the distribution of *M. alcicornis* ($h = 0\text{--}0.982$), *M. braziliensis* ($h = 0.286\text{--}0.702$) and *M. nitida* ($h = 0.255\text{--}0.667$). The results of genetic structure analyses suggest that the plume of the Amazon-Orinoco Rivers (AOP) and the stretch of open ocean dividing eastern and western Atlantic, also known as Mid-Atlantic Barrier (MAB), impose major barriers to gene flow of *M. alcicornis* across the Caribbean, Brazil and Eastern Atlantic. The São Francisco River plume (SFP) seems to restrict the dispersal of the endemic species, whereas it is permeable for the widespread species *M. alcicornis*. The loss of diversity towards the edges of the distribution may be responsible for the loss of resilience capacity in peripheral populations when facing environmental disturbances. Thus, peripheral populations of the widespread species (*M. alcicornis*) and central populations of the endemic species (*M. braziliensis* and *M. nitida*) deserve a special attention from conservation efforts.

Keywords: Gene flow. Genetic diversity. Biogeographic barriers. Peripheral populations. Morphometric.

LISTA DE FIGURAS

- Figura 1 – Distribuição geográfica de Milleporidae no Brasil segundo Amaral et al. (2008). Fonte: Amaral et al. (2008).....31
- Figura 2 – Barreiras biogeográficas à distribuição de corais escleractínios no Oceano Atlântico. As barreiras indicadas são: AOP, Amazon-Orinoco Plume (Pluma dos rios Amazonas-Orinoco); SFP, São Francisco River Plume (Pluma do rio São Francisco); MAB, Mid-Atlantic Barrier (Barreira do Atlântico Central). Adaptado de Luiz et al. (2012).....33

CAPÍTULO ÚNICO/SINGLE CHAPTER

- Figure 1 – Fire coral species in Brazil: (A) *Millepora alcicornis* from Tamandaré in Pernambuco State, (B) *M. braziliensis* from Tamandaré in Pernambuco State, (C) ramified morphotype of *M. nitida* from Porto Seguro in Bahia State, (D) encrusting morphotype of *M. nitida* from Guarapari Islands in Espírito Santo State, and (E) *M. laboreli* from Manuel Luiz Coral Banks in Maranhão State. Photos: (A,B, D) Júlia Souza, (C) Emiliano Calderon and (E) Luiz Rocha.41
- Figure 2 – *Left*: Map of the Atlantic Ocean showing major Atlantic biogeographic barriers for corals (AOP, Amazon-Orinoco Rivers Plume; SFP, São Francisco River Plume and MAB, Mid-Atlantic Barrier) and the distribution of the Atlantic *M. alcicornis* is depicted in orange. *Right*: distribution of Brazilian Milleporidae (A, *M. alcicornis*; B, *M. braziliensis*; C, *M. nitida* and D, *M. laboreli*; *denotes endemic species; light grey squares refer to Laborel 1970 and Belém et al. 1986; and dark grey squares refer to Amaral et al. 2006, 2007). Illustration on the right modified from Amaral et al. (2008).45
- Figure 3 – Sampling sites of milleporids in the Atlantic Ocean. Colors represent regions: North Atlantic in blue, Caribbean in red, Brazil in green and Eastern Atlantic in yellow.....46
- Figure 4 – Measurements performed in colonies of Atlantic *Millepora* species. G, gastropore; D, dactylopore; i, gastropore diameter; ii, dactylopore diameter; iii, distance between gastropores; iv, distance from gastropore to nearest dactylopore; v: distance between dactylopores. Photo: Júlia Souza.50
- Figure 5 – Bayesian consensus tree build for Atlantic milleporids. Numbers represent the support values: posterior probability/bootstrap for an equivalent Maximum Likelihood tree. Colors on the tips of the branches

indicate regions of origin of samples: red denotes Great Caribbean (Bermuda + Caribbean), green denotes Brazil and yellow denotes Eastern Atlantic. Numbers in brackets indicate the number of individuals with the same haplotype. The rectangular orange highlights the unique encrusting colonies of *M. nitida* described for Guarapari Islands, Espírito Santo.....53

- Figure 6 – Bayesian consensus tree build for Brazilian endemic Milleporidae emphasizing the area covered by *Millepora nitida* and *M. braziliensis* samples from Pernambuco to Espírito Santo. The red bar indicates the barrier imposed by São Francisco Plume to the dispersal of these two species.54
- Figure 7 – Boxplot of morphological variables measured in the following morphotypes: Mb, *Millepora braziliensis*; Mn, *M. nitida* ramified and Mne, *M. nitida* encrusting.....56
- Figure 8 – Discriminant Function Analysis of the following *Millepora* morphotypes: *M. braziliensis* (Mb) in blue, *M. nitida* ramified (Mn) in red and *M. nitida* encrusting (Mne) in green.57
- Figure 9 – Haplotype network of *Millepora alcicornis* from the Atlantic Ocean. Colors illustrate major regions of fire coral sampling localities, black circles represent ancestral or not sampled haplotypes and black dashes correspond to mutational events.60
- Figure 10 – Haplotype network of *Millepora alcicornis* from the Atlantic Ocean. Colors illustrate populations of fire coral set by localities, black circles represent ancestral or not sampled haplotypes and black dashes correspond to mutational events. Populations are classified according to major regions: NA, North Atlantic; CA, Caribbean; EA, Eastern Atlantic; and BR, Brazil.61
- Figure 11 – Haplotype networks of Brazilian endemic Milleporidae. Colors illustrate populations of fire coral set by localities, black circles represent ancestral or not sampled haplotypes and black dashes correspond to mutational events.....62

LISTA DE TABELAS

CAPÍTULO ÚNICO/SINGLE CHAPTER

- Table 1 – Summary statistics of morphological variables of the following morphotypes: Mb, *Millepora braziliensis*; Mn, *M. nitida* ramified; and Mne, *M. nitida* encrusting. Six variables were measured: G, diameter of gastropores; D, diameter of dactylopores; G-G, distance between gastropores; D-D, distance between dactylopores; G-D, distance between gastropores to nearest dactylopores; D/G, and number of dactylopores per gastropores.....55
- Table 2 – Standardized coefficients of canonical variables. The variables evaluated were G, diameter of gastropores; D, diameter of dactylopores; G-G, distance among gastropores; G-D, distance from gastropore to nearest dactylopores; D-D, distance among dactylopores; and D/G, number of dactylopores per gastropore.57
- Table 3 – Molecular diversity indices and neutrality tests for 16S rDNA gene in Atlantic *Millepora*. Codes used are N, number of individuals; H, number of haplotypes; s, number of segregating sites; *h*, gene diversity; π , average nucleotide diversity; and *k*, average number of nucleotide differences.....63
- Table 4 – Analyses of Molecular Variance (AMOVA) of the Atlantic *Millepora* using data from 16S rDNA gene. Regions used in the analyses consisted of NA, North Atlantic Ocean; CA, Caribbean; EA, Eastern Atlantic; BRN, Northern/Northeastern Brazil; and BRS, Eastern/Southern Brazil.64
- Table 5 – Pairwise Φ_{st} for *Millepora alcicornis* populations inferred from data of 16S rDNA gene.65
- Table 6 – Pairwise Φ_{st} for *Millepora braziliensis* and *M. nitida* inferred from data of 16S rDNA gene.65
- Table 7 – Fixation indices and correspondent population groups inferred by SAMOVA algorithm for Atlantic *Millepora* by using data from 16S rDNA gene. Localities corresponding to populations used in the analysis were BD, Bermuda; PA, Panama; FL, Florida; CO, Colombia; CV, Cape Verde; MA, Maranhão; RN, Rio Grande do Norte; FN, Fernando de Noronha; PE, Pernambuco; AN, Northern Alagoas; AS, Southern Alagoas; BN, Northern Bahia; BP, Porto Seguro in Southern Bahia; BA, Abrolhos in Southern Bahia; ES, Espírito Santo; and RJ, Rio de Janeiro.....67

Table S1 – Description of samples of *Millepora* species collected in the Atlantic Ocean. The type of analysis (genetic or morphological) used is also designated.....87

LISTA DE ABREVIATURAS E SIGLAS

Barreiras biogeográficas

AOP – Amazon-Orinoco Plume (Pluma dos Rios Amazonas-Orinoco)

MAB – Mid-Atlantic Barrier (Barreira do Atlântico Central)

SFP – São Francisco Plume (Pluma do Rio São Francisco)

Morfotipos de *Millepora* spp. endêmicas

Mb – Morfotipo *Millepora braziliensis*

Mn – Morfotipo *Millepora nitida* ramificada

Mne – Morfotipo *M. nitida* incrustante

Caracteres morfométricos

G – Diâmetro de gastróporos

D – Diâmetro de dactilóporos

G-G – Distância entre gastróporos

G-D – Distância entre o gastróporo e o dactilóporo mais próximo

D-D – Distância entre dactilóporos

D/G – Número de dactilóporos por gastróporo

Regiões biogeográficas segundo Veron (1995)




NA – North Atlantic (Atlântico Norte) (Bermuda foi separada do Caribe em Atlântico Norte para fins de análises de dados)

CA – Caribbean (Caribe)

EA – Eastern Atlantic (Atlântico Oriental)

BR – Brazil (Brasil)

LISTA DE SÍMBOLOS

-  *Millepora braziliensis* morphotype (Mb)
-  *Millepora nitida* ramified morphotype (Mn)
-  *Millepora nitida* encrusting morphotype (Mne)

SUMÁRIO

1	INTRODUÇÃO	29
1.1	OBJETIVOS	33
1.1.1.	Objetivo Geral	33
1.1.2.	Objetivos Específicos	33
2	CAPÍTULO ÚNICO/SINGLE CHAPTER	35
2.1	INTRODUCTION	39
2.2	MATERIAL AND METHODS	46
2.2.1	Coral sampling	46
2.2.2	DNA extraction, amplification and sequencing	47
2.2.3	Genetic data analyses	47
2.2.4	Morphological analyses	49
2.3	RESULTS	51
2.3.1	Data set	51
2.3.2	Phylogenetic analyses	51
2.3.3	Morphological analyses	52
2.3.4	Haplotype network distribution and age estimation	58
2.3.5	Genetic diversity and neutrality	58
2.3.6	Population genetic structure and genetic barriers	60
2.4	DISCUSSION	68
2.4.1	The Atlantic widespread fire coral species, <i>Millepora alcicornis</i>	68
2.4.2.1	Molecular phylogeny	68
2.4.2.2	Genetic connectivity	68
2.4.2.3	Genetic diversity	73
2.4.2	The Brazilian endemic fire coral species, <i>Millepora braziliensis</i> , <i>M. nitida</i> and <i>M. laboreli</i>	74
2.4.2.1	Morphometry, molecular phylogeny, and connectivity	74
2.4.2.2	Genetic diversity	75
2.5	ACKNOWLEDGMENTS	76
2.6	REFERENCES	76

2.7	SUPPLEMENTARY MATERIAL.....	87
3	CONCLUSÃO	95
	REFERÊNCIAS.....	97
	ANEXO A – Instruções para preparação de manuscritos estabelecidas pelo periódico internacional “Coral Reefs”	111

1 INTRODUÇÃO

Os ecossistemas recifais estão entre os ecossistemas mais ricos do planeta. Os níveis de diversidade em recifes de corais são tão altos que esses podem ser comparados a florestas tropicais no meio marinho (Connell 1978, Maragos et al. 1996, Jackson 1991, Knowlton 2001, Knowlton & Jackson 2008). A diversidade total de espécies estimada para esses ambientes situa-se entre 1 e 9 milhões de espécies (Reaka-Kudla 1997), sendo que, aproximadamente 835 correspondem a espécies de corais escleractínios estruturadoras de recifes (Veron 1995). Apesar disso, os recifes estão entre os habitats mais ameaçados mundialmente (Carpenter et al. 2008). Dentre as principais causas de declínio dos ambientes recifais destacam-se a sobrepesca (Jackson et al. 2001, Pandolfi et al. 2003), poluição (McCulloch et al. 2003), doenças (Harvell et al. 2002, Francini-Filho et al. 2008) e mudanças climáticas (Hughes et al. 2003, Gardner et al. 2003). Duas importantes características ecológicas a serem acessadas de forma a promover o conhecimento e a conservação a cerca desses habitats e de seu funcionamento são a biodiversidade em si (Plaisance et al. 2011) e a capacidade de resiliência desses sistemas (Bellwood et al. 2004). Nesse caso, ferramentas moleculares são extremamente apropriadas e podem auxiliar no manejo e delimitação de áreas marinhas protegidas a partir dos conhecimentos por elas gerados (Plaisance et al. 2011).

A resiliência de um sistema, isto é, sua capacidade de absorver impactos, resistir a mudanças de fases e de se regenerar após distúrbios de origem natural ou antropogênica (Nyström et al. 2000), pode estar relacionada com a diversidade genética dos principais grupos funcionais presentes nesse habitat. Ehlers et al. (2008), por exemplo revelam um forte efeito negativo do aquecimento e um efeito positivo da diversidade genotípica nas densidades de brotos da grama marinha *Zostera marina* Linnaeus, 1753 em experimentos de mesocosmos. Nesse sentido, outro ponto importante que merece fundamental consideração é a questão da conectividade populacional, pois a resiliência das espécies aos impactos depende de um espectro mais amplo também de sua capacidade de dispersão. Conhecer até que ponto as populações marinhas conectam-se por dispersão larval é de extrema valia para se compreender os impactos pretéritos e futuras prospecções para sustento da biodiversidade. Por exemplo, espécies com ampla capacidade de dispersão podem estar menos suscetíveis à extinção global devido às suas grandes áreas de distribuição, múltiplas populações, e potencial para recuperação local

através do transporte larval (Jones et al. 2007). Em contraste, para espécies com distribuição restrita, ditas endêmicas, as ameaças ambientais podem ser muito mais devastadoras.

Dentre a fauna de corais escleractínios (Classe Anthozoa) do Oceano Atlântico tropical, a diversidade de espécies é maior no Caribe se comparada às populações periféricas do Brasil e África Ocidental (Nunes et al. 2011). Já os corais-de-fogo do gênero *Millepora* (Classe Hydrozoa) no Oceano Atlântico se destacam pela coocorrência de espécies endêmicas do Brasil e uma espécie de ampla distribuição ao longo de sistemas de recifes de corais e de costões rochosos: *Millepora alcicornis* (Figura 1). Contudo, até o momento apenas os corais caribenhos desse gênero foram estudados sob o prisma molecular (Ramos 2009) e nada se sabe acerca da conectividade populacional e diversidade genética desses corais em escala regional, ou seja, abrangendo as espécies brasileiras e as demais populações de *M. alcicornis* no Atlântico. Uma vez que a conectividade é reconhecida como um elemento-chave da conservação devido à sua importância para a persistência populacional e recuperação pós-distúrbios (Roberts et al. 2006, Salm et al. 2006), é vital que se compreenda o seu mecanismo em um gênero de coral tão importante para os ecossistemas recifais e que ainda é relativamente pouco estudado.

Os corais-de-fogo são um dos principais responsáveis pela complexidade tridimensional dos recifes do Brasil, pois são os únicos corais ramificados nessa região (Leão et al. 2003), e, portanto, cumprem o papel funcional exercido por corais escleractínios nos recifes do Caribe e Indo-Pacífico. Esses organismos coloniais apresentam zooides que secretam um esqueleto de carbonato de cálcio e que podem ser responsáveis pela defesa da colônia, nesse caso conhecidos como dactilozoides, ou por sua alimentação, sendo esses os gastrozoides (Moseley 1880, Moschenko 1993). Esses hidrocorais reproduzem-se sexuadamente através de medusoides liberados de estruturas na colônia denominadas ampolas (Quelch 1884, Hickson 1891, 1899a). As colônias são gonocóricas e a liberação das medusas é sazonal, iniciando com o aparecimento das ampolas e liberação das medusas em abril e maio em Taiwan, entre abril e julho em Barbados e de junho a março em Curaçao (Lewis 2006). No Brasil, a liberação de medusas por *M. alcicornis* Linnaeus 1758 foi registrada por Amaral et al. (2008) durante a estação chuvosa, mais especificamente de junho a agosto. Os mesmos autores

descreveram a liberação de medusas por *M. braziliensis* Verrill 1868 como sendo de março a julho, também na estação chuvosa.

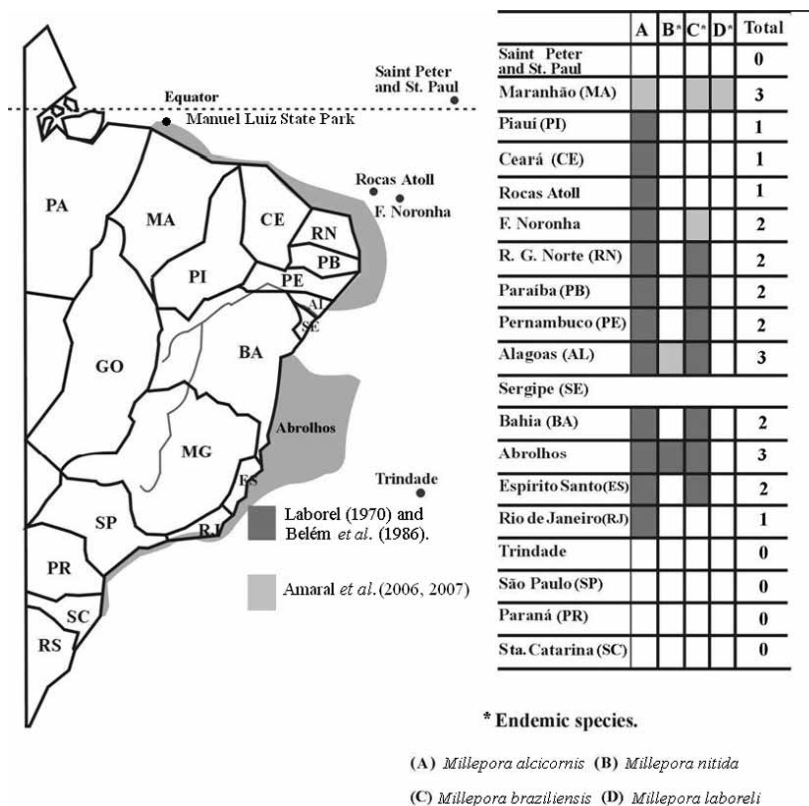


Figura 1. Distribuição geográfica de Milleporidae no Brasil segundo Amaral et al. (2008). Fonte: Amaral et al. (2008).

O gênero *Millepora* distribuiu-se mundialmente nos oceanos tropicais, com um total de 18 espécies (Lewis 2006, Amaral et al. 2008). No oceano Atlântico, apresentam-se as espécies *Millepora alcicornis* Linnaeus 1758, *M. squarrosa* Lamarck 1816, *M. complanata* Lamarck 1816, *M. striata* Duchassing and Michelotti 1864, *M. braziliensis* Verrill 1868, *M. nitida* Verrill 1868 e *M. laboreli* Amaral 2008 in Amaral et al.

(2008). As quatro primeiras espécies foram avaliadas por Ramos (2009) em estudo molecular e morfológico, conforme mencionado previamente. No entanto, pouco se conhece sobre os corais-de-fogo endêmicos do Brasil (*M. braziliensis*, *M. nitida* e *M. laboreli*) e sobre *M. alcicornis*, pois sua distribuição ultrapassa as fronteiras caribenhas e se estende desde Bermuda, no Atlântico Norte, até o estado do Rio de Janeiro, no Brasil, e ainda alcança o Atlântico Oriental, ocorrendo em Cabo Verde e nas Ilhas Canárias (Clemente et al., 2011), no oeste da África.

Muito embora os aspectos biogeográficos e filogeográficos de mileporídeos careçam de serem estudados, a fauna de corais escleractínios do Atlântico tem recebido maior atenção nesse sentido. Na maior compilação de estudos biogeográficos com corais escleractínios, Veron (1995) subdivide o Atlântico em Oriental e Ocidental segundo a biogeografia desses corais. De acordo com Veron (1995) a fauna de escleractínios está distribuída em duas grandes regiões no Atlântico Ocidental, a primeira estendendo-se de Bermuda ao sul do Caribe, e a segunda sendo o Brasil. Todavia, dentro do Brasil, a pluma do Rio São Francisco (São Francisco Plume, SFP) se constitui em uma barreira à dispersão desses organismos, e com isso provoca a divisão dessa região em duas, uma ao norte e outra ao sul de seu deságue (Figura 2). Outras barreiras biogeográficas observadas por Nunes et al. (2009) para o coral *Montastraea cavernosa* Linnaeus, 1767 dentro do Oceano Atlântico, incluem a pluma dos rios Amazonas-Orinoco (Amazon-Orinoco Plume, AOP), que separa o Caribe do Brasil, e a extensão de águas profundas dividindo os dois lados do Oceano Atlântico, também conhecida como Barreira do Atlântico Central (Mid-Atlantic Barrier, MAB) (Figura 2). Sob o contexto evolutivo, essas barreiras podem eventualmente promover a especiação de populações isoladas de uma espécie ancestral com distribuição originalmente ampla, conforme observado para os peixes recifais *Clepticus* (Beldade et al. 2009), cujas três espécies endêmicas correspondentes a cada uma das três principais regiões biogeográficas (Caribe, Brasil, Atlântico Oriental) evoluíram provavelmente como consequência da restrição pelas barreiras AOP e MAB.

Sendo assim, tendo em vista a escassez de conhecimento a cerca dos aspectos biogeográficos dos corais-de-fogo do Oceano Atlântico, este estudo visou ampliar o entendimento sobre os processos ecológicos e evolutivos responsáveis pelos padrões de conectividade e diversidade genética encontrados nesses corais com distribuição sobreposta.

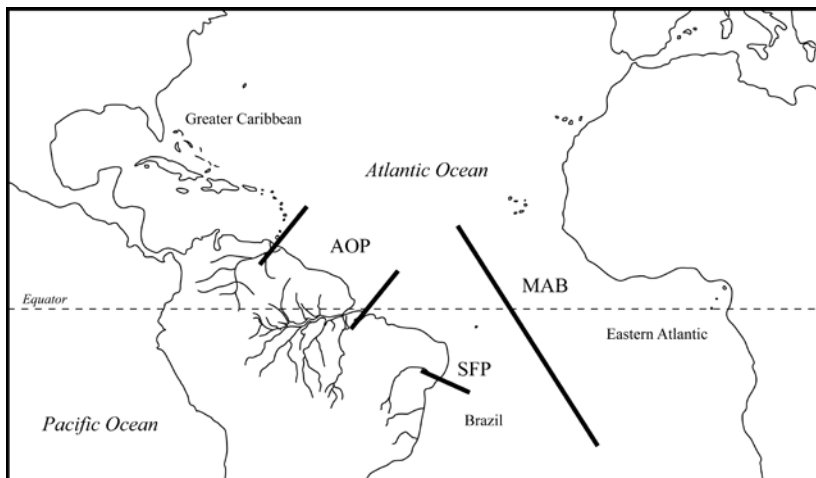


Figura 2. Barreiras biogeográficas à distribuição de corais escleractínios no Oceano Atlântico. As barreiras indicadas são: AOP, Amazon-Orinoco Plume (Pluma dos rios Amazonas-Orinoco); SFP, São Francisco River Plume (Pluma do rio São Francisco); MAB, Mid-Atlantic Barrier (Barreira do Atlântico Central). Adaptado de Luiz et al. (2012).

1.1 OBJETIVOS

1.1.1. Objetivo Geral

Este estudo teve como objetivo principal avaliar os padrões de conectividade e diversidade genética tanto dos corais-de-fogo endêmicos do Brasil (*M. braziliensis*, *M. nitida* e *M. laboreli*) quanto do coral de mais ampla distribuição no oceano Atlântico (*M. alcicornis*), bem como verificar caracteres micromorfológicos possivelmente diagnósticos das espécies endêmicas simpátricas *M. braziliensis* e *M. nitida*.

1.1.2. Objetivos Específicos

(1) Testar a existência de uma quebra filogeográfica em *M. alcornis* concordante com as barreiras biogeográficas formadas pela pluma dos rios Amazonas-Orinoco (Amazon-Orinoco Plume, AOP) e pela barreira do Atlântico Central (Mid-Atlantic Barrier, MAB);

(2) verificar se o deságue do Rio São Francisco (São Francisco Plume, SFP) impõe-se como uma barreira à dispersão para alguma das espécies de corais-de-fogo;

(3) identificar se os corais endêmicos simpátricos *M. braziliensis* e *M. nitida* podem ser corretamente distinguidos entre si a partir de algum caráter micromorfológico.

2 CAPÍTULO ÚNICO/SINGLE CHAPTER

Endemismo e Conectividade de Corais-de-Fogo
(*Millepora* spp.) no Oceano Atlântico



Endemism and Connectivity of Fire Corals
(*Millepora* spp.) in the Atlantic Ocean

Targeted journal: Coral Reefs

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Endemism and connectivity of fire corals (*Millepora* spp.) in the Atlantic Ocean

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2.1 INTRODUCTION

The levels of endemism in reef environments have important ecological applications as they stand, for example, among the main criteria used to identify biodiversity hotspots and to delineate biogeographic regions/provinces (e.g. Roberts et al. 2002, Floeter et al. 2008). From a conservational point of view, Hughes et al. (2002) have expanded the original idea of focusing on biodiversity hotspots as conservational units in a way that it should contemplate the connectivity and genetic diversity of the widely dispersed species and embrace local protection of peripheral areas that have proportionately higher numbers of endemics. In this sense, molecular approaches can provide insights into the ecological processes operating over different time scales, and can be applied to either endemic or widespread species and help set conservation efforts.

The tropical Atlantic Ocean is a closed ocean basin, with relatively stable currents, and that configures a perfect stage for studying patterns of connectivity among coral populations. One peculiarity in this region is the sympatric distribution of closely related endemic and widespread species of fire corals (*Millepora* spp.) combined with the presence of biogeographic barriers to coral dispersal. Based on the biogeography of the Scleractinia, Veron (1995) recognizes four regions in the Atlantic Ocean: the Caribbean, Northern Brazil, Southern Brazil and Eastern Atlantic. Nunes et al. (2009, 2011) found evidence for the existence of two biogeographic barriers to dispersal of corals, separating the regions of Caribbean and Brazil, and the Eastern and Western Atlantic. The Amazon-Orinoco plume (AOP) and the separation between Eastern and Western Atlantic, conventionally called Mid-Atlantic barrier (MAB), which are widely recognized as barriers to dispersal for reef fishes (Floeter et al. 2008, Luiz et al. 2012), were identified by Nunes et al. (2009, 2011) as the majors barriers constraining gene flow among populations of six scleractinian corals. According to Veron (1995) and Leão et al. (2003), a third barrier occurs within the Brazilian coast, the freshwater plume of the São Francisco River (SFP). Despite the potential biogeographic importance of this barrier, no studies have focused on testing its effectiveness over coral dispersal and distribution.

Brazilian and Caribbean reef ecosystems harbor high levels of endemism (Budd 2000, Leão et al. 2003, 2010). The Brazilian

hydrocoral fauna is composed of five species (Figure 1), of which three are endemic: *Millepora braziliensis* Verrill 1868, *Millepora nitida* Verrill 1868 and *Millepora laboreli* Amaral 2008 in Amaral et al. (2008). Among the 23 stony corals recognized in this region, six are considered endemics: *Mussismilia braziliensis* Verrill 1868, *M. hispida* Verrill 1868, *M. harttii* Verrill 1868, *M. leptophylla* (Verrill 1868), *F. gravida* Verrill 1868 and *Siderastrea stellata* Verrill 1868 (Maida and Ferreira 1997, Castro and Pires 2001, Leão et al. 2003, 2010, Nunes et al. 2008, Budd et al. 2012). In the Atlantic Ocean, the phylogenetic status of endemic Brazilian Scleractinia has been evaluated using DNA sequences (Nunes et al. 2008), but hydrocorals remain to be studied. An interesting biogeographic scenario can be drawn from Brazilian endemic milleporids, since these corals occur on either side, northern and southern, of the SFP.

Endemic and widespread species basically differ in their range. The geographic range of a species is a result of the interaction between ecological and evolutionary forces. The characteristics of past environments select the biological traits of a species and influence events of colonization, speciation and extinction, which will determine its present geographical distribution (Brown et al. 1996). Speciation is responsible for generating “new” diversity (The Marie Curie SPECIATION Network 2012). Furthermore, extinction events can promote the differentiation among populations by creating gaps in the geographic range and reducing the chances of mating between the new forming species (Norris and Hull 2012). Evolutionarily, the range of a species may change and in order to expand its range, a species must be able to (1) reach a new area, (2) cope with unfavorable conditions along the way, and (3) establish a viable population upon its arrival. The dispersal between populations is necessary to keep the species cohesion throughout its range. Once populations are interconnected by regular dispersal and gene flow, they behave as large populations. These interconnected populations tend to preserve more genetic diversity than smaller populations that are more prone to the effects of genetic drift (Wright 1931), but these small populations at the same time contribute to the overall diversity through local adaptation.

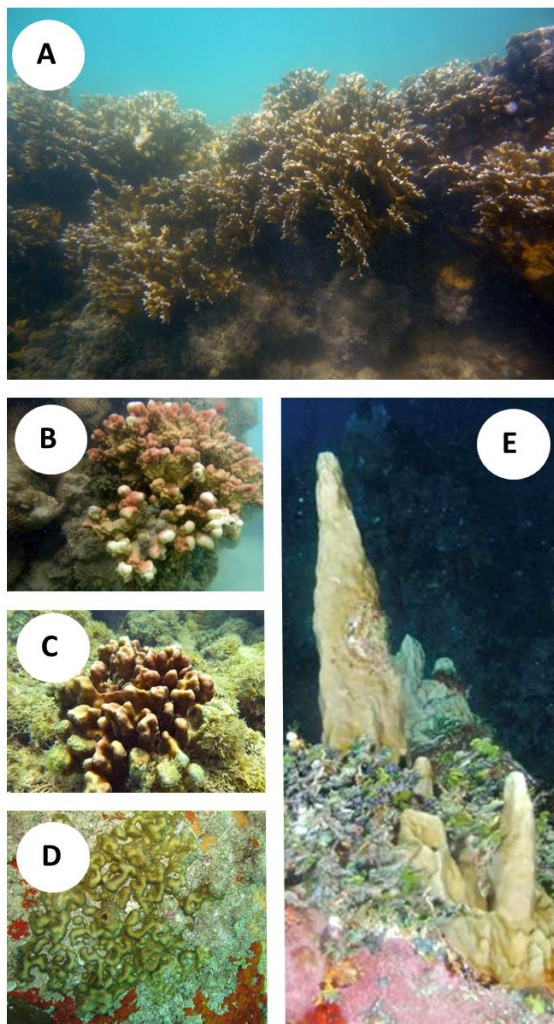


Figure 1. Fire coral species in Brazil: (A) *Millepora alcicornis* from Tamandaré in Pernambuco State, (B) *M. braziliensis* from Tamandaré in Pernambuco State, (C) ramified morphotype of *M. nitida* from Porto Seguro in Bahia State, (D) encrusting morphotype of *M. nitida* from Guarapari Islands in Espírito Santo State, and (E) *M. laboreli* from Manuel Luiz Coral Banks in Maranhão State. Photos: (A,B, D) Júlia Souza, (C) Emiliano Calderon and (E) Luiz Rocha.

According to Grosberg and Cunningham (2001: 61), “virtually all species of marine organisms...are patchily distributed, consisting of local populations connected to a greater or lesser extent by dispersal”. Understanding in which moment and by what route they were connected is important to gain an understanding about the evolution and ecology of these reef animals (Hellberg 2007). The concept of population connectivity emerges when dispersal is combined with factors leading to survival of the dispersed organisms. Various physical drivers, such as water flow, wind, temperature and salinity, can interact to influence nutrient availability, physiological rates, and behavioral response of dispersers. Other factors, either biotic or abiotic, such as feeding interactions, settlement habitat availability, and postsettlement survivorship, can drive dispersors to decrease in numbers, partially due to the conditions of larvae while settling (Cowen and Sponaugle 2009). Many shallow-water marine organisms achieve long-distance dispersal by rafting (Jokiel 1984), and for reef fish it may be an essential mechanism facilitating the dispersal of multiple life stages (juveniles and adults). *Millepora* corals are known to use pumice, for example, as rafting substrate (Jokiel 1989), which may explain their presence in the Canary Islands (Hoeksema et al. 2012).

Revealing the patterns of connectivity is especially important for “designing management strategies to restore and conserve reef populations” (Hellberg 2007). Population connectivity can be indirectly assessed by the employment of molecular markers as tags for identifying migrants. Since the discovery of restriction endonuclease in the 1960s, there has been a burst in population genetic studies using mitochondrial DNA (mtDNA) as a tool for connectivity studies, as long as they are suited markers for phylogeographic analyses (Avice 2000). However, some organisms exhibit extremely slow rates of nucleotide substitution for the mtDNA. In Scleractinians, for example, these rates are about 100 times slower than those for most animals (Hellberg 2006). Hydrocorals, instead, profit from the higher rates of nucleotide substitution if compared to those from Scleractinians (e.g. $0.1-0.6 \times 10^{-9}$ 16S rRNA substitution site⁻¹ year⁻¹ in scleractinian corals [Govindarajan et al. 2005] compared to 7.71×10^{-9} substitutions site⁻¹ year⁻¹ in stylasterid hydrocorals [Lindner et al. 2008]), and thus hydrocorals can have their connectivity inferred by the use of mitochondrial DNA.

Fire corals (*Millepora* spp.) are the only extant branching corals in Brazil (Figure 1), since all scleractinians in this region have massive

morphology (Leão et al. 2003). For this reason, they replace the functional role played by branching scleractinians in other regions, acting as one of the primary ecosystem engineers of shallow reefs and, thus, providing the three-dimensional structural complexity to the environment. Millepores are often called “stinging corals” or “fire corals” due to the numerous defensive polyps that protrude through pores in the skeleton carrying highly toxic substances (Lewis 2006). The genus occurs worldwide in tropical seas and inhabits coral reefs at depths of 1 to ~40 m (Boschma 1948). To date, *Millepora* is comprised by 18 extant species, of which seven are present in the Atlantic: *Millepora alcicornis* Linnaeus 1758, *M. squarrosa* Lamarck 1816, *M. complanata* Lamarck 1816, *M. striata* Duchassing and Michelotti 1864, *M. braziliensis* Verrill 1868, *M. nitida* Verrill 1868 and *M. laboreli* Amaral 2008 in Amaral et al. (2008). Among Atlantic hydrocorals, *M. alcicornis* is the one with the widest distribution, ranging from Bermuda to Brazil (up to Rio de Janeiro State) in the Western Atlantic, and occurring in Cape Verde, Canary Islands in the Eastern Atlantic (Figure 2). In Brazil, this species cooccur with all other Brazilian endemic species at some point of their distribution (Amaral et al. 2008) showing gaps around the Atlantic biogeographic barriers, and this turn makes them valuable to pursue for phylogeographic correspondences. Among the endemics, *M. braziliensis* covers the greatest area, ranging from Maranhão to Espírito Santo with a gap between Piauí State and Rocas Atoll. *Millepora nitida* ranges from Alagoas to Abrolhos Archipelago (Bahia), whereas *M. laboreli* has the most restricted range, occurring only off Maranhão.

Millepora reproduces asexually throughout a well-developed polypoid generation that buds off planktonic medusoids (the planktonic sexual stage). The colonies are gonochoristic and medusoid release initiates with the appearance of ampullae and then the release of medusae during the rainfall season, between June and August for *M. alcicornis* and between March and July for *M. braziliensis*, both registered in Brazil by Amaral et al. (2008). In Taiwan, synchronism of medusa release was detected between colonies of the same species, but not between different species, what may prevent hybridization as proposed in some stony corals (Harrison et al. 1984; Soong and Cho 1998). Medusae have been reported to possess a short life span in the Caribbean *M. complanata* (Lewis 1991). Molecular studies with the Atlantic Milleporidae include the phylogeny and connectivity of Caribbean milleporids inferred by Ramos (2009), though this study did

not include populations of *M. alcicornis* from Brazil and Africa, and the molecular systematics of *M. alcicornis* and *M. braziliensis*, by using allozymes (Amaral et al. 1997). So, to date there is a gap in the knowledge of Brazilian and Eastern Atlantic Milleporids concerning their phylogenetic relationship and phylogeographic patterns. Additionally, for long the identification of *Millepora* has been deemed by several researches as being problematic (see Dana 1846; Quelch 1884, 1885, 1886, Hickson, 1891, 1897, 1898a, 1898b, 1899a, 1899b, Duerden 1899). And although many efforts have been employed by H. Boschma (1949, 1950, 1951, 1961, 1962, 1966) to address the systematics of millepores, a number of taxonomic problems have yet to be tackled. Amaral et al. (2002) evaluated the morphology of *Millepora alcicornis*, *M. braziliensis* and *Millepora nitida* and found significant differences between *M. alcicornis* and the endemics, but no differences were found between the endemics.

Herein, we aimed to examine, by means of mitochondrial DNA, the connectivity and the phylogenetic status of the Brazilian endemic fire corals *M. braziliensis*, *M. nitida* and *M. laboreli* and the Atlantic widespread species *M. alcicornis* by testing (1) if the Amazon-Orinoco plume (AOP) and the Mid-Atlantic barrier (MAB) restrict the gene flow of *M. alcicornis*, (2) if the São Francisco River's plume (SFP) represents a barrier to any of the Brazilian fire corals, and (3) if the genetically confirmed species *M. braziliensis* and *M. nitida* can be discriminated from each other by morphometric characters.

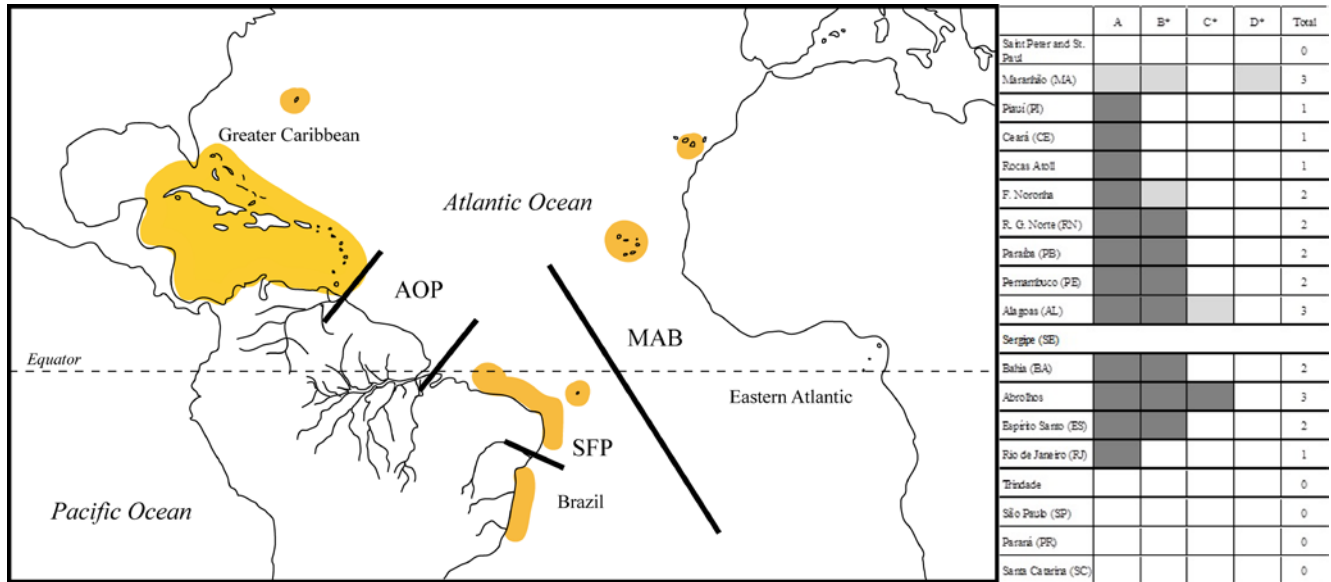


Figure 2. *Left:* Map of the Atlantic Ocean showing major Atlantic biogeographic barriers for corals (AOP, Amazon-Orinoco Rivers Plume; SFP, São Francisco River Plume and MAB, Mid-Atlantic Barrier) and the distribution of the Atlantic *M. alcicornis* (depicted in orange). *Right:* distribution of Brazilian Milleporidae (A, *M. alcicornis*; B, *M. braziliensis*; C, *M. nitida* and D, *M. laboreli*; *denotes endemic species; light grey squares refer to Laborel 1970 and Belém et al. 1986; and dark grey squares refer to Amaral et al. 2006, 2007). Illustration on the right modified from Amaral et al. (2008).

2.2 MATERIAL AND METHODS

2.2.1 Coral sampling

Tissue samples of *Millepora alcicornis*, *M. braziliensis*, *M. nitida* and *M. laboreli* were obtained from a total of 270 colonies (see supplementary material, Table S1) collected across the tropical Atlantic Ocean. Sampling of clones was avoided by outlining a fixed minimum distance of 3 meters between colonies of the same species. Sixteen study sites were established in the Atlantic, encompassing four regions: one site in the North Atlantic (Bermuda), three sites within the Caribbean (Panama, Florida, Colombia), eleven sites in Brazil (Maranhão, Fernando de Noronha Archipelago, Rio Grande do Norte, Pernambuco, Northern Alagoas, Southern Alagoas, Northern Bahia, Southern Bahia Porto Seguro, Southern Bahia Abrolhos Archipelago, Espírito Santo and Rio de Janeiro) and one site in the Eastern Atlantic (Cape Verde) (Figure 3). Tip branches were broken off from *M. alcicornis* colonies in order to get the samples, while a hammer and a chisel were used to break a small piece (~ 5 – 7 cm²) off the endemic colonies, taking care to minimize damage to the overall colony. Samples were stored in ethanol 92 – 98% and kept at room temperature.

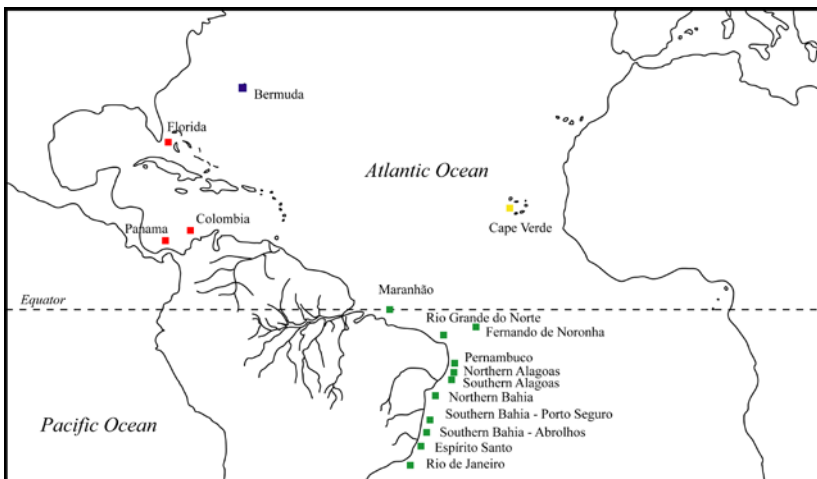


Figure 3. Sampling sites of milleporids in the Atlantic Ocean. Colors represent regions: North Atlantic in blue, Caribbean in red, Brazil in green and Eastern Atlantic in yellow.

2.2.2 DNA extraction, amplification and sequencing

DNA was extracted using the DNeasy Blood & Tissue Kit (QIAGEN®) or according to the standard protocol described by Sambrook et al. (1989) for phenol-chloroform extraction.

The large ribosomal subunit of the mitochondrial RNA (16S rRNA, 16S) was amplified by polymerase chain reaction (PCR) consisting of 2 μL of 10X buffer (200 mM Tris-HCl, 500 mM KCl), 2 μL of dNTP (4 x 2 mM), 2 μL of BSA (bovine serum albumin), 1 μL of MgCl_2 (50 mM), 0,3 μL Taq polymerase (5 U. μL^{-1}), 2 μL of primers, 1 μL of template and water to 20 μL . The thermal cycler conditions included an denaturation step at 94° for 1 minute followed by 35 cycles of 94° for 15 s, 50° for 1 min and 30 s, 72° for 2 min and 30 s, and a final extension step at 72° for 5 min. Forward (SHA - ACGGAATGAACTCAAATCATGT) and reverse (SHB - TCGACTGTTTACCAAAAACATA) primers used were previously published by Cunningham & Buss (1993). Amplified products were purified using the QIAquick PCR Purification Kit (QIAGEN®) and DNA sequencing was performed in forward and reverse directions, using an automated ABI3500 Genetic Analyzer.

2.2.3 Genetic data analyses

In order to infer phylogeographic patterns of fire corals in the Atlantic, a sequence length of approximately 600 base pairs of 16S rDNA gene was amplified for 270 colonies belonging to 16 populations. Sequences were edited with Sequencher v5.0 (Gene Codes Corporation® 1991-2011). A maximum-likelihood (ML) phylogenetic tree was inferred using an online version of PhyML v3.0 (Guindon et al. 2010) after the sequences had been aligned using ClustalW available in MEGA v5.05 (Tamura et al. 2011). The selection of the nucleotide-substitution model was performed in jModelTest v2.0 (Darriba et al. 2012), and the resulted model for the dataset according to the AIC criterion was HKY + G. Statistical support for the nodes was estimated using a bootstrap analysis that consisted of 100 replicates (identical haplotypes were excluded to save computational time). A Bayesian inference for evolutionary relationship of *Millepora* spp. was performed in Beast v2.0.1 (Drummond and Rambaut 2007, Bouckaert et al. 2013). Under the same model of evolution (HKY) computations in BEAST encompassed a chain length of 30,000,000 sampled every 3,000 steps.

The first 300 steps were discarded as burn in. Both ML and Bayesian consensus trees were generated using FigTree v1.4.0 (Rambaut 2006-2012).

A median-joining haplotype network (Bandelt et al. 1999) was constructed for the 16S rRNA gene using NETWORK[®] v4.6.1.1 (Fluxus Technology Ltd). Networks were classified according to regions for i) *M. alcicornis*; and also according to populations for ii) *M. alcicornis*, iii) *M. braziliensis*, iv) *M. nitida* and v) *M. laboreli*. Nodes in the network configured ancestral or not sampled haplotypes. The age of a clade can be estimated with the method implemented by Saillard et al. (2000). If the ancestral haplotype and its descendants form a perfect star phylogeny, the age is easily estimated according to coalescent theory (Govindarajan et al. 2005). According to Govindarajan et al. (2005), “The more these haplotypes depart from a star phylogeny, the wider the confidence limits. The method consists in estimating the divergence ρ , which is the average number of links in terms of observed mutations between the observed haplotypes and the ancestral haplotype, following the equation:

$$\rho = (n_1 l_1 + n_2 l_2 + \dots + n_m l_m) / n \quad (1)$$

where n is the number of individuals with a given haplotype, l is the number of steps (links) of a given haplotype to the ancestral haplotype, and m is the number of haplotypes. The equation of variance σ is describes as:

$$\sigma^2 = (n_1^2 l_1 + n_2^2 l_2 + \dots + n_m^2 l_m) / n^2 \quad (2)$$

In order to express ρ in terms of a per-locus rate of substitution, we multiplied the substitution rate available for other Hydrozoa (Hydractinia [Cunningham et al. 1991] and Conopora [Lindner et al. 2008]) times the number of positions in 16S rRNA data.

The number of haplotypes (H), segregating sites (s), standard measures of genetic diversity (gene diversity [h] and nucleotide diversity [π]), average of nucleotide changes (k) and statistics for neutral sequence evolution (Tajima’s D and Fu’s F_s) were calculated both for populations, regions and species using Arlequin v3.5.1.2 (Excoffier and Lischer 2010). Gene diversity is characterized by the probability that two randomly chosen haplotypes differ in the sample, whereas the

probability that two randomly chosen homologous sites difference characterizes the nucleotide diversity. Biogeographic regions adopted for *M. alvicornis* to perform genetic analyses were North Atlantic (Bermuda), Caribbean (Florida, Panama and Colombia), Eastern Atlantic (Cape Verde), Northern/Northeastern Brazil (Fernando de Noronha, Rio Grande do Norte, Pernambuco and Southern Alagoas) and Eastern/Southern Brazil (Northern Bahia, Southern Bahia [Porto Seguro and Abrolhos], Espírito Santo and Rio de Janeiro).

Genetic differentiation among populations/regions was tested using an analysis of molecular variance (AMOVA) performed in Arlequin v3.5.1.2. Hierarchical AMOVA is comprised by levels of genetic differentiation among populations (Φ_{st}), between groups of populations or regions (Φ_{ct}), and between populations within regions (Φ_{st}). This index ranges from 0 to 1, in which 0 corresponds to a lack of differentiation and, conversely, 1 corresponds to maximum differentiation between populations or regions. Furthermore, in order to define groups of populations that are geographically homogeneous and maximally differentiated from each other, a spatial analysis of molecular variance (SAMOVA) was performed (Dupanloup et al. 2002). As a by-product, SAMOVA approach also leads to identification of genetic barriers between groups. This analysis does not require groups formed a priori, because the method implemented in the analysis enables to find a group structure based exclusively on genetic data.

2.2.4 Morphological analyses

After the recognition of the genetic clades, the endemic species *M. braziliensis* and *M. nitida* were classified in three morphotypes in order to evaluate morphological variation among them. The morphotypes delineated were *M. braziliensis* (Mb), *M. nitida* ramified (Mn) and *M. nitida* encrusted (Mne). A fixed number of 10 colonies per morphotype were bleached with 30% sodium hypochlorite, dried and analyzed under a stereoscopic microscope connected to a PC-integrated camera. The program used for measurements of the colonies was AxioVision LE (Carl Zeiss MicroImaging GmbH[®], 2010). Morphological variation was achieved by the following measures (Figure 4): i) gastropore diameter, ii) dactylopore diameter, iii) distance between gastropores, iv) distance from gastropore to nearest dactylopore, v) distance between dactylopores and vi) number of dactylopores per gastropore. Measures i, ii and vi were previously

undertaken by Amaral et al. (2002) in Brazilian fire corals using univariate statistics analyses, whereas the first five measurements were applied by Ramos (2009) in Caribbean fire corals using univariate and multivariate statistics analyses.

Up to 60 measurements were taken per colony per character, resulting in a range of 448 – 600 measurements per morphotype per character. Data were tested for normality and homocedasticity. Even after transformation, the data did not meet normality. Univariate statistics in this study were achieved by means of non-parametric Kruskal-Wallis analyses to test the hypothesis that there were no significant morphological differences between the three taxa for each analyzed character. Afterwards, a multivariate analysis was applied using a reduced number of measurements ($n = 40$ per colony per trait) to avoid missing data. A Discriminant Function Analysis (DA) was used to test the utility of the six morphological traits to distinguish the identified morphotypes. Statistical analyses were performed in STATISTICA v10 (StatSoft.Inc[©] 2011).

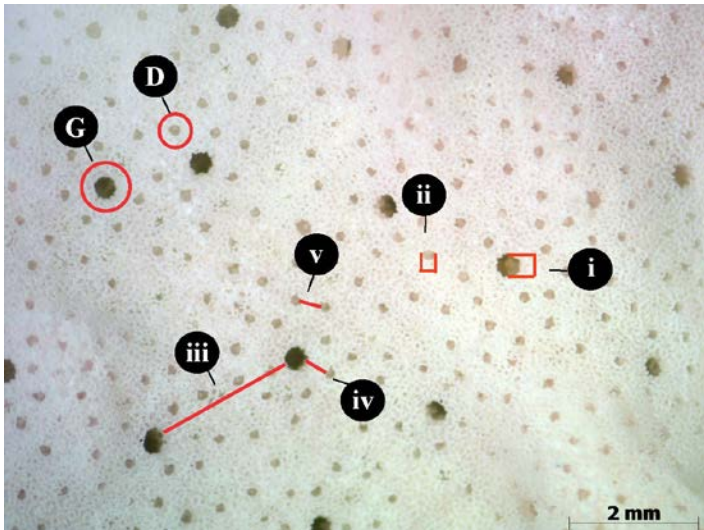


Figure 4. Measurements performed in colonies of Atlantic *Millepora* species. G, gastropore; D, dactylopore; i, gastropore diameter; ii, dactylopore diameter; iii, distance between gastropores; iv, distance from gastropore to nearest dactylopore; v, distance between dactylopores. Photo: Júlia Souza.

2.3 RESULTS

2.3.1 Data set

The resulting sequences (after being edited, aligned and had the tips cut) were 535 base pairs long. A total of 163 coral colonies of *M. alcicornis* were genetically analyzed as well as 52 colonies of *M. nitida*, 46 colonies of *M. braziliensis* and 9 colonies of *M. laboreli*.

2.3.2 Phylogenetic analyses

Consensus tree built with the Bayesian method is shown in Figure 5. The Bayesian tree is supported by posterior probability and also shows the bootstrap values from the ML inference. According to the phylogeny inferred the four species formed well supported clades with bootstrap values > 0.85. The phylogenetic tree revealed reciprocal monophyly that may result in range change in the endemic species *M. braziliensis*, because in the literature this species occurs from Northern to Southern Brazil contrasting with our findings: this species was restricted to Northern/Northeastern Brazil in this study. Morphotypes ascribed as *M. braziliensis* that were collected in Espírito Santo (22 colonies) fell into the monophyletic clade of *M. nitida*. Additionally, all three Brazilian endemic fire corals have shown to be sister species, in which the closer clades were *M. laboreli* + *M. nitida*, and these two were sister clades of *M. braziliensis*, the most basal clade among endemic species. This molecular marker did not have enough resolution to clearly distinguish among populations of *M. alcicornis*, and this may represent a signal of recent divergence of its populations.

These results may have profound implications in the area of distribution covered by the endemic fire corals *M. braziliensis* and *M. nitida* for three main reasons: (1) a good sampling (~50 sampled colonies of each species) was carried out, and they did not co-occur at any single point of their distributions; (2) all northern SFP samples of fire corals that did not belong to *M. alcicornis* clade, clustered in a second clade, here named *M. braziliensis* (based on the type-location described by Verrill 1868); and (3) all southern SFP samples that did not belong to *M. alcicornis* clade, clustered in a third group, here named *M. nitida* (based on the type-location described by Verrill 1868). These results indicate that differently from the literature, which states that they are sympatric species in Alagoas and Abrolhos (Amaral et al. 2008),

they are actually allopatric species that may have been evolutionarily isolated by the barrier formed by the SFP (Figure 6). SAMOVA results below provide additional support for this assumption.

2.3.3 Morphological analyses

All morphometric measurements differed significantly among morphotypes according to the Kruskal-Wallis results ($P < 0.05$). Summary statistics are presented in Table 1, and the boxplot diagrams are available in Figure 7. Diameter of gastropores (G) and dactylopores (D) were larger in encrusting *M. nitida* (Mean \pm SE: G = 0.304 ± 0.001 , D = 0.141 ± 0.001). The distance between gastropores and between dactylopores were greater in average in branching *M. nitida* (Means \pm SE: G-G = 1.243 ± 0.016 , D-D = 0.227 ± 0.002). Also, the distance from gastropore to the nearest dactylopores and the number of dactylopores per gastropore were higher in *M. braziliensis* (Mean \pm SE: G-D = 0.172 ± 0.002 , D/G = 6.494 ± 0.046).

The Discriminant Function Analysis (Wilk's $\lambda = 0.219$, $F = 197.550$, $P < 0.0001$) corroborated the a priori assigned groups, with 76.426% ($n = 804$) of the replicated measurements correctly classified (Figure 8). The canonical plot showed one distinct group, corresponding to encrusting *M. nitida*, and two other groups superimposed, corresponding to ramified *M. nitida* and *M. braziliensis*. Major morphological variation among morphotypes occurred along root 1 (eigenvalue = 3.364) (Table 2). The highest standardized canonical coefficients for variables associated to root 1 were diameter of gastropores and dactylopores, which, as a consequence, were the variables that better discriminated the encrusting *M. nitida* from the branching *M. nitida* and *M. braziliensis*.

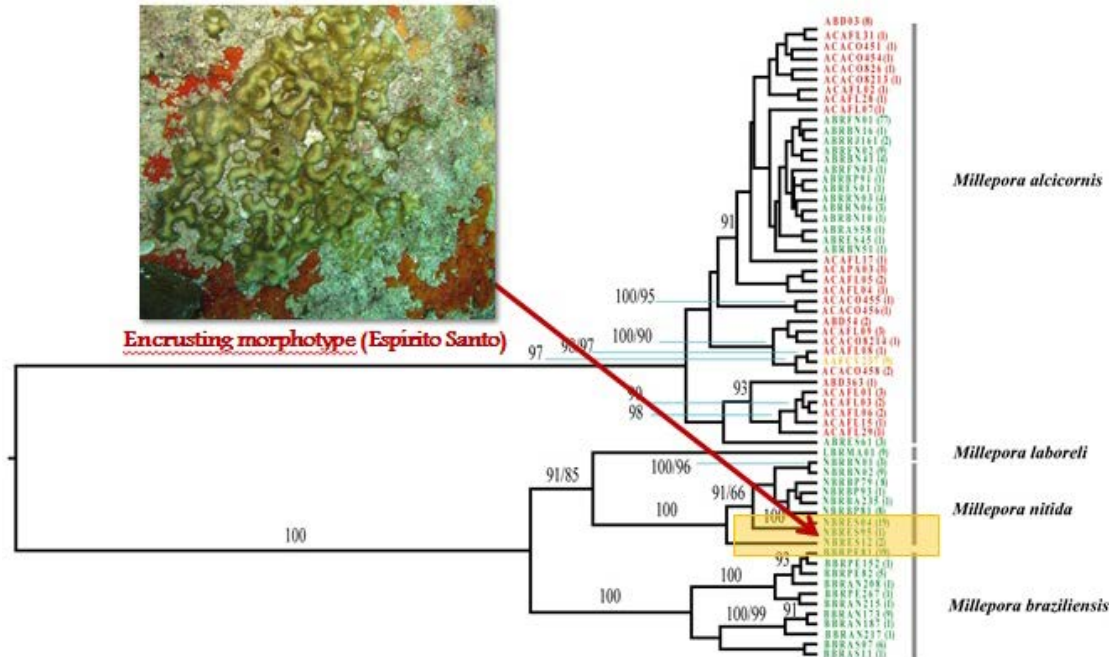


Figure 5. Bayesian consensus tree build for Atlantic milleporids. Numbers represent the support values: posterior probability/bootstrap for an equivalent Maximum Likelihood tree. Colors on the tips of the branches indicate regions of origin of samples: red denotes Great Caribbean (Bermuda + Caribbean), green denotes Brazil and yellow denotes Eastern Atlantic. Numbers in brackets indicate the number of individuals with the same haplotype. The rectangular orange highlights the unique encrusting colonies of *M. nitida* described for Guarapari Islands, Espírito Santo.

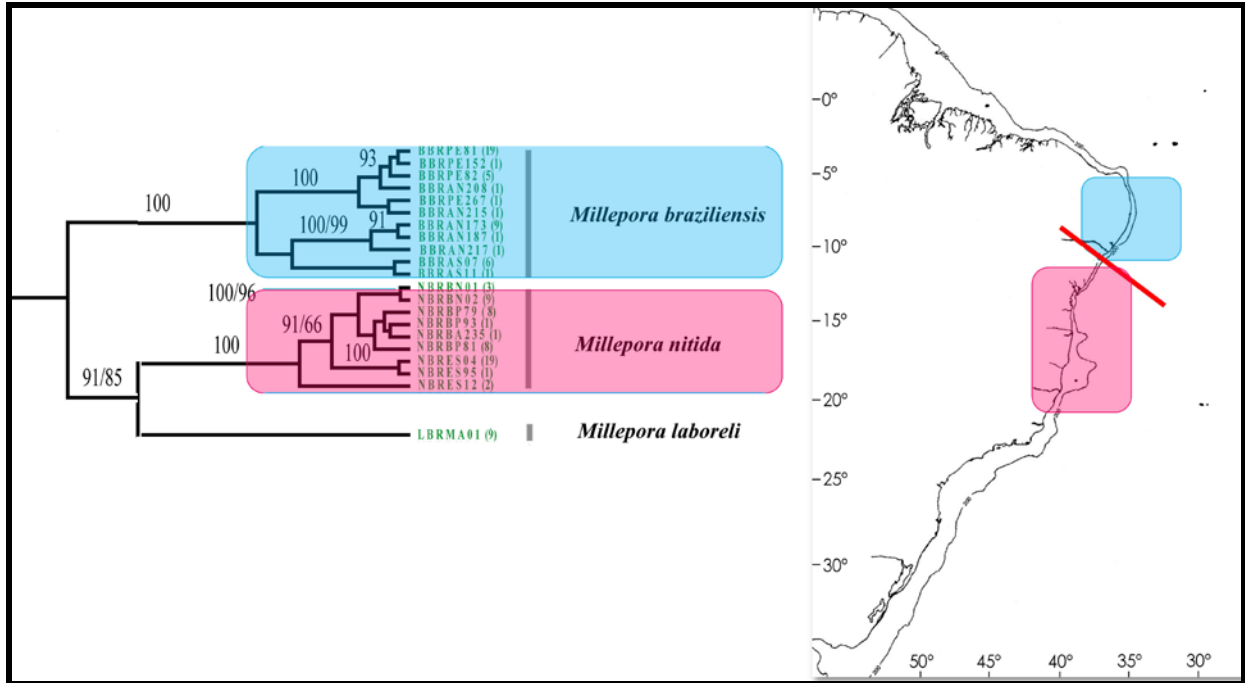


Figure 6. Bayesian consensus tree build for Brazilian endemic Milleporidae emphasizing the area covered by *Millepora nitida* and *M. braziliensis* samples from Pernambuco to Espírito Santo. The red bar indicates the barrier imposed by the São Francisco Plume to the dispersal of these two species.

Table 1. Summary statistics of morphological variables of the following morphotypes: Mb, *Millepora braziliensis*; Mn, *M. nitida* ramified; and Mne, *M. nitida* encrusting. Six variables were measured: G, diameter of gastropores; D, diameter of dactylopores; G-G, distance between gastropores; D-D, distance between dactylopores; G-D, distance between gastropores to nearest dactylopores; D/G, and number of dactylopores per gastropores.

Morphotype	Variable	N	Median	Mean	S.E.	Min	Max
Mb	G	600	0.200	0.200	0.001	0.080	0.310
Mn	G	600	0.210	0.216	0.001	0.130	0.330
Mne	G	590	0.310	0.304	0.001	0.170	0.480
Mb	D	600	0.090	0.091	0.001	0.050	0.140
Mn	D	600	0.100	0.102	0.001	0.060	0.150
Mne	D	600	0.141	0.141	0.001	0.090	0.210
Mb	G-G	600	1.130	1.178	0.016	0.160	2.600
Mn	G-G	600	1.220	1.243	0.016	0.250	2.600
Mne	G-G	588	0.960	0.960	0.012	0.150	1.920
Mb	G-D	600	0.160	0.172	0.002	0.050	0.420
Mn	G-D	600	0.160	0.162	0.002	0.070	0.310
Mne	G-D	574	0.160	0.159	0.002	0.030	0.330
Mb	D-D	600	0.240	0.242	0.002	0.120	0.500
Mn	D-D	600	0.230	0.227	0.002	0.120	0.430
Mne	D-D	600	0.210	0.219	0.002	0.090	0.480
Mb	D/G	555	6.000	6.494	0.046	4.000	11.000
Mn	D/G	448	6.000	6.172	0.045	4.000	9.000
Mne	D/G	498	6.000	5.737	0.050	3.000	8.000

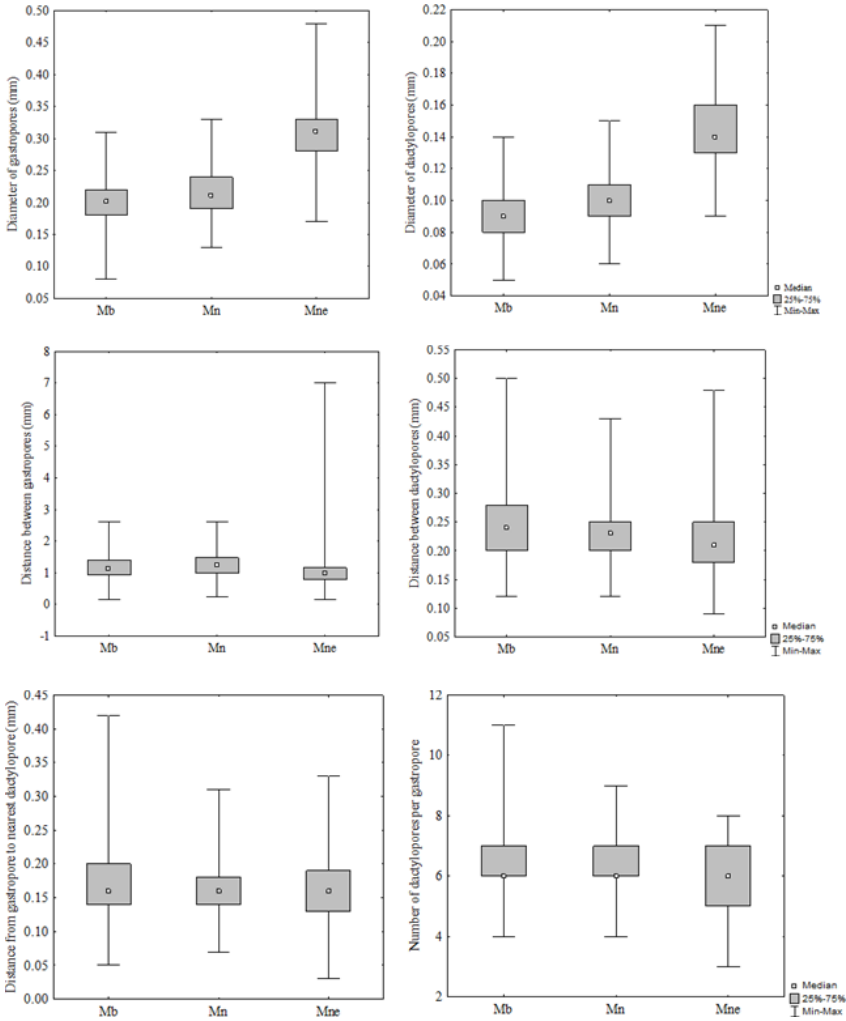


Figure 7. Boxplot of morphological variables measured in the following morphotypes: Mb, *Millepora braziliensis*; Mn, *M. nitida* ramified and Mne, *M. nitida* encrusting.

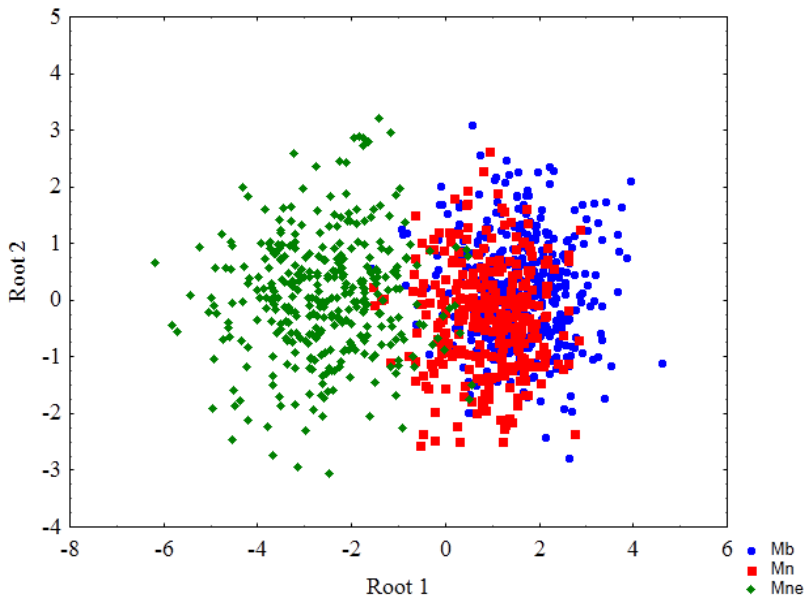


Figure 8. Discriminant Function Analysis of the following *Millepora* morphotypes: *M. braziliensis* (Mb) in blue, *M. nitida* ramified (Mn) in red and *M. nitida* encrusting (Mne) in green.

Table 2. Standardized coefficients of canonical variables. The variables evaluated were G, diameter of gastropores; D, diameter of dactylopores; G-G, distance among gastropores; G-D, distance from gastropore to nearest dactylopores; D-D, distance among dactylopores; and D/G, number of dactylopores per gastropore.

Variable	Root 1	Root 2
G	-0.641	0.397
D	-0.617	-0.347
G-G	0.102	-0.589
G-D	-0.088	0.407
D-D	0.166	0.357
D/G	0.154	0.533
Eigenvalue	3.364	0.045

2.3.4 Haplotype network distribution and age estimation

A total of 42 haplotypes were observed for *M. alpicornis*, 11 for *M. braziliensis*, 9 for *M. nitida* and only one for *M. laboreli* (Figure 7—9). One haplotype was shared between the North Atlantic and the Caribbean, whereas one haplotype was shared between Northern/Northeastern and Eastern/Southern Brazilian regions. On the other hand, among Brazil, the Caribbean and Eastern Atlantic no haplotypes were shared (Figure 9). A star phylogeny was detected for the Brazilian populations as a whole, in which all sampled sites in Brazil shared a major haplotype, and almost all the other haplotypes have been recently emerged. An exception to this rule was a divergent haplotype from Espírito Santo that seems to be more closely related to the Caribbean samples. Haplotypes of the endemic species *M. laboreli* and *M. braziliensis* were confined to Northern/Northeastern Brazil, whilst *M. nitida* was observed in the Eastern/Southern Brazilian region, showing no overlap across their ranges (Figure 11). Central populations of *M. braziliensis* and *M. nitida* shared haplotypes with only one adjacent population for each of these species. In general, the endemic species exhibited restricted haplotypes.

For age estimation of Brazilian populations of *M. alpicornis*, different rates of substitution were used. Using the slowest rate belonging to *Hydractinia* spp. (1.25×10^{-9} substitution site⁻¹ year⁻¹), the analysis resulted in an estimate of 846 ± 202 thousand years for the formation of the Brazilian populations, including the most divergent haplotype from Espírito Santo. Considering that the divergent haplotype probably had appeared in Brazil in a time different than the other haplotypes, which are much more similar among each other, we performed an estimate excluding this haplotype from analysis. If the divergent haplotype is excluded from the analysis, the estimative falls down to 421 ± 158 thousand years. The higher rate of substitution used from *Conopora* spp. (7.71×10^{-9} substitution site⁻¹ year⁻¹) resulted in an age estimation of 141 ± 33 thousand years, considering all Brazilian haplotypes. The last estimative, removing the most divergent haplotype, corresponded to 70 ± 26 thousand years.

2.3.5 Genetic diversity and neutrality

Molecular diversity indices for each population, region and species are shown in Table 3. All haplotypes were identical for the

Eastern Atlantic populations (*M. alcicornis*), Southern Bahia and Maranhão (*M. laboreli*) and for this reason no genetic diversity or neutrality were recorded for these sites. Conversely, for *M. alcicornis*, high genetic diversity was found in the Caribbean region ($h = 0.975 \pm 0.012$, $\pi = 0.010 \pm 0.005$), contrasting with lower gene diversity found in the North Atlantic ($h = 0.556 \pm 0.165$, $\pi = 0.008 \pm 0.005$) and Brazil ($h = 0.503 \pm 0.058$, $\pi = 0.002 \pm 0.001$). Gene diversity also differed among regions of Brazil, with higher values in the Northern/Northeastern region ($h = 0.696 \pm 0.060$) if compared to the Eastern/Southern region ($h = 0.359 \pm 0.071$). The same pattern was observed for the average nucleotide changes (k) and also for the number of haplotypes (H) and segregating sites (s): greater values being found in the Caribbean, intermediate values in Northern Brazil and lowest values in Southern Brazil. For the endemic species *M. braziliensis* the highest gene diversity was assigned to the Northern Alagoas population ($h = 0.702 \pm 0.080$, $\pi = 0.007 \pm 0.004$), the central population in the covered range. *M. nitida* followed a similar pattern, with a central population holding the greatest gene diversity ($h = 0.667 \pm 0.314$), but a peripheral population holding the highest nucleotide diversity ($\pi = 0.003 \pm 0.002$). In general, populations of fire corals did not show a significant departure from neutrality ($P > 0.05$). However, considering *M. alcicornis*, significant values ($P < 0.05$) were recorded for Caribbean ($F_s = -12.032$, $P = 0.000$), Eastern/Southern Brazil ($D = -2.083$, $P = 0.002$; $F_s = -4.039$, $P = 0.038$), the Brazilian region as a whole ($D = -2.264$, $P = 0.000$; $F_s = -9.415$, $P = 0.002$), and for the entire species ($D = -1.615$, $P = 0.014$; $F_s = -24.020$, $P = 0.000$). It is important to state that negative values of F_s are indicative of an excess number of alleles, as a result of recent population expansion or genetic hitchhiking (Fu, 1997). Thereafter, according to the F_s values plus the star phylogeny found for Brazilian *M. alcicornis*, there is solid evidence of recent population expansion for this species.

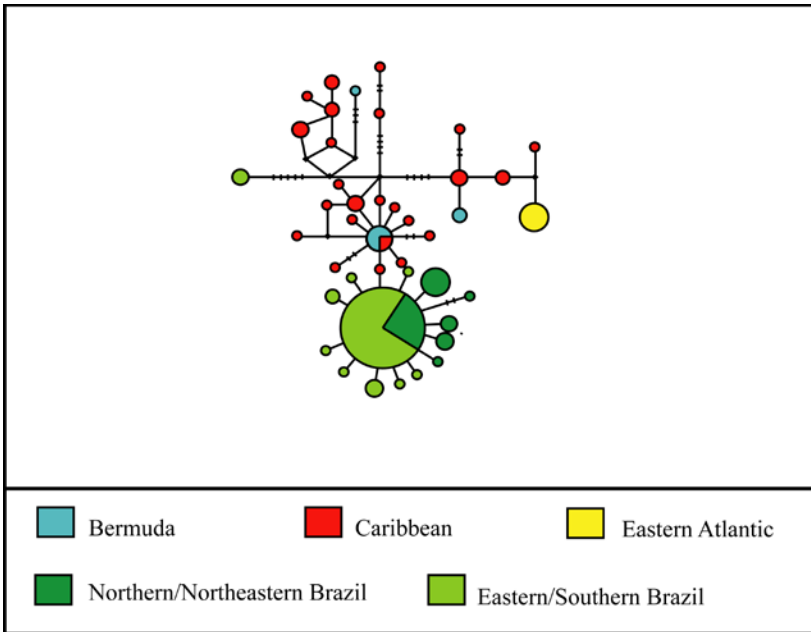


Figure 9. Haplotype network of *Millepora alcicornis* from the Atlantic Ocean. Colors illustrate major regions of fire coral sampling localities, black circles represent ancestral or not sampled haplotypes and black dashes correspond to mutational events.

2.3.6 Population genetic structure and genetic barriers

Populations of *Millepora alcicornis*, *M. braziliensis* and *M. nitida* exhibited significant genetic structure throughout the tropical Atlantic Ocean (Table 4). Hierarchical AMOVA revealed significant genetic structure at all levels for *M. alcicornis*: between populations ($\Phi_{st} = 0.596\text{--}0.680$), among populations within regions ($\Phi_{sc} = 0.067\text{--}0.079$) and between regions ($\Phi_{ct} = 0.567\text{--}0.652$). Fixation indices increased substantially once Northern and Southern Brazil were grouped together. On the other hand, when grouping North Atlantic with the Caribbean, the results did not differ much. Variation among groups was the most prominent among all, accounting for 56.69–65.21%, followed by a variation of 32.30–40.43% within populations and by 2.55–3.24% of

variation among populations within regions. Genetic subdivision in the endemic species was very strong (*M. braziliensis*, $\Phi_{st} = 0.689$; *M. nitida*, $\Phi_{st} = 0.828$), and the major portion of the differentiation occurred among populations for both species (*M. braziliensis* = 68.89%; *M. nitida* = 82.85%).

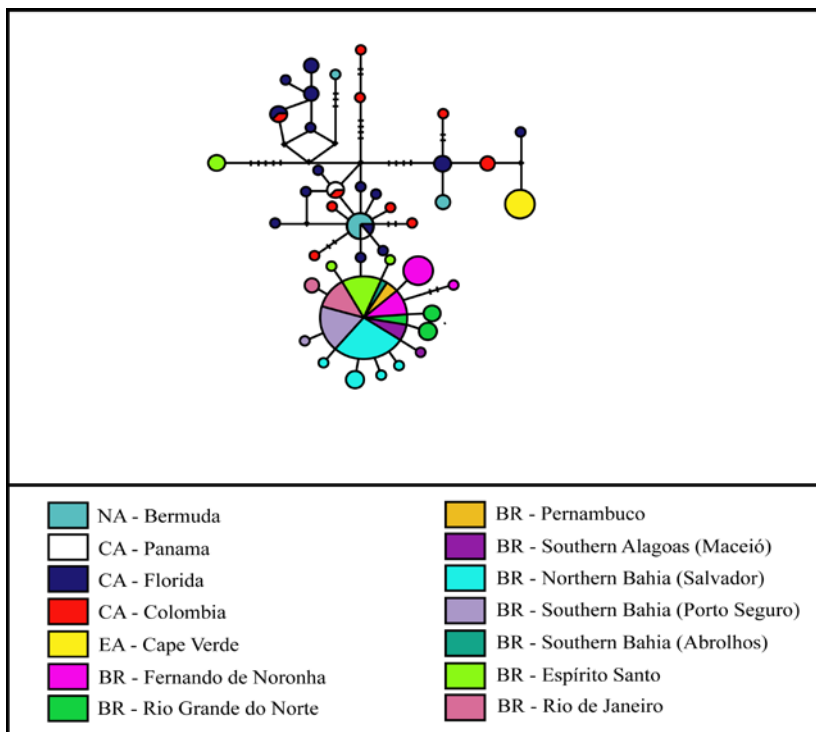


Figure 10. Haplotype network of *Millepora alcicornis* from the Atlantic Ocean. Colors illustrate populations of fire coral set by localities, black circles represent ancestral or not sampled haplotypes and black dashes correspond to mutational events. Populations are classified according to major regions: NA, North Atlantic; CA, Caribbean; EA, Eastern Atlantic; and BR, Brazil.

Pairwise Φ_{st} data for *M. alcicornis* are presented in Table 5. In terms of pairwise Φ_{st} , strong genetic differentiation was observed in the population from Eastern Atlantic against all others ($\Phi_{st} = 0.569$ –1.000). Moreover, significant differentiation was found in populations from

Brazil in relation to the Caribbean ($\Phi_{st} = 0.276\text{--}0.931$) and the North Atlantic ($\Phi_{st} = 0.340\text{--}0.629$). Overall, no significant pairwise Φ_{st} values were observed among Brazilian populations, except for populations from Fernando de Noronha ($\Phi_{st} = 0.191\text{--}0.414$), Rio Grande do Norte ($\Phi_{st} = 0.130\text{--}0.390$) and from Espírito Santo compared to Northern Bahia ($\Phi_{st} = 0.106$). Among the *Millepora* endemic species, high genetic differentiation was observed among almost all populations, with the exception of Porto Seguro compared to Abrolhos (Table 6). Significant pairwise Φ_{st} values ranged from 0.499 to 0.934 in *M. braziliensis* and from 0.768 to 0.857 in *M. nitida*.

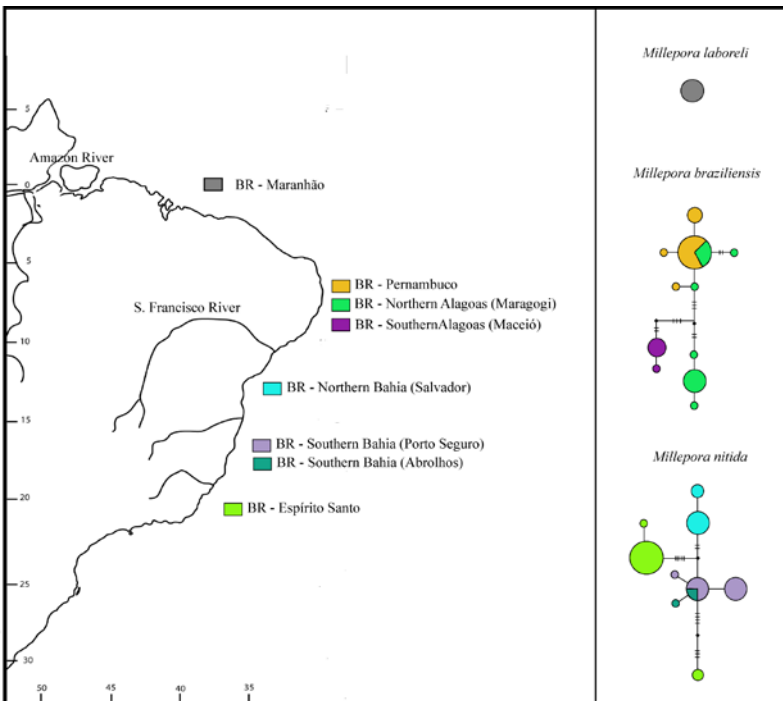


Figure 11. Haplotype networks of Brazilian endemic Milleporidae. Colors illustrate populations of fire coral set by localities, black circles represent ancestral or not sampled haplotypes and black dashes correspond to mutational events.

Table 3. Molecular diversity indices and neutrality tests for 16S rRNA gene in Atlantic *Millepora*. Codes used are *N*, number of individuals; *H*, number of haplotypes; *s*, number of segregating sites; *h*, gene diversity; π , average nucleotide diversity; and *k*, average number of nucleotide differences.

	Molecular diversity indices (16S - 535bp)						Neutrality	
	<i>N</i>	<i>H</i>	<i>s</i>	<i>h</i>	π	<i>k</i>	Tajima's <i>D</i>	Fu's <i>F_s</i>
<i>M. alvicornis</i>	163	42	50	0.768 ± 0.035	0.008 ± 0.004	4.12 ± 2.06	-1,615	-24,020
North Atlantic (Bermuda)	9	3	12	0.556 ± 0.165	0.008 ± 0.005	4.06 ± 2.23	-0.386	3,751
Caribbean	34	23	29	0.975 ± 0.012	0.010 ± 0.005	5.39 ± 2.66	-0.845	-12,032
Florida	21	15	20	0.967 ± 0.024	0.010 ± 0.006	5.33 ± 2.68	-0.152	-5,629
Panama	3	2	1	0.667 ± 0.314	0.001 ± 0.001	0.67 ± 0.67	0	0.201
Colombia	11	10	22	0.982 ± 0.046	0.012 ± 0.007	6.51 ± 3.33	-0.608	-3,548
Eastern Atlantic (Cape Verde)	9	1	0	0	0	0	0	N.A.
Brazil	110	15	25	0.503 ± 0.058	0.002 ± 0.001	1.10 ± 0.73	-2,264	-9,415
<i>Northern/Northeastern Brazil</i>	35	6	7	0.696 ± 0.060	0.002 ± 0.001	0.99 ± 0.69	-1,188	-1,474
Fernando de Noronha	15	3	4	0.562 ± 0.095	0.002 ± 0.001	0.92 ± 0.67	-0.823	0.736
Rio Grande do Norte	9	3	2	0.722 ± 0.097	0.002 ± 0.002	1.06 ± 0.77	1,494	0.453
Pernambuco	5	1	0	0	0	0	0	N.A.
Southern Alagoas - Maceio	6	2	1	0.333 ± 0.215	0.001 ± 0.001	0.33 ± 0.38	-0.933	-0.003
<i>Eastern/Southern Brazil</i>	75	10	18	0.359 ± 0.071	0.002 ± 0.001	1.09 ± 0.73	-2,083	-4,039
Northern Bahia - Salvador	24	5	4	0.486 ± 0.113	0.001 ± 0.001	0.53 ± 0.46	-1,356	-2,538
Southern Bahia - Porto Seguro	17	2	1	0.118 ± 0.101	0.000 ± 0.000	0.12 ± 0.20	-1,164	-0.748
Southern Bahia - Abrolhos	3	1	0	0	0	0	0	N.A.
Espírito Santo	20	4	12	0.432 ± 0.126	0.005 ± 0.003	2.88 ± 1.58	-0.529	3,034
Rio de Janeiro	11	2	1	0.327 ± 0.153	0.001 ± 0.001	0.33 ± 0.36	-0.100	0.356
<i>M. laboreli</i> (Maranhão)	9	1	0	0	0	0	0	N.A.
<i>M. braziliensis</i>	46	11	17	0.776 ± 0.047	0.009 ± 0.005	4.75 ± 2.37	0.723	0.716
Pernambuco	20	4	4	0.537 ± 0.104	0.001 ± 0.001	0.69 ± 0.55	-1,111	-0.831
Northern Alagoas - Maragogi	19	6	10	0.702 ± 0.080	0.007 ± 0.004	3.91 ± 2.05	1,296	1,629
Southern Alagoas - Maceió	7	2	1	0.286 ± 0.196	0.000 ± 0.001	0.29 ± 0.34	-1,006	-0.095
<i>M. nitida</i>	52	9	18	0.798 ± 0.035	0.009 ± 0.005	4.52 ± 2.26	0.420	2,214
Northern Bahia - Salvador	12	2	1	0.409 ± 0.133	0.001 ± 0.001	0.41 ± 0.40	0.540	0.735
Southern Bahia - Porto Seguro	15	3	2	0.590 ± 0.077	0.001 ± 0.001	0.67 ± 0.54	0.221	0.105
Southern Bahia - Abrolhos	3	2	1	0.667 ± 0.314	0.001 ± 0.002	0.67 ± 0.67	0	0.201
Espírito Santo	22	3	10	0.255 ± 0.116	0.003 ± 0.002	1.65 ± 1.01	-1,360	2,715

* Statistically significant values ($\alpha = 0.05$) are highlighted in bold

Table 4. Analyses of Molecular Variance (AMOVA) of the Atlantic *Millepora* using data from 16S rRNA gene. Regions used in the analyses consisted of NA, North Atlantic Ocean; CA, Caribbean; EA, Eastern Atlantic; BRN, Northern/Northeastern Brazil; and BRS, Eastern/Southern Brazil.

<i>Millepora alvicornis</i>							
Regions	Source of variation	Variance components	% of variation	Regions	Source of variation	Variance components	% of variation
NA, CA, EA, BRN, BRS				NA, CA, EA, BR			
	Among groups	1.415	56.69		Among groups	1.972	64.46
	Among populations within regions	0.072	2.89		Among populations within regions	0.078	2.55
	Within populations	1.009	40.43		Within populations	1.009	32.99
	Fixation indices				Fixation indices		
	ϕ_{ct}	0.567			ϕ_{ct}	0.645	
	ϕ_{sc}	0.067			ϕ_{sc}	0.072	
	ϕ_{st}	0.596			ϕ_{st}	0.670	
NA, CA, EA, BRN, BRS				NA, CA, EA, BR			
	Among groups	1.452	57.07		Among groups	2.055	65.21
	Among populations within regions	0.082	3.24		Among populations within regions	0.087	2.76
	Within populations	1.009	39.68		Within populations	1.009	32.3
	Fixation indices				Fixation indices		
	ϕ_{ct}	0.571			ϕ_{ct}	0.652	
	ϕ_{sc}	0.075			ϕ_{sc}	0.079	
	ϕ_{st}	0.603			ϕ_{st}	0.680	
<i>Millepora braziliensis</i>							
Populations	Source of variation	Variance components	% of variation	Populations	Source of variation	Variance components	% of variation
PE, ALN, ALS				BAN, BASP, BASA, ES			
	Among populations	2.195	68.89		Among populations	2.506	82.85
	Within populations	0.991	31.11		Within populations	0.519	17.15
	Fixation indices				Fixation indices		
	ϕ_{st}	0.689			ϕ_{st}	0.828	

*Statistically significant values ($\alpha = 0.05$) are highlighted in bold

Table 5. Pairwise Φ_{st} for *Millepora alcicornis* populations inferred from data of 16S rRNA gene.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 Bermudas	0.000													
2 Cape Verde	0.753	0.000												
3 Panama	-0.070	0.984	0.000											
4 Florida	0.053	0.608	0.013	0.000										
5 Colombia	-0.016	0.569	-0.068	0.036	0.000									
6 Fernando de Noronha	0.570	0.951	0.749	0.505	0.507	0.000								
7 Rio Grande do Norte	0.500	0.955	0.722	0.452	0.426	0.382	0.000							
8 Pernambuco	0.438	1.000	0.915	0.392	0.354	0.281	0.221	0.000						
9 Southern Alagoas - Maceió	0.455	0.988	0.847	0.407	0.376	0.291	0.227	-0.034	0.000					
10 Northern Bahia - Salvador	0.629	0.965	0.812	0.545	0.568	0.351	0.301	-0.052	0.016	0.000				
11 Southern Bahia - Porto Seguro	0.628	0.993	0.931	0.519	0.541	0.414	0.390	-0.102	0.067	0.047	0.000			
12 Southern Bahia - Abrolhos	0.358	1.000	0.875	0.339	0.276	0.210	0.125	0.000	-0.154	-0.138	-0.195	0.000		
13 Espírito Santo	0.340	0.829	0.338	0.363	0.347	0.191	0.130	-0.029	0.001	0.106	0.082	-0.115	0.000	
14 Rio de Janeiro	0.544	0.984	0.862	0.465	0.463	0.346	0.298	-0.004	0.053	0.068	0.098	-0.100	0.057	0.000

* Statistically significant values ($\alpha = 0.05$) are highlighted in bold

Table 6. Pairwise Φ_{st} for *Millepora braziliensis* and *M. nitida* inferred from data of 16S rRNA gene.

<i>Millepora braziliensis</i>				<i>Millepora nitida</i>				
Population	1	2	3	Population	1	2	3	4
1 Pernambuco	0.000			1 Northern Bahia - Salvador	0.000			
2 Northern Alagoas - Maragogi	0.499	0.000		2 Southern Bahia - Porto Seguro	0.857	0.000		
3 Southern Alagoas - Maceió	0.934	0.675	0.000	3 Southern Bahia - Abrolhos	0.873	0.286	0.000	
				4 Espírito Santo	0.842	0.816	0.768	0.000

* Statistically significant values ($\alpha = 0.05$) are highlighted in bold

The resulting fixation indices corresponding to population groups inferred by the SAMOVA analyses are presented in Table 7. The strongest genetic structure assigned for *M. alcicornis* corresponded to two groups of populations ($F_{ct} = 0.700$), with the Eastern Atlantic Ocean

population isolated from the Brazilian and Caribbean (Western Atlantic Ocean) ones. In addition, the presence of a genetic barrier between East and West Tropical Atlantic was inferred for this species. In contrast, endemic species were highly structured, showing seven groups of populations as the result with the highest F_{ct} ($F_{ct} = 0.925$). Barriers to gene flow were inferred among almost all populations, except between Porto Seguro and Abrolhos populations (Southern Bahia). Isolation of *M. laboreli* and the Espirito Santo population of *M. nitida* from the others were consistent in all SAMOVA analyses. The groups inferred from SAMOVA corroborate the assumption based on phylogenetic analysis that the São Francisco Plume imposes a barrier to the distribution of *M. braziliensis* and *M. nitida* populations.

Table 7. Fixation indices and correspondent population groups inferred by SAMOVA algorithm for Atlantic *Millepora* by using data from 16S rRNA gene. Localities corresponding to populations used in the analysis were BD, Bermuda; PA, Panama; FL, Florida; CO, Colombia; CV, Cape Verde; MA, Maranhão; RN, Rio Grande do Norte; FN, Fernando de Noronha; PE, Pernambuco; AN, Northern Alagoas; AS, Southern Alagoas; BN, Northern Bahia; BP, Porto Seguro in Southern Bahia; BA, Abrolhos in Southern Bahia; ES, Espírito Santo; and RJ, Rio de Janeiro.

Species	Number of groups	Group composition	F _{sc}	F _{st}	F _{ct}
<i>Millepora alcicornis</i>					
	Two groups	1. CV	0.407	0.822	0.700
		2. BD, PA, FL, CO, FN, RN, PE, AS, BN, BP, BA, ES, RJ			
	Three groups	1. BD, PA, FL, CO	0.079	0.680	0.652
		2. CV			
		3. FN, RN, PE, AS, BN, BP, BA, ES, RJ			
	Four groups	1. PA	0.757	0.676	0.649
		2. BD, FL, CO			
		3. CV			
		4. FN, RN, PE, AS, BN, BP, BA, ES, RJ			
<i>Millepora</i> spp. endemics					
	Three groups	1. MA	0.776	0.948	0.767
		2. PE, AN, AS			
		3. BN, BP, BA, ES			
	Five groups	1. MA	0.644	0.938	0.826
		2. PE, AN			
		3. AS			
		4. BN, BP, BA			
		5. ES			
	Seven groups	1. MA	0.085	0.931	0.925
		2. PE			
		3. AN			
		4. AS			
		5. BN			
		6. BP, BA			
		7. ES			

* Statistically significant values ($\alpha = 0.05$) are highlighted in bold

2.4 DISCUSSION

2.4.1 The Atlantic widespread fire coral species, *Millepora alcicornis*

2.4.2.1 Molecular phylogeny

This is the first study that evaluates the molecular status of fire corals in the South Atlantic Ocean by means of nucleotide sequences. Our current findings suggest the existence of four well-delimited species in the South Atlantic Ocean: *Millepora alcicornis*, *M. braziliensis*, *M. nitida* and *M. laboreli*. In contrast, the study of Ramos (2009) examined Caribbean samples of *M. squarrosa*, *M. alcicornis*, *M. complanata* and *M. striata* and indicated the existence of a species complex formed by the three latter species. High genetic differentiation was found within Caribbean populations rather than between the morphotypes of *M. alcicornis*, *M. complanata* and *M. striata*, which provided evidence for a species complex formed by these three taxa. Herein, *M. alcicornis* was easily distinguished from its Brazilian endemic congeners, based on support values (bootstrap and posterior probability) of 100%. These results corroborate the study performed by Amaral et al. (1997), in which *M. alcicornis* and *M. braziliensis* were depicted as valid taxonomic species as revealed by allozyme markers.

2.4.2.2 Genetic connectivity

Exploring the extent to what peripheral populations are interconnected, as well as, connected to more central populations, can provide knowledge on the ability of dispersal of corals, and also on the barriers that constrain the movements of their dispersors (Nunes et al. 2009). Our findings reveal significant genetic structure for *M. alcicornis* across major regions of the Atlantic separated by at least 2,500—5,000 km, the Caribbean, Brazil and Eastern Atlantic. Furthermore, no haplotypes were shared among these three regions, suggesting that AOP and MAB can represent effective barriers to gene flow for this species. Similar findings were reported by Nunes et al. (2009), with significant genetic structure being found among populations of the scleractinian coral *Montastraea cavernosa* spanning the same regions. The authors attributed the observed differences to the operation of the two aforementioned barriers to gene flow (Nunes et al. 2009). Additionally, other broadcasting, *Siderastrea siderea* (Ellis & Solander, 1768), and

brooding, *Favia fragum* (Esper, 1795) + *Favia gravida* Verrill, 1868 and *Siderastrea radians* (Pallas, 1766), species of corals also have gene flow restricted by the low salinity waters of the Amazon, Orinoco and other numerous rivers along the coast of northern South America between Caribbean and Brazil, and by the stretch of open water dividing the western and eastern Atlantic (Nunes et al. 2011).

The Amazon is the largest river system in the world, comprising about 16% of the annual discharge into the world's oceans (Muller-Karger et al. 1988). Patches of low salinity (~32-34) in surface waters floats as far as 2,000 km away from the mouths of the Amazon and Orinoco Rivers, and the average depth of the Amazon plume for sea-surface salinity was found to be 20-30 m (Hu et al. 2004). Since corals are very sensitive to changes in salinity (Vermeij et al. 2006) and soft sediment bottoms are unsuitable for larval settlement, we can assume by the results obtained, that these great rivers and its associated high sedimentation and low salinity waters, have the potential to constrain the movement of coral dispersers between the Caribbean and Brazil.

Considering other Atlantic marine invertebrates, the patterns of connectivity vary even among closely related genera, and, consequently, phylogeographic inferences are discordant. In the case of sea urchins, which share similar life history traits and pelagic larval duration, trends of differentiation vary across the Atlantic for different species. For instance, restricted gene flow caused by the freshwater outflow of Amazon-Orinoco rivers is evident in *Echinometra lucunter*, *Diadema antillarum* and *Tripneustes ventricosus* (McCartney et al. 2000, Lessios et al. 2001, Lessios et al. 2003). Conversely, *Eucidaris tribuloides* keeps high levels of gene flow between the Caribbean and Brazil (Lessios et al. 1999). Amongst tropical reef fishes, a survey has demonstrated that there is a poor correlation between pelagic larval duration and genetic structure in the squirrelfishes *Myripristis jacobus* ($\Phi_{st} = 0.008$, $P = 0.228$) and *Holocentrus ascensionis* ($\Phi_{st} = 0.091$, $P < 0.001$) (Bowen et al. 2006). Larval behavior, rather than larval duration, may be the key to understand patterns of connectivity in these reef fishes, since distinct levels of genetic structure were found among species of the genus *Acanthurus*, and these were attributed to the adult habitat specificity (Rocha et al. 2002). This provides evidence that reproductive traits solely do not explain gene flow among populations of reef dwellers, but instead, ecological aspects also play a role in this process.

Our results are in agreement with the findings by Nunes et al. (2009, 2011). Great stretches of open water seemed to impose a barrier for the hydrocoral *M. alcicornis* and for scleractinian corals spanning the Caribbean, Brazil and Eastern Atlantic. Nunes et al. (2011) suggested that mid-Atlantic islands could have served as stepping-stones for dispersal across the Atlantic Ocean, decreasing dispersal distances by 50%. Corals, instead, did not seem to be able to cope with long distances of water in order to maintain gene flow between populations. On the other hand, similarly to *Montastraea cavernosa* and *M. faveolata* (Ellis & Solander, 1786) (Severance and Karl 2006, Nunes et al. 2009), *M. alcicornis* from Bermuda could maintain connectivity with Caribbean populations separated by at least 1,500 km. This is probably due to the fast moving currents of the Gulf Stream that supplies Bermuda populations with larvae, although a number of scleractinian species have shown fragmented gene flow even within the Caribbean (Baums et al. 2005, Brazeau et al. 2005, Severance and Karl 2006, Vollmer and Palumbi 2007, Goodbody-Gringley et al. 2010). We can conclude that *M. alcicornis* exhibits population connectivity comparable to *M. cavernosa* and *M. faveolata* in the central Atlantic and that in concordance with Nunes et al. (2011), a sum of distance and physical oceanography may have played a role in isolating the Eastern Atlantic populations.

Contrasting to the restricted gene flow throughout major regions of the Atlantic, high levels of gene flow were encountered within Brazilian regions. Brazilian populations of *M. alcicornis* separated by ~2,000 km were connected by gene flow, and thus, the SFP did not represent a dispersal barrier for this species. Thus, how can we explain such wide distribution strengthened by high levels of connectivity? As mentioned above, sexual reproductive traits poorly correlates to levels of connectivity, although Nunes et al. (2011) have found different result for scleractinians in Brazil. According to these authors, brooding and broadcasting corals exhibit differential levels of gene flow that vary with reproductive modes. They concluded that Brazilian broadcasters have more connected populations than brooders. Meanwhile, this is not the case for Indo-Pacific Scleractinia: a number of studied cases have provided evidence that reproductive modes fails to predict connectivity and genetic structure in corals (Ayre and Hughes 2000, 2004, Miller and Ayre, 2008).

However, compared to scleractinians, milleporids have a different life history, since they exhibit a medusoid planktonic stage. The duration of the medusae in the plankton could explain the successful distribution of *M. alcicornis* in the Atlantic Ocean. However, Lewis (1991) suggested that the medusae of *M. complanata* are typically short-living. Thus, considering that these two species are actually clustered together in a species complex (Ramos, 2009), we can assume that their medusae traits, if not exactly the same, should be very similar. In this sense, the duration of the medusae in the water column is a weak predictor for the long distance dispersal of *M. alcicornis*. Nevertheless, little is known about the biology of their larvae. The single description of a zooxanthellate larva of *Millepora* was provided by Bourmaud et al. (2013) after a successful egg fertilization in the laboratory. The authors demonstrated that the planula larvae of *M. exaesa* Forsskal, 1775 do not swim, but they do crawl several weeks until settling. Interestingly, these planula did not settle on clean corals, but required encrusted stone to initiate their metamorphosis. Considering this panorama, it could be hypothesized that *M. alcicornis* developed a prolonged planktonic larval stage, which could explain why *M. alcicornis* is a widespread species in the Atlantic. On the other hand, this is a weak explanation and it would contradict the great amount of evidence provided for marine organisms showing that reproductive traits solely do not explain patterns of connectivity.

Finally, we now raise and discuss the three main explanations for the wide range of *M. alcicornis* in the Atlantic: (1) long distance dispersal/establishment are primarily achieved by rafting; (2) large colony size enhance the potential of dispersal by producing more propagules and/or asexually developing fragments; and (3) this species is generalist in the use of resources and consequently is an efficient competitor. Rafting is considered an important barrier-crossing mechanism for Atlantic tropical reef fishes. The coastal geography of the Atlantic Ocean facilitates the rafting along the MAB due to the presence of large continental landmasses on each side of MAB, which are a potential source of plant debris that can be used as substrate for rafting fishes (Luiz et al. 2012). Corals also benefit from floating objects of natural or anthropogenic origin to achieve long-distance dispersal. In 2009, colonies of *Favia fragum* were found attached to a gas cylinder in the Netherlands. The floating object probably initiated its trajectory 3 years before in Florida, until they reached the coast of Europe and they were probably alive right before being washed ashore (Hoeksema et al.

2012). There is a report of *Millepora* rafting on a ship hull that was moved from Bermuda to Copenhagen (Bertelsen and Ussing 1936), and also using pumice as rafting substrata (Jokiel 1989), the latter possibly explaining their presence in the Canary Islands (Hoeksema et al. 2012).

The evidence of rafting among corals strongly supports the idea that this is a mechanism that enhances the chances of crossing large and quite effective barriers such as AOP and MAB in the Atlantic, as observed for *M. alcicornis*. However, a wide range hardly is just a matter of successful rafting, but it also depends on some ecological traits exhibited by the species to succeed in the colonized environment. Luiz et al. (2012) found a positive correlation between adult body size and the occurrence of fishes on both sides of the AOP and MAB and this reflects that large-bodied tropical reef fish have advantage at colonizing new habitats and expanding their ranges across marine barriers. Although the effect of such correlation have not been tested for corals, our results, combined with empirical observations, strongly suggest that fire coral species with larger colony size (Brazilian colonies of *M. alcicornis* can easily reach > 2 m in width) are better at expanding their ranges than do their congeners with smaller colonies size (colonies of *M. braziliensis* reach only up to 60 cm in width). Larger sizes may result in the release of more propagules, either sexual or asexually. As stated by Edmunds (1999), most of the fragments of *M. alcicornis* can recover after a disturbance. Thus, we can assume that the appropriate ecological traits presented by *M. alcicornis* combined with the proposed mechanism of rafting may help explain such wide distribution in the Atlantic and their highly connected populations in Brazil.

The success of a species cannot be only reasoned by its ability to disperse, but also to cope with the new environment. A colonizer must be competitively superior to many other organisms in order to survive in the new environment. In reef environments, an important resource subject to competition is space in well-lit habitats, because the available suitable substrate is often scarce (Connell 1973, Lang 1973). *M. alcicornis* is much more generalist in the use of substrate than the other Brazilian endemic species (personal observation). This species grows over other organisms and even covers artificial substrates. The other species seem to need only natural substrate for settlement and grow. It is thus possible that *M. alcicornis* is found in a wider range of habitats than the the endemic species of *Millepora*. Additionally, in case of disturbances as storms, some man-made substrates, covered by colonies

of *M. alcicornis*, may break apart and float with fragments of the colony, forming new rafting objects that may allow the process of long-distance dispersal. Further studies dealing with the differential use of environmental resources might help to better understand the differential competitive ability of these species of fire corals.

2.4.2.3 Genetic diversity

Intra-specific genetic variation provides the basis for any evolutionary change and, thus, is the most fundamental level of biodiversity (May and Godfrey 1994). Patterns of genetic diversity in *M. alcicornis* followed the pattern for the Atlantic broadcast spawning coral *M. cavernosa* (Nunes et al. 2009) and for the Indo-Pacific brooding coral *Seriatopora hystrix* Dana, 1846 (Noreen et al. 2009). The Caribbean central populations held the greatest diversity values, whereas the peripheral ones (Brazil, West Africa and North Atlantic) showed lower values. This may indicate that the central populations are also the center of origin of the populations of *M. alcicornis* in the Atlantic.

The resilience of ecosystems is defined as the capacity of complex ecosystems with multiple stable states to absorb disturbance, reorganize and adapt to change (Nyström and Folke 2001). The ability of an ecosystem to cope with disturbance may be influenced by the genetic diversity of the resident species. In a scenario of reduced diversity, organisms may struggle to adapt to new selective pressures, such as climate change and environmental contamination (Johannesson and André 2006). Although isolated populations of South Eastern and Western Atlantic are isolated from central populations and exhibited lower genetic diversity, this does not mean that they are evolutionary dead ends that do not deserve the attention of conservational efforts. Bowen et al. (2013) stressed the importance of peripheral areas as potential sources of biological and genetic diversity for central areas. Brazilian marine fauna are exposed to a variety of stressors, including high rates of sedimentation and high winds that combined could have played a selective pressure in the evolution of corals. A scenario that gathers differential environmental conditions with reduced gene flow between peripheral and central populations may result in divergence between regions followed by local adaptation and allopatric speciation. In this sense, conservation of peripheral areas should not be neglected, since they embrace possible sites for speciation that may increase diversity and act as refuge for unique evolutionary lineages.

2.4.2 The Brazilian endemic fire coral species, *Millepora braziliensis*, *M. nitida* and *M. laboreli*

2.4.2.1 Morphometry, molecular phylogeny, and connectivity

In this study, two endemic species of *Millepora* could not be correctly identified by morphological characters, as revealed by the molecular analyses. *Millepora nitida* showed a prominent phenotypic plasticity including encrusting and branching forms. Amaral et al. (2002) suggested that the higher sedimentation rates in coastal localities might have had a greater effect on the development of the gastrozooids of *M. alcicornis* and *M. braziliensis*. It is possible that the highest gastropore and dactylopore mean diameter assigned to the encrusting form of *M. nitida* in this study could be explained by environmental conditions and by the lowest mean sea surface temperature at Guarapari Islands (Espírito Santo). In the Western Pacific, for example, the zebra coral *Oulastrea crispata* (Scleractinia: Faviidae) was suggested to present morphological characters that varied latitudinally (Chen et al. 2011). On the opposite extreme, the branching morphs of *M. nitida* and *M. braziliensis* converged in their gastropore and dactylopore characteristics. Amaral et al. (2002) have previously demonstrated that there was no significant morphological difference between these two species/morphotypes, highlighting the importance of molecular systematics in delineating species of this genus.

Verrill (1868) first described *M. nitida* for Abrolhos in Bahia and *M. braziliensis* for Pernambuco, whilst Amaral et al. (2008) described *M. laboreli* for Parcel do Manuel Luiz off Maranhão. In contrast with previous studies, even with the extensive sampling carried out in our work (~100 specimens for *M. braziliensis* and *M. nitida*), there was no record of *M. braziliensis* occurring in Espírito Santo or Bahia, (e.g. Amaral et al. 2008). All samples from this region were genetically assigned to *M. nitida*, despite the fact that some samples, when collected, had been identified as *M. braziliensis*. Similarly, *M. nitida* was not encountered North of the São Francisco River, as previously registered for Alagoas (Amaral et al. 2008). This raises the hypothesis that the endemic *M. braziliensis* is more narrowly distributed than previously thought and, conversely, *M. nitida* is more widely distributed than mentioned in the literature, reaching southward the state of Espírito Santo). They can be considered allopatric and the range described for these two species should be altered to incorporate such changes.

This finding contrasts with the results for *M. alcicornis*, whose distribution reaches both sides of the river plume, with a gap along the coast of Sergipe. The SAMOVA analyses reinforces the evidence inferred from the phylogenetic tree that the SFP play major role in restricting the movement of the endemic fire corals. We can assume that these species are allopatric in their range due to the constrained imposed by the SFP. Thereafter, it is likely that the ancestral species' distribution ranged from northern to southern SFP, before its geological formation (formation date for SFP is not mentioned in the literature). This strengthens the idea that probably the populations of the endemic species (*M. braziliensis* and *M. nitida*) are much older than the widespread species (*M. alcicornis*) in the Brazilian coast, whose populations are in process of expansion.

Peripherally isolated endemics seem to have restricted ability for range expansion (Hodge et al. 2012). Additionally, as these species bear limited range and small population sizes, they are more prone to extinction threats, especially if they are specialists (Hawkins et al. 2000, Munday 2004). Thus, since the risk of extinction implies in global loss, it would be important to incorporate the distribution attributes of these endemic species in local conservational decisions, such as implementations of Marine Protected Areas (MPAs) and management strategies concerning coral reefs.

2.4.2.2 Genetic diversity

The trend of genetic diversity observed for *M. alcicornis* was also observed for the endemics *M. braziliensis* and *M. nitida*. This means that the more central populations held the highest values of genetic diversity, whereas the peripheral populations experienced a decline in diversity values. Similarly to *M. alcicornis*, it may be an indicative of the populations' origins, that is, they probably originated in the central areas of their distribution and spread to adjacent areas in the past. On the other hand, colonies of the endemic *M. laboreli* were genetically identical to each other. This is not attributed to a problem in the molecular marker used but rather it is probably due to the sampling effort, as only one population of this species could be sampled.

Given our results, we propose that management planning should focus on the central populations of *M. braziliensis* (Maragogi, northern Alagoas) and *M. nitida* (Porto Seguro and Abrolhos, southern Bahia).

This argument is based on the fact that these species cover a much more narrow range than *M. alcicornis*, and that peripheral populations are more prone to the effects of extinction due to the small area covered and to the reduced levels of diversity. For this reason, focusing on peripheral populations rather than the central ones would imply losing additional diversity. Thus, we proposed different conservation strategies for the endemics in comparison to the widespread species mainly due to the difference in their size ranges.

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2.7 SUPPLEMENTARY MATERIAL

Table S1. Description of samples of *Millepora* species collected in the Atlantic Ocean. The type of analysis (genetic or morphological) used is also designated.

#	Sample name	Species	Place of origin	Genetic analyses	Morphological analyses
1	ABD03	<i>M. alcicornis</i>	North Atlantic, Bermuda	X	
2	ABD16	<i>M. alcicornis</i>	North Atlantic, Bermuda	X	
3	ABD23	<i>M. alcicornis</i>	North Atlantic, Bermuda	X	
4	ABD51	<i>M. alcicornis</i>	North Atlantic, Bermuda	X	
5	ABD54	<i>M. alcicornis</i>	North Atlantic, Bermuda	X	
6	ABD218	<i>M. alcicornis</i>	North Atlantic, Bermuda	X	
7	ABD282	<i>M. alcicornis</i>	North Atlantic, Bermuda	X	
8	ABD363	<i>M. alcicornis</i>	North Atlantic, Bermuda	X	
9	ABD382	<i>M. alcicornis</i>	North Atlantic, Bermuda	X	
10	ACAPA03	<i>M. alcicornis</i>	Caribbean, Panama	X	
11	ACAPA04	<i>M. alcicornis</i>	Caribbean, Panama	X	
12	ACAPA05	<i>M. alcicornis</i>	Caribbean, Panama	X	
13	ACAFL01	<i>M. alcicornis</i>	Caribbean, Florida	X	
14	ACAFL02	<i>M. alcicornis</i>	Caribbean, Florida	X	
15	ACAFL03	<i>M. alcicornis</i>	Caribbean, Florida	X	
16	ACAFL04	<i>M. alcicornis</i>	Caribbean, Florida	X	
17	ACAFL05	<i>M. alcicornis</i>	Caribbean, Florida	X	
18	ACAFL06	<i>M. alcicornis</i>	Caribbean, Florida	X	
19	ACAFL07	<i>M. alcicornis</i>	Caribbean, Florida	X	
20	ACAFL08	<i>M. alcicornis</i>	Caribbean, Florida	X	
21	ACAFL09	<i>M. alcicornis</i>	Caribbean, Florida	X	
22	ACAFL10	<i>M. alcicornis</i>	Caribbean, Florida	X	
23	ACAFL11	<i>M. alcicornis</i>	Caribbean, Florida	X	
24	ACAFL12	<i>M. alcicornis</i>	Caribbean, Florida	X	
25	ACAFL15	<i>M. alcicornis</i>	Caribbean, Florida	X	
26	ACAFL16	<i>M. alcicornis</i>	Caribbean, Florida	X	
27	ACAFL17	<i>M. alcicornis</i>	Caribbean, Florida	X	
28	ACAFL18	<i>M. alcicornis</i>	Caribbean, Florida	X	
29	ACAFL27	<i>M. alcicornis</i>	Caribbean, Florida	X	
30	ACAFL28	<i>M. alcicornis</i>	Caribbean, Florida	X	
31	ACAFL29	<i>M. alcicornis</i>	Caribbean, Florida	X	
32	ACAFL30	<i>M. alcicornis</i>	Caribbean, Florida	X	
33	ACAFL31	<i>M. alcicornis</i>	Caribbean, Florida	X	
34	ACACO451	<i>M. alcicornis</i>	Caribbean, Colombia	X	
35	ACACO454	<i>M. alcicornis</i>	Caribbean, Colombia	X	
36	ACACO455	<i>M. alcicornis</i>	Caribbean, Colombia	X	
37	ACACO456	<i>M. alcicornis</i>	Caribbean, Colombia	X	
38	ACACO458	<i>M. alcicornis</i>	Caribbean, Colombia	X	
39	ACACO4512	<i>M. alcicornis</i>	Caribbean, Colombia	X	
40	ACACO4514	<i>M. alcicornis</i>	Caribbean, Colombia	X	
41	ACACO826	<i>M. alcicornis</i>	Caribbean, Colombia	X	
42	ACACO8211	<i>M. alcicornis</i>	Caribbean, Colombia	X	
43	ACACO8213	<i>M. alcicornis</i>	Caribbean, Colombia	X	
44	ACACO8214	<i>M. alcicornis</i>	Caribbean, Colombia	X	

45	AAFCV237	<i>M. alcicornis</i>	West Africa, Cape Verde	X
46	AAFCV239	<i>M. alcicornis</i>	West Africa, Cape Verde	X
47	AAFCV241	<i>M. alcicornis</i>	West Africa, Cape Verde	X
48	AAFCV243	<i>M. alcicornis</i>	West Africa, Cape Verde	X
49	AAFCV245	<i>M. alcicornis</i>	West Africa, Cape Verde	X
50	AAFCV247	<i>M. alcicornis</i>	West Africa, Cape Verde	X
51	AAFCV249	<i>M. alcicornis</i>	West Africa, Cape Verde	X
52	AAFCV251	<i>M. alcicornis</i>	West Africa, Cape Verde	X
53	AAFCV253	<i>M. alcicornis</i>	West Africa, Cape Verde Brazil, Fernando de	X
54	ABRFN01	<i>M. alcicornis</i>	Noronha Brazil, Fernando de	X
55	ABRFN02	<i>M. alcicornis</i>	Noronha Brazil, Fernando de	X
56	ABRFN03	<i>M. alcicornis</i>	Noronha Brazil, Fernando de	X
57	ABRFN04	<i>M. alcicornis</i>	Noronha Brazil, Fernando de	X
58	ABRFN05	<i>M. alcicornis</i>	Noronha Brazil, Fernando de	X
59	ABRFN06	<i>M. alcicornis</i>	Noronha Brazil, Fernando de	X
60	ABRFN07	<i>M. alcicornis</i>	Noronha Brazil, Fernando de	X
61	ABRFN08	<i>M. alcicornis</i>	Noronha Brazil, Fernando de	X
62	ABRFN09	<i>M. alcicornis</i>	Noronha Brazil, Fernando de	X
63	ABRFN10	<i>M. alcicornis</i>	Noronha Brazil, Fernando de	X
64	ABRFN109	<i>M. alcicornis</i>	Noronha Brazil, Fernando de	X
65	ABRFN111	<i>M. alcicornis</i>	Noronha Brazil, Fernando de	X
66	ABRFN113	<i>M. alcicornis</i>	Noronha Brazil, Fernando de	X
67	ABRFN115	<i>M. alcicornis</i>	Noronha Brazil, Fernando de	X
68	ABRFN117	<i>M. alcicornis</i>	Noronha	X
69	ABRRN01	<i>M. alcicornis</i>	Brazil, Rio Grande do Norte	X
70	ABRRN02	<i>M. alcicornis</i>	Brazil, Rio Grande do Norte	X
71	ABRRN03	<i>M. alcicornis</i>	Brazil, Rio Grande do Norte	X
72	ABRRN04	<i>M. alcicornis</i>	Brazil, Rio Grande do Norte	X
73	ABRRN05	<i>M. alcicornis</i>	Brazil, Rio Grande do Norte	X
74	ABRRN06	<i>M. alcicornis</i>	Brazil, Rio Grande do Norte	X
75	ABRRN08	<i>M. alcicornis</i>	Brazil, Rio Grande do Norte	X
76	ABRRN09	<i>M. alcicornis</i>	Brazil, Rio Grande do Norte	X
77	ABRRN10	<i>M. alcicornis</i>	Brazil, Rio Grande do Norte	X
78	ABRPE92	<i>M. alcicornis</i>	Brazil, Pernambuco	X
79	ABRPE123	<i>M. alcicornis</i>	Brazil, Pernambuco	X
80	ABRPE124	<i>M. alcicornis</i>	Brazil, Pernambuco	X
81	ABRPE212	<i>M. alcicornis</i>	Brazil, Pernambuco	X
82	ABRPE263	<i>M. alcicornis</i>	Brazil, Pernambuco	X

83	ABRAS06	<i>M. alcicornis</i>	Brazil, Southern Alagoas (Maceio)	X
84	ABRAS09	<i>M. alcicornis</i>	Brazil, Southern Alagoas (Maceio)	X
85	ABRAS10	<i>M. alcicornis</i>	Brazil, Southern Alagoas (Maceio)	X
86	ABRAS31	<i>M. alcicornis</i>	Brazil, Southern Alagoas (Maceio)	X
87	ABRAS55	<i>M. alcicornis</i>	Brazil, Southern Alagoas (Maceio)	X
88	ABRAS58	<i>M. alcicornis</i>	Brazil, Southern Alagoas (Maceio)	X
89	ABRBN04	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
90	ABRBN05	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
91	ABRBN08	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
92	ABRBN10	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
93	ABRBN12	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
94	ABRBN16	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
95	ABRBN17	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
96	ABRBN20	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
97	ABRBN24	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
98	ABRBN25	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
99	ABRBN26	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
100	ABRBN28	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
101	ABRBN34	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
102	ABRBN35	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
103	ABRBN41	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
104	ABRBN42	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
105	ABRBN44	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
106	ABRBN46	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
107	ABRBN51	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
108	ABRBN57	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
109	ABRBN67	<i>M. alcicornis</i>	Brazil, Northern Bahia	X

			(Salvador)	
110	ABRBN71	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
111	ABRBN75	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
112	ABRBN79	<i>M. alcicornis</i>	Brazil, Northern Bahia (Salvador)	X
113	ABRBP87	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
114	ABRBP89	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
115	ABRBP91	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
116	ABRBP199	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
117	ABRBP201	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
118	ABRBP203	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
119	ABRBP205	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
120	ABRBP207	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
121	ABRBP301	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
122	ABRBP306	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
123	ABRBP319	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
124	ABRBP401	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
125	ABRBP406	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
126	ABRBP419	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
127	ABRBP501	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
128	ABRBP506	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
129	ABRBP606	<i>M. alcicornis</i>	Brazil, Southern Bahia (Porto Seguro)	X
130	ABRBA209	<i>M. alcicornis</i>	Brazil, Southern Bahia (Abrolhos Archipelago)	X
131	ABRBA211	<i>M. alcicornis</i>	Brazil, Southern Bahia (Abrolhos Archipelago)	X
132	ABRBA213	<i>M. alcicornis</i>	Brazil, Southern Bahia (Abrolhos Archipelago)	X
133	ABRES01	<i>M. alcicornis</i>	Brazil, Espirito Santo	X
134	ABRES03	<i>M. alcicornis</i>	Brazil, Espirito Santo	X
135	ABRES13	<i>M. alcicornis</i>	Brazil, Espirito Santo	X
136	ABRES18	<i>M. alcicornis</i>	Brazil, Espirito Santo	X
137	ABRES21	<i>M. alcicornis</i>	Brazil, Espirito Santo	X
138	ABRES27	<i>M. alcicornis</i>	Brazil, Espirito Santo	X

139	ABRES29	<i>M. alcicornis</i>	Brazil, Espirito Santo	X	
140	ABRES45	<i>M. alcicornis</i>	Brazil, Espirito Santo	X	
141	ABRES52	<i>M. alcicornis</i>	Brazil, Espirito Santo	X	
142	ABRES57	<i>M. alcicornis</i>	Brazil, Espirito Santo	X	
143	ABRES61	<i>M. alcicornis</i>	Brazil, Espirito Santo	X	
144	ABRES64	<i>M. alcicornis</i>	Brazil, Espirito Santo	X	
145	ABRES65	<i>M. alcicornis</i>	Brazil, Espirito Santo	X	
146	ABRES66	<i>M. alcicornis</i>	Brazil, Espirito Santo	X	
147	ABRES71	<i>M. alcicornis</i>	Brazil, Espirito Santo	X	
148	ABRES96	<i>M. alcicornis</i>	Brazil, Espirito Santo	X	
149	ABRES97	<i>M. alcicornis</i>	Brazil, Espirito Santo	X	
150	ABRES101	<i>M. alcicornis</i>	Brazil, Espirito Santo	X	
151	ABRES151	<i>M. alcicornis</i>	Brazil, Espirito Santo	X	
152	ABRES155	<i>M. alcicornis</i>	Brazil, Espirito Santo	X	
153	ABRRJ51	<i>M. alcicornis</i>	Brazil, Rio de Janeiro	X	
154	ABRRJ52	<i>M. alcicornis</i>	Brazil, Rio de Janeiro	X	
155	ABRRJ159	<i>M. alcicornis</i>	Brazil, Rio de Janeiro	X	
156	ABRRJ161	<i>M. alcicornis</i>	Brazil, Rio de Janeiro	X	
157	ABRRJ163	<i>M. alcicornis</i>	Brazil, Rio de Janeiro	X	
158	ABRRJ166	<i>M. alcicornis</i>	Brazil, Rio de Janeiro	X	
159	ABRRJ167	<i>M. alcicornis</i>	Brazil, Rio de Janeiro	X	
160	ABRRJ169	<i>M. alcicornis</i>	Brazil, Rio de Janeiro	X	
161	ABRRJ173	<i>M. alcicornis</i>	Brazil, Rio de Janeiro	X	
162	ABRRJ175	<i>M. alcicornis</i>	Brazil, Rio de Janeiro	X	
163	ABRRJ177	<i>M. alcicornis</i>	Brazil, Rio de Janeiro	X	
164	LBRMA01	<i>M. laboreli</i>	Brazil, Maranhão	X	
165	LBRMA02	<i>M. laboreli</i>	Brazil, Maranhão	X	
166	LBRMA03	<i>M. laboreli</i>	Brazil, Maranhão	X	
167	LBRMA04	<i>M. laboreli</i>	Brazil, Maranhão	X	
168	LBRMA05	<i>M. laboreli</i>	Brazil, Maranhão	X	
169	LBRMA06	<i>M. laboreli</i>	Brazil, Maranhão	X	
170	LBRMA07	<i>M. laboreli</i>	Brazil, Maranhão	X	
171	LBRMA08	<i>M. laboreli</i>	Brazil, Maranhão	X	
172	LBRMA09	<i>M. laboreli</i>	Brazil, Maranhão	X	
173	BBRPE81	<i>M. braziliensis</i>	Brazil, Pernambuco	X	X
174	BBRPE82	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
175	BBRPE84	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
176	BBRPE85	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
177	BBRPE87	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
178	BBRPE89	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
179	BBRPE91	<i>M. braziliensis</i>	Brazil, Pernambuco	X	X
180	BBRPE132	<i>M. braziliensis</i>	Brazil, Pernambuco	X	X
181	BBRPE133	<i>M. braziliensis</i>	Brazil, Pernambuco	X	X
182	BBRPE134	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
183	BBRPE136	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
184	BBRPE152	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
185	BBRPE153	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
186	BBRPE154	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
187	BBRPE156	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
188	BBRPE157	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
189	BBRPE159	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
190	BBRPE160	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
191	BBRPE161	<i>M. braziliensis</i>	Brazil, Pernambuco	X	

192	BBRPE164	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
193	BBRPE267	<i>M. braziliensis</i>	Brazil, Pernambuco	X	
194	BBRAN93	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	
195	BBRAN173	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	
196	BBRAN174	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)		X
197	BBRAN175	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	
198	BBRAN176	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	
199	BBRAN177	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	
200	BBRAN178	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	
201	BBRAN179	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	X
202	BBRAN180	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	
203	BBRAN183	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	
204	BBRAN184	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	X
205	BBRAN185	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	
206	BBRAN186	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	
207	BBRAN187	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	
208	BBRAN208	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	
209	BBRAN210	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	X
210	BBRAN211	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)		X
211	BBRAN212	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	
212	BBRAN215	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	
213	BBRAN217	<i>M. braziliensis</i>	Brazil, Northern Alagoas (Maragogi)	X	
214	BBRAN219	<i>M. braziliensis</i>	Brazil, Southern Alagoas (Maragogi)	X	X
215	BBRAS07	<i>M. braziliensis</i>	Brazil, Southern Alagoas (Maceio)	X	
216	BBRAS08	<i>M. braziliensis</i>	Brazil, Southern Alagoas (Maceio)	X	
217	BBRAS11	<i>M. braziliensis</i>	Brazil, Southern Alagoas (Maceio)	X	
218	BBRAS56	<i>M. braziliensis</i>	Brazil, Southern Alagoas (Maceio)	X	
219	BBRAS59	<i>M. braziliensis</i>	Brazil, Southern Alagoas	X	

			(Maceio)		
220	BBRAS60	<i>M. braziliensis</i>	Brazil, Southern Alagoas (Maceio)	X	
221	BBRAS61	<i>M. braziliensis</i>	Brazil, Southern Alagoas (Maceio)	X	
222	NBRBN01	<i>M. nitida</i>	Brazil, Northern Bahia (Salvador)	X	
223	NBRBN02	<i>M. nitida</i>	Brazil, Northern Bahia (Salvador)	X	
224	NBRBN07	<i>M. nitida</i>	Brazil, Northern Bahia (Salvador)	X	
225	NBRBN13	<i>M. nitida</i>	Brazil, Northern Bahia (Salvador)	X	
226	NBRBN15	<i>M. nitida</i>	Brazil, Northern Bahia (Salvador)	X	
227	NBRBN18	<i>M. nitida</i>	Brazil, Northern Bahia (Salvador)	X	
228	NBRBN22	<i>M. nitida</i>	Brazil, Northern Bahia (Salvador)	X	
229	NBRBN23	<i>M. nitida</i>	Brazil, Northern Bahia (Salvador)	X	
230	NBRBN29	<i>M. nitida</i>	Brazil, Northern Bahia (Salvador)	X	
231	NBRBN38	<i>M. nitida</i>	Brazil, Northern Bahia (Salvador)	X	
232	NBRBN43	<i>M. nitida</i>	Brazil, Northern Bahia (Salvador)	X	
233	NBRBN45	<i>M. nitida</i>	Brazil, Northern Bahia (Salvador)	X	
234	NBRBP79	<i>M. nitida</i>	Brazil, Southern Bahia (Porto Seguro)	X	
235	NBRBP80	<i>M. nitida</i>	Brazil, Southern Bahia (Porto Seguro)	X	
236	NBRBP81	<i>M. nitida</i>	Brazil, Southern Bahia (Porto Seguro)	X	
237	NBRBP93	<i>M. nitida</i>	Brazil, Southern Bahia (Porto Seguro)	X	
238	NBRBP229	<i>M. nitida</i>	Brazil, Southern Bahia (Porto Seguro)	X	
239	NBRBP301	<i>M. nitida</i>	Brazil, Southern Bahia (Porto Seguro)	X	X
240	NBRBP302	<i>M. nitida</i>	Brazil, Southern Bahia (Porto Seguro)	X	X
241	NBRBP303	<i>M. nitida</i>	Brazil, Southern Bahia (Porto Seguro)	X	X
242	NBRBP304	<i>M. nitida</i>	Brazil, Southern Bahia (Porto Seguro)	X	X
243	NBRBP305	<i>M. nitida</i>	Brazil, Southern Bahia (Porto Seguro)	X	X
244	NBRBP306	<i>M. nitida</i>	Brazil, Southern Bahia (Porto Seguro)	X	X
245	NBRBP307	<i>M. nitida</i>	Brazil, Southern Bahia (Porto Seguro)	X	X

246	NBRBP308	<i>M. nitida</i>	Brazil, Southern Bahia (Porto Seguro)	X	X
247	NBRBP309	<i>M. nitida</i>	Brazil, Southern Bahia (Porto Seguro)	X	X
248	NBRBP310	<i>M. nitida</i>	Brazil, Southern Bahia (Abrolhos Archipelago)	X	X
249	NBRBA231	<i>M. nitida</i>	Brazil, Southern Bahia (Abrolhos Archipelago)	X	
250	NBRBA233	<i>M. nitida</i>	Brazil, Southern Bahia (Abrolhos Archipelago)	X	
251	NBRBA235	<i>M. nitida</i>	Brazil, Southern Bahia (Abrolhos Archipelago)	X	
252	NBRES04	<i>M. nitida</i>	Brazil, Espirito Santo	X	
253	NBRES05	<i>M. nitida</i>	Brazil, Espirito Santo	X	X
254	NBRES07	<i>M. nitida</i>	Brazil, Espirito Santo	X	
255	NBRES12	<i>M. nitida</i>	Brazil, Espirito Santo	X	
256	NBRES14	<i>M. nitida</i>	Brazil, Espirito Santo	X	
257	NBRES17	<i>M. nitida</i>	Brazil, Espirito Santo	X	
258	NBRES20	<i>M. nitida</i>	Brazil, Espirito Santo	X	
259	NBRES25	<i>M. nitida</i>	Brazil, Espirito Santo	X	X
260	NBRES31	<i>M. nitida</i>	Brazil, Espirito Santo	X	
261	NBRES35	<i>M. nitida</i>	Brazil, Espirito Santo	X	X
262	NBRES36	<i>M. nitida</i>	Brazil, Espirito Santo	X	
263	NBRES38	<i>M. nitida</i>	Brazil, Espirito Santo	X	X
264	NBRES44	<i>M. nitida</i>	Brazil, Espirito Santo	X	X
265	NBRES47	<i>M. nitida</i>	Brazil, Espirito Santo	X	
266	NBRES48	<i>M. nitida</i>	Brazil, Espirito Santo	X	X
267	NBRES50	<i>M. nitida</i>	Brazil, Espirito Santo	X	X
268	NBRES56	<i>M. nitida</i>	Brazil, Espirito Santo	X	X
269	NBRES58	<i>M. nitida</i>	Brazil, Espirito Santo		X
270	NBRES59	<i>M. nitida</i>	Brazil, Espirito Santo	X	X
271	NBRES63	<i>M. nitida</i>	Brazil, Espirito Santo	X	
272	NBRES67	<i>M. nitida</i>	Brazil, Espirito Santo	X	
273	NBRES88	<i>M. nitida</i>	Brazil, Espirito Santo	X	
274	NBRES95	<i>M. nitida</i>	Brazil, Espirito Santo	X	

Total	274 samples	4 species	16 sampling sites	270 colonies	30 colonies
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3 CONCLUSÃO

Os dados moleculares sustentam a hipótese de existência de quatro clados reciprocamente monofiléticos no oceano Atlântico Sul, sendo eles *M. alcicornis*, *M. braziliensis*, *M. nitida* e *M. laboreli*. As espécies endêmicas estiveram mais proximamente relacionadas entre si. Ainda é provável que *M. braziliensis* e *M. nitida* tenham se originado por especiação alopátrica devido à barreira formada pelo deságue do rio São Francisco. Os dados morfológicos estiveram pouco integrados com a informação gerada pela árvore filogenética, demonstrando que os caracteres micro-morfológicos avaliados não são adequados para distinguir entre essas duas espécies. Com relação às inferências populacionais, alta estruturação foi observada entre as regiões do Caribe, Brasil e África Ocidental no que se refere às populações de *M. alcicornis*. Os dados indicam que muito provavelmente o aporte de água doce pelos rios Amazonas-Orinoco devam restringir a dispersão desses corais entre o Brasil e o Caribe, e ainda a existência de uma barreira biogeográfica formada pela extensão de oceano aberto que separa os lados ocidental e oriental do Atlântico. Dentro do Brasil, pelo contrário, os resultados sugerem que as populações de *M. alcicornis* encontram-se em expansão demográfica com alta conectividade, contrastando com o fluxo gênico restrito entre as populações das espécies endêmicas. Nessa região, o deságue do rio São Francisco limita a dispersão, e, portanto, a distribuição, da espécie *M. braziliensis* ao norte de sua afluência, e de *M. nitida* ao sul de sua afluência. Já para a espécie de ampla distribuição, *M. alcicornis*, essa barreira é permeável à sua dispersão. As explicações para os padrões de conectividade contrastantes entre essas espécies provavelmente se devem a diferenças em características morfológicas e ecológicas e principalmente no que se refere ao uso do “rafting”. Em geral, as populações apresentaram diminuição da diversidade genética das populações mais centrais em direção às regiões mais periféricas. Desse modo, tendo vista a diferenciação entre espécies endêmicas e espécie de mais ampla distribuição estar embasada no tamanho da área de distribuição, nós propomos diferentes estratégias de conservação para essas espécies. Atenção especial deve ser dada às populações periféricas de *M. alcicornis*, ao passo que as populações mais centrais de *M. braziliensis* e *M. nitida* devem ser foco prioritário de manejo e conservação. Cabendo ressaltar que a distribuição periférica de *M. alcicornis* coincide com a distribuição de ambas as endêmicas, o que facilita o emprego dos esforços conservacionistas.

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ANEXO A – Instruções para preparação de manuscritos estabelecidas pelo periódico internacional “Coral Reefs”

Coral Reefs – Instructions to authors

Careful adherence to these instructions will avoid delays and extra work for authors. Poorly prepared submissions will be returned for correction and may well result in immediate rejection.

1. Copyright declaration

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2. Copyright Form

When authors receive their proofs from the publisher they will be required to sign the copyright declaration.

3. Submission procedure

Manuscripts must be submitted online at the journal website www.editorialmanager.com/core/. Authors using the Editorial Manager site for *Coral Reefs* for the first time will need to complete a simple registration procedure to obtain an account. They will then be led through a series of menus which will help them submit their manuscript. Authors will be able to check the progress of the review by logging on to their account. They will also be notified by e-mail when the review is complete.

Peer review will involve the following steps:

1. Author submits manuscript.
2. Editorial Office checks for completeness and formatting (manuscript may be returned for corrections at this stage).
3. Editor-in-Chief assigns a Topic Editor.
4. Manuscript is subject to Pre-Review (manuscript may be returned for corrections at this stage).
5. Topic Editor assigns Reviewers.
6. Reviewers submit reviews.
7. Topic Editor makes recommendation.
8. Editor-in-Chief makes final decision.

9. Author is informed of decision.

The manuscript and all accompanying tables, figures and supplementary information must conform to the style and formatting detailed in these instructions. Errors will be returned by the Editorial Office for correction before the manuscript is sent for review.

If any data in the manuscript were previously published or are used in another manuscript presently under consideration elsewhere, describe the extent of the overlap in the cover letter and include copies of the relevant papers. Similarly, include PDF copies of related manuscripts that are 'in press', submitted to another journal, or that reviewers are likely to have difficulty locating.

Pre-Review

Manuscripts will be subject to a pre-review on the following criteria:

1. Does the paper fall within the scope of *Coral Reefs*?
2. Does the paper have the potential to make a substantial contribution to the field of research?
3. Is the subject area of potential interest to a wide readership?
4. Is the paper in the correct style and format for *Coral Reefs*?
5. Are the figures appropriate and well presented – is there unnecessary use of color?
6. Is the manuscript well written?
7. Authorship should be limited to those that significantly participated in the research and/or made a substantial intellectual contribution to the manuscript. In cases of a disproportionate number of authors, an explanation of coauthor specific contributions will be requested. An excessive number of authors is not acceptable.

Either the Editor-in-Chief or a Topic Editor undertakes the pre-review assessment. A manuscript may be returned to the authors for revision or rejected without further review at this stage.

4. Revisions and Resubmissions

Requests for revision of a manuscript are common. *Coral Reefs* normally only allows one major revision of any submission. When submitting a revised manuscript authors must explain how they have responded to editor and reviewer comments in an accompanying cover letter. If the revision of a paper is still not acceptable the manuscript may be rejected. Rejection of a manuscript typically arises because of flaws in experimental design or methodology, or if the writing is unclear, the manuscript poorly organized, incomplete, or deviates significantly from the *Coral Reefs* style. In certain circumstances, the editor may invite an author to resubmit a paper for consideration as a new manuscript after further research or analyses have been undertaken. Resubmissions must represent a profound rewrite and not merely cosmetic changes.

5. Decisions

The decision to accept or reject a manuscript is made by the Editor-in-Chief. The comments and recommendations by the Topic Editors and the reviewers are carefully assessed in each case. However, authors should be aware that other considerations such as publication space and the relative importance of the work are also factors that the Editor-in-Chief will take into account. The Editor-in-Chief may also seek additional reviews and defer decision until those reviews are

received. The decision by the Editor-in-Chief is final and appeals will only be considered in exceptional circumstances.

6. Manuscript Types

Reports are full length papers that should not exceed 8000 words, including Abstract, References, Acknowledgements and Figure Legends (approximately 10-12 printed pages).

Reviews may be invited by the Editor-in-Chief or proposed by authors. Authors who wish to write a review must submit a proposal to the Editor-in-Chief for approval. Proposals should be limited to 1000 words, and should include the following:

- a provisional title
- a list of authors and roles, including all institutional affiliations
- an explanation of material to be covered and excluded
- a statement indicating why the review is both timely and needed
- an explanation of the approach to be taken
- an explanation of the overall novelty of the approach and its likely impact on practice or the field.

The proposal may be sent out to experts in the field for comment before the Editor allows submission.

The length of Reviews will normally be the same length as Reports and any deviation from those. Since a Review is intended to be an authoritative statement, a very high standard of presentation will be required both in language and style, and figures.

Notes are short papers up to 2900 words including Abstract, References, Acknowledgements and Figure Legends (2-4 printed pages). They should combine the results and discussion into a single section, and have an abstract of no more than 150 words.

Perspectives are short papers that present opinions or novel interpretation of existing ideas. They may be submitted in Note format, but will be as 'Perspectives'. Headings may be chosen to suit the style of the Perspective.

Comments and Responses –*Coral Reefs* welcomes constructive comments and criticism of papers already published in the journal, where these are in the interests of science. Such manuscripts should be structured and styled in a manner similar to a full-length paper, modified to suit the circumstances. They will be subject to the normal review process. Comments and Responses concerning papers published elsewhere will not be considered, they should properly be sent to the publishing journal.

Reef Sites -Reef Sites are not small articles. The emphasis is on high quality photographic images that illustrate a topic of interest to the *Coral Reefs* readership. The topic must be scientifically interesting (e.g., an unusual event, observation or phenomenon).

Reef Sites include the image(s) and a short explanatory text (including no more than 3 references). The length of a Reef Site should not exceed 450 words, including title, references,

acknowledgements, and author details. Authors must supply a text file excluding the figures in Word.DOC (or Word.DOCX) format, and a proposed layout in Word or PDF format with the figures embedded. The purpose of the layout file is to demonstrate to the Editor that the Reef Site will fit on a single page. The figures must be provided as TIF or EPS files as appropriate (see the instructions on figure preparation below). Digital images are required as these can be uploaded to the website. Authors should contact the Editorial Office for advice where prints or transparencies are involved.

7. Cover Letter – Include a cover letter that identifies the important findings of your submission and the audience that you believe will find your submission most interesting. Any additional information, such as the presence of other relevant submissions and in press manuscripts, or other pertinent information should also be discussed in the cover letter. Material that you wish to be seen by the editors alone should be placed in “Enter Comments” field when submitting the manuscript. Cover letters for revisions or resubmissions should explain how you have addressed the editor’s and reviewers’ comments.

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This will help to avoid their manuscript being returned for incorrect formatting. The template can be used with MSWord for Windows and Macintosh. All manuscripts are subject to final copy editing by the publisher, after acceptance.

Language – Manuscripts should be in English. If English is not your first language we suggest that the text is edited before submission, by an English speaker. Poorly drafted manuscripts will be returned immediately.

Style – Manuscripts may be written in either first person or third person.

Spelling – Coral Reefs accepts both English and American variants of spelling, but manuscripts must be consistent throughout.

Configuration and Layout -

- **Format** - Word.doc or Word.docx
- **Font** - Times New Roman is preferred as this converts best to the PDF proof.
- **Line Spacing** - double space the abstract, main text, and figure legends. Single space and use hanging indents in the reference list. Single space the body of Tables.
- **Running head and Headers and Footers should not be used in the manuscript.**
- **Line and Page Numbering** – Do not use line and page numbering as they will be automatically created in the online PDF proof.
- **Headings**: If you do not use the template, your main headings should be in lowercase bold letters using a large font. Use bold normal sized font for sub-headings and further subheadings may be used as long as their order is clear (e.g., use italics or bold italics). Headings should be kept short. Do not number headings and subheadings.
- **Cite each figure and table** at the appropriate point, numbered consecutively.
- **Species names**: must be in italics; the genus is written in full at the first mention in the Abstract and again in the main text and the figure and table legends, and abbreviated thereafter.

- **All Abbreviations and Acronyms:** Should be defined at first mention in the Abstract, and again in the main body of the text, and thereafter use only the abbreviation. Do not use a list of abbreviations at the beginning of the manuscript. In exceptional cases abbreviations may be included as one of the Tables.
- **Appendices:** If there is more than one appendix, they should be numbered consecutively.
- **Footnotes:** Essential footnotes to the text should be numbered consecutively and placed at the bottom of the page to which they refer. In general, avoid the use of footnotes.
- **Units:** Use standard SI units. Relations or concentrations (e.g. mg per l) must be given as 'mg l⁻¹' (not 'mg/l'). This applies to text, tables and graphs. Units of time should be expressed as s, min, h, d, yr; do not abbreviate 'week' or 'month'. Use hhmm for time of day. Do not use 'am' or 'pm', e.g., use 0900 hrs, 2300 hrs, not 9:00 AM and 11:00 PM.
- **Means and Standard deviations / standard errors:** If you present results as means with either the standard deviation or standard error make it clear which you are reporting. E.g., 300 ± 20.5 cm (mean ± SD). Similarly if you are reporting a range or a confidence interval say so.
- **Date Format:** Use English date formats, i.e., 3rd March 2005; 1–3 March 2003, between 1 and 3 March 1980, 1 March to 1 April (not 1 March–1 April), March 1980 to August 1981, March–April 1991.

9. Order of Manuscript/ Section Formats

The Word file must contain all of the following sections. (Information such as Author details, Abstract and Keywords, that you provide on the manuscript submission site is used as 'metadata' and should be repeated, as directed below in the manuscript file.)

Title Page – The first page should contain the following information:

- A concise and informative title. (Titles should accurately reflect the content of the paper. *Coral Reefs* is a scientific publication, and 'eye catching' titles are generally inappropriate.)
- Names of the authors
- Affiliations and addresses of authors
- E-mail address, telephone and fax numbers of communicating author
- Keywords - 4 to 6 key words are required for indexing purposes.

Abstract - Reports and Reviews must be preceded by a single paragraph abstract presenting the most important results and conclusions in **no more than 300 words**. **Notes should have a short abstract of up to 150 words**. Abstracts should state what was explicitly found in the study. Abstracts must not contain literature cited, or excessive data. Omit 'introductory' statements that summarize previous work and avoid statements that do not identify actual findings. Summarize, rather than advertise both the important findings and their significance.

Introduction - The Introduction should state the purpose of the investigation in the context of earlier studies. A short review of the pertinent literature is generally appropriate, not a dissertation. The Introduction should conclude with a statement of the scientific hypothesis to be tested / purpose of the study. Do not report the findings of the study in the Introduction.

Materials and methods - This section should give sufficient information for the methodology to be critically evaluated. Descriptions of the study site should appear in this section.

Results -The Results should describe the outcome of the study.

Discussion -The Discussion should explain and interpret the results in the context of other published research and may be used to present concepts or theories based upon the findings.

Do not use a 'Conclusion' or 'Summary' section.

Acknowledgements - These should be brief. Any grant that requires acknowledgement may be mentioned. The names of funding organizations should be written in full. Authors are responsible for ensuring that all persons named in the Acknowledgments section know and agree to being identified there (since it may be interpreted as endorsement of the data or conclusions).

Reference List - see below for further details

Figure Legends – see below for further details

10. References

Text Citations -In the manuscript text, references should be cited by the author and year e.g., (Hammer et al. 1993; Hammer 1994; Hammer and Sjöquist 1995; Jones 1995 a, 1995b; Brown 2000, 2004) and listed in year order first, followed alphabetically for references with the same year. Please note the correct punctuation; use 'and' where there are two authors and 'et al.' for multiple authors. Do not use italics. References should be separated by semicolons, multiple references by the same author are separated by a comma. Where a cite needs to be differentiated in the text, use 'a' or 'b': e.g., Smith et al. 1999a, 1999b, and include the 'a' or 'b' in the Reference List.

Reference List -The list of References should only include works that are cited in the text and that have been published or accepted for publication. It should be in alphabetical order. Where there are multiple papers by the same first author, the correct order is; single author papers, followed by two author papers, three author papers, etc., and by year within each group. Always show all the names of the authors in the Reference List – do not abbreviate to 'et al.'

Citations of Abstracts, or 'In preparation', 'submitted' or 'in review' manuscripts are not acceptable; all are 'unpublished'. Do not include unpublished citations in the Reference List.

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Commercial Software - do not list normal commercial software in the Reference List; however, include unusual or specialist software that is otherwise difficult to locate.

Personal communications - should only be mentioned in the text, together with the initials and name of the correspondent. Keep personal communications to a minimum. They are the exception rather than the rule.

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Theses - Ph.D. theses may be cited, but the inclusion of unpublished data from these sources will be carefully reviewed, and may not be acceptable. M.Sc. theses may only be cited if they have been subjected to external examination and are readily available either on the internet on a permanent website or through Inter Library Loan. Undergraduate theses are not acceptable. Authors must give full details of the availability of any thesis in the covering letter.

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ENDNOTE bibliography users - an output style is available for download. Please use this. Authors should still check the format of both the in text cites and the Reference List because the EndNote styles cannot be made comprehensive. **Please remove EndNote file codes before submission. Manuscripts with EndNote field codes will be returned to authors for correction.**

Journals - Coral Reefs uses abbreviated journal titles according to the BIOSIS abbreviations. Standard abbreviation of a journal's name is usually derived from the ISSN "List of Title Word Abbreviations" (www.issn.org/2-22661-LTWA-online.php). Where a journal is not listed the name is given in full. Do not include issue numbers in the citations unless each issue is paginated separately from the volume.

Risk MJ, Sammarco PW, Edinger EN (1995) Bioerosion in *Acropora* across the continental shelf of the Great Barrier Reef. *Coral Reefs* 14:79-86

Conley DJ, Schelske CL, Stoermer EF (1993) Modification of the biogeochemical cycle of silica with eutrophication. *Mar Ecol Prog Ser* 101:179-192

Goreau TJ (1977) Coralskeletal chemistry: physiological and environmental regulation of stable isotopes and trace metals in *Montastrea annularis*. *Proc R Soc Lond B Biol Sci* 196:291-315

Articles with a Digital Object Identifier (DOI) but not yet published in print or where there are no page numbers

Gooseff MN, McKnight DM, Lyons HJ, Blum RJ (2002) Weathering reactions and hyporheic exchange controls on stream water chemistry in a glacial meltwater stream in the McMurdo Dry Valleys. *Water Resour Bull* 38 [doi: 10.1029/2001WR000834]

Books and Technical Reports

Sorokin YI (1993) Coral reef ecology. Springer, Heidelberg

Food and Agriculture Organization (FAO).2001. Global forest resource assessment 2000: Main report. FAO Forestry paper 240, FAO, Rome

Do not capitalize book titles: i.e., "Coral reef ecology" NOT "Coral Reef Ecology". Capitals should only be used for proper names or where convention requires. You should aim to provide sufficient detail to enable a reader to locate the book/report. Each publication will have been produced in a different way so there can be no hard and fast rule.

Theses and dissertations (use the term thesis)

Coppard SE (2002) Morphological and ecological differences between species of the echinoid genera. Ph.D.thesis, University of London, p 82

Chapters

Bosence DWJ (1991) Coralline algae: mineralization, taxonomy, and palaeoecology. In: Riding R, Jones FR(eds) Calcareous algae and stromatolites. Springer, New York, pp 98–113

International Coral Reef Symposia -The International Coral Reef Symposia are treated as a special case by the journal and are shown as follows:

Steven ADL, Broadbent AD (1997) Growth and metabolic responses of *Acropora paliferata* long-term nutrient enrichment. Proc 8th Int Coral Reef Symp 1:867–872

Other Conference Proceedings -For conference proceedings other than the Coral Reef Symposia, please give the title of the proceedings in full, e.g.,

Lough JM (2001) Perspectives on global climate change and coral bleaching: 1997-1998 sea surface temperatures at local to global scales. Proceedings JAMSTEC International Coral Reef Symposium: Coral Reef Biodiversity and Health as Indicators of Environmental Change. Science and Technology Agency, Japan Marine Science & Technology Center, Tokyo, pp 215-229

You should aim to provide sufficient detail to enable a reader to locate the proceedings. Each conference will have been published in a different way so there can be no hard and fast rule.

Websites -The citing of websites should be avoided because of their temporal nature. The Editorial Office will verify that websites are sufficiently permanent as part of the reference check carried out after submission.

Stewart SR (2005) Tropical cyclone report: Hurricane Ivan. National Hurricane Center. <http://www.nhc.noaa.gov/2004ivan.shtml>

Articles In Press -If a reference does not yet have page numbers, the DOI may be added in lieu of volume and page numbers (see above), or the paper should be cited as 'in press' as follows:

Authors will be expected to provide the publication details when they receive the proofs for correction from the publisher.)

Simberloff D (2006) Flagships, umbrellas, and keystones: is single-species management passé in the landscape era? *BiolConserv* 'in press'

When the year is not known)

Simberloff D (in press) Flagships, umbrellas, and keystones: is single-species management passé in the landscape era? *BiolConserv*

12. Reporting Statistical Results

- **Significance and threshold values** -A result is *significant* if the P value is less than a preset threshold value α . In Coral Reefs α should normally be set at 0.05 or lower. If a result is *significant*, the P value can be reported in the text or a table, together with the F-stat, e.g., $F_{1,24}=5.6, p=0.001$. It is equally acceptable to use $p<0.05$, or <0.01 etc.
- **Terminology** - do not use terms such as *highly* and *extremely significant* where $p<0.01$ or 0.001 respectively, it will be for the reader to assess the relative importance of the result. Also do not use *almost significant* or *just not significant* where $p>0.05$.
- **Non significant P values** – authors should consider whether a non significant result may have been the result of a lack of statistical power and whether this might affect the interpretation of their results. In these circumstances it is acceptable to report a p value greater than 0.05, at the same time you must report the power and explain the context of the result. In all other cases, simply report "X was not significantly different from Y".
- **Beyond significance** -think beyond 'significance', by asking the question: "My results are statistically significant but are they scientifically important or even interesting?" For example statistics will detect extremely small differences where large Ns have been used, e.g., in flow cytometry, but do these very small differences have any meaning?

13. Figures

General Remarks – Please consult the Springer Artwork page for a more detailed guide to figure formats and general information about Artwork submission:

<http://www.springer.com/authors/manuscript+guidelines?SGWID=0-40162-12-331200-0>

Do not embed your figures in the manuscript.

Please prepare your figures very carefully; poor figures are a principal source of delay and additional work in the review and production process. Each figure will be checked by the Editorial Office before the manuscript enters the review process. Obvious errors or poor quality will be returned to the author at this stage. Manuscripts will only be accepted into review when the Editorial Office is satisfied that they are publication quality, this avoids unnecessary work for Editors and Reviewers and unnecessary delays for authors.

Figures must be presented, each in its own file, correctly formatted (see below).

Figure Legends should be typed at the end of the manuscript, and must be brief, self-sufficient explanations of the illustration. Always give any species name or acronyms in full where they first appear in each legend. Do not include 'results' in the legend.

Graphs and Line Drawings

Prepare as VECTOR format line graphics and store in EPS format (with TIF preview). Adobe Illustrator is the preferred program to create EPS files. Please do not draw with hairlines; the minimum line width is 0.2 mm (i.e., 0.567pt) relative to the final size. Use Open Type fonts or convert fonts to paths or outlines. If a line drawing cannot be computer generated it may be captured by scanning and inserted as a bitmap into an EPS file. The scan should be composed at 800dpi or greater relative to final size, before inserting in the EPS file. Please bear in mind that any bitmap images embedded in an EPS file cannot be edited by the journal office, or the printer. Any alterations required, however minor, will have to be returned to the author. Where possible any text, etc., should be added in vector EPS format. Programs such as Adobe Illustrator and Corel Draw can also be used to vector trace and vector paint embedded bitmaps, but the end result will depend on the initial bitmap quality. Graphs should be prepared using a good scientific graphing program which can save/export the graph direct to an EPS file. We cannot accept scanned graphs other than in exceptional circumstances with prior approval from the Editorial Office. Figures composed of part photograph and extensive line art (whether color or black and white) should be submitted in EPS format where the photograph will be in bitmap format (at 300dpi) and the line art/text in vector. If the figure only contains a very small amount of line art, e.g., scale bars and labels, it may be prepared as a photograph (see below). In EPS figures, the edges of lines and text should be crisp when viewed at high magnification 'onscreen'. Pixelly or fuzzy edged line art will not be suitable.

Maps

These should be prepared as for Line Drawings. They should include a scale and a compass orientation. Latitude and Longitude should be expressed in degrees, minutes, and where necessary, seconds together with N/S and E/W. They must include some text mark up to identify important features.

Photographs

Black and White, and Color photographs should be saved in TIF format (Color as RGB 8 bits per channel, B&W as greyscale). Scan/save the image at 300dpi. Use scale bars and embedded text and annotations as required. Several figures or figure parts should be grouped in a plate on one page. Where there is extensive annotation it is better to use an EPS format (see above) adding the mark up in VECTOR text. TIF figures are preferred at the size of one or two column widths (either 3.6 or 17.6 cm). The maximum printed page height is 23.6 cm. Authors should prepare any TIF figures at the intended final size, and view and run a test print prior to submission. If all parts of the figure can be clearly seen in the printed version this will be a good indication that the figures will be acceptable.

Composite Figures

Where a figure contains several parts, these must always be presented as a single composite. Lowercase letters (a, b etc.) must be used to identify figure parts in the figure, in the manuscript text, and in the figure legend. If the composite involves both halftones (e.g., photographs) and

line art (graphs or line drawings) the correct format is an EPS file with the halftone as an embedded raster (bitmap) image and the remaining line art and text mark up in VECTOR format. Vector drawing programs such as Adobe Illustrator and Corel Draw should be used to do this. Composites containing halftones with limited additions of text and markers, such as labels, arrows, and scale bars, can be prepared in image processing software (e.g., Photoshop) and submitted as TIF files.

Color Figure Printing Costs

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14. Tables

Tables must be submitted separately from the text in a single Word.DOC file containing all of the tables.

Table legends must be included in the table file, each legend next to each table to which it refers. Legends should be brief and include any species names or acronyms in full together with an explanation of any abbreviation or symbol (e.g., asterisks for significance value) used in that table. Footnotes to tables should be indicated by superscript lower-case letters. Double line space the legends and single space the table content. Care should be taken to produce well designed layouts.

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Electronic supplementary material (ESM) for an article printed in this journal will be published and be permanently available to subscribers on Springer's website. Prepare any ESM in a similar format to the main manuscript. In the printed article, reference should be given to the ESM and vice versa. ESM may consist of:

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- Information that is more convenient in electronic form.
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Authors will be notified by e-mail once the printer's proofs are available online. Full instructions are provided at the time. The author is entitled to formal corrections only. Substantial changes in content, e.g., new results, corrected values, title and authorship are not allowed without approval. In such circumstances please contact the Editor-in-Chief before returning the proofs to the publisher. After online publication, further changes can only be made in the form of an Erratum. An Erratum will only be allowed to correct substantive errors in the content of a published manuscript.

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