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REPORT

Contrasting patterns of connectivity among endemic and widespread fire coral species (*Millepora* spp.) in the tropical Southwestern Atlantic

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Abstract Fire corals are the only branching corals in the South Atlantic and provide an important ecological role as habitat-builders in the region. With three endemic species (*Millepora brazilensis*, *M. nitida* and *M. laboreli*) and one amphi-Atlantic species (*M. alcicornis*), fire coral diversity in the Brazilian Province rivals that of the Caribbean Province. Phylogenetic relationships and patterns of population genetic structure and diversity were investigated in all four fire coral species occurring in the Brazilian Province to understand patterns of speciation and biogeography in the genus. A total of 273 colonies from the four species were collected from 17 locations spanning their geographic ranges. Sequences from the 16S ribosomal DNA (rDNA) were used to evaluate phylogenetic relationships. Patterns in genetic diversity and connectivity were inferred by measures of molecular diversity, analyses of molecular variance, pairwise differentiation, and by spatial analyses of molecular variance. Morphometrics of the endemic species *M. braziliensis* and *M. nitida* were evaluated by discriminant function analysis; macro-morphological characters were not sufficient to distinguish the two species. Genetic analyses showed that, although they are closely related, each species forms a well-supported clade. Furthermore, the endemic species characterized a distinct biogeographic barrier: *M. braziliensis* is restricted to the north of the São Francisco River, whereas *M. nitida* occurs only to the south. *Millepora laboreli* is restricted to a single location and has low genetic diversity. In contrast, the amphi-Atlantic species *M. alcicornis* shows high genetic connectivity within the Brazilian Province, and within the Caribbean Province (including Bermuda),

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despite low levels of gene flow between these populations and across the tropical Atlantic. These patterns reflect the importance of the Amazon–Orinoco Plume and the Mid-Atlantic Barrier as biogeographic barriers, and suggest that, while *M. alcicornis* is capable of long-distance dispersal, the three endemics have restricted ranges and more limited dispersal capabilities.

Keywords Biogeographic barriers · Endemics · Genetic diversity · Genetic structure · Peripheral populations · Phylogenetics

Introduction

Reef corals in the tropical Atlantic Ocean have been reported from Bermuda (32°N) to southern Brazil (27°S) as well as along the tropical west coast of Africa (Laborel 1969a, 1974; Boekschoten and Borel Best 1988; Castro and Pires 2001; Capel et al. 2012). However, within this broad distribution, individual coral species in the tropical Atlantic are generally restricted to one or two biogeographic provinces. For example, of the 81 Atlantic reef-building scleractinian species (Cnidaria: Anthozoa: Scleractinia) only nine are amphi-Atlantic, spanning all three major biogeographic provinces in the tropical Atlantic Ocean (sensu Briggs and Bowen 2012): the Caribbean Province, the Brazilian Province, and the Tropical Eastern Atlantic (Nunes et al. 2011). The small proportion of widespread species is associated with two major biogeographic barriers to dispersal: (1) the expanse of open ocean that separates the western and eastern Atlantic, known as the Mid-Atlantic Barrier (MAB); and (2) the 2000-km freshwater outflow of the Orinoco and the Amazon Rivers, known as the Amazon–Orinoco plume (AOP), that separates the Caribbean and Brazilian Provinces (Fig. 1).

The Brazilian Province, also referred to as the Southwestern Atlantic (SWA), extends from the mouth of the Amazon River to the state of Santa Catarina in southern Brazil, and includes the oceanic islands of Atol das Rocas, Trindade and Martin Vaz, Fernando de Noronha Archipelago, and St. Peter and St. Paul Rocks (Briggs and Bowen 2012). Recent taxonomic and geographic range revisions indicate that the Brazilian Province hosts 18 species of zooxanthellate scleractinian corals (Castro and Pires 2001; Neves et al. 2006, 2008, 2010; Nunes et al. 2008; Budd et al. 2012), 63 azooxanthellate scleractinians (Kitahara 2007; Pires 2007; Neves and Johnsson 2009; Cordeiro et al. 2012) and four species of fire coral (Hydrozoa: Milleporidae; Amaral et al. 2008). Among these, six scleractinians (*Mussismilia braziliensis*, *M. harttii*, *M. hispida*, *M. leptophylla*, *Meandrina brasiliensis,* and *Siderastrea stellata*) and three fire corals (*Millepora braziliensis*, *M. nitida*, and *M. laboreli*) are considered shallow-water endemic species of the Brazilian Province (Laborel 1969a, b; Leão et al. 2003; Amaral et al. 2008; Pinzón and Weil 2011), and one additional scleractinian species (*Favia gravida*) is found only in the Brazilian, Ascension, and Tropical Eastern Atlantic provinces; Hoeksema 2012). Some of these coral endemics show extremely limited distributions, such as the scleractinian *Mussismilia braziliensis*, known only from the states of Bahia and Espírito Santo in Brazil (Leão et al. 2003), and the fire coral *Millepora laboreli*, known only from the Parcel de Manuel Luís reefs off NE Brazil (Amaral et al. 2008).

Recent examinations of genetic diversity and connectivity of tropical South Atlantic scleractinian corals have revealed greater gene flow in broadcasting than in brooding species, and generally lower levels of genetic diversity in the Brazilian Province and in the Tropical Eastern Atlantic (Nunes et al. 2009, 2011). However, it is unclear whether these patterns are representative of all reef-building cnidarians, in particular for species that differ in their mode of reproduction and dispersal. Fire corals are the only extant branching corals in Brazil, and therefore play an important role in creating the three-dimensional diversity and complexity of Brazilian reefs (Pereira et al. 2012; Coni et al. 2013; Leal et al. 2015). They are usually abundant and important reef builders, providing habitat for many associated species (Lewis 1989, 2006; Pereira et al. 2012; Leal et al. 2013). *Millepora* reproduces sexually through a well-developed polypoid generation that buds off planktonic medusoids (the planktonic sexual stage). The colonies are gonochoristic and, in Brazil, medusoids are released during the rainy season by *M. alcicornis* and *M. braziliensis* (Amaral et al. 2008). The genus is comprised of seven species in the Atlantic, three of which are endemic to the Caribbean Province (*M. squarrosa* Lamarck, 1816, *M. complanata* Lamarck, 1816, and *M. striata* Duchassing and Michelotti, 1864) and three that are endemic to the Brazilian Province (*M. braziliensis* Verrill, 1868, *M. nitida* Verrill, 1868, and *M. laboreli* Amaral, 2008) [but see Boschma (1962) for a possible occurrence of *M. squarrosa* in Brazil]. One species, *M. alcicornis*, is amphi-Atlantic, known from the Caribbean, Brazil (including the oceanic islands of Fernando de Noronha and Atol das Rocas), Ascension Island, Bermuda, Cape Verde, and the Canary Islands (Boschma 1948; de Weerdt 1984; Lewis 2006; Clemente et al. 2011; Hoeksema et al. 2014).

Here, we present the first evaluation of phylogenetic relationships, population structure, genetic diversity and connectivity of the four milleporids known from the tropical SWA: the amphi-Atlantic *M. alcicornis* and the endemic species *M. braziliensis, M. nitida*, and *M. laboreli*. Our results, based on 16S rDNA (a useful marker for phylogenetic and phylogeographic inference within

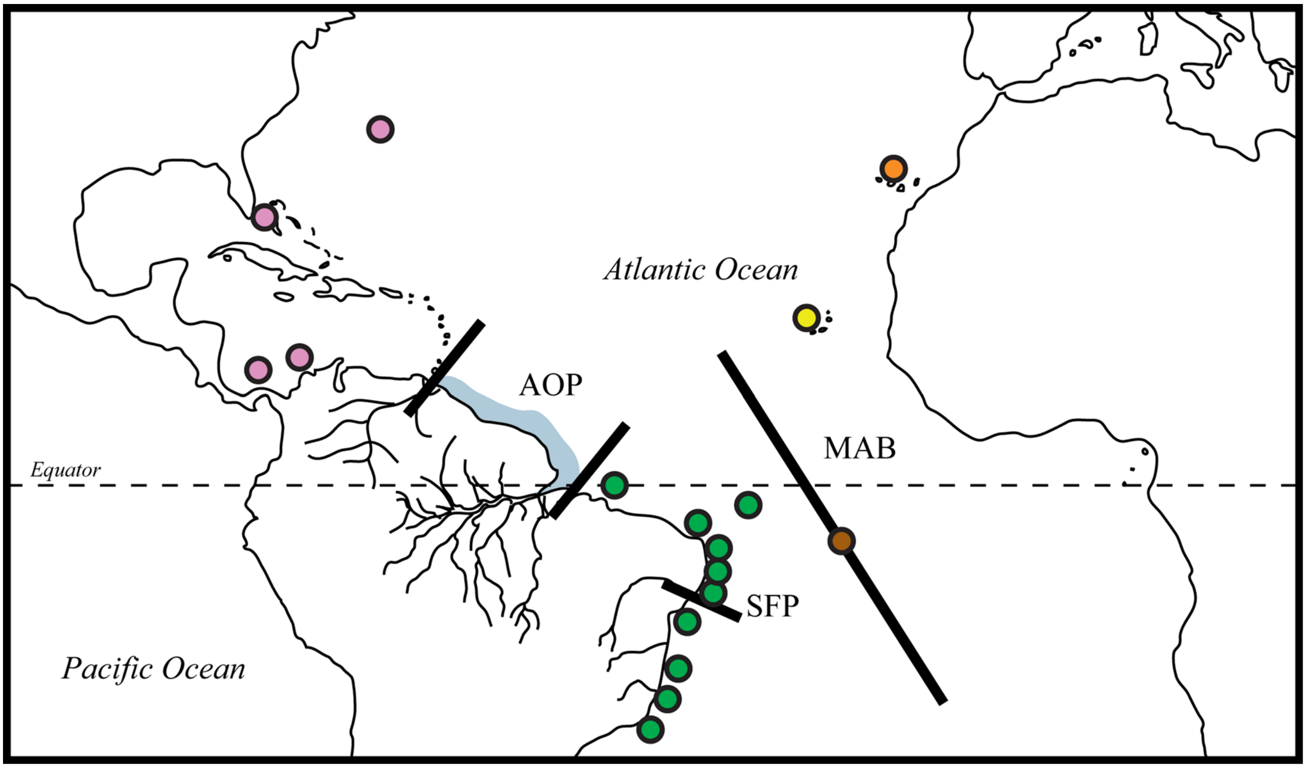

Fig. 1 Major biogeographic regions and barriers in the tropical Atlantic Ocean with reference to the sampling sites (*colored circles*): Caribbean Province (*pink*), Brazilian Province (*green*), Ascension Province (*brown*), Tropical Eastern Atlantic Province (*yellow*), and Lusitania Province (*orange*). Barriers illustrated as black lines correspond to the Amazon–Orinoco Plume (AOP); São Francisco River Plume (SFP), and mid-Atlantic Barrier (MAB). *Light blue shaded area* corresponds to the zone under the influence of the AOP. Modified from Luiz et al. (2012)

Hydrozoa; e.g., Govindarajan et al. 2005; Moura et al. 2011, 2012), reveal that the genetic diversity of *M. alcicornis* is greater in the Caribbean Province than in the Brazilian Province, and indicate that SWA populations originated from Caribbean ancestors. Our results also confirm the distinctiveness of the three endemic species from Brazil and provide some of the first evidence that the freshwater plume of the São Francisco River, in NE Brazil, may represent a barrier for dispersal in the SWA.

Materials and methods

Coral sampling

Tissue samples of *M. alcicornis*, *M. braziliensis*, *M. nitida*, and *M. laboreli* were obtained from a total of 273 colonies collected across seventeen sampling sites in five biogeographic regions: four sites in the Caribbean Province (Bermuda, Florida, Panamá, Colombia); ten sites in the Brazilian Province (Maranhão, Fernando de Noronha Archipelago, Rio Grande do Norte, Pernambuco, northern Alagoas, southern Alagoas, northern Bahia, southern Bahia, Espírito Santo, and Rio de Janeiro), one site in the Tropical Eastern Atlantic Province (Cape Verde), one site in the Lusitania Province (Canary Islands) and one site in the Ascension Province (Ascension Island) (see Electronic Supplementary Material, ESM Table S1; Fig. 1). To minimize the likelihood of sampling clones, colonies of *M. alcicornis* were collected at a minimum distance of 5 m (following Ruiz-Ramos et al. 2014), but due to their lower abundances, the endemic (and much smaller) species were collected at distances of 3–5 m. Branch tips of *M. alcicornis* colonies were broken off manually, while a hammer and a chisel were used to break a small piece (~5–7 cm^2) off the colonies of the three endemic species. Fire corals have small and abundant gastropores and dactylopores (Fig. 2), therefore the size of these specimens was adequate as vouchers for species identification. Samples were stored in >90% ethanol at room temperature.

DNA extraction, amplification and sequencing

DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen) or according to a DNA extraction protocol based on either phenol-chloroform (Fukami et al. 2004) or cetrimonium-bromide (CTAB) (Coffroth et al. 1992). A 532-bp fragment of the large ribosomal subunit of the mitochondrial RNA was amplified using previously published primers (forward primer SHA: ACGGAATGAACTCAAATCATGT; reverse primer SHB: TCGACTGTTTACCAAAAACATA) (Cunningham and Buss 1993) by polymerase chain reaction (PCR) consisting of 2 µL of 10× buffer (200 mM Tris–HCl, 500 mM KCl), 2 µL of dNTP (4 × 2 mM), 2 µL of bovine serum albumin (BSA), 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 a denaturation step at 94 °C for 1 min, followed by 35 cycles of 94 °C for 15 s, 50 °C for 90 s, 72 °C for 2 min and 30 s, and a final extension step at 72 °C for 5 min. Amplified products were purified using the QIAquick PCR Purification Kit (Qiagen) and DNA sequencing was performed in both forward and reverse directions, using an automated ABI3500 genetic analyzer.

Genetic data analyses

Phylogenetic relationships were evaluated using sequences from 273 colonies. Additional sequences of *Millepora* from the Pacific Ocean and of other closely related hydrozoans (*Zanclea prolifera* and *Asyncoryne ryniensis*; see Nawrocki et al. 2010) were included in the analysis to verify whether Atlantic *Millepora* form a monophyletic clade. Sequences were edited and aligned with Sequencher v5.0 (Gene Codes Corporation) (alignment available in ESM *Millepora* alignment). The nucleotide substitution model was selected according to the AIC criterion in jModelTest v2.0 (Darriba et al. 2012). A maximum-likelihood (ML) tree was inferred with PhyML v3.0 (Guindon et al. 2010), using the HKY model of evolution with the gamma shape parameter (0.231) as determined by jModelTest. Statistical support for the nodes was estimated using 1000 bootstrap pseudoreplicates. Bayesian inference for phylogenetic relationships was performed in Beast v2.1.3 (Bouckaert et al. 2014) under the HKY (gamma) model, using empirical base frequencies and four gamma categories. A strict clock model and a Yule process tree prior were used. The MCMC had a chain length of 20 million generations sampled every 1000 steps. The results of three runs were compared in Tracer to check for convergence and then combined, with the initial 4000 trees being discarded as burn-in. An 85% majority-rule consensus tree was visualized and edited using FigTree v1.4.0.

A median-joining haplotype network was constructed using NETWORK v4.6.1.1 (Fluxus Technology Ltd). In this study, an ancestral haplotype from the Brazilian Province and its descendants formed a star phylogeny whose age could be estimated by coalescent theory (see Govindarajan et al. 2005). A per-locus rate of substitution was estimated for *Millepora* by multiplying the substitution rate available for other hydrozoans [*Hydractinia* (Cunningham et al. 1991) and *Conopora* (Lindner et al. 2008)] by the number of positions in the 16S rDNA sequence. To our knowledge, *Hydractinia* and *Conopora* show the lowest and highest substitution rates available thus far for hydrozoans, therefore providing likely minimum and maximum age estimates for *Millepora*. The number of haplotypes (*H*), segregating sites (*s*), gene diversity (*h*), nucleotide diversity (π), average of nucleotide changes (*k*), and statistics for neutral sequence evolution (Tajima's *D* and Fu's *Fs*) were calculated for populations, regions, and species using Arlequin v3.5.1.2 (Excoffier and Lischer 2010).

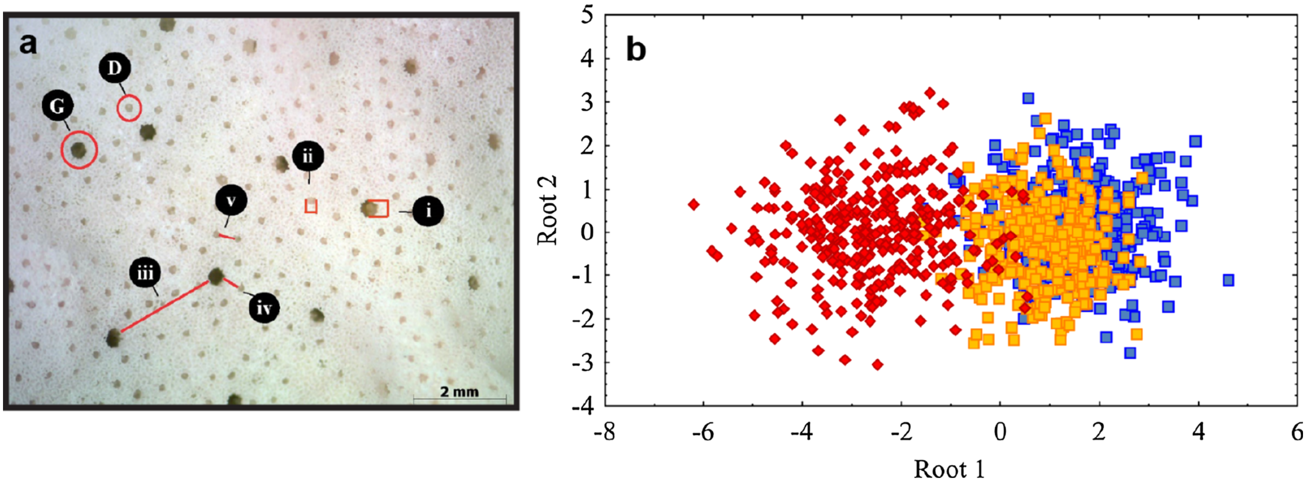

Fig. 2 **a** *Millepora* spp. colony surface, gastropores (G), dactylopores (D) and measurements: gastropore diameter (*i*), dactylopore diameter (*ii*), distance between gastropores (*iii*), distance from gastropore to nearest dactylopore (*iv*), and distance between dactylopores (*v*). **b** Discriminant function analysis of the following *Millepora* morphotypes: *Millepora braziliensis* (*blue squares*), *M. nitida* with branching colonies (*orange squares*) and *M. nitida* with encrusting colonies (*red diamonds*)

Genetic differentiation among the four regions was tested using analysis of molecular variance (AMOVA), and differentiation among populations was tested by pairwise ϕ_{st}, in Arlequin v3.5.1.2. Both tests were performed for populations of *M. alcicornis*. For the species restricted to Brazil, *M. braziliensis* and *M. nitida*, differentiation among populations was evaluated only by pairwise ϕ_{st}. 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) (Dupanloup et al. 2002) was performed for *M. alcicornis*.

Morphological analyses

Two endemic species from Brazil were classified into three morphotypes to evaluate morphological variation among them: branching *M. braziliensis*, branching *M. nitida*, and encrusting *M. nitida*. Ten colonies per morphotype were bleached with 30% sodium hypochlorite, dried and measured under a stereoscopic microscope. The following characters were measured (Fig. 2): (1) gastropore diameter, (2) dactylopore diameter, (3) distance between gastropores, (4) distance from gastropore to nearest dactylopore, (5) distance between dactylopores, and (6) number of dactylopores per gastropore. Measurements (1), (2), and (6) were undertaken according to Amaral et al. (2002) and the remaining measurements according to Ruiz-Ramos et al. (2014). Measurements per colony varied from 16 up to 40 for a single trait. A discriminant function analysis (DA) was performed in Statistica v10 (StatSoft Inc.) to test the utility of the six morphological traits to distinguish the morphotypes.

Results

Phylogenetic analysis

Our results show that the endemic fire corals of the Brazilian Province (*M. braziliensis, M. nitida*, and *M. laboreli*) are closely related and confirm that they are distinct from the amphi-Atlantic *M. alcicornis*. Despite limited sampling in the Indo-Pacific, the results also indicate that Atlantic milleporids form a monophyletic clade (bootstrap support of 100%; Fig. 3). The phylogeny not only confirms the identity of the three SWA endemics, but also reveals that *M. braziliensis* and *M. nitida* form distinct clades and do not occur in sympatry, as previously thought. Although the topology of the maximum-likelihood tree places the root of the endemic clade within *M. braziliensis* (Fig. 3), this topology is not supported statistically (bootstrap) and results of the Bayesian analysis strongly support this species as a distinct monophyletic clade (posterior probability of 1.00; ESM Fig. S1). Our results indicate that *M. braziliensis* is restricted to the north of the São Francisco River, whereas *M. nitida* is restricted to the states of Bahia and Espírito Santo, south of the river's outflow (Figs. 3, 4). *Millepora laboreli* was only recorded from the Parcel de Manuel Luís reefs off the state of Maranhão (Fig. 4). Our results also indicate that an unidentified *Millepora* (GenBank accession number EU876551) previously used in phylogenetic analyses of the Class Hydrozoa (e.g., Nawrocki et al. 2010) should be identified as *M. alcicornis* (see "MACF" in Fig. 3g).

Haplotype networks

A total of 44 haplotypes were observed for *M. alcicornis*, 11 for *M. braziliensis*, nine for *M. nitida*, and only one for *M. laboreli* (Figs. 3, 4). No haplotypes of *M. alcicornis* were shared among individuals from the Caribbean, Brazilian, Tropical Eastern Atlantic, Lusitania, or Ascension Provinces (Fig. 5). Haplotypes from the Tropical Eastern Atlantic, Lusitania, and Ascension Provinces were nested within those from the Caribbean Province. A star phylogeny was detected in the Brazilian Province, with all sampled sites in Brazil sharing one common haplotype, and remaining haplotypes differing by a single mutational step (with the exception of Espírito Santo and Fernando de Noronha Archipelago). Average substitution rates from *Hydractinia* spp. (1.25×10^{-9} substitution site^{-1} yr^{-1}) and *Conopora* spp. (7.71×10^{-9} substitution site^{-1} yr^{-1}) were used to estimate the age of this lineage. The *Hydractinia* rate resulted in an age estimate of 450 ± 164 kyr, while the *Conopora* rate resulted in an age estimate of 73 ± 27 kyr. Populations of the three endemic species are weakly distinguished based on the haplotype network but exhibited abundant private alleles (i.e., haplotypes restricted to only one population), with the exception of the geographically "central" populations of *M. braziliensis* and *M. nitida* that shared haplotypes with adjacent populations.

Genetic diversity indices

Gene diversity (h) for *M. alcicornis* was highest in the Caribbean Province ($h = 0.957 \pm 0.019$), followed by intermediate levels in the Brazilian Province ($h = 0.503 \pm 0.058$). This pattern was observed for all molecular diversity indices (Table 1). Haplotypes sampled within the Tropical Eastern Atlantic, Ascension, and Lusitania Provinces were identical, but low sample numbers in Cape Verde, Ascension, and the Canary Islands may have underestimated diversity in the eastern and mid-Atlantic. For the SWA-endemic species *M. braziliensis*, the highest gene diversity was found in northern Alagoas

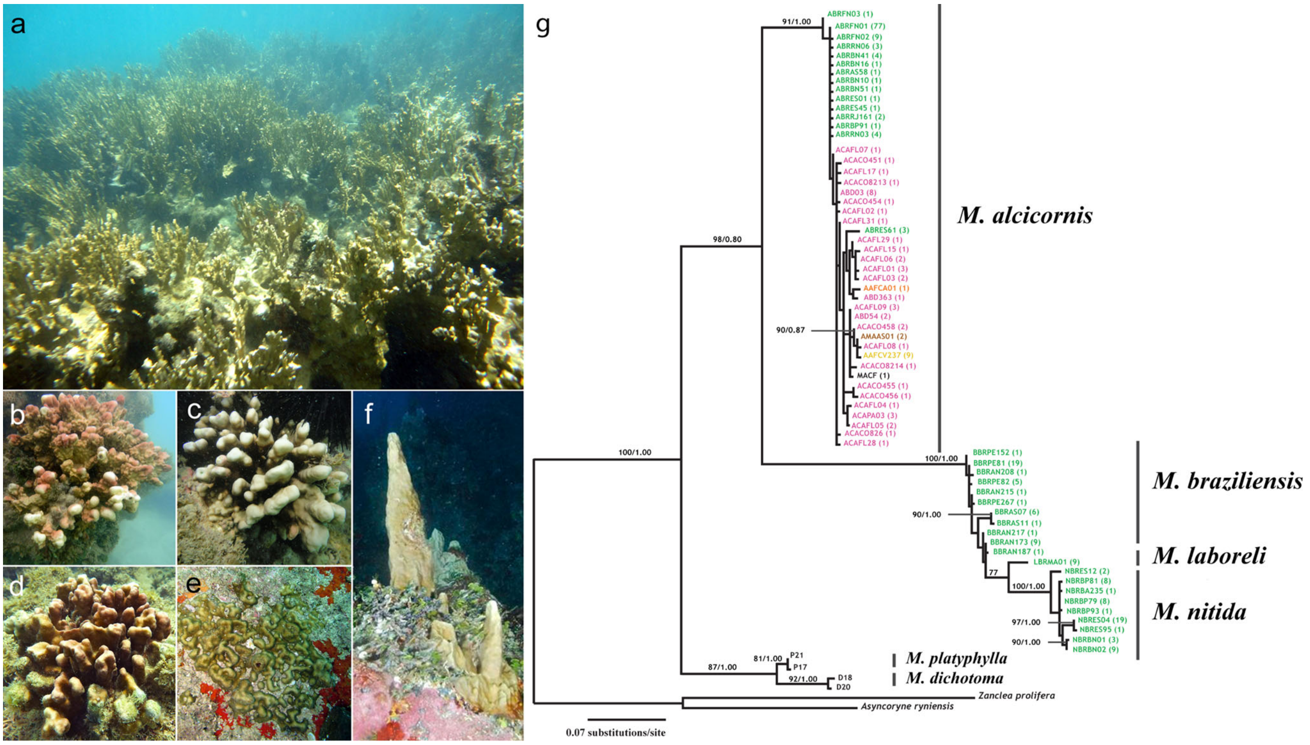

Fig. 3 Tropical Southwestern Atlantic milleporids and their phylogenetic relationships. **a** *Millepora alcicornis*; **b**, **c** *M. braziliensis*; **d**, **e** *M. nitida*; **f** *M. laboreli*; **g** Phylogenetic relationships inferred using maximum likelihood. *Numbers in parentheses* correspond to the number of individuals with identical sequences. *Numbers* represent bootstrap support values (>75%)/posterior probabilities (>0.85) (for the Bayesian tree, see ESM Fig. S1). Tip label colors correspond to Caribbean Province (*pink*), Brazilian Province (*green*), Ascension Province (*brown*), Tropical Eastern Atlantic Province (*yellow*), and Lusitania Province (*orange*)

($h = 0.702 \pm 0.080$), the central population in the covered range. *Millepora nitida* exhibited a similar pattern, with the central population having the highest gene diversity ($h = 0.634 \pm 0.069$). For *M. laboreli*, all haplotypes sampled were identical ($n = 9$). In general, populations of fire corals did not show significant departures from neutrality ($P > 0.05$). However, two populations of *M. alcicornis* in the Caribbean had significant values of Fu's *Fs*: Florida ($Fs = -5.629$) and Colombia ($Fs = -3.548$).

Population differentiation

Hierarchical AMOVA revealed significant genetic structure ($P < 0.05$) at all levels for *M. alcicornis*: among populations ($\Phi_{ST} = 0.693$), among populations within regions ($\Phi_{SC} = 0.089$) and among regions ($\Phi_{CT} = 0.663$) (Table 2). Variation among regions accounted for 66.30% of the total genetic variation. Genetic subdivision in two of the endemic species was also significant (*M. braziliensis*, $\Phi_{ST} = 0.689$; *M. nitida*, $\Phi_{ST} = 0.832$), with most variation attributed to differentiation among populations for both species (*M. braziliensis* = 68.89%; *M. nitida* = 83.21%). Differentiation was not tested for *M. laboreli* because this species is known to occur in only one location.

In terms of pairwise population differentiation for *M. alcicornis*, no significant population differentiation was observed among sites in the Caribbean Province (Bermuda, Florida, Panamá, Colombia). Similarly, most pairwise ϕ_{st} showed no significant differentiation among populations in the Brazilian Province, except the oceanic island of Fernando de Noronha, and Rio Grande do Norte (Table 3). In contrast, significant differentiation was found in all pairwise comparisons between populations from different biogeographic regions (Table 3), with the exception of the Canary Islands (possibly due to the small sample size; $n = 1$). The genetic structure of *M. alcicornis*, according to the SAMOVA, was similarly significant for both sets of two ($F_{CT} = 0.694$), three ($F_{CT} = 0.691$), and four groups of populations ($F_{CT} = 0.663$) (Table 4). For both *M. braziliensis* and *M. nitida*, all values of pairwise ϕ_{st} were significant (Table 3). For *M. braziliensis,* the highest pairwise differentiation ($\phi_{st} = 0.934$) occurred between populations at the edges of their distribution, i.e., Pernambuco and southern Alagoas. The same pattern was observed in *M. nitida* populations, in which maximum differentiation was observed between the marginal populations of northern Bahia and Espírito Santo ($\phi_{st} = 0.842$).

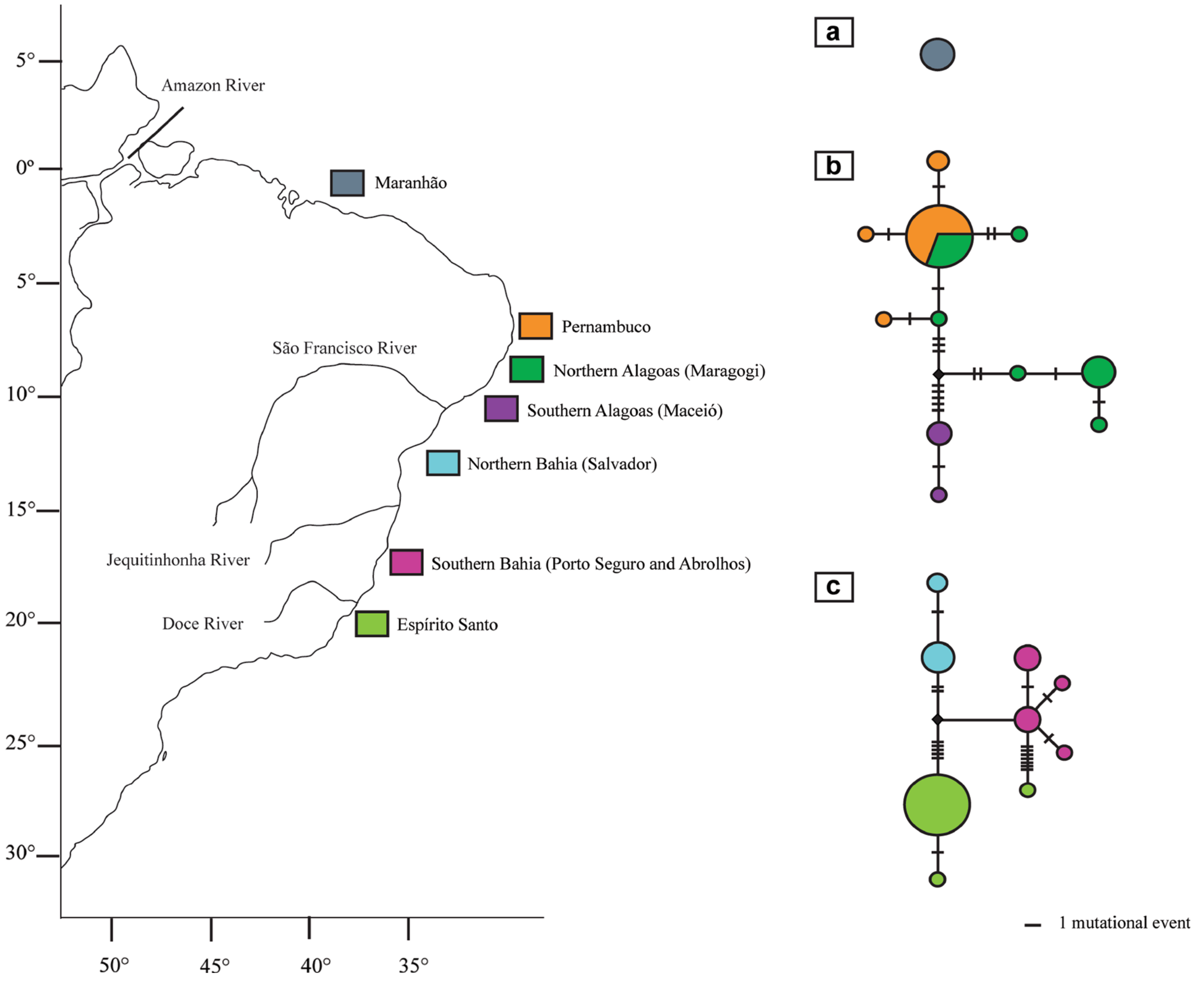

Fig. 4 Haplotype networks of Brazilian endemic milleporids. **a** *Millepora laboreli*; **b** *M. braziliensis*; **c** *M. nitida*. *Colors* illustrate each population. *Black dashes* correspond to mutational events

Morphological analyses

The DA corroborated the groups assigned a priori (Wilk's $\lambda = 0.219$, $F = 197.550$, $P < 0.0001$), with 76.4% ($n = 804$) of the replicated measurements correctly classified (Fig. 2). The canonical plot showed one distinct group, corresponding to encrusting *M. nitida*, and two other overlapping groups, corresponding to branching *M. nitida* and *M. braziliensis*. Major morphological variation among morphotypes occurred along root 1 (eigenvalue = 3.364). The highest standardized canonical coefficients for variables associated with root 1 were the diameter of gastropores and dactylopores, which, as a consequence, were the variables that best discriminated the encrusting *M. nitida* from the branching *M. nitida* and *M. braziliensis*.

Discussion

Our results reveal contrasting patterns of connectivity and population structure among three fire coral species endemic to the tropical SWA (*M. braziliensis*, *M. nitida*, and *M. laboreli*) and the amphi-Atlantic congener *M. alcicornis*. Previous studies of *M. alcicornis*, based on cytochrome c oxidase subunit 1, revealed high genetic diversity in the Caribbean (Ruiz-Ramos et al. 2014) and found that populations in the Cape Verde and Canary Islands likely descended from Caribbean ancestors (López et al. 2015). The data presented here agree with these findings, with haplotypes from other peripheral populations being either nested within the Caribbean network (Ascension Island) or closely related to the Caribbean populations (SWA, separated by 1–2 mutations). Furthermore, populations of *M. alcicornis*

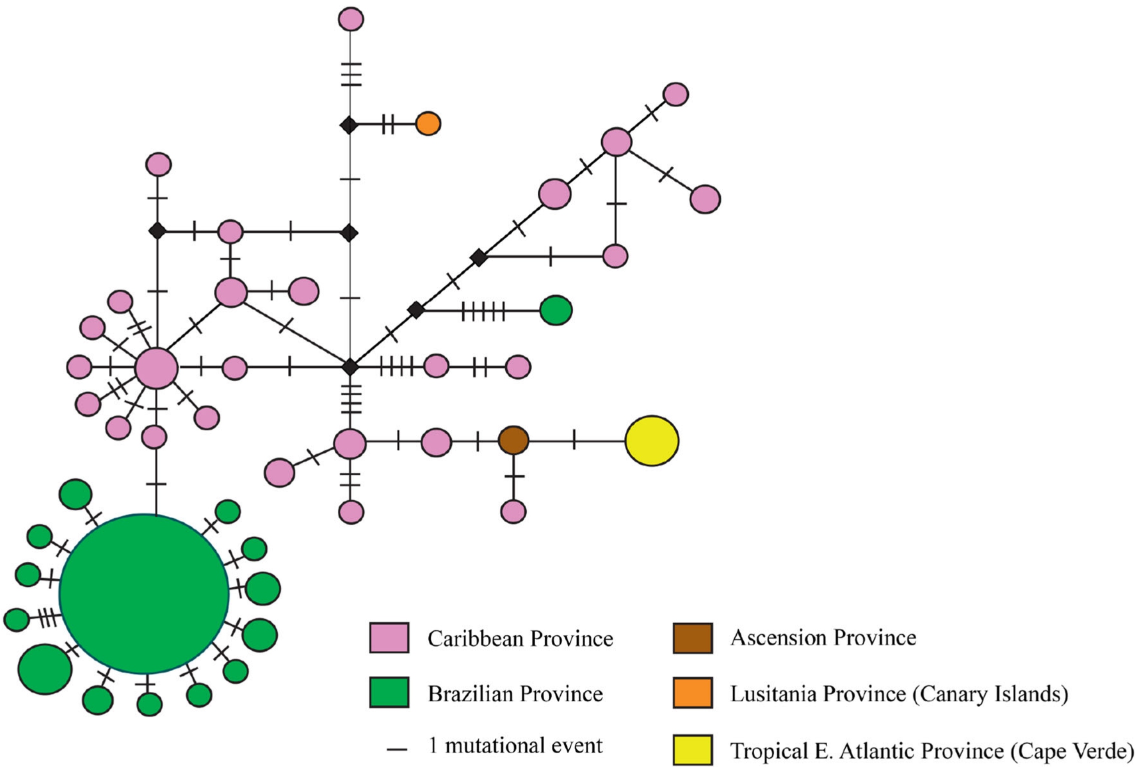

Fig. 5 Haplotype network of *Millepora alcicornis* in the Atlantic Ocean. *Colors* illustrate major regions of populations' occurrence. *Black diamonds* represent ancestral or not sampled haplotypes and *black dashes* correspond to mutational events

are significantly structured between the Caribbean and Brazilian Provinces, which are separated by >2000 km. While recently discovered mesophotic coral communities offshore of the Amazon River outlet include occurrences of *M. alcicornis* and *M. braziliensis* (Cordeiro et al. 2015; Moura et al. 2016), genetic exchange may not necessarily be facilitated by these deeper reefs. Indeed, genetic structure has been found between shallow and deep habitats for at least two Caribbean coral species (Serrano et al. 2014, 2016). Significant genetic structure across these three biogeographic provinces has also been observed for two broadcast-spawning and four brooding corals (Nunes et al. 2009, 2011). These findings suggest a general pattern of gene flow restricted by the low-salinity surface waters of the Amazon–Orinoco Plume for shallow-water reef corals. Low-salinity surface waters are found as far as 2000 km from the mouths of the Amazon and Orinoco Rivers at an average depth of up to 20–30 m (Hu et al. 2004). Since coral larvae are sensitive to changes in salinity (Vermeij et al. 2006), these large rivers have the potential to limit dispersal between the Caribbean and Brazilian Provinces. No shared haplotypes were found between the Caribbean and the Cape Verde Islands in the present study, whereas a single shared haplotype was reported by López et al. (2015). Additional studies are needed to evaluate population structure for corals across the MAB, but it is possible that gene flow between the western Atlantic and Cape Verde (López et al. 2015) is less restricted than between the western Atlantic and the Gulf of Guinea (Nunes et al. 2009, 2011; see also Laborel 1974).

Caribbean populations of *M. alcicornis* showed higher genetic diversity than peripheral populations, similar to patterns found for *Montastraea cavernosa* and *S. siderea* (Nunes et al. 2011). In the Brazilian Province, *Millepora alcicornis* showed low genetic structure (Table 3) and low-to-intermediate genetic diversity ($h = 0$–0.722), with one abundant haplotype found in all Brazilian populations (Fig. 5). The age of this lineage was estimated at 72 ± 27 to 450 ± 164 kyr. Although further analyses may reveal more accurate estimates once rates for *Millepora* become available, our results indicate that this major lineage arose long after the formation of the Amazon River in the late Miocene, ~5–10 Ma (Hoorn et al. 1995). Moreover, because the haplotypes found in the peripheral populations (Brazil, Ascension, Canary, and Cape Verde Islands) are not connected in the haplotype network, they indicate that the colonization of each location most likely occurred as independent events, as suggested for the Canary and Cape Verde Islands (López et al. 2015). Similarly, the identification of a divergent haplotype in Brazil (Fig. 5) may indicate an independent colonization event in the Brazilian Province.

Table 1 Molecular diversity indices and neutrality tests for 16S rDNA gene in Atlantic *Millepora*, showing number of individuals (N), number of haplotypes (H), number of segregating sites; (s), gene diversity (h), average nucleotide diversity (π), and average number of nucleotide differences (k)

	Molecular diversity indices						Neutrality	
	N	H	s	h	π	k	Tajima's D	Fu's Fs
M. alcicornis	166	44	50	0.777 ± 0.034	0.008 ± 0.004	4.28 ± 2.13		
Caribbean Province	44	26	33	0.957 ± 0.019	0.010 ± 0.005	5.22 ± 2.57		
Bermuda	9	3	12	0.556 ± 0.165	0.008 ± 0.005	4.06 ± 2.23	−0.386	3.751
Florida	21	15	20	0.967 ± 0.024	0.010 ± 0.006	5.33 ± 2.68	−0.152	**−5.629**
Panamá	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**
Ascension Province	2	1	0	0	0	0		
Ascension Island	2	1	0	0	0	0	0	N.A.
Tropical Eastern Atlantic Province	9	1	0	0	0	0		
Cape Verde	9	1	0	0	0	0	0	N.A.
Lusitania Province	1	1	0	N.A.	N.A.	N.A.		
Canary Islands	1	1	0	N.A.	N.A.	N.A.	N.A.	N.A.
Brazilian Province	110	15	25	0.503 ± 0.058	0.002 ± 0.001	1.10 ± 0.73		
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 (Maceió)	6	2	1	0.333 ± 0.215	0.001 ± 0.001	0.33 ± 0.38	−0.933	−0.003
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 and Abrolhos)	20	2	1	0.100 ± 0.088	0.000 ± 0.000	0.10 ± 1.17	−1.164	−0.879
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
M. laboreli	9	1	0	0	0	0		
Brazilian Province	9	1	0	0	0	0		
Maranhão	9	1	0	0	0	0	0	N.A.
M. braziliensis	46	11	17	0.776 ± 0.047	0.009 ± 0.005	4.75 ± 2.37		
Brazilian Province	46	11	17	0.776 ± 0.047	0.009 ± 0.005	4.75 ± 2.37		
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
M. nitida	52	9	18	0.798 ± 0.035	0.009 ± 0.005	4.52 ± 2.26		
Brazilian Province	52	9	18	0.798 ± 0.035	0.009 ± 0.005	4.52 ± 2.26		
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 and Abrolhos)	18	4	3	0.634 ± 0.069	0.001 ± 0.001	0.74 ± 0.58	−0.404	−0.782
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

In the Caribbean Province, high levels of connectivity were observed for *M. alcicornis* among sites separated by as much as 1500 km. Connectivity between the central Caribbean sites and Bermuda has been attributed to the rapid currents of the Gulf Stream that supply Bermuda with larvae (Nunes et al. 2009; Goodbody-Gringley et al. 2012; Serrano et al. 2014). Although connectivity across the Caribbean has been observed in some coral species (Severance and Karl 2006; Nunes et al. 2009; Serrano et al. 2014, 2016), most scleractinians show more fragmented patterns of gene flow within the Caribbean (Baums et al. 2005; Brazeau et al. 2005; Severance and Karl 2006; Vollmer and Palumbi 2007; Goodbody-Gringley et al. 2010, 2012; Foster et al. 2012). *Millepora alcicornis* thus appears to have higher levels of connectivity within the Caribbean Province than most species, although greater

Table 2 Analyses of Molecular Variance (AMOVA) of the Atlantic *Millepora* using data from the 16S rDNA gene

Regions	Source of variation	Variance components	% of variation
Millepora alcicornis			
CA, ASC, TEA, BR, LUS	Among regions	2.151	66.30
	Among populations within regions	0.097	2.99
	Within populations	0.996	30.71
	Fixation indices		
	Φct	**0.663**	
	Φsc	**0.089**	
	Φst	**0.693**	

Populations	Source of variation	Variance components	% of variation
Millepora braziliensis			
PE, ALN, ALS	Among populations	2.195	68.89
	Within populations	0.991	31.11
	Fixation indices		
	Φst	**0.689**	
Millepora nitida			
BN, BS, ES	Among populations	2.620	83.21
	Within populations	0.529	16.79
	Fixation indices		
	Φst	**0.832**	

The regions assigned for *Millepora alcicornis* populations were *CA* Caribbean Province, *ASC* Ascension Province, *TEA* Tropical Eastern Atlantic Province (Cape Verde), *BR* Brazilian Province, and *LUS* Lusitania Province (Canary Islands). The populations analyzed of *M. braziliensis* were *PE* Pernambuco; *ALN* Northern Alagoas (Maragogi), and *ALS* Southern Alagoas (Maceió). For *M. nitida*, the populations evaluated were *BN* Northern Bahia (Salvador), *BS* Southern Bahia (Porto Seguro and Abrolhos), and *ES* Espírito Santo. Statistically significant values ($\alpha = 0.05$) are highlighted in bold

sampling within the Caribbean may reveal additional population structure. High levels of gene flow for *M. alcicornis* within the Caribbean are consistent with observations in the SWA, where Brazilian populations separated by ~2000 km also show little genetic structure.

High connectivity in *M. alcicornis* within the Caribbean and Brazilian Provinces is unexpected given its mode of reproduction. Milleporids have different life history traits from scleractinians, with a medusoid planktonic stage in addition to planula larvae. The medusoid stage of *M. alcicornis* is thought to be short-lived (Lewis 1991), lasting just 5–6 h, with the release of gametes into the water shortly after medusae are released (Mayer 1910). Medusae of three Pacific species (*M. dichotoma*, *M. murrayi*, and *M. platyphylla*) may last up to 12 h (Soong and Cho 1998). The only description of a milleporid larva (for the Indo-Pacific species *M. exaesa* Forsskål. 1775), suggests that planulae do not swim, but crawl for several weeks before settlement (Bourmaud et al. 2013). Although observations of the planulae of *M. alcicornis* are needed, the information available for the medusoid stage of this species and its congeners are at odds with the successful dispersal of *M. alcicornis*. Indeed, sexual reproductive traits do not always correlate with high connectivity in corals (Ayre and Hughes 2000; Miller and Ayre 2008).

One reason why *M. alcicornis* may have greater dispersal capability compared to the three endemic species is the ability of its initial encrusting growth form to settle on diverse substrates, which confers a competitive advantage (Connell 1973; Lang 1973; Wahle 1980; Dubé et al. 2016). In particular, *M. alcicornis* is capable of growing on artificial substrates (de Souza, Lindner and Hoeksema, pers. obs.), a trait that has not been observed in the endemic species. This ability may increase the availability of suitable habitat and may also promote long-distance dispersal by allowing *M. alcicornis* to recruit to floating objects of natural or anthropogenic origin and disperse over long distances (e.g., Hoeksema et al. 2012). Indeed, *Millepora* has been reported to raft on ship hulls (Bertelsen and Ussing 1936) and pumice (Jokiel 1989); the more generalist settlement behavior of *M. alcicornis* may pre-adapt this species for dispersal by rafting, particularly on non-natural objects. Post-settlement transportation via rafting provides a means of overcoming natural barriers to dispersal such as the MAB and AOP, as has been observed for other invertebrates (Hoeksema et al. 2012; Cabezas et al.

Table 3 Pairwise ϕ_{st} for *Millepora alcicornis* and for the Brazilian endemics *M. braziliensis* and *M. nitida* inferred from 16S rDNA sequence data

Province	Population		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Millepora alcicornis																	
CA	1	Bermuda	–	0.487	0.146	0.495	**0.020**	0.999	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**
CA	2	Panamá	−0.070	–	0.367	0.785	0.099	0.999	**0.005**	**0.001**	**0.005**	**0.017**	**0.012**	**0.000**	**0.001**	**0.039**	**0.003**
CA	3	Florida	0.053	0.013	–	0.147	**0.013**	0.999	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
CA	4	Colombia	−0.016	−0.069	0.036	–	**0.026**	0.999	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
ASC	5	Ascension Island	**0.545**	0.940	**0.430**	**0.287**	–	0.999	**0.018**	**0.008**	**0.020**	**0.049**	**0.034**	**0.003**	**0.003**	**0.003**	**0.013**
LUS	6	Canary Islands	0.381	0.875	0.194	0.148	1.000	–	0.999	0.064	0.098	0.163	0.144	**0.042**	**0.045**	0.187	0.074
TEA	7	Cape Verde	**0.753**	**0.984**	**0.608**	**0.569**	**1.000**	1.000	–	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
BR	8	Fernando de Noronha	**0.570**	**0.749**	**0.505**	**0.507**	**0.922**	0.896	**0.951**	–	**0.000**	**0.040**	**0.015**	**0.000**	**0.000**	**0.001**	**0.002**
BR	9	Rio Grande do Norte	**0.500**	**0.722**	**0.452**	**0.427**	**0.914**	0.880	**0.955**	**0.382**	–	0.090	**0.028**	**0.000**	**0.000**	**0.024**	**0.000**
BR	10	Pernambuco	**0.438**	**0.915**	**0.392**	**0.354**	**1.000**	1.000	**1.000**	**0.281**	0.221	–	0.999	0.752	0.999	0.570	0.541
BR	11	Southern Alagoas	**0.455**	**0.847**	**0.407**	**0.376**	**0.973**	0.959	**0.988**	**0.291**	**0.227**	−0.034	–	0.502	0.420	0.390	0.267
BR	12	Northern Bahia	**0.629**	**0.812**	**0.545**	**0.568**	**0.950**	**0.934**	**0.965**	**0.352**	**0.301**	−0.052	0.016	–	0.110	**0.008**	0.082
BR	13	Southern Bahia	**0.656**	**0.940**	**0.541**	**0.570**	**0.991**	**0.988**	**0.994**	**0.440**	**0.422**	−0.105	0.087	0.055	–	0.230	0.112
BR	14	Espírito Santo	**0.340**	**0.338**	**0.363**	**0.347**	**0.751**	0.644	**0.829**	**0.191**	**0.130**	−0.029	0.001	**0.106**	0.096	–	0.073
BR	15	Rio de Janeiro	**0.544**	**0.862**	**0.466**	**0.463**	**0.971**	0.960	**0.984**	**0.346**	**0.298**	−0.004	0.053	0.068	0.114	0.057	–

Province	Population		1	2	3
Millepora braziliensis					
BR	1	Pernambuco	–	0.000	0.000
BR	2	Northern Alagoas	**0.499**	–	0.000
BR	3	Southern Alagoas	**0.934**	**0.675**	–

Province	Population		1	2	3
Millepora nitida					
BR	1	Northern Bahia	–	0.000	0.000
BR	2	Southern Bahia	**0.840**	–	0.000
BR	3	Espírito Santo	**0.842**	**0.815**	–

Both values for pairwise ϕ_{st} and for correspondent associated P values are presented, respectively, below and above the main diagonal. The provinces assigned for each population were *CA* Caribbean Province, *ASC* Ascension Province, *LUS* Lusitania Province (Canary Islands), *TEA* Tropical Eastern Atlantic Province (Cape Verde), and *BR* Brazilian Province. Statistically significant values ($\alpha = 0.05$) are highlighted in bold

Table 4 Fixation indices and correspondent population groups inferred by SAMOVA algorithm for *Millepora alcicornis*

Number of groups	Group composition	F_{SC}	F_{ST}	F_{CT}
Two groups	(1) AS, CV	**0.421**	**0.822**	**0.694**
	(2) BD, PA, FL, CO, CA, FN, RN, PE, ALS, BN, BS, ES, RJ			
Three groups	(1) AS, CV	**0.410**	**0.818**	**0.691**
	(2) CA			
	(3) BD, PA, FL, CO, FN, RN, PE, ALS, BN, BS, ES, RJ			
Four groups	(1) AS, CV	**0.091**	**0.693**	**0.663**
	(2) CA			
	(3) BD, PA, FL, CO			
	(4) FN, RN, PE, ALS, BN, BS, ES, RJ			

Populations used in the analysis were *BD* Bermuda, *PA* Panamá, *FL* Florida, *CO* Colombia, *AS* Ascension Island, *CA* Canary Islands, *CV* Cape Verde, *FN* Fernando de Noronha, *RN* Rio Grande do Norte, *PE* Pernambuco, *ALS* Southern Alagoas (Maceió), *BN* Northern Bahia (Salvador), *BS* Southern Bahia (Porto Seguro and Abrolhos), *ES* Espírito Santo, and *RJ* Rio de Janeiro. F_{ST}, F_{SC}, and F_{CT} represents the extent of genetic differentiation within populations, among populations within groups, and among groups of populations, respectively. Statistically significant values ($\alpha = 0.05$) are highlighted in bold

2013; López-Gappa and Liuzzi 2016; Ros et al. 2016) and vertebrates (Luiz et al. 2012).

Another reason why *M. alcicornis* may disperse more effectively is that its larger colony size enhances dispersal. Colony size has been shown to be positively correlated with fertility in several Atlantic coral species (Soong and Lang 1992). Colonies of *M. alcicornis* reach several meters in diameter and are much larger than endemic milleporids (<1 m in diameter; Fig. 3). Large colony size likely results in more propagules, either sexual or asexual, which enhances dispersal. Moreover, and in contrast to the endemic species, colonies of *M. alcicornis* have slender (instead of massive) branches that easily break and reattach, which favors asexual reproduction (Edmunds 1999; Lewis 2006). Although larval biology and ecology do influence dispersal of corals (Connolly and Baird 2010), traits such as colony size and the ability to raft may have a greater influence on population structure of fire corals at both regional and ocean-wide scales.

In contrast to high connectivity among populations of *M. alcicornis* in the SWA, our results show that the three endemic species are highly structured, and call for a reassessment of species boundaries and distribution ranges of two species: *M. braziliensis* and *M. nitida*. *Millepora braziliensis* was originally described by Verrill (1868) as occurring in Pernambuco, north of the São Francisco River, whereas *M. nitida* was described for the Abrolhos reefs off Bahia, south of the São Francisco River. However, subsequent studies reported *M. braziliensis* also off Bahia and *M. nitida* off Pernambuco, rendering them sympatric (e.g., Leão et al. 2003; Amaral et al. 2008). *Millepora braziliensis* was previously identified as having predominantly larger and more massive or encrusting colonies (Amaral et al. 2002, 2008), whereas colonies of *M. nitida* were often considered to be smaller and branching. Indeed, Verrill's original description stated that "in texture and the character of the cells [*M. braziliensis*] resembles [*M. nitida*], and possibly it may eventually prove to be only a variety of it. It differs, however, very remarkably in the mode of growth and form of the branches" (Verrill 1868). Our results support Verrill's qualitative observations and indicate that neither colony shape nor the arrangement and size of pores are diagnostic morphological characters distinguishing either species (Figs. 2, 3). Among scleractinian corals, recent work has shown that macro-morphological characters are usually homoplastic (Fukami et al. 2004; Gittenberger et al. 2011; Benzoni et al. 2012; Huang et al. 2014a), but that micromorphology and microstructure provide additional informative characters for taxonomy (Budd and Stolarski 2009, Budd et al. 2012; Arrigoni et al. 2014; Huang et al. 2014b). The data presented here indicate that *M. braziliensis* and *M. nitida* can only be distinguished with molecular data. However, future work that examines fine-scale morphological traits in hydrozoans may reveal additional morphological characters that distinguish the two species.

Our results also reveal that small encrusting colonies found off Espírito Santo [formerly identified as *M. braziliensis* (Amaral et al. 2002)] are clearly populations of *M. nitida* (Fig. 3). Our results indicate that *M. braziliensis* is restricted to the north of the São Francisco River and *M. nitida* to the south (Fig. 4). This is some of the first evidence that the São Francisco outflow may be a barrier for dispersal and is potentially associated with speciation of marine organisms in the Brazilian Province. Further evidence is the restricted geographic distribution of at least six reef fish species that occur only south of the São Francisco River (Guimarães and Bacellar 2002; Gasparini et al. 2003; Caires et al. 2008; Carvalho Filho and Ferreira 2013) and divergent patterns in *Symbiodinium* clades associated with *M. hispida* on either side of the São Francisco River (Picciani et al. 2016).

The third endemic fire coral species, *M. laboreli*, occurs only at the Parcel de Manuel Luís in northern Brazil, and has unique massive conical colonies. This species has the narrowest range among SWA corals and has one of the most restricted geographic distributions among any coral species worldwide. For this reason, this species could potentially be listed in the IUCN Red List as critically endangered, as it fulfills criterion B1a: "extent of occurrence to be <100 km^2 and known to exist at only a single location" (IUCN Red List Categories and Criteria v3.1), although additional data are required to identify a second criterion for listing. Thus far, it is listed as "vulnerable" in the list of endangered species from Brazil (Brasil 2014). Although only nine individuals of *M. laboreli* were sampled in the present study, the lack of genetic variation in 16S rDNA and its remarkably limited geographic distribution suggests it may be especially vulnerable due to inbreeding depression or other deleterious effects associated with low genetic diversity. *Millepora braziliensis* and *M. nitida* show highly structured populations (Fig. 4), suggesting little genetic exchange even across their restricted distributional ranges. For both species, populations show the highest genetic diversity at the center of their ranges, with a decline toward the edges of the distribution (Fig. 4). For *M. laboreli*, all individuals were genetically identical.

Our results confirm that *Millepora* is an important component of Atlantic coral reef diversity, being one of the most species-rich ($n = 6$) genera of Atlantic zooxanthellate corals, along with *Agaricia*, *Madracis*, and *Porites*. Research on the phylogeny, phylogeography and taxonomy of Brazilian fire corals can be expanded in the future by adding other Atlantic species (de Weerdt 1984) and those from the Indo-Pacific (e.g., Razak and Hoeksema 2003), as well as assessing the genetic diversity of the algal symbionts (*Symbiodinium* spp.). This may show whether additional areas of endemism exist among fire corals, such as has recently been demonstrated for fishes in the Red Sea (DiBattista et al. 2013).

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