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Postglacial north–south expansion of populations of *Rhizophora mangle* (Rhizophoraceae) along the Brazilian coast revealed by microsatellite analysis[1]

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- Premise of the study: Red mangrove (*Rhizophora mangle*) dominates tropical tidal areas along both sides of the Atlantic, yet little is known about its degree of population differentiation over large geographical scales. Information on the genetic variability of mangrove species along the Brazilian coast is important not only for understanding the recent gene flow dynamic between populations, but also to evaluate models of evolutionary diversification and develop effective strategies for conservation. We investigated the genetic variability of the red mangrove along the Brazilian coast.
- Methods: Eight microsatellite loci were used to genotype 145 individuals across 10 populations spanning more than 4500 km of coast line. We estimated the genetic variability and structure of the populations and the historical gene flow between them.
- Key results: The level of genetic variability was low, with only 27 different alleles being detected and allele richness between 1.25 and 2.75. On the other hand, there was substantial population differentiation ($R_{st} = 0.48$; $P < 0.001$), especially between the northern and southern populations. The populations from Pará and Maranhão had significantly greater genetic variability than did the remaining locations.
- Conclusions: This difference might reflect the older age of the northern mangroves, which likely remained stable during the Quaternary glaciations. The lowest variability observed in the southern populations of the red mangrove most likely reflects their recent age, associated with allelic reduction, resulting from the consecutive founder events that followed subsequent colonization of estuaries during the gradual warming by the end of the last glacial period.

Key words: genetic structure; genetic variability; mangrove; microsatellites; propagule dispersal; *Rhizophora mangle*; Rhizophoraceae.

Mangroves compose a unique ecosystem comprised of intertidal marine/estuarine plants, mostly trees, predominantly bordering margins of tropical coastlines around the world. These ecosystems are characterized by low plant diversity and include only species resistant to high salinity (Maia et al., 2006). In Brazil, mangroves are present in a significant fraction of the coast—around 92% of the shore line—encompassing approximately 6800 km from the north, in the Oiapoque region, state of Amapá, to the south of the country, in the state of Santa Catarina (Schaeffer-Novelli et al., 1990). The distribution of mangrove ecosystems in the Brazilian coast is directly associated with estuarine regions, which are located at variable distances of each other, conferring a patchy distribution pattern.

Mangroves play an important role in the geomorphic stability of the coast line, in the preservation of the biodiversity, in the maintenance of fishery resources, and as nursery grounds that shelter juveniles of a variety of economically important fisheries (Maia et al., 2006). Because of its great ecological value, the Brazilian legislation considers mangroves areas of permanent preservation (Conama Resolution 303/02). Despite the efforts for their preservation, mangroves are constantly threatened by numerous anthropogenic activities such as the construction of river dams, agriculture, aquaculture, urbanization, and direct deforestation (Maia et al., 2006). Understanding the geography of genetic variability of mangrove species and the historical patterns of gene flow can provide insights to effective strategies for conservation of the environment and its inhabitants.

There are only 28 genera and ca. 70 species of mangrove plants in the world, of which 17 are present exclusively in mangroves (Duke et al., 1998). The species richness is low and probably influenced by the severe conditions found in those environments, including tidal oscillations, low oxygen levels in the soil and high salinity. These conditions apparently reduce opportunities for the establishment of species that are less fit and for posterior diversification of characteristic lineages of mangroves (Duke et al., 1998). In Brazil, mangrove forests are characterized by species of four genera: *Rhizophora* L., *Avicennia* L., *Laguncularia* C. F. Gaertn, and *Conocarpus* L. (Schaeffer-Novelli et al., 1990).

Rhizophora mangle L. (Rhizophoraceae), the red mangrove, subject of this study, is one of the most prevalent species present in this ecosystem. It is a subtropical/tropical tree, which colonizes

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coastlines and brackish water habitats below the 20° isotherm in both the northern and southern hemispheres. It is characterized by aerial roots, which allow better support in unconsolidated soils (Maia et al., 2006). The species is wind-pollinated, and its reproduction appears to result mainly from self-pollination, although cross-pollination might occur (Menezes et al., 1997). *Rhizophora mangle* has a wide distribution, occurring from east Africa to the Atlantic and Pacific coast of America (Tomlinson, 1986). The origin of the disjunctive occurrence between the neotropics and an outlier in the southwest Pacific has resulted in speculations for over a century (Ellison, 1991). In Brazil, the species occurs along the entire distribution of mangroves in the country. However, there is a general trend of increased scarcity and smaller size with increasing latitude, possibly because in the south the tidal amplitude and temperature are lower and the coast lowland is restricted by a mountain range, the Serra do Mar (Schaeffer-Novelli et al., 1990).

The red mangrove, as the other arboreal species of this ecosystem, is dispersed by water. It is a viviparous species with slightly curved propagules, that float on water before establishment. These structures are able to disperse for long distances since they may remain viable for more than a year (Rabinowitz, 1978). Because propagule dispersal has an important role in the colonization of new environments, the dispersal ability has a particularly strong influence in the structuring and distribution of a species (Cain et al., 2000).

Studies on the genetic variability of *R. mangle* are still scarce. Núñez-Farfán et al. (2002) have analyzed populations in the Atlantic and Pacific coast of Mexico. They showed that populations of these two regions are genetically distinct. Floral morphology data also indicated differentiation among Mexican populations of *R. mangle* (Domínguez et al., 1998). Another study, conducted by Arbeláez-Cortes et al. (2007), demonstrated that populations of *R. mangle* in the Pacific coast of Colombia are genetically structured and have high genetic variability. Understanding the geography of genetic variability of *R. mangle* and the historical patterns of gene flow can provide insights to effective strategies for conservation of the species and of the environment it inhabits. In the present study, we provide a comprehensive assessment of the degree of population differentiation of *R. mangle* along the Brazilian coast using high-resolution molecular data. We also discuss how climatic and oceanographic patterns of the West Atlantic coast may have influenced the observed genetic scenario.

MATERIALS AND METHODS

DNA isolation and amplification—Leaves of 145 individuals of *R. mangle* were collected from 10 populations distributed along the Brazilian coast (Fig. 1, Table 1). A voucher of the species collected in Ajurutea (Pará, Brazil) can be found at the UPCB Herbarium under access code 59292. Populations from Pará and Maranhão are referred to in this study as the northern populations, while the other eight are referred to as the southern ones. Sampled individuals were at least 10 m apart from each other. The leaves were dehydrated in silica gel and then pulverized with liquid nitrogen and frozen at −20°C until processed. The DNA was extracted following the standard procedure of the DNeasy Plant Mini (Qiagen, Valencia, California, USA), and preserved at −20°C in AE buffer, provided with the kit.

The genetic variability of the *R. mangle* populations was analyzed through the amplification of eight nuclear microsatellite regions previously developed specifically for *R. mangle*: RM19, RM38, RM41, RM46 (Rosero-Galindo et al., 2002) and RM05, RM50, RM 52, RM86 (Takayama et al., 2008a). The forward primers of each pair were labeled with fluorescent dyes. The PCR was conducted on a total reaction volume of 25 μL, containing 0.2 ng of DNA, 1× Buffer without Mg, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTP (Biotools, Jupiter, Florida, USA), 1 pmol/μL of each primer and 0.625 U Taq Polymerase Platinum (Invitrogen, Carlsbad, California, USA). All reactions were prepared with a pipetting robot CAS-1200 (Qiagen, Valencia, California, USA). The amplification conditions were as follows: 3 min at 94°C; followed by 35 cycles of 30 s at 95°C, 60 s at 52°C, 60 s at 70°C; and a final extension of 1 h at 70°C. Following amplification, the microsatellite alleles of each marker were defined by electrophoresis in an ABI 3130 automated DNA sequencer (Applied Biosystems, Carlsbad, California, USA), and visualized and scored in the program GeneMapper v.3.7 (Applied Biosystems).

TABLE 1. Localization, municipality, geographic coordinates, and number of individuals (N) in the sampled *Rhizophora mangle* populations.

State	Municipality	Coordinates	N
Pará (PA)	Bragança	0°50′19″S, 46°36′44″W	15
Maranhão (MA)	Barreirinhas	2°36′43″S, 42°41′38″W	15
Rio Grande do Norte (RN)	Natal	5°45′40″S, 35°12′45″W	12
Pernambuco (PE)	Jaboatão dos Guararapes	8°13′36″S, 34°56′11″W	15
Sergipe (SE)	Aracajú	11°5′52″S, 37°9′12″W	13
Bahia (BA)	Ilhéus	14°48′39″S, 39°2′54″W	15
Rio de Janeiro (RJ)	Barra de Guaratiba	23°2′56″S, 43°33′30″W	15
São Paulo (SP)	Bertioga	23°49′S, 46°09′W	15
Paraná (PR)	Ilha do Mel	25°32′2″S, 48°17′40″W	15
Santa Catarina (SC)	Palhoça	27°49′33″S, 48°37′17″W	15

Data analysis—Linkage disequilibrium between loci was analyzed using the software Arlequin ver. 3.1 (Excoffier et al., 2005), through the maximum expectation algorithm, with 10 000 permutations (Slatkin and Excoffier, 1996; Excoffier and Slatkin, 1998). Genetic diversity was estimated within and between populations of *R. mangle* through estimates of proportion of polymorphic loci, number of allele per locus, allele richness per population (A), expected (H_e) and observed (H_o) heterozygosity using the program POPGENE ver. 1.32 (Yeh et al., 1997). Hardy–Weinberg equilibrium per locus and per population was calculated from a Markov chain of 100 000 steps using Arlequin. For these tests, a Bonferroni (1936) correction was used to adjust the level of significance.

We used an analysis of molecular variance (AMOVA) in Arlequin to analyze the genetic structure of the populations of *R. mangle* sampled along the Brazilian coast. Analyses of population structure were performed including all populations, as well as without the Pará and Maranhão populations, after we noticed that the presence of these populations in the analysis significantly altered the results. An AMOVA with two hierarchical levels was also performed, where populations from Pará and Maranhão formed one group and the remainder populations formed the other. The analyses performed with and without the Pará and Maranhão populations provided a better understanding of the data. Geographical differentiation between pairs of populations was also analyzed using the genotypic frequencies (Goudet et al., 1996) and 10 000 steps of the Markov chain, but with nominal significance level of 0.0006, following a Bonferroni correction. The genetic differentiation was calculated using the program FSTAT ver. 2.9.3.2 (Goudet, 2001) by estimating R_{st}, which is analogous to Wright's F_{st}. However, R_{st} is more appropriate to the analysis of microsatellite because it takes into consideration high levels of mutation and the stepwise mutation model to explain the changes in allele sizes that occur in those repetitive regions of the genome (Slatkin, 1995).

The genetic distances between populations were calculated as Nei's (1972) estimates using POPGENE. These distances were used to construct a neighbor-joining dendrogram (Saitou and Nei, 1987) using POPTREE (Takezaki, 2001). The significance of the best topology tree was estimated by 1000 bootstrap replicates. A Mantel (1967) test was performed using Arlequin to test the correlation between genetic and geographic distances of the *R. mangle* sampled populations. The geographic distances were calculated following the coastlines, given that it is the distance the propagule of the species would have to float. A Mantel test was performed also with the populations of Pará and Maranhão separately.

Coalescence methods for analyzing genetic differences among individuals and populations allow us to explore evolutionary processes and demographic events. These methods work backward in time and allow time dimensions to be added to the analyses. Consequently, they are more powerful than conventional

analyses that use only current distribution and patterns of genetic differences (Frankham et al., 2004). Long-term gene flow (M = m/μ, where m = migration rate and μ = mutation rate) between populations and θ, a measure of effective population size (θ = $4N_e$ μ; where N_e = effective population size, and μ = mutation rate) were estimated using the program MIGRATE, version 3.1.6 (Beerli and Felsenstein, 1999, 2001). This program estimates historical migration rates and effective population sizes using coalescence theory and Markov chain Monte Carlo techniques. Parameter distributions were estimated using the Bayesian implementation of the program Migrate (Beerli, 2006). Our interest was to infer the historical gene flow between the structure clusters associated with the northern and the southern populations and to infer both effective population sizes. Although it would be interesting to see the relationships between all populations, preliminary analyses suggested that a robust estimate of the population parameters was not possible. Therefore, we only analyzed the relationship between the northern locations (Pará and Maranhão) and the remaining populations. Microsatellite mutation was modeled as a continuous Brownian process and was allowed to have different rates among loci. Following a burn-in of 5×10^4 iterations, 2×10^5 genealogies were recorded at a sampling increment of 50 iterations. Uniform priors (minimum, maximum, delta) were placed for both θ (0, 2, 0.02) and *M* (0, 500, 25). Four replicates of single long Markov chains were implemented using different random number seeds.

RESULTS

Genetic diversity—Given that there was no statistically significant linkage disequilibrium or departures from Hardy–Weinberg equilibrium, all eight loci were used in further analyses. Every locus was polymorphic and, in combination, generated a total of 27 alleles. Loci RM05, RM41 and RM46 produced two alleles each; loci RM50 and RM52 produced three each; locus RM38 resulted in four; locus RM19, five; and locus RM86 produced six alleles. The population of Pará presented the highest number of alleles (22), followed by the population of Maranhão (18). In the remaining populations, the number of alleles did not exceed 14. The population of Santa Catarina depicted the lowest genetic diversity, with only 10 alleles.

Private alleles were found in the populations of Pará, Maranhão, Pernambuco, and Sergipe (Table 2). Moreover, the populations of Pará and Maranhão shared four alleles that were not present in the other populations. The allele richness per population varied from 2.75 in Pará to 1.25 in Santa Catarina (Table 2). Table 2 also shows the average expected and observed heterozygosity of the studied populations of *R. mangle*. Prior to Bonferroni correction, the vast majority of the populations presented fewer heterozygotes than expected, with the only exception of the population from the state of São Paulo, suggesting the occurrence of inbreeding. There is an overall reduction in heterozygosity from the northern populations (Pará and Maranhão) to the populations in estuaries further south (Fig. 1). The average expected heterozygosity of the Pará and Maranhão populations is H = 0.38, whereas the average for the estuaries further south is 68.4% lower (H = 0.12). The total number of private alleles in northern populations (5) is also higher when compared to the total in southern populations (2).

Genetic structure—The AMOVA indicated statistically significant geographical structure among populations of *R. mangle*, either when including all populations or when excluding Pará and Maranhão from the analysis (F_{st}= 0.297, P < 0.001, F_{st}= 0.146, P < 0.01, respectively). When populations from the northern states above were considered as a separate hierarchical level in relation to the remaining populations, the percentage variation was greater between these two groups (39.17%) than between populations of the same group (13.09%)

TABLE 2. Allele richness (A), number of private alleles (A_p), expected heterozygosity (H_e), and observed heterozygosity (H_o) for each sampled population of *Rhizophora mangle*. Mean expected and observed heterozygosity are also shown.

Populations	A	A_p	H_e	H_o
Pará	2.75	4	0.38	0.24
Maranhão	2.25	1	0.38	0.26
Rio Grande do Norte	1.75	—	0.19	0.07
Pernambuco	1.37	1	0.09	0.01
Sergipe	1.62	1	0.16	0.13
Bahia	1.62	—	0.12	0.09
Rio de Janeiro	1.37	—	0.13	0.06
São Paulo	1.37	—	0.10	0.12
Paraná	1.37	—	0.11	0.06
Santa Catarina	1.25	—	0.05	0.03
Mean			0.17	0.11

(Table 3), evidencing the differentiation between the northern populations (Pará and Maranhão) and those of the remaining areas.

The R_{st} value considering all populations was 0.48, which indicates a very high degree of differentiation. However, when removing the two northern populations from the analysis, the value of R_{st} (0.08) indicates low differentiation. Thus, populations of Pará and Maranhão are genetically similar to each other and different from all remaining populations analyzed. Pairwise AMOVA comparisons between populations south to Pará and Maranhão indicate that only the population of Santa Catarina differs significantly from most others (Table 4).

Nei's (1972) genetic distances between the sampled *R. mangle* populations further support the differentiation of the populations of Pará and Maranhão from the remaining sampled populations (Table 4). The genetic distances found between these two populations and the others are much higher than the distances among the southern populations themselves. The dendrogram drawn from these distances (Fig. 2) and the result of the Mantel test indicate a relationship between genetic and geographic distances (R^2 = 0.50, P < 0.05) only when populations of Pará and Maranhão are present in the analysis.

Coalescence analyses of migration rate and effective population size—Results of different replicates of the Bayesian coalescent analyses of θ (Fig. 3A, 3B) and M (Fig. 3C, 3D) showed consistent results, suggesting that Markov chains had converged on the stationary distribution, except for θ of the combined southern populations, which showed a bimodal posterior probability distribution in three of the four replicates. This instability occurred despite effective sample sizes of exceeding 40 000. We ran two additional chains, 10 times longer, and the results were similar to the original chain with a unimodal posterior distribution, which seems, therefore, to be the most robust estimate. The median of the estimated posterior probabilities of M indicate migration is very limited between the northern and southern populations. It also indicates that migration is slightly higher from the northern populations to the south than the estimate in the opposite direction (1.31 from north to south, 1.18 from south to north). However, the posterior distribution of the Bayesian analysis shown by the gray shades in Fig. 3C and 3D indicate there is an overlap between the two measurements. On the other hand, there is a dramatic difference in the effective population sizes (θ) of more than an order of magnitude (1.59 and 0.09 for the northern and southern populations, respectively) and their posterior distribution (confidence intervals) do not overlap.

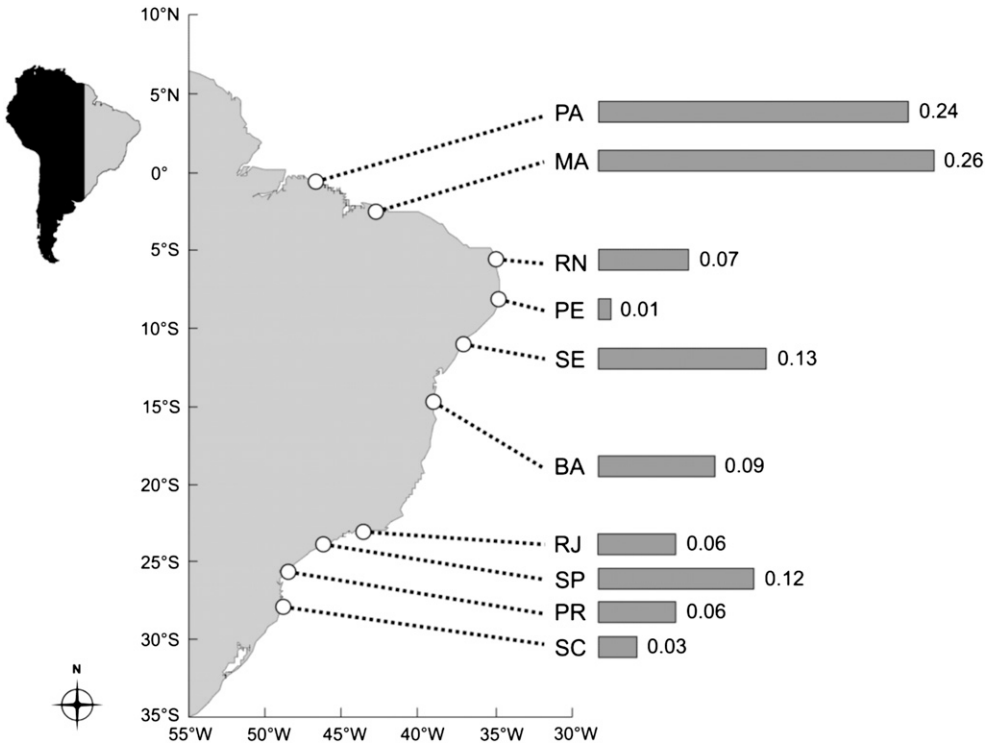

Fig. 1. Locations of the samples of *Rhizophora mangle* populations along the Brazilian coast. PA = Pará; MA = Maranhão; RN = Rio Grande do Norte; PE = Pernambuco; SE = Sergipe; BA = Bahia; RJ = Rio de Janeiro; SP = São Paulo; PR = Paraná; SC = Santa Catarina. The bars are a scaled representation of the expected heterozygosity values for each population.

DISCUSSION

Genetic variability and population structure—The small number of alleles and the low heterozygosity found in this study reveal a comparatively low genetic variability of *R. mangle* along most of the Brazilian coast. This result is likely not an artifact associated with inadequate marker choice because Arbeláez-Cortes et al. (2007) used three of the same microsatellite loci of the present study (RM19, RM41, and RM46) to find a much higher genetic variability in populations of the same species in five populations of the Pacific coast of Colombia. These authors detected a total of 17 alleles, while we detected only nine for the same set of loci. Likewise, the allelic richness reported for the Colombian *R. mangle* populations was higher, varying from 4.3 to 5.7. Allelic richness of the Brazilian populations of the present study varied from only 1.25 to 2.75. There is a small difference in the number of individuals sampled per population between their study and ours (mean of 18.4 and 14.5, respectively). We used a smaller number, but we do not believe it influences our results or comparisons with other studies because of the low variability found within those individuals. Núñez-Farfán et al. (2002) reported a similar difference in genetic variability between populations from the Atlantic and the Pacific coasts. These authors estimated genetic diversity indices twice as high in populations of *R. mangle* from the Pacific coast of Mexico in relation to the ones on the Atlantic coast. They used 10 isoenzyme markers in 14 populations of *R. mangle*. They also detected genetic differentiation among populations ($F_{st} = 0.287$) and high inbreeding ($F_{is} = 0.428$). In general, previous studies revealed that low genetic diversity is a common feature in mangrove species (Lakshmi et al., 1997; Duke et al., 1998; Sun et al.,1998; Ge and Sun, 1999; Jian et al., 2010).

Our results further suggest that the red mangrove populations of the Brazilian coast do not compose a single panmictic population. This differentiation is especially evident between populations sampled in the northern coast (Pará and Maranhão) and the rest of the populations, located to the south of Rio Grande do Norte (Fig. 1).

TABLE 3. Group AMOVA One of the group is formed by the Pará and Maranhão populations and the other group consists of the remaining populations.

Source of variation	df	Sum of squares	Variance components	% variation
Among groups	1	42.87	0.54	39.17
Among populations within groups	8	32.72	0.18	13.09
Within populations	280	143.36	0.66	47.74
Total	289	218.95	1.38	

Inferred populations history—A likely scenario to explain the differences and genetic structuring between the northern and southern populations is associated with the climatic changes that occurred in the last thousands of years, the behavior of coastal oceanographic currents, and the biological characteristics of *R. mangle*. During the Quaternary, climate in the western Atlantic coast was colder, with frequent temperature oscillation that increased in amplitude, consequence of a series of ice ages. The last peak of ice was about 22 000 to 18 000 yr ago (Hewitt, 2000, 2004; Yokoyama, et al., 2001). During these glaciations, in the northern hemisphere, the ice sheets expanded

TABLE 4. Nei's (1972) pairwise genetic distances between populations (Pop.) of *Rhizophora mangle* (above diagonal) and genetic structure between *R. mangle* populations based on AMOVA (below diagonal).

Pop.	PA	MA	RN	PE	SE	BA	RJ	SP	PR	SC
PA		0.1150	0.2616	0.2791	0.2477	0.2741	0.3062	0.3186	0.3004	0.3337
MA	—		0.2356	0.2039	0.2789	0.2344	0.2010	0.3315	0.3266	0.3573
RN	+	+		0.0444	0.0176	0.0301	0.0179	0.0558	0.0420	0.0782
PE	+	+	—		0.0561	0.0120	0.0239	0.1052	0.1062	0.0860
SE	+	+	—	+		0.0513	0.0551	0.0686	0.0775	0.0943
BA	+	+	+	—	—		0.0184	0.0779	0.0557	0.0528
RJ	+	+	—	—	—	—		0.0769	0.0620	0.0846
SP	+	+	—	—	—	—	—		0.1131	0.0214
PR	+	+	—	—	—	—	—	—		0.1045
SC	+	+	+	—	+	—	+	+	+	

Notes: +, significant genetic structure ($P < 0.001$); —, no structure.

considerably, and the vegetation zones were limited to the equatorial regions (Hewitt, 2000, 2004). In the southern hemisphere, however, the ice sheet was not as extensive, and most of the South America suffered mainly temperature and humidity reduction during this period (Ab'Saber, 2000; Hewitt, 2000). Since the latitudinal distribution of mangroves is strongly determined by temperature and humidity (Duke et al., 1998), their distribution was likely restricted to equatorial regions during the cooling of the Quaternary (Saenger, 1998). Furthermore, mangroves depend on low-wave-energy areas to establish and develop; therefore, changes in the sea level are also an important additional factor influenced by glacial cycles (Woodroffe and Grindrod, 1991).

With the increasing temperatures and humidity following the postglacial period, mangrove species could gradually expand their range of distribution to southern coastal areas. This recolonization process can be seen through palynological and genetic evidences and are widely recorded for temperate species in North America and Europe (Petit et al., 2003; Hewitt, 2004). For the tropical regions, little is known about patterns of recolonization and the subsequent genetic architecture of species (Hewitt, 2004). However, available palynological dating of mangroves in Brazil supports the above hypothesis. Mangroves of the northern areas sampled in this study are older (between 7250 and 5600 yr old in areas of Pará and Maranhão; Behling and Costa, 1997, 2001; Behling, 2001; Vedel et al., 2006) than southern mangroves (mangroves in the state of São Paulo are only 1300 yr old; Amaral et al., 2006). Thus, northern mangroves likely represent descendent of mangroves located in older, and presently deeper, coastal lines during the last glacial period.

Rhizophora mangle populations in the equatorial region indeed depict greater genetic variability (this study), and the mangrove plant community, greater taxonomic richness. *Rhizophora* is represented in northern mangroves by three species: *R. mangle, R. harisonii,* and *R. racemosa* (Menezes et al., 2008), unlike the remaining southern estuaries of the coast of Brazil, in which only one species occurs, *R. mangle*. Cerón-Souza et al. (2010) have recently studied the evolutionary history of the New World *Rhizophora* to conclude that *R. harrisonii* is not a distinct species but rather a product of ongoing hybridization and backcrossing between *R. mangle* and *R. racemosa*. The existence and maintenance of the putative hybridization in the northern part of the Brazilian coast—predicted solely by the presence of the three alleged species—further corroborate with the hypothesis presented herein. This diversification process exists only in the northern coast and not in any other areas studied, where we postulate populations to be much more recent.

Indeed, a more recent colonization of the coastal areas south of Rio Grande do Norte may explain the reduced genetic diversity of the red mangrove populations and the richness of the *Rhizophora* component of the mangrove forests. The first scenario would be the result of consecutive founder effects associated to a stepping-stone dispersion pattern (see Palumbi, 2003) because *R. mangle* colonized estuaries farther away from the equatorial refuge (see Petit et al., 2003). The sequential founder effects seem to have resulted in the loss of alleles and consequent elevated homozygosity observed in these populations. Nettel and Dodd (2007) provided analogous explanation for the low genetic variability and structure found in populations of *A. germinans* in some regions of the northern Atlantic Ocean.

While it is reasonably easy to understand how a stepping-stone model (see Hastings and Harrison, 1994) of dispersion from the northern refuge populations to the south would initially result in subpopulations with significantly lower variability toward the limits of distribution of the species, it is not as clear how the observed low variability is maintained. Several studies (Lowenfeld and Klekowski, 1992; Klekowski et al., 1994;

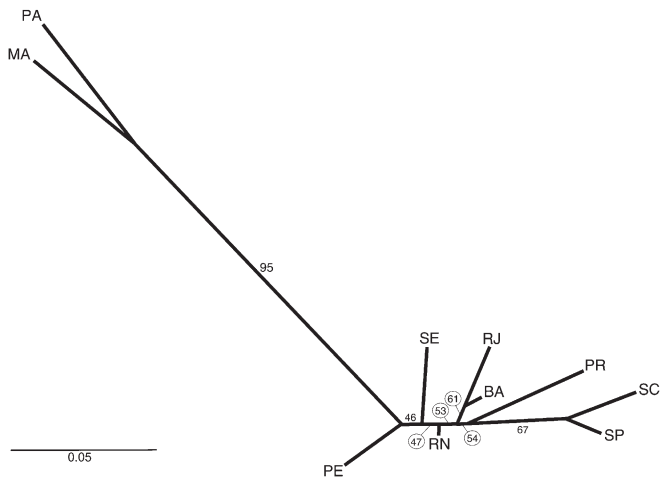

Fig. 2. Neighbor-joining dendrogram drawn using Nei's genetic distances between *Rhizophora mangle* populations with the respective bootstrap values (1000 replicates). PA = Pará; MA = Maranhão; RN = Rio Grande do Norte; PE = Pernambuco; SE = Sergipe; BA = Bahia; RJ = Rio de Janeiro; SP = São Paulo; PR = Paraná; SC = Santa Catarina.

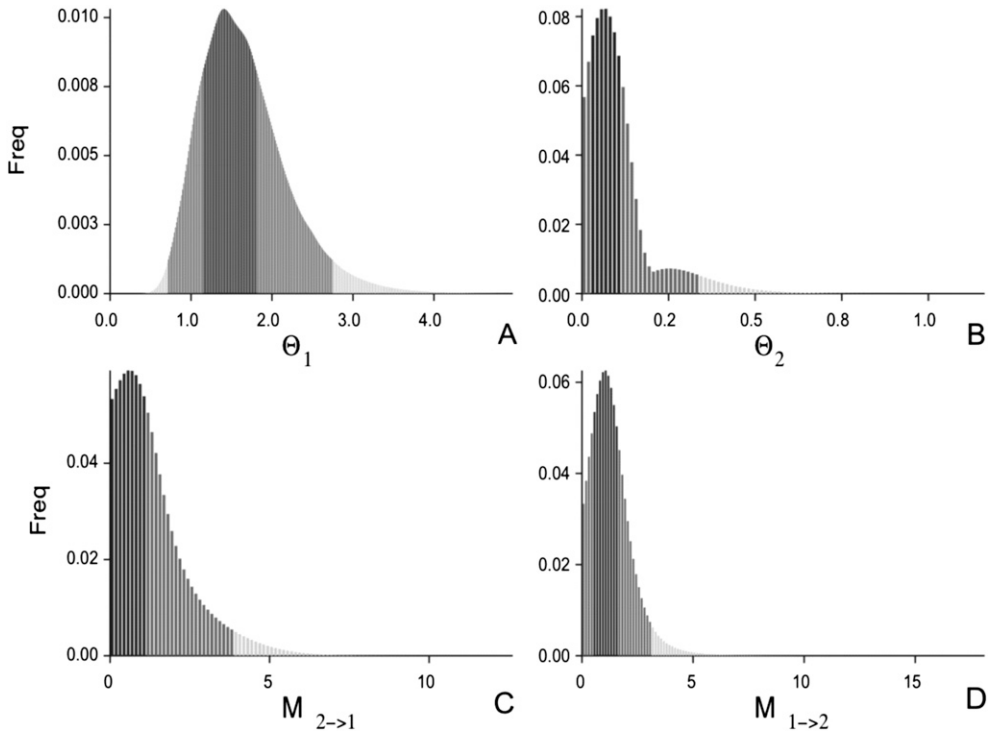

Fig. 3. Posterior distribution (Freq) over all loci for the θ values of the (A) northern (θ1) and the (B) southern (θ2) population clusters and long-term gene flow from the southern to the northern cluster of populations (M 2→1; C) and from the northern to the southern clusters of populations (M 1→2; D) based on Bayesian analysis of the program Migrate (see Material and Methods for parameters). Different shades of gray indicate estimates in the different percentiles of the distribution (2.5%, 25%, mode, 75% and 97.5%).

Núñez-Farfán et al., 2002; Arbeláez-Cortes et al., 2007) indicate that endogamy is a common characteristic in *R. mangle* populations. The autofertilization ability of the species (Lowenfeld and Klekowski, 1992) makes it able to rapidly occupy and colonize new areas. Consequently, even with a low rate of propagule arrival in the estuary, the species is able to establish a viable

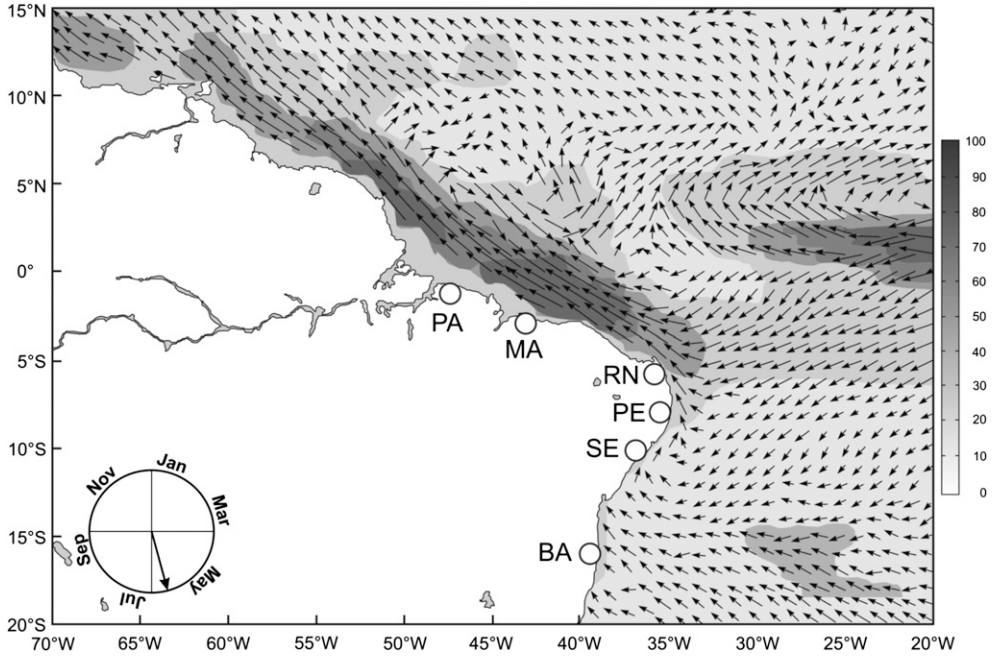

Fig. 4. Climatological currents of the Brazilian coast on 30 April and 31 August. Darker shades of gray indicate stronger currents. White circles represent the six sampled population included within the latitude represented in the map. Source: http://www.aoml.noaa.gov.

population. Even few surviving propagules are able to establish colonies distant from their point of origin, but the resulting populations will, inevitably, have low genetic variability. And once the population is established, posterior migrants contribute little to the reproductive pool of the species. The reproduction rate of these migrants is logistically low, while the one of the original colonizers is exponentially high (Hewitt, 1996). This may be one of the descriptive scenarios to explain the maintenance of low genetic variability in the southern populations of *R. mangle*.

Another explanation for the persistent low variability of populations and likely, also, for the reduced richness in species of *Rhizophora* in the southern Brazilian coast may reside in the dispersion constraint of propagules from the northern Quaternary refuge to the available estuarine areas in the south. The apparently abrupt break in the variability (and species richness) between the northern and southern population clusters is consistent with the bifurcation of climatological and superficial currents (NOAA, 2010; Lumpkin and Garraffo, 2005; Lumpkin and Pazos, 2006; Fig. 4) observed in the region that persisted likely for most of the Quaternary. The South Equatorial Current originates in the African coast and flows west until it reaches the Brazilian coast, off the states of Rio Grande do Norte and Paraíba, when it splits into two other currents that flow in distinct directions, the Brazil Current (southward) and the Guiana Current (northwestward) (Stramma and England, 1999). The associated climatological currents in the region depict elevated velocity throughout the year (Fig. 4), several times faster than the corresponding southern branch.

Thus, dispersion of propagules from populations of the equatorial zone to the southern coastal region following postglacial warming was, and still is, most probably constrained by the prevailing fast northwest superficial currents of the northern coastal areas of the country. This constraint would amplify the founder effect on the putative stepping-stone-colonizing dispersion that results in the low variability of the newly colonized regions, as observed in this study. Indeed, coalescence measure of migration indicates there was little migration between the northern and southern populations of *R. mangle*. The consistently low rate of propagule mixing between these two regions (northern and southern coast) probably prevents homogenization, maintaining the genetic difference between them. Probably as a result of the same dynamics and chance, specimens of *R. racemosa* and of the alleged hybrid, *R. harrisonii*, did not colonize estuaries south of Rio Grande do Norte. Another source of propagules could be from populations of the West African continent because of the South Equatorial Current that originates off the African coast. On the basis of data from ITS sequences, chloroplast DNA (cpDNA), and amplified fragment length polymorphisms (AFLPs), Nettel and Dodd (2007) found that populations of *Avicennia germinans* sampled from the Brazilian coast, near our sampled population of Rio Grande do Norte, are genetically similar to populations of the West African coast (Senegal, Guinea-Bissau, and Angola). This similarity could be an indication that there can be gene flux among mangrove populations between those two continents.

If the described scenario is correct, the postglacial history of other western Atlantic species of other organisms that disperse through the oceans should show the same general influence of the currents and of the temperature and humidity elevation of the areas in the southern coast. Indeed, the same differentiation pattern observed for *R. mangle* was reported by Takayama et al. (2008b) for *Hibiscus pernambucensis* Arruda. These authors found an evident genetic differentiation between populations located to the north and to the south of the state of Rio Grande do Norte, as we have for *R. mangle*. Further, other marine organisms show the same genetic structuring between populations that are also to the north and south of Rio Grande do Norte (Santos et al., 2003; Muss et al., 2001).

The phylogeography of *Rhizophora mangle* along the Brazilian coast provides knowledge to support management plans and conservation efforts of the species in the country. The genetic structure found between the populations suggests that the efforts to conserve the species should not be based only on preserving large areas, but also on small and separate ones, to encompass the different genetic patterns found between the northern and southern regions. However, this information is for only one species; to generate substantial benefits in management and conservation plans based on the genetic variability of species in an area, we need to have a broader understanding of the genetic structure of additional species.

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