Geographic and environmental contributions to genomic divergence in mangrove forests

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ABSTRACT

Assessing the relative importance of geographic and environmental factors to the spatial distribution of genetic variation may provide relevant information about the processes that maintain genetic variation in natural populations. With a globally wide but very restricted habitat distribution, mangrove trees are an interesting model for studies aiming to understand the contributions of these factors. Mangroves occur along the continent-ocean interface of tropical and subtropical latitudes, regions considered inhospitable to many other plant types. Here, we used landscape genomics approaches to investigate the relative contributions of geographic and environmental variables to the genetic variation of two black mangrove species, *Avicennia schaueriana* and *Avicennia germinans*, along the South American coast. Using single nucleotide polymorphisms, our results revealed an important role of ocean currents in the gene flow of *A. schaueriana* and isolation by environment as the pattern that best explains the genetic differentiation of *A. germinans*. Additionally, for both species, we observed significant correlations between genetic variation with evidence of selection and precipitation regimes, tidal variation, solar radiation and temperature patterns. These discoveries expand our knowledge about the evolution of mangrove trees and provide important information to predict future responses of coastal species to the expected global changes for this century.

KEYWORDS: adaptation of mangroves - coastal ecology - environmental gradient - isolation by barrier - isolation by distance - molecular ecology

INTRODUCTION

Environmental and geographic variations are often determinants of the distribution of allele frequencies in natural populations by facilitating or limiting gene flow across space and influencing the selection of certain genotypes (Wang *et al.*, 2013; Sork, 2016; Murray *et al.*, 2019). For example, under an isolation-by-distance (IBD) model (Wright, 1943), geographic distance may limit dispersal, leading to the accumulation of allele frequency divergences by genetic drift (Bradburd *et al.*, 2013). Furthermore, under the isolation-by-barrier (IBB) model, a barrier to gene flow may abruptly reduce or even disrupt connectivity between individuals of a species (Barton, 1979). In addition to these models, genetic differentiation can increase in response to environmental differences, regardless of the geographic distance. This pattern, described by the isolation-by-environment (IBE) model (Wang & Bradburd, 2014) can be generated by a variety of ecological processes, such as selection against immigrants, leading to the evolution of locally adapted populations (Bradburd *et al.*, 2013).

These models, IBD, IBB, and IBE, are not mutually exclusive and often co-occur in nature (Wang, 2013; Sexton *et al.*, 2014). Studies aiming to determine the factors that control the distribution patterns of genetic variation across space may provide relevant information about the underlying processes that generate and maintain genetic variation in natural populations (Lee & Mitchell-Olds, 2011; Wang & Bradburd, 2014). This knowledge is essential to predict future responses of current populations to environmental changes (Vincent *et al.*, 2013) and may contribute to decision-making processes aiming to minimize future biodiversity loss (Kovach *et al.*, 2012; Muñoz *et al.*, 2015; Wee *et al.*, 2019).

The field of research that seeks to clarify the roles of these factors in the distribution of the neutral and adaptive genetic variability of a species over space is known as landscape genomics (Joost *et al.*, 2007; Lowry, 2010; Schoville *et al.*, 2012; Vincent *et al.*, 2013). Recently, this approach has been increasingly applied to the study of non-model organisms

(Storfer *et al.*, 2018). Nevertheless, landscape genomic studies are mostly limited to animal species, whereas studies on plants, especially tropical trees (Storfer *et al.*, 2010), remain very limited despite their fundamental roles in global biogeochemical cycles (Jasechko *et al.*, 2013) and as habitat providers to most terrestrial biodiversity (Mannion *et al.*, 2014).

As sessile organisms, trees respond directly to the environment in which they live (Holderegger *et al.*, 2010). Conversely, they often have high levels of gene flow, which tend to reduce the strength of natural selection (Savolainen *et al.*, 2007). Thus, tree species that often occur across wide latitudinal ranges along environmental gradients, such as mangrove forests (Tomlinson, 1986), represent a promising biological model to investigate and understand the effects of the environment on microevolutionary processes and population dynamics.

Mangrove trees occur in a narrow area along the continent-ocean interface (Tomlinson, 1986; Hamilton, 2020) of tropical and subtropical latitudes of the world, mainly between 30°N and 30°S (Giri *et al.*, 2011). These species produce floating seeds or fruits, referred to as "propagules" (Tomlinson, 1986), which can disperse over long distances via rivers and ocean surface currents (Van der Stocken *et al.*, 2019b). Their geographic distribution is limited by the topography of the intertidal zone (Middleton, 2012) and frequently by the occurrence of low temperatures (Morrisey *et al.*, 2010) and precipitation patterns (Spalding *et al.*, 1997). However, environmental variations in their boundaries are associated with different climatic thresholds (Osland *et al.*, 2017; Cavanaugh *et al.*, 2018) and oceanographic conditions (Soares *et al.*, 2012; Saintilan *et al.*, 2020).

Avicennia L. (Acanthaceae) is one of the most diverse and widely distributed mangrove genera globally (Duke, 1991; Li *et al.*, 2016) and is very abundant on the Western Atlantic coastline (Schaeffer-Novelli *et al.*, 1990). In this region, *Avicennia* is mainly represented by two of the three New World *Avicennia* species, namely, *Avicennia germinans*

and *Avicennia schaueriana* (Schaeffer-Novelli *et al.*, 1990; Duke, 1991). These species present a partially sympatric distribution on the South American coast, where they share a remarkable north-south pattern of genetic divergence, as revealed by selectively neutral microsatellites (Mori *et al.*, 2015) and single nucleotide polymorphisms (SNPs) (Cruz *et al.*, 2019, 2020). A similar pattern of putatively neutral genetic diversity has also been observed in other coastal species, such as *Rhizophora mangle* (Pil *et al.*, 2011; Francisco *et al.*, 2018) and the mangrove-associated tree *Hibiscus pernambucensis* (Takayama *et al.*, 2008). These findings likely indicate a prominent role of the dispersal of floating propagules in shaping the overlapping north-south genetic divergence of these trees, given the direction of the South Equatorial current (SEC) along the Atlantic coast of South America (Lumpkin & Johnson, 2013).

In addition to neutral processes that shape the diversity of *Avicennia* species in this region, recent studies have identified various genomic regions possibly associated with adaptive processes relevant to the environmental context of mangroves (Cruz *et al.*, 2019, 2020). These variations have been attributed to climatic and oceanographic factors that vary widely along the latitudinal gradient of the species distribution. Although these studies provide insights into the role of the environment in the organization of the adaptive genetic variation in *A. schaueriana* and *A. germinans*, the relative importance of neutral and nonneutral abiotic factors remains unknown. The present study aimed to investigate the relative contributions of geographic and environmental variables to the organization of the genetic diversity of these two dominant species of the genus *Avicennia* that are found on the Atlantic coast of South America.

MATERIAL AND METHODS

Biological materials and genotyping of SNP markers

Biological materials were collected and SNP markers were identified as described in previous studies by Cruz *et al*. (2019) for *A. schaueriana* and Cruz *et al*. (2020) for *A. germinans*. Briefly, seventy-seven *A. schaueriana* individuals were sampled from ten different locations and 48 *A. germinans* individuals were sampled from six different locations along the Brazilian coast (Figure 1 and Table 1). Assembly, mapping and SNP locus identification were performed using custom scripts SNPsaurus (SNPsaurus LLC, Institute of Molecular Biology, Eugene, OR, USA), which created a reference catalogue of abundant reads, retaining biallelic loci present in at least 10% of the samples. High-quality sequences were used, allowing a maximum of 65% of missing data and one SNP per sequence and requiring a minimum coverage of 8× and a minor allele frequency ≥ 0.05 using vcftools v.0.1.12b (Danecek *et al.*, 2011). A maximum reading cover of 56 was used (resulting from the product with the average read depth and a standard deviation of 1.5 from the average) to reduce the paralogy or low-quality genotype calls. In total, 6,170 and 2,297 SNP markers were identified for *A. schaueriana* and *A. germinans*, respectively.

Detection of SNP loci with signatures of natural selection

SNP loci with evidence of natural selection were identified previously by Cruz *et al.* (2019, 2020). For *A. schaueriana*, 86 loci showed considerable deviations from neutral expectations of interpopulation divergence. They were detected using two methods to minimize false positives: LOSITAN (Antao *et al.*, 2008), with a confidence interval of 0.99 and a false-discovery rate (FDR) of 0.1, and pcadapt (Luu *et al.*, 2017), with an FDR of 0.1. For *A. germinans*, 25 loci showed considerable deviations from the neutral expectations of interpopulation divergence. For the latter species, in addition to LOSITAN (a confidence interval of 0.99 and an FDR of 0.05) and pcadapt (an FDR of 0.05), SNP loci associated with

ecological variables were detected using Latent Factor Mixed Models (LFMM) implemented in the *LEA* package (Frichot & François, 2015).

Estimation of genetic distances

To analyze the importance of geographic distance and environmental variables in spatial genetic divergence, we evaluated which model — IBD, IBB or IBE — best described the distribution of the genetic diversity of each species based on genome-wide SNP markers. To that end, we estimated the pairwise genetic differentiation (Wright's *F*ST) (Wright, 1949) for the total set of SNP molecular markers and the set of SNP markers with evidence of selection using the *Hierfstat* (Goudet, 2005) package for R v. 3.6.2 (R Core Team, 2019).

Geographic and environmental distances and the oceanographic barrier

Pairwise geographic distances among populations were measured using the geographic coordinates of the sampling sites (Table 1) with the global positioning system (Garmin 76CSx, WGS-84 standard, Garmin International Inc., Olathe, KS, USA). Distances between points were estimated based on the contour of the Brazilian coast; thus, we considered floating propagule-mediated dispersal (Van der Stocken *et al.*, 2019a). A binary matrix (0 or 1) was constructed based on the presence (1) or absence (0) of the supposed oceanographic barrier between each pair of sampling sites to determine the relative significance of the split pattern of the SEC into the Brazil current (BC) and the North Brazil current (NBC) (Lumpkin & Johnson, 2013) (Figure 1) for *A. schaueriana* and *A. germinans* propagules.

We obtained 42 environmental variables for each sampling site, with a resolution of 30 arc-seconds (~ 1 km in Ecuador), to evaluate the overall effect of the environment on the distribution of genetic variation. In our dataset, we included 27 climatic variables derived from the WorldClim temperature and precipitation datasets (version 1.4 and 2.0) (Fick &

Hijmans, 2017), 10 oceanographic variables derived the Marspec ocean surface salinity and temperature datasets (Sbrocco & Barber, 2013) and five variables related to tidal variations retrieved from the Environmental Climate Data Sweden (ECDS) platform (Klein *et al.*, 2013). We removed variables that showed a high correlation ($R^2 > 0.8$) (Supporting Information, Figure S1), as measured by the *removeCollinearity* function of the *virtualspecies* package (Leroy *et al.*, 2016) in R (R Core Team, 2019), to avoid nonindependence between environmental variables. We extracted the values of the environmental variables for our sample points (Supporting Information, Tables S1 and S2) using the *raster* package (Hijmans, 2017) in R (R Core Team, 2019). All occurrence data have been carefully inspected to detect and correct problems associated with inconsistent records (Chapman, 2005). We transformed this environmental data matrix using a principal component analysis (PCA). We used the most representative scores from the PCA to calculate Euclidean distances between population pairs (Lira-Noriega & Manthey, 2014). The PCA and environmental distance measurements were all performed in R (R Core Team, 2019).

Association tests

We performed Multiple Matrix Regression with Randomization (MMRR) to determine which model best explained our genetic data (both neutral and putatively nonneutral) (Wang, 2013). We employed this method to estimate the independent effect of each factor (geographic or environmental) on a single response variable, typically the genetic distance. The analysis was performed using the *MMRR* function in R with 10,000 permutations. This function calculates the coefficient of determination and the regression coefficients (β), and estimates significance values for all parameters, which has been shown to be accurate for several types of conditions (Wang, 2013). The following comparisons were performed: (I) geographic distance (km) and

environmental distance, (II) geographic distance (km) and pairwise *F*ST, (III) environmental distance and pairwise *F*ST, (IV) geographic distance (km) in conjunction with environmental distance and pairwise *F*ST and (V) the presence/absence of the supposed oceanographic barrier and pairwise *F*ST. These comparisons were also performed separately for the set of samples located northerly from the SEC and for the set of samples located southerly from the SEC because of previous genetic structure evidences (Takayama *et al*., 2008; Pil *et al*., 2011; Mori *et al*., 2015; Francisco *et al*., 2018). Finally, we tested for correlations between the genetic distances based on the SNP marker set with selection evidence for each environmental variable separately.

RESULTS

Genetic, geographic and environmental distances

For the total set of SNP markers, we obtained *F*ST values ranging from 0.019 to 0.189 for pairs of *A. schaueriana* populations and from 0.047 to 0.387 for pairs of *A. germinans* populations (Figure 2). For the set of markers with evidence of selection, the *F*ST ranged from 0.02 to 0.36 for *A. schaueriana* (Supporting Information, Table S3) and from 0.03 to 0.88 for *A. germinans* (Supporting Information, Table S4). The pairwise geographic distances ranged from approximately 5000 km between Bragança (SB) and Laguna (SL) to 77 km between Cananéia (SC) and Pontal do Paraná (SP) for *A. schaueriana* (Supporting Information, Table S5) and from 2100 km between Ilha de Marajó in Soure (GM) and Tamandaré (GT) to 222 km between Ilha de Marajó in Soure (GM) and Bragança (GB) for *A. germinans* (Supporting Information, Table S6).

After removing highly correlated environmental variables ($R^2 > 0.8$), 23 variables were retained for analyses of environmental distances (Supporting Information, Tables S7

and S8). The first five and the three most representative axes of the PCA used to calculate the environmental distance between the sampling sites (Supporting Information, Tables S9 and S10) retained 97% and 92% of the variation for *A. schaueriana* and *A. germinans*, respectively. The first two PCA axes for *A. schaueriana* represented 80% of the variance and were mainly explained by the variations in air temperature and sea surface temperature, the solar radiation and tidal cycles (Figure 3A). For *A. germinans*, the first two PCA axes represented approximately 80% of the variance and were mainly explained by the air temperature variation, precipitation regimes, vapor pressure deficit (VPD) and solar radiation (Figure 3B).

Association tests

For *A. schaueriana*, considering all SNP loci, we observed that the geographic distances between pairs of sampling sites played the greatest role in the describing genetic distances (R^2 = 0.81, $P < 0.001$; $\beta = 0.90$, $P = 0.001$; Figure 4A), but environmental distance also contributed significantly to the genetic distance ($R^2 = 0.54$, $P < 0.001$; $\beta = 0.73$, $P < 0.01$; Figure 4B). However, a strong and significant correlation was observed between the geographic distance and environmental difference ($R^2 = 0.82$, $P < 0.05$; $\beta = 0.88$, $P = 0.001$; Figure 4C), implying that the farther the geographic distance, the greater the environmental difference. When tests were performed separately for sampling locations located north and south from the SEC, we only observed significant correlations between genetic and geographic distances ($R^2 = 0.81$, $P < 0.001$; $\beta = 0.89$, $P = 0.01$) for sampling sites located south of the SEC. A multivariate regression analysis combining the effects of geographic and environmental distances on genetic distance provided a slight increase in the fit of the model ($R^2 = 0.86$, $P < 0.05$); however, the effects of geographic ($\beta_{geographic} = 0.13$, $P = 0.001$) and environmental ($\beta_{environment} = -0.48$, $P = 0.01$) distances were lower than the regression

analyses including only one predictor variable. The variable that best explained the genetic differentiation observed in *A. schaueriana* was the presence or absence of the oceanographic barrier ($R^2 = 0.92$, $P < 0.01$; $\beta = 0.96$, $P < 0.01$).

For *A. germinans*, geographic ($R^2 = 0.61$, $P < 0.05$; $\beta = 0.78$, $P = 0.05$; Figure 5A) and environmental ($R^2 = 0.67$, $P < 0.05$; $\beta = 0.81$, $P = 0.01$; Figure 5B) distances played significant roles in the genetic differentiation between sampling sites in the entire SNP dataset. However, when we removed the samples from GT, which is the only sampling site south of the SEC, the genetic divergence was not correlated with either the geographic distance ($R^2 = 0.006$, $P = 0.97$; $\beta = 0.07$, $P = 0.88$) or the environmental distance ($R^2 = 0.03$, $P = 0.70$; $\beta = 0.17$, $P = 0.68$). The correlation between geographic and environmental distances was significant ($R^2 = 0.74$, $P < 0.01$; Figure 5C) but less pronounced than for *A. schaueriana*, probably due to the lower latitudinal amplitude between their sampling sites and consequently lower environmental variation compared to the latter species. When both types of distances were included in the model, we obtained significant values only for the environmental distance, which exerted an almost two-fold greater effect than the geographical distance ($R^2 = 0.69$, $P = 0.03$; $\beta_{environment} = 0.55$, $P < 0.05$, $\beta_{geographic} = 0.28$, $P = 0.34$). The presence or absence of the oceanographic barrier did not explain the genetic divergence among *A. germinans* sampling locations ($R^2 = 0.91$, $P = 0.15$; $\beta = 0.95$, $P = 0.12$).

We identified a significant environmental contribution to the genetic differentiation based on the putative nonneutral SNPs for both species. This contribution was more pronounced for *A. germinans* ($R^2 = 0.62$, $P < 0.05$; $\beta = 0.78$, $P < 0.05$; Figure 5D) than for *A. schaueriana* ($R^2 = 0.51$, $P < 0.01$; $\beta = 0.7$, $P = 0.001$; Figure 4D). When we analyzed each environmental variable separately, we found significant correlations of putative nonneutral genetic differentiation among *A. schaueriana* sampling locations with variables that vary

along the latitudinal gradient of the species, such as the annual mean temperature (R^2 = 0.45, P < 0.001; β = 0.67, P = 0.001), isothermality (R^2 = 0.75, P < 0.001; β = 0.86, P = 0.001), precipitation seasonality (R^2 = 0.56, P < 0.001; β = 0.75, P < 0.01), minimum solar radiation (R^2 = 0.49, P < 0.001; β = 0.70, P = 0.001), the annual average cycle of tidal amplitudes (R^2 = 0.62, P < 0.001; β = 0.79, P < 0.05) and the mean annual sea surface temperature (R^2 = 0.46, P < 0.001; β = 0.68, P = 0.01). For *A. germinans*, the nonneutral variation was related to variations in the annual mean temperature (R^2 = 0.81, P = 0.01; β = 0.88, P < 0.05), maximum temperature of the warmest month (R^2 = 0.80, P < 0.05; β = 0.88, P < 0.05), precipitation in the warmest quarter (R^2 = 0.91, P < 0.05; β = 0.95, P = 0.05), maximum solar radiation (R^2 = 0.96, P = 0.01; β = 0.98, P = 0.01) and the annual average cycle of tidal amplitudes (R^2 = 0.12, P < 0.01; β = 0.35, P = 0.01) (Table 2).

DISCUSSION

The analysis of the geographic and environmental factors shaping neutral and adaptive genetic variation in heterogeneous environments is one of the main approaches used to understand the dynamics and evolutionary potential of natural populations (Schoville *et al.*, 2012; Li *et al.*, 2017). In the present study, we analyzed the relative contributions of environmental and geographic distances and the presence of an oceanographic barrier to the genetic differentiation of populations of two dominant mangrove species in the Neotropics. We identified the relative importance of the main environmental variables that generate adaptation in these species, providing relevant information for decision makers who will plan future efforts targeting conservation and the recovery of coastal vegetation in the face of increasing challenges resulting from anthropogenic, environmental and climate changes in this century.

Pairwise *F*ST results revealed a variable degree of genetic divergence in both species, indicating the existence of substantially structured populations, particularly when samples to the north and south of the SEC are considered. These results corroborate the genetic structure patterns reported in previous studies conducted with neutral molecular markers (Takayama *et al.*, 2008; Pil *et al.*, 2011; Mori *et al.*, 2015; Francisco *et al.*, 2018) and indicate that regardless of the characteristics of these markers with high (microsatellite) (Vieira *et al.*, 2016) or low (SNPs) (Morin *et al.*, 2004) mutation rates, the evolutionary processes that led to this divergence must be quite intense or ancient.

The geographic distances between sampling sites contributed significantly to the genetic divergence of *A. schaueriana* (R^2: 0.81, $P < 0.001$; $\beta = 0.90$, $P = 0.001$) and *A. germinans* ($R^2 = 0.61$, $P < 0.05$; $\beta = 0.78$, $P = 0.05$), suggesting that spatial distance plays a fundamental role in the genetic divergence of populations of these species. This model appears to be common in studies of plants (Sexton *et al.*, 2014; Segarra-Moragues *et al.*, 2016; Cruz-Nicolás *et al.*, 2019) and mangroves (Cerón-Souza *et al.*, 2010; Sandoval-Castro *et al.*, 2014; Kennedy *et al.*, 2016; Binks *et al.*, 2019; Ochoa-Zavala *et al.*, 2019). Although evidence of water dispersion over long distances exists for mangrove species (Nettel & Dodd, 2007; Takayama *et al.*, 2013; Mori *et al.*, 2015; Van der Stocken *et al.*, 2019b), our results indicate that the large geographic extension and major oceanic currents of the Brazilian coast physically limit the dispersal of *Avicennia* species. Although these correlations were significant for the total set of sampling locations, they were not significant when considering only the set of *A. schaueriana* and *A. germinans* sampling locations north of the SEC. For the five sampling locations of *A. germinans* in this region, which are distributed in a fairly geographically continuous habitat, the model was not explained by the coefficient of determination values ($R^2 = 0.006$, $P = 0.97$). We hypothesized that the genetic differentiation ranging from 0.047 to 0.387 observed among sampling locations may actually result from an

IBB effect caused by the SEC acting as a barrier to the dispersal of propagules. However, we did not find a significant correlation between genetic differentiation and the presence of the SEC, probably because we were not able to sufficiently sample *A. germinans* south of the SEC, as only a single location, GT, was used.

In contrast, we obtained statistical evidence for the action of the SEC as a barrier to gene flow in *A. schaueriana*. For this species, the IBB is the model that best explains the genetic differentiation between their populations. This model has also been shown in populations of *A. germinans* and *R. mangle* in Central America, whose patterns of genetic diversity were consistent with the patterns of ocean circulation in the east tropical Pacific (Cerón-Souza *et al*., 2015). Our results corroborate the findings reported by Mori *et al.* (2015), who suggested that the neutral genetic divergence observed for *A. schaueriana* may have been shaped by marine currents. Based on our results, the bifurcated flow of marine currents along the Atlantic coast of South America may play a key role as a driver of the genetic differentiation observed in other species of mangrove or species associated with this ecosystem, such as *R. mangle* (Pil *et al.*, 2011; Francisco *et al.*, 2018) and *H. pernambucensis* (Takayama *et al.*, 2008). Our findings statistically showed that the SEC is an important driver of the genetic structure of mangrove species; however, coastal and ocean currents vary temporally in strength and directionality (Van der Stocken *et al.*, 2019a). For example, the SEC splits into the BC and the NBC, which have different speeds and directions (Lumpkin & Johnson, 2013) (Figure 1). The NBC is faster than the BC, favoring the spread of propagules from south to north, as observed in previous studies (Mori *et al.*, 2015; Francisco *et al.*, 2018). Additionally, the direction of flow of coastal currents may influence the direction of gene flow among populations. Therefore, future investigations of the dynamics of these currents, not as a static barrier but including different levels of resistance to gene flow, may

provide more realistic insights into their effects on the distribution of the genetic variation of species dispersed by sea currents.

We also identified an IBE pattern in the structure of the genetic diversity of *A. schaueriana* and *A. germinans*. For *A. germinans*, the IBE model explained the genetic differentiation between their populations better than the neutral processes. Many species under IBE showed this same pattern (Mitchell-Olds *et al.*, 2007; Byars *et al.*, 2009; Barker *et al.*, 2011; Vernesi *et al.*, 2012; Shafer & Wolf, 2013; Dennenmoser *et al.*, 2014; Sexton *et al.*, 2014; Manthey & Moyle, 2015; Rodríguez-Zárate *et al.*, 2018; Jiang *et al.*, 2019), indicating that environmental heterogeneity may be the main factor underlying the geographic distance (Shafer & Wolf, 2013; Sexton *et al.*, 2014; Beheregaray *et al.*, 2015). Notably, the environmental differences of both species were correlated with the geographic distance. Therefore, we recommend future investigations that control the effect of environmental predictors on geographic distance.

Given the significant results for IBE, we also estimated the contribution of each environmental variable to nonneutral SNP genetic variation, allowing us to determine the degree to which individual environmental variables contribute to these results. For *A. schaueriana*, we observed significant correlations with variations in the tidal range regimes, air and ocean surface temperatures, VPD, solar radiation, and precipitation (Table 2). Our results corroborate the findings reported by Cruz *et al.* (2019), who performed a functional annotation of these loci showing deviations from neutral expectations based on allele frequency variations and associated these loci with biological processes related to responses to environmental variables, such as ultraviolet radiation (UV), tidal amplitude and drought, characterized by patterns of precipitation, temperature and VPD. Under contrasting latitudes, Cruz *et al.* (2019) also observed transcriptomic changes in the genes involved in responses to variations in temperature, solar radiation, precipitation and VPD. Additionally, using a

common garden experiment, individuals from contrasting latitudes diverged in ecophysiological traits involved in carbon and water balance of these plants, suggesting local adaptation to the observed traits (Cruz *et al.*, 2019).

Conversely, for *A. germinans*, genetic variation with evidence of selection was correlated with variations in atmospheric temperature patterns, precipitation regimes, solar radiation and tidal amplitude cycles (Table 2). These results corroborate the findings reported by Cruz *et al*. (2020), who performed a functional characterization of putative loci under selection and suggested that differential precipitation regimes play a fundamental role in genetic divergence between populations of this species. Additionally, our findings support the results reported by Bajay *et al.* (2018), who found different profiles of gene expression among populations of *Rhizophora mangle* located at contrasting latitudes of the Brazilian coast, with differentially expressed genes putatively involved in the responses to variations in temperature, solar radiation, precipitation and flood frequency. The results obtained for both species corroborate the data from other studies reporting that precipitation and air and sea temperature variables are limiting factors regulating the distribution of coastal species (McKee *et al.*, 2012; Soares *et al.*, 2012; Osland *et al.*, 2016; Duke *et al.*, 2017; Cavanaugh *et al.*, 2018; Ximenes *et al.*, 2018). Although we acknowledge the need for complementary studies that avoid molecular spandrels (Barrett & Hoekstra, 2011), our findings, together with the results published by Cruz *et al.* (2019, 2020), provide new evidence of the role of local adaptation in the distribution of the genetic diversity of these species.

Our results are particularly relevant in light of the climate changes that have been occurring in the last few decades. With them, further investigations on the responses of these species to future changes in predicted increases in average annual air and ocean temperatures and sea level rise (IPCC, 2014)(IPCC, 2014) can be directed. These results also have implications for conservation management and planning decisions (Friess *et al.*, 2019). With

a better understanding of the role of environmental variables in the modeling of genetic variation in *Avicennia* species on the South American coast, our results contribute to the definition of evolutionarily significant units (ESUs) for these species, thus maintaining their evolutionary potential (Fraser & Bernatchez, 2001). In addition, based on the results showing the remarkable pattern of genetic divergence of *A. schaueriana*, which results in part from the action of the current of the marine surface studied here, we suggest the consideration of genetic banks north and south from the SEC as conservation measures and the maintenance of biodiversity.

CONCLUSIONS

Our results provide novel evidence that the combined actions of the geographic distance, ocean currents and environmental gradient contribute to the evolution of spatial genetic divergence within two Neotropical *Avicennia* species growing on the Atlantic coast of South America. Our findings reveal the major role of ocean currents as barriers to gene flow in *A. schaueriana* and the environmental gradient as the main abiotic factor contributing to the genetic differentiation of *A. germinans*. For both species, we found new evidence of adaptation to climatic and oceanographic variables that have changed rapidly in recent decades. In this context, our results provide a basis for understanding the role of environmental variables in shaping genetic variation in *Avicennia* species of the South American coast, contributing to improving our understanding of the evolution of genetic diversity within this genus and providing information that may be relevant to other coastal organisms. Additionally, our findings will guide further research on the responses of these species to climate change and contribute to effective conservation plans for mangroves and other species with similar dispersion mechanisms.

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CONFLICT OF INTEREST

The authors have no competing interests to declare.

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SUPPORTING INFORMATION

Additional Supporting Information is available in the online version of this article on the publisher's web-site:

Figure S1. Groups (red boxes) of correlated environmental variables retrieved from the public data platforms WorldClim (Fick & Hijmans, 2017), Marspec (Sbrocco & Barber, 2013), and ECDS (Klein *et al.*, 2013). The cutoff value for Pearson's correlation coefficient was set to 0.8. *Environmental variables retained for subsequent analysis.

Table S1. Environmental data matrix for the ten sampling points of *A. schaueriana*. Sample codes are denoted as in Table 1.

Table S2. Environmental data matrix for the six sampling points of *A. germinans*. Sample codes are denoted as in Table 1.

Table S3. Interpopulation genetic differentiation based on the set of markers with evidence of natural selection estimated by F_{ST} (Wright) pairs for the *A. schaueriana* collection. Sample codes are denoted as in Table 1.

Table S4. Interpopulation genetic differentiation based on the set of markers with evidence of natural selection estimated by F_{ST} (Wright) pairs for the *A. germinans* collection. Sample codes are denoted as in Table 1.

Table S5. Pairwise geographic distances for *A. schaueriana*. Sample codes are denoted as in Table 1.

Table S6. Pairwise geographic distances for *A. germinans*. Sample codes are denoted as in Table 1.

Table S7. Pairwise environmental distances based on each environmental variable for *A. schaueriana*. Sample codes are denoted as in Table 1.

Table S8. Pairwise environmental distances based on each environmental variable for *A. germinans*. Sample codes are denoted as in Table 1.

Table S9. Pairwise environmental distances for *A. schaueriana*. Sample codes are denoted as in Table 1.

Table S10. Pairwise environmental distances for *A. germinans*. Sample codes are denoted as in Table 1.

SHARED DATA

A. germinans SNP genotype data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.pk0p2nghr. *A. schaueriana* SNP genotype data are available from the article: doi:10.1038/s41598-019-56469-w (supplementary datasets 1 and 2).

TABLES

Table 1. Sites at which the samples of *Avicennia schaueriana* and *A. germinans* were collected along the South American coast.

A. schaueriana	***A. germinans***	**Locality (City/State)**	**Latitude**	**Longitude**
	GM	Soure, Pará	-0.723	-48.490
	GB	Bragança, Pará	-0.904	-46.687
SB		Bragança, Pará	-0.820	-46.615
SA	GA	Alcântara, Maranhão	-2.410	-44.406
	GN	Parnaíba, Piauí	-2.778	-41.822
SR	GR	Paracuru, Ceará	-3.413	-39.056
	GT	Tamandaré, Pernambuco	-8.526	-35.013
SV		Vera Cruz, Bahia	-12.983	-38.684
SG		Guapimirim, Rio de Janeiro	-22.701	-43.007
SU		Ubatuba, São Paulo	-23.489	-45.164
SC		Cananéia, São Paulo	-25.020	-47.918
SP		Pontal do Paraná, Paraná	-25.575	-48.352
SF		Florianópolis, Santa Catarina	-27.576	-48.518
SL		Laguna, Santa Catarina	-28.445	-48.840

Sample populations of *A. schaueriana* and *A. germinans* are indicated by two capital letters, as described in Figure 1.

Table 2. Results of the MMRR between the genetic distances based on the set of nonneutral molecular markers and each environmental variable for *A. schaueriana* and *A. germinans*.

Environmental variables	***A. schaueriana***				***A. germinans***			
	β	*P*	R^2	*P*	β	*P*	R^2	*P*
Annual mean temperature (°C)	0.67	0.001	0.45	**0.0009**	0.88	0.02	0.81	**0.01**
Annual temperature range (°C)	0.60	0.005	0.36	**0.0004**	0.02	0.91	0.04	0.63
Isothermality (°C)	0.86	0.001	0.75	**0.0005**	0.72	0.06	0.50	0.09
Min temperature of the coldest month (°C)	0.69	0.001	0.49	**0.002**	0.86	0.08	0.75	0.07
Max temperature of the warmest month (°C)	0.05	0.63	0.003	0.63	0.88	0.02	0.80	**0.03**
Mean temperature of the coldest quarter (°C)	0.72	0.001	0.53	**0.002**	0.90	0.08	0.82	0.07
Precipitation seasonality (mm)	0.75	0.01	0.56	**0.0004**	0.65	0.05	0.42	**0.05**
Precipitation in the warmest quarter (mm)	0.56	0.006	0.31	**0.008**	0.95	0.05	0.91	**0.04**
Precipitation in the coldest quarter (mm)	0.62	0.01	0.39	**0.01**	-0.09	0.88	0.008	0.88
Mean solar radiation (kJ m^{-2} day^{-1})	0.66	0.001	0.44	**0.0005**	0.68	0.05	0.46	**0.04**
Min solar radiation (kJ m^{-2} day^{-1})	0.70	0.001	0.49	**0.0001**	-0.26	0.37	0.06	0.36
Max solar radiation (kJ m^{-2} day^{-1})	0.48	0.005	0.23	**0.006**	0.98	0.01	0.96	**0.01**
Wind speed mean (m s^{-1})	0.19	0.36	0.04	0.39	0.27	0.45	0.07	0.41
Max VPD (kPa)	0.46	0.02	0.22	**0.01**	0.45	0.22	0.20	0.24
Mean annual SSS (psu)	0.009	0.96	0.00008	0.96	-0.17	0.67	0.03	0.68
SSS in the saltiest month (psu)	0.12	0.47	0.01	0.48	-0.31	0.22	0.09	0.23
Annual variance in SSS (psu)	0.19	0.48	0.03	0.50	-0.22	0.39	0.05	0.39
Mean annual SST (°C)	0.68	0.01	0.46	**0.001**	-0.08	0.82	0.007	0.81
SST in the coldest month (°C)	0.64	0.002	0.42	**0.001**	0.08	0.78	0.006	0.76
Annual range of SST (°C)	0.59	0.003	0.36	**0.006**	0.88	0.13	0.78	0.15
Annual average cycle amplitude (cm)	0.79	0.002	0.62	**0.007**	0.35	0.007	0.12	**0.006**
Annual average duration of tidal cycles (h)	-0.32	0.1	0.1	0.12	-0.18	0.73	0.03	0.74
Annual number of cycles	-0.29	0.22	0.08	0.23	-0.16	0.84	0.02	0.82

The results are presented by the overall fit of the model (R^2), regression coefficient (β) and significance (*P*). Models with significant values are presented in bold. SST = sea surface temperature. SSS = sea surface salinity.

FIGURE LEGENDS

Figure 1. Geographic distribution and characterization of the *A. schaueriana* and *A. germinans* sampling sites along the South American coast. The blue area represents the geographical distribution of *A. schaueriana* and its sympatric region with *A. germinans* (blue and yellow). Black circles and triangles represent the sampling sites of the plant material used for the genotyping of genomic DNA from A. *schaueriana* and A. *germinans*, respectively (Cruz *et al.*, 2019, 2020). Sampling locations, which are indicated by two letters, are displayed as described in Table 1. Arrows represent the direction of the main ocean currents acting on the Brazilian coast. Arrow widths illustrate the mean current speed (Lumpkin & Johnson, 2013).

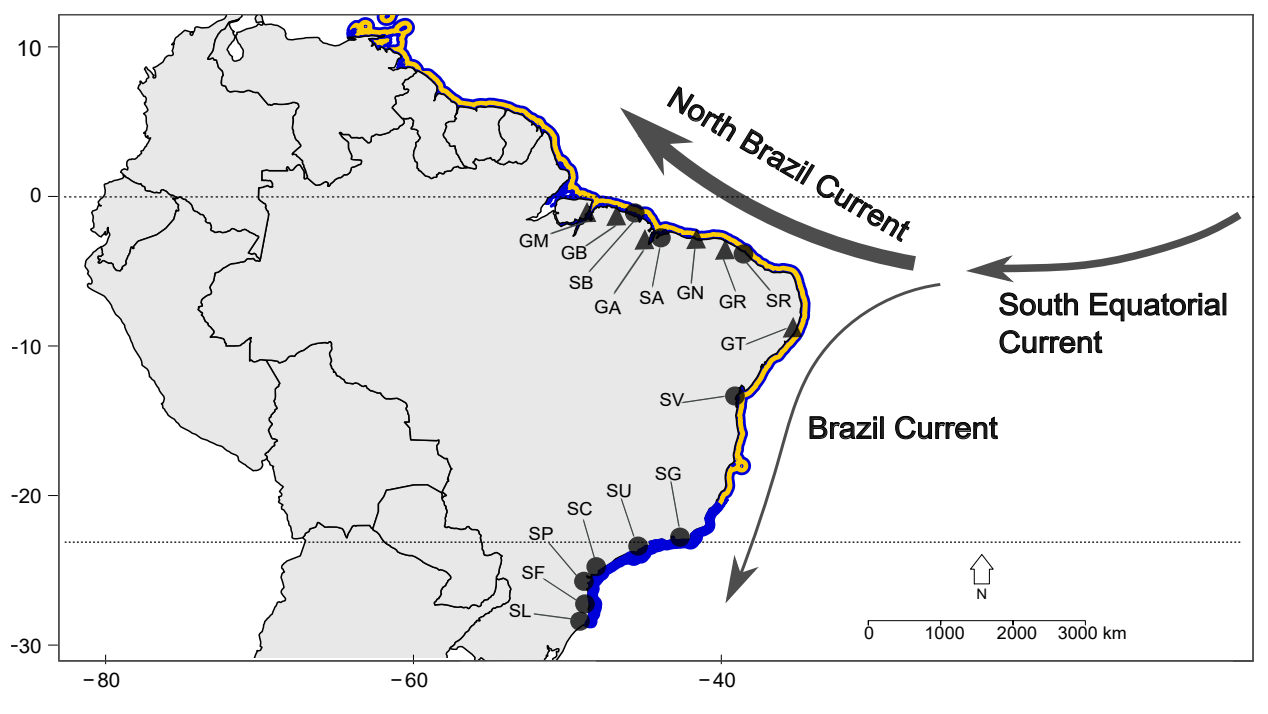
Figure 2. Heatmaps describing interpopulation genetic differentiation based on the total set of SNP molecular markers estimated by pairwise *F*ST (Wright) for (A) *A. schaueriana* (black circles) and (B) *A. germinans* (black triangles) collection sites. Sample codes are denoted as in Table 1.

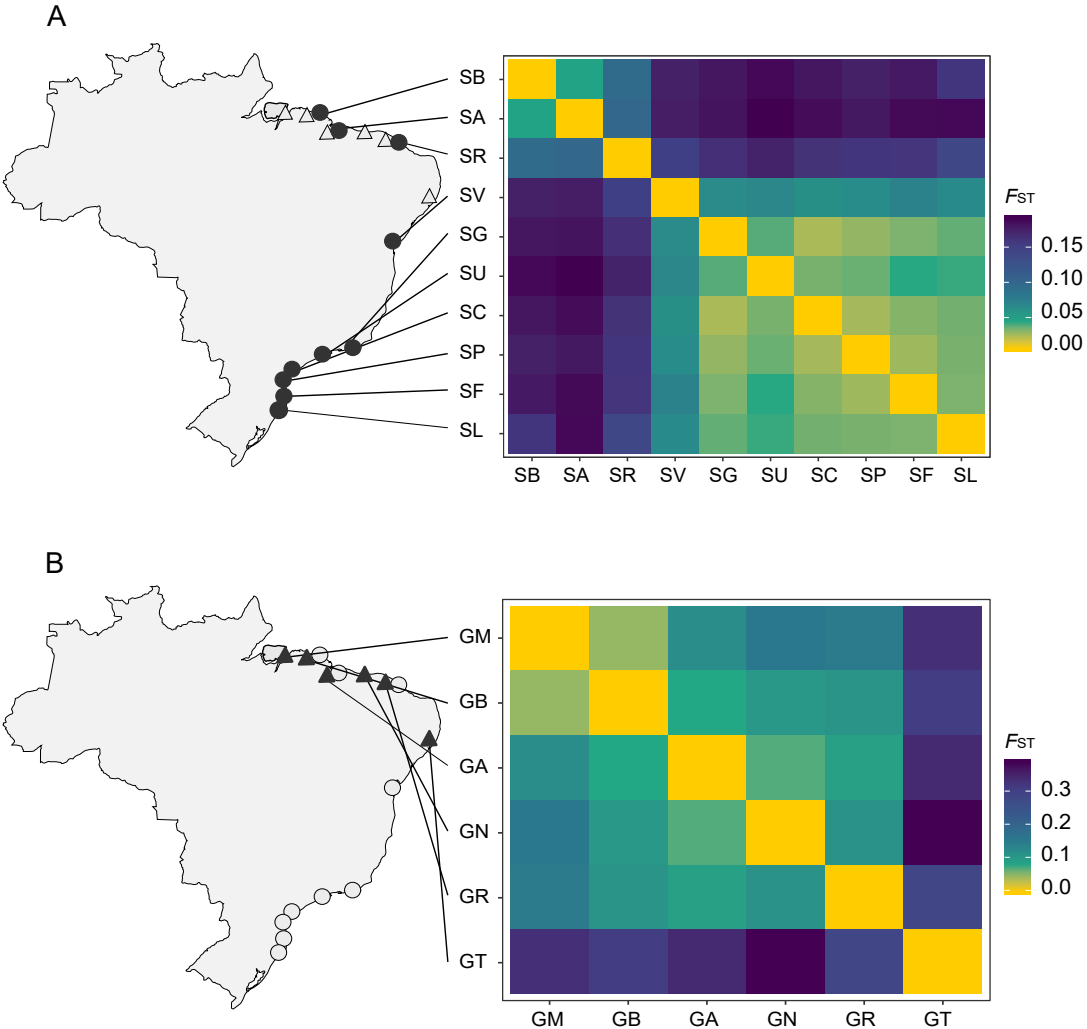
Figure 3. Locations of populations of (A) *A. schaueriana* and (B) *A. germinans* in a bidimensional projection of the PCA used to calculate the environmental distance. The acronyms for sampling locations, which are indicated by two letters, are the same as those listed in Table 1. The color gradient represents the contribution of each environmental variable to PC1 and PC2. V1: Annual mean temperature (°C). V2: Annual temperature range (°C). V3: Isothermality (°C).V4: Min temperature of the coldest month (°C). V5: Max temperature of the warmest month (°C). V6: Mean temperature of the coldest quarter (°C).V7: Precipitation seasonality (mm). V8: Precipitation in the warmest quarter (mm). V9: Precipitation in the coldest quarter (mm). V10: Mean solar radiation (kJ m^{-2} day^{-1}). V11: Min solar radiation (kJ m^{-2} day^{-1}). V12: Max solar radiation (kJ m^{-2} day^{-1}). V13: Wind speed mean (m s^{-1}). V14: Max VPD (kPa). V15: Mean annual SSS (psu). V16: SSS in the saltiest month

(psu). V17: Annual variance in SSS (psu). V18: Mean annual SST (°C). V19: SST in the coldest month (°C). V20: Annual range in SST (°C). V21: Annual average cycle amplitude (cm). V22: Annual average duration of tidal cycles (h). V23: Annual number of cycles. SST = sea surface temperature. SSS = sea surface salinity.

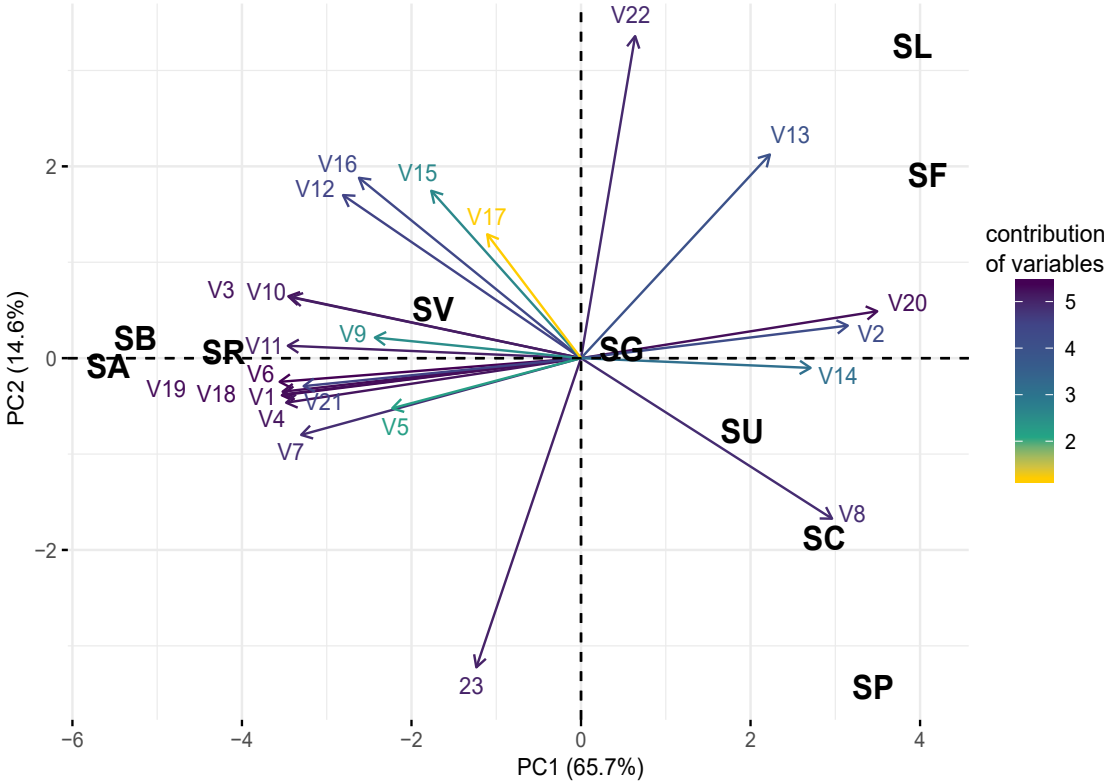
Figure 4. Plot of linear regression analyses showing the correlations among genetic, geographic, and environmental distances for *A. schaueriana*. Pairwise comparisons were performed between (A) geographic and genetic distances, (B) environmental and genetic distances, (C) geographic and environmental distances, and between (D) environmental and genetic distances based on the putative nonneutral SNPs. The R^2 and *P*-values are included.

Figure 5. Plot of linear regression analyses showing the correlations among genetic, geographic, and environmental distances for *A. germinans*. Pairwise comparisons were performed between (A) geographic and genetic distances, (B) environmental and genetic distances, (C) geographic and environmental distances, and between (D) environmental and genetic distances based on the putative nonneutral SNPs. The R^2 and *P*-values are included.

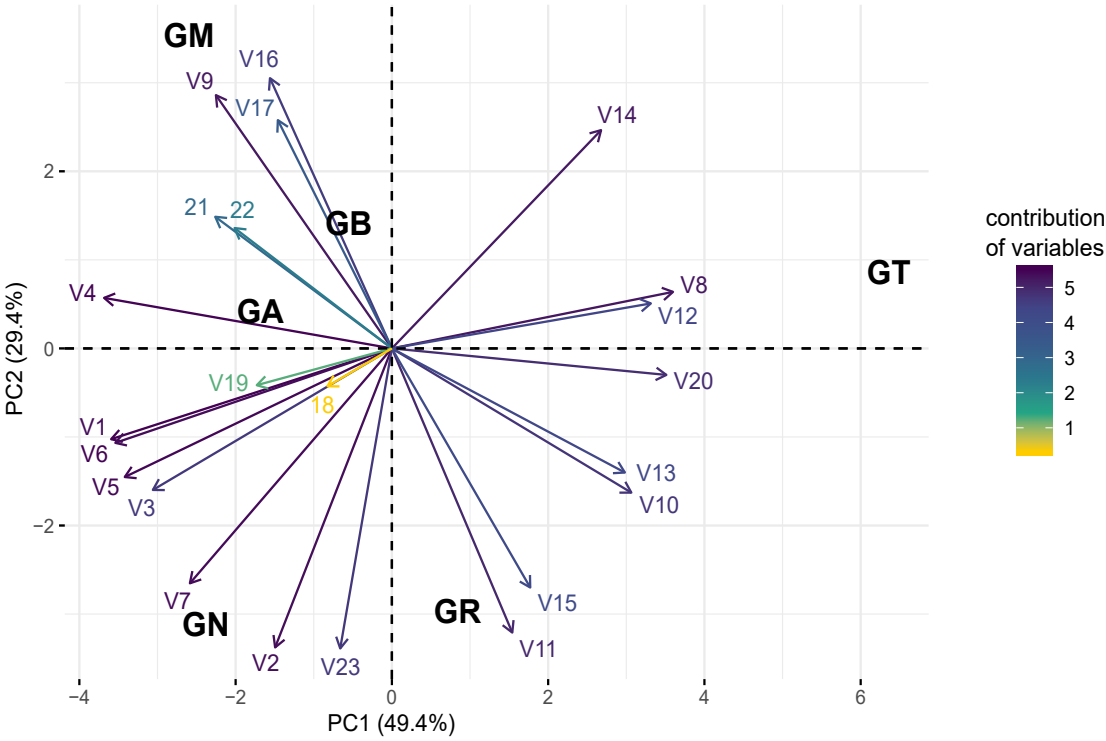

North Brazil Current
South Equatorial Current
Brazil Current
GM
GB
SB
GA
SA
GN
GR
SR
GT
SV
SG
SU
SC
SP
SF
SL
N
0
1000
2000
3000 km
10
0
-10
-20
-30
−80
−60
−40


A
SB
SA
SR
SV
SG
SU
SC
SP
SF
SL
FST
0.15
0.10
0.05
0.00
B
GM
GB
GA
GN
GR
GT
FST
0.3
0.2
0.1
0.0

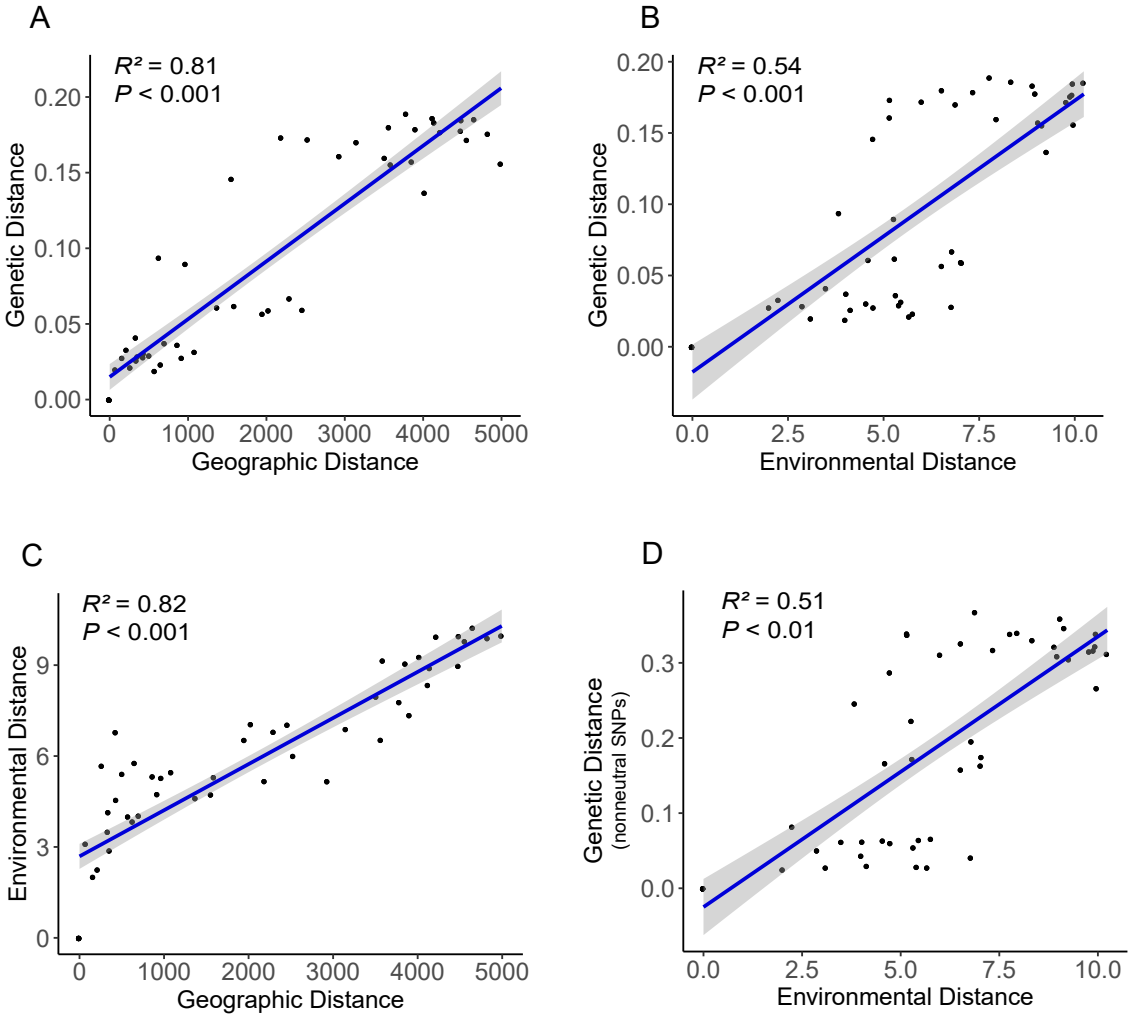
A


V22
SL
V13
SF
V16
V12
V15
V17
V3
V10
SV
V20
V2
SB
SR
V11
V9
SG
SA
V6
V14
V19
V18
V1
V21
V4
V5
SU
V7
V8
SC
23
SP
contribution of variables
5
4
3
2
PC2 (14.6%)
PC1 (65.7%)
−6
−4
−2
0
2
4

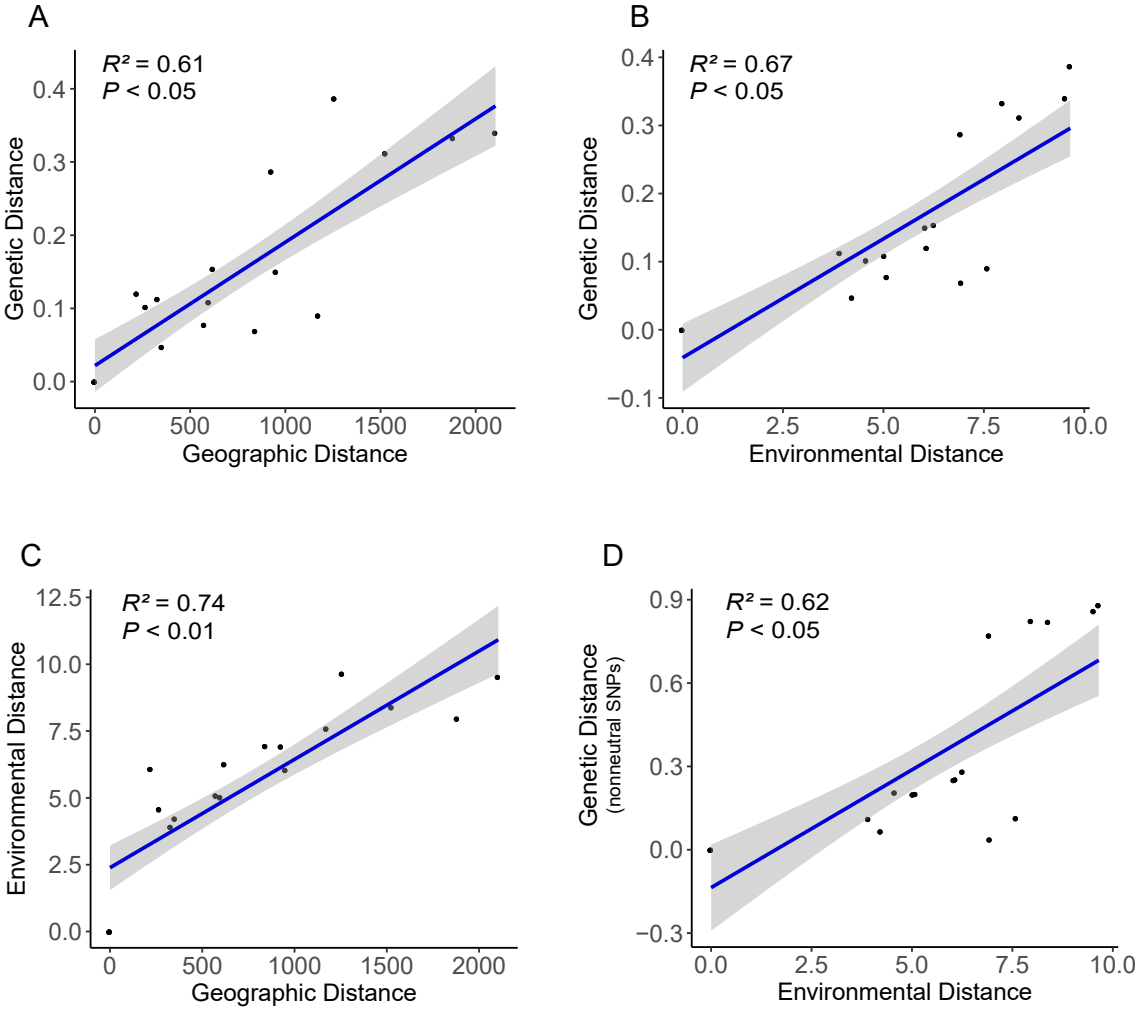
B


GM
V16
V9
V17
V14
21
22
GB
V4
GA
V8
GT
V12
V19
V20
18
V1
V6
V5
V3
V13
V10
V7
GN
V15
GR
V2
V23
V11
contribution of variables
5
4
3
2
1
PC2 (29.4%)
PC1 (49.4%)
−4
−2
0
2
4
6

A. schaueriana



A. germinans


A
$R^2 = 0.61$
$P < 0.05$
Genetic Distance
Geographic Distance
B
$R^2 = 0.67$
$P < 0.05$
Genetic Distance
Environmental Distance
C
$R^2 = 0.74$
$P < 0.01$
Environmental Distance
Geographic Distance
D
$R^2 = 0.62$
$P < 0.05$
Genetic Distance
(nonneutral SNPs)
Environmental Distance