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Assessing Genetic Diversity after Mangrove Restoration in Brazil: Why Is It So Important?

Renan Granado [1,2], Luiza C. Pinto Neta [1,3], André F. Nunes-Freitas [3,4], Carolina M. Voloch [2] and Catarina F. Lira [1,3,*]

1 Diretoria de Pesquisa, Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Laboratório de Biologia Molecular, Rua Pacheco Leão, 915, Rio de Janeiro RJ 22460-030, Brazil; granado.renan@gmail.com (R.G.); luizacallado.nt@gmail.com (L.C.P.N.)

2 Laboratório de Virologia Molecular Animal, Departamento de Genética, Universidade Federal do Rio de Janeiro, sala a2-121, UFRJ-Av. Carlos Chagas Filho, 373-Cidade Universitária, Rio de Janeiro RJ 21941-902, Brazil; carolvoloch@gmail.com

3 PPGPDS—Programa de Pós-Graduação em Práticas em Desenvolvimento Sustentável, Instituto de Florestas, Universidade Federal Rural do Rio de Janeiro, Rua Anfilófio de Carvalho, 29 salas 901/902 e 1003/1004, Rio de Janeiro RJ 20030-901, Brazil; afnfreitas@gmail.com

4 Laboratório de Ecologia Florestal e Biologia Vegetal (LEFBV), Departamento de Ciências Ambientais, Instituto de Florestas, Universidade Federal Rural do Rio de Janeiro, Rodovia BR-465 Km 7, Seropédica RJ 23897-055, Brazil

* Correspondence: catarina@jbrj.gov.br; Tel.: +55-21-3204-2135

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Abstract: Vital for many marine and terrestrial species, and several other environmental services, such as carbon sink areas, the mangrove ecosystem is highly threatened due to the proximity of large urban centers and climate change. The forced fragmentation of this ecosystem affects the genetic diversity distribution among natural populations. Moreover, while restoration efforts have increased, few studies have analyzed how recently-planted areas impact the original mangrove genetic diversity. We analyzed the genetic diversity of two mangroves species (*Laguncularia racemosa* and *Avicennia schaueriana*) in three areas in Brazil, using inter-simple sequence repeat (ISSR) markers. Using the local approach, we identified the genetic diversity pool of a restored area compared to nearby areas, including the remnant plants inside the restored area, one well-conserved population at the shore of Guanabara Bay, and one impacted population in Araçá Bay. The results for *L. racemosa* showed that the introduced population has lost genetic diversity by drift, but remnant plants with high genetic diversity or incoming propagules could help improve overall genetic diversity. *Avicennia schaueriana* showed similar genetic diversity, indicating an efficient gene flow. The principal component analysis showing different connections between both species indicate differences in gene flow and dispersal efficiencies, highlighting the needed for further studies. Our results emphasize that genetic diversity knowledge and monitoring associated with restoration actions can help avoid bottlenecks and other pitfalls, especially for the mangrove ecosystem.

Keywords: ISSR; mangroves; restoration; genetic diversity; conservation

1. Introduction

The mangrove forests are widely distributed in the tropical and subtropical areas of the world, where they occupy muddy intertidal environments [1]. They are also one of the most important ecosystems globally, and serve as nurseries for many marine and terrestrial species [2]. However, they have been identified as one of the critical systems that would be affected by global change [3], and could even disappear within the next 100 years [4]. The mangrove ecosystem, including the flora

and fauna, is an important source and sink for sediments, organic matter and nutrients [1]. It is among the most carbon-rich biomes, being important in the atmospheric carbon sequestration/capture [5,6].

Mangrove soil is strictly related to organic decomposition, making this system crucial to primary production in coastal zones and erosion control [7]. Due to the type of soil—which is flooded, salty, and poorly oxygenated—and the brackish water as a transitional ecosystem between freshwater and saltwater, few plant species have adapted to survive in it [8,9]. True mangrove species, from a physiological perspective, are facultative halophytes [10]. Up to 70 true mangrove species worldwide belong to 17 families, of which 11 species qualified for a Red List threatened category, according to different classifications [11,12].

Nevertheless, anthropic activities have dramatically reduced the mangrove areas globally, due to excessive pressure from industrial development, urban growth on the coasts, use of natural resources, and even for the eviction of garbage and sewer in water bodies [13–15]. The primary threat to mangroves is habitat destruction, given the conversion to aquaculture, agriculture, urban and coastal development, and overexploitation [11], reducing and fragmenting the mangrove areas and increasing the isolation of the remnant fragments [16–19]. Other threats are based on climatic changes, such as sea-level rise, high water events, and change in weather conditions, such as storm frequency and intensity, precipitation, temperature, and atmospheric CO_2 concentration [20].

One of the problems with fragmentation is the isolation of the populations, causing a reduction of the gene flow and increasing endogamy, which in turn causes the decrease of genetic variation and may have significant long-term evolutionary consequences [19,21]. In short, genetic variation is an important aspect to analyze within and between populations, mainly whether the populations are constantly affected by natural stressors—such as floods, storms, and cyclones—but also by anthropogenic stressors, such as oil spills and industrial chemical contaminations, like mangroves. Genetic diversity is related to the species persistence, or avoiding extinction, since the population with higher genetic variability will likely adapt and survive [22,23]. However, because the application of genetics in the management of threatened species in the wild is in its infancy, stakeholders and decision-makers fail to consider genetic issues in wild management [24].

Many studies have evaluated genetic diversity within mangrove populations with different molecular markers, successfully leading the discussion about mangrove gene flow in a broader sense [25]. However, local approaches are important for assessing the genetic diversity of a specific area and therefore, for improving conservation planning, especially when dealing with restoration efforts where genetic pollution can occur [26].

The molecular marker inter-simple sequence repeat (ISSR) can be employed for studying genetic variation within and among populations, because of its high levels of polymorphism [27–29]. Many studies have shown genetic and morphological variation in mangroves using other molecular markers, such as microsatellite (SSR) [30,31], allozymes [32], and random amplified polymorphic DNA (RAPD) [33]. However, ISSR has also been shown to be useful in evaluating genetic diversity in many plant species, including mangrove species [33–39].

In this study, ISSR markers were used to elucidate the genetic diversity of *Laguncularia racemosa* (L.) C.F. Gaertn and *Avicennia schaueriana* Stapf and Leechm. ex Moldenke within and among three areas of mangroves in Brazil. The Guanabara Bay has one of the biggest mangrove areas in Rio de Janeiro State, and is very emblematic because of its history and beauty, although environmental degradation and water pollution have increased in recent years, affecting many protected areas around the bay. The main objective of this work was to monitor the genetic diversity pool of Guanabara Bay mangrove populations, considering an area being restored. We assessed the genetic diversity of the oldest planted trees, adult trees aged 7 to 10 years old, that survived the first restoration efforts in the area of Mauá Beach. For comparison, we included individuals originally from Mauá Beach (remnant plants), from a well-conserved and protected area on the shore of Guanabara Bay, and from a third area outside Guanabara Bay. The planted individuals of the species *L. racemosa* suffered genetic diversity loss, possibly by genetic drift, but remnant plants had the highest diversity levels.

On the other hand, *Avicennia schaueriana* had similar genetic diversity levels within all investigated populations, showing more efficient gene flow than *L. racemosa*, and no visible genetic loss within populations. Although Mauá Beach site was successfully restored, the genetic diversity of the plants was initially lacking. Now this work will subsidize the genetic monitoring and management of this area, improving the chances of long-term survival of the restored mangrove.

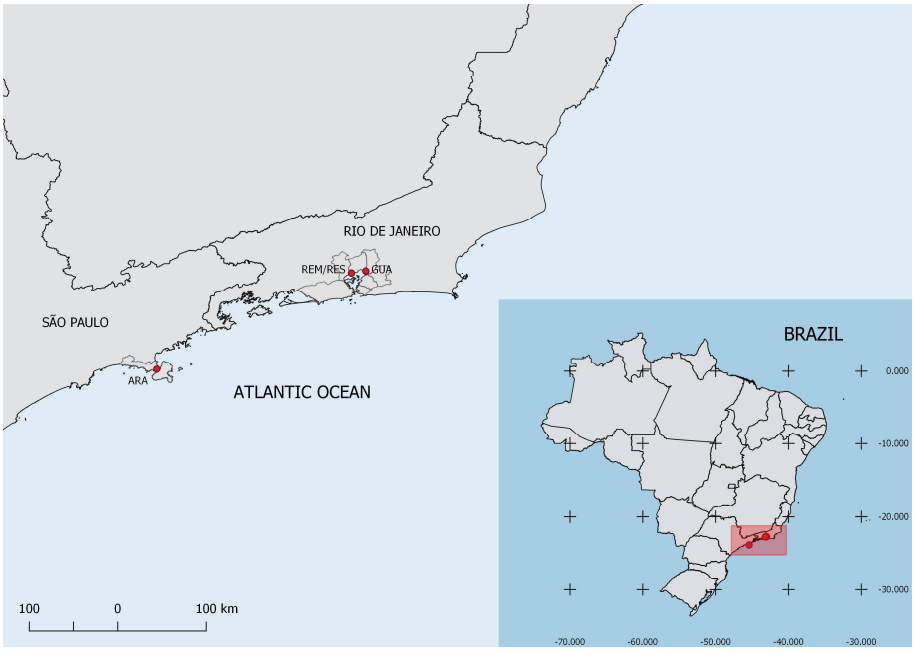
2. Materials and Methods

2.1. Studied Areas and Plant Material

The studied areas were two areas around Guanabara Bay, in the state of Rio de Janeiro (RJ), and one area in the State of São Paulo (SP). One of the areas in RJ was Mauá Beach, a previously highly-degraded mangrove area with few remnant adult plants. It was restored into a 12-ha mangrove forest through a 10-year initiative called the Mangue Vivo project by the OndAzul Institute, which is still in progress. The restoration started with the transplantation of plants from a nearby area, known as Remanso, which has no further information or studies. The Remanso is located a few hundred meters away from the restored area; it is a very small and modified mangrove area in the region of Mauá Beach.

The other area was Guanabara Ecological Station (ESEC Guanabara) inside Guapimirim Protected Area (APA Guapimirim), a well-conserved mangrove area on the other side of Guanabara Bay in RJ. The third area was at the Araçá Bay in São Sebastião (SP), which is a very degraded and low-numbered population but has high genetic diversity levels within its remnant mangrove plants, making it vital to conserve [40].

For practical purposes, we considered our dataset as four distinct populations: two in Mauá Beach, called Remnant (REM), which represents the remnant autochthonous plants originally from that area, and Restored (RES), which represents the allochthonous plants used in the beginning of the restoration; sampled individuals from inside ESEC Guanabara (GUA); and individuals from the Araçá Bay (ARA) (Figure 1).



(**a**)

Figure 1. *Cont.*

(**b**)

(**c**)

Figure 1. (**a**) Location of the studied areas in the states of Rio de Janeiro (RJ) and São Paulo (SP) within Brazil; (**b**) map showing Araçá Bay area. The red circle represents the collection site ARA; (**c**) map showing Guanabara Bay in detail. The location of the restored site in Mauá Beach (Restored (RES) and Remnant (REM)) is represented by a red circle on the left. The conserved mangrove in the Ecological Station (ESEC) Guanabara (GUA) is represented by a red circle on the right.

This work studied two of the three mangrove species found in these areas: *Laguncularia racemosa* and *Avicennia schaueriana*. The leaf material was collected and immediately stored in silica gel. We collected up to 30 plants of each population (REM, RES, GUA, and as many as possible in ARA) and of each species for DNA extraction. The population RES was represented by plants previously planted at the site at least 7 to 10 years ago, based on their trunk size, height, and personal communication of the person had been doing the plantings since the beginning of the project in 2001. For this study, we did not collect samples from any plants recently planted or naturally recruited within the area.

2.2. DNA Isolation and PCR Amplification

The DNA extraction followed the protocol of Lira-Medeiros et al. [41]. After the extraction, the samples were checked and quantified using 1% agarose gel and Nanodrop^{TM} 2000 (Thermo Fisher Scientific, Waltham, MA, USA), then diluted into 12.5 ng/µL. The PCR amplification reactions were performed in 20 µL final volume with 2 µL buffer 1X (KCl 500 mM, Tris-HCl 100 mM, pH 8.5), 3.2 µL of $MgCl_2$ (25 mM), 0.4 uL of dNTP (10 mM), 2 µL of primer (10 uM), 0.2 µL of *Taq* polymerase (5 U/µL; Promega), 0.4 µL of formamide, 0.02 µL of Triton X-100, 12.8 µL of milliQ autoclaved water and 2 µL of DNA, following Ge et al. [34].

Of the 15 primers from the University of British Columbia dataset that were tested, seven produced clear and reproducible fragments for *L. racemosa* (808, 809, 811, 834, 840, 841, 842) and eight for *A. schaueriana* (808, 809, 810, 811, 834, 835, 840, 842), as shown in Table 1. The PCR amplifications were carried out in a Veriti thermal cycler (Applied Biosystems, Waltham, MA, USA) with an initial denaturation of 95 °C for 5 min, followed by 40 cycles of 2 min at 95 °C, specific annealing temperature (Table 1) for 1 min, 2 min at 72 °C, and a final 7-min extension at 72 °C. The PCR products (samples) were separated by gel electrophoresis on 1.2% agarose gels for *L. racemosa*, and 1.8% for *A. schaueriana*, in 0.5X TBE buffer and visualized using UV light. We used 4 µL of sample, with 2 µL of GelRed®(Biotium, Fremont, CA, USA) and 1 µL of carrying buffer (30% glycerol with xylene cyanol and bromophenol blue). Also, a 100 bp ladder (Ludwig Biotec, Alvorada, RS, Brazil) was used to estimate the fragment sizes, and the gels were run in 135 V for 25 min for *L. racemosa*, and in 100 V for 1 h for *A. schaueriana*.

Table 1. Inter-simple sequence repeat (ISSR) primers used for the mangrove populations in the PCR reactions, with their sequences and their annealing temperature (T_A) for each specie, *Laguncularia racemosa* and *Avicennia schaueriana*. No annealing temperature means that primer did not amplify for that species.

Primer	Primer Sequence	T_A for *L. racemosa*	T_A for *A. schaueriana*
808	5′ [AG]8C 3′	46 °C	46 °C
809	5′ [AG]8G 3′	46 °C	50 °C
810	5′ [GA]8T 3′	-	52 °C
811	5′ [GA]8C 3′	48 °C	50 °C
834	5′ [AG]8YT 3′	46 °C	52 °C
835	5′ [AG]8YC 3′	-	48 °C
840	5′ [GA]8YT 3′	48 °C	52 °C
841	5′[GA]8YC 3′	46 °C	-
842	5′ [GA]8YG 3′	48 °C	54 °C

2.3. Data Analysis

The fragments were recorded in a binary matrix as present (1) or absent (0) for each DNA sample (individual). The dataset was cleaned by excluding loci with high amounts of missing data. The binary matrix created was used in Hickory v-1.1 [42], which is a Bayesian method that calculates deviation of the Hardy–Weinberg equilibrium by the Markov chain Monte Carlo (MCMC), so it does not calculate using allele frequency (details in [41]). The Bayesian differentiation index, θ_{ST}, was calculated with the *f*-free model: 250,000 runs and 50,000 burn-ins in the Hickory software. We selected this program because it does not assume the Hardy–Weinberg equilibrium. When assuming it, the results might not be trustworthy, since the Hardy–Weinberg equilibrium considers infinite populations and completely random mating. The Hickory program generated the following genetic diversity indexes: the percentage of polymorphic loci (P), the genetic differentiation index (θ_{ST}), the total genetic diversity of the specie (H_T), the genetic diversity within a population or population heterozygosity (H_S), and the inbreeding coefficient (f).

The data was also analyzed through a principal component analysis (PCA) using the Ade4 package [43], and graphics were generated by Factoextra and ggplot2 packages in R software [44]. The between-class principal component analysis, followed by Monte-Carlo test based on 999 replicates, was used to calculated B_{ST}, a differentiation index between populations based on multivariate analysis and its significance.

3. Results

3.1. Genetic Diversity of Laguncularia racemosa

Using seven ISSR primers to amplify *L. racemosa* DNA samples, we obtained 41 loci, of which only 27 were polymorphic, leading to a polymorphic percentage (P) of 65.85%. The studied populations of *L. racemosa* showed low overall polymorphism, and amplified fewer fragments per primer than *A. schaueriana*.

The H_S varied from 0.108 (SD = 0.020) of the allochthonous individuals to 0.239 (SD = 0.017) of the autochthonous individuals from the Mauá Beach area (Table 2), indicating a very low genetic diversity of the introduced plants inside an area with originally greater genetic diversity. The H_T of *L. racemosa* was 0.271 (SD = 0.013), showing that the studied areas have low representativity of the genetic diversity of this plant.

Table 2. Within-population genetic diversity index (H_S) calculated for the four studied populations of *Laguncularia racemosa* and the mean value. RES—restored plants in Mauá Beach; REM—remnant plants in Mauá Beach; GUA—ESEC Guanabara; ARA—Araçá Bay; SD—standard deviation.

Populations	H_S (SD)
RES	0.108 (0.020)
REM	0.239 (0.017)
GUA	0.185 (0.021)
ARA	0.157 (0.018)
Mean	0.173

The Bayesian differentiation index θ_{ST} was 0.365 (SD = 0.032), and multivariate differentiation index B_{ST} was 0.315 ($p < 0.001$), showing high genetic differentiation between the studied populations, considering that they are very close geographically. The likely genetic isolation of these populations is also corroborated by high inbreeding value (f) of 0.302 (SD = 0.224), although it is not completely accurate for ISSR markers.

The PCA showed the spatial association of the genetic diversity of the species *L. racemosa* in the four studied populations (Figure 2). The individuals of the RES plants did not overlap with individuals from other studied areas. Individuals of ARA were also slightly isolated, although the confidence interval showed some overlap with GUA and REM. The REM plants showed the most diverse pattern on the PCA.

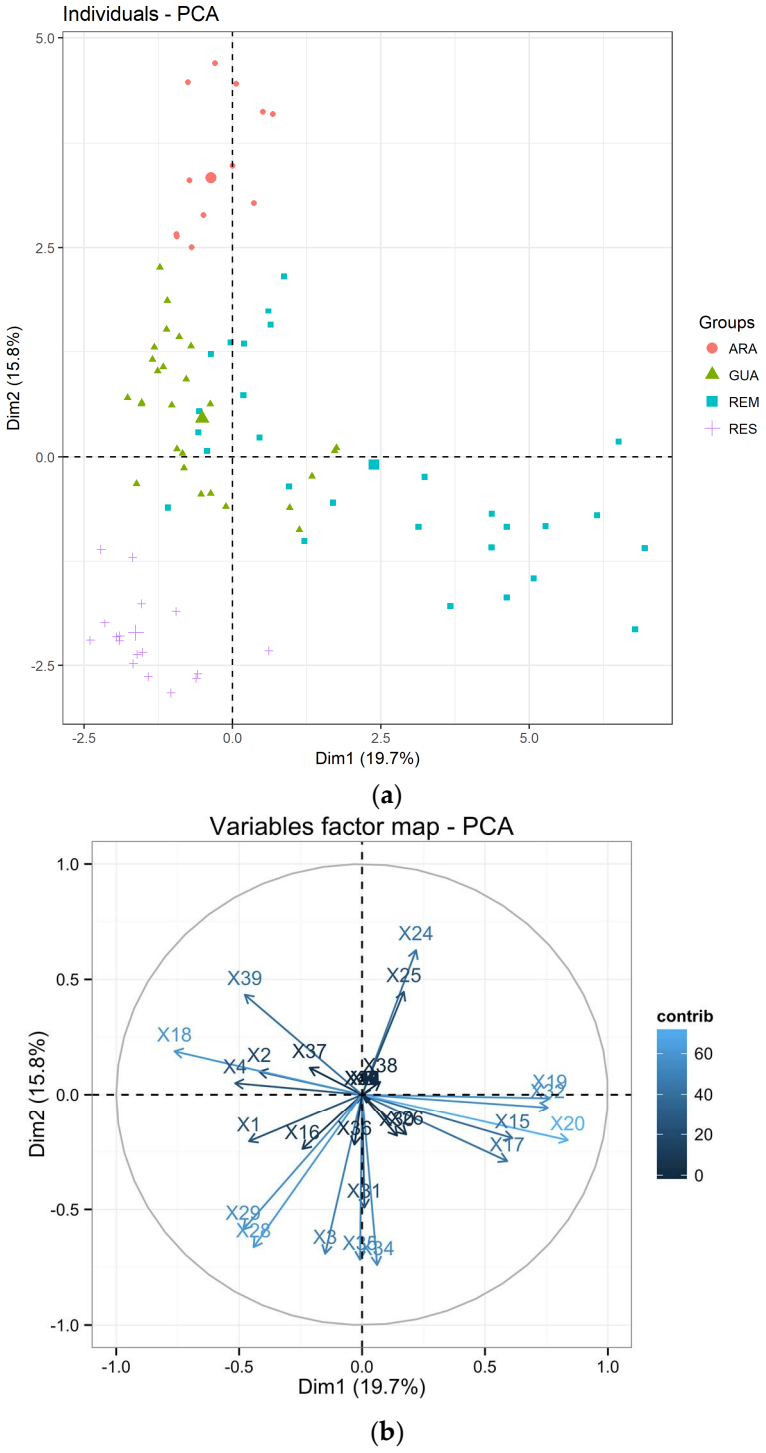


Figure 2. (**a**) PCA of *Laguncularia racemosa* individuals: RES—plants from restored area in Mauá Beach; REM—remnant plants in Mauá Beach; GUA—ESEC Guanabara; ARA—Araçá Bay. (**b**) Graphic showing the contribution of each ISSR loci in the PCA of *L. racemosa* individuals. The legend shows a gradient color map from blue (no contribution) to red (high contribution).

3.2. Genetic Diversity of *Avicennia schaueriana*

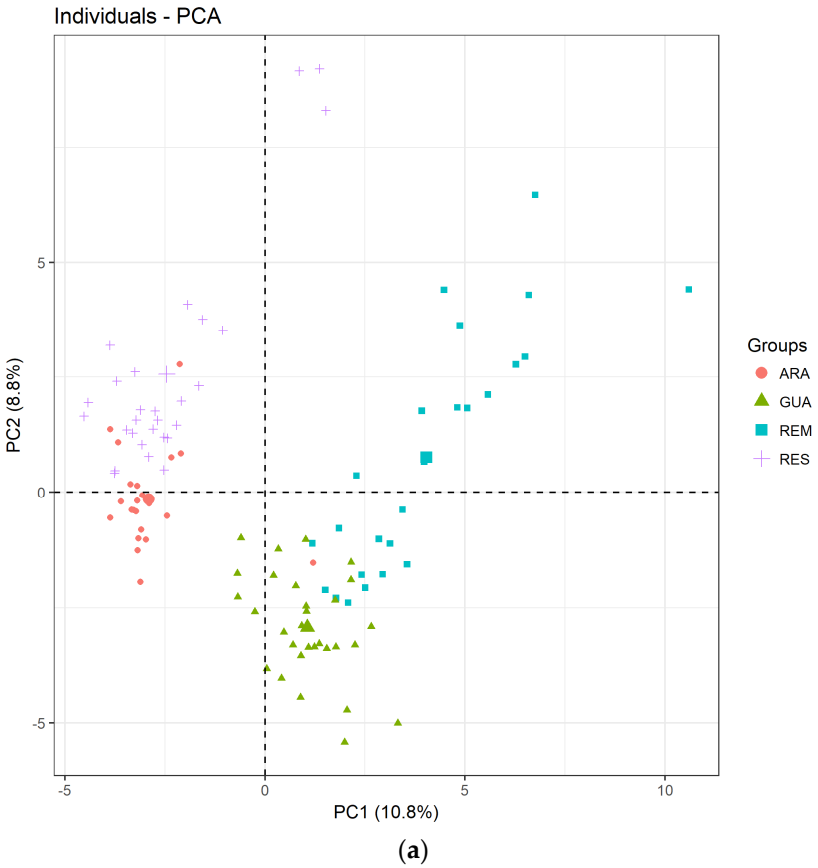
Using eight ISSR primers to amplify *A. schaueriana* DNA samples, we obtained 102 loci, of which 90 were polymorphic (p = 88.23%), a high rate of polymorphism. The genetic diversity index H_S was similar in all four populations with mean value of 0.246 (Table 3). This result indicates that

A. schaueriana has been able to maintain a fairly high genetic diversity despite facing habitat destruction and fragmentation in the studied region of SP and RJ State. This was also corroborated by the H_T of 0.308 (SD = 0.010).

Table 3. Within-population genetic diversity index H_S calculated for the four studied populations of *Avicennia schaueriana* and its mean value. RES—restored plants in Mauá Beach; REM—remnant plants in Mauá Beach; GUA—ESEC Guanabara; ARA—Araçá Bay; and SD—standard deviation.

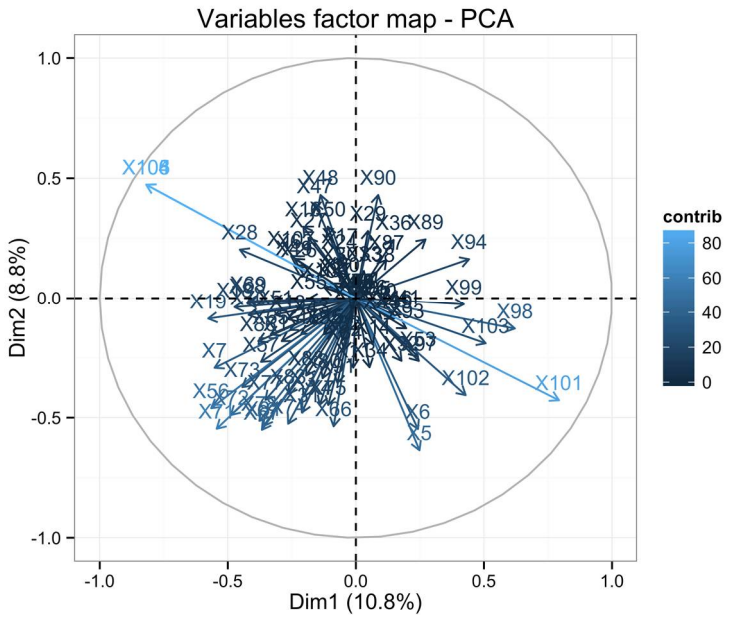
Populations	H_S (SD)
RES	0.242 (0.015)
REM	0.261 (0.012)
GUA	0.238 (0.012)
ARA	0.243 (0.014)
Mean	0.246

The θ_{ST} was 0.203 (SD = 0.013), the B_{ST} was 0.178 ($p < 0.001$), and inbreeding (f) was 0.152 (SD = 0.083). The PCA with *A. schaueriana* data showed little divergence of the genetic diversity between the studied populations (Figure 3). The RES and REM individuals were more dispersed on the PCA and did not overlap. The ARA individuals overlapped with RES, and GUA individuals overlapped with REM.



(a)

Figure 3. *Cont.*



(**b**)

Figure 3. (**a**) PCA of *Avicennia schaueriana* individuals. RES—plants from restored area in Mauá Beach: REM—remnant plants in Mauá Beach; GUA—ESEC Guanabara; ARA—Araçá Bay. (**b**) Graphic showing the contribution of each ISSR loci in the PCA of *A. schaueriana* individuals. The legend shows a gradient color map from blue (no contribution) to red (high contribution).

4. Discussion

4.1. Mangrove Restoration Practices Caused Bottleneck in Introduced Laguncularia racemosa

The results showed overall low genetic diversity of *L. racemosa* in the studied populations (mean H_S = 0.173). A previous work showed an even lower H_T of 0.219 and mean H_S of 0.157 for *L. racemosa* in Rio de Janeiro State, analyzing seven other populations with ISSR markers [45]. The expected low genetic diversity of mangroves in the southern and southeastern regions of Brazil is explained by younger colonization, with a founder effect due to the dispersal of few propagules from older and more diverse regions [46]. In addition, *Lumnitzera racemosa*, a mangrove species from the same taxonomic family of *Laguncularia racemosa,* had very low mean within-population genetic diversity (0.097) and similar H_T (0.260) [47], showing a similar pattern that could be associated with shared taxonomic characteristics in both genera.

Although the H_S index is expectedly low, we observed differences in the H_S of each analyzed population. The genetic diversity was the lowest (H_S = 0.108) among all studied areas, suggesting that the genetic pool of the introduced plants in Mauá Beach has lost genetic diversity through genetic drift during the restoration process. The first possible cause of the genetic drift in this area is the low survival rate of the first introduced *L. racemosa* plants in the area (Silva A., personal communication). Poor translocation methods added to bad environmental conditions can cause poor survival rates in the beginning of the restoration efforts [48]. The other possible cause is the use of plants with no genetic background, which can lead to low genetic diversity, due to genetic drift or high genetic load of source population [49].

On the other side, the autochthonous plants in Mauá Beach had the highest genetic diversity index (0.239). Given this, the genetic diversity within this area could still improve in future generations. However, the restricted gene flow between populations, suggested by the high genetic differentiation and inbreeding indexes found in this study, could complicate the admixture of these populations, especially due to population size and fragmentation [50], such as in the southeast region of Brazil.

We suggest that genetic monitoring is necessary to evaluate the evolution of the genetic diversity of *L. racemosa* in the next generations.

The Araçá Bay population of *L. racemosa* also has very low genetic diversity levels, and requires urgent management to avoid the effects of genetic drift and to successfully conserve the remnant genetic diversity of this area. Araçá Bay is highly threatened, its population significantly reduced by habitat destruction and human pressure; it should be urgently preserved and restored considering its high genetic diversity [40].

This study employed a local approach, using a few populations geographically close to each other, and showed a highly structured genetic diversity for *L. racemosa*. The differentiation index was higher than a previous study of *L. racemosa* in Rio de Janeiro State that found a G_{ST} of 0.285 [45]. The G_{ST} was also high for *Lumnitzera racemosa*, a sister species of the Indo-Pacific mangroves assessed with the same markers, in the South China Sea (0.337) and in the East Indian Ocean (0.402) [47]; however, our work focused on a more limited area, which is very alarming for the conservation of *Laguncularia racemosa* in the southeastern region of Brazil.

The PCA of *L. racemosa* explains 35.5% of the variation found in the sampled populations. The genetic structure of the populations grouped GUA and REM as more similar, and ARA and RES as more distinct and with lower within-population diversity. This corroborates our theory that genetic drift has caused a loss of genetic diversity, not only in RES plants but also in ARA plants. However, it is also possible that the genetic diversity of the original RES plants were already eroded. On the other hand, GUA and REM showed some genetic similarity within the genetic structure of *L. racemosa*, which can be explained by the long-term exchange of propagules by the Guanabara Bay tide or pollen, based on their geographical proximity.

4.2. Avicennia schaueriana Has Similar Within-Population Genetic Diversity in Fragmented, Impacted, and Restored Areas

Avicennia schaueriana showed similar within-population genetic diversity for all studied populations. Other work with the same markers showed higher diversity in seven populations of *A. schaueriana* in the state of Rio de Janeiro (H_T = 0.413 and mean H_S = 0.331) [45]. However, a study using ITS markers had similar or even lower values of genetic diversity than our results in other populations of *A. schaueriana* in the São Paulo and Rio de Janeiro States [51]. Thus, we believe that ISSR markers provide a good and low-cost molecular tool for evaluating the genetic diversity of *A. schaueriana* populations in Brazil.

The genetic differentiation index was much lower for *A. schaueriana* than *L. racemosa*, although still significant, showing a structure in the genetic diversity of this species. Previous works showed similar differentiation index values for *A. schaueriana* in Rio de Janeiro State (G_{ST} = 0.2) [45]. Since the species has maintained similar diversity in all studied populations until now, we believe that gene flow is effective, even with relatively high differentiation between populations. However, it is important to emphasize that the inbreeding rate, coupled with the differentiation index, could lead to a much worse scenario for genetic diversity in the future generations of this species. Management and improvement of connectivity between the mangrove areas are needed to avoid further habitat destruction.

The allochthonous plants of *A. schaueriana* in Mauá Beach do not show effects of genetic drift. These plants were last introduced when environmental conditions were not as unfavorable as during the very beginning of the restoration (Silva A., personal communication). Although the overall diversity of all studied sites was similar, and gene flow apparently still effective, future monitoring is necessary to avoid any genetic diversity loss. *Avicennia* species generally has an effective gene flow, maintaining the connectivity between mangroves, even when fragmented and degraded, since *A. germinans* also has a low differentiation index [52].

The PCA of *A. schaueriana* had a lower percentage of variation explained (19.6%), possibly due to higher genetic diversity within the studied sites and the closeness of the sites. The fact that ARA and RES were more similar between them requires further investigation with other sites. However,

it is clear that the similarity between GUA and REM, such as the one found in *L. racemosa* samples, exists because of the proximity of the Guanabara Bay, which facilitates the propagule dispersal by the tides. The distribution of REM samples in the bPCA showed how the autochthonous plants within Mauá Beach are important for the genetic diversity of the species. Thus, conservation efforts with monitoring and management are highly needed, to avoid losing the genetic diversity in this area.

4.3. Pitfalls of Mangrove Restoration

Different species occurring in the mangrove ecosystem have been studied around the world. The genetic diversity of mangrove species found in the American and African coasts—*Rhizophora mangle*, *Laguncularia racemosa*, and several *Avicennia* species—were studied mainly in the United States and Central America [32,52–54], but not as much in South America [47,54]. Brazil has the fourth-largest area of mangroves, distributed all along the coast, but it has little estimation of mangrove forest loss since 1980 [11]. Further, little scientific literature exists on the genetic diversity knowledge of mangrove species in Brazil, although such studies are urgently needed to subsidize mangrove conservation and future or in-progress mangrove restoration efforts.

In general, successful mangrove restoration and rehabilitation efforts were observed in the last decades in the Northern Hemisphere [55]. Empirical data of the genetic diversity of a species is important for the conservation and restoration of any ecosystem. The higher the genetic diversity of planted individuals, the longer they live and with higher fitness [48]. However, this work is one of the few research studies that compared the genetic diversity of mangrove plant species in both natural and restored areas.

The Mauá Beach area is an example of successful restoration, but with no prior genetic diversity study, so further management might be necessary. A mangrove area can fully recover by secondary succession if, after mangrove plantation, there is an availability of propagules and proper hydrology conditions [56]. The lack of genetic diversity awareness in the restoration process could bring negative effects, such as founder effects, genetic swamping, heterosis, and outbreeding depression [57].

In Mauá Beach, the lack of prior genetic diversity knowledge resulted in different diversity levels of the restored individuals compared to local remnant individuals for *L. racemosa*. The restored plants showed half the genetic diversity of the local remnant plants, but there is a natural re-colonization observed by the presence of seedlings and the continuation of the restoration project mainly using local propagules. Thus, new individuals in this area might contribute to improve the genetic diversity of the restored area by successful gene flow and dispersal from other populations, such as the autochthonous plants or plants from GUA. Further studies are necessary to monitor the genetic diversity of this area for a longer period of time, in order to determine whether genetic restoration is needed or whether the genetic diversity of the restored population can occur naturally.

On the other hand, *A. schaueriana* individuals of the restored area showed similar levels of genetic diversity compared to the autochthonous plants, with no need for future management in the restoration site for this species. Thus, even after full mangrove rehabilitation, monitoring and management might be needed for species with genetic diversity loss caused by restoration, in order to improve the long-term resilience of the restored area.

5. Conclusions

Genetic diversity studies are important to measure the success of environmental restoration, not only during or after restoration, but also before, in order to identify new and better strategies for genetic enrichment and avoid unwanted effects due to genetic drift, such as bottlenecks and the founder effect. Our results indicate the importance of conserving fragmented mangrove populations, since they may provide an important pool of genetic diversity to maintain the species in the long term, especially populations like ARA, where diversity can be a source to restore other fragmented populations. However, more in-depth studies are needed to understand the correlation between pioneer species and genetic bottlenecks. Further studies are also needed to monitor the genetic

diversity of the current generations, to determine whether or not restoration improved the genetic enrichment of the Mauá Beach area.

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