# Transferability of microsatellite markers among Myrtaceae species and their use to obtain population genetics data to help the conservation of the Brazilian Atlantic Forest

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**Research Article**

# Transferability of microsatellite markers among Myrtaceae species and their use to obtain population genetics data to help the conservation of the Brazilian Atlantic Forest

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**Abstract**

Myrtaceae species play an important role in maintaining the biodiversity of the Atlantic Forest. However, most species of this biome have not been studied. In this work we examined whether microsatellite markers developed for other Myrtaceae species are useful in genetic studies of native species of the Atlantic Forest. We evaluated the transferability of 21 microsatellite markers derived from *Eucalyptus* sp., *Eugenia uniflora*, and *Melaleuca alternifolia* Cheel to eight Atlantic Forest species (*Campomanesia xanthocarpa* (guabiroba do mato), *Campomanesia adamantium*, *Eugenia uniflora* (surinam cherry), *Eugenia involucrata* (cherry of the Rio Grande), *Myrcianthes pungens* (guabiju), *Plinia cauliflora* (jaboticaba), *Psidium guajava* (guava), and *Psidium* sp.(araçá) and the utility of the transferred markers in obtaining genetic data from Eugenia uniflora. The transferability of microsatellite primers pairs was high (>52%) in seven of the eight species studied. In three populations of *E. uniflora*, the transferred primers amplified the same average number of alleles, and the same expected heterozygosity was obtained with species-specific primers. The genetic parameters $F_{IS}$, Shannon diversity, genetic distance of Nei, $F_{ST}$ and $F_{IT}$, AMOVA, and the PCoA were calculated using data from four transferred microsatellites. Results were similar to those obtained using species-specific primers. Both datasets (from transferred and species-specific primers) indicated that *E. uniflora* populations of the Atlantic Forest are unstructured and have high genetic diversity. The results of our study indicate that transferability of microsatellite markers is an economic and powerful way to obtain genetic information of Myrtaceae species of the Atlantic Forest, and consequently, will aid in the conservation of these species and the biome.

**Keywords:** Atlantic Forest; SSR; heterologous amplification; *Eugenia uniflora*

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## Introduction

Among the most threatened biomes in Brazil urgently in need of conservation strategies is the Atlantic Forest. The second largest tropical rainforest in America occurs in this biome, and is one of the world's biodiversity "hotspots" [1]. The Atlantic Forest has been dramatically reduced by human activities, and currently only about 8.5% of its original size remains [2]. Approximately 54% of the plant species of the Atlantic Forest are endemic [2] and many of these have not been studied, which increases their risk of genetic erosion. These species also include several members of the Myrtaceae family [3].

The Myrtaceae family is distributed throughout the southern hemisphere; the highest numbers of species occur in Western Australia and in the Cerrado and Atlantic Forest of Brazil [4-5]. In the Atlantic Forest, approximately 650 species are known, many of them endemic [6]. The abundance of Myrtaceae in this biome suggests that they play an important role in restoring and maintaining the community's biodiversity. Most of these species are pioneer and fruit-bearing species, which provide food for wildlife. Therefore, many species of this family are used in reforestation projects for the regeneration of degraded areas in Brazil and in ecological corridors that connect the remaining forest fragments [7-8]. The fruit-bearing Myrtaceae of the Atlantic Forest include *Campomanesia xanthocarp*a O. Berg. (guabiroba do mato), *Campomanesia adamantium* (Cambess) O. Berg., *Eugenia uniflora* L. (surinam cherry), *Eugenia involucrata* D.C. (cherry of the Rio Grande), *Myrcianthes pungens* O. Berg. (guabiju), *Plinia cauliflora* O. Berg (jaboticaba), and *Psidium* sp. (araçá) [9-14, 5, 15]. The vernacular names written in brackets are like the species are known in English or Portuguese language and are valid according to the Encyclopedia of Life site (http://eol.org/ Accessed 30 november 2015).

Besides their ecological importance, all these species have the potential for commercial exploitation in the food and cosmetic industries. *E. uniflora*, *C. xanthocarpa*, *C. adamantium*, and *P. cauliflora* are widely used in the manufacture of ice cream and candies, and *E. uniflora* is often used in the cosmetic industry for the manufacture of shampoos and hydrating creams. Commercial exploitation, if not coupled with conservation strategies, creates a risk for these species. Despite the ecological importance and economic potential of the Myrtaceae species, molecular genetics data only of *E. uniflora* [16] and *P. cauliflora* [17] are available.

Most studies on genetic diversity and structure of natural populations use microsatellite markers, also known as SSR (simple sequence repeat). Microsatellites are highly polymorphic and codominant, facilitating the study of detailed patterns of genetic diversity of natural populations [18]. Microsatellite markers have not been developed for Myrtaceae species of the Atlantic Forest. The development of specific primers for microsatellite locus amplification is an expensive and time-consuming process involving cloning and sequencing of the DNA fragments [19]. However, transferability of microsatellite primers between species in the same genus or between different genera is possible (20-24).

Currently, 366 microsatellite markers have been developed for the genus *Eucalyptus* [25-31], 28 for *Corymbia* [32-33], 113 for *Melaleuca* [34-35), 24 for *Metrosideros* [36, 37], nine for *Eugenia* [16], 14 for *Myrtus* [38], 10 for *Calothamnus* [39] and 13 for *Acca* [40]. These markers may be an important tool in the genetics studies of other species belonging to the Myrtaceae family.

Our objective was to determine whether microsatellite markers developed for other Myrtaceae species are applicable to population genetics studies of the species native to the Atlantic Forest. We tested the transferability of 21 microsatellite primers pairs (from *Eucalyptus* sp., *Eugenia uniflora* L., and *Melaleuca alternifolia* Cheel) in seven native species from the Atlantic Forest (*Campomanesia xanthocarpa*, *Campomanesia adamantium*, *Eugenia uniflora*, *Eugenia involucrata*, *Myrcianthes pungens*, *Plinia cauliflora*, and *Psidium* sp.) and in one native of Cerrado with natural occurrence in the Atlantic Forest (*Psidium guajava* L. [guava]). Furthermore, we tested the utility of the transferred microsatellite markers to obtain population genetics data from one of the species (*Eugenia uniflora*).

## Methods

Plant material for the transferability test

To select species for tests, we explored the Guarapuava region, State of Paraná, Southern Brazil (Fig. 1) to determine populations of Myrtaceae fruit trees common in this region. During these collections, we identified eight species: *Campomanesia xanthocarpa*, *Campomanesia adamantium*, *Eugenia uniflora*, *Eugenia involucrata*, *Myrcianthes pungens*, *Plinia cauliflora*, *Psidium* sp. and *Psidium guajava*. Young leaves of each species from five different plants were collected and kept in liquid nitrogen until DNA extraction.

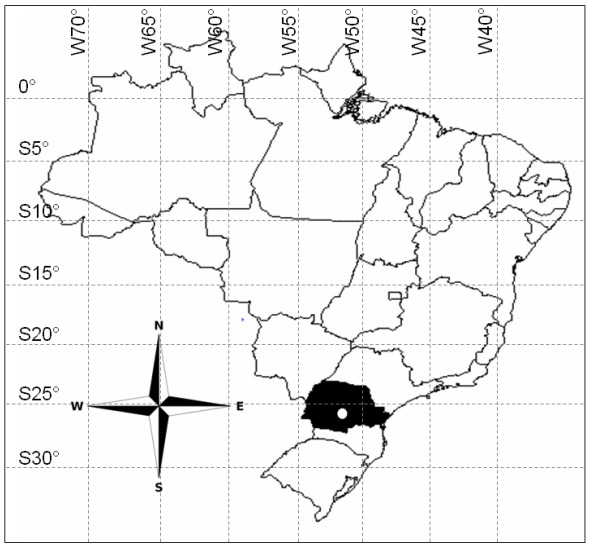


**Fig. 1. Map of Brazil showing the region where *Campomanesia xanthocarpa* O. Berg., *Campomanesia adamantium* (Cambess) O. Berg., *Eugenia uniflora* L., *Eugenia involucrata* D.C., *Myrcianthes pungens* O. Berg., *Plinia cauliflora* O. Berg, *Psidium* sp., *Psidium guajava* and three populations of *Eugenia uniflora* L. were sampled.**

*Eugenia uniflora* material for population genetics study

*Eugenia uniflora* samples were collected from three populations in the Guarapuava region, state of Paraná, southern Brazil for population genetics analysis (Fig. 1). In each population, we sampled 20 adult trees from the same generation. The populations were named A, B, and C. These populations were separated from one another by a distance of 12 km. The collected leaves were stored in liquid nitrogen until DNA extraction.

DNA extraction

The DNA of all species was extracted following the protocol proposed by Sharma *et al*. [41]. One ml of extraction buffer (20 mM EDTA, 100 mM Tris-HCl pH 8.0, 2 M NaCl, 2% CTAB, 2% PVP, 2% β-mercaptoethanol) was added to a tube containing 100 mg of tissue previously ground in liquid nitrogen. The tubes were placed in a water bath at 65°C for 30 min. DNA was separated from the extraction solution by precipitation with phenol:chloroform:isoamyl alcohol (25:24:1) and centrifugation. Next, DNA was precipitated with PEG 4000 (polyethylene glycol) and successively washed with ethanol to obtain DNA of high purity. After extraction, the DNA was resuspended in TE (10 mM Tris-HCl, pH 8.0; 1 mM EDTA), treated with RNase at 37°C for 30 min, and stored at -20°C until use.

Transferability test for microsatellite markers

For transferability tests we selected 21 pairs of primers from published literature. Thirteen of these loci were identified in *Eucalyptus* spp. [25], seven in *E. uniflora* L. [16], and one in *M. alternifolia* Cheel [35] (Appendix 1). The microsatellite loci were named Embra, Eun, and Scutt, derived from *Eucalyptus* spp., *E. uniflora*, and *M. alternifolia*, respectively. For the selection of microsatellite primers, we considered the number of amplified alleles. Preference was given to primer pairs that amplified more alleles. Except *E. uniflora*, which was tested for 13 loci, all the other species were tested for all 21 loci. *E. uniflora* was not tested for the seven Eun loci because these loci were developed for this species.

For Embra and Eun loci, the reactions were conducted in a final volume of 10 µL containing 25 ng DNA, 2.5 µM of each primer, 250 µM of each dNTP, 1.0 mM of $MgCl_2$, 1 U of Taq DNA polymerase, and 1X PCR buffer. For the marker Scutt052, reactions were conducted in a final

volume of 15 uL containing 30 ng DNA, 2.5 uM of each primer, 250 uM each dNTP, 1.5 mM $MgCl_2$, 1 U of Taq DNA Polymerase, and 1X PCR buffer. For DNA amplification by PCR the thermal cycler was programmed for initial denaturation at 96°C for 3 min, followed by 35 cycles of 94°C for 30 s, primer annealing temperature (Appendix 1) for 1 min, and 72°C for 1 min, and a final extension at 72°C for 15 min. After amplification, the fragments were separated on a 3% agarose gel in a constant 110 V current for 4 h and visualized by staining with ethidium bromide (0.5 µg $mL^{-1}$) in UV light. The size of amplified DNA fragments was estimated by comparison with a molecular weight marker 100-bp DNA ladder.

For each primer pair, the first thermocycler program started with the annealing temperature described in the literature. After obtaining the results of this PCR, three outcomes were possible: 1) the expected size fragment amplification; 2) nonspecific amplification; or 3) no amplification. Given scenario 1, if any genotype did not exhibit amplification, the reaction was repeated to be sure of the null allele. When scenario 2 occurred, new PCR programs were run and temperature was increased by 2 °C until the specific amplification was obtained or 60°C was reached. Given scenario 3, new PCR programs were run and temperature was lowered by 2 °C until the amplification was obtained or 45 °C was reached.

Evaluation of the utility of transferred microsatellite primers in the population-level study

Three populations of *E. uniflora* were studied with the transferred microsatellite primers to evaluate the utility of these markers for population-level studies. In addition to transferring the primer pairs, we used four primer pairs developed from the species itself (Table 1). Twenty trees from each population were individually genotyped for each microsatellite loci. The number of alleles per locus, observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), coefficient of inbreeding ($F_{IS}$), and Nei genetic distance [42] were estimated using the GDA (Genetic Data Analysis) software package [43] according to the following equations.

Observed heterozygosity:

$$\mathrm{Ho} = 1 - \sum \mathrm{P}ii$$

where $P_{ii}$ is the observed frequency of homozygous genotypes of allele *i*.

Expected heterozygosity:

$$\mathrm{He} = 1 - \sum \mathrm{p}i^2$$

where $p_i$ is the frequency of the $i^{th}$ allele.

Coefficient of inbreeding:

$$\mathrm{F_{IS}} = (\mathrm{He\text{-}Ho})/\mathrm{He}$$

Gene flow between populations was estimated using POPGENE [44] based on F statistics [45] and following Nei 1978 [42]. The hierarchical distribution of genetic variation among and within populations was determined by analysis of molecular variance (AMOVA) [46,47] using the GENALEX 6 software package [48]. The genetic structure of populations was visualized by principal coordinate analysis (PCoA) using the GENEALEX 6 software package [48]. The linkage disequilibrium and the Hardy-Weinberger Equilibrium (HWE) were tested for all loci using the Genepop package [49].

**Table 1.** Parameters of the four heterologous and five own species microsatellite loci in *Eugenia uniflora* L. based on the analysis of three populations. A - number of alleles; $H_o$ - observed heterozygosity; $H_e$ - expected heterozygosity.

| Locus | GenBank code | Fragment size (pb) | A | $H_o$ | $H_e$ |
|---|---|---|---|---|---|
| Embra14[a] | G74881 | 95 – 170 | 5 | 0.10 | 0.73 |
| Embra123 | BV682244 | 180 – 300 | 2 | 0.23 | 0.20 |
| Embra148[a] | BV682248 | 200 – 320 | 2 | 0.00 | 0.37 |
| Embra166[e] | BV682113 | 130 – 300 | 4 | 0.66 | 0.71 |
| Mean of transferred primers | | | 3.2 | 0.24 | 0.50 |
| Eun2[b] | EU000456 | 182 – 208 | 4 | 0.30 | 0.52 |
| Eun5[c] | EU000458 | 140 – 162 | 4 | 0.21 | 0.40 |
| Eun11[d] | EU000460 | 162 – 182 | 3 | 0.71 | 0.66 |
| Eun21[a] | EU000462 | 198 – 212 | 3 | 0.53 | 0.65 |
| Eun121 | EU000464 | 205 – 215 | 2 | 0.26 | 0.23 |
| Mean own primers | | | 3.2 | 0.40 | 0.49 |
| Mean of the specie | | | 3.2 | 0.33 | 0.50 |
| Total of alleles | | | 29 | | |

[a, b, c, d, e] locus with significant ($P$<0.05) deviation from Hardy-Weinberg Equilibrium in populations A, B and C; A and B; B and C; and C, respectively.

## Results

Microsatellite primer transferability

There was variation in the best annealing temperature among species for most primer pairs (Appendix 1). The primer Embra14 showed the greatest variation in temperature, with the best temperature of 49°C in *E. uniflora* and 58°C in *P. guajava* (Appendix 1). Primer pairs that produced fragments of the expected size and did not produce nonspecific amplification were considered transferred. Based on this criterion, the percentage of transferability was 38% (5 of 13) in *E. uniflora*, 57% (12 of 21) in *E. involucrata*, 62% (13 of 21) in *M. pungens*, 67% (14 of 21) in *C. xanthocarpa*, 71% (15 of 21) in *C. adamantium*, 57% (12 of 21) in *P. guajava*, 57% (12 of 21) in *Psidium sp.*, and 52% (11 of 21) in *P. cauliflora*. The microsatellite primers Embra123, Eun5, and Eun11 were transferred to all species (Appendix 1). The primer pairs Embra99, Embra108, and Embra267 did not amplify in any of the tested species. The primer pair Eun22 amplified only in *P. guajava* and was monomorphic. The primer pair Embra265 amplified only in *C. adamantium* and exhibited a null allele. A large number of alleles were observed for the Embra14 locus with five alleles in *C. xanthocarpa* and *E. uniflora*, and Embra148 with five alleles in *C. xanthocarpa* (Appendix 1).

*Utility of transferred microsatellite primers in the population-level genetics study of E. uniflora*

Analysis of the five microsatellite primers transferred to *E. uniflora* and the four developed for this species (species-specific) in three populations generated 29 alleles, with an average of 3.2 alleles per locus (Table 1). The average number of alleles for the transferred microsatellite primers and species-specific primers was the same (Table 1). The lowest and the highest number of alleles was two for Embra123, Embra148, and Eun121 loci, and five for the Embra14 locus (Table 1). Of the five alleles amplified with primer pair Embra14, three were unique in populations B and C. Observed heterozygosity ($H_o$) ranged from 0.00 (Embra148 locus) to 0.71 (Eun11 locus) (Table 1), and expected heterozygosity ($H_e$) from 0.20 (Embra123 locus) to 0.71

(Embra166 locus). The average of $H_o$ and $H_e$ for all loci was 0.33 and 0.50, respectively (Table 1). The mean of $H_e$ was the same for transferred microsatellite and species-specific ones (Table 1). A significant ($P < 0.05$) deviation from HWE was observed in five loci in populations A and B, and six loci in the population C (Table 1). Pairwise comparisons after applying the Bonferroni correction (95%, $\alpha = 0.05$) for multiple comparisons indicated linkage disequilibrium between Eun2 and Eun11 primers.

The $H_e$ and $H_o$ values for the species, considering the three populations studied, was 0.33 and 0.47, respectively (Table 2). The Shannon diversity index was 0.51, and ranged from 0.38 to 0.47 among populations (Table 2). The genetic distance of Nei [42] ranged from 0.25 to 0.31 among populations and was 0.33 overall. Gene flow was 5.02, 3.21, and 4.73 between populations A and B, A and C, and B and C, respectively. Among all populations, gene flow was 3.12. AMOVA indicated differentiation among populations of 7%, within populations of 26%, and within individuals of 67% (Table 3). The inbreeding coefficient ($F_{IS}$) for each population ranged from 0.25 to 0.32, with a value of 0.29 for all populations (Table 2). The fixation index ($F_{ST}$) average of the three populations was 0.074, and $F_{IT}$ was 0.331. The first three axes of the principal coordinates analysis (PCoA) accounted for approximately 39% of accumulated variability (Fig. 2).

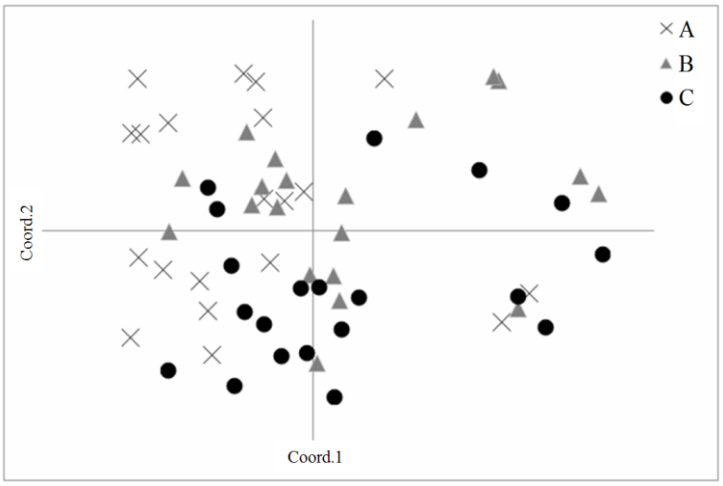


Fig. 2. Dispersion of three populations of *Eugenia uniflora* (A, B and C) according to principal component analysis (PCoA) based on the molecular variations in microsatellite markers.

**Table 2.** Genetic parameters of three populations of *Eugenia uniflora* L. based on analysis of the nine microsatellite loci. N - number of trees sampled; $H_o$ - observed heterozygosity; $H_e$ - expected heterozygosity; $F_{IS}$ - inbreeding coefficient; H - Shannon diversity; Nei - genetic distance of Nei (1978).

| Population | N | $H_o$ | $H_e$ | $F_{IS}$ | H | Nei |
|---|---|---|---|---|---|---|
| A | 20 | 0.35 | 0.46 | 0.25 | 0.47 | 0.31 |
| B | 20 | 0.35 | 0.49 | 0.29 | 0.40 | 0.26 |
| C | 20 | 0.31 | 0.31 | 0.32 | 0.38 | 0.25 |
| All | 60 | 0.33 | 0.47 | 0.29 | 0.51 | 0.33 |

**Table 3.** Analysis of molecular variance (AMOVA) based on the data of nine microsatellite loci in three populations of *Eugenia uniflora* L.

| Source of variation | d.f. | Sum of squares | Variance components | % of variation | p |
|---|---|---|---|---|---|
| Among Populations | 2 | 19.13 | 9.56 | 7 | < 0.001 |
| Within Populations | 57 | 155.37 | 2.726 | 26 | < 0.001 |
| Within individuals | 60 | 92.5 | 1.542 | 67 | |
| Total | 119 | 267.008 | | 100 | |
| $F_{ST}$: 0.074 | | | | | |

## Discussion

The transferability of microsatellite markers as a strategy for studying the native species of the Atlantic Forest

Several studies have demonstrated the successful transferability of microsatellite primers between tree species [21, 22, 50, 51, 19, 52, 53]. However, most of these are species with high commercial value and/or are domesticated. We transferred microsatellite primers for native and uncultivated Myrtaceae species of the Atlantic Forest, which had no developed microsatellite markers (except *E. uniflora*). The microsatellite primers evaluated in species showed high rates of transferability, with an overall rate >52% (57% in *E. involucrata*, 62% in *M. pungens,* 67% in *C. xanthocarpa*, 71% in *C. adamantium*, 57% in *P. guajava*, 57% in *Psidium sp.*, and 52% in *P. cauliflora*). Some microsatellite primers evaluated here have already shown good transferability rates for other species of Myrtaceae. The microsatellite primers *Eucalyptus* spp. and *M. alternifolia* exhibited 44.5% and 60% transferability to *Acca sellowiana* and other species of *Melaleuca*, respectively [54, 55]. Further, Ferreira-Ramos *et al*. [24] evaluated the *E. uniflora* primers in *Eugenia pyriformis*, *E. brasiliensis,* and *E. francavilleana* and obtained transferability rates above 60% for all species.

The potential of transferability of each microsatellite primer pair depends on the conservation of flanking regions on primer binding sites [23]. Many studies have demonstrated the high conservation of these flanking regions of microsatellites in cultivated tree species, which has resulted in high transferability indexes [50, 56, 57, 52, 51, 53]. The high transferability rates obtained in the 10 species tested (seven in this study and three in the study by Ferreira-Ramos *et al*. [24]) indicate that the flanking regions of microsatellites are also highly conserved in the genome of uncultivated Myrtaceae species native to the Atlantic Forest. Thus, microsatellite transferability is an effective method for obtaining molecular markers for use in native Myrtaceae species of the Atlantic Forest to obtain molecular genetics data. The data that can be generated using these markers can help to develop more effective strategies for the conservation and/or sustainable exploitation of these species.

Utility of heterologous microsatellite markers in population genetics studies of *E. uniflora*

After the transfer of the microsatellite primer pair into a species, its ability to differentiate genotypes and populations needs to be evaluated. This is necessary because a transferred primer pair will only be useful if it is robust and capable of detecting differences between

individuals and populations. We used the microsatellite primers transferred to *E. uniflora* to evaluate them in generating population genetics data.

The evaluation of four heterologous loci together with five species-specific ones showed that $H_o$ (0.33) in *E. uniflora* populations was low compared to other tree species with cross-fertilization such as *Vitellaria paradoxa* (0.74), *Araucaria angustifolia* (0.52), *Eugenia pyriformis* (0.72), *Eugenia brasiliensis*, and *Eugenia francavilleana* (0.57) [58-60, 24). This low $H_o$ appears to be intrinsic to *E. uniflora*, and was also observed in natural populations from the regions of the states of São Paulo and Rio de Janeiro ($H_o$ = 0.30 for both) [61, 24]. Its rate of self-fertilization, which according to Almeida *et al*. [8] is approximately 10%, can help explain this low $H_o$. Expected heterozygosity was above $H_o$ in populations A and B, and on average, the populations were lower than the expected in HWE. Furthermore, the observation of only two primers in HWE in all populations supports this conclusion. This result may be due to several factors, such as self-fertilization, limited sample size, Wahlund effect, and presence of null alleles, the latter being a common factor in transferability work [62-64].

The AMOVA results showed that most variability of *E. uniflora* populations from Paraná State is within populations, and according to PCoA and $F_{ST}$, these populations are not structured. *Eugenia uniflora* populations from the states of São Paulo and Rio de Janeiro also did not present genetic structure ($F_{ST}$ values of 0.03 and 0.04, respectively) [62, 16]. The non-genetic structure of populations of the three states indicates that most variability is maintained within populations. Hamrick *et al*. [65] and Ferreira-Ramos *et al*. [16] stated this is a characteristic of tropical forest tree populations. From the Shannon diversity and the genetic diversity of Nei [42] of each population, and overall values (Table 3), we conclude that even with low differentiation among populations, high variation within populations is sufficient to maintain high genetic diversity in the species.

High diversity and lack of genetic structure observed in *E. uniflora* can be explained by the gene flow observed among populations. All the pairs of populations showed gene flow higher than 3.2 (5.02, 3.21, and 4.73 between populations A and B, A and C, and B and C, respectively). These significant gene flow rates can be explained by the dispersal of the fruits of this species, which are eaten by birds and mammals [66]. By eating the fruit, animals disperse seeds from one population to another. Thus, birds with high foraging rates probably play an important role in maintaining high homogeneity and genetic diversity among populations of this species. Furthermore, bees (Family Apidae) are the main pollinators of this species [66], and the high mobility of bees may have facilitated pollen flow among populations.

## Implications for Conservation

The results observed for *E. uniflora* support the hypothesis that the microsatellite primers analyzed in this study may produce the same robustness in obtaining population genetics data for other Myrtaceae species. This hypothesis is based on the observation that even by using only five plants of most species evaluated, the microsatellite primers amplified more than one allele. The expectation is that when used in populations with 20 or more plants, this number of alleles will increase significantly, and even those that were monomorphic may show polymorphism. Our results clarify the hypothesis that microsatellite markers of other Myrtaceae are useful in obtaining population genetics data of native species from the Atlantic Forest. To this end, these new heterologous microsatellites are an important tool for obtaining genetic data needed in conservation strategies of native Myrtaceae. The high genetic diversity observed in *E. uniflora*

is important for t the development of conservation strategies, sustainable commercial exploitation, and breeding programs of this species. The data observed for *E. uniflora* show that the species is not suffering genetic erosion and even in fragmented environments is maintaining high genetic diversity. Apparently, for the populations of *E. uniflora* studied to date, no conservation effort is necessary.

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**Appendix 1.** Analysis parameters for 21 heterologous microsatellite loci in eight native species of Myrtaceae from Atlantic Forest. Ta: annealing temperature; A: number of alleles observed by primer.

| *Locus* | Fragment range (pb) | *Eugnia uniflora* | | *Eugenia involucrata* | | *Myrcianthes pungens* | | *Campomanesia xanthocarpa* | | *Campomanesia adamantium* | | *Psidium guajava* | | *Psidium sp.* | | *Plinia cauliflora* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ta °C | A | Ta° C | A | Ta °C | A | Ta °C | A | Ta °C | A | Ta °C | A | Ta °C | A | Ta °C | A |
| Embra14 | 95-170 | 49 | 5 | 50 | 1b | 50 | 2 | 49 | 5 | 50 | - | 58 | 1[a] | 50 | - | 50 | - |
| Embra26 | 155-320 | 50 | - | 50 | - | 50 | 4 | 50 | 3 | 56 | 1[b] | 56 | 2 | 50 | 2[b] | 50 | - |
| Embra63 | 200-290 | 49 | - | 50 | 2 | 50 | 4 | 49 | 2 | 56 | 1[a] | 56 | 2 | 50 | - | 50 | 2 |
| Embra69 | 200-290 | 50 | - | 50 | - | 50 | 4[b] | 50 | 3 | 56 | 2[b] | 50 | 1[a] | 50 | 1[b] | 50 | 1[b] |
| Embra85 | 140-300 | 50 | - | 50 | 1[a] | 50 | 1[b] | 50 | 1[a] | 56 | 1[a] | 50 | 1[a] | 50 | 1[b] | 50 | 1[a] |
| Embra 99 | 230-250 | 50 | - | 56 | - | 56 | - | 50 | - | 56 | - | 56 | - | 50 | - | 56 | - |
| Embra108 | 150-160 | 50 | - | 56 | - | 56 | - | 50 | - | 56 | - | 56 | - | 56 | - | 56 | - |
| Embra123 | 180-300 | 50 | 2 | 50 | 2 | 50 | 3 | 50 | 3 | 56 | 3 | 50 | 2 | 50 | 2[b] | 50 | 1[b] |
| Embra148 | 200-320 | 50 | 2 | 54 | - | 54 | - | 50 | 5 | 53 | - | 50 | - | 54 | - | 54 | - |
| Embra166 | 130-300 | 50 | 4 | 53 | - | 53 | - | 50 | 4 | 56 | 2 | 58 | 1[b] | 56 | 1[b] | 56 | 1[a] |
| Embra210 | 195-210 | 50 | 1[a] | 53 | 1[b] | 53 | 1[b] | 50 | 3 | 56 | 2[b] | 50 | 2[b] | 53 | 2[b] | 53 | - |
| Embra265 | 200-250 | 50 | - | 50 | - | 50 | - | 50 | - | 56 | 1[b] | 56 | - | 50 | - | 50 | - |
| Embra267 | 120-140 | 50 | - | 56 | - | 50 | - | 50 | - | 56 | - | 56 | - | 56 | - | 56 | - |
| Eun2 | 182-208 | x | x | 56 | 1[a] | 56 | 1[a] | 56 | - | 56 | 2[b] | 56 | - | 56 | 1[b] | 56 | 1[a] |
| Eun5 | 140-162 | x | x | 50 | 3 | 50 | 1[a] | 54 | 3 | 54 | 2 | 54 | 1[a] | 50 | 1[b] | 50 | 1[a] |
| Eun7 | 196-202 | x | x | 54 | 1[b] | 50 | - | 52 | 3 | 54 | 2 | 54 | - | 50 | - | 50 | - |
| Eun11 | 162-182 | x | x | 52 | 1[a] | 52 | 1[a] | 52 | 4 | 52 | 3 | 52 | 1[a] | 52 | 2[b] | 52 | 1[a] |
| Eun21 | 198-212 | x | x | 55 | 1[a] | 55 | 1[b] | 52 | - | 54 | 3 | 52 | - | 55 | 1[b] | 55 | 1[b] |
| Eun22 | 175-183 | x | x | 48 | - | 48 | - | 52 | - | 52 | - | 52 | 1[a] | 48 | - | 48 | - |
| Eun121 | 205-215 | x | x | 52 | 1[a] | 52 | 1[a] | 54 | 1[b] | 52 | 1[a] | 52 | - | 52 | 1[b] | 52 | 1[a] |
| Scutt052 | 305-345 | 49 | - | 56 | 3 | 56 | 3 | 49 | 4 | 56 | 2 | 56 | 1[a] | 56 | 2[b] | 56 | 1[a] |

- No amplification, [x] not evaluated, [a] monomorphic, [b] presence of null alleles;