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Genetic structure and diversity of native *Guadua* species (Poaceae: Bambusoideae) in natural populations of the Brazilian Amazon rainforest

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Abstract: The Southwestern Region of the Brazilian Amazon is formed by forests dominated by bamboos. The genus *Guadua* is endemic to the Americas, and little is known about the genetic diversity and structure of species of this genus. This study aimed to evaluate the genetic diversity and structure of two native *Guadua* species in natural populations in the Southwestern region of the Brazilian Amazon. Therefore, the genetic diversity and structure of *Guadua* aff. *chaparensis* and *Guadua* aff. *lynnclarkiae* were evaluated with the use of microsatellite molecular markers (SSR). It was verified that the average genetic diversity for the populations studied was considered high ($\widehat{H}_e$ =0.5) compared to other species of bamboo. All populations had rare and private alleles, and none of them presented significant values of inbreeding. The populations were divergent ($\hat{G}_{ST}$ = 0.46), resulting in a low apparent gene flow. The Bayesian analysis showed that among the 350 individuals analyzed, five groups (K=5) were formed, with little similarity among the groups (Populations), although two of them presented clonal individuals. According to the results obtained, it can be conclude that populations should be treated as having unique characteristics, mainly when accessed for management and for *in situ* and *ex situ* conservation studies.

Key words: Amazon Rainforest, Genetic diversity, *Guadua*, Bamboo, native population, underutilization plants.

INTRODUCTION

Bamboo is a plant that belongs to the family Poaceae (Gramineae), subfamily Bambusoideae (Calderon & Soderstrorn 1980). It is predominantly tropical, perennial, renewable, fast growing, and has a high production of biomass. Bamboo has been used in several activities ranging from landscaping to construction (Paraskeva et al. 2017). Moreover, it is a source of raw material for food products for humans and animals, and is used in the recovery of degraded areas (Bhatt et al. 2005, Moktan et al. 2009). It can also be an important alternative crop for carbon sequestration (Nath et al. 2015). Several research groups have concentrated efforts on various aspects to establish the production chain of bamboo, aiming at the best use of the products and by-products of this crop (Rao & Sastry 1990, Azmy 1996, Diab & Mohaned 2008, Nirala et al. 2017).

Guadua Kunth is an endemic genus of the Americas (Soderstrom & Londoño 1987, Londoño & Clark 2002). Brazil, Peru, Bolivia, Ecuador, Colombia and Venezuela are considered centers of origin of this genus, and there is a great diversity of species (Londoño & Peterson 1992). They are arborescent and, in general, have thorns on the colms and branches. Like other bamboos

in this genus, most species are semelparous (a single event of sexual reproduction) and monocarpic (they die after this event), and flowering is in waves, followed by the death of the clump (gregarious).

Open *Guadua*-dominated bamboo forests cover about 165,000 to 180,000 square kilometers that extend though the southwestern Amazon Basin, including southeastern Peru, northern Bolivia to western Brazil (Silveira 2005, Smith & Nelson 2011). The state of Acre is located in the Southwestern region of the Brazilian Amazon, with forests dominated by bamboo that are considered as the planet's largest natural reserves with *Guadua* (Pereira & Beraldo 2007, Silva 2015). In these areas, the dominant species are *Guadua weberbaueri*, *Guadua sarcocarpa* and *Guadua superba* (Silveira 2005).

The species of this study were recently described for the flora of Acre. *Guadua* aff. *chaparensis* Londoño and Zurita is a woody, arboreal bamboo with pachymorph rhizome and hollow cylindrical colms (stalks), erect at the base and arching from the middle to the apex, measuring 18–25 meters in height and 7–12 cm in diameter (Londoño & Zurita 2008). *Guadua* aff. *lynnclarkiae* is a species of arborescent, woody and thorny bamboo, with a pachymorph rhizome. The colms are cylindrical and hollow, measuring 20–27 meters in height and 9–17 cm in diameter (Londoño 2013). Each species has long reproductive cycles and similar morphological characteristics, which often hinder identification in the field.

The use of molecular markers for these species is a valuable tool that supports the classification and identification of bamboo around the world (Rugeles-Silva et al. 2012), in addition to demonstrating the genetic characteristics of the species. Estimating diversity depends on a variety of indices that represent the informative content of a site. Such an estimate may have different applications, such as individual identification and kinship analysis, estimation of gene flow, definition of the crossing-breeding system, and determination of spatial genetic structure (Sujii et al. 2015, Silva et al. 2016, Zhu et al. 2016, Nilkanta et al. 2017). For most species of *Guadua*, few studies on the genetic diversity and structure have been conducted. Studies such as these may provide genetic data for these species, which may help in understanding the genetic structure and diversity, among and within populations, in the selection of the best genotypes for maintaining species within ecosystems, especially those that suffer anthropogenic actions (Terranova 2011, Yang et al. 2012, Attigala et al. 2017) and identification of clonal individuals. The assessment of genetic variation within and between populations of natural bamboo is important for developing effective conservation methods (Liu et al. 2013, Nilkanta et al. 2017, Yeasmin et al. 2015).

This study aims to evaluate the genetic diversity and structure of native species *Guadua* aff. *chaparensis* and *G.* aff. *lynnclarkiae* in natural populations in the southwestern region of the Brazilian Amazon, through the transferability of microsatellite loci (SSR) of genetically correlated species.

MATERIALS AND METHODS

Study area and sampling

The study was carried out in the Brazilian Southwestern Amazon based on collections made between August 2015 and April 2016. The four native populations of *Guadua* aff. *chaparensis* (Fig. 1) were sampled in a fragmented patch in the municipalities of Bujari and Sena Madureira, throughout the Antimary State Forest and in the areas known as Ramal do

Figure 1. (a–b) Culms of *Guadua* aff. *chaparensis* in Sena Madureira – AC/BR.

Ouro and Ramal Toco Preto (Table I and Fig. 1). The surrounding areas of where the populations are located are cattle pasture, old secondary woodlands, and forest edge, as well as in forests that were subjected to commercial logging. On the other hand, the population of *Guadua* aff. *lynnclarkiae* (Fig. 2), located in the municipality of Porto Acre, is situated in an area of old secondary woodlands and forest, with cattle pasture in the immediate surroundings (Table I and Fig. 3). The populations are approximately 150 meters above sea level. The types of soils in these areas are argisol and luvisol. The climate is humid equatorial. Annual precipitation for these areas is around 2200 to 2500 mm, and the rainy season is from November to April. The mean temperature ranges from 22 °C to 26 °C.

Sampling of the clumps was carried out based on their distribution. Because it is a species with vegetative growth and with large number of colms, each clump found was considered an individual. For the *G.* aff. *chaparensis* species, two approaches were considered: for population 1 (P1), 100 individuals (clumps) were collected, in which all the individuals found in the delimited area were identified and mapped using the Global Positioning System (GPS), model MAP 76CSX, by Garmin. For populations 2, 3 and 4 (P2, P3 and P4), the sample included 50 individuals (clumps). This sampling occurred randomly and individuals were collected with a greater geographical space between them (distances of 250 to 500 meters). For the species *G.* aff. *lynnclarkiae*, 100 individuals (clumps) were collected for population P5, in which all the individuals found in the delimited area were identified and mapped using GPS. Dried plant samples were assembled from all of the populations, which were sent to the Biology and Plant Ecology Laboratory of the Federal University of Acre, Brazil, under numbers 7685 and 7686.

For the genetic analyses, leaf tissue was collected from the individuals, which were identified and stored in paper bags with silica gel and 1.5-ml microtubes containing transport buffer (2% CTAB/70% ethanol), the samples in the transport buffer were stored at -20°C for subsequent DNA extraction.

Figure 2. (a–b) Culms of *Guadua* aff. *lynnclarkiae* in Porto Acre – AC/BR.

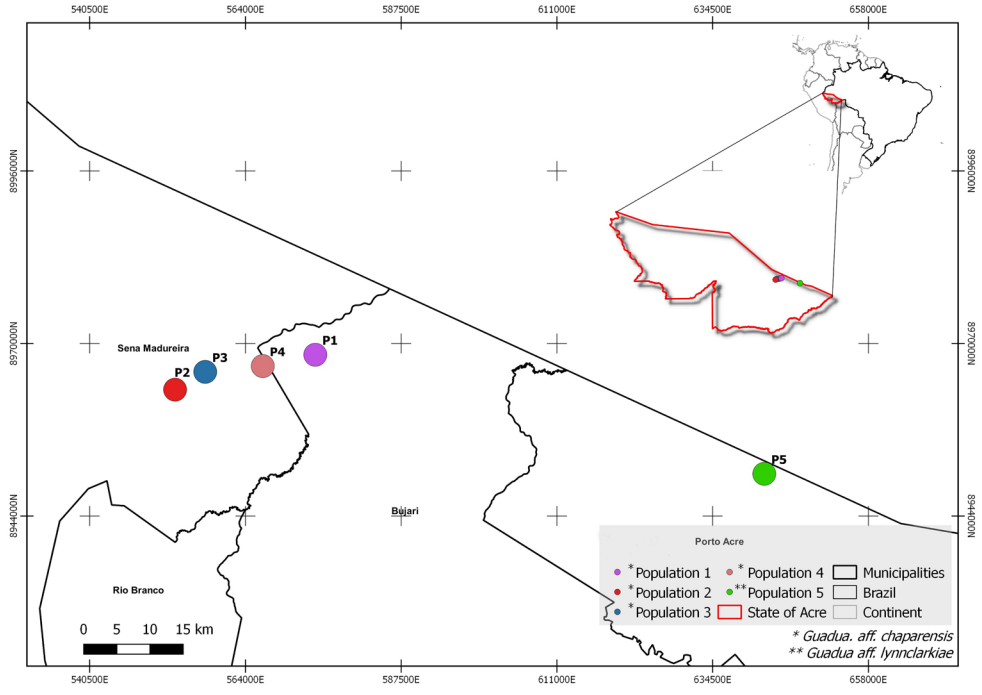

Figure 3. Location of the studied populations in the state of Acre, including the municipalities of Bujari, Sena Madureira and Porto Acre.

Table I. Characterization of the sample collection areas of the study with the species of *Guadua* aff. *chaparensis* and *G.* aff. *lynnclarkiae* in the southwest region of the Brazilian Amazon, in the state of Acre.

Population	Species	Municipality	Geographic position	Total area (ha^{-1})	Collection Area (ha^{-1})	Number of plants collected
1	*G.* aff. *chaparensis*	Bujari	19L 574447 UTM 8968267	76000	12.4	100
2	*G.* aff. *chaparensis*	Sena Madureira	19L 552777 UTM 8962628	8000	63.18	50
3	*G.* aff. *chaparensis*	Sena Madureira	19L 557700 UMT 8965024	8000	69.45	50
4	*G.* aff. *chaparensis*	Sena Madureira	19L 567010 UTM 8967696	-	111	50
5	*G.* aff. *lynnclarkiae*	Porto Acre	19L 642225 UTM 8950325	100	2.3	100

Genetic analyses

DNA extraction

Genomic DNA extraction and genotyping of the individuals was done according to a CTAB 2% procedure (Doyle & Doyle 1987), adapted for the species *Guadua angustifolia* Kunt (personal communication with Paula Andrea Rugeles – Colombia, via email). After extracting the genomic DNA, it was quantified by comparative analysis of each sample with phage DNA λ of a known molecular weight (10 to 200 ng), after underwater electrophoresis on agarose gel (1%). The gels were visualized through UV transilluminator (UVP), then stained with Red Gel and subsequently photographed. After quantification, the DNA was diluted to 25 ng/µL, stored at –20 °C.

Microsatellite Markers

Genetic analyses were conducted using transfer of microsatellite loci developed for the following species: *Guadua angustifolia* Kunt (nine loci) (Pérez-Galindo et al. 2009); *Saccharum* spp. (five loci) (Oliveira et al. 2009), and *Oriza sativa* L. (three loci) (Temnykh et al. 2000) (Table II). Reactions were made containing 25 ng of genomic DNA; 10 mM Tris-HCl, 50 mM KCl; 0.25 mM of each dNTP; 0.25 mg/mL BSA (Bovine Serum Albumin); 2.0 mM $MgCl_2$; 0.2 µM of each primer and 2.5 U of Taq DNA polymerase and sterile ultrapure water, as the final volume of 13 µL. Amplifications were done in Analitik Jena thermocycler with the following conditions: 94 °C for 1 minute, followed by 30 cycles of 94 °C for 1 minute, annealing temperature defined for each primer for 1 minute and 72 °C for 1 minute, followed by a final extension phase at 72 °C for 5 minutes. The PCR product was quantified on agarose gel (3%). After the PCR reaction, the amplified fragments were separated on denaturing polyacrylamide gel (5%) in a vertical vial containing 1X TBE buffer. After electrophoresis, the samples were stained with silver nitrate (Creste et al. 2001).

Interpretation of the amplified fragments was performed by comparison with a standard molecular weight marker (10-bp Ladder Life Technologies®). Fragments presenting different molecular weights were considered different alleles.

Table II. SSR loci developed by Pérez-Galindo et al. (2009)[1], Oliveira et al. (2009)[2] Temnykh et al. (2000)[3] and Chen et al. (1997)[4] used for the analysis of transferability in *Guadua* aff. *chaparensis* and *G.* aff. *Lynnclarkiae*.

Loci	Repetitions	Sequence (5'-3')	Size of fragments (pb)	Species
FJ444930[1]	$(GATA)_8$	R: CCTTCACATGGTCTCACAAG F: CAGTCTAGCAATCAATTTGAAG	225-270	*G. angustifolia*
FJ444929[1]	$(GATA)_{10}$	R: CTAGATCTTCCTAATCAAAGTGG F: TACTAACCGATTGTCCCGTCTAG	240-260	*G. angustifolia*
FJ444932[1]	$(CTAT)_{10}$	R: CGCCACGTTAATCCCAGTTAGG F: CTATACATATATGCATTGTGTGG	450-500	*G. angustifolia*
FJ476075[1]	$(CTAT)_{13}$	R: GTTCCTACATGTAGACATATCC F: CTCTTGGGAGTGAGCATGGTGAC	175-195	*G. angustifolia*
FJ444934[1]	$(GATA)_{16}$	R: CCCGACAGATAGATGGTCAAA F: CTCATTTCTCAATTGCGCAAGAG	170-190	*G. angustifolia*
FJ444931[1]	$(GATA)_{16}$	R: GTCAATCACGCCAGCTCTAACA F: CTCTGACATGTATGGATCTTGCA	225-275	*G. angustifolia*
FJ444936[1]	$(GATA)_9$	R: CCCAACAAAGATGGTCAGAT F: CAGGAGATGAGCCTGTTAGT	180-220	*G. angustifolia*
FJ444935[1]	$(CTAT)_8$	R: CTAGGCCCACTCCTATCCCA F: AGCTTCCTCAGAATGCCTAATTA	210-260	*G. angustifolia*
FJ476076[1]	$(GATA)_8$	R: CCTTCAATTAGTACATAGATAG F:GTACAGAACCATCTCATCCT	230-256	*G. angustifolia*
RM31[4]	-*	F: GATCACGATCCACTGGAGCT R: AAGTCCATTACTCTCCTCCC	-*	*Oriza sativa*
RM309[3]	$(GT)_{13}$	F: GTAGATCACGCACCTTTCTGG R: AGAAGGCCTCCGGTGAAG	132-146	*Oriza sativa*
RM332[3]	$(CTT5)_{12}$ – $(CTT)_{14}$	F: GCGAAGGCGAAGGTGAAG R: CATGAGTGATCTCACTCACCC	162-183	*Oriza sativa*
ESTB41[2]	$(CGA)_8$	F: CATGGAGAGCTGGGCGACCTG R: GGCGGCGGCGACGATGA	81–165	*Saccharum* spp.
ESTB60[2]	$(TTG)_{10}$	F: AGCCGCAATCCAACTG R: CTCTAGCTCCGATGATACCTC	159–272	*Saccharum* spp.
ESTC45[2]	$(ATTG)_5$	F: GCCGCCGTCGCTGGATTG R: ATGGATCCCCGCCTACCCTACAC	106–168	*Saccharum* spp.
ESTC66[2]	$(CCGC)_3$	F: AGTACAGGCTGCTCTCAATCAA R: TCTGCTATCTGTGTTCGTTCTG	102–265	*Saccharum* spp.
ESTC119[2]	$(AAGC)_4$	F: GAATTAAGCTTTGCCGACACCAC R: GGCAGCACCTCCCCTTCACC	84–326	*Saccharum* spp.

***not informed by the authors.**

Data analyses

Genetic diversity

The genetic diversity of the species was analyzed by observed heterozygosity($\hat{H}_o$), heterozygosity expected according to the Hardy-Weinberg equilibrium ($\hat{H}e$), average number of alleles per locus ($\hat{A}$), and Wright's fixation index ($\hat{f}$). These estimates were obtained from the GDA and Cervus programs (Lewis & Zaykin 2002, Kalinowki et al. 2007). The polymorphism information content (PIC) for each locus was calculated using the Cervus software (Kalinowski et al. 2007). Allelic Richness (R_S), proposed by El Mousadick & Petit (1996) was calculated using the FSTAT program (Goudet 1995). The effective number of alleles ($\hat{A}_e$) was calculated based on:

$$\hat{A}_e = {}^{1}\!/\!_{(1-\hat{H}e)}$$

Confidence intervals at the 95% probability level for $\hat{f}$ were obtained through the 10,000 bootstrap re-sampling procedure on the loci, using the GDA program (Lewis & Zaykin 2002). The statistical significance of the values of $\hat{f}$ was tested by the G-Test with 1,000 permutations, using a Bonferroni correction (95%, a = 0.05) using FSTAT (Goudet 1995). When there are excess homozygotes and they are distributed homogeneously in all classes of homozygotes (for all alleles), the presence of null alleles is confirmed. For these cases, the frequency of null alleles (r) was calculated using the Cervus program (Kalinowski et al. 2007), with the Bonferroni correction.

Spatial genetic structure

The spatial distribution of the genotypes within the populations was characterized based on the average estimates of coancestry coefficients ($\hat{\theta}_{xy}$) (Loiselle et al. 1995), among pairs of individuals grouped into spatial distance classes with constant intervals. The number of classes and intervals were defined according to the minimum number of 50 pairs of individuals compared in each class. The aforementioned coancestry coefficient is not biased by the presence of rare alleles in the population.

For each distance class, the confidence interval at 95% probability of the $\hat{\theta}_{xy}$ was obtained using 10,000 permutations on the location of each genotype. For the analysis of the spatial genetic structure (SGS) within the populations, the SPAGeDi 1.3.a program was used, developed by Hardy & Vekemans (2002).

Identification of clones and parameters for identity analysis

Due to the vegetative growth of the species under study, the presence of clonal individuals was evaluated using Genclone 2.0 software (Arnaud-Haond & Belkhir 2007). Another parameter that evaluates the identification of similarity between the individuals of the species being studied was analyzed using Cervus software (Kalinowski et al. 2007). In this parameter, identity among the individuals was analyzed, aimed at finding genetically similar individuals within the populations. Similarity was evaluated based on eight equal alleles between individuals.

Genetic structure

The genetic structure of the populations was estimated by $\hat{G}_{ST}$ ', proposed by Hedrick (2005):

$$\hat{G}_{ST}' = \frac{\hat{G}_{ST}(1+\hat{H}_S)}{(1-\hat{H}_S)}$$

Where $\hat{G}_{ST}$ is the genetic divergence between populations and $\hat{H}_S$ is the average intrapopulation diversity, according to Nei (1978). To obtain these estimates, the FSTAT program was used (Goudet 1995). Values close to zero indicate low genetic divergence between

populations and values close to one indicate high genetic divergence among populations.

The apparent gene flow ($\widehat{Nm}$) was estimated indirectly according to the islands model proposed by Crow & Aoki (1984), which corrects the analysis for a small number of populations:

$$\widehat{Nm} = \left(\frac{1}{4\alpha}\right)\left[\left(\frac{1}{\hat{F}_{ST}}\right) - 1\right]$$

Where:

$\hat{F}_{ST}$ is the genetic divergence between populations.

α the correction for number of populations, where $\alpha = [n/(n-1)]^2$;

n is the number of populations.

The estimator $\hat{G}_{ST}$ ' was used instead of $\hat{F}_{ST}$ to know the magnitude of the gene flow carried out and the variation given by these statistics.

The structure of the populations was also evaluated with the Structure 2.3.4 program (Pritchard & Stephens 2000), whose statistical model groups together individuals in Hardy-Weinberg equilibrium and linkage disequilibrium. The number of genetically distinct populations (K) represents the groupings of individuals with different allelic frequencies. The simulations were performed with an estimated number of K from 1 to 6, with five interactions based on the Bayesian model. This analysis considers the separation of the total number of individuals analyzed in clusters, giving a value of K that represents the number of different sets of genes. The Burn-in was 10,000 and the MCMC (Monte Carlo and Markov Chain) was 100,000. The admixture model was used, which assumes that each individual may have ancestors of more than one population, and correlated allelic frequencies among the subpopulations, which increases the probability of grouping closely related populations. The number of population clusters was tested using Structure Harvester software (Earl & Von Hold 2012). The most probable ΔK was estimated according to the Evanno method (Evanno et al. 2005). The individuals were allocated to each cluster according to the probability of each individual belonging to each cluster.

RESULTS AND DISCUSSION

Genetic diversity

This is the first study carried out with the aforementioned species in the southwestern Brazilian Amazon, and demonstrated that the microsatellite sequences developed for *G. angustifolia, Oriza sativa*, and *Saccharum* spp. used in this study were useful for accessing the genetic diversity of the species being studying (Table III). Of the 17 loci used, six were monomorphic (Table III; Fig. 4a), and one (ESTB60) did not amplify (Table III). Ten loci showed polymorphism and were used for genetic diversity studies in this study (Fig. 4b).

According to Maralunda et al. (2007), several species of the *Guadua* genus were also tested using *Oriza sativa* and *Saccharum* spp primers, with a high degree of transferability. Transferability studies using SSR loci of the grass species *Brachypodium distachyon* to a species of the same genus, *Brachypodium hybridum*, were highly informative and deemed a powerful tool for genetic characterization (Neji et al. 2015). For forage species of the Poaceae family, SSR markers developed for *Urochloa humidicola* were transferred to *U. brizantha*, *U. decumbens*, *U. ruziziensis* and *U. dictyoneura*, showing major potential for use in genetic studies as a basis for breeding and conservation (Santos et al. 2015). The conservation of microsatellite sites between related species makes it possible to transfer these markers to other species (Azevedo et al. 2016). By using polymorphic loci, it was possible to obtain a total number of 169 alleles identified in the 250 individuals of the populations of *G.*

Table III. Loci transferred to the species *Guadua* aff. *chaparensis* and *G.* aff. *lynnclarkiae*.

Loci	Species	Ta °C	Allele size (pb)	Amplification	Polymorphism
FJ444930	*G. angustifolia*	52.1	22 - 284	A	P
FJ444929	*G. angustifolia*	52.1	180 - 302	A	P
FJ444932	*G. angustifolia*	55.4	268 - 330	A	P
FJ476075	*G. angustifolia*	55.4	136 - 320	A	P
FJ444934	*G. angustifolia*	60	200	A	M
FJ444931	*G. angustifolia*	52.1	246 - 280	A	P
FJ444936	*G. angustifolia*	52.1	156 - 200	A	P
FJ444935	*G. angustifolia*	55.4	100 - 170	A	P
FJ476076	*G. angustifolia*	52.1	102	A	M
RM31	*Oriza sativa*	45	224	A	M
RM309	*Oriza sativa*	45	156 - 188	A	P
RM332	*Oriza sativa*	52.1	176	A	M
ESTB41	*Saccharum* spp.	52.1	180	A	M
ESTB60	*Saccharum* spp.	48.5	-	NA	-
ESTC45	*Saccharum* spp.	46.8	136 - 224	A	P
ESTC66	*Saccharum* spp.	46.8	96 - 192	A	P
ESTC119	*Saccharum* spp.	46.8	154	A	M

Ta: Annealing temperature A: Amplification; NA: Not amplified; P: Polymorphic; M: Monomorphic.

aff. *chaparensis*, ranging from 6 to 32 alleles per locus, with an average of 16.9 alleles (Table IVa).

The gene diversity expressed by the expected heterozygosity ($\widehat{H}_e$) was high for most of the loci (Table IVa). The average for all loci was 0.80 and the population average was 0.50 (Table V), which can be considered a good diversity value, because it is a species with peculiar reproductive biology (Ramanayake 2006).

The observed heterozygosity values ($\widehat{H}_o$) for most of the loci were low, with an average of 0.43 (Table IVa), indicating a greater number of homozygous individuals in the loci and in the populations. For the species *G.* aff. *lynnclarkiae*, the total number of alleles found for the 100 individuals analyzed was 40 alleles, ranging from 2 to 8 alleles per locus, with an average of 4 alleles per locus (Table IVb). The gene diversity expressed by the expected heterozygosity ($\widehat{H}_e$) was low for most of the loci (Table IVb) and the species average of 0.46 could also be considered a good diversity value, since most loci demonstrated low $\widehat{H}_e$.

The observed heterozygosity values ($\widehat{H}_o$) for the population of *G.* aff. *lynnclarkiae* were variable between the loci and the average for the population was 0.39, indicating a larger number of homozygous individuals in the population, as observed for *G.* aff. *chaparensis*. The average values$\widehat{H}_e$ and $\widehat{H}_o$ found for the populations (Table V) of both species in this study were low, which may also be related to the transferability of the loci, since they are not specific loci, the number of alleles and genetic diversity tend to be lower (Gaiotto et al. 2001).

Studies conducted in Colombia with *G. angustifolia* using Random Amplified Microsatellite (RAM) molecular markers found

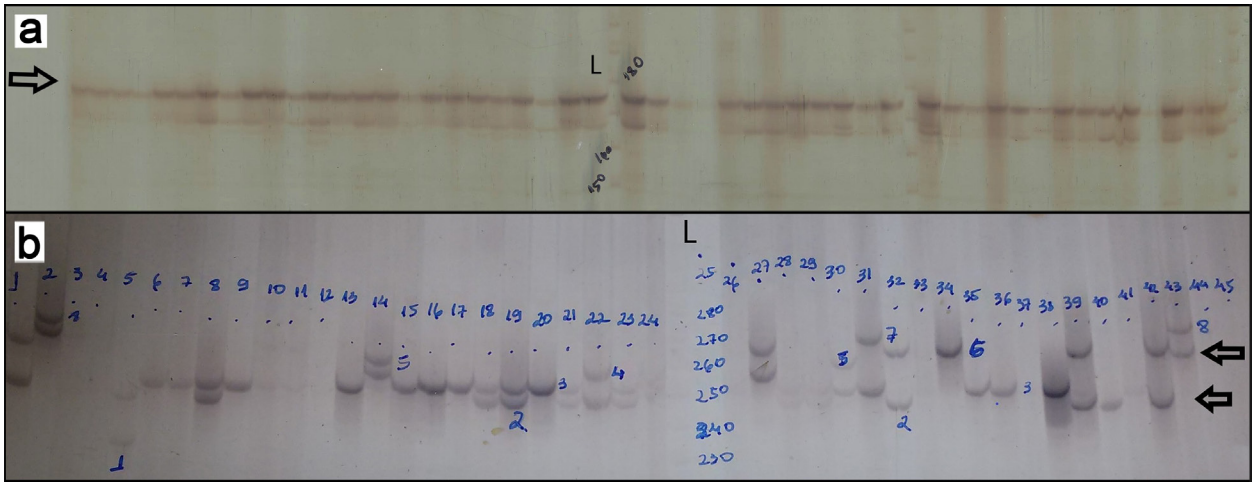

Figure 4. (a) Monomorphic profile of locus RM332 for population 2 of *Guadua* aff. *chaparensis* (L: Ladder 10 pb LifeTechnologies®). The arrow in the image indicates the presence of an allele in all individuals evaluated.). (b) Polymorphic profile of locus FJ444930 for population 3 of *G.* aff. *chaparensis* (L: Ladder 10 pb Life Technologies®). The arrows in the image indicate the presence of two alleles in individual 42).

values for $\widehat{H}_e$ ranging from 0.19 to 0.37 (Rugeles-Silva et al. 2012). In natural populations in China, the species *Dendrocalamus membranaceus* showed a $\widehat{H}_e$ of 0.169 using ISSR markers (Yang et al. 2012). In natural populations in Sri Lanka, the species *Kuruna debilis* exhibited $\widehat{H}_e$ of 0.709 using SSR markers (Attigala et al. 2017).

The effective number of alleles (A_e) was lower than the average number of alleles for all populations analyzed (Table V). The values ranged from 1.73 (P1) to 2.18 (P3). For populations of *G. angustifolia* and *Kuruna debilis*, the A_e varied from 1.56 to 3.56 for the first species, and from 3.33 to 4.75 for the second one (Terranova 2011, Attigala et al. 2017), showing values close to those found in the present study.

The values of Allelic Richness show that populations P5 and P2 exhibited lower allelic richness compared to the other populations, and P3 exhibited the highest allelic richness, which indicates a greater number of alleles in this population compared to the others. For populations of *Kuruna debilis*, the average allelic richness was 4.58 (Attigala et al. 2017). The index of fixation ($\hat{f}$) was rather variable between loci, observing a trend of excess homozygosity within the species (Tables IVa and b). In the populations, it ranged from 0.05 to 0.167 (Table V) and, even with values considered high for certain populations, was not significantly different from zero, indicating that populations are not inbreeding.

The excess of homozygotes may be the result of demographic processes, which may be related to unsynchronized flowering events, aside from the presence of null alleles, as seen for some loci. The high frequency of null alleles was estimated for loci FJ444935 (0.341), ESTC66 (0.377), FJ444931 (0.78), FJ444932 (0.46), and FJ444929 (0.79); the other loci have frequencies below 0.3. For populations of *G. angustifolia*, locus FJ444935 presented a low frequency (0.035) of null alleles (Terranova 2011). The presence of null alleles may occur by the preferential amplification of small alleles, and by genotyping errors due to the presence of stutter (shaded bands or bands resulting from DNA polymerase slippage).

The value of polymorphism information content (PIC) was higher for population P3

Table IV. Characterization of the SSR loci for the populations of *Guadua* aff. *chaparensis* in the municipality of Sena Madureira, AC (a) and *G.* aff. *lynnclarkiae* in the municipality of Porto Acre, AC (b).

***Guadua* aff. *chaparensis* (a)**					
Locos	**N**	**A**	**H_o**	**H_e**	$\hat{f}$
FJ476075	199	29	0.47	0.88	0.47
FJ444935	193	32	0.36	0.87	0.59
FJ444931	242	8	0.12	0.77	0.85
FJ444932	143	12	0.41	0.65	0.38
FJ444929	208	13	0.08	0.73	0.89
FJ444930	235	26	0.63	0.91	0.31
FJ444936	232	16	0.63	0.77	0.19
ESTC66	244	6	0.90	0.72	-0.25
ESTC45	239	12	0.25	0.76	0.67
RM309	240	15	0.45	0.91	0.50
Average	218	16.9	0.43	0.80	$0{,}46^{ns}$ (IC -0.277 – 0.353)
***G.* aff. *lynnclarkiae* (b)**					
Locos	N	A	H_o	H_e	
FJ476075	93	2	0.20	0.18	-0.11
FJ444935	69	5	0.33	0.71	0.53
FJ444931	83	8	0.89	0.80	-0.12
FJ444932	87	4	0.17	0.48	0.64
FJ444929	97	3	0.00	0.17	1.00
FJ444930	98	6	0.73	0.73	0.00
FJ444936	97	2	0.56	0.43	-0.30
ESTC66	94	4	0.26	0.57	0.55
ESTC45	94	3	0.04	0.04	-0.01
RM309	95	3	0.68	0.51	-0.34
Average	90.7	4	0.39	0.46	0.162^{ns} (IC -0.151 – 0.509)

N = Number of individuals sampled; A = number of alleles per locus; $\hat{H}_o$ = Observed Heterozygosity; $\hat{H}_e$ = Expected Heterozygosity; $\hat{f}$ = fixation index; IC = Confidence Interval.

(0.50), indicating that the loci were more highly polymorphic and with higher information content. For *G. angustifolia*, the average value of observed PIC was 0.5 (Terranova 2011). Chen et al. (2010), evaluating several species of bamboo from several genera, observed PIC that ranged from 0.48 to 0.987, with values close to those of the populations studied in the present study.

Spatial genetic structure

The populations of *G.* aff. *chaparensis* exhibited a weak spatial genetic structure at distances up to 500 meters. Population P1 (Fig. 5) has a spatial genetic structuring of up to 400 meters between individuals, being first-degree cousins up to around 200 meters.

Table V. Genetic diversity of populations of *Guadua* aff. *chaparensis* and *G.* aff. *lynnclarkiae* evaluated in the municipalities of Sena Madureira, AC and Porto Acre, AC.

Species	Popul.	N	A	A_e	R_s	PIC	H_o	H_e	$\hat{f}$
G. aff. *chaparensis*	1	90	7.3 (4.9)	1.73	4.96 (2.73)	0.40	0.36 (0.29)	0.42 (0.28)	0.141^{ns}
G. aff. *chaparensis*	2	47	5 (2.8)	2	3.95 (2.66)	0.44	0.49 (0.34)	0.50 (0.18)	0.05^{ns}
G. aff. *chaparensis*	3	38	6.2 (3.92)	2.18	5.85 (3.13)	0.5	0.51 (0.38)	0.54 (0.28)	0.074^{ns}
G. aff. *chaparensis*	4	46	5.4 (3.77)	2.12	5.2 (3.12)	0.48	0.50 (0.21)	0.53 (0.28)	0.167^{ns}
G. aff. *lynnclarkiae*	5	90	4 (1.81)	1.85	3.33 (1.69)	0.4	0.38 (0.28)	0.46 (0.27)	0.162^{ns}

N = Number of individuals sampled; A = average number of alleles per locus; A_e= effective number of alleles; $\hat{H}_o$ = Observed Heterozygosity; $\hat{H}_e$= Expected Heterozygosity; $\hat{f}$ = fixation index; R_S= Allelic richness; PIC: Polymorphism Information Content.

P2 showed individuals correlated as second-degree cousins at a distance of 300 meters. For P3, genetic correlation was low within 500 meters. In P4, at a distance of 500 meters individuals can be considered first cousins (Fig. 4). In these populations, individuals were collected at distances greater than those of P1. Even with the difference in collection methods, there were no major differences in the level of kinship between them. Population P5 of *G.* aff. *lynnclarkiae* showed strong spatial genetic structuring of full siblings up to 75 meters away (Fig. 6). In this population, the individuals were distributed very near, with short distances between most of them.

Genetic structuring is mainly associated with the characteristics of the reproduction system of the species (Loveless & Hamrick 1984). The reproductive biology and ecology of these species can justify the high degree of kinship within some populations. The species of the *Guadua* genus have gregarious flowering, but often the flowering space within and between populations is not synchronized (Ramanayake 2006), leading to a short dispersion of pollen, which in these species is made mainly by wind (anemophily), while seeds and fruits are dispersed by anemochory and zoochory (Janzen 1976, Reid et al. 2004, Lebbin 2007). Dispersion events are responsible for variation within and between populations (Loiselle et al. 1995). More geographically distant individuals, as is the case of individuals of *G.* aff. *chaparensis*, tend to have a lower spatial genetic correlation (Loiselle et al. 1995). In the *G.* aff. *lynnclarkiae* population, individuals are much closer geographically, leading to greater kinship and increased coancestry coefficient (Fig. 6). The dispersing agents of this population, which are birds, rodents, wind and rain, may have dispersed in a restricted way, facilitating the establishment of siblings and cousins. This is generally consistent with the prediction that plant populations often have a spatial genetic structure at short distances (Ennos 2001).

The spatial organization of local populations and their concomitant patterns of gene flow are important factors for a species to become genetically different throughout its geographical distribution. *Oriza officinalis*, which belongs to the same family (Poaceae) as the species under study, exhibited a spatial genetic structure of up to 17 meters, leading to a more clustered distribution of related genotypes (Zhao et al. 2012). For populations of grasses, such as *Triticum diccocoides*, the populations analyzed demonstrated strong spatial genetic structuring at distances up to 20 meters in isolated populations and from 1 to 5 meters in central populations (Volis et al. 2014). For populations

of *Sorghum bicolor*, strong spatial genetic structuring was observed up to 180 km away between cultivated and wild populations in Africa (Mutegi et al. 2011).

Clonal identity

Bamboo species normally have vegetative growth throughout their life cycle, with uncertain flowering and seed production (Janzen 1976, Ramanayake 2006). Even with seed germination, individuals grow by sprouting, and it is often a difficult task to determine who an individual is. Based on the programs for clonal identification and genetic similarity among individuals within the populations of the species under study, two genetically equal individuals were identified in P1 of *G.* aff. *chaparensis*, with a distance of 30 meters between them. Ten individuals with major genetic proximity were identified, among them with an allelic similarity of six to eight loci with equal alleles, with a geographic distance ranging from 66 to 384 meters (Fig. 7a). For P2, ten individuals were identified with major genetic similarity, exhibiting five to nine loci with equal alleles, with the geographical distance between them ranging from 470 to 1220 meters (Fig. 7b).

For P3 and P4 of *G.* aff. *chaparensis*, no clonal individuals were detected. P5 (*G.* aff. *lynnclarkiae*) – because it is another species with greater density and shorter distance between the individuals collected – was the one that showed the largest number of clonal individuals. Twenty-two individuals were identified as clones, such as individuals 20 and 21 (group 1); 19,17 and 22 (group 2); 13 and 14 (group 3); 32, 33, 34, 28 and 29 (group 4); 40 and 44 (group 5); 86, 87, 88, 89, 90, 91, 92 (group 6). Of the 22 individuals, six groups were formed, giving rise to only six genetically distinct individuals. The average distance between the genetically identical individuals was 15 meters between the clumps (Fig. 8). About the similarity between individuals, this population had the highest number of individuals with equal loci and alleles. Twenty-eight individuals had eight to nine loci with equal alleles within this population.

P1 and P5 showed low numbers of clonal individuals, but some individuals with high genetic similarity, such as the population of *G.* aff. *lynnclarkiae*. In the total of 100 individuals collected, 88 are of different genotypes and

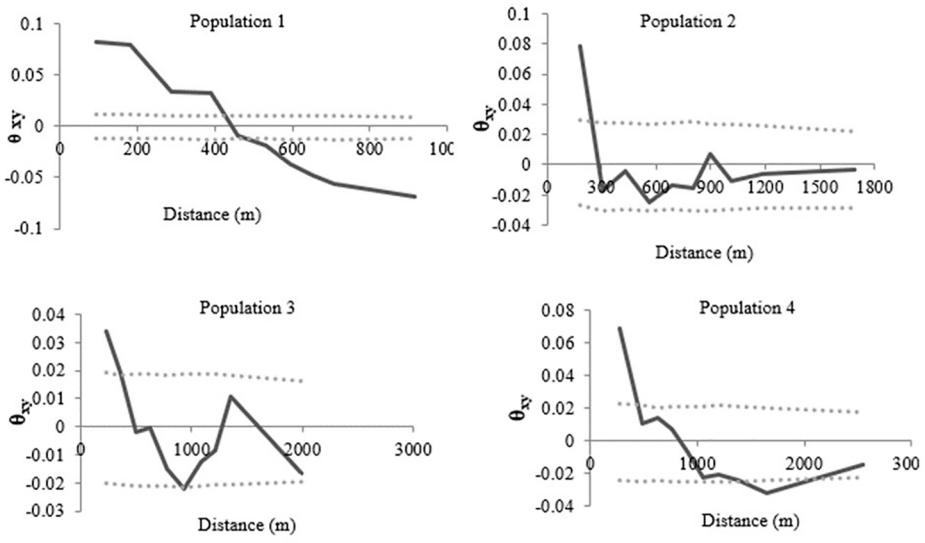

Figure 5. Graphical representation of the coancestry coefficient. Dashed lines correspond to the 95% confidence interval, and solid line shows the coancestry coefficient in populations of *Guadua* aff. *chaparensis*.

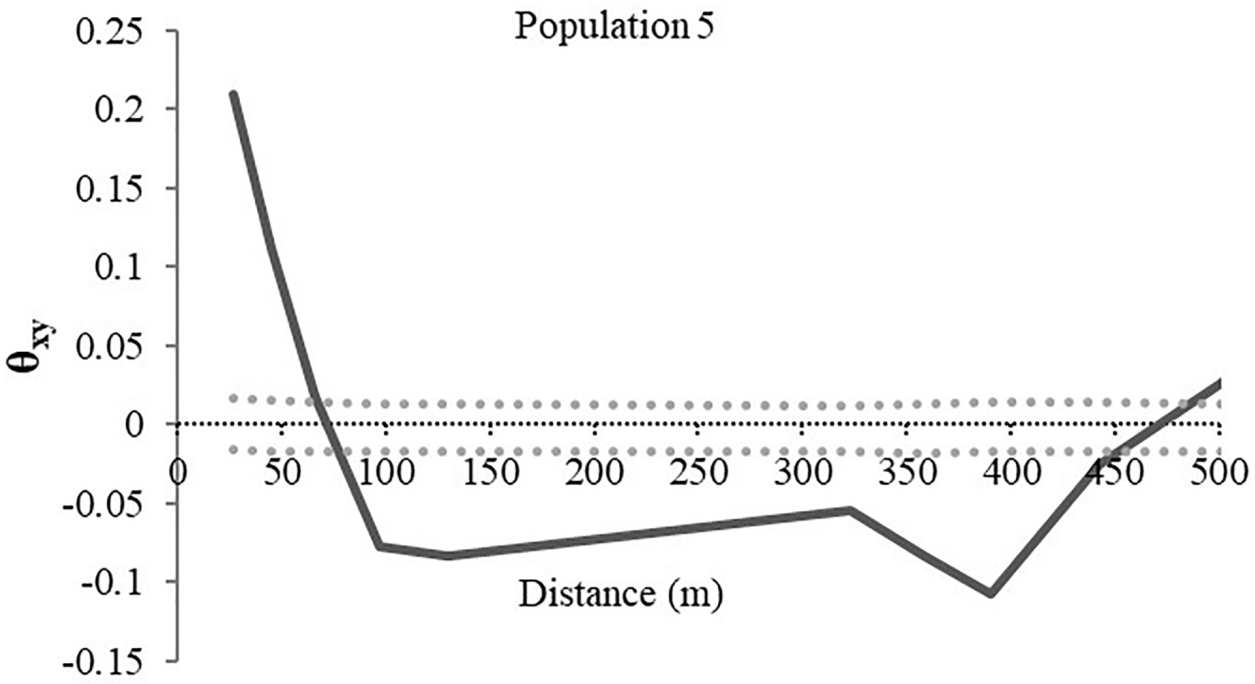

Figure 6. Graphical representation of the coancestry coefficient. Dashed lines correspond to the 95% confidence interval, and solid line shows the coancestry coefficient in the population of *Guadua* aff. *lynnclarkiae*.

out of these, 28 are highly similar. Since the species under study have vegetative growth via pachymorph rhizome (clumping) (Londoño & Zurita 2008), it is common to issue several colms, causing them to be densified in an individual. Bamboos can sustain themselves within the patches for several decades through a habit of rhizomatic growth (Janzen 1976). For these populations, the number of clonal individuals may be considered low compared to other studies, such as the dwarf bamboo species *Sasa kurilensis*, *Sasa palmata* and *Sasa senanensis* – out of the 439 colms in 24 populations analyzed, only 96 were genetically distinct (Mizuki et al. 2014). However, for four populations of *Aulonemia aristulata*, a bamboo species of the Brazilian Atlantic Forest, no clonal individuals were observed (Abreu et al. 2014).

Species of sexually reproducing plants and regenerative systems with vegetative growth, such as bamboo, can ensure the establishment of offspring in new environments asexually – rhizomatous vegetative reproduction – and thereby ensure that genetic diversity is maintained through sexual reproduction (Kitamura & Kawahara 2011).

Genetic structure

The value of $\hat{G}_{ST}$, which is the genetic divergence between populations of *G.* aff. *chaparensis* was 0.46, and $\hat{H}_S$, which is the average intrapopulation diversity, was 0.56. This value indicates a high genetic divergence between populations, even if they are geographically close. Through the use of satellite images, it was possible to see death events in the populations of *G.* aff. *chaparensis*. The events began in 1994 and ended in 1995 (Fig. 9a and b). These images may help in the understanding of such high genetic divergence, since it was possible to see that for

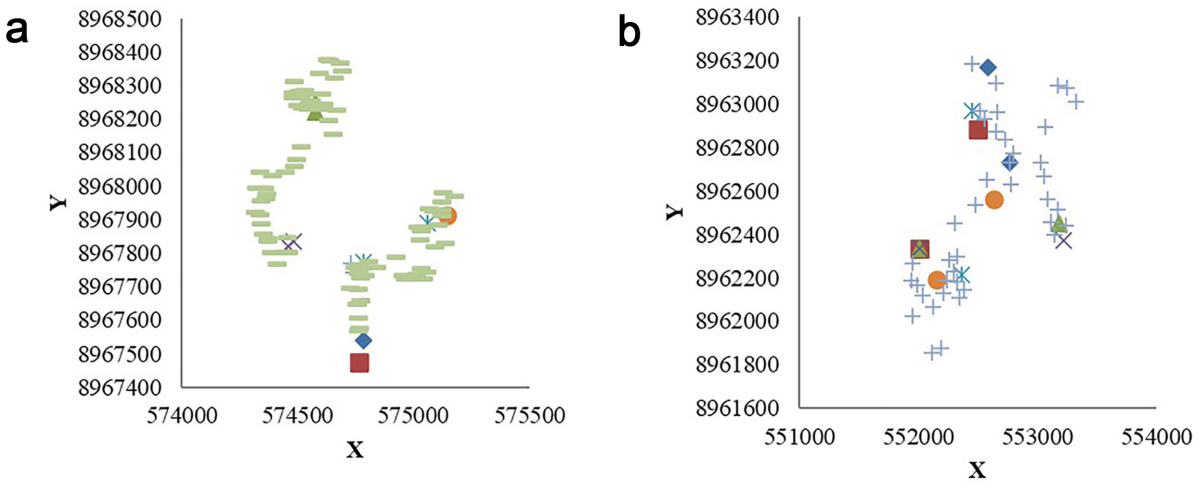

Figure 7. Geographic distribution of clonal individuals within populations 1 (a) and 2 (b) of *Guadua* aff. *chaparensis*. The Y and X axes represent the geographical coordinates; (a) the light-green dots (–) represent the non-clonal individuals (b) the light-blue dots (+) represent non-clonal individuals.

these populations, the death event occurred at different periods, being unsynchronized flowering. Other factors related to fragmentation, restricted gene flow, and mutations may also have interfered with the high divergence. For a wide range of plant species, the crossbreeding system affects patterns of genetic variation between and within populations (Maguire et al. 2000). For other bamboo species, such as *Dendrocalamus membranaceus*, *G. angustifolia*, *Dendrocalamus giganteus* and *Phyllostachys edulis*, values of 0.252, 0.185, 0.84 and 0.162 were found, respectively (Terranova 2011, Yang et al. 2012, Tian et al. 2012, Jiang et al. 2017), indicating moderate to high divergence values, which was also found in the present study.

The estimated gene flow ($\widehat{Nm}$) among populations of *G.* aff. *chaparensis* was 0.522, indicating the migration of one individual every two generations. Values lower than one reveal a low gene flow and may indicate differentiation in populations and genetic isolation (Slatkin 1985). For values higher than 1, low differentiation is expected between populations, by drift or selection (Wright 1931, Slatkin & Barton 1989). Considering that the populations have a life cycle of approximately 30 years, and because this is a species that rapidly becomes established, the historical gene flow may be related to past events of synchronized flowering, such as the results of gene exchanges during the generations when the populations were possibly interconnected by practically continuous forests (Kageyama et al. 2003).

The Bayesian analysis of the Structure program showed that there are 5 defined groups within the 347 individuals analyzed (Fig. 10) (K=5) demonstrating little similarity between the groups (populations). This analysis confirms the existence of genetic divergence and the formation of five gene pools between the populations, showing a clear difference between the species *G.* aff. *chaparensis* and *G.* aff. *lynnclarkiae*. Within the groups formed, little information is shared between them, mainly concerning P5 (Fig. 10). Differentiation between geographically close populations suggests that they were geographically isolated at some point in the past, and/or the gene flow between them is restricted at present (Aboukhalid et al. 2017). The

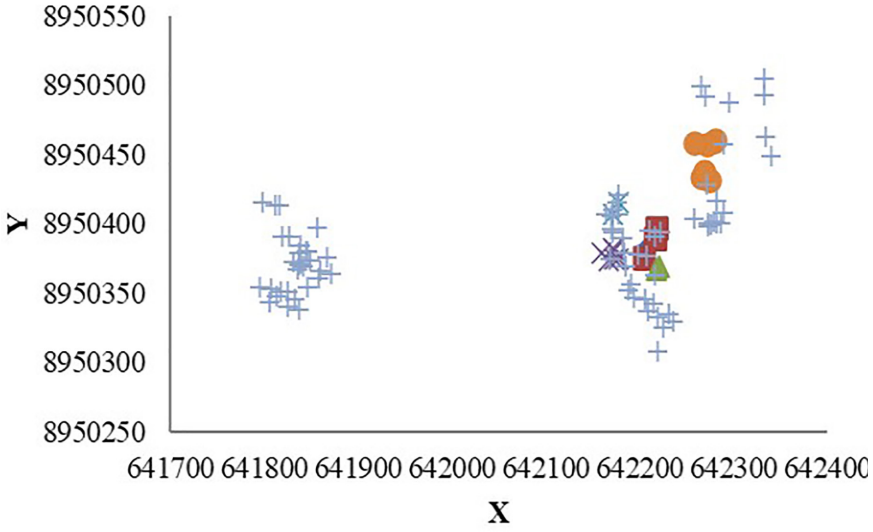

Figure 8. Geographic distribution of individuals with high genetic similarity within population 5 (*Guadua* aff. *lynnclarkiae*). The Y and X axes represent the geographical coordinates; the blue dots (+) represent non-clonal individuals.

analysis has led to the belief that there is little connectivity between these populations. One of the barriers of gene flow among the populations of *G.* aff. *chaparensis* is the uncertain flowering, which results in the difference between the flowering events (sporadic flowering), as seen in 1994 and 1995 for these populations and the low pollen dispersion caused by the scarcity of flowering (McClure 1966, Tian et al. 2012). Another factor involved is fragmentation due to the presence of a geographical barrier, Highway BR 364, which divides the populations into two parts. Moreover, deforestation and conversion of forest land into pasture are other major factors. These results may aid in the *in situ* conservation of each population (Martins et al. 2015). Each gene pool must be conserved to maintain genetic diversity within and between populations. With these analyses, it was possible to observe that part of the bamboo patch in which the populations of *G.* aff. *chaparensis* are located is not homogeneous and must be conserved as such, each population having its own genetic characteristics. The same holds true for the population of *G.* aff. *lynnclarkiae*.

CONCLUSION

Transferability was an efficient tool, and the loci used from other species resulted in good genetic diversity values within populations. The genetic divergence found among the populations clearly showed little connectivity between them. This is not normally expected for populations of the same species with short geographic distances. Both species exhibited spatial genetic structure. Geographically close individuals are genetically correlated. Some clonal individuals were found in both species, but only in the larger populations (P1 and P5), which shows that in these bamboo massifs that make up these natural populations, most individuals come from the germination of seeds and not from vegetative growth, as was expected. In general, the populations were quite distinct, with little sharing of genetic information between them. This may be a genetic trait, as flowering in unsynchronized waves may interfere directly. With these results, it is possible to conclude that the populations should be treated as areas with unique genetic characteristics, mainly for *in situ* and *ex situ* management and conservation.

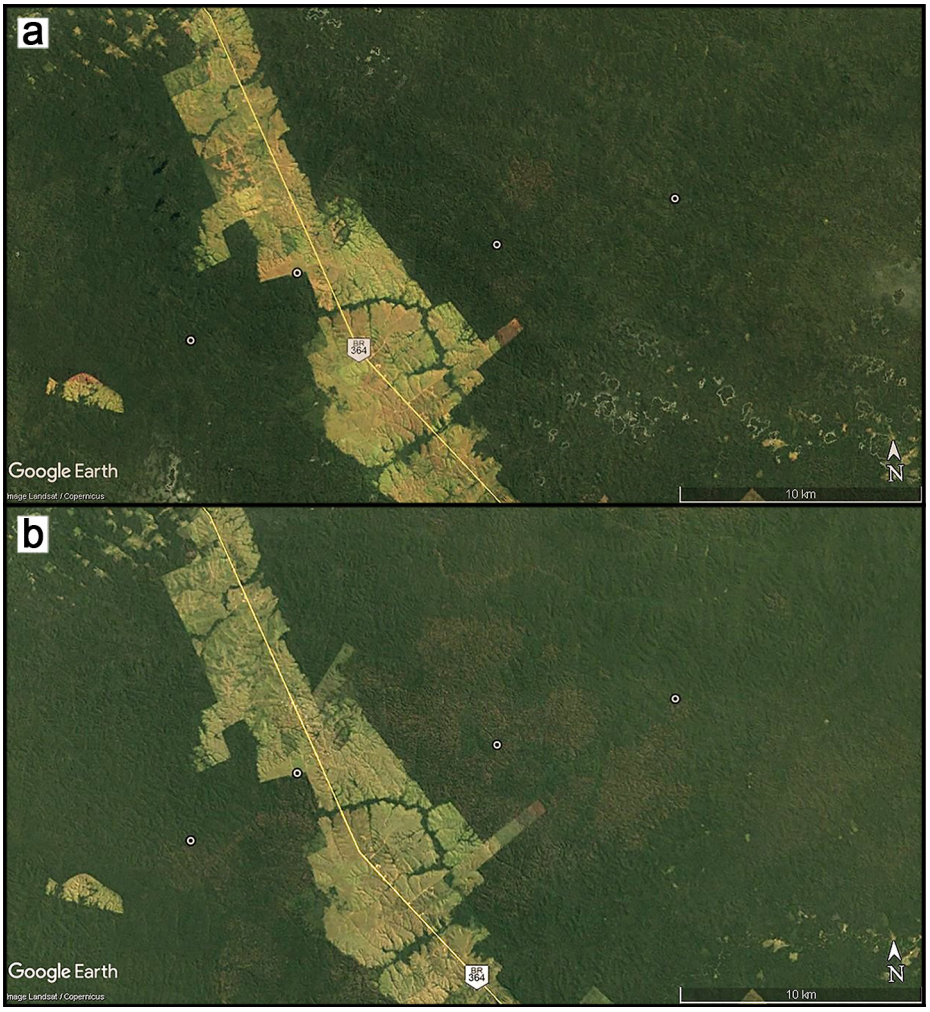

Figure. 9. The dots in the images represent the populations, showing populations P2, P3, P4 and P1 from left to the right, respectively. (a) Onset of mortality in the area of P1 and P4 of *Guadua* aff. *chaparensis*, in the municipality of Sena Madureira, Acre, Brazil, in 1994) (b) Continuation of mortality in P1 and P4, and onset of mortality in P2 and P3, in the municipality of Sena Madureira, Acre, Brazil, in 1995. The lighter spots in the image indicate the mortality of bamboo in the scenario.

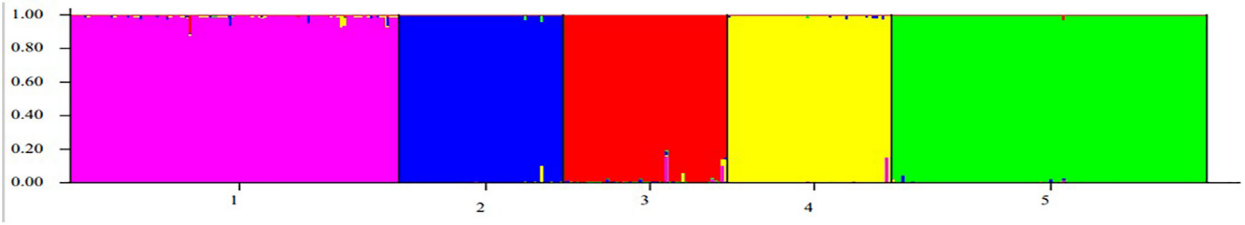

Figure 10. Representation of the populations of *Guadua* aff. *chaparensis* and *G.* aff. *lynnclarkiae* using the Structure 2.3.4 program. Each color represents a population, Pink: P1; Blue: P2; Red: P3; Yellow: P4 and Green: P5 (*G.* aff. *lynnclarkiae*).

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All authors contributed with the discussion and writing original draft. Susana M. M. Silva performed the literature review, experiments, data acquisition, data analysis and wrote the first version. Tatiana de Campos, Karina Martins and Jonny E. Scherwinski-Pereira contributed in the design of methodology, data analysis and manuscript review. Susana M. M. Silva and Frederico H. S. Costa collected the plant material.