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Population genetic structure of three species in the genus *Astrocaryum* G. Mey. (Arecaceae)

N.P. Oliveira[1], M.S.P. Oliveira[2], L.C. Davide[1] and S. Kalisz[3]

[1]Laboratório de Citogenética, Universidade Federal de Lavras, Lavras, MG, Brasil
[2]Laboratório de Genética Molecular, Embrapa Amazônia Oriental, Belém, PA, Brasil
[3]Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN, USA

Corresponding author: N.P. Oliveira
E-mail: naty_poliveira@hotmail.com

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ABSTRACT. We assessed the level and distribution of genetic diversity in three species of the economically important palm genus *Astrocaryum* located in Pará State, in northern Brazil. Samples were collected in three municipalities for *Astrocaryum aculeatum*: Belterra, Santarém, and Terra Santa; and in two municipalities for both *A. murumuru*: Belém and Santo Antônio do Tauá and *A. paramaca*: Belém and Ananindeua. Eight microsatellite loci amplified well and were used for genetic analysis. The mean number of alleles per locus for *A. aculeatum*, *A. murumuru*, and *A. paramaca* were 2.33, 2.38, and 2.06, respectively. Genetic diversity was similar for the three species, ranging from H_E = 0.222 in *A. aculeatum* to H_E = 0.254 in *A. murumuru*. Both F_{ST} and AMOVA showed that most of the genetic variation was found within populations for all three species, but high genetic differentiation among populations was found for *A. aculeatum*. Three loci were not in Hardy-Weinberg equilibrium, with populations of *A. paramaca* showing a tendency for the excess of heterozygotes (F_{IS} = -0.144). Gene flow was

high for populations of *A. paramaca* (N_m = 19.35). Our results suggest that the genetic diversity within populations followed the genetic differentiation among populations due to high gene flow among the population. Greater geographic distances among the three collection sites for *A. aculeatum* likely hampered gene flow for this species.

Key words: Genetic diversity; Gene flow; Microsatellite markers; Amazonia

INTRODUCTION

The palm genus *Astrocaryum* G. Mey. (Arecaceae) comprises 40 species with primary distributions in tropical ecosystems of South and Central America (Kahn, 2008). The genus is comprised of many life forms, from large woody species, such as *A. aculeatum* G. Mey. and *A. murumuru* Mart., to small acaulescent palms, such as *A. paramaca* Mart. Because they produce fruits throughout the year, like other species in the Arecaceae, *Astrocaryum* spp are considered key economic species in the environments where they occur (Dransfield et al., 2008).

Although almost all species of *Astrocaryum* are used by humans, according to Kahn (2008) only a few have real economic potential and significant importance in local and regional trades. Of these, *A. aculeatum* stands out due to its abundant fruits that are widely consumed by humans in the Amazon region and to its potential to become an important crop, especially in North of Brazil (Clement et al., 2005; Kahn, 2008). Likewise, *A. murumuru* has been suggested by Clement et al. (2005) as an important palm for the cosmetics industry because of the high quality of its oil. In contrast with the large literature available on these two palm species, there is not much information on *A. paramaca*. However, because *A. paramaca* does not produce a central stem, its low stature could facilitate fruit harvest and could be useful in crossbreeding programs.

There are few areas with *Astrocaryum* plantations, and these only grow *A. aculeatum.* Fruit harvesting of both *A. aculeatum* and *A. murumuru* is based on the exploitation of natural populations (Clement et al., 2005; de Macêdo et al., 2015). Ferreira and Gentil (2006) suggest that difficulty in seed germination due to seed dormancy and, in the case of *A. aculeatum*, the inability to use vegetative propagation, contribute to a lack of interest by farmers to develop these species as crops. However, the absence of adequate management strategies for any species can have a great impact on the genetic diversity of its populations and therefore compromise its long-term survival (Booy et al., 2000).

The genetic diversity of a species can be assessed by quantifying morphological characters and biochemical or molecular markers. Of these, molecular markers offer numerous advantages over the other two types. First, they are stably expressed and detectable in all tissues regardless of growth and cell differentiation (Hamrick et al., 1992; Mondini et al., 2009). Moreover, molecular markers are not influenced by the environment, as are morphological traits (Hamrick et al., 1992). Importantly, microsatellites are especially good for studies of population genetic structure because they are highly polymorphic and allow for the final scale-resolution of population genetic parameters.

Studies of genetic diversity in palm species have been increasing in the last decades and, in most of them, the level of genetic diversity was high, with the percentage of polymorphic loci ~100%, the number of alleles per locus greater than four, and heterozygosity

higher than 0.5 (e.g., Conte et al., 2003; Meerow et al., 2003; Choo et al., 2010; Gaiero et al., 2011; Gomes et al., 2011). Other population genetic structure parameters, such as Wright's inbreeding coefficient and gene flow, have shown that, in general, genetic differentiation (F_{ST}) is low, indicating genetic variation higher within - rather than among - populations and a high gene flow among populations (Elshibli and Korpelainen, 2008; Martins-Corder et al., 2009; Shapcott et al., 2009; Namoff et al., 2011; Silva et al., 2011; Giovino et al., 2014; Nazareno and dos Reis, 2014; Oliveira et al., 2014; Moura et al., 2015). Recently, Ramos et al. (2016) found a high level of genetic diversity, mostly within populations, for adults and seedlings in populations of *A. aculeatum*, and along with results for spatial genetic structure indicated short-distance seed dispersal and modest levels of pollen flow. To date, there is no information regarding the level and distribution of genetic diversity for *A. murumuru* and *A. paramaca*, which is needed to evaluate the potential of them as potential crop species. Here, we present molecular genetic data to evaluate the level and distribution of genetic diversity within and among natural populations of *A. aculeatum*, *A. murumuru*, and *A. paramaca*.

MATERIAL AND METHODS

Study species, sampling sites, and plant material

A. aculeatum is a large palm growing up to 35 m, *A. murumuru* is classified as a stemmed palm with a height up to 10 m, and *A. paramaca* is an acaulescent species (Kahn, 2008). Both *A. aculeatum* and *A. paramaca* have single stem habit, whereas *A. murumuru* is multi-stemmed. All three species are monoecious, perennial, produce fleshy fruits containing a single seed, and their inflorescences are composed of separate male and female flowers distributed in dyads and triads (Kahn, 2008). The mating system of *A. aculeatum* was classified as outcrossing (Ramos et al., 2011). To date, there is no information on the mating system of the other two species studied here. All three species have tropical distribution with common occurrence in Brazil, Guyana, and Suriname. *A. murumuru* and *A. paramaca* are also found in French Guiana, while *A. aculeatum* occur in Bolivia, Trinidad, and Venezuela (Kahn, 2008). In Brazil, these species are restricted to the Amazon region, in the northern part of the country (Leitman et al., 2015). Only *A. aculeatum* and *A. murumuru* have economic importance in the Amazon region, the first due to its edible fruits largely consumed in Amazonas State, and the second for its oil utilized in the national cosmetic industry (Clement et al., 2005).

We carried out sampling expeditions in three municipalities for *A. aculeatum* - Belterra (2°41'54"S and 54°53'18"W, 146 m above sea level, asl), Tapajós National Forest, in Santarém (2°26'22"S and 54°41'55"W, 43 m asl) and Terra Santa (2°6'16"S and 56°29'15"W, 19 m asl) - and two municipalities for both *A. murumuru* - Cotijuba Island (1°13'04"S and 48°32'44"W, 10 m asl) in Belém and Santo Antônio do Tauá (1°9'9"S and 48°7'45"W, 24 m asl) - and *A. paramaca* - Experimental fields located in Belém (1°27'18"S 48°30'9"W, 6 m asl) and Ananindeua (1°21'59"S and 48°22'20"W, 2 m asl) - all in Pará State, Brazil (Figure 1). According to Köppen's classification, the climate in Belterra, Santarém, and Terra Santa is type Ami, Equatorial monsoon, with a minimum temperature higher than 18°C and variation of less than 5°C, and annual precipitation of 1820 mm. The climate in Belém, Santo Antônio do Tauá, and Ananindeua is classified as Afi, Equatorial rainforest (fully humid), differing from the Ami only in the annual precipitation at ~2800 mm (Kottek et al., 2006).

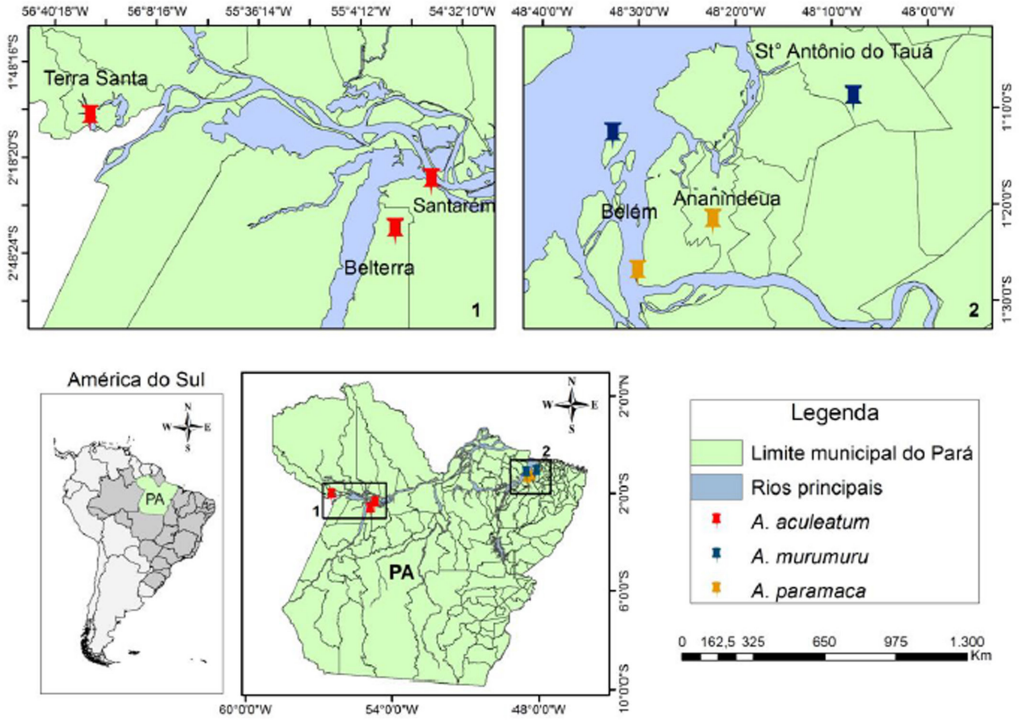

Figure 1. Geographic coordinates of sampling sites of plant material for *Astrocaryum* species in Pará State.

For the genetic diversity and population structure analyses, we collected leaves from a minimum of 20 randomly chosen adult individuals in each site. Plant material was kept on ice and transported to the Laboratory of Molecular Genetic at EMBRAPA Western Amazon, Belém, Pará, where they were stored at -20°C until DNA extraction.

DNA extraction and PCRs

For DNA extraction, leaves stored were rinsed in a 10% sodium hypochlorite solution for approximately 1 min. Total genomic DNA of each was extracted using ~100 mg leaf tissue following Costa and Oliveira (2002). Thirteen microsatellite markers developed by Ramos et al. (2012) were tested and PCR conditions were adapted for duplex and triplex reactions (**Table S1**). Combinations of multiplexes are shown in **Table S1**. PCR mix (11 µL) contained 10 ng total genomic DNA, 1.4X PCR buffer, 1.5 mM $MgCl_2$, 0.8 µg BSA, 200 µM of each dNTP, 0.16 µM forward and M13 labeled primer (NED, FAM, and VIC), 0.32 µM reverse primer, 0.4 U Taq (5 U/µL), and milli-Q water to complete the final volume. The fragment amplifications were performed using the GeneAmp PCR System 9700 from Applied Biosystems and Mastercycler Ep gradient on Eppendorf thermal cyclers. The PCR programs were composed by two steps. The first step consisted of denaturation (68°C for 2 min and 94°C for 30 s), followed by 30 cycles [30 s at 92°C, 35 s at the primer combination-specific annealing temperature (**Table S1**), and 30 s at 68°C]. The second step consisted of 15 cycles

(30 s at 92°C, 30 s at 53°C, and 30 s at 72°C), and a final extension at 72°C for 30 min as suggested by Ramos et al. (2012).

After PCRs had been completed, plates were prepared to contain a mix of 0.5 to 2 µL of three different multiplex products labeled with different dyes, 0.2 µL Liz 500 size standard and 10 µL Hi-Di Formamide and submitted to electrophoresis in an automated ABI 3730xl Genetic Analyzer to determine the size of the DNA fragments obtained.

Data analysis

For population genetic diversity analyses, mean number of alleles per locus (N_A), mean effective number of alleles (N_E), which is the number of alleles equally frequent needed to obtain the same level of homozygosity found for the real population, percentage of polymorphic loci (P%), expected heterozygosity (H_E), observed heterozygosity (H_O), and fixation index (F) were estimated. Estimates were obtained for each locus and means for each population and species calculated based on the average over all loci. For population genetic structure analysis, Wright F-statistics and gene flow (N_m) were computed. Deviation from Hardy-Weinberg equilibrium (HWE) was assessed for each locus, in each population, for each species using the chi-square test. Analysis of molecular variance (AMOVA) among the populations of each species was performed with two hierarchical levels (among and within populations). Principal coordinate analysis (PCoA) was performed among all individuals, for each species, after conversion of the individual-by-individual genetic distance matrix to a covariance matrix and data standardization. When applicable, a Mantel test was conducted to estimate the correlations between genetic and geographic distances. The above analyses were all conducted using the GenAlEx v.6.501 package software (Peakall and Smouse, 2006, 2012).

The assignment of genotypes to genetic clusters for each species was assessed by a Bayesian cluster analysis using the Structure 2.3.3 software (Pritchard et al., 2000). The Structure software identifies genetic clusters and assigns genotypes to those clusters without a priori information on their geographical origin. Both the admixture model and the independent allele model were run. Following a burn-in period of 100,000 runs, ten independent runs were carried out for each value of K (from 1 to 7) with 100,000 repeats. The choice of the most likely number of clusters (K) was carried out by calculating the statistics ΔK, which is based on the rate of change in the log probability of the data between successive K values, as described by Evanno et al. (2005) using Structure Harvester (Earl and Von Holdt, 2011). Among the 10 runs per K, the one with the highest maximum likelihood was used to assign individual genotypes to clusters.

RESULTS

Genetic diversity

The test for HWE suggests the occurrence of assortative mating, especially in *A. paramaca* populations. The chi-square test values for populations of the three species studied are shown in **Table S2**. Significantly deviation from HWE was found for three loci, Aac04 in populations 1 and 2 of *A. aculeatum* and populations 2 of *A. murumuru* and *A. paramaca*; Aac06 deviated from HWE in populations 1 and 2 of *A. aculeatum* and in population 1 of *A.*

murumuru and population 2 of *A. paramaca*; and Aac12 deviated from HWE in population 3 of *A. aculeatum* and both populations of *A. murumuru* and *A. paramaca*.

Eight of the thirteen primers tested produced positive peaks allowing their fragment sizing. Considering all three species together, most primers were polymorphic. The most polymorphic primers, across all populations of the three species, were Aac04, Aac06, and Aac12 (**Table S3**). However, one (Aac13) was monomorphic across all species, and two were monomorphic in some species (Aac02 in *A. aculeatum* and *A. murumuru*; Aac11 in *A. paramaca*). The total number of alleles varied among species, ranging from 18 in populations of *A. paramaca* to 24 for *A. aculeatum* (**Table S3**).

The average P%, for all three species, varied from 62.5% for *A. aculeatum* to 75% for *A. murumuru* (Table 1). The mean N_A ranged from 1.75 to 2.88 and the mean N_E ranged from 1.32 to 1.85; all these values found for populations 1 and 2 of *A. aculeatum*, respectively. When the average among populations within species was considered, *A. aculeatum* and *A. murumuru* showed the highest value and similar estimates for both mean N_A and N_E (Table 1). Estimates of H_O and H_E were higher for populations 1 of *A. paramaca* and *A. aculeatum*, respectively. The mean H_O and H_E were higher for *A. paramaca* with 0.285 and *A. murumuru* with 0.254, respectively (Table 1). Populations 2 and 3 of *A. aculeatum*, 1 and 2 of *A. paramaca*, and population 2 of *A. murumuru* showed values of H_O higher than the H_E. The *F*, equivalent to F_{IS}, varied from positive and negative among populations, being statistically different from zero for populations 1 and 3 of *A. aculeatum*, populations 1 and 2 of *A. paramaca* and population 2 of *A. murumuru*. When means for species are considered, only *A. paramaca* showed a negative *F*, significantly different from zero, suggesting a tendency for the excess of heterozygotes and other species that did not statistically deviate from HWE (Table 1).

Table 1. Mean estimates of genetic diversity parameters and fixation index (*F*) estimated on eight loci (standard deviation) for adult individuals from natural populations of three species of *Astrocaryum*.

Species/Pop	N	N_A	N_E	P (%)	H_E	H_O	*F* (SD)
A. aculeatum							
Belterra	27	2.875	1.852	62.5	0.313	0.239	0.149 (0.133)
Santarém	19	1.750	1.322	62.5	0.175	0.185	0.163 (0.194)
Terra Santa	30	2.375	1.369	62.5	0.179	0.221	-0.139 (0.083)
Mean	25.333	2.333	1.514	62.5	0.222	0.215	0.058
SD	1.013	0.322	0.159	0	0.052	0.055	0.082
A. murumuru							
Cotijuba Island	27.375	2.500	1.568	75	0.239	0.178	0.132 (0.130)
St. Ant. do Tauá	30.375	2.250	1.542	75	0.269	0.272	-0.060 (0.124)
Mean	28.875	2.375	1.555	75	0.254	0.225	0.036
SD	0.576	0.287	0.182	0	0.062	0.060	0.089
A. paramaca							
Belém	28.625	2.125	1.500	75	0.244	0.291	-0.123 (0.061)
Ananindeua	30	2.000	1.453	62.5	0.206	0.279	-0.154 (0.145)
Mean	29.313	2.063	1.477	68.75	0.225	0.285	-0.137
SD	0.198	0.232	0.164	6.25	0.060	0.086	0.072

N is the number of adult individuals used for the parameters estimated; N_A is the mean number of alleles per locus; N_E is the effective number of alleles; H_E is the expected heterozygosity; H_O is the observed heterozygosity; and *F* is the fixation index (standard error for each population) and calculated as $F = (H_E - H_O) / H_E$, representing the within-population inbreeding.

Genetic structure and gene flow

Mean values for Wright's F-statistics provide insight into the distribution of

genetic variation from individuals to among populations (Table 2). The mean reduction of heterozygosity within individuals in a subpopulation, F_{IS}, was due to non-random mating. F_{IS} was negative and statistically different from zero only for populations of *A. paramaca*, indicating an excess of heterozygotes. Our relatively low estimates of F_{ST} for all three species examined indicated that populations are not differentiated genetically.

In fact, both Wright's inbreeding coefficient and AMOVA for *A. paramaca* pointed to genetic differentiation among populations of only 1%. *A. aculeatum* and *A. murumuru* showed higher percentages of genetic differentiation among populations with AMOVA (Table 2). Estimates of *N*m varied significantly among species, ranging from 2.57 for *A. aculeatum* to 19.35 for *A. paramaca*, suggesting a high level of migration constituted of pollen and seed dispersal, for *A. paramaca*. The low level of *N*m found especially for *A. aculeatum* helps explain the higher degree of genetic differentiation among populations of this species (Table 2).

Table 2. Estimates of Wright's F-statistics, N_m, and partitioning of genetic variation (standard deviation) across the eight microsatellite loci for three species of *Astrocaryum* calculated using the GenAlEx v.6.501 package software (Peakall and Smouse, 2006, 2012).

Statistics	Species		
	A. aculeatum	*A. murumuru*	*A. paramaca*
F_{IS}	0.029 (0.115)	0.033 (0.123)	-0.144 (0.089)
F_{IT}	0.107 (0.114)	0.065 (0.131)	-0.130 (0.090)
F_{ST}	0.089 (0.014)	0.053 (0.031)	0.013 (0.007)
*N*m	2.070 (0.843)	22.641 (14.231)	70.527 (28.277)
AMOVA			
Variation among populations	19%	13%	1%
Variation within populations	81%	87%	99%

F_{IS} is the inbreeding coefficient calculated within populations; F_{IT} is the overall inbreeding coefficient related to the total sample; F_{ST} is the genetic differentiation among populations; N_m is the gene flow and estimates the proportion of migrants entering a population per generation. The results of AMOVA are percentages based on 999 permutations.

The Bayesian analysis of Structure 2.3.3 assigned genotypes to two populations only (K = 2), for all three species analyzed of *Astrocaryum* (Figure 2). In the case of *A. aculeatum* (Figure 2A), the number of genetic clusters found (K) was lower than the number of sampled populations, which suggests that populations may reflect a common evolutionary lineage (i.e., subdivision from a single large population) or historically high levels of N_m. The occurrence of genotype admixture is also noted for populations of *A. murumuru* and *A. paramaca* (Figure 2B and C, respectively), with *A. paramaca* individuals sharing around 50% of their genomes. This result can be explained by the high gene flow among populations in the genetic structure analysis for this species (Table 2).

According to the PCoA analysis performed for *A. aculeatum*, the first principal coordinate explained 72.41% of the genetic variance, and the second principal coordinate explained 27.59%, totalizing together 100% of the genetic variance, and population 3, Terra Santa, was more genetically divergent from the other two populations (Figure 3).

The Mantel test for three populations of *A. aculeatum* suggests that isolation by distance can influence the genetic divergence among the populations sampled (Figure 4).

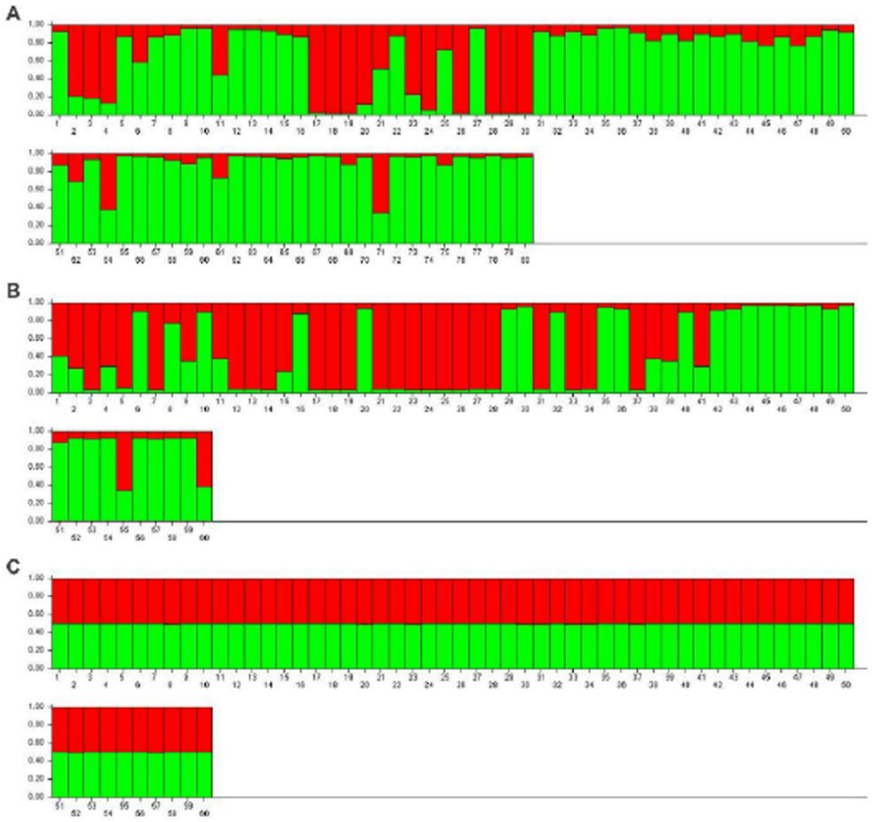

Figure 2. Assignment of samples of three *Astrocaryum* species by Structure version 2.3.3. Each bar represents a different genotype. **A.** *A. aculeatum*; **B.** *A. murumuru*; **C.** *A. paramaca*.

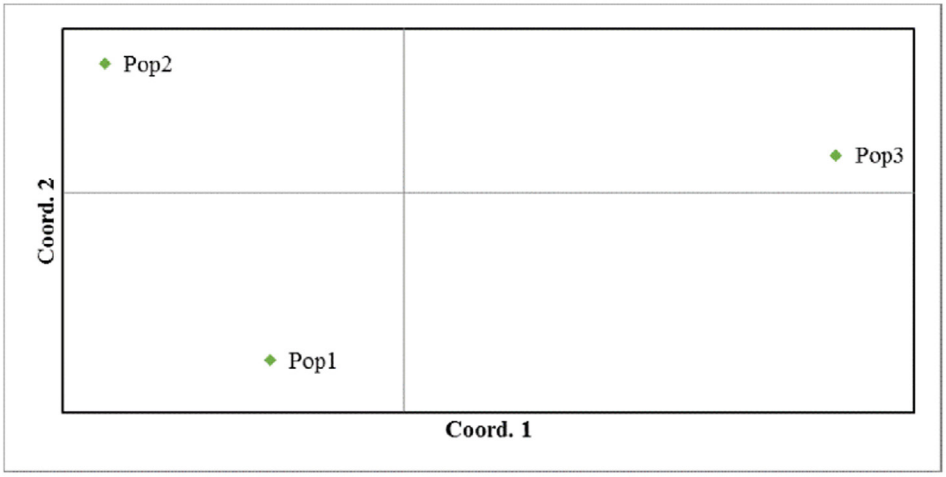

Figure 3. Principal coordinate analysis of the three populations of *Astrocaryum aculeatum*.

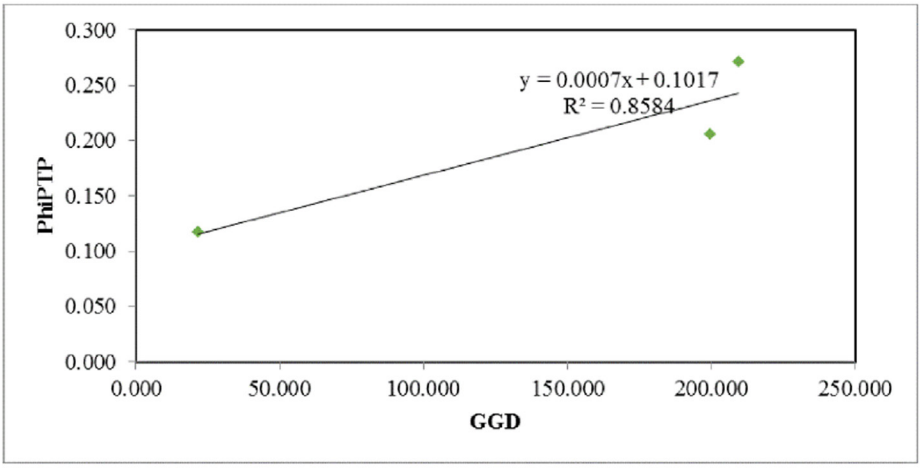

Figure 4. Mantel test for correlation between genetic distance (PhiPTP) and geographic distance (GGD) for populations of *Astrocaryum aculeatum*.

DISCUSSION

The number of alleles per locus found for the populations of *A. aculeatum* and *A. murumuru* species differed from that published by Ramos et al. (2012) (Table 3). For *A. aculeatum*, we found more monomorphic loci while for *A. murumuru* we found more polymorphism than the previous report, likely because our sample size was much higher. This is the first report for *A. paramaca*, and the number of alleles was similar to that of *A. murumuru* found in our study. Besides, samples from the two studies were collected in different states, and it is worth noting that some unique alleles for some loci (e.g., Aac01, Aac02, and Aac04) were found for *A. aculeatum*. The occurrence of these private alleles and the higher level of polymorphism were expected because our study included more sampling sites, a greater number of individuals/site, and a greater geographic extent sampled.

Table 3. Comparison of the number of alleles and allele size per locus found for species by Ramos et al. (2012) and in the present study.

Locus	*A. aculeatum*	*A. murumuru*	*A. aculeatum*	*A. murumuru*	*A. paramaca*
	Ramos et al. (2012)		Present study		
Aac01	2 (332-362)	1 (338)	1 (354)	2 (339-354)	2 (342-354)
Aac02	2 (282-342)	1 (291)	1 (306)	1 (306)	2 (298-306)
Aac03	3 (137-167)	1 (145)	3 (157-181)	2 (157-162)	2 (157-162)
Aac04	6 (212-242)	4 (207-227)	7 (207-274)	3 (228-258)	4 (231-263)
Aac06	8 (132-192)	5 (142-162)	6 (141-196)	5 (134-197)	3 (143-156)
Aac11	2 (202-228)	1 (205)	2 (201-223)	3 (215-252)	1 (221)
Aac12	5 (167-181)	4 (158-178)	3 (163-195)	5 (172-205)	3 (179-195)
Aac13	3 (182-202)	2 (182-206)	1 (204)	1 (204)	1 (204)
N	40	4	80	60	60

N is the total number of individuals sampled. Allele ranges (bp) are given in parentheses.

The percentage of polymorphic loci found for the three *Astrocaryum* species was lower than that reported in other studies involving other palm species and using SSR (Conte et al., 2008; Elshibli and Korpelainen, 2008; Choo et al., 2010; Namoff et al., 2011; Ottewell et

al., 2012; Giovino et al., 2014; Lanes et al., 2015; Nazareno and dos Reis, 2014). An extremely low value of polymorphism (6.17%) was reported for *Livistona carinensis*, an endangered palm species (Shapcott et al., 2009). However, given the wide geographic distribution of the palm species investigated in our study, we would have expected higher levels of polymorphism. Even wider geographic sampling is necessary to determine the level of polymorphism for *A. aculeatum*, *A. murumuru*, and *A. paramaca*.

Levels of genetic diversity (H_E and H_O) found for the species of *Astrocaryum* presented here were also lower than most of the literature available for other palm species using microsatellite markers, which showed a minimum estimate for H_E of 0.5 (Conte et al., 2008; Elshibli and Korpelainen, 2008; Choo et al., 2010; Namoff et al., 2011; Ottewell et al., 2012; Nazareno and dos Reis, 2014; Ramos et al., 2016). Nevertheless, similar results for H_E and H_O were found for *Chamaerops humilis*, with a mean N_A equal to 3.67 and H_E and H_O equal to 0.374 and 0.366, respectively (Giovino et al., 2014). It is worth mentioning that studies with other congeners of *Astrocaryum* (Oliveira et al., 2014; Ramos et al., 2012, 2016) that used the same microsatellite set used here, excluded monomorphic loci from the estimation of genetic diversity; this automatically increased the estimates for these parameters [given the formula used to calculate heterozygosity ($H = 1 - \Sigma_{(i=1)}^{m} p^2$)]. Genetic diversity is dependent on the number of alleles and the evenness of their frequencies. In fact, Conte et al. (2003) demonstrated that considering only polymorphic loci in the estimation of genetic diversity parameters drastically affects their values. If we considered only polymorphic loci, H_E and H_O estimates would represent an increase of ~60% for *A. aculeatum* and ~33% for *A. murumuru* and *A. paramaca* (Table 4).

Table 4. Means for the number of alleles (N_A), the effective number of alleles (N_E), and expected and observed heterozygosities (H_E and H_O) for *Astrocaryum* species when only polymorphic loci are considered.

Species	N_A	N_E	H_E	H_O
A. aculeatum	3.133 (0.389)	1.823 (0.220)	0.356 (0.061)	0.344 (0.069)
A. murumuru	2.833 (0.271)	1.740 (0.219)	0.338 (0.067)	0.300 (0.066)
A. paramaca	2.417 (0.229)	1.635 (0.199)	0.300 (0.067)	0.380 (0.101)

Standard deviations are shown in parentheses.

If the low levels of genetic diversity exhibited in the studied species are a result of stochastic events, such as genetic drift, population bottlenecks, or inbreeding, we would expect to find a higher proportion of homozygotes with positive values for F_{IS} (Namoff et al., 2011). However, fixation index and Wright's inbreeding coefficients suggested an excess of heterozygotes for *A. paramaca*. Other studies conducted with palm species also found a tendency for a higher proportion of heterozygotes within populations (Conte et al., 2003; Elshibli and Korpelainen, 2008); this could be the result of early-acting inbreeding depression mechanisms. A study with *Vaccinium corymbosum* (Ericaceae) found that heterozygosity increases due to the abortion of most self-fertilized seeds, a post-zygotic mechanism of high inbreeding depression (Krebs and Hancock, 1990). In a review, Husband and Schemske (1996) found general support for the theoretical prediction that, for outcrossing species, inbreeding depression tends to be expressed at early life stages. Although, there is no information on the mating system of *A. murumuru* and *A. paramaca*, close relatives, including *A. mexicanum* (Eguiarte et al., 1992) and *A. aculeatum*, are outcrossing (Ramos et al., 2011), and Oliveira NP, Oliveira MSP, Davide LC, Kalisz S (unpublished results) indicate that *A. vulgare* is

outcrossing as well. Given the life history, floral and ecological traits shared by *A. murumuru* and *A. paramaca* with *A. vulgare* are likely all outcrossing.

According to our estimates of F_{ST} and AMOVA, most of the genetic variation is found within populations for all three species of *Astrocaryum*, following expectation for woody species (Hamrick et al., 1992). Nevertheless, genetic differentiation among *A. aculetaum* populations was significantly higher than for the other two species (Figure 1) likely explained by the greater distance between sample sites for *A. aculetaum*. Estimated gene flow for this species was much lower than for *A. murumuru* and *A. paramaca*. Similar results were found for *A. aculeatum* (Namoff et al., 2011) with a percentage of genetic variation within and among populations of 83.96 and 16.04, respectively. Even higher genetic differentiation among populations was found for *Oenocarpus mapora* (~36%), suggesting small sample sizes within populations as one possible factor to explain such variation.

CONCLUSION

Most of the genetic variation within the economically important palm species, *Astrocaryum aculeatum*, *A. murumuru*, and *A. paramaca*, is found within populations. This result is expected given their life history, floral, and ecological traits. Low levels of inbreeding occur for these species, with the proportion of heterozygotes being higher than expected by HWE in *A. paramaca*. Genetic differentiation among populations is strong correlated to geographic distance, with gene flow between distant populations being probably restricted by low levels of animal-mediated seed dispersal and pollen flow.

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Supplementary material

Table S1. Characterization of multiplex sets with primer combinations used for PCR reactions of *Astrocaryum* species.

Table S2. Chi-square test for Hardy-Weinberg Equilibrium (HWE) in populations sampled of three *Astrocaryum* species.

Table S3. Comparison of number of alleles found per locus for the genetic diversity analysis of three *Astrocaryum* species.