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Genetic diversity and structure of *Melocactus conoideus* Buin. & Bred (Cactaceae), a critically endangered species endemic to southwestern Bahia State (Brazil)

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ABSTRACT. According to the International Union for Conservation of Nature [IUCN], *Melocactus conoideus* Buin. & Bred is a critically endangered cactus species, and genetic studies are still needed to support conservation strategies. Thus, this study aimed to characterize the genetic diversity and structure of *M. conoideus* using Inter Simple Sequence Repeats (ISSR) markers. We amplified the genomic DNA of 126 *M. conoideus* genotypes from the municipality of Vitória da Conquista, state of Bahia (Brazil), obtained within and outside the limits of the *M. conoideus* Environmental Reserve. To this end, 13 ISSR primers were used, and the genetic amplification profile was subjected to statistical analysis of genetic diversity and structure. One hundred and ninety-one markers were analyzed, 188 of which (97.7%) were polymorphic. Moderate genetic diversity (h = 0.29) was observed, with a significant variation when considering protected and unprotected regions. The set of markers varied between informative and uninformative (mean PIC = 0.24), and genetic differentiation ranged from moderate to high (Gst = 0.32) between populations inside and outside the environmental reserve. Gene flow between populations (Nm) was estimated at 1.02. Analysis of molecular variance revealed 33% of genetic variation between populations and 67% within populations. Bayesian analysis and principal coordinate analysis (*PCoA*) confirmed the existence of two groups (K=2), with individuals from the reserve showing homogeneity for a single gene pool. This result highlights the influence of exclusive genomic regions on the genetic structure of the species. These results suggest that the auto-ecology of *M. conoideus* influences variability and differentiation levels, besides contributing to genetic structuring in subpopulations. Our findings may help in genetic conservation and management planning, as well as in the *in situ* demographic expansion used by the bodies responsible for *M. conoideus* population maintenance.

Keywords: Molecular Markers; Genetic Conservation; ISSR.

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Introduction

Conservation biology was created to prescribe conservation management plans for specific situations through multi and interdisciplinary scientific knowledge, incorporating ideas and specificities from various fields of research, especially ecology, biogeography, and population genetics (Soulé, 1985; Primack and Rodrigues, 2006). Their actions have been focused on species under different levels of threat, such as those listed in Red Data Books and Red Lists of the International Union for Conservation of Nature [IUCN]. These groups are related to general conditions of vulnerability, such as species with a limited area of occurrence, endemic or occurring in a geographic area with specific conditions under environmental pressures, in addition to having reduced populations (Primack and Rodrigues, 2006; [IUCN], 2012).

Half of the species of the genus *Melocactus* Link & Otto are endangered according to the [IUCN] threat criteria (Goettsch et al., 2015). The genus has 38 accepted described species distributed throughout Central and South America, from coastal sandbanks near the state of Rio de Janeiro to the northeastern states of Brazil, reaching Mexico and the Caribbean Islands (Taylor, 1991; Zappi and Taylor, 2022). In Brazil, there are 24 native species, 22 of which are endemic to the national territory. In the Brazilian Northeast, there are 21

species, with the state of Bahia deemed as the center of diversity and endemism of the genus in the country, with a total of 19 species, 10 of which are endemic and classified as endangered (Zappi and Taylor, 2022).

Among the endemic and threatened species is *Melocactus conoideus*. It was described by Albert Buining and Arnold J. Brederoo in 1974 in Morro do Cruzeiro, a transition region between Atlantic Forest, Vine Woodland, Cerrado, and Caatinga. This area is also known as Serra do Periperi and is located at 1,000 m altitude. Estimation indicates that the area of occurrence and propagation of this species is less than 10 Km2 and has been in a condition of endemism since 1989 (Taylor, 1992). Among its morphological characteristics are a globular-conical body shape with round buds, whitish apical cephalium, pinkish-brown marginal spines, solitary central spine, lilac fruit, and shiny seeds (Rizzini, 1982; Taylor, 1992). This species is known as the "*cabeça-de-frade-do-Periperi*" and is classified as critically endangered (CR) by the [IUCN] (Rizzini, 1982; Taylor, 1992; Machado, 2009). The species has been considered endemic to southwest Bahia State since 1989, with higher predominance in regions close to the municipality of Vitória da Conquista (Rizzini, 1982; Taylor, 1992).

Melocactus conoideus has suffered environmental pressures due to illegal markets for ornamental purposes, environmental fragmentation, and anthropic pressure due to irregular urbanization (Taylor, 1992; Cerqueira-Silva and Santos, 2008). For this reason, in recent years, conservation actions and population maintenance have been applied to the species, e.g., measures against illegal trades established by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), as well as breeding and allocation of a specific area (115,644 m^2) for its conservation within the Conservation Unit "*Parque Municipal da Serra do Periperi*" (Serra do Periperi Municipal Park, in English). In this area, studies have been carried out, and seedlings have been produced in the *Herbário Sertão da Ressaca* to increase the population within the protected area (Cerqueira-Silva and Santos, 2008).

Few genetic and auto-ecological studies have been carried out to support *M. conoideus* management practices focused on reducing its risk of extinction (Cerqueira-Silva and Santos, 2008). In this regard, conservation genetics has been inserted through a research center that deals with genetic factors affecting the risk of extinction, with the ability to quantify genetic variability through molecular analysis and describe how natural forces shape genetic information distribution dynamics in plant populations (Frankham, Ballou, Briscoe, & Ballou, 2002; Frankham, 2003).

This study aimed to characterize the genetic diversity and structure of *M. conoideus* populations through the Inter Simple Sequence Repeats (ISSR) molecular markers. In addition to describing genetic variability distribution, we also discuss the potential implications of genetic data, aiming to provide information for *M. conoideus* population management.

Material and methods

Sampling design and DNA extraction

Samples of *M. conoideus* fruit were used as a source of genomic DNA. All samples were collected from two sampling groups in the municipality of Vitória da Conquista, state of Bahia (Brazil). One population is located in a protected area at *Parque Municipal da Serra do Periperi* - PMSP (14°49'49" S; 40°50'0.3" W), inside the *Reserva Ambiental do Melocactus conoideus* – RAMc (*Melocactus conoideus* Environmental Reserve), hereafter referred to as PMSP-RAMc. Another encompasses three populations near the highway BA-265 (14°52'21.7"S 40°43'42.1"W; 14°52'01.7"S 40°44'04.9"W; and 14°52'46.2"S 40°44'04.6"W). These sites are outside the limits of the PMSP (non-protected area) and are located on private properties 12.5 km from the first point (Figure 1a).

Genomic DNA was extracted from fruit pericarp using the Cetyl Trimethyl Ammonium Bromide (CTAB) protocol, according to the routine proposed by Doyle & Doyle (1990). Extraction was performed at the Laboratory of Applied Molecular Genetics (LGMA), *Universidade Estadual do Sudoeste da Bahia* (UESB), Campus in Itapetinga, state of Bahia. Altogether, 126 genotypes were used for analysis. Of these, 53 were from inside PMSP-RAMc (protected area), 43 were from outside PMSP-RAMc (non-protected area) in site 1, 17 were from sites 2, and 13 were from site 3, totaling 73 genotypes outside the limits of PMSP-RAMc (Figure 1a). The quality and quantity of DNA samples were evaluated by electrophoretic running on a 1% (m/v) agarose gel immersed in 1X TBE running buffer at 70 volts for 2 hours. Images were captured using an L-Pix EX (Loccus) under ultraviolet light and stained with GelRed™ (BIOTIUM).

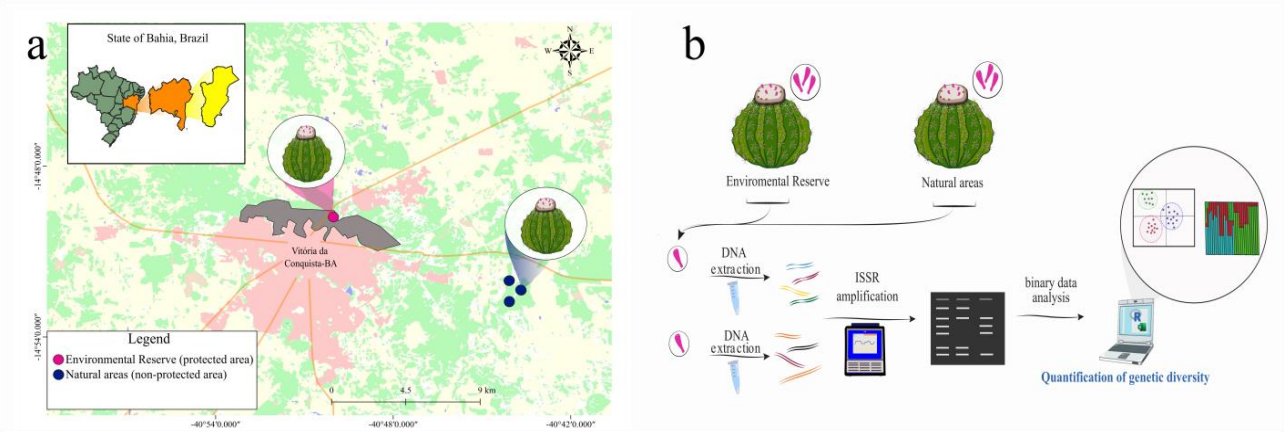

Figure 1. Experimental design of the study. a) Geographic location of the samples sites of *Melocactus conoideus* Link & Otto in the municipality of Vitória da Conquista, state of Bahia (Brazil). The area related to the Serra do Periperi Municipal Park (PMSP) is highlighted in gray, where the Reserva Ambiental do *Melocactus conoideus* (RAMc) is located (pink spot) as well as the natural sites sampled outside the PMSP-RAMc (blue spots). QGIS.org, 2021. QGIS *Geographic Information System*. QGIS Association. http://www.qgis.org and MapBiomas (Souza et al., 2020). b) Simplified scheme showing the strategy applied in our work, including DNA extraction, molecular genotyping, and statistical analysis.

Molecular genotyping

For molecular characterization, we used the dominant Inter Simple Sequence Repeat (ISSR) markers, a technique developed by Zietkiewicz, Rafalski, and Labuda (1994). Thirteen ISSR primers were preselected by Vieira, Cardoso, Silva, Cerqueira-Silva, and Santos (2019). PCR amplifications were performed in a Veriti™ 96-Well Thermal Cycler (Applied Biosystems™), using a final volume of 16 µL containing 15 ng genomic DNA, 50 mM 10X buffer, 1.2 mM MgCl2 (50 mM), 2 mM of each deoxynucleotide triphosphate (dNTPs), 0.2-unit Taq DNA polymerase (Invitrogen, Carlsbad, California, USA), and 1.0 µM primer. Temperature settings for amplification of primers were: 5 minutes at 95°C, followed by 34 cycles (50 seconds at 94°C, 50 seconds at 48°C, 1 minute at 72°C), and a final extension of 5 minutes at 72°C, following the protocol used by Dos Santos; De Oliveira; Dos Santos et al. (2011).

Amplification products were electrophoretically run on a 2% (m/v) agarose gel immersed in 1X TBE running buffer for 120 minutes. The resulting images were captured using an L-Pix EX (Loccus) under ultraviolet light and stained with GelRed™ (BIOTIUM). An overview of methods is shown in Figure 1b.

Statistical analysis

The agarose gel images were analyzed by two researchers, serving as a basis for a binary data matrix built in Excel™ (0 for absence of markers, 1 for presence, and 9 for inconclusive data). Descriptive statistical analyses for the characterization of primer amplification profiles in the population, such as percentage of polymorphic loci (PPL), average markers per primer, and percentage of rare markers in up to 5% of the sample (RM) and exclusive markers (ME) of each population studied were conducted in Excel™.

Population diversity, differentiation, and genetic structure were estimated using the following parameters: Nei's diversity index (*h*) (Nei, 1978), wherein: $h = 1 \quad \sum_{i=1}^{k} x_i^{\,2}$. Genetic differentiation (G_{st}) (Nei, 1987), wherein the total diversity (H_t) and variability within populations (H_s) were subjected to the equation: $Gst = \frac{(HT \quad HS)}{HT} = 1 \quad \frac{HS}{HT}$, and number of migrants: $Nm = 0.5(1 \quad Gst)/Gst$, analyzed by the POPGENE software version 1.32 (Yeh, Yang, Boyle, Ye, & Mao, 1997).

The PIC (Polymorphism Information Content) (Botstein, White, Skolnick, & Davis, 1980) was calculated using the GENES platform (Cruz, 2006), following the equation: $PIC = 1 \quad \left[\sum_{i=1}^{k} x_i \right]^2 \quad \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} 2x^2 i 2x^2 j.$ PIC quantifies marker locus information, *i.e.*, a parameter directly related to alleles and their frequencies. Indices below 0.25 are not very informative, between 0.25 and 0.50 are informative, and above 0.50 is very informative, with 0.50 being the maximum reached in dominant markers such as ISSR (Botstein et al., 1980; Chesnokov and Artemyeva, 2015).

Analysis of Molecular variance (AMOVA) (Excoffier, Smouse, & Quattro, 1992) was performed using the GenALEX v.6.5 (Peakall and Smouse, 2012) considering 999 permutations of the data set to partition the

general genetic variation into different hierarchical levels of population differentiation, based on the mean to fixation index of Wright - *Fst* (Wright, 1965). *Fst* above 0 indicates genetic differences among populations. *Fst* ⩾ 0.25 indicates great differentiation among subpopulations, from 0.15 to 0.25 means a moderate differentiation, and below 0.05 is non-significant differentiation. The Principal Coordinate Analysis (*PCoA*) plots were generated to visualize genetic relationships between populations.

Bayesian analysis was used to allocate clusters into a predetermined number of populations (K). The k value was chosen based on the method described by Evanno, Regnaut, and Goudet (2005), using the Structure Selector (Li and Liu, 2018). A model that predicts gene flow or miscegenation between populations (admixture model) was used in 20 simulations for each of the possible "K" gene pools tested, with burn-in of 100,000 and 1,000,000 randomizations collected via Markov and Monte Carlo Chains (MCMC). The analyses were run in the STRUCTURE version 2.3.4 (Pritchard, Stephens, & Donnelly, 2000). Admixture indices derived from STRUCTURE for each sample were visualized through bar plots using the 'StructuRly' package in R (Criscuolo and Angelini, 2020).

Results

Genetic diversity and differentiation

We observed a total of 191 amplification products related to 13 ISSR primers used. The level of polymorphism detected by the primers was high (97.76%). Only three primers did not achieve 100% polymorphic markers (DiGA3'C, TRIAGA3'RC, and TRIGGA 3' RC). The average number of markers generated per primer was 14.7, with a maximum of 23 markers (TRICGA3'RC and TRIAAC3'RC) and a minimum of 11 (TRIGGA 3' RC). Considering rare markers, such as those with low frequency of occurrence in sampling, in up to 5% of the analyzed genotypes, 18 of these (9.4%) could be detected (Table 1).

Table 1. Genomic amplification profile for ISSR primers used to characterize *Melocactus conoideus* genetic diversity. PMSP-RAMc = Parque Municipal da Serra do Periperi – Reserva Ambiental do *Melocactus conoideus*, N = number of markers, PPL = percentage of polymorphic loci, RM= rare markers, ME = exclusive markers, and PIC (Polymorphic Information Content) (Botstein et al., 1980).

		Total			PMSP-RAMc				Outside PMSP-RAMc			
ISSR markers	N	PPL	RM	*PIC*	PPL	RM	*PIC*	EM	PPL	RM	*PIC*	EM
DICA3'G	13	13 (100%)	2	0.21	11 (84.6%)	0	0.10	0	13 (100%)	2	0.22	4
DiCA3' RG	11	11(100%)	3	0.16	9 (81.8%)	0	0.14	0	11 (100%)	2	0.15	2
DiGA3'C	15	14 (93%)	3	0.21	10 (66.6%)	0	0.11	0	13 (86.6%)	2	0.20	4
DIGA3'RC	14	14 (100%)	2	0.19	10 (71.4%)	3	0.11	2	14 (100%)	0	0.21	0
DiGA3'T	10	10 (100%)	0	0.30	10 (100%)	0	0.28	0	10 (100%)	2	0.28	0
TriTGT3`YC	17	17 (100%)	0	0.25	13 (76.4%)	1	0.21	2	17 (100%)	1	0.19	1
TRIAAC3'RC	23	23 (100%)	4	0.23	19 (82.6%)	3	0.14	3	23 (100%)	0	0.24	3
TRIAAG 3`RC	14	14 (100%)	0	0.24	14 (100%)	0	0.24	0	11 (78.5%)	0	0.20	0
TRIAGA3'RC	11	10 (91%)	2	0.21	8 (72%)	1	0.10	1	11 (100%)	1	0.21	2
TRITGG 3' RC	12	12 (100%)	1	0.20	10 (83%)	2	0.10	0	11 (91.7%)	1	0.23	2
TRICGA3'RC	23	23 (100%)	1	0.27	21 (91.3%)	3	0.21	1	23 (100%)	1	0.28	0
TRICGC 3' RC	19	19 (100%)	0	0.30	19 (100%)	1	0.26	0	19 (100%)	1	0.30	1
TRIGGA 3' RC	9	8 (89%)	0	0.26	3 (33%)	1	0.06	0	8 (88.9%)	0	0.27	0
TOTAL	191	188 (97.7%)	18		137 (71.3%)	15		9	173 (90.58%)	13		19
MEAN	14.7			0.24			0.24				0.23	

Remarkably, we observed a distinction in genomic amplification profiles between genotypes sampled inside and outside the PMSP-RAMc (Table 1). We noticed that 71.3% of polymorphism for the 53 genotypes from inside the reserve, and 90.58% for the 73 genotypes sampled outside its limits. Indeed, these results indicate a higher number of polymorphic regions accessed in genotypes from outside the reserve.

The number of primers that reached 100% polymorphism was contrasting between regions, with three primers for genotypes inside the reserve and nine for genotypes outside of it. The primer TRIGGA 3' RC showed the lowest percentage of polymorphism for the inside of the reserve (33%) and the TRIAAG 3'RC with 78.5% for the outside. Regarding rare markers, 15 were observed inside the reserve and 13 outside it. Exclusive markers for each region were also counted, with 9 markers sampled exclusively in genotypes inside PMSP-RAMc and 19 outside (Table 1).

Overall, the mean PIC was 0.24, which did not change significantly among regions of occurrence. Primers DiGA3'T and TRICGC 3' RC stood out for being informative (PIC= 0.30), which was the same for genotypes

from both localities, inside and outside PMSP-RAMc limits, respectively (DiGA3' T PIC= 0.28; TRICGC 3' RC PIC= 0.26 / 0.30). This indicates the good quality of the genomic marker regions explored by the primers for population genetic studies on the studied species. The other PIC values varied between locations within an uninformative range, except for TRICGA3'RC (PIC = 0.27, 0.21, and 0.28, for total values inside and outside PMSP-RAMc, respectively).

Nei's genetic diversity (h) was estimated at 0.29 and varied for the genotypes inside (0.20) and outside (0.28) the PMSP-RAMc. The total genetic diversity (H_t = 0.28) was slightly higher than the genetic diversity within populations (H_s = 0.25). The relative magnitude of genetic differentiation (G_{st}) was 0.32 for *M. conoideus*, and N_m was 1.02. The latter represents a minimum rate of gene flow for the species, considering that one migrant per generation is an indicative criterion for dominance of the dispersal process in a metapopulation, and values < 5 indicate negligible differentiation (Mills and Allendorf, 1996; Wang, 2004).

AMOVA evidenced a greater percentage of variation within populations (67%) than between populations (33%). Table 2 lists the summary of calculations based on data related to the generation of molecular variation percentage, which was based on the mean F_{st} value (0.33).

Table 2. Analysis of molecular variance (AMOVA) based on ISSR markers for *Melocactus conoideus*. Note: DF - degrees of freedom, SS – sum of squares, MS – mean square, E. Var – estimated variance, F_{st} - value for genetic variability for dominant markers, P (rand ⩾ data) Probability for F_{st} based on standard permutation across the entire dataset.

	DF	SS	MS	E. Var	%
Among populations	3	1089.6	363.2	11.9	33
Within populations	122	2891.7	23.7	23.7	67
Total	125	3981.4		35.6	100

F_{st} = 0.33 P (rand ⩾ data) 0.001

AMOVA also provided F_{st} according to the regions of occurrence of *M. conoideus*. We also analyzed the G_{st} and N_m for each region (Table 3). In this sense, genetic divergence between PMSP-RAMc and sampling sites in an unprotected environment could be compared. We found high levels of genetic differentiation between genotypes from the reserve and between sites that have a smaller geographic distance outside the reserve limits.

Table 3. Genetic differentiation in each *Melocactus conoideus* occurrence site. PMSP-RAMc = Parque Municipal da Serra do Periperi – Reserva Ambiental do *Melocactus conoideus*. 1, 2, and 3 are sampling sites.

Site 1	Site 2	F_{st}	G_{st}	N_m
PMSP-RAMc	Outside PMSP-RAMc 1	0.247	0.147	2.90
PMSP-RAMc	Outside PMSP-RAMc 2	0.433	0.281	1.27
Outside PMSP-RAMc 1	Outside PMSP-RAMc 2	0.428	0.284	1.26
PMSP-RAMc	Outside PMSP-RAMc 3	0.341	0.242	1.56
Outside PMSP-RAMc 1	Outside PMSP-RAMc 3	0.331	0.250	1.49
Outside PMSP-RAMc 2	Outside PMSP-RAMc 3	0.318	0.251	1.49

Genetic structure

The Bayesian analyses allowed us to identify two clusters that correspond to the most likely hierarchical level of genetic structure between populations. The model of Evanno et al. (2005) estimated the most likely value of ΔK (561.59) for *M. conoideus*. We observed a general histogram for ΔK (K=2), then the distribution of genotypes within the gene pool followed by the admixture index. This, in turn, represents the level of correlation between allele frequencies of each gene pool. An overview of Bayesian model results can be seen in Figure 2.

The pool was homogeneous for genotypes inside the PMSP-RAMc (pink color); however, genotypes collected at site 1 (outside PMSP-RAMc) showed similar homogeneity with the PMSP-RAMc. Therefore, there might be occurred allelic sharing between both sites. Interestingly, genotypes from site 2 were, for the most part, a distinct group, with their gene pool (blue color) different from that of PMSP-RAMc (Figure 2b). Finally, the third sampling site showed a genotype with a mix of gene pools (admixture index values ⩽ 0.7 for any of the gene pools) without any specific identity to a gene pool.

The *PCoA* distributed genotypes as a function of genetic distance into four axes (Figure 3). There was a relationship of genetic similarity between individuals from within the reserve and from sampling site 1 outside it, i.e., they had a similar gene pool. Genetic variability among sites outside the reserve proportionally affected genotype distribution. Individualized plots show the behavior of genotypes in a two-dimensional plane.

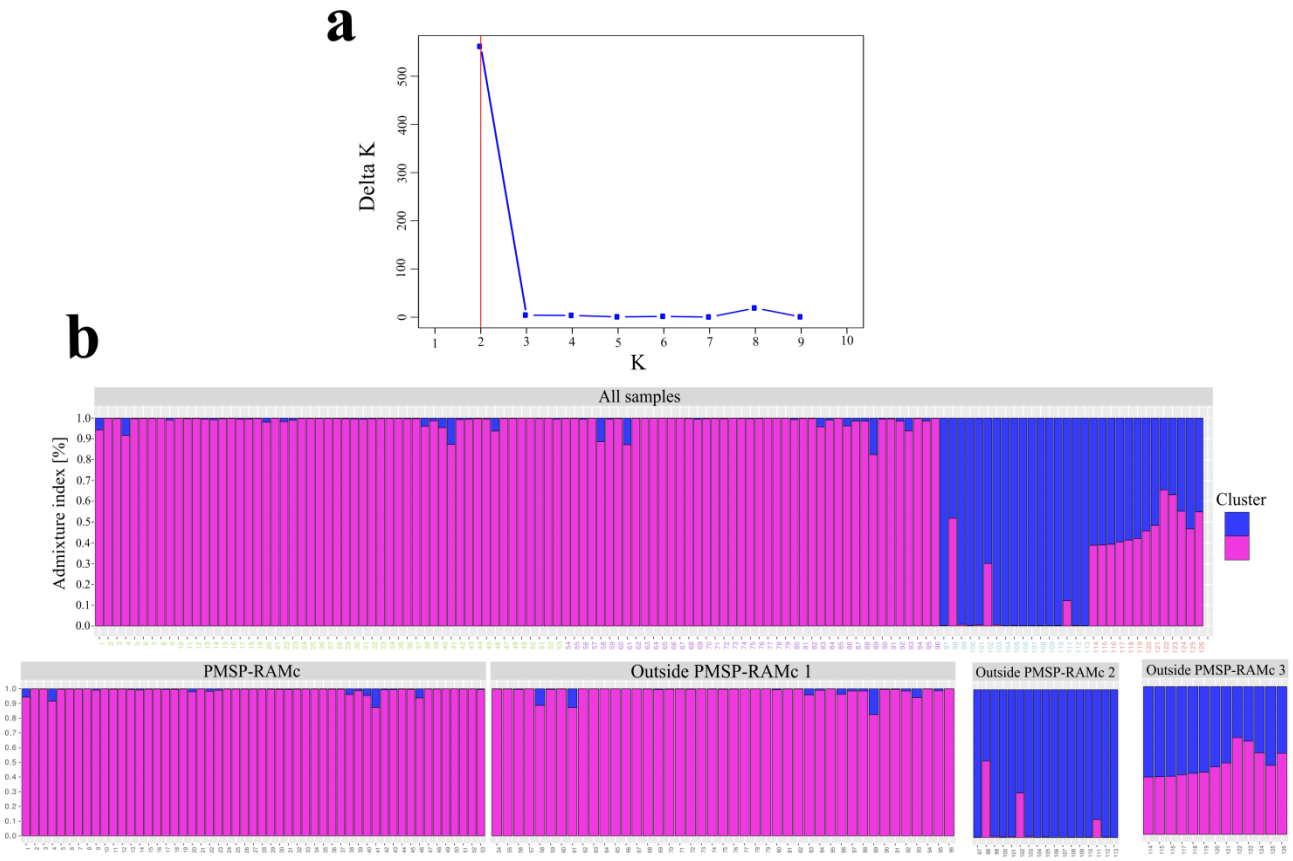

Figure 2. Results of average logarithmic probability analysis of structure by cluster based on the Bayesian model. a) The estimated K value by Delta K method of Evanno et al. (2005); b) Histogram for K=2.

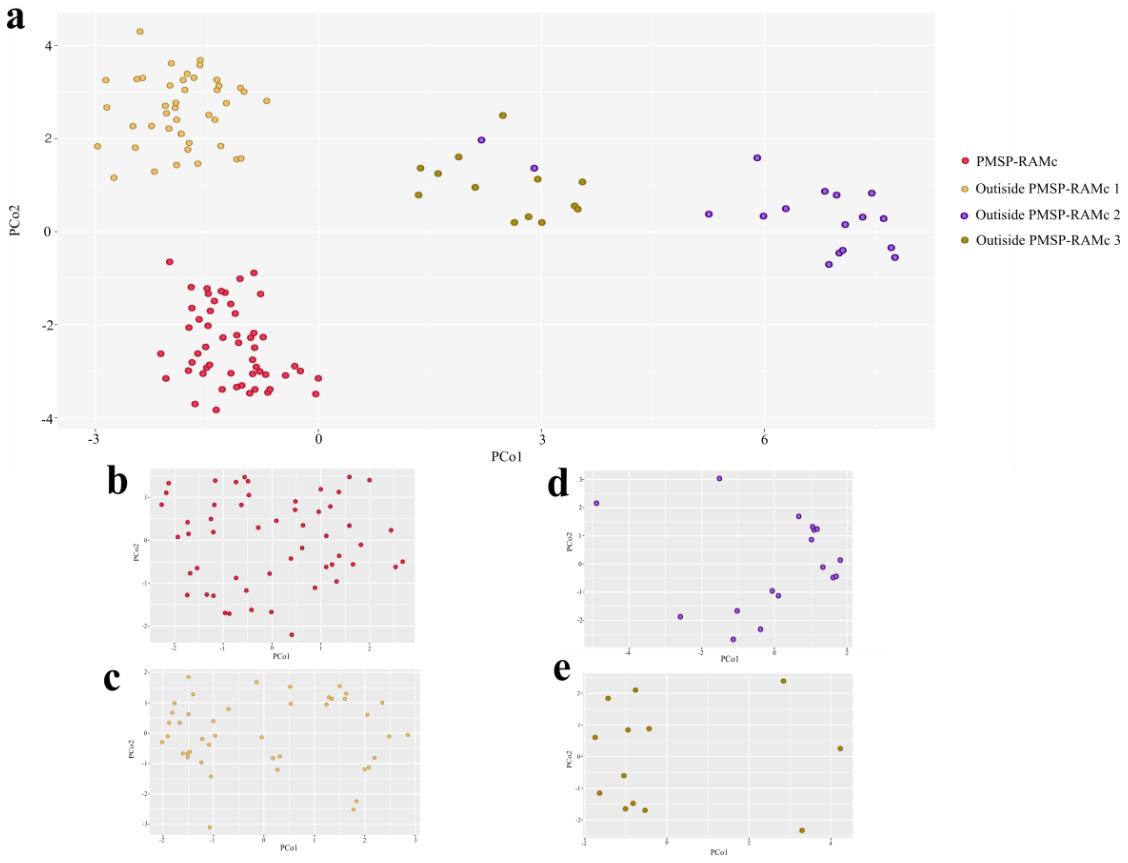

Figure 3. Distribution of *Melocactus conoideus* genotypes by Principal Coordinate Analysis (*PCoA*). A - Distribution of the analyzed genotypes; B - Distribution of the genotypes from PMSP-RAMc; C- Distribution of genotypes (Outside PMSP-RAMc 1); D - Distribution of genotypes (Outside PMSP-RAMc 2); E - Distribution of genotypes (Outside PMSP-RAMc 3).

Discussion

In this study, we identified genomic regions exclusive to *M. conoideus* outside a protected area, as well as their impact on *M. conoideus* population dynamics and potential genetic erosion in case of local extinction or intensification of genetic drift. The analyses also pointed out how management policies related to demographic expansion in the reserve region, when using individuals with high genetic similarity as sampled in this study, increase the risks of inbreeding depression (Frankham, 2005, 2010).

Genetic-population studies on cacti using different molecular marking techniques have shown natural trends in genetic variability distribution among Cactaceae from North, Central, and South America, including threatened species. Globular cactus genera, such as *Melocactus*, have often shown moderate or low intrapopulation diversity and moderate to high population differentiation levels (Solórzano and Dávila, 2015; Solórzano, Arias, & Dávila, 2016). In this study, *M. conoideus* had an intrapopulation genetic diversity (0.29) higher than other species of the genus, such as *M. concinnus*, *M. ernestii*, *M. glaucescens*, *M. paucispinus*, and *M. zehntneri*. These species had their genetic diversity estimated by SNP markers, which varied (*H*) between 0.005 and 0.23, and genetic differentiation (F_{st}) between 0.46 and 0.70 (Khan et al., 2020). Indices similar to the analysis by allozyme-based markers showed similar variation ([*H*; between 0.009 and 0.14], and [F_{st}; between 0.045 and 0.34]) (Nassar, Hamrick, & Fleming, 2001; Lambert, Borba, Machado, & Andrade, 2006b; Lambert, Borba, & Machado, 2006a). The higher diversity of *M. conoideus* than those species can be explained by its adaptive advantage to colonize regions far from hybrid zones with sympatric species, thus preventing interspecific crosses in such locations (Khan et al., 2020).

Melocactus conoideus also shows moderate to high genetic diversity and high genetic differentiation (G_{st} = 0.32; F_{st} = 0.33). The genetic variability and differentiation of *M. conoideus* may be associated with the overestimation of parameters due to its tetraploid condition (4n = 44) (Assis, Oliveira, Resende, Senra, & Machado, 2003). Polyploidy is highly present in Cactaceae species, and chromosomal marker tests may show duplications in genomic region numbers and positions. In other words, sites occurring at two sites in diploids were present at four sites in polyploid species, including representatives of the genus *Melocactus* (Castro et al., 2019). Even though the knowledge of how to assess genetic distribution patterns is not entirely clear for polyploids, these processes occur differently than in diploids, leading to interpretation biases in genetic data analysis (Meirmans, Liu, & Van Tienderen, 2018). In this context, some studies have highlighted that a higher number of chromosome copies reduces genetic drift effects and statistically increases detected variability. Thus, markers with low mutation rates could show up to twice as much variability as in a diploid population (De Silva, Hall, Rikkerink, McNeilage, & Fraser, 2005; Meirmans et al., 2018).

According to the evolutionary population paradigm, studies on genetic structure have revealed the existence of two gene pools for populations of *M. conoideus* in the municipality of Vitória da Conquista, state of Bahia (Waples and Gaggiotti, 2006). The genetic distribution dynamics analyzed by molecular variance, Bayesian clusters, and principal coordinates suggest a potential vicarious effect as a microevolutionary mechanism at the sampling sites. Three genetic compositions were formed by two extremely homogeneous groups and one transition zone. Thus, the low genetic variability of genotypes inside the reserve denotes the environmental pressure effects on the genetic composition of these genotypes over 60 years. Such stresses include the construction of the BR-116 highway, land invasions, illegal urban clusters, and natural resource extraction for civil construction, in addition to fires (Cerqueira-Silva and Santos, 2008). Thus, markers considered non-informative amplify conserved regions with little variability, reducing the occurrence of rare and exclusive marker regions between sampling sites. This may explain the results of the Bayesian analysis, which considered genotypes from regions inside and outside the reserve as a single gene pool. However, the low mixing between the gene pools detected in clusters on private properties suggests a significant genetic differentiation between regions further away from urban areas. The same was observed in greater detail for molecular variance between individuals distant at about 1 Km (Table 3).

Population behavior often tends to reflect species auto-ecology, especially among Cactaceae, which depend on pollinator and biotic disperser guilds. Species of the genus *Melocactus* are predominantly allogamous, although some representatives perform self-pollination (Taylor, 1991; Colaço et al., 2006; Lambert et al., 2006a; Romão, Hughes, Vieira, & Fontes, 2007; Machado, 2009). These species are pollinated by territorial and competitive species, such as hummingbirds, and may have their seeds dispersed by lizards, which restrict pollen and seed flows between populations, leading to their reproductive isolation (Taylor, 1991; Colaço et al., 2006; Lambert et al., 2006a; Romão et al., 2007; Machado, 2009). Despite scarce, studies

characterizing *M. conoideus* reproductive ecology are in line with the scientific consensus on the genus (Romão et al., 2007; Brito and Corrêa, 2012).

The three points sampled outside the reserve may be part of a single gene pool. Added to this, the genetic structure of this species is associated with ecological factors. One is related to its survival mode based on high edaphic specificity, which was evidenced in the *PCoA* (Luz-Freire, Trindade, Sá-Neto, & Corrêa, 2014). Another factor is regarding its self-pollination compatible reproduction mode. Finally, other factors like restrictions on cross-pollination and dispersal by territorial species of hummingbirds and insects (Romão et al., 2007; Brito and Corrêa, 2012). However, the species is also sensitive to environmental fragmentation caused by gravel extraction in these regions; therefore, the growing gap between patches of quartz gravel further limits the movement of alleles by cross-pollination, thus contributing to genetic drift and isolation (Cerqueira-Silva and Santos, 2008). Regarding statistical biases in estimating *M. conoideus* genetic structure, the Bayesian clustering model established by STRUCTURE has been recognized as ideal for polyploid analysis (Meirmans et al., 2018; Stift, Kolář, & Meirmans, 2019).

Population genetic data complement species auto-ecology information, wherein clusters of individuals tend to isolate themselves reproductively. Therefore, by analyzing distant *M. conoideus* clusters increases the chances of finding genetic variability, and there is a need to preserve more natural areas of *M. conoideus* occurrence. In this sense, conservation actions should be intensified in *M. conoideus* patches far from urban areas. However, considering the lack of evidence of habitat differences and reproductive incompatibility between protected and non-protected areas (Luz-Freire et al., 2014), to avoid further loss of heterozygotes and the increase in genetic differentiation of *M. conoideus* populations from protected areas compared to non-protected areas, the translocation of individuals or pollen from non-protected areas in demographic management inside the PMSP-RAMc may represent a suitable genetic conservation measure for *M. conoideus*, aiming to artificially stimulate gene flow, introducing rare alleles within the environmental reserve. Conservation translocations may be a strategy for restoration that combines demographic benefits and genetic management (Frankham et al., 2017), suitable for use in PMSP-RAMc, which must serve as shelter for *M. conoideus*, including its genetic diversity, as provided for in the decree of the municipality of Vitória da Conquista 10.999/2002. Thus, further conservation genetics studies on *M. conoideus* are required for implementing conservation activities and whose efforts are likely to succeed.

Conclusion

The characterization of *Melocactus conoideus* genetic diversity and structure using ISSR molecular markers evidences the need for genetic management measures in an *in situ* conservation model for this species. Our genetic-population findings should be prioritized to guide genetic management plans focused on increasing *M. conoideus* conservation efficiency.

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