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BIODIVERSITY AND CONSERVATION - ORIGINAL ARTICLE

Diversity and genetic structure of mariri tucunacá (*Banisteriopsis caapi*) populations in the southern Brazilian Amazon rainforest

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ABSTRACT

The Brazilian Amazon plays a vital role in global biodiversity, harboring species such as *Banisteriopsis caapi*, known for its cultural and medicinal significance. This study aimed to analyze the genetic diversity, with the use of ISSR (inter-simple sequence repeat) markers, of natural populations of mariri (*B. caapi* ethnovariety Tucunacá) in the Southern Amazon, as well as cultivated populations in agroforestry systems in Mato Grosso. The analysis revealed 92.3% polymorphism, with an average of 11.55 amplified bands per primer and a mean PIC of 0.466, considered moderately informative. Natural populations had greater genetic diversity (H = 0.323; I = 0.468) than cultivated ones (H = 0.164; I = 0.241), highlighting their role as genetic reservoirs. AMOVA indicated that 58% of the genetic variation occurs between populations, with low gene flow (Nm = 0.485) and strong genetic structuring (Fst = 0.582). The UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram grouped populations into two main clusters, while Bayesian analysis identified three genetic clusters, reflecting geographic isolation and human management. Cultivated populations, particularly those at the Xingu Park Indigenous Territory, maintained significant genetic diversity, likely due to the use of seedlings from different origins. This study reinforces the importance of conserving natural populations, given increasing deforestation, and promoting sustainable management of cultivated populations to ensure species adaptability. Strategies such as *in situ* conservation and genetic diversity enhancement should be integrated to safeguard the ecological and cultural viability of *B. caapi*, ensuring the continuity of Ayahuasca's traditional use by the communities dependent on this ethnovariety.

KEYWORDS: biodiversity; ISSR molecular marker; Ayahuasca

Diversidade e estrutura genética populacional de Mariri tucunacá (*Banisteriopsis caapi)* no sudoeste da Amazonia brasileira

RESUMO

A Amazônia brasileira desempenha um papel vital na biodiversidade global, abrigando espécies como *Banisteriopsis caapi*, reconhecida por sua importância cultural e medicinal. Este estudo teve como objetivo analisar a diversidade genética, com uso de marcadores moleculares ISSR (inter-simple sequence repeat), de populações nativas de mariri (*B. caapi* ethnovariety Tucunacá) na Amazônia Meridional, bem como de populações cultivadas em sistemas agroflorestais em Mato Grosso. A análise revelou 92,3% de polimorfismo, com uma média de 11,55 bandas amplificadas por primer e um PIC médio de 0,4662, classificado como moderadamente informativo. As populações nativas apresentaram maior diversidade genética (H = 0,323; I = 0,468) do que as cultivadas (H = 0,164; I = 0,241), reforçando seu papel como reservatórios genéticos. A AMOVA indicou que 57,98% da variação genética ocorre entre as populações, com baixo fluxo gênico (Nm = 0,485) e forte estruturação genética (Fst = 0,582). O dendrograma UPGMA (Unweighted Pair Group Method with Arithmetic Mean) agrupou as populações em dois clusters principais, enquanto a análise Bayesiana identificou três clusters genéticos, refletindo isolamento geográfico e práticas de manejo humano. Populações cultivadas, especialmente Xingu (Território Indígena do Parque do Xingu), mantiveram diversidade genética significativa, possivelmente devido ao uso de mudas de diferentes origens. Este estudo reforça a importância da conservação das populações nativas, diante do avanço do desmatamento, e a necessidade de um manejo sustentável para garantir a adaptabilidade da espécie. Estratégias como conservação *in situ* e aumento da diversidade genética devem ser integradas para preservar a viabilidade ecológica e cultural de *B. caapi*, assegurando a continuidade do uso tradicional da Ayahuasca pelas comunidades que dependem dessa variedade.

PALAVRAS-CHAVE: biodiversidade; marcador molecular ISSR; Ayahuasca

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INTRODUCTION

The Brazilian Amazon, recognized for its vast biodiversity, plays a crucial role in providing ecosystem services, including climate and water regulation, the provision of genetic resources, and the availability of valuable plant species for medicinal, food, and ritualistic use. The southern region of the Amazon, encompasses the southern part of the State of Amazonas, the entire territory of Rondônia, and part of Mato Grosso, which originally had a forest cover of 54% (Rodrigues et al. 2015). The Amazon rainforest represents 49.29% of Brazil's territory but has lost more than 21.97% of its primary forest (INPE 2021; IMAZON 2025) due to the increasing conversion of natural areas for deforestation, mining, and monocultures, exerting pressure on ecosystem integrity. This habitat loss threatens numerous species with ecological, economic, and cultural significance (Costa et al. 2024), many of which remain insufficiently studied, including species of the genus *Banisteriopsis*.

The genus *Banisteriopsis*, within the Malpighiaceae family, comprises approximately 62 species predominantly found in the tropical regions of the New World, with 48 species recorded in Brazil (Francener and Almeida 2025). Some species, such as *B. adenopoda* and *B. hatschbachii*, are endemic to Brazil (Gates, 1982), while others are widely distributed across various ecosystems. *Banisteriopsis caapi* (Spruce ex Grisebach) Morton, commonly known as mariri, occurs naturally in the Amazon rainforest, where it holds important biocultural value, both for its botanical importance and its spiritual use. Its extra-floral nectaries attract ants for protection against herbivory and predation. The vine connects trees in the forest canopy, facilitating the movement of primates. Flowering occurs from July to August, and seed dispersal occurs through anemochory. Flowers store starch and lipids, which serve as food for insects (Carvalho et al. 2023), contributing to pollination and increasing the likelihood of genetic recombination.

A decoction made from parts of the *B. caapi* stem combined with the leaves of *Psychotria viridis* Ruiz & Pavón, known as chacrona, produces the Ayahuasca brew, widely used in religious rituals by indigenous communities and syncretic groups in Brazil (Morales-García et al. 2017; Dos Santos and Hallak 2021; Santos et al. 2022). The traditional knowledge of indigenous peoples attributes a meaning to *B. caapi* that transcends its botanical characteristics, emphasizing its central role in cultural and spiritual practices (Chavarro-Mesa et al. 2024). This psychoactive effect highlights the relevance of Ayahuasca for mental health (De Oliveira et al. 2021; Palhano-Fontes et al. 2022; Santos and Garcia 2024).

In the face of rapid environmental changes, studies focused on the conservation of plant resources are becoming increasingly necessary. Understanding the genetic diversity of a species is essential for its conservation and sustainable use and molecular genetic techniques, such as ISSR (inter-simple sequence repeat) markers, have proven effective in assessing the genetic diversity of plant species within and between populations, contributing to the development of conservation strategies (Brandão et al. 2023; Das Chagas et al. 2023; Mendonça et al. 2023). Luz et al. (2023) studied the genetic diversity of the mariri caupuri and tucunacá ethnovariety of *Banisteriopsis caapi*, emphasizing the need for further research to elucidate genetic and structural variations in the genome, aiming at strategic planning for the conservation of both natural and cultivated populations of mariri.

The increasing interest in the conservation of sacred plants by religious institutions such as União do Vegetal, Barquinha, and Santo Daime, which cultivate *B. caapi*, has driven research focused on the genetic analysis of the species, with the goal of promoting its rational use through sustainable management. The preservation of mariri (*B. caapi*) and chacrona (*P. viridis*) also involves *in situ* management and conservation practices, which are essential for maintaining the balance of the Amazon biome, particularly in terms of preserving and sustainably cultivating sacred plants that support their faith (Thevenin et al. 2021). These species are typically cultivated in agroforestry systems. In this context, this study aimed to analyze the genetic diversity and structure of natural populations of mariri (*B. caapi* ethnovariety Tucunacá) from the Southern Amazon, in the states of Mato Grosso and Rondônia, as well as cultivated populations in agroforestry systems in Mato Grosso. The hypothesis is that the management practices of agroforestry systems, including the *ex situ* genotypic conservation of mariri (*B. caapi*), can contribute to the species perpetuation through the exchange of seedlings as a means of conserving its genetic resources.

MATERIAL AND METHODS

The research was approved by the Research Ethics Committee (review no. 5,166,391) and registered in the National Genetic Heritage Management System – SisGen (AB63C5D).

Study area

Five areas were sampled, each area being considered a population of *B. caapi*, including two natural and three cultivated populations (Figure 1). The first natural population

(NAF = natural to Alta Floresta) is located within the urban perimeter, in vestigial forest fragments, and in the rural area along the 4ª Leste road in the municipality of Alta Floresta, Mato Grosso (38 individuals). The second natural population (NRO = natural to Rondônia) was sampled in the rural area of the municipality of Ouro Preto do Oeste, Rondônia (28 individuals). The cultivated populations were sampled from three different municipalities: population 1 (CAF = cultivated in Alta Floresta) at Sítio Florestal (rural area) in the Santíssima Trindade community along the Aurora vicinal road in Alta Floresta, Mato Grosso (28 individuals); population 2 (CCBA= cultivated in Cuiabá) at Sítio Luz Sublime (rural area) in the farmhouse sector of Coxipó do Ouro in Cuiabá, Mato Grosso (28 individuals); and population 3 (CXG = cultivated in Xingu), cultivated by the indigenous people of the Yudjá (Juruna) ethnic group in the Xingu Indigenous Territory, Tuba Tuba village, within the municipality of Marcelândia, Mato Grosso (38 individuals). A minimum distance of 50 meters was established between sampled individuals.

Leaf collection and processing

Leaves were collected at an intermediate stage of maturation, free from injuries or diseases. The leaf material was stored in plastic Ziploc bags containing silica gel, labeled with their respective codes while still in the field, and later stored in a freezer at -20 °C.

DNA extraction was performed at the Laboratory of Plant Genetics and Molecular Biology of the Southern Amazon Technology and Research Center (CEPTAM) at Mato Grosso State University, Alta Floresta Campus. DNA was extracted from approximately 100 mg of *B. caapi* leaf tissue using the CTAB (cetyl-trimethyl ammonium bromide) protocol described by Doyle and Doyle (1987), with the following modifications: polyvinylpyrrolidone (PVP) was added at a concentration of 1% to 2%; the concentrations of CTAB were increased from 2% to 5%, β-mercaptoethanol from 0.2% to 2% in the extraction buffer, and the incubation temperature was raised from 60 to 65 °C for 30 min. The quality and concentration of DNA were verified by electrophoresis in a 1% agarose gel and compared with a lambda DNA marker (50 and 100 ng μL^{-1}). The gel was stained with ethidium bromide (0.6 $\mu g\ mL^{-1}$) and photographed using a photodocumentation system. After quantification, DNA samples were diluted to approximately 20 ng μL^{-1} for use in Polymerase Chain Reaction (PCR) amplifications.

Ten ISSR markers developed by the University of British Columbia, Vancouver, Canada (UBC 810, 811, 825, 826, 834, 840, 856, 868, 880) were used. Amplifications were performed in a total volume of 15 µL, containing: 1.5 µL of 10× buffer (1 M KCl, 1 M Tris pH 8.3, 1 M $MgCl_2$, 10% Tween 20), 3 µL of $MgCl_2$ (25 mM), 2.3 µL of primer (1 mM), 3 µL of dNTPs (1 mM of each dNTP), 0.75 µL of DMSO, 0.15 µL of Taq polymerase (5 U μL^{-1}), and 4 µL of DNA (±80 ng). Amplifications were performed using an Aeris™ Thermal Cycler under the following conditions: initial denaturation at 94 °C for 4 min, followed by 35 cycles

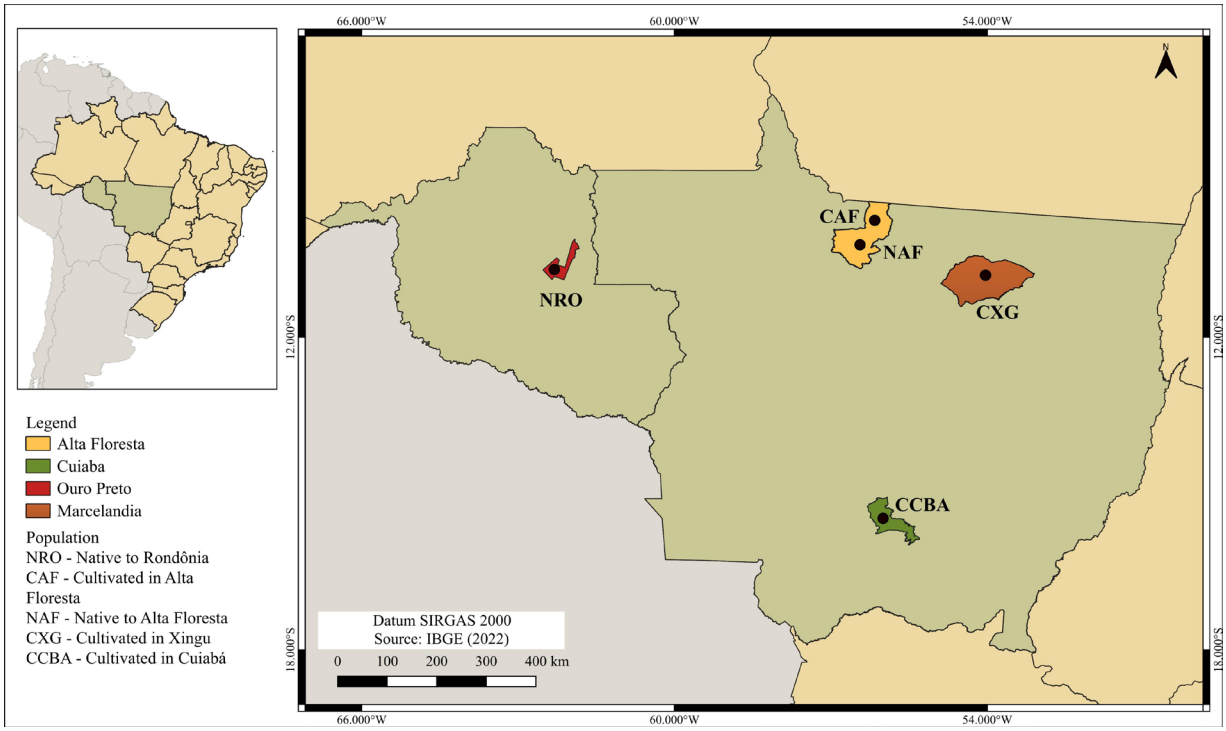

Figure 1. Sampling areas of *Banisteriopsis caapi* natural populations in the states of Rondônia and Mato Grosso and cultivated populations in Mato Grosso, Brazil.

consisting of denaturation at 94 °C for 30 s, annealing at 46-52 °C (depending on the primer used) for 35 s, extension at 72 °C for 2 min, and a final extension at 72 °C for 7 min. The amplification products were separated by electrophoresis in a 1.5% agarose gel with 1× TBE buffer (89.15 mM Tris base, 88.95 mM boric acid, and 2.23 mM EDTA). The gel was photographed under ultraviolet light using the LTB-20×20 STi transilluminator system, a photodocumentation system, and L-Pix STi software (Loccus Biotecnologia®).

Data analysis

The amplified ISSR fragments were coded as binary characters, with the presence of bands recorded as 1 and their absence as 0. Only robust and unequivocal bands were evaluated, while bands with weak intensity or those coalescing with others were excluded. Descriptive analyses of the molecular characterization included the total number of amplified fragments (TNF), number of polymorphic fragments (NPF), percentage of polymorphism (%P), and polymorphic information content (PIC), the latter calculated according to Rezende et al. (2009). Genetic diversity was assessed using the Shannon and Nei indexes, gene flow, and Gst, obtained with the PopGene program.

Analysis of molecular variance (AMOVA) was used to infer the genetic structure of the populations by decomposing total genetic variance into components between and within populations. This analysis was performed according to Excoffier et al. (1992) using Arlequin 3.01 software (Excoffier et al. 2006). The PopGene 1.32 program (Yeh et al. 1999) was used to calculate the gene flow value (Nm).

Genetic comparisons between populations were performed with Nei's 1978 genetic distance matrix, obtained through the PopGene software. A dendrogram based on these distances was then constructed using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method in the Genes software (Cruz 2016). This clustering method was chosen as it best represented the genetic variation under study, based on the cophenetic correlation coefficient (CCC), stress and distortion values, and the cutoff point determined by the Mojena method (1977).

The Structure program (Pritchard et al. 2000), based on Bayesian statistics, was used to infer the number of genetic groups (K). Twenty independent runs were performed for each K value (1 to 8), with 250,000 burn-ins and 500,000 Markov Chain Monte Carlo (MCMC) simulations. To determine the most likely K value among those tested, the criteria proposed by Pritchard and Wen (2004) and Evanno et al. (2005) were applied, and the results were analyzed using the Structure Selector website (Li & Liu 2018).

To determine whether there was a correlation between genetic similarity and geographic distance of the different populations analyzed, the Mantel test was performed with 1,000 permutations. This analysis was performed using the Genes program (Cruz 2016). The genetic relationships between all individuals evaluated were visualized through Principal Coordinate Analysis (PCoA), obtained by genetic distance, using the GenAIEx program (Peakall and Smouse 2006, 2012).

RESULTS

Genetic diversity

The molecular analysis using ISSR markers resulted in the amplification of 104 bands, 92.3% of which were polymorphic. The average number of amplified bands per primer was 11.55, while the polymorphic information content (PIC) ranged from 0.333 to 0.589, with an overall mean of 0.466 (Table 1). The Primers UBC811, UBC826, UBC834, and UBC856 stood out with a PIC above 0.50, considered highly informative (Botstein et al. 1980).

The parameters of intrapopulation genetic diversity of *B. caapi* indicate variation among the evaluated populations, with higher values observed in natural populations compared to cultivated ones (Table 2). The natural populations NAF and NRO exhibited the highest levels of genetic diversity among the studied samples, as indicated by the Nei and Shannon indices.

Table 1. Total number of amplified bands (TAB), number of polymorphic bands (NPB), percentage of polymorphism (%P), and Polymorphic Information Content (PIC) of the nine ISSR markers used in the molecular characterization of five populations of *Banisteriopsis caapi* in the Brazilian southern Amazon.

Primer	Primer sequence 5′- 3′	TºC	TAB	NPB	%P	PIC
UBC - 810	$(GA)_8$ T	46	16	15	93.75	0.418
UBC - 811	$(GA)_8$ C	50	10	10	100	0.547
UBC - 825	$(AC)_8$ T	48	12	12	100	0.495
UBC - 826	$(AC)_8$ C	50	15	15	100	0.519
UBC - 834	$(AG)_8$ YT	48	12	10	83.33	0.540
UBC - 840	$(GA)_8$ YT	50	9	9	100	0.398
UBC - 856	$(AC)_8$ YA	52	11	11	100	0.589
UBC - 868	$(GAA)_6$	46	8	7	87.5	0.352
UBC - 880	$(GGAGA)_3$	50	11	7	57.14	0.333
Total			104	96	92.30	-
Mean			11.55	10.66	91.30	0.466

Table 2. Intrapopulation genetic diversity of mariri (*Banisteriopsis caapi*) from five populations in the Brazilian southern Amazon.

Population	N	Na	Ne	P (%)	H	I
NRO	28	1.485	1.350	48.54	0.197	0.287
NAF	38	1.514	1.376	51.46	0.210	0.304
CAF	28	1.436	1.289	43.69	0.164	0.241
CXG	38	1.524	1.336	52.43	0.193	0.285
CCBA	28	1.456	1.299	45.63	0.173	0.255
Mean	32	1.483	1.330	48.35	0.187	0.274
Species	160	1.932	1.684	93.20	0.378	0.549

N = number of individuals in the population; Na = number of alleles observed; Ne = effective number of alleles; P (%) = percentage of polymorphism; H = Nei's genetic diversity; I = Shannon index; NRO = natural to Rondônia; NAF = natural to Alta Floresta; CAF = cultivated in Alta Floresta; CXG = cultivated in Xingu; and CCBA = cultivated in Cuiabá.

Among the cultivated populations, CXG stood out, displaying genetic diversity levels similar to those of natural populations. This diversity may be linked to the management practices of the Yudjá indigenous community, which involve receiving seedlings from different origins as gifts and cultivating species introduced into the natural forest. Genetic diversity indices revealed higher variability in natural populations compared to cultivated ones (Table 3).

Population genetic differentiation and genetic structure

Analysis of molecular variance revealed that 0.25% of the molecular variation derives from differences between natural and cultivated populations. AMOVA also showed that 57.98% of the total variance occurs between populations and 41.77% within populations, demonstrating that genetic differentiation is more pronounced at the interpopulation level than within populations (Table 4). The Fst value (0.582) indicates genetic structuring between populations, suggesting low gene flow among them (Nm = 0.485). The Nei's (1978) genetic distance matrix showed greater genetic proximity between the CXG and CCBA populations (genetic distance of 0.262), while the greatest distance was observed between CXG and NRO (0.487) (Table 5).

High genetic structuring among the five populations of *Banisteriopsis caapi* was found, with Fst values ranging from 0.52 to 0.65 (Table 6). The Gst value of 0.508 (values greater than 0.25 suggest a strong genetic structure, according to Wright 1978) reinforces the existence of genetic differentiation between populations (Table 7). This result can be explained by the low gene flow observed between *B. caapi* populations (Nm = 0.485).

The dendrogram obtained using the UPGMA clustering method, with a cutoff point of 99.25% established by Mojena (1977), revealed the formation of two distinct groups (Figure 2). Group I (GI) comprises the CXG, CCBA, NAF, and CAF populations, further subdivided into two subgroups, while Group II (GII) consists solely of the NRO population. Bayesian analysis (Figure 3a) divided the five populations into three genetic groups (K = 3), represented by the colors blue, red, and green (Figure 3b). The vertical lines along the X-axis represent *B. caapi* individuals, while the colored segments (blue, red, and green) along the Y-axis indicate the association coefficient of each individual assigned to each K. The clusters formed by the Bayesian analysis in Structure (Figure 3b) correspond to the UPGMA clusters (Figure 2), reinforcing the genetic isolation of the NRO population and the genetic proximity between the NAF and CAF populations, as well as between the CXG and CCBA populations. The blue genetic group consists of the natural population of Rondônia (NRO), with no admixture from the other genetic groups. The red genetic group comprises the NAF (natural to Alta Floresta) and CAF (cultivated in Alta Floresta) populations, while the green group encompasses the CXG and CCBA populations.

The Mantel test showed that genetic distance is not related to geographic distance (r = 0.339, not significant), based on the matrix of pairwise geographic distances (Table 5), since the closest genetically were the CXG and CCBA (576 km)

Table 3. Intrapopulation genetic diversity of mariri (*Banisteriopsis caapi*) from five populations in the Brazilian southern Amazon.

Population	N	P (%)	H	I
Natural	66	77.67	0.323	0.468
Cultivated	94	43.69	0.164	0.241
Species	160			

N: number of individuals; P (%) = percentage of polymorphism; H = Nei's gene diversity index (1973); I = Shannon & Weaver index (1949).

Table 4. Analysis of molecular variance (AMOVA) of the five populations of *Banisteriopsis caapi* studied using nine ISSR molecular markers.

Source of variation	DF	SS	VC	TV (%)	P-value
Between groups (NAT and CULT)	1	338.982	0.0434	0.25	<0.001
Among populations	3	957.905	9.996	57.98	
Within populations	154	1109.196	7.202	41.77	
Total	158	2406.082	17.242		

Degrees of freedom (DF), sum of squares (SS), variance component (VC), total variance (TV) and P are the likelihood of a variance component greater than observed by chance. The probabilities were calculated by 1023 random permutations. Fst = 0.5823. NAT = natural; CULT = cultivated.

Table 5. Nei's (1978) genetic distance matrix above the diagonal and geographic distance (km) below the diagonal among the five populations of *Banisteriopsis caapi*.

	NRO	NAF	CAF	CXG	CCBA
NRO	---	0.325	0.412	0.487	0.386
NAF	698	---	0.277	0.337	0.287
CAF	681	25	---	0.340	0.396
CXG	325	306	331	---	0.262
CCBA	631	613	620	576	---

Approximate geographic distances obtained by Google Earth Pro version 7.3.3.

Table 6. Pairwise genetic distance (Fst) among five populations of populations of *Banisteriopsis caapi*.

	NRO	NAF	CAF	CXG	CCBA
NRO	---				
NAF	0.525	---			
CAF	0.607	0.520	---		
CXG	0.631	0.590	0.637	---	
CCBA	0.548	0.532	0.650	0.555	---

Table 7. Population genetic parameters of *Banisteriopsis caapi*. Total heterosigosity (Ht); Average genetic diversity (Hs); Genetic divergence between populations (Gst); Gene flow (Nm).

	Ht	Hs	Gst	Fst	Nm*
Mean	0.381	0.187	0.507	0.582	0.485
Standard deviation	0.022	0.013			

*Nm = 0.5(1 - Gst)/Gst.

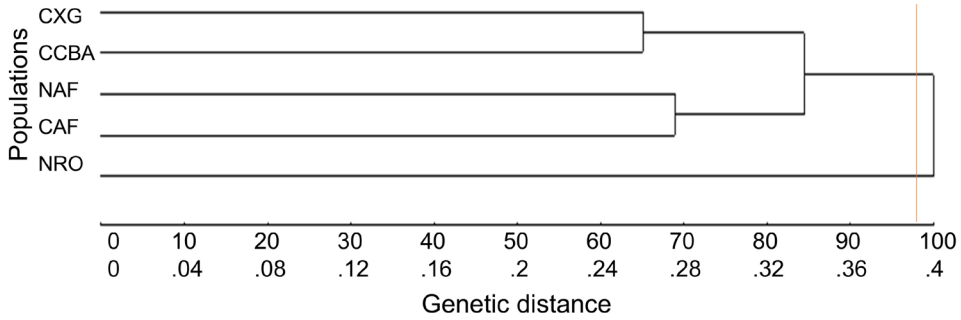

Figure 2. Genetic distance dendrogram generated by the UPGMA method and based on the Jaccard dissimilarity matrix for five *Banisteriopsis caapi* populations from the southern Brazilian Amazon. Groups generated with a cutoff point of 99.25% using the Genes software (Nei 1978). CCC = 0.7449.

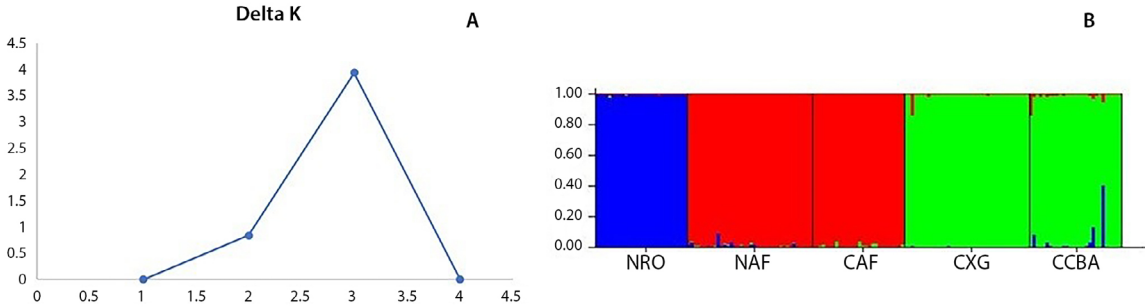

Figure 3. Population structure of *Banisteriopsis caapi* samples from Brazilian southern Amazon. (**A**) Estimation of the number of clusters (K) from 20 independent runs for K = 1–8, which best fits the *B. caapi* dataset, according to Evanno et al. (2005). Cluster analysis generated by Structure software (K = 3). (**B**) Vertical lines along the x-axis represent the individuals, and colored segments along the y-axis demonstrate the association coefficient of each individual attributed to each of the inferred K. Blue NRO = natural to Rondônia; red NAF = natural to Alta Floresta and CAF = cultivated in Alta Floresta; green CXG = cultivated in Xingu and CCBA = cultivated in Cuiabá.

populations, whereas the closest geographically were NAF and CAF (25 km). The most genetically distant populations are NRO and CXG (325 km), which indicates that it is unlikely that the CXG population received seedlings from NRO. The relationship between individuals and populations is summarized in the PCoA scatterplot (Figure 4). This analysis indicated a structure similar to that found in the Structure Bayesian analysis and the UPGMA dendrogram. The first three principal coordinates explain 40.53% of the genetic variation among individuals.

DISCUSSION

The high percentage of polymorphism observed in this study highlights the potential of *B. caapi* for genetic conservation and sustainable management programs, both in natural populations and agroforestry systems. The values indicate genetic variability in *B. caapi* populations, which is essential for the species' adaptation to different environments. The average number of amplified bands per primer suggest that *B. caapi* populations maintain genetic variability even under different management and use conditions. High genetic variability is an important indicator of a population's ability to adapt to environmental changes (Sebbenn 2023) and is crucial for preserving the plant's pharmacological properties, which are of great cultural and medicinal significance (Miranda 2021).

When comparing natural and cultivated *B. caapi* populations, cultivated populations exhibit lower diversity rates. This may be attributed to the widespread use of vegetative propagation (cloning), a common cultivation practice. *B. caapi* propagates both sexually and asexually (Carvalho et al. 2023), with vegetative reproduction from stem or xylopodium cuttings, leading to genetically identical clones (Corrêa 1994). While this practice is efficient for short-term reproduction, it reduces genetic diversity over time, making populations more vulnerable to environmental changes and diseases. Silva et al. (2024) emphasize the crucial role of scientific knowledge, shaped by traditional knowledge, in preserving the genetic diversity of plants through seed use, which reflects biocultural memory and ancestral knowledge that should be safeguarded and valued. The observed reduction in genetic diversity in cultivated populations highlights the need for management strategies that incorporate genetic variability from natural populations. Sustainable management strategies, such as using seeds from diverse populations and incorporating agroforestry practices, are essential for maintaining genetic diversity.

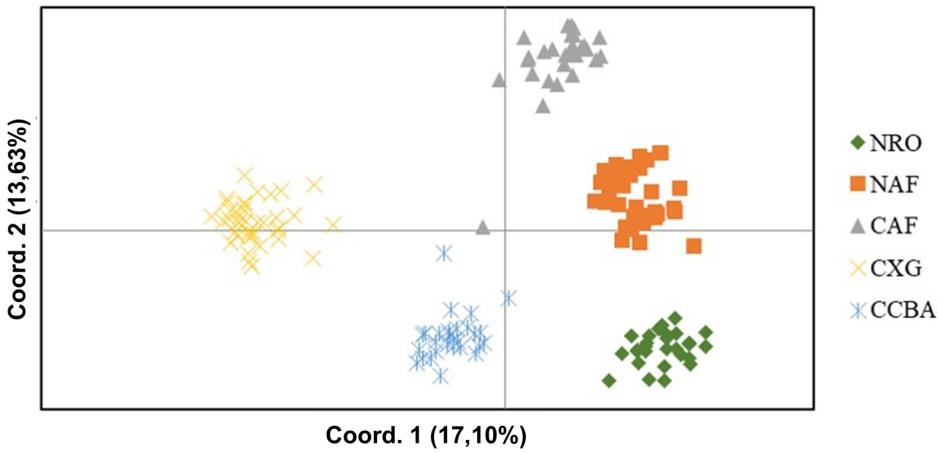

Figure 4. Geographic distance analysis of the main coordinates of 160 *Banisteriopsis caapi* individuals from the southern Brazilian Amazon. NRO = native to Rondônia; NAF = native to Alta Floresta and CAF = cultivated in Alta Floresta; CXG = cultivated in Xingu and CCBA = cultivated in Cuiabá, using Mantel.

The results confirm the importance of natural populations as essential genetic reservoirs for the conservation of the species. Integrating sustainable management practices with *in situ* conservation is essential to preventing genetic erosion in cultivated systems. Using seeds from different fragments can enhance gene flow and help maintain genetic diversity in agroforestry systems, particularly for culturally and medicinally significant species such as *B. caapi*. Preserving genetic diversity is vital to ensuring the species' adaptability and the continuity of sustainable use (Reyes-García et al. 2023).

Population genetic differentiation and genetic structure

Analysis of molecular variance revealed that cultivated populations maintain some of the genetic diversity of the species; however, the use of seeds may be required to maintain genetic diversity in the long-term. The AMOVA results indicate that genetic differentiation is more pronounced at the interpopulation level than within populations. The Gst value suggests a strong genetic structure, which can be attributed to limited gene flow among *B. caapi* populations. Such a pattern is commonly found in species affected by habitat fragmentation (França et al. 2022), where limited genetic exchange promotes structuring and increases genetic differentiation among populations. Nm values below 1 indicate that gene flow is insufficient to counteract the effects of genetic drift, fostering increased differentiation between populations over time (Wright 1951), thus the natural population of Rondônia (NRO) belongs to a distinct genetic group with no admixture, suggesting geographic isolation or minimal genetic exchange. The grouping of NAF and CAF populations into the same genetic cluster indicates gene flow between natural and cultivated populations, likely due to their geographic proximity and the use of cuttings for propagation from these places. Similarly, the clustering of CXG and CCBA populations suggests the influence of human-mediated genetic mixing, reinforcing the need for seed-based propagation strategies to maintain genetic diversity (Silva and Vargas 2023) and search for specimens from different origins. Finally, the cultivated populations of Xingu (CXG) and Cuiabá (CCBA) were assigned to the green genetic group. This grouping pattern reflects the influence of human management, such as cloning practices and the selection of specific genetic materials for plantation establishment. Genetic material from the blue and red groups is also present within this group, highlighting the need for seed-based propagation. These findings highlight the importance of traditional management practices - seed guardians for example, maintain more than a functional relationship with their territories; their seed-safeguarding practices play a critical role in preserving biodiversity (Silva and Vargas 2023).

Despite the large geographic distance between CXG and CCBA, they show lower genetic divergence according to Nei (1978). This is due to human management, as according to reports from people who cultivate the species, they seek individuals to propagate other plantations, with seedlings being taken from a few individuals or even a single specimen divided into several parts, giving rise to several individuals (clones). People who make seedlings do not consider seeking individuals with greater geographical distance. This genetic structure is thus related to the way the crop is cultivated. These findings suggest that geographic distance alone does not fully explain the observed genetic structure and that cultural and ecological factors associated with management practices may also play a significant role.

Limited gene flow ($Nm < 1$), geographic isolation, founder effects associated with the establishment of cultivated stands from few genotypes, and the widespread use of vegetative propagation, help explain the high genetic structuring observed in this study ($Fst = 0.582$). The high genetic

differentiation observed suggests that there is significant reproductive isolation among the evaluated populations, which may be a consequence of limitations in gene flow. The lower distance between NAF and CAF may reflect a common origin or more recent exchanges of propagules between these populations, while the high divergence between CAF and CCBA indicates independent management. These results reinforce the importance of conserving each population separately, given their unique contribution to the species' genetic diversity.

The differentiation between NAF and NRO is expected, even though this is a cross-pollinated species, given that gene flow is restricted due to geographic distance. The genetic differentiation between native and cultivated populations clearly demonstrates, in addition to the absence of gene flow, the action of genetic drift through founder effect, which tends to increase differentiation among populations. Even if native propagules were used in the establishment of cultivated populations, the new populations do not encompass all the variation present in natural populations and tend to become genetically distant from them. This intrinsic divergence within the species has also been reported in previous studies. Using DNA barcoding and ISSR markers, Luz et al. (2023) identified two genetically distinct lineages of *Banisteriopsis caapi* (tucunacá), but without associating this differentiation with human management or domestication processes. The observed distinction was attributed primarily to previously documented morphological and biochemical differences. Thus, the divergence identified reflects intrinsic variability between the lineages rather than direct effects of management.

In a complementary perspective, recent genomic studies indicate that *Banisteriopsis caapi* exhibits substantial molecular plasticity. The analysis of the mitogenome from a single accession revealed extensive structural rearrangements, integrated plastid pseudogenes, and trans-splicing events (Chavarro-Mesa et al. 2024). Although the study did not assess genetic differentiation among lineages and does not allow population-level inferences, it uncovers genomic features that may support future research in evolutionary ecology. In this context, such evidence reinforces the potential of the species to generate genetic variation, even though the mechanisms detected in the mitogenome do not directly explain the nuclear patterns observed here using ISSR markers.

CONCLUSIONS

The present study demonstrates that *Banisteriopsis caapi* populations from the southern Amazon are genetically structured, with differentiation shaped by both geographic isolation and human management practices. Although natural populations harbor greater genetic diversity, cultivated populations still retain a substantial portion of this variability, indicating their relevance for conservation strategies. The predominance of vegetative propagation may contribute to long-term genetic erosion if not complemented by practices that promote genetic recombination. These findings expand current knowledge on the genetic dynamics of this culturally important species and highlight the need for integrated conservation approaches that combine the protection of natural populations with improved management of cultivated systems. This approach is crucial both ecologically and culturally, safeguarding the traditional use of Ayahuasca by communities.

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