

Research Article

Paleogenetic Studies in Guajajara Skeletal Remains, Maranhão State, Brazil

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In the early 17th century, French and Portuguese colonizers and Jesuit priests settled in the state of Maranhão and made contact with the Guajajara, an ethnic group that lived along the margins of the Pindaré River. The Guajajara maintained contact with Brazilian national society over the centuries, including with Brazilian admixed populations, and with African slaves that flocked towards the region from the 18th century onwards. The present study investigates the origins of this admixture using mitochondrial genetic variability. The bones of 12 individuals investigated, which are currently part of the collection of the *National Museum*, were tested for genetic diversity. aDNA was extracted by the phenol-chloroform method and by DNA IQ (Promega, Madison, WI, USA). Amplification of the HVS I region was performed by PCR, followed by direct sequencing using the Big Dye kit (Life Technologies, Foster City, CA, USA). This region was found to represent haplogroups of Amerindians (A, C, and D) and Africans (L, L1b, L1c, and L3). The presence of African haplogroups in Guajajara bones from as early as the 18th century is consistent with historical and anthropological data, suggesting the admixture with Africans and/or Afrodescendants. Therefore, this study demonstrates that women with African haplogroups were introduced into the Guajajara population.

1. Introduction

1.1. The Guajajara. Among the indigenous peoples that still inhabit the vast South American continent are the Guajajara, an example of ethnic resistance after four centuries of contact with European, Brazilian, and African populations. The Guajajara are also referred to as the Tenetehara-Guajajara of Maranhão to distinguish them from the Tenetehara-Tembé of Pará, and they belong to the Tupi linguistic family. According to Nimuendaju [1], during the 19th century, the Guajajara lived along the Pindaré, Grajaú, and Mearim rivers (Figure 1), an area that they had occupied since pre-Columbian times according to Wagley and Galvão [2].

A more detailed analysis of the population's demography revealed that although their population size had been severely reduced in the past, it recovered significantly during at least two historical periods and is an extraordinary example of demographic expansion. Another particularity of these groups is their substantial level of admixture [3], both with Africans and Europeans. Additionally, in the contemporary demographic process, it is noticeable that more Guajajara men have married women of the surrounding national society than women have married non-Guajajara men. The greater mobility of men in search of work and the social pressure for the permanence of women within the matrilineal villages help to explain this pattern. According to Wagley and

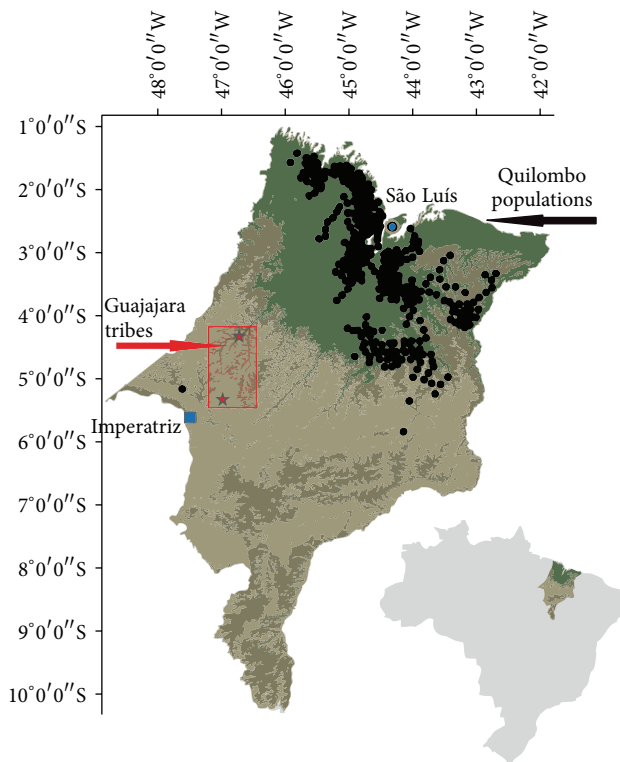


FIGURE 1: Current distribution of the studied Guajajara villages of Kamiranga and Januária (in red) and the quilombola populations (in black) in the state of Maranhão, Brazil (adapted from [59]).

Galvão [2] and Gomes [3], the Guajajara's traditional kinship system is flexible enough to accommodate the need for seeking marriages in more distant and less related communities, thereby acting as a buffer during the most critical demographic periods. The historical conditions of admixture with other ethnic groups throughout four centuries of contacts are mapped in the historical-anthropological revision by Gomes [3]. However, the precise time that the first events of contact with African and/or Afrodescendants started is not clear in the historical data (Box 1).

1.2. Bioanthropology and Bioarchaeology of Guajajara. The Guajajara are currently one of the most documented ethnic groups of Brazil. Many studies have recorded the ethnography, demography, history, bioanthropology, and bioarchaeology of the Guajajara [4–7]. Lima [4] documented the interesting practice of intentional dental modification prevalent among the Guajajara, and it was confirmed in some exhumed skulls. The dental modification was characterized by symmetric cuts of the lateral and medial angles of the incisors, called “piranha teeth,” and was attributed to the adoption of African habits. This interpretation was based on oral information about Afrodescendants from some of the Guajajara in the villages. Other biological features, other than some adopted material culture, music, and customs of these Indians, were also recognized as being of Afro-American origin. In the past, dental modification was found in several regions of Africa. In Brazil, other indigenous groups, such

as the Tucuna, Makuxi, Galibi, Karipuna, Pauaxiana, Jauari, and Kaingang, also adopted similar dental modifications for the same reasons since the practice of intentional dental modification has never been demonstrated in Brazilian archeological sites prior to contact [4, 8–10]. This practice has already been described among Africans and Afrodescendants in Brazil [9, 11, 12].

The distribution of genetic patterns of the ancestral mtDNA complements and confirms the results obtained by morphological and archeological studies; it has enabled the formulation of a more adequate model to explain prehistoric diversity and the peopling of the Americas. These characteristics confer significant advantages of the use of the mitochondrial genome for the research in the present study. However, one must not forget a significant limitation: from a biological perspective, it only narrates one part of human history, that of the maternal lineage.

The specificity of mtDNA lineages among different populations and groups has favored their use as ancestry markers over the past few decades [13–20]. Among Amerindian populations, five main haplogroups are prevalent (A, B, C, D, and X—[21, 22]) [13, 23–27]. Asian populations share American haplogroups (A, B, C, and D), as well as other haplogroups (M, N, G, F, Z, Y, and R) that are described as specific to the Asian continent [28, 29]. European and Euro-Asian populations also possess more frequent haplogroups (I, J, K, H, T, U, V, and W) [15, 28, 29]. In contrast, African populations have a large amount of exclusive haplogroups and subhaplogroups (L0, L1, L2, L3, L4, L5, and L6) [18, 30, 31].

This study is a first step toward elucidating the genetic characteristics of the Guajajara individuals. Our objective is to characterize the admixture with non-Amerindian populations. In addition to contributing to our understanding of the history of this ethnic group, this study aims to corroborate previous bioanthropological studies and to contribute to the knowledge of regional population dynamics during the centuries that followed colonization until the final formation of what is today the Brazilian national society. The present study uses skeletal samples from the Guajajara cemeteries to investigate aDNA mitochondrial haplogroups and to contribute to the reconstruction of part of its history.

2. Materials and Methods

2.1. Subjects. The bone samples belong to the Biological Anthropology collection of the National Museum, Rio de Janeiro. The samples were obtained from 12 different individuals exhumed from cemeteries of the Guajajara villages of Januária and Kamiranga (Figure 1). These skeletons are part of the collection brought by Pedro Lima and his collaborators in 1945.

The bone samples were collected from ribs removed from each individual. The ribs were selected following criteria of integrity, absence of pathological signs, anomalies, or severe taphonomic changes that could compromise future investigations about the health state of other parts of the skeletons (bioarchaeological studies, such as age and sex assessment or

First colonization try at Maranhão	1553
French invade São Luiz island, they contact “Pinariens” (Maranhão)	1612
Portuguese expel the French	1613
Portuguese refer the Guajajara Amerindians (Maranhão)	1615
Jesuits fund the first catechist missions (Maranhão)	1653
Ships bringing the first Africans slaves, São Marcos bay (Maranhão)	century VII
Foundation Mission Carará (Maranhão)	century XVIII.
First slave ships officers arrive (Maranhão)	1761
Refuge of Cabanagem soldiers (Grão-Pará)	1835–1841
Foundation of the original village, Januária (Maranhão)	1854
Migration of refugees from drought in the Northeast	1877–1880
Foundation of the Quilombos in Imperatriz (Maranhão)	century XIX
Foundation of the present village, Januária	century XX

Box 1: Chronology related to contact with the Guajajara (upper Pindaré River).

TABLE 1: Sex, age, and village of origin of the studied samples and a description of analyses performed in each methodological stage.

Sample	Sex	Age	Number of extractions	Number of sequences	Sequences obtained	Village
GAJ 700	Female?	18/20	2	21	11	Kamiranga
GAJ 701	Female?	20/25	2	17	4	Kamiranga
GAJ 709	Female	+50	2	21	6	Januária
GAJ 713	Undetermined	6/12	2	13	6	Januária
GAJ 715	Female	+20	2	21	6	Januária
GAJ 717 ¹	Male?	20/49	2	10	11	Januária
GAJ 718 ²	Male?	20/34	2	15	7	Januária

¹Skeleton number GAJ 717-MN-RJ provided a 19th century chronology of 140 ± 30 BP (BETA-291714-AMS, C13 corrected). ²Skeleton number GAJ 718-MN-RJ provided an 18th century chronology of 210 ± 40 BP (GEOCHRON MA GX31824-AMS, C13 corrected).

other additional information). The upper ribs, in particular the fourth rib, were preserved as they are used for age assessment and therefore contain additional bioarchaeological data. All of the selected bone samples were photographed and described prior to being sent to the laboratory for analysis.

Osteometric and osteological data, as well as sex and age, were already estimated in previous studies, and the information in the National Museum archives was used for the present study (Table 1). The material was also dated for the purpose of the present investigation; the skeleton number 717-MN-RJ provided a 19th century chronology of 140 ± 30 BP (BETA-291714-AMS, C₁₃ corrected), and the skeleton number 718-MN-RJ provided an 18th century chronology of 210 ± 40 BP (GEOCHRON MA GX31824-AMS, C₁₃ corrected).

2.2. Extraction, PCR, and Sequencing. The extraction and treatment of the DNA samples were performed in the Paleogenetic section of the Human and Medical Genetics Laboratory at the Federal University of Pará (UFPA) according to the established protocol [25, 32]. The DNA was extracted either with the phenol-chloroform method [33] with modifications or with the DNA IQ System kit (Promega, Madison, WI, USA). The method used depended on the state of preservation of the samples. The extracted material was then stored in a freezer until PCR amplification of the hypervariable region I (HVS-I, ranging from 15920 to 16498).

For this purpose, two sets of primers pairs with overlapping regions were selected, which together amplified a 578-bp region [25, 32]. A negative control containing all necessary components for a reaction except DNA was used in all the stages of the amplification.

The PCR amplification product was sequenced following the method of Sanger et al. [34], with the *Big Dye Terminator Cycle Sequence* kit, which uses *AmpliTaq DNA Polymerase* (Life Technologies, Foster City, CA, USA). The direct sequencing of mtDNA was performed in an ABI Prism-3130 genetic analyzer (Life Technologies). These sequences were exported and printed using the Chromas v1.4 software.

All steps from extraction to sequencing were repeated between two and five times. To confirm the results and to assess the reliability of the sequencing, each sample was on average extracted 1.8 times, enlarged by PCR 4.6 times, and sequenced 15.7 times using the two sets of primers in both directions (forward and reverse).

The result of each sample was compared to the mtDNA reference sequence [35] to identify the haplogroups. Comparisons were also made with the sequences of each research participant to discard the possibility of contamination from inside the laboratory.

2.3. Data Analysis. Neighbor-joining (NJ) trees of mtDNA sequences were constructed with MEGA version 5.0 using the Kimura two-parameter distance method and the NJ method of Saitou and Nei [36] bootstrapped with 10,000 replicates.

In addition, the samples were subjected to the analysis of inconsistent mutations (phantoms) using the *Netmat* and *Network* v. 4.1.0.9 programs [37–39], which were also used to assess the quality and reliability of the obtained results.

3. Results and Discussion

The total number of analyzed samples generated 73 sequences, 23 of which were excluded either because of contamination or because they did not generate a conclusive result concerning the HVS-I region of the mtDNA. Among the 12 individuals analyzed in the present study, five were excluded by contamination or by inconclusive results (GAJ 699; GAJ 702; GAJ 704; GAJ 705; GAL 711) (Table 1).

To ensure the reliability of the results, we followed the authentication criteria suggested by Cooper and Poinar [40], Gilbert et al. [41], Hofreiter et al. [42], and Pääbo et al. [43], for results obtained in ancient DNA sequencing. They are (1) isolation of the work area: the DNA extraction must be in a separate area from the PCR amplification; (2) there must be negative control samples to detect the presence of contaminants; (3) respect the molecular state of the sample: amplify small fragments, due to the difficulty of amplifying large fragments; (4) reproducibility: extractions and PCRs must be repeated; (5) cloning: to evaluate the presence of contaminants; (6) independent replication of the results in another laboratory; (7) morphological and biochemical preservation of the material: bone structure and biomolecule preservation aspects that relate to the integrity of the DNA (collagen, amino acids, etc.); (8) quantification to assess the quantity of the extracted DNA. Out of these criteria, the only one we did not follow was item number 6 (independent replication of the results in another laboratory).

In the seven samples that allowed sequencing analysis, haplogroups characteristic of Amerindian and African populations was observed. Of the Amerindian individuals, one belonged to haplogroup A, another to haplogroup C1, and one to haplogroup D. Of the individuals of African origin, one belonged to haplogroup L, two to haplogroup L1 (L1b e L1c), and another to haplogroup L3e (Table 2). None of the individuals studied belonged to Amerindian haplogroup B or to the European haplogroups.

A reticulation was obtained without filter application and can be explained either by the existence of two different ancestries (Amerindian and African) or by the presence of hotspot mutations. This result was confirmed when the same samples were analyzed after application of a hotspot mutation filter (in the *Netmat* program) as suggested by Bandelt et al. [44], followed by the analysis of the *Network* program.

In the present study, the samples were aligned and subjected to phylogenetic analyses with other groups of samples from previous mitochondrial studies [24, 25, 45–47]. An NJ tree (Figure 2) of this data set gives tenuous support for separation into seven clusters. There is good bootstrap index (10,000 replicates) for the Amerindians and Africans haplogroups. The extended sequence information clarifies the admixture pattern evident in the Guajajara. These results were consistent; the sample GAJ 701 divided the

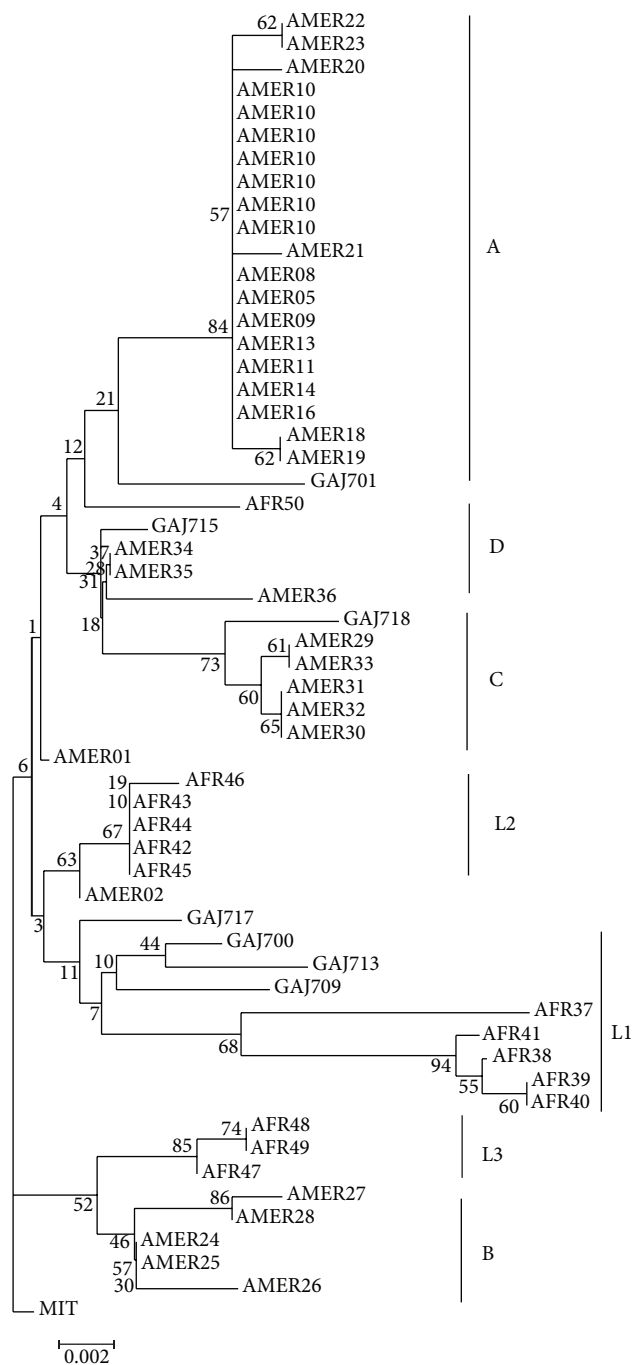


FIGURE 2: NJ Phylotree bootstrapped with 10,000 replicates.

same branch with contemporaneous Amerindian samples that belong to haplogroup A; the samples GAJ 715 and GAJ 718 cluster with Amerindian samples according to their respective haplogroups D and C1; and the samples GAJ 700, GAJ 709, GAJ 713, and GAJ 717 cluster with African samples, according to their respective haplogroups L, L3e, L1c, and L1b.

The absence of Amerindian haplogroup B in this series is probably due to the reduced number of examined specimens [13, 24, 27, 48]. However, it may also reflect the underrepresentation of this haplogroup in the formation

TABLE 2: Mutations assessed in the present study according to their nucleotide position, their haplogroup, and their respective intentional dental modification (IDM).

Sample	IDM***	Nucleotide position in the mitochondrial genome																HAPLOG
		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
		6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	
		0	1	1	1	2	2	2	2	2	3	3	3	3	3	3	3	
Sequence reference*	—	5	0	2	8	2	5	7	9	9	9	1	2	2	2	3	6	H
		1	4	6	4	3	7	8	0	4	8	1	0	5	7	4	2	
		A	C	T	C	C	C	C	C	C	T	T	C	T	C	T	T	
		G	—	—	—	—	T	—	—	—	—	C	—	—	—	—	—	
GAJ 700	Present	G	—	—	—	—	T	—	—	—	—	C	—	—	—	—	—	L
GAJ 701	Present	G	—	—	T	—	—	T	T	—	—	—	—	—	—	—	C	A
GAJ 709	Absent	G	—	—	T	T	—	T	—	—	—	C	T	—	—	—	—	L3e
GAJ 713	Absent	—	—	—	—	—	T	T	—	T	—	C	—	—	—	C	—	L1c
GAJ 715	Undet.	—	—	—	—	—	—	—	—	—	—	—	C	—	—	—	C	D
GAJ 717	Undet.	—	—	C	—	—	—	T	—	—	—	C	—	—	—	—	C	L1b
GAJ 718	Absent	—	T	—	—	T	—	—	—	—	C	C	—	C	T	—	—	C1

* Anderson et al. 1981 [35].

*** IDM: Intentional dental modification.

of the community, in particular due to the mechanisms of fission-fusion [49] that rule the population growth of indigenous groups. The absence of haplogroup B in ancestral Amerindian populations is not exclusive to this study [32, 50–53].

In the lineage classified as haplogroup A, five mutations were observed (GAJ 701: **16,051**, 16,184, 16,278, **16,290**, and **16,362**). Only the mutations in bold are consensually accepted as motifs of haplogroup A. The presence of the other mutations can be justified as hotspot positions or private mutations.

Each one of the lineages classified as haplogroups C1 and D was observed in a single sample and presented six and two mutations, respectively (GAJ 718: 16,104, **16,223**, **16,298**, 16,311, **16,325**, and **16,327**; GAJ 715: **16,325**, and **16,362**). Of these, only the mutations in bold are accepted as motifs of haplogroups C1 and D.

Each of the lineages classified as haplogroups L, L1b, L1c, and L3e was represented by a single sample and presented three, four, five, and six mutations, respectively (GAJ 700: 16,051, **16,257**, and 16,311; GAJ 717: **16,126**, **16,278**, **16,311**, and **16,362**; GAJ 713: 16,257, **16,278**, **16,294**, **16,311**, and **16,334**; GAJ 709: 16,051, 16,184, **16,223**, **16,278**, **16,311**, and 16,320). Of these, only the mutations in bold are consensually accepted as haplogroup motifs. In order to assign the haplogroups, scientific articles [24, 25, 45, 46, 54–56] and databases specialized in mtDNA analysis HaploGrep (<http://haplogrep.uibk.ac.at/>) and Phylotree (<http://www.phylotree.org/>) were used.

Analysis of mitochondrial DNA extracted from Guajajara ribs revealed African haplogroups in higher proportion (57.14%) than indigenous haplogroups. Although the number of successful extractions was low, this result raises an important issue to be confirmed in future studies. The possible penetration of African haplogroups among the Guajajara Indians suggests that women of African ancestry have been incorporated into the Native American group

and have reproduced. One cultural marker from the same period as the samples from this study is the intentional dental modification, which has never been recorded as an original practice of the groups of the Tupi linguistic family in Brazil and which was still present among the Guajajara in the middle of the 20th century [4].

During the period when the Guajajara bones from this study originated, historical documents report a decrease in contact with Europeans and a strong African and admixed population presence in the Sertão of Maranhão, an area towards the East to where runaway slaves from the agricultural region fled to [57] and mixed with *sertanejos* and *quilombolas* who were living in an increasingly Africanized Maranhão. Therefore, it is likely that the social circumstances and interests among African, Afrodescendant, and Indigenous groups resulted in admixture.

As mentioned earlier, the results obtained here are consistent with the data provided by Wagley and Galvão [2], Consiglio [58], Gomes [3], and other authors on the Guajajara and their admixture with Africans. The process of admixture was possibly initiated in the 17th century, and it is important to stress that at least four slave ships arrived in Maranhão between 1630 and 1693, not to mention the ones that may have clandestinely ported on the Maranhão shores. Therefore, contact and occasional admixture with Africans could have started very early and could have been favored by the practice of enslaving Indigenous people, who provided cheaper labor than Africans at the time.

The beginning of the slave trade in Maranhão during the 17th and 18th century brought Africans from the Benin (West Africa) region and more recently from the Central African coast. The presence of L1b, a haplogroup mostly restricted to West Africa [46, 55, 56], is consistent with our findings, which identified four African individuals, one of which belonging to L1b haplogroup.

During the colonial period in Brazil, both African and indigenous populations occupied the basis of the social pyramid. These groups were regarded as nothing more than manual laborers in an economy that was rural and slave-based. This social and economic profile could be found throughout Brazil. Slaves and Amerindians shared a common fate, which they both attempted to escape. These shared life experience spontaneously introduced different nonindigenous ethnical elements into the indigenous villages, which was also promoted by the Empire's strategy of mixing different ethnic groups within the colonies. This process also led to the appearance of the *quilombos* or multiethnic communities formed by Amerindians and Africans [46, 54, 57], as seen in the present study.

One of the hypotheses explaining admixture is the Africanization of Maranhão villages after the trade activities of the General Company of Grão-Pará and Maranhão (Figure 1). Although reports on the first *quilombos* of Maranhão date back to the end of the 19th century, there are a number of documents that address the concerns of the *Diretório dos índios* with the Guajajara, due to their mixture with "deserters and fugitive slaves" [3]. Only two *quilombola* communities were close to the Alto Pindaré region, Mandi dos Pretos and Buritirama [59], but there are no documents to confirm their contact with Guajajara. The first census performed in the state of Maranhão in the 19th century (1872) measures in 52.48% (2.749/5.238) the contribution of foreign individuals of African origin (non-Amerindians) (IBGE).

A second wave of contact, which involved admixed Brazilian people, might have occurred in the Pindaré area beginning from the first half of the 19th century onwards. The *Cabanos* came from the state of Pará between 1835 and 1841, as well as the northeastern *sertanejos* who were fleeing from the severe drought in the northeastern region between 1877 and 1880. These new waves of people may have introduced African or people of Afrodescent to the Guajajara and would revitalize villages whose populations were decreasing. The *sertanejos* had a significant number of women who practiced intentional dental modifications similar to those observed among the Guajajara [11], making the *sertanejos* women the probable vector of this cultural practice.

Another work suggests the contact between the African and Amerindian populations in Brazil. Gonsalves et al. [60] described an unexpected mitochondrial lineage, traditionally considered Polynesian, among teeth samples obtained from two Amerindian skulls (Botocudos, who lived in the southwest Brazil at XIX century). The authors presented several possible scenarios to explain the admixture process among Polynesian and Amerindian.

Within this context, the African haplogroups found in this sample are of greater interest as they suggest the important role of African or Afrodescent women who left their mitochondrial evidence. While the common sense constructed around the process of admixture tends to emphasize the role of enslaved men fleeing captivity and introducing themselves into indigenous communities, it is also important to consider other possibilities, such as the role of women. Although less numerous than men in the slave squads, the presence of fugitive women is documented and proven

[57]. Furthermore, the hypothesis of the kidnapping and/or conquest of women should also be considered. Additionally, the current literature on Guajajara demography draws attention to the fact that men had greater mobility while in search of work, and they probably brought back nonindigenous women to the villages for the purpose of marriage. This scenario undermines the stereotype that most of the sex dynamic was between Brazilian men that married indigenous women. It is therefore reasonable to assume that when the Guajajara men returned to their villages after escaping from oppression or captivity, some of them brought along slaves or admixed women with whom they had established a relationship. The chronology of Guajaras is summarized in Box 1.

Conflict of Interests

The authors declare no competing interests.

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