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CONTENT

- Abstracts of the 18th International Symposium on Dental Morphology and the 3rd congress of the International Association for Paleodontology, August 15th – 19th, 2022, Frankfurt, Germany 33
- Zama Moosvi, Scheila Mânica, Gavin Revie / **Enamel thickness of human mandibular canine: A radiographic study** 221
- Maria Vitória Lameiro, Mariana Correia, Patrícia Antunes, Raquel Carvalho, Tatiana Major, Rui Santos, Cristiana Palmela Pereira / **Odontometrics analysis from a commingled archaeological human population related to 1755 Lisbon's earthquake** 230
- Anahit Yu. Khudaverdyan / **Bioarcheology of bone remains from medieval burials from Armenia** ... 239
- Arofi Kurniawan, An'nisaa Chusida, Mieke Sylvia Margaretha, Beta Novia Rizky, Beshlina Fitri Widayanti Prakoeswa, Patricia Shankar Jethani, Intan Puspa Ramadani, Ahmad Yudianto, Anand Marya / **Tooth evolution and its effect on the malocclusion in modern human dentition** 262
- Giusy Capasso / **Evidence of dental anomalies from prehistoric Eastern Sudan: two cases from the Mesolithic graveyard UA 50** 267
- Ana Solari, Nathalie Antunes-Ferreira, Anne Marie Pessis, Gabriela Martin, G. Richard Scott / **Kinship analysis using rare nonmetric dental traits in a prehistoric cemetery from Northeastern Brazil** 276
- Renate Rabenstein, Dagmar Stiefel / **Rare dental anomalies in two sympatric European bat species (Pipistrellus spp.)** 284
- News: **Alt KW, Al-Ahmad A, Woelber JP. Nutrition and Health in Human Evolution-Past to Present. Nutrients. 2022 Aug 31;14(17):3594.** 292

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Kinship analysis using rare nonmetric dental traits in a prehistoric cemetery from Northeastern Brazil*

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Abstract

This study evaluates whether three prehistoric burials containing 12 individuals (three adults and nine subadults) could represent family relationships in a small cemetery from a Middle Holocene site in Northeastern Brazil. In the absence of collagen for aDNA analysis, the hypothesis of genetic kinship relationship was verified through the analysis of nonmetric dental traits. Two rare nonmetric dental traits were analyzed: barrel-shaped upper lateral incisors (grades 6 and 7 on UI2 shoveling scale) and premolar odontomes. The relative frequencies of these traits were high in the sample, and statistical interpretation of the data revealed that the co-occurrence of these rare traits is unlikely to happen at random. Thus, their presence in individuals from the burial site of Toca do Enoque suggest plausible intracemetery genetic kin relationships.

Keywords: Odontologic kinship analysis; kin-structured cemetery; rare nonmetric dental traits

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Introduction

Small-scale societies are often organized along kinship lines (1). In those societies, social and biological kinship are commonly expressed in the funerary rituals that can be reconstructed through bioarchaeological approaches (2-5). Biodistance analysis can use phenotypic data from the skull or dentition to estimate genetic similarity among individuals within a sample (6) in an intracemetery study that uses the site or cemetery as the unit of analysis (4). As the central goal of kinship analysis is to infer biological relationships from human skeletal remains (2, 3, 7, 8), we present an analysis of two rare dental traits from a small cemetery from Brazil (Toca do Enoque). Even with a small sample, the co-occurrence of rare heritable dental traits shows the possibility of evaluating presumed genetic relationships in a kin-structured cemetery.

The archaeological site of Toca do Enoque is located in the Serra das Confusions National Park, in Northeastern Brazil (9-11) (Figure 1). Archaeological research at the site was conducted between 2008 and 2009 by a FUMDHAM team. The sandstone wall of the shelter contains rock representations of geometric, zoomorphic, and anthropomorphic figures painted in red associated with the Northeast 'Agreste' and Geometric traditions. Human burials and large bonfire structures associated with the funerary practices by small hunter-gatherer preceramic groups were also identified.

The excavations uncovered three burials that included a total of 12 individuals, with a predominance of subadults (three adults and nine subadults) (9-11). Two primary individual burials were identified (#1 and #3), along with a complex multiple/collective burial (#2) containing ten individuals (Figure 2). Burial 3 shows evidence of post-burial disturbance (natural and cultural), compromising its integrity. Detailed data about each burial and taphonomic processes can be found in previous works (10-11).

Funerary practices in the region throughout the Holocene are characterized by individual primary burials in pits (preceramic groups) or in funerary ceramic urns (ceramic groups), with few or no funeral accompaniments (12). Toca do Enoque stands out for the atypical characteristics of a multiple/collective burial (#2), presenting many individuals buried in the same funerary space associated with abundant funerary accompaniments. These atypical burial characteristics suggest the possibility of kinship

relationships among the individuals buried alongside and in relation to the other individuals buried at Toca do Enoque.

Chronologically, the approximate funerary use of the site during the Middle Holocene was estimated at c. 5900 – 6600 years BP, according to indirect radiocarbon dates obtained from charcoal samples retrieved in the inhumation contexts. Attempts to directly date human bones and teeth, or any funerary accompaniments (fauna teeth and bones), did not offer satisfactory results due to the lack of collagen. Therefore, a wide chronological range indicates the potential period this site was used as a cemetery.

Despite good preservation of organic materials in the burials, the absence of collagen in the human remains made it impossible to conduct genetic studies about the individuals to establish possible kinship relationships. The alternative to aDNA analysis was an odontologic kinship analysis of rare nonmetric dental traits (2-3).

The methodology of odontologic kinship analysis was applied (2, 3, 7, 8) to establish presumptive genetic relationships based on the observation of rare heritable dental traits. These traits are anatomical variants and anomalies that are identified more frequently among family members than among randomly selected individuals (4). They must be selected for their high heritability, low frequency, distinct expression, age and sex independence, and low degree of intercorrelation with other traits (6,13). Odontologic traits are particularly well-suited for the purpose of testing hypothesis of genetic relationships in skeletal remains (8).

Material and Methods

Of the 12 individuals recovered in the excavations of three burials from Toca do Enoque, we analyzed the permanent teeth of seven individuals safeguarded in the reserve of organic materials of the FUMDHAM (Piauí, Brazil). The exclusion criteria were age-at-death below one year old without erupted teeth and deciduous dental crowns in formation (Individuals #6, #9 and #10) and the absence of observable teeth due to high fragmentation and poor preservation (Individuals #3 and #13). Due to the fragmentation of the mandibles and maxillae, unerupted permanent tooth crowns in subadult individuals could be observed. Thus, the permanent teeth of one adult (Individual #8) and six subadult individuals (Individuals #1, #2, #4, #5, #7 and #11) from Burials 1 and 2 were analyzed.

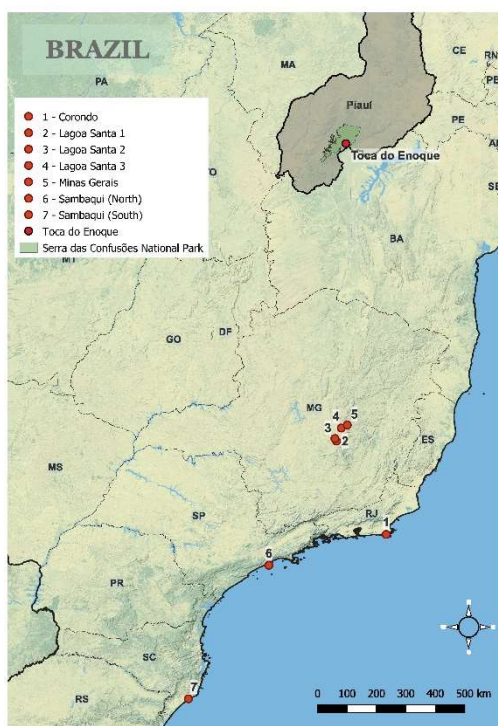


Figure 1. Geographic location of Toca do Enoque, Serra das Confusões National Park, Piauí, Northeastern Brazil, and the location of the comparative sites according to C.G. Turner II Database.

The crowns of the permanent teeth were observed macroscopically, through visual inspection using an artificial light and a magnifier lens (10 X). No signs of occlusal wear were identified on the teeth. Nonmetric dental traits were recorded for all complete crowns of the permanent teeth, erupted or unerupted (2-3). The traits were recorded as present, absent, or indiscernible (e.g., no registered traits in cases of missing, incomplete, or badly preserved teeth, as well as in individuals below one year old). The bilateral or unilateral expressivity of the traits was recorded. If the trait was present bilaterally, it was counted only once in the dataset. If the trait occurred on one side, it was scored as present. The frequencies for each analyzed trait are based on individuals, not number of teeth.

The less common a trait is in a given population, the more informative it can be establishing kinship (2-3). Two rare nonmetric dental traits were selected as the most useful traits: shoveling in the form of barrel-shaped upper lateral incisors (grades 6 and 7 on ASUDAS scale) and premolar odontomes (14).

In particular, shoveling grade 6 or semi-barreled occurs when “the marginal ridges wrap around and contact at a low point on the basal eminence”

while shoveling grade 7 or barreling occurs when, “the marginal ridges are so pronounced that they contact at almost the incisal surface of the basal eminence, assuming a full barrel-shape” (15:33). Odontomes are tubercles of conical shape expressed in the central sulcus of crowns on both upper and lower premolars (15).

The frequency of each trait was compared to the frequencies of other preceramic indigenous populations that inhabited the southeastern region of Brazil during the Holocene, including Corondo/n=103, Sambaqui North/n=94, Sambaqui South/n=119, Lagoa Santa 1/n=41, Lagoa Santa 2/n=98, Lagoa Santa 3/n=59 and Minas Gerais/n=44; Figure 1). These data were obtained from the C.G. Turner II unpublished Database (GRS personal communication). Following common practice for kinship analysis in small samples (2, 3), a Fisher's Exact test was employed to compare those frequencies to the Toca do Enoque frequencies to evaluate statistical significance.

Sex and age-at-death were previously assessed using standard anthropological methods (16-18).

Results

The biological profile of the individuals, as well as the presence or absence of rare nonmetric dental traits, are summarized in Table 1. Counts were generated for barrel-shaped upper lateral incisors (UI2) of grade 6-7 (Figure 3), and premolars odontomes (Figure 4) (14). Odontomes were exclusively identified in unerupted permanent upper premolars (UP).

The relative frequencies of rare nonmetric dental traits observed in the burials from Toca do Enoque were high (Table 2). Shared expression of barrel-shaped upper incisors (33.3 %) and upper premolars odontomes (16.7 %) support the hypothesis of genetic relationship between individuals that may have constituted a kin group. Statistical analysis showed that the co-occurrence of these rare traits is unlikely to happen at random. The hypothesis was assessed through odontologic kinship analysis and validated with archaeological data such as spatial distribution and funeral treatment.

The relative frequencies for other Brazilian preceramic archaeological sites (Table 3) were much lower than those for Toca do Enoque: barrel-shaped upper incisors IU2 (4.5 %) and premolar odontomes (6.4 %). Comparisons between the population groups show a $p < 0.05$

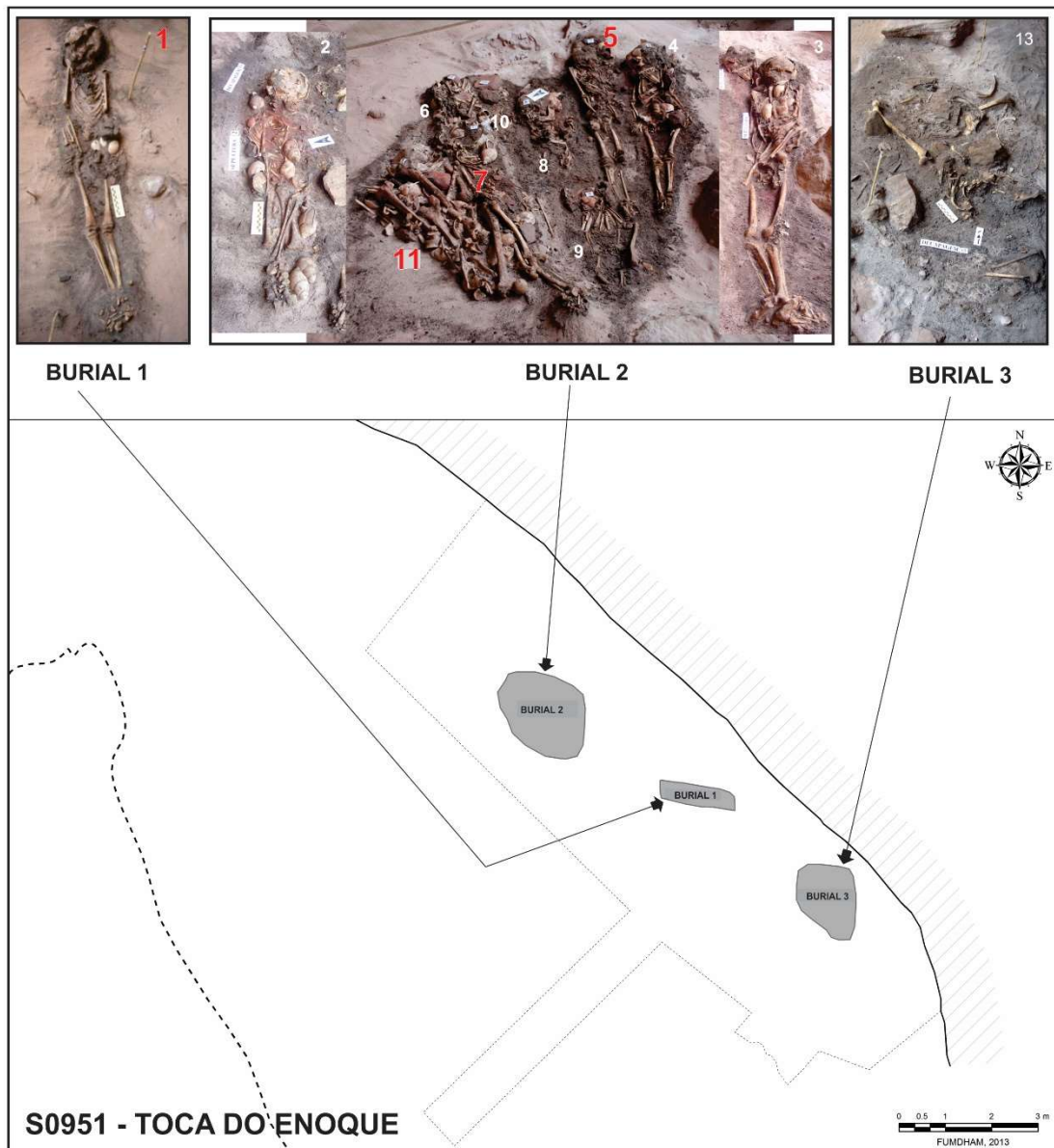


Figure 2. Burials from Toca do Enoque. Top from left to right, Burial 1 - Individual #1, Burial 2 - Individuals #2 to #11, and disturbed Burial 3 - Individual #13 (highlighted in red, individuals with rare dental traits). Below the drawing with the location of burials on the site.

for the UI2 barrel shape (grades 6 and 7), but a $p > 0.05$ for the premolar odontomes. Despite the statistically nonsignificant difference in odontome frequencies between groups, the presence of two individuals exhibiting this rare trait is suggestive of kinship ties between some individuals from Toca do Enoque, not contradicting the barreled UI2 results.

Discussion

The study of dental traits with high heritability, low frequency in the general population, unequivocal expression, and low variation according to demographic parameters like age and sex, enable biological kinship assessment in past populations (5). In that sense, North Asian and Native American populations have high frequencies of incisor shoveling with grades 3

and 4 as the modal expressions while grades 6 and 7 are rare (15). Furthermore, odontomes are a rare trait that occur most commonly in Asian and Asian-derived populations (15). Nonmetric dental traits used in population studies are usually relatively common and are found in frequencies between 10% and 90%. Traits like two-rooted lower canines are rare, or uncommon,

by the standards of those used in anthropological studies, falling below 10% in all world populations (19). A rare trait occurs in a frequency of 0-5% of the population, relative to other traits in the ASUDAS (14). So, barrel-shaped UI2 and premolar odontomes can be included in the category of rare or uncommon traits.

Table 1. Biological profile and rare dental traits distribution of the individuals from Toca do Enoque (*).

Burial #	Individual #	Age	Sex	Barrel-shape IU2	UP odontomes
1	1	7 y ± 24 m	-	Present (grade 7), unilateral	Absent
2	2	10 y ± 30 m	-	Absent	Indiscernible
	3	Young adult	Female (?)	Indiscernible	Indiscernible
	4	6 y ± 24 m	-	Absent	Absent
	5	6 y ± 24 m	-	Present (grades 6 and 7), bilateral	Absent
	6	7 – 9 m	-	Indiscernible	Indiscernible
	7	6 y ± 24 m	-	Present (grade 7), unilateral	Present, unilateral
	8	Young adult	Female (?)	Absent	Absent
	9	9 m ± 3 m	-	Indiscernible	Indiscernible
	10	32-34 weeks (gestational age)	-	Indiscernible	Indiscernible
	11	6 – 7 y	-	Present (grade 7), bilateral	Present, bilateral
3	13	Middle aged adult	Male	Indiscernible	Indiscernible

*Due to changes in the numbering of the individuals, there is no Individual # 12.

Table 2. Rare dental traits and their relative frequency in Toca do Enoque.

Dental traits	Present	Absent	Indiscernible	Relative frequency (%)
UI2 barrel shape (grades 6 and 7)	4	3	5	33.3
UP odontomes	2	4	6	16.7

Table 3. Rare dental traits in samples from southern Brazil, comparative frequency with Toca do Enoque and statistical significance (P).

Dental traits	Corondo	Sambaqui North	Sambaqui South	Lagoa Santa 1, 2, 3 / Minas Gerais	Comparative frequency (%)	p
UI2 barrel shape (grades 6 and 7)	6.25 (1/16)	16.7 (3/18)	2.0 (1/50)	0.0 (0/28)	4.5 (5/112)	0.0005
Premolar odontomes	4.0 (1/25)	0 (0/9)	7.7 (3/39)	7.7 (4/52)	6.4 (8/125)	0.0669

Following the odontologic kinship analysis proposal for small populations (2, 3, 7, 8), we know that if the trait is rare, its observation may be important in indicating family relationships, since finding a rare trait at a high frequency is unlikely outside of a group of genetically related individuals. Thus, in small samples, the key to

kinship analysis is the comparison of the frequency of each trait with the comparative frequency in related populations. A high frequency of a single rare trait may suggest family relationships between individuals. Also, high-



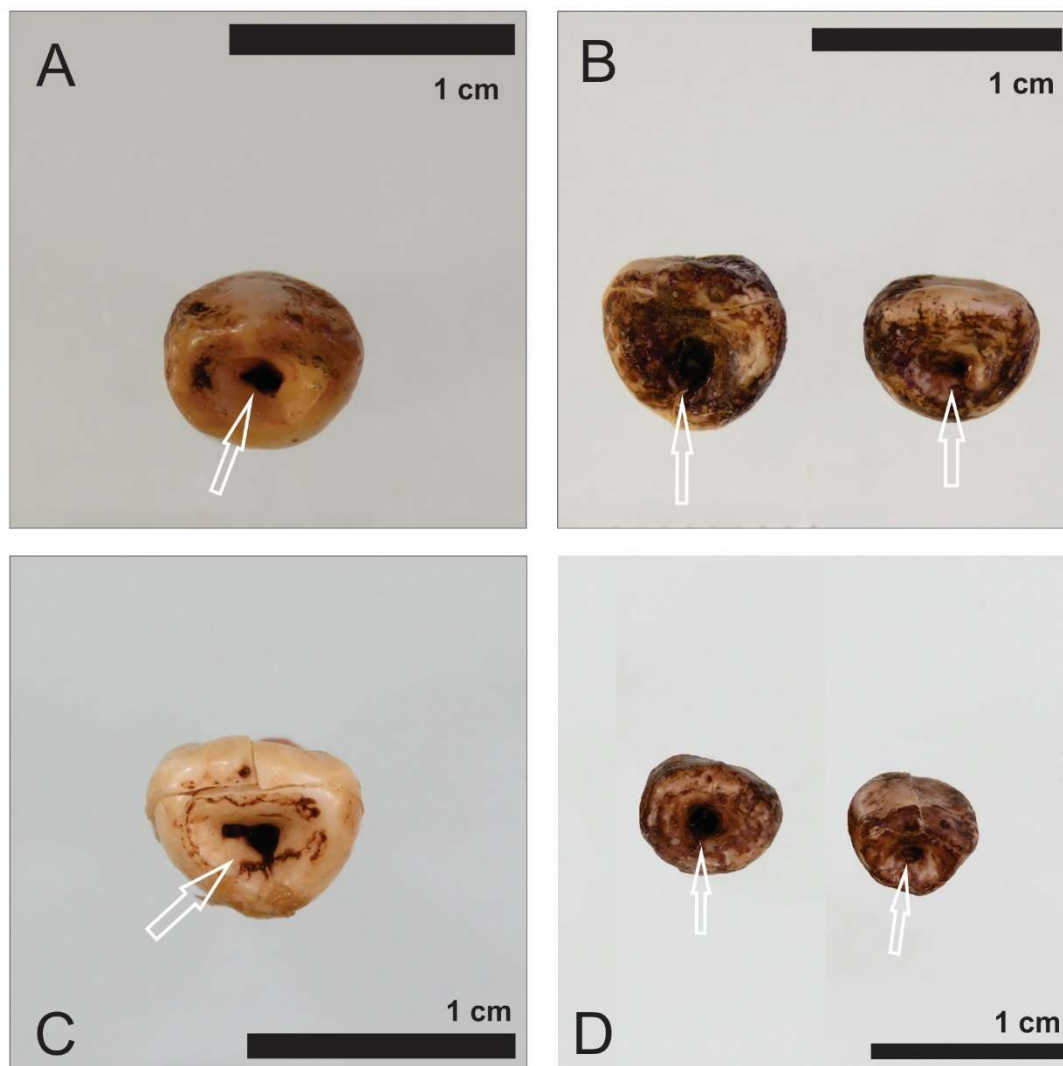


Figure 3. Rare nonmetric dental traits from Toca do Enoque with arrows pointing the traits. Shoveling barrel-shaped upper lateral incisors (UI2 - grades 6 and 7): A- Burial 2 (Individual #7); B- Burial 2 (Individual #11); C- Burial 1 (Individual #1); D- Burial 2 (Individual #5).

value traits with small p values are more important than low-value traits with small p values (7). The highest value is ascribed to traits which have high heritability and are rare in the population.

Lastly, the hypothesis of family relationships based on dental traits must be validated by external information (age, sex, chronology and spatial distribution, burial characteristics, among others) (5). In our case study, the validation was given by the similarity of the funerary practices observed in undisturbed Burials 1 and 2 (as Burial 3 suffered for post-depositional

perturbation), the position and orientation of the individuals in both burials, the similar mortuary treatments and grave goods in both burials, and finally by the spatial proximity of the individuals in the multiple/collective Burial 2 and individual Burial 1 (9). Unfortunately, the absence of direct dating on the human remains made it impossible to determine the exact temporal proximity between each burial, an important datum for interpreting the familial relationships between individuals. However, the wide chronological range of the indirect dates does not invalidate our hypothesis since it only gives a chronological

framework to place these funerary practices in the past.

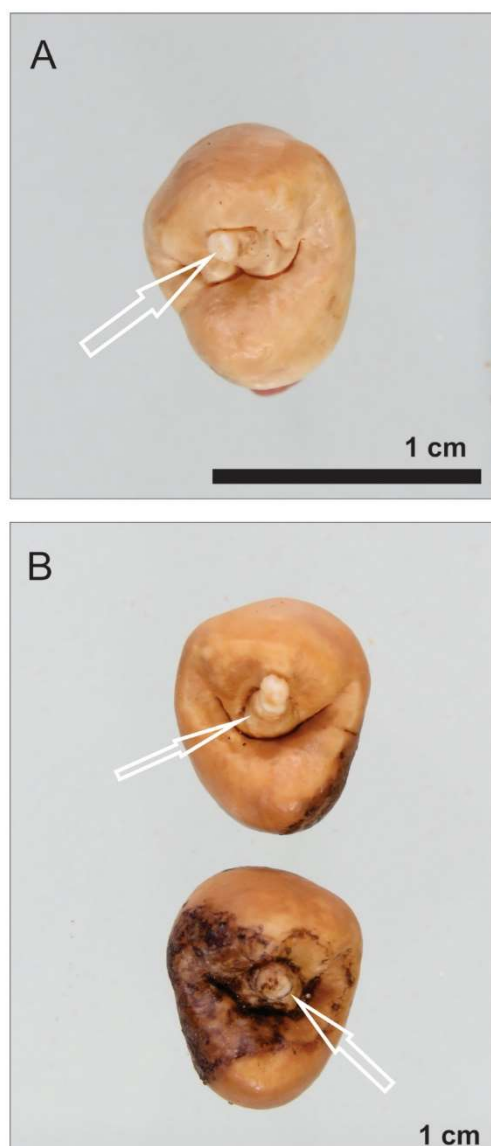


Figure 4. Rare nonmetric dental traits from Toca do Enoque, with arrows pointing the traits. Upper premolars odontomes: A- Burial 2 (Individual #7); B- Burial 2 (Individual #11).

Conclusion

In the odontologic analysis of the individuals buried at the Toca do Enoque funerary prehistoric site in Northeastern Brazil, two rare heritable traits (barrel-shaped upper lateral incisors and upper premolars odontomes) were identified (14). The rarer a trait occurs in a given population, the greater its value is for kinship

analysis (7). Even if kinship analysis does not identify the exact genealogical nature of family relationships between individuals, the high frequency of these two rare dental traits in this small cemetery, suggests genetic relationships between some of the individuals who are likely members of a family group, although it is possible to find this co-occurrence in small endogamous groups as a consequence of population inbreeding.

The young age of three individuals and the absence or high fragmentation of teeth in two others, did not allow us to evaluate the hypothesis in five of the 12 individuals that could also have been relatives. The validation of our findings by external archaeological information, even with the absence of direct dates and a wide temporal framework of the use of the site, shows no contradictions with our proposal of presumed genetic relationships in the spatially kin-structured cemetery of Toca do Enoque.

Finally, this case report, in addition to showing an approach to the study of kinship even without DNA data, is also important for the study of funerary practices among the ancient inhabitants of Brazil and the way in which the living structured the burial areas for their dead relatives in terms of family relationships.

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Declarations of interest

None

Author contributions

AS, NAF and GRS contributed to the conception, design of the study and analysis of data. NAF contributed to the statistical analysis. AMP and GM contributed to drafting and critical review of the article. AS and GRS are the supervisors of the present study. All authors have read and approved the final manuscript.

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