Endocranial morphological affinities of the Early Holocene individuals from Lagoa Santa and implications for the peopling of the Americas

Afinidades morfológicas endocranianas de indivíduos do Holoceno inicial de Lagoa Santa e suas implicações para o povoamento das Américas

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Abstract: The ectocranial morphology of early Holocene humans from Lagoa Santa, Brazil has been described as exhibiting a 'Paleoamerican' form with affinities to African and Australasian individuals, in contrast to past and present Native Americans, who predominantly exhibit 'Amerindian' form. While this interpretation remains disputed, its led to the 'Two Main Biological Components Model', which proposes separate dispersals from Asia into the Americas. This study aims to assess whether the endocranial morphology of Lagoa Santa individuals similarly reflects these affinities. For this study, the overall endocranial morphology, as well as the temporal bones of the crania of twenty individuals from Lagoa Santa, were analyzed and compared with five population samples from the Americas, Australia, Africa, and Asia using computed tomography scans and geometric morphometric methods. Our analyses revealed that, while the overall endocranial morphology of the Lagoa Santa individuals exhibits shape affinities with Australians and Africans, the surface morphology of the temporal bones displays a wider range of variation across individuals.

Keywords: Endocranium. Geometric morphometrics. Paleoamerican. Amerindian.

Resumo: A morfologia ectocraniana da população humana do Holoceno inicial de Lagoa Santa, Brasil, apresenta uma forma 'paleoamericana' com afinidades com indivíduos africanos e australianos, ao contrário dos nativos americanos do passado e do presente, que possuem morfologia 'ameríndia'. Embora contestada, essa observação levou à formulação do 'modelo dos dois componentes biológicos principais', o qual propõe que duas populações morfologicamente distintas se dispersaram da Ásia para as Américas. O presente estudo tem como objetivo avaliar se a morfologia endocraniana de indivíduos de Lagoa Santa apresenta afinidades semelhantes à morfologia ectocraniana. Para isso, os crânios de vinte indivíduos de Lagoa Santa foram analisados por meio de tomografia computadorizada e morfometria geométrica, sendo comparados com cinco amostras populacionais das Américas, da Austrália, da África e da Ásia. Nossas análises demostraram que, enquanto a morfologia endocraniana geral dos indivíduos de Lagoa Santa apresenta afinidades formais com populações australianas e africanas, a morfologia ectocraniana dos ossos temporais apresenta maior variação entre todos os indivíduos.

Palavras-chave: Endocrânio. Morfometria geométrica. Paleoamericano. Ameríndio.

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INTRODUCTION

The Lagoa Santa region is a karstic area located in the state of Minas Gerais, Brazil, near the city of Belo Horizonte (Figure 1). Given the abundance of rock shelters and the late Pleistocene/early Holocene sites dated to as early as 12.5 ka (Bueno et al., 2013; Da-Glória et al., 2018; Strauss et al., 2015, 2016, 2020), as well as the faunal and human remains that have been recovered there since the 1830s (Lund, 1840, 1844), this region has been important for the paleontology and archaeology of Brazil, as well as for the investigation and development of new research regarding how and when humans dispersed into the American continent (e.g. Azevedo et al., 2011; González-José et al., 2008; Hubbe et al., 2010; Menéndez, 2024; Neves & Hubbe, 2005; Neves & Pucciarelli, 1989; Strauss et al., 2020). Different hypotheses, drawing on the study of the morphological variation among Lagoa Santa individuals, have been proposed to clarify the processes of dispersal and peopling of the New World. While some authors consider that there is no morphological differentiation between early Holocene individuals and late or recent Native Americans (Kuzminsky et al., 2017), other scholars contend that two distinct morphological patterns, each characterized by specific traits, define these populations (Neves & Hubbe, 2005; Neves & Pucciarelli, 1989, 1991; von Cramon-Taubadel et al., 2017). Therefore, research on craniofacial morphology to date can be divided into two main perspectives: the first suggests that South American populations share a common ancestral population or descend from closely related groups, whereas the second proposes that morphological variation is better explained by means of multiple migratory waves or distinct ancestral lineages (reviewed in Perez et al., 2009; Skoglund & Reich, 2016).

The idea of the presence of two distinct morphologies dates back to the 19th century (Lacerda & Peixoto, 1876; Moreno, 1882), and became a classical question frequently examined throughout the 20th century (Menéndez, 2024). One of the most influential studies, performed by Neves and Pucciarelli (1989), introduced the first multivariate analyses examining the morphological variation among early South American populations. This work provided

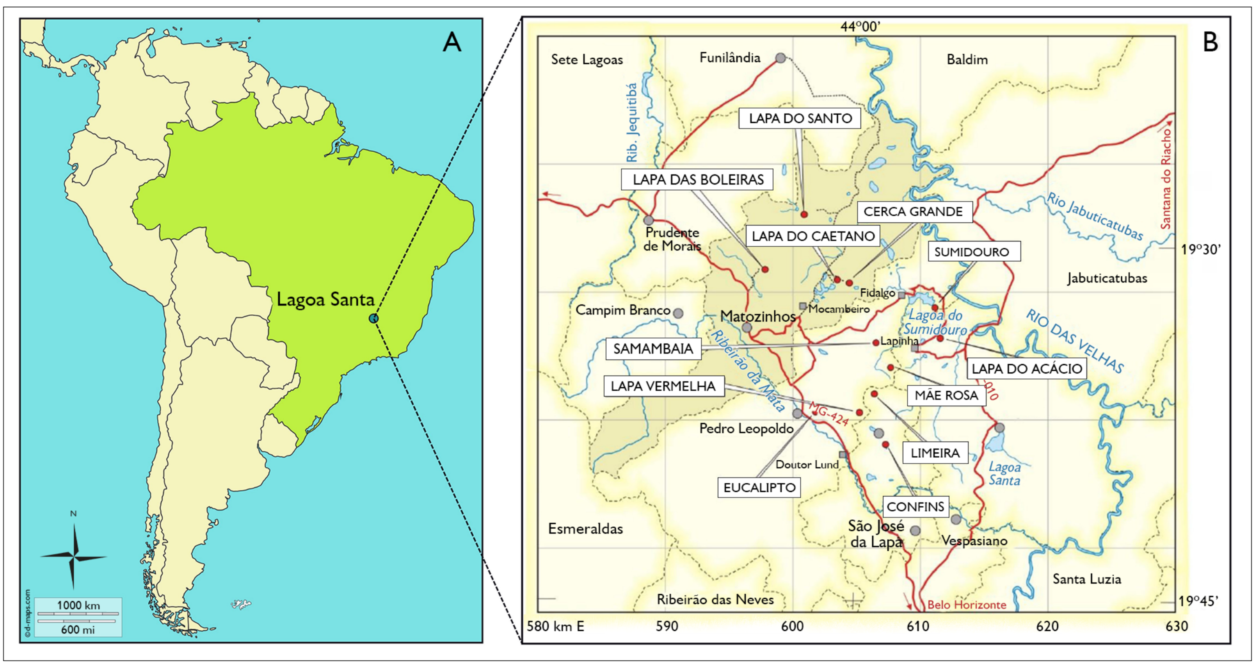

Figure 1. A) Map of South America showing the location of Lagoa Santa; B) detail of the Lagoa Santa region and the location of the sites from where the samples analyzed in this study stem. Source: d-maps (2026) edited by López-Sosa (2021) (A) and Strauss et al. (2015) (B).

support to the migratory hypothesis now referred to as the Two Main Biological Components Model (TMBCM) (Neves et al., 1996, 2003). Neves and Pucciarelli (1989) interpreted their results as confirming morphological differentiation between Lagoa Santa individuals and recent Native Americans, explaining this cranial variation as the result of two successive migratory waves from Asia, in which the first component is represented by the so-called 'Paleoamericans' and the second component by the so-called 'Amerindians.' The Paleoamerican morphology has been described as exhibiting long and narrow neurocrania, low and prognathic faces, and low and wide orbits and noses – anatomical characteristics found at high frequency also in recent populations from Australia, Melanesia, and sub-Saharan Africa, which suggested a common ancestral population shared with the Australasian groups and the retention of shared ancestral morphological traits (Neves & Hubbe, 2005; Skoglund et al., 2015; von Cramon-Taubadel et al., 2017). The Amerindian morphology, on the other hand, is characterized by short and wide neurocrania, high and retracted faces, and narrow orbits and noses. This derived anatomical pattern is present at high frequencies in recent populations from northeastern Asia and in most recent individuals indigenous to the Americas, although some recent Native American populations inhabiting high latitudes, such as Fuegian and Inuit groups, have also been argued to retain plesiomorphic traits and a morphology adapted to cold environments (Evteev et al., 2024; Hernández et al., 1997; Mirazón Lahr, 1995).

The TMBCM suggests that the first component was introduced by an initial population in the late Pleistocene and the second by a subsequent population in the early Holocene (Neves & Hubbe, 2005; Neves & Pucciarelli, 1989, 1991; von Cramon-Taubadel et al., 2017). To evaluate this migratory hypothesis, researchers have sought, for example, to increase sample sizes, explore other morphological traits or methods, and analyze diverse Native American and Asian populations for comparative purposes (e.g., González-José et al., 2008; Herrera et al., 2017; Kuzminsky et al., 2017; Menéndez et al., 2017; Neves & Hubbe, 2005; Perez et al., 2009; Pucciarelli et al., 2006, 2008; Seguchi et al., 2011; Strauss et al., 2015; von Cramon-Taubadel et al., 2017). However, when assessing skeletal samples, only the ectocranial morphology has been analyzed thus far.

The study of endocranial structures has been proposed as an alternative to ectocranial form. For example, endocranial morphology, which reflects brain shape, has been explored because brain morphology is predictive of genetic ancestry at regional (Bakken et al., 2011) and global (Fan et al., 2015; von Cramon-Taubadel, 2009) scales. In addition, large-scale biomedical efforts characterize genomic and phenotypic variation in brain morphology while controlling for population-specific patterns (Altmann & Mourao-Miranda, 2019; Fan et al., 2015; Zhao et al., 2021). Furthermore, the temporal bone has been shown to be particularly informative regarding modern human population history and genetic affinities in both its ectocranial (Harvati & Weaver, 2006; Reyes-Centeno et al., 2014; Smith et al., 2013) and endocranial (Fan et al., 2015) morphology, to a greater extent than facial or neurocranial anatomy. Using cortical surface data derived from magnetic resonance imaging (MRI) of modern human brains, Fan et al. (2015) demonstrated that brain shape (and not size or volume) shows a significant correspondence with genetic variation. An alternative approach to analyzing brain surfaces in individuals from paleontological or archaeological contexts is to examine their morphology either through the use of reconstructed brain endocasts (e.g. Balzeau & Pagano, 2022; Bruner, 2008) or the endocranial surface as a proxy (e.g. Rosas et al., 2014; Scott et al., 2014). Since the endocranium has not yet been used to assess the TMBCM, this study aims to analyze the endocranial morphology of Lagoa Santa individuals, as well as their affinities with populations from the Americas, Australia, Africa, and Asia, which represent the morphological variation proposed by the migratory models. Thus, we conducted our analyses with three objectives: 1) to examine the morphological variation in the endocranium among populations from different continents, and hence; 2) to assess whether the morphometric variation in the endocranium produces population affinities similar explained by the TMBCM, as has been observed for ectocranial morphology; and finally, 3) to determine whether there are differences between overall endocranial variation and variation in the temporal bone region specifically. In line with previous ectocranial analyses and previous results on the temporal bone and endocranial shape, we propose that the endocranial shape of the Lagoa Santa sample will either show stronger affinities with Australasians and Africans, supporting the two-wave model or, alternatively, stronger affinities with other Native American and Asian individuals, supporting the one-wave model. In light of the strong association between genomic variation and temporal bone and temporal lobe cortical surface shape, we expect that the endocranial surface of the temporal bone may further support one of these models.

MATERIALS AND METHODS

A total of 20 individuals from several sites in Lagoa Santa were analyzed in this study. The crania were selected from three different collections. The first is the Harold Walter Collection, from which eight individuals (Da-Glória, 2012; Walter, 1958; Walter et al., 1937) that are known to come from various Lagoa Santa sites, were selected (Lapa Mortuaria, Lapa do Caetano, Mãe Rosa, among others; Neves & Hubbe, 2005; Strauss, 2010; Walter, 1958; Figure 1; Table 1), although without precise provenance. The only exception is 'Confins Man,' excavated from the homonymous rockshelter. In June 2020 this collection was lost following a fire at the host repository. The second collection consists of individuals from the archaeological sites of Lapa do Acácio, Lapa da Samambaia and Sumidouro, all located on the central-eastern area of Lagoa Santa (Bányai, 1997; Strauss, 2010; Figure 1; Table 1). From these, seven individuals were selected, which will be studied in the present paper for the first time. The third (Figure 1; Table 1) contains crania from Lapa do Santo, which is an archaeological site in the northern region of the Lagoa Santa karst. Its oldest occupation dates to at least 11.7-12.7 ky cal BP (Strauss et al., 2015, 2016; Villagran et al., 2017, 2019). For this study, five adult individuals from different burials (11, 14, 15, 21 and 26) were selected (Neves et al., 2014; Posth et al., 2018; Strauss et al., 2015, 2016; von Cramon-Taubadel et al., 2017). We note that while not all of these individuals have yielded direct dates, several others from Sumidouro and Lapa do Santo have been directly dated to the early Holocene (ca. 9.4-10.4 cal BP; Moreno-Mayar et al., 2018; Posth et al., 2018). Those with known provenance have an average radiocarbon age of ca. 8 ky cal BP, based on direct dates obtained from human bones and/or charcoal directly associated to the burials, as well as indirect stratigraphic estimates (Table 1; Araujo et al., 2012; Da-Glória et al., 2018; Neves & Hubbe, 2005; Strauss et al., 2016, 2020). We selected crania based on their state of preservation (fragmentation, taphonomy, etc.) and those that could be directly measured without reconstruction.

The comparative sample consists of 123 individuals from five regions in South America, Northeast Asia, Oceania, and Africa (Table 2). The South American samples derive from recent populations from Buenos Aires and Tierra del Fuego. The Buenos Aires sample stem from central-eastern Argentina, while Tierra del Fuego is located in the southernmost region of South America, covering modern Argentine and Chilean territory (Gusinde, 1931; Murdock, 1962). The Northeast Asian sample consists of individuals from a recent population from Mongolia, and the Oceanian sample comprises recent crania from several locations in Australia (Noback, 2014; Spencer & Gillan, 1966; Vreeland, 1973). Finally, a Nubian sample was selected from Northeast Africa, which consists of archaeological crania dating to the late Holocene (Noback, 2014; Wilhelm, 1953). The comparative samples were chosen primarily based on their provenance, representing geographical regions that encompass the morphological variation expressed by the competing migratory models and populations comparable to those used in previous studies testing these models.

Table 1. Accession ID, institutional repository and provenance of the Lagoa Santa individuals used in this study. Legends: [1] = Natural History Museum and Botanical Garden of the Federal University of Minas Gerais – Harold Walter Collection; [2] = from Lagoa Santa region, but unknown exact location of site of origin; [3] = neither direct nor stratigraphic dates are available – nevertheless, as the great majority of skeletons from Lagoa Santa properly dated are early Holocene, it is assumed all skeletons are from roughly the same age; [4] = a radiocarbon date of was obtained, but it is likely contaminated; [5] = Archaeological Museum of Lagoa Santa of the *Instituto Estadual de Florestas of Minas Gerais* (a.k.a. Castelinho); [6] = same locality explored by Peter Lund during the 19th century; [7] = Institute of Bioscience, University of São Paulo; n/a = not available; HD = Hélio Diniz Collection and MB = Mihály Bányai Collection.

ID this study	Accession ID	Collection	Archaeological site	Radiocarbon Date (non-cal BP)
LS1	HW-002	NHMBG-HW[1]	n/a[2]	n/a[3]
LS2	HW-003	NHMBG-HW	n/a[2]	n/a[3]
LS3	HW-004	NHMBG-HW	n/a[2]	n/a[3]
LS4	HW-005	NHMBG-HW	n/a[2]	n/a[3]
LS5	HW-293	NHMBG-HW	n/a[2]	8,020 ± 40
LS6	HW-Confins	NHMBG-HW	Lapa Mortuária Confins	n/a[4]
LS7	HW-010	NHMBG-HW	n/a[2]	n/a[3]
LS8	HW-014	NHMBG-HW	n/a[2]	n/a[3]
LS9	Acacio2	AMLS-MB[5]	Lapa do Acácio	n/a[3]
LS10	DinizAdulto196	AMLS-HD[5]	Lapa do Sumidouro[6]	n/a[3]
LS11	DinizAdulto198	AMLS-HD	Lapa do Sumidouro[6]	n/a[4]
LS12	DinizSumidouro2	AMLS-HD	n/a[2]	n/a[3]
LS13	DinizSumidouro3	AMLS-HD	n/a[2]	n/a[3]
LS14	SamambaiaAdultoF	AMLS-MB	Lapa da Samambaia	n/a[3]
LS15	SamambaiaAdultoM	AMLS-MB	Lapa da Samambaia	n/a[3]
LS16	Lst-Bur-11	IB-USP[7]	Lapa do Santo	5,990 ± 40
LS17	Lst-Bur-14	IB-USP	Lapa do Santo	8,230 ± 40
LS18	Lst-Bur-15	IB-USP	Lapa do Santo	9,550-9470 cal BP
LS19	Lst-Bur-21	IB-USP	Lapa do Santo	8,584 ± 33
LS20	Lst-Bur-26	IB-USP	Lapa do Santo	8,540 ± 50

Table 2. Samples, number of individuals, antiquity, location, and type of scans of the populations used for analyses. Legends: AMNH = American Museum of Natural History; NHM-Wien = Natural History Museum of Vienna; μCT = micro-Computed Tomography; * = the approximated antiquity of the Lagoa Santa series is based on the dating of the osteological remains of several individuals from this region.

Series	Number	Antiquity	Institutional repository	References	Scan
Lagoa Santa	20	~8,000 BP*	See Table 1	Neves & Hubbe (2005); Strauss (2010)	CT
Buenos Aires	19	Late Holocene	Museo de La Plata	Lehmann-Nitsche (1911)	CT
Tierra del Fuego	24	Late Holocene	AMNH; NHM-Wien	Noback (2014)	CT, μCT
Mongolia	26	Late Holocene	Smithsonian Institution	Noback (2014)	CT
Nubia	28	2,300-1,200 BC/350 BC-350 AD	Copenhagen University	Noback (2014)	CT
Australia	26	Late Holocene	AMNH	Noback (2014)	CT

Each of our population samples comprises between 19 and 28 individuals (Table 2), with approximately equal numbers of males and females (Table S1 in López-Sosa et al., 2026). For individuals whose sex was not known or recorded in institutional catalogues, this was determined by one of us (MCL-S) following the criteria of Martin and Saller (1957) based on cranial dimorphic features (Table S1 in López-Sosa et al., 2026).

To achieve these objectives, the principles and methods of virtual anthropology, including geometric morphometrics and multivariate statistics, were applied (Weber & Bookstein, 2011). First, using Viewbox 4 Cephalometric Software (v. 4.0.1.7, dHAL), a total of 26 landmarks on the endocranial surface of the cranial base, and 26 curve semilandmarks along the midsagittal plane were collected by one of the authors (MCL-S) for each cranial surface (Table 3; Figure 2; for further details see Methods in López-Sosa et al., 2026). Interobserver error testing for variation in landmark registration was found to be negligible (see Methods and Table S3 in López-Sosa et al., 2026).

Table 3. Set of landmarks (landmarks 1-9, 11-19 used in temporal bone data subset).

1.	Right opening of the cochlear aqueduct
2.	Right internal acoustic meatus (anterior)
3.	Right internal acoustic meatus (posterior)
4.	Right opening of the vestibular aqueduct (anterior)
5.	Right opening of the vestibular aqueduct (posterior)
6.	Right angle between Sigmoid Sinus and petrous portion
7.	Right endoasterion
8.	Right arcuate eminence
9.	Right fallopian hiatus
10.	Right endopterion
11.	Left opening of the cochlear aqueduct
12.	Left internal acoustic meatus (anterior)
13.	Left internal acoustic meatus (posterior)
14.	Left opening of the vestibular aqueduct (anterior)
15.	Left opening of the vestibular aqueduct (posterior)
16.	Left angle between Sigmoid Sinus and petrous portion
17.	Left endoasterion
18.	Left arcuate eminence
19.	Left fallopian hiatus
20.	Left endopterion
21.	Foramen caecum (inferior)
22.	Foramen caecum (superior)
23.	Lowest point of the crista frontalis
24.	Endobregma
25.	Endolambda
26.	Internal occipital protuberance
C1. Curve 1 (8 semilandmarks from landmark 23 to landmark 24)	
C2. Curve 2 (12 semilandmarks from landmark 24 to landmark 25)	
C3. Curve 3 (6 semilandmarks from landmark 25 to landmark 26)	

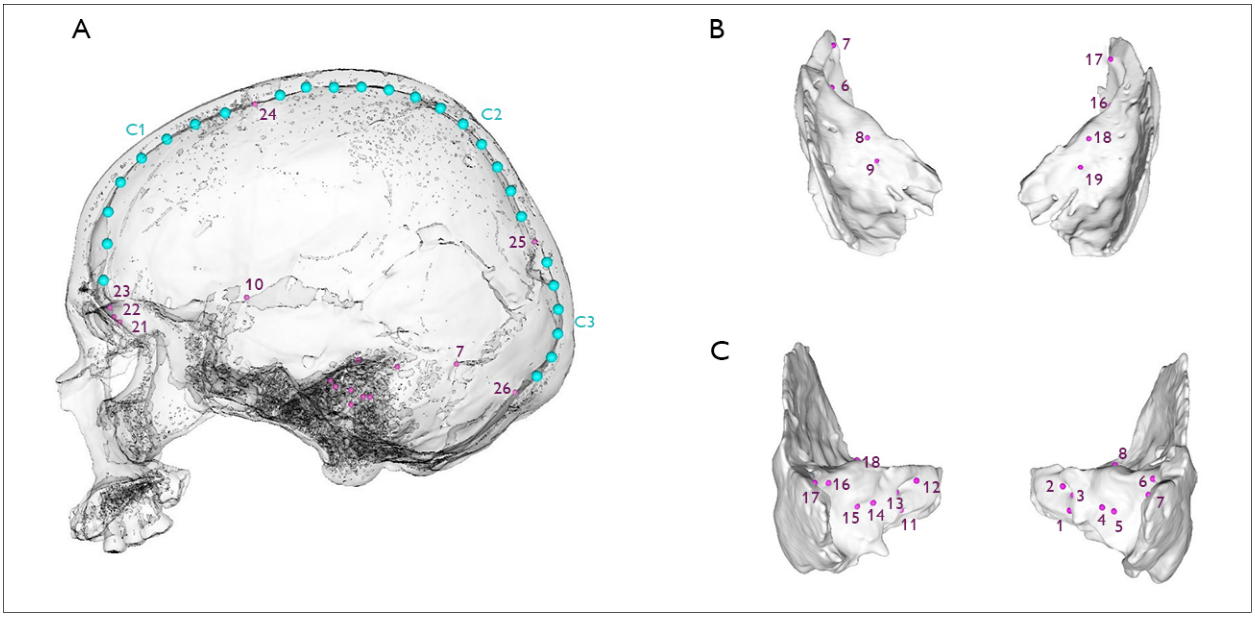

Figure 2. Landmark and semilandmark set (see Table 3): A) lateral view of the landmark (pink) and semilandmark (blue) set; B) detail of the landmarks of the temporal bones in superior view; C) detail of the landmarks in posterior view. Source: López-Sosa (2026).

In addition, we subsetted the dataset to capture the endocranial regions of the temporal bone in two ways: first maintaining both bilateral regions as in previous studies of cortical shape (Fan et al., 2015) and then using only the left temporal bone as in previous ectocranial studies (Reyes-Centeno et al., 2014; Smith, 2009). After applying a Generalized Procrustes Analysis (GPA) to the landmark configurations of the endocranium and separately for the temporal bones, we obtained the shape variables to perform two multivariate statistical methods: 1) Principal Component Analysis (PCA), and 2) Discriminant Function Analysis (DFA). Because the number of landmark coordinates exceeds that of individuals per group, we reduced the dimensionality of the dataset to PC scores and determined the number of PCs to include in the DFA by applying a three-step 'stopping rule' (Jackson, 1993; Reyes-Centeno et al., 2016; for more details see Methods in López-Sosa et al., 2026). In all cases, the number of PC variables was lower than the number of individuals in each sampled population. We therefore performed DFAs with these PC scores in order to assess how accurately individuals were classified into pre-assigned groups, first by including Lagoa Santa as a pre-assigned group in order to test whether they would be classified within the group with high probability. The purpose of this analysis was to compare the classification accuracy of the Lagoa Santa group with that of the comparative groups, thereby providing a relative measure of the homogeneity and morphological variation within the Lagoa Santa sample. Subsequently, a second DFA was performed, this time excluding Lagoa Santa as an *a priori* classification group, to assess whether these individuals would be classified as Australians and/or Nubians, as predicted under the two-wave model. In all cases, DFA priors assumed equal group probabilities and additional DFAs were performed with a leave-one-out cross-validation procedure. To assess the degree of bias resulting from unequal group sizes in our analyses, we computed the *Tau* statistic (Klecka, 1980; Kovarovic et al., 2011), which is a chance-corrected measure of classification accuracy when prior probabilities are not proportional. Both the DFA and *Tau* statistic results provide indication of endocranial morphological variation among population samples from different continents (objective 1), as well as an indication of whether there are differences between the endocranium as a whole and the temporal bone subsets (objective 3). Specifically, high classification accuracies for all data subsets would indicate consistent support for the TMBCM for endocranial data, as previously shown for ectocranial data (objective 2).

Additionally, Procrustes distances were calculated and the statistical significance of pairwise differences in mean shapes among the six studied groups was evaluated using permutation tests (10,000 replications). These distances are used to assess shape differences because the p-values can differ due to the anisotropy effect (i.e., variation in different properties across examined directions inherent to shape data) that shape variation has (Klingenberg & Monteiro, 2005). They have already been applied to evaluate craniometric differences among different South American groups and have proven effective (e.g., Perez et al., 2007; von Cramon-Taubadel et al., 2017). We note that permutation tests are not appropriate for estimating confidence intervals since the standard deviation of the parameter estimates (in this case, the mean shape) does not reliably estimate the standard error of that parameter (Zelditch et al., 2012, pp. 216-217). Sample distribution in PCA shape space was visualized along the axes explaining the majority of variation in the dataset. Shape variation was graphically represented by 'warping' the surfaces of the neurocranium and temporal bones of one Lagoa Santa individual (HW-010) along the first two PC axes, using the TPS algorithm. Finally, Minimum Spanning Trees (MST) were calculated using the mean group Procrustes Distances and superimposed on the PCA plots. All analyses were subsequently conducted using a subset of the dataset, which captures the shape of the endocranial structure of the temporal bones specifically. The PCA and surface warpings were conducted using the EVAN Toolbox (v. 1.71, by the Evan Society, n.d.), while the DFA, MST, as well as the Procrustes distances calculation and the Repeated Measures ANOVA were performed with the JMP Pro (v. 16, by the SAS Institute Inc., 2021), MorphoJ (Klingenberg, 2011), and PAST (v. 2.17, Hammer et al., 2001) software. *Tau* statistics were calculated using OpenOffice.

RESULTS

ENDOCRANIAL VARIATION

The PCA conducted on all shape variables places Lagoa Santa individuals close to Australians and Nubians along the first two PCs (35.5%; Figure 3). Fuegians appear with them on the right side of the plot, although closer to Mongolians. The individuals from Buenos Aires occupy primarily the upper-left quadrant of the graph. To visualize endocranial surface shape changes along the first two PCs, we warped a surface using as reference the mean landmark configuration and as a target the landmark configuration at approximately three standard deviations from the mean (along PC1 $x = -0.09, 0.09$, along PC2 $y = -0.06, 0.06$ of Figure 3). These visualizations emphasize variation in endocranial length along PC1 and width along PC2. The cases located on the positive side of PC1, comprising mostly individuals from Lagoa Santa, Australia, Nubia, and Tierra del Fuego, exhibit a more antero-posteriorly elongated endocranium while those on the negative side of the axis, comprising primarily individuals from Mongolia and Buenos Aires, exhibit a shorter endocranium. Along PC2, the cases located on the positive side exhibit taller and narrower endocrania than the ones located on the negative side, which tend to be shorter and wider. The MST shows that the mean of the Lagoa Santa sample is directly linked to the Nubian, as well as the Fuegian sample means.

Regarding the DFA, when performed with Lagoa Santa included as a classification group, seventeen of these individuals (85% of the total) were correctly classified, whereas two were classified as Nubians and one as Fuegian (Table 4). The overall correct classification rate across the dataset was ~85%, or ~71% after cross-validated (Tables S4 and S5 in López-Sosa et al., 2026). The chance-corrected classification rates,

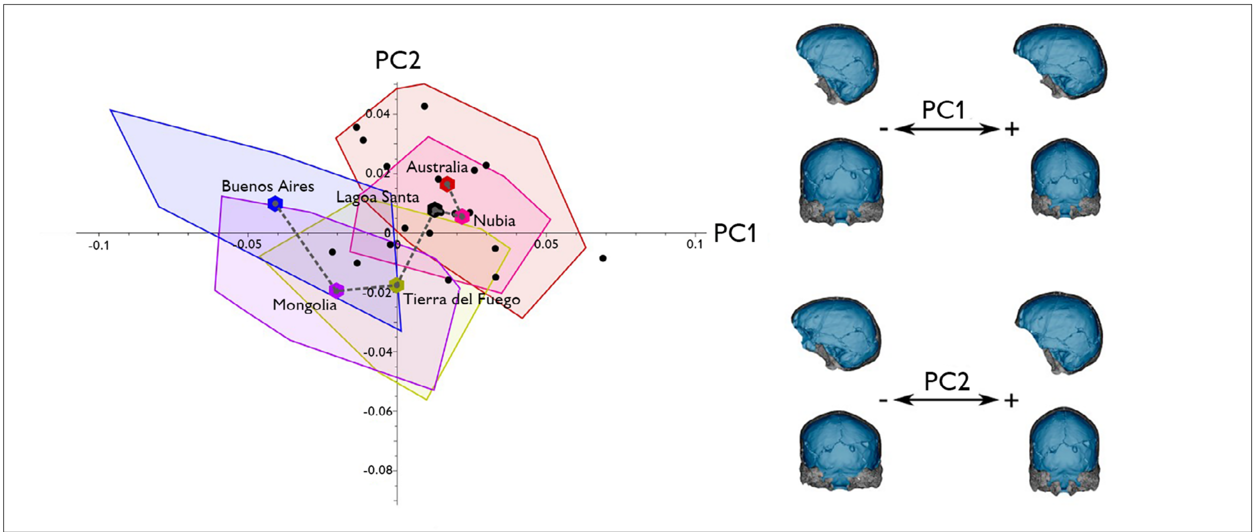

Figure 3. PCA of the shape variables of the endocranium. PC1 (23.7%) *vs*. PC2 (11.8%). The Lagoa Santa individuals are depicted by the black circles. Large polygons represent population means. The dotted line connecting the polygons represents the MST based on Procrustes distances among the group means. Crania in anterior and lateral view represent the mean surface configuration warped at approximately three standard deviations from the mean along PC1, at x = -0.09, 0.09, and approximately three standard deviations along PC2, at y = -0.06, 0.06. Source: López-Sosa (2026).

Table 4. DFA results including Lagoa Santa as a classification group (Endocranium) (~85% overall correct classification rate, ~71% cross-validated – *Tau* ~81% and ~65%, respectively).

Actual individual	Predicted	Posterior probability	Other posterior probabilities
LS1	Lagoa Santa	0.9672	
LS2	Lagoa Santa	0.9570	
LS3	Lagoa Santa	0.9928	
LS4	Lagoa Santa	0.8101	Fuegian 0.19
LS5	Lagoa Santa	0.8500	Nubian 0.14
LS6	Nubian	0.5189	Australian 0.38
LS7	Lagoa Santa	0.9101	
LS8	Lagoa Santa	0.6542	Nubian 0.17 Fuegian 0.13
LS9	Fuegian	0.5427	Australian 0.12
LS10	Lagoa Santa	0.9554	
LS11	Lagoa Santa	0.8642	Australian 0.11
LS12	Lagoa Santa	0.5586	Nubian 0.23 Fuegian 0.13
LS13	Lagoa Santa	0.8723	Nubian 0.11
LS14	Lagoa Santa	0.5390	Fuegian 0.46
LS15	Lagoa Santa	0.9277	
LS16	Lagoa Santa	0.9770	
LS17	Nubian	0.6057	Australian 0.18
LS18	Lagoa Santa	0.8770	
LS19	Lagoa Santa	0.9238	
LS20	Lagoa Santa	0.9054	

as indicated by the *Tau* statistics, were ~81% and ~65%, respectively. In the second DFA excluding Lagoa Santa as a classification group, twelve Lagoa Santa individuals were assigned to Nubia, six to Tierra del Fuego, and two to Australia (Table 5). In this case, ~80% of the total sample was correctly classified after cross-validated (Tables S6 and S7 in López-Sosa et al., 2026; *Tau* ~74%). The Procrustes distances show significant differences between all pairwise comparisons (< 0.01). The group pair showing the greatest difference is Nubian-Buenos Aires, while the most similar pair is Nubian-Australian (Table 6). The mean endocranial shape of Lagoa Santa individuals is similar to that from Nubians, followed by the one from Australians, while it is most distant in Procrustes shape from the Buenos Aires population sample.

We also conducted additional analyses for comparative purposes using only cranial vault data (i.e., the endocranial landmark configuration at the exclusion of the temporal bone landmarks but the inclusion of endoasterion), which yielded classification rates between ~62-68% after cross-validated. However, since this was beyond the scope of our objectives, the results of these DFA are summarized in Tables S19 and S20 in López-Sosa et al. (2026).

TEMPORAL BONE VARIATION

The PCA of temporal bone endocranial surface shape places Lagoa Santa individuals at the center of shape space, between the other samples along the first two PCs (ca. 33%; Figure 4). To visualize shape changes, we also warped the mean surface configuration of the temporal bones at approximately three standard deviations from the mean along each

Table 5. DFA results without Lagoa Santa as a classification group (Endocranium) (~86% overall classification rate, ~80% cross-validated – *Tau* ~83% and ~74%, respectively).

Actual individual	Predicted	Posterior probabilitiy	Other posterior probabilities
LS1	Nubian	0.8861	Australian 0.11
LS2	Nubian	0.4534	Fuegian 0.43
LS3	Nubian	0.8787	Australian 0.12
LS4	Fuegian	0.9999	
LS5	Nubian	0.9727	
LS6	Australian	0.5883	Nubian 0.41
LS7	Nubian	0.5559	Australian 0.26 Fuegian 0.18
LS8	Fuegian	0.4987	Australian 0.20 Nubian 0.29
LS9	Fuegian	0.9674	
LS10	Nubian	0.8132	Australian 0.18
LS11	Australian	0.8308	Nubian 0.17
LS12	Nubian	0.4792	Australian 0.24 Fuegian 0.28
LS13	Nubian	0.8209	Australian 0.18
LS14	Fuegian	1.0000	
LS15	Fuegian	0.9203	
LS16	Nubian	0.7898	Fuegian 0.11
LS17	Nubian	0.8435	Australian 0.15
LS18	Fuegian	0.5893	Nubian 0.36
LS19	Nubian	0.6568	Australian 0.34
LS20	Nubian	0.9575	

Table 6. Procrustes distances among groups and P-values from permutation tests (10,000 permutation rounds) for endocranial shape.

	Australian	Fuegian	Lagoa Santa	Buenos Aires	Mongolian	Nubian
Australian	0	<.0001	<.0001	<.0001	<.0001	0.0088
Fuegian	0.0436	0	<.0001	<.0001	<.0001	<.0001
Lagoa Santa	0.0339	0.0380	0	<.0001	<.0001	<.0001
Buenos Aires	0.0635	0.0544	0.0609	0	<.0001	<.0001
Mongolian	0.0540	0.0369	0.0552	0.0455	0	<.0001
Nubian	0.0211	0.0397	0.0298	0.0665	0.0513	0

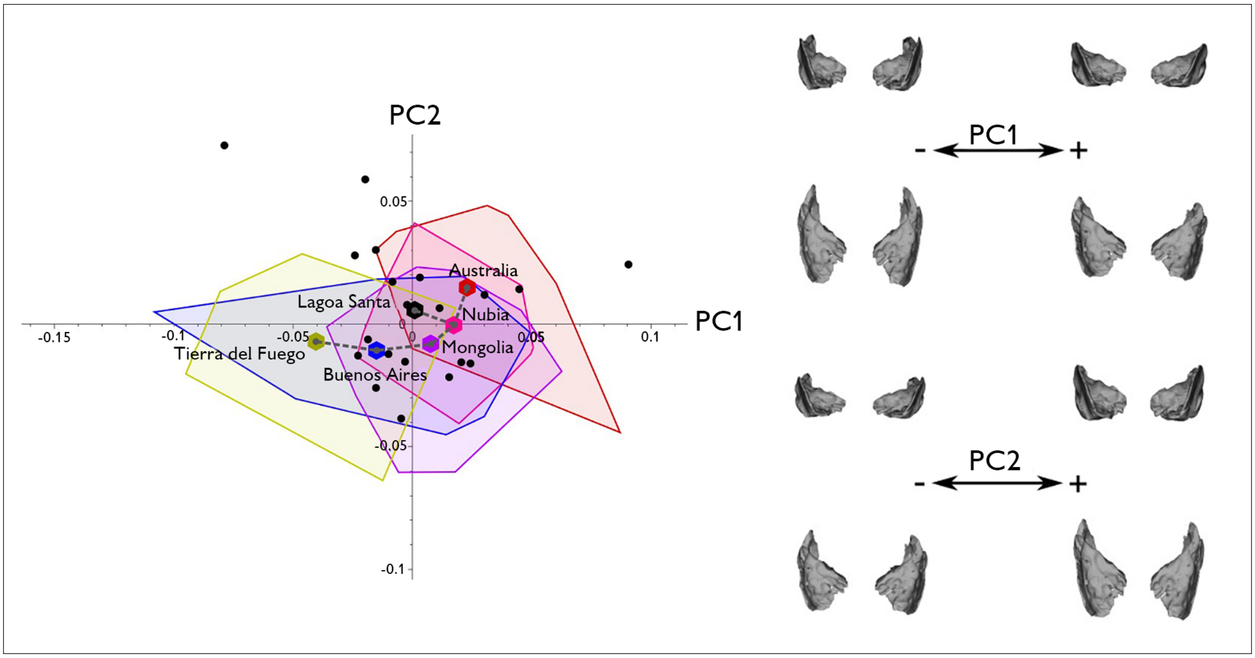

Figure 4. PCA of the shape variables of the temporal bones. PC1 (22.7%) *vs*. PC2 (9.9%). The Lagoa Santa individuals are depicted by the black circles. Large polygons represent population means. The dotted line connecting the polygons represents the MST based on Procrustes distances among the group means. Temporal bones in anterior and superior view represent the mean surface configuration warped at approximately three standard deviations from the mean along PC1, at x = -0.1, 0.1, and approximately three standard deviations along PC2, at y = -0.07, 0.07. Source: López-Sosa (2026).

PC (PC1 axis x = -0.1, 0.1 and PC2 axis y = -0.07, 0.07 of Figure 4). This visualization emphasizes variations in the length, width, and height of the temporal bones along the first two PCs. Significant variation on the posterior region of the temporal bones is observed along PC1, whereas PC2 expresses variation in the relative length and height of the bone as a whole. When analyzing only the left temporal bone, Lagoa Santa individuals are distributed along PC1 (21.9%) and across the positive side of PC2 (12.8%), largely overlapping with the individuals from Nubia, Australia, and Buenos Aires. In this case we warped the mean surface configuration of the left temporal bone as well, at approximately three standard deviations from the mean along each PC (PC1 axis x = -0.18, 0.18 and PC2 axis y = -0.13, 0.13 of Figure S2 in López-Sosa et al., 2026). This highlights variation in length along PC1 and height along PC2. When examining t the MST of the means of each population sample for both temporal bones, the mean from Lagoa Santa is directly connected to Nubia (Figure 4), whereas it is linked to both Nubia and Mongolia when only the left temporal bone is considered (Figure S2 in López-Sosa et al., 2026).

In the first DFA, including Lagoa Santa as an *a priori* category fifteen Lagoa Santa individuals (75%) were correctly classified (which comprise twelve of the individuals that were correctly classified with the endocranium variables). Of the remaining individuals, one was classified as Fuegian, two as Nubians, one as Australian, and one as Mongolian (Table 7). The overall classification rate across the dataset was ~73% and ~52% when cross-validated (Tables S8 and S9 in López-Sosa et al., 2026; *Tau* ~67% and ~43%, respectively). In the second analysis (excluding Lagoa Santa as a pre-assigned category), ten Lagoa Santa individuals were classified as Nubians, four as Australians, three as Fuegians, and three as Mongolians (Table 8). Across all samples in the dataset ~80% were correctly classified and ~65% after cross-validated (Tables S10 and S11 in López-Sosa et al., 2026; *Tau* ~74% and ~56%, respectively).

The DFA conducted using shape variables of the left temporal bone and including Lagoa Santa as a classification group correctly classified eleven Lagoa Santa individuals (55% of the total); two were classified as Fuegians, two as Australians, two as Nubians, and three as Mongolians (Table S12 in López-Sosa et al., 2026). The overall correct classification rate was ~60%, or ~51% after cross-validated (Tables S13 and S14 in López-Sosa et al., 2026; *Tau* ~52% and ~41%, respectively). When excluding Lagoa Santa as a pre-assigned category, five Lagoa Santa individuals were classified as Mongolians, nine as Nubians, two as Australians, two as Fuegians, and two as from

Table 7. DFA results including Lagoa Santa as a classification group (Temporal Bones) (~73% overall classification rate, ~52% cross-validated – *Tau* ~67% and ~43%, respectively).

Actual individual	Predicted	Posterior probability	Other posterior probabilities
LS1	Lagoa Santa	0.8221	Nubian 0.17
LS2	Lagoa Santa	0.5537	Nubian 0.42
LS3	Lagoa Santa	0.5049	Australian 0.24 Nubian 0.26
LS4	Nubian	0.4175	Fuegian 0.11
LS5	Lagoa Santa	0.9470	
LS6	Lagoa Santa	0.5612	Australian 0.11 Nubian 0.30
LS7	Lagoa Santa	0.7203	Nubian 0.15 Fuegian 0.11
LS8	Lagoa Santa	0.6158	Mongolian 0.16 Nubian 0.12
LS9	Lagoa Santa	0.6464	Fuegian 0.20
LS10	Lagoa Santa	0.8969	
LS11	Lagoa Santa	0.8969	
LS12	Mongolian	0.4122	Nubian 0.23
LS13	Nubian	0.5897	Australian 0.13
LS14	Fuegian	0.7251	
LS15	Australian	0.5102	
LS16	Lagoa Santa	0.5972	Nubian 0.21 Fuegian 0.17
LS17	Lagoa Santa	0.6577	Nubian 0.30
LS18	Lagoa Santa	0.9341	
LS19	Lagoa Santa	0.4703	Nubian 0.28 Fuegian 0.19
LS20	Lagoa Santa	0.7540	Nubian 0.19

Table 8. DFA results without Lagoa Santa as a classification group (Temporal Bones) (~80% overall classification rate, ~65% cross-validated – *Tau* ~74% and ~56%, respectively).

Actual individual	Predicted	Posterior probability	Other posterior probabilities
LS1	Mongolian	0.7791	Nubian 0.22
LS2	Nubian	0.9102	
LS3	Australian	0.8001	Nubian 0.20
LS4	Nubian	0.5889	Mongolian 0.11 Fuegian 0.28
LS5	Nubian	0.7665	Australian 0.21
LS6	Nubian	0.5735	Mongolian 0.34
LS7	Nubian	0.6228	Fuegian 0.32
LS8	Mongolian	0.7704	Nubian 0.13
LS9	Fuegian	0.6965	Nubian 0.17
LS10	Australian	0.3941	Mongolian 0.24 Nubian 0.35
LS11	Australian	0.7924	Nubian 0.19
LS12	Mongolian	0.7443	Nubian 0.22
LS13	Nubian	0.7408	Australian 0.25
LS14	Fuegian	0.9841	
LS15	Australian	0.9618	
LS16	Fuegian	0.7796	Buenos Aires 0.11 Nubian 0.11
LS17	Nubian	0.9204	
LS18	Nubian	0.7884	Fuegian 0.14
LS19	Nubian	0.6242	Fuegian 0.26
LS20	Nubian	0.7876	Australian 0.17

Buenos Aires (Table S15 in López-Sosa et al., 2026). Across all samples ~67% were correctly classified and ~56% after cross-validated (Table S16 and S17 in López-Sosa et al., 2026; *Tau* ~58% and ~45%, respectively).

The Procrustes distances show significant differences among all pairwise comparisons (< 0.01) (Table 9 and S18 in López-Sosa et al., 2026). The results for both temporal bones show that the group pairs that differ the most are Australian-Fuegian, while the most similar pair is Nubia-Lagoa Santa (Table 9). The mean bilateral temporal bone shape of Lagoa Santa individuals is most similar to that of the Nubian sample, followed by those of Mongolia and Australia, and differs most from the Fuegian sample. For the left temporal bone, the group

Table 9. Procrustes distances among groups and P-values from permutation tests (10,000 permutation rounds) for the temporal bones.

	Australian	Fuegian	Lagoa Santa	Buenos Aires	Mongolian	Nubian
Australian	0	<.0001	0.0001	<.0001	<.0001	0.0002
Fuegian	0.0693	0	<.0001	0.0008	<.0001	<.0001
Lagoa Santa	0.0407	0.0521	0	0.0003	0.0001	0.0252
Buenos Aires	0.0566	0.0393	0.0451	0	0.0008	0.0001
Mongolian	0.0418	0.0561	0.0407	0.0371	0	<.0001
Nubian	0.0333	0.0633	0.0291	0.0487	0.0395	0

pair showing the greatest difference is Nubian–Fuegian, whereas the most similar pair is Buenos Aires–Mongolian. The mean shape of the left temporal bone of Lagoa Santa individuals likewise most closely resembles that of the Nubian sample and differs most from the Fuegian sample (Table S18 in López-Sosa et al., 2026).

DISCUSSION

This study sought to explore whether endocranial shape, as captured through 3D landmark data, can be reliably used to classify modern human populations in multivariate analyses (objective 1), and if so, whether it conforms to the TMBCM, originally proposed based on multivariate analyses of ectocranial variables (objective 2), thereby offering an explanation for cranial variation of South American populations. In addition, this study sought to assess whether the temporal bone region of the endocranium would yield similar results to those obtained for the endocranium as a whole, given previous work demonstrating that the temporal lobe and temporal bone are particularly informative for human population history (objective 3). Given that the characteristic features of Paleoamerican morphology can be observed in recent populations from Melanesia, Australia, and sub-Saharan Africa, shared affinities with individuals from these regions may indicate a shared ancestry with Australasians. Conversely, similarities with individuals from Mongolia and recent Native Americans, which frequently exhibit traits also described for the Amerindian morphology, would challenge this view. The implications suggest that Eurasian and Amerindian populations exhibit a derived cranial morphology and that Paleoamericans therefore retain the ancestral condition shared with African and Australian populations. Such a pattern has been explained in terms of demographic conditions resulting from population structure, where ancestral morphologies will be differentially retained through a palimpsest of global human dispersals since the Pleistocene (Mirazón Lahr, 1995; von Cramon-Taubadel et al., 2017). Accordingly, we expected that our results would either (1) lend support to the two-wave model for the peopling of the Americas if Lagoa Santa individuals would show more affinities with Nubians and/or Australians or, to the contrary, (2) would support a single founder population model if the Lagoa Santa individuals showed closer affinities with other Native American population samples and/or with Mongolians. We also sought to assess whether the subset analyses of the temporal bone endocranial regions would support one of the models, given the temporal bone's high association with genetic variation in modern human populations.

With regard to the first objective of this study, we could observe that the analyses performed using the full endocranial surface dataset showed a high classification rate. However, we did not obtain similarly high classification rates from the subset dataset comprising only temporal bone data. As shown in Tables 4 and 5, classification rates are higher when using the whole endocranial dataset. It is also higher when the Lagoa Santa series is not included as a pre-assigned group, which may suggest relatively high morphological variation within this sample. The temporal bones' results, on the other hand, yielded lower classification rates, indicating that they may not be suitable for this purpose and should therefore be interpreted with caution. We note that in this case the distribution of Lagoa Santa individuals along the major axes of variation in the PCA plot is also more scattered by comparison with the PCA of the whole dataset (Figures 3, 4 and S2 in López-Sosa et al., 2026). Hence, regarding the second objective of this work, our results, particularly those for the overall endocranial surface, show that the Lagoa Santa individuals do share the most affinities with Nubian and Australian individuals, in agreement with the TMBCM. With regard to our third objective, the results of the temporal bone analyses provide more conflicting support for either the two-wave or one-wave migration model, indicating that the Lagoa Santa individuals also share affinities with Mongolian and Native American populations.

With respect to endocranial variation, the affinities described for Paleoamerican ectocranial morphology

(Neves & Hubbe, 2005; Neves & Pucciarelli, 1989, 1991) are supported by the DFA, PCA, and significance tests of Procrustes distances. First, both PC1 and PC2 express the variance related to the relative length and width of the crania and position the Lagoa Santa series close to Australian and Nubian population samples, tending toward a morphology that is narrow and elongated, as shown by the warped surface. The MST and the DFA also support affinities between Lagoa Santa and Nubian individuals, and the Procrustes distances indicate the strongest morphological affinities among the Lagoa Santa individuals and those from Nubia and Australia. Nevertheless, it is worth noting that the absence of facial data may have influenced our results since the facial skeleton plays an important role in defining the diagnostic features of Paleoamerican or Amerindian morphology (Neves & Pucciarelli, 1991). Furthermore, we note that interpretations regarding the width of the crania are based solely on four landmarks (right and left endoasterion and right and left endopterion), and we caution that the warped surfaces should only be interpreted for landmarked regions.

It has been previously hypothesized that Paleoamerican morphology may be indicative of the earliest human occupation of the Americas and possibly related to an Australo-melanesian ancestral lineage. This has often been questioned given that some recent populations such as Fuegian and Patagonian (Chile/Argentina), Pericú (Mexico), and Botocudo (Brazil) groups have been described as presenting a Paleoamerican cranial pattern but lack genomic affinities to Australo-melanesians (Raghavan et al., 2015; Skoglund & Reich, 2016). The morphology has therefore been explained as either a relict ancestral morphology retained under conditions of geographic isolation, as a case of convergence resulting from climatic adaptations, or a combination thereof (González-José et al., 2003; Lucea & Turbón, 2019; Mirazón Lahr, 1995; Perez et al., 2007; Strauss et al., 2015). Thus far, only one of the twelve DNA-sampled Lagoa Santa individuals (not available for this study) shows Australasian genetic affinity (Moreno-Mayar et al., 2018; Posth et al., 2018). Nevertheless, our endocranial analyses consistently show an affinity of the Lagoa Santa series mean landmark configuration to that of the Nubian and Australian sample means, followed by Fuegians. This suggests that while Fuegians might retain aspects of the Paleoamerican morphology, they also have a more derived Amerindian anatomy that is likely associated with climatic adaptations (Evteev et al., 2024; Hernández et al., 1997; Mirazón Lahr, 1995; Perez et al., 2007).

In contrast, PCA of the temporal bones (both the bilateral and the left temporal bone analyses) places the Lagoa Santa series closest to the Nubian and the Mongolian samples. This result is corroborated by the evaluation of pairwise comparisons for Procrustes distances between samples, where Lagoa Santa individuals present the strongest similarities with Nubia, as well as with Mongolia, albeit to a lower degree. On the other hand, they show the greatest differences with Tierra del Fuego. It is important to note that the variance expressed by PC1 appears to be strongly influenced by the endoasterion landmark (Figure 4), as the posterior portion of the temporal bones, and with it, their length, shows notable differences across negative and positive PC values. This can be explained by the fact that endoasterion is affected by the occipital and the parietal bone morphologies and therefore also reflects changes related to these structures. Hence, the changes we observe on the warped surfaces vary in relation not only to the temporal bones, but also to their articulation with the other adjacent bones. In this case, PC1 is not only informative about the length of the temporal bones themselves, but also about the width of the occipital bone, as appreciated in the full landmark configuration (Figure 2). PC2, on the other hand, actually expresses the variability related to the length of the temporal bones (see Figure S3 in López-Sosa et al., 2026). The MST results based on both temporal bones place the Lagoa Santa series directly linked to the Nubian sample, whereas analyses using only the left temporal bone also link it to Mongolia. Moreover, the DFA of the bilateral variables yielded an estimated classification, where fourteen

Lagoa Santa individuals were assigned to Nubia and Australia, whereas 6 were classified as other Native Americans. The DFA based on the left temporal bone yielded a more balanced classification, where eleven of the Lagoa Santa individuals were classified as Nubians or Australians, and nine as Mongolians, Fuegians or from Buenos Aires.

As shown above, there are differences between the results obtained from the overall endocranial surface shape variables and those from the temporal bones' endocranial surface structure, which suggests that the observed morphological affinities may vary depending on the anatomical structure analyzed. Our results from the temporal bones' analyses may be more indicative of affinities associated with population history, as indicated in previous studies. In particular, previous work on brain cortical surface, Fan et al. (2015) found that the posterior-temporal region is particularly variable between individuals of different continental ancestry. At the same time, correct classification rates for our sampled populations were as low as ~41% in the temporal bone analyses when correcting for chance classification due to unbalanced sample sizes (*Tau* statistic). This contrasts with previous work showing that temporal bone shape shows relatively high correct population classification in a linear discriminant analysis framework (Timbrell & Plomp, 2019). We note that the subsampling of variables for these analyses, as well as the reduced number of cases, may account for the lower classification rate. However, the analyses using only the data from the cranial vault yielded higher classification rates than that obtained using solely the landmarks of the temporal bone (Tables S19 and S20 in López-Sosa et al., 2026 report the DFA results from 14 PCs), thus casting doubt on the validity of the temporal bone results. Hence, when comparing South American individuals with populations from other continents, the endocranial surface of the temporal bone landmark configuration used in this study may not constitute the most suitable proxy for inferring population history. However, we emphasize that the landmark configuration in our study differs from that used in previous research validating the temporal bone's utility in population history reconstruction. Further work is therefore necessary to validate whether the population history signature previously observed for the temporal bone cortical surface can be replicated.

While this paper is the first to address the peopling of the Americas through the analysis of endocranial morphology in Native American populations with a global comparative database, it presents some important limitations. Methodologically, our semi-landmark sampling strategy was limited to the midsagittal line due to differential preservation of the bilateral surfaces across the samples. We did not employ a denser semi-landmark configuration due to missing areas along bilateral endosurfaces. In addition, the justification for using endocranial morphology as a reliable proxy for population history remains to be tested at deeper temporal scales with the inclusion of ancient DNA and paleoenvironmental data. For example, it is well established that the cranium is differentially affected by environmental effects (e.g., Harvati & Weaver, 2006; Roseman, 2004) especially among South American populations (Menéndez et al., 2014; Perez & Monteiro, 2009), and that the cranial morphology of some of the populations we sampled, such as the Fuegians, likely differentiated under strong adaptive pressures (Evteev et al., 2024; Hernández et al., 1997; Perez et al., 2007). It is therefore possible that the morphological affinities between early Holocene Paleoamericans and more recent populations is, at least in part, a result of convergent morphological adaptations that may differentially affect the cranium, although this point should be further explored. Irrespective of the evolutionary and environmental factors that may differentially affect endo- and ectocranial shape, a crucial consideration for contextualizing our results with previous studies is the geographical and temporal scope of the comparative samples. For example, Seguchi et al. (2011) found support for stronger affinities of a subset of the Lagoa Santa series to prehistoric hunter-gatherer populations from the Americas and East Asia, greater than to recent Australo-melanesians. Such results underscore the importance of including other prehistoric samples as comparative material, particularly pene-contemporaneous early Holocene populations from the Americas (Hubbe et al., 2007; Menéndez et al., 2015, 2022; Neves et al., 2003; Pucciarelli et al., 2006). Some of the most widely accepted models for the peopling of South America favors an expansion through North America (Azevedo et al., 2017; Moreno-Mayar et al., 2018; Posth et al., 2018); therefore, including and analyzing early Holocene samples from this region would shed light on the debate regarding the number of migratory waves. Also, the individuals from Nubia and Lagoa Santa are the most ancient ones within our samples; therefore, the affinities observed between Lagoa Santa and Nubia may, at least to some extent reflect the fact that they are the geologically oldest samples in our dataset, and as such, share plesiomorphic features. Future research addressing Paleoamerican and Amerindian endocranial morphology and its relationship to the peopling of the Americas should therefore focus on increasing the number of prehistoric populations in order to achieve better spatial and temporal coverage. For example, we emphasize that the two recent (late Holocene) South American samples used in this study are not enough to adequately represent the morphological variation present in the continent. In this study, as well as in previous studies that compare the morphologies of recent and ancient archaeological samples, a central limitation is ascertaining that they adequately represent the geographical and temporal diversity of the populations under study. It is therefore important to expand the number and diversity of the comparative samples across time and space for future studies.

Overall, our results based on the endocranial surface lend support to the TMBCM but at the same time are more nuanced when considering only the temporal bone. We note that several recent morphological and genetic studies support the 'Beringian Standstill' hypothesis, which considers that all Native Americans descend from a founder population isolated in Beringia for thousands of years prior to entering the Americas (Fagundes et al., 2008; González-José et al., 2008; Kitchen et al., 2008; Kuzminsky et al., 2017; Meltzer, 2009; Moreno-Mayar et al., 2018; Pitblado, 2011; Tamm et al., 2007). Our results may be compatible with this model if the Beringian founder population was morphologically and genetically heterogeneous, relative to Native American populations today, as previously suggested (González-José et al., 2008; Hubbe et al., 2020; Pucciarelli et al., 2003; Sardi et al., 2004, 2005). Under this model, descendants of that ancestral population retain morphological characteristics not found at high frequency today. This agrees with the largest ancient DNA study conducted on individuals from Brazil, which suggests that early Holocene individuals did not contribute substantially to the biological variation of more recent coastal groups (also known as *Sambaqui* societies; Ferraz et al., 2023). Our results are also compatible with recent genomic studies that find an Australasian genetic signal detectable in one individual from Lagoa Santa (the geologically oldest of five sampled from Sumidouro Cave: Moreno-Mayar et al., 2018) but not in others (none of the seven individuals sampled from Lapa do Santo, which are geologically younger: Posth et al., 2018). Thus far, the Australasian genetic signal has not been detected in other early Holocene individuals exhibiting a Paleoamerican morphology (Moreno-Mayar et al., 2018), but has in some extant indigenous South Americans, most of whom inhabit the Amazonian region of central Brazil and northeast Peru today (Silva et al., 2021; Skoglund et al., 2015), and in an ancient ~1.000 yBP individual from Panama (Santos et al., 2022). While it is unlikely that extant individuals who retain an Australasian genetic signal also retain the full suite of Paleoamerican morphological traits, no morphometric analyses have yet been conducted to assess this (Moreno-Mayar et al., 2018). Taken together, the current consensus is that an Australasian genetic signal does not necessarily correspond to a Paleoamerican morphology, implying that the morphological affinities of Paleoamericans and recent

Africans and Australians are not a direct signal of common ancestry. Nevertheless, the limited morphometric analyses available for late Holocene Amazonian populations from central Brazil (Hubbe et al., 2015; Strauss et al., 2015) show affinities with Lagoa Santa and other early Holocene Paleoamerican samples, particularly when considering neurocranial form. Future studies should therefore focus on neurocranial form and particularly the temporal bone region (Menéndez & Pasqualini, 2020).

Perhaps most importantly, there is now genomic evidence supporting multiple dispersals into South America during the late Pleistocene (Ionnidis et al., 2020; Moreno-Mayar et al., 2018; Posth et al., 2018). In particular, in addition to the earliest Sumidouro individual's retention of the Australasian genetic signal, the Lapa do Santo series (including the oldest individual analyzed here, LS18) has genomic affinities with the terminal Pleistocene Anzick-1 individual from North America, whilst later individuals show affinities with recent Native American populations (Posth et al., 2018). In light of this, morphological differences between early South Americans, including the Lagoa Santa individuals, and later populations can be explained, at least in part, by multiple dispersals originating not only from East Asia or Beringia, but also from North and Central America, as well as by the partial replacement of the early Holocene gene pool (Posth et al., 2018) and of the full suite of traits of the Paleoamerican morphology in the Lagoa Santa region, as it has been proposed earlier in morphometric studies (*sensu* Hubbe et al., 2015; Kuzminsky, 2013; Pucciarelli, 2009). Finally, we stress that genetic and morphometric data should be integrated coherently in a common analytical framework and discussed together in order to arrive at more robust interpretations.

CONCLUSIONS

The endocranial morphology of the Lagoa Santa individuals exhibits a range of features that attest to shared morphological affinities with Africans and Australo-melanesian populations, as previously described for the ectocranium, thus supporting the TMBCM. However, affinities with other Native American and East Asian population samples were also observed, particularly when analyzing the endocranial region of the temporal bone, which has previously been shown to be informative for reconstructing population history. Our results are consistent with the hypothesis of multiple migration waves into South America. Given that this is the first study to use endocranial morphology as an approach to assess competing dispersal hypotheses, we expect that future research will improve upon these findings by incorporating additional early Holocene samples from North, Central, and South America.

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AUTHORS' CONTRIBUTION
M. C. López-Sosa contributed to conceptualization, data curation, formal analysis, investigation, methodology, project administration, validation, view, and writing (original draft, review, and editing); H. Reyes-Centeno contributed to conceptualization, methodology, project administration, supervision, validation, and writing (original draft, review, and editing); L. P. Menéndez contributed to formal analysis, investigation, methodology, supervision, validation, and writing (review, and editing); R. E. Oliveira contributed to funding acquisition, investigation, and resources; C. C. Castro contributed to funding acquisition, investigation, and resources; and A. Strauss contributed to conceptualization, funding acquisition, investigation, project administration, resources, supervision, and writing (review, and editing).

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