

The Cranial Morphology of the Botocudo Indians, Brazil

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ABSTRACT The Botocudo Indians were hunter-gatherer groups that occupied the East-Central regions of Brazil decimated during the colonial period in the country. During the 19th century, craniometric studies suggested that the Botocudo resembled more the Paleoamerican population of Lagoa Santa than typical Native Americans groups. These results suggest that the Botocudo Indians might represent a population that retained the biological characteristics of early groups of the continent, remaining largely isolated from groups that gave origin to the modern Native South American variation. Moreover, recently, some of the Botocudo remains have been shown to have mitochondrial and autosomal DNA lineages currently found in Polynesian populations. Here, we explore the morphological affinities of Botocudo skulls within a worldwide context. Distinct multivariate analyses based on 32 craniometric

variables show that 1) the two individuals with Polynesian DNA sequences have morphological characteristics that fall within the Polynesian and Botocudo variation, making their assignation as Native American specimens problematic, and 2) there are high morphological affinities between Botocudo, Early Americans, and the Polynesian series of Easter Island, which support the early observations that the Botocudo can be seen as retaining the Paleoamerican morphology, particularly when the neurocranium is considered. Although these results do not elucidate the origin of the Polynesian DNA lineages among the Botocudo, they support the hypothesis that the Botocudo represent a case of late survival of ancient Paleoamerican populations, retaining the morphological characteristics of ancestral Late Pleistocene populations from Asia. *Am J Phys Anthropol* 157:202–216, 2015. © 2015 Wiley Periodicals, Inc.

The way craniometric variation is structured over time in the New World has been the focus of intensive analysis in the last 20 years. Neves et al. have documented that the cranial pattern of the human groups inhabiting the Lagoa Santa region in East-Central Brazil during the Pleistocene/Holocene transition did not resemble that shared by most late and recent Native Americans (Neves and Pucciarelli, 1989, 1991; Neves et al., 2004a, 2007a,b; Neves and Hubbe, 2005; Hubbe et al., 2010, 2011). Contrary to the cranial morphology that characterizes these late/recent Native Americans (Amerindians) that varies around short and wide neurocrania, orthognathic high faces with relatively high and narrow orbits and noses, the pattern seen in early remains (Paleoamericans) is characterized by narrow and long neurocrania, prognathic low faces with relatively low and broad orbits and noses (Neves and Hubbe, 2005; Neves et al., 2007a,b; but see González-José et al. 2008, for a critique on the use of the Paleoamerican term).

Further studies have shown that Lagoa Santa is not an isolated case. The Paleoamerican cranial pattern was present all over the New World during Early Holocene: in East-Central Brazil (Santana do Riacho; Neves et al., 2003), in Northeast Brazil (Toca das Onças; Hubbe et al., 2004, and Serra da Capivara; Hubbe et al., 2007), in Southern Brazil (Capelinha; Neves et al., 2005), in the interior of Rio Grande do Sul (Neves et al., 2004b), in Colombia (Sabana de Bogotá; Neves et al., 2007a), in the pampa region of Argentina (Pucciarelli et al., 2010),

in the very southern tip of the continent in Palli Aike (Neves et al., 1999), in Mexico (González-José et al., 2005), and in North America (Chatter et al., 1999; Jantz and Owsley, 2001).

Therefore, in spite of the ongoing debate concerning the reasons behind the origin of the New World's morphological variability (Neves and Hubbe, 2005; Powell, 2005; Sardi et al., 2005; González-José et al., 2008; Hubbe et al., 2010, 2011; de Azevedo et al., 2011), there is general consensus that the Early Americans exhibited

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a cranial pattern not represented today among most Native Americans (Jantz and Owsley, 2001; González-José et al., 2005; Neves and Hubbe, 2005; Neves et al., 2007b; Hubbe et al., 2011). On the other hand, it is still debated for how long this Paleoamerican morphology remained in the continent. Evidence of a Middle/Late Holocene survival of Paleoamerican morphology has been reported for Sabana de Bogotá, Colombia (Neves et al., 2007a), and for the interior of Southern Brazil (Neves et al., 2004b). González-José et al. (2003) were able to extend the survival of the Paleoamerican morphological pattern even later in time, by documenting its presence among the Pericú group from Baja California, Mexico, which were extinct by the 19th century. According to the authors this geographically isolated group could represent a morphological relic of the first humans who settled the Americas. Together, these recent findings suggest that the nature of the transition between predominantly Paleoamericans groups, by the end of Pleistocene, toward a majority of groups sharing the Amerindian pattern by the end of the Holocene was more complex than first envisioned.

In Brazil, late 19th century scholars from the National Museum in Rio de Janeiro suggested that the Botocudo Indians from East-Central Brazil shared the same cranial morphology as the sympatric Lagoa Santa Paleoamerican population. In the typological framework that prevailed during the late 19th century, Lacerda and Peixoto (1876) described the Botocudo Indians as belonging to the Lagoa Santa "race." Subsequently, other scholars such as Imbelloni (1938), Rivet (1942), and Pucciarelli et al. (2003) made similar claims about the Botocudo, while Mello e Alvim (1963) challenged the idea of a close resemblance between these two populations. Recently, a couple of molecular studies (Gonçalves et al., 2013; Malaspina et al., 2014) conducted on the same material studied by these early scholars kindled again the discussion about the biological characteristics of the Botocudo Indians. These articles report that two of the Botocudo skulls collected and studied by Lacerda and Peixoto (1876) have mitochondrial and autosomal DNA lineages that are not Native American. The mitochondrial haplogroups reported by Gonçalves et al. (2013) are common in nowadays Polynesia and are also found in lower frequencies in Indonesia and Madagascar populations. The study was recently complemented by a study of autosomal DNA sequences of the two Botocudo skulls that had the Polynesian mtDNA motif (Malaspina et al., 2014), which concluded that these individuals have exclusive Polynesian sequences, with no contribution of Native American sequences.

To explain the presence of these unique haplogroups among the Botocudo Indian, Gonçalves et al. (2013) suggest three possible scenarios. The first one is that Botocudo Indians are descendants of the Paleoamerican groups from Lagoa Santa, following the early studies from the 19th century (Lacerda and Peixoto, 1876). Under this scenario, the Polynesian motif would be already present among the Lagoa Santa populations, and the Botocudo Indian would have inherited them from the early populations. This scenario, however, is not supported by the mtDNA, because the estimated time of origin of the Polynesian motif is not old enough to be among the earliest occupants of South America. Moreover, the autosomal DNA extracted later from these individuals show no evidence of admixture with Native Americans (Malaspina et al., 2014). The second hypoth-

esis is that recent gene flow from Polynesia introduced the haplogroups to the Botocudo Indian. Although this scenario could explain the presence of Polynesian individuals (as inferred from the autosomal DNA) among Botocudo groups, it also seems unlikely given that the Botocudo lived in east South America, and no other evidence of the reported mitochondrial haplogroups has been yet found among native groups located in South American regions closer to Polynesia, despite evidence of contact between Polynesia and the Pacific coast of South America by the end of the Holocene (Yen, 1974; Green, 2000; Storey et al., 2007; Gongora, 2008; Gongora et al., 2008). The third hypothesis suggested by the authors is that these haplogroups are the results of the admixture between Botocudo groups and African slaves in the country. Under this hypothesis, slaves from Madagascar would have been captured by Mozambique slave traders and had their mtDNA haplogroups introgressed into the Botocudo gene pool, who for some period coexisted with slaves in East-Central Brazil. This is also not a very probable scenario, given that the autosomal DNA data reported by Malaspina et al. (2014) show no evidence of African admixture. In addition, no other evidence of more common African slave mtDNA sequences is found among the Botocudo, an unlikely fact given that Madagascar was not the major source of slaves for Brazil during the colonial period.

Therefore, the biological variation observed among the Botocudo cannot be easily explained given the actual knowledge about the biological diversity of past and present Native American groups, especially when it comes to the origin of the Polynesian molecular lineages among them. To contribute to the discussion on the origin of the biological variation of the Botocudo Indians, we reassess the morphological affinities of Botocudo Indians using modern multivariate approaches focusing on two complementary goals: 1) to explore if the two individuals with Polynesian DNA can be assumed to belong to the Botocudo population (as far as cranial morphological variation is concerned), and 2) to test the hypothesis proposed by Lacerda and Peixoto (1876) that the Botocudo Indians retain the same morphological pattern as early Paleoamerican groups in the continent.

THE BOTOCUDO INDIANS

Botocudo is a Portuguese term established during the 18th century to refer to a series of different native groups such as the Krenak, Naknenuk, Nakrehé, Etwet, and Takruk-karak that used to occupy the eastern part of Brazil in the area delimited by the Una river to the north and the Doce River to the south. Those groups spoke different languages belonging to the Macro-Jê, considered a Paleo-indian linguistic family according to Rodrigues (2002). The term Botocudo derives from the wood disks and plugs worn on lips and ears, which were commonly used by many of the groups occupying the Atlantic Forest in East and South Brazil. The Portuguese associated those ornaments to corks that were called *botoques* in their own language. Before the term Botocudo became widely used those groups were referred to as Aimorés or Tapuiás, names that are still used in present time.

The specific sample used in this study includes groups of Botocudo who lived in the Brazilian Atlantic Forest and associated ecotones during the 19th century. The Botocudo skulls analyzed here come mostly from the margins of the

TABLE 1. Information on the Botocudo specimens that comprise the series used in this study

ID	Sex	Details ^a
MN-003	M	Skull—Botocudo Indian. Poaia e Mutum Village. Rio Doce—Minas Gerais, Brazil.
MN-004	F	Skull—Botocudo Indian, with mandible. Mutum Village—Espírito Santo, Brazil.
MN-006	M	Skull of Botocudo Indian, with mandible. S. Matheus—Espírito Santo, Brazil.
MN-007	F	Skull—Botocudo Indian. Poaia e Mutum Village—Espírito Santo, Brazil.
MN-008	M	Skull—Botocudo Indian. Rio Doce—Minas Gerais, Brazil.
MN-009	F	Skull—Poxixa Indian, with mandible. Mucuri River—Espírito Santo, Brazil.
MN-011	F	Skull—Botocudo Indian, with mandible. Rio Doce—Minas Gerais, Brazil.
MN-012	F	Skull—Botocudo Indian. Mucuri River—Espírito Santo, Brazil.
MN-013	F	Skull—Botocudo Indian, with mandible. Mutum Village—Espírito Santo, Brazil.
MN-014	F	Skull—Botocudo Indian, with mandible. Mutum Village—Espírito Santo, Brazil.
MN-015	M	Skull—Botocudo Indian. Rio Doce—Minas Gerais, Brazil.
MN-017	M	Skull—Botocudo Indian. Rio Doce—Minas Gerais, Brazil.
MN-020	M	Skull—Botocudo Indian. Mucuri River—Espírito Santo, Brazil.
MN-021	F	Skull—Botocudo Indian, with mandible. Mutum Village—Espírito Santo, Brazil.
MN-023	F	Skull—Poxixa Indian. Mucuri River—Espírito Santo, Brazil.
MN-026	M	Skull—Nak-Nanuk Indian, with mandible. Bahia, Brazil.
MN-039	F	Skull—Botocudo Indian, with mandible. Poaia e Mutum Village—Espírito Santo, Brazil.
MN-053	M	Skull—Botocudo Indian, with mandible. Babylonia Cave—Fazenda de Sant'Ana—Minas Gerais, Brazil.
MN-055	M	Skull—with mandible. Found in cave near Itapemirim—Espírito Santo, Brazil.
MN-056	F	Skull—Botocudo Indian. Mucuri River—Espírito Santo, Brazil.
MN-062	M	Skull—Botocudo Indian, with mandible. Mucuri River—Espírito Santo, Brazil.
MN-063	F	Skull—Botocudo Indian, with mandible. S. Matheus—Espírito Santo, Brazil.
MN-064	F	Skull—Botocudo Indian, with mandible. Rio Doce—Minas Gerais, Brazil.
MN-065	M	Skull—Botocudo Indian, with mandible. Itapemirim—Espírito Santo, Brazil.
MN-066	F	Skull—Botocudo Indian, with mandible. Mutum Village—Espírito Santo, Brazil.
MN-067	M	Skull—Botocudo Indian, with mandible. Minas Gerais, Brazil.
MN-068	M	Skull—Botocudo Indian, with mandible. Mutum Village—Brazil.
MN-069	M	Skull—Botocudo Indian. Mucuri River—Espírito Santo, Brazil.
MN-088	M	Skull—Poxixa Indian, with mandible. Mucuri River—Espírito Santo, Brazil.
MN-118	F	Skeleton—articulated, incomplete. Botocudo Indian. Rio Doce—Minas Gerais, Brazil.
MN-119	M	Skeleton—articulated, incomplete. Botocudo Indian. Rio Doce—Minas Gerais, Brazil.
MN-120	F	Skeleton—articulated, incomplete. Botocudo Indian. Rio Doce—Minas Gerais, Brazil.

^a Translated from the Catalogue of the National Museum of Rio de Janeiro by the authors.

Rio Doce and from the Babylonia Cave (Lacerda and Peixoto, 1876; Table 1). Some of the Rio Doce individuals come from unknown localities and others were recovered from a mass grave where individuals of the Poté tribe were buried after being murdered by the Brazilian army during a 19th century “pacification” mission. The individuals from Babylonia Cave are inhumations probably from the Coropó and Goyatacazes tribes, who were expelled from the coast by Portuguese settlers during the 16th and 17th century (Lacerda and Peixoto, 1876). By the 19th century the Rio Doce region was already a well-explored area of Brazil with an expressive presence of non-natives. Between 1800 and 1850 approximately 73 villages and 87 military outposts were established in the area (Paraíso, 2002).

Ethnographic information on Botocudo comes mainly from 19th century travelers such as Briard (1986, first edition from 1855), Rugendas (1979, first edition from 1928), Expilly (1977, first edition from 1857), Freyre (1900, first edition from 1814), Jomarb (1847), Mawe (1944, first edition from 1810), Wied-Neuwied (1958, first edition from 1815 to 1817), Saint Hilaire (1823), and Spix and Martius (1976, first edition from 1823). The social organization of the Botocudo Indians is characterized by frequent group splitting and by a religious system centered on the figure of the enchanted spirit of the dead (Paraíso, 2002). By the time of European contact, they were seminomadic and still relied heavily on a hunting-fishing-gathering subsistence strategy. Hunting and fishing were typical male activities while gathering was a strictly female occupation (Paraíso, 2002). Hunting was the most important economic activity and was carried out within well-defined territories that were

frequently the subject of intergroup disputes. The Botocudo had few nonportable items in their material culture allowing for fast movement when enemies approached (Paraíso, 2002). For the same reason, their campsites and huts were very simple and readily taken down and rebuilt. Although it is known that marriage would preferentially occur among cross-cousins and never between parallel cousins, no details are known about their postmarital residence practices.

Europeans and other native groups, especially the expanding tribes of Tupi speakers, surrounded the Botocudo populations considered here. The Tupi as they are known in the Brazilian archeological literature, were mostly slash-and-burn horticulturalists, and represented the most widespread ethnic group within Brazilian territory at the time of European conquest (Brochado, 1989; Noelli, 1998).

MATERIALS AND METHODS

Cranial morphology was assessed through 32 metric variables following Howells (1973, 1989) protocol (Table 2). The Botocudo material included here is comprised of 16 male and 16 female skulls of adult individuals curated at the National Museum of Rio de Janeiro (see Table 1 for details on the individuals, as presented in the Catalog of the National Museum). The morphological affinities of the Botocudo specimens were assessed by comparing them to early and mid-Holocene South American series and to Late Holocene series representing the worldwide modern human morphological variation (Table 3). The Paleoamerican series include

TABLE 2. Craniometric measurements included in each anatomical region

Major anatomical regions	Minor anatomical regions	Variables included ^a
Neurocranium	Frontal	Maximum frontal breadth (XFB) Frontal cord (FRC) Frontal subtense (FRS) Frontal fraction (FRF)
	Parietal	Maximum cranial breadth (XCB) Parietal cord (PAC) Parietal subtense (PAS) Parietal fraction (PAF)
	Occipital	Biasterionic breadth (ASB) Occipital cord (OCC) Occipital subtense (OCS) Occipital fraction (OCF)
	Not included in minor regions	Glabello-occipital length (GOL) Nasio-occipital length (NOL) Basion-nasion length (BNL) Basion-bregma height (BBH) Biauricular breadth (AUB)
Face	Nasal	Nasion-prosthion height (NPH) Nasal height (NLH) Nasal breadth (NLB)
	Orbit	Orbit breadth (OBB) Orbit height (OBH)
	Upper face breadth	Bijugal breadth (JUB) Bizygomaxillare breadth (ZMB) Bifrontomallare breadth (FMB) Biorbital breadth (EKB)
	Malar	Malar length, inferior (IML) Malar length, superior (XML) Cheek height (WMH)
	Not included in minor regions	Interorbital breadth (DKB) Subspinale subtense (SSS) Nasion subtense (NAS)

^a Measurement definitions according to Howells (1973, 1989).

specimens from Lagoa Santa, Brazil, and Sabana de Bogotá, Colombia. These series have been extensively described by Neves and Hubbe (2005) and Neves et al. (2007a) and further details will not be given here. The other South American series include a mid-Holocene shellmound series (Cabeçuda) associated with pre-ceramic, pre-agriculture, fisher-hunter-gatherers from the Archaic Period of the southern Brazilian Coast; two late Holocene coastal series (Base Aérea and Tapera) representing ceramic fisher-hunter-gatherers; Tupi-Guarani composed of North Brazilian recent native groups speaking mainly Tupi languages (Hubbe et al., 2014); and Howells (1996) modern Peruvian series. With the exception of Peru, all South American series were measured by WAN following Howells' (1973, 1989) protocol. The South American series were complemented by 30 series from Howells (1996) database that together represent the morphological variation seen among modern humans worldwide (Table 3).

Given the presence of the two individuals with Polynesian DNA in our sample (MN-15 and MN-17), we initially explored the morphological affinities of these

specimens in two complementary ways. First, we analyzed their morphological affinities in relationship to the remaining Botocudo specimens according to the first two principal components extracted from the covariance matrix calculated for the Botocudo series. Second, we used linear multivariate discriminant analyses to classify the Botocudo individuals in the reference series. Classification was done based on posterior probabilities, with three different datasets: all reference series; only American series (minus Botocudo); and American + Polynesian series (minus Botocudo). In all analyses, MN-15 and MN-17 were not included in the calculation of the linear discriminant functions.

The morphological affinities of the Botocudos series as a whole were initially assessed based on the Mahalanobis' distance matrix (D^2 ; Mahalanobis, 1936) between series. To represent the D^2 matrix and explore the morphological affinities among series, the distances were plotted in a scatterplot generated by a Kruskal's nonmetric multidimensional scaling (MDS; Cox and Cox, 2010). To explore the expected distribution of the observed distances, 100 new distance matrices were

TABLE 3. Series included in the study

Series	Region	Sample size	Chronology	Percentage of missing values	Reference
Central Brazil Botocudo	South America	32	Sub-recent	1.52	This study
Lagoa Santa	South America	19	11.0–7.5 kyr	10.69	Neves and Hubbe, 2005
Early Colombia	South America	14	11–6.5 kyr	10.27	Neves et al., 2007a
Archaic Colombia	South America	33	5–3 kyr	11.55	Neves et al., 2007a
Tupi-Guarani	South America	23	Sub-recent	4.62	This study
Base Aérea	South America	18	~1.0 kyr	7.81	Neves and Hubbe, 2005
Tapera	South America	47	~1.0 kyr	6.58	Neves and Hubbe, 2005
Cabeçuda	South America	19	~1.0 kyr	12.99	This study
Peru	South America	110	Sub-recent	0.00	Howells, 1973, 1989
Arikara	North America	69	Sub-recent	0.00	Howells, 1973, 1989
Eskimo	North America	108	Sub-recent	0.00	Howells, 1973, 1989
Santa Cruz	North America	102	Sub-recent	0.03	Howells, 1973, 1989
Ainu	East Asia	86	Sub-recent	0.00	Howells, 1973, 1989
Buriat	East Asia	109	Sub-recent	0.23	Howells, 1973, 1989
Hainan	East Asia	83	Sub-recent	0.00	Howells, 1973, 1989
North Japan	East Asia	87	Sub-recent	0.07	Howells, 1973, 1989
South Japan	East Asia	91	Sub-recent	0.00	Howells, 1973, 1989
Anyang	East Asia	42	Sub-recent	0.00	Howells, 1973, 1989
Andaman	Southeast Asia	70	Sub-recent	0.00	Howells, 1973, 1989
Atayal	Southeast Asia	47	Sub-recent	0.00	Howells, 1973, 1989
Guam	Southeast Asia	57	Sub-recent	0.00	Howells, 1973, 1989
Philippines	Southeast Asia	50	Sub-recent	0.06	Howells, 1973, 1989
Australia	Australo-Melanesia	101	Sub-recent	0.00	Howells, 1973, 1989
Tasmania	Australo-Melanesia	87	Sub-recent	0.00	Howells, 1973, 1989
Tolai	Australo-Melanesia	110	Sub-recent	0.00	Howells, 1973, 1989
Teita	Sub-Saharan Africa	83	Sub-recent	0.04	Howells, 1973, 1989
Dogon	Sub-Saharan Africa	99	Sub-recent	0.00	Howells, 1973, 1989
Zulu	Sub-Saharan Africa	101	Sub-recent	0.00	Howells, 1973, 1989
Bushman	Sub-Saharan Africa	90	Sub-recent	0.00	Howells, 1973, 1989
Egypt	North Africa	111	Sub-recent	0.03	Howells, 1973, 1989
Berg	Europe	109	Sub-recent	0.03	Howells, 1973, 1989
Zalavar	Europe	98	Sub-recent	0.00	Howells, 1973, 1989
Norse	Europe	110	Sub-recent	0.00	Howells, 1973, 1989
Moriari	Polynesia	108	Sub-recent	0.00	Howells, 1973, 1989
Mokapu	Polynesia	100	Sub-recent	0.02	Howells, 1973, 1989
Easter island	Polynesia	86	Sub-recent	0.00	Howells, 1973, 1989
North Maori	Polynesia	10	Sub-recent	0.00	Howells, 1973, 1989
South Maori	Polynesia	10	Sub-recent	0.00	Howells, 1973, 1989
Total		3,024			

calculated from bootstrapping the original data within groups, respecting the original sample sizes. This procedure generated new distance matrices that take into consideration the variation expected in the distance between series due to population estimation errors associated with the sample sizes. The representation of the error distribution was also done via MDS. In this case for each of the 100 bootstrapped D^2 matrices, MDS coordinates were calculated and then superimposed on the original MDS using a Procrustes analysis (Bookstein, 1996), to minimize the differences in rotation and translation between the observed and the bootstrapped MDS results.

To test the hypothesis that Botocudo share the same morphological pattern as Paleoamerican groups, further comparisons between series were conducted using minimum F_{st} estimates (Relethford and Blangero, 1990; Relethford et al., 1997), obtained by averaging the principal diagonal of the R -matrix (rii) extracted from the phenotypic data. Minimum F_{st} estimates can be calculated from phenotypic data and represent a measurement of the amount of the variance seen in the data that is due to differences between groups (Williams-Blangero and Blangero, 1989, 1990; Relethford and Blangero, 1990; Relethford et al., 1997). F_{st} estimates for metric data are minimum estimates and can greatly

underrepresent intergroup variation apportionment if the heritability values of the additive characters (measurements) are low (Relethford and Blangero, 1990; Relethford, 1994). Heritability values for human cranial dimension range from moderate to high (Devor, 1987) although different traits show very distinct heritability levels (Carson, 2006; Martínez-Abadías et al., 2009). The variability differences between traits pose a problem, because F_{st} estimates as proposed by Relethford and Blangero (1990) cannot include differential heritabilities for each trait, and instead require an average heritability for all traits. However, assuming mean heritability values of 0.55 in the past produced similar apportionment values to neutral molecular data (Relethford, 1994, 2002), showing that even when using average heritability values, craniometric data generate comparable F_{st} estimates. In this case, the assumed heritability values are of minor concern, because correcting for heritability does not change the hierarchy of F_{st} values obtained for different sets of series, as long as the same variables are used in all sets of comparisons (as is the case here). Therefore, all F_{st} estimates calculated here assume a constant heritability of 1.0, and therefore represent the minimum possible value of F_{st} (i.e., they assume that the totality of observed phenotypic variation is produced by genetic variation).

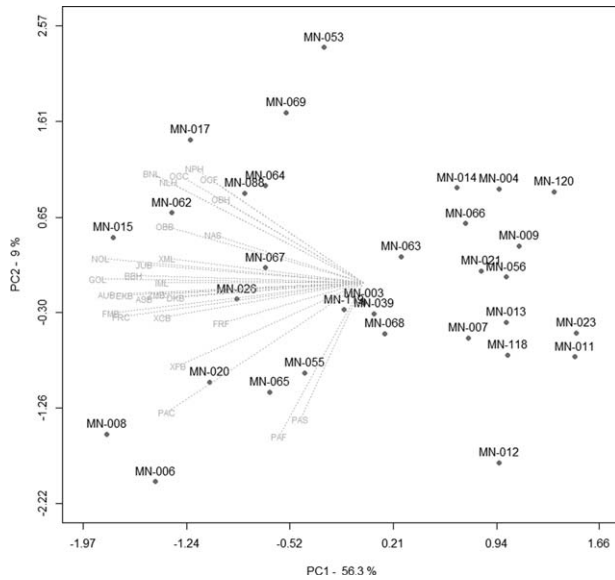


Fig. 1. Morphological affinities among the Botocudo specimens based on the first two principal components extracted from the covariance matrix between the series. The dashed gray lines show the relative contribution of the variables (correlation coefficient scaled to the range of the axes) to each PC. Only correlations larger than 0.5 are shown.

Fst estimates were calculated among the three Paleoamerican series alone and then with each of the South American series added one at a time, to measure how much of the between-group variance is contributed by each South American series independently. Fst values between Paleoamericans and South American series were compared to Fst estimates for series worldwide, as a reference of the magnitude of between-group variance that is observed among modern human groups in the planet. The error distribution of the Fst estimates was calculated as the standard deviation from the Fst values calculated in a thousand bootstraps of the data respecting the original sample sizes of the series.

Given the modular nature of the human skull (Lieberman et al., 2000; Collard and Wood, 2007; Bastir and Rosas, 2008; Klingenberg, 2008, 2013; Mitteroecker and Bookstein, 2008; Paschetta et al., 2010), and the fact that functional anatomical regions can present distinct evolutionary trajectories and respond differently to evolutionary forces, prior to Fst calculations the craniometric measurements were grouped in different anatomical regions (Table 2). The selection of anatomical regions tried to focus on clear functional regions of the skull; however, a real functional division of the skull is impossible with the linear variables available from Howells protocol. Nonetheless, the analyses within South America were conducted for two major anatomical regions (Neurocranium and Face) and seven minor anatomical regions (Frontal, Parietals, Occipital, Upper Face, Nasal, Orbit, and Malar). By dividing the skull in major and minor anatomical regions, and analyzing them separately a more informative profile of the morphological differences and similarities between Paleoamericans and the rest of the South American series is possible. In that way, it is possible to compare the associations between Botocudo and Early Americans within the framework of differences observed in South America for each anatomical region.

For all analyses, sexes were pooled together to maximize sample size in the archaeological series. However, all series (with the exception of Howells' South and North Maori) have balanced sex distributions, so that sexual dimorphism will not influence the results significantly. Missing values (see Table 3 for details on the amount of missing values in each series) in the prehistoric and recent native South American series were replaced through multiple regression of the variable total mean (i.e., including all the series available and both sexes), using the remaining measurements of each individual as independent variables (see Hubbe et al., 2011, for a detailed justification of the missing values replacement method chosen here). As size can be an important source of change across time, and this is an important component to explore the morphological affinity between Botocudo and Paleoamerican series, no size correction was applied to the craniometric data in this study. However, size-corrected analyses of morphological affinities (MDS based on D^2 ; data not shown) produced a nearly identical topology as the one presented here, indicating that size is not an important source of morphological variation in this database. All analyses were done in R (R Core Team, 2014), with functions written by MH, complemented by functions from packages MASS (Venables and Ripley, 2002) and vegan (Oksanen et al., 2013).

RESULTS

Figure 1 shows the morphological affinities among the Botocudo specimens according to the first two principal components, which together explain 65.3% of the variation in the series. The first PC is largely reflecting differences in size between the specimens and separates males (to the left) from females (to the right). PC2 does not show any clear pattern of separation. From the perspective of this study, the two specimens with Polynesian DNA (MN-15 and MN-17) fall within the dispersion of other Botocudo individuals, among the largest specimens in the series. Table 4 shows the results of the classification analyses and posterior probabilities for each of the Botocudo specimens. The two individuals with Polynesian DNA (MN-15 and MN-17) show different tendencies of classifications. MN-15 classifies with very high posterior probability to Botocudo Indians and Early Americans (in the analyses where Botocudo were not included as an option). MN-17, on the other hand, is classified as a Polynesian in both analyses where Polynesian groups were included (Mori and Easter Island), although with relatively lower posterior probabilities in both cases. The remaining Botocudo individuals show very coherent results, classifying mostly as Botocudo in the first analysis or as Early Americans in the second and third analyses, which is in accordance with the analyses done with the entire series (see below).

Figure 2 shows the morphological affinities of the Botocudos in relation to the comparative series included in this study. The MDS shows a close affinity between the centroids of all Paleoamerican groups (Lagoa Santa, Paleo Colombia, and Early Colombia), who appear in an intermediate position between the cluster of African (Zulu, Teita, Dogon, and Bushman) and Australo-Melanesian (Tolai, Australia, and Tasmania) series and the Polynesian series of Easter Island. These results reflect well the pattern of affinities reported in previous

TABLE 4. Classifications and posterior probabilities of the Botocudo specimens based on linear discriminant functions for three different datasets

ID	All series		American series		American and Polynesian series	
	Classification	Posterior probability	Classification	Posterior probability	Classification	Posterior probability
MN-015	Botocudo	0.9937	Archaic Colombia	0.5443	Archaic Colombia	0.3818
MN-017	Moriore	0.3634	Paleo Colombia	0.7512	Easter Island	0.5434
MN-006	Botocudo	0.9999	Lagoa Santa	0.7844	Lagoa Santa	0.9393
MN-008	Botocudo	0.7799	Lagoa Santa	0.9283	Lagoa Santa	0.9743
MN-026	Botocudo	0.9999	Lagoa Santa	0.7548	Lagoa Santa	0.8766
MN-062	Botocudo	1	Lagoa Santa	0.8198	Lagoa Santa	0.4894
MN-020	Botocudo	0.9999	Lagoa Santa	0.9969	Mokapu	0.9545
MN-065	Lagoa Santa	0.6027	Lagoa Santa	0.999	Lagoa Santa	0.9996
MN-088	Botocudo	0.9992	Paleo Colombia	0.6399	Paleo Colombia	0.5445
MN-067	Botocudo	0.5903	Paleo Colombia	0.599	Paleo Colombia	0.4066
MN-119	Botocudo	0.9612	Lagoa Santa	0.6458	Lagoa Santa	0.7844
MN-069	Botocudo	1	Paleo Colombia	0.4876	Mokapu	0.706
MN-068	Tupi-Guarani	0.7277	Tupi-Guarani	0.9498	Tupi-Guarani	0.9542
MN-053	Botocudo	0.98	Paleo Colombia	0.9338	Paleo Colombia	0.679
MN-003	Botocudo	0.9616	Cabeçuda	0.6355	Lagoa Santa	0.5975
MN-055	Botocudo	0.999	Tupi-Guarani	0.9931	Tupi-Guarani	0.9935
MN-064	Botocudo	0.9997	Tapera	0.9626	Tapera	0.5288
MN-039	Botocudo	0.9803	Tupi-Guarani	0.9819	Tupi-Guarani	0.9811
MN-056	Botocudo	0.9998	Lagoa Santa	0.7961	Lagoa Santa	0.9103
MN-063	Botocudo	0.9194	Lagoa Santa	0.4003	Tapera	0.4655
MN-066	Botocudo	0.9999	Paleo Colombia	0.8733	Paleo Colombia	0.7533
MN-007	Botocudo	0.4687	Peru	0.4785	Peru	0.4369
MN-014	Botocudo	0.992	Eskimo	0.4826	Paleo Colombia	0.4497
MN-021	Botocudo	0.9504	Lagoa Santa	0.8526	Mokapu	0.9478
MN-004	Botocudo	1	Tupi-Guarani	0.4679	Tupi-Guarani	0.5122
MN-012	Lagoa Santa	0.6881	Lagoa Santa	0.7788	Lagoa Santa	0.7987
MN-013	Lagoa Santa	0.3831	Lagoa Santa	0.8987	Lagoa Santa	0.9192
MN-120	Botocudo	0.9863	Lagoa Santa	0.6127	Lagoa Santa	0.6932
MN-118	Botocudo	0.9839	Lagoa Santa	0.7654	Lagoa Santa	0.7926
MN-023	Botocudo	0.9991	Lagoa Santa	0.963	Lagoa Santa	0.9719
MN-009	Botocudo	0.9963	Paleo Colombia	0.7966	Paleo Colombia	0.7463
MN-011	Botocudo	0.8887	Paleo Colombia	0.636	Paleo Colombia	0.6455

Specimens in bold represent the individuals with Polynesian DNA lineages.

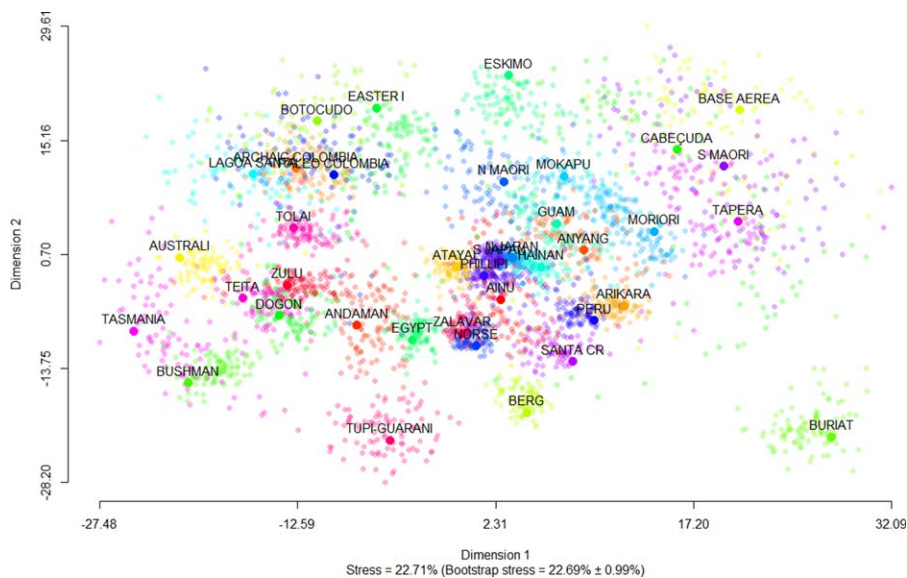


Fig. 2. Morphological affinities among the series included in this study represented by nonmetric multidimensional scaling (MDS) of the Mahalanobis distances matrix. The solid dots represent the MDS results obtained for the original data, and the transparent dots represent MDS results for each of the 100 bootstraps of the samples superimposed on the original MDS results. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 5. *Fst* for Early American series and each of the South American series, in comparison to the *Fst* of series worldwide

Series	Major regions					Minor regions				
	Neurocranium	Face	Frontal	Parietal	Occipital	Orbit	Nasal	Upper face	Malar	
Early Americans	0.038 ± 0.026	0.045 ± 0.02	0.034 ± 0.025	0.018 ± 0.016	0.024 ± 0.022	0.103 ± 0.033	0.045 ± 0.029	0.025 ± 0.016	0.026 ± 0.022	
+ Botocudo	0.056 ± 0.018	0.06 ± 0.016	0.045 ± 0.02	0.038 ± 0.018	0.057 ± 0.02	0.152 ± 0.042	0.054 ± 0.025	0.03 ± 0.015	0.038 ± 0.018	
+ Tupi-Guarani	0.106 ± 0.026	0.067 ± 0.017	0.093 ± 0.033	0.099 ± 0.028	0.072 ± 0.025	0.12 ± 0.032	0.035 ± 0.023	0.054 ± 0.019	0.098 ± 0.03	
+ Cabeçuda	0.107 ± 0.03	0.087 ± 0.024	0.136 ± 0.044	0.146 ± 0.034	0.072 ± 0.031	0.158 ± 0.04	0.097 ± 0.031	0.062 ± 0.022	0.055 ± 0.024	
+ Tapera	0.09 ± 0.024	0.141 ± 0.026	0.115 ± 0.038	0.116 ± 0.027	0.135 ± 0.028	0.196 ± 0.035	0.097 ± 0.03	0.121 ± 0.023	0.054 ± 0.02	
+ Base Aérea	0.113 ± 0.033	0.152 ± 0.037	0.112 ± 0.039	0.084 ± 0.026	0.193 ± 0.04	0.19 ± 0.038	0.079 ± 0.032	0.129 ± 0.036	0.048 ± 0.025	
+ Peru	0.09 ± 0.017	0.071 ± 0.014	0.08 ± 0.033	0.118 ± 0.026	0.021 ± 0.015	0.111 ± 0.027	0.039 ± 0.022	0.064 ± 0.019	0.036 ± 0.018	
World	0.171 ± 0.004	0.172 ± 0.004	0.177 ± 0.008	0.192 ± 0.007	0.158 ± 0.008	0.198 ± 0.01	0.2 ± 0.007	0.173 ± 0.008	0.164 ± 0.007	

This article is dedicated to the 19th century physical anthropologists of the National Museum in Rio de Janeiro, in memoriam, who were right from the start. André Strauss and Mark Hubbe contributed equally to the preparation of this article.

studies (e.g., Neves and Hubbe, 2005; Neves et al., 2007a; Hubbe et al., 2010). The Botocudo centroid appears located close to the Paleoamerican series and Easter Island. The other Polynesian series (Mokapu, Moriori, S Maori, and N Maori) appear associated with Southeast Asian (Guam and Anyang) and the Brazilian coastal series (Cabeçuda, Base Aérea, and Tapera). The remaining Native American series are located far away from the cluster of Paleoamericans and Botocudo, further supporting their strong morphological affinities. Howells' American series (Arikara, Peru, and Santa Cruz) appear closely associated with some East Asian (Ainu) and European (Zalavar and Norse) series, a pattern of association also previously observed (Howells, 1973, 1989). The Brazilian series (Base Aérea, Cabeçuda, Tapera, and Tupi Guarani), on the other hand, show a larger range of morphological distribution, supporting recent studies that suggest high morphological diversity in South America (e.g., Hubbe et al., 2014). The error distributions observed from the bootstrapped data indicate, as expected, larger variation in the archaeological series, owing to their smaller sample sizes. However, despite this larger error distribution, the patterns of associations described above hold true, even when this source of error is taken into account.

Table 5 and Figure 3 show the minimum *Fst* values calculated between early Americans and the other South American series for the two major and seven minor anatomical regions of the skull. In all anatomical regions, the three early American series show low between-group variances, especially when compared with the *Fst* observed worldwide. When any of the later South American series is included in the analyses, there is an increase in the observed *Fst*, as would be expected. However, only in a few cases the *Fst* reach values comparable to the worldwide variation. The overall pattern that arises from this analysis is that the Botocudo Indians are more similar to Paleoamericans when the neurocranium is considered. When the major neurocranium component and the minor frontal and parietal components are considered, Botocudo is the only series that, when added to the Paleoamericans, does not raise the *Fst* observed above the 95% confidence interval of Paleoamericans alone. When the facial regions are considered (major and minor components), the addition of Botocudo individuals still is within the 95% CI of Paleoamericans alone, but this is also observed for all other Late series, which indicates that in these cases the Botocudo are not particularly closely related to the Paleoamerican series, and low differentiation between groups is observed in Late South America as a whole. Interestingly, the coastal series show significant increase in *Fst* values when added to Paleoamerican groups, which fits previous results that suggest strong barriers between coastal and inland Brazil during most of the Holocene (see Hubbe et al., 2014, for an extended discussion on the topic). The only anatomical region that is characterized by high between-group variances in South America is the orbit, where even the *Fst* among Paleoamericans is ~0.10. This is also the only region where the addition of Botocudo results in a considerable increase in minimum *Fst* values.

DISCUSSION AND CONCLUSIONS

Our results support the earlier 19th century observation that the Botocudo Indians present morphological

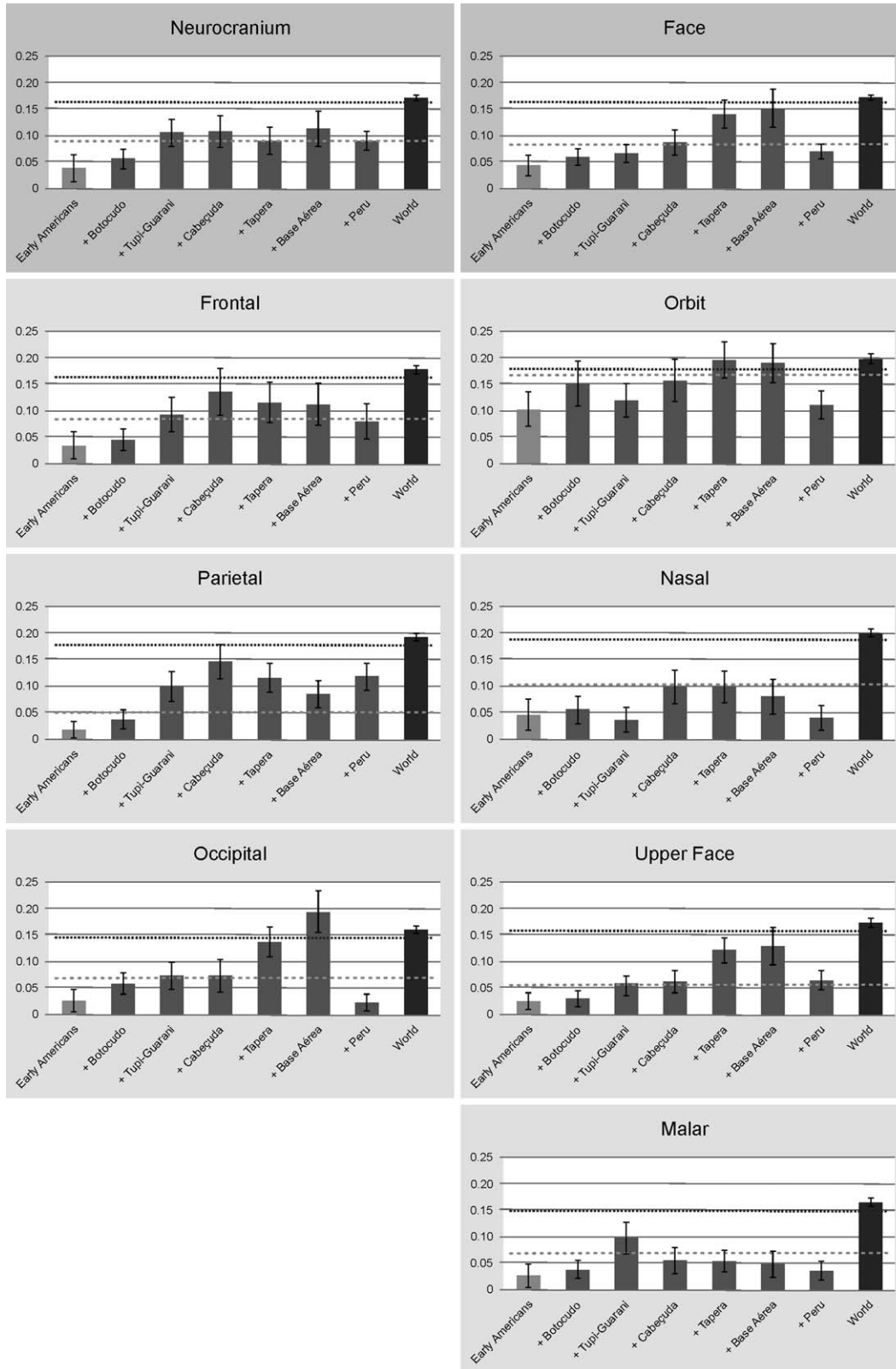


Fig. 3. Minimum F_{st} estimates obtained for the Early South American series by themselves and when each of the late South American series is analyzed together with them. Error bars represent 1 SD calculated from 1,000 bootstraps of the series. The light gray dashed line represents the upper confidence interval of the Paleoamerican F_{st} (Paleoamerican $F_{st} + 1.96*SD$), and the dark dashed line represents the lower 95% confidence interval of the worldwide F_{st} (World $F_{st} - 1.96*SD$).

affinities with early South American groups (Lacerda and Peixoto, 1876). Indeed, Botocudo can be considered as retaining the cranial morphological pattern observed among Paleoamerican groups, particularly when the neurocranium is taken into account. This in itself is an interesting observation, given that it suggests the maintenance of the Paleoamerican morphology throughout the Holocene, despite the existence of groups sharing considerable morphological differences occupying Brazil during this period. Remarkable in this case is the fact that Tupi-Guarani groups coexisted in the same region as the Botocudo, and yet low morphological affinities are found between them (see Fig. 2), suggesting limited gene flow between these groups. The coastal groups in this study (Base Aérea, Tapera, and Cabeçuda) present a very different morphological pattern from Botocudo and Paleoamerican groups as well, both in the neurocranium and in the face. These differences may be the result of two dispersal waves into the continent, as recently suggested by Hubbe et al. (2014).

However, these morphological affinities do not help us elucidate the presence of Polynesian DNA lineages found in two of the Botocudo specimens analyzed. From the morphological perspective, there is no strong evidence that these individuals differ from the remaining individuals in the series, i.e., that they were intrusive specimens among the Botocudo collection. The classifications based on discriminant function are equally puzzling, because the two Polynesian individuals have different pattern of association, one of them (MN-15) closely resembling the Botocudo and Paleoamerican morphological characteristics, and the other (MN-17) classifying primarily as Polynesian. These results present themselves as an interesting conundrum: on one hand, there is no strong evidence from the morphological point of view that these individuals (but especially MN-15) do not belong to the Botocudo populations (who show unique cranial morphological patterns themselves). On the other hand, it is exceedingly hard to accept, from a genetic point of view, that these individuals belong to a Native American population (Malaspina et al., 2014).

Given these uncertainties, we separated our discussion into two parts. The first addresses the origin of the morphological pattern that characterizes the Botocudo specimens analyzed here. Given that strong morphological affinities were observed among the Botocudo specimens as a whole, it is safe to assume that these results were not influenced by the presence of the two skulls presenting Polynesian DNA (MN-15 and MN-17), and therefore their presence in the sample does not affect the implications of our results. The second part addresses the presence of the Polynesian DNA lineages in our Botocudo series and how the morphological analyses can contribute to the discussion of the origin of these particular lineages in the continent.

The retention of Paleoamerican morphology among the Botocudo

Three different explanations can be proposed to account for the morphological similarities found between Paleoamericans and Botocudos: adaptive (genetic) convergence to similar climatic and environmental conditions; developmental plasticity to similar lifestyles (nongenetic convergence); or a direct ancestral-descendant relationship. The first possibility, adaptive convergence due to similar climatic and environmental

conditions, is in our opinion hard to sustain owing to two independent sources of information. The first concerns the environmental information available for early Lagoa Santa, early Colombia, and Botocudo. East-Central Brazil (where Lagoa Santa and Botocudo were established) can be characterized as a subtropical setting, exhibiting a seasonal, low-land landscape (around 600 m altitude), covered by a mosaic of savanna, semideciduous forests, and rain forests, never subjected to cold temperatures (Araujo et al., 2005; Oliveira et al., 2005). On the other hand, the first Colombians were settled in the Andean highlands (~2,000 m a.s.l.), a region covered by parklands and subjected to low temperatures during the year, especially in the winter months (van der Hammen, 1988). During the Pleistocene/Holocene transition, when the climate was colder than today, differences were probably even more accentuated (van der Hammen, 1988; Oliveira et al., 2005). As the same cranial pattern is observed in different environments their similarities cannot be explained by convergent adaptation to comparable local conditions.

The second line of evidence suggesting that convergent natural selective pressures could explain the similarities found among the Lagoa Santa, Sabana de Bogotá, and Botocudo groups comes from contemporary understandings of the forces governing the evolution of human cranial morphology. Recent literature strongly supports that the action of natural selection on human cranial morphology is restricted to harsh cold conditions (Relethford, 2002; Roseman and Weaver, 2004; Harvati and Weaver, 2006; Betti et al., 2009; Hubbe et al., 2009; von Cramon-Taubadel, 2009), which is clearly not the case in East-Central Brazil, or in the Sabana de Bogotá, during the entire Holocene (Oliveira et al., 2005). It is now well accepted that the evolution of human skull shape involves mainly stochastic processes of differentiation (see von Cramon-Taubadel, 2013 for a comprehensive review). Also, the strongest evidence of morphological retention in the Botocudo Indians is observed in the neurocranium, which has been shown to better reconstruct the phylogenetic relationships among modern humans when compared with other regions of the skull (Harvati and Weaver, 2006, 2008; Hubbe et al., 2009).

The second explanation that could be proposed for the similar morphological pattern of early Paleoamericans and Botocudo relies on developmental plasticity to similar lifestyles. Indeed, the three series showing Paleoamerican morphology in this study share the same broad subsistence pattern: hunting and gathering. However, contrary to what was defended in the past (Boas, 1912; Carlson and Van Gerven, 1977), current investigations have shown that although an influence on cranial morphology, plastic responses are very limited when the skull is considered as a whole (Sparks and Jantz, 2002; González-José et al., 2005; Paschetta et al., 2010). The results obtained in this study seem to point to this direction. In fact, the largest differences observed between Paleoamerican and Botocudo are associated with the face, which is the region that is most affected by adaptation to different subsistence patterns (González-José et al., 2005). Some authors have suggested that the Amerindian morphology could be the result of adaptation to regular plant cultivation and consumption since the Middle Holocene (Perez and Monteiro, 2009; Perez et al., 2011). The idea behind this scenario is that with plant cultivation (and pre-oral processing of vegetal food), much of the mastication stress generated by a hunter-gatherer life-

style was dissipated. Recently, Menéndez et al. (2014) proposed an alternative pathway in which subsistence strategy can influence cranial morphology that is not focused on the differential levels of mechanic stimuli (i.e., bite force) but on the differences in the composition of the diet itself (i.e., carbohydrate and protein intake). According to those lines of thought, the Amerindian morphological pattern that succeeded Paleoamerican morphology could have resulted from a plastic response to chewing stress relaxation or to a change in diet composition.

However, our results speak against the correlation between life-style change and cranial morphological differentiation. The shellmounds of Southern Brazil represented in our analysis by the Cabeçuda series exhibit a very distinct skull pattern from Early Americans and Botocudo (Figs. 2 and 3). However, it is well recognized in the local archaeological literature that the shellmound builders relied on fishing, shell-fishing, and hunting (Lima, 1999; Gaspar et al., 2008). These specialized hunter-gatherers seldom consumed plant items and there is no evidence that plant cultivation was among their regular subsistence activities. The high degree of dental attrition (Neves and Wesolowski, 2002) also attests to the chewing of hard and abrasive food items, with meager pre-oral processing, if any. Moreover, when facial anatomy is considered, there are more differences between Paleoamerican and the coastal sites, than between the former and Late Native Americans that relied on agriculture (Tupi-Guarani and Peru; Fig. 3). In addition, a recent isotopic study (Colonese et al., 2014) has pointed to a strong dietary similarity between coastal and inland groups, in spite of the accentuated morphological differences between them reported here. Therefore, subsistence change and phenotypic plasticity, in the context of this study, are poor candidates for either the origin of the Amerindian cranial pattern, or the similarities between Paleoamericans and Botocudos.

In view of the fact that our result do not conform easily to developmental hypotheses to explain the morphological affinities between Early Americans and Botocudo Indians, we propose that the morphological affinities of the 19th century Botocudos from East-Central Brazil and the Paleoamerican populations included in this study are best explained as a case of direct ancestor–descendant relationship between them, with the latter retaining to a large degree the morphological pattern that characterized the former. The Botocudo would not be the first example of late groups presenting Paleoamerican morphology in the continent. The Pericú Indians from Baja California (González-José et al., 2003) and Patagonian groups from the southernmost part of South America (Lahr, 1995) have also been shown to present the same morphological pattern as early American groups in modern times. Both these groups inhabited remote and isolated areas in the continent, and therefore the retention of the Paleoamerican morphology has been suggested to have occurred only on regions where groups bringing the Amerindian morphology did not easily reach (González-José et al., 2003). The Botocudo, however, do not fit well in this rule, given that they inhabited an accessible region in East-Central Brazil, and coexisted with Tupi-Guarani groups that show no morphological affinities with Paleoamerican groups. Hubbe et al. (2014) explored possible scenarios to explain the structure of this morphological variation among Brazilian groups during the Holocene, finding support for a strong isolation between

coastal and inland groups. The existence of a strong gene flow barrier, either due to cultural differences or environmental aspects, is supported by the data in this study, given the differences between the coastal series and other Brazilian groups (Figs. 2 and 3). Future studies will need to explore more specifically the relationship between the indigenous groups in East-Central Brazil to solve for the causes behind the barrier between these groups.

The origin of the biological variation of Botocudo Indians

The results presented here, in particular the retention of Paleoamerican morphology among Botocudo Indians and the close morphological affinities between Paleoamerican groups and Easter Island, allow us to explore and contribute to the discussion of the origins of the biological diversity observed among the Botocudo. As reviewed in the introduction, three hypotheses were proposed to explain the presence of Polynesian haplogroups among the Botocudo Indians (Gonçalves et al., 2013; Malaspinas et al., 2014): 1) they are the direct descendants of Lagoa Santa groups; 2) they are directly connected with Polynesian populations via trans-pacific migration; and 3) they inherited this DNA lineages from genetic exchange with African slaves during the colonial period in Brazil. From an exclusive point of view of cranial morphology, we would argue that our results are not in accordance with what would be expected under Hypotheses 2 and 3, offering support only to Hypothesis 1. However, as detailed below, the first hypothesis is the least supported by the mitochondrial and autosomal genetic data.

The results of the craniometric analyses done here suggest that the Botocudo Indians represent a case of late retention of the Paleoamerican morphological pattern, as discussed above. Although the Botocudo also share strong morphological affinities with one of the Polynesian series (Easter Island), a direct connection between Polynesian and Botocudo Indians due to trans-pacific migration is hard to sustain, for several reasons: 1) most of the Botocudo specimens analyzed show the typical C1 haplogroup of Native America groups (Gonçalves et al., 2013), and therefore cannot be considered as individuals who migrated from Polynesia. Also, the two specimens (MN-15 and MN-17) that show Polynesian autosomal and mtDNA lineages (Gonçalves et al., 2013; Malaspinas et al., 2014) show no evidence of admixture with Native Americans, making it very unlikely that the morphological affinities between Botocudo and Easter Island are due to the introgression of Polynesian biological diversity into the Native American gene pool. Second, the uniqueness of the Botocudo Indians, especially given their geographic location, speaks strongly against the idea of Late Holocene trans-pacific migrations as the cause for their biological characteristics. Polynesia was settled only in the last 3.5 thousand years (Weisler and Woodhead, 1995; Burley and Dickinson, 2001; Collerson and Weisler, 2007; Finney, 2007), and therefore any gene flow from Polynesia directly into the Americas (e.g., Yen, 1974; Green, 2000; Storey et al., 2007; Gongora, 2008; Gongora et al., 2008) must have occurred during the Late Holocene, when the continent was already densely populated and occupied by human groups. In this scenario, it is very unlikely that the only groups sharing lineages from such gene-flow event would be located on

the eastern fringe of the continent, with no evidence of similar lineages occurring more frequently among series closer to the point of insertion of Polynesian genetic diversity into the continent, namely the Pacific coast. Third, Botocudo shows high morphological affinities with only the most outlier (and geographically remote) of the Polynesian series included in the analyses (Easter Island), suggesting they do not present morphological affinities with the typical Polynesian morphology (see Fig. 2).

Similarly, it is hard to envision a situation where admixture with African slaves could explain the Botocudo biological diversity (Hypothesis 3). Although this is the favored hypothesis of Gonçalves et al. (2013), it has been deemed as unlikely by the autosomal analyses, which show no evidence of admixture with Native Americans or African populations (Malaspina et al., 2014). Moreover, it is extremely unlikely that only the relatively uncommon mtDNA frequencies from relatively uncommon Madagascar slaves would be the only African lineages introduced into the Botocudo gene pool. The morphological analyses presented here add further support to this, as the sub-Saharan African series included in the comparative analyses (Teita, Dogon, Zulu, and Bushman; Fig. 2) do not present strong morphological affinities with the Botocudo.

Although our results go against what would be expected for Hypothesis 2 and 3, we believe they conform well to the expectations of Hypothesis 1, namely that the Botocudo are the biological descendants of the Early American groups that inhabited Lagoa Santa by the end of the Pleistocene and beginning of the Holocene. As demonstrated by Hubbe et al. (2011), the early American groups from South America show very similar morphologies when compared to Late Pleistocene groups from East Asia and North Europe. This strong morphological affinity among Late Pleistocene/Early Holocene groups worldwide suggests that Early Americans share the generalized morphology that predates the morphological diversification that characterizes populations nowadays in the planet. The dispersion scenario defended by previous studies to explain these morphological relationships (Hubbe et al., 2011; Neves et al., 2013) considers that Paleoamericans present the morphological pattern that was observed in Southeast Asia by the end of the Pleistocene, when groups carrying this morphology moved northward toward Northeast Asia and subsequently into the Americas, and southward toward Indonesia, Australia, and Melanesia. Later during the Holocene, the morphological differentiation that gave rise to the typical modern East Asian and Amerindian morphology largely replaced traces of the previous morphology in these regions, resulting in visible affinities among Paleoamericans and recent Australo-Melanesians (e.g., Hubbe et al., 2010), but not with Asians and most Native Americans. The presence of some generalized groups in East Asia as well (e.g., the Ainu; Seguchi et al., 2011) supports the idea of a strong morphological change in Asia. Evidence of two morphological patterns associated with distinct dispersion waves has been previously used to describe the morphological diversity within the Americas (Neves and Hubbe, 2005; Hubbe et al., 2010, but see de Azevedo et al., 2011 for a counter-argument). Recently, a similar argument has been made to explain the morphological diversity among South and Southeast Asian groups (Reyes-Centeno et al., 2014), further supporting the idea that

Southeast Asia experienced a significant shift in morphology during the Holocene.

By extension then, our present results show that among the South American groups the Botocudo largely retained the same generalized morphology until the end of the Holocene, and similarly among the Polynesian series, Easter Island also retained a similar morphological pattern. Easter Island is one of the most remote locations within Polynesia, and the fact that it could have retained a more generalized morphology can be a result of their geographic isolation from groups introducing recent Asian morphology to the region, in the same way that it has been suggested that the Pericú and Patagonian groups in the America retained the Paleoamerican morphology due to their geographic isolation until recent times (Lahr, 1995; González-José et al., 2003).

Therefore, this scenario would explain the morphological affinities observed here. However, although this hypothesis fits well with the morphological variation observed among our series, it fails to explain the presence of Polynesian DNA lineages in individuals MN-15 and MN-17. As highlighted by Gonçalves et al. (2013), such scenario can be discarded to explain the two individuals with Polynesian mtDNA because the time to the most recent common ancestor of the Polynesian motif is too young to accommodate the expansion into the Americas, and because the mtDNA motif observed in these individuals lacks any private polymorphisms, which is not compatible with the scenario presented above. The lack of Native American admixture in the autosomal DNA of these two individuals (Malaspina et al., 2014) gives further support to Gonçalves et al.'s (2013) interpretation. On top of this evidence, to date no early Holocene skeleton that had DNA extracted shows mtDNA haplogroups that are not Native American (e.g., Kemp et al., 2007; Gilbert et al., 2008; Chatters et al., 2014; Rasmussen et al., 2014; see also Raff et al., 2011 for a comprehensive review of aDNA studies in the Americas). Therefore, although a model of retention of the morphological pattern present in the Old World by the end of the Pleistocene could explain the morphological association between Botocudo Indians, Early Americans, and Easter Island, it fails to explain the presence of the Polynesian DNA lineages in two of the Botocudo specimens available to study, whose explanation remains elusive.

A fourth hypothesis is that these specimens represent Polynesian skulls that were mixed with the National Museum collection during the past century. Although we find this unlikely given the fact that the individuals are clearly labeled and well referenced in the catalogue (See Malaspina et al., 2014, for photographs of the specimens). However, due to the fact that one of the individuals (MN-17) classifies primarily as Polynesian in our analysis (see Table 4), this possibility cannot be ruled out. Indeed, there are Polynesian crania housed in the National Museum that were acquired in the late 19th century for the "Brazilian Anthropological Exhibition" (e.g., specimen MN-111 was brought from the Chatam Islands in 1872 and specimens MN-104 and MN-105 were brought from the Marquesas Islands-Fatu Hiva, precise date unknown). For MN-111 this means that the specimen was acquired only 2 years before the first Botocudo arrived at the National Museum in 1874 (Malaspina et al., 2014). The museum catalogue, however, only came into existence more than three decades later, in 1906.

Taking into account how hard it is at this point to harmonize the data concerning specimens MN-15 and MN-17, further scrutinization to eliminate the possibility of mislabeling would be worth pursuing. In particular, it would be necessary for DNA samples to be extracted from the Polynesian specimens and their sequences compared to MN-15 and MN-17, to ascertain they come from different backgrounds. Complementarily, these two individuals have considerably older age (~AD 1420–1510, although marine reservoir effect could be causing an older date for the specimens; Malaspinas et al., 2014) than the other Botocudo specimens dated so far, and generating a better chronological context for the Botocudo and Polynesian series of the museum is required to try to confidently rule out specimen mislabeling.

In conclusion, our analyses of the morphological affinities of the Botocudo Indians within a worldwide context support the hypothesis proposed by Lacerda and Peixoto (1876) that these groups from East-Central Brazil retained the same morphological pattern from the early inhabitants from Lagoa Santa. Indeed, we were able to further clarify that these similarities are best observed in the neurocranium, which is an anatomical region that is usually less affected by adaptive responses to climate or diet change, while in the face some variations occur, particularly in the orbit. From a morphological point of view, our results fit better a scenario where the Botocudo are biological descendants from early Paleoamerican groups (e.g., Lagoa Santa), who share they last common ancestor with Polynesian groups probably in Southeast Asia, before the morphological differentiation that resulted in the present-day morphological pattern seen in Asia and most of the Americas occurred. However, this scenario fails to shed light on the origin of the Polynesian DNA lineages found in the two Botocudo specimens collected from Rio Doce.

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