

**Paleoamerican origins and behavior: a multidisciplinary  
study of the archaeological record from Lagoa Santa  
region (east-central Brazil)**

**Dissertation**

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**NO OVERLAP STATEMENT IN REFERENCE TO PHD PROJECT IN LEIPZIG  
UNIVERSITY**

Tübingen, 1. August 2016

André Strauss is enrolled as a doctoral candidate in the Fakultät für Biowissenschaften, Pharmazie und Psychologie of the Leipzig University. His PhD project in Leipzig University is entitled 'Late juvenile, early adult cranial growth in hominids' and is supervised by Prof. Dr. Jean-Jacques Hublin and co-supervised by Prof. Dr. Fred Spoor and Dr. Philipp Gunz. The area of the PhD project in Leipzig (i.e. Fachgebiet der Dissertation) is biology.

We hereafter declare that the two PhD projects are unrelated and that there is no overlap between the dissertations. This applies to its general topic, field of investigation, content, collaborators, dataset and methods. We also assure that an equivalent statement will be included in the dissertation to be submitted in Leipzig University.

**Prof. Dr. Katerina Harvati**

**Prof. Dr. Joachim Wahl**

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## List of publications for cumulative dissertation

### a) Published papers

#### *Appendix I:*

Hubbe M, Strauss A, Hubbe A, Neves WA. 2015. *Early South Americans cranial morphological variation and the origin of American biological diversity*. PlosOne 10:e0138090. USA. English

#### *Appendix II:*

Strauss A, Hubbe M, Bernardo DV, Neves WA, Atui JPV. 2015. *The cranial morphology of the Botocudo Indian. Brazil*. American Journal of Physical Anthropology: 157:202-216. USA. English.

#### *Appendix V:*

Strauss A, Oliveira RE, Bernardo DV, Salazar-García DC, Talamo S, Jaouen K, Hubbe M, Black S, Wilkinson C, Richards MP, Araujo AGM, Kipnis R, Neves WA. 2015. *The oldest case of decapitation in the New World (Lapa do Santo east-central Brazil)*. PlosOne 10:e0137456. USA. English.

### b) Accepted paper

#### *Appendix III:*

Villagran X, Strauss A, Miller C, Ligouis B, Oliveira RE. (accepted). *Buried in the ashes: formation processes of an early South American site (Lapa do Santo, Brazil)*. Journal of Archaeological Sciences. USA. English.

### c) Paper under review

#### *Appendix IV:*

Strauss A, Oliveira RE, Villagran X, Bernardo DV, Salazar-Garcia DC, Bissaro MC, Pugliese F, Hermenegildo T, Santos R, Barioni A, de\_Oliveira EC, Souza JCM, Jaouen K, Ernani M, Hubbe M, Inglez M, Gratao M, Rockwell HM, Machado MC, Souza GN, Chemale F, Kawashita K, O'Connell T, Israde I, Black S, Feathers J, Castro C, Richards MP, Kipnis R, Wahl J, Araujo A, Neves WA. (under review). *Early Holocene ritual complexity in South America: the archaeological record of Lapa do Santo (east-central Brazil)*. Antiquity. UK. English.

### d) Manuscript (literature review) to be published as a book chapter

#### *Included as part of the introduction:*

Strauss A. *Cranial morphological diversity in South American*. In: Pearce A. Beresford-Jones, D., Heggarty P. (Eds.). *Rethinking the Andes-Amazonia divide: a cross-disciplinary exploration*. Oxford University Press. English.

## ABSTRACT

By the very end of the Pleistocene the exploratory phase of the settlement of the Americas was over and by the beginning of the Holocene most landscapes were occupied by human populations well-adapted to their local environments. This is a crucial period in the process of cultural and biological differentiation of human groups in the continent and is the focus of the present dissertation. Using multivariate statistics and craniometric data the first two studies presented here investigate the debated hypothesis that those groups were not directly ancestral to recent Amerindians. Results of the first study indicate that during early Holocene the magnitude of morphological variation was not higher than observed among extant populations as was previously proposed. Therefore, *in loco* differentiation by drift is unlikely to be the major micro-evolutionary agent explaining modern patterns of variation in South America. Results of the second study confirm the century-old hypothesis that the Botocudo Indians (east-central Brazil) have strong morphological affinities with early Holocene groups from Lagoa Santa region constituting a potential case of late survival. The previously reported presence of Polynesian DNA in Botocudo is suggested to reflect the mixing of museum collections.

The remaining three studies focus on the early Holocene archaeological record of Lapa do Santo (east-central Brazil) aiming to characterize life style and ritual practices. The third study establishes a formation process model for the site identifying an expressive component of anthropogenic sediments produced after repeated combustion activities accumulating at extremely high rates. The fourth study presents the excavation protocol, chronological Bayesian model and the overall archaeological record with emphasis on the human burials. Accordingly, lithic technology, zooarchaeology, and multi-isotopic analyses indicate foraging groups with low mobility and a subsistence strategy focused on gathering plant foods and hunting small and mid-sized animals. Lapa do Santo was first occupied between 11.7-12.7 cal kyBP and its use as an interment ground started between 10.3-10.6 cal kyBP with primary burials. Between 9.4-9.6 cal kyBP the reduction of the body by means of mutilation, decapitation, defleshing, tooth removal, exposure to fire and possibly cannibalism, followed by the secondary burial of the remains according to strict rules, became a central element in the treatment of the dead. In the absence of monumental architecture or grave goods, these groups were using parts of fresh corpses to elaborate their rituals, showing this practice was not restricted to the Andean region at the beginning of the Holocene as previously thought. Between 8.2-8.6 cal kyBP another change occurred whereby pits were instead filled with disarticulated bones of a single individual without signs of body manipulation. Those changes show that during the early Holocene Lagoa Santa was a region inhabited by dynamic groups that were in constant transformation over a period of centuries. In the fifth study the oldest case of decapitation in the New World is investigated. Confocal microscopy confirms the presence of flake induced cut-marks and strontium isotope analysis indicates the individual was a local member of the group. It is proposed that this decapitation does not reflect punishment or war trophy but instead veneration as part of funerary rituals.

In conclusion, this dissertation supports that the groups inhabiting Lagoa Santa during the early Holocene are not the direct ancestral to the majority of the Native Americans. Nevertheless, cases of late survival such as the Botocudo do exist. Future studies based on aDNA will allow testing if these propositions are correct. The archaeological record of Lapa do Santo depicts with unprecedented detail the life of the groups inhabiting east-central Brazil during this period. Sophisticated funerary rituals, high reliance on vegetable items, reduced mobility, dynamic transformations through time, ample cultural diversity and early ethnogenesis are now new hallmarks characterizing those who were among the first to systematically occupy the savannah-like landscapes of east-central Brazil.

## ABSTRACT

Im auslaufenden Pleistozän endete die Erschließung der Besiedlung Amerikas und zu Beginn des Holozäns wurden die meisten Landschaften von menschlichen Populationen besiedelt, die gut an ihre lokale Umwelt angepasst waren. Dies stellt einen entscheidend wichtigen Zeitraum für den Prozess kultureller und biologischer Differenzierung von Menschengruppen auf diesem Kontinent und den Schwerpunkt der vorliegenden Dissertation dar. Die ersten beiden hier vorgelegten Studien untersuchen durch den Einsatz multivariater Statistik und kranio-metrischer Daten die umstrittene Hypothese, dass diese Gruppen nicht die direkten Vorfahren rezenter indigener Völker Amerikas darstellen. Die Ergebnisse der ersten Studie weisen darauf hin, dass die Variationsbreite im frühen Holozän nicht höher war als unter bestehenden Populationen beobachtet wurde, wie zuvor vorgeschlagen. Daher erscheint eine *in loco* Differenzierung durch Drift unwahrscheinlich als mikroevolutionäre Hauptursache, um moderne Variationsmuster in Südamerika zu erklären. Die Resultate der zweiten Studie bestätigen die jahrhundertalte Hypothese, dass die Botokuden (Ost-Zentralbrasilien) starke morphologische Ähnlichkeiten mit frühholozänen Gruppen aus der Region Lagoa Santa aufweisen, wodurch ein potenzieller. Es wird darauf hingedeutet, dass das zuvor berichtete Vorkommen polynesischer DNS in Botokuden eine Vermischung von Museumssammlungen widerspiegelt.

Die übrigen drei Studien befassen sich mit den frühholozänen archäologischen Zeugnissen von Lapa do Santo (Ost-Zentralbrasilien) mit dem Ziel, die Lebensweise und rituellen Praktiken zu beschreiben. Die dritte Untersuchung erarbeitet ein Modell zur Fundstellengenese, um die expressive Komponente der anthropogenen Sedimente, die durch wiederholte, extrem hohe Frequenz akkumulierende Verbrennungstätigkeiten fabriziert wurden, zu ermitteln. Die vierte Studie stellt die Grabungsmethodik, das chronologische Bayes'sche-Modell und die archäologischen Daten allgemein mit besonderer Hervorhebung der menschlichen Bestattungen vor. Dementsprechend zeigen die Steintechnologie, die Zooarchäologie und die Multiisotopenanalysen an, dass es sich um Jäger- und Sammlergruppen mit geringer Mobilität und einer Subsistenzstrategie, die auf dem Sammeln pflanzlicher Nahrung und dem Jagen kleiner und mittelgroßer Tiere beruht, handelt. Lapa do Santo wurde zuerst zwischen 11.7-12.7 ka kal. BP besiedelt und die Nutzung als Bestattungsgelände begann zwischen 10.3-10.6 ka kal. BP mit den Primärbestattungen. Zwischen 9.4-9.6 ka kal. BP wurde die Reduzierung des Körpers mittels Verstümmelung, Dekapitation, Dekarnation, Zahnentfernung, Feuereinwirkung und möglicherweise Kannibalismus, gefolgt von der Sekundärbestattung der Überreste gemäß strikter Regeln, ein zentrales Element in der Totenbehandlung. Diese Gruppen nutzten mangels monumentaler Architektur oder Grabbeigaben Teile der frischen Leichen, um ihre Rituale sorgfältig auszuführen, womit belegt wird, dass diese Vorgehensweise am Beginn des Holozäns nicht auf die Andenregion beschränkt ist, wie zuvor angenommen wurde. Zwischen 8.2-8.6 ka kal. BP trat eine andere Veränderung auf, wobei stattdessen Gruben mit disartikulierten Knochen von einzelnen Individuen ohne Anzeichen auf Körpermanipulation gefüllt wurden. Diese Änderungen deuten darauf hin, dass während des frühen Holozäns Lagoa Santa eine Region darstellt, die von dynamischen Gruppen, die in konstantem Wandel über einen Zeitraum von Jahrhunderten war, bevölkert wurde. In der fünften Studie wird der älteste Beleg für eine Dekapitation in der Neuen Welt untersucht. Konfokale Mikroskopie bestätigt die Präsenz von, durch Abschlüge verursachten, Schnittspuren und Strontiumisotopenanalyse weist darauf hin, dass das Individuum ein lokales Mitglied der Gruppe war. Es wird davon ausgegangen, dass die Enthauptung keine Bestrafung widerspiegelt oder eine Trophäe war, sondern stattdessen Verehrung als Teil des Totenrituals.

Zusammenfassend untermauert diese Dissertation, dass die Gruppen, die Lagoa Santa während des frühen Holozäns besiedelten, nicht die direkten Vorfahren der Mehrheit der indigenen Völker Amerikas

sind. Dennoch existieren Fälle, die ein Spätüberleben belegen, wie die Botokuden. Zukünftige, auf alter DNS basierend Untersuchungen werden es ermöglichen, zu testen, ob diese Aussagen korrekt sind. Die archäologischen Zeugnisse von Lapa do Santo beschreiben mit beispielloser Detailliertheit das Leben der in diesem Zeitraum in Ost-Zentralbrasilien lebenden Gruppen. Komplexe Bestattungsriten, ausgeprägte Abhängigkeit von pflanzlichen Produkten, reduzierte Mobilität, dynamischer Wandel durch die Zeit hindurch, reichliche kulturelle Diversität und frühe Ethnogenese sind nun neue Merkmale, die diejenigen charakterisieren, die unter den ersten waren, welche systematisch die savannengleiche Landschaft von Ost-Zentralbrasilien besiedelten.



## **1. Introduction**

The ancestral-descendent relationship between early Americans and Amerindians is a topic of debate for more than a century (e.g. Ten Kate, 1885; Hrdlíčka, 1912; Imbelloni, 1938; Rivet, 1942) and constitute the focus of the first part of this dissertation (Appendixes I and II). The evidence so far available is relatively scarce, and has often been invoked to support opposing models. Nevertheless, in spite of the multiple hypotheses on the time and mode of the settlement of America (detailed below) there is an overall agreement that early Americans shared a morphological pattern (effectively, a cranium shape) distinct from that seen among most Native Americans of late and recent periods. This distinctive pattern, dubbed 'Paleoamerican morphology', is known from several sites across South America: in East-Central Brazil at Santana do Riacho (Neves et al., 2003); in Northeast Brazil at Toca das Onças (Hubbe et al., 2004) and Serra da Capivara (Hubbe et al., 2007); in Southern Brazil at Capelinha (Neves et al., 2005) and in the interior of Rio Grande do Sul (Neves et al., 2004); at Sabana de Bogotá in Colombia (Neves et al., 2007); in the rockshelter of Lauricocha (Fehren-Schmitz et al., 2015) and the sites associated with the Paiján tradition in Peru, in the Pampas region of Argentina (Pucciarelli et al., 2010) and at the very southern tip of the continent in Palli Aike (Neves et al., 1999).

While it has been noted that early South Americans (and to a certain degree early North Americans too) differ significantly from their late and recent counterparts in cranial morphology among present-day Amerindians, meanwhile, diversity was commonly assumed to be low. Hrdlicka's concept of an American homotype (Fewkes et al., 1912:11), according to which indigenous groups were physically similar to each other, associated with a putative linguistic homogeneity embracing the entire continent (e.g. Greenberg et al., 1986) favoured the view of a 'biologically homogenous megapopulation' (Pucciarelli et al., 2006). Following initial observations by Neumann (1942, 1952) and Bass (1964), however, recent studies on late/recent Native South American populations (Ross et al., 2002, 2008; Sardi et al., 2005; Pucciarelli et al., 2006; Perez et al., 2009; Hubbe et al., 2014) have revealed greater diversity, indicating that cranial morphology in South America varies significantly not just over time but also between

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contemporary populations. Similarly, most linguistic studies now strongly contradict the hypothesis of homogeneity and depict South America as one of the most diverse of all continents as far as native language lineages are concerned (Nichols, 1990; Campbell, 1997; Nettle, 1999). Nettle (1999), for example, proposes a simulation model in which high linguistic diversity would be a consequence of rapid group fission and relative isolation once people arrived in the continent.

As for genetic data, the general picture remains one of overall homogeneity and of a single founding population to all Amerindians (Reich et al., 2012; but see Skoglund et al., 2015). It has also been recognized, however, that although there is little genetic diversity *within* any given population in South America, the differences *between* some groups can actually be rather high. Wang et al., (2007: 2052), for example, report that in Eastern South America 14.7% of the total genetic variance is found between populations while the remaining variance is found within populations. This proportion is almost twice as high as in other continents and points to significant processes of between-group genetic differentiation in South America.

This high diversity in cranial morphology among recent South American groups is all the more interesting given how starkly it contrasts with the pattern in genetics, where diversity generally decreases with distance from Africa (Cavalli-Sforza et al., 1994; Prugnolle et al., 2005; Wang et al., 2007). Of all continents, it is the Americas whose native populations present the lowest genetic diversity within any one population group. Similar patterns have been reported for worldwide diversity in cranial morphology (Manica et al., 2007; Betti et al., 2009). Nonetheless, this largely refers just to low *average* within-group diversity, and is a function of serial founder effects and range expansion as populations migrated out of Africa. On the other hand, differences *between* population groups are actually high in South America compared to other regions of the world. As Howells puts it: "*intraregional heterogeneity is greatest in Polynesia and the Americas, the two regions we can certify as the latest to be occupied. This goes counter to any expectation that such recency would be expressed in cranial homogeneity*" (Howells, 1989:83). In order to better understand the nature of this uniquely high level of morphological variation present in the New World is crucial to determine if this was already present among early Americans or if

instead was generated *in situ* during the Holocene. The study presented in Appendix I of this dissertation addresses this topic specifically and the results are discussed in the frame of the three main models commonly used to explain the settlement of the continent.

One of these models postulates that the high levels of morphological variation observed among recent Native Americans is mainly the result of intense drift, given the small population sizes of the founder groups. Powell (2005), for instance, presents a scenario favouring microevolution within the New World to explain the marked differences in cranial morphology between early and late/modern Native Americans. This is based on assumptions that the first Americans exhibited an especially high degree of genetic diversity, and that this highly variable source population was then subject to strong genetic drift, mainly due to group fission keeping population sizes small, factors that together would explain the morphological diversity of late Native Americans. This scenario, however, is based on the scant early material available in North America, a limiting factor also confronted by Jantz and Owsley (2001).

Sardi et al. (2005) suggest a similar scenario. Recognizing that late/modern Native South Americans display very different cranial patterns, they do not dismiss the possibility that the morphological pattern of late Holocene populations was generated *in situ* from the early pattern by local stochastic processes of differentiation. In their opinion, however, the local differentiation scenario would be feasible only if Early South Americans had displayed an uncommonly high degree of biological diversity, which has not been properly evaluated to date. A similar scenario has been proposed to reconcile the contrasting degrees of diversity in genetics (low) and in cranial morphology (high) observed across the continent. According to Gonzalez-José et al. (2008), this unexpected combination would be explained if, in the early stages of settlement, the population of the continent was highly diverse morphologically, and maintained continuous gene-flow with Asia (González-José et al., 2008; de Azevedo et al., 2011).

A second line of reasoning sees diversity in cranial morphology as a product of non-genetic shape changes during the growth of each individual during its youth (i.e. developmental plasticity) under the influence of different environments and/or subsistence strategies. Some authors have suggested that the Amerindian morphology could be the result of

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adaptation to regular plant cultivation and consumption from the Middle Holocene onwards, either as a result of reduced mechanical stress during mastication (Perez and Monteiro, 2009; Perez et al., 2011) or as a result of nutritional differences in diet itself, i.e. carbohydrate and protein intake (Menéndez et al., 2014). In a change from past thinking on this (e.g. Boas, 1912; Carlson and Van Gerven, 1977), however, current research has shown that although plastic responses do have localized influence on cranial morphology, this is very limited in the cranium as a whole and across samples taken on a broad geographical scale (detailed below).

A third possible explanation sees the high morphological diversity of recent Amerindians as resulting from a late survival of the Paleoamerican morphology into recent times. This hypothesis assumes that the continent was populated by two distinct human groups by the end of the Pleistocene (Model of Two Main Biological Components as defined by Neves and Hubbe, 2005). Evidence of a Middle/Late Holocene survival of Paleoamerican morphology has been reported for Sabana de Bogotá, Colombia (Neves et al., 2007), and for the interior of Southern Brazil (Neves et al., 2004). González-José et al. (2003) were able to extend the survival of the Paleoamerican morphologic pattern even later in time, by documenting its presence among the Pericú group from Baja California, Mexico, which were extinct by the 19<sup>th</sup> 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, towards a majority of groups sharing the Amerindian pattern by the end of the Holocene was more complex than first envisioned. The study presented in Appendix II of this dissertation evaluates this possibility.

In Brazil, late 19<sup>th</sup> century scholars from the National Museum in Rio de Janeiro suggested that the Botocudo of east-central Brazil shared the same cranial morphology as the Lagoa Santa Paleoamerican population. In the typological framework that prevailed during the late 19<sup>th</sup> century, Lacerda and Peixoto (Lacerda and Peixoto, 1876) described the Botocudo as belonging to the Lagoa Santa 'race'. Subsequently, other scholars such as José Imbelloni (1938), Paul Rivet (1942) and Pucciarelli et al. (2003) made similar claims about the Botocudo, while

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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; Malaspinas 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 autosomal and mitochondrial DNA lineages that are not Native American. The other Botocudo skulls (two for autosomal and 12 for mtDNA), however, have typical Amerindian DNA. The non-Amerindian 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 of the autosomal DNA sequences of the two Botocudo skulls that had Polynesian mtDNA (Malaspinas et al., 2014) supports 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, Gonçalves et al. (2013) suggest three possible scenarios. The first one is that Botocudo are descendants of the Paleoamerican groups from Lagoa Santa, following the early studies from the 19<sup>th</sup> century (Lacerda and Peixoto, 1876). Under this scenario, the Polynesian motif would be already present among the Lagoa Santa populations, and the Botocudo 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 (Sapfo et al., 2014). The second scenario is that recent gene flow from Polynesia introduced the haplogroups to the Botocudo. 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 et al.,

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2008). The third scenario 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 Sapfo et al. (2014) show no evidence of African admixture. In addition, no other evidence of more common African slave mtDNA sequences are found among the Botocudo, an unlikely fact given that Madagascar was not the major source of slaves for Brazil during the colonial period.

In this dissertation these competing models for the micro-evolution of humans in America are addressed by two multivariate studies (Appendixes I and II) in which cranial morphology is used as a proxy for neutral molecular markers. As any phenotypic trait cranial morphology is influenced by the interaction of the genes with the environment and is potentially capable of tracking the impact of migration, drift, selection, climate, diet and subsistence strategy in the differentiation of human populations along time. It is not surprising, thereafter, that the study of diversity in cranial morphology among Native Americans, past and present, has been central to debates on when the New World was first settled, and by whom.

Contrary to standard thinking for most of the twentieth century, there is in fact a close link between cranial morphology and population history (Harvati and Weaver, 2006; Roseman and Weaver, 2007). This association was first recognized by studies demonstrating that craniometric traits, like many other phenotypic traits, are in fact heritable, although some cranial traits more so than others (Carson, 2006; Sherwood et al., 2008; Martínez-Abadías et al., 2009). Cranial morphology does, therefore, presents a genetic base and can potentially be used as a proxy for ancestry (Cheverud, 1988; Roseman and Weaver, 2004). This perception has made it possible to extrapolate certain concepts from population genetics and apply them to cranial morphology using a quantitative genetics framework (e.g. Konigsberg and Ousley, 1995; Relethford, 2002; Ackermann and Cheverud, 2004; Sherwood et al., 2008; von Cramon-Taubadel, 2009; Martínez-Abadías et al., 2009; Perez and Monteiro, 2009; Smith, 2009; Strauss

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and Hubbe, 2010; Reyes-Centeno et al., 2014). As well as statistics such as *F<sub>st</sub>*, a measure of inter population differentiation (Williams-Blangero and Blangero, 1989; Relethford, 1994; Relethford and Harpending, 1994), there are also now techniques for inferring how far natural selection and/or stochastic evolutionary processes can influence cranial morphology (Ackermann and Cheverud, 2004). Together, these advances have significantly improved prospects for exploring diversity in cranial morphology patterns on a global scale, so that it can be compared and contrasted with neutral genetic markers.

The patterns of global variation in cranial morphology (Relethford, 2002, 2004) are very similar to those observed for neutral genetic markers (Lewontin, 1972; Bowcock et al., 1991; Barbujani et al., 1997; Rosenberg et al., 2002): differences between groups account for only around 15% of total worldwide variation. Neutral genetic markers (Ramachandran et al., 2005; Liu et al., 2006) and cranial morphology (Manica et al., 2007) both show declining diversity with distance from Africa. Moreover, the genetic architecture that determines cranial morphology appears to be governed, at least to a certain extent, by what is known as an additive polygenetic system (Martínez-Abadías et al., 2009). This means that when two different populations intermix, their hybrid offspring will have cranial morphology intermediate between them, so that it remains possible to recover their population history.

It is important to stress, however, that all evidence in favour of a neutral evolutionary basis for the diversity in cranial morphology among modern human populations seems to hold only across wide geographical ranges. In more localized studies, it has been suggested that selection or environmental plasticity has a more determining role in morphological differentiation (Relethford, 2004). Specific studies have suggested that some craniometric measurements and anatomical regions may be under long-term selection, in response to climatic conditions, especially populations adapted to extreme cold (Beals et al., 1984; Harvati and Weaver, 2006; Hubbe et al., 2009; Noback et al., 2011). Significant correlations have also been reported between specific craniometric measurements and environmental factors such as altitude (Guglielmino-Matessi et al., 1979; Rothhammer and Silva, 1990) and life-style (Carlson and Van Gerven, 1977; González-José et al., 2005; Paschetta et al., 2010; Noback and Harvati,

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2015). These may have played a role in how crania became so differentiated across South America and have been taken by some to argue in favour of cranium shape being highly responsive to local environmental conditions. In conjunction, the craniometric studies forming the first part of this dissertation (Appendixes I and II) contribute to evaluating competing models for the settlement of the New World. In a broader perspective, they inform on the relative role that different micro-evolutionary processes had in shaping modern phenotypic diversification.

In contrast to the first part of this dissertation, which deals with a century-old anthropological debate, the second one addresses a topic traditionally neglected in Brazilian archaeology. Based on the archaeological record of Lapa do Santo the three studies presented in Appendixes III to V provide a detailed characterization of hunter-gatherers behavior and life-style in Lagoa Santa region during the early Holocene period. In doing so, this part of the dissertation goes beyond 'Lund's Dilemma' (see below) and operates a necessary paradigmatic shift in the archaeological research of the region.

The Danish naturalist Peter Wilhelm Lund is considered the founder of Brazilian archaeology and paleontology. Between 1835 and 1843, during excavations in Lagoa Santa region, he found human remains in close proximity with fossils of the extinct mega-fauna (Lund, 1844; Cartelle, 1994; Piló and Auler, 2002; Araujo et al., 2005; Luna, 2007). Although recognizing that alternative hypothesis could account for such association he nevertheless postulated that humans and extinct animals likely coexisted. A revolutionary proposition at the time this idea became known as 'Lund's dilemma'. As a result Lagoa Santa became well-known for 19<sup>th</sup>-century scholars (e.g. Kollman, 1884; Ten Kate, 1885; Hansen, 1888; Hrdlička, 1912) and during the 20<sup>th</sup>-century several teams went to the region in search of evidence that could support the coexistence hypothesis (e.g. Walter et al., 1937; Walter, 1958; Hurt and Blasi, 1969; Laming-Emperaire, 1979; Bányai, 1997).

After more than 170 years of excavations a large collection of early Holocene skeletons and associated archaeological remains from Lagoa Santa was formed. A detailed depiction of how human groups inhabiting Lagoa Santa during early Holocene used to live should,



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therefore, be available. However, topics such as dietary habits, mobility patterns and ritualistic practices were of little relevance for those addressing Lund's dilemma. In addition, these excavations were conducted at a time when proper documentation was not available and, therefore, they often lack associated contextual information. The second part of this dissertation addresses these two topics. On one hand, it focuses on behavioral aspects of past populations from Lagoa Santa region, with emphasis on ritualistic practices. On the other hand, it provides detailed contextual information of new excavations conducted using appropriate documentation techniques (e.g. spatial control, three-dimensional model, flotation, etc.) in the archaeological site of Lapa do Santo.

Lapa do Santo, located in the Lagoa Santa karst in east-central Brazil, consists of a wide sheltered area of approximately 1300m<sup>2</sup> at the base of a ca. 30m-high limestone massif that rises from a doline valley. The Lagoa Santa karst is formed in Upper Pre-Cambrian metasedimentary rocks of the Bambuí Group, with a basal metacalcareous body corresponding to the Sete Lagoas Formation (dated to  $740 \pm 22$  Ma, Babinski et al., 2006) covered by metapellitic rocks (siltstones and claystones) of the Serra de Santa Helena Formation (IBAMA-CPRM, 1998). Dissolution of the Sete Lagoas limestone resulted in several karstic features on the landscape, such as caves and doline lakes. The soil cover is dominated by clayey, hematite-rich, red Oxisols (*latossolos* in the Brazilian Soil System) over yellow, goethite-rich oxisols developed on the Serra de Santa Helena metapellites (Piló, 1998; Araujo et al., 2013). Oxisols are highly weathered soils, rich in Fe-(hydr)oxides (e.g. hematite, goethite), Al-(hydr)oxides (e.g. gibbsite) and resistant clay minerals (e.g. kaolinite) (Schaeztz and Anderson, 2005).

Excavations in Lapa do Santo first took place between 2001 and 2009 under the coordination of Renato Kipnis, Astolfo Gomes Mello Araujo and Danilo Bernardo. Starting in 2001 several units were opened in distinct areas of the shelter. It became apparent that the densest archaeological deposits were located in the southern part of the shelter, immediately in front of the cave's entrance. An ample excavation surface was established in this region and a total of 26 early Holocene human burials were exhumed. Excavations ended in 2009 when, according to Brazilian laws, the excavated area was filled with sediments in order to reconstruct

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the original topography of the shelter's floor. Excavations at the site resumed in 2011 through the project 'Life and Death in Lapa do Santo', coordinated by the author of the present dissertation. Two new excavation areas were opened: a main area of 10,5m<sup>2</sup> to the east of the 2001-2009 excavation and a second area of 2m<sup>2</sup> to the south. During three field seasons (2011, 2012 and 2014) seven human burials were exhumed from the new excavation area.

Of particular interest is the mortuary record of Lapa do Santo as in the task of reconstructing the life of past populations human burials are highly informative of symbolic and ritual behavior. In several societies, bones and body parts constituted a potent cultural resource and their manipulation and organization into meaningful arrangements were commonly used to reify cosmological ideas and beliefs system (Goldstein, 2000; Brown, 2010). This kind of practice is usually considered part of a broader system of ceremonies that reflect a high degree of symbolic complexity and is therefore of great interest for archaeologist all over the world (Sofaer, 2006). Since rock art is usually difficult to date with precision (Neves et al., 2012; Pessis, 2013) most of the information on symbolic behavior during the early Holocene in South America is left to the analysis of human burials.

In the western portion of the continent substantial efforts have been made both in the sense of increasing the number of available sites with human skeletons and of providing new theoretical frameworks for its interpretations (Santoro et al., 2005). In a synthesis of the available evidence on early Holocene mortuary rituals in the Andean region, Santoro and collaborators (2005:330) concluded that *"the manipulation and transformation of dead bodies was more common than previously thought in the study of Archaic mortuary rituals"* and that despite considerable diversity of mortuary practices, a common emphasis on the preservation or reduction of the body can already be discerned.

In the eastern part of the continent, on the other hand, archaeological sites containing skeletal remains dating to the early Holocene are extremely rare, precluding the proper study of their ritual dimensions. One exception is precisely the region of Lagoa Santa in central Brazil where hundreds of early Holocene human skeletons have been exhumed in almost two centuries of research (Neves and Hubbe, 2005). According to the sparse descriptions available

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the mortuary practices in Lagoa Santa were simple and homogenous, including nothing but primary interments without grave goods (Walter, 1958; Neves et al., 2003). It contrasted, therefore, with the elaborated burials described for the western part of South America during the same period (Santoro, 2015). However, this depiction of the mortuary practices might not be accurate as for more than a century the investigations in Lagoa Santa were solely focused around 'Lund's dilemma' (see above). In this context, the findings from Lapa do Santo become particularly valuable as they allow to test if this traditional perspective is correct. In the studies presented in Appendixes IV and V of this dissertation the ritualistic aspects of the groups inhabiting Lagoa Santa at the beginning of the Holocene are discussed in details.

Another traditional assumption about the early Holocene hunter-gatherers of South America is that they constituted static societies that would not change over the course of millennia. In Brazil, specific techno-functional complexes such as the Itaparica (limaces)(Lourdeau, 2015), the Umbu (projectile points)(Schmitz, 1987) and the Lagoasantanse (flakes and cores)(Pugliese, 2008; Moreno de Sousa, 2014) occurred over extensive geographic areas and remained unchanged for thousands of years (Bueno et al., 2013). Such diachronically stable strategies of producing stone tools (Palmer, 2010) was proposed to reflect long-term cultural stability resulting from the presence of novelty-avoidance mechanism among early Holocene hunter-gatherers populations (Okumura and Araujo, 2014). The reasoning behind this interpretation is that even in the absence of an intentional attempt to innovate the intrinsic errors that are part of any (re)production process (Eerkens and Lipo, 2005, 2007) would result in the accumulation of modifications over time (Eerkens and Bettinger, 2013).

However, techno-functional constraints are not necessarily the best proxy for overall cultural stability (Deetz and Dethlefsen, 1971; Rogers, 2003; Kimmelmeier and Kühnen, 2012) and the proclaimed novelty-avoidance mechanisms might well be exclusively directed to preserve the functionality of utilitarian artifacts. In face of the current absence of information on the diachronic behavior of other cultural markers such as burials or rock art, it is hard to evaluate if this temporal continuity also applies to less utilitarian components and if this

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stability was not strictly techno-functional instead of cultural. In this frame, the characterization of the temporal variability of Lapa do Santo's mortuary behavior provides a rare opportunity in which an independent assessment of this asserted cultural stability can be established.

Finally, in addition to its immediate relevance for characterizing life style in Lagoa Santa region during early Holocene the second part of this dissertation also contributes for a broader comprehension of how tropical foragers adapted to their local environments. During most of its existence, humans were hunter-gatherers and, therefore, a proper comprehension of this lifestyle is of crucial importance (Kuhn and Stiner, 2001). Based on ethnographic accounts, for example, hunter-gatherer mortuary practices were sometimes considered not to go much beyond the necessities of getting rid of a rotting corpse (Woodburn, 1982). Although highly informative, however, ethnographic reports are limited by subjective bias and by the limited amount of time that they usually cover (Wobst, 1978; O'Shea, 1984). Additionally, foragers groups today usually occupy marginal environments and represent only a limited fraction of the total variation that once characterized this lifestyle (Deetz, 1968; Schrire, 1984; Hodder, 1986). To a great extent, this is a result of the European invasions (Stannard, 1992) as well of the earlier expansions of autochthonous pastoralist and agriculturalist groups (e.g. Bantu in Africa (Li et al., 2014; Grollemund et al., 2015) and Tupi in South America (Noelli, 1998)). The archeological record is free from those particular biases and, therefore, is an important complementary source of information to characterize hunter-gathers lifestyle, in general, and their mortuary practices, in particular.

## **2. Objectives**

The objectives of this dissertation are:

- Test competing models of cranial morphological differentiation in the New World.
- Provide a detailed account of context, dating, formation processes and excavation protocols for the archaeological site of Lapa do Santo.
- Characterize the behavior (emphasis on funerary rituals) of early Holocene human groups in east-central Brazil based on the archaeological record of Lapa do Santo.

More specifically, these objectives were divided into the following research topics:

2.1 Appendix I – Early Holocene morphological variation in South America.

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This study is designed to evaluate if the high cranial morphological variation seen among recent Native Americans was already present among the continent's early human groups. To achieve this goal estimative of within and between-group variances of Early South Americans are compared to modern human population values. Morphological variances within and between populations were assessed based on 23 linear craniometric measurements from Howells' protocol (Howells, 1973, 1989). Two series were used to represent early morphological variability in South America: Lagoa Santa (11.5-7.5 kyr BP) from east-central Brazil and Sabana de Bogotá (10.5-7.0 kyr BP) from central Colombia. Within and between group variance apportionment of Early Americans was contrasted with the values obtained for series representing recent Native Americans, East Asians, Europeans, Sub-Saharan Africans, Australo-Melanesians, and Polynesians from Howells database. Within group variance was estimated using the trace of the covariance matrix (VCV) of the series after standardizing all variables into z-scores. Inter-group morphological variability between regions and among series within each region was quantified by means of  $F_{st}$  estimates, obtained by averaging the principal diagonal of the R-matrix ( $r_{ii}$ ) extracted from the phenotypic data. The pairwise  $F_{st}$  matrix was represented graphically with a Kruskal non-metric Multidimensional Scaling (MDS). To explore the confidence of the affinities observed in this case, the analysis was repeated with 100 bootstraps of the data, respecting the original sample size of the series. The bootstrapped MDS configurations were then superimposed on the original data using Procrustes Analysis, allowing the results to be combined in a single scatterplot. The implications of distinct magnitudes of early morphological variation to different settlement scenarios are discussed within a micro-evolutionary framework.

### 2.2 Appendix II – Late survival of Paleoamerican morphology: the Botocudo of east-central Brazil.

To contribute to the discussion on the origin of the biological variation of the Botocudo Indian, this study evaluates their cranial morphological affinities using multivariate statistics focusing on two complementary goals: 1) to test the hypothesis proposed by Lacerda and Peixoto (1876) that the Botocudo Indians retain the same morphological pattern as early

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Paleoamerican groups in the continent and 2) to explore if the two individuals previously identified as having Polynesian DNA can be assumed to belong to the Botocudo population (as far as cranial morphological variation is concerned). Cranial morphology was assessed by 32 metric variables following Howells (1973, 1989) protocol. The Botocudo material included here is comprised of 16 male and female skulls of adult individuals curated at the National Museum of Rio de Janeiro in Brazil. 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 from Howell's database representing the worldwide modern human morphological variation. The Paleoamerican series include specimens from Lagoa Santa, Brazil, and Sabana de Bogotá, Colombia. The other South American series include a mid-Holocene shellmound series (Cabeçuda) associated to 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 (Howells, 1996) modern Peruvian series.

The morphological affinities of the Botocudos series as whole were assessed based on the Mahalanobis' Distance matrix (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 non-metric Multidimensional Scaling (MDS; Cox and Cox, 2010). To explore the expected distribution of the observed distances, 100 new distance matrices were 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 to 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 (same procedure described for Appendix I).

### 2.3 Appendixes III and IV – Lapa do Santo archaeological record

Even though dozens of archaeological sites have been excavated in the Lagoa Santa region, most of them lack proper contextual information making the available archaeological material of limited use. For this reason, the present dissertation emphasizes documentation and contextualization. In Appendix III and IV the relevant information related to the archaeological deposits of Lapa do Santo is provided. This includes a review of the history of research in Lagoa Santa region and Lapa do Santo, a detailed account of the excavation/exhumation protocols, a multiproxy model of the site formation processes and a chronological Bayesian model.

A total of 53 charcoal samples were selected for radiocarbon dating. The samples were sent to the Beta Analytic AMS system in Miami where they have been pretreated with the ABA method. Fifty-eight human bone and teeth sampled from Lapa do Santo's burials were also sent to Beta Analytic between and pretreated without ultrafiltration method. Nine samples provided collagen and carbon for accurate measurement. The measured ages were then corrected according to the  $^{13}\text{C}/^{12}\text{C}$  sample ratio, from which the conventional age was derived. Twenty-one fragments of human bone from Lapa do Santo were pretreated at the Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology (MPI-EVA), Leipzig, Germany, using the method described by Talamo and Richards (Talamo and Richards, 2011). For acceptable quality collagen, the atomic C:N ratio should be between 2.9 and 3.4 and a collagen yield of more than 1% of weight (DeNiro, 1985; Ambrose, 1990; van Klinken, 1999). The samples satisfying these conditions and with enough collagen for radiocarbon dating and were sent to the Klaus-Tschira-AMS facility of the Curt-Engelhorn Centre in Mannheim, Germany, where they have been graphitized and dated (Kromer et al., 2013). The dates from MPI-EVA were corrected for a residual preparation background estimated from pretreated  $^{14}\text{C}$  free bone samples. The radiocarbon dates were calibrated using the SHcal13 (Hogg et al., 2013) curve and modeled into three contiguous phases using OxCal 4.2 (Ramsey and Lee, 2013). Lapa do Santo deposit has a very expressive anthropogenic component and all charcoals are assumed to derive from combustion structures. Discounted their *terminus post quem* nature, the charcoals from Lapa do Santo are themselves the events to be dated. Therefore, the t-type outlier model is not

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appropriate and instead we used a s-type outlier model (Ramsey, 2009), with prior probabilities set at 0.05.

Hypothesis about site formation processes were formulated in the field and further evaluated by a micro-contextual approach (Goldberg and Berna, 2010), integrating micromorphology,  $\mu$ FTIR and organic petrology. Micromorphology is the study of intact blocks and thin sections of sediment and soil, often under magnification (Courty et al., 1989; Courty, 2001). This approach allows to identify the components of deposits and soils, and also study their spatial and stratigraphic relationship to one another (Goldberg and Arpin, 1999; Karkanas, 2000; Goldberg and Sherwood, 2006). FTIR analyses are able to identify diagenetic minerals and heated materials (Weiner et al., 2002; Berna et al., 2007; Miller et al., 2013; Stahlschmidt et al., 2015b). Organic petrology allows the precise identification of burned organic material within the sediments and the classification of the remains according to the type of tissue, degree of burning, weathering and permineralization (Ligouis, 2006; Goldberg et al., 2009; Villagran et al., 2013; Stahlschmidt et al., 2015a). Diatom analyses were performed to investigate the potential flooding of the site by a pond that existed north of the site, and whose presence is indicated by watermarks in the limestone wall. Analyses were conducted on loose sediments collected in aseptic conditions from the second excavation area. Additionally, the stratigraphic integrity of the deposits was accessed by the spatial analysis of dated samples. Accordingly, the residuals from a linear regression of age and depth (i.e. z-value) were computed and interpreted as indicative of mean stratigraphic errors. Finally, Lapa do Santo depositional regime is compared to other sites in Lagoa Santa region in order to characterize formation processes diversity within a single karstic terrain.

### 2.4 Appendix IV – Life during the early Holocene period in Lagoa Santa

This study provides a synthesis of past behavior in Lagoa Santa during early Holocene based on the re-evaluation of pre-existing data and performance of new analyses. Carbon and nitrogen isotopes data is generated for skeletons from Lapa do Santo and added to pre-existing data (generated by Tiago Hermenegildo) for other skeletons of the site. Carbon and nitrogen stable isotope analysis on bone collagen is widely used in archaeology for reconstructing



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ancient diets (Vogel and van der Merwe, 1977; van der Merwe and Vogel, 1978). Since almost all of the carbon in the biosphere is fixed by autotrophs (DeNiro and Epstein, 1978), stable carbon isotopic ratios ( $^{13}\text{C}/^{12}\text{C}$ ) can distinguish between plants that fixate carbon using  $\text{C}_3$  and  $\text{C}_4$  photosynthetic pathways (O'Leary, 1988).  $\text{C}_4$  type plants are mostly grasses, such as maize, sorghum and sugarcane, which have  $\delta^{13}\text{C}$  values ranging from -9 to -14‰.  $\text{C}_3$  type plants made up of some grasses (e.g. oats, wheat, rice) and virtually all other non-grasses, have  $\delta^{13}\text{C}$  values ranging from -20 to -35‰ (Deines, 1980). Stable nitrogen isotopes ( $^{15}\text{N}/^{14}\text{N}$ ) accumulate throughout successive trophic levels in which primary producers (plants) have the lowest ratios and each subsequent level in the trophic chain is enriched in approximately 3-5‰ (Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984; Hedges and Reynard, 2007) in  $\delta^{15}\text{N}$  values. Study of modern-day plants occurring in the Brazilian savanna, in which Lapa do Santo is located, have  $\delta^{15}\text{N}$  values that range from -5.0‰ to +7.9‰ (Bustamente et al., 2004).

Pre-existing unpublished zooarchaeological data (generated by Marcos Bissaro Jr.) are integrated with the isotope data to provide an overall synthesis of dietary habits. Faunal analyses at Lapa do Santo followed standard zooarchaeological methodology (Klein and Cruz-Uribe, 1984; Lyman, 1994, 2008). The Number of Identified Specimens (NISP) and Minimum Number of Individuals (MNI) was computed for a sub-sample of faunal assemblage from Lapa Santo constituted by the material coming from units: L7, L8, L10, M3, M4, M5, M6 (archaeological strata contemporary to the early Holocene human remains).

Mobility patterns are investigated by the analysis of strontium isotopes from dental enamel of individuals from Lapa do Santo. The results are compared to pre-existing unpublished data (generated by Marcia Machado) on shells collected from the site that characterize regional levels of strontium bioavailability. Strontium isotopic analysis ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) of skeletal material is a commonly employed method for detecting provenance and mobility amongst mammals, including humans (Price et al., 2002, 2004), because tooth enamel from individuals records the isotopic signal during its formation at the earliest stages of life (Humphrey et al., 2008). Since radiogenic isotope  $^{87}\text{Sr}$  forms by radioactive decay from rubidium ( $^{87}\text{Rb}$ ), the  $^{87}\text{Sr}/^{86}\text{Sr}$  signature of a specific location is determined by the underlying

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bedrock age and its content of Rb. Younger geological formations like volcanic rocks have lower  $^{87}\text{Sr}/^{86}\text{Sr}$  values than older geological formations such as granite. A specific geological strontium signature is incorporated into body hard tissues by substituting for calcium (Ericson, 1985; Price et al., 2002; Bentley, 2006), since strontium enters the ecosystems without fractionation (Faure and Powell, 1972; Graustein, 1989). Amongst skeletal tissues, to date, tooth enamel is the preferred substrate for this analysis, due to its greater resistance to diagenesis in the burial environment (Budd et al., 2000; Hoppe et al., 2003). Within a single archaeological population,  $^{87}\text{Sr}/^{86}\text{Sr}$  analyses of individuals' teeth can potentially detect those who were born on differing geological substrates ('non-locals'). However, environmental background studies are needed to assess the local bioavailable  $^{87}\text{Sr}/^{86}\text{Sr}$  signature from the different geologies in the study region (Price et al., 2002; Evans et al., 2010), in order to assess possible provenance and territorial mobility.

Previous technological studies are complemented by macro-wear analysis and a detailed description of the curated artifacts. A parametric model is used to determine the date when allochthonous raw material stops being used on the site (a pattern identified by Pugliese, 2008). The mortuary patterns previously presented in Strauss (2010) are redefined upon new direct dates based on ultrafiltered collagen extracted from bone or dental remains (see previous section for description). Sex estimation for Lapa do Santo skeletons was based on different anatomical regions: analysis of the skull (Walker, 2008), the ischium-pubic region (Phenice, 1969), the pelvis (Bruzek, 2002), the proximal region of the ulna (Cowan and Pastor, 2008), and the femoral diaphysis (Black, 1978). Confocal and scanning electron microscopy are used for the diagnosis of cut-marks in human bones. A detailed literature review is provided and the implications of Lapa do Santo in local, regional and continental scales discussed.

## 2.5 Appendix V – Defeated enemy or venerated ancestral? The oldest case of decapitation in the New World

Few Amerindian habits impressed the European colonizers more than the taking and displaying of human body parts, especially when decapitation was involved. Although disputed by some authors, it has become widely accepted that decapitation was common

among Native Americans across the entire continent and the archaeological evidence confirms that the practice has deep chronological roots. In South America, the oldest decapitation is reported for the Andean region and dates to ca. 3000 BP at the site of Asia 1, Peru. Since all other South American archaeological cases occur in the Andes (e.g., Nazca, Moche, Wari, Tiwanaco) it was assumed that decapitation was an Andean phenomenon in both its origins and in its most unambiguous expression.

However, in 2007 a case of decapitation was found in the early Holocene component of Lapa do Santo. It was therefore, potentially much older than any of the known cases in South America. For this reason it was decided that this particular burial deserved special investigation. Particularly, three studies were conducted in order to elucidate the nature of Lapa do Santo's decapitation. First, the chronology of the interment was determined using ultra-filtration methods of collagen extraction. Second, confocal microscopy is applied for the diagnosis of cut-marks. Finally, strontium isotope analysis was performed in an attempt to determine if the decapitated individual was a local member of the group or an outsider. This information can contribute evaluating if this case of decapitation reflects inter personal violence or punishment rather than a funerary ritual. In addition, a comprehensive review of all archaeological cases of decapitation in South America is provided.

### **3. Results and Discussion**

#### **3.1 Appendix I – Magnitude of morphological variation among early Holocene South American populations.**

Results from this study indicate the high cranial morphological diversity seen among Late/Recent Native Americans was not present among the early American populations. Regarding within-group variability, early Americans did present average variability, comparable to modern populations, despite the fact that these series represent more than three thousand years of human occupation in the continent. Therefore, the morphological pattern that characterized early American groups remained unchanged for long periods of time, highlighting the importance of the differences seen among South American groups in modern times. These results also support the use of the early American series included here as valid

units of analysis from the perspective of morphological affinities, i.e. the use of such collections as possible 'demes' is warranted, despite their chronological span.

When between-group diversity is considered the Early Americans'  $F_{st}$  estimate is considerably low, falling well within the range of the other continents and macro-regions, (Europe, Australo-Melanesia, sub-Saharan Africa), especially when series that have shown strong adaptive responses to climate are removed (East Asia without Buriat, and North America without Eskimo). Indeed, the only two regions that show particularly high  $F_{st}$  estimates are South America and Polynesia. Polynesia is expected to show increased between-group variance apportionments due to the fact that islands have stronger natural barriers to gene-flow in the form of the ocean stretches separating them. South America, however, not only does not present the same level of natural barriers as the deep ocean islands of Polynesia, but also shows a larger proportion of the variance due to differences between groups than Polynesia. Thus, South America is particularly interesting in terms of the development of modern human cranial morphological diversity, especially given that our results suggest that the high diversity seen among Late Native South Americans was not present among early groups entering the continent, as proposed before (e.g. Powell, 2005; González-José et al., 2008; de Azevedo et al., 2011).

In conclusion, the results of this study contribute to our growing knowledge about the origins of the biological diversity of Native American groups during the Holocene, by showing significant differences in the apportionment of variation across time in the continent. Under this scenario, the biological diversity that characterizes New World populations originated only during the Holocene, much later than the initial human occupation of the New World, and most probably required the entrance of additional morphological diversity from regions outside the continent (Northeast Asia).

### 3.2 Appendix II – Late survival of the Paleoamerican morphology: the Botocudo of east-central Brazil.

The morphometric study of the Botocudo's crania supports the hypothesis that groups in east-central Brazil retained the Paleoamerican morphology characteristic of the early Holocene

inhabitants of Lagoa Santa region. 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 the results fit better a scenario where the Botocudo are descendants from early Paleoamerican groups (e.g. Lagoa Santa), who share they last common ancestor with groups in SE Asia before the morphological differentiation that resulted in the present day morphological pattern seen in Asia and most of the Americas occurred.

However, morphological data fail to explain the presence of Polynesian DNA lineages in individuals MN-15 and MN-17 previously reported by Gonçalves et al., (2013) and Sapfo et al. (2014). As highlighted by Gonçalves et al. (2013) the time to the most recent common ancestor of the Polynesian lineage is too young to accommodate the expansion into the Americas, and because the mtDNA lineage observed in these individuals lack any private polymorphisms, which is not compatible with the scenario presented above, a deep ancestral link must be discarded. The lack of Native American admixture in the autosomal DNA of these two individuals (Sapfo et al., 2014) gives further support to Gonçalves et al. (2013) interpretation. On top of this evidence, to date no early Holocene skeleton that had DNA extracted shows the 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; Prufer and Meyer, 2015; see Raff et al., 2011 for a comprehensive review of aDNA studies in the Americas). Therefore, while 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 possibility is that these specimens represent Polynesian skulls that were mixed with the National Museum collection during the past century. This possibility was previously discarded as the crania are clearly labeled and well referenced in the catalogue (see Sapfto et al., 2014, for photographs of the specimens). Still, it is important to bear in mind that there are indeed Polynesian crania housed in the National Museum that were acquired in the late 19<sup>th</sup>

century for the 'Brazilian Anthropological Exhibition' (e.g. specimen MN-111 was brought from the Chatham 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 two years before the first Botocudo's crania arrived at the National Museum in 1874 (Sapfo et al., 2014). The museum catalogue as we know it today, 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 genetic 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. This means that the nuclear DNA study available failed to sample the true Botocudo individuals.

### 3.3 Appendixes III and IV – Excavation protocol, chronology and formation processes

In conjunction these two papers provide a detailed description of the history of research, excavation protocol, chronological context and formation processes of Lapa do Santo. A Bayesian model based on 21 OSL dates, 67 radiocarbon dates on charcoal and 13 radiocarbon dates on collagen extracted from human bone indicate three distinct periods of occupation (95.4% interval): 12.7-7.9 cal kyBP, 5.4-3.9 cal kyBP and 2.1-0.0 cal kyBP. Parametric evaluation of the residual distributions of calibrated age against depth (i.e. z-values) indicates 95% uncertainty of  $\pm 500$  years for any given vertical position. At the same time, in accordance with the good preservation of human skeletons, multi-proxy formation processes analysis indicates an overall integrity of the deposits. It is proposed that the high frequency of chronological inversions mainly results from grave digging disturbance.

Based on field observations, it was postulated that anthropogenic sediments produced after repeated combustion was the main constituent of the deposits at Lapa do Santo. In comparison with other sites in east-central Brazil Lapa do Santo is unique in presenting elevated depositional rates. In conjunction, these two observations led to the proposition that Lapa do Santo was more intensively occupied than other localities. However,

micromorphological evaluation (conduct by Prof. Villagran) identified a high component (ca. 50%) of clay aggregates of geogenic origins as part of the site matrix. Therefore, the initial hypothesis of high-depositional rate reflecting intense human occupation could not be supported.

#### 3.4 Appendix IV – Archaeological record of Lapa do Santo

Lapa do Santo is an archaeological site located in the northern part of the Lagoa Santa karst (see Appendix III and IV for detailed characterization of the archaeological context). It is a cave with an associated sheltered area of ca. 1300 m<sup>2</sup> developed under the negative slope of a 30 meters high limestone massif. The southern region of the sheltered area is relatively flat, high and dry, and is located immediately in front of the cave's entrance. The floor of the shelter has a strong descending inclination towards the north, which becomes flat again near a natural sinkhole located in the northern extreme of the sheltered area.

Zooarchaeological analysis indicates the presence of fish, lizards, rodents, armadillos, peccaries and deer that were brought in single pieces from the killing site (Bissaro Jr., 2008). Carbon and nitrogen stable isotope analysis show a slightly enriched  $\delta^{13}\text{C}$  and low  $\delta^{15}\text{N}$  values in the adult population. Nitrogen values are distinct from the carnivores and similar to the herbivores from Lagoa Santa region thus suggesting a heavy reliance in C<sub>3</sub> plant resources. Together with dental caries frequencies comparable to those observed among agricultural populations (Da-Gloria, 2012), the emerging picture is of an economy structured around plant resources that were probably rich in carbohydrates complemented by hunting of small and mid-sized animals.

The lithic assemblage is dominated by small flakes and cores (Pugliese, 2008). Crystal quartz was by far the dominant raw material, but silex, quartzite and silicified sandstone were also present. There is no clear division between artifact and *debitage* in an industry where every flake was a potential tool. With the exception of a single hematite axe blade and an arrow point, formal artifacts made of stone are inexistent in the early Holocene deposits. Flakes were discarded when their edge became dull and most of them were used only a few times. Feather scars, occasional scaler and snap fractures were identified by preliminary use-wear analysis of

## *Results and Discussion*

flakes from Lapa do Santo indicating they were used to cut soft materials such as hides, meat, cordage and grasses (Pugliese, 2008). While lithic types were constant through time, the use of raw materials varied and around 9.9 cal kyBP the exploitation of non-local sources such as silexite was drastically reduced with the locally available crystal quartz becoming dominant (Pugliese, 2008). The bone artifacts from Lapa do Santo are very similar to what is observed in other parts of central Brazil during the same timeframe. They contrast sharply with the expedient technological approach adopted for the production of lithic artifacts. A total of 198 bone artifacts or fragments of bone artifacts were found on the site, including spatulas (71%), burins (25%) and fishhooks (0.01%).

The 26 human burials from Lapa do Santo were divided into six different mortuary patterns based on their chronology and shared features. Lapa do Santo Mortuary Patterns (LSMP) 1, 2 and 3 were the focus of the study presented in Appendix IV. LSMP-1 is dated to 9.7-10.6 cal kyBP and is characterized by two primary single burials in flexed position (Burials 1 and 27).

Lapa do Santo Mortuary Pattern 2 (LSMP-2) is dated to 9.4-9.6 cal kyBP and can be further subdivided into three categories: LSMP-2a (Burial 21 and 26), LSMP-2b (Burials 9, 14, 17, 18 and 23) and LSMP-2c (isolated bones). LSMP-2a is characterized by fully-articulated partial skeletons with cutting and chopping marks. In Burial 21, the midshafts of both tibiae and fibulae were chopped and removed while soft tissue was still present. Burial 26 is a decapitated head with the first six cervical vertebrae articulated in anatomical position (see Appendix V for details). The hyoid bone was missing and both amputated hands were laid over the face.

LSMP-2b is characterized by graves filled with the fully disarticulated bones of up to five individuals presenting a strong selection of anatomical parts. Some bones show evidence of exposure to fire, application of red pigment, defleshing, cutting, chopping and removal of teeth. Burials 14, 17 and 18 were composed of a bundle of long bones from one or two individuals, deposited with the individualized cranium and/or mandible of a different individual. Bundles comprising infant post-cranial bones were found next to adult crania (Burials 14 and 17), and bundles comprising adult post-cranial bones were found next to an infant cranium (Burial 18).



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The long bones of the bundles had been chopped and segregated into extremities and midshafts and in some cases the latter were further chopped into smaller sections. The cranium of Burial 17 was used as a funerary receptacle and filled with chopped burnt bones some of which present defleshing cut-marks. Black burn marks limited to the anterior portion of the external maxillary alveolar margin indicate exposure to fire while soft tissue was still present. The co-occurrence of chopped and defleshed bones with signs of burning with soft tissues suggests that LSMP-2 may have involved some form of cannibalism.

In Burials 17 and 18, all teeth were intentionally removed and the coronoid processes of Burial 18's mandible were drilled. Red pigment was abundantly applied to the bones of Burial 14 and Burial 18. Burial 23 was composed of a cranium *calotte* filled with 54 permanent and 30 deciduous teeth, some of which belonged to the skull of Burial 17. Burial 9 was an individualized child skull placed near the pelvis of an individual of similar age. The deciduous dentition was removed and an assemblage of human teeth and chopped midshafts (accession code: LSt-2253) were deposited next to Burial 9. LSMP-2c is defined by isolated burnt chopped bones that were not part of any formal burial and the presence of rodent gnaw marks could indicate they were subject to scavenging and not immediately buried.

LSMP-3 is dated to 8.2-8.6 cal kyBP and includes nine burials: 6, 7, 10, 11, 12, 13, 15, 19 and 22. Burials are characterized by shallow circular pits completely filled with mostly disarticulated bones of single individuals of various ages and sexes. Circular stone structures covered some of the burials, but also occur independently of them. Anatomical selection was not observed and, with the exception of some small bones, most elements of the skeleton were present. The midshafts of long bones of adult individuals were in some cases intentionally broken in the central region before deposition, resulting in butterfly fractures with impact points indicating the use of some percussion instrument. The burials belonging to LSMP-3 are very similar to each other, contrasting the larger variability observed within LSMP-2. Furthermore, characteristic elements of the latter, such as cut-marks, chop-marks, absence of dentition, red pigment, and burnt marks are not present in the former. The presence of diverse and elaborated mortuary practices in Lapa do Santo supports the notion that the traditional

view of Lagoa Santa's mortuary practices as simple and homogenous results from misinterpreting the archaeological record. On the contrary, in the absence of monumental architecture or grave goods, these groups were using parts of fresh corpses to elaborate their rituals, showing this practice was not restricted to the Andean region at the beginning of the Holocene.

The mortuary record from Lapa do Santo also indicates that the groups inhabiting Lagoa Santa during the beginning of the Holocene were dynamic and facing constant transformation through time. Therefore, the results presented here show that techno-functional constraints are not necessarily the best proxy for overall cultural stability. In this frame, the characterization of the temporal variability of mortuary behavior in Lapa do Santo provides an independent assessment of this asserted cultural stability.

### 3.5 Appendix V – Defeated enemy or venerated ancestral? The oldest case of decapitation in the New World.

This study focused on Burial 26 from Lapa do Santo which is constituted by a case of *perimortem* decapitation. An ultra-filtered AMS age determination on a fragment of the sphenoid provided an age range of 9.1-9.4 cal kyBP (95.4% interval) making this the oldest case of decapitation in the New World. The interment was composed of an articulated cranium, mandible and first six cervical vertebrae. Visualization of cut-marks with confocal microscopy identified a v-shaped profile and parallel micro-striations indicating the decapitation was made with stone tools. The right hand was amputated and laid over the left side of the face with distal phalanges pointing to the chin and the left hand was amputated and laid over the right side of the face with distal phalanges pointing to the forehead. Strontium analysis comparing Burial 26's isotopic signature to other specimens from Lapa do Santo suggests this was a local member of the group. Therefore, Burial 26 likely constitute a ritualized decapitation instead of trophy-taking, testifying for the sophistication of mortuary rituals among hunter-gatherers in the Americas during the early Archaic period.

The early Holocene age of Burial 26 extends the timeline of decapitation in South America by more than 4500 years. Geographically, the archaeological record of North America

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and Mesoamerica shows a more widespread occurrence of decapitation compared to South America, with cases occurring from the Arctic to southern Mexico. Our findings suggest that South America had the same spatially widespread distribution observed for North America, making the occurrence of decapitation widespread across the whole continent since the beginning of the Holocene. In addition, they confirm that the vast territorial range of decapitation behavior described in ethnohistorical and ethnographic accounts for the New World has deeper chronological roots. Until now, every archaeological site in South America where evidence of decapitation was observed was related to the so-called Pan-Andean societies. Lapa do Santo, located in the lowlands of east-central South America, indicates that decapitation does not necessarily have a restricted Pan-Andean distribution as previously suggested.

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## Appendix I

### **Early South Americans cranial morphological variation and the origin of the American biological diversity**

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## ABSTRACT

Recent South Americans have been described as presenting high regional cranial morphological diversity when compared to other regions of the planet. This high diversity is in accordance with linguistic and some of the molecular data available to date for the continent, but its origin has not been satisfactorily explained yet. Here we explore if this high morphological variation was already present among Early groups from the continent, in order to refine our knowledge about the timing and place of the origins of the morphological diversity observed during recent times in the continent. Between-group ( $F_{st}$  estimates) and within groups variances (trace of within groups covariance matrix) of two early South American groups (Lagoa Santa and Sabana de Bogotá) were estimated based on linear craniometric measurements and compared to modern human reference populations representing six regions of the planet, including the Americas. The results show that Early Americans present moderate within-groups diversity, falling well within the range of modern human groups, despite representing almost three thousand years of human occupation. Between-group variance is very low between Early Americans, but is high when recent American groups are included in the analysis, being similar to values for the entire planet. These results support the hypothesis that the high morphological diversity of Native Americans was not present among the first human groups arriving in the continent and must have originated during the Middle Holocene, possibly due to the arrival of new morphological diversity coming from Asia during the Holocene.

The cranial morphological diversity of Native American groups over time has been an important source of information about the processes of human occupation of the New World, and has often been used to support different settlement scenarios for the Americas<sup>1-20</sup>. Although distinct scenarios for the occupation of the New World have been supported through the study of local cranial morphological diversity, most rest on the notion that early Americans shared a distinct morphological pattern from the one seen among most late/recent Native Americans. These differences suggest high diachronic morphological diversity<sup>8</sup>, where early South Americans (and to a certain degree early North Americans<sup>21</sup>) differed significantly in terms of cranial morphology from their late and recent counterparts<sup>5-6,9,16,22-23</sup>. However, recent studies have revealed a high degree of biological variability even when only late/recent Native South Americans are considered<sup>11,14,24-27</sup>, indicating that the high morphological diversity in South America is not only restricted to differences over time.

A high morphological diversity among recent South American groups is unexpected when contrasted to the molecular studies that demonstrate a general loss of genetic diversity associated with increased distance from Africa<sup>28-30</sup>, with Native American populations presenting the lowest within-group biological variances among all continents. Although there is no reason to expect that phenotypic variance may be correlated to genetic variance at the loci influencing the phenotype<sup>31</sup>, a similar decrease in variance with increased distance from Africa was also reported for worldwide cranial morphological diversity<sup>32-33</sup>. However, this loss of within-group variance, explained as the result of multiple founder effects and expansion range effects from populations migrating out of Africa, is not correlated with the degree of population structure, or differences between groups. When the apportionment of the variation due to between-group differences is considered, South America has been described as highly diverse. Linguistic studies, for instance, demonstrate that South America is impressively diverse as far as native languages are concerned<sup>34-35</sup>. Nettle<sup>35</sup> defends the idea that high linguistic diversity is a consequence of the rapid group fission and relative isolation once people arrived in the unoccupied South American lowlands. Similarly, although South Americans present low overall within-group molecular variance, differences between group, as measured by  $F_{st}$  values, of eastern South Americans has been reported to be high. Wang et al.<sup>30</sup> (p. 2052), for example, report  $F_{st}$  values for Eastern South America (14.7%) more than twice as high as  $F_{st}$  for series worldwide (7.1%), indicating high population structure among recent eastern Native South Americans.

Consequently, the high levels of cranial morphological differences between groups reported for recent South Americans is in accordance with the idea of high between-group differentiation, despite the loss of intra-group variation associated with distance from Africa. Distinct, however, to molecular and linguistic data, which are largely restrained to recent samples, cranial morphology allows us to investigate the origin of the high inter-group

diversity seen in the continent by assessing the within and between-group variation of Early South American samples.

Despite the large number of studies demonstrating the high cranial morphological differences between early and late Native American groups, the morphological variance present among the first humans who occupied the New World has been scarcely studied (see 21 and 36 for exceptions). Powell<sup>10</sup>, for instance, presented a scenario favoring microevolution within the New World to explain the marked differences in terms of cranial morphology between early and late/modern Native Americans, based on the assumptions that the first Americans exhibited an especially high degree of biological diversity and that genetic drift (mainly due to group fission) acting on the highly variable mother population could explain the origin of the morphological differentiation observed among late Native American populations. However, his scenario is based on the scant early material available in North America which is entirely composed of isolated specimens and no population parameter estimates are possible for this material. This is a limiting factor also confronted by other studies dealing with the early North American remains (e.g., 21).

Sardi et al.<sup>26</sup> also recognized that Early and Late/Modern Native South Americans display very different cranial patterns. Moreover, they do not dismiss the possibility that the morphological pattern of Late Holocene populations was generated *in situ* from the early morphological pattern by means of local stochastic processes of differentiation. In their opinion, however, the local differentiation scenario would be feasible only if early South Americans displayed an uncommonly high degree of biological variance within-groups, that could then be later partitioned in structured in differences between group by genetic drift and group fissions during the Holocene. A similar scenario is also proposed by Gonzalez-José et al.<sup>8</sup>, to accommodate both the molecular and morphological diversity observed in the continent. According to these authors, a highly morphologically diverse population was present in the early stages of the settlement of the continent, who maintained continuous gene-flow with Asia (see also 19), which could explain the high cranial morphological diversity and low molecular diversity in the continent.

Consequently, understanding if the high cranial morphological variation seen among recent Native Americans was already present among the continent's early human groups is crucial to discuss the processes of morphological diversification and human dispersion in the continent. Here we address this question by estimating within and between-group variances of Early South Americans comparatively to modern human population values, and explore the consequences of this information for our understanding of the processes by which the Americas were settled during the end of the Pleistocene.



## MATERIALS AND METHODS

Morphological variances within and between populations were assessed based on 23 linear craniometric measurements from Howells' protocol (Table 1<sup>37,38</sup>). Two series were used to represent early morphological variability in South America (Table 2; Dataset S1): Lagoa Santa (11.5-7.5 kyr BP) from east-central Brazil and Sabana de Bogotá (10.5-7.0 kyr BP) from central Colombia. The morphological affinities and archaeological context of these series have been extensively described elsewhere<sup>5,9</sup>. Despite spanning over three thousand years of human occupation, these two collections represent the only skeletal series in the continent with enough individuals recovered to allow the estimation of within-population parameters, and therefore offer a unique opportunity to explore the early American groups based on population estimates. All other early skeleton remains in the continent are represented by isolated or few specimens (e.g., 10, 21).

**Table 1.** Craniometric variables used in this study.

Variables included*
Glabella-occipital length (GOL)
Nasio-occipital length (NOL)
Basion-bregma height (BBH)
Maximum cranial breadth (XCB)
Maximum frontal breadth (XFB)
Biauricular breadth (AUB)
Biasterionic breadth (ASB)
Nasion-prosthion height (NPH)
Nasal height (NLH)
Orbit height (OBH)
Orbit breadth (OBB)
Bijugal breadth (JUB)
Nasal breadth (NLB)
Bizygomaxillare breadth (ZMB)
Bifrontomallare breadth (FMB)
Nasion Subtense (NAS)
Biorbital breadth (EKB)
Malar length, inferior (IML)
Malar length, superior (XML)
Cheek height (WMH)
Frontal cord (FRC)
Parietal cord (PAC)
Occipital cord (OCC)

Within and between group variance apportionment of Early Americans was contrasted with the values obtained for series representing recent Native Americans, East Asians, Europeans, Sub-Saharan Africans, Australo-Melanesians, and Polynesians from Howells database<sup>39</sup> (Table 2). Within group variance was estimated using the trace of the covariance matrix (VCV) of the series after standardizing all variables into z-scores. VCV trace was calculated for each series independently. Since variance estimations are affected to some extent by small sample sizes, to compare the VCV trace of the early South American series with the worldwide series, random subsets with the same number of individuals as the early series were selected a thousand times from each series and the results were used to build the variance distributions for each one. Consequently, the comparisons with Lagoa Santa were based on 1000 within-group variances calculated from subsets of 29 individuals for each series, and for the Colombian series the same number of variances was calculated from subsets of 14 individuals per series. The variances observed within the early series were plotted in a graph with the distribution of the random sets of each series to compare the results visually.

Inter-group morphological variability between regions and among series within each region was quantified by means of  $F_{st}$  estimates, obtained by averaging the principal diagonal of the R-matrix ( $r_{ii}$ ) extracted from the phenotypic data.  $F_{st}$  gives an estimation of the apportionment of between-group genetic variation<sup>40-43</sup>.  $F_{st}$  estimates for metric data are minimum estimates and can greatly underrepresent inter-group variation apportionment if the heritability values of the traits (measurements) are low<sup>42,44</sup>. Heritability values for human cranial dimension range from moderate to high<sup>45</sup>, although different traits show very distinct heritability levels<sup>46-47</sup>. However, assuming mean heritability values of 0.55 in the past produced similar apportionment values to neutral molecular data<sup>44,48</sup>, showing that even when using average heritability values, craniometric data generates comparable  $F_{st}$  estimates. Therefore, all  $F_{st}$  estimates calculated here assume a constant heritability of 0.55 to improve comparability of the results with previous studies<sup>44-45,48</sup>.

Initially,  $F_{st}$  was calculated between all pairs of series, using the pooled within group covariance matrix (VCV) for all groups to calculate the C matrix. The use of the pooled VCV among all groups was required because of the small sample size of some of the archaeological series, which resulted in non-reliable (i.e., weakly correlated) VCVs between groups and therefore biased the values of  $F_{st}$  between pairs of groups.  $F_{st}$  in this case can be considered a measurement of distance, since it will reflect the distance between each group centroid to the overall centroid (i.e., if the data were not divided into groups). Still, the pairwise calculations allow exploring the morphological affinities between series included in the study. The pairwise  $F_{st}$  matrix was represented graphically with a Kruskal non-metric Multidimensional Scaling (MDS<sup>49</sup>). To explore the confidence of the affinities observed in this case, the analysis was repeated with 100 bootstraps of the data, respecting the original sample size of the series. The

bootstrapped MDS configurations were then superimposed on the original data using Procrustes Analysis<sup>50</sup>, allowing the results to be combined in a single scatterplot.

**Table 2.** Craniometric series included in the analyses.

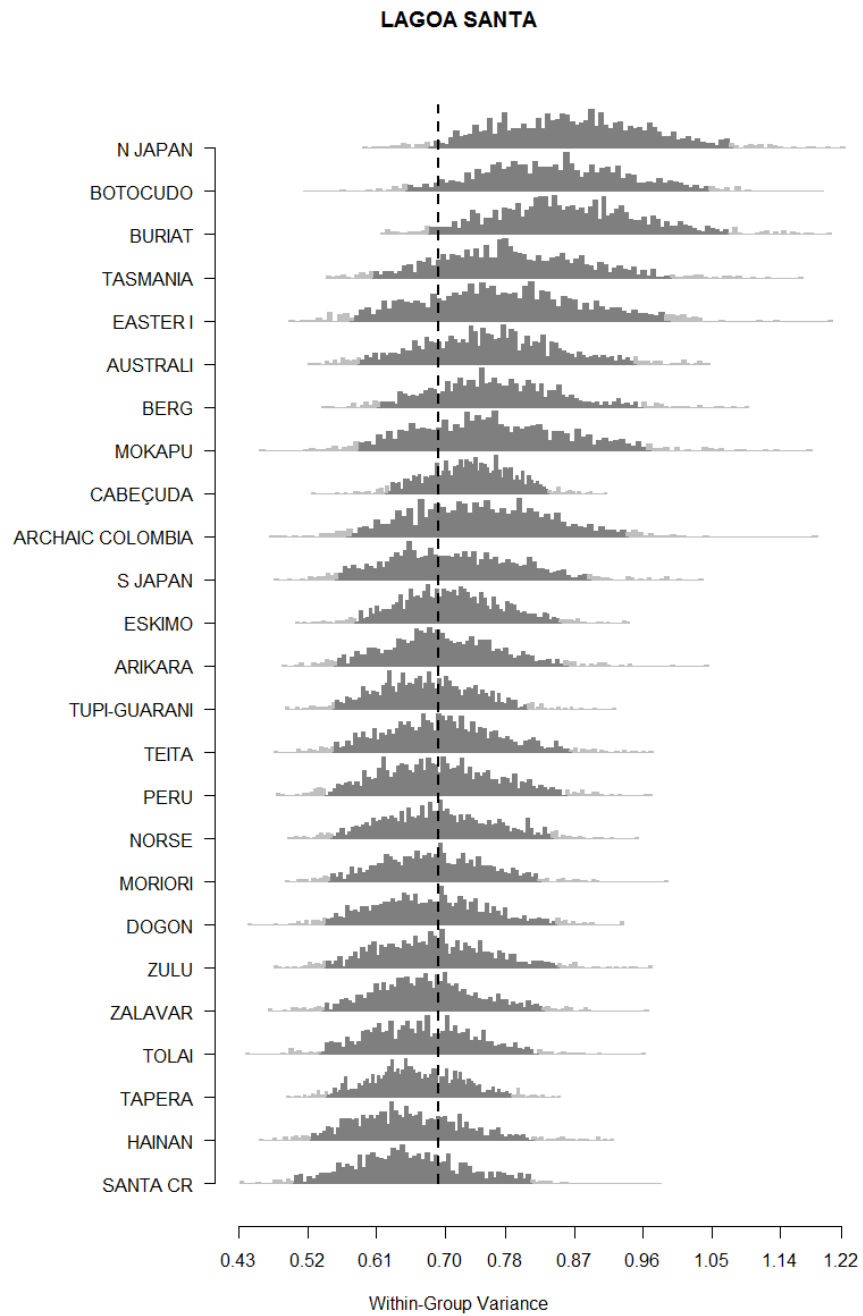
Population	Regional/chronological affiliation	Sample size	Males/Females ratio	% Missing values	Reference
Lagoa Santa	Early America	29	18/11	16.94	9
Early Colombia	Early America	14	6/8	6.52	6
Peru	South America	110	55/55	0	37, 38
Botocudo	South America	32	16/16	1.90	25
Archaic Colombia	South America	33	12/21	11.86	6
Tapera	South America	47	26/21	5.28	25
Cabeçuda	South America	19	12/7	13.50	25
Tupi-Guarani	South America	23	14/9	2.65	25
Arikara	North America	69	42/27	0	37, 38
Santa Cruz	North America	102	51/51	0	37, 38
Eskimo	North America	108	53/55	0	37, 38
North Japan	East Asia	87	55/32	0	37, 38
South Japan	East Asia	91	50/41	0	37, 38
Hainan	East Asia	83	45/38	0	37, 38
Buriat	East Asia	109	55/54	0	37, 38
Australia	Australo-Melanesia	101	52/49	0	37, 38
Tasmania	Australo-Melanesia	87	45/42	0	37, 38
Tolai	Australo-Melanesia	110	56/54	0	37, 38
Berg	Europe	109	56/53	0	37, 38
Norse	Europe	110	55/55	0	37, 38
Zalavar	Europe	98	53/45	0	37, 38
Zulu	Sub-Saharan Africa	101	55/46	0	37, 38
Dogon	Sub-Saharan Africa	99	47/52	0	37, 38
Teita	Sub-Saharan Africa	83	33/50	0	37, 38
Easter Island	Polynesia	86	49/37	0	37, 38
Mokapu	Polynesia	100	51/49	0	37, 38
Moriori	Polynesia	108	57/51	0	37, 38

Complementing the pairwise analysis,  $F_{st}$  estimates and their standard errors<sup>40-42</sup> were also calculated for series within each of the large regions in the dataset. For the American series,  $F_{st}$  were calculated once with all series, and then for the early series alone, for all late American series, and for all South American Late series separately, to explore the impact that diachronic changes have in the apportionment of the variation in the New World. Also, given that our series include two groups that lived in extreme cold environments (Eskimo and Buriat), which have been shown to have cranial morphology responding adaptively to this environmental factor<sup>51-52</sup>,  $F_{st}$  estimates were also calculated for the Americas and East Asia without these groups.

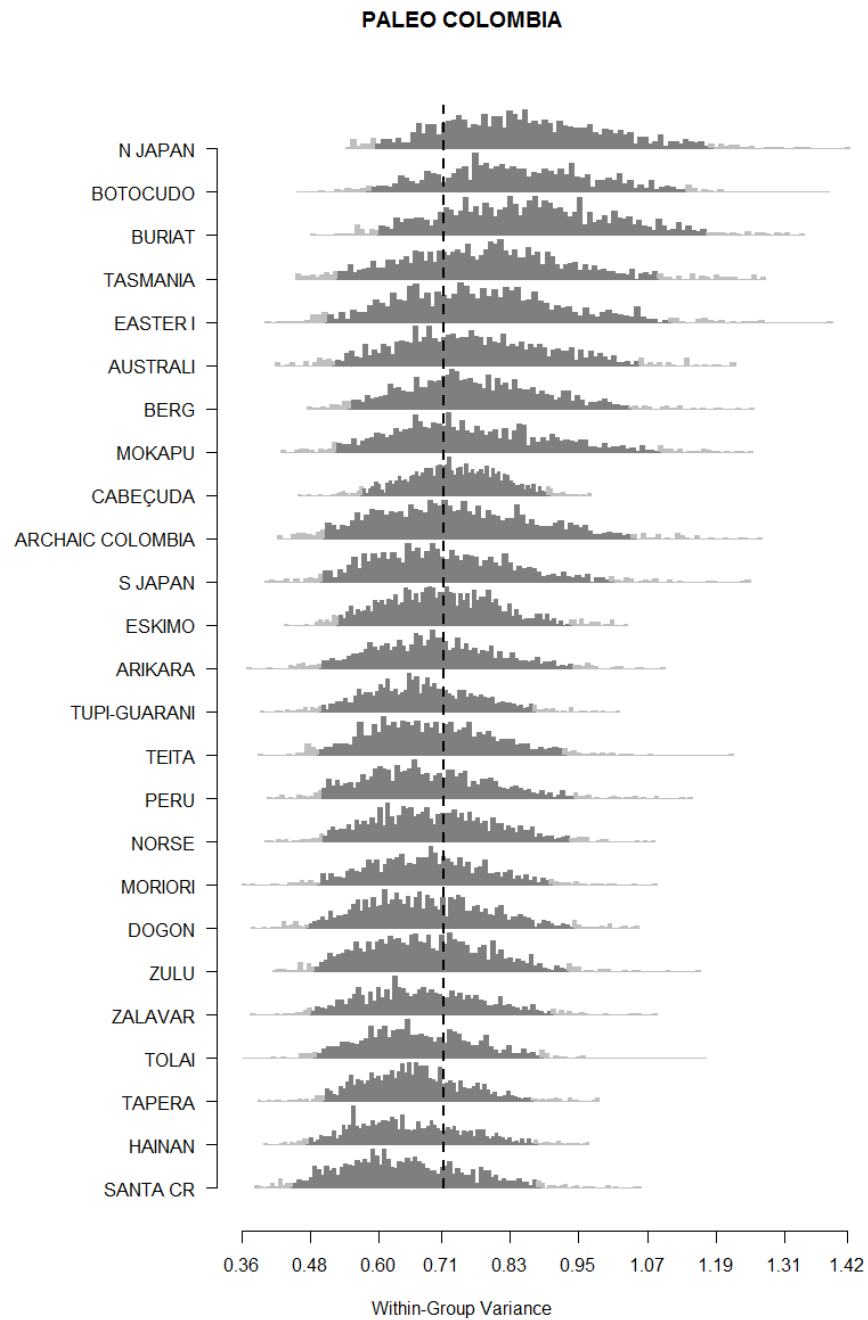
Prior to the analyses, missing values in the Paleoamerican series (see Table 2 for details) were estimated through multiple regressions, using the overall mean of the missing variables as the dependent value and the individual's remaining variables as independent variables (the reasoning behind this replacement has been covered elsewhere<sup>17</sup> and consequently we will not elaborate on it here). All analyses pooled males and females together, to maximize sample sizes of the early American series. Although pooling sexes together will inflate the within group variances, this is unavoidable in this case, since a subdivision of the prehistoric series would result in very unreliable estimates of within group variances due to low sample sizes. However, the proportion of males and females in the series is roughly similar (Table 2), so sexual dimorphism should not affect the comparative results significantly. Nonetheless, the within-group variances reported here must be considered as overestimations since they include the sexual dimorphism within series. All analyses were done in R<sup>53</sup>, with functions written by MH, complemented by functions from packages MASS<sup>54</sup> and vegan<sup>55</sup>.

## RESULTS

Figures 1 and 2 show the comparison between the within group variances of Lagoa Santa and Colombia, the two early South American series included in this study, to the distributions generated from the bootstraps of the worldwide modern reference series. In both cases, the worldwide within-group variances overlap considerably, with North Japan, Botocudo and Buriat showing a slightly larger variance distribution. In the context of the reference series, both Early American groups have moderate within-group variances falling well inside the expected variance for modern human populations, with Lagoa Santa and Colombia presenting remarkably similar within group variances.



**Figure 1.** Comparison between the within-group variance of Lagoa Santa and the variance distributions generated for the reference series. The dashed line indicates the variance calculated for Lagoa Santa, and each of the grey histograms show the distribution of variances based on 1,000 random selections of 29 individuals from the reference series.



**Figure 2.** Comparison between the within-group variance of Colombia and the variance distributions generated for the reference series. The dashed line indicates the variance calculated for Colombia, and each of the grey histograms show the distribution of variances based on 1,000 random selections of 14 individuals from the reference series.

Table 3 presents the  $F_{st}$  estimates used to assess between group differences in the data.  $F_{st}$  were calculated for different combinations of the series in the study. With the exception of the Americas, all regions in the planet show  $F_{st}$  values considerably lower than the  $F_{st}$  observed among series worldwide. In the Americas, the  $F_{st}$  observed is similar (0.24) to the worldwide one (0.27). This increased differentiation between groups is not present among Early Americans, since the  $F_{st}$  for these groups (0.07) is closer to the  $F_{st}$  observed for the other regions in the planet in modern times. When Early Americans are removed,  $F_{st}$  estimate among the American series is still high (0.24), even when Eskimos are removed ( $F_{st}=0.23$ ). When only South American series are included in the analysis, the  $F_{st}$  estimate still is remarkably high (0.22), showing high levels of between group differentiation in the continent, corroborating previous studies<sup>26</sup>. These results suggest that the high population structure described for the American series in the past was not present among early groups in the continent. Figure 3 shows the MDS scatterplot representing the pairwise  $F_{st}$  matrix between series, which permits to explore how the variance apportionment worldwide is distributed in terms of morphological affinities among series. The MDS plot shows that most of the diversity seen is due to differences among regions. With the exception of the Americas and Polynesia, series within regions overlap when the bootstrap distribution is taken into account, with Australo-Melanesians showing high Affinities with Sub-Saharan Africans, Europeans overlapping with the North American series (with the exception of Eskimos), and East Asians being close to some of the Polynesian series (Mokapu and Moriori). The only truly outlier population in our analysis is Buriat, a NE Asia series that has been shown to have a peculiar cranial morphology probably due to the adaptive responses to extreme cold climates<sup>51-52</sup>. Corroborating the  $F_{st}$  values by region (Table 3), the differences in the Americas are very marked, especially when the South American series are taken into account. However, these differences are not due to the chronology of the series, since Early Americans show a great overlap among themselves and with Archaic Colombia. In other words, the differences among South American series are as high as the one seen between continents (e.g., Australo-Melanesia and East Asia) in present times.

## DISCUSSION AND CONCLUSIONS

Our results corroborate previous studies that show that South America is characterized by high levels of cranial morphological differentiation between groups<sup>14,24,26-27</sup>. However, our results also suggest that the high cranial morphological diversity seen among Late/Recent Native Americans was not present among the early American populations. Regarding within-group variability, Early Americans did present average variability, comparable to modern populations, despite the fact that these series represent more than three thousand year of human occupation in the continent. These results suggest that the morphological pattern that characterized Early American groups remained unchanged for long periods of time, which

highlight even more the importance of the differences seen among South American groups in modern times. Incidentally, these results also support the use of the early American series included here as valid units of analysis from the perspective of morphological affinities, i.e. the use of such collections as representatives of a single biological populations is valid, despite their chronological span.

When between-group diversity is considered the Early Americans'  $F_{st}$  estimate is considerably low, falling well within the range of the other continents and macro regions, (Europe, Australo-Melanesia, Sub-saharan Africa), especially when series that have shown strong adaptive responses to climate are removed (East Asia without Buriat, and North America without Eskimo). Indeed, the only two regions that show particularly high  $F_{st}$  estimates are South America and Polynesia. Polynesia is expected to show increased between-group variance apportionments due to the fact that islands have stronger natural barriers to gene-flow in the form of the ocean stretches separating them. South America, however, not only does not present the same level of natural barriers as the deep ocean islands of Polynesia, but also shows a larger proportion of the variance due to differences between groups than Polynesia. As mentioned before, South America is the only continent that has between-group differences on a similar scale as when we consider all populations worldwide. Thus, South America is particularly interesting in terms of the development of modern human cranial morphological diversity, especially given that our results suggest that the high diversity seen among Late Native South Americans was not present among early groups entering the continent, as proposed before<sup>8,10,19</sup>. In other words, we argue that the high diversity seen in South America today must have been generated after the Pleistocene/Holocene transition, long after the arrival of the first humans on the continent.

**Table 3** -  $F_{st}$  values ( $h^2 = 0.55$ ) within regions and chronological period in the study.

Region/Chronological period	$F_{st}$	SE
World	0.276	0.002
All America	0.235	0.005
Early America	0.068	0.011
Late America	0.239	0.005
Late North America	0.205	0.006
Late North America without Eskimos	0.105	0.006
Late South America	0.224	0.007
East Asia	0.170	0.005
East Asia without Buriat	0.041	0.004
Australo-Melanesia	0.096	0.005
Sub-Saharan Africa	0.089	0.005
Europe	0.058	0.004
Polynesia	0.158	0.005





**Figure 3.** Multidimensional scaling (MDS) of the pairwise  $F_{st}$  matrix between series. The solid dots represent the MDS for the  $F_{st}$  matrix of the original data, and the transparent dots represent the MDS from bootstrapped data superimposed on the original MDS with Procrustes Analysis. Series from the same region have been represented with the same colors, following the assignment given in Table 3.

Recently, Hubbe et al.<sup>17</sup> suggested, based on analyses of cranial morphological affinities, that the morphological pattern seen among Early South Americans is a retention of the morphological pattern that characterized other human groups by the end of the Pleistocene in the Old World (specifically in Europe and East Asia). Populations worldwide retained a similar morphological pattern throughout most of the modern human dispersion across the World, and fast changes occurred during the end of the Pleistocene and across the Holocene, especially in Europe and Asia<sup>56-58</sup>, and consequently changes observed in South America can then be seen as an extension of what happened in the rest of the planet. However, South America is different from the processes observed elsewhere in two aspects: first, the transition from the Paleoamerican morphology to the modern morphological variation seems to have occurred faster there than in the other continents. To date there is no evidence of changes in the overall cranial morphological pattern or in its variance before 7.5 kyr BP<sup>2-6</sup>, and our results strongly support this since both our Early American series do not show morphological change nor increase in within-sample variance despite representing over three thousand years of human occupation in each region. Second, in the regions where a strong morphological differentiation

process is observed in the Old World (Europe and East Asia), the modern populations included in our analysis do not show strong differences between group (i.e., the regional  $F_{st}$  estimates are low), while South America presents a different pattern, where there is an extreme increase of morphological differences between groups by the end of the Holocene, some of them retaining a similar morphological pattern as the early Americans (e.g. the Archaic Colombia series<sup>6</sup>, included in this study, the Pericu Indians from Baja California<sup>59</sup> and the Botocudo Indians to some extent<sup>60</sup>), some of them diverging considerably from it (e.g., Peru and the coastal shellmound series). Yet, this has to be seen with caution at the moment, since our analyses only include a few series from each of the macro regions explored here and it is possible that they are underrepresenting the local morphological diversity in these regions. Nonetheless, South America shows as much between group variance apportionment as seen worldwide and this is indicative of a strong process of morphological differentiation in the continent, even if the values observed for the reference regions are underestimated.

However, this does not contribute necessarily to our understanding of how this morphological variation originated. Elucidating the causes behind this process is a complex endeavor, since *Homo sapiens* skull seems to be less evolutionarily constrained than other mammal skulls and as such can accumulate phenotypic changes quickly under either stochastic or non-stochastic evolutionary pressures. This relative constraint release is due to the human skull, as the skull of many if not all mammals, being organized in development modules<sup>61-65</sup>, which can be defined as sets of highly intercorrelated traits that are less correlated with other such sets<sup>66-70</sup>. Modularity is a population's property represented in its additive genetic variance and covariance matrix and may enhance evolvability<sup>70</sup>, which is the capacity of a given population to evolve in the direction of any given selective pressure<sup>71</sup>, since modules allow the genetic architecture to interact with selection to produce an evolutionary response<sup>72</sup>. When compared to the modular organization of other mammals, ours is one of the most flexible to respond in the same direction of natural selection<sup>64,73</sup>, due to the fact that our modules are less strongly integrated with other modules in the skull. However this increased evolvability is not only restricted to selection. Genetic drift is also dependent on the genetic variance and covariance matrix, and it is expected that under drift the amount of traits divergence among populations will be proportional to the pattern and magnitude of variation in the ancestral population<sup>62-63,72</sup>. Among mammals, *H. sapiens* has a high number of dimensions in the morphospace defined by the genetic covariance matrix when compared to other mammals, which allows drift to accumulate morphological change in many potential directions, when in mammals the higher integration between modules restrict the directions that phenotypic change can be channeled by drift.

The high evolvability potential in the *H. sapiens* skull makes formal tests of the evolutionary forces behind the origin of morphological differentiation in South America hard to

be conducted. Hubbe et al.<sup>16</sup> attempted to test the best dispersion model to explain the diachronic morphological differences observed in the continent, and their results supported a dual dispersal model into the continent, with early American and Late American groups sharing a common ancestor in East Asia at least 25 thousand years ago. This model presented a stronger fit to the morphological differences observed than any model assuming *in situ* evolutionary processes. However, these results rested on the assumption that the rate of morphological differentiation under neutral evolutionary forces for humans was of a similar scale than to other mammals, which as detailed above is probably not true<sup>64,73</sup>. De Azevedo et al.<sup>19</sup> tried to replicate the results obtained by the previous study using different measurements and samples and also failed to find support for a dual-dispersal model, favoring instead a model of recurrent gene-flow between Asia and North America. Taken together, this information suggests that at the moment attempts to formally test the forces behind the morphological diversity in the continent are not possible, especially given the limited samples available to date.

Nonetheless, the seemingly unique process of morphological differentiation observed in South America when compared to other regions of the world, and the fact that such high morphological diversity was not present among early South American populations, must have been a result of two distinct (and complementary) processes, namely a strong *in situ* microevolutionary process (by random and non-random forces<sup>10,14</sup>) or the migration of populations carrying new morphological diversity into the continent after its initial settlement<sup>6,8-9,16,19</sup>. While it is hard to test the relative contribution of each of these processes in the shaping of morphological variation in the continent, we argue that *in situ* processes can be tentatively excluded as a strong component of the morphological differentiation in South America and that the entrance of extra-continental morphological diversity (either through discrete dispersals or recurrent gene-flow) is more parsimonious given our current knowledge on modern human morphological variation.

Morphological changes associated to adaptive responses to climate<sup>51-52</sup> (see also Table 3) and life-style change<sup>14,74</sup> have been described among modern humans, although these seem to be localized to specific anatomical regions or restricted to populations inhabiting extremely cold environments. However, South America does not present the extreme climatic range to explain the morphological diversity seen in the continent as a result to adaptation to cold climate, and although some of the changes observed in the continent are correlated with the adoption of agriculture<sup>14,74</sup>, contradictory evidence in this regards exist when the continent is seen as a whole. In Brazil for example<sup>60</sup> the shellmound populations show strong departure from the morphological pattern that characterizes Early Americans (see also Figure 3), despite maintaining a fisher-hunter-gatherer life style.

Neutral evolutionary processes resulting from genetic drift and strong and long-lasting gene-flow barriers in the continent are also hard to sustain at this moment to explain the origin of the morphological diversity in the continent. First, the time for such amount of changes to be developed in South America seems to be too short, even assuming the highly evolvable nature of the human skull. Since our results suggest Early Americans did not present uniquely high within-group variances and very low differentiation between regions and across time, that implies that during the Holocene a similar degree of morphological differentiation between groups appeared as the one that occurred between continents worldwide in a much longer period of time (see  $F_{st}$  estimates in Table 3). Second, if we assume that the Early American series used here represent the variability of the groups who entered the continent and that late American groups descend directly from them, resulting from multiple founder effects associated with range expansions from the mother populations (Early Americans), a general loss of variability would be expected, similar to what is seen associated with distance from Africa<sup>32-33,75-76</sup>. Although our study is limited to a few American series, our results do not favor a loss of variability between early and late Native American groups, but rather the opposite.

Thus, unless new evidence appears in the future refuting our current understanding of how modern human cranial morphological diversity evolved, it is hard to defend exclusively local processes as responsible for the unique level of morphological differentiation seen between groups in South America. Therefore, our results would favor a scenario where additional diversity arrived in the continent after its first occupation, either through discrete waves of human dispersion into the continent<sup>9,16</sup> or through a constant or semi-constant gene-flow with outside regions<sup>8,19</sup> (see also Ray et al.<sup>77</sup> for molecular data suggesting a similar scenario). Evidently, our results at present are limited by the few recent American samples available in this study and the formal testing of this hypothesis will demand the inclusion of more South American series in the future.

Although the notion of external diversity influx into the continent during the Holocene has not found support in most of the molecular studies concerning Native American biological diversity conducted in the past decade<sup>30, 78-80</sup> (but see Reich et al.<sup>81</sup> for a more complex scenario), recent studies based on rare alleles have suggested that a single dispersion wave might not be enough to explain their presence in the continent<sup>82-83</sup>. As such, the molecular data available to date does not eliminate the possibility of external diversity influx into the continent during the Holocene.

In conclusion, the results presented here contribute to our growing knowledge about the origins of the biological diversity of Native American groups during the Holocene, by showing significant differences in the apportionment of variation across time in the continent. Under this scenario, the biological diversity that characterizes New World populations originated only during the Holocene, much later than the initial human occupation of the New World, and most

probably required the entrance of extra morphological diversity from regions outside the continent (Northeast Asia).

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## Appendix II

### **The cranial morphology of the Botocudo Indians, Brazil.**

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## ABSTRACT

The Botocudo Indians were hunter-gatherers groups that occupied the East-Central regions of Brazil and went extinct during the colonial period in the country. During the 19<sup>th</sup> century craniometric studies suggested that the Botocudo resembled more the Paleoamerican population of Lagoa Santa than typical Native Americans groups. These results suggest 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 share a similar morphological pattern when compared to the other Botocudo Indians in the series; 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. While 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 of ancestral Late Pleistocene populations from Asia.

The way craniometric variability is structured over time in the New World has been the focus of intensive analysis in the last twenty years. Neves and collaborators 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), and in the interior of Rio Grande do Sul (Neves et al., 2004 b), 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 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 morphologic pattern even later in time, by documenting its presence among the Pericú group from Baja California, Mexico, which were extinct by the 19<sup>th</sup> 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, towards a majority of groups sharing the Amerindian pattern by the end of the Holocene was more complex than first envisioned.

In Brazil, late 19<sup>th</sup> 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 19<sup>th</sup> century, Lacerda and Peixoto (1876) described the Botocudo Indians as belonging to the Lagoa Santa “race”. Subsequently, other scholars such as José Imbelloni (1938), Paul 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; Sapfo 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 (Sapfo 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 19<sup>th</sup> 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 (Sapfo et al., 2014). The second hypothesis 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 Sapfo et al. (2014) show no evidence of African admixture. In addition, no other evidence of more common African slave mtDNA sequences are 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, here, 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**

Botocudo is a Portuguese term established during the 18<sup>th</sup> century to refer to a series of different native groups such as the Krenak, Naknenuk, Nakrehé, Etwet and Takruk-krak 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 wore 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 19<sup>th</sup> century. The Botocudo skulls analyzed here come mostly from the margins of the Rio Doce and from the Babylonia Cave (Lacerda e Peixoto, 1876; Table 1). Some of the Rio Doce individuals come from unknown locality 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 19<sup>th</sup> 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 16<sup>th</sup> and 17<sup>th</sup> Century (Lacerda and Peixoto, 1876). By the 19<sup>th</sup> century the Rio Doce region was already a

well explored area of Brazil with an expressive presence of non-natives. Between 1800 and 1850 approximate 73 villages and 87 military outposts were established in the area (Paraíso, 2002).

Ethnographic information on Botocudo come mainly from 19<sup>th</sup> century travelers such as Briard (1986, first edition from 1855), Rugendas (1979, first edition from 1928), Expilly (1977, first edition from 1857), Freyreys (1900, first edition from 1814), Jomarb (1847), Mawe (1944, first edition from 1810), Wied-Neuwied (1958, first edition from 1815-1817), 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 semi-nomadic 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 inter-group disputes. The Botocudo had few non-portable 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 post-marital 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 horticulturists, and represented the most widespread ethnic group within Brazilian territory at the time of European conquest (Brochado, 1989; Noelli, 1998).



**Table 1** – Information on the Botocudo specimens that comprise the series used in this study.

ID	Sex	Details <sup>1</sup>
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 – Espirito Santo, Brazil.
MN-006	M	Skull of Botocudo Indian, with mandible. S.Matheus - Espirito Santo, Brazil.
MN-007	F	Skull – Botocudo Indian Poaia e Mutum Village – Espirito Santo, Brazil.
MN-008	M	Skull – Botocudo Indian. Rio Doce – Minas Gerais, Brazil.
MN-009	F	Skull – Poxixa Indian, with mandible. Mucuri River – Espirito Santo, Brazil.
MN-011	F	Skull – Botocudo Indian, with mandible. Rio Doce – Minas Gerais, Brazil.
MN-012	F	Skull – Botocudo Indian. Mucuri River – Espirito Santo, Brazil.
MN-013	F	Skull – Botocudo Indian, with mandible. Mutum Village – Espirito Santo, Brazil.
MN-014	F	Skull – Botocudo Indian, with mandible. Mutum Village – Espirito 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 – Espirito Santo, Brazil.
MN-021	F	Skull – Botocudo Indian, with mandible. Mutum Village – Espirito Santo, Brazil.
MN-023	F	Skull – Poxixa Indian. Mucuri River – Espirito 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 - Espirito Santo, Brazil.
MN-053	M	Skull – Botocudo Indian, with mandible. Babylonia Cave - Fazenda de Sant'Ana
MN-055	M	Skull – with mandible. Found in cave near Itapemirim – Espirito Santo, Brazil.
MN-056	F	Skull – Botocudo Indian. Mucuri River – Espirito Santo, Brazil.
MN-062	M	Skull – Botocudo Indian, with mandible. Mucuri River – Espirito Santo, Brazil.
MN-063	F	Skull – Botocudo Indian, with mandible. S. Matheus – Espirito Santo, Brazil.
MN-064	F	Skull – Botocudo Indian, with mandible. Rio Doce - Minas Gerais, Brazil.
MN-065	M	Skull – Botocudo Indian, with mandible. Itapemirim – Espirito Santo, Brazil
MN-066	F	Skull – Botocudo Indian, with mandible. Mutum Village – Espirito 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 – Espirito Santo, Brazil.
MN-088	M	Skull – Poxixa Indian, with mandible. Mucuri River– Espirito 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.

<sup>1</sup> translated from the Catalogue of the National Museum of Rio de Janeiro by the authors.

## **MATERIALS AND METHODS**

Cranial morphology was assessed by 32 metric variables following Howells (1973, 1989) protocol (Table 2). The Botocudo material included here is comprised of 16 males and 16 females 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 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 to 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 the analyses MN-15 and MN-17 were not included in the calculation of the Linear Discriminant Functions.

**Table 2** – Craniometric measurements included in each anatomical region.

Major Anatomical Regions	Minor Anatomical Regions	Variables included*
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) Bifrontomalare 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)

\* - measurement definitions according to Howells (1973, 1989)

**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
Phillipines	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-Sahara Africa	83	Sub-recent	0.04	Howells, 1973, 1989
Dogon	Sub-Sahara Africa	99	Sub-recent	0.00	Howells, 1973, 1989
Zulu	Sub-Sahara Africa	101	Sub-recent	0.00	Howells, 1973, 1989
Bushman	Sub-Sahara 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
Moriori	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
<b>TOTAL</b>		<b>3024</b>			

The morphological affinities of the Botocudos series as whole were initially assessed based on the Mahalanobis' Distance matrix (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 non-metric Multidimensional Scaling (MDS; Cox and Cox, 2010). To explore the expected distribution of the observed distances, 100 new distance matrices were 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 to 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 ( $r_{ii}$ ) 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 inter-group 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 poses a problem, since  $F_{st}$  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 generates comparable  $F_{st}$  estimates. In this case the assumed heritability values are of minor concern, since 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).

$F_{st}$  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.  $F_{st}$  values between Paleoamericans and South American series were compared to  $F_{st}$  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  $F_{st}$  estimates were calculated as the standard deviation from the  $F_{st}$  values calculated in a thousand bootstraps of the data respecting the original sample sizes of the series.

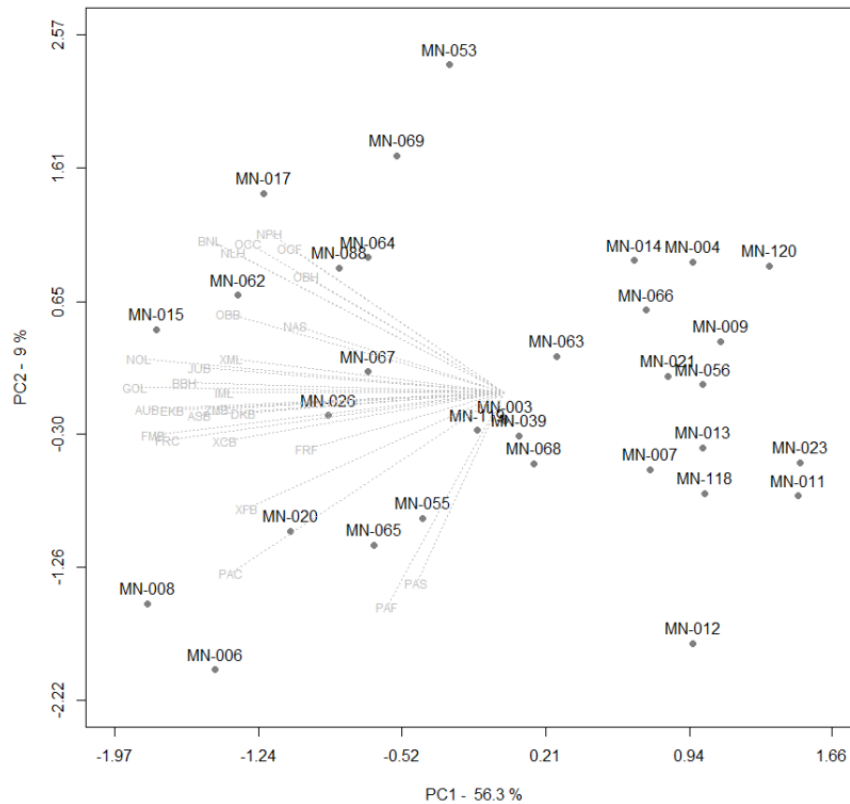
Given the modular nature of the human skull (Lieberman et al., 2000; Collard and Wood, 2007; Bastir and Rosas, 2008; Klingenberg, 2008, 2013; Mitteroecker and Bookstein, 2008; Paschetta et al., 2010), and the fact that functional anatomical regions can present distinct evolutionary trajectories and respond 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, 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). Since 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 the present 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 Polynesia in both analyses where Polynesian groups were included (Mori and Easter Island), although with relatively low 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 analysis, which is in accordance with the analyses done with the entire series (see below).



**Figure 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.

**Table 4** – Classifications and Posterior Probabilities of the Botocudo specimens based on Linear Discriminant Functions for three different datasets.

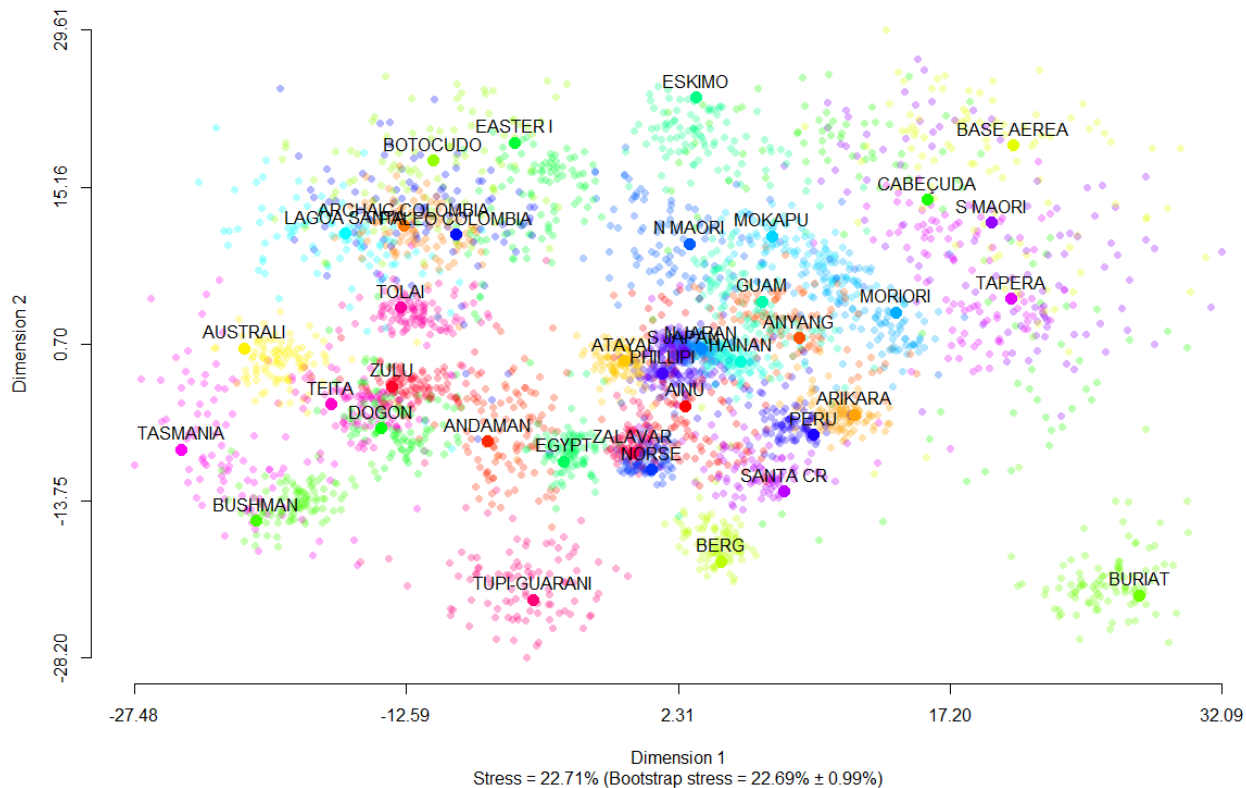
ID	All Series		American Series		American and Polynesian Series	
	Classification	Post. Prob.	Classification	Post. Prob.	Classification	Post. Prob.
MN-015	<b>Botocudo</b>	<b>0.9937</b>	<b>Archaic Colombia</b>	<b>0.5443</b>	<b>Archaic Colombia</b>	<b>0.3818</b>
MN-017	<b>Moriori</b>	<b>0.3634</b>	<b>Paleo Colombia</b>	<b>0.7512</b>	<b>Easter Island</b>	<b>0.5434</b>
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

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, Bushman) and Australo-Melanesian (Tolai, Australia, Tasmania) series and the Polynesian series of Easter Island. These results reflect well the pattern of affinities reported in previous studies (eg. 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 to 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, Santa Cruz) appear closely associated to 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, due 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.

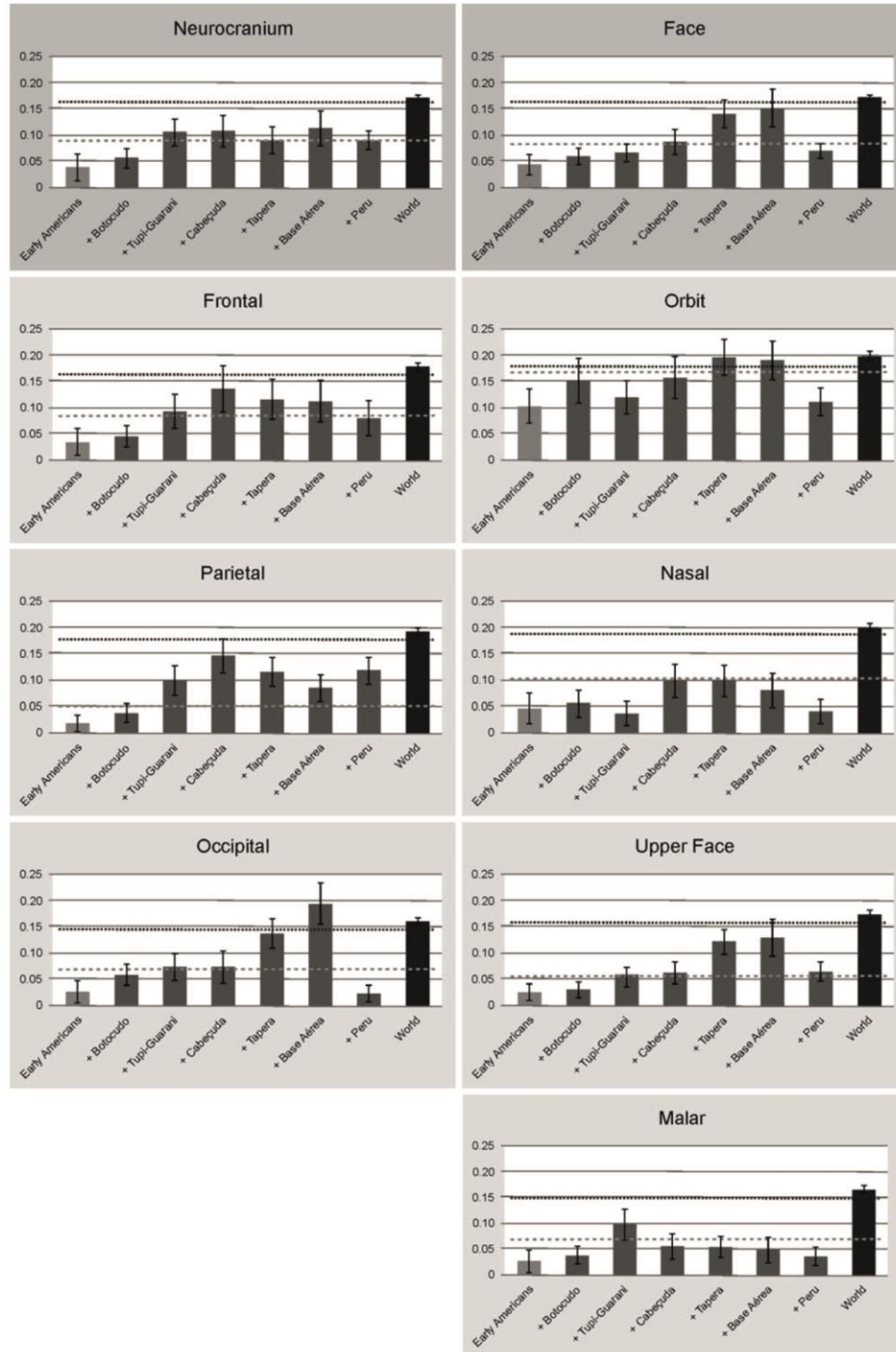
Table 5 and Figure 3 show the minimum  $F_{st}$  values calculated between early Americans and the other South American series for the two major and seven minor anatomical regions of the skull. In all anatomical regions the three early American series show low between-group variances, especially when compared to the  $F_{st}$  observed worldwide. When any of the later South American series is included in the analyses, there is an increase in the observed  $F_{st}$ , as would be expected. However only in a few cases the  $F_{st}$  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  $F_{st}$  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  $F_{st}$  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  $F_{st}$  among Paleoamericans is  $\sim 0.10$ . This is also the only region where the addition of Botocudo results in a considerable increase in minimum  $F_{st}$  values.



**Figure 2.** Morphological affinities among the series included in this study represented by non-metric 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.

## DISCUSSION AND CONCLUSIONS

Our results support the earlier 19<sup>th</sup> century observation that the Botocudo Indians present morphological 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 Figure 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).



**Figure 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 one standard deviation calculated from 1000 bootstraps of the series. The light gray dashed line represents the upper confidence interval of the Paleoamerican  $F_{st}$  (Paleoamerican  $F_{st} + 1.96 \cdot sd$ ), and the dark dashed line represent the lower 95% confidence interval of the worldwide  $F_{st}$  (World  $F_{st} - 1.96 \cdot sd$ ).

**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
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
+ 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
+ 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
+ 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
+ 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
+ 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
+ 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
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

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, since 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 as Polynesian. These results present themselves as an interesting conundrum: on the one hand, there is no strong evidence from the morphological point of view that these individuals 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 (Sapfo et al., 2014).

Given these uncertainties, we separated our discussion in 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 (non-genetic 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 due 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 sub-tropical setting, exhibiting a seasonal, low-land landscape (around 600 meters altitude), covered by a mosaic of savanna, semi-deciduous 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 (~ 2000 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). Since 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 to 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 taken 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 is associated to 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 (ie. bite force) but on the differences in the composition of the diet itself (ie. 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 (Figures 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; Figure 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 19<sup>th</sup> 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 been shown to also 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 their 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 the present study, given the differences between the coastal series and other Brazilian groups (Figures 2 and 3). Future studies will need to explore more specifically the relationship between the indigenous groups in East-Central Brazil to solve 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; Sapfo 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), 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; Sapfo 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 molecular 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; Price et al., 2004; 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. And third, Botocudo show 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 Figure 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 (Sapfo 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, since the sub-Saharan African series included in the comparative analyses (Teita, Dogon, Zulu, and Bushman; Figure 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 SE Asia by the end of the Pleistocene, when groups carrying this morphology moved northward towards NE Asia and subsequently into the Americas, and southwards towards 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) support the idea of a strong morphological change in Asia. Evidence of two morphological patterns associated to 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 SE 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 lack 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 (Sapfo et al., 2014) give further support to Gonçalves et al. (2013) interpretation. On top of this evidence, to date no early Holocene skeleton that had DNA extracted show the 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, while 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. We find this an unlikely scenario, because 1) the individuals are clearly labeled and well referenced in the catalogue (See Sapfo et al., 2014, for photographs of the specimens), and 2) from a morphological point of view there is no strong evidence that these individuals differ from the remaining Botocudo specimens.

Still, it is important to bear in mind that there are indeed Polynesian crania housed in the National Museum that were acquired in the late 19<sup>th</sup> century for the “Brazilian Anthropological Exhibition” (e.g., specimen MN-111 was brought from the Chatham 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 two years before the first Botocudo arrived at the National Museum in 1874 (Sapfo 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; Sapfo 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 are 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 their last common ancestor with Polynesian groups probably in SE 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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## Appendix III

### **Buried in the ashes: formation processes of an early South Americans' site (Lapa do Santo, Brazil).**

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## ABSTRACT

Few archaeological sites in the Americas contain high concentrations of human burials dating back to the early Holocene. The tropical karstic region of Lagoa Santa, in central Brazil (state of Minas Gerais) is one of the richest bioanthropological records available to study the behaviors and funerary practices of early Holocene South Americans, with more than 200 skeletons found so far. One of the key locations to examine the history of human settlement in Lagoa Santa is the site of Lapa do Santo, a rockshelter known to contain the oldest rock art and the earliest evidence of funerary complexity in the continent. In this geoarchaeological investigation we focus on the early Holocene settlement at Lapa do Santo (7.9 – 12.7 cal kyBP) applying high-resolution geoarchaeological techniques, such as micromorphology, organic petrology and  $\mu$ FTIR, on both archaeological, modern reference and experimental samples. This is the first time that a micro-contextual approach integrated with experimental geoarchaeology has been applied to study the formation of rockshelter deposits in a tropical setting. Our results show that the stratigraphic sequence formed under the dual influence of anthropogenic sedimentation—through continuous combustion activities—and geogenic sedimentation in the form of oxisol aggregates which fell from above the limestone cliff into the rockshelter. Intact hearths and remobilized combustion debris, possibly hearth rake-out, are close to the graves suggesting repeated burning activities as part of the ritual behavior of early Holocene South Americans. Large amounts of ash are intermixed with heated and unheated oxisol aggregates. Heated termite mound fragments were also found mixed within the sediments. Post-depositional alteration of the site includes limited bioturbation and localized, low energy surface water and sub-surface concentrations of moisture, leading to precipitation of dense, secondary carbonates. The age inversions can be attributed to the human action of reworking the ashy sediments and not to post-abandonment processes. Despite this, the overall preservation of the sediments is good and most human burials can be considered to be in primary context.

The karstic region of Lagoa Santa in east-central Brazil (state of Minas Gerais) is an important location to understand the activities and behaviors of early Holocene populations in South America. An astonishing amount of well-preserved human remains were found, the oldest being the female skeleton of “Luzia” stratigraphically dated ca. 11.3- 15.1 cal kyBP (Araujo et al., 2012; Feathers et al., 2010; Neves and Hubbe, 2005; Neves et al., 1999). Peter Lund, a Danish naturalist, conducted the first research in the area in the 19<sup>th</sup> century. Lund’s observations on the skeletal remains led to bioanthropological research focused on the study of a skeletal/cranial type named “Paleoamerican”. Morphologically distinct from most archaeological and present-day Native Americans, Paleoamericans are characterized by a morphological affinity with modern populations from Africa and the South Pacific (Neves et al., 1999, 2003, 2004, 2007; Powell and Neves, 1999; Neves and Hubbe, 2005; Hubbe et al., 2011).

Most of the 200 skeletons recovered from the region date to the early Holocene, when systematic burial inside rockshelters first appears. The burial practices of early Holocene South Americans from Lagoa Santa were traditionally thought to be simple and homogeneous, involving primary deposition of flexed bodies in shallow graves (Araujo et al., 2012; Neves and Hubbe, 2005; Neves et al., 2012, 2002; Prous and Fogaça, 1999). Recent studies demonstrate low mobility among these groups (Da-Gloria, 2012) and novel funerary practices since early Holocene times, including complex rituals involving the *perimortem* reduction of the dead body (Neves et al., 2002; Strauss, 2010, 2016; Strauss et al., 2015).

The settlement of Lagoa Santa dates from the Paleoindian to Archaic periods (Araujo et al., 2012, 2008; Feathers et al., 2010; Laming-Emperaire, 1979; Neves et al., 2004, 1999; Prous and Fogaça, 1999; Prous, 1992). The stone tool technology typically consists of small flakes and informal tools made of local quartz and quartzite, with rare bone tools and bifacial points (Araujo and Pugliese, 2009; Araujo et al., 2012; Moreno de Souza, 2015; Pugliese 2008). Despite the technological differences between Lagoa Santa and other sites in central Brazil (Bueno, 2005; Bueno et al., 2013a, 2013b; Dias, 2004; Kipnis, 1998; Prous and Fogaça, 1999; Prous, 1992), the subsistence in the earliest settlements is persistently characterized by broad-spectrum strategies, including plants (roots and fruits) and small and medium-sized game (Bissaro Jr., 2008; Bueno et al., 2013a; Dias, 2004; Hermenegildo, 2009; Kipnis, 1998; Strauss et al., in press).

Adding to the long history of archaeological and bioanthropological research in Lagoa Santa, geoarchaeological studies have provided essential information on local sedimentation processes inside several local caves and rockshelters (e.g. Piló et al., 2005; Araujo et al., 2008, 2013; Feathers et al., 2010; Hubbe et al., 2011).

The site of Lapa do Santo, a rockshelter with deposits dating to the Pleistocene/Holocene transition, offers one of the most complete stratigraphic sequences available to study the chronology, subsistence, funerary ritual and artistic expressions of early South Americans. The site is located in the north of the Lagoa Santa karstic region (Figure 1A), where 33 human

interments have been recovered so far. Field observations suggested that the almost 5m of powdery grey sediments could be ash from anthropogenic fires, pointing to rapid accumulation and intensive occupation of the site. However, the possible human origin for the sediments at Lapa do Santo has until now been unconfirmed. Few data and analyses exist that explicitly study the relation between the ashy sediments and the multiple human burials.

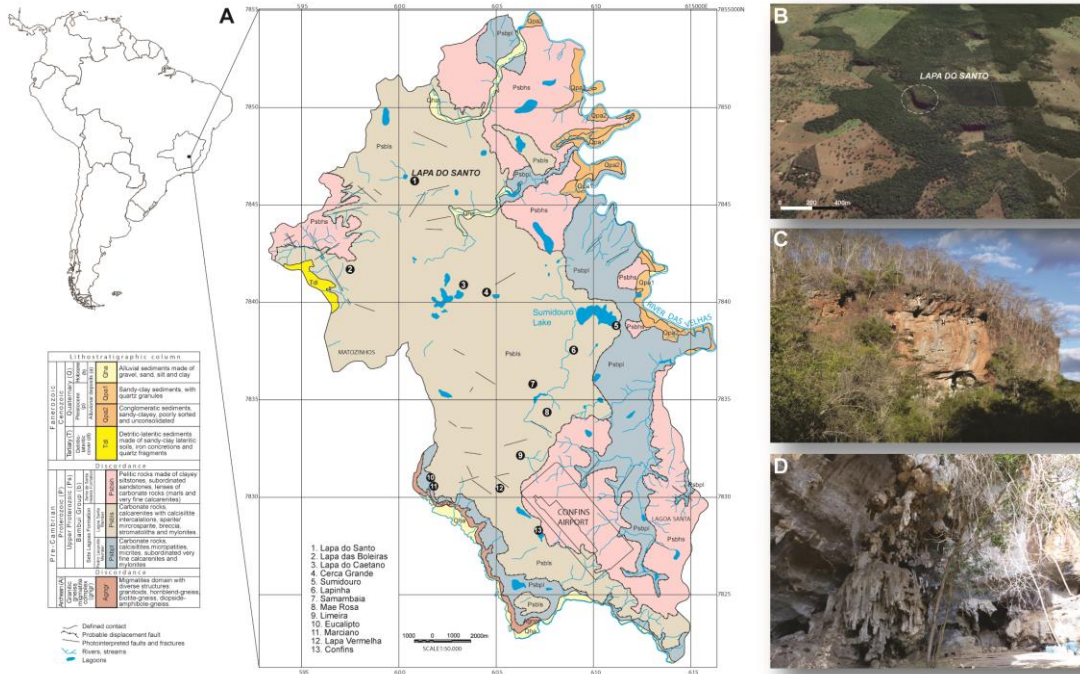
High-resolution geoarchaeological analysis, including micromorphology, FTIR analysis (Fourier Transform Infrared Spectrometry) and FTIR microspectroscopy ( $\mu$ FTIR) have proven their potential to study archaeological sites in caves and rockshelters (e.g., Berna et al., 2004, 2012; Goldberg and Sherwood, 2006; Schiegl and Conard, 2006; Karkanas and Goldberg, 2007; Goldberg et al., 2009; Mallol et al., 2009; Feathers et al., 2010; Goldberg and Berna, 2010; Wadley et al., 2011; Miller et al., 2013). Microstratigraphic analyses are especially suitable to evaluate the relative input of geogenic vs. anthropogenic sediments in archaeological deposits. The use of high-resolution techniques also allows us to identify past human activities that are only recorded in the sediments themselves.

In this paper, we focus on the early Holocene occupation of the site where most of the human remains are found. We conducted high-resolution geoarchaeological analyses with the goal of understanding the nature and mode of accumulation of the sediments that contain the interments and archaeological remains. We present data from micromorphology,  $\mu$ FTIR and organic petrology, testing the utility of a micro-contextual approach for the study of rockshelters in tropical settings. The data are combined with a complete set of reference samples of local soils and sediments and with experimental heating studies for a comprehensive interpretation of the archaeological sediments. The wide-scale sampling strategy aims at covering all the possible components involved in site formation, from natural to anthropogenic ones to investigate the occupation and ritual use of the rockshelter.

## LAPA DO SANTO AND THE KARST OF LAGOA SANTA

Lapa do Santo consists of a wide sheltered area of approximately 1300m<sup>2</sup> at the base of a ca. 30m-high limestone massif that rises from a doline valley (Figure 1A-D). The karst is formed in Upper Pre-Cambrian metasedimentary rocks of the Bambuí Group, with a basal metacalcareous body corresponding to the Sete Lagoas Formation (dated  $740 \pm 22$  Ma, Babinski et al., 2006) covered by metapellitic rocks (siltstones and claystones) of the Serra de Santa Helena Formation (IBAMA-CPRM, 1998) (Figure 1A). Dissolution of the Sete Lagoas limestone resulted in several karstic features on the landscape, such as caves and doline lakes. The soil cover is dominated by clayey, hematite-rich, red Oxisols (*latossolos* in the Brazilian Soil System) over yellow, goethite-rich oxisols developed on the Serra de Santa Helena metapellites (Araujo et al., 2013; IBAMA-CPRM, 1998; Piló, 1998). Oxisols are highly weathered soils, rich in Fe-

(hydr)oxides (e.g. hematite, goethite), Al-(hydr)oxides (e.g. gibbsite) and resistant clay minerals (e.g. kaolinite) (Embrapa, 2006; Schaetzl and Anderson, 2005; Soil Survey Staff, 2003).



**Figure 1.** Map with location of the site of Lapa do Santo in the state of Minas Gerais, central Brazil: A) Geological map of the karst of Lagoa Santa with location of Lapa do Santo and other archaeological sites in rock shelters (geological map adapted from IBAMA-CPRM, 1998); B) Satellite image of the limestone massif where Lapa do Santo is located (image from GoogleEarth); C) Photograph of the limestone wall above the site; D) Photograph inside the rock shelter.

The modern climate is tropical semi-humid, with mean annual temperature of 22<sup>o</sup> C, rainy summers and dry winters. The vegetation cover is dominated by *Cerrado* (woody savanna) and semi-deciduous forests, although in most flat areas the natural vegetation has been replaced by crops and pastures (CPRM, 2010).

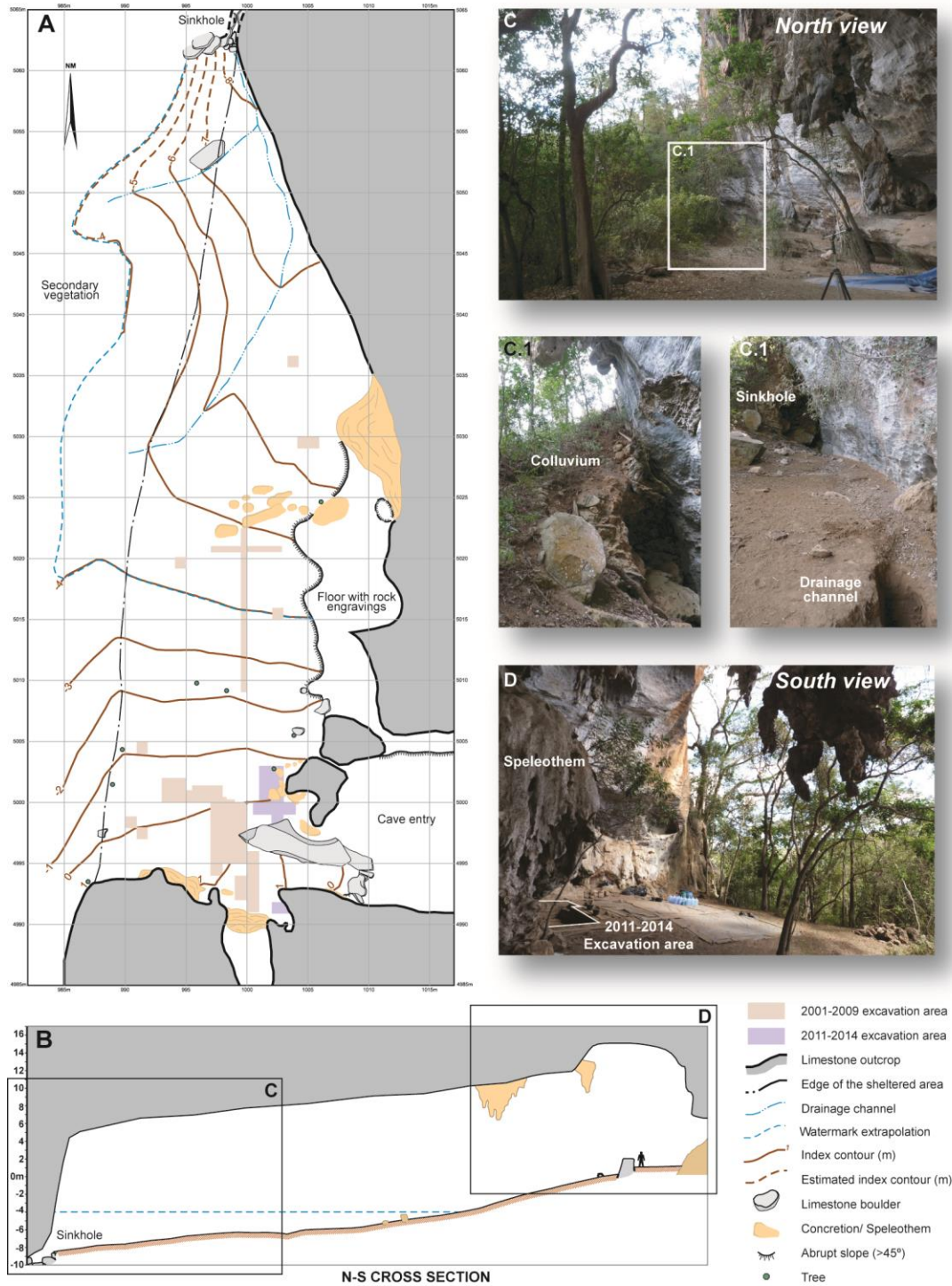
Lapa do Santo was excavated between 2001 and 2009 as part of a project entitled “Origins and microevolution of man in America: a paleoanthropological approach” coordinated by Prof. Walter Neves (FAPESP 99/0670-7). The site is currently protected and no visitors are allowed. The work exposed an area of 30.5 m<sup>2</sup> in the southern portion of the site containing 26 well-preserved human burials (Figure 2A). A total of 52 radiocarbon ages were determined on charcoal fragments and ten on human bones (Araujo et al., 2012; Strauss et al., in press). The radiocarbon ages cluster in three distinct and non-overlapping chronological periods

corresponding to the early, mid and late Holocene: 1) 7.9 – 12.7 cal kyBP; 2) 3.9 – 5.4 cal kyBP; 3) 0.0 – 2.1 cal kyBP (intervals of 95.4 %) (Strauss et al., in press).

The human burials are dated between ca. 8.2-10.6 cal kyBP and are stratigraphically restricted to the uppermost 1 m of sediment. They include primary and secondary burials, some of them with evidence for decapitation, defleshing and tooth removal (Strauss 2010; 2016; Strauss et al., 2015, in press). A 30 x 20cm petroglyph consisting of an anthropomorphic figure occurs at the base of the sedimentary succession, reaching 4m below the surface. The petroglyph was dated by radiocarbon and OSL between ca. 9200 and 12.500 BP, making it the oldest evidence of rock art in the Americas (Neves et al., 2012).

Excavations at the site resumed in 2011 through the project “The mortuary rituals of the first Americans”, coordinated by André Strauss (Department of Human Evolution, MPI-EVA). Two new excavations were opened: a main area of 10,5m<sup>2</sup> to the east of the 2001-2009 excavation and a second area of 2m<sup>2</sup> (Figure 2A and D, Figure 4A). During three field seasons (2011, 2012 and 2014) seven human burials were exhumed from the main area (Figure 4A). The chronology of the archaeological record in the areas of the new excavation is based on 14 radiocarbon ages, 13 of which are between 9.4 – 10.1 cal kyBP (Figure 4B) (see supplementary material online 1 for the complete list of dates). An understanding of the sedimentary dynamics inside the rockshelter is essential for reconstructing the formation processes of Lapa do Santo. The topography of the sheltered area shows a south-north slope of approximately 10 degrees that dips into a sinkhole (Figure 2A-C). Watermarks on the limestone wall about 4m above the modern surface suggest an area of intermittently ponded water (see Figure 2A and B). A colluvial fan in the northwest part of the site dips into the sinkhole and consists of soil material derived from the oxisol on top of the limestone (Figure 2C.1). The rockshelter shows an east-west slope of approximately 45 degrees near the edges that flattens to the east.

Most excavations are located to the south of the rockshelter, on its highest part. Neither colluvial fans, nor chimneys were observed in this location (Figure 2D). About 6m south of the 2011-2014 excavation, an outcrop of reddish, loamy breccia consisting of limestone fragments fills a dissolution pipe (Figure 3D and F). Large speleothems occur over the excavation area and several speleothem fragments have fallen to the ground and are now contained within the archaeological sediments.



**Figure 2.** Topography and photographs of Lapa do Santo rockshelter: A) Topography of the site with location of the two main excavation areas; B) Schematic cross-section of the rock shelter from north to south; C) North view of the rock shelter with detail of the red colluvium and sinkhole (C.1); D) South view of the rock shelter with indication of the 2011-2014 excavation area.

## MATERIALS AND METHODS

In this work we focus on the 2011-2014 excavation area, using a micro-contextual approach (Goldberg and Berna, 2010), integrating micromorphology,  $\mu$ FTIR and organic petrology to better understand the sediments in Lapa do Santo and study the context of the archaeological findings (artifacts and human interments). Micromorphology is the study of intact blocks and thin sections of sediment and soil, often under magnification (Courty, 2001; Courty et al., 1989). This approach allows us to identify the components of deposits and soils, and also study their spatial and stratigraphic relationship to one another.

This approach is especially useful at archaeological sites found in caves and rockshelters, where it can provide high-resolution data that can help resolve complex stratigraphic issues and formation processes (Goldberg and Arpin, 1999; Goldberg and Sherwood, 2006; Goldberg, 2000; Homsey and Capo, 2006; Karkanis, 2000; Mallol et al., 2010; Schiegl, 1996; Weiner, 2009).

In archaeological contexts, FTIR analyses have proven especially useful for the identification of diagenetic minerals and heated materials (Weiner et al., 2002; Berna et al., 2007; Mallol et al., 2009; Miller et al., 2013; Stahlschmidt et al., 2015). Recently,  $\mu$ FTIR has become an ideal, complementary technique for micromorphological studies, as it allows for the identification of minerals and heated materials directly in the thin section (Berna and Goldberg, 2007; Berna et al., 2012; Goldberg and Berna, 2010).

Organic petrology is also a useful, complementary technique to soil micromorphology. It allows not only the precise identification of burned organic material within the sediments, but also the classification of the remains according to the type of tissue, degree of burning (from slightly heated to completely carbonized), weathering and permineralization (Goldberg et al., 2009; Ligouis, 2006; Villagran et al., 2013; Stahlschmidt et al., 2015a; Stahlschmidt et al., 2015b).

For this work, two sets of samples were collected for analysis, in order to cover all the possible sedimentary agents involved in the formation of the deposit: 1) archaeological samples, taken from the excavation surfaces and stratigraphic profiles. 2) Reference and experimental samples from local sediments, soils, termite mounds and an experimental hearth to identify the effects of surface heating on tropical oxisols.

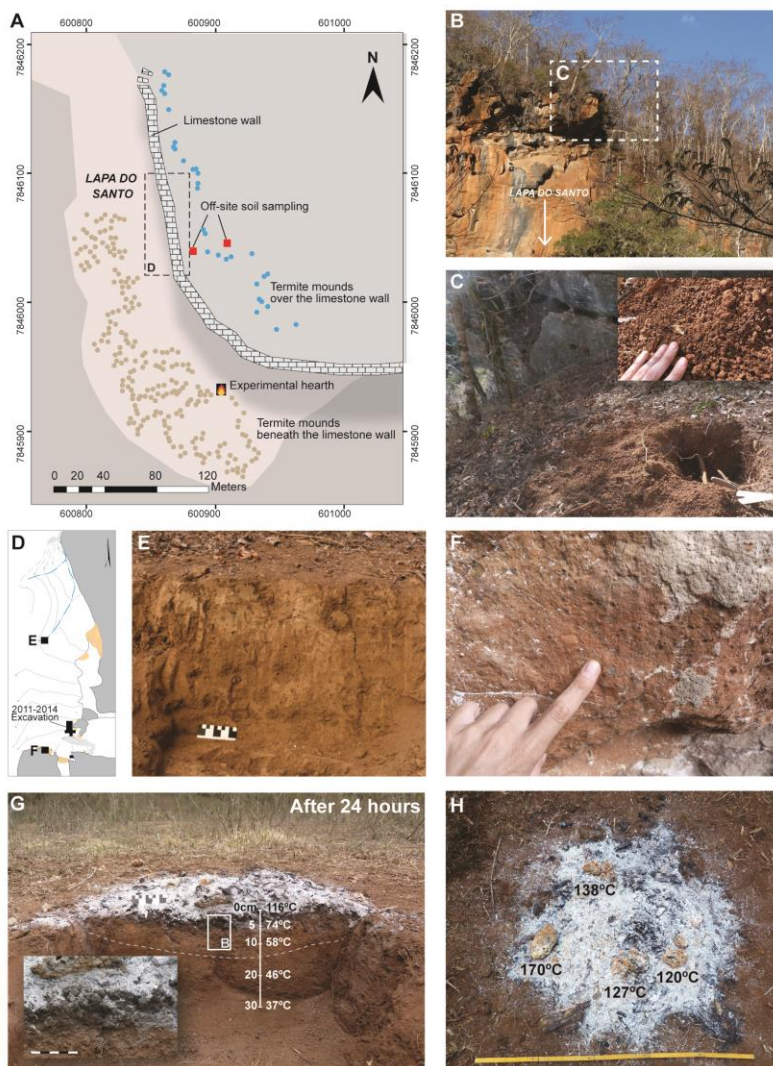
### *Reference and experimental sampling*

To assess the input of local geogenic sediments and soils, bulk samples and undisturbed blocks for micromorphological and  $\mu$ FTIR analysis were collected from: 1) the red oxisol developed over the limestone outcrop (Figure 3A-C); 2) the colluvial deposits in the northern portion of the site (Figure 3D-E); 3) the reddish breccia south of the 2011-2014 excavation area (Figure 3D and F).

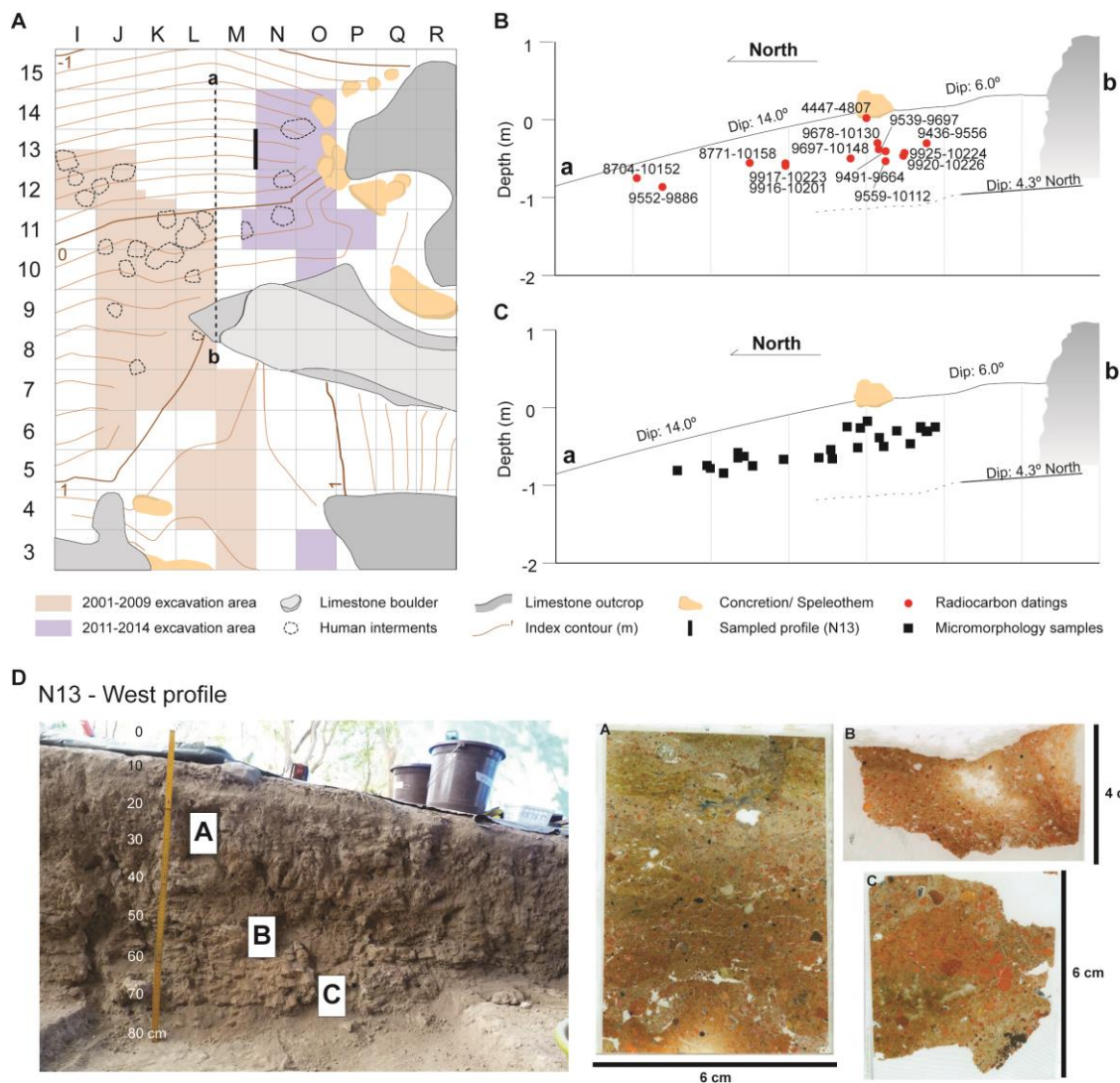
To serve as reference for identifying the thermal alteration of local clay, red oxisol aggregates were heated in a porcelain crucible for 4 hours using a muffle furnace at



temperatures of 200°C, 300°C, 400°C, 500°C, 600°C, 700°C, 800°C, 900°C and 1000° C, following the protocol of Berna et al. (2007). The heated aggregates were impregnated, cut into 30µm-thick thin sections and analyzed with a petrographic microscope and µFTIR following Miller et al. (in prep.).



**Figure 3.** Off-site sampling for reference and experimental studies: A) Schematic view of the limestone massif from above with location of the site (D), sampling locations for the red oxisol, the experimental hearth and the termite mounds; B) View of the topmost part of the limestone wall above the site, where the oxisol samples were collected; C) Photograph of the trench dug to collect the oxisol sample, with details of characteristic granular structure; D) Topography of the rock shelter with indication of the locations where samples from the colluvium (E) and red breccia (F) were collected; E) Detail of the colluvium deposits north of the site; F) Detail of the red breccia south of the 2011-2014 excavation; G) Experimental hearth lit on red oxisol substrate, with indication of the temperature of the substrate at various depths; H) The experimental hearth with the heated termite mound fragments and their temperatures after the fire extinguished.



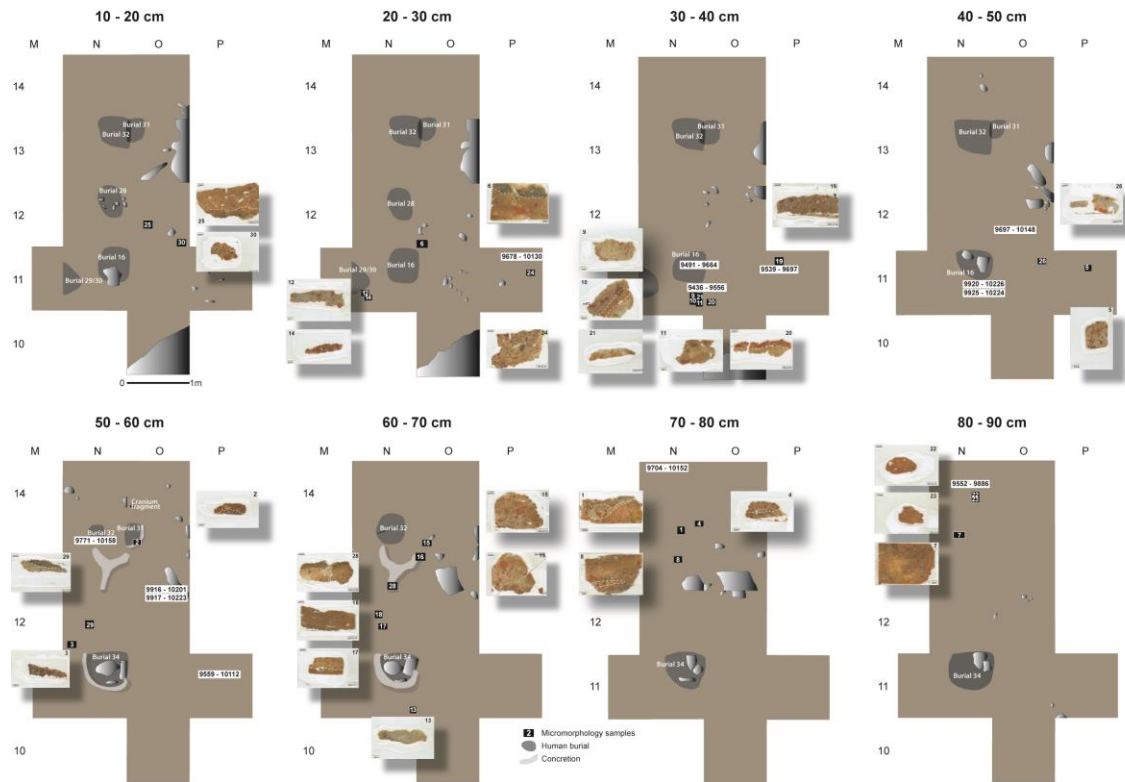
**Figure 4.** Sampling for micromorphology and radiocarbon dating: A) Close-up of the topography with the two main excavations at Lapa do Santo (2001-2009 and 2011-2014) showing the location of human burials; B) Cross section of the 2011-2014 excavation with vertical location of samples for radiocarbon dating (age intervals of 95,4% in cal yrsBP); C) Same as B with vertical location of micromorphology samples; D) Profile sampled for micromorphology with location of sampling boxes and scanned view of the thin sections.

Another set of samples was taken from an experimental hearth lit over the red oxisol, about 80m southeast of the site (Figure 3A and G). The experiment was made to observe the properties of thermally altered oxisols and the extremely abundant termite mounds in simulated field conditions. Logs, branches and dry grasses from the local vegetation were used as fuel, as well as nuts from palm trees and fragments of termite mounds (Figure 3A). The fire was kept for 2 hours and the temperature of the flames, the ground surface and the substrate (ca. 2-5 cm below the surface) was measured using a thermocouple and a digital thermometer.

The temperature of the remaining ashes and the substrate was measured for 2 hours after the fire was extinguished and 24 hours later during sampling (Figure 3G). The temperature inside the termite mound fragments was measured during the fire and after it extinguished (Figure 3H).

*Archaeological sampling*

Undisturbed blocks of sediment were taken at depths between 10 and 90 cm (Figures 4C and 5). Sample collection focused on sedimentary changes seen in the excavation surfaces, as opposed to profile sampling (n=30). This was done to identify potential differences in sedimentation or weathering related with activity areas and/or taphonomic processes, respectively. However, the horizontal collection indirectly favored sampling of compacted layers over friable sediments. Therefore, another three undisturbed blocks were collected from an excavation profile to serve as stratigraphic reference (Figure 4D). Figure 5 shows the location of the micromorphology samples at different depths.



**Figure 5.** Schematic view of the excavation surface at the different depths where micromorphology samples were collected, including scanned view of the thin sections and location of the radiocarbon datings (age intervals of 95.4% in cal yrsBP).

*Analysis – Micromorphology, organic petrology and  $\mu$ FTIR*

All micromorphology blocks were oven-dried and impregnated with a mixture of polyester resin (Viscovoss N5), diluent (Styrene) and catalyst (MKEP). Thin sections of  $9 \times 6$  cm and 30  $\mu$ m thick were made out of the impregnated blocks and analyzed with a Stemi 2000-C stereomicroscope and Zeiss Axio Imager A2 petrographic microscope under plane polarized light (PPL), cross-polarized light (XPL) and fluorescent light (blue light) at magnifications ranging from x8.0 to x500. Micromorphological descriptions followed the guidelines of Stoops (2003).

All the thin sections from Lapa do Santo were used for  $\mu$ FTIR analyses. Analyses were done with a Cary 610 Series FTIR microscope with Resolutions Pro software (Agilent Technologies). Spectra were collected both in transmission (64 scans), and attenuated total reflectance using a germanium crystal (ATR – 32 scans), with wavenumbers ranging between 4000 and 400  $\text{cm}^{-1}$  at 4  $\text{cm}^{-1}$  resolution. Transmission measurements have high resolution at the hydroxyl (OH) region of clays (wavenumbers between 3550 and 3750  $\text{cm}^{-1}$ ), allowing for a more precise identification of changes in the spectra due to heating. In this region of the spectrum the glass slides do not interfere with the measurements, since amorphous silicates are transparent to the IR radiation at wavenumbers above 2500  $\text{cm}^{-1}$  (Beauvais and Bertaux, 2002). For transmission measurements, the background was collected on a portion of the slide with spectra resulting exclusively from the absorption of the glass and the resin used for impregnation. For ATR spectra background measurements were taken on air.

Of the 33 undisturbed archaeological samples nine were selected for analysis with organic petrology. Five of the impregnated blocks were dry-polished and analyzed with reflected white light (RLo) and incident ultra violet light (RVLo) under oil immersion (Taylor et al., 1998) using a Leica DMRX/MPV-SP microscope photometer (50 $\times$  to 500 $\times$  magnifications). Complementary analyses were done with PPL and XPL. Four thin sections were dry polished for the same analysis. The description and classification of organic micro-components followed the nomenclature for brown coal and coal (ICCP, 2001, 1998; Sykorová et al., 2005; Taylor et al., 1998). Reflectance measurements were done on plant tissues identified in the polished blocks and one polished thin section to determine the degree of humification and/or carbonization (Borrego et al., 2006; Guo and Bustin, 1998; Jacob, 1980; Jones et al., 1991; Schwaar et al., 1990). The random reflectance in oil (mean %Rr) of the organic micro-components was measured following the procedure in Taylor et al. (1998).

## REFERENCE AND EXPERIMENTAL RESULTS

### *Local oxisol, red breccia and colluvium*

Red oxisols near Lapa do Santo are made of homogeneous, red colored (PPL), coalesced granules, with mammilated chamber and channel voids, and undifferentiated b-fabric due to

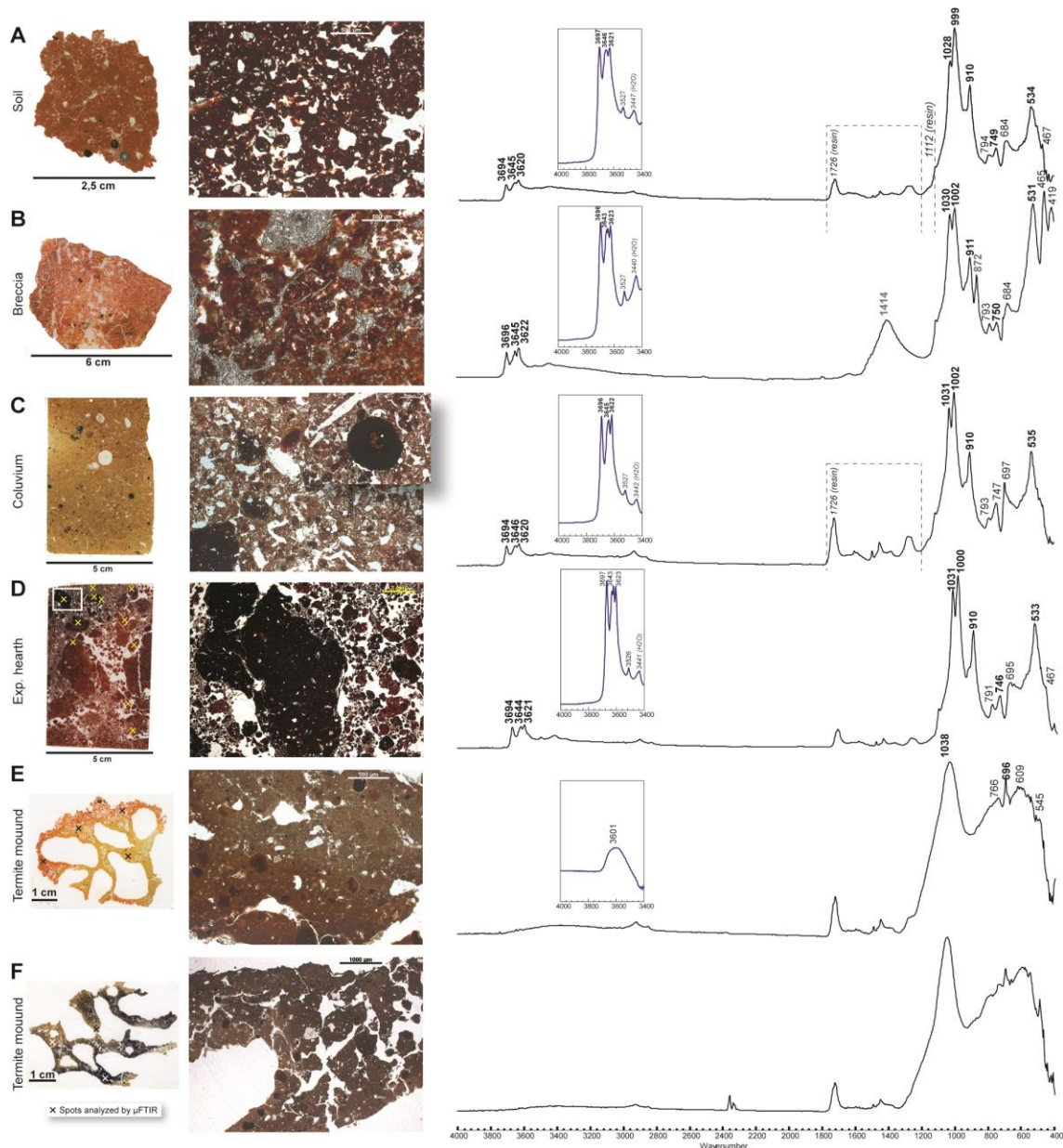
high Fe-oxide content: all typical micromorphological attributes of tropical oxisols (see Marcelino et al., 2010) (Figure 6A). Fine to very fine sand-sized grains of quartz are frequent, large Fe(hydr)-oxide nodules, rock fragments and opaque grains are rare. The ATR spectrum of the oxisol shows the presence of kaolinite in the clay fraction (absorption bands at 3694, 3645, 3620, 1028, 999, 910, 749, 534  $\text{cm}^{-1}$ ), with small amounts of quartz (794 and 684  $\text{cm}^{-1}$ ). The kaolinite bands at the OH region are also readily seen in the transmission spectrum (3697, 3646, 3621  $\text{cm}^{-1}$  and shoulder at 3666  $\text{cm}^{-1}$ ) (see Beauvais and Bertaux, 2002), together with an absorption band corresponding to gibbsite (3527  $\text{cm}^{-1}$ ) (Figure 6A). The main interference of the resin is seen between 1200 and 1800  $\text{cm}^{-1}$ , with only minor interference between 800 and 700  $\text{cm}^{-1}$ . In general, the low porosity of the material prevents the resin to completely mask the main clay peaks in the spectra, although slight shifts in the main clay peaks are sometimes observed.

The red breccia that outcrops south of the 2011-2014 excavation area shows a clayey red matrix (PPL), with undifferentiated b-fabric and dense sparite infillings.  $\mu\text{FTIR}$  analysis shows kaolinite and calcite in its composition, with small amount of quartz and gibbsite (Figure 6B). The colluvial deposit north of the excavation area has an heterogeneous composition made of speckled to cloudy clay with weak stipple-speckled b-fabric, coarse Fe-(hydr)oxide nodules, plant remains, rock fragments and charcoal fragments. Channel and chamber voids are dominant, indicating intense bioturbation. Surface crusts are frequent, suggesting reworked surfaces by colluvial transport. As in the oxisol and red breccia, kaolinite is the predominant clay mineral with some gibbsite and quartz.  $\mu\text{FTIR}$  analysis also showed that several of the Fe(hydr)-oxide nodules are goethite (Figure 6C).

#### *Heated clay from local oxisols*

A distinct change in color is the most evident macroscopic alteration of the heated oxisol aggregates (Figure 7). Kaolinite is identified in the soil aggregates heated from 200 $^{\circ}$  C to 500 $^{\circ}$  C, both in the ATR and transmission spectra, at the main Si-O-Si peak ( $\sim$ 1030  $\text{cm}^{-1}$ ) and the OH region, respectively (Figure 7). The interference of the resin is mainly seen in the absorption band at 1045  $\text{cm}^{-1}$  in the sample heated at 200 $^{\circ}$ C, and in the broad peak at 1038  $\text{cm}^{-1}$  for the sample heated at 300 $^{\circ}$  C; both caused by a main resin peak at  $\sim$ 1066  $\text{cm}^{-1}$ . The spectra for the aggregates heated above 600 $^{\circ}$ C are consistent with previous FTIR studies on heated kaolinite (Berna et al., 2007; Friesem et al., 2013; Karkanis and Koumouzelis, 2004; Shoval and Beck, 2005; Shoval, 1994; Shoval et al., 2011) which show temperature-induced changes in the OH region and the main Si-O-Si peak (Figure 7). Changes in the OH region are caused by the dehydroxylation of the clay minerals and loss of structurally bound water. The changes in the main Si-O-Si band are associated to the destruction of the clay minerals, formation of an amorphous phase (named metakaolinite) and crystallization of new minerals at higher

temperatures (Berna et al., 2007; Friesem et al., 2013; Karkanis and Koumouzelis, 2004; Shoval and Beck, 2005; Shoval, 1994; Shoval et al., 2011).



**Figure 6.** Reference and experimental data: Scanned thin sections from the red oxisol (A), red breccia (B), colluvium deposit (C), experimental hearth on oxisol substrate (D), and heated termite mound fragment with orange (E) and black (F) colors, with photomicrograph and ATR- $\mu$ FTIR spectra, including detail of transmission spectra at the hydroxyl region of clays (blue line).

The transmission spectra show a broad absorbance peak in the OH region above  $600^{\circ}\text{C}$ , which progressively decreases in intensity at higher temperatures and shifts to higher wavenumbers (Figure 7). Similar abrupt changes at  $600^{\circ}\text{C}$  are also seen in the ATR spectra at

the main Si-O-Si peak and the Al-O-H absorbance peak ( $\sim 910\text{ cm}^{-1}$ ): the Si-O-Si peak broadens and the Al-O-H peak disappears. The shift to lower wavenumbers seen at the main Si-O-Si peak is caused by interference of the resin. The shift to higher wavenumbers that characterizes heated kaolinite (see Shoval, 1994; Shoval & Beck, 2005; Shoval et al, 2011; Friesem, et al. 2013) is only seen above  $800^\circ\text{C}$ . The results of the  $\mu\text{FTIR}$  analysis of heated oxisol aggregates indicate that clear alterations of the clay due to heating (at the OH region, main Si-O-Si and Al-O-H bands) will only be identifiable after exposure to temperatures above  $500\text{-}600^\circ\text{C}$ .

#### *Hearth over red oxisol substrate and heated termite mounds*

The maximum temperature reached by the experimental fire was  $875^\circ\text{C}$ , with a mean temperature of  $\sim 650^\circ\text{C}$  in the flames. The maximum temperature in the substrate (2-5 cm deep) reached  $236^\circ\text{C}$ , with a mean of  $\sim 110^\circ\text{C}$  (see supplementary material online 2 for further information on the experimental hearth).

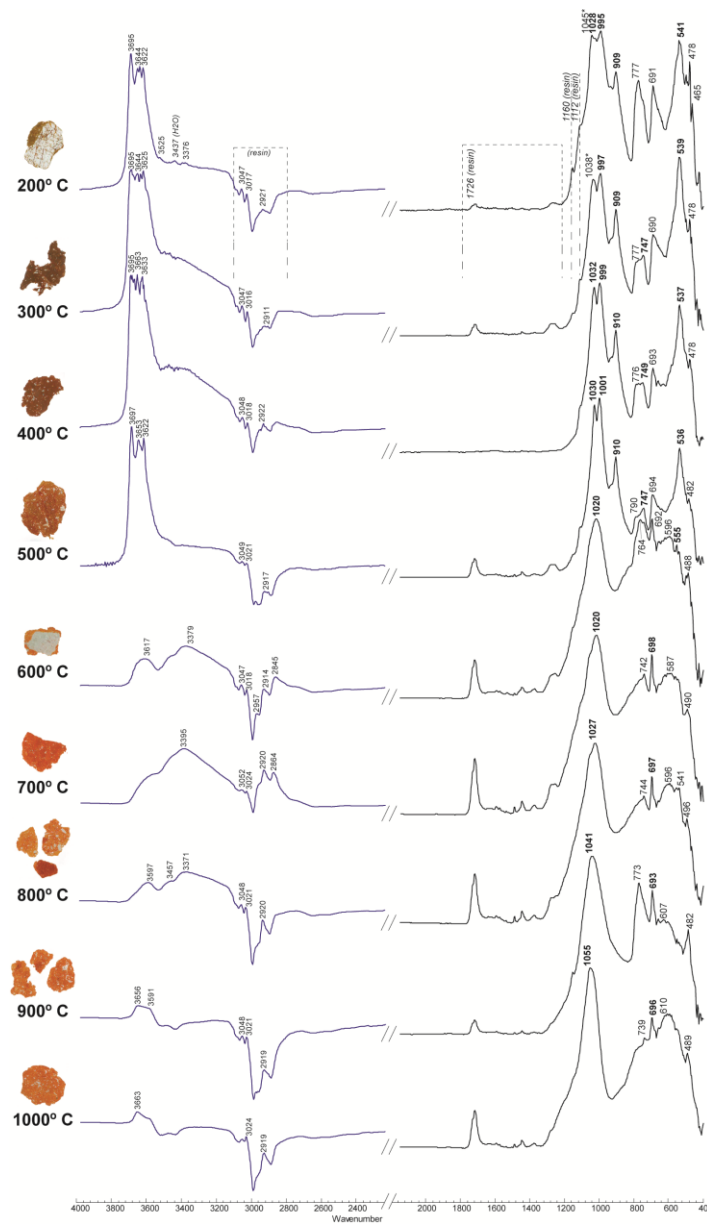
The first 5cm of heated soil beneath the ashes showed dark brown to black color with coarse granular structure, turning gradually red with increasing depth (Figure 3G). No rubefication occurred, since the soil is naturally rich in red iron oxides. Despite the color changes underneath the fire,  $\mu\text{FTIR}$  analysis shows no signs of alteration of the clays (Figure 6D). Even the clay at the topmost part of the blackened lens was not affected. This is consistent with the temperatures reached by the substrate, below the threshold of heat-induced alteration of kaolinite ( $500\text{-}600\text{ C}$ ).

The heating of termite mound fragments in the fire proved their capacity to attain high temperatures and preserve heat for long periods of time. During the experiment the temperature inside the termite mound fragments varied from  $609$  to  $430^\circ\text{C}$ , according to the proximity to the flames. After the fire extinguished, the temperature inside the fragments varied from  $120^\circ\text{C}$  to  $170^\circ\text{C}$  (Figure 3H). The next day all fragments had temperatures around  $30^\circ\text{C}$ . Most termite mound fragments turned bright orange after heating, showing a color gradient from yellow to orange when sectioned (Figure 6E). ATR- $\mu\text{FTIR}$  and transmission spectra are consistent with the transformation of kaolinite above  $600^\circ\text{C}$  (Figure 8E). Other aggregates showed a dark brown core and yellow rims, with spectra also showing heat-altered kaolinite (Figure 8F).

## ARCHAEOLOGICAL RESULTS

The stratigraphic profiles from the 2011-2014 excavation at Lapa do Santo show little variation and are mostly composed of tabular, grey, centimetric layers (5YR 6.1) of powdery carbonate-rich sediments, with common sand grains, frequent clay aggregates (20-40 %) and dispersed charcoal. Some red centimetric lenses (5YR 5.6) of indurated clay are also present, as well as black, millimetric and centimetric lenses of black silty-clay sediments. Some areas of grey, cemented sediments were discovered during excavation (see Figure 5). Besides this,

sediments are mostly loose and dusty, showing slight variations in color and texture more visible in the excavation surfaces than in the profiles.



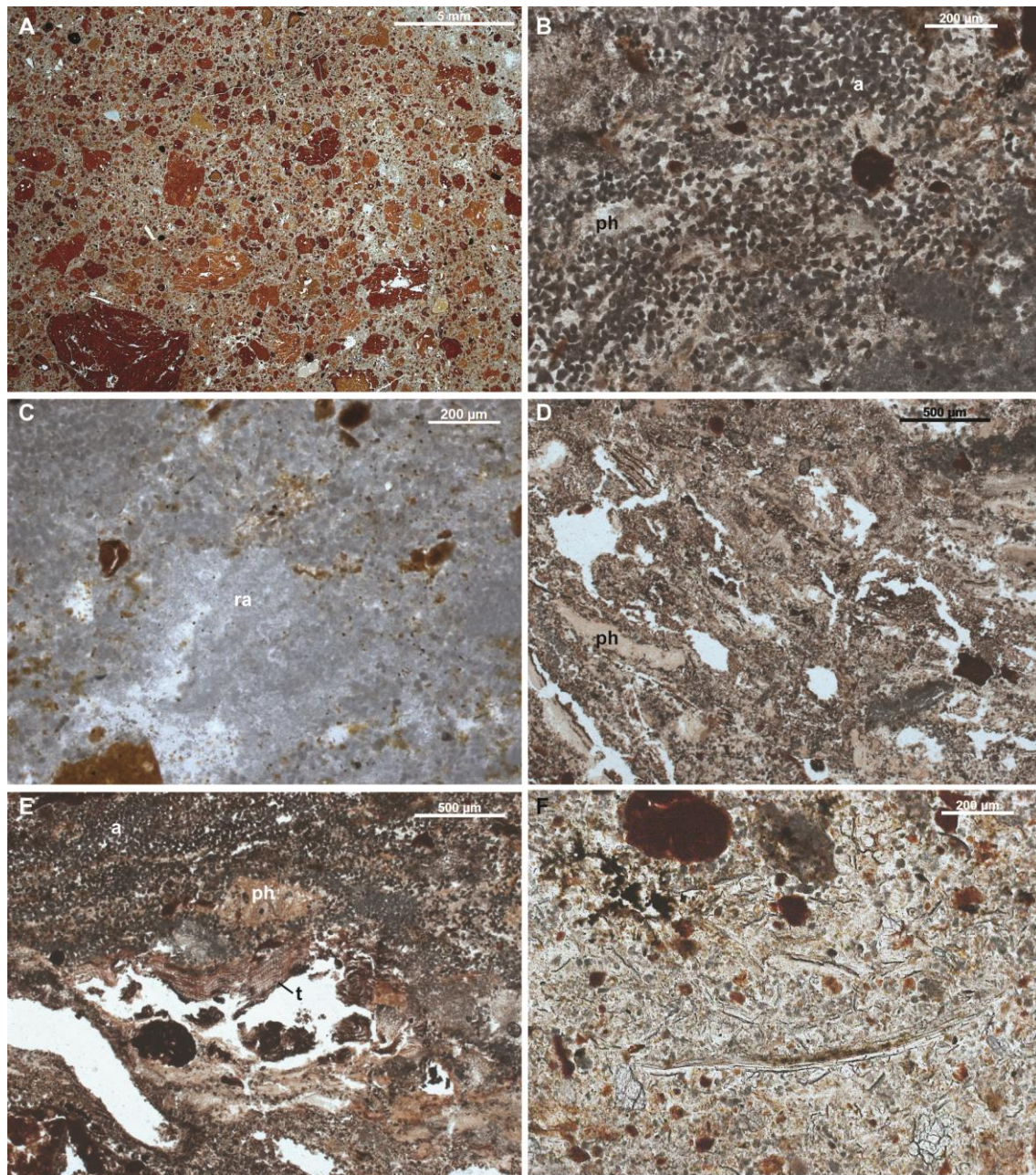
**Figure 7.** Heated oxisol aggregates from 200 to 1000° C with ATR-μFTIR spectra (black line) and transmission spectra at the hydroxyl region of clays (blue line). The peak numbers in bold are diagnostic of kaolinite. According to Beauvais and Bertraux (2002), the absence of the 3668 cm<sup>-1</sup> band and the good resolution of the 3695, 3654 and 3622 cm<sup>-1</sup> bands are indicative of small-size, poorly ordered kaolinite. As also described by Friesem et al (2013), the Si-O-Al band at ~540 cm<sup>-1</sup> shifts to 550 cm<sup>-1</sup> at 600°C. The Kaolinite band at ~1110 cm<sup>-1</sup> is masked by a resin band at 1112 cm<sup>-1</sup>. Absorption bands at 3525 and 3376 cm<sup>-1</sup> indicate gibbsite.



More than 90% of the thin sections analyzed show similarities in coarse fraction and micromass: the coarse fraction is always made of clay aggregates with random distribution and poorly sorted (sizes from 30  $\mu\text{m}$  to 1 cm); and the micromass consistently includes well preserved ash rhombs (see supplementary material online 3 for tables with micromorphological descriptions) (Figure 8A and B). Besides clay aggregates and ashes, the sediments at Lapa do Santo include minor concentrations (below 5%) of other components such as: charcoal (unsorted); limestone fragments from the cave walls; quartzite micro-artifacts (stone flakes); opaque minerals and quartz grains detached from the clay aggregates; fine bone (mostly fish bones) and shell fragments, frequently burned; articulated ashes; partially carbonized tissue; tissue residues; and loose and articulated phytoliths. The low amount of charcoal in the samples may result from a sample bias, since high quantities of charcoal were recovered during excavation.

Ashes are certainly the predominant component at the site. Random accumulations of ashes make up the fine fraction of all the analyzed samples. Ashes form when calcium oxalate crystals within the plant tissue decompose through heating. At lower temperatures (<500C) calcite forms spontaneously in solid state, whereas at higher temperatures (above 740°C), the oxalates first transform to calcium oxide (CaO) which, after cooling, can transform to calcite through re-carbonization (Regev et al., 2010; Shahack-Gross and Ayalon, 2013; Shahack-Gross et al., 2008). Under the microscope ashes frequently appear as rhombs (10-30  $\mu\text{m}$ ) of micro-crystalline calcite. Because they often preserve the original form of the oxalate druses, they are called pseudomorphs of calcite after calcium oxalates (POCC) (Brochier, 1983; Canti, 2003; Courty et al., 1989). The ashes in Lapa do Santo are generally well preserved, though areas of cemented ashes are also present (Figure 8C).

In almost all the samples the ashes are embedded in a pale yellow (PPL) undifferentiated (XPL) phosphatic micromass (Figure 8D). Phosphate concentrations and nodules are frequently associated with tissue residues, phytoliths and plant pseudo-voids (Figure 8E and F). This association indicates that phosphates could derive from the ashes and/or the decay of plant tissue (Karkanas et al., 2002; Weiner et al., 2002). Another possible source may be bat guano, a common input in caves and rockshelters (Goldberg and Nathan, 1975; Karkanas, 2000; Shahack-Gross et al., 2004). However, the overall good preservation of the ash crystals indicates low action of acidic waters, like the ones produced after passing through guano (Karkanas et al., 2002). The few bones in the sediments, combined with the pH conditions favorable for hydroxyapatite preservation (alkaline pH), preclude bone dissolution as a source for the authigenic phosphates. Though plant residues and ashes seem to be the main source of phosphates, the fact that the excavation contains at least seven human burials suggests another likely input of phosphates: the byproducts of the decomposing human bodies.



**Figure 8.** Photomicrographs of microfacies (mF) from Lapa do Santo, with phosphates and plant remains (PPL): A) Predominant microfacies (mF I) with clay aggregates in between ashy micromass, sample A from profile; B) Detailed view of well-preserved ash crystals (a) in between a phosphatic matrix (ph), sample 21; C) Recrystallized ash crystals (ra), sample 1; D) Ash crystals, dense phosphates, tissue residues and chamber voids, sample 21; E) Phosphatic nodule (ph) in association with a plant tissue fragment (t) partially removed during sectioning, in an ashy matrix (a), sample 21; F) Articulated and dispersed phytoliths in between ashes and phosphates, sample A.

Besides ashes, clay aggregates are the most conspicuous and frequent component in the sediments. The aggregates are mostly blocky, varying from angular to sub-rounded, with undifferentiated b-fabrics (XPL) due to high iron content. They show four distinct colors in PPL: red (Figure 9A), orange (Figure 9B), yellow (Figure 9C) and, less frequently, dark brown (Figure 9D). All clay aggregates show coalesced granular to massive microstructures, with star-shaped voids, chamber voids and fissures. Some aggregates contain limpid and laminated clay coatings and infillings suggesting they derive from a Bt horizon of a soil outside the shelter. The yellow clay aggregates have weak interference color and massive or striated microstructures and sometimes show a color gradient from yellow to bright orange (Figure 9G) or from dark brown to yellow (Figure 9H); Other yellow aggregates have a distinctive 100-200  $\mu\text{m}$  red or dark red rim (Figure 9I-L). Some orange aggregates show a distinct morphology, with smooth curved edges that differ from the predominant blocky shape (Figure 9E and F) and resemble the termite mound fragments (see Figure 6E and F). Clay aggregates may show a dense rim of recrystallized ashes (Figure 9K and L), whose sharp edges and disconnection with the surrounding powdery matrix suggest remobilization of the ash-cemented aggregates.

Changes in porosity and incidence of pedofeatures cementing the sediments reflect post-depositional alterations. Pedofeatures include mostly dense sparitic coatings and infillings (Figure 10D and E). Secondary sparite implies slow water passage through the sediments and/or episodes of water saturation and drying, also suggested by the few iron (hydr)oxide hypocoatings and nodules and manganese dendrites (Figure 10F) in the micromass. A few channel and chamber voids suggest that some bioturbation influenced the deposits.

#### *Microfacies identification*

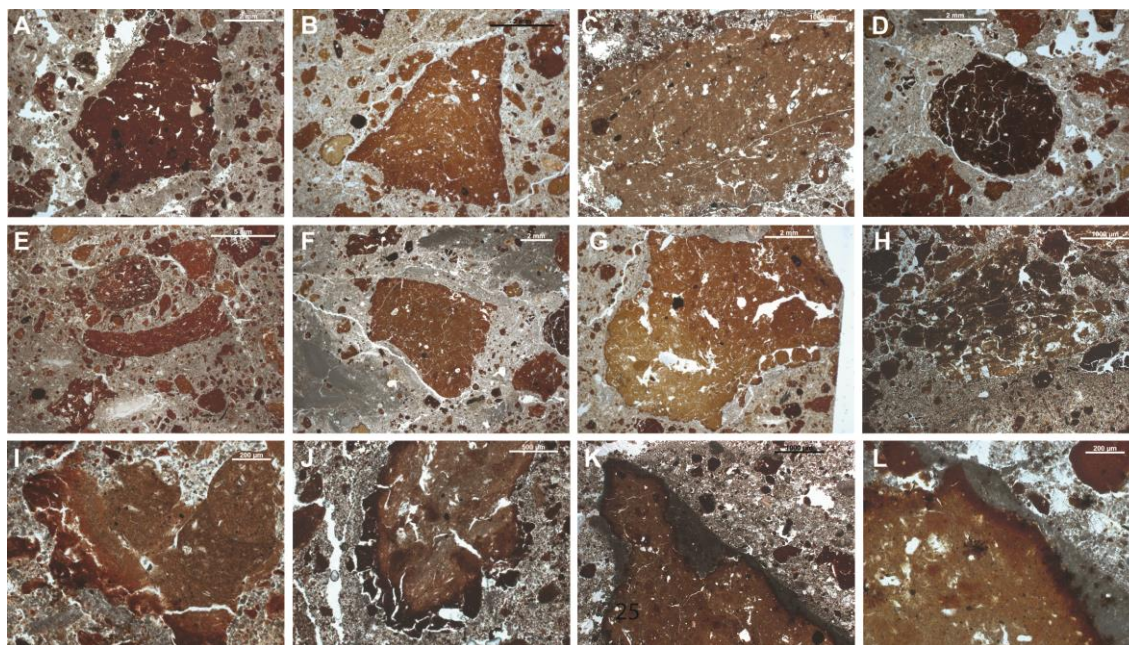
A total of 44 microfacies units (mF) was described in the 30 samples collected from the excavation surfaces. Slight changes in the relative frequency of clay aggregates vs. ash crystals (i.e. variations in the c/f ratio), and the presence/absence of organic elements (charcoal, articulated ashes, partially carbonized tissue, tissue residues and phytoliths) express variations between microfacies. Three main microfacies types characterize the deposit: mF I (~85% of the microfacies), containing ashes (randomly distributed and articulated), clay aggregates and organic remains (charcoal, tissue, bone etc) (Figure 10A); mF II (~10%), made of a massive, highly compacted dark grey micromass (PPL) with ashes and phosphates (Figure 10B); and mF III (~5%), with only red oxisol material.

#### *$\mu\text{FTIR}$ analysis of clay aggregates*

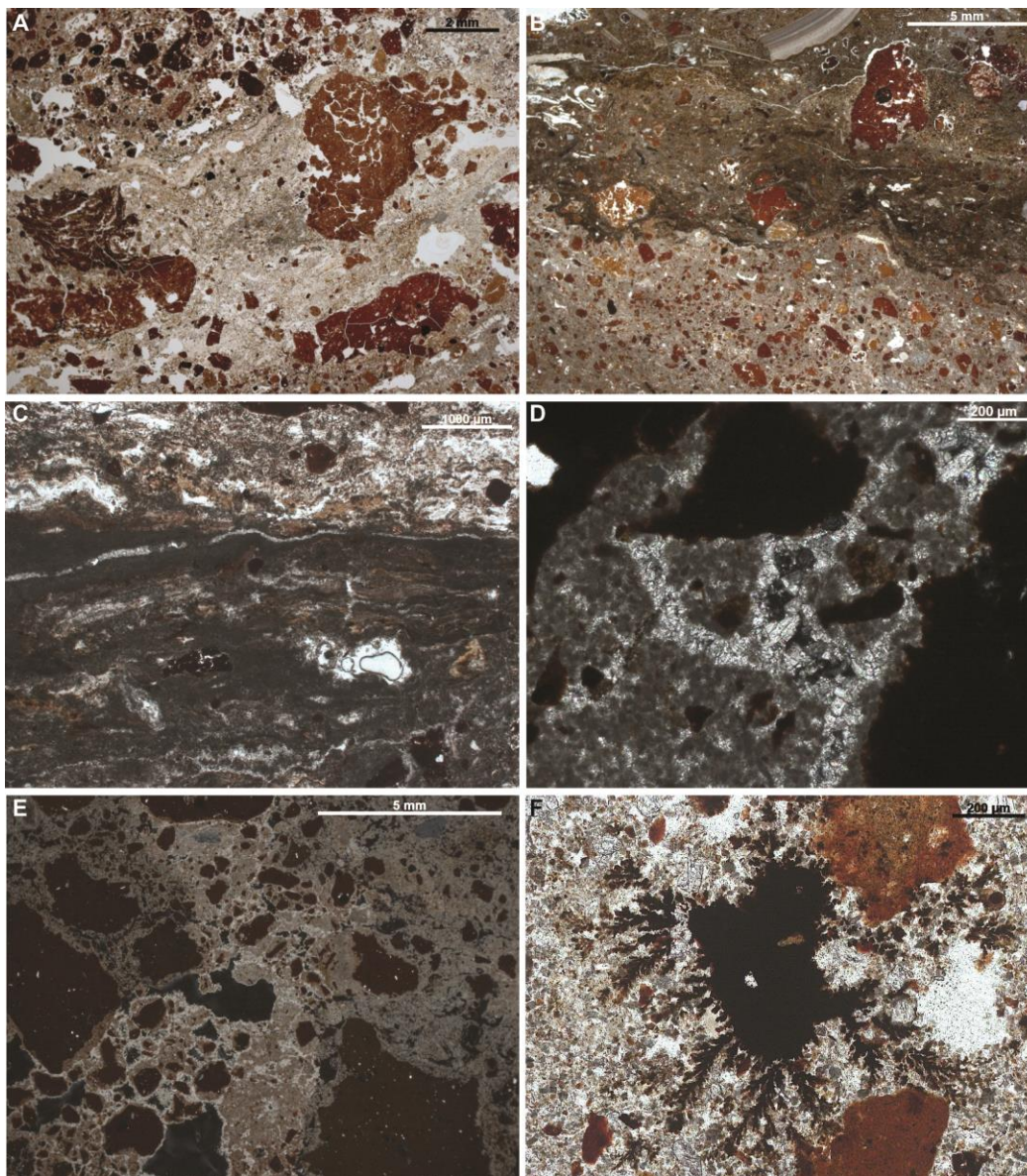
Clay aggregates in the thin sections were analyzed for the identification of heated clay in the site by comparing the spectra with the results of the experimental heating of oxisol aggregates. A total of 30 thin sections were studied: 28 from the excavation surfaces, and one

from the profiles. Table 1 summarizes the results and shows that the majority of the analyzed clay aggregates are in fact heated above 500-600° C. In the non-heated aggregates the kaolinite peaks in the OH region are clearly visible, as well as the main Si-O-Si peak at  $\sim 1030\text{ cm}^{-1}$  and the Al-O-H peak at  $\sim 910\text{ cm}^{-1}$ . In the heated samples, the OH region shows no peaks, the main Si-O-Si peak becomes broad and the Al-O-H peak disappears. The shift to lower wavenumbers in the main Si-O-Si peak was also described in the experimental test as being produced by interference of a main resin peak. Only at above 800°C does the main Si-O-Si peak shift to higher wavenumbers, indicating that clay aggregates at the site were heated above 500-600°C, but below 800°C.

Also noticeable is the fact that most red clay aggregates are non-heated or heated at temperatures below 500°C, while orange, orange-yellow, yellow and brown aggregates are always heated, with only few exceptions (seven aggregates, mostly orange). Figure 11 presents four examples of thin sections with the analyzed clay aggregates and selected spectra. The thin sections show the mix of non-heated and heated aggregates that characterize the sediments at Lapa do Santo, with most samples containing aggregates heated above 500-600° C.



**Figure 9.** Photomicrographs of different types of clay aggregates identified at Lapa do Santo (PPL): A) Red clay aggregate, sample 5; B) Orange clay aggregate, sample 1; C) Yellow clay aggregate, sample 8; D) Brown clay aggregate, sample 1; E) Orange clay with smooth edges resembling termite mound fragments, sample 15; F) Same as E, sample 1; G) Clay aggregate with transition from yellow to orange color, sample 1; H) Clay aggregate with transition from yellow to dark brown color, sample 25; I) Yellow clay aggregate with red rim, sample 7; J) Yellow clay aggregate with dark red rim, sample 30; K) Clay aggregate with dark rim and coated with recrystallized ashes, sample A; L) Closer view of K showing sharp edge of the micrite coating.



**Figure 10.** Photomicrographs of pedofeatures and microfacies from Lapa do Santo (PPL): A) Detail of typical mF I with clay aggregates embedded in a phosphatic matrix with ash crystals, sample 8; B) mF II (top) made of massive, highly compacted dark grey micromass consisting of permineralized plant remains with ashes and phosphates, sample 6 (note sharp boundary with underlying sediments); C) Laminated organic tissue remains cemented with ashes, phosphates and sparitic infillings, sample 10; D) Detail view of sparitic infillings in XPL, sample 3; E) Area of sparitic cementation (XPL), sample 16; F) Manganese dendrites, frequently found in the groundmass, in the clay aggregates and even on bone fragments, sample A.

### *Organic petrology*

Petrographic analysis confirmed that plant tissue remains are only a minor component of the sediments, randomly embedded in the ashy matrix with sizes that vary from 1 µm to 200

µm. Woody tissue prevail over herbaceous tissue. Plant remains are mostly permineralized (Figure 12A), especially with phosphate replacement (Figure 12C and F), Mn-oxide replacement and silicification (Figure 12D). Small plant remains described as cell detritus were also found inside the clay aggregates, confirming the association of the aggregates with soil material.

As described in the micromorphology section, secondary phosphates are frequent in the matrix (Figure 12B, E and F). The dense, dark grey (PPL) micromass described for samples 06, 28 and 29 is made of dense metal oxides (possibly Fe/Mn-oxides) (Figure 12G) phosphates and secondary carbonates embedding the ash rhombs and the tissue remains. Charcoal fragments of white color in RLo (fusinite and inertodetrinite) show high reflectance values (0.8-1.746 %Rr) (Figure 13A) and are sometimes complete or partially permineralized by Mn-oxides (Figure 13B). However, most plant detritus seems oxidized and/or degraded by fungi (see supplementary material online 4 for the complete list of reflectance measurements). Despite their low reflectance (0.3-0.7 %Rr) indicative of humified plant tissue (Jacob, 1974; Teichmüller, 1961, 1950), they show no fluorescence, have the same morphology as the charcoal particles and occur together with charcoal and ashes. Also, some detritus show domains of variable reflectance (ranging from 0.45 to 1.10 %Rr) indicating different degrees of thermal alteration (Figure 13C). Thus, the data suggest that most plant remains in the sediments are in fact low reflecting charcoal particles (semifusinite) (Figure 13D) produced after the incomplete combustion of wood.

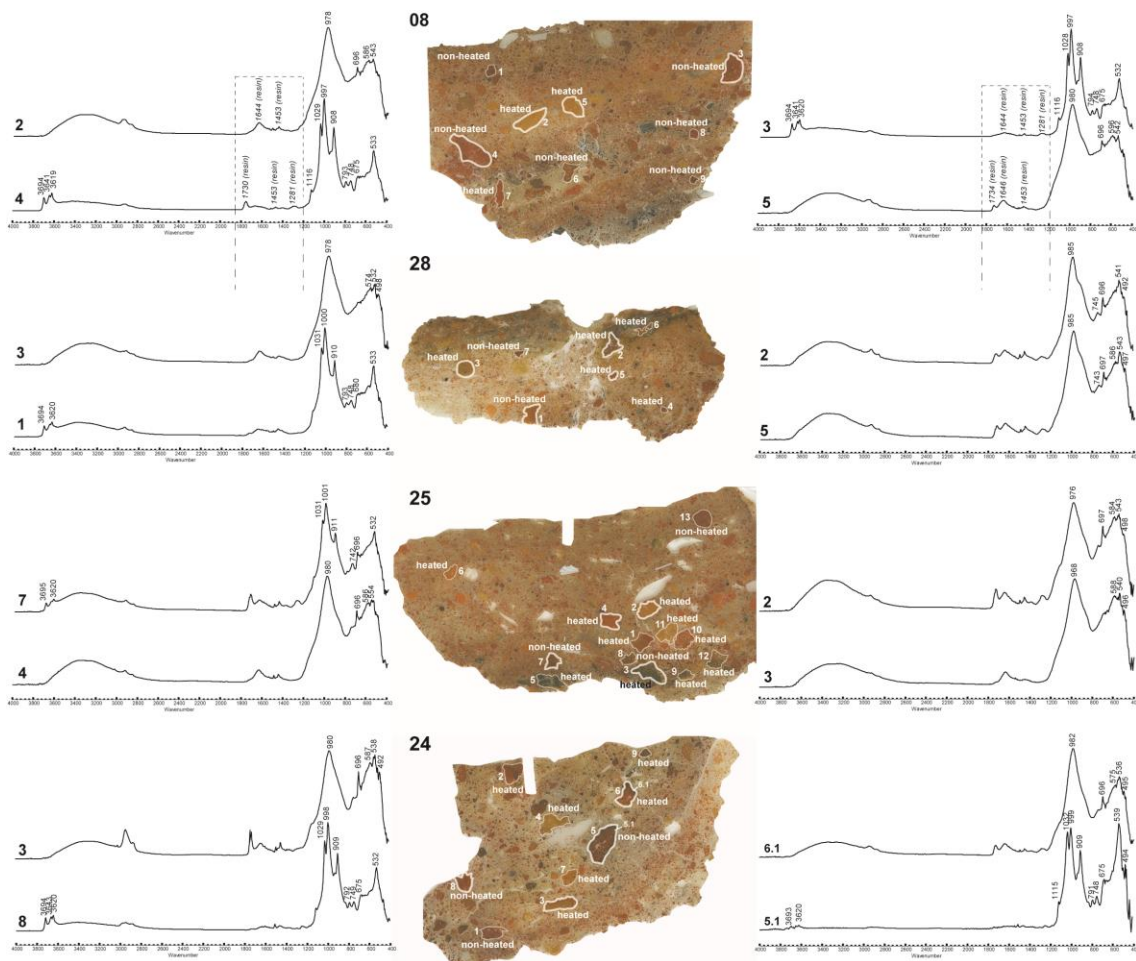
Moreover, a frequent phenomenon is the presence of well-preserved cell fillings (secretinite: organic gels that naturally fill cell voids in plant tissue), both isolated in the groundmass or inside the charcoal (semifusinites) and permineralized tissue (Figure 13E). Since wood charcoal does not contain secretinite (Braadbaart and Poole, 2008; Braadbaart et al., 2012; Guo and Bustin, 1998; Jones and Lim, 2000; Scott et al., 2000; Taylor et al., 1998), the preservation of organic gels in the charcoal indicate that the tissue underwent advanced humification prior to charring. This is also suggested by the swollen appearance of the cell walls (Diessel, 1992) showing shrinkage cracks and corroded edges (Figure 13F). Both the cell walls and cell fillings have fungal borings (Figure 13G), also suggesting biological weathering of wood prior to burning (Guo and Bustin, 1998; Jones and Lim, 2000). These results imply that mostly decayed wood (e.g. forest litter), was used to light the fires at Lapa do Santo (Figure 13H).

## DISCUSSION

### *Geogenic sedimentation*

The results of the micromorphological and µFTIR analyses show there is no similarity between the clay aggregates in the archaeological sediments and both the two possible geogenic sources near the excavation area: the red breccia and the colluvial deposits. Both the

composition and groundmass of the red clay aggregates are similar to the oxisol samples analyzed in this study and described in previous works (see Piló, 1998; Piló et al., 2005; Araujo et al., 2008, 2013). The yellow clay aggregates, which are much less in number and show different micromorphological features (massive microstructure and weak interference color), may derive from the yellow, goethite-rich oxisol that underlies the red oxisols, as suggested for other sites in the region (Araujo et al., 2008; Piló et al., 2005). However, both orange or dark brown clay aggregates are not observed in the natural soils. Neither are soil aggregates with color gradients or colored rims, as described for many of the yellow clay aggregates. Bright orange clay could only be observed in the heated clay experiment, while a dark brown color was only seen in the burned termite mound fragments, as will be discussed in the following sections.

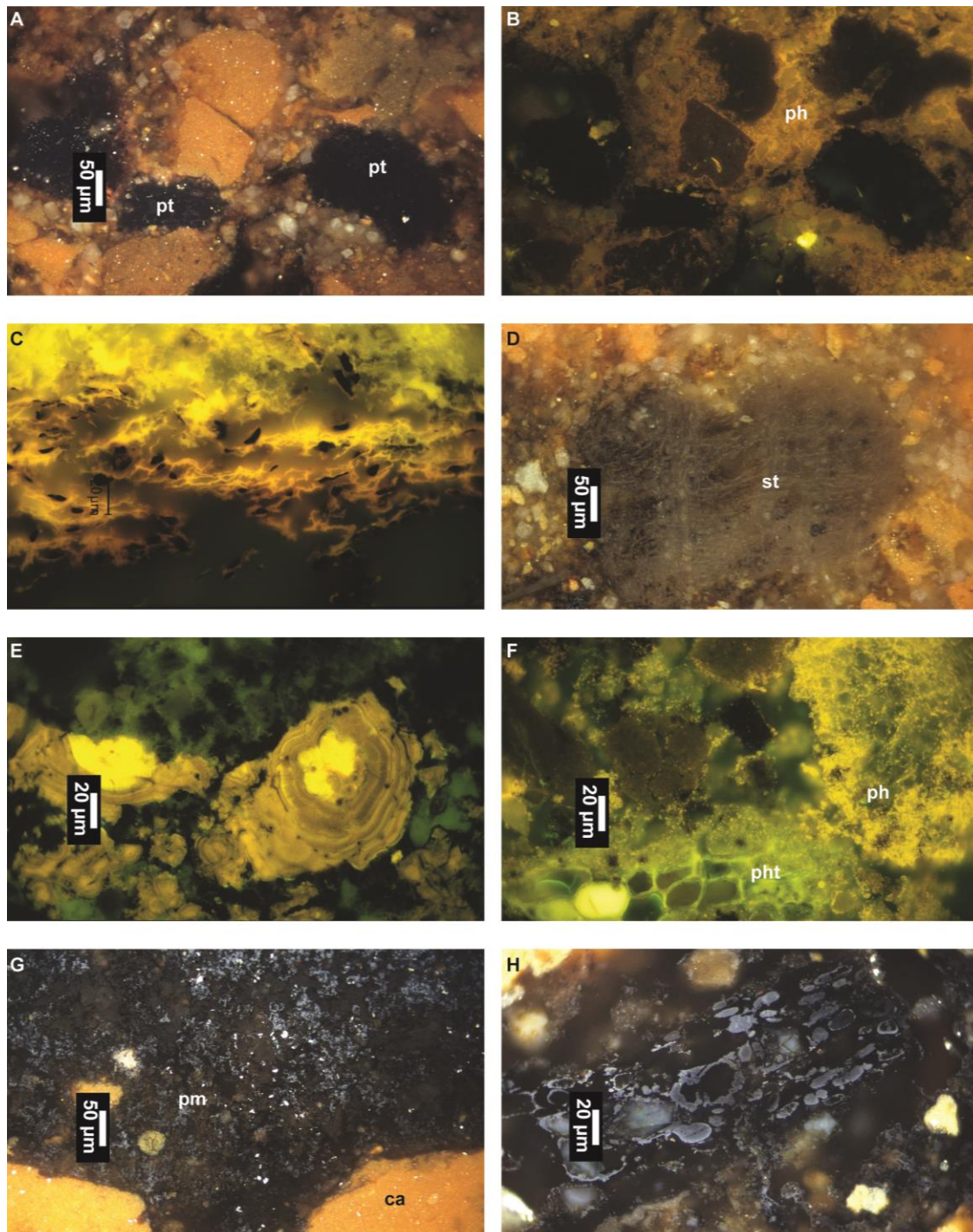


**Figure 11.** Selected thin sections with ATR- $\mu$ FTIR spectra of analyzed clay aggregates proving the presence of a mixture of unheated and heated clay in the archaeological sediments.

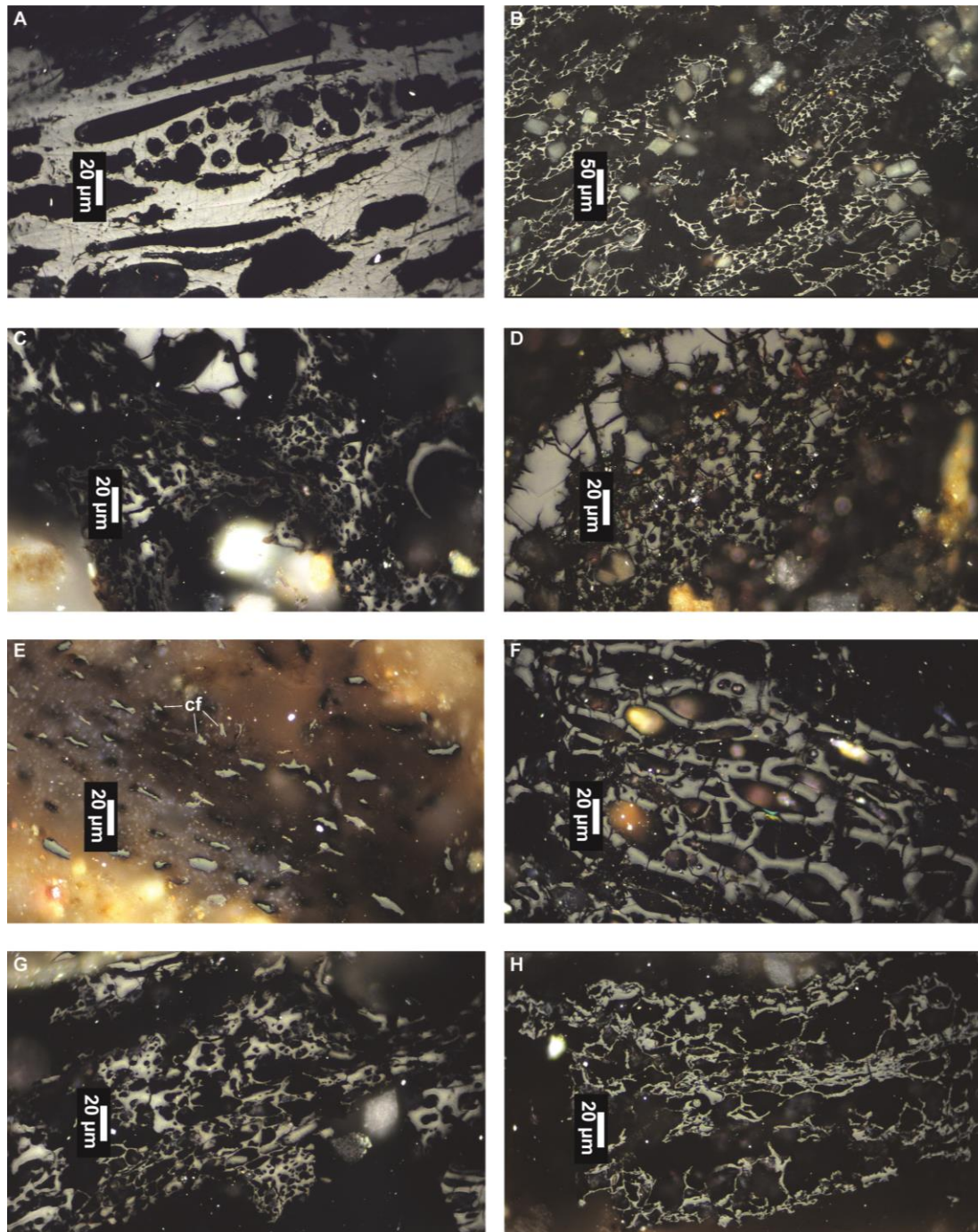
**Table 1.** Clay aggregates (discriminated by color) analyzed in the thin sections from Lapa do Santo with  $\mu$ FTIR (ATR and transmission). The aggregates were chosen trying to sample at least one of the different colors seen in each slide. The black dots showed no signs of heating at above 500-600° C, the red dots are heated above this temperature and the grey dots gave ambiguous results (spectra that does not allow a clear differentiation between heating and non-heating). The slides where fewer aggregates were studied are those that contain clay of only one color.

Sample	Color of clay aggregates									Total
	Red		Orange		Orange-yellow	Yellow		Brown		
	non-heated	heated	non-heated	heated	heated	non-heated	heated	heated		
LDS-01	•				••					3
LDS-02	•						•	•		3
LDS-03	•									1
LDS-04	•			•						2
LDS-05		•		•						2
LDS-06			••		••		••••	•		9
LDS-07		•			•			•		3
LDS-08	•••		••	•		•	••			9
LDS-10	••						•			3
LDS-11	••						•			3
LDS-12		•					•			2
LDS-14	•									1
LDS-15	•			•••			•			5
LDS-16				•	•		•			3
LDS-17		•		•			•			3
LDS-18	•	•			•					3
LDS-19	•••			•						4
LDS-20	••			•	•					4
LDS-21				•			••			3
LDS-22	•••									3
LDS-23	•									1
LDS-24	••	••	•	•			•••			9
LDS-25	•••	••		•	••		•	••••		13
LDS-26				•			•			2
LDS-28	•	•••						••		7
LDS-29				••						2
LDS-30	••	•	•		•					5
LDS-C	•••	•		•			•			6
Total	34	15	6	17	11	1	22	8	114	





**Figure 12.** Organic petrology of samples from Lapa do Santo: A) Completely permineralized plant tissue (pt) (RLo), sample 18; B) Same as A with phosphates (ph) visible under RVLo; C) Bright fluorescing phosphatized tissue (RVLo), sample 21; D) Silicified tissue (st) (RLo), sample 18; E) Colloform texture in phosphates (RVLo), sample 28; F) Mineral micromass rich in phosphates (ph) with phosphatized tissue fragments (pht) (RVLo), sample 8; G) Contact between red clay aggregates (ca) and permineralized ashy matrix (pm) (RLo), sample 28; H) Permineralized tissue with Mn-oxides (RLo), sample 28.



**Figure 13.** Organic petrology of samples from Lapa do Santo (RLo): A) Wood-derived fusinite tissue, sample 8; B) Partially permineralized fusinite tissue in ash matrix (ash crystals seen as grey rombs), sample 18; C) Semifusinite with heterogeneous reflectance, sample 11; D) Poorly preserved semifusinite tissue, sample 8; E) Relicts of cell fillings (cf) in a permineralized tissue, sample 25; F) Semifusinite tissue with swollen cell wall, sample 11; G) Semifusinite tissue with holes in cell fillings from fungal attack, sample 11; H) Fusinitized humified tissue with droplets of humic colloids droplets attached to cell walls, sample 21

The absence of cracks, conduits or chimneys bringing soil material to the rockshelter, and the similarity in composition, color and structure with the local oxisols suggests that clay aggregates fell from the red oxisol developed directly over the limestone massif. The steep slope on top of the limestone promotes the downhill creep of eroded and loosened soil aggregates. In fact, observations during fieldwork attest to the continuous fall of soil material into the rockshelter, especially in the dry season, when vegetation cover is minimal and soil erosion is naturally enhanced.

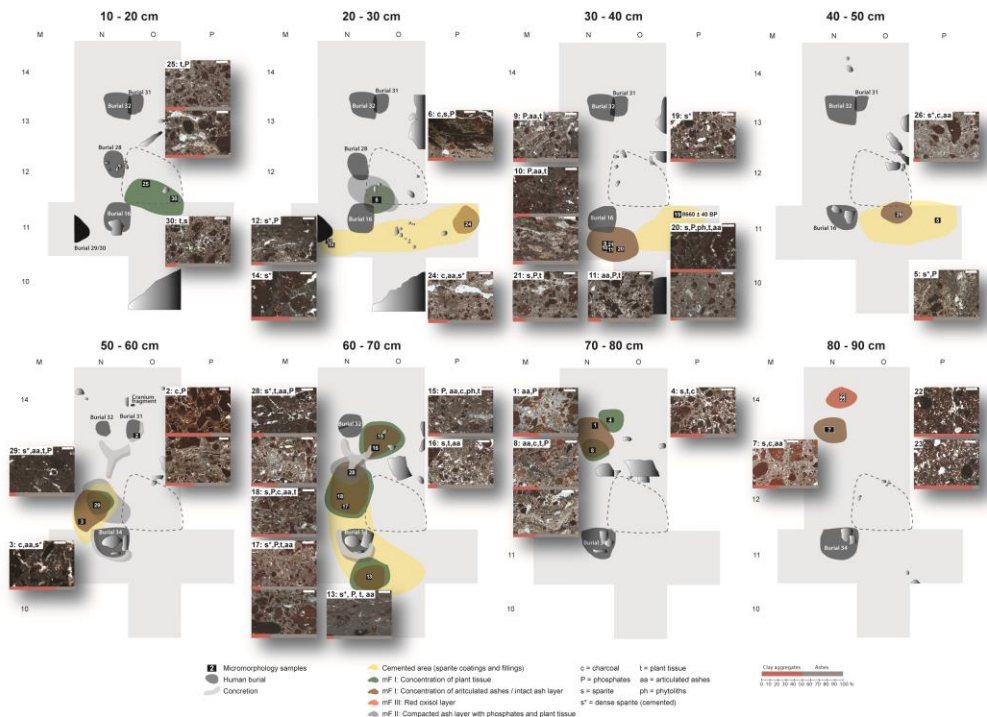
*Anthropogenic sedimentation and recurrent burning activities*

Our study demonstrated that human-made fires and combustion activities were key sedimentary processes at the early Holocene burial area in Lapa do Santo. Anthropogenic burning promoted the accumulation of ashes that mixed with soil continuously falling from above the limestone cliff (Figure 15). By extrapolating the results of this study to the whole site, we could expect this mixing process to be responsible for the accumulation of almost 5m of sediments during a time span of almost 5000 years. Decayed wood, possibly coming from forest litter, was used as fuel to light the fires. This observation could serve as indirect evidence of seasonality of site occupation. Forest litter in the *Cerrado* decays at a higher rate during the rainy season (from October to March) (Peres et al., 1983) or the transition from the dry to rainy seasons (September to November) (Sanches et al., 2008). However, careful analysis of the charcoal remains are needed in order to confirm this, since dry wood may have burned completely, thereby fully converting to the ashes found in the deposit.

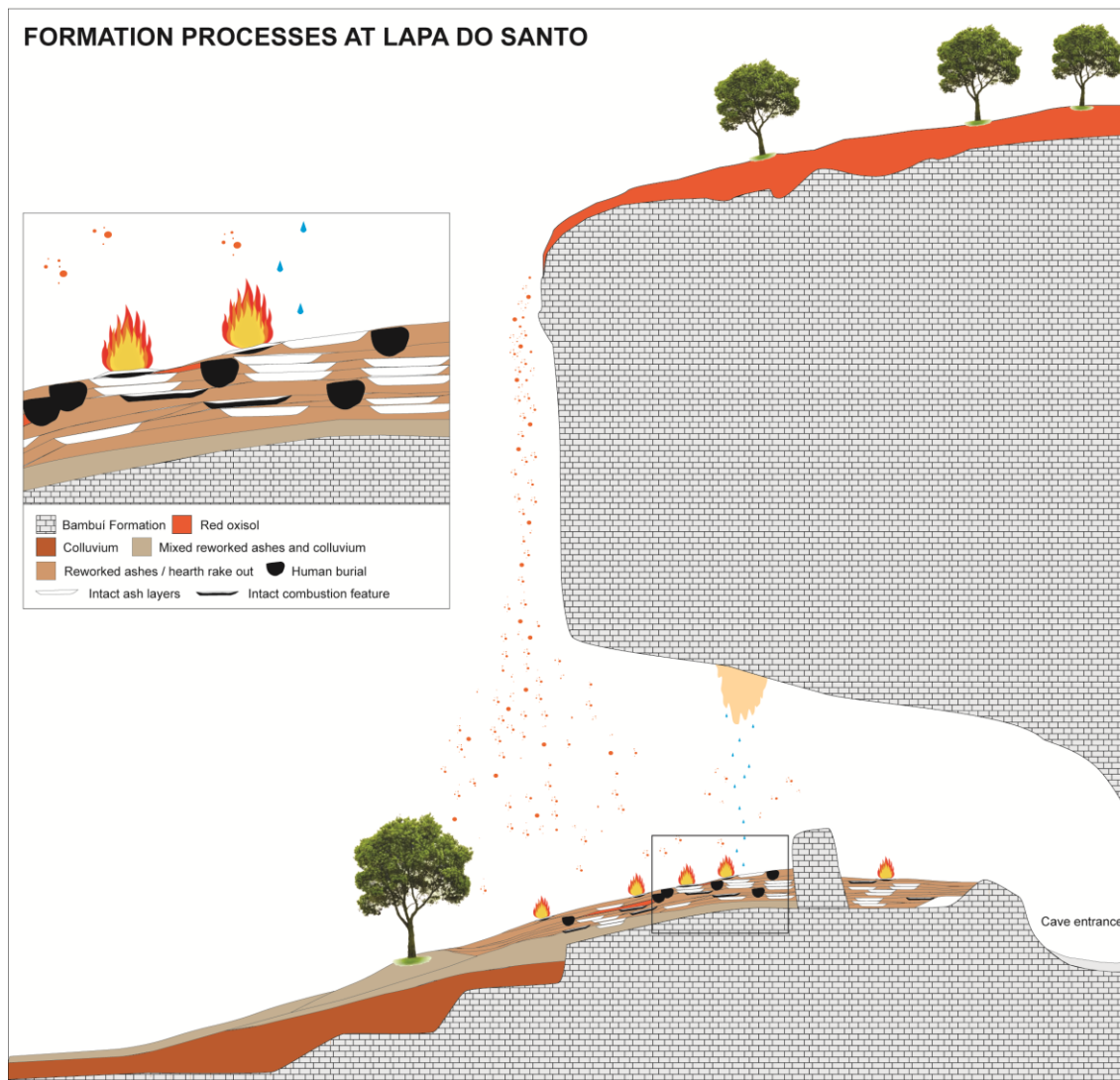
Thick concentrations of ashes have been identified at least in another rockshelter in Lagoa Santa, at the site of Lapa das Boleiras (Araujo et al., 2008), and recently at Lapa Grande de Taquaraçu (Villagran, 2013). The sediments at Lapa das Boleiras were interpreted as reworked hearths, mobilized by humans as part of site maintenance activities/ trampling, or as wind blown particles transported by local air currents (Araujo et al., 2008). At Lapa do Santo the ashes and byproducts of combustion activities seem to include both intact and reworked combustion structures. On the one hand, the random distribution of unsorted coarse fraction components (clay aggregates, charcoal, tissue residues etc.) and the absence of micro-stratification and/or clear boundaries between the ash layers are indicative of continuous fire-building at a location (see Karkanas et al., 2007; Mallol et al., 2013). A similar arrangement of components has been interpreted as resulting from dumped, burned materials or hearth rake-outs (Aldeias et al., 2012; Goldberg, 2003; Goldberg et al., 2009; Meignen et al., 2007; Mentzer, 2011; Miller et al., 2013; Vallverdu, 2002) and interpreted as the result of dumping and sweeping of hearth components for site maintenance and cleaning.

However, some of the microfacies units with high frequency of partially carbonized tissue and articulated ashes at Lapa do Santo (see Figure 14) indicate low reworking of parts of

the deposit and possible ash lenses from intact combustion features (see Mentzer, 2014). Thin ash lenses (~1cm) containing articulated ashes and partially carbonized tissue can also correspond to intact hearths, as described by Mentzer (2011) for the Üçağızlı I site. Both components are very fragile structures and minimal disturbance, or only short-distance transport, is necessary for their preservation. They have been described in ethnographic and experimental hearths (Mallol et al., 2007; Villagran et al., 2011) and also in archaeological contexts where they are interpreted as intact combustion structures (Homsey and Capo, 2006; Karkanas, 2010; Karkanas et al., 2007; Mentzer, 2011; Shahack-Gross et al., 2008).



**Figure 14.** Schematic view of the excavation surfaces at different depths where micromorphology samples were taken, with representative photomicrographs. In the photomicrographs it is indicated the percentage of clay aggregates vs. ashes and the presence of phosphates and organic remains in the samples. Micromorphological analysis allowed mapping of the approximate location of: 1) cemented areas within the excavation, surrounding the large speleothem fragment that covered a portion of the excavation surface (indicated with a dashed line), where sparitic coatings and infillings are prevalent; 2) concentrations of plant tissue remains and articulated ashes in mF type I; 3) the location of mF type III, composed of pure red oxisol at about 80-90 cm depths; 4) and the location of an ancient depression within the site where Mn-water dripping from the roof cemented an area rich in plant tissue remains (described as mF type II). Scale bar in samples 4, 8-top, 9, 10, 11, 15, 16, 17-top, 18, 19, 20-bottom, 21, 22, 23, 24, 25-top, 28-top and 29 is 1000 µm. Scale bar in samples 1, 3, 5, 7, 8-bottom, 10-bottom, 12, 13, 14, 17-bottom, 20-top, 25-bottom, 26, 28-bottom and 30 is 500 µm. Scale bar in sample 2 (top and bottom) is 200 µm and in sample 6 is 2 mm.



**Figure 15.** Formation processes at Lapa do Santo. The combined action of geogenic and anthropogenic sedimentation produced the archaeological sediments described in this study, which contain a mixture of soil aggregates eroding and falling from the top of the limestone massif into the rock shelter, with the reworked residues of hearths and other burning activities. Sediments mostly contain reworked ashes and successive layers of hearth rake-out that mixed with the soil falling from above. Thin layers of intact ashes are also described, indicating the presence of in situ synchronic and diachronic hearths throughout the studied time-span of human settlement. Ash layers combined with charcoal lenses have also been described during excavation. Human interments are frequently close to the fires; the graves were dug in the ashy matrix.

Articulated ashes and partially carbonized tissue exist at Lapa do Santo and are especially abundant in the samples that contain higher amount of heated clay aggregates. This association allowed the mapping of intact ash layers in the excavation area, which frequently

coincide with areas of higher concentration of plant tissue remains (see Figure 14, samples 24, 25). One exception is sample 06, which contains a concentration of plant tissue but no articulated ashes. However, the sample was taken from underneath the area covered by a large speleothem fragment (square O12, see Figure 4).

Traditionally, a tripartite sequence of rubified sediment, charcoal and ash is used to interpret intact combustion structures (see Mentzer, 2014, for a complete review of the micromorphological characteristics of combustion features). The absence of this sequence at Lapa do Santo, or at least in the 2011-2014 excavation area, can be explained in different manners: 1) the lack of rubified sediments may be due to the fact that hearths are stacked and successively lit over each other (i.e. over the ash lenses of previous hearths) lacking a clayey sedimentary substrate to be rubified in the first place; 2) the low amount of charcoal in the micromorphology samples is a product of sampling bias, since thin and sparse charcoal lenses are visible during the excavation.

Thus, the sediments at Lapa do Santo include few stacked combustion features, containing superimposed ash lenses, and dumped burned materials or hearth rake-out. The geoarchaeological evidence points to the recurrent use of the site for combustion activities, with some combustion features maintained intact while others were remobilized (see Figure 14). The dual presence of intact and remobilized combustion features explains the inversions in the radiocarbon ages from the 2011-2014 excavation area (see supplementary material online 1).

The high concentration of human burials at the site, both surrounded and covered by combustion residues, suggests that the lighting of fires and the dumping of hearths was done close to the interments. A possible explanation for the dumped hearths could be to accommodate the human interments, since highly manipulated human bodies were buried in the ashy sediments and covered by them.

#### *Human-made fires and their effects on geogenic sediments*

The  $\mu$ FTIR analysis showed that more than half of the clay aggregates analyzed in 28 thin sections was heated at temperatures above 500-600° C (68 aggregates of 119). Heating also explains the different colors described in the aggregates during the micromorphological study. Orange, yellow and brown aggregates consistently show signs of heating as opposed to red colored aggregates, which are mostly non-heated. As shown by the experimental heating of oxisol material, the clay in the local soils will only present orange color and signs of heat-induced alterations when exposed to temperatures above 500-600° C (see Figure 7). However, the experimental hearth on oxisol substrate demonstrated that such high temperatures are not attained in the substrate of the fire, even close to the surface with flames reaching temperatures above 800° C. This means that clay aggregates must be either in direct contact with the flames to be altered at temperatures above 500-600° C.

This is consistent with the provenance of geogenic sediments, eroding from above the rockshelter and partially falling inside the human-made fires. The clay aggregates may have been also heated already after deposition, by being beneath the fires lit on the ashy substrate. Low-density ashy sediments may transfer heat more readily than solid soil, thus heating the clay aggregate already contained within the ashes. Whatever the possibilities, our study shows that heated soil aggregates make up more than half of the clay aggregates at the site and they likely do not come from fires lit outside the rockshelter on natural soils. Hearths appear to have only been built inside the rockshelter and in close association with the human interments.

*The presence of termite mound fragments in the sediments*

Human selection of materials may also account for the presence of some of the heated aggregates within the site. For instance, several clay aggregates whose heating was demonstrated by  $\mu$ FTIR are similar to the heated termite mound fragments. Similarities include: yellow and dark brown-colored aggregates with signs of heating; the color gradient seen in some heated aggregates; the orange and red rims that do not exist in the natural soils; and the massive microstructure of the aggregates with weak interference color. Termite mound fragments would not fall naturally into the site like the soil aggregates, since termites do not build their nests on shallow loose soil. Above the site, the termite mounds appear on the limestone massif only over flat terrain and far from the cliff (Figure 3A).

The use of termite mounds by the prehistoric inhabitants of Lagoa Santa should not come as a surprise. Termite mounds are extremely frequent in the region: near the site we noted the presence of 256 nests identified at the elevation of the site and 41 above the limestone massif. Termites have existed for millions of years before the human settlement in the area and some authors believe that termite activity since the Paleogene/ Neogene (former Tertiary) is responsible for the characteristic granular microstructure of Brazilian oxisols (Sarcinelli et al., 2009; Schaefer, 2001).

Termite mounds are dense and compact (Cosarinsky and Roces, 2007) and our experimental heating studies show they can retain heat for long periods of time. Because of its characteristics, the ancient and modern populations of Minas Gerais used termite mounds as a type of natural clay oven for food preparation (Nunes & Nunes, 2001). It is also known that ethnographic Xavante groups from central Brazil use termite mounds to build small ovens (Prous, 1992) and for the fires used to cook their traditional maize cake (Lewis, 1967). The termite mound fragments at Lapa do Santo are possible evidence for the use of such resources by early Holocene human groups in South America. However, our analyses cannot determine at this point whether the termite mounds were in fact carried to the site or unintentionally brought as attached fragments to firewood.

*The post-depositional alteration of the site*

The horizontal strategy of sampling for micromorphology revealed that spatial differences within the excavation area are mostly due to post-depositional processes, caused by water passage through the sediments. Dense sparite infillings are mostly concentrated around the large speleothem fragment that existed in the excavation area (see Figure 4 and 14) (see supplementary material online 5 for 3D view of the excavation area with the speleothem). These pedofeatures attest to water accumulation and slow drainage in this area of the site.

## **CONCLUSIONS**

The site of Lapa do Santo contains one of the thickest archaeological deposits of the rockshelters in Lagoa Santa, a region well-known for its human remains dating back to the early Holocene. The site has gained recognition for its earliest evidence of rock art in the Americas and the complexity of the funerary practices (Araujo et al., 2012; Neves et al., 2012; Strauss, 2016; Strauss et al., 2015, in press), both opposing the traditional expectations of cultural “simplicity” for the early Holocene populations of the continent. By studying the stratigraphic sequence at Lapa do Santo using a micro-contextual approach (Goldberg and Berna, 2010)—integrating micromorphology with  $\mu$ FTIR analysis, organic petrology and experimental studies—our study provides key information on 1) the human activities at the site; 2) the influence of natural processes in site formation; 3) the potential use of local resources (e.g. termite mounds); 4) and intensity of occupation.

The hearths that people lit during occupation of the site contributed significantly to the formation of the archaeological deposit. The ashes and other combustion remains are spread across the southern portion of the rockshelter, which was used as multi-functional space including a burial ground. Micromorphological evidence points to a combination of intact hearths and remobilized hearths through site maintenance activities. The remobilization and lateral reworking of sediments, which explains the age inversions reported for the 2011-2014 excavation, may be related to the funerary practices of the site inhabitants, since all the interments were dug into the ashy sediments and later covered by them. This opens a venue for future studies, focusing on understanding the causal or intentional relation between the anthropogenic sediments and the funerary practices of the site inhabitants.

Micromorphology showed a dual influence in sediment formation that is not evident in the field. There is a noticeable input of geogenic sediments in the form of clay aggregates derived from soil eroding from a steep slope over the limestone cliff and falling into the rockshelter. The clay aggregates are unsorted and vary from rounded to angular. They show different colors which  $\mu$ FTIR studies proved to be related with their thermal alteration: red clay



aggregates are mostly non-heated fragments of oxisols, whereas orange aggregates are mostly heated at temperatures above 500-600° C.

This type of anthropogenic infilling is documented around the world, in areas as distant as the Levant (Mentzer, 2011) and South Africa (Miller, personal observation). However, this work is the first micro-contextual approach applied to fully understand the natural and anthropogenic sedimentary dynamics behind the formation of thick ash deposits containing oxisol aggregates. The results of this work indicate that the techniques and approaches here should be applied at other sites to unravel the full set of information contained in mixed ash/oxisol deposits in rockshelters, despite their geographic and/or climatic context. Besides micromorphology, which has long proved its efficacy in site formation studies, complimentary techniques such as  $\mu$ FTIR and organic petrology should be included as sources of data not easily obtained through standard, optical microscopic observations, such as: the heat-induced alteration of sediments; temperatures attained by human-made fires; and fuel sources.

Other components identified in the sediments at Lapa do Santo have a less straightforward association with local soils. These aggregates are heated and show resemblance in shape, color and micromorphology to heated termite mound fragments. This observation suggests the use of termite mounds by the early inhabitants of Lagoa Santa, which appear in dense concentrations around this and other sites in the region. Their presence at the site may be the first potential evidence for the use of this local resource by early South Americans, possibly for heating and/or cooking. However, further experimental studies are needed to refute a possible natural cause for the presence of this resource at the site.

Despite the high amount of ashes and charcoal, fresh plant remains are scarce and, when present, are persistently permineralized with Mn-oxides, silicified or phosphatized. The high concentration of secondary phosphates in the sediments seems to derive from the ashes and the charred plant remains, as indicated by the association of concentrated plant residues and phosphate nodules and the ash crystals persistently embedded in a phosphatic micromass. However, the possible relation of secondary phosphates with decaying human bodies must be further investigated. The charred plant remains in the sediments derived from decomposed wood (degraded by fungi) point at the use of decayed forest litter as fuel.

Understanding the dual composition of the sediments at Lapa do Santo (i.e. ashes and oxisol aggregates) is essential when discussing the intensity of occupation. The thick archaeological deposit resulted from the mixed input of anthropogenic sediments from intact and remobilized hearths, and from the constant fall of soil aggregates into the rockshelter. This indicates that thickness in the archaeological deposit does not necessarily correlate with a more intensive occupation of the site, and that local sedimentation processes must be taken into consideration for comparison. However, the recurrent use of Lapa do Santo and other sites for

almost 5000 years certainly reflects the central position of the region in the cultural landscape of the early Holocene.

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## Appendix IV

### **Early Holocene ritual complexity in South America: the archaeological record of Lapa do Santo (east-central Brazil)**

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## ABSTRACT

In reconstructing the life of past populations, human burials are highly informative of symbolic and ritual behavior. In eastern South America, however, skeletal remains dating to the early Archaic are rare, precluding the proper study of their ritual dimensions. Here we report 26 human burials from this period found in Lapa do Santo (eastern Brazil) and their associated archaeological context. Lithic technology, zooarchaeology, and multi-isotopic analyses indicate groups of hunter-gathers with low mobility and a subsistence strategy focused on gathering plant foods and hunting small and mid-sized animals. The use of Lapa do Santo as an interment ground started between 10.3-10.6 cal kyBP with primary burials. Between 9.4-9.6 cal kyBP the reduction of the body by means of mutilation, defleshing, tooth removal, exposure to fire and possibly cannibalism, followed by the secondary burial of the remains according to strict rules, became a central element in the treatment of the dead. In the absence of monumental architecture or grave goods, these groups were using parts of fresh corpses to elaborate their rituals, showing this practice was not restricted to the Andean region at the beginning of the Holocene. Between 8.2-8.6 cal kyBP another change occurred whereby pits were instead filled with disarticulated bones of a single individual without signs of body manipulation. Those changes show that during the early Archaic, Lagoa Santa was a region inhabited by dynamic groups that were in constant transformation over a period of centuries.

In reconstructing the life of past populations, human burials are highly informative of symbolic and ritual behavior. In preterite societies, bones and body parts constituted a potent cultural resource and their manipulation and organization into meaningful arrangements were commonly used to reify cosmological ideas and beliefs system (J. Brown, 2010; Goldstein, 2000). This kind of practice is usually considered part of a broader system of ceremonies that reflect a high degree of symbolic complexity and is therefore of great interest for archaeologists all over the world (Sofaer, 2006).

In South America, despite recent studies revisiting the possibility of occupations in the continent going back to 28,000 years or more (Boëda et al., 2013, 2014; Dillehay et al., 2015; Lahaye et al., 2013), human skeletal remains only appear in the archaeological record from the early Holocene onwards (Lucas Bueno, Dias, et al., 2013; W.A. Neves et al., 2013). In this period, known as the early Archaic, most landscapes of the sub-continent were already occupied and human populations thrived (Aceituno et al., 2013; Lucas Bueno, Dias, et al., 2013; Capriles & Albarracín-Jordan, 2013; Martínez et al., 2013; Mazz, 2013; Melgar, 2013; Prates et al., 2013; Rademaker et al., 2013). These groups are relatively well studied in regard to their mobility patterns and subsistence strategies being characterized as generalist foragers with a strong emphasis on the exploitation of vegetable and maritime items when available (Scheinsohn 2003; Kipnis 1998, 2002; Borrero 2015). Considering ideational, symbolic or ritualistic aspects, however, the available information is limited (Dillehay, 1997). Since rock art is usually difficult to date with precision (W.A. Neves et al., 2012; Pessis, 2013) most of the information on symbolic behavior during the early Holocene is left to the analysis of human burials.

In the western portion of the continent substantial efforts have been made both in the sense of increasing the number of available sites and of providing new theoretical frameworks (Santoro, Standen, Arriaza, & Dillehay, 2005). In a synthesis of the available evidence on the Andean region, Santoro and collaborators (2005:330) have concluded that *the manipulation and transformation of dead bodies was more common than previously thought in the study of Archaic mortuary rituals* and that despite considerable diversity of mortuary practices, a common emphasis on the preservation or reduction of the body can already be discerned during the early Archaic.

In the eastern part of the continent, however, archaeological sites containing skeletal remains dating to the early Archaic are extremely rare, precluding the proper study of their ritual dimensions. One exception is the region of Lagoa Santa in central Brazil where hundreds of early Holocene human skeletons have been exhumed in almost two centuries of research (W.A. Neves & Hubbe, 2005). According to the classic descriptions, the mortuary practices in Lagoa Santa were simple and homogenous, including nothing but primary interments without grave goods (W.A. Neves et al., 2003; Walter, 1958). It contrasted, therefore, with the elaborated burials described for the western part of South America during the same period (Santoro 2015).

In 2001, however, excavations restarted in Lagoa Santa and findings from Lapa das Boieiras and Lapa do Santo engendered a transformation in our understanding of the mortuary rituals in the region (Araujo et al., 2008, 2012; Araujo, 2010; Strauss et al., 2015). The abundance of well-preserved early Holocene burials in those sites offered a rare window into the commonly inaccessible world of the funerary rituals of the early Archaic hunter-gatherers in eastern South America (Astolfo G.M. Araujo et al., 2012). Here we report burials from Lapa do Santo and their associated archaeological context discussing their implication for a proper understanding of the mortuary landscape of South America during the early Holocene. Far from simple, the burials of Lapa do Santo points to an unexpected sophistication based on the manipulation of the fresh corpse as a key element of the rituals.

### LAPA DO SANTO ARCHAEOLOGICAL CONTEXT

Lapa do Santo is an archaeological site located in the northern part of the Lagoa Santa karst (Araujo et al., 2012)(see SI-1 for a detailed description). Lagoa Santa is located in east-central Brazil (Fig. 1a) and is well known among archaeologist and paleontologists since the 19<sup>th</sup> century. The first human skeletons were found by the Danish naturalist Peter Lund between 1835 and 1844 in the Sumidouro cave in association with bones of extinct megafauna (Araujo et al., 2005; Cartelle, 1994; Luna, 2007; Lund, 1844; Piló & Auler, 2002). Due to this putative coexistence of man and megafauna Lagoa Santa became the focus of many 19<sup>th</sup> century scholars (Hansen, 1888; Hrdlička, 1912; Kollman, 1884; Ten Kate, 1885). During the 20<sup>th</sup> centuries, different teams went to the region pursuing to find evidence that could confirm the coexistence hypothesis (Bányai, 1997; Hurt & Blasi, 1969; Laming-Emperaire, 1979; Walter et al., 1937; Walter, 1958). As a result of more than 170 years of excavations a large collection of early Holocene skeletons was formed (Neves *et al.* 2013; Bernardo *et al.* 2011; Da-Gloria 2012). However, all those excavation were done in a time when proper documentation was not available and, therefore, they considerably lack contextual information. The project “Origins and Microevolution of Man in America: a Paleoanthropological Approach” aimed to overcome this situation by identifying and excavating new sites in Lagoa Santa region. Lapa do Santo was found in the frame of those efforts.

Lapa do Santo is a cave with an associated sheltered area of ca. 1300 m<sup>2</sup> (Fig. 2a) developed under the negative slope of a 30 meters high limestone massif (Fig. 1b). The southern region of the sheltered area is relatively flat, high and dry, and is located immediately in front of the cave’s entrance. The floor of the shelter has a strong descending inclination towards the north, which becomes flat again near a natural sinkhole located in the northern extreme of the sheltered area (Figs. 2c-f). The chronology of the site is based on OSL (Tables S1-S5) and radiocarbon dating (Fig. 3a, Table S6, and SI-2) and it defines three distinct periods of human occupation with the oldest one starting at 12.7-11.7 cal kyBP (all chronological ranges

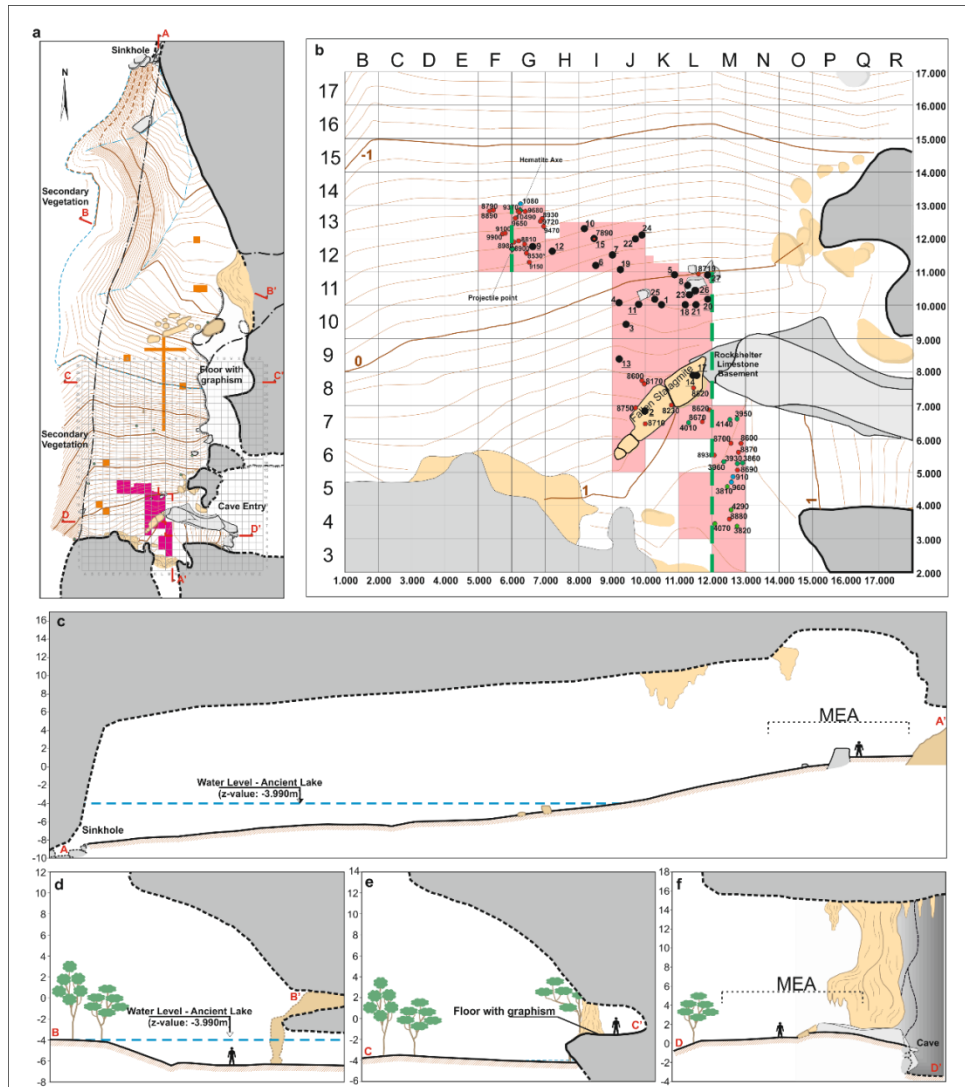
are based on a 95.4% interval)(Table 1). When the three periods are considered, there is a good agreement between depth (i.e., z-value) and dated charcoals, showing the stratigraphic integrity of the deposits (Fig. 3b). Site formation process analysis concluded that Lapa do Santo contains an expressive component of anthropogenic sediments produced after repeated combustion activities (Fig. 4; SI-3 and SI-4)(X. Villagran, Strauss, Miller, Ligouis, & Oliveira, 2016).

**Table 1.** Chronological periods for the 2001-2009 excavations in Lapa do Santo.

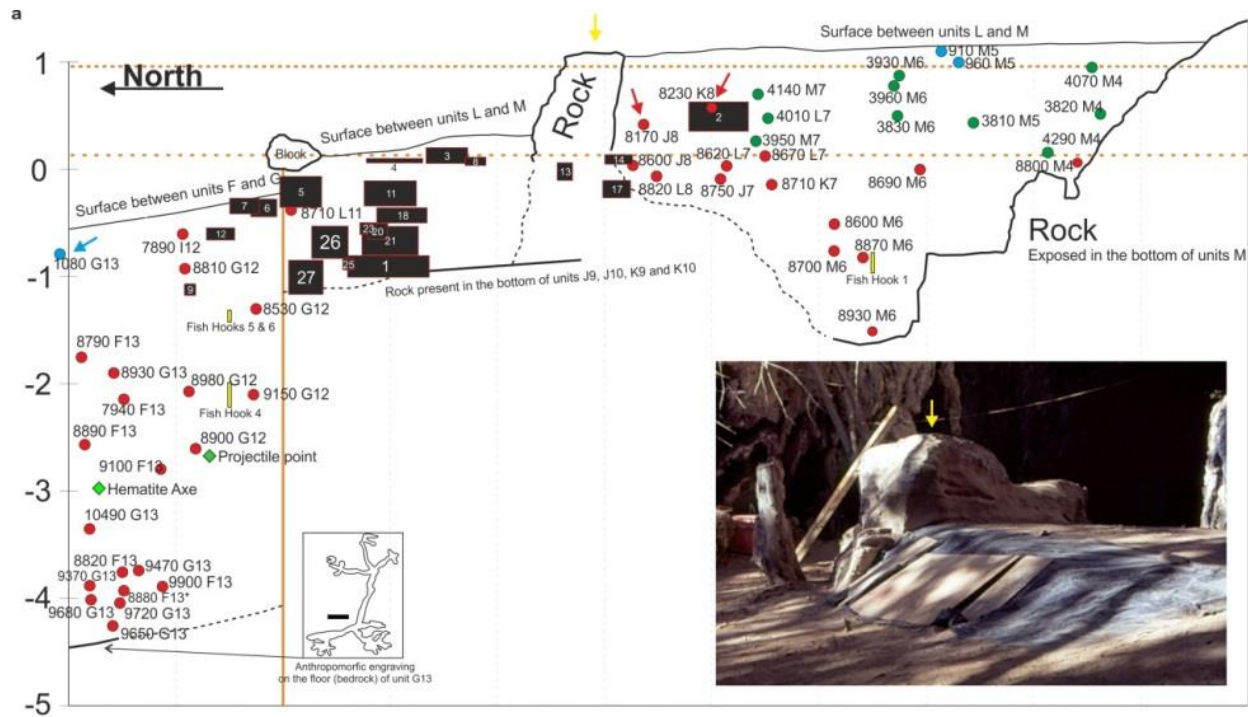
	68.2% interval	95.4% interval	Vertical interval (z-value in meters)
Lapa do Santo Period 3	0.7-1.1 cal kyBP	0-2.1 cal kyBP	> 0.947
Lapa do Santo Period 2	4.0-5.2 cal kyBP	3.9-5.4 cal kyBP	0.137-0.947
Lapa do Santo Period 1	8.1-12.5 cal kyBP	8.0-12.7cal kyBP	<0.137



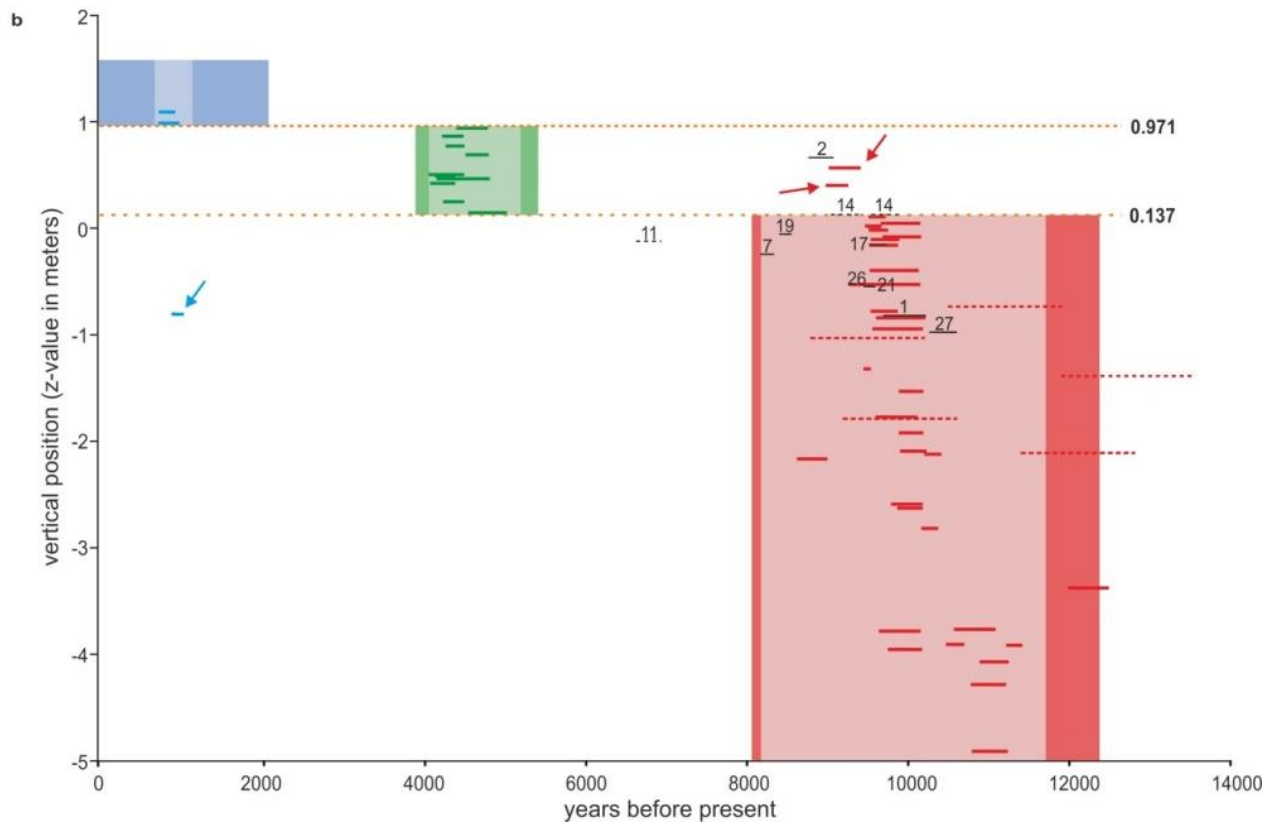
**Figure 1: Geographic location and aerial view of Lapa do Santo:** Left, Map of South America showing the location of Lagoa Santa region (red star) and other sites mentioned in the text: Baño Nuevo (yellow diamond), Huchichocana Cave (black diamond), La Chimba (purple diamond), La Fundición (orange diamond), Lauricocha (light blue diamond), Pampa de los Fósiles (light green diamond), Tequendama (white diamond), Capelinha (black square), Justino (green square), Loca do Suim (yellow square), Santana do Riacho (white square) and Toca dos Coqueiros (blue square). **Right,** Aerial view of the Lapa do Santo massif. The black arrow points to the sheltered area where the archaeological site is located.



**Figure 2: Plan and sections of Lapa do Santo.** a, Plan of Lapa do Santo. The grid corresponds to 1 square meter units. Pink and orange indicate, respectively, excavated surfaces. Pink indicate the main excavation area (MEA). The bedrock is depicted in gray and secondary deposits such as breccia and stalagmites in beige. The topographic lines are 10 cm equidistant and the associated values correspond to the z-value of the site coordinate system. The red letters indicate the start and end of the sections depicted in “c”, “d”, “e” and “f”. b, Detail of the MEA area. Red, green and blue dots are, respectively, early, middle and late Holocene dated charcoal samples. The number next to the dots indicates the radiocarbon non-calibrated date. Black disks indicate the position of the human burials and their correspondent number is indicated by the underlined numbers. The green diamond indicates where the hematite blade was found (Lst-6410). Numbers in the lower and right margin indicate the x and y values, respectively, from the coordinate system of site. The dashed green lines indicate the surfaces of the profiles in Figure 3. c, profile from points A to A’ as indicated in “a”. d, Section from points B to B’ as indicated in “a”. e, Section from points C to C’ as indicated in “a”. f, Section from points D to D’ as indicated in “a”.

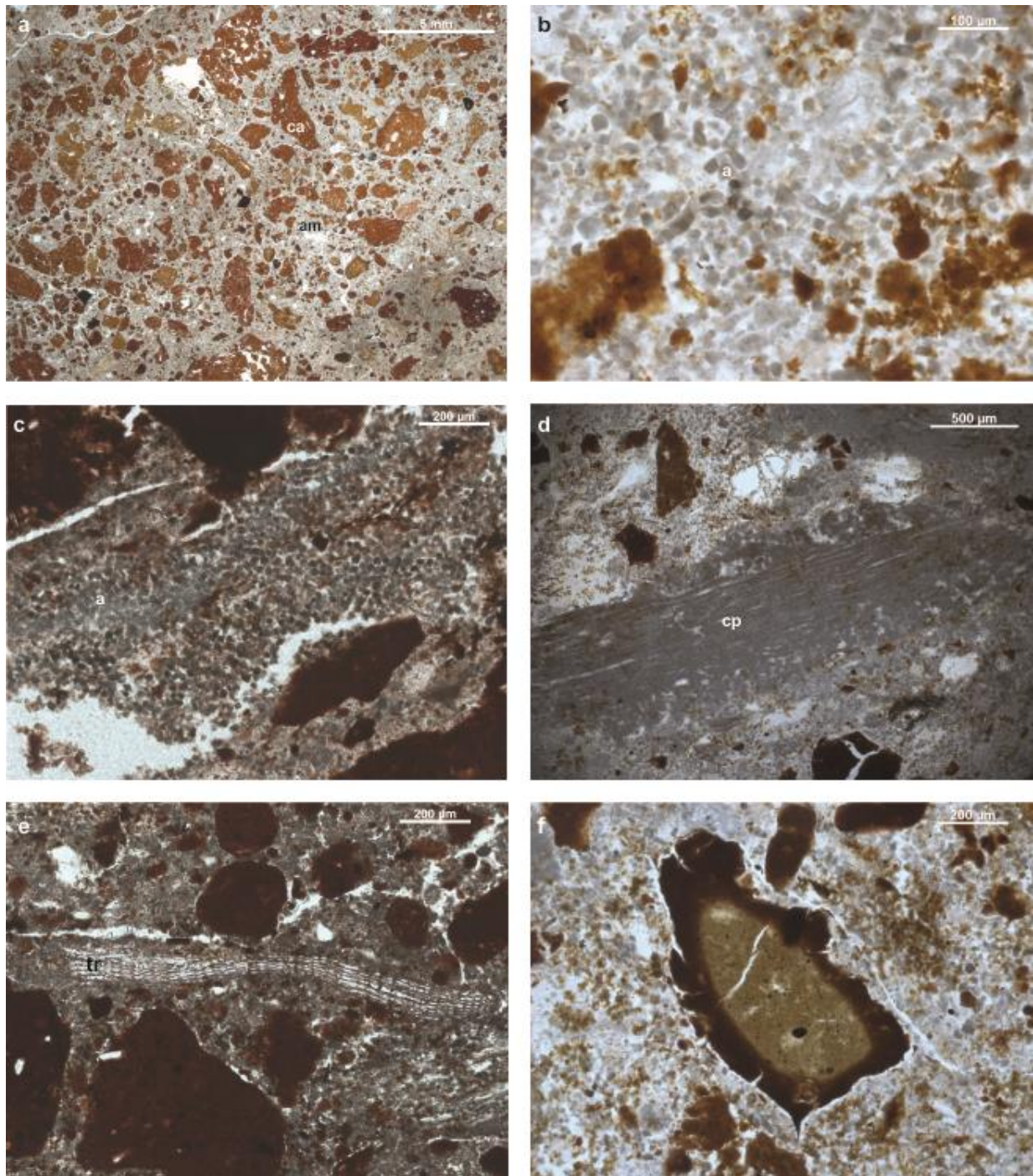


**Figure 3a.** Chronology of Lapa do Santo. a, South-North section following the surfaces indicated with the dashed green line in the Figure S1. The continuous thin black line indicates the original surface of the archaeological deposit before excavations. The continuous and pointed thick black line indicates observed and inferred rockshelter’s floor and walls, respectively. Red, green and blue dots are dated charcoal belonging to Lapa do Santo Period (LSP) 1, 2 and 3, respectively. The number next to the dots indicates the radiocarbon non-calibrated age and the unit where the charcoal was located. The numbers in the left frame indicate the z-values in meters. Black rectangles show the position and dimensions of the burials and their respective identification is indicated by the white number. The picture in the detail was taken from a similar angle as the one of the profile and the yellow arrows point to the rock that is also represented in the schematic profile. Note the presence of a steep transition in the surface of the site between the south (right side from the rock in the picture) and the north (left side of rock in the picture) regions. This is exactly where the fallen stalagmite was located, which was removed for excavation under it. See legend of “b” for meaning of the blue and red arrows and also for dashed and dotted orange lines. The green diamond indicates the position of the hematite axe (Lst-6410). The vertical yellow bar indicate the z-values’ range of levels in which fishhooks were sieved from.



**Figure 3b.** Chronology of Lapa do Santo. Scatterplot showing the relationship between vertical position (z-value) and age for different components of Lapa do Santo. Red, green and blue horizontal continuous bars indicated the 95.4% interval of the modelled calibrated radiocarbon dates obtained from charcoal samples. Black and dashed horizontal continuous bars indicate the 95.4% interval of the modelled and non-modelled calibrated radiocarbon dates obtained from bone collagen, respectively. The associated number indicates the burial accession number. Red horizontal dashed bars indicate the 95.4% interval of the OSL dates. The blue, green and red zones indicate the 68.2% (light color) and 95.4% (strong color) intervals of LSP-1, LSP-2 and LSP-3 (see Supplementary Information for details). The orange dotted and dashed lines indicate, respectively, the average z-value that set apart LSP-3 from LSP-2 and LSP-2 from LSP-1. The blue and red arrows point to the three charcoals that present z-values incompatible with the boundaries defined between LSPs. They are correspondent to the arrows in “b”. Note that Burial 11 does not belong to any of the defined periods.



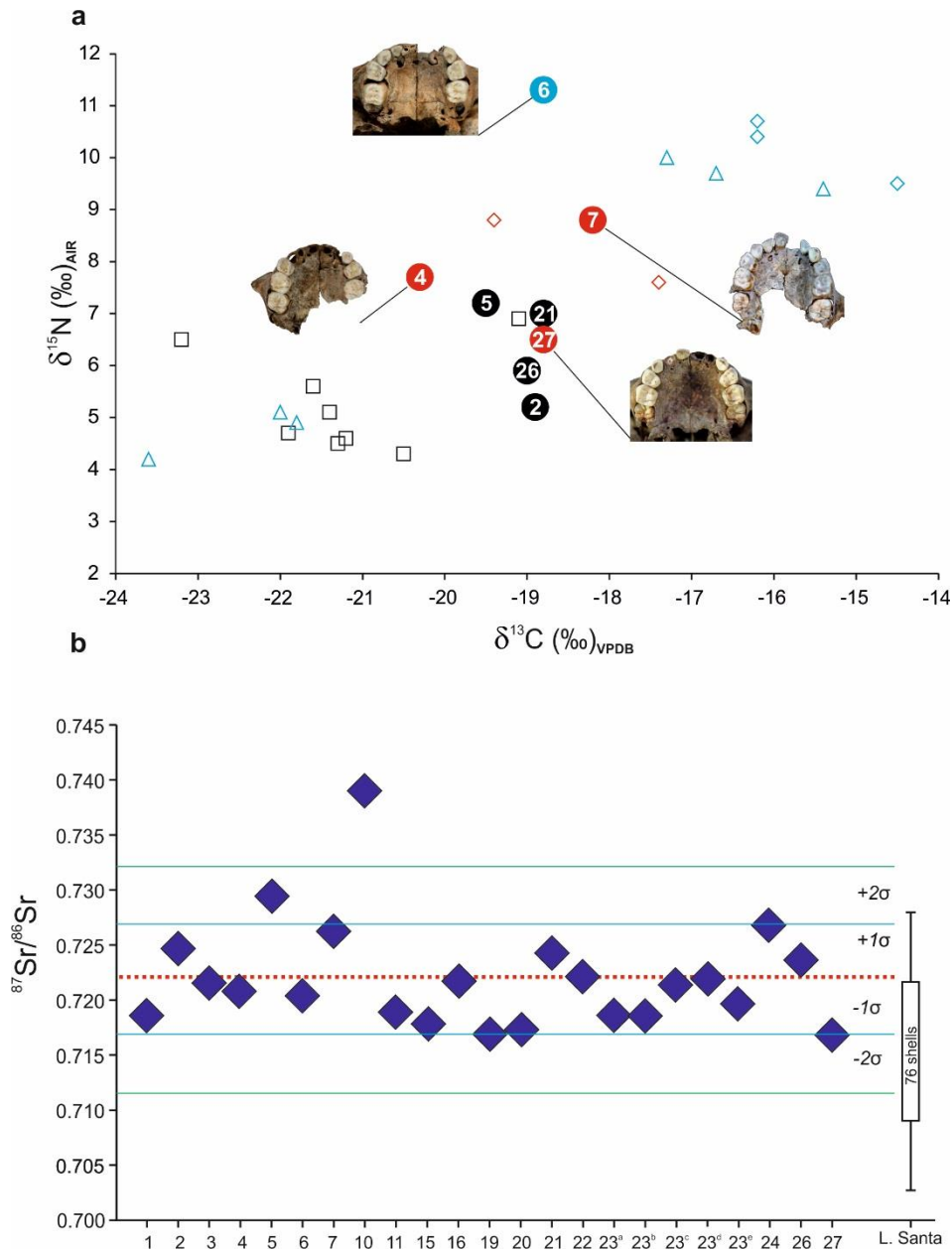


**Figure 4.** Photomicrographs of thin sections from Lapa do Santo. **a**, groundmass made of clay aggregates (ca) in micromass consisting of plant ashes. **b**, well-preserved ash crystals (a). **c**, layer of ash crystals in-between red clay aggregates. **d**, articulated ashes (cp) made of arranged ash crystals that maintain the cell structure of the charcoal. **e**, silicified tissue residue (tr). **f**, yellow clay aggregate with dark red rim suggesting burning.

Zooarchaeological analysis indicates the presence of fish, lizards, rodents, armadillos, peccaries and deer that were brought in single pieces from the killing site (Table 2 and Tables S7-S8, Spearman rho for %MAU vs FUI [n=25] = 0.34; p=0.09; see SI-5). Carbon and nitrogen stable isotope analysis show a slightly enriched  $\delta^{13}\text{C}$  and low  $\delta^{15}\text{N}$  values in the adult population. Nitrogen values are distinct from the carnivores (t=4.50; p=0.001) and similar to the herbivores from Lagoa Santa region (t=0.25; p=0.400), thus suggesting a heavy reliance in  $\text{C}_3$  plant resources (Fig. 5, Tables S9-S10 and SI-6). Together with dental caries frequencies comparable to those observed among agricultural populations (Da-Gloria 2012), the emerging picture is of a typical early Archaic economy structured around plant resources that were probably rich in carbohydrates complemented by hunting of small and mid-sized animals.

**Table 2:** Taxonomic identification of faunal remains from Lapa Santo, Number of Identified Specimens (NISP) and Minimum Number of Individuals (MNI).

Taxon	Common name	NISP	MNI
Amphibia	Frogs	55	5
Aves	Birds	33	4
Fish	Fishes	127	5
Mammals			
<i>Mazama</i> sp.	Brocket deer	137	4
<i>Tayassu</i> sp.	Peccaries	10	1
Carnivora	Carnivore	4	1
<i>Dasybus novemcinctus</i>	Nine-banded long-nose armadillo	31	4
<i>Euphractus sexcinctus</i>	Six-banded long-nose armadillo	6	1
<i>Sylvilagus brasiliensis</i>	Brazilian rabbit	4	1
Didelphidae	Common opossum	13	4
Primate	Non-human primates	3	1
<i>Agouti paca</i>	Paca	6	1
Rodentia	Small rodents	129	23
Reptile			
<i>Chelonia chelidae</i>	Turtle	6	1
	Lizards	54	4



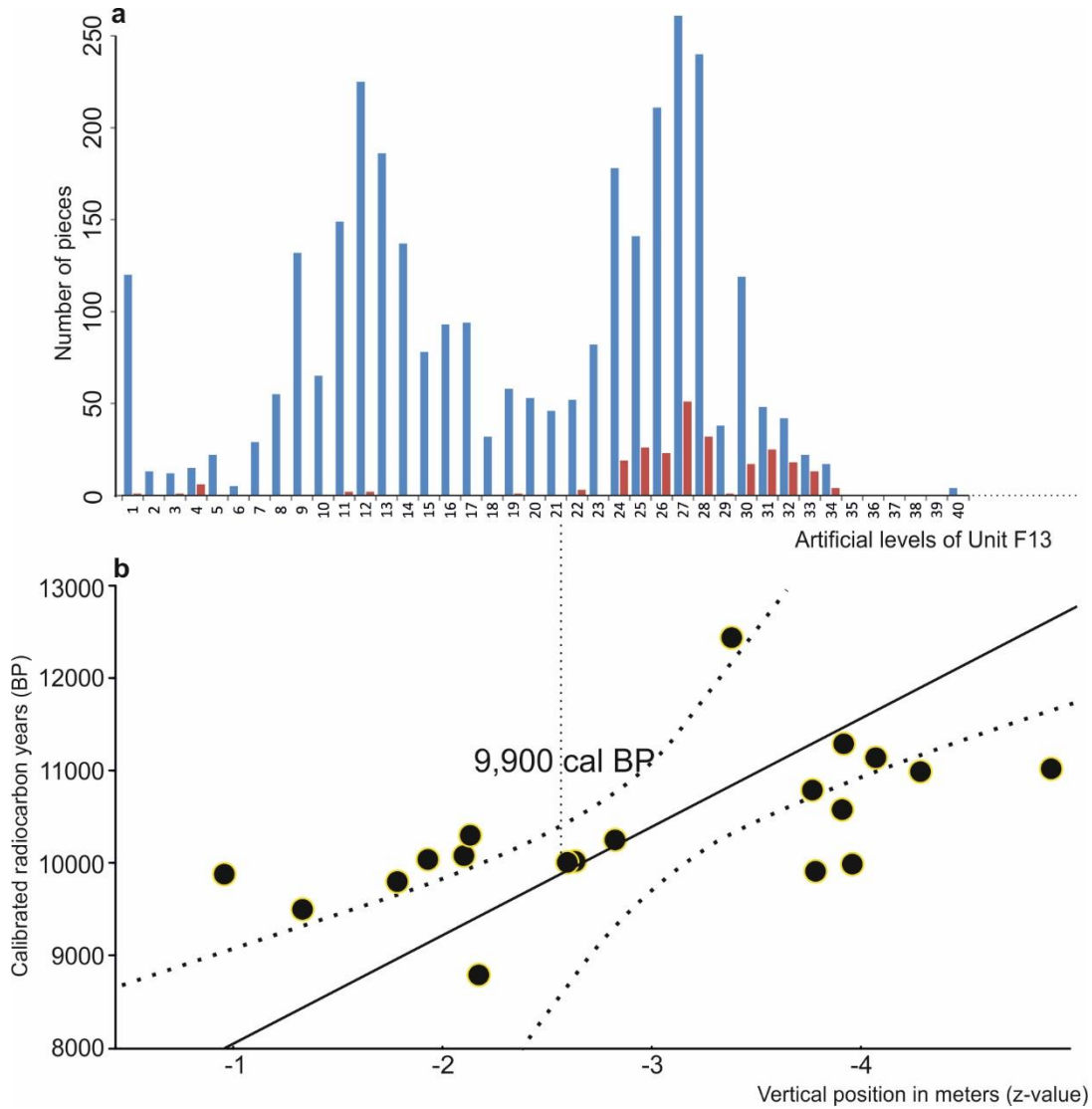
**Figure 5.** Isotopes analyses. **a**,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of bone collagen from humans and animals from Lagoa Santa region. Black squares: *Mazama* sp.; blue triangles: Tayassuidae; blue diamonds: *Dasypus novencintus*; red diamonds: *Euphractus sexcinctus*; black disks: adult humans with fully occluded permanente dentition; red disks: sub-adult humans with erupted permanent first molar; blue disk: sub-adult human with non-erupted permanent dentition. The number inside the black disks indicates the identification number of the burial. Maxilla's photos depict dental development stage for the non-adult individuals. **b**, Enamel's  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio values from the individuals of Lapa do Santo (blue diamonds), plotted on  $^{87}\text{Sr}/^{86}\text{Sr}$  mean ratio value (red dashed line), mean ratio  $\pm 1\sigma$  values (area between blue lines), and mean ratio  $\pm 2\sigma$  values (area between green lines) of the entire human population. The box plot indicates the descriptive statistics (mean, standard deviation and two standard deviations) for the 76 samples of shells characterizing Lagoa Santa strontium bioavailability.

The lithic assemblage is dominated by small flakes and cores (Fig. S1, SI-7). Crystal quartz was by far the dominant raw material, but silex, quartzite and silicified sandstone were also present. There is no clear division between artifact and *debitage* in an industry where every flake was a potential tool. With the exception of a single hematite axe blade (Fig. S2, Table S11) and an arrow point (Fig. S3), formal artifacts made of stone are inexistent in the early Holocene deposits. Flakes were discarded when their edge became dull and most of them were used only a few times. Feather scars, occasional scaler and snap fractures were identified by preliminary use-wear analysis of flakes from Lapa do Santo indicating they were used to cut soft materials such as hides, meat, cordage and grasses (Fig. S6, Table S13 and SI-8). While lithic types were constant through time, the use of raw materials varied and around 9.9 cal kyBP the exploitation of non-local sources such as silexite was drastically reduced with the locally available crystal quartz becoming dominant (Pugliese, 2008)(Fig. 6).

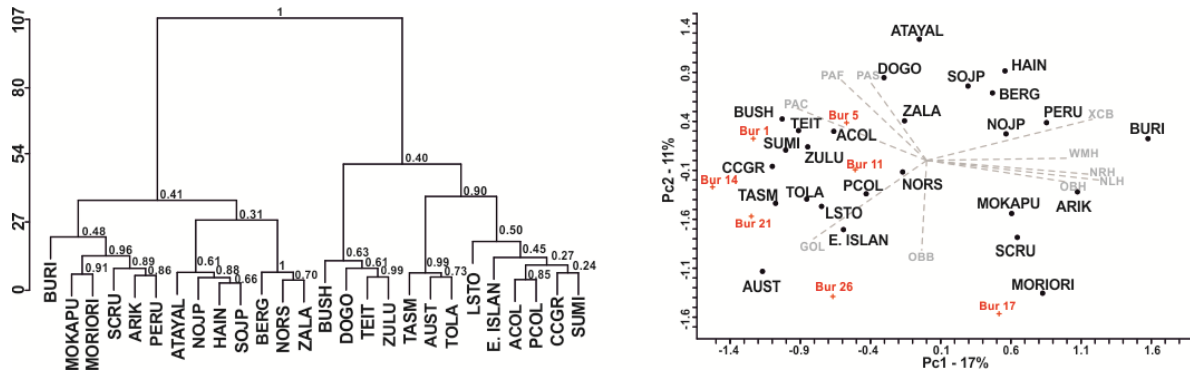
The bone artifacts from Lapa do Santo are very similar to what is observed in other parts of central Brazil during the same timeframe (R. Souza, 2011). They contrast sharply with the expedient technological approach adopted for the production of lithic artifacts. A total of 198 bone artifacts or fragments of bone artifacts were found on the site, including spatulas (71%), burins (25%) and fishhooks (0.01%) (SI-7) (Fig. S4-S5, Table S12).

Human enamel strontium isotopic values that are close to the range of the local bioavailable signature (Fig. 6b, Tables S14-S15 and SI-9) are consistent with a subsistence strategy based on immediately available local resources. Low levels of mobility are also supported by previous studies of the femur's midshaft morphology (Da-Gloria 2012). The aforementioned abandonment of allochthonous raw material for lithic production after 9.9 cal kyBP could indicate the moment when this less mobile mode of life began. Lapa do Santo is also known for presenting an early Holocene low relief rock art record including a pictorial tradition that depicts phallic imagery and birth scenes suggesting the existence of fertility rituals (W.A. Neves et al., 2012).

Analysis of morphological affinities shows that individuals from Lapa do Santo shares a typical Palaeoamerican cranial morphology with other groups of Lagoa Santa region (Fig. 7, Tables S16-S17, and SI-10) and can be, therefore, characterized as typical early Holocene groups from Lagoa Santa.



**Figure 6.** Chronological behavior of lithic abundance and type of raw material in Lapa do Santo during early Holocene. **a**, Histogram of quartz (blue bars) and silexite (red bars) abundance along Lapa do Santo's unit F13 stratigraphy. Note that after level 22 silexite, which is an allochthonous raw material, is no longer used in the site. **b**, The event identified in the histogram are dated by comparing its vertical position (z-value) with the calibrated radiocarbon dates from charcoals (black disks) from unit F13 and the adjacent unit G13 in correspondent vertical position. The x-axis of both graphs are in aligned and in the same scale.



**Figure 7.** Morphology of Lapa do Santo crania. Left: Wards cluster based on Mahalanobis distance between craniometric data from worldwide populations and Lapa do Santo (see Supplementary Material for details). The frequencies associated to each branch represent how strong the clusters are, based on 1000 bootstraps of the data. Right: Scatterplot of the series according to the first two Principal Components extracted from the complete dataset. Centroids are plotted in black; Lapa do Santo individuals are plotted in red. The gray lines and labels represent the correlations between the original variables and each of the PCs. Only correlations larger than 0.5 were plotted. Correlations are scaled to the axis dimensions, i.e., perfect correlations ( $r=1.0$  or  $r=-1.0$ ) would touch the limits of the graph.

### THE MORTUARY PATTERNS OF LAPA DO SANTO

The 26 human burials from Lapa do Santo were divided into six different mortuary patterns based on their chronology and shared features. Lapa do Santo Mortuary Patterns (LSMP) 1, 2 and 3 are the focus of this contribution. Age and sex estimation were done by a single trained observer (Tables S18; SI-11). LSMP-1 is dated to 9.7-10.6 cal kyBP and is characterized by two primary single burials in flexed position (Burials 1 and 27) (Fig. 8).



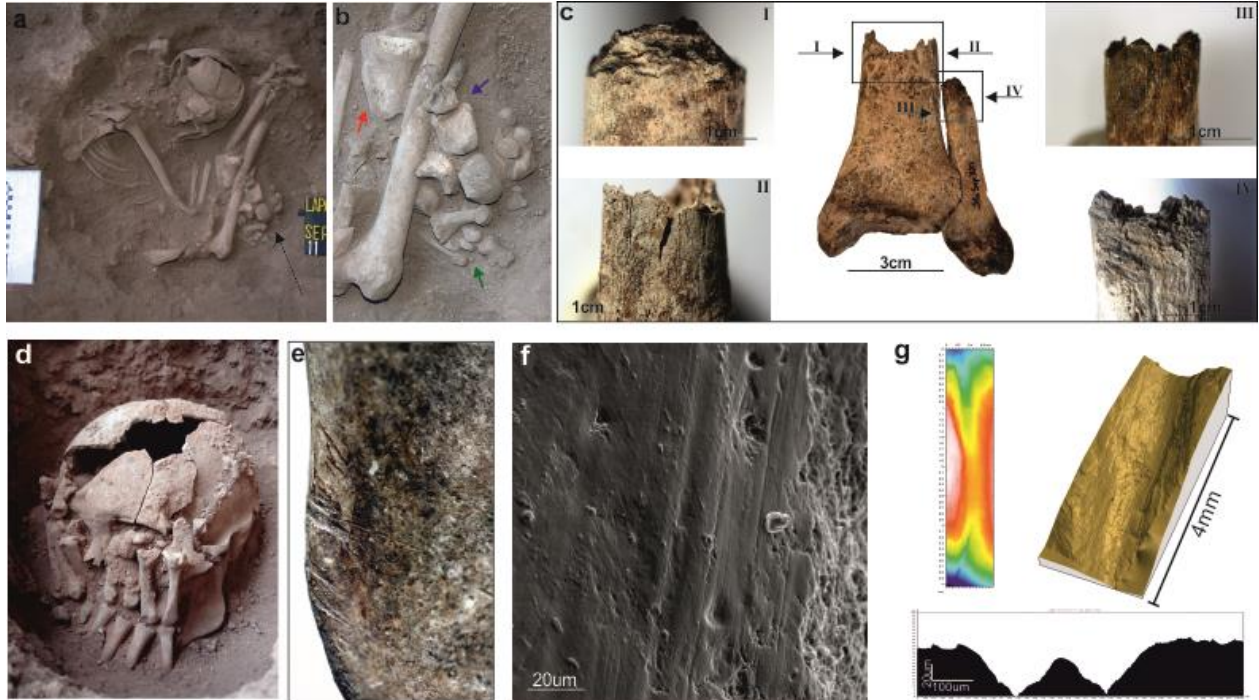
**Figure 8.** Lapa do Santo Mortuary Pattern 1. a, Picture of Burial 1 after the stones that were covering the skeleton were removed. b, Picture of the north-east corner of unit L11 during the initial stages of exhumation of Burial 27. c, Picture of Burial 27 with an open mandible indicating the grave was not fully filled with sediments after inhumation.

Lapa do Santo Mortuary Pattern 2 (LSMP-2) is dated to 9.4-9.6 cal kyBP and can be further subdivided into three categories: LSMP-2a (Burial 21 and 26), LSMP-2b (Burials 9, 14, 17, 18 and 23) and LSMP-2c (isolated bones). LSMP-2a is characterized by fully-articulated partial skeletons with cutting and chopping marks. In Burial 21, the midshafts of both tibiae and fibulae were chopped and removed while soft tissue was still present (Fig. 9a-c). Burial 26 is a decapitated head with the first six cervical vertebrae articulated in anatomical position. The hyoid bone was missing and both amputated hands were laid over the face (Fig. 9d). Scanning electron microscopy and confocal microscopy of the cut-marks (Fig. 9e) show the presence of parallel micro-striations and a V-shaped transversal profile, indicating the use of stone flakes as the cutting agent (Fig. 9f-g). In conjunction, the evidence shows LSMP-2a involved the mutilation of fresh corpses.

LSMP-2b is characterized by graves filled with the fully disarticulated bones of up to five individuals presenting a strong selection of anatomical parts. Some bones show evidence of exposure to fire, application of red pigment, defleshing, cutting, chopping and removal of teeth. Burials 14, 17 and 18 were composed of a bundle of long bones from one or two individuals, deposited with the individualized cranium and/or mandible of a different individual. Bundles comprising infant post-cranial bones were found next to adult crania (Burials 14 and 17, Fig. 10a-b), and bundles comprising adult post-cranial bones were found next to an infant cranium (Burial 18, Fig. 10c). The long bones of the bundles had been chopped and segregated into extremities (Fig. 10d) and midshafts (Fig. 10e) and in some cases the latter were further burned and chopped into smaller sections (Fig. 10f). The calvaria of Burial 17 was used as a funerary receptacle (Fig. 10g) and filled with chopped burnt bones (Fig. 10f) some of which present defleshing cut-marks (Fig. 10h). Black burn marks limited to the anterior portion of the external maxillary alveolar margin indicate exposure to fire while soft tissue was still present in the face (Fig. 10i). The co-occurrence of chopped and defleshed bones with signs of burning with soft tissues suggests that LSMP-2 may have involved some form of cannibalism.

In Burials 17 and 18, all teeth were intentionally removed (Fig. 10j) and the coronoid processes of Burial 18's mandible were drilled (Fig. 10k). Red pigment was abundantly applied to the bones of Burial 14 (Fig. 10l) and Burial 18. Burial 23 was composed of a cranium *calotte* filled with 54 permanent and 30 deciduous teeth, some of which belonged to the skull of Burial 17. Burial 9 was an individualized child skull placed near the pelvis of an individual of similar age. The deciduous dentition was removed and an assemblage of human teeth and chopped midshafts (accession code: LSt-2253) were deposited next to Burial 9 (Fig. S7). LSMP-2c is defined by isolated burnt chopped bones that were not part of any formal burial (Fig. 11). In the case of the distal left femur (LSt-3083), the burning mark exclusively affect the area close to the chopped extremity of the bone (Fig. 11a), a feature indicative of exposure to fire while meat was still adhering to it (known as 'Bratenspur' in the Germanic specialized literature (Kokabi,

Amberger, & Wahl, 1994; Wahl, 1994)). The same bone presents gnaw marks (Fig. 11e-11f) suggesting it was subject to scavenging and not immediately buried.

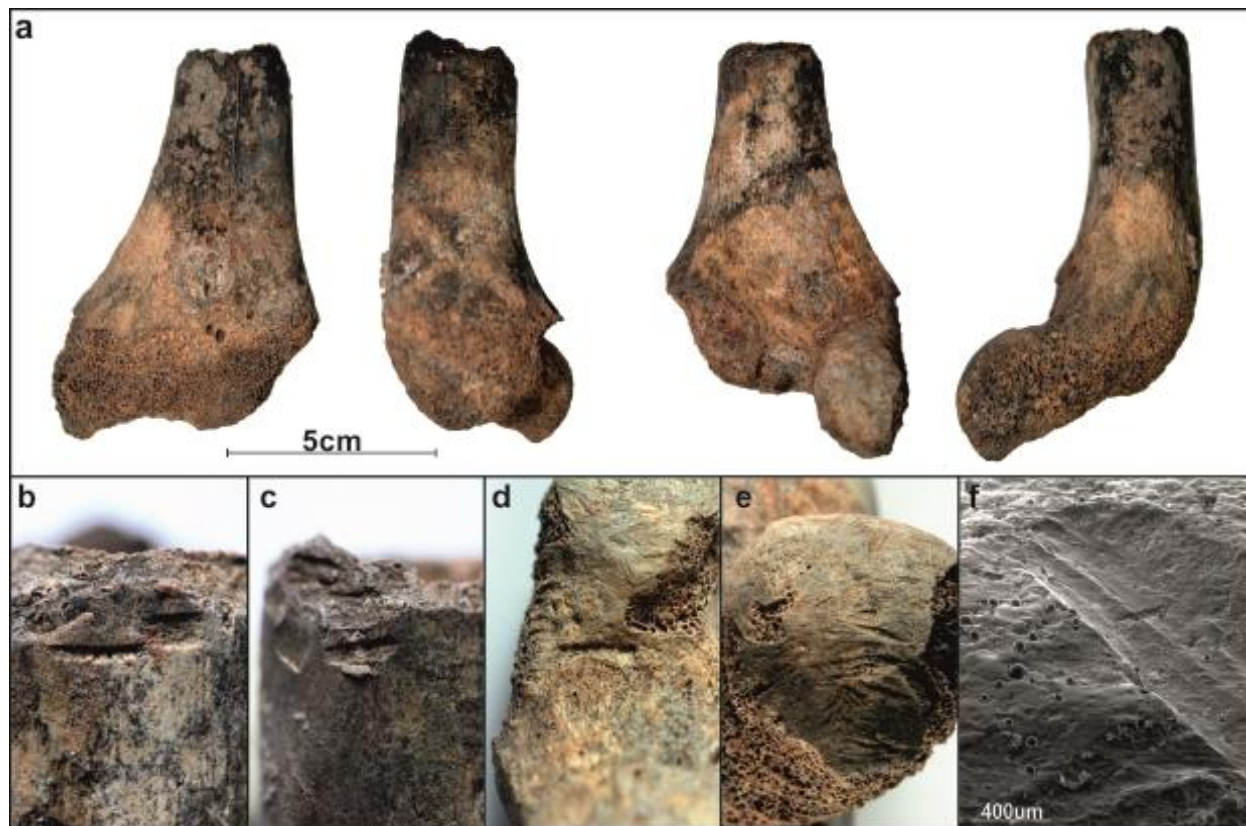


**Figure 9:** Lapa do Santo Mortuary Pattern 2a: **a**, Burial 21 from Lapa do Santo after removing stones covering the grave. Note that although fully articulated the midshafts of the lower legs were absent (black arrow). **b**, Detail of Burial 21's bones that were directly involved in the mutilation processes. The distal extremities of the chopped left tibia and fibula (blue arrow) and the bones of the feet (green arrow) were articulated. The proximal extremity of the right tibia (red arrow), however, was not in connection with the distal extremity of the right femur. **c**, Distal extremities of the articulated left tibia and fibula. Note that only the portion of the bones that would have been in contact with the skin (I and IV) presents chop marks confirming cutting took place while soft tissues were still present. **d**, Burial 26. Picture of the decapitated head with the hands resting over the face. **e**, Burial 26's right mandibular ramus exemplifying the cut marks found in the bones. **f**, Scanning electron microscopy of the cut-mark in Burial 26's mandible showing parallel micro-striation. **g**, Top: Topography and digital 3D model of a cut-mark in Burial's 26 mandible generated using confocal microscopy. The parallel micro-striations can also be observed. Bottom: Cut-mark transection depicts a V-shaped profile.



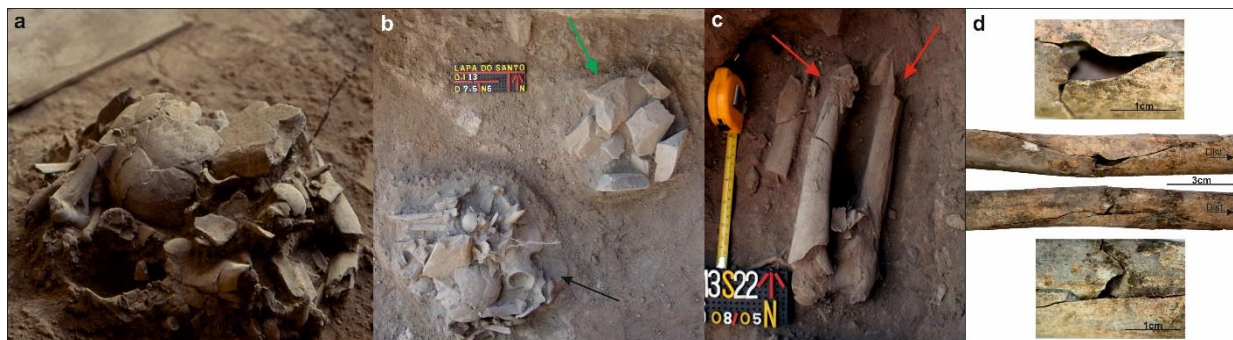


**Figure 10: Lapa do Santo Mortuary Pattern 2b:** **a**, Field picture of Burial 14 showing the individualized adult cranium (yellow arrow) next to a bundle of post-cranial bones of two infants (black arrow) **b**, Field picture of Burial 17 showing the individualized adult cranium (yellow arrow) next to a bundle of post-cranial bones of infants (black arrow). The purple and green arrows indicate the left ulna and humerus that are shown in “d” and “h”, respectively. Note that inside the cranium several bones can be observed. **c**, Field picture of Burial 18 showing a bundle composed of four chopped midshafts from adult long bones (same as in “e”) that was deposited within the mandible of an infant (same as in k). **d**, sectioned distal extremity of left ulna found inside Burial 17’s cranium. In the detail, chop marks associated with sectioning processes. **e**, from left to right sectioned midshafts of the left humerus, the right humerus and the right radius. **f**, chopped sections of burnt long bones that were deposited inside Burial 17’s neurocranium. **g**, The neurocranium of Burial 17 was used as a funerary receptacle inside which chopped and/or burnt bones of the same individual were deposited. **h**, Mutilated distal extremity of the left humerus from Burial 17 presenting defleshing marks (I-IV) and chop marks (V- VI). **i**, Burial 17 maxilla has burn marks concentrated in the anterior portion of the external alveoli margin. **j**, Burial 17’s maxillary dentition was intentionally removed prior to the interment. **k**, Mandible of Burial 18 had teeth removed and holes drilled in its coronoid processes (see detail). **l**, Calvarium of Burial 14 with red pigment and missing face.



**Figure 11: Lapa do Santo Mortuary Pattern 2c.** Chopped distal extremity of left femur (LSt-3083) found isolated from other human remains. **a**, From left to right anterior, lateral, posterior and medial views, respectively. **b,c**, detail of chopping marks in the anterior and posterior portion of the sectioned margin, respectively. **d**, incisions present in the posterior region near the articular surface. **e**, gnawing marks in the articular surface of the medial condyle. **f**, scanning electron microscopy of the gnawing marks.

LSMP-3 is dated to 8.2-8.6 cal kyBP and includes nine burials: 6, 7, 10, 11, 12, 13, 15, 19 and 22. Burials are characterized by shallow circular pits completely filled with mostly disarticulated bones of single individuals of various ages and sexes (Fig. 12a). Circular stone structures covered some of the burials, but also occur independently of them (Fig. 12b). Anatomical selection was not observed and, with the exception of some small bones, most elements of the skeleton were present. The midshafts of long bones of adult individuals were in some cases intentionally broken in the central region before deposition (Fig. 12c), resulting in butterfly fractures with impact points indicating the use of some percussion instrument (Fig. 12d). The burials belonging to LSMP-3 are very similar to each other, contrasting the larger variability observed within LSMP-2. Furthermore, characteristic elements of the latter, such as cut-marks, chop-marks, the absence of dentition, red pigment, and burnt marks are not present in the former.



**Figure 12.** Lapa do Santo Mortuary Pattern 3. a, LSMP-3 was characterized by circular pits completely filled with the non-articulated bones of a single individual. b, Burial 15 (black arrow) is typical of LSMP-3. The picture was taken after the circular structure composed of stones covering the grave was removed. The green arrow points to a circular structure of stones that was not directly associated with any burial. c, Long bones at the bottom of Burial 22's grave exemplify the practice of breaking the midshafts (midshafts). d, Humerus of Burial 22 exemplifying butterfly fracture and impact points (details above and below).

## DISCUSSION

### *Lapa do Santo Mortuary Record*

During the Archaic period in South America, burying the dead within the domestic space was a common practice (Rossen & Dillehay, 2001). Accordingly, Lapa do Santo was not exclusively used as a burial site and cannot be classified as a formal cemetery (Goldstein, 1976; Pardoe, 1988; Saxe, 1970). The accumulation of burials there must be understood as a long-term process resulting from the association between formal prescriptions for treating the dead and the nature of Lapa do Santo as a “persistent place” (*sensus* Schlanger (1992)) that was repeatedly visited due to its unique features in the landscape (Littleton & Allen, 2007). This rockshelter is better classified as an area intentionally maintained for burying the dead (*sensus* Littleton and Allen 2007) rather than a formal cemetery.

Nevertheless, the funerary agents were following formal prescriptions in dealing with death. Therefore, Lapa do Santo presents a scenario where highly formalized mortuary prescription occurs without the existence of formal cemeteries. Since non-articulated burials are rarely related to social organization or economic demands (Binford, 1971; Saxe, 1970; Tainter, 1978), Lapa do Santo mortuary record should not be seen as determined by the social organization of these groups but rather as a response to unique ideational and metaphysical concepts (Carr, 1995; Goldstein, 2000; Schroeder, 2001).

More specifically, LSMP-2 can be interpreted as the result of a funerary ritual based on principles of secundarization (Hertz, 1907; Huntington & Metcalf, 1979), in which LSMP-2a represents the initial phase, LSMP-2b the final disposal of the remains and LSMP-2c the by-product of the dismemberment process. The careful organization of bones and body parts in deliberate arrangements is compatible with an intermediate phase in which curation and public

display were essential elements to enhance the visual communication of the ceremonies (Chesson, 1999; Kuijt, 1996; Rakita & Buikstra, 2005). This kind of ritual is traditionally understood as a mechanism to increase social cohesion during times of disruption.

The strict observation of such specific and technically demanding procedures suggests the existence of specialized funerary agents. In ancient societies, hard organic residues constituted a potent cultural resource, and the manipulation and organization of bones and body parts were commonly used to reify cosmological principles (Goldstein 2000; Brown 2010). In LSMP-2 this seems to have been centered on a “dichotomic principle” that was expressed by pairs of opposition among abstract categories such as “adult” and “sub-adult”, “cranium” and “post-cranium”, “diaphysis” and “extremities”, and “teeth” and “empty alveoli”.

The organization of disarticulated bones into meaningful arrangements or conceptual designs as observed in LSMP-2b probably mirrors key features of *the cosmology of those groups concerning the origin and organization of their universe* (Brown 2010:32). We may never be able to unveil what precisely these cosmological principles are but being able to visualize their reification with so many details is already a privilege that has not been frequently granted to early Holocene archaeologists in South America until this point. Complex mortuary rituals as those of LSMP-2 allows to approximate much as possible the plotline contained in myths that give access to the cosmological world of those past societies (J. Brown, 2010; Goldstein, 2000). It seems that the “dualistic” principle that has been long recognized as central in both the Andes (Rostworowski, 1983) and the Amazon region (Maybury-Lewis, 1979; Viveiros de Castro, 1992) was already present among the earliest inhabitants of South America.

LSMP-3, on the other hand, is believed to result from the practice of “delayed burials”. The body would have been allowed to decompose elsewhere until almost complete decay. The bones were then brought to Lapa do Santo and deposited in a circular pit. In the case of adults, some long bones required breaking to ensure that the skeleton would fit inside the grave. LSMP-3 hardly fits any available model relating secondary burials and mobility patterns in hunter gatherers. In principles, the high frequency of secondary burials in LSMP-3 (100%) would point to a logistic organization in which seasonal collectors explored vast territories (Charles & Buikstra, 1983). However, in LSMP-3 secondary burial was as mandatory for logistically active individuals as it was for the group as a whole, making the causal link between the first and the second feeble. Therefore, LSMP-3 seems to be a case in which the delay of interment is not related to “situational circumstances” but with formal mortuary prescriptions applied to the entire group. One possible interpretation is that the delay was due to the intent of using the funerary rituals within rockshelters as special events of aggregation at a certain periodicity of time.

*Diachronic changes the mortuary practices*

It has been traditionally assumed that all early Holocene burials from the Lagoa Santa region belonged to a single highly homogenous mortuary pattern (W.A. Neves & Hubbe, 2005). The findings from Lapa do Santo show this is an incorrect assumption resulting from the lack of a proper chronology (Chapman, 2005; O’Shea, 1984) for the skeletons of the region. On the contrary, when the variety of burials is properly partitioned, it reveals a short-term succession of funerary patterns over time.

Recently, it has been argued, based on the analysis of stone tools, that some prehistoric foragers groups in South America were remarkably static through time. Accordingly, this would be compatible with the existence of a “novelty-avoidance” mechanism among those groups (Okumura & Araujo, 2014). In this regard, Lapa do Santo is an enlightening case. Considering stone tool types alone, one could reach the same conclusions about cultural stasis. It is only when the Lapa do Santo mortuary record is considered, however, that the dynamic nature of these groups, in constant transformation over a period of centuries, is perceived (Cannon et al., 1989; Chesson, 1999; Kuijt, 1996; O’Shea, 1984; Okumura & Araujo, 2014). At this point, however, it is not possible to assert whether these changes were the result of internal transformations in the social organization of these groups or the arrival of newcomers to the Lagoa Santa region or even whether they reflect a process of cultural drift.

Others sites in South America such as OGSE-80 (Stothert, 1985); Cuchipuy (Kaltwasser, Medina, & Munizaga, 1986), Arroyo Seco 2 (Pucciarelli et al., 2010) and Justino (Vergne, 2002) seem to present a very similar pattern where one locality is used for millennia by different and successive groups as a mortuary site. In other cases, however, the same mortuary pattern is observed for long periods of time as is the case for Baño Nuevo-1 (Mena, Reyes, Stafford, & Southon, 2003), Paloma (Quilter, 1989) and the Chinchorro (Arriaza, 1995). This variability in the chronological behavior of the South American archaeological record helps explain why the old debate around the temporal stability of the mortuary rituals has never been adequately settled (Cannon et al., 1989; Chesson, 1999; Deetz & Dethlefsen, 1971; Kroeber, 1927; Kuijt, 1996; Metcalf, 1981; O’Shea, 1984; Saxe & Gall, 1977; Ucko, 1969; Walthall, 1999).

*Lapa do Santo in the regional landscape*

After more than 170 years of archaeological research in the region, with hundreds of exhumed early Holocene skeletons, a consensus was reached in the literature according to which the mortuary rituals of Lagoa Santa during that period were characterized for their simplicity, homogeneity and stability through time (W.A. Neves & Hubbe, 2005; W.A. Neves, González-José, et al., 2004; Walter, 1958). Based on this traditional view of Lagoa Santa’s

mortuary rituals, one could postulate that Lapa do Santo was a regional center where particularly elaborate funerals were reserved for special individuals. In this scenario, the other sites in the region would represent locations where more common burials took place.

However, when the sparse descriptions of the burials made by previous scholars are scrutinized it becomes apparent that they were misleading. In the Cerca Grande complex, excavated in 1956 by Wesley Hurt and Oldemar Blasi, only primary single burials were originally reported (Hurt & Blasi, 1969). However, researchers who later studied the collection spotted several cut-marks in the human bones (Messias & Mello e Alvim, 1961) and reported burials with multiple individuals (Da-Gloria, 2012a). Similarly, it is likely that the isolated skull with a huge fracture exhumed from Lapa das Limeiras (Walter, 1958) and the “molehills” composed of the skull of a child and the skeleton of an elderly woman found in the second chamber of Lapa da Samambaia (Bányai, 1997) are analogous to the individualized skulls described here for LSMP-2b (i.e. burials 9, 14, 17, 18 and 23).

In the site of Confins, “pipes” crafted from a burned human femur were reported (Walter, 1958). Notwithstanding, from the available pictures (Walter, 1958) it is clear that these pipes are identical to the isolated burnt bones from LSMP-2c. Corroborating this interpretation, a total of 23 chopped adult bones identical to those of LSMP-2 were identified in the Harold Walter collection (Da-Gloria *et al.* 2011). In the case of Confins, Cerca Grande and for the bones of the Harold Walter collection this association is further supported by the existence of directly dated bones that are peri-contemporaneous with LSMP-2. This supports the notion that the pattern described for LSMP-2 was relatively widespread in Lagoa Santa and not restricted to Lapa do Santo at 9.4-9.6 cal kyBP.

Besides these cases of chopped or isolated bones, descriptions of highly fragmented skeletons within a small grave covered by a layer of stones are also known from the literature (e.g. Lapa da Mãe Rosa, Lapa da Limeira, Terraço do Sumidouro, Cerca Grande 5 and Cerca Grande 6)(Hurt & Blasi, 1969; Walter, 1958). It was always assumed that such high levels of fragmentation were a by-product of the overload due to the heavy limestone blocks. However, in the face of the findings from Lapa do Santo it might well be the case that in many sites these “highly fragmented” burials were analogous to LSMP-3. At least in some of these sites, direct dating of bones from these sites roughly falls in the same chronological range of LSMP-3. Future studies looking for *perimortem* fractures among skeletons from Lagoa Santa will allow to define if this association is indeed correct.

The presence of diverse and elaborated mortuary practices in other sites in the region other than Lapa do Santo supports the notion that the traditional view of Lagoa Santa’s mortuary practices as simple and homogenous results from misinterpreting the archaeological record. At the same time, this makes it unlikely that Lapa do Santo was any type of ceremonial center in the mortuary landscape of the region.

*Continental perspective of Lapa do Santo*

In South America, during the early Holocene, funerary rituals focusing on manipulating and transforming the human body are better characterized in the Andean region where a relatively abundant number of sites is known (Santoro et al., 2005). In the east of the continent, however, the evidence is scarcer. For this reason, sometimes the manipulation of the body as part of funerary rituals was assumed to be an Andean phenomenon preceding the rituals arising later with the emergence of complex societies (Cordy-Collins, 1992; Ferguson, 1990; Verano et al., 1999). Lapa do Santo location in eastern Brazil, however, contributes evidence for expanding the geographical range of such practices to the Brazilian lowlands and suggests a more complex scenario for its development in the continent (Llagostera, 2003).

Compared to the closest sites, it seems that Lagoa Santa constituted a coherent and localized cultural unity. Santana do Riacho (ca. 150 km distant from Lapa do Santo) is well known for presenting burials estimated to be ca. 9.0kyBP (non-calibrated) and, therefore, contemporaneous with LSMP-1 and slightly older than LSMP-2. The burials from Santana do Riacho are characterized by fully articulated skeletons in flexed position. The comparison, however, is contested due to different lines of interpretation of the burials of Santana do Riacho. Some authors state that there was some sort of body manipulation (Lavallée, 1995) while others categorically disagree with this (Prous, 1992b).

Farther away from Lagoa Santa the site of Capelinha (Vale do Ribeira - São Paulo)(Eggers, Parks, Grupe, & Reinhard, 2011) and Toca dos Coqueiros (Serra da Capivara - Piauí)(A. R. Nelson, 2005) have burials roughly contemporaneous with LSMP-1 (although the last was not directly dated). In both cases fully articulated flexed skeletons were found in association with projectile points. In Justino (Xingó - Sergipe) four articulated and one 'secondary burial' were associated with a charcoal dated to 8.9kyBP (non-calibrated) (Vergne, 2002). These sites are too far from each other and from Lagoa Santa to allow for any kind of direct connection to be postulated. Nevertheless, in this period rock art from sites as distant as those mentioned above already shared very clear stylistic elements (W.A. Neves et al., 2012) and therefore it is not impossible that the similarities between mortuary practices reflect some kind of shared cultural ancestry. Anyhow, although the limited available evidence must be interpreted with caution, it seems that during the early Holocene, Lagoa Santa was the only place where funerary rituals such as those represented by LSMP-2 and LSMP-3 were taking place in Brazil.

After 8.0kyBP some eastern South Americans sites do show evidence of rituals involving body manipulation and reduction. In Burial 1 from Lapa do Malhador (Vale do Peruaçu - Minas Gerais)(Prous & Schlobach, 1997) the right arm of an entirely articulated skeleton was amputated leaving a profusion of cut-marks in the bones. This burial is assumed to be middle

Holocene in age and therefore younger than LSMP-2 but in the absence of a direct date, an earlier date cannot be ruled out. Loca do Suin (PAINS – Minas Gerais) has two burials directly dated to 7.4 and 7.5 kyBP including a case of cremation and of the presence of multiple individuals (A. M. Strauss et al., 2011).

From a broader continental perspective the presence of mortuary rituals focusing on reducing the body in sites thousands of kilometers from each other such as Arroyo Seco 2 (Pucciarelli et al., 2010), Pampa de los Fósiles (Chauchat & La Combe, 1984), Lauricocha (Cardich, 1964; Fehren-Schmitz et al., 2015), Huchichocana Cave, La Chimba and La Fundación (Costa-Junqueira, 2001), Baño Nuevo (Mena et al., 2003), Tequendama (W.A. Neves, Hubbe, & Correal, 2007) and Lapa do Santo shows that this was a common practice in the continent dating as far back as the early Holocene.

More specifically, LSMP-2 finds similarities with the mortuary record of the sites of the Sabana de Bogotá region in Colombia, especially Aguazuque (Correal, 1990). In this site, the funerary rituals are also characterized by a strong selection and re-allocation of different parts of the human body, including the individualization of human skulls and the sectioning of long bones to separate the diaphysis from its extremities. It is hard to appreciate to what extent such similarities are the result of random factors and to what extent they reflect a common cultural background. It is worth remembering that craniometric studies point to a very close genetic relationship between Lagoa Santa and Sabana de Bogotá populations, suggesting that some link or shared ancestry should be at least considered as a possibility.

## CONCLUSION

Lagoa Santa is known since the first half of the 19<sup>th</sup> century when the Danish naturalist Peter Lund first explored the caves of the region. This is the only location in Brazil and one of the few in the entire continent where well-preserved, securely dated and abundant human burials – as well as rock art - from the early Holocene occur in association with more ubiquitous archaeological reminiscent such as stone tools, animal bones and combustion structures. Research in the region in the last 170 years has focused on two topics: i) co-existence of humans and megafauna and ii) distinct cranial morphology when compared to Native Americans.

Without denying the relevance of these topics, other aspects of the archeological record were neglected and, sometimes, even damage as a result of this narrow perspective. The result was a limited and biased view of the groups inhabiting the region during the early Holocene, which were characterized as simple groups that were homogeneous through time. The present contribution is part of a broader effort to promote a paradigmatic shift in the archeological research in Lagoa Santa in which the reconstruction of human behavior and its diversity among past hunter-gatherers is emphasized (Neves *et al.* 2012; Aruajo *et al.*, 2013; Da-Gloria and Larsen 2014).



The archaeological record of Lapa do Santo indicates that between 12.7-11.7 cal kyBP and 8.3-8.0 cal kyBP the region was occupied by early Archaic generalized foragers. Lithic technology, zooarchaeology, and multi-isotopic analyses indicate groups of hunter gathers with low mobility and a subsistence strategy focused on gathering plant foods and hunting small and mid-sized animals. Their technology and subsistence strategy were very efficient as is shown by their long temporal continuity over more than three millennia. The use of Lapa do Santo as an interment ground started between 10.3-10.6 cal kyBP with primary burials. Between 9.4-9.6 cal kyBP the reduction of the body by means of mutilation, defleshing, tooth removal, exposure to fire and possibly cannibalism, followed by the secondary burial of the remains according to strict rules, became a central element in the treatment of the dead. In the absence of monumental architecture or grave goods, these groups were using parts of fresh corpses to elaborate their rituals, showing this practice was not restricted to the Andean region at the beginning of the Holocene. Between 8.2-8.6 cal kyBP another change occurred whereby pits were instead filled with disarticulated bones of a single individual without signs of body manipulation.

The stability over time and the monotony of lithic types identified in Lapa do Santo and in other sites of the region were considered to indicate a low degree of cultural diversity. This view was epitomized when the term 'People of Luzia' was coined to refer to all groups that inhabited the region during the early Holocene. However, the shared use of a specific techno-functional strategy might not be the best proxy of cultural identity. In Lapa do Santo, as described here, the mortuary practices were in constant transformations along relatively short periods of time. It shows how a purely technologically oriented interpretation of the past can obliterate the elevated cultural diversity that was already established in the region by the beginning of the Holocene. It is proposed, therefore, that the traditional notion of a single 'people of Luzia' should be abandoned. Instead, during the early Holocene Lagoa Santa region was inhabited by 'many peoples' that were culturally diverse and, tentatively, indicative of the early occurrence of regional processes of ethnogenesis.

These groups were also traditionally depicted as presenting simple and expedient funerary rituals. However, the evidence from Lapa do Santo and the critical reappraisal of the literature presented here show that in the absence of monumental architecture or grave goods they elaborated their funerary rituals through the manipulation and organization of body parts. In a regional perspective Lagoa Santa is unique in presenting these elaborated rituals and there is no other location in Brazil where similar practices can be found for this timeframe. In a broader continental perspective similar instances are known to occur both along the Andes as well as in the Pampas.

Nevertheless, the scarcity of Brazilian sites presenting early Holocene human skeletons prevents any further attempt of identifying significant regional patterns. Hence, it is crucial to

change this scenario by identifying and excavating such sites. This general paucity of skeletal remains has traditionally been attributed to acidic soils precluding their preservation. Although this must certainly be taken into account, there are other important factors too, not least the vast scale of the region and, most importantly, the relatively recent beginnings of systematic research. If this gap is to be filled over coming decades research in the region must expand and intensify.

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## SUPPORTING INFORMATION<sup>1</sup>

- SI 1 - Location and excavations methods
  - SI 2 - Chronology
  - SI 3 - Formation Processes
  - SI 4 - Diatom analysis
  - SI 5 - Zooarchaeology
  - SI 6 - Carbon and nitrogen isotope data
  - SI 7 - Lithic and bone technology
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  - SI 10 - Cranial morphological affinities
  - SI 11 - Sex and age estimation
- 

<sup>1</sup> Tables and figures are displayed after the supplementary text.

## 1. LOCATION AND EXCAVATIONS METHODS

Lapa do Santo is located in Lagoa Santa region, eastern central Brazil (Figure 1). Lagoa Santa is an environmentally protected area comprising 360 km<sup>2</sup>. The vegetation is dominated by savanna (*cerrado*) and semi-deciduous forest. The rivers Mocambo, Samambaia, Jaguará and Gordura make up a tributary net that flows west to east to Velhas River, the main river in the area. Geomorphologically Lagoa Santa is a karstic terrain that can be divided into four distinct domains: 1) below 660 meters above sea level (masl) the terrain is characterized by a fluvial plain connected with the regional base level (Velhas River); 2) between 660 and 750 masl there is a karstic plain with dolines and lakes 3) between 750 and 850 masl there are karstic plateaus characterized by the presence of limestone outcrops (reaching up to 75 meters in height); 4) above 850 masl residual peaks composed of the non-soluble metasedimentary rocks from the Serra da Santa Helena Formation.

The Lagoa Santa region geology comprises Sete Lagoas Formation and Serra da Santa Helena Formation, both part of the Upper Proterozoic metasediments of the Bambuí Group (Viana, Kohler, & Tavares, 1998) of the São Francisco craton. This cratonic cover metamorphosed during the Brazilian Cycle (700-450 million years ago) in a process that resulted in planar structures, such as lineation and foliation, and sub-vertical structures, such as normal and revert faults. The combination of these structures provides the path for the geomorphologic evolution that leads to the rockshelter configurations found in the region. The regional rockshelters and outcrops are developed in the limestone of the Sete Lagoas Formation. More specifically, Lapa do Santo rockshelter developed in the Member Pedro Leopoldo that is composed by very pure limestones with more than 90% calcite (Viana et al., 1998).

The annual mean temperature is 23°C, with lower temperatures (11°C) occurring between June and July and higher temperatures (35°C) occurring between October and November. The average humidity is around 65% in the dry season, from May to September, and around 85% on the rainy season, from November to April, with a pluviometrical mean of 1,400 mm/year. The major climatic characteristic of this region is the high concentration of rain during the rainy season (93% of total volume). When evaporation is analyzed, the region presents an annual deficit of 176 mm (Piló, 1998b). Despite these particular variations, the regional climate is classified as tropical, with a rainy summer and dry winter (Nunes, Vicente, & Candido, 2009). During dry periods, the above ground water sources can become very scarce although underground drainages are capable of keeping the discharge in Velhas River.

The first human bones from Lagoa Santa were found by Peter Lund between 1835 and 1843 (Astolfo G.M. Araujo et al., 2005; Cartelle, 1994; Luna, 2007; Lund, 1844; Piló & Auler, 2002). Due to the putative coexistence of man and megafauna Lagoa Santa became a well-known region for 19<sup>th</sup>-century scholars (Hansen, 1888; Hrdlička, 1912; Kollman, 1884; Ten Kate,



1885). During the 20<sup>th</sup>-century, different teams went to the region in order to find evidence that could confirm the coexistence hypothesis (Bányai, 1997; Hurt & Blasi, 1969; A Laming-Emperaire, 1979; Walter et al., 1937; Walter, 1958). As a result of more than 170 years of excavations a large collection of early Holocene skeletons was formed. However, all those excavation were done in a time when proper documentation was not available and, therefore, they considerably lack contextual information. Coordinate by WAN and funded by São Paulo State Grant Foundation (FAPESP) the project “Origins and Microevolution of Man in America: a Paleoanthropological Approach” aimed to overcome this problem by identifying and excavating new sites in Lagoa Santa region.

Lapa do Santo was found in the frame of those efforts. Excavations took place between 2001 and 2009 under the coordination of RK, AGMA and DVB. Starting in 2001 several units were open in distinct areas of the shelter. It became apparent that the densest archaeological deposits were located in the south part of the shelter, immediately in front of the cave’s entrance. An ample excavation surface was established in this region becoming the Main Excavation Area (MEA, the pink area in Fig. 2). All human burials were found in the MEA. Excavations ended in 2009 when, according to Brazilian laws, the excavated area was filled with sediments recomposing the original topography of the shelter’s floor.

In 2011, a new excavation area was open in Lapa do Santo as part of another research project. Entitled “The Mortuary Rituals of the First Americans” and coordinated by AS this is a joint venture between the Department of Human Evolution of the Max Planck Institute for Evolutionary Anthropology (Germany) and the Laboratório de Estudos Evolutivos e Ecológicos Humanos da Universidade de São Paulo (Brazil). This new excavation is currently on-going and will not be discussed in the present contribution. The only exception to this concerns the undeformed samples for micromorphology. Since no undeformed samples were collected between 2001 and 2009 from the levels where the burials were found, the micromorphological analysis presented here is based on samples collected from the new excavation’s area at levels compatible with those from which the burials from LSMP-1, LSMP-2 and LSMP-3 come from. A detailed account on the formation processes studies on Lapa do Santo can be found in Villagran et al., (2016).

“Lapa” and “Santo” are the Portuguese words for, “rockshelter” and “saint”. Lapa do Santo is a cave with an associated sheltered area of ca. 1300 m<sup>2</sup>. The southern region of the sheltered area has a relatively flat, height and dry area located immediately in front of the cave’s entrance. The floor of the shelter has a strong descending inclination towards the north, which becomes flat again near a natural sinkhole located at the northern extreme of the sheltered area (Figs. 2c-d).

A three-dimension coordinate system (x,y,z) was established in Lapa do Santo (Fig. 2a-b). The y-axis was conveniently oriented following the longer dimensions of the sheltered area,

which is, in turn, roughly aligned with the geographic north-south axis (increasing towards the north). Therefore, the y-axis is also referred to as the north-south or N-S axis. The x-axis is perpendicular to the y-axis and is therefore roughly aligned with geographic east-west (increasing towards east). The x-axis is also referred to as the east-west or E-W axis. X-axis and y-axis define a horizontal plane. The z-axis is perpendicular to the plane defined by x-axis and y-axis and is therefore also referred to as the vertical axis or absolute depth (decreasing with progressive depth). The origin of the coordinate system (i.e. x=0, y=0, z=0) was conveniently positioned outside the sheltered area (see Figure 2). An arbitrary grid with squares of 1 meter per side was established starting from the origin of the coordinate system. In the x-axis, each one-meter interval was sequentially labeled with letters (A,B,C,D, etc.) and in the y-axis, each one-meter interval was sequentially labeled with numbers (1, 2, 3, 4, 5, etc.). The excavation of the site followed this grid and the unit's code refers to this system (e.g. L11, B13, Z14).

The excavations were based on natural depositional levels further divided into lithostratigraphic units (Gasche & Tunca, 1983; Stein, 1987, 1992), which are composed of *facies*, to assure stratigraphic and spatial control of the archaeological remains (Stein, 1987). A *facies* is the smallest stratigraphic/excavation unit, defined by its color, texture, hardness and inclusions. This concept of *facies* emphasizes the visible lithological changes that occur vertically and horizontally, representing any event over time resulting from the action of carriers bringing similarly sourced material and depositing them on the site (Stein & Rapp, 1985). The depositional event that led to the formation of certain *facies* may have occurred on any time scale. The time of formation of the *facies* is not central to the definition of the term, only the fact that the same lithology was deposited continuously or sporadically brought no changes.

The lithostratigraphic unit hierarchically above the *facies* is defined by "*natural stratigraphic level*". This unit refers to the layer defined by the difference in color, texture and compositions observable in the field (bones, plants, rocks, etc.) representing qualitative differences along the strata excavation. A *natural level* may be composed of only one or more than one *facies*, with or without predominance between the *facies* (Stein & Rapp, 1985). For each excavation unit, the *facies* were sequentially numbered (*facies 1, facies 2, facies 3...*).

The stratigraphic levels were also identified ordinarily with the surface level receiving the number 1. The change from one level to another was determined by changes in color, texture, hardness or composition of its constituent *facies*. However, in cases in which no changes occurred after excavating 10 cm, the level number was automatically changed. At the beginning of the excavation of each level, the z-value of the corners and the central portion of the correspondent unit was recorded. Within each level, their constituent *facies* were excavated separately. A sample of four liters of sediment was collected for flotation from each *facies* in each level. Flotation separated the material that sunk (heavy fraction) and that floated (light fraction). This material is currently under study.

All material of archaeological interest, i.e., lithic materials, bone, seeds, fruits, artifacts made of organic materials (wood, bone, plant) and charcoal, greater than 1 cm, were plotted using a total station according to the site's coordinate system. Each plotted piece received an identification number preceded by the prefix "St-". For each plotted piece, provenance information (unit, level and *facies*) was recorded. The material that could not be plotted (pieces smaller than 1cm found *in situ* or any piece recovered from the sieve) did not receive an identification number and can be tracked only based on its provenance information (unit, level and *facies*). The material was stored individually in plastic bags with their identification cards. During the excavation all structured occurrences were excavated separately by stripping layers of archaeological material.

Archaeological excavation and documentation followed an Excavation Protocol created by one of the authors (RK) specifically for the project, so to ensure standardization of procedures within and between excavation teams. The excavations were documented independently for each unit using a Unit Excavation Form (UEF), Unit Schematic Drawing (USD), Unit Field Pictures (UFP), Worksheet of Spatial Registration (WSR), Worksheet of Volume of *Facies* (WVF), Notebooks of Excavation (NE) and Field Director's Diary (FDD).

Each level of each unit has a UEF. In this form the opening and closing z-values of the level were registered as well as the description of the excavated sediment (color, texture, hardness, inclusions). The USD is complementary to the UEF. Any noticeable feature or structure is registered in the USD (e.g., pebbles and/or boulder concentrations, fire hearths, sediment lens, roots, and animal holes). The USD is of particular importance since it is the most reliable tool to determine the spatial position of the burials in relation to the entire site.

Each unit has a WSR in which the x, y and z values of the plotted pieces are registered. In addition, the identification number and the provenance information were also recorded. This information is also available on the Identification Card that is kept together with the plotted piece. By duplicating the documentation, we minimized the risk of losing information. Each unit has a WVF in which the volume of each bucket of sediment is registered. This allows computing the exact volume of each excavated *facies* and levels. Each unit has an NE in which the excavator can note all his subjective impressions.

Concerning the exhumation of human burials, we adopted a method based on consecutive expositions. The exposition is the unit of the exhumation process. Each exposition must uncover the largest number of bones as possible, without implying the removal of any bone. When this stage is achieved no more sediment is removed and a detailed drawing (BSD) aided by the total station is made, each bone is individually identified, and photographic recording is done. After the BSD is done, the exposed bones are removed and the exhumation moves on, repeating the process until it is no longer possible to remove sediments without further removing bones. This is a very meticulous process and sometimes a single burial might

take as much as 20 days to be fully exhumed. The final result is a high-resolution documentation that allows a full reconstitution of the burial after its removal from the site.

In addition to the already mentioned sources of primary documentation, the burial exhumation processes also included the Burial Exhumation Form (BEF), Burial's Schematic Drawing (BSD), Burial Field Pictures (BFP) and Laboratory Pictures.

The BEF is composed of two pages. In the first one information as the name of the responsible for the exhumation and the date of its occurrence is inserted. In addition, there are specific fields where information about the disposition of the skeleton should be added (flexed or extended, single or multiple, degree of articulation, angle of joints, etc.) The second page of the BEF has a schematic drawing of a skeleton used to indicate which bones were present. In addition, there is a field for the excavator to make a detailed description of the burial while it is being exhumed. The primary documentation was done in Portuguese and is available in digital format upon request.

## 2. CHRONOLOGY

Lapa do Santo chronology is based on OSL dates from sediment, radiocarbon dates on charcoal and radiocarbon dates on human bone collagen. All samples were spatially controlled using a total station.

### OSL

In total 21 sediment samples were collected for luminescence dating, including two outside the shelter in the lake basin (Table S1). The samples were mainly collected from test unit Q48 in the northern part of the shelter, from a T-shaped trench extending from the northern to southern part of the shelter, and from test units F13 and M6 in the south part of the shelter. Because this paper is focused on the burials coming from the main area of excavation, only the samples from F13 and M6 will be considered.

Samples were collected by driving light-tight cylinders into exposed profiles. After retrieval the ends were capped, and the samples shipped to Seattle. The caps were removed in subdued red/orange light. The end portions of the sample were used for dose rate measurements and the middle portion for luminescence measurements. Separate samples were collected to measure current moisture content.

*Dose rate* – Radioactivity was measured by alpha counting in conjunction with flame photometry for K. For alpha counting the pairs technique was employed to separate the U and Th decay series. Total K was measured on the flame photometer and converted to  $^{40}\text{K}$  by natural atomic abundance. All samples were measured plus some additional strata or rocks that were near the samples and contributed to their dose rate. Radioactivity was also measured as a check by beta counting. Four assays were averaged and converted to beta dose rate (Bøtter-Jensen & Mejdahl, 1988). This was compared with the beta dose rate calculated from the alpha counting

and flame photometry results. CaSO<sub>4</sub>:Dy dosimeters were placed at some of the sample locations but only a few were retrieved, because some of the excavation areas were not reopened. Dose rate information is given in Tables S2 and S3.

The beta dose rates, computed in the two ways mentioned, are compared in Table S3. A significant difference is only observed for UW1376, possibly because of some disequilibrium in the U decay chain. For this sample, the beta dose rate from beta counting, as a more direct measure, was used in age calculations. External dose rates from lab and dosimeter measurements are also compared in Table S3. For UW1376 there is a no significant difference. For UW1374, the difference is just barely significant at one-sigma. For UW1375, the difference was significant and the dosimeter was taken as the best measure of external dose rate. Current moisture content varied considerably throughout the shelter. For age calculation, the current content was used as the best estimate with a  $\pm 5\%$  error bar.

Although dose rates vary from different parts of the site, they are broadly similar within a single area. The samples from F13 have dose rates of about 1.8-2.3 Gy/ka, and the sample from M6 about 1.2 Gy/ka.

*Equivalent dose* – For luminescence measurements, the unexposed material from the samples is sieved, both wet and dry, to obtain appropriate size fractions. Both 150-180 $\mu$ m and 180-212 $\mu$ m fractions were used. These were treated with HCl and H<sub>2</sub>O<sub>2</sub> to remove carbonates and organics. They were then etched for 40 minutes in 48% HF and rinsed with water, HCl and water again. Any grains remaining were passed through the 150 $\mu$ m or 180 $\mu$ m screen again to remove degraded feldspar. The material caught in the screen was density separated using a lithium metatungstate solution of 2.67 specific gravity to remove heavy minerals.

Grains were placed in specially-manufactured disks for single-grain measurement. Luminescence was measured on a Risø TL-DA-15 reader with single-grain attachment. Stimulation was by a 532nm laser delivering 45 W/cm<sup>2</sup>. Detection of light was through 7.5 mm U340 (ultraviolet) filters. Exposure was for 0.8s on each grain at 125°C. The first 0.06s was used for analysis and the last 0.15s for background.

Equivalent dose ( $D_e$ ), the laboratory estimate of total absorbed dose through time, was determined using the single-aliquot regenerative dose (SAR) protocol (Murray & Wintle, 2000; Wintle & Murray, 2006). The SAR method measures the natural signal and the signal from a series of regeneration doses on a single aliquot. The method uses a small test dose (about 3 Gy in this case) to monitor and correct for sensitivity changes brought about by preheating, irradiation or light stimulation. SAR consists of the following steps: 1) preheat, 2) measurement of natural signal (OSL or IRSL), L(1), 3) test dose, 4) cut heat, 5) measurement of test dose signal, T(1), 6) regeneration dose, 7) preheat, 8) measurement of signal from regeneration, L(2), 9) test dose, 10) cut heat, 11) measurement of test dose signal, T(2), 12) repeat of steps 6 through 11 for various regeneration doses. A growth curve is constructed from the L(i)/T(i) ratios and the

equivalent dose is found by interpolation of  $L(1)/T(1)$ . A zero regeneration dose and a repeated regeneration dose are employed to insure the procedure is working properly. The preheat removes unstable signal. Both a 180°C and a 240°C preheat for 10 s were employed; no systematic differences in results were observed.

An advantage of single-grain dating is the opportunity to remove from analysis grains with unsuitable characteristics by establishing a set of criteria grains must meet. Grains were eliminated from analysis if they (1) had poor signals (as judged from errors on the test dose greater than 30 percent or from net natural signals not at least three times above the background standard deviation), (2) did not produce, within 20 percent, the same signal ratio (often called recycle ratio) from identical regeneration doses given at the beginning and end of the SAR sequence, suggesting inaccurate sensitivity correction, (3) yielded natural signals that did not intersect saturating growth curves, (4) had a signal larger than 10 percent of the natural signal after a zero dose, (5) produced a zero  $D_e$  (within 1-sigma of zero), or (6) contained feldspar contaminates (judged visually on growth curves by a reduced signal from infrared stimulation before the OSL measurement; done on two doses to lend confidence the reduction in signal is due to feldspar contamination). A dose recovery test was performed on some grains. The luminescence of the grains was first removed by exposure to the laser (using the same parameters mentioned earlier). A dose of known magnitude was then administered. The SAR procedure was then applied to see if the known dose could be obtained. Successful recovery is an indication that the procedures are appropriate.

$D_e$  values were obtained on both 150-180 $\mu\text{m}$  and 180-212 $\mu\text{m}$  grains. The former sacrifices single-grain resolution because more than one grain will fit in the measuring holes, but comparison of  $D_e$  distributions between the two grain sizes yielded no differences that could not be accounted for by differential sample size. Using the rejection criteria described earlier, an acceptance rate for all samples in the shelter of 10.9% was obtained for the 180-212 $\mu\text{m}$  fraction. It is 29.2% for the smaller size fraction, but the probability of both grains in the same hole having a usable signal is only 1.2%, and only 3.6% for one out of three. Averaging of signals from two or three grains is thus not expected to be significant, so the results from the two size fractions were pooled for further analysis. The most common reason for rejection was lack of measurable signal (91% of those rejected from the 180-212 $\mu\text{m}$  fraction). No other reason constituted more than 4% of the rejections.

The SAR protocol is designed for the fast component in quartz. Quartz is known to contain multiple traps that bleach at different rates. The fast component is the only stable one that bleaches rapidly. If grains are dominated by slower bleaching components, the SAR protocol may not yield the correct  $D_e$  value. One way to visualize component structure is to utilize linear modulated OSL (LM-OSL). Most OSL is measured in what is called continuous wavelength mode, where the power and the wavelength of the stimulating source are kept

constant. In LM-OSL, the wavelength is kept constant, but the stimulating power is ramped from zero to full power. Electrons from rapidly bleaching peaks are preferentially released early, when power is still low, while electrons from slower bleaching peaks are released later. This provides a peak structure where the components can be visually distinguished.

LM-OSL was measured for several samples, including four under consideration here, at the end of an SAR sequence, following a dose approximately the size of the equivalent dose. The laser power was ramped from 0 to 90% power in 30s. A comparison was made among grains of the intensity ratio of the curve at 16% power and that at about 5.5% power. Those values were chosen as the point of the fast component peak (5.5%) and the point where the fast component drops to background (16%), as concluded from grains where the signal dropped the quickest. Grains with a ratio less than 0.2 were compared with those with a ratio greater than 0.6, the former taken as grains dominated by the fast component and the latter taken as grains with a significant medium or slow component. For all samples, grains with ratios less than 0.2 are much more common than those with ratios more than 0.6. Comparing the central age  $D_e$  values for each group shows either no significant difference or a smaller  $D_e$  for the higher ratio, which is the opposite one would expect if the slower components had much effect. Quartz in these samples appears then to be dominated by the fast component, and in those few grains where slower components are present, the  $D_e$  values did not differ significantly from those dominated by the fast component.

Dose recovery was performed on 12 samples from the entire shelter with a total number of accepted measurements at 399. The central tendency (as measured by the central age model, discussed later) of the normalized results (obtained dose/given dose) is  $1.03 \pm 0.01$  with an over-dispersion of  $13.5 \pm 1.4\%$ . This suggests the procedures are satisfactory. Over-dispersion in dose recovery reflects only intrinsic causes (machine error, variation in luminescence properties of grains, etc.). All external causes, differential dose rate or differential age, are controlled. This means that for a single-aged sample, an over-dispersion of at least 15% should be expected. This is within the range reported for single-aged samples from many areas.

A  $D_e$  value is obtained for each suitable grain. Because of varying precision from grain to grain, the same value is not obtained for each grain even if all are of the same age. Instead a distribution is produced. The common age model and central age model of Galbraith (Galbraith & Roberts, 2012) are statistical tools used in evaluation of  $D_e$  distributions. These models are used in reference to  $D_e$  and not "age" per se, although dividing the  $D_e$  values by the bulk dose rate provides an "age" for each grain (not accounting for differential dose rates for individual grains). The common age model controls for differential precision by computing a weighted average using log  $D_e$  values. The central age model is similar except rather than assuming a single true value it assumes a natural distribution of  $D_e$  values, even for single-aged samples, because of non-statistical sources of variation. It computes an over-dispersion parameter ( $\sigma_b$ ) or

that deviation beyond what can be accounted for by measurement error. For samples of mixed ages a finite mixture model is employed for evaluation (Galbraith & Roberts, 2012). This uses maximum likelihood to separate the grains into single-aged components based on the input of a given  $\sigma_b$  value and the assumption of a log normal distribution of each component. The model estimates the number of components, the weighted average of each component, and the proportion of grains assigned to each component. The model provides two statistics for estimating the most likely number of components, maximum log likelihood (l<sub>lik</sub>) and Bayes Information Criterion (BIC).

Table S4 gives the equivalent dose central age value and over-dispersion for each sample. Over-dispersion is higher than 15% for all samples, although not by much for some. This suggests, overall, that some of the scatter in the distributions of  $D_e$  values has extrinsic causes.

To look at the structure of these distributions, a finite mixture model was applied, using 15% as the assumed minimum over-dispersion for a single-aged sample. The number of components detected and the percentage of the most common component are given in Table S4. Two samples, UW861 and UW1375 are consistent with a single age. The other samples appear more mixed.

One extrinsic cause of over-dispersion is differential dose rate, mainly affecting the beta dose rate because of heterogeneous distribution of either uncommon K-feldspars (the K from which can contribute a large part of the beta dose rate) or carbonate materials (such as speleothems) which contain little radioactivity. Grains close to K-feldspars or speleothem fragments will experience a different dose rate than grains further away. While no measurements of spatial distribution of radionuclides has been done, it is possible to model the possible effect to see if it could cause the observed  $D_e$  distributions. The effect of heterogeneous distribution of K was modeled following Mayya et al. (2006) using the procedure of delineated by David et al. (2007), while the effect of speleothems was modeled by just assuming that grains next to speleothem fragments would experience half the dose rate of other grains. In both cases, the ages of lower components are increased by assuming a minimum dose rate as determined from the models and comparing them with adjusted ages of higher components. Comparisons were between the two most common components. The difference between assuming K hotspots (i.e., areas of high radioactivity) or speleothem cold spots (low radioactivity) was not substantial, so either has about the same effect. The results show that these models can explain the difference in  $D_e$  for the two most common components for all of these samples. This is not to say that the models are an accurate description of the dose rate distribution. It is to say that beta dose rate heterogeneity cannot be ruled out for explaining the over-dispersion.



Given that beta heterogeneity can explain the over-dispersion and no obvious agent for large scale mixing presents itself, the central age model appears to represent the best estimator for determining equivalent dose. If beta heterogeneity is present, the central age model allows for averaging out the effects. It could also take into account small scale mixing, where there was movement both up and down, which might occur with trampling, for example.

*Ages* – Age is determined by dividing the central age equivalent dose by the bulk dose rate. Table S5 gives the ages for each sample. Single-, or near single-aged samples are italicized. As can be seen in Figure 3 samples UW861 and UW1377 are in complete stratigraphic agreement with the dated charcoals (see next section). The other three samples, however, are consistently older than charcoals found in equivalent depth (ie. z-values). No good explanation is available for that. According to the OSL samples the earliest human occupation appears to date from about 9.0 to 12.0 kyBP, consistent with other paleoindian occupations in Lagoa Santa. In the unit F13 dates from UW1374 and UW1375 are at least two thousand years older than the dates obtained from radiocarbon. In addition, there is a general lack of agreement between the vertical position of the OSL sample and the radiocarbon dates (see below). Therefore, we consider the OSL dates as generally supporting the early Holocene chronology of the oldest deposits but they cannot be relied on for more refined estimation of age of different strata.

#### *Radiocarbon*

At Lapa do Santo a total of 53 charcoal samples were selected for radiocarbon dating. The samples were sent to the Beta Analytic AMS system in Miami where they have been pretreated with the ABA method. Fifty-eight human bone and teeth sampled from Lapa do Santo's burials were also sent to Beta Analytic between 2001 and 2009 and pretreated without ultrafiltration method. Nine samples provided collagen and carbon for accurate measurement. The measured ages were then corrected according to the  $^{13}\text{C}/^{12}\text{C}$  sample ratio, from which the conventional age was derived.

Twenty-one fragments of human bone from Lapa do Santo were pretreated at the Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology (MPI-EVA), Leipzig, Germany, using the method described by Talamo and Richards (Talamo & Richards, 2011). The outer surface of the bone samples are first cleaned by a shot blaster and then 500mg of bone powder is taken. The samples are then decalcified in 0.5M aq. HCl at room temperature until no  $\text{CO}_2$  effervescence is observed, usually for about 4 hours. 0.1M aq. NaOH is added for 30 minutes to remove humics. The NaOH step is followed by a final 0.5M HCl step for 15 minutes. The resulting solid is gelatinized in a pH3 solution in a heater block at 75°C for 20h, following Longin et al., (Longin, 1971). The gelatin is then filtered in an Ezee-Filter™ (Elkay Laboratory Products (UK) Ltd.) to remove small (<8 μm) particles. The gelatin is then ultrafiltered with Sartorius "Vivaspin 15" 30 KDa ultrafilters (T. A. Brown, Nelson, Vogel, &

Southon, 1988). Prior to using the filter is cleaned to remove carbon-containing humectants (Higham, Jacobi, & Ramsey, 2006). The samples are then lyophilized for 48 hours.

C:N ratios, %C, %N,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were measured at Max Planck using a Thermo Finnigan Flash EA coupled to a Delta V isotope ratio mass spectrometer. For acceptable quality collagen, the atomic C:N ratio should be between 2.9 and 3.4 and a collagen yield of more than 1% of weight (Ambrose, 1990; DeNiro, 1985; van Klinken, 1999). For Lapa do Santo all the isotopic results, C:N ratios and collagen yields are well within the accepted ranges (Table S6, attached as Excel File). Three out of 21 samples obtained enough collagen for radiocarbon dating and were sent to the Klaus-Tschira-AMS facility of the Curt-Engelhorn Centre in Mannheim, Germany, where they have been graphitized and dated (Kromer, Lindauer, Synal, & Wacker, 2013). The three dates from MPI were corrected for a residual preparation background estimated from pretreated  $^{14}\text{C}$  free bone samples, kindly provided by the ORAU.

The radiocarbon results are listed in Table S6 (attached as Excel File). Burial 14 was dated twice at Beta laboratory, but unfortunately, the ages are statistically incompatible. We cannot discard one of the two dates, because not enough information about the collagen preservation is available. Until a third date is produced with more accurate and detailed information we will consider them not to be reliable, and for this reason, they are not included in the Bayesian model (see below). Burial 17 has one date from the adult individual and one date from the sub-adult individual. The two ages are compatible according to the R\_Combine function of Oxcal (Burial 17, R\_Combine,  $^{14}\text{C}$  Age 8,620 $\pm$ 36).

Based on the distribution of radiocarbon dates (Fig. 3b) it is clear that they cluster into three distinct groups roughly corresponding to the early, mid and late Holocene. From this initial assessment, the radiocarbon dates were calibrated using the SHcal13 (Hogg et al., 2013) curve and modelled into three contiguous phases using OxCal 4.2 (Ramsey & Lee, 2013) (see Supplementary Data 1 for the OxCal script). Lapa do Santo deposit has a very expressive anthropogenic component (see section 7 on formation processes) and all charcoals are assumed to derive from combustion structures. Discounted their *terminus post quem* nature, the charcoals from Lapa do Santo are themselves the events to be dated. Therefore, the t-type outlier model (Ramsey, 2009) is not appropriate and instead we used a s-type outlier model (Ramsey, 2009), with prior probabilities set at 0.05.

The agreement of this model is above the recommended threshold of 60% ( $A_{\text{overall}} = 82.1\%$ ). According to the utilized model Lapa do Santo's Period 1 (LSP-1) starts at 12.5 cal kyBP and ends at 8.1 cal kyBP, Lapa do Santo's Period 2 (LSP-2) starts at 5.2 cal kyBP and ends at 4.0 cal kyBP; Lapa do Santo's Period 3 (LSP-3) starts at 1.1 cal kyBP and ends at 0.7 cal kyBP (all 1 $\sigma$  interval) (see light shaded areas in Fig. S2b). If we consider the 2 $\sigma$  interval Lapa do Santo's Period 1 (LSP-1) starts at 12.7 cal kyBP and ends at 7.9 cal kyBP, Lapa do Santo's Period 2 (LSP-2) starts at 5.4 cal kyBP and ends at 3.9 cal kyBP; Lapa do Santo's Period 3 (LSP-3) starts at 2.1

cal kyBP and ends at 0.0 cal kyBP (see dark shaded areas in Fig. 3b). The chronology of Lapa do Santo will be based on these modelled calibrated ages.

According to the model two radiocarbon dates are outliers. The first is the oldest dated charcoal from the site (Beta-280489,  $^{14}\text{C}$  Age 10,490 $\pm$ 50). However, we decided to keep this date in the model for two reasons. First, in Lagoa Santa region there is well-documented occupation at correspondent period testified by other sites (Astolfo G.M. Araujo et al., 2012). Second, the oldest OSL dates of Lapa do Santo are roughly from the same timeframe as this charcoal. The second outlier is the date of Burial 11's collagen (Beta-215195,  $^{14}\text{C}$  Age 5,990 $\pm$ 40). This date is chronologically intermediate between LSP-1 and LSP-2 and is considered an outlier if included in any of the two periods. Therefore, either Burial 11's date is to be discarded because it is incorrect, or because it represents an event of occupation that was not preserved in any other part of Lapa do Santo's archaeological record. Taking into account the strong similarities Burial 11 shares with Burials 7 and 19, that date to more than a thousand years earlier, we favor the interpretation this is a wrong date that must be discarded.

The chronological range of each Lapa do Santo Mortuary Pattern (LSMP) was defined by the oldest extreme of the 95.4% interval of its oldest directly dated burial and the youngest extreme of the 95.4% interval of its youngest directly dated burial. Both burials from LSMP-1 could be directly dated resulting in a range of 9.7-10.6 cal kyBP for this mortuary pattern. For LSMP-2 a total of six direct dates were obtained. Of those, two were discarded as not reliable (Burial 14) and two were combined since they referred to the same burial (Burial 17). LSMP-2 is dated to between 9.4-9.6 cal kyBP. For LSMP-3 a total of three direct dates on bone is available. Of those, one was discarded as an outlier (Burial 11). Based on the two remaining dates LSMP-3 is dated to 8.2-8.6 cal kyBP.

From the total of 61 radiocarbon dates included in the model 56 come from the main excavation area where the human remains were found (pink area in Fig. 2). Contrary to what is observed for the OSL dates (see previous section), in the main excavation area charcoals and burials from the different periods are not randomly vertically distributed. They are mostly restricted to specific vertical intervals, meaning absolute depth (i.e. z-values) and radiocarbon age are strongly correlated. In fact, in 93% of the cases (four out of 56; Burial 2 and the three charcoals indicated by blue and red arrows in Figure 3a-b) it is possible to correctly predict the chronological period to which a radiocarbon date belongs solely based on its vertical position. More specifically, radiocarbon dates located below the z-value of 0.137 belong to LSP-1, between 0.137 and 0.971 to LSP-2 and above 0.947 to LSP-3 (these are the limits indicated by the dotted and dashed lines in Fig. S2). Among the burials belonging to LSMP-1, LSMP-2 and LSMP-3 the highest z-value is 0.059 (Burial 13). Since this value is inferior to the limit of 0.137 separating LSP-1 from LSP-2 we estimated that all these burials belong to LSP-1.

As discussed in section 7 of this Supplementary Information, allochthonous raw material was used for lithic production since the beginning of the occupation of Lapa do Santo. However, as indicated by the histogram depicted in the Figure 6a, after Level 20 of Unit F13 allochthonous raw material disappears from Lapa do Santo archaeological record. Unit F13's Level 20 has an average z-value of -2.738. We used the equation of a linear regression of z-value against calibrated radiocarbon dates for the charcoals in units F13 and G13 (Figure 6b). Those are the charcoals located closest to the lithic assemblage analyzed (Unit F13) and, therefore, most suitable to estimate their chronology. When the z-value -2.738 is applied to the equation of the linear regression a date of 9.9 cal kyBP is obtained with a 95% confidence interval going from 8.5 cal kyBP to 10.4 cal kyBP. However, the lower limit of this estimation must be seen as very conservative since it is heavily influenced by the presence of a single charcoal sample that is younger than the others at corresponding depth (Beta-15924). In the future, a more appropriate chronological model for the different levels based on Bayesian statistics should be prepared to improve this estimation.

The same linear equation is also appropriate to present a conservative chronological estimation for the hematite axe, projectile point and two fish-hooks that were found in units F12, F13, G12 and G13 (Table x, see section 7 for a description of stone and bone artifacts).

### 3. FORMATION PROCESSES

Here we present the preliminary results of micromorphological analyses from a second excavation area opened at Lapa do Santo in 2011. This area corresponds to the same levels where burials from LSMP-1, LSMP-2 and LSMP-3 were found. For the complete geoarchaeological study, the reader is referred to Villagran et al., (*in press*). A total of 30 undisturbed blocks for micromorphological analyses were taken from the exposed surfaces during excavation. The horizontal strategy of sample collection aimed at covering the lateral and vertical variation in sedimentary facies observed in the excavation levels. The blocks were oven-dried for one week at 50° C and impregnated with a mixture of polyester resin (Viscovoss N5 S, 700 ml), styrene (300 ml) and hardener (MKEP, 5-7 ml). Analyses were done with a Stemi 2000-C stereomicroscope and Zeis Axio Imager A2 petrographic microscope in plane polarized light (PPL), and cross-polarized light (XPL). Samples were prepared and analyzed at the Institute for Archaeological Sciences, Eberhard Karls Universität Tübingen.

The macro stratigraphy of Lapa do Santo shows an intercalation of layers with diffuse to sharp boundaries that can be divided into three main categories, from the more to the less frequent: 1) tabular, grey, centimetric layers (5YR 6.1) of powdery carbonate sediments, with common sand grains and frequent to common red clay aggregates and charcoal (20-40 %); 2) lenticular, red centimetric layers (5YR 5.6) of indurated clay minerals with rare charcoal

fragments; 3) lenticular, black, milimetric and centimetric layers, with high concentration of charcoal and microcharcoal.

Of the 30 thin sections analyzed, 27 show a similar composition characterized by: 1) coarse fraction made of randomly distributed clay aggregates of diverse size (from 30  $\mu\text{m}$  to 1 cm) (Fig. 4a); 2) micromass made of densely packed ash crystals (Fig. 4b-c). The remaining 3 thin sections are exclusively red clayey sediments (2 samples) and a fragment of limestone from the cave walls (1 sample). In compositional terms, variations in the c/f ratio seen as changes in the relative frequency of ash crystals vs. clay aggregates describe the differences between the samples. Other differentiating features are: variations in porosity; frequency of organic elements, such as charcoal, articulated ashes (Fig. 4d) or tissue residues (Fig. 4e); and the presence of micro-stratifications (several microfacies in one sample) vs. samples made of one single microfacies.

The ash crystals are described as rhombohedric micro-crystalline calcite crystals (10-30  $\mu\text{m}$ ) (Fig. 4b). The crystals develop after burning of the calcium oxalates that naturally appear in the plant cells at temperatures around 400-600° C. Ash crystals, also described as pseudomorphs of calcium oxalate into calcite (POCC), are the diagnostic micromorphological trait of plant ashes (Brochier, 1983; Canti, 2003; Courty, Goldberg, & Macphail, 1989). Ash crystals have an overall good preservation. However, there are evidences of ash dissolution, such as areas of recrystallized ash, sparitic coatings and infillings inside voids, around coarse fraction components and also cementing the ashes. These pedofeatures suggest water passage through the sediments and vadose conditions, with slow water percolation and/or episodes of water saturation. The presence of iron (hydr)oxide hypocoatings and nodules also suggests this.

The clay aggregates are always blocky, angular to sub-rounded, with undifferentiated b-fabrics (XPL). They appear in frequencies from 10-70% and show three distinct colors in PPL: red, orange and yellow. The differences could be related with the natural  $\text{Fe}_2\text{O}_3$  content or to the anthropic modification of the clay aggregates (by human fires), as suggested by the dark red rims (Fig. 4f). The microstructure inside the clay aggregates is close granular to massive, with star-shaped voids (from coalesced granules), chamber voids (from biological activity) and fissures. Laminated clay coatings and infillings have been observed inside the clay aggregates, suggesting provenance from B-horizons. Very fine sand and fine sand sub-rounded quartz grains are embedded in the clay aggregates (frequency below 5%), with random distribution and good selection.

The coarse fraction is also made of other organic and inorganic components that appear in concentrations below 5%, such as: limestone fragments (from the cave walls); microlithic flakes; opaque minerals and quartz grains (detached from the clay aggregates); bone fragments (sometimes burned); shell fragments; charcoal; articulated ashes; tissue residues; and phytoliths. Besides the ash crystals, the micromass also includes pale yellow (PPL), undifferentiated or low

birefringence (XPL) material possibly derived from neoformed phosphates. Given the low amount of bones in the sediment and the good conditions for bone preservation in the deposit (alkaline pH), phosphates could derive from bird guano and/or plant decay. The common association of phosphate layers with tissue residues, silicified tissue and plant pseudo-voids in numerous thin sections suggests that at least some portion of the secondary phosphates derives from plants.

Bioturbation of the sediments is indicated by channel and chamber voids, seen in most of the thin sections, passage features and large faunal channels. However, bioturbation was generally not intense since fragile components, such as tissue residues, laminations of fine plant tissue and articulated ashes show good integrity.

The mixing of geogenic (clay aggregates) and anthropogenic sediments (ashes) seen in Lapa do Santo is a frequent characteristic in rockshelters of Lagoa Santa. Ashes from anthropic fires have been described in the sediments of Lapa das Boleiras (Araujo *et al.* 2008) and Lapa Grande de Taquaraçu. Although no micromorphological analysis have been done on other sites, the macroscopic and micromorphological similarity between the greyish fine sediments in Lapa do Santo, Lapa das Boleiras and Lapa Grande de Taquaraçu, indicate that plant ashes could be a frequent component in other archaeological sites in rockshelters from the region.

Geogenic clay aggregates making up the site stratigraphy and frequently mixed with plant ashes have also been reported at the Sumidouro cave (Piló *et al.* 2005) and Lapa das Boleiras (Araujo *et al.* 2008). At the Sumidouro cave, the red and yellow sediments were interpreted as soil aggregates from the oxisol developed from the Serra de Santa Helena Formation (Araujo *et al.* 2005). At Lapa das Boleiras the clay aggregates are described as red colluvium derived from the red oxisols in the vicinity of the sites (Araujo *et al.* 2008).

At Lapa do Santo, the morphological and compositional characteristics of the clay aggregates also fit to what has been described for the red and yellow oxisols of the region, such as coalesced granules and angular blocky aggregates; presence of quartz grains (1-5% frequency); microstructure made of indurated aggregates of clay. Because of a lack of cracks and conduits naturally bringing soil material to the rockshelter, one possibility is that material came from the oxisol developed over the limestone shelter. Soil material loosened by rain, plant-root, etc. continuously falls down the cliff above the rockshelter, and is later redistributed by human trampling within the site and mixed with the ashes from combustion features. Lateral colluvial reworking of oxisol fragments would have also taken place within the rockshelter.

Another possibility for the presence of soil material inside the rockshelter is human transport. In fact, the differences in color of the clay aggregates may relate with their provenance from different soils or soil horizons. For example, the orange clay aggregates are texturally similar to the yellow oxisol aggregates described at the Sumidouro site (Araujo *et al.* 2013). Yellow oxisol fragments have also been interpreted as making part of the sediments at

Lapa Vermelha (Feathers et al., 2010). Another possibility is related to a hypothesis currently being tested by experimental archaeology and spectroscopic techniques. The hypothesis states that the different colors resulted from the in situ burning of clay aggregates in the anthropic fires that produced the extraordinary amount of ashes that make up the site (see Villagran et al., submitted).

#### 4. DIATOM ANALYSIS

Diatom analyses were done to investigate the potential flooding of the site by a pond that existed north of the site, and whose presence is indicated by watermarks in the limestone wall. Analyses were done on loose sediments collected in aseptic conditions from the second excavation area.

For the identification of siliceous microfossils 12 samples of loose sediment were dried in an oven at 40°C. One gram of sample was placed in a 250 ml beaker, with 30 ml of hydrochloric acid to remove the carbonates. The solution was warmed on a hot plate for six hours under 100°C. After carbonate removal, 30 ml of hydrogen peroxide were added to remove organic matter. Samples were successively rinsed with distilled water and decanted six times until solution reached a neutral pH. Afterwards, samples were diluted to 100 ml and 3 ml of the solution placed on a microscope slide and mounted with Nafrax.

Diatoms were identified in 70% of the analyzed samples but at a very low frequency. *Eunotia*, a genus generally preferring marshes, rivers, and lake habitats were the most common type of diatom. The genus lives in epilithic habitats (attached to rocks) in rivers and springs at a pH of 4.3 (slightly acid water), at water temperature of 26.3°C, variable concentration of dissolved oxygen (2-10 mg/L), and null turbidity and salinity (Burlinga et al., 2007). *Hantzschia amphioxys* was also identified. It inhabits temporal bodies of water or periodically emerging areas associated with ephemeral drainage channels (Camburn et al., 2010). This assemblage is compatible with a lacustrine origin for the diatoms. The very low frequency of diatoms indicates that a water body was not the main agent in the deposition of the sediments. However, the presence of reworked diatoms, frustules and sponge spicules suggest some sort of water input to the site. This may be associated with the seasonal pond that was active in the past in the northern part of the rockshelter. Alternatively, it could be the result of water being brought to the site by its dwellers from the nearby lakes. Future work characterizing diatom assemblages in other locations in Lagoa Santa region will allow a more precise interpretation of the assemblage reported here for Lapa do Santo.

#### 5. ZOOARCHAEOLOGY

Zooarchaeological analyses are key to better understand the relationship between humans and other animal populations, specifically for studies on diet and hunting strategies

(Reitz & Wing, 2008). A central point, however, is how we can interpret the zooarchaeological record to generate unbiased information about those relationships. Wrong interpretations can derive from the incorrect understanding of the role that human and natural agencies play in constituting the faunal assemblage recovered in archaeological sites (Bissaro Jr., 2008; Kipnis, 2002; Lyman, 1994; C. P. Perez, 2009).

For the faunal analyses at Lapa do Santo we followed standard zooarchaeological methodology (Klein & Cruz-Urbe, 1984; Lyman, 1994, 2008). The Number of Identified Specimens (NISP) and Minimum Number of Individuals (MNI) was computed for a sample of faunal assemblage coming from units: L7, L8, L10, M3, M4, M5, M6, and from archaeological strata contemporary to the early Holocene human remains. The results point to a faunal assemblage dominated by small and medium-sized mammals, as well as reptiles, birds and fishes (Table 2). Bigger mammals, such as deer and peccaries are also present in the faunal assemblage, but not as predominantly as one would expect based on their much higher return rate when compared to medium-sized animals; but in accordance with evolutionary ecology models for prehistoric foraging societies in Central Brazil (Kipnis, 2002).

The fact that almost all the anatomical parts of deer are represented (Table S7) indicates a subsistence/dietary strategy that is not targeting specific body parts when analyzed against Food Utility Index (Metcalf & Jones, 1988) (FUI). The game was not dismembered in the killing site, but brought as entire piece to the dwelling camp (see examples on the use of utility index in Binford (1978), Brink (1997), Brink and Dawe (1989), Lyman *et al.* (1992), Metcalfe and Jones (1988), Savelle *et al.* (1996), Savelle and Friesen (1996), Kipnis (2002); Fernández-Jalvo *et al.* (2002), Denys (2002), Bissaro-Júnior (2008), Couso *et al.* (2011), Manne (2014)).

However, the signature of hunting strategies based on Food Utility Indexes can be biased by the differential weathering of bones with varying densities (Rogers, 2000). To verify if the anatomical representation of *Mazama* sp. in Lapa do Santo is reflecting dietary/hunting practices, instead of resulting from taphonomic processes, we evaluated the differential preservation of *Mazama* sp. bones according to density-mediated attrition (Grayson, 1989; Lyman, 1985, 1992).

For that, taphonomic signatures of natural and human agency were generated from paleontological and ethnoarchaeological assemblages as control studies for comparing with the zooarchaeological record from Lapa do Santo (Bissaro Jr., 2008).

For the paleontological sample we used bones recovered from the Gruta Cuvieri, a paleontological site located few kilometers from Lapa do Santo, with similar chronology, and rich in bones of *Mazama* sp. Previous studies showed the lack of anthropic inputs in the formation of Gruta Cuvieri fossil record (A. Hubbe *et al.*, 2011), making it an ideal control sample of a non-human-mediated assemblage. For the ethnoarchaeological samples, we used bones recovered by Renato Kipnis in the early 1990's at a Guajá site. The Guajá is one of the last



hunter-gatherers society of South America lowlands (Forline, 1997). They live in Brazilian Amazon (state of Maranhão) and commonly hunt *Mazama* sp., among other species (for a detailed description of the Guajá subsistence strategy see Prado (2009)). The bones used in this study were collected after discard and were an ideal control sample for a human-mediated assemblage.

For the taphonomic study, deer bones from other excavated units at Lapa do Santo were also included as to have a more significant sample size. The relative skeletal abundance of *Mazama* sp. in Lapa do Santo, Gruta Cuvieri and the Guajá assemblage was quantified using the Standardized Minimum Number of Animal Unit (%MAU, Binford 1984) for each anatomical part. Because there are no specific utility indices for *Mazama* sp. the bone density of the body parts considered in this study was obtained from Lyman (Lyman, 1984) reports on *Odocoileus virginianus* (Artiodactyla, Cervidae). Table S8 presents the correlation between %MAU and bone density for the three sites.

In the paleontological context (Gruta Cuvieri) the positive and significant correlation between %MAU and bone density supports the survivorship of higher density anatomical parts due to density-mediated attrition (e.g. post-depositional). The absence of any significant correlation between %MAU and bone density in the ethnoarchaeological assemblage (Guajá) indicates that whole deer skeletons were entering the Guajá settlement.

In Lapa do Santo, the non-significant correlation between %MAU of *Mazama* sp. and bone density indicates that density-mediated attrition was not central to the pattern observed. The low depositional rates at Gruta Cuvieri would have given enough time for density-mediated attrition to take place before the bones were completely buried, resulting in their differential preservation. In Lapa do Santo, however, the fast depositional rates associated with anthropogenic sediments that contain high degree of ashes and calcium, good for bone preservation, would have precluded the differential preservation of the bone assemblage triggered by density-mediated attrition.

Thus, we interpret that the representation of bones in Lapa do Santo did not result from differential preservation, but is indeed reflecting a hunting strategy in which *Mazama* sp. was not dismembered in the killing site, but brought as an entire piece to the dwelling camp. This is supported by the lack of significant statistical correlation between %MAU and FUI for *Mazama* sp. in Lapa do Santo ( $n=25$ , Spearman  $\rho=0.344$ ;  $p=0.09$ ). Similar values are observed for the Guajá ethnoarchaeological collection ( $n=27$ , Spearman  $\rho = 0.268$ ;  $p=0.17$ ) attesting this is a common hunting strategy in the tropics, where even the largest game, tapir, can still be carried without dismembering (Kipnis 2002). In conclusion, the faunal assemblage from Lapa do Santo is an adequate sample upon which to base inferences about prehistoric human faunal exploitation, and that the inferences point towards a hunting strategy of small and medium-size

animals as the primary prey items, and less frequent the inclusion of bigger animals upon encounter (Bissaro Jr., 2008; Kipnis, 2002).

## 6. CARBON AND NITROGEN ISOTOPES

Carbon and nitrogen stable isotope analysis on bone collagen is widely used in archaeology for reconstructing ancient diets (van der Merwe & Vogel, 1978; Vogel & van der Merwe, 1977). Since almost all of the carbon in the biosphere is fixed by autotrophs (DeNiro & Epstein, 1978), stable carbon isotopic ratios ( $^{13}\text{C}/^{12}\text{C}$ ) can distinguish between plants that fixate carbon using  $\text{C}_3$  and  $\text{C}_4$  photosynthetic pathways (O'Leary, 1988).  $\text{C}_4$  type plants are mostly grasses, such as maize, sorghum and sugarcane, which have  $\delta^{13}\text{C}$  values ranging from -9 to -14‰.  $\text{C}_3$  type plants made up of some grasses (e.g. oats, wheat, rice) and virtually all other non-grasses, have  $\delta^{13}\text{C}$  values ranging from -20 to -35‰ (Deines, 1980).

Stable nitrogen isotopes ( $^{15}\text{N}/^{14}\text{N}$ ) accumulate throughout successive trophic levels in which primary producers (plants) have the lowest ratios and each subsequent level in the trophic chain is enriched in approximately 3-5‰ (Hedges & Reynard, 2007; Minagawa & Wada, 1984; Schoeninger & DeNiro, 1984) in  $\delta^{15}\text{N}$  values. Modern day studies show that plants in the Brazilian savanna, in which Lapa do Santo is located, have  $\delta^{15}\text{N}$  values that range from -5.0‰ to +7.9‰ (Bustamente et al., 2004).

Stable carbon and nitrogen isotopes can also be used to identify marine components in the diet. Marine organisms usually have a carbon enrichment to the order of 7‰ since their primary source of carbon comes from dissolved carbonates (0‰) and not atmospheric  $\text{CO}_2$  (-7‰) like terrestrial carbon fixers (Chisholm, Nelson, Hobson, Schwarcz, & Knyf, 1983; Chisholm, Nelson, & Schwarcz, 1982; Tauber, 1981). Nitrogen also has enriched values in this context as marine trophic chains tend to be longer and leading to a more pronounced cumulative effect of  $\delta^{15}\text{N}$  (Schoeninger & DeNiro, 1984).

A total of 17 human bones and 51 faunal bones were processed. Due to poor preservation conditions only eight human (Table S9) and 22 faunal samples (Table S10) provided collagen with an acceptable C:N ratio between 2.9 and 3.6 (DeNiro, 1985). Since the faunal material from Lapa do Santo was extremely fragmented and poorly preserved, with only seven reliable results, the assemblage was complemented with material from Gruta Cuvieri, a paleontological cave of compatible chronology located 3 km away from Lapa do Santo (A. Hubbe et al., 2011).

The material was analyzed in three different laboratories: the Dorothy Garrod Laboratory for Isotopic Analysis of the McDonald Institute for Archaeological Research, University of Cambridge (UC); the Laboratório de Ecologia Isotópica (Isotope Ecology Lab) at the Escola Superior de Agricultura Luis de Queirós, University of São Paulo (ESALQ-USP); and the isotope facilities at the Max-Planck Institute for Evolutionary Anthropology (MPI-EVA).

This variation of laboratories also made for a slight difference in methodologies. As the data were produced in three different labs, there are differences in the methods employed.

The samples analyzed at the University of São Paulo (USP) and the University of Cambridge (UC) used the same collagen extraction methodology. Following Longin (1971), samples of bones ranging from 500mg to 1000mg were sandblasted using aluminium oxide and then demineralised in a 0.5M aq. solution of hydrochloric acid (HCl). Once demineralised, the bone material was rinsed 3 times in deionised water and gelatinized in a pH3 aq. solution at 75°C for 48 hours. Then supernatant collagenous solution was removed from the test tube using an Evergreen Sera-Separa®, 4¼" long, 9 ml capacity filter and freeze dried for 3 days. Once the collagen was dry, individual samples were sub-sampled in triplicates of 0.7 to 0.9mg and finally sent to be analysed in the mass spectrometer. At the University of São Paulo samples were analysed in a Carlo Erba EA1100-CHN elemental analyzer coupled to a Thermo Finnigan Delta Plus isotope ratio mass spectrometer, while at the University of Cambridge they were run in a Costech elemental analyser coupled to a Thermo Finnigan MAT253 isotope ratio mass spectrometer. All results were calibrated with reference to international and laboratory standards. The samples analyzed in MPI-EVA were treated according the protocols of Talamo and Richards (Talamo & Richards, 2011) that are described in detail in section 3. Figure S6b shows the results of all analyses.

Results for the fauna are mostly consistent with their dietary habits. Deer (*Mazama* sp.; Cervidae, Goldfuss 1820) are browsing animals, and in the savannas of central Brazil normally feed on more <sup>13</sup>C-depleted gallery forest plants (Marinho-Filho, Rodrigues, & Juarez, 2002). Their predominantly C<sub>3</sub> plant based diet is compatible with the observed collagen isotope values of δ<sup>13</sup>C -20.7±1.6‰ and δ<sup>15</sup>N 6.0±1.9 ‰ (1 sigma interval, n=10). The presence of an outlier (CvL2-6333) presenting high values of δ<sup>13</sup>C could be the result of incorrect taxonomic classification.

Tayassuidae (Palmer 1897) have generalist omnivore diets (Barreto, Hernandez, & Ojasti, 1997; Bodmer, 1991; March, 1993). The total of 6 analyzed collagen samples clustered into two groups of three samples each. One group has a typical C<sub>3</sub> type herbivore diet, very similar to that found in deer, averaging δ<sup>13</sup>C -22.5±1.0‰ and δ<sup>15</sup>N 4.7±0.5‰; whilst the other group has a more carnivore-like diet, averaging δ<sup>13</sup>C -16.5±1.0‰ and δ<sup>15</sup>N 9.7±0.3‰. This difference could be due to a number of reasons such as their broad alimentary range, inter-species variation, environmental changes, or the small sample size.

Both armadillo species, *Dasypus novencinctus* and *Euphractus sexcinctus* (Dasypodidae, Gray 1821) are omnivores with a tendency to carnivory (Bezerra, Rodrigues, & Carmignotto, n.d.; Breece & Dusi, 1985; McBee & Baker, 1982; McDonough & Loughry, 2003). Both species have collagen isotope values compatible with a carnivorous behavior averaging δ<sup>13</sup>C -14.9±1.7‰, δ<sup>15</sup>N 8.2±0.5‰ (n=3) and δ<sup>13</sup>C -18.4‰, δ<sup>15</sup>N 8.2‰ (n=2), respectively. These results

might seem unusually enriched, but similar ones have been found in armadillos from pre-classic Mayan sites (van der Merwe, Tykot, Hammond, & Oakberg, 2000).

The human results are invariant in their  $\delta^{13}\text{C}$  values, displaying a mean of  $\delta^{13}\text{C} - 19.0 \pm 0.6\text{‰}$  and, therefore, indicating a predominately  $\text{C}_3$  based diet. The  $\delta^{15}\text{N}$  values range from 5.3 to 11.3‰. The most enriched  $\delta^{15}\text{N}$  value comes from an infant with non-erupted permanent dentition and probably reflects breastfeeding (Fuller, Fuller, Harris, & Hedges, 2006) (blue disks in Figure 5a). Sub-adults with erupted permanent deciduous already have  $\delta^{15}\text{N}$  values more similar to adults (red disks in Figure 5a). The low  $\delta^{15}\text{N}$  values in the adult population is distinct from the carnivores ( $t=4.50$ ;  $p=0.001$ ) and similar to the herbivores from Lagoa Santa region ( $t=0.25$ ;  $p=0.400$ ), suggesting a diet based on plants and supplemented by fauna (O'Connell & Hedges, 1999). This is consistent with zooarchaeological studies from central Brazil pointing to a generalist diet mostly based on the gathering of plants but supplemented by small game (Kipnis, 2002).

## 7. LITHIC AND BONE TECHNOLOGY

Lapa do Santo presents abundant lithic assemblage with tens of thousands of pieces collected. A complete study of this material is yet to be realized, but the content of Unit F13 was analyzed to offer a preliminary characterization. This unit is a good representative of the early Holocene phase of Lapa do Santo, presenting an approximately 4-m thick deposit of archaeological sediments that accumulated between 12.7-11.7 cal kyBP and 8.3-8.0 cal kyBP. A total of 3589 lithics were analyzed (Pugliese, 2008). Of those 212 were plotted and 3377 recovered from the sieving. Among the plotted material, 84% are flakes (178), 11% cores (24) and the remaining 5% are composed of unused raw material, flaked pebbles, one hematite axe and one preform of a projectile point that based on its small size and the presence of a peduncle is assumed to be an arrowhead. This assemblage is dominated by small flakes and small cores and formal tools are very rare (see Figure S1 for selected examples of typical elements of Lapa do Santo industry). A total of 126 complete flakes were measured yielding the following average and standard deviation of length, width and thickness, respectively:  $25.27 \pm 12.12\text{mm}$ ;  $21.18 \pm 9.66\text{mm}$ ;  $9.45 \pm 6.48\text{mm}$ .

There is almost no preparation of cores, and flakes were obtained by a reduction strategy resulting in 70% of amorphous cores. The flakes are rarely retouched (among the 212 pieces, only one case was identified), but they sometimes present macroscopic use-wear traces. Flakes with and without macroscopic use wear have similar morphologies. It is proposed that the flakes were the final goal of the reduction strategy being used as instruments of some kind of specific activity such as cutting or scraping. It is proposed that the reduction strategy aimed for flakes with this morphological characteristics to be used directly for some kind of specific activity such as cutting or scraping without any further retouching. Experiments with crystal

quartz flakes show they quickly become dull and since retouching is not observed it is assumed that they were used only a few times before being discarded (Pugliese, 2008).

The lithic assemblage of Lapa do Santo has a low variability in raw material, forms and types. The incidence of curated vestiges is low and formal tools are virtually absent. Another characteristic of Lapa do Santo is the exhaustive exploitation of cores in which all planes of crystals are flaked. Altogether, this supports the hypothesis that specific activities were executed on the site (Binford, 1982; Panja, 2003; Parry & Kelly, 1987). The constancy of the lithic types through time attests to the stability of the related activities and the efficiency of this technology. It is plausible that there is a strong correlation between the lithic industry and the processing of vegetables at Lapa do Santo, especially considering that this kind of vestige is very frequent at the site and that the characteristics of the used flakes indicate the potential employment of composite instruments for this sort of use (Pugliese, 2008).

While lithic types were constant, the use of raw material varied through time (Fig. 6). Crystal quartz was by far the dominant raw material, but silex, quartzite and silicified sandstone also appears as a minor component during specific periods of occupation. Crystal quartz commonly occurs within the karstic region but the other three are allochthonous, being found in the vicinities of the karst in locations such as Jaboticatubas River (ca. 25 km) and Espinhaço Mountains (ca. 60 km). Silex, although scarcer and far easier to knap, was used in a similar way as crystal quartz, to produce flakes and cores. Thermal treatment of silex was common.

Although in low frequency, allochthonous raw material was used since the beginning of the occupation of Lapa do Santo (Unit F13, Level 40), but at approximately 9.9 cal kyBP (Fig. 6) it almost disappears from the site remaining present only at small quantities (see section on chronology for the basis upon which this date was obtained). The same pattern of drastic reduction in the use of exogenous raw material around 9.9 cal kyBP has been described in other sites in Lagoa Santa region, such as the Lapa das Boeliras rockshelter (Astolfo G.M. Araujo et al., 2008; Astolfo G.M. Araujo & Pugliese Jr., 2010; Pugliese, 2008) and also in open air sites in the Lagoa Santa region (Astolfo G.M. Araujo et al., 2012). The abandonment of allochthonous raw material around 9.9 cal kyBP is interpreted as reflecting a shift towards a subsistence strategy more focused on local items immediately available in the karst. Taking into account how close to Lagoa Santa the sources of the allochthonous raw material were, if this interpretation is correct, it implies a very limited territorial range for these hunter-gatherer groups. This shift towards more expedient practices based on easily available raw material could imply a change in the mobility pattern related to the increased stability in the environment conditions with the advance of the early Holocene.

In addition to the tens of thousands of flakes and cores recovered from Lapa do Santo, one hematite "axe" blade (PN: Ls-6410; Fig. S2) and one projectile point (PN: Ls-5534; Fig. S3)

were also found in the deposits belonging to LSP-1 (i.e. early Holocene). Other blades were found in Lapa do Santo but they are either not from the early Holocene component of the site or of unknown stratigraphic provenience and will not be described here. Even so, for sake of completeness we provide basic contextual information for all blades from Lapa do Santo in Table S11.

The piece identified with accession code St-6410 is the proximo-mesial part of a broken hematite 'axe'-blade and it weights 375g. It was found on level 31 of unit G13 of Lapa do Santo ( $x = 6.621$ ,  $y = 12.726$ ,  $z = -2.998$ , see the green diamond in Figures 2 and 3). It was recovered in August 2008. When this z-values is applied to the linear regression proposed in section 2 the estimated chronology for the hematite axe blade is 10.4 cal kyBP, ranging from 9.7 cal kyBP to 11.0 cal kyBP within a 95% parametric confidence interval.

The presence of knapping stigmas in the ventral face indicates this blade was produced from a thick flake and that it was most probably the head of an axe. Since no agriculture is known among the groups inhabiting Lagoa Santa during the early Holocene is unlikely this blade was the head of an adze (Fig. S2). The straight and planar breakage at the distal part is commonly observed in other blades from Brazil and is probably the result of a strong impact during use. The hematite blade from Lapa do Santo presents some polishing, mostly concentrated near the butt. The grinding seen in some parts of the artifact are the result of attrition with some vegetal material, probably the wooden shaft.

Both sides of the blade show technological signs of being produced over an anvil (Fig. S2). After the breakage, there was an attempt to reshape the blade, trough flaking the ventral side and using the flat broken surface as the striking platform. This process, however, was unable to properly reshape the blade, possibly leading to its abandonment/discard. The hematite blade from Lapa do Santo can be tentatively classified as cordiform following the typological scheme proposed by Prous et al., (2003). Cordiform blades are characterized by i) being completely bifacially flaked, ii) usually presenting the active edge narrower than the butt, iii) having an asymmetric beveled edge and, iv) presenting an aspect of an overall lack of aesthetic refinement. They contrast with the more symmetrical and fully polished axes blades that are more commonly found in Brazil.

Hematite blades are rare in Brazil. In the state of Minas Gerais (586,528 km<sup>2</sup>), where Lapa do Santo is located, from a total of 155 known blades only 10 were produced over hematite (Ott, 1958). The non-hematite blades are usually associated with ceramist occupations and are assumed to be more recent than those made over hematite (G. N. Souza, 2013). However, this is a working hypothesis deserving further testing.

Among all hematite blades from Brazil only two were found in context: the one described in the present contribution and one from the archaeological site of Lapa das Boieiras (PN: Bi-2179)(see Araujo *et al.* 2008 for a detailed description of the site). Similar to the one from

Lapa do Santo, the hematite blade from Lapa das Boleiras was found within the early Holocene component of the site. Although a sample size of two is far from enough to propose a general pattern, it seems that the hematite axe blades were a common element of the early Archaic tool-kit.

The findings from Lapa do Santo and Lapa das Boleiras support *Prous et al.*, (2003) original suggestion that the so-called “cordiform blades” are related to the earlier periods of occupation in central Brazil. A third cordiform hematite blade was found in the site of Santana do Riacho (ca. 40 km from Lapa do Santo) and might belong to the early Archaic period as well. However, its chronology is ambiguous since it was found between layer 2 and layer 4 that are dated to 5.0-8.0kyBP and 8.0-10kyBP, respectively.

A detailed study on the provenience of the hematite used in Lagoa Santa region (i.e. Lapa do Santo and Boleira) is not yet available. The nearest known ferrous outcrop is located ca. 55 km to the south (the Cauê formation on the Curral Hills) but it might be possible that cobbles of hematite were available in the beds of the Velhas River, less than 10km distant from Lapa do Santo. Therefore, it is not possible to be sure if the hematite was immediately accessible for the production of the blades or not.

Therefore, it is possible that the groups inhabiting Lagoa Santa during the early Holocene were intentionally targeting hematite as one of their favorite raw material for the production of axe blades. This might be explained by the greater hardness of hematite when compared to other raw materials commonly used for blade production and it has been shown (G. N. Souza, 2013) that hematite blades have sharper cutting edges when compared to non-hematite blades. Besides, its red color and metallic shine can be considered as aesthetic appealing characteristics and, therefore, non-utilitarian reasons might explain the selection of hematite as raw material (G. N. Souza, 2013).

The projectile point (PN: Ls-5534, Fig. S3) was recovered in July of 2002 in the level 28 of unit G12 (x=6.013, y=11.686, z=-2.673). When this z-values is applied to the linear regression proposed in section 2 the estimated chronology for the projectile point is 10.0 cal kyBP, ranging from 8.9 cal kyBP to 10.5 cal kyBP within a 95% parametric confidence interval. The projectile point is made out of silicified limestone, using a natural original plaque as a blank. It presents ground technique at one face. This is the most ancient evidence of the ground technique for lithic tools production (at reduction/*façonage* stage) in Americas to be recorded. The grounding technique is not usual for lithic points production in Brazilian industries, not even at Late Holocene. This projectile point has no technological similarity to the Early Holocene lithic points found at southern Brazil, related to the Umbu tradition industries (Moreno de Sousa, 2014).

There are three possible explanation for the rare presence of these formal artifacts (i.e. hematite axe and projectile point): 1-) formal tools were commonly produced outside the

rockshelters by the same groups that produced the flakes and cores commonly found in Lapa do Santo, but were almost never discarded within the rockshelters, 2) that groups with distinct lithic technology would eventually pass through Lagoa Santa or, 3-) that the groups from the karst were part of a broader socio-economic network and that these rare elements were obtained by some sort of interregional contact (Pugliese, 2008). These are hypothesis deserving future investigation.

The bone artifacts from Lapa do Santo are very similar to what is observed in other parts of central Brazil during this chronological timeframe. A total of 198 bone artifacts or fragments of bone artifacts were found on the site, including spatulas (71%) and burins (25%)(Fig. S4). Highly standardized and by far the most common type of artifacts occurring in Lapa do Santo, the spatulas' functions remain unknown. The manufacturing of these artifacts required considerable time investment and involved distinct types of techniques. The rare presence of elaborate fishhooks (six in total, Table S12 and Fig. S5) is at odds with the rare presence of fish remains at the site. All fishhooks were part of LSP-1. The fish hooks were all retrieved from the sieve and therefore did not received a provenience number. Later in laboratory they received a special numeration from 1 to 6. Projectile points made from bone were not found on the site. Fifty-seven percent of the bone artifacts were made of deer bone, which was also by far the most commonly consumed animal in the site. A total of 49% of the artifacts were carved from metapodial deer bones (R. O. Santos, 2011).

## **8. MICROWEAR ANALYSIS OF LAPA DO SANTO LITHIC**

Archaeological artifacts function, within archaeological research, is often interpreted from its morphology. This creates a problem when dealing with archaeological assemblages which do not have formalized tools such as in Lapa do Santo. Microwear analysis offers a method to overcome such limitation by directly assessing the uses of lithic tools.

Properly conducted microwear research protocols involve three key steps: experimentation, blind testing, and analysis. Over the course of the previous four years one of the authors (HMR) has collected and created a collection of over 100 experimental tools. These tools were used for a broad range of activities including scraping, chopping, drilling, cutting, and butchery. Contact materials included wood, bone, antler, plant remains, animals, leather, hides, and shell. Microwear experiments are necessary in order to create comparative collections for use in analysis as well as for use in blind testing (Odell, 1985; Tringham, Cooper, Odell, Voytek, & Whitman, 1974).

Blind tests are used to assess an analyst's preparedness for conducting work upon archaeological assemblages. These tests are conducted using experimental artifacts for whose use is known. Generally, ten to twenty experimental pieces are chosen by an outside party for the analyst to examine. Analysts must identify the location of utilization, relative action, relative



hardness of contact material, exact action and exact contact material for each artifact selected in the examination. Scores are then calculated to determine the accuracy of the analyst. It should be noted that blind test scores often underestimate the skills of an analyst. When examining archaeological assemblages, for any tool for which information is indeterminate analysts do not propose a guess, however when conducting a blind test analysts must put a precise answer to all questions, any blanks or indeterminate information is simply marked incorrect. This ensures that analyst cannot artificially inflate their scores by leaving blank answers but tends to negatively skew results. Scores for the most recent blind test for HMR are listed in Table S13.

In the course of analysis of archaeological collections, artifact uses are identified using a constellation of traits including edge scarring, striations, polish, and edge rounding. Since this analysis utilized primarily the low-power method, which utilizes magnification between 10-120x, edge scarring was of particular importance. Certain scar types, distributions, and sizes are associated with particular activities, which allow an analyst to identify both the action of the tool and the potential hardness of the contact material. For instance, a tool used in a longitudinal pattern will display scars on both edge surfaces, while a tool used for transverse activities will tend to have scarring on a single surface. The relative hardness of the contact materials also affects what kind of scars will be present, for instance use of softer materials, such as flesh or hides, tends to produce small scars, generally with feather terminations. Harder materials such as antler or bone will produce larger scars which often have step terminations and edge crushing. Comparisons with experimentally used pieces are vital during the course of analysis as some lithic raw materials will vary in the intensity of the edge scarring depending upon the brittleness of the raw material.

To explore the potential use of micro-wear analysis in the Lapa do Santo assemblage a sub-sample of nine lithics were chosen for a preliminary study. They were examined using a Nikon SMZ 800 microscope with a microscopic range of 10-120x magnification power. The nine lithic artifacts examined were made of quartz crystal which can be difficult to analyze due to its translucent crystalline structure. Artifacts were cleaned using water and toothbrush, a sonic cleaner was not utilized as quartz will occasionally break apart when subjected to this kind of cleaning. Some of the more stubborn soils were not removed as they were cemented to surface and their removal may have caused damage to the surface of the tool. When necessary powdered-paint was applied to the surface of the artifacts to improve the visibility of scar patterns, all artifacts were washed again after examination to remove all residue from the surface application.

From a total of nine analyzed lithics only one had definitive evidence of utilization that could be detected by microwear analysis. This lithic (St-7865) was recovered in situ on the 7<sup>th</sup> of May 2012 from Unit O3, Level 3, Facies 4. Its exact location within the excavation unit is  $x=14.325$ ,  $y=2.154$ ,  $z=0.416$ . The utilized piece is a small flake made on translucent crystal quartz,

measuring 33.15mm long, 18.70mm wide, and 6.88mm thick. St-7865 has light utilization on polar coordinate seven. The scar patterns are bifacial and close together including scaler and feather scars and some snap fractures (Fig. S6a). The scars are small indicating the artifact was used as cutting implement of soft materials (Fig. S6b). Given the entire lack of polish development and striations it was not possible to assign a more exact material. Soft materials might include vegetal, hides, meat, cordage or grasses.

## 9. STRONTIUM ISOTOPIC DATA

Strontium isotopic analysis ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) of skeletal material is a commonly employed method for detecting provenance and mobility amongst mammals, including humans (Price, Burton, & Bentley, 2002; Price, Knipper, Grupe, & Smercka, 2004), because tooth enamel from individuals record the isotopic signal of when it is formed during the earliest stages of life (Humphrey, Dean, Jeffries, & Penn, 2008). Since radiogenic isotope  $^{87}\text{Sr}$  forms by radioactive decay from rubidium ( $^{87}\text{Rb}$ ), the  $^{87}\text{Sr}/^{86}\text{Sr}$  signature of a specific location is determined by the underlying bedrock age and its content of Rb. Younger geological formations like volcanic rocks have lower  $^{87}\text{Sr}/^{86}\text{Sr}$  values than older geological formations such as granite. A specific geological strontium signature is incorporated into body hard tissues by substituting for calcium (Bentley, 2006; Ericson, 1985; Price et al., 2002), since strontium enters the ecosystems without fractionation (Faure & Powell, 1972; Graustein, 1989). Amongst skeletal tissues, to date, tooth enamel is the preferred substrate for this analysis, due to its greater resistance to diagenesis in the burial environment (Budd, Montgomery, Barreiro, & Thomas, 2000; Hoppe, Koch, & Furutani, 2003). Within a single archaeological population,  $^{87}\text{Sr}/^{86}\text{Sr}$  analyses of individuals' teeth can potentially detect those who were born on differing geological substrates ("non-locals"). However, environmental background studies are needed to assess the local bioavailable  $^{87}\text{Sr}/^{86}\text{Sr}$  signature from the different geologies in the study region (Evans, Montgomery, Wildman, & Boulton, 2010; Price et al., 2002), in order to assess possible provenance and territorial mobility.

The human teeth were prepared in solution and analysed in a MC-ICP-MS for strontium isotope in the lab facilities of the Department of Human Evolution from the Max-Planck Institute for Evolutionary Anthropology (MPI-EVA) in Leipzig, Germany (Copeland et al., 2008). Solid pieces of enamel weighing approximately 20 mg were drilled from the crown of each of the teeth, spanning from the cement-enamel junction to the occlusal surface, and cleaned thoroughly on all sides under a magnifying lens with a diamond drill bit to ensure no dentine or other material remained attached to it. After the drilling and cleaning, the pieces of enamel were sonicated for at least 15 minutes in high purity deionized water, before they were taken to the MPI-EVA clean lab facility (PicoTrace GmbH, Bovenden, Germany). The samples were then rinsed three times with high purity deionized (18.2 M $\Omega$ ) water (Milli-Q<sup>®</sup> Element A10

ultrapure water purification system, Millipore GmbH, Schwalbach, Germany), rinsed once with ultrapure acetone (GR for analysis grade,  $\geq 99.8\%$ , Merck KGaA, Darmstadt, Germany), and dried overnight.

Further preparation of the enamel samples followed a modified version of the method described by Deniel and Pin (Deniel & Pin, 2001). Each enamel sample was weighed into clean 3 mL Savillex™ (Minnetonka, MN, USA) vials and closed-vessel digested on a heating block at 120 °C in 1 mL of 14.3M nitric acid (HNO<sub>3</sub>) before being evaporated to dryness at around 90-120 minutes. The resulting residue was then re-dissolved in 1 mL 3M HNO<sub>3</sub> in order to pass its solution through ion exchange chromatography using 50-100 μm bead size Sr-spec™ resin (EiChrom Technologies, Inc., Darien, USA) suspended in ultrapure deionized water (Horwitz, Chiarizia, & Dietz, 1992) and previously cleaned following the procedure delineated by Charlier and collaborators (Charlier et al., 2006). Several washes were carried out with 3M HNO<sub>3</sub> before the Sr in the sample was eluted with ultrapure deionized water, dried down, and redissolved in 3% HNO<sub>3</sub> prior to MC-ICP-MS analysis.

A standard with known strontium isotope values (Bone Meal SRM 1486, National Institute of Standards & Technology, USA) and a blank sample were prepared parallel to the samples. Thus, one preparation batch was formed by 13 samples, 1 standard, and 1 blank. All acids used were made from SupraPur® grade (Merck KGaA) stock solutions and diluted using ultrapure deionized water.

A Thermo Fisher Neptune™ (Thermo Fisher Scientific Inc., Dreieich, Germany) MC-ICP-MS instrument at the MPI-EVA facilities (see Table S14 for operational parameters) was used to obtain the strontium isotope measurements. This mass spectrometer is a high-resolution double-focusing one, equipped with nine Faraday detectors fitted with 10<sup>11</sup> Ω resistors (four movable detectors H1-H4/L1-L4 on either side of a fixed axial detector) and a Virtual Amplifier™ system which eliminates possible amplifier-detector bias and provides a dynamic range of 5 mV to 50 V on each detector (Batey et al., 2005; Nowell, Pearson, Ottley, Schweiters, & Dowall, 2003). A 100 μL/min self-aspirating capillary and MicroFlow PFA (perfluoroalkoxy) ST-nebulizer (Elemental Scientific Inc., Omaha, USA) was used to introduce the solutions, diluted in 3% HNO<sub>3</sub> to give <sup>88</sup>Sr signal intensities of 20-25 V into the plasma.

A static mode using a collector configuration similar to that described by Batey and collaborators (Batey et al., 2005) was used to measure <sup>87</sup>Sr/<sup>86</sup>Sr strontium isotope values. The analysis of each sample was divided in two consecutive parts: a first baseline measurement at half mass positions (85.6 and 86.5) of the axial cup mass (<sup>86</sup>Sr) for 30s (20 cycles each 1.05 s), and secondly data collection involving a block of 50 cycles of 2 s integrated time. Interferences by Kr in the carrier gas (argon) and by Rb in both the carrier gas and samples were corrected, same as mass bias normalization (using <sup>88</sup>Sr/<sup>86</sup>Sr=8.375209, exponential law), following an inverse mass bias correction procedure described by Nowell and collaborators (Nowell et al., 2003).

A regression equation described by Copeland and collaborators (Copeland et al., 2008) was used for estimating the strontium concentration (ppm) of the enamel solution runs, based on the  $^{88}\text{Sr}$  signal intensity (V) of three solutions with known strontium concentrations (100, 400 and 700 ppb). We used the strontium carbonate isotopic standard SRM 987 (NIST, USA) SRM 987 as working standard during the measurement, standard SRM 1486 as prepared external standard, and blanks as controls for contamination during the preparation. Thus, one analytical session was composed of 24 samples, 2 prepared blanks, 2 prepared standards SRM 1486, and 8 working standards SRM 987 with 16 blanks (one before and one after the working standard). Samples of this study were measured in two different analytical sessions.

Repeated  $^{87}\text{Sr}/^{86}\text{Sr}$  measurements of working standard SRM\_987 resulted in a mean of  $0.710287 \pm 0.000010$  ( $1\sigma$ ,  $n=16$ ) during the analytical sessions and were corrected to the accepted value of  $0.710240 \pm 0.00004$  (Terakado et al., 1988; Johnson et al., 1990). The long-term average for  $^{87}\text{Sr}/^{86}\text{Sr}$  of the external standard SRM 1486 is  $0.709297 \pm 0.000024$  ( $n=68$ ). The measurements of standard SRM 1486 resulted in a mean of  $0.709297 \pm 0.000011$  ( $1\sigma$ ,  $n=2$ ) during the analytical sessions. All procedural blanks were considered negligible ( $^{88}\text{Sr} < 0.040$  V) at  $<0.4\%$  of the analyte signal intensity ( $^{88}\text{Sr} \approx 20\text{V}$ ).

To provide a preliminary background of the local level of strontium bioavailability in Lagoa Santa region forty-two shell samples from Lapa do Santo and 34 shell samples from Lapa das Boieiras (Astolfo G.M. Araujo et al., 2008) were also analyzed. Geologically Lagoa Santa region is fairly homogeneous being largely dominated by late Neo-Proterozoic sedimentary rocks (see section 1). Therefore, the level of strontium bioavailability is not expected to vary widely within the region. These samples were prepared and analysed in the lab facilities of the Universidade de Brasília, in Brasília, Brazil. The mechanical cleaning was done with a brush with soft plastic bristles to remove superficial impurities and then followed by ultrasonic cleaning during 5 minutes in ultra-pure water. After this step, the samples were dried down in an oven at room temperature. The chemical cleaning involved 3 different sequences of rinsing operations to remove any organic remains: diluted peroxide oxygen ( $\text{H}_2\text{O}_2$ ), 0.1M glacial acetic acid and ultrapure water. In the case this treatment was not enough to fully dissolve the sample they were further subjected to 0.1M hydrochloric acid treatments during a few seconds. After being dried, the fragment was grinded manually using agate mortar and pestle. Usually, aliquots of 0.3 to 20 mg of powdered samples were used depending on sample availability.

The Sr for isotope analysis was separated using Teflon Eichrom microcolumns with specific Sr resin (EiChrom Technologies, Inc., Darien, USA) after it was dissolved using 500  $\mu\text{l}$  of 5N  $\text{HNO}_3$ . The separated Sr was dissolved using 5ml of 3% nitric acid and the isotopic composition of strontium was determined on an MC-ICP-MS (Neptune from Thermo Instruments) in the Laboratory of Geochronology of Universidade de Brasília. The concentration of Sr in the solution was usually higher than 200 ppb, allowing it to minimize the

isobaric interferences from Kr which are always present in the Argon gas necessary to cool the plasma source. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios were corrected for isotope fractionation to  $^{88}\text{Sr}/^{86}\text{Sr} = 0.1194$ , as is currently adopted when using the thermal ionization mass spectrometry. The SRM 987 Sr isotope standard was analyzed 20 times and routinely interspersed every 5 samples in the course of this analysis. Its average value and standard deviation were 0.710300 and 0.000013, respectively. Since this value is slightly higher than the expected 0.710248 described by McArthur (McArthur, 1994), all sample ratios were corrected by subtracting a fixed value of 0.000052.

Strontium  $^{87}\text{Sr}/^{86}\text{Sr}$  values from the 23 enamel samples (Table S15) were successfully measured. The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio measured in human enamel has a mean value of  $0.722 \pm 0.005$  ( $1\sigma$ ) and  $\pm 0.001$  ( $2\sigma$ ), with minimum and maximum values of 0.717 and 0.739 respectively. The values of all but one, Burial 10 (0.739), fall well within the  $2\sigma$  mean value of the population (Figure S5b). Burial 10 is the only one that was cremated and this could explain its anomalous behaviour by indicating that the individual in it buried was an outlier coming from terrains presenting higher  $^{87}\text{Sr}/^{86}\text{Sr}$  values. This suggests that the majority of the individuals lived on a same type of terrain, at least during the major time of the crown formation of their respective analysed teeth: P4 (n=11, 6-7 years old), M2 (n=2, 7-8 years old), M3 (n=2, variable, normally during early adulthood) and deciduous M2 (n=8, 10 months; if breastfed, representing their mothers' values). Furthermore, these values are close to the bioavailable strontium signature of the region, as indicated by the analysis of the 76 shells. Together, this is compatible with a scenario of low territorial mobility and a subsistence strategy probably based on local items. Future studies focusing on better characterising the strontium bioavailability within Lagoa Santa and nearby regions will further improve our capacity of understanding the significance of the Sr ratios reported here.

## 10. CRANIAL MORPHOLOGICAL AFFINITIES

The seven most complete adult skulls from Lapa do Santo (Burials 1, 5, 11, 14, 17, 21, 26) were measured by DVB following Howells protocol (Howells, 1973, 1989). The Lapa do Santo skulls were then compared to reference samples representing other Lagoa Santa populations (Cerca Grande (W.A. Neves, González-José, et al., 2004) and Sumidouro (W.A. Neves, Hubbe, & Piló, 2007)), Early and Archaic Colombia Series (W.A. Neves, Hubbe, & Correal, 2007) and reference populations from Howells database (Howells, 1996). Together they create an appropriate framework to interpret the morphological affinities between the Lapa do Santo Individuals and the morphological variation in the America across time in relation to the modern human worldwide cranial morphological variation (see Table S16 for details on the series included). The morphological affinities analyses were based on 22 variables (Table S17), selected to minimize a number of missing values among the prehistoric individuals. The

remaining missing values were replaced by means of multiple regressions (M. Hubbe, Harvati, & Neves, 2011). For all analyses, size was removed from the individuals by calculating a double z-score of the values (W.A. Neves et al., 2013; Relethford, 1994).

To explore the morphological affinities of the series, we realized two analyses. First, Mahalanobis  $D^2$  distances (Mahalanobis, 1936) were calculated between series. The relationship between series given by the resulting matrix was then graphically represented through a Ward's Hierarchical Cluster (Ward Jr., 1963), which combine series into clusters that minimize within-cluster variation while maximizing between-cluster variation. To test the impact that subsampling has in the patterns of association between Lapa do Santo and the remaining series, the cluster was repeated 1000 times, each time on a different permutation with the original data, respecting the initial sample sizes. The frequency of times the same topologies occurred between each branch of the original cluster in each of the permutations was used as a measurement of the strength of the morphological associations between series. In other words, this indicates how well the average morphology obtained in a series represents the real morphological affinities of the population represented by the skull sample.

Second, morphological affinities were also represented in the first two Principal Components (PCs) extracted from the covariance matrix between series. PCs were calculated from the covariance matrix instead of the correlation matrix since the size correction procedure adopted here (see below) standardizes the variance of the variables to a large degree (C. Roseman & Weaver, 2004). PC scores were calculated for all individuals in the dataset. To facilitate visualization, the individuals from Lapa do Santo were plotted together with the centroids of the series in a scatterplot based on the first two Principal Components. In this way, it is possible to compare results from a dimension reduction analysis (Principal Components Analysis), where only the major axes of variation between series are represented, with a distance matrix analysis (Wards Cluster), which considers the totality of the differences between the groups, weighted in this case by the covariance between variables.

Figure 7a (left) shows the results of the cluster analysis. Lapa do Santo appears in a cluster together with the other Lagoa Santa Series, the Colombians series, and Easter Island. The associations between this cluster are not very stable ( $p=0.50$ ), however this cluster, in general, has strong affinities with the cluster composed by Australo-Melanesians ( $p=0.90$ ), which reinforces the idea that all Lagoa Santa groups share a common morphological pattern, similar to Australo-Melanesians and quite distinct from late Native Americans.

Figure 7a (right) shows the morphological affinities among the series based on the first two Principal Components, which together explain 29.1% of the original variation. The centroids, plotted in black, show a similar pattern of affinities from the one obtained by the cluster analysis. Lapa do Santo share the same position in the morphospace with other Lagoa Santa populations, the Colombian series, Australo-Melanesians, and Sub-Saharan Africans.

However, in this analysis, the Lapa do Santo centroid appears closer to Australo-Melanesians and Easter Island, than to other Early South Americans series, which appear closer to the Sub-Saharan African series. Most of the Lapa do Santo individuals, plotted in red, share the same general morphology. The only exception is Burial 17, who appears as an outlier to the series, more closely associated to one of the Polynesia series (Moriuri). The main difference between Early Americans and Late Americans is given by differences in the first Principal Component, which is positively correlated with cranial breadth (XCB) and total and midfacial height (NPH, NLH, OBH, WMH), and negatively correlated with cranial length (GOL, PAC, PAF). Therefore, series on the left side of the graph show longer and narrower neurocrania with relatively shorter faces, while series on the right side of the chart show the opposite pattern. The main difference between the Lapa do Santo centroid and the other early American series is due to the fact that Lapa do Santo tend to have wider orbits (OBB) and shorter Parietals (PAC, PAF).

In conclusion, the morphological analyses of the Lapa do Santo material show that they share the same morphological pattern with other Paleoamericans, which is quite distinct from the morphology shared by the Late Native Americans included in this analysis. This result is entirely consistent with previous studies of the Lagoa Santa material (M. Hubbe, Neves, & Harvati, 2010; W.A. Neves, Hubbe, & Piló, 2007; W.A. Neves & Hubbe, 2005). The one outlier (Burial 17) is hard to explain at this point, and future studies will have to address this individual for more details.

## **11. ESTIMATION OF SEX AND AGE AT DEATH**

Sex estimation for Lapa do Santo skeletons was based on different anatomical regions: analysis of the skull (Walker 2008), the ischium-pubic region (Phenice, 1969), the pelvis (Bruzek, 2002), the proximal region of the ulna (Cowan & Pastor, 2008), and the femoral diaphysis (Black, 1978). These sex evaluations were done through a systematic study, by a single observer (Mariana Inglez). The observer tested her estimation skills in skeletons of the osteological collection of the Museum of Human Anatomy, at University of São Paulo, (MAH-USP), whose demographic profile is known. The accuracy of sex estimation varied from 74% for the femoral diaphysis to 85% for the pelvis (for details, see Inglez (2010). This range of accuracy is within those reported in the literature attesting the observer of the present study is properly skilled. The estimated sex and age at death for the individuals for Lapa do Santo are reported in Table S18. Although this particular publication was focused on the burials allocated to LSMP-1, LSMP-2 and LSMP-3 for the sake of completeness in Table S17 sex and age at death estimation is provided for all individuals from Lapa do Santo available at the date of this publication.

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## SUPPLEMENTARY TABLES

**Table S1:** Basic information of the sediment samples.

<b>Lab #</b>	<b>OSL #</b>	<b>Year collected</b>	<b>Provenance</b>	<b>Depth from surface (cm)</b>
UW861	3	2002	Unit F13, red layer	132
UW1374	18	2005	Unit F13, red layer below hard white layer	164
UW1375	19	2005	Unit F13, red layer above whitish layer	92
UW1376	20	2005	Unit F13, red layer	27
UW1377	21	2005	Unit M6, above rocks at base of excavation	214

**Table S2:** Concentrations relevant to dose rate.

<b>Sample</b>	<b><sup>238</sup>U (ppm)</b>	<b><sup>232</sup>Th (ppm)</b>	<b>K (%)</b>	<b>Measured moisture (%)</b>
UW861	1.31±0.30	30.89±2.56	0.63±0.01	21.2
UW1374	2.72±0.25	17.52±1.66	0.71±0.02	13.7
UW1375	2.11±0.17	9.57±1.07	0.32±0.01	11.5
UW1376	2.31±0.24	17.52±1.67	0.28±0.01	14.1
UW1377	2.69±0.20	8.89±1.24	0.25±0.01	21.6
Rock below UW1374	0.47±0.04	0.20±0.15	0.02±0.01	-
White layer above UW1374	1.15±0.22	21.08±2.00	0.45±0.01	-
White layer near UW861	1.94±0.18	11.32±1.26	0.29±0.01	-
White layer below UW1376	2.23±0.19	9.27±1.26	0.24±0.01	-

**Table S3:** Dose rate information.

<b>Sample</b>	<b><math>\beta</math> dose rate (Gy/ka)</b>		<b>External dose rate (Gy/ka)</b>		<b>Total dose rate (Gy/ka)*</b>
	<b><math>\beta</math>-counting</b>	<b><math>\alpha</math>-counting/flame photometry</b>	<b>dosimeter</b>	<b>laboratory</b>	
UW861	1.42±0.15	1.53±0.08			2.31±0.13
UW1374	1.38±0.12	1.43±0.06	0.94±0.03	1.05±0.07	1.97±0.07
UW1375	0.96±0.11	0.82±0.04	1.17±0.08	0.74±0.06	1.77±0.09
UW1376	1.31±0.20	1.03±0.06	1.02±0.11	1.03±0.08	1.90±0.10
UW1377	0.86±0.14	0.83±0.04			1.22±0.07

\* Total dose rate reflects corrections for moisture content, which are not taken into consideration in the beta dose rates listed. The total dose rate also includes a small alpha contribution. It also depends on whether the dosimeter or laboratory measurements of external dose rate are used.

**Table S4:** Equivalent dose central age value and over-dispersion for each sample.

<b>Sample</b>	<b>N</b>	<b><math>D_e</math> (Gy)</b>	<b>Over-dispersion (%)</b>	<b># of components</b>	<b>Proportion of main component (%)</b>
UW861	312	22.8±0.4	19.3±1.6	1	100
UW1374	452	23.8±0.5	32.3±1.7	2	54.4
UW1375	398	22.5±0.4	19.8±1.7	1	100
UW1376	285	21.4±0.4	21.0±1.9	2	53.0
UW1377	401	11.7±0.3	47.8±2.3	3	53.3

**Table S5:** Date and information on vertical position of the OSL samples from Lapa do Santo.

<b>Lab Id</b>	<b>Unit</b>	<b>Depth (cm)</b>	<b>Inferred z-value</b>	<b>Age (years BP)</b>	<b>Error</b>
UW1376	F13	27	-0.72	11200	700
UW1375	F13	92	-1.37	12700	800
UW1374	F13	164	-2.09	12100	700
UW861	F13	132	-1.77	9900	700
UW1377	M6	214	-1.015	9500	700

**Table S6:** Radiocarbon dates for Lapa do Santo (table available as a .xls file).



**Table S7:** Skeletal representation of *Mazama* sp. at Lapa do Santo.

<b>Skeletal Part</b>	<b>NISP</b>
Calcaneus	3
Carpal	1
Tarsal	1
Horn	3
Rib	25
Innominate	2
Cranium	6
Scapula	9
Proximal phalanx	5
Medial phalanx	3
Distal phalanx	4
Femur	3
Mandible	10
Maxilla	1
Metapodial	5
Metacarpal	7
Metatarsal	26
Navicular	4
Carpal radial	1
Radius	7
Talus	10
Tibia	16
Ulna	3
Humerus	5
Cervical vertebrae	2
Thoracic vertebrae	7
Lumbar vetebrae	1

**Table S8:** Correlations between skeletal abundance (%MAU) and bone density for *Mazama* sp.

<b>SITE</b>	<b>n</b>	<b>Spearman rho</b>	<b>p-value</b>
Guajá	90	-0.047	0.66
Gruta Cuvieri	94	0.699	<0.001
Lapa do Santo	85	0.205	0.06

**Table S9:** Human bone samples from Lapa do Santo. Sample analysed at: (UC) Dorothy Garrod Laboratory for Isotopic Analysis, McDonald Institute for Archaeological Research, University of Cambridge; (MPI) Isotope facilities at the Max-Planck Institute for Evolutionary Anthropology. Mean values of analyses runs given. Sample variation was <0.6‰ .

<b>Code</b>	<b><math>\delta^{13}\text{C}</math> (‰)</b>	<b><math>\delta^{15}\text{N}</math> (‰)</b>	<b>C:N</b>	<b>Dental Development</b>	<b>Analysis</b>	<b>Runs</b>
Burial 2	-18.9	5.2	3.1	Fully erupted	MPI	2
Burial 4	-20.3	7.7	3.5	Erupted first molar	UC	1
Burial 5	-19.5	7.2	3.2	Fully erupted	UC	3
Burial 6	-18.8	11.3	3.5	Non-erupted permanent dentition	UC	2
Burial 7	-18.2	8.8	3.1	Erupted first molar	UC	3
Burial 21	-18.8	7.0	3.2	Fully erupted	MPI	2
Burial 26	-19.0	5.9	3.0	Fully erupted	MPI	1
Burial 27	-18.8	6.5	3.1	Erupted first molar	MPI	1

**Table S10:** Faunal bone samples from Lagoa Santa. Sample analysed at: (UC) Dorothy Garrod Laboratory for Isotopic Analysis, McDonald Institute for Archaeological Research, University of Cambridge; (USP) Laboratório de Ecologia Isotópica, Escola Superior de Agricultura Luis de Queirós, University of São Paulo. Mean values of analyses runs given. Sample variation was <0.7‰.

Code	Site	Species	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N	Analysis	Runs
St-3354	Lapa do Santo	<i>Mazama</i> sp.	-20.5	4.3	3.2	UC	3
St-996	Lapa do Santo	<i>Mazama</i> sp.	-21.9	4.7	3.5	UC	3
St-578	Lapa do Santo	<i>Euphractus sexcinctus</i>	-17.4	7.6	3.4	UC	3
St-743	Lapa do Santo	<i>Dasypus novencinctus</i>	-12.6	10.6	3.2	UC	3
St-548	Lapa do Santo	<i>Mazama</i> sp.	-21.2	4.6	3.2	USP	3
St-2248	Lapa do Santo	<i>Mazama</i> sp.	-21.6	5.6	3.2	USP	3
St-284	Lapa do Santo	<i>Dasypus novencinctus</i>	-14.5	9.5	3.1	USP	3
CvL2-1239	Gruta Cuvieri	<i>Mazama</i> sp.	-21.4	5.1	3.2	USP	2
CvL2-2066	Gruta Cuvieri	<i>Mazama</i> sp.	-19.1	6.9	3.2	USP	3
CvL2-3419	Gruta Cuvieri	<i>Mazama</i> sp.	-18.3	9.1	3.2	USP	1
CvL2-3419b	Gruta Cuvieri	<i>Mazama</i> sp.	-23.2	6.5	3.3	USP	1
CvL2-6333	Gruta Cuvieri	<i>Mazama</i> sp.	-18.6	9.4	3.3	USP	3
CvL2-6333b	Gruta Cuvieri	<i>Mazama</i> sp.	-21.3	4.5	3.2	USP	1
CvL2-1372	Gruta Cuvieri	Tayassuidae	-15.4	9.4	3.1	USP	2
CvL2-4443	Gruta Cuvieri	Tayassuidae	-16.7	9.7	3.2	USP	3
CvL2-2779	Gruta Cuvieri	Tayassuidae	-17.3	10.0	3.2	USP	1
CvL2-4448	Gruta Cuvieri	Tayassuidae	-22.0	5.1	3.2	USP	3
CvL2-5687	Gruta Cuvieri	Tayassuidae	-21.8	4.9	3.3	USP	2
CvL2-7185	Gruta Cuvieri	Tayassuidae	-23.6	4.2	3.3	USP	2
CvL2-7391	Gruta Cuvieri	<i>Dasypus novencinctus</i>	-16.2	10.4	3.2	USP	1
CvL2-5873	Gruta Cuvieri	<i>Dasypus novencinctus</i>	-16.2	10.7	3.2	USP	2
CvL2-5652	Gruta Cuvieri	<i>Euphractus sexcinctus</i>	-19.4	8.8	3.1	USP	3

**Table S11.** Contextual information of all blades found in Lapa do Santo.

<b>Id</b>	<b>Raw Material</b>	<b>Unit</b>	<b>x</b>	<b>y</b>	<b>z</b>	<b>Period</b>	<b>Date of disc.</b>
Ls-00597	?	G13	6.300	12.690	-1.013	Unkown	2002
Ls-02367	?	M05	12.305	4.868	0.756	Unkown	2002
Ls-02976	Igneous	M04	12.945	3.187	0.118	LSP2	2003
Ls-03560	Igneous	L29	11.844	28.417	-5.970	Unkown	2005
Ls-06410	Hematite	G13	6.621	12.726	-2.998	LSP1	2005
Ls-09903	Igneous	O14	14.434	13.619	-0.827	LSP1(?)	2012
Ls-11607	Igneous	P13		Surface find		Unknown	2014
Ls-11608	Igneous	Q12		Surface find		Unknown	2014

**Table S12.** Contextual information of all fishhooks found in Lapa do Santo.

<b>Hook ID</b>	<b>Unit</b>	<b>Level</b>	<b>Raw Material</b>	<b>z-value</b>	<b>Date of recovery (dd/mm/yyyy)</b>
<b>1</b>	M6	26	Bone	-0.778 to -0.968	14/07/2005
<b>2</b>	L23	220-230cm	Bone	-4.900 to -5.400	na/08/2005
<b>3</b>	L22	130-140cm	Bone	-3.700 to -4.100	na/07/2005
<b>4</b>	G12	22	Bone	-1.802 to -2.031	15/07/2005
<b>5</b>	G12	16	Bone	-1.315 to -1.425	15/07/2003
<b>6</b>	G12	16	Bone	-1.315 to -1.425	14/07/2003

**Table S13:** Blind Test Results for Heather M. Rockwell, administered December 2013.

<b>Type of blind test</b>	<b>Frequency of correct identification</b>
Location	19/20= <b>95%</b>
Relative Action	16/20= <b>80%</b>
Relative Material	18/20= <b>90%</b>
Exact Action	15.5/20= <b>77.5%</b>
Exact Material	12.5/20= <b>62.5%</b>
<b>Total Score</b>	<b>81/100=81%</b>

**Table S14:** Operation parameters for MC-ICP-MS solution analysis used at the Max-Planck Institute for Evolutionary Anthropology (Leipzig, Germany).

MC-ICP-MS	Thermo Fisher Neptune™
Forward power	1200 W
Reflected power	<4 W
Interface cones	Nickel
Sample cones	Nickel
Skimmer cones	Nickel (X-cone)
Coolant argon gas flow	15 L/min
Auxiliary argon gas flow	0.8 L/min
Sample gas Argon gas flow	1.17 L/min
Mass resolution	Low (400)
Lens settings	Optimized for maximum signal intensity
Nebulizer	Elemental Scientific Inc., Microflow 100µL/min, perfluoroalkoxy (PFA)
Sensitivity on <sup>88</sup> Sr	50 V/ppm
Cup configuration	L4 ( <sup>82</sup> Kr); L3 ( <sup>83</sup> Kr); L2 ( <sup>84</sup> Sr); L1 ( <sup>85</sup> Rb); Ax ( <sup>86</sup> Sr); H1 ( <sup>87</sup> Sr); H2 ( <sup>88</sup> Sr)
Data collection	1 block, 50 cycles, 2 s integrations

**Table S15:** S-EVA number, archaeological code,  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio,  $^{84}\text{Sr}/^{86}\text{Sr}$  ratio, Sr concentration (ppm) and voltage ( $^{88}\text{Sr}$ ) from enamel of the human teeth prepared in solution and analysed in the MC-ICP-MS.

S-EVA	Bur. #	Tooth	Start mass (mg)	$^{87}\text{Sr}/^{86}\text{Sr}$	$^{84}\text{Sr}/^{86}\text{Sr}$	Sr conc (ppm)	$^{88}\text{Sr}$ (V)
26019	Bur. 1	Inferior Right M3	23.2	0.719	0.0565	123.5	15.7
26020	Bur. 2	Superior Right P4	10.4	0.725	0.0565	181.5	15.7
26021	Bur. 3	Inferior Right P4	33.9	0.722	0.0565	41.4	17.5
26022	Bur. 4	Inferior Right dM2	21.7	0.721	0.0565	58.1	15.7
26023	Bur. 5	Superior Right M3	24	0.729	0.0565	169.9	18.4
26024	Bur. 6	Inferior Left dM2	23	0.720	0.0565	69.3	15.9
26025	Bur. 7	Inferior Left dM2	20.9	0.726	0.0565	87.3	18.1
26026	Bur. 10	Inferior Right P4	29.3	0.739	0.0564	123.3	18.0
26027	Bur. 11	Inferior Right P4	15.1	0.719	0.0565	152.9	16.4
26028	Bur. 15	Inferior Right P4	21.4	0.718	0.0564	155.4	18.2
26029	Bur. 16	Inferior Right P4	24.8	0.722	0.0565	82.8	17.1
26030	Bur. 19	Inferior Left dM2	19.7	0.717	0.0564	88.7	17.4
26031	Bur. 20	Inferior Left dM2	16	0.717	0.0565	136.7	18.2
26032	Bur. 21	Inferior Left M2	21.3	0.724	0.0564	99.7	21.3
26033	Bur. 22	Inferior Right P4	34.5	0.722	0.0564	122.6	21.2
26034	Bur. 23a	Inferior Right dM2	19.9	0.719	0.0565	65.1	21.6
26035	Bur. 23b	Inferior Right dM2	9.2	0.719	0.0565	126.5	19.4
26036	Bur. 23c	Superior Right P3	16.7	0.721	0.0564	216.5	20.1
26037	Bur. 23d	Superior Right P4	14.3	0.722	0.0565	171.4	20.5
26038	Bur. 23e	Inferior Left M2	13.4	0.720	0.0565	96.3	21.5
26039	Bur. 24	Superior Right P4	9.5	0.727	0.0565	105.8	16.8
26041	Bur. 27	Inferior Right P4	20.6	0.717	0.0565	113.5	19.6
26040	Bur. 26	Inferior Left dM2	18.9	0.724	0.0564	163.8	19.4

**Table S16:** Series included in the morphological affinity analyses.

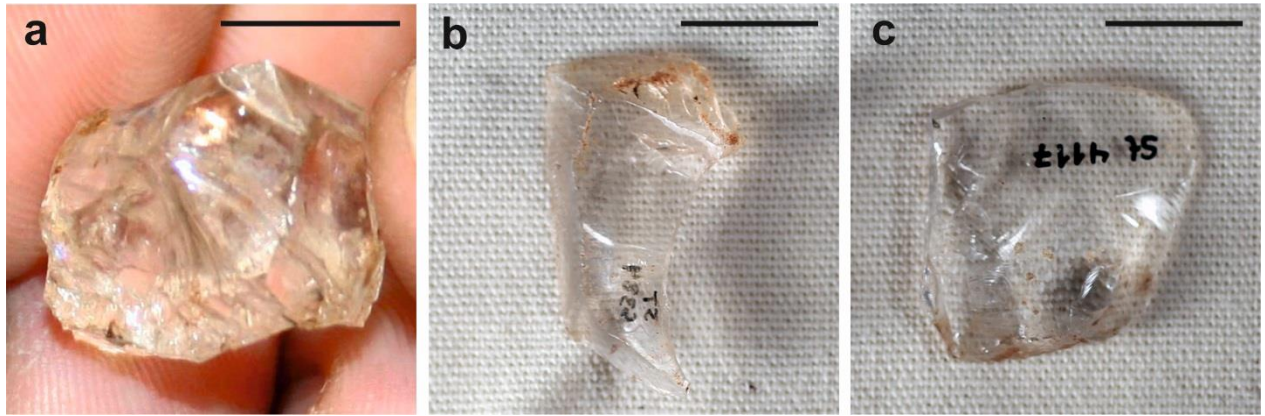
<b>Series</b>	<b>Region/Chronologic Affiliation</b>	<b>N</b>	<b>Reference</b>
Lapa do Santo	Early Lagoa Santa	7	This paper
Cerca Grande	Early Lagoa Santa	5	Neves et al. 2004
Sumidouro	Early Lagoa Santa	13	Neves et al. 2007b
Early Colombia	Early Colombia	38	Neves et al. 2007a
Archaic Colombia	Archaic Colombia	14	Neves et al. 2007a
Peru	South America	110	Howells 1973, 1989
Arikara	North America	69	Howells 1973, 1989
Santa Cruz	North America	102	Howells 1973, 1989
Buriat	NE Asia	109	Howells 1973, 1989
Anyang	East Asia	42	Howells 1973, 1989
Atayal	East Asia	47	Howells 1973, 1989
Hainan	East Asia	83	Howells 1973, 1989
Northern Japan	East Asia	87	Howells 1973, 1989
Southern Japan	East Asia	91	Howells 1973, 1989
Australia	Australo-Melanesia	101	Howells 1973, 1989
Tasmania	Australo-Melanesia	87	Howells 1973, 1989
Tolai	Australo-Melanesia	110	Howells 1973, 1989
Berg	Europe	109	Howells 1973, 1989
Norse	Europe	110	Howells 1973, 1989
Zalavar	Europe	98	Howells 1973, 1989
Bushman	Sub-saharan Africa	90	Howells 1973, 1989
Dogon	Sub-saharan Africa	99	Howells 1973, 1989
Teita	Sub-saharan Africa	83	Howells 1973, 1989
Zulu	Sub-saharan Africa	101	Howells 1973, 1989
Easter Island	Polynesia	86	Howells 1973, 1989

**Table S17:** Craniometric measurements used in the morphological affinity analyses.

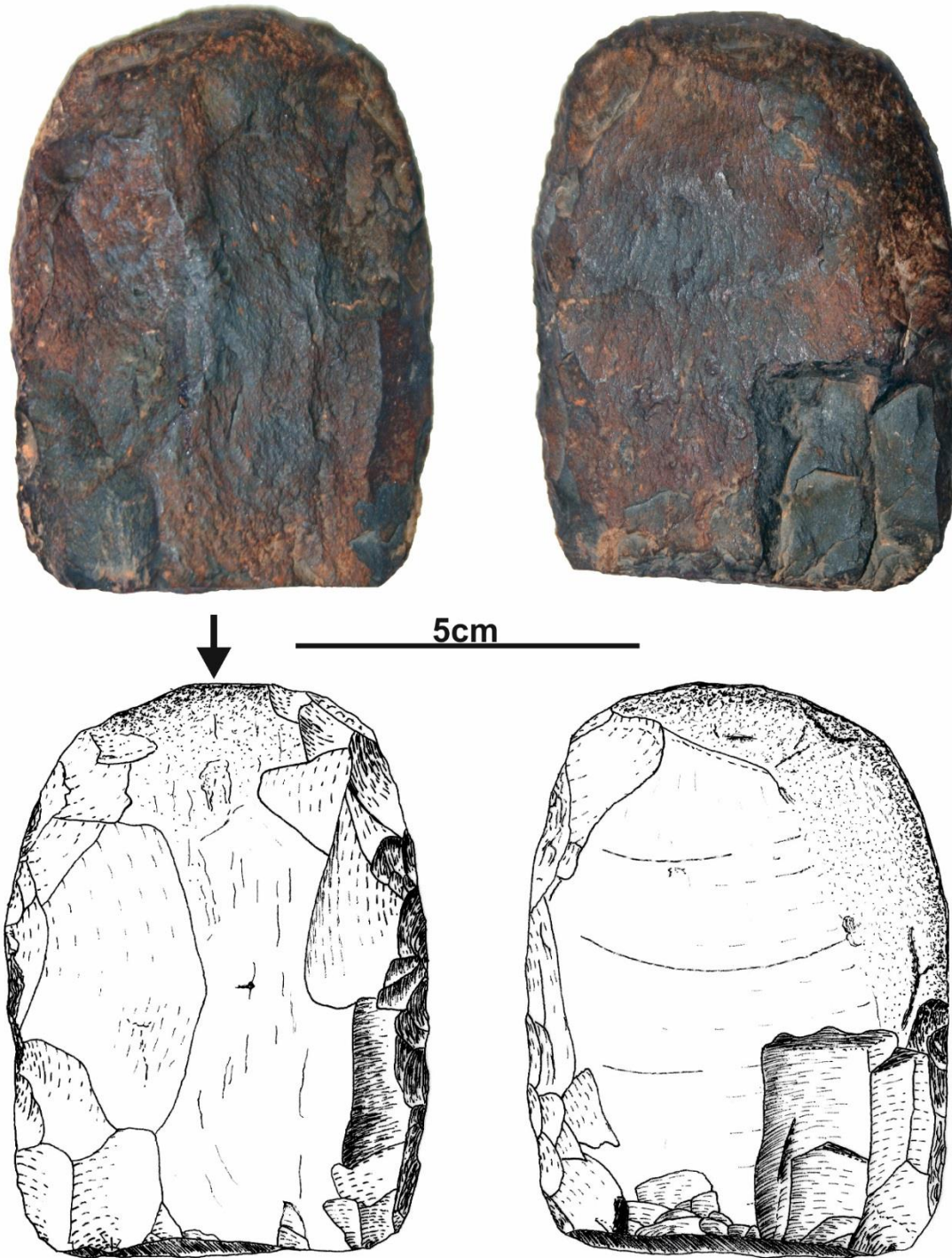
Glabella-occipital length (GOL)	Nasion Subtense (NAS)
Nasio-occipital length (NOL)	Interorbital breadth (DKB)
Maximum cranial breadth (XCB)	Cheek height (WMH)
Maximum frontal breadth (XFB)	Supraorbital subtense (SOS)
Nasion-prosthion height (NPH)	Glabella subtense (GLS)
Nasal height (NLH)	Frontal cord (FRC)
Orbit height (OBH)	Frontal subtense (FRS)
Orbit breadth (OBB)	Frontal fraction (FRF)
Mastoid Height (MDH)	Parietal cord (PAC)
Mastoid Breadth (MDB)	Parietal subtense (PAS)
Bifrontomallare breadth (FMB)	Parietal fraction (PAF)



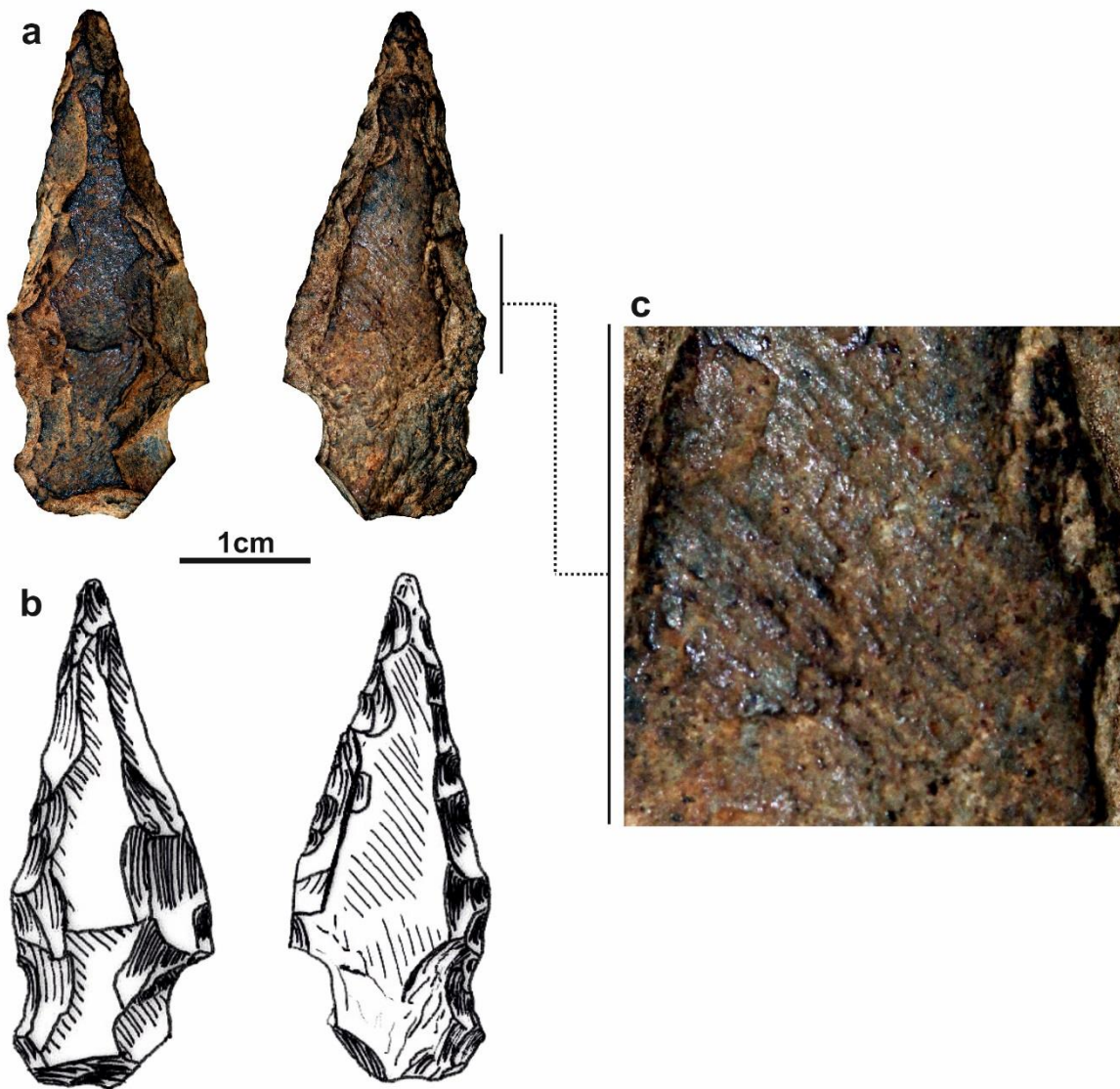
SUPPLEMENTARY FIGURES



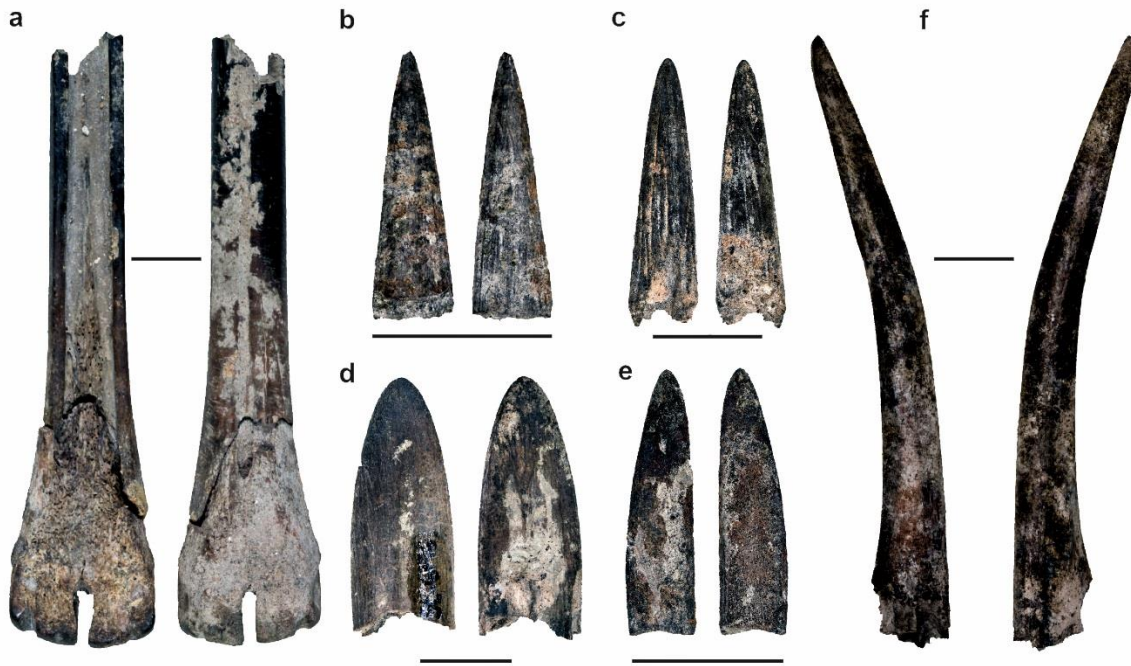
**Figure S1: Selected cores and flakes from Lapa do Santo.** Examples of typical lithic material recovered from Lapa do Santo. The scale bars are 1 cm length. Photos by Francisco Pugliese and André Strauss.



**Figure S2: Hematite blade from Lapa do Santo (PN-6410).** Left and right depicts the ventral and dorsal faces, respectively. It weight is 375g. Photo by André Strauss and drawing by João Carlos Moreno de Souza.



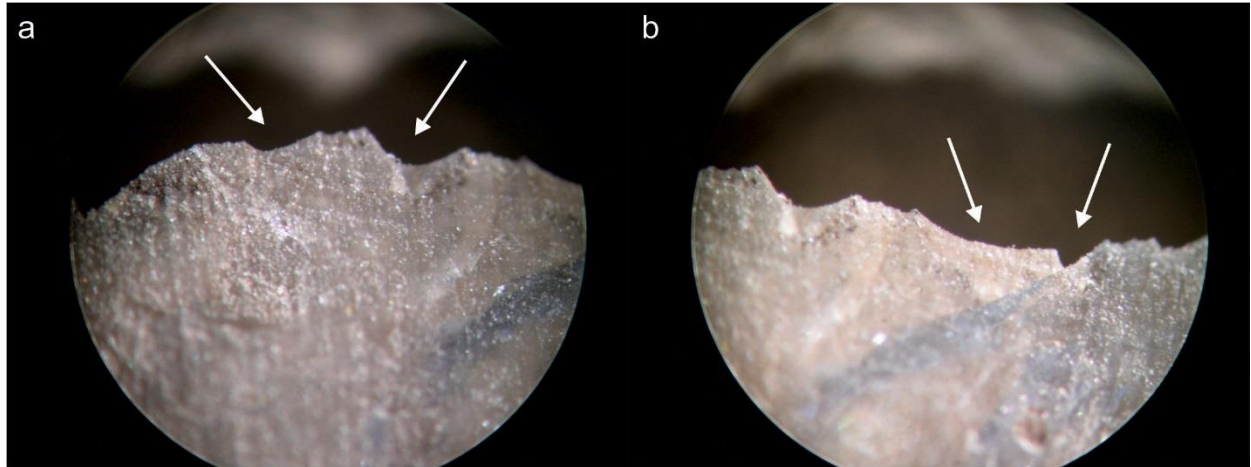
**Figure S3:** Projectile point from Lapa do Santo (PN-5534). Photo by André Strauss and drawing by João Carlos Moreno de Souza.



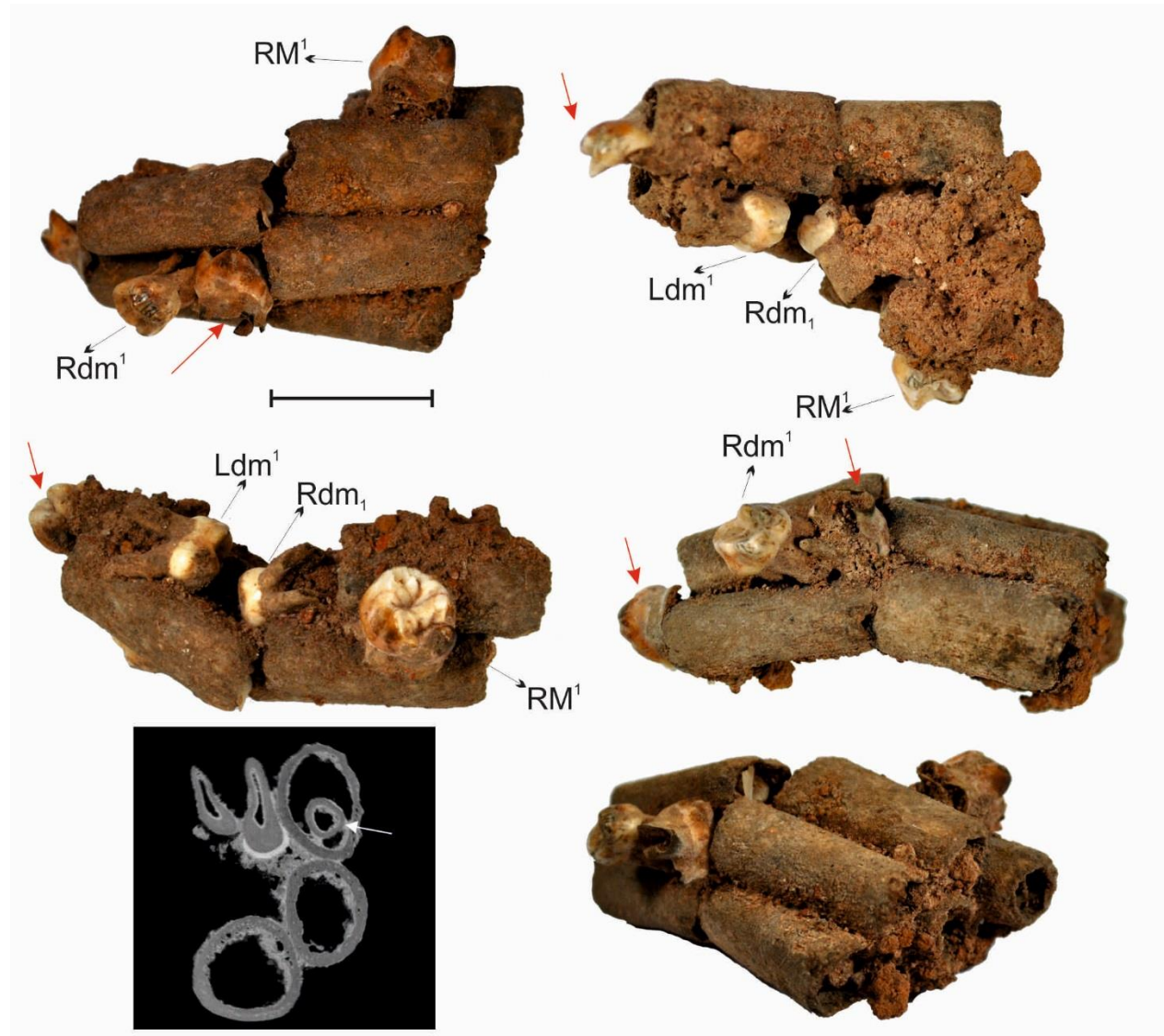
**Figure S4:** Bone artifacts from Lapa do Santo. Each artifact is shown in two different views. The scale bar corresponds to 1 cm. Photos by André Strauss.



**Figure S5:** Fish hooks from Lapa do Santo. From left to right they refer to the numbers 1 to 6, as indicated in Table S11 Photos by André Strauss.



**Figure S6: Microwear analysis of flake St-7865 from Lapa do Santo.** Photo of flake St-7865: 50x magnification of polar coordinate 7, location of utilization. **a**, Feather scar located on the dorsal surface(right arrow) and the base of a removal occurring on the ventral surface (left arrow). **b**, Snap fracture (left arrow) and the edge of a scalar scar at the far right (right arrow). Photo by Heather Marie Rockwell.



**Figure S7:** Assemblage (PN-2253) composed of human bone and teeth found near the sub-adult cranium of Burial 9. This assemblage of cut diaphysis and teeth was found in level 10 of unit G12 and was collected with provenience number 2253. This is most probably associated to Burial 9. The red arrows points to a tooth that was fractured in its half (likely resulting from thermal exposure) and each of the halves were located in a different position of the assemblage. The detail in the left bottom is a CT-scan of the assemblage and the white arrow points to a tooth located inside one of the cut diaphysis. Photo and CT-image by André Strauss.

## Appendix V

### **The oldest case of decapitation in the New World (Lapa do Santo east-central Brazil)**

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## ABSTRACT

We present here evidence for an early Holocene case of decapitation in the New World (Burial 26), found in the rock shelter of Lapa do Santo in 2007. Lapa do Santo is an archaeological site located in the Lagoa Santa karst in east-central Brazil with evidence of human occupation dating as far back as 11.7-12.7 cal kyBP (95.4% interval). An ultra-filtered AMS age determination on a fragment of the sphenoid provided an age range of 9.1-9.4 cal kyBP (95.4% interval) for Burial 26. The interment was composed of an articulated cranium, mandible and first six cervical vertebrae. Cut marks with a v-shaped profile were observed in the mandible and sixth cervical vertebra. The right hand was amputated and laid over the left side of the face with distal phalanges pointing to the chin and the left hand was amputated and laid over the right side of the face with distal phalanges pointing to the forehead. Strontium analysis comparing Burial 26's isotopic signature to other specimens from Lapa do Santo suggests this was a local member of the group. Therefore, we suggest a ritualized decapitation instead of trophy-taking, testifying for the sophistication of mortuary rituals among hunter-gatherers in the Americas during the early Archaic period. In the apparent absence of wealth goods or elaborated architecture, Lapa do Santo's inhabitants seemed to use the human body to express their cosmological principles regarding death.



Few Amerindian habits impressed the European colonizers more than the taking and displaying of human body parts, especially when decapitation was involved [1]. Although disputed by some authors [2], it has become widely accepted that decapitation was common among Native Americans across the entire continent and the archaeological evidence confirms that the practice has deep chronological roots [3]. In South America, the oldest decapitation is reported for the Andean region and dates to ca. 3000 BP at the site of Asia 1, Peru. Since all other South American archaeological cases occur in the Andes (e.g., Nazca, Moche, Wari, Tiwanaco) it was assumed that decapitation was an Andean phenomenon in both its origins and in its most unambiguous expression. In the present contribution we review the available evidence on decapitation in South America and report the discovery in east-central Brazil of a case of human decapitation directly dated to 9127-9438 cal BP (all chronological ranges reported here are based on a 95.4% interval). Excavated at the Lapa do Santo rock shelter in Lagoa Santa, Central Brazil, this is the oldest case of decapitation found in the New World, leading to a re-evaluation of the previous interpretations of this practice, particularly with regards to its origins and geographic dispersion.

#### **DISEMBODED HEADS AND DECAPITATION IN SOUTH AMERICA**

In South America, the practice of decapitation is reported in both the ethnographic and archaeological literature. Tupinamba groups from coastal Brazil, famous for their rituals, including exo-cannibalism [4], used to collect body parts, including heads, as war trophies [5]. The Arara Indians, in the Brazilian Amazon, performed the Ieipari ceremony in which the cranium of the defeated enemy, also used as a musical instrument, was displayed on the top of a pole [6]. Among the Uru-Uru Chipayas, in Bolivia, skulls were used as part of a syncretic Christian liturgy [7]. Among the Inca, decapitation was a common means of establishing and reinforcing positions of status and power. The head of important enemies were turned into trophies and the skulls into drinking jars in a clear message of military supremacy [8]. However, among the ethnographic examples in which decapitation was prominent, the trophy heads made by the Munduruku and Jivaros are the most famous.

The Munduruku Indians from the Tapajós River in northern Brazil used to behead the defeated enemy immediately after death [9–16]. The spine was sectioned near the foramen magnum and the head removed. The internal muscles, brain, eyes and tongue were then removed [16] and the head mummified through immersion in hot oil and subsequent smoking [15]. The trophy would be brought to the village and designated as the focus of a series of ceremonies over several years. At first, the ritual involved the cultural appropriation of the trophy by adding ornaments and tattoos to it. Subsequently, as the power of the head faded away, the skin and the ornaments were removed. Finally, the dentition was extracted from the

skull and attached to a cotton belt that would remain with the owner of the head indefinitely, while the skull itself would be left in some corner of his habitation to be forgotten [13].

For the Munduruku, the head of the defeated enemies clearly served the role of a war trophy and symbol of belligerent superiority [14,16]. The head was sometimes positioned on the end of a long pole [11] or carried by strings attached to the cranium, clearly characterizing the importance of public display [13]. At the same time, the head was an empowering object capable of increasing success in hunting and incorporating a female semiology of fertility. Although the Munduruku would remove other body parts of their own dead, they only produced trophy heads with enemies. The enemy's children were commonly captured and incorporated into the community but never used to generate trophy heads [15].

In Ecuador, the Jivaros produced shrunken heads (*tsantsa*) from dead enemies. The head was quickly removed from the body with a "v-shaped" incision made above the clavicles. Later, in a safer location, the skin of the head was removed from the skull. This scalp was then washed with boiling water for 15-30 minutes resulting in a 50% reduction of the head's dimensions. The shrunken head was equipped with cords to facilitate transport and handling [17]. Jivaro's *tsantsa* had the power to imprison the soul of the dead enemy precluding it from perpetrating any vengeance [18–21] (but see Fausto and Rodgers (1999) [22] for a broader perspective on the meaning of *tsantsa*).

Some authors suggested that the practices of head-hunting were not a truly indigenous phenomena but a result of the western commercial demands for trophy heads [2]. However, although the European market certainly catalyzed the practice of head-hunting in South America, leading to a transformation of the reasoning behind it, archaeological evidence confirms that similar practices were common long before the arrival of the European colonizers [23,24].

The Chimus (900AD-1470AD) in Peru incorporated decapitation as a standard procedure in human sacrifices. In the Huaca 1 Complex of Pacatnamu, the mutilated skeletons of 14 individuals were found within a defensive trench of three meters deep. The ubiquitous presence of young males, many of which were tied and left exposed after death, suggests that these were sacrificed defeated warriors. Among the diverse types of mutilation to which they were subjected, decapitation was one of them [25]. Chimu human sacrifices also took place in the Temple of the Sacred Stone in Tucume [26]. Osteological analysis suggests a ritual sequence starting with throat cutting followed by heart extraction and ending with decapitation (a total of 72 individuals presented explicit osteological evidence of decapitation). The severed heads were buried in the same pit with the correspondent headless body. The presence of children among the sacrificed individuals makes it unlikely that these were defeated warriors, pointing to a different sort of sacrificial ritual compared to Pacatnamu. Disembodied skulls of both adults and children were also used as dedicatory offerings and were included in tombs as

individualized objects wrapped in textile accompanying the remains of sacrificed individuals [27].

Among the Chachapoyas from the Peruvian Amazon, disembodied skulls are found on top of elaborated anthropomorphic sarcophagi used as funerary monuments (e.g., Karajia) [28]. Disembodied skulls were also found in the walled city of Kuelap. In either case, detailed osteological analyses are not available, and the interpretations about the disembodied skulls range from them being considered simply delayed burials to being war trophies [29,30].

In the Wari Empire (600AD-1100AD), in southern Peru, disembodied heads were transformed into trophies and played a central role in ritualistic traditions [31]. In the site of Conchopata at least 31 trophy heads were recovered from ritual structures (EA143 and EA72) [32]. The skulls show drill holes near the bregma and, sometimes, at the occipital bone [7]. The demographic profile of Wari's trophy heads shows a predominance of male individuals of all ages, including children [32]. Isotopic analyses suggest a non-local origin for some of the decapitated individuals and osteological evidence points to high levels of inter-personal violence [33]. Altogether, and including the practice of child abduction, decapitation in Wari is understood as a strategy adopted by military and ritual elites to legitimate their authority in the eyes of their enemies. However, not all disembodied skulls found in Wari contexts were trophy heads. In the site of Wari, a non-modified skull wrapped in cloth and pinned with four copper *tupus* was found under the floor of an architectural construction and was probably a dedicatory offering [34].

During the Tiwanaku period (300AD-1000AD), in the Titicaca basin in Bolivia, scenes involving decapitation or disembodied human heads were a common theme in the etchings of their rock sculptures and panels [35]. The osteoarchaeological record for the corresponding period confirms that these were indeed a real practice. In the high-status residential complex of Putuni (west to the Kalasasaya) a total of fifteen articulated and disarticulated individuals were buried as a dedicatory offering to the building, including a disembodied human skull [36,37]. In the pyramid of Akapana, a site of communal ritual in the core of the Tiwanaku complex, isolated human bones or partially articulated skeletons were recovered from the base of some of the excavated pyramid's walls. Several skulls were found isolated (in one case, three skulls were grouped together), and eighteen skeletons lacked their skulls [35]. In the absence of cut marks, the skulls must have been removed from the skeleton in secondary contexts, which has been suggested to be a part of an "esoteric cult of the head" [36]. In the site of Wata Wata, human heads were presented as dedicatory offerings [38]. Three disembodied skulls were found displaying different signs of *perimortem* violence, including beheading, cranial and facial fracturing, defleshing, jaw removal, and possible eye extraction. The extreme violence characterizing these findings suggests this was done to remove power from those individuals and legitimize the authority of the expanding influence of Tiwanaku into the region [38].

Head removal is a common theme in Moche (100AD-700AD) iconography, in northern Peru [39–42], and archaeological and osteological evidence abound to confirm this was not merely figurative but a real practice. In Plaza 3A and Plaza 3C of Huaca de la Luna [34,43–50], articulated severed heads and decapitated bodies were found in a context of generalized sacrifice of defeated warriors [49,51,52]. In Plaza 3C, in addition to the ritual of sacrifice, the severed skulls were also subject to both *peri* and *postmortem* intentional manipulation which could imply some sort of ritual cannibalism [49]. Nearby, at the complex named ZUM 8, two disembodied skulls altered to function as jars show the diversity of purposes head removal had among the Moche, going beyond the immediate needs of sacrificing defeated warriors [45,48]. In Huaca Dos Cabezas, a cache of 18 severed skulls with cut marks on the anterior portion of the cervical vertebrae was found [41]. Nearby, the complete skeleton of a tall man was found with a *tumi* (ceremonial axe characterized by a semi-circular blade) in his left hand and a pottery human head in his right hand, suggesting he was an actual decapitator. In San José del Moro (tomb M-U1221), seven individuals were buried together and eight disembodied skulls were placed on top of the burial [53]. The presence of several pottery artifacts related to shamanistic activities [54] suggests that the skulls are grave offerings, possibly holding some supernatural power. During the Moche period, human bones from reopened tombs were used as dedicatory offerings. Skulls were the most commonly selected anatomical part and therefore not all disembodied heads or headless bodies are a product of decapitation (i.e., *perimortem* removal of the head) [55,56]. In addition to humans, llamas' decapitated heads were also included in tombs and graves (e.g., Huaca Rajada Sipán [57] and Dos Cabezas Tomb 2 [58,59]). During the earlier Gallinazo period, in Huacas de Moche, a single case of skull removal is known for burial G2. The skull was removed and replaced by a pottery jar with the figure of a human head stamped on it. It is not possible, however, to determine if this was a *peri* or *postmortem* removal [60].

The Nazca (100BC-800AD), in southern coastal Peru, produced elaborate trophy heads that were characterized by a drill hole in the front of the head and an enlargement of the foramen magnum [61–67]. The lips and eyes were usually sealed with spines and the head was equipped with a carrying string [34]. The available iconography and the predominance of adult males among trophy heads [34] indicates that decapitation took place in the battlefield, and that the severed head functioned as a trophy of war. Isotopic analysis indicates that these were intra-valley battles involving local Nazca warriors [68,69]. The heads were commonly interred in caches in numbers ranging from three to groups of 40 or more [66,70]. Therefore, their significance went far beyond signaling military supremacy, and it is assumed they were a central element in rituals aiming to control the forces of nature, particularly concerning crop fertility [64,71–73].

In the site of Chavín de Huantar (1200BC-500BC), in the northern Peruvian highlands, four disembodied skulls were found on a platform (Urabarriu phase, 900BC-500BC). Since the skulls were from an old adult male, a young adult male, an adolescent female and an infant, they are sometimes thought to represent an extended family [74]. The skulls show no signs of modification. Another isolated skull in Chavin de Huantar was recovered from the Galeria de Ofrendas and, although a precise date is not available, this could represent the earliest modified trophy head in the Andes [31,75].

During the Formative period, five disembodied skulls were found in the site of Wichquana, in Peru. Buried in individual pits within a ceremonial structure these skulls still had the cervical vertebrae articulated to them supporting the interpretation that they were decapitated when soft tissue was still present, which suggests that they were sacrificed [76]. The site of Asia 1 [77], in central coastal Peru, is usually considered the oldest possible case of decapitation in South America (ca. 3000 BP) [31,45]. However, in the absence of a detailed osteological description accounting for the presence of cut marks in the cranium and associated cervical vertebrae, it is not possible to determine if this is indeed a case of decapitation. The findings consisted of three wrapped bundles containing a total of eight disembodied heads that were found in separate graves. In addition, two headless bodies were also present. One skull had cut marks on the frontal bone that were interpreted as resulting from the scalping of the face [77]. The funerary context included several textiles, a necklace of bone disks, shell pendants, a bone pin, feathers, red pigment and an “engraved tray holding a mirror” [77]. Such an elaborate treatment indicates that the practice of removing skulls in Asia 1 could have been reserved to individuals of special status. Altogether, and considering the lack of any further modification to the skulls, it seems they were less likely trophy heads, but instead venerated members of this society. Accordingly, it has been suggested that the flayed skull might represent a local individual who was mutilated somewhere else and later brought back to Asia 1 [34].

The site of Asia 1 is commonly mentioned as the first appearance of disembodied heads in the South American archaeological record. However, Aguazuque (5025-2725 BP) might be a better candidate. Located in Sabana de Bogotá, Colombia, at least two cases of disembodied skulls and one headless body were identified among a total of 59 burials. The site presents one of the most elaborate funerary records of the Archaic period and the disembodiment of the skulls were part of a broader mortuary context that was focused on the manipulation of bones and body parts [78–80]. Long bones, for example, were sectioned into diaphyses and epiphyses and further painted with geometric motifs. Once again, in the absence of a detailed osteological description accounting for the presence or absence of cut marks, it is not possible to determine if these were true cases of decapitations. Notwithstanding, the fact that one of the disembodied

skulls was articulated with the cervical vertebrae is highly suggestive that the removal occurred while soft tissue was still present and therefore characterizes a case of decapitation.

In Brazil, as far as we could determine, there is only one single case of a possible decapitation reported for the entire pre-history of the country. This finding comes from the shellmound of Forte Marechal Luz [81], but no detailed chronology or osteological descriptions are available. Therefore, it is clear that almost all reported archaeological cases of decapitation and disembodied heads in South America are concentrated in the Andean region [82]. For this reason it is commonly assumed that this was an Andean phenomenon in both its origins and in its most unambiguous expression [2,24,40,72]. The purpose of the present publication is to contribute to the field by reporting an early Holocene case of decapitation found in Lagoa Santa, east-central Brazil.

### THE LAGOA SANTA REGION

Lagoa Santa is an environmentally protected area comprising 360 km<sup>2</sup> located in east-central Brazil (Fig. 1). The vegetation is dominated by *cerrado* (a savannah-like vegetation) and semi-deciduous forest. The rivers Mocambo, Samambaia, Jaguará and Gordura make up a tributary net that flows west to east towards the Velhas River, the main river in the area. Geomorphologically, Lagoa Santa is a karstic terrain that can be divided into four distinct domains [83]: 1) below 660 meters above sea level (masl), the terrain is characterized by a fluvial plain connected with the regional base level (Velhas River); 2) between 660 and 750 masl, there is a karstic plain with dolines and lakes; 3) between 750 and 850 masl, there are karstic plateaus characterized by the presence of limestone outcrops (reaching up to 75 meters in height); 4) above 850 masl, residual peaks composed of the non-soluble meta-sedimentary rocks from the Serra da Santa Helena Formation.

The region's geology comprises the Sete Lagoas Formation and the Serra da Santa Helena Formation, both part of the Upper Proterozoic meta-sediments of the Bambuí Group [84] of the São Francisco craton. This cratonic cover metamorphosed during the Brazilian Cycle (700-450 million years ago) in a process that resulted in planar structures, such as lineation and foliation, and sub-vertical structures, such as normal and revert faults. The combination of these structures provides the path for the geomorphologic evolution that leads to the rock shelter configurations found in the region. The regional rock shelters and outcrops are developed in the limestone of the Sete Lagoas Formation. More specifically, Lapa do Santo rock shelter developed in the Member Pedro Leopoldo that is composed of very pure limestone with more than 90% calcite [84].



**Figure 1.** Map of South America. The location of Lagoa Santa is indicated by the dot.

The annual mean temperature is 23°C, with lower temperatures (11°C) occurring between June and July and higher temperatures (35°C) occurring between October and November. The mean humidity is around 65% in the dry season, from May to September, and around 85% in the rainy season, from November to April, with a pluviometric mean of 1,400 mm/year. The major climatic characteristic of this region is the high concentration of rain during the rainy season (93% of total volume). When evaporation is analyzed, the region presents an annual deficit of 176 mm [85]. Despite these particular variations, the regional climate is classified as tropical, with a rainy summer and a dry winter [86]. During the dry period, the above ground water sources can become very scarce, although underground drainages are capable of preserving the discharge in the Velhas River.

The first prehistoric human bones in Lagoa Santa were found by the Danish naturalist Peter Lund between 1835 and 1844 [87–91]. Due to the putative coexistence of humans and megafauna, Lagoa Santa became a well-known region for 19<sup>th</sup> century scholars [92–95]. During the 20<sup>th</sup> century different teams went to the region to find evidence that could confirm the coexistence hypothesis [96–100]. As a result of more than 170 years of excavations, a large collection of early Holocene skeletons was gathered [101–103]. However, all those excavations were done without proper documentation and therefore they lack detailed contextual information. Coordinated by WAN and funded by the São Paulo State Grant Foundation

(FAPESP), the project “Origins and Microevolution of Man in America: a Paleoanthropological Approach” aimed to overcome this problem by identifying and excavating new sites in the Lagoa Santa region. Lapa do Santo was excavated within the midst of these efforts.

### LAPA DO SANTO ARCHAEOLOGICAL RECORD

Lapa do Santo (“Saint’s rock shelter”) is an archaeological site located in the northern part of the Lagoa Santa karst (city of Matozinhos, state of Minas Gerais, Brazil, coordinates of the site 19°28'37.86"S and 44° 2'17.00"W) (Fig. 2) [104]. The site has an associated sheltered area of ca. 1300 m<sup>2</sup> (Fig. 3a) developed under the negative slope of a 30-meter high limestone massif (Fig. 4). The southern region of the sheltered area has a relatively flat, high and dry area located immediately in front of the cave’s entrance. The floor of the shelter has a strong descending inclination towards the north, which becomes flat again near a natural sinkhole located in the northern extreme of the sheltered area.

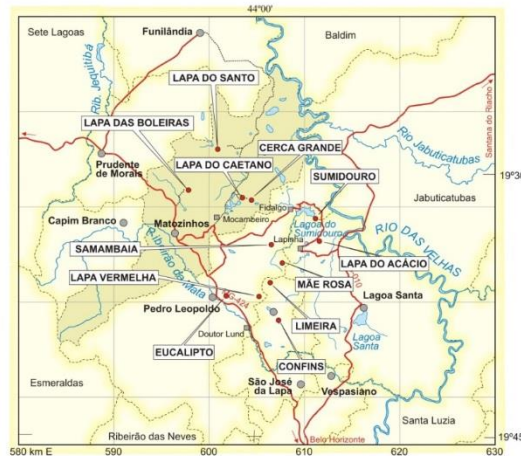
Excavations took place between 2001 and 2009 under the coordination of RK, AGMA and DVB (Fig. 5). Starting in 2001 several units were opened in distinct areas of the shelter, which showed that the richest archaeological deposits were located in its southern part, immediately in front of the cave’s entrance. An ample excavation surface was established in this region, becoming the Main Excavation Area (MEA, the highlighted area in Fig. 3b). Excavations ended in 2009 when, in accordance to Brazilian laws, the excavated area was filled with sediments to reconstitute the original topography of the shelter’s floor. In 2011 a new excavation area was opened as part of a new research project (“The Mortuary Rituals of the First Americans”), coordinated by AS, and a joint venture between the Department of Human Evolution of the Max Planck Institute for Evolutionary Anthropology (Germany) and the Laboratório de Estudos Evolutivos Humanos da Universidade de São Paulo (Brazil).

The chronology of the site is based on OSL and radiocarbon dates and points to the human presence starting at 12.7-11.7 cal kyBP (95.4% interval). Three distinct periods of occupation were determined based on the radiocarbon dates. Lapa do Santo’s Period 1 (LSP-1) starts at 12.7 cal kyBP and ends at 7.9 cal kyBP; Lapa do Santo’s Period 2 (LSP-2) starts at 5.4 cal kyBP and ends at 3.9 cal kyBP; Lapa do Santo’s Period 3 (LSP-3) starts at 2.1 cal kyBP and ends at 0.0 cal kyBP (see [105] for a detailed account on the site chronology).

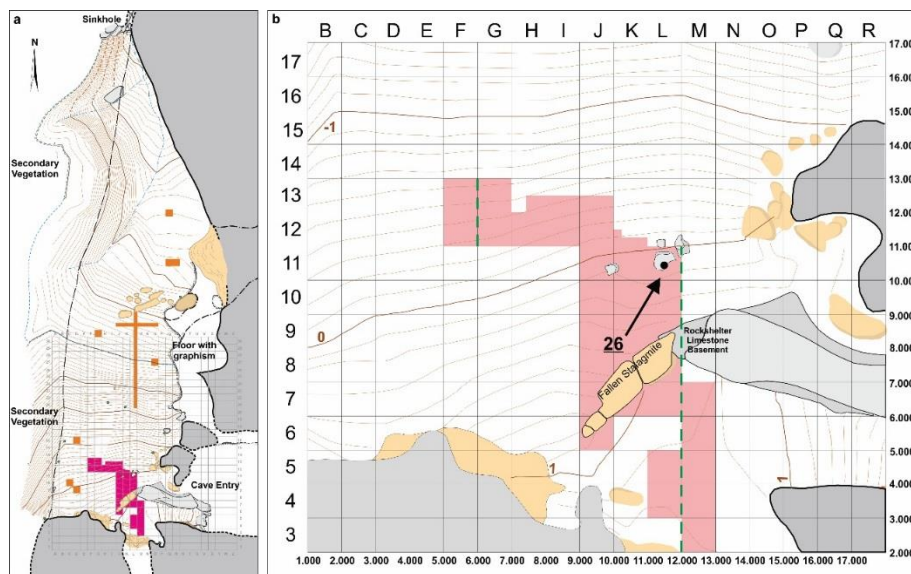
Lithic technology [106,107], zooarchaeology [108], and multi-isotopic analyses [109] indicate typical early Archaic groups of hunter-gathers with low mobility and a subsistence strategy focused on gathering plant foods and hunting small and mid-sized mammals [104]. Together with reported frequencies of dental caries comparable to those observed among agricultural populations [103,110,111], the emerging picture for Lagoa Santa during early Holocene is an economy structured around staple carbohydrates complemented by hunting of small and mid-sized animals. Formation process analysis characterizes the Lapa do Santo’s



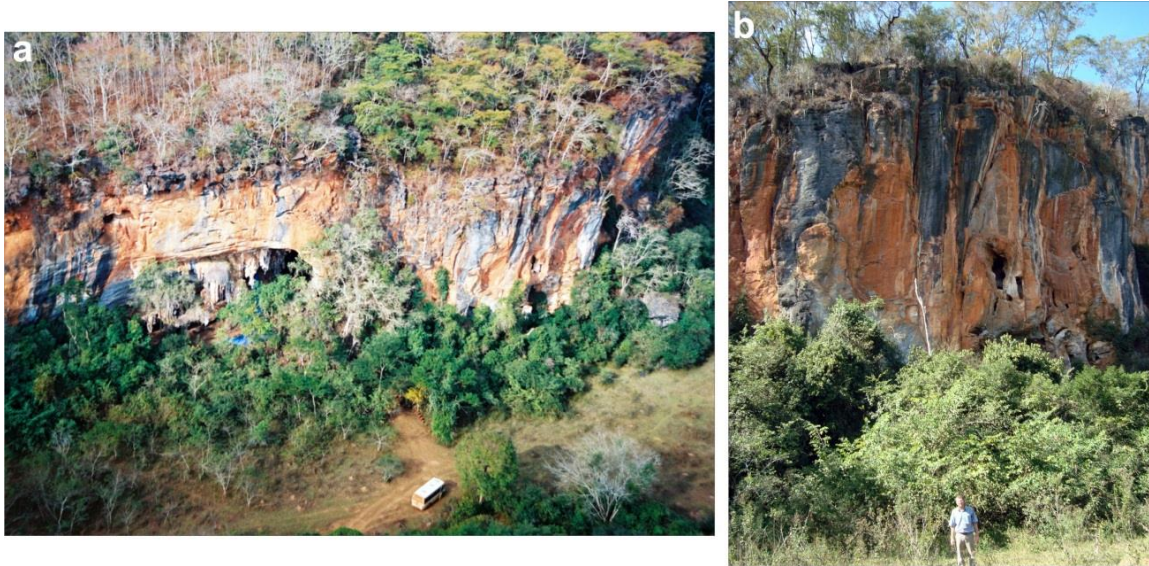
deposits as mainly anthropogenic and composed of repeated combustion activities, indicating an intense occupation of the same locality. The oldest evidence of rock art in South America, including a pictorial tradition that depicts phallic imagery, was also found engraved on the bedrock of Lapa do Santo, under four meters of excavated sediments [112].



**Figure 2.** Map of the Lagoa Santa region. The dots indicate all early Holocene sites where human skeletal remains were found.



**Figure 3.** Plan of Lapa do Santo. a) The grid corresponds to 1 square meter units. Purple and orange areas indicate excavated surfaces. Pink area indicates the main excavation area (MEA). The bedrock is depicted in gray, and secondary deposits such as breccia and stalagmites in beige. The topographic lines are 10 cm equidistant and the associated values correspond to the z-value of the site coordinate system. b) Detail of the MEA area. Black disk and the black arrow indicate the position of Burial 26. Numbers in the lower and right margin indicate the x and y values, respectively, from the coordinate system of site.



**Figure 4.** Lapa do Santo massif. a) Aerial view of the massif in which the rock shelter is located; b) ground view of the massif, the site is located just behind the vegetation.



**Figure 5.** Field pictures of excavation progress in Lapa do Santo. a) 2001 field season, b) 2003 field season, c) 2005 field season, d) 2008 field season.

A total of 26 human burials dating to early Holocene (LSP-1) were exhumed from Lapa do Santo between 2001 and 2009 (see [105] for a comprehensive depiction of the mortuary practices in Lapa do Santo and the Lagoa Santa region). The use of Lapa do Santo as an interment ground started between 10.3-10.6 cal kyBP. Lapa do Santo Mortuary Pattern 1 (LSMP-1) was characterized by articulated skeletons in flexed position buried in shallow graves and covered by limestone blocks and occurred between 9.7-10.6 cal kyBP. Lapa do Santo Mortuary Pattern 2 (LSMP-2) took place between 9.4-9.6 cal kyBP and was characterized by an emphasis on the reduction of the body by means of mutilation, defleshing, tooth removal and exposure to fire followed by the secondary burial of the remains according to specific rules. The case of decapitation reported here is part of LSMP-2. In the absence of monumental architecture or grave goods, during this period the local groups elaborated their funerary rituals through the use of the human body as a symbol [113]. Lapa do Santo Mortuary Pattern 3 (LSMP-3) took place between 8.2-8.6 cal kyBP when another change occurred whereby pits were instead filled with disarticulated bones of a single individual without signs of body manipulation. In some cases the long bones were highly comminuted in order to fit the small pit.

#### **THE DECAPITATION OF LAPA DO SANTO'S BURIAL 26**

The decapitation case that is the focus of the present contribution (accession ID Burial 26, Fig. 6) was exhumed from Lapa do Santo in July 2007. The site was excavated under the authorization of the Instituto do Patrimônio Histórico e Artístico Nacional (IPHAN processes: 01514.000329/2000-51, 01516.000236/2005-11, 01514.002967/2011-97) and of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio processes: 29395-2 and 29395-3). Burial 26 is today housed in the Laboratory for Human Evolutionary Studies (Department of Genetics and Evolutionary Biology, Instituto de Biociências, Universidade de São Paulo). Permission to study the specimen was granted by the curator of the collection (WAN).

Burial 26 was found on level 10 of unit L11 at 55 cm below the surface (Fig 3a and Fig. 7). This area of the site was extensively used for interments and several pits surrounded the grave of Burial 26 but without intercepting it. Burial 26 was composed of three distinct groups of fully articulated bones found as a single interment. The first group comprised the skull with its mandible in occlusion and the first six cervical vertebrae (C1-C6) (Fig. 8). The hyoid bone was absent. The second group of articulated bones was composed of the bones of the left hand and the third group consisted of all bones of the right hand and the distal extremity of the right radius (Fig. 9). The palms of the hands were positioned over the face of the skull. The right hand was laid over the left side of the face with distal phalanges pointing down (i.e., to the chin), while the left hand was laid over the right side of the face with distal phalanges pointing up (i.e. to the forehead). This assemblage was found within a circular grave of ca. 40cm in diameter filled with loose sediment, which was distinct from the remaining matrix of the site. Five

Appendix V

limestone cobbles were found above the bones, but still within the grave's borders. Using cranial morphology and tooth wear (see SI for details), this individual was estimated to be a young adult male.

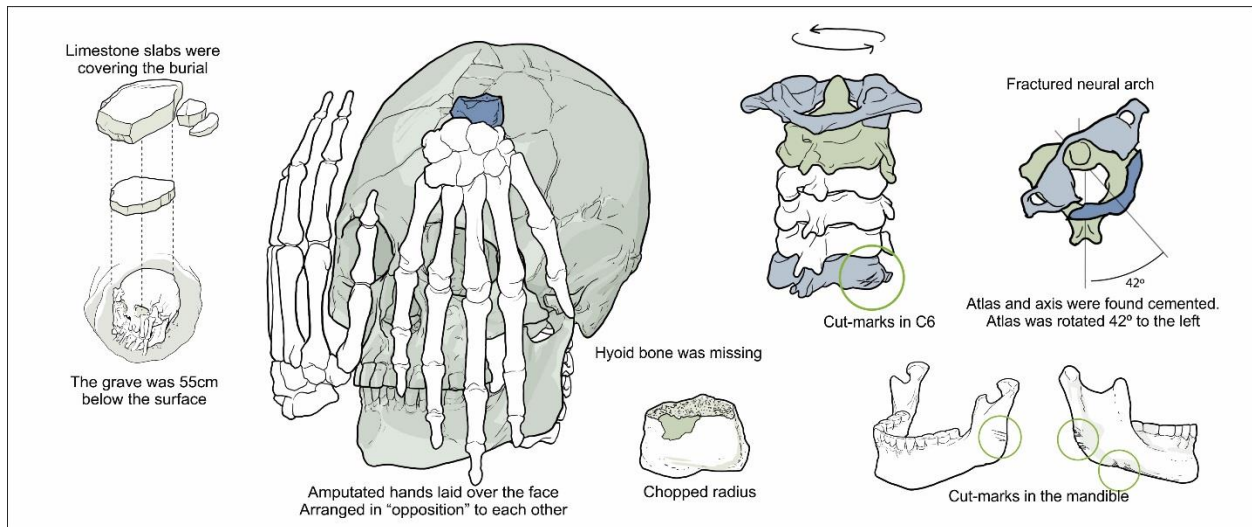


Figure 6. Schematic representation of Burial 26 from Lapa do Santo. Drawing by Gil Tokyo.

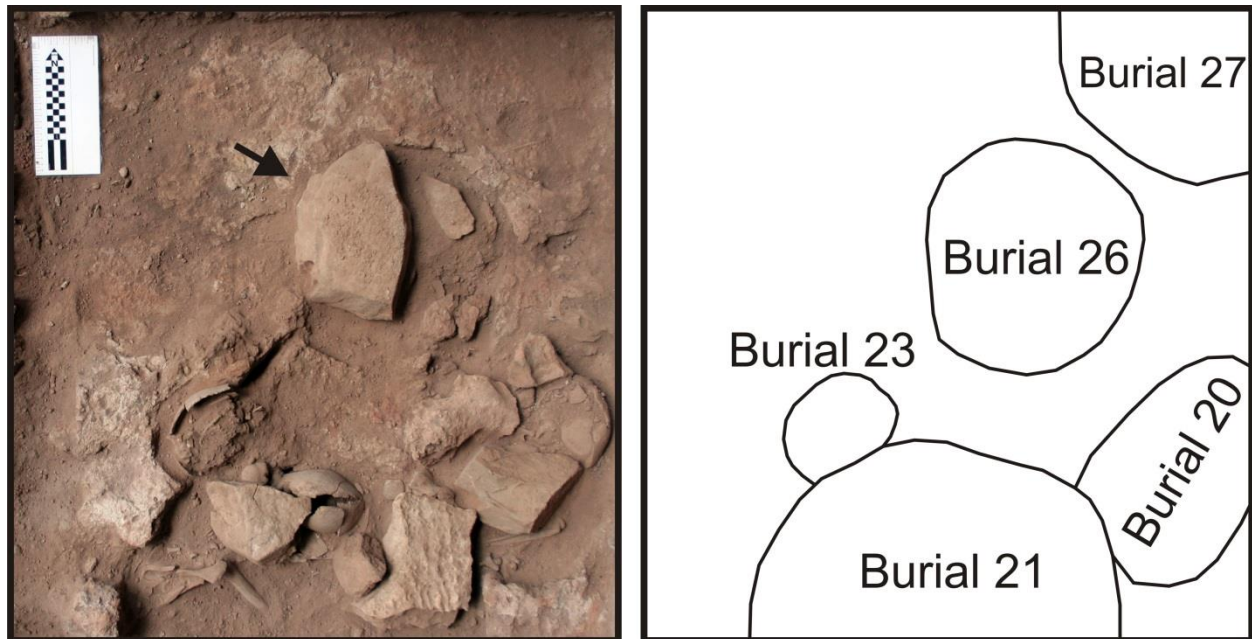
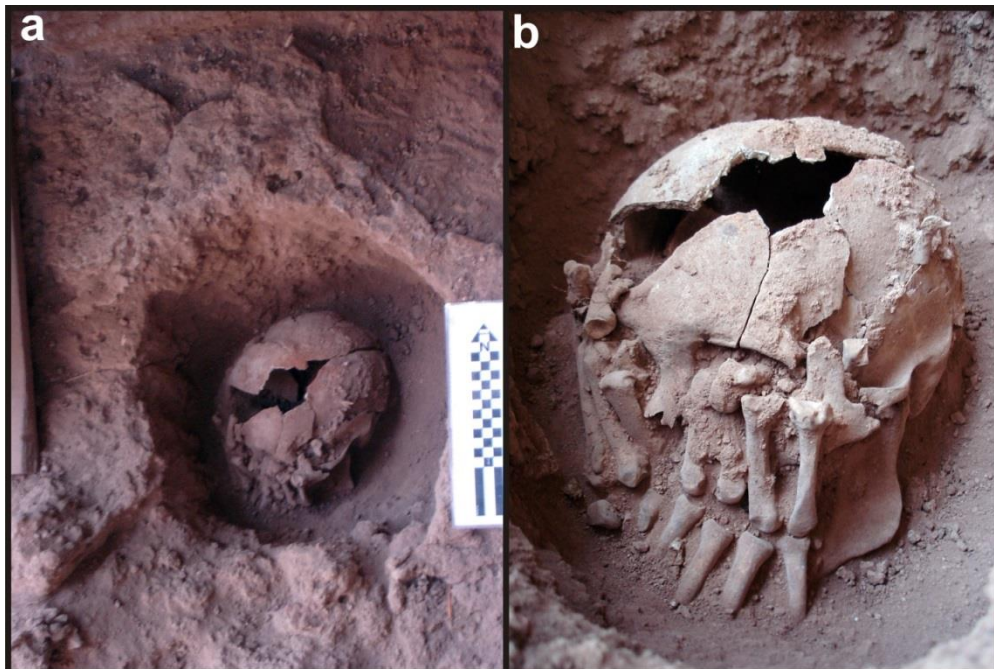


Figure 7. Lapa do Santo unit L11 at level 10. a) Field picture. The black arrow points to the block that marks the upper limit of the pit of Burial 26; b) schematic representation of Unit L11's level 10, the black contours indicate the approximate limit of each burial.



**Figure 8.** Burial 26. Arrangement of the cervical vertebrae. a) infero-lateral view; b) infero-anterior view; c) the left part of face and neurocranium were removed to allow the view of the relative position of atlas and foramen magnum; d) detail of the relationship of atlas, axis, and the other cervical vertebrae.



**Figure 9.** Burial 26. a) Pit shape; b) Arrangement of the hands over the skull.

Several cut marks were observed on the cranial and vertebral elements of Burial 26 (see SI for a detailed description). The mandible showed a number of parallel cut marks on the inferior and posterior margins of the right ramus and on the posterior margin of the left ramus (Fig. 10). Two parallel incisions were also identified on the right zygomatic bone. Concerning the neurocranium, a single vertical incision was found in the right side of the frontal bone. The incisions in the zygomatic and frontal bone are not, however, cut marks but result from taphonomic processes (see SI for cut mark analysis). In addition, parallel incisions were found near the mastoid angle of the right parietal bone and along the right lambdoidal suture of the occipital bone. The atlas and axis were cemented together by carbonate concretion in such an anatomical position that the C1 was rotated by 42° in relation to C2 (Fig. 11). Two oblique and fibrous-like fractures were found in the atlas' posterior arch, suggesting green bone breakage.

In the vertebrae, cut marks were observed at the right column of the articular processes of C6, where the zygapophysial joint capsule would be located (Fig. 12). Concerning the hands, the distal segment of the right radius was clearly sectioned in a plane perpendicular to the long axis of the bone, as is made evident by a hack mark near the cut surface (Fig. 13). These marks indicate that an implement was used to separate the hands forcibly from the arms. No cut marks were observed on the bones of the left hand, although the left radius and ulna were not recovered during the excavation.

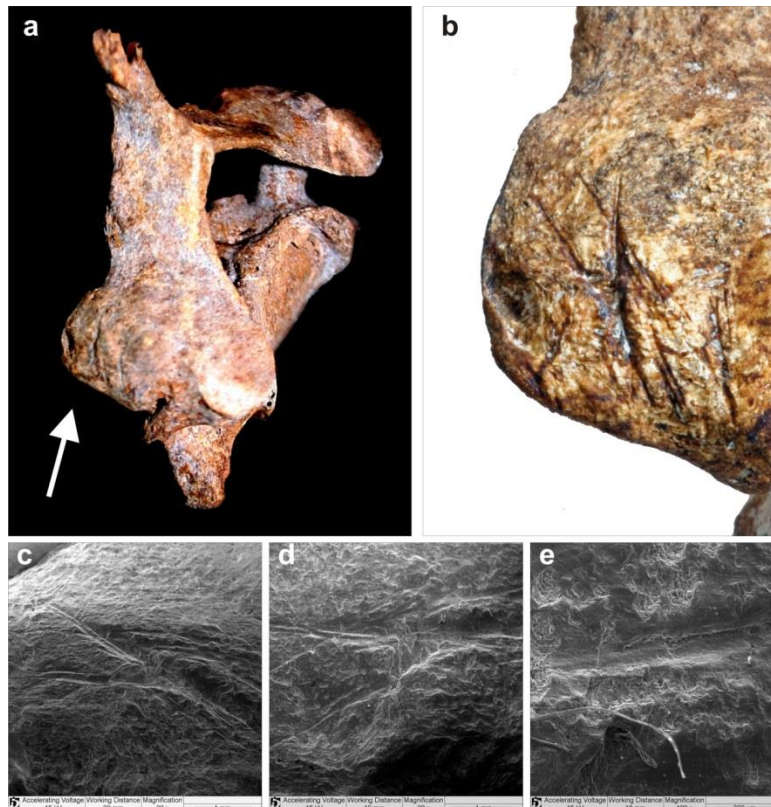
Taken together, this assemblage suggests that two different procedures were applied to the skull of Burial 26: soft tissue removal and decapitation. Cut marks on the articular process of C6 point to the sectioning of the neck between C6 and C7. Cut marks on the posterior and inferior parts of the mandible are likely related to cutting of soft tissue in the floor of the mouth, the neck and the pharynx, respectively. The fracture of the atlas is in accordance with vertical pressure followed by hyperextension of the head [114], while the rotation of the atlas on axis may be related to head torsion. It is possible that multiple forces were applied to the head to detach it from the neck. Vault and zygomatic cut marks are attributed to soft tissue removal in the right side of the skull. Therefore, Burial 26 constitutes a clear case of decapitation (see SI).



**Figure 10.** Mandible of Burial 26. a) The arrows point the location of the incisions; b) Incisions on the lateral surface of the left ramus; c) Incisions on the posterior margin of right ramus; e) Incisions in the lower margin of the right ramus; e); f) and g) SEM of the incisions on the inferior margin of the right ramus.



**Figure 11.** Atlas and axis of Burial 26. Although in anatomical position due the presence of carbonate cement, the posterior arch of the atlas was broken. a) Picture taken immediately after exhumation; the arrow indicates the point where the neural arch is attached to atlas by means of carbonatic concretion; b) Atlas was rotated 42 degrees in relation to the axis.



**Figure 12.** Burial 26's sixth cervical vertebra. a) Carbonatic concretion was still present making the incisions in the column of the right inferior articular processes, indicated by white arrow, very subtle; b) detail of the right column of articular processes after removal of concretion; c); d) and e) SEM of the incisions.



## DATING

A fragment of the sphenoid from Burial 26 was pretreated at the Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology (MPI-EVA), Leipzig, Germany, using the method described by Talamo and Richards [115]. The outer surface of the bone sample was first cleaned by a shot blaster and then 500mg of bone powder was removed. The sample was then decalcified in 0.5M aq. HCl at room temperature for about 4 hours, until no CO<sub>2</sub> effervescence was observed. 0.1M aq. NaOH was added for 30 minutes to remove humic acids. The NaOH step was followed by a final 0.5M HCl step for 15 minutes. The resulting solid was gelatinized in a pH3 solution in a heater block at 75°C for 20h, following Longin et al., [116]. The gelatin was then filtered in an Ezee-Filter™ (Elkay Laboratory Products (UK) Ltd.) to remove small (<8 μm) particles, and then ultra-filtered with Sartorius “Vivaspin 15” 30 KDa ultra-filters [117]. Prior to use, the filter was cleaned to remove carbon containing humectants [118]. The sample was then lyophilized for 48 hours.

C:N ratios, %C, %N, δ<sup>13</sup>C and δ<sup>15</sup>N values were measured at the MPI-EVA using a Thermo Finnigan Flash EA coupled to a Delta V isotope ratio mass spectrometer. For acceptable quality collagen, the atomic C:N ratio should be between 2.9 and 3.4 and a collagen yield of more than 1% of weight [119–121]. For Burial 26, the isotopic results, C:N ratios and collagen values are well within the accepted ranges (Table 1). The samples provided enough collagen for radiocarbon dating and were sent to the Klaus-Tschira-AMS facility of the Curt-Engelhorn Centre in Mannheim (MAMS), Germany, where they were graphitized and dated [122]. The resulting date was corrected for a residual preparation background estimated from pretreated <sup>14</sup>C-free bone samples, kindly provided by the Oxford Radiocarbon Accelerator Unit (ORAU). The radiocarbon dates were calibrated using OxCal 4.1 [123] and SHcal13 [124] (Table 1).

**Table 1.** Isotopic values, C:N ratios, amount of collagen extracted (%Coll) refer to the >30 kDa fraction. δ<sup>13</sup>C values are reported relative to the vPDB standard and δ<sup>15</sup>N values are reported relative to the AIR.

MPI Code	Type	%coll	δ <sup>13</sup> C	δ <sup>15</sup> N	%C	%N	C:N	AMS Nr	<sup>14</sup> C Age	1σ err	Cal BP 68.2%	Cal BP 95.4%
S-EVA 26436	Sphenoid fragment	0.81	-19.03	5.86	3.00	1.17	3.00	MAMS-16368	8331	44	9146-9407	9127-9438

In addition to the date obtained at the MPI-EVA, another date was obtained from Beta Analytic. Despite the excellent preservation of Burial 26, small fragments of bone from the nasal cavity and sphenoid could not be reassembled to the cranium. A portion of 8.707 grams of this highly fragmented material was sent to Beta Analytic Laboratories in Miami in December 2008

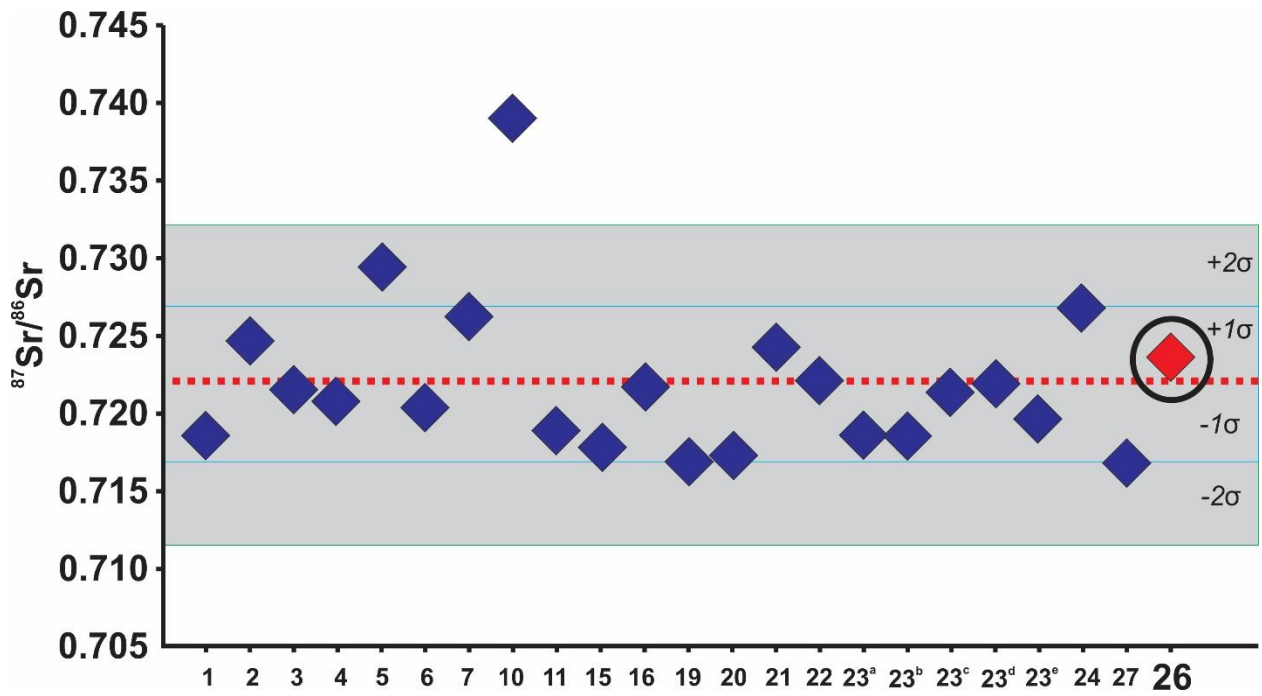
(Beta# 253511). The final age result was  $8540 \pm 50$   $^{14}\text{C}$  BP, the calibration age range was obtained with OxCal 4.1 [123] and SH13 [124] which resulted in an interval between 9.47 and 9.54 cal kyBP (68.2%) and between 9.43 and 9.55 cal kyBP (95.4%). Since the date from the Beta Analytic did not follow the same quality control parameters we adopted for bones at the MPI-EVA, we consider the latter as more accurate for dating Burial 26.

## STRONTIUM ISOTOPIC ANALYSIS

Strontium isotopic analysis ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) of skeletal material is commonly employed to detect geographic provenance and mobility among mammals, including humans [125,126], because tooth enamel from individuals records the isotopic signal of when it was formed during the earliest stages of life, whereas bone isotopic signal reflects a period closer to the time of the death of the individual [127]. Since radiogenic isotope  $^{87}\text{Sr}$  forms by radioactive decay from rubidium ( $^{87}\text{Rb}$ ), the  $^{87}\text{Sr}/^{86}\text{Sr}$  signature of a specific location is determined by the underlying bedrock age and its content of Rb. Younger geological formations like volcanic rocks have lower  $^{87}\text{Sr}/^{86}\text{Sr}$  values than older geological formations such as granite. A specific geological strontium signature is incorporated into hard body tissues by direct substituting for calcium [125,128,129], since strontium enters the ecosystems without fractionation [130,131].

Among skeletal tissues, tooth enamel is the preferred substrate for this analysis, due to its greater resistance to diagenesis in the burial environment [132,133]. Within a single archaeological population,  $^{87}\text{Sr}/^{86}\text{Sr}$  analyses of individuals' teeth can potentially detect those who were born in the same geological substrates ("locals") and those who were born in different geological substrates ("non-locals"). However, environmental background studies are needed to assess the local bioavailable  $^{87}\text{Sr}/^{86}\text{Sr}$  signature from the different geologies in the study region [125,134] in order to assess possible provenance and mobility. The use of strontium isotopes to investigate questions relating to the identity (local versus foreign) of disembodied heads is a well-established field in the Andes [32,33,68,135].

Strontium  $^{87}\text{Sr}/^{86}\text{Sr}$  values from 23 enamel samples (Table 2) were successfully measured (see SI for methodological details). The  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio measured in human enamel has a mean value of  $0.722 \pm 0.005$  ( $1\sigma$ ) and  $\pm 0.001$  ( $2\sigma$ ), with minimum and maximum values of 0.717 and 0.739 respectively. The value from the decapitated human Burial 26, (0.724) falls well within the  $1\sigma$  range of the population (Fig. 14), suggesting that at the time of its lower right P2 crown formation (3.6-6.6 years old [136]) this individual lived in a locality with similar strontium isotope values as the region where most of the others individuals of the population lived during their childhood, and therefore he was probably a local individual.



**Figure 14.** Strontium isotopic analysis.  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio enamel values from the individuals of Lapa do Santo, plotted on  $^{87}\text{Sr}/^{86}\text{Sr}$  mean ratio value (red dashed line), mean ratio  $\pm 1\sigma$  values (area between blue lines), and mean ratio  $\pm 2\sigma$  values (area between green lines) of the entire sample. A black circle marks the decapitated individual.

**Table 2:** S-EVA number, archaeological code,  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio,  $^{84}\text{Sr}/^{86}\text{Sr}$  ratio, Sr concentration (ppm) and voltage ( $^{88}\text{Sr}$ ) from enamel of the human teeth prepared in solution and analyzed in the MC-ICP-MS.

S-EVA	Burial	Tooth	Start mass (mg)	$^{87}\text{Sr}/^{86}\text{Sr}$	$^{84}\text{Sr}/^{86}\text{Sr}$	Sr conc (ppm)	$^{88}\text{Sr}$ (V)
26019	1	Inferior Right M3	23.2	0.719	0.0565	123.5	15.7
26020	2	Superior Right P4	10.4	0.725	0.0565	181.5	15.7
26021	3	Inferior Right P4	33.9	0.722	0.0565	41.4	17.5
26022	4	Inferior Right dM2	21.7	0.721	0.0565	58.1	15.7
26023	5	Superior Right M3	24	0.729	0.0565	169.9	18.4
26024	6	Inferior Left dM2	23	0.720	0.0565	69.3	15.9
26025	7	Inferior Left dM2	20.9	0.726	0.0565	87.3	18.1
26026	10	Inferior Right P4	29.3	0.739	0.0564	123.3	18.0
26027	11	Inferior Right P4	15.1	0.719	0.0565	152.9	16.4
26028	15	Inferior Right P4	21.4	0.718	0.0564	155.4	18.2
26029	16	Inferior Right P4	24.8	0.722	0.0565	82.8	17.1
26030	19	Inferior Left dM2	19.7	0.717	0.0564	88.7	17.4
26031	20	Inferior Left dM2	16	0.717	0.0565	136.7	18.2
26032	21	Inferior Left M2	21.3	0.724	0.0564	99.7	21.3
26033	22	Inferior Right P4	34.5	0.722	0.0564	122.6	21.2
26034	23a	Inferior Right dM2	19.9	0.719	0.0565	65.1	21.6
26035	23b	Inferior Right dM2	9.2	0.719	0.0565	126.5	19.4
26036	23c	Superior Right P4	16.7	0.721	0.0564	216.5	20.1
26037	23d	Superior Right P4	14.3	0.722	0.0565	171.4	20.5
26038	23e	Inferior Left P4	13.4	0.720	0.0565	96.3	21.5
26039	24	Superior Right P4	9.5	0.727	0.0565	105.8	16.8
26041	27	Inferior Left dM2	20.6	0.717	0.0565	113.5	19.6
26040	26	Inferior Right P4	18.9	0.724	0.0564	163.8	19.4

## MORPHOLOGICAL AFFINITIES

A complementary approach to strontium isotope in determining whether Burial 26 was a local or foreigner involves determining its genetic resemblance with the other individuals from Lapa do Santo. If genetically more distinct from the other individuals than the average, this would be compatible with Burial 26 being a foreigner to that group. Molecular data, however, is not yet available for the individuals from Lapa do Santo. Alternatively, cranial morphology can be used as a proxy to infer genetic relationships (see [51] for an analogous application of the method using dental traits), since there is a close link between cranial morphology and population history. This association was first recognized by studies demonstrating that craniometric traits, as many other phenotypic traits, present a moderate heritability [137–145], even though the heritability of each craniometric trait can vary

considerably [143,145]. Under this assumption, genetic information can be estimated from phenotypic traits determined, at least partially, by quantitative genetic loci [144,146–152].

Linear measurements were extracted from the 3D digital cast of Burial 26 using Landmark 3.0. Linear measurements followed Howells protocol to allow the comparison of this specimen with Howells series [153,154], as well as Lagoa Santa and Colombian remains [78,155]. Only landmarks that could be easily identified in the cast were used for measurements. Measurements that required projections (e.g., maximum cranium breadth) were not taken, due to the difficulties to achieve similar results from measurements with calipers. In total, 24 of Howells variables were extracted from the virtual cast (Table S2). However, the skull had an unusually long frontal (FRC) and high skull (BBH), outside of the 99% confidence interval of modern humans. Therefore, these variables were removed and all analyses were performed with the remaining 22 variables. Although the Howells database includes series from all continents, we selected here only the series from the Americas, Asia and Australo-Melanesia, due to its demonstrated relationship with the Lagoa Santa remains (e.g., Hubbe et al., 2010 [156]). Including series from regions that had no direct biological relationship with the Americas would add noise to the analyses, rendering the morphological affinities between Burial 26 and the other series harder to assess.

Since Burial 26 is a male, comparisons were made only with male specimens of the reference database. Only specimens that had at least 75% of the variables present were included in the analysis. This reduced the sample size of early Lagoa Santa and Archaic Colombia remains, but it minimized the frequency of missing values in the data (less than 6% of the total measurements in each series; Table S3). Missing values were replaced via multiple regressions, following the same protocol and reasoning adopted by Hubbe et al. [156].

Analyses were performed on the raw measurements and subsequently on the measurements corrected for size differences between specimens. Size correction was accomplished by dividing each measurement by the geometric mean of the individual [157]. The geometric mean was also used as a proxy to overall cranium size of the individuals. All analyses were done for the original and the size corrected data. Burial 26 was compared to the reference series via a series of multivariate analyses.

Initially, to check if Burial 26 showed an unusual size, its geometric mean was compared to the geometric means of other Lagoa Santa remains, via a box-plot. Secondly, we compared its morphological affinities using a principal component analysis (PCA), based on the overall correlation matrix between the variables. PCA was calculated using the individual data and Burial 26 morphological affinities was contrasted with the 95% confidence ellipsis of the comparative regions according to the first two PCs. To simplify the reading of the plots, series were grouped according to their geographic regions (Table S3).

Finally, Burial 26 was included in a Discriminant Functions Analysis (DFA) and classified according to its posterior probabilities to the comparative series. To complement the posterior probabilities, typicalities based on the Mahalanobis distances between Burial 26 and each of the reference series centroids were also calculated. All statistical analyses were performed in Statistica 7 (Statsoft Inc).

The boxplot comparing the overall size of Burial 26 to other Lagoa Santa crania can be seen in Figure 15. Although above average in size, Burial 26 falls well within the distribution of Lagoa Santa. The PCA analysis of the raw data (Fig. 16) and size corrected data (Fig. 17) show similar results. In both plots, Burial 26 occupies a central position in the morphospace, falling inside the confidence ellipses of Lagoa Santa, Archaic Colombia and many of the comparative series included here.

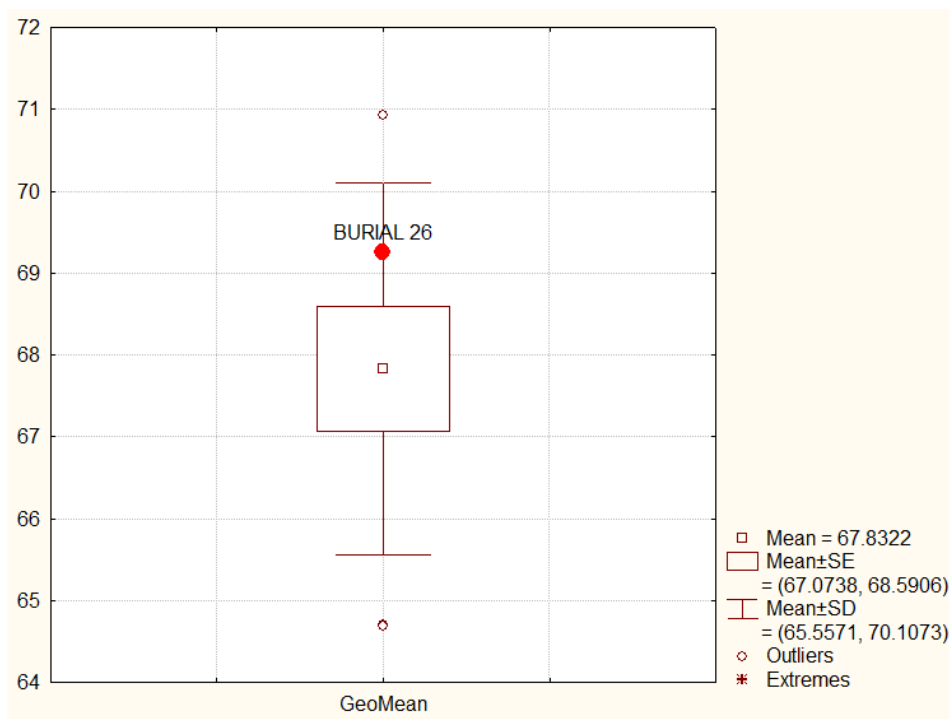
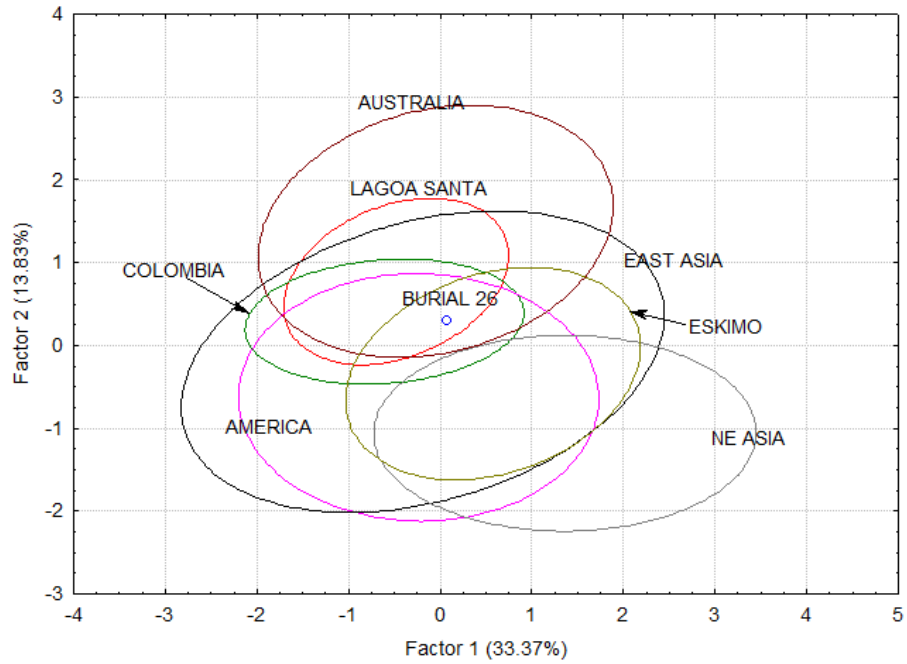
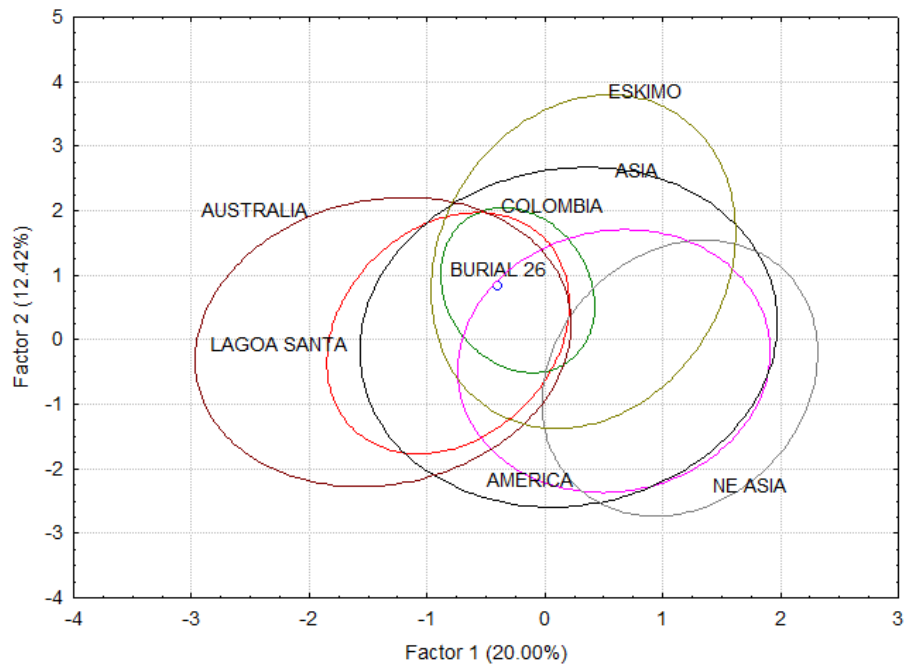


Figure 15. Boxplot of the geometric mean of Burial 26 compared to Lagoa Santa skulls.



**Figure 16.** Morphological affinities of Burial 26 compared to the variation of the reference series, based on original variables (size and shape).



**Figure 17.** Morphological affinities of Burial 26 compared to the variation of the reference series, based on size corrected variables (shape alone).

The DFA also show similar results for both size and shape, and size corrected analyses (Table S4). When either posterior probabilities or typicalities are taken into account, Burial 26 classifies clearly with Australia, which has been shown in the past to share high morphological affinities with Early South Americans [155,156]. Yet, interestingly, in none of the analyses Burial 26 appear close to the other Lagoa Santa remains. When typicalities are taken into account, in both analyses, Burial 26 is statistically different ( $p < 0.05$ ) from Lagoa Santa's centroid. However, these results may be influenced in this case by the reduced number of individuals in the Lagoa Santa sample, which is probably biasing the population estimates in these analyses.

The results do not indicate Burial 26 from Lapa do Santo presents a distinct morphology compared to other specimens from the Lagoa Santa region, thus supporting the notion he was a local individual and not an outsider.

## **DISCUSSION**

The early Holocene age of Burial 26 extends the timeline of decapitation in South America by more than 4500 years. As far as we could evaluate, in North America the oldest reported cases include the inferred decapitation from Windover Pond, Florida (8120-6990 cal BP)[158] and the demonstrated cases from the tributaries of the Ohio River in Illinois, Kentucky and Tennessee (6000-3000 cal BP)[159-161], which are also younger than Burial 26 from Lapa do Santo.

Geographically, the archaeological record of North America and Mesoamerica shows a more widespread occurrence of decapitation compared to South America, with cases occurring from the Arctic to southern Mexico [1]. Our findings suggest that South America had the same spatially widespread distribution observed for North America, making the occurrence of decapitation widespread across the whole continent since the beginning of the Holocene. In addition, they confirm that the vast territorial range of decapitation behavior described in ethnohistorical and ethnographic accounts for the New World has deeper chronological roots. Until now, every archaeological site in South America where evidence of decapitation was observed was related to the so-called Pan-Andean societies. Lapa do Santo, located in the lowlands of east-central South America, indicates that decapitation does not necessarily have a restricted Pan-Andean distribution.

Although the Eurocentric view has always understood decapitation in the context of inter-group violence, the archaeological and ethnographic record points to a more complex scenario in the New World [82]. In some cases, decapitation and the subsequent public exhibition of the severed head was indeed used as a punitive mean to subjugate rebellious groups (e.g., European colonizers and Inca). In some occasions, decapitation was just one among several other means of mutilating defeated enemies as part of sacrificial rituals and the



disembodied head received little or no attention (e.g., Plaza 3A and 3C of Huaca de la Luna, Pacatnamu). In other cases, the heads of the enemies themselves were the main reason behind decapitation and they would be further transformed into valuable objects. Beyond memorializing victory those trophy heads were also symbolically embedded with signs of fertility and rebirth (e.g., Jivaro, Munduruku, Nazca). The commoditization of human heads was also common as part of an ancestral cult where the beheaded one was not the enemy but instead a member of the group (e.g., Asia 1, Aguazuque). The focus around the head or the skull would sometimes result in the explicit transformations of those body parts into material culture (e.g., the jar's skulls from the Incas or Moches). Decapitation was not the only mean of obtaining a human head or skull. In some cases, usually related to ancestral cults, they were removed from previously interred individuals in advanced stages of decomposition.

Although no straightforward method is available to determine the nature of a severed head, the analysis of its context can provide relevant information. Trophy heads, for example, usually present the drilling of the skulls for carrying, or enlargement of the foramen magnum for brain removal [162]. At Lapa do Santo, neither drill holes nor an enlargement of the foramen magnum were observed in the skull, making it unlikely that this was a trophy head.

Determining the identity of the decapitated individual can also contribute to understanding the broader cultural context in which decapitation practices are inserted. A common parameter used in this task is the demographic profile of the samples. It is usually assumed that a sample composed of young males is more likely to reflect the execution of a group of defeated warriors instead of regular mortuary practices. Burial 26 was a young male. However, in the absence of other decapitated individuals in Lapa do Santo, it is hard to determine whether this indeed reflects a regional pattern.

The status of Burial 26 as a local or an outsider to the group is another relevant point. If an outsider, he might in fact represent an enemy. If local, he could represent an individual of unique status in the groups, like a venerated ancestral [30,66,135]. The results of the strontium isotope analysis for Lapa do Santo show a very similar  $^{87}\text{Sr}/^{86}\text{Sr}$  value to almost all other individuals, offering no support to the notion that Burial 26 was an outsider. Additionally, the cranial morphological affinities of Burial 26 compared with other specimens from the same region provide no evidence that he was an outsider. Together with the osteological evidence indicating low levels of inter-group conflict in Lagoa Santa during the early Holocene [103], the result from the strontium isotope analysis is compatible with a scenario in which the ritualized decapitation of Burial 26 was not a violent act against the enemy but instead part of a broader set of mortuary rituals involving a strong component of manipulation of the body. The careful arrangement of the hands over the face is compatible with an important public display component in the ritual that could have worked to enhance social cohesion within the community. This ritualized burial attests to the early sophistication of mortuary rituals among

hunter-gatherers in the Americas. In the apparent absence of wealth goods or elaborate architecture, Lagoa Santa's inhabitants seemed to be using the human body to reify and express their cosmological principles concerning death. A more detailed evaluation of this matter will depend on further work in the region. After all, the findings at Lapa do Santo opens the possibility that similar practices occurred in other parts of east South America among other early Holocene hunter-gatherer societies.

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## Appendix V

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## SUPPORTING INFORMATION

### ARCHAEOLOGICAL CONTEXT

#### *Burial 26's grave*

Burial 26 was located in the center of unit L11 (Fig. 7). At the same level other five burials were found in this unit (Burials 18, 20, 23 and 27). At the surface, the z-value at the center of the unit was 0.108. The z-value at the top of Burial 26 was -0.443, corresponding to the highest point of the biggest limestone block that was above the grave (see arrow in Fig. 7). The z-value at the top of the skull was -0.684 and at the base of the grave -0.684. Therefore, top and the base of Burial 26 were located, respectively, at 56 and 79 centimeters below the surface.

The grave was circular with a diameter of ca. 40 centimeters. It was excavated within the harder matrix of the site and filled by soft and friable sediment. Above the skeleton, five blocks of limestone of different sizes were deposited (Fig. 7). The blocks were completely within the grave boundaries.

*Sex and age at death estimation*

The sex of Burial 26 was estimated using the standard craniometric method described in Buikstra and Ubelaker [1]. Five traits were utilized to characterize its cranial morphology. Burial 26 scored 4 and 5 in all traits, indicating a male morphology. In addition, this cranium shows high general robusticity in relation to other skulls from the same site. Da-Gloria [2] estimated the age at death of this individual using permanent molar tooth wear. The method is an adaptation of the work of Miles [3]. Da-Gloria [2] used 30 sub-adult individuals (under 18 years old) from Lagoa Santa as the population baseline. The ages of the sub-adults were estimated by Da-Gloria [2] using the dental developmental chart of Ubelaker [4] and tooth wear was scored using the method of Scott [5]. Instead of using the seriation process of Miles method, a best-fit regression curve was applied. The regression curve of the sub-adult baseline (molar wear versus age) was used to infer the unknown age of Burial 26. The method resulted in the age of 32 years old at death for Burial 26 [2]. The method of cranial suture closure was inapplicable to Burial 26 due to the lack of suture visibility.

*The Skull*

The cranium and mandible were in occlusion and facing southwest. The cranium was almost fully reassembled in the laboratory (Fig. S1). Incisions were observed in three different regions of the cranium. In the right side of the frontal bone a single incision of five centimeters long was observed (Fig. S2). This incision is very linear and homogeneous through its extensions, with parallel margins. It is also relatively wide and presents a transversal section that is “U” shaped. In the scanning electron microscope (SEM) (Fig. S2b-2d) and confocal microscope (CM) (Fig. S3), the flat bottom of this incision is evident and micro-striations are not present. In the right zygomatic bone two very thin and barely distinguishable incisions parallel to each other were observed (Fig. S4). Through SEM and CV microscopy (Fig. S5) it is possible to see that they present less than 80 microns breadth but do present a v-shaped profile. Finally, near the right asterion on the occipital and parietal bones, a profusion of sub-parallel incisions are present (Fig. S6). As can be seen in the SEM images (Fig. S7) these incisions are short with less than 1 centimeters of length and while some look like incisions with a v-shaped profile, others resemble broader striations.

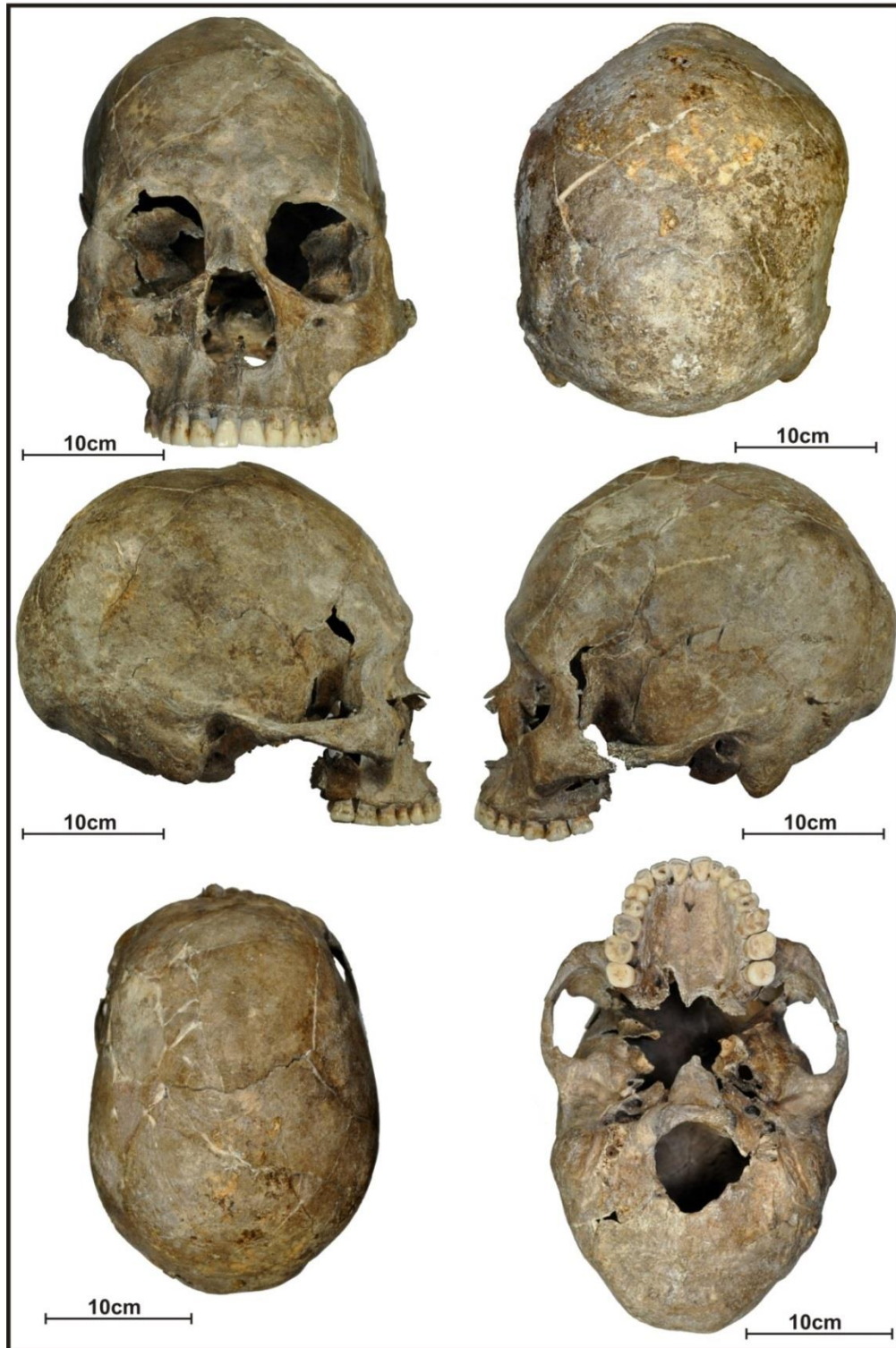
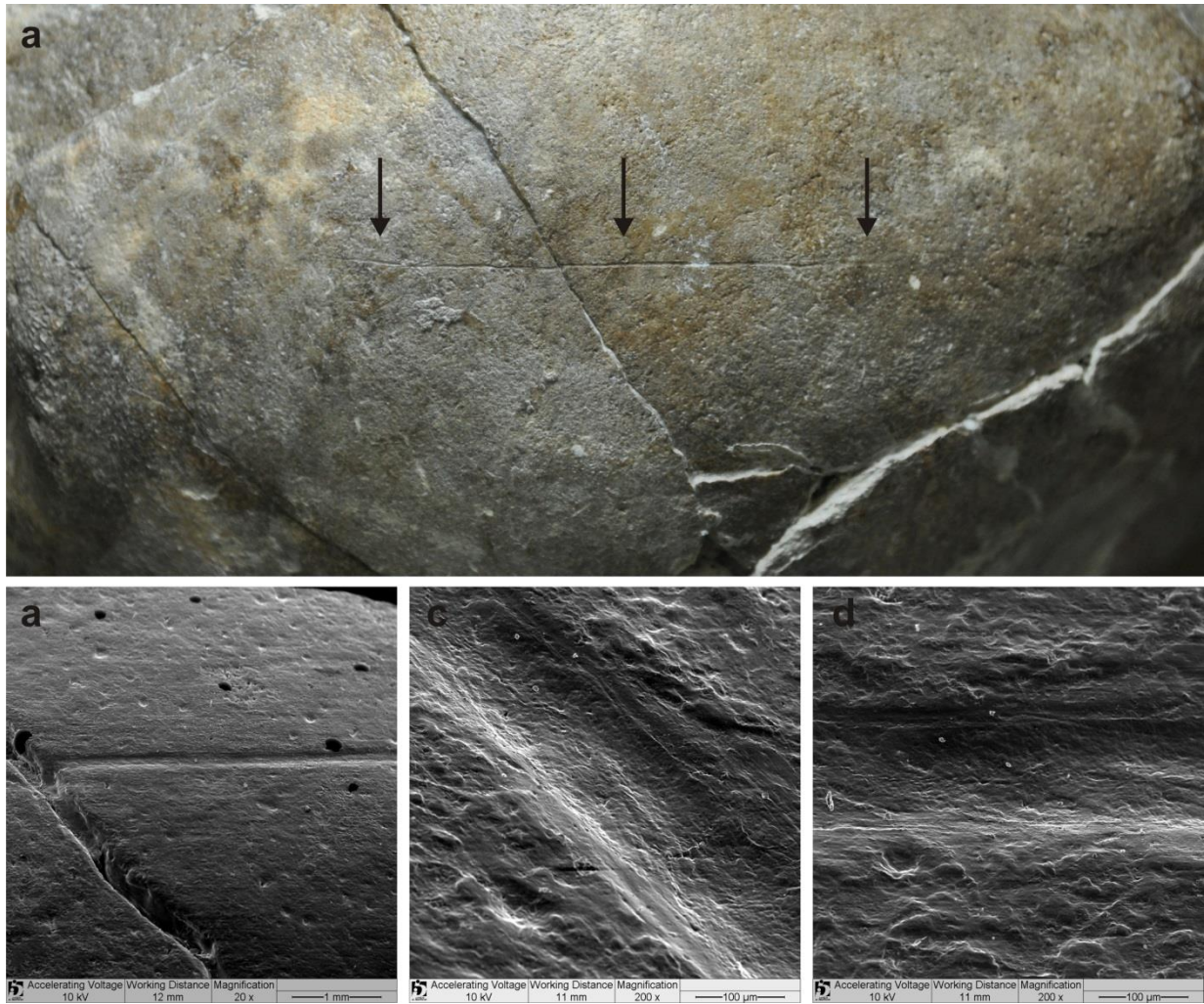
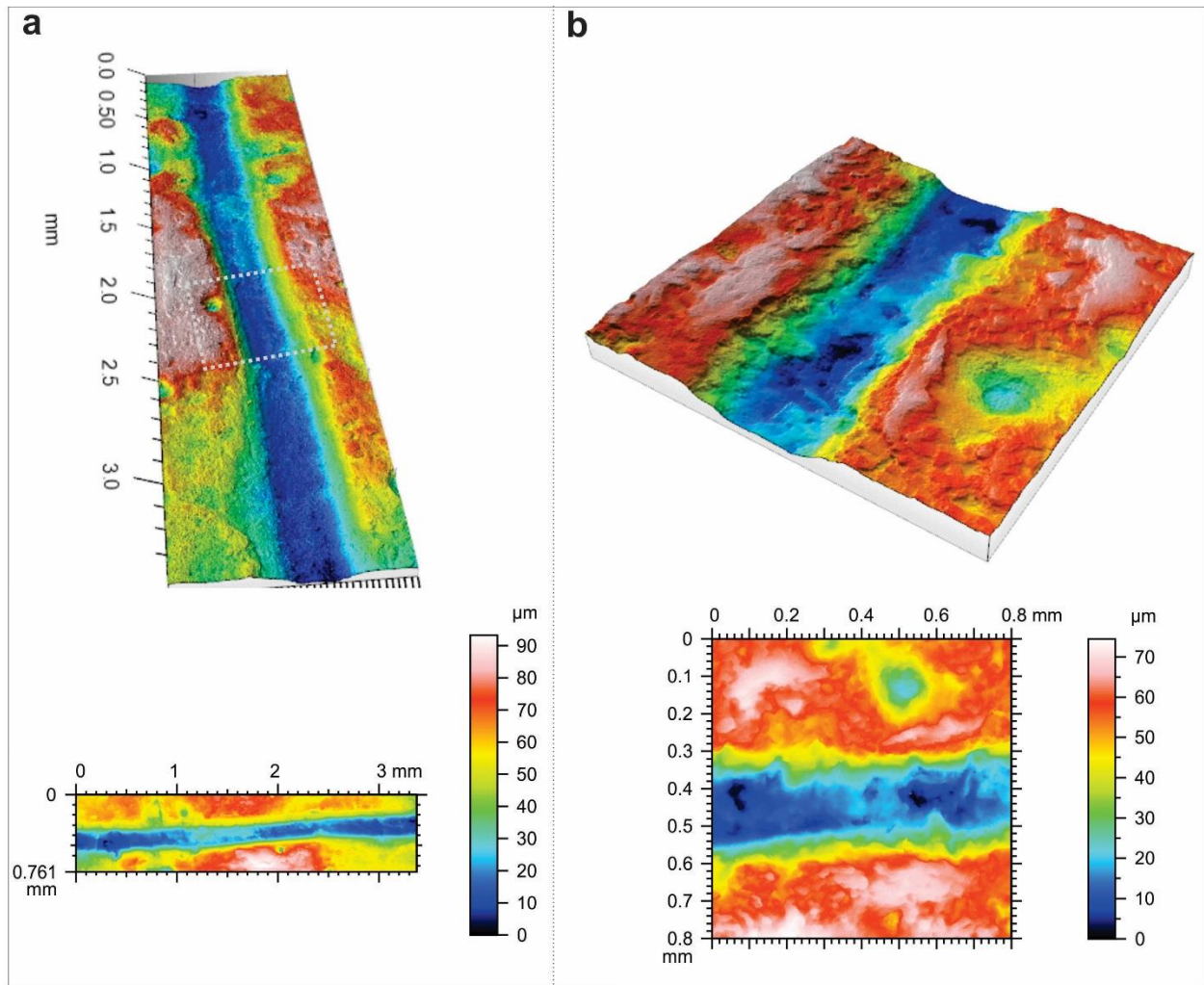


Figure S1. Cranium of Burial 26.



**Figure S2.** Frontal bone of Burial 26. a) Picture of the right region of the frontal bone. The arrows point the incision; b); c) and d) SEM of the incision.



**Figure S3.** Confocal imaging of the incision located in the frontal bone (same as depicted in Figure 7). a) Three-dimensional model (above) and topography (bottom) based on the 20x lens (resolution of  $\mu\text{m}$ ). The white dotted rectangle delimits the area shown in “b”; b) Three-dimensional model (above) and topography (bottom) based on the 50x lens (resolution of  $1.57\mu\text{m}$ ). Note how the incision has a flat bottom not compatible with a cut mark.



Figure S4. Right zygomatic of Burial 26. Yellow arrows indicate the very thin incisions in the malar bone.

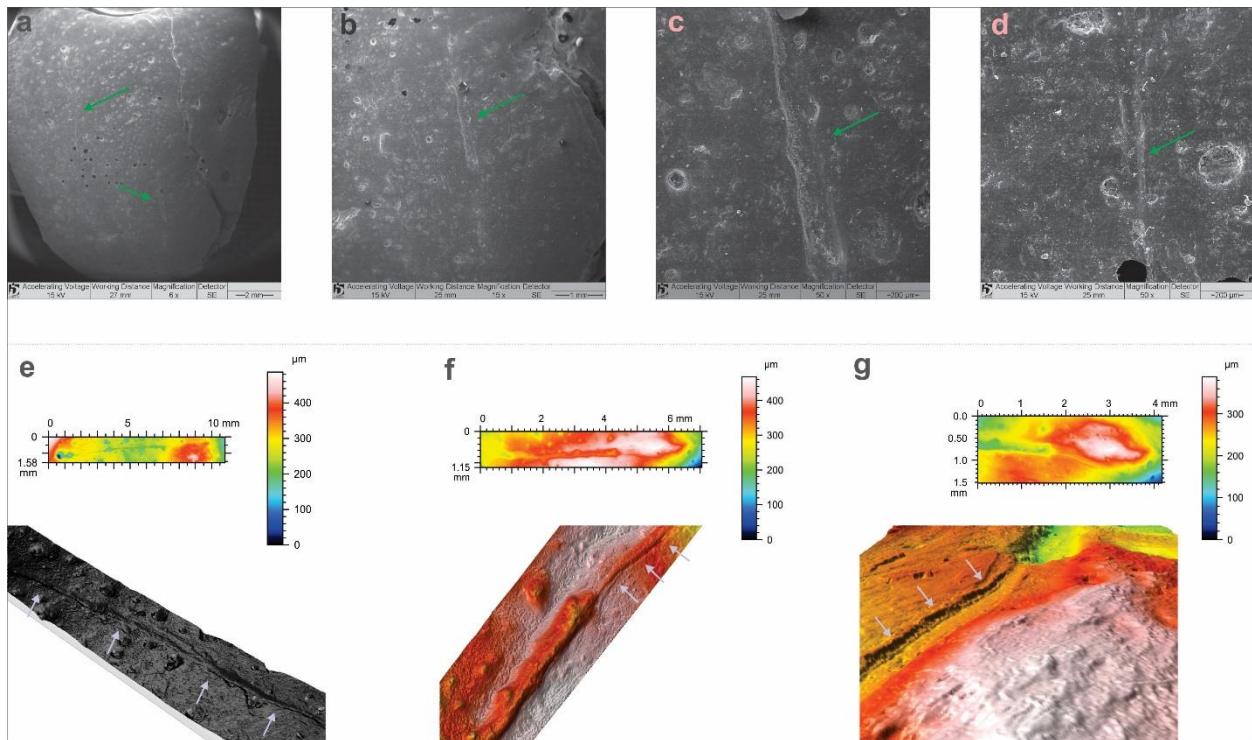
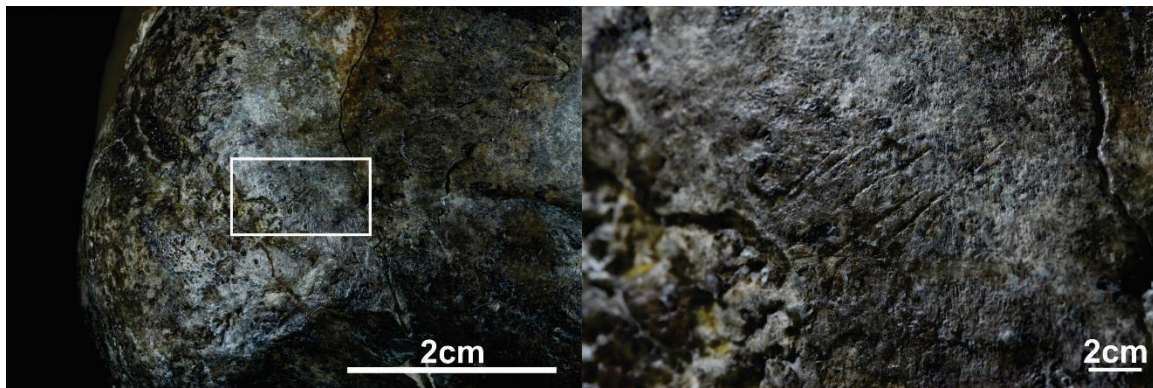
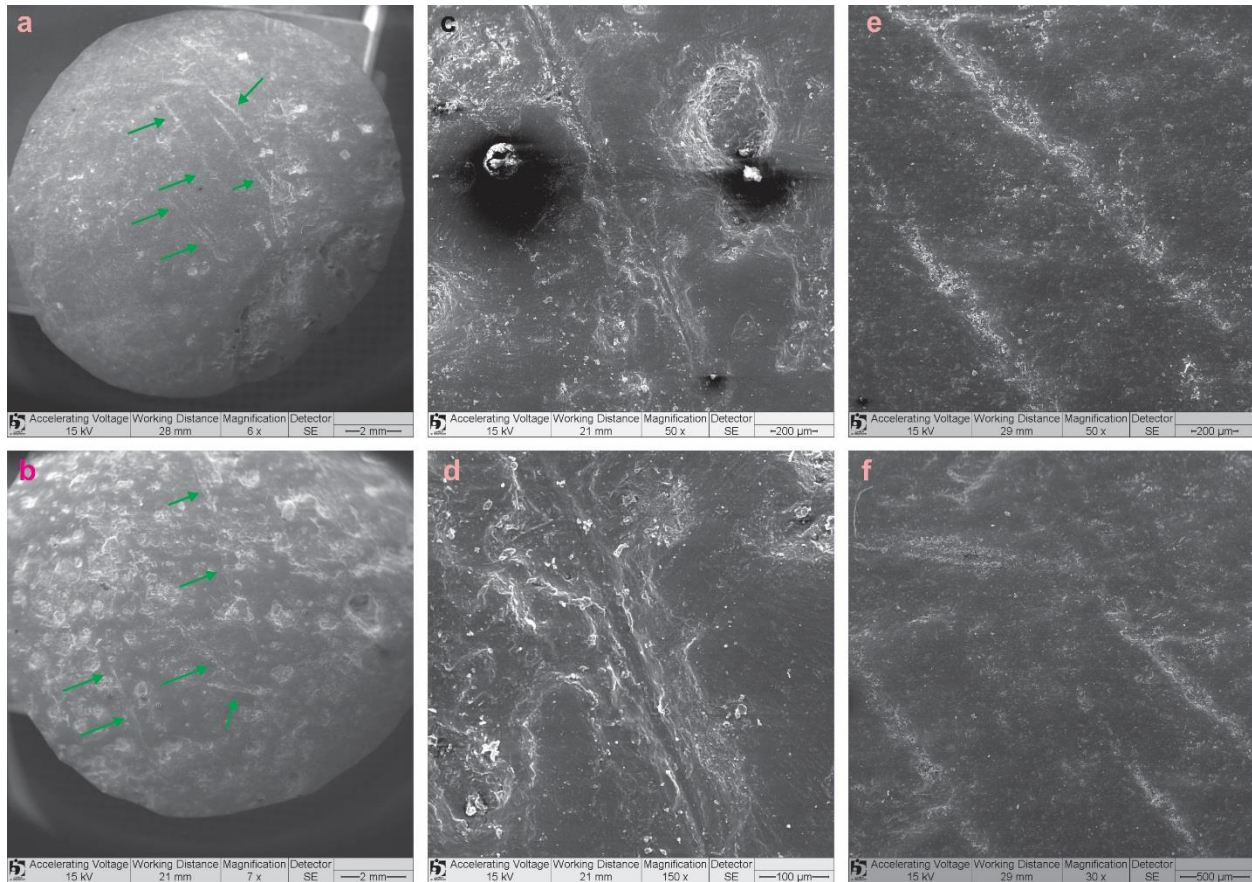


Figure S5. SEM and confocal microscopy of the incisions (green and white arrows) observed in the region of right zygomatic.



**Figure S6.** Right asterionic region of the cranium of Burial 26. a) Picture of the posterior right portion of the cranium where incisions are present near the right asterion.



**Figure S7.** SEM of the right asterionic region of the cranium of Burial 26 (same as in figure S6). In low magnification (“a” and “b”) is possible to observe the sub-parallel orientation of the possible cut marks (indicated by the green arrows). In higher magnification some look more like v-shaped incisions (“c” and “d”) while others look more like broad striation (“e” and “f”).

The mandible was covered by a thin layer of calcium carbonate that was removed with the assistance of acetic acid solution in concentration of 10%. After removal, incisions were evident in the inferior and posterior margins of the right ramus and in the posterior region of the lateral surface of the left ramus (Fig. 10). Within each of these anatomical regions, the incisions occur in a sub-parallel cluster. They vary in width going from 0.05 to 0.1 millimeters. In the SEM images it is possible to observe very fine parallel striation within the incisions (Fig. 10), a diagnostic characteristic of stone flake cut marks.

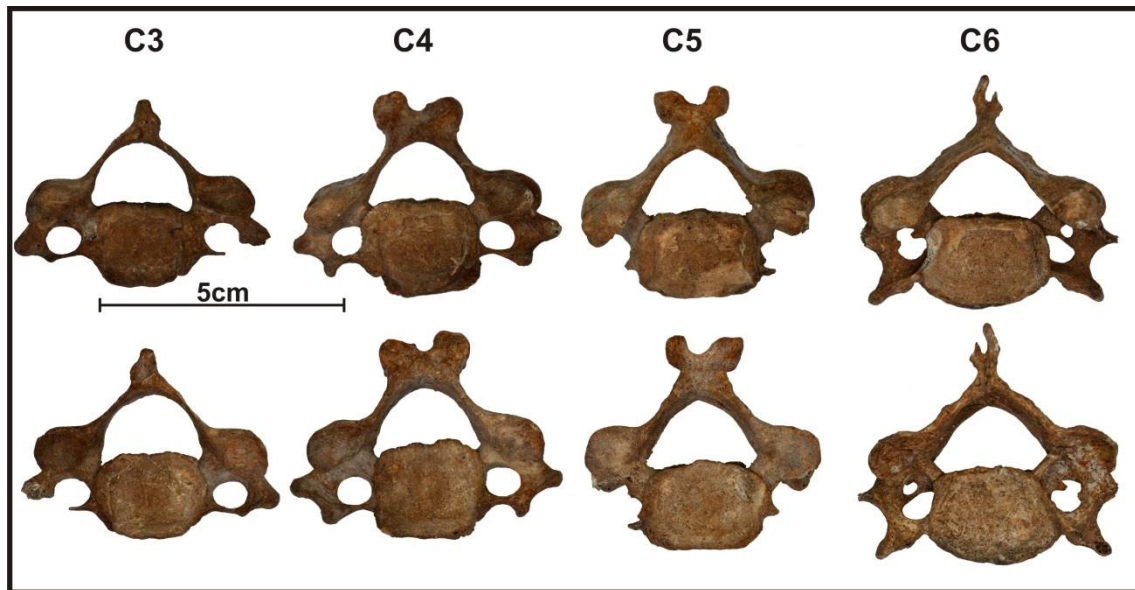
#### *The Cervical Vertebrae*

Only the first six cervical vertebrae (C1-C6) were found. Except for the atlas (see below), the vertebrae show no signs of breakage or fracture (Fig. S8). They were articulated with each other (Fig. 8a-8b). However, the whole set of vertebrae was anteriorly dislocated. The atlas, for example, was not in direct articulation with the occipital condyles, but anteriorly dislocated by approximately two to three centimeters (Fig. 8c-8d). The third to sixth cervical vertebrae were located within the mouth and oriented perpendicular to the basicranium in such a way that C3 was very close to the palate and the body of the sixth cervical vertebra was located between the posterior part of the mandibular corpus (i.e., on the line between the molars, see Fig. 8a). The atlas and axis, on the other hand, were aligned perpendicularly to the coronal plane and, therefore, they were also perpendicular relative to the other cervical vertebrae (Fig. 8d). In addition, the atlas was rotated by 42° to the left with respect to the axis (Fig. 11b). They were found cemented to each other in this disposition (see Fig. 11a for field picture immediately after recovery) and were not separated later on during laboratory work. The posterior arch of the atlas was broken. Two oblique and fibrous-like fractures, typical of green bone breakage, characterize the breakage of the posterior arch (Figure 11).

Among the vertebrae, incisions were observed only at the right column of the articular processes of C6, where the zygapophysial joint capsule would be located (Fig. 12). These incisions were originally covered by carbonate cement and were very subtle (Fig. 12a). After treatment with acetic acid in concentration of 10%, the carbonate was removed and the incisions fully exposed (Fig. 12b). These incisions present a “V” shape transversal profile, are of no more than 1 cm of length and 0.5 cm of width. Parallel to the main grooves, very fine striation can be observed by naked eyes. These incisions are clearly cut marks made by flakes.

The hyoid bone was not found and there is no reason to postulate a taphonomic or post-depositional reason for this absence.





**Figure S8.** Cervical vertebrae. They were complete and presented no signs of fracture or breakage.

#### *The hands*

Both hands were found lying over the skull with the palmar surfaces in contact with the face. The right hand was laid over the left side of the face with distal phalanges pointing down (i.e., to the chin), while the left one was laid over the right side of the face with distal phalanges pointing up (i.e., to the forehead) (Fig. 9). All bones from both hands were found. The distal part of the right radius was the only bone present from the lower arm. In general, the bones were in direct anatomical articulation. Still, in the superior region of the grave, just above the calvaria, where the distal phalanges of the left hand and the wrist bones of the right hand were located, some perturbation was observed. A distal phalanx, for example, was cemented to the left parietal bone. The distal extremity of the radius was found within the left orbital cavity and some of the carpal bones of the right hand were within the left temporal fossa.

The distal extremity of the radius was clearly sectioned in a transversal plane a few centimeters before the distal end of the bone. A chop mark parallel to the sectioned surface can be observed in the lateral side of the bone (Fig. 13). On the hand bones, no incisions were observed.

#### **DECAPITATION PROCESS AND SOFT TISSUE MANIPULATION**

In the archaeological literature decapitations are classified as inferred or demonstrated. Demonstrated cases consist of injuries or mutilations which show unhealed cut marks, while inferred cases show headless or dismembered skeletons in undisturbed context but with no reported cut marks. Burial 26's fully articulated nature and the presence of cut marks clearly indicate that this is a demonstrated case of decapitation. However, the scarcity of cut marks in

the vertebrae does not correspond to the more flagrant cut marks usually associated with unequivocal cases of decapitation. The proper interpretation of this process depends on a great extent on defining how many cut marks are found and which of those cut marks are directly related to the process of decapitation.

The incisions in the mandible and in the sixth cervical vertebrae are clear evidence of cut marks made by stone flakes, as demonstrated by the morphology of the cut seen under the SEM and CM. Cut marks in the posterior region of the mandible are common on cases of decapitation. However, in such cases the plane of cut is usually much closer to the nuchal plane than in Burial 26, resulting in cut marks on the mastoid and mandible. In Burial 26, on the other hand, the cut plane was between the C6 and C7 at the shoulders height, well below the nuchal plane. As expected, there are no signs of cut marks near the mastoids. Still, one possible way of making the cut marks in the mandible compatible with the decapitation process is to postulate that the head was hyperflexed (chin touching the rib cage) when the cervical spine was cut. In this position, the ramus of the mandible would have been in the same plane as the last cervical vertebrae.

Even assuming that both cut marks in the sixth cervical vertebrae and in the mandible are directly related to the decapitation procedure, Burial 26 still shows few cut marks when compared to other unequivocal cases of decapitation reported in the literature. Two explanations for this scarcity of marks are 1) an advanced degree of decomposition of soft tissues minimized the necessity of cutting and/or 2) that the strategy adopted to remove the head was one that minimized the presence of cut marks. Concerning the first possibility, the absence of some bones like the hyoid and hand/wrist bones could be interpreted as supporting a somewhat advanced degree of decomposition. However, the distal and intermediate phalanges were articulated, showing that, at the moment of interment, even the most delicate labial articulations were still fully preserved. The articulations between the hand phalanxes are among the first parts of the human body to start decomposing [6]. A picture in which the soft tissues attached to the hyoid bone or the ones that connect the radius and ulna to the hand were decomposed while the soft tissues that hold the intermediate and distal phalanxes together is unlikely. Regarding the second explanation, it is possible that the procedure applied for removing the head was not solely based on cutting. This possibility finds support on the unique arrangement of atlas and axis (i.e., anterior dislocation in relation to the foramen magnum, fracture of posterior arch and rotation in relation to axis). One explanation is that the position of the atlas in relation to axis was the result of an excessive rotation of the head around the cervical axis and the fracture of the posterior arch as a consequence of vertical compression of the vertebral column followed by hyper-extension of the head [7]. Such extreme forces that are well beyond the normal anatomical limits are compatible with a scenario in which the head was

pulled away. The relative importance of force and cutting to remove the head remains to be further investigated through experimental work on cadavers.

As a working hypothesis, we postulate that this case of decapitation involved two consecutive steps. First the cervical spine was exposed by the removal of the main muscles and ligaments of the neck and adjacent areas using cutting stone flakes. Muscles such as splenius capitis and sternocleidomastoid at the back of the head, and mylohyoid and digastric muscles between the hyoid and mandible were cut in the processes. This procedure resulted in the observed cut marks in the sixth cervical vertebrae and in the mandible. The separation of the head from the body, however, was not achieved by means of cutting instruments alone, but by pulling and rotational forces. These forces resulted in the last stage of individualization of the head, also causing the fracture of the atlas, its rotation in relation to axis and the anterior displacement of the vertebral column to the foramen magnum.

In addition to the process of decapitation, the incisions observed in the cranium might point to a secondary manipulation of the skull. If these incisions are indeed cut marks they are anatomically compatible with a process of soft tissue manipulation in the right side of the skull. Among the three regions of the cranium where incisions were observed the group near the right asterion is the one that most closely resembles cut marks associated with defleshing. They occur in the form of sub-parallel clusters and are "V" shaped in transversal section. The incisions in the frontal and in the zygomatic bone, on the other hand, do not present typical features of defleshing cut marks. The first one is broad, the margins are linear, there are no striations on the walls and the bottom is flat and smooth. These characteristics are not typical of marks made by flakes. Furthermore, there is a single incision, which is not compatible with the cluster of sub-parallel cut marks usually associated with defleshing cut marks.

The incisions in the maxilla are very thin, being incompatible with a process of substantial skin removal. Taken together, the morphology of the cut marks does not point to a single and uniform process of skin manipulation. Indeed, there is no undisputable evidence of defleshing of the skull. Experimental work needs to be done to determine the nature of the manipulation and the object utilized on the skull.

The chop mark in the right radius is a clear evidence that the amputation of the hands were achieved by sectioning the bone. However, the absence of both ulnas and the left radius, in one hand, and the absence of cut marks in any of the bones of the left hand point to a more complex scenario. Assuming these bones were not missing as a consequence of high levels of decomposition of the soft tissues, their absence might indicate that forceful movements (i.e., pulling, shearing and twisting) played an additional role in these dismemberments.

Finally, a comparison with a recent forensic case of decapitation supports that the case from Lapa do Santo is indeed a decapitation done while soft tissues were fully present and also points to the high levels of anatomical expertise demanded by the task. Before presenting the

forensic case, however, it is important to keep in mind that none of the modern classifications for dismemberment or decapitation leaves room for the possibility of a ritual rationale that engenders respect, since according to modern law, mutilation of the deceased is a crime in most countries. In modern forensic investigations, dismemberment is classified into four general groups: defensive, aggressive, offensive and necromaniacal. Within these, defensive dismemberment is the most common representing an act undertaken to facilitate transportation of the remains, cover up traces of a crime or hinder identification of the deceased. Aggressive mutilation is where anger is expressed by the perpetrator on the victim after death. Offensive mutilation relates to a lust or necrosadistic murder and is performed usually to release sexual pressure or undertake sexual activities. Necromaniacal mutilation occurs when the perpetrator keeps a part of the remains as a trophy.

Therefore, to relate decapitation to modern practices is difficult with regards to motive but not necessarily with regards to the expertise of the perpetrator. Jeffrey Howe was murdered in the UK in March of 2009. His head was found in Leicestershire and his torso, his right leg, his left leg and his right forearm were each found in different locations throughout Hertfordshire. What was unusual about the dismemberment was the skill with which the body parts were removed, as if the perpetrator had training or extensive anatomical knowledge. In particular, the marks seen on the skull and the cervical vertebrae resonate with the remains found in Burial 26.

Removal of the head cleanly from the rest of the body is a difficult task as the overlapping nature of the vertebrae generally prevents a clean cutting action. Most dismemberments occur between C3 and C6 and in forensic cases are generally traumatic causing extensive bone splintering either through the use of a saw, an axe, a meat cleaver or some other heavy implement. However in the case of Jeffrey Howe (known colloquially as the Jigsaw Murder), the marks were restricted to areas on the mandible and the sides of the vertebral column and his hyoid was not found. Stephen Marshall was convicted of the murder and sentenced to life in prison. After sentencing, he admitted to being a cutter for a London drug gang, and he had dismembered many bodies and was therefore skilled in his trade. What he did was:

- Cut around the margins of the mandible and remove the floor of the mouth as one would in a *postmortem* examination.
- Reflect the tongue, pharynx and larynx away from the vertebral column via the pre-vertebral lamina of the cervical fascia (thereby removing the hyoid bone too).
- This allowed the position of the intervertebral discs to be seen anteriorly and permit a sharp blade to be inserted into the space between the two vertebral bodies.

- With a twisting of the neck, subluxation of the superior and inferior articular facets can occur, allowing the sharp blade to cut through the remaining soft tissue without resulting in extensive fracturing to either vertebra.

Therefore, in similarity with Burial 26, Jeffrey Howe showed cut marks around the inferior and posterior regions of the rami of the mandible. The hyoid bone was absent. Cut marks were noted only around the articular pillar region of the vertebral column in the region of separation between the two vertebrae where decapitation occurred. Twisting of the head to generate the subluxation of the joints can cause fracturing primarily of the C1 vertebra, which is attached firmly to the skull base. Further, there was removal of both the superficial and deep muscles of the face and back of the neck in the case of Howe's murder, in an attempt to conceal other forensic evidence. Removal of the skin and muscles attached to the skull could result in the marks seen on the frontal, parietal, zygomatic and temporal bones seen in Burial 26. The features seen in the Jeffrey Howe and the Burial 26 cases are strikingly similar and suggest an element of skill and expertise in the decapitation process.

## **STRONTIUM ISOTOPES**

The human teeth from Lapa do Santo were prepared and analysed for solution MC-ICP-MS strontium isotope analysis in the lab facilities of the Department of Human Evolution from the Max-Planck Institute for Evolutionary Anthropology (MPI-EVA) in Leipzig, Germany [8]. Solid pieces of enamel weighting approximately 20 mg were drilled from the crown of each of the teeth, spanning from the cement-enamel junction to the occlusal surface, and cleaned thoroughly on all sides under a magnifying lens with a diamond drill bit to ensure no dentine or other material remained attached to it. After the drilling and cleaning, the pieces of enamel were sonicated for at least 15 minutes in high purity deionized water, before they were taken to the MPI-EVA clean lab facility (PicoTrace GmbH, Bovenden, Germany). The samples were then rinsed three times with high purity deionized (18.2 M $\Omega$ ) water (Milli-Q® Element A10 ultrapure water purification system, Millipore GmbH, Schwalbach, Germany), rinsed once with ultrapure acetone (GR for analysis grade,  $\geq$  99.8 %, Merck KGaA, Darmstadt, Germany), and dried overnight.

Further preparation of the enamel samples followed a modified version of the method described by Deniel and Pin [9]. Each enamel sample was weighed into clean 3 mL Savillex™ (Minnetonka, MN, USA) vials and closed-vessel digested on a heating block at 120 °C in 1 mL of 14.3M nitric acid (HNO<sub>3</sub>) before being evaporated to dryness at around 90-120 minutes. The resulting residue was then re-dissolved in 1 mL 3M HNO<sub>3</sub> in order to pass its solution through ion exchange chromatography using 50-100  $\mu$ m bead size Sr-spec™ resin (EiChrom Technologies, Inc., Darien, USA) suspended in ultrapure deionized water [10] and previously

cleaned following the procedure delineated by Charlier and collaborators [11]. Several washes were carried out with 3M HNO<sub>3</sub> before the Sr in the sample was eluted in ultrapure deionized water, dried down, and re-dissolved in 3% HNO<sub>3</sub> prior to MC-ICP-MS analysis.

A standard with known strontium isotope values (Bone Meal SRM 1486, National Institute of Standards & Technology, USA) and a blank sample were prepared parallel to the samples. Thus, one preparation batch was formed by 13 samples, 1 standard, and 1 blank. All acids used were made from SupraPur® grade (Merck KGaA) stock solutions and diluted using ultrapure deionized water.

A Thermo Fisher Neptune™ (Thermo Fisher Scientific Inc., Dreieich, Germany) MC-ICP-MS instrument at the MPI-EVA facilities (see Table S1 for operational parameters) was used to obtain the strontium isotope measurements. This mass spectrometer is a high-resolution double-focusing one, equipped with nine Faraday detectors fitted with 10<sup>11</sup> Ω resistors (four movable detectors H1-H4/L1-L4 on either side of a fixed axial detector) and a Virtual Amplifier™ system which eliminates possible amplifier-detector bias and provides a dynamic range of 5 mV to 50 V on each detector [12,13]. A 100 μL/min self-aspirating capillary and MicroFlow PFA (perfluoroalkoxy) ST-nebulizer (Elemental Scientific Inc., Omaha, USA) was used to introduce the solutions, diluted in 3% HNO<sub>3</sub> to give <sup>88</sup>Sr signal intensities of 20-25 V into the plasma.

A static mode using a collector configuration similar to that described by Batey and collaborators [12] was used to measure <sup>87</sup>Sr/<sup>86</sup>Sr strontium isotope values. The analysis of each sample was divided in two consecutive parts: a first baseline measurement at half mass positions (85.6 and 86.5) of the axial cup mass (<sup>86</sup>Sr) for 30s (20 cycles each 1.05 s), and secondly data collection involving a block of 50 cycles of 2 s integrated time. Interferences by Kr in the carrier gas (argon) and by Rb in both the carrier gas and samples were corrected, same as mass bias normalization (using <sup>88</sup>Sr/<sup>86</sup>Sr=8.375209, exponential law), following an inverse mass bias correction procedure described by Nowell and collaborators [13].

A regression equation described by Copeland and collaborators [8] was used to estimate the strontium concentration (ppm) of the enamel solution runs, based on the <sup>88</sup>Sr signal intensity (V) of three solutions with known strontium concentrations (100, 400 and 700 ppb). We used the strontium carbonate isotopic standard SRM 987 (NIST, USA) as working standard during the measurement, standard SRM 1486 as prepared external standard, and blanks as controls for contamination during the preparation. Thus, one analytical session was composed of 24 samples, 2 prepared blanks, 2 prepared standards SRM 1486, and 8 working standards SRM 987 with 16 blanks (one before and one after the working standard). Samples of this study were measured in two different analytical sessions.

Repeated <sup>87</sup>Sr/<sup>86</sup>Sr measurements of working standard SRM\_987 resulted in a mean of 0.710287 ± 0.000010 (1σ, n=16) during the analytical sessions and were corrected to the accepted

value of  $0.710240 \pm 0.00004$  [14,15]. The long-term average for  $^{87}\text{Sr}/^{86}\text{Sr}$  of the external standard SRM 1486 is  $0.709297 \pm 0.000024$  ( $n=68$ ). The measurements of standard SRM 1486 resulted in a mean of  $0.709297 \pm 0.000011$  ( $1\sigma$ ,  $n=2$ ) during the analytical sessions. All procedural blanks were considered negligible ( $^{88}\text{Sr} < 0.040$  V) at  $<0.4\%$  of the analyte signal intensity ( $^{88}\text{Sr} \approx 20\text{V}$ ).

**Table S1.** Operation parameters for MC-ICP-MS solution analysis used at the Max-Planck Institute for Evolutionary Anthropology (Leipzig, Germany).

<b>MC-ICP-MS</b>	Thermo Fisher Neptune™
<b>Forward power</b>	1200 W
<b>Reflected power</b>	<4 W
<b>Interface cones</b>	Nickel
<b>Sample cones</b>	Nickel
<b>Skimmer cones</b>	Nickel (X-cone)
<b>Coolant argon gas flow</b>	15 L/min
<b>Auxiliary argon gas flow</b>	0.8 L/min
<b>Sample gas Argon gas flow</b>	1.17 L/min
<b>Mass resolution</b>	Low (400)
<b>Lens settings</b>	Optimized for maximum signal intensity
<b>Nebulizer</b>	Elemental Scientific Inc., Microflow 100 $\mu$ L/min, perfluoroalkoxy (PFA)
<b>Sensitivity on <math>^{88}\text{Sr}</math></b>	50 V/ppm
<b>Cup configuration</b>	L4 ( $^{82}\text{Kr}$ ); L3 ( $^{83}\text{Kr}$ ); L2 ( $^{84}\text{Sr}$ ); L1 ( $^{85}\text{Rb}$ ); Ax ( $^{86}\text{Sr}$ ); H1 ( $^{87}\text{Sr}$ ); H2 ( $^{88}\text{Sr}$ )
<b>Data collection</b>	1 block, 50 cycles, 2 s integrations

**Table S2.** Craniometric variables used in this study.

<b>Variables included<sup>a</sup></b>
Basion-Nasion length (BNL)
Basion-bregma height (BBH) <sup>b</sup>
B-zygomatic breadth (ZYB)
Biauricular breadth (AUB)
Minimum cranial breadth (WCB)
Biasterionic breadth (ASB)
Basion-prosthion length (BPL)
Nasion-prosthion height (NPH)
Nasal height (NLH)
Orbit height (OBH)
Orbit breadth (OBB)
Bijugal breadth (JUB)
Nasal breadth (NLB)
Palate breadth, external (MAB)
Bizygomaxillare breadth (ZMB)
Bifrontomallare breadth (FMB)
Biorbital breadth (EKB)
Interorbital breadth (DKB)
Malar length, inferior (IML)
Malar length, superior (XML)
Foramen magnun length (FOL)
Frontal cord (FRC) <sup>b</sup>
Parietal cord (PAC)
Occipital cord (OCC)

<sup>a</sup> - measurement definitions according to Howells (1973, 1989)

<sup>b</sup> - measurements not included in the analyses, due to outlier values in Burial 26.



**Table S3.** Comparative series included in the craniometric analyses.

Series	Region	Sample Size	% of missing values
Lagoa Santa	Early South America	9	4.04
Sabana de Bogotá	Early South America	14	7.14
Peru	South America	55	-
Arikara	North America	43	-
Santa Cruz	North America	51	-
Eskimo	North America	53	-
Ainu	East Asia	48	-
Anyang	East Asia	42	-
Atayal	East Asia	29	-
Hainan	East Asia	45	-
North Japan	East Asia	55	-
South Japan	East Asia	50	-
Buriat	Northeast Asia	55	-
Australia	Australo-Melanesia	52	-
Tasmania	Australo-Melanesia	45	-
Tolai	Australo-Melanesia	56	-

**Table S4.** Classifications of Burial 26 according to Discriminant Function Analysis.

Reference series	Size and Shape			Reference series	Shape alone		
	Posterior Probability	D <sup>2</sup>	Typicality		Posterior Probability	D <sup>2</sup>	Typicality
Australia	0.902	22.779	0.356	Australia	0.884	22.227	0.387
Tolai	0.031	29.694	0.098	Santa Cruz	0.036	28.606	0.124
Eskimo	0.024	30.054	0.091	Tolai	0.033	28.966	0.115
Santa Cruz	0.017	30.654	0.08	Peru	0.019	29.978	0.092
Tasmania	0.01	31.532	0.065	Tasmania	0.008	31.299	0.069
Ainu	0.007	32.207	0.056	Ainu	0.008	31.587	0.064
South Japan	0.003	34.458	0.032	Atayal	0.005	31.413	0.067
Peru	0.002	34.872	0.029	South Japan	0.004	33.164	0.044
Lagoa Santa	0.001	32.759	0.049	Eskimo	0.002	34.389	0.033
Atayal	0.001	35.215	0.027	North Japan	0.001	36.014	0.022
North Japan	0.001	37.021	0.017	Arikara	0.001	36.388	0.02
Arikara	0	37.554	0.015	Hainan	0	40.326	0.007
Hainan	0	43.366	0.003	Lagoa Santa	0	37.525	0.015
Sabana Bogotá	0	41.882	0.004	Anyang	0	43.13	0.003
Anyang	0	44.947	0.002	Sabana de Bogotá	0	41.237	0.005
Buriat	0	52.443	0	Buriat	0	51.349	0

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Jean-Jacques Hublin's early support was a turning point in my carrier and for his trust I am gratefull. I thank Fred Spoor and Philipp Gunz for their support during this processes. In the Konrad Lorenz Institute (Klosterneuburg) I found the perfect environment to write-up my dissertation. Yogi Jaeger and Gerd Müller built up a unique atmosphere in which science and philosophy harmoniously coexist. While in Austria I enjoy the company of my colleagues from the KLI and of the University of Vienna. Thank you all.

In Tübingen I had the privilege to work with Joachim Wahl and his expertise in skeletal remains inspired new interpretations for the Brazilian archaeological record. Katerina Harvati enthusiasm for the Lapa do Santo project was fundamental in making it viable. I hope this already long collaboration will continue for many years to come. The splendid group of people at the INA and in the castle warmly welcomed me in Tübingen and I soon felt home here. I thank you all for being so generous. Special thanks for Viola Schmid who not only provided me shelter but also translated the abstract of this dissertation to German.

Finally, I thank my mother for her unconditional support during all these years. I hope that in this journey I developed at least a fraction of her courage and sense of dignity.

## André Strauss - Curriculum Vitae

**Date of birth:** April 7, 1984

**Place of birth:** São Paulo, Brazil

**Nationality:** Brazilian

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### University Education

- 2010 -**            **Doctoral candidate of the Leipzig School of Human Origins**  
Max Planck Institute for Evolutionary Anthropology, Department of Human Evolution, Leipzig, German  
Universität Leipzig, Fakultät für Biowissenschaften, Pharmazie und Psychologie, Leipzig, German  
Title of the thesis: *Late juvenile, early adult cranial growth in hominids*  
Supervisors: Fred Spoor, Philipp Gunz and Jean-Jacques Hublin
- 2011 - 2016**       **Doctoral candidate in Natural Scientific Archaeology**  
Eberhard Karls Universität Tübingen, Mathematisch-Naturwissenschaftliche Fakultät, Tübingen, German  
Title of the thesis: *Paleoamerican origins and behavior: a multidisciplinary study of the archaeological record from Lagoa Santa region (east-central Brazil)*  
Supervisors: Katerina Harvati, Joachim Wähl
- 2008 - 2010**       **Master in Evolutionary Biology**  
São Paulo University, Bioscience Institute, Department of Genetics and Evolutionary Biology, IB-USP, São Paulo, Brazil.  
Title of the dissertation: *The mortuary practices of pre-historic hunter-gatherers from Lagoa Santa region: a case study of the Santo Rockshelter archaeological site.*  
Supervisors: Walter Alves Neves, Renato Kipnis
- 2004 - 2008**       **Bachelor in Geology**  
São Paulo University, Geoscience Institute, IGC-USP, São Paulo, Brazil.  
Title of the monograph presented to obtain the bachelor degree: *Early Holocene human occupation around a karstic lake of the Lagoa Santa region – MG: an interdisciplinary approach.*  
Supervisor: Astolfo Gomes Araujo and Joel Sígolo
- 2004 - 2007**       **Bachelor in Social Science (Anthropology)**  
Pontifical Catholic University of São Paulo, PUC-SP, São Paulo, Brazil.  
Title of the monograph presented to obtain the bachelor degree: *Under the sign of Darwin.*

### Complementary Formation

- 2016**            **Radiocarbon dating**  
University of Oxford, UK.  
Coordination: Christopher Ramsey and Thomas Higham
- 2015**            **Geometric morphometrics**  
Bioscience Institute, São Paulo University, Brazil.

- Coordination: Kris Klingenberg
- 2015 **Skeletal trauma in forensic context**  
International Committee of the Red Cross, Brazil.  
Coordination: José Pablo Baraybar
- 2012 **3D Geometric Morphometrics Workshop**  
Instituto Catalã de Paleontologia, Sabadell, Spain.  
Coordination: Melissa Tallman
- 2011 **Geometric Morphometrics Workshop**  
Centre for Anatomical and Human Sciences, Hull York Medical School, Univ. of York.  
Coordination: Paul O'Higgins and Andrea Cardini
- 2009 **Koobi Fora Field School**  
Rutgers University, New Jersey, USA.  
National Museum of Kenya, Nairobi, Kenya.  
Coordination: Jack Harris and David Braun
- 2006 **Introduction to Geometric Morphometrics**  
Centro Nacional Patagónico, CENPAT, Puerto Madryn, Argentina.  
Coordination: Rolando González-José
- 2006 - 2007 **Scientific Initiation Project**  
São Paulo University, Bioscience Institute, Department of Genetics and Evolutionary  
Biology, IB-USP, São Paulo, Brazil.  
Title of the project: *The cranial morphology of the Lagoa Santa region Paleoindians as seen by  
3D geometric morphometrics.*  
Coordination: Walter Alves Neves and Rolando González-José

#### **Scholarship and Fellowships**

- 2016 Writing-up fellowship from the Konrad Lorenz Institute. Austria.
- 2015 - 2016 Teaching Fellowship from the Eberhards Karl Universität Tübingen (DFG Teach@Tübingen program). Germany.
- 2010 - 2014 PhD research scholarship funded by the Max Planck Institute for Evolutionary Anthropology. Germany.
- 2009 - 2010 Master research scholarship funded by the São Paulo State Research Foundation – FAPESP (process N<sup>o</sup> 08/51747-0). Brazil.
- 2009 Travel grant from the National Program for Support of Graduate Students (CAPES-PROAP). Brazil.
- 2009 Teaching Assistant Grant from the Teaching Development Program of the São Paulo University (process N<sup>o</sup> 110956). Brazil.
- 2008 Master research scholarship funded by the Brazilian Science Foundation - CNPq.
- 2006 - 2007 Scientific Initiation research scholarship funded by the São Paulo State Science Foundation – FAPESP (process N<sup>o</sup>: 05/60319-4). Brazil.

#### **Work as Forensic Anthropologist**

**2014-2015** Forensic anthropologist of the United Nations Development Programme (UNPD, process nº2014/000286) and of the Brazilian Bureau of Human Rights (SDH, process nº139554) in the project: “Transitional justice: Identifying the missing victims of the military dictatorship in the clandestine mass grave of the Dom Bosco Cemetery (São Paulo – Brazil)”.

### **Teaching Experience**

For all disciplines I have developed the syllabus as well as prepared the classes.

**2015 - 2016** Discipline: “An introduction to human evolution”. Eberhards Karl Universität in Tübingen, Germany. Total of hours: 30 hours. Undergraduate course. English.

**2015** Discipline: “An introduction to Bioarchaeology”. Federal University of Minas Gerais, Brazil. Total of hours: 20 hours. Undergraduate course. Portuguese.

**2015** Discipline: “The archaeology of mortuary practices”. São Paulo University, Brazil. Total of hours: 20hours. Undergraduate course. Portuguese.

### **Teaching Assistance Experience**

**2009** Discipline: Anthropology: biology and culture. São Paulo University, Institute of Bioscience. Total of hours: 20hours. Portuguese.

**2008** Discipline: Philosophy of biological sciences. São Paulo University. Institute of Bioscience. Total of hours: 20hours. Portuguese.

**2008** Discipline: Evolutionary Biology for Geologists. São Paulo University, Institute of Geoscience. Total of hours: 40hours. Portuguese.

### **Experience in co-supervising students<sup>2</sup>**

**2015 - present** Student: Daniela Ortega. Type: Master’s thesis. Language: Portuguese.  
Title: Analysis of micro-residuals from stone tools artifacts from Lapa do Santo  
Institution: São Paulo University, Museum of Archaeology and Ethnology  
Co-supervised by: Ximena Villágran and Célia Boyadijian

**2015 - present** Student: Maria Lopez Sosa. Type: Master’s thesis. Language: English  
Title: Global apportionment of modern human endocranial morphological variation  
Institution: Eberhards Karl Universität in Tübingen, Germany.  
Co-supervised by: Katerina Harvati and Hugo Reyes-Centeno

**2013 - 2015** Student: Mariana Inglez. Type: Master’s thesis. Language: Portuguese.  
Title: Hybrid cranial morphology in modern human populations.  
Institution: São Paulo University, Dpt. of Genetics and Evolutionary Biology  
Co-supervised by: Walter Neves and Danilo Bernardo.

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<sup>2</sup> Non-officially

### Field Work

As part of my education I have been trained in both geological and archaeological field work. My experience on archaeological sites was mainly focused on the meticulous exhumation of well-preserved human burials found within rockshelter. However I also had the opportunity to work for long periods at open air sites where more straightforward excavation methods were used. Archaeological documentation is one of my field of specialization. I had the opportunity to work in several countries such as Brazil, Peru, Kenya and Jordan and since 2011 I am the field director of the excavations in the site "Lapa do Santo". More specifically I participated on the following field seasons:

- 2016** Archaeological site: **Lapa do Santo** (Brazil, 40 days)  
Type of participation: Field director  
Field director: **André Strauss** (Eberhard Karls Universität Tübingen)
- 2015** Archaeological site: **Shellmiddens of Espírito Santo** (Brazil, 15 days)  
Type of participation: Responsible for excavation protocol and documentation.  
Field director: Ximena Villagran (São Paulo University)
- 2015** Archaeological site: **Toca da Janela da Barra do Antônio** (Brazil, 30 days)  
Type of participation: Physical Anthropologist  
Field director: Eric Böeda (Université Paris X - Nanterre)
- 2014** Archaeological site: **Lapa do Santo** (Brazil, 40 days)  
Type of participation: Field director  
Field director: **André Strauss** (Eberhard Karls Universität Tübingen)
- 2014** Archaeological site: **Zarqa Valley** (Jordan, 15 days)  
Type of participation: Excavator  
Field director: Walter Neves (São Paulo University) and Fábio Parenti (UFPR)
- 2013** Archaeological site: **Toca da Janela da Barra do Antônio** (Brazil, 15 days)  
Type of participation: Physical Anthropologist  
Field director: Eric Böeda (Université Paris X - Nanterre)
- 2012** Archaeological site: **Lapa do Santo** (Brazil, 55 days)  
Type of participation: Field director  
Field director: **André Strauss** (Max Planck Institute for Evolutionary Anthropology)
- 2011** Archaeological site: **Lapa do Santo** (Brazil, 40 days)  
Type of participation: Field director  
Field director: **André Strauss** (Max Planck Institute for Evolutionary Anthropology)
- 2009** Koobi Fora Field School (Kenya): **Fwjj 14 E, Fwjj 25 W** and **Gaji 10** (45 days, total)  
Type of participation: Student  
Field director: Jack Harris (Rutgers University)
- 2008** Archaeological site: **Taquaraçu Rockshelter** (Brazil, 20 days)  
Type of participation: Student  
Field director: Astolfo Araújo (Museum of Archaeology and Ethnology, São Paulo University)
- 2008** Archaeological site: **Coqueirinho** (Brazil, 15 days)  
Type of participation: Student

- Field director: Lucas Bueno (Laboratory for Human Evolutionary Studies, São Paulo University)
- 2008 Archaeological site: **Sumidouro** (Brazil, 30 days)  
Type of participation: Student  
Field director: Lucas Bueno (Laboratory for Human Evolutionary Studies, São Paulo University)
- 2008 Archaeological site: **Taquaraçu Rockshelter** (Brazil, 20 days)  
Type of participation: Student  
Field director: Astolfo Araújo (Museum of Archaeology and Ethnology, São Paulo University)
- 2007 Archaeological site: **Lapa do Santo** (Brazil, 30 days)  
Type of participation: Student  
Field director: Renato Kipnis (Laboratory for Human Evolutionary Studies, São Paulo University)
- 2007 Geological Mapping of the **Campo Alegre neoproterozoic Basin** (Brazil, 30 days)  
Type of participation: Student  
Coordinator: Miguel Basei (Geoscience Institute, São Paulo University)
- 2007 Geological Mapping of the **Camacã neoproterozoic Basin** (Brazil, 20 days)  
Type of participation: Student  
Field director: Antonio Romalino Fragoso (Geoscience Institute, São Paulo University)
- 2006 Archaeological site: **Buritizeiros Caixa d'Água** (Brazil, 25 days)  
Type of participation: Student  
Field director: André Prouss (Dpt. Anthropology, Federal Univeristy of Minas Gerais)
- 2005 Archaeological site: **Lapa do Santo** (Brazil, 30 days)  
Type of participation: Student  
Field director: Renato Kipnis (Laboratory for Human Evolutionary Studies, São Paulo University)

#### **Publications in peer-reviewed journals**

1. Von Cramon-Taubadel N, **Strauss A**, Hubbe M. (submitted). Evolutionary population history of early Paleoamerican cranial morphology.
2. Villagran X, **Strauss A**, Miller C, Ligouis B, Oliveira R. (*accepted*). *Buried in the ashes: formation processes of an early South Americans' site (Lapa do Santo, Brazil)*. **Journal of Archaeological Sciences**. USA. English.
3. Hattori ML, **Strauss A**. (*in press*). *Kiju-Sakai – The Japanese anthropologist who dedicated his life to study Brazil during the first half of the 20<sup>th</sup> century*. **Bulletin of the Emílio Goeldi Museum of Para: Human Sciences**. Belém, Brazil. Portuguese.
4. **Strauss A**, Oliveira RE, Villagran X, Bernardo DV, Salazar-Garcia DC, Bissaro MC, Pugliese F, Hermenegildo T, Santos R, Barioni A, Oliveira EC, Souza JCM, Jaouen K, Ernani M, Hubbe M, Inglez M, Rockwell HM, Machado MC, Souza GN, Chemale F, Kawashita K, O'Connell T, Israde I, Black S, Feathers J, Castro C, Richards MP, Kipnis R, Wahl J, Araujo A, Neves WA. (*under review*). *Early Holocene ritual complexity in South America: the archaeological record of Lapa do Santo (east-central Brazil)*.



**Antiquity.** UK, English.

5. **Strauss A.** 2016. *The burial patterns in the archaeological site of Lapa do Santo (early Holocene, east-central Brazil).* **Bulletin of the Emílio Goeldi Museum of Para: Human Sciences** 11:243-276. Belém, Brazil. Portuguese.
6. Martinez-Maza C, Friedline S, **Strauss A**, Nieto-Diaz M. 2016. *Bone growth dynamics of the facial skeleton and mandible in Gorilla gorilla and Pan troglodytes.* **Evolutionary Biology** 43:1-21.
7. **Strauss A**, Oliveira RE, Bernardo DV, Salazar-García DC, Talamo S, Jaouen K, Hubbe M, Black S, Wilkinson C, Richards MP, Araujo AGM, Kipnis R, Neves WA. 2015. *The oldest case of decapitation in the New World (Lapa do Santo east-central Brazil).* **PlosOne** 10:e0137456.USA. English.
8. Hubbe M, **Strauss A**, Hubbe A, Neves WA. 2015. *Early South Americans cranial morphological variation and the origin of American biological diversity.* **PlosOne** 10:e0138090.USA. English
9. **Strauss A**, Hubbe M, Bernardo DV, Neves WA, Atui JPV. 2015. *The cranial morphology of the Botucudo indian.Brazil.* **American Journal of Physical Anthropology** 157:202-216. USA. English.
10. **Strauss A.** 2015. *The mortuary practices of the first South Americans.* **Arquivos do Museu de História Natural e Jardim Botânico da UFMG** 23:89-154. Brazil. Portuguese.
11. Neves WA, Hubbe M, **Strauss A**, Bernardo DV. 2014. *Cranial morphology of the human skeletal remains from Lapa do Santo, Lagoa Santa, Minas Gerais, Brazil: implications for the settlements of the Americas.* **Bulletin of the Emílio Goeldi Museum of Para: Human Sciences** 9:715-740. Belém, Brazil. Portuguese.
12. Araujo A, **Strauss A**, Feathers J, Paisani JC, Schrage T. 2013. *Paleoindian open-air sites in tropical settings: a case study in formation processes, dating methods and paleoenvironmental models.* **Geoarchaeology** 28:195-220.
13. Wenceslau JFC, **Strauss A.** 2012. *Bioanthropology and the Incest tabu.* **Cadernos de Campo** 21:13-30. São Paulo, Brazil. Portuguese.
14. **Strauss A.** 2012. *Interpretative possibilities and limitations of Saxe/Goldstein hypothesis.* **Bulletin of the Emílio Goeldi Museum of Para: Human Sciences** 7:525 Belém, Brazil. Portuguese.
15. **Strauss A**, Koole Edward, de-Oliveira R, Da-Gloria PT, Nunes T, Robazzini A, Walter F, Neves W. 2012. *Two directly dated Early Archaic burials from Pains, State of Minas Gerais, Brazil.* **Current Research on Pleistocene.** Texas, United States of America. English.
16. **Strauss A.** 2011. *The mortuary practices of pre-historic hunter-gatherers from Lagoa Santa region: a case study of the Santo Rockshelter archaeological site.* **Revista Brasileira de Arqueologia** 24:136-139. São Paulo, Brazil. Portuguese.
17. Hubbe A, Haddad-Martim PM, Hubbe M, Mayer EL, **Strauss A**, Auler AS, Piló LB, Neves WA. 2011. *Identification and importance of critical depositional gaps in pitfall cave environments: the fossiliferous deposit of Cuvieri Cave, eastern Brazil.* **Palaeogeography, Palaeoclimatology, Palaeoecology** 312:66-78. United States of America. English.
18. Neves WA, Bernardo DV, Okomura MMM, Almeida T, **Strauss A.** 2011. *Origin and dispersion of Tupiguarani: what the cranial morphology says about it.* **Bulletin of the Emílio Goeldi Museum of Para: Human Sciences** 6:95-122. Belém, Brazil. Portuguese.
19. **Strauss A**, Hubbe M. 2010. *Craniometric similarities within and among human populations.* **Journal of Human Biology** 82:1-29. Michigan, United States of America. English.
20. Hubbe M, Neves WA, de Oliveira EC, **Strauss A.** 2009. *Postmarital residence practice in southern*

*Brazilian coastal groups: continuity and change.* **Latin American Antiquity** 20:267-278. Washington, United States of America. English.

21. **Strauss A**, Waizbort R. 2008. *Under the sign of Darwin? On the misuse of a chimer.* **Brazilian Journal of Social Science** 23:125-134, São Paulo, Brazil. Portuguese.

#### Book chapters

1. **Strauss A.** (*in prep*). *The Lagoa Santa region archaeological record.* In: Auler A, Pessoa P. (Eds.). **Lagoa Santa Karst.** Springer. English.
2. Hubbe M, Neves WA. **Strauss A**, Bernado D. (*in press*). *Hector Pucciarelli and the morphological diversity of early Humans in the Americas.* In: **Hector Pucciarelli and the development of Latin American bioanthropology.** Spanish.
3. **Strauss A.** (*in press*). *Cranial morphological diversity in South America.* In: Pearce A, Beresford-Jones D, Heggarty P. (Eds.). **Rethinking the Andes-Amazonia divide: a cross-disciplinary exploration.** Oxford University Press. English.
4. **Strauss A.** (*in press*). *The mortuary practices in the Lagoa Santa region: history of research.* In: Da-Gloria P, Hubbe M, Neves WA. (Eds.). **The history of research in the Lagoa Santa region.** Anna-Blume. Portuguese.
5. Neves WA, Hubbe M, Bernardo D, **Strauss A**, Araujo A, Kipnis R. 2013. *Early human occupation of Lagoa Santa, Eastern Central Brazil: Craniometric variation of the initial settlers of South America.* In: Graf KE, Ketron CV, Waters MR. (Eds.). **Paleoamerican Odyssey.** Center for the Study of First Americans. English.
6. Bernardo D, **Strauss A**, Neves WA, Okomura MMM. 2011. *Measuring skulls – getting into the biological realm of the Settlement of the New World.* In: Vialou D. (Ed.). **Peuplements et Préhistoire de l'Amérique.** CTHS, Paris. English.

#### Publications in not peer-reviewed journals

1. Bernardo DV, Neves WA, **Strauss A**, Hubbe M. 2016. *The people from Lagoa Santa [O povo de Lagoa Santa].* **Scientific American Brasil.** Portuguese, Brazil.
2. **Strauss A**, Olivera RE, Gratao M. 2015. *The human remains from Serra da Capivara: critical review and new evidence.* In: Boëda E. (Ed.). **Scientific Report of the Mission franco-brésilienne du Piauí – Recherches sur le peuplement pléistocène et holocène du Nordeste (Brésil), CNRS.** English, France.
3. **Strauss A.** 2013. *New human remains from Toca da Janela da Barra do Antonião.* In: Boëda E (Ed.) **Scientific Report of the Mission franco-brésilienne du Piauí – Recherches sur le peuplement pléistocène et holocène du Nordeste (Brésil), CNRS.** English, France.
4. Abreu R, Hatori, ML, Fischer P, Inglez M, DiGiusto M, **Strauss A.** 2015. *The clandestine mass grave from Perus. Report to the Comissão Estadual da Verdade Rubens Paiva.* Portuguese, Brazil.
5. Strauss A. 2015. *With limit proposals, Napoleon Chagnon new book contributes to further increasing the distance between human and biological science.* **A Folha de São Paulo** (21<sup>st</sup> of February). Portuguese, Brazil.
6. Neves WA, **Strauss A**, Bernardo D, Araujo A, Kipnis, R. 2013. *As Promessas de Lagoa Santa [The promises of Lagoa Santa region].* 2013. **Scientific American Brasil.** Portuguese, Brazil.
7. Strauss, A. 2013. *Lapa do Santo: as praticas mortuarias dos primeiros americanos.* **Informativo do**

*Centro de Arqueologia Annette L. Imperaire 3*. Portuguese, Brazil.

**Works presented on conferences**

1. Hubbe M, **Strauss A**, Von Cramon-Taubadel N. 2016. Paleoamerican cranial variation in global microevolutionary perspective: implications for the settlement of the Americas (Contributed paper, poster presentation in English). In: **85<sup>th</sup> annual meeting of the American Association of Physical Anthropology**. Atlanta, USA.
2. **Strauss A**. 2015. New bioarchaeology (and aDNA) samples from north coast Peru, including high-profile individuals. Early human remains from eastern, lowland South America (Invited paper, Oral presentation in English). In: **A Cross-disciplinary prehistory of Andean Civilisation. Max Planck Institute for the Science of Human History** Jena, Germany.
3. **Strauss A**. 2015. Bones, genes, and tools in the South American past (Invited paper, Oral presentation in English). In: **Inaugural symposium: words, bones, genes, tools. Center for Advanced Studies**. University of Tübingen, Germany.
4. Von Cramon-Taubadel N, **Strauss A**, Hubbe M. 2015. Multivariate cranial affinity of Paleoamerican crania from Lagoa Santa, Brazil in comparative global context: a 3D geometric morphometric analysis. (Contributed paper, poster presentation in English). In: **84<sup>th</sup> annual meeting of the American Association of Physical Anthropology**. Saint Louis, USA.
5. **Strauss A**, Olivera R, Villagran X, Bernardo D, Biassaro Jr M, Pugliese F, Hermenegildo T, Santos R, Inglez M, Talamo S, Kipnis R, Araujo A, Neves W, Hublin J-J. 2014. Early Holocene technical innovations in Lagoa Santa region, central Brazil. (Invited paper, oral presentation in English). In: **17<sup>th</sup> World UISPP Congress**. Burgos, Spain.
6. Scott N, **Strauss A**, Neubauer S, Hublin JJ, Gunz P. 2014. Exploring developmental cranial integration in great apes. (Contributed paper, poster presentation in English). In **5<sup>th</sup> Meeting of the European Society for Evolutionary Developmental Biology**. Vienna, Austria.
7. Scott N, **Strauss A**, Neubauer S, Hublin JJ, Gunz P. 2014. Developmental and evolutionary cranial integration in extant great apes. (Contributed paper, poster presentation in English). In **74<sup>th</sup> Annual Meeting of the Society of Vertebrate Paleontology**. Berlin, Germany.
8. Neves WA, Hubbe M, Bernardo DV, **Strauss A**. 2014. Cranial morphology of the human skeletal remains from Lapa do Santo, Lagoa Santa, Brazil: Implications for the peopling of the New World. (Invited paper, poster presentation in English) In: **83<sup>th</sup> annual meeting of the American Association of Physical Anthropology**. Calgary, Canada.
9. **Strauss A**, De-Oliveira R, Bernardo D, Salazar-Garcia DC, Wilkinson C, Black S, Talamo S, Gunz P, Richards M, Hubbe M, Araujo A, Kipnis R, Hublin J-J, Neves W. 2013. The oldest case of decapitation in the New World. (Contributed paper, poster presentation in English). In **Paleoamerican Odyssey**. Santa Fe, USA.
10. Hubbe M, Neves W, Bernardo D, **Strauss A**, Araujo A, Kipnis R. 2013. Early human occupation of Lagoa Santa, Central Brazil: Implications for the dispersion and adaptation of early human groups in South America (Invited paper, podium presentation in English). In **Paleoamerican Odyssey**. Santa Fe, USA.
11. **Strauss A**, Gunz P, Benazzi E, Spoor F. 2013. Late juvenile cranial growth in hominids. (Contributed paper, poster presentation in English). In 2<sup>th</sup> Meeting of the **European Society for the study of Human Evolution (ESHE)**, Bordeaux (France).

12. Bernardo D, Hubbe M, **Strauss A**, Neves WA. 2012. Morfología craneana de los esqueletos humanos tempranos de Lagoa Santa, Brasil Central: Implicaciones para el poblamiento de las Américas. (Contributed paper, Oral presentation in Spanish). 6th International Symposium “**El poblamiento temprano de América: Modelos de Poblamiento y Aportes desde las Territorialidades Tropicales**”. Pereira, Colombia.
13. Hubbe M, **Strauss A**, Bernardo D, Neves WA. 2012. Diferenciación morfológica diacrónica en Sudamérica. (Contributed paper, Oral presentation in Spanish). **12th Meeting of the Latin American Society of Bioanthropology Abstracts**, Costa Rica.
14. **Strauss A**, Da-Gloria P. 2012. Paradigm shift in Lagoa Santa’s research tradition. **12th Meeting of the Latin American Society of Bioanthropology Abstracts**, Costa Rica.
15. DeOliveira R, **Strauss A**, Da-Gloria P, Inglez M. 2012. Nuevos entierros de Lapa do Santo y sus implicaciones para reevaluar el registro arqueológico del Holoceno temprano de Lagoa Santa. **12th Meeting of the Latin American Society of Bioanthropology Abstracts**, Costa Rica.
16. Araujo A, **Strauss A**, Schrage T. 2012. Tropical geomorphology, soils, and archaeology: towards a tropical geoarchaeology. **34th International Geological Congress**, Brisbane, Australia.
17. **Strauss A**, Araujo A. 2012. The use of sedimentary depositional rates as an interpretative tool in geoarchaeology: a case study of the archaeological region of Lagoa Santa, central Brazil. **AK Geoarchaeologie, Leipzig (Germany)**. [pdf]
18. **Strauss A**, Gunz P, Spoor F. 2012. *Late juvenile, early adult cranial growth in Pan*. (Contributed paper, poster presentation in English). **2012 Paleoanthropology Society meeting**.
19. **Strauss A**, DaGloria P, Neves WA. 2012. *Beyond Lund’s dilemma: new perspectives from Lagoa Santa region, central Brazil*. (Invited paper, Oral presentation in English). In: 77<sup>th</sup> annual meeting of the Society of American Archaeology, Memphis (USA). **SAA 77<sup>th</sup> Anniversary Meeting Abstracts**.
20. Hubbe M, **Strauss A**. 2012. *The initial Settlement of South America: Perspectives from bioanthropology* (Invited paper, Oral presentation in English). In: 77<sup>th</sup> annual meeting of the Society of American Archaeology, Memphis (USA). **SAA 77<sup>th</sup> Anniversary Meeting Abstracts**.
21. DeOliveira, **Strauss A**, DaGloria P, Inglez M, Neves WA. 2012. *Secondary ritual or peri-mortem body manipulation during early Holocene in South America: the case of Burial 21 from the site of Lapa do Santo, Lagoa Santa region, Brazil: Implications for the settlement of the New World* (Invited paper, Oral presentation in English). In: 81<sup>th</sup> annual meeting of the American Association of Physical Anthropology, Portland (USA). **American Journal of Physical Anthropology**.
22. Hubbe M, **Strauss A**. 2012. *Diachronic cranial morphological diversity in South America: a functional approach* (Invited paper, Oral presentation in English). In: 81<sup>th</sup> annual meeting of the American Association of Physical Anthropology, Portland (USA). **American Journal of Physical Anthropology**.
23. Bernardo D, Neves WA, **Strauss A**, Almeida T, DeOliveira R. 2012. *Cranial morphology of early human skeletal remains from Lapa do Santo, Lagoa Santa, Brazil: Implications for the settlement of the New World* (Invited paper, Oral presentation in English). In: 81<sup>th</sup> annual meeting of the American Association of Physical Anthropology, Portland (USA). **American Journal of Physical Anthropology**.
24. **Strauss A**, Kipnis R, de Oliveira RE, DaGloria PT, Bernardo DV, Araujo A, Neves WA. 2011. *The mortuary practices in Lagoa Santa region: implications of the new findings at Lapa do Santo rockshelter* (Contributed paper, Oral presentation in Portuguese). In: **16<sup>th</sup> World Congress of the International**

- Union for Prehistoric and Protohistoric Sciences (IUSPPS)/ 16<sup>th</sup> Meeting of the Society for Brazilian Archaeology**, Florianópolis (Brazil).
25. **Strauss A**, Gunz P, Spoor F. 2011. *Late juvenile, early adult cranial growth in a chimpanzee population from the Tai forest* (Contributed paper, Poster presentation in English). In: **Inaugural Meeting of the European Society for the study of Human Evolution (ESHE)**, Leipzig (Germany).
  26. **Strauss A**, DaGloria PT, de Oliveira RE, Bernardo DV, Araujo AG, Kipnis R, Neves WA. 2011. *Lapa do Santo rockshelter: New evidence of perimortem body manipulation in Early Holocene South America* (Contributed paper, Oral presentation in English). In: 80<sup>th</sup> annual meeting of the American Association of Physical Anthropology, Minneapolis (USA). **American Journal of Physical Anthropology**, 144(S52), p.287
  27. DeOliveira RE, **Strauss A**, DaGloria PT, Kipnis R, Neves WA. 2011. *Burial 21 from Lapa do Santo rockshelter (8500BP): first case of peri-mortem body manipulation during Early Holocene in Lagoa Santa region* (Contributed paper, Oral presentation in Portuguese). In: **16<sup>th</sup> World Congress of the International Union for Prehistoric and Protohistoric Sciences (IUSPPS)/ 16<sup>th</sup> Meeting of the Society for Brazilian Archaeology**, Florianópolis (Brazil).
  28. DaGloria PT, **Strauss A**, Neves WA. 2011. *Mortuary rituals in the Early Holocene population of Lagoa Santa: The Harold Walter collection* (Contributed paper, Poster presentation in English). In: 80<sup>th</sup> annual meeting of the American Association of Physical Anthropology, Minneapolis (USA). **American Journal of Physical Anthropology** 144(S52), p.119
  29. Hubbe A, **Strauss A**, Hubbe M, Bernardo D. 2011. *Understanding Fst and Discriminant Analysis Classifications by means of resampled datasets: the influence of different variables and the extent to which they actually reflect sub-population divergence* (Contributed paper, Poster presentation in English). In: 80<sup>th</sup> annual meeting of the American Association of Physical Anthropology, Minneapolis (USA). **American Journal of Physical Anthropology**, 144(S52), p.169
  30. Neves WA, Bernardo DV, Okomura MMM, Almeida T, **Strauss A**. 2011. *Origin and dispersion of the Tupiguarani: what does cranial morphology say?* (Contributed paper, Poster presentation in English). In: 80<sup>th</sup> annual meeting of the American Association of Physical Anthropology, Minneapolis (USA). **American Journal of Physical Anthropology**, 144(S52), p.225
  31. **Strauss A**, DaGloria PT, Bernardo DV, Kipnis R, Neves WA. 2010. *High Variability of Paleoamerican Mortuary Practices in Lagoa Santa Region, Central Brazil* (Contributed paper, Oral presentation in English). In: 75<sup>th</sup> meeting of the Society of American Archaeology, Saint Louis (USA). **SAA 75<sup>th</sup> Anniversary Meeting Abstracts**, p.238 – 238.
  32. **Strauss A**, Hubbe M, Neves WA, Nunes T. 2010. *Morphological diversity of Early South-Americans: Testing hypothesis for the origin of the high regional diversity of recent Americans* (Contributed paper, Poster presentation in English). In: 79<sup>th</sup> meeting of the American Association of Physical Anthropology, Albuquerque (USA). **American Journal of Physical Anthropology**, v.141, p.225 – 225.
  33. Oliveira E, **Strauss A**, DaGloria P, Bernardo DV, Araujo A, Kipnis R, Neves W. 2010. *Paleoamerican mortuary practices in Lagoa Santa region – the use of the body as a symbol* (Contributed paper, Oral presentation in Spanish). In: **5<sup>th</sup> International Symposium “Early Man in America: 100 years of the Ameghino-Hrdlicka debate**, La Plata (Argentina).
  34. **Strauss A**, Hubbe M. 2009. *Dissimilarity fraction for metric traits of human skull: comparison with genetic studies* (Contributed paper, Oral presentation in English). In: 78<sup>th</sup> meeting of the American

- Association of Physical Anthropology, 2009, Chicago. **American Journal of Physical Anthropology** , 2009. v.138. p.250 – 250.
35. **Strauss A**, Araujo A. 2009. *Gearqueologia na Lagoa: um estudo exploratório do sítio arqueológico do Sumidouro (Lagoa Santa - MG)* (Contributed paper, Oral presentation in Portuguese). In: XV Congresso da Sociedade de Arqueologia Brasileira, Belém (Brazil). **15<sup>th</sup> Meeting of the Society for Brazilian Archaeology (SAB) Abstracts**, p.45 – 45.
  36. **Strauss A**, Nunes T, Neves WA. 2009. *Partindo do princípio: impacto de distintos métodos de correção de dados em análise de afinidade morfocraniana* (Contributed paper, Oral presentation in Portuguese). In: XV Congresso da Sociedade Brasileira de Arqueologia, Belém (Brazil). **15<sup>th</sup> Meeting of the Society for Brazilian Archaeology (SAB) Abstracts**, p.46 – 46.
  37. **Strauss A**, Hubbe M. 2008. *Estructuración de la variabilidad morfológica mundial: comparación con los datos moleculares* (Contributed paper, Oral presentation in Spanish). In: X Congreso de la Asociación Latinoamericana de Antropología Biológica, La Plata (Argentina). **10<sup>th</sup> Meeting of the Latin American Society of Bioanthropology Abstracts**.
  38. Hubbe M, **Strauss A**, Neves WA. 2008. *Supervivencia y dispersión geográfica de la morfología paleoamericana en el Holoceno de Sudamérica* (Contributed paper, Oral presentation in Spanish). In: X Congreso de la Asociación Latinoamericana de Antropología Biológica, La Plata (Argentina). **10<sup>th</sup> Meeting of the Latin American Society of Bioanthropology Abstracts**.

#### Organized Symposiums

1. Hubbe M, **Strauss A**. 2012. *Early Man in South America: New paradigms in Late Pleistocene/ Early Holocene biological anthropology*. In: 81<sup>th</sup> annual meeting of the American Association of Physical Anthropology, Portland (USA). **American Journal of Physical Anthropology**.
2. Hubbe M, **Strauss A**. 2012. *Early Man in South America: New paradigms in Late Pleistocene/ Early Holocene archaeology*. In: 77<sup>th</sup> annual meeting of the Society of American Archaeology, Memphis (USA). **SAA 77th Anniversary Meeting Abstracts**.

#### Invited Academic Lectures

1. Konrad Lorenz Institute. Colloquium. “Emerging *Homo* and the diagnosis of *Australopithecus sediba*”. English.
2. São Paulo University, Institute of Bioscience, Department of Genetics and Evolutionary Biology, Brazil – 2015. Title of the lecture: “*The mortuary practices in Lapa do Santo – Central Brazil*”. Portuguese.
3. Senckenberg Center for Human Evolution and Paleoecology, Eberhard-Karls-Universität Tübingen, Tübingen, Germany. 2014. Title of the lecture: “*Late cranial growth among Hominoid*”. English.
4. Université Bordeaux Montaigne, Bordeaux, France. 2014. Title of the lecture: “*The First Americans*”. English.
5. Unisaesianos, Lins, Brazil. 2008. Title of the lecture: “*The Kiju Sakai archaeological collection*”. Portuguese.

#### Technical Reports

1. Neves W, Bernardo D, Hubbe A, **Strauss A**. 2009. Report (final) of the thematic project “Origins and Microevolution of Man in the New World: a paleoanthropological approach”. Portuguese. 455p.

## Curriculum Vitae

2. **Strauss A**, 2009. Report (FAPESP, final) of the master project “*The mortuary practices of pre-historic hunter-gatherers from Lagoa Santa region: a case study of the Santo Rockshelter archaeological site*” 570p.
3. **Strauss A**, 2009. Report (FAPESP, partial) of the master project “*The mortuary practices of pre-historic hunter-gatherers from Lagoa Santa region: a case study of the Santo Rockshelter archaeological site*” 100p.
4. **Strauss A**, 2009. Report (CNPQ, partial) of the master project “*The mortuary practices of pre-historic hunter-gatherers from Lagoa Santa region: a case study of the Santo Rockshelter archaeological site*” 35p.
5. **Strauss A**, 2007. Report (FAPESP, partial) of the scientific initiation project “*The cranial morphology of the Lagoa Santa region Paleindians as seen by 3D geometric morphometrics*”. 64p.
6. **Strauss A**, 2006. Report (FAPESP, partial) of the scientific initiation project “*The cranial morphology of the Lagoa Santa region Paleindians as seen by 3D geometric morphometrics*”. 74p.

### Laboratory Hands-on Experience with Skeletal Remains

2006 - 2010	Responsible for the curation of the human skeletal remains of the Kiju Sakai Collection (including at least 27 individuals of both historical Native Americans and archaeological Shellmound builders)
2007	Responsible for the curation of the human skeletal remains from the archaeological site Loca do Suin, located in central Brazil (at least 4 individuals dated on ca. 8000 RDYBP).
2006	Co-responsible for the organization of the human skeletal remains from Santo Rockshelter, an archaeological site in the Lagoa Santa region (27 burials dated to ca. 8000 RCYBP)
2005	Assistant on the organization of the megafauna remains from the paleontological site Cuvieri, located in central Brazil (including extinct giant sloth dated to ca. 12000 BP)
2004	Assistant on the organization of the “ <i>South American zooarchaeological reference collection</i> ”, hosted by the Laboratory for Human Evolutionary Studies.

### Computer Programming Experience

1. **Strauss A**, Caparica JF. 2006. **Analytical package for Anthropometrical Analysis** (open source).

Developed using C and VB language this package provides a friendly visual interface that allows for, among others things, to do Principal Component Analysis or calculate Mahalanobis Distances using modified var/covar matrixes, correct metric traits for the effects of allometry, do Principal Coordinate Analysis, calculate Qst values for a large number of groups, replace missing values of large databases using multiple regression techniques and implement bootstrap routines allowing for hypothesis testing. I am also well experienced with other languages such as R and Mathematica as well as with softwares for 3d-manipulation such as Avizo and Geomagic.

Some examples of studies that used this software are:

- Hubbe M, Hanihara T, Neves W. 2011. Paleoamerican morphology in the context of European and East Asian Late Pleistocene Variation: Implications for Human Dispersion into the New World. *Am J Physical Anthropology* 144:442-453.

Hubbe M, Hanihara T, Harvati K. 2009. Climate Signatures in the Morphological Differentiation of Worldwide Modern Human Populations. *Anat Rec* 292:1720-1733.

- Hubbe M, Neves WA, de Oliveira EC, **Strauss A**. 2009. Postmarital residence practice in southern Brazilian coastal groups: continuity and change. *Latin American Antiquity* 20:267-278.
- **Strauss A**, Hubbe M. 2009. Dissimilarity fraction for metric traits of human skull: comparison with genetic studies. *American Journal of Physical Anthropology* 138 (Supplement 48).
- Nunes T, Neves W, Hanihara T. 2009. Afinidades biológicas extra-continentais dos paleoíndios tardios de lagoa santa e suas implicações para a questão do povoamento do novo mundo: uma abordagem odontométrica.
- Hubbe A. 2008. Taxonomic, taphonomic and morphometric contextualization of the skeletal remains of the Cuvieri Rockshelter megafauna. Master dissertation. São Paulo University.

2. **Strauss A**, Caparica JF. 2005. **Geometric morphometrics data acquirement and management** (open source).

Developed mainly on VBA language it works together with a Microscribe arm (or similar) allowing for strict geometric rules to be applied at the moment of data acquisition.

#### Teaching Experience

- 2016            Discipline: "Human Evolution". Eberhard Karls Universität Tübingen, Mathematisch-Naturwissenschaftliche Fakultät. English.
- 2015            Discipline: "An introduction to Bioarchaeology". Federal University of Minas Gerais. Total of hours: 20 hours. Portuguese.
- 2015            Discipline: "The archaeology of mortuary practices". São Paulo University. Total of hours: 20hours. Portuguese.

#### Teaching Assistance

- 2009            Discipline: Anthropology: biology and culture. São Paulo University, Institute of Bioscience. Total of hours: 20hours. Portuguese.
- 2008            Discipline: Philosophy of biological sciences. São Paulo University. Institute of Bioscience. Total of hours: 20hours. Portuguese.
- 2008            Discipline: Evolutionary Biology for Geologists. São Paulo University, Institute of Geoscience. Total of hours: 40hours. Portuguese.

#### Organization of exhibition

- 2016            Curator of the exhibition "**Life and death in Lapa do Santo: an archaeological biography of the people of Luzia**". Lagoa Santa, Brazil.
- 2009 - 2011    Curator (together with Walter Neves and Danilo Bernardo) of the exhibition "**From ape to man**". São Paulo, Brazil.
- 2008            Curator of the exhibition "**Kiju Sakai the archaeologist**". Lins, Brazil.

#### Languages

**Portuguese:** Native language

**English:** Fluent



**Spanish:** Fluent

**German:** Intermediate

**Scientific work featured in media**

1. Discovery News 23<sup>st</sup> September 2015. "Oldest case of decapitation in the New World" [[Link](#)]. English. Repórter: Rossella Lorenzi.
2. Science 23<sup>st</sup> September 2015. "Oldest decapitation in New World dates back 9000 years" [[Link](#)]. English. Repórter: Michael Balter.
3. LiveScience 23<sup>st</sup> September 2015. "Grisly discovery: 9,000-year-old decapitated skull covered in amputated hands" [[Link](#)]. English. Repórter: Charles Q. Choi.
4. NewScientist 23<sup>st</sup> September 2015. "America's oldest decapitated head reveals strange burial ritual" [[Link](#)]. English. Repórter: Andy Coghlan.
5. VICE Motherboard 23<sup>st</sup> September 2015. "9,000 years ago, someone chopped off this person's head and hands" [[Link](#)]. English. Repórter: Emiko Jozuka.
6. International Business Times 23<sup>st</sup> September 2015. "Brazil: 9,000-year old ritually decapitated skull unearthed" [[Link](#)]. English. Repórter: Sean Martin.
7. National Geographic 23<sup>st</sup> September 2015. "Oldest decapitated head in the New World found in "Vogue" pose" [[Link](#)]. English. Repórter: Erika Engelhaupt.
8. ScienceNews 23<sup>st</sup> September 2015. "More than 9,000-year-old decapitated head discovered in Brazil" [[Link](#)]. English. Repórter: Bruce Bower.
9. CBS News 23<sup>st</sup> September 2015. "Evidence of New World's oldest decapitation" [[Link](#)]. English. Repórter: Michael Casey.
10. Revista Pesquisa FAPESP 24<sup>st</sup> September 2015. "Pesquisadores encontram crânio decapitado em Minas Gerais" [[Link](#)]. Portuguese. Repórter: Rodrigo de Oliveira Andrade.
11. Newspaper (Brazil). "O Estado de Minas" from September 24 2015. "Crânio de 9 mil anos escondido por mãos amputadas é achado em Minas Gerais" [[Link](#)]. Portuguese. Reporter: Isabela de Oliveira.
12. Newspaper (Brazil). "O Estado de Minas" from September 24 2015. "Decapitação era usada por vários povos" [[Link](#)]. Portuguese. Reporter: Isabela de Oliveira.
13. Newspaper (Brazil). "Folha de São Paulo" from September 24 2015.
14. La Vanguardia 23<sup>st</sup> September 2015. "Hallan en Brasil la decapitación más antigua de América" [[Link](#)]. Spanish. Repórter: Cristina Saez.
15. El País 23<sup>st</sup> September 2015. "El extraño caso del decapitado más antiguo de América" [[Link](#)]. Spanish. Repórter: Nuño Rodriguez.
16. El Mundo 23<sup>st</sup> September 2015. "La decapitación más antigua?" [[Link](#)]. Spanish. Repórter: Miguel Corral.
17. Scientific American Español 23<sup>st</sup> September 2015. "La decapitación como ritual se realizaba en Suramérica hace al menos 9.000 años" [[Link](#)]. English. Repórter: Martín Cagliani .
18. CTV News 23<sup>st</sup> September 2015. "9,000-year-old skull may be oldest case of decapitation in New World" [[Link](#)]. English. Repórter: Josh Elliot.
19. Globo G1 Portal 23<sup>st</sup> September 2015. "Crânio em Minas é registro mais antigo de decapitação nas Américas" [[Link](#)]. Portuguese. Repórter: Rafael Garcia.
20. Estado de São Paulo OnLine 23<sup>st</sup> September 2015. "Crânio de 9 mil anos revela decapitação mais antiga das Américas" [[Link](#)]. Portuguese. Repórter: Fábio de Castro.
21. Circular da Prefeitura de Pains (Brazil) – 13<sup>st</sup> May 2015 – "MAC recebe arqueólogo André Strauss para datação de esqueleto do acervo". Reporter: Elaine Martins [[Link](#)].

22. Globo channel (Brazil) – Fantástico from 14th December 2014 - “‘Jornada da Vida’ mostra origem dos brasileiros há 13 mil anos”. Reporter: Sônia Bride [\[Link\]](#).
23. Globo channel (Brazil) – “Jornal Nacional” from 7th November 2014 - “‘Crânios humanos que viveram há mais de 10 mil anos em Minas estão passando por exames’”. Reporter: Ricardo Soares [\[Link\]](#).
24. Magazine (Brazil). “Planeta” from November 2014. Cover article: “Em busca do Brasil ancestral”. Reporter: Rodrigo Cardoso [\[Link\]](#).
25. Magazine (Brazil). “Terra da Gente” from October 2014. “Lagoa Santa”. Reporter: Adriano Gambarini.
26. Newspaper (Brazil). “O Estado de Minas” from 26th September 2014. “Os rituais dos primeiros brasileiros” (The rituals of the first Brazilians). Journalist: Isabela de Oliveira.
27. Newspaper (Brazil). “O Correio Braziliense” from 21st September 2014. “Os rituais dos primeiros brasileiros” (The rituals of the first Brazilians). Journalist: Isabela de Oliveira.
28. Newspaper (Brazil). “O Estado de Minas” from 1st August 2014. “Descoberta de esqueletos põe Minas no mapa da arqueologia” (Discovery of skeletons put Minas in the focus of archaeological research). Journalist: Gustavo Werneck [\[Link\]](#).
29. Newspaper (Brazil) “O Estado de Minas” from 31st July 2014. “Descoberta de sítio arqueológico em Matozinhos vai revelar pré-história mineira” (Discovery of archaeological site in Matozinhos will unveil pre-history)”. Journalist: Pedro Ferreira [\[Link\]](#).
30. Newspaper (Brazil) “O Estado de Minas” from 31st July 2014. “Fosseis localizados no exterior em Matozinhos são analisados no exterior” (Human fossils discovered in Matozinhos are analyzed abroad)”. Journalist: Pedro Ferreira [\[Link\]](#).
31. Newspaper (Brazil) “O Correio Braziliense” from 8th August 2014. “Vida em comunidade suavizou feição humana” (Life in community made human features more gracile)”. Journalist: Isabela de Oliveira [\[Link\]](#).
32. Newspaper (Brazil) “Folha de São Paulo” from 4th July 2014. “Pesquisa relaciona brigas à evolução humana” (Research relates fight with the evolution of the human face)”. Journalist: Ricardo Aguiar [\[Link\]](#)
33. Newspaper (Brazil) “O Estado de Minas” from 20th June 2014. “História do neandertal é reescrita” (The history of neanderthal was rewritten)”. Journalist: Isabela de Oliveira
34. Newspaper (Brazil) “Correio Braziliense” from 20th June 2014. “História do neandertal é reescrita” (The history of neanderthal was rewritten)”. Journalist: Isabela de Oliveira
35. Globo Channel – Terra de Minas – 8th December 2012 – “Excavations at Lapa do Santo” – Part 1 [\[Link\]](#). Portuguese.
36. Globo Channel – Terra de Minas – 8th December 2012 – “Excavations at Lapa do Santo” – Part 2 [\[Link\]](#). Portuguese.
37. Boletim do Parque Estadual do Sumidouro (State Park of Sumidouro Bulletin). 16th July 2012. “Parque Estadual do Sumidouro recebe arqueólogo pesquisador de Universidade Alemã” [\[Link\]](#). Portuguese. Journalist: Luisa Costa.
38. Globo G1 Portal 3rd August 2012. “Arqueólogos desenterram passado de mais de 8 mil anos na Grande BH (Archaeologist unveil 8000 year past near Belo Horizonte)” [\[Link\]](#). Portuguese.
39. Globo Channel “Jornal Nacional” 3rd August 2012 “Arqueólogos decifram como nossos antepassados enterravam os mortos (Archaeologist decipher how our ancestors buried their deads)” [\[Link\]](#). Portuguese.
40. Globo Minas Channel “MG-TV” 24th July 2012. “Grupo de estudiosos encontra relíquias de ritual de sepultamento (Scholars found vestiges of funerary rituals)” [\[Link\]](#). Portuguese.

## *Curriculum Vitae*

41. Newspaper (Brazil) "O Estado de Minas" from 1st July 2012. "Ossadas valiosas (Valuable bones)" [[Link](#)]. Journalist: Paulo Henrique Lobato
42. Newspaper (Brazil) "O Estado de São Paulo" from 19<sup>th</sup> September 2011. "Como vivia um paulista pré-histórico (How was the life of early people from São Paulo state)" [[Link](#)]. Portuguese. Journalist: Alexandre Gonçalves.
43. Newspaper (Brazil) "O Estado de São Paulo" from 9<sup>th</sup> September 2011. "Estudo aponta que fóssil deve ter pertencido a ancestral humano (Study indicates fossil was a human ancestral)" [[Link](#)]. Portuguese.
44. GloboNews Channel – Jornal das 10 – 15<sup>th</sup> of August 2011. "Archaeologist recreates First Americans Rituals" (2 and half minute) [[Link](#)]. Portuguese.
45. Newspaper (Brazil) "Boletim Agência Fapesp" of 18<sup>th</sup> April 2011. "The Body Speaks" [[Link](#)]. Portuguese. Journalist: Elton Alisson.
46. Newspaper (Brazil) "Folha de São Paulo" of 22<sup>th</sup> February 2011. "Pre-historic Brazilians made art with the deads" [[Link](#)]. Portuguese. Journalist: Reinaldo Lopes.