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Title

Validation of a standard forensic anthropology examination protocol by measurement of applicability and reliability on exhumed and archive samples of known biological attribution

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Abstract

Forensic anthropology makes an important contribution to human identification and assessment of the causes and mechanisms of death and body disposal in criminal and civil investigations, including those related to atrocity, disaster and trafficking victim identification. The methods used are comparative, relying on assignment of questioned material to categories observed in standard reference material of known attribution. Reference collections typically originate in Europe and North America, and are not necessarily representative of contemporary global populations. Methods based on them must be validated when applied to novel populations. This study describes the validation of a standardized forensic anthropology examination protocol by application to two contemporary Brazilian skeletal samples of known attribution. One sample (n=90) was collected from exhumations following 7-35 years of burial and the second (n=30) was collected following successful investigations following routine case work. The study presents measurement of 1) the applicability of each of the methods used and 2) the reliability with which the biographic parameters were assigned in each case. The results are discussed with reference to published assessments of methodological reliability regarding sex, age and—in particular—ancestry estimation.

Highlights

Validation of forensic examination protocol applied to exhumed and archived skeletal samples of known biographic profile

Measurement of methodological applicability and reliability

Issues of methodological reliability and validation in Brazilian and other novel populations discussed

Keywords

Forensic Anthropology, Osteology, Validation, Applicability, Reliability, Brazil

1. Introduction

The methods used in forensic anthropology involve estimation of biographic parameters—age, sex, ancestry, handedness and stature, identification of pathology or trauma, and assessment of the means of disposal in cases involving skeletonized or partially-skeletonized human remains (1-7). These methods are comparative, in that they rely on the classification of unknown or questioned skeletal material into categories determined from standard reference material of known attribution—that is, where the ante mortem biological profile of the deceased is available for each skeleton. Reference collections can only be assumed to be representative of the population from which they were derived, however. When applied to populations that are geographically or temporally distant, standard methods derived from this reference material need to be validated. This study describes the validation of a forensic anthropology examination protocol based on methods developed in the United States and Europe for use in a population in Brazil.

Brazil is an example of a region where forensic anthropology is a relatively nascent discipline (8, 9), principally as a consequence of its emergence from the state control of forensic science, which was imposed during the military governments of 1964-1985. It is applied in routine criminal and civil investigations (10-12), as well as in inquiries relating to alleged human rights abuses that occurred during the dictatorship (13, 14). Like many contemporary populations, the Brazilian population is demographically complex, resulting from migration and intermarriage arising from Native American, African, European, Middle Eastern and—more recently—Japanese settlement, which has considerable historic and prehistoric time depth, and varies substantially by region (15-17).

The approach used in validation is a 'blind study' of two contemporary Brazilian skeletal samples of known attribution, allowing the results of the examinations to be compared with the ante mortem information. For each method used in the protocol, the study assesses 1) applicability—that is, whether or not each method can be applied; and 2) reliability—that is, whether or not the classification for each skeleton, if obtained, corresponded to the ante mortem value where known.

The results are discussed in relation to the utility of the protocol when applied to Brazilian populations, priorities for further work, and the wider implications for the validation of existing forensic anthropology examination protocols in novel populations.

2. Material and methods

2.1. Skeletal samples

Two skeletal samples were investigated. The first consisted of the remains of 90 individuals exhumed from the municipal cemetery of Bom Pastor in Ribeirão Preto, SP. The remains had been interred between 1979 and 2007, and were exhumed between 2012 and 2014 as part of an urban redevelopment project. Following careful exhumation avoiding loss or damage, the skeletal remains were packaged and labelled prior to transportation to the Forensic Anthropology Laboratory, Medico-Legal Centre, Ribeirão Preto Medical School—University of São Paulo, SP (LAF/CEMEL).

The second skeletal sample consisted of the remains of a further 30 individuals held in the LAF/CEMEL archive, obtained as a consequence of local investigative casework undertaken between 2004 and 2015.

2.2. Ante mortem data collection

In the exhumed skeletal sample, known values of age, sex and ancestry were obtained from administration forms documenting each individual at the time of burial. In the archive sample, known values of age, sex, ancestry, handedness and stature were obtained from ante mortem data in cases solved following successful criminal or civil investigations. In the latter sample, age and sex were the classifications officially assigned in the deceased's identity document the Cartão de Identidade or Registro Geral (RG) card. The ancestry, handedness and stature, however, were provided by family members at the time of a "missing person" report.

The ante mortem attributes of the two skeletal samples are given in the Supplementary Data (Tables S1 and S2, respectively). In summary, where an ante mortem value was assigned, the exhumed skeletal sample (n=90) consisted of 74 males and 13 females of 10 to 86 years of age, of which 33 were White, 15 African, and 20 of Mixed African and White ancestry. The archive sample (n=30) consisted of 28 males and 2 females of 14 to 65 years of age, of which 13 were White and 13 of Mixed African and White ancestry.

2.3. Post mortem data collection

In the exhumed sample, subsampling for complementary studies of DNA and bone diagenesis was undertaken—including, for example, for investigations of microstructure and DNA survival (18) and of taphonomic processes examined in 3-D using tissue microarray analysis (19). Subsampling was undertaken by recovery of a single tooth and section of femoral mid-shaft, respectively, following a protocol intended to optimally combine the requirements of genetic and anthropological investigations (20), after which the skeletal material was carefully cleaned. Removal of material adhering to the skeleton was achieved using soft bristle nylon brushes under running water, applying a mild neutral detergent when necessary. In the archive sample, a similar process had been undertaken prior to archiving.

Each skeleton was laid out in anatomical order and a comprehensive examination completed commencing with a full and detailed inventory of the skeletal elements and dentition, and recording findings indicative of pathology or trauma (21).

Estimation of biographic parameters followed standard analytical methods described in both technical reference (1-7) and primary scientific (22-39) literature combined in the examination protocol—the 'LAF/CEMEL protocol'—completed in the form of formal report. The methods for age estimation (1-7, 22-29) are summarized in Table 1, for sex estimation (1-7, 30-34) in Tables 2a and 2b, for ancestry estimation (1-7, 34, 35) in Table 3, and for handedness estimation (2, 3, 36, 37) in Table 4. For stature estimation (4, 38, 39) regression formulas derived from adult long bone length were used, adjusted for sex and ancestry as given in Table 5.

A maximum age range was assigned, based on a multifactorial approach (40) using the methods summarized in Table 1, following any required sex assignment (see below) where possible. Firstly, an age range was estimated using each available method. Then, from these, an age range was assigned corresponding to the maximum consensus or 'overlap' between each individual range. Where this could not be achieved, a conservative age range was assigned based on the overall minimum and maximum. Similarly, where a required sex assessment was not possible, a maximum range was assigned. Where the method permitted only a distinction between an approximate minimum or maximum age—for example, in the case of only a single clavicle being available—then only this minimum or maximum value, and not a range, was assigned.

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For sex estimation, separate assessments were made derived from the skull (Table 2a) and pelvis (Table 2b) following any required ancestry assignment (see below), and a classification assigned based on the predominant and unweighted indication of all features examined, that is, whichever sex assignment scores most when the nine features of the pelvis and seven features of the skull are considered. The features of the skull and pelvis were scored separately, excluding ambiguous or indeterminate assignments, and a sex assigned for each based on whichever score—male or female—was greater. Where no score predominated, the sex was assigned as indeterminate.

Ancestry (Table 3) and handedness (Table 4) were similarly based on the predominant indication of separate assignments. Ancestry was assigned using the nomenclature 'White', 'African', 'Oriental' and 'Indigenous' or a mixture of these contributions. These are the commonplace terms used in Brazil, which has a large multiplicity of ancestries and mixtures thereof—the term 'mixed' is used when the ancestry cannot be assigned to one of the major groups. Where it could be assigned, handedness was estimated as 'Right' or 'Left' handed, or 'Ambidextrous'.

Stature was recorded as a range based on the first standard deviation a value calculated from long bone length (Table 5). Where possible, the stature estimation was based on the assigned age, sex and ancestry of the individual. Where this was not possible, the adult White male values were used.

The forensic anthropology examination protocol is part of a standard step-wise procedure summarized as a flow chart in the Supplementary Data (Figure S1). The entire recording process including inventory completion, assessment of features, and classification of biographic parameters was completed digitally, using desktop applications and a supporting online system (41), and uploading of digital photographic images, including those of the full skeleton and any distinctive features—such as indications of pathology, trauma or surgery. A proforma of the tangible 'output' from the protocol—a forensic anthropology examination report—is included in the Supplementary Data. Detailed discussion of the development of the protocol and its application to the Brazilian population is described in Francisco (42). Adjustments made to standard methods developed using European and North American reference material—particularly in relation to ancestry assignment—are considered in Discussion.

2.4. Measurement of applicability

The number and percentage of cases in which each method used in the assessment of age, sex, ancestry, handedness and stature could be applied—that is allowed an assignment to be made irrespective of the result—were calculated for each skeletal sample and for the two samples combined. While the study is therefore focused on absolute applicability—that is, irrespective of the cause of attrition that prevented assignment from the relevant feature. The number of cases where the relevant skeletal element or feature was present were also scored, however, in order that this issue could be considered in further detail.

2.5. Measurement of reliability

Comparison of known and assigned values of biographic parameters was undertaken as a 'blind study'. Cases where the ante mortem value was unknown or the post mortem value could not be assigned were excluded. The number and percentage of cases where age, sex, ancestry, handedness and stature were or were not in agreement were calculated. For age and stature, this required the known age to be within the classified range. For sex and handedness, this required the assigned classification to correspond with the known value. For ancestry, this required the assigned classification to fully or partially correspond to the known ancestry (see Results and Discussion).

3. Results

The classifications of age, sex, ancestry, handedness and stature assigned for each skeleton in the exhumed and archive sample are given in the Supplementary Data (Tables S3 and S4, respectively).

3.1. Measurement of applicability

The number and percentage of cases in which each method used in the assessment of age, sex, ancestry, handedness and stature could be applied—that is, an assignment could be made irrespective of the result—are given in Tables 6 to 10, respectively. This value makes no distinction regarding why the method could not be applied: whether, for

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example, it was because of complete absence of the relevant skeletal element or because of taphonomic erosion obliterating the feature concerned. For comparison, however, the number of cases where the relevant element or feature was present is also given.

The frequency with which the methods used in age estimation could be applied varies from 10.0 to 76.7 percent of cases in the exhumed sample and 53.3 to 90.0 percent of cases in the archive (Table 6). Overall applicability varies from 39.2 to 75.0 percent.

The frequency with which the methods used in sex estimation from the pelvis could be applied varies from 38.9 to 88.9 percent of cases in the exhumed sample and 80.0 to 93.3 percent of cases in the archive (Table 7a). Overall applicability varies from 52.5 to 90.0 percent. Methods used in sex estimation from the skull could be applied in from 58.9 to 85.6 percent of cases in the exhumed sample and 63.3 to 73.3 percent of cases in the archive (Table 7b). Overall applicability varies from 60.8 to 82.5 percent.

The frequency with which the methods used in ancestry could be applied varies from 17.8 to 87.8 percent of cases in the exhumed sample and 46.7 to 80.0 percent of cases in the archive (Table 8). Overall applicability varies from 46.7 to 85.0 percent.

Methods used to assign handedness could be applied in 33.3 to 68.9 % of cases in the exhumed sample and 40.0 to 66.7 % of cases in the archive, and 38.3 to 65.8 percent of cases overall (Table 9).

The methods used to estimate stature were applied in 92.2 percent of cases in the exhumed sample and 83.3 percent of cases in the archive (Table 10). Although alternative long bones were available, the femur was used in preference in every case where the other elements were present.

3.2. Measurement of reliability

The known and assigned values of biographic parameters are given in the Supplementary Data (Tables S1 to S4).

The numbers and percentages of parameters assigned that correspond with the known values are given in Table 11.

Correspondence between known and assigned ages was high in the archive sample (n=30), with the known age falling within the estimated range in 28 out of 29 cases in which the methods could be applied. Correspondence in the exhumed sample (n=90) was poor,

however, with the known age falling within in the estimated age range in only 18 out of 50 cases in which the methods could be applied.

Assessment of sex was more reliable in both samples. In the exhumed sample, the assigned value was in agreement with the known sex in 76 out of 81 cases in which the methods could be applied. In the archive sample, the sex was assigned correctly in all 29 cases in which the methods could be applied.

In the exhumed sample, assignment of ancestry precisely matched the recorded value in 21 out of 65 cases where the methods could be applied and, in the archive sample, in 22 out of 23 cases where the methods could be applied.

Reliability of handedness and stature assignment could not be assessed in the exhumed sample as these parameters are not recorded as part of the post mortem administrative procedure. In the archive sample, assignment of handedness agreed with the recorded value in 14 out of 18 cases where the methods could be applied. In one of the four incorrectly classified cases, an individual believed to be left-handed was classified as ambidextrous. Assignment of stature agreed with the recorded value in 12 out of 22 cases where the methods could be applied.

4. Discussion

4.1. Applicability

Variation in applicability of the methods within and between the exhumed and archive samples may be attributable to the relative degree of decomposition.

The age estimation methods used (Table 1), rely on examination of relatively friable features of the skeleton, such as the extremities of the fourth rib and clavicle, and articular surfaces of the pelvis. Applicability of these methods will substantially diminish in eroded and incomplete skeletons, as is evident in Table 6. For example, the fourth sternal rib end is present in 79 out of 90 cases in the exhumed sample, but can only be assessed in 9 of those; whereas the pubic symphysis surface is present in 51 out of 90 cases, and can be assigned in 21.

By contrast, the methods used in the assignment of sex (Tables 2a and 2b) and ancestry (Table 3) are based on classification of relatively gross morphological features less affected by diagenetic processes, unless the skull or—in the case of sex assessment—

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skull and pelvis are absent. These factors are anticipated to account for the greater applicability of the methods used in sex (Tables 7a and 7b) and ancestry (Table 8) estimation, compared to those used in age (Table 6) estimation. Ancestry assignment could be made in every case in which the relevant feature was present (Table 8).

These factors are also anticipated to account for the generally lower applicability of all methods in the exhumed sample, which consisted of skeletons interred for an average period of 15 years in an aggressive tropical climate known to promote rapid bone diagenesis (19). The archive sample, by contrast, consists of remains many of which were found complete in deposits typically detected within twelve months of their deposition (10, 43). Total values are skewed, however, due to the three times larger size of the exhumed sample.

Methods used in the estimation of handedness (Table 4) rely on assessment of proportions as well as individual features, and depends on bilateral comparisons that cannot be applied unless the left and right sides of the skeletal elements concerned are present. Hence, these methods show the lowest applicability (Table 9), frequently as only one element of a paired feature may be present.

Methods for stature estimation can be extrapolated from measurements of several of the long bones (Table 5) taken from either the left or right side. Stature assessment therefore has considerable redundancy, meaning it is not so susceptible to taphonomic processes, even in the exhumed sample (Table 10). In a small proportion of each sample, however, none of the three preferred long bones were available for measurement.

The measure of applicability chosen—that is, disregarding whether feature absence or taphonomic erosion is the cause of a non-assignment—is conservative. Applicability is greater, if applicability to the proportion of features actually present is measured.

4.2. Reliability

Reliability of age estimation in the exhumed sample may be a reflection of the extent of erosion due to taphonomic factors, as well as absence of material. Assignment of age frequently relied on less reliable or precise methods (see Table 1), such as those based on assessment of arthritic changes to the vertebrae or the status of the medial clavicle epiphysis, respectively. Age assessment was not possible at all in 28.9 percent of cases (Table 6). Unfortunately, the relative frequency with which methods based on these

features could be applied is the inverse of their precision and utility. Assessment of the medial clavicle epiphysis (22) essentially yields a 'younger than' or 'older than' classification, rather than an age or bracket. Assessment of changes to the vertebrae is relatively lacking in precision (29) and, while assessment of the fourth sternal rib end is reported to be a more reliable method in the older age ranges (27), it could only be applied in 10.0 % of cases in the sample. There was little correlation between period of burial and age at death, however, suggesting taphonomic damage was not compromising only older material (see Supplementary Data Figure S2). Although the age range is greater in the exhumed sample (22 to 80 years) than in the archive sample (34 to 61 years), the mean ages are similar in each—49.8 and 47.6 years, respectively.

In the archive sample, an age range assignment was possible in 29 out of 30 cases, and in agreement with the known age in 28 of those 29 cases. In this sample, the more precise methods based on the features of the pelvis and rib (Table 6) could applied with much greater frequency relative to the exhumed sample—where assignment relied on less precise methods, such as those using developmental changes to the vertebrae or fusion of the medial clavicle epiphysis. The sample consisted of well-preserved and near-complete remains arising from surface deposits on local agricultural land or in forests, or found submerged in lakes and rivers (43).

Assignment of sex showed high levels of agreement in both samples, at 84.4 % in the exhumed sample and 96.7 % in the archive (Table 11). Although a range of applicability was evident in the methods used to assign sex (Tables 7a and 7b), several features of both the skull and pelvis were used. This conferred some redundancy, in that sex could be assigned from features available, buffering sex assignment from taphonomic affects that would reduce the applicability of methods used based on individual features alone. While the pelvis is regarded to be more reliable than the skull in sex estimation—especially in non-White populations, combined use of both the skull and pelvis is optimal (1-7).

Exact concordance of the assigned and recorded ancestry in 22 out of 23 cases in the archive sample (Table 11), which is encouraging. Agreement in the exhumed sample was much poorer, however. Ancestry could not be assigned in 20 percent of cases and estimated and known values could only be compared in 61 percent of the sample. Exact concordance of the assigned and recorded values was obtained in only 21 out of 55 cases. This is a disappointing result, which may be influenced by the large proportion of individuals of mixed ancestry.

Assessment of ancestry is widely understood to be problematic, even in apparently straight-forward cases (28). Assignment is complex, as a consequence of fundamental semantic and theoretical problems (44, 45), and contemporary and historical admixture (15-17). It is this biographic parameter that was found most challenging with regard to adjustment of standard methods based on European and North American reference material for the Brazilian population (42). A number of issues were encountered in attempts to optimize the protocol and assess its reliability. In this study the deceased individual´s ancestry is subjectively assigned by family, friends or cemetery administrators and is classified by terms describing skin colour or race—as opposed to ancestry. These assignments may be affected by socio-cultural perceptions held by the deceased or the assigner. For example, in Brazilian law (46) it would be possible for an individual to self-declare themselves as 'black', 'brown' or 'indigenous' and for this information to be unintentionally, but technically false—precluding scientific agreement. These factors are anticipated to have less influence on the archive sample, where the assignments are made following the successful conclusion of a criminal or civil investigation. Nevertheless, it is necessary to consider how to address reliability within the context of the investigation of unidentified skeletal remains in Brazil, where the risks of miscommunication and misunderstanding of what is meant in ancestry assignment may easily arise. For these reasons, two measures of reliability were offered—one assessing precise assignment (see above) and the second including partial agreement. The reason for this is to reduce the prospect of making an exclusion for semantic reasons.

In cases where either the assigned or known value corresponds to one of the components of a mixed ancestry classification, then a partial agreement was considered to have occurred. For example, if an individual of known African ancestry is classified as mixed, this may be regarded as technically in error. However, in Brazil it may be anticipated that local 'black' people are all in reality of mixed ancestry and, in that respect, the assignment is correct. Similarly, a skeleton that can be clearly assigned as African osteologically, may have belonged to a person who may well have described themselves as 'black' or 'mixed'. If these cases are included in the totals, then in the exhumed sample, assignment of ancestry was concordant with the recorded value in 51 out of 65 cases where the methods could be applied and, in the archive sample, in all 23 cases where the methods could be applied. It is imperative, however, that this information is conveyed meaningfully to investigators, in order that apparent non-correspondence between 'black' or

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'mixed' assignments—whether arising from ante mortem or post mortem classifications—may be less likely to lead to a false exclusion. The accurate assignment of 'mix' of ancestry in a person of mixed ancestry in Brazil is likely to be the subject of much further research, requiring access to contemporary skeletal material of known genetic background.

Reliability of handedness and stature assignment could only be assessed in the archive sample (Table 11). Assignments of handedness were made in 20 out of 30 cases (Table 9), and were in exact agreement with known values in 14 of those cases (Table 11). Handedness could not be assigned in 45.6 percent of cases in the exhumed sample (Table 9), and the low and variable frequency with which different methods in handedness assignment were applied (Table 9) suggests taphonomic processes causing the loss of paired bone material and erosion of individual features are implicated in the poor success rate. It can be anticipated that assessment of the reliability of handedness assignment will be complicated by the heavy predominance of righthandedness in the general population. Whilst the results do not show a preponderance of mis-assignments of any particular kind, further research on a larger sample of confidently known handedness attributions would be required to unequivocally establish the reliability of the handedness assignment methods used, as assessed against chance agreements, for example.

Methods for the estimation of stature were applied in 25 out of 30 cases (Table 10), and were in agreement with known values 12 of those cases (Table 11). If partial overlaps between stature ranges are included, however, this figure rises to 15 out of 22. A further two incorrect classifications were within 1 cm of the known range assigned. If this margin of error is tolerated, then the figure rises to 17 out of 22. These assignments were reliable, even though typically based primarily on stature estimation from the femur (42)—see Table 10—and on the use of the adult White male regression equations (Table 5) where the age, sex or ancestry could not be assigned.

The substantial differences evident in reliability between the exhumed and archive samples can readily be attributed to the circumstances of disposal and preservation. The exhumed sample consisted of skeletonised remains recovered from long periods of interment in an environment hostile to preservation. The archive material typically consisted of surface deposits recovered after relatively short periods, carefully defleshed prior to examination.

4.3. Validity of the protocol

The LAF/CEMEL laboratory arose as part of a scientific collaboration between Ribeirão Preto Medical School—University of São Paulo, SP (FMRP-USP) and the University of Sheffield, United Kingdom funded by the UK Foreign and Commonwealth Office Global Opportunities Fund. Following the establishment of the laboratory, a forensic anthropology protocol was developed incrementally (47-50) to meet international standards and the demands of the local environment for rapid and reliable examinations, and to permit recording of ante mortem as well as post mortem data. The resultant desktop proforma (see Supplementary Data) extends to 11 pages prior to completion, including contingency tables, regression equations and descriptive anthroscopic and anthropometric characteristics, and is fully bilingual in Portuguese and English, but efficiency in its use is greatly facilitated by digitization (41).

Furthermore, the utility of the protocol in Brazil is promising in as much as the archive sample in particular shows a case demography that appears to follow consistent trends across jurisdictions internationally (51, 52), subject to the influence of two key factors. These are homicide rate—in particular, the elevated use of firearms—and environment—distinguished by parameters of mild or extreme climate and density or sparsity of population (43).

Forensic anthropologists commonly recognize the need for validation studies based on samples representative of the populations concerned (1-7, 51-58). Attempts to systematically assess error or precision are rare, however, and tend to be restricted to assignment of sex and age (21-34). Opportunities to access contemporary skeletal collections of known attribution are uncommon and this 'blind study' therefore represents an unusual attempt at validation. Reliability in sex estimation is comparable with reported values from the literature, and reliability in age estimation is good when applied to the archive sample of skeletal material recovered within about twelve months post mortem. Although less precise when applied to skeletons exhumed after 7 to 35 years of burial, the poorer results in age estimation in this sample are attributable to taphonomic attrition of features used in classification rather than the methods themselves.

Assignment of ancestry is complicated by the high level of admixture encountered, associated semantic issues, and limited precision in the assignment of contributions of African, White and Indigenous origin.

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Utility may be improved by careful explanation of findings, given in relation to common usage and understanding of the associated terms that will have meaning to investigators and the public.

4.4. Further research

Recent academic discourse has examined the role of forensic anthropology as a complement to forensic pathology, odontology, archaeology and other sub-disciplines in multi-disciplinary medico-legal investigations, in crime prevention and detection, and in promoting public health and safety (43, 53-57). As a consequence, new priorities for education and training, research, and professional practice have been proposed. Furthermore, mass fatality incidents—such as air crashes—are not unknown in Brazil. Forensic anthropology plays an important role in the investigation of such fatalities with a cross- or extra-jurisdictional component, such as atrocity victim identification (AVI), disaster victim identification (DVI) and trafficking victim identification (TVI) relating to missing persons (60-62).

Under the system of regulation and accreditation followed in Brazil, forensic anthropology and odontology are separate disciplines requiring independent validation. Age estimation from the dentition was not part of this study—but dental material was present in 92.2 percent of cases in the exhumed sample and 83.3 percent in the archive sample. Dental age estimation would be anticipated to substantially improve the reliability of age estimation particularly in juveniles. In these samples, however, the benefits would be small as the minimum age in the exhumed sample is 22 years and in the archive sample 34 years. Furthermore, ante mortem dental records are rarely available for the socio-economic groups encountered in the samples studied, which tend to be marginal.

Ancestry assignment in Brazilian populations may be improved via further research (63), although previous results suggest anthroscopic methods perform better than anthropometric ones in this population, whether applied to ancestry or sex (47-49). Further research targeting the estimation of biographic parameters from features on the skeleton less liable to erosion may also be beneficial.

Where sex and ancestry could not be assigned, stature was estimated using standard White male regression equations (38-39) adopting an approach that has been followed in Brazil for some decades, which might be improved by further research.

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Similarly, the reliability of age estimation was somewhat skewed by offering large age ranges. For example, of 50 cases in the exhumed sample where the age was known ante mortem and could be assigned from the skeleton, ages assigned within 4 to 5 year ranges were correct in 1 out of 6 cases (17 percent), whereas ages assigned within 15 to 25 year ranges were correct in 10 out of 26 cases (38 percent)—see Tables S1 and S3.

While the aim of the study was to establish if the protocol and supporting methods were valid, rather than new methodological development, it is possible that further research on a larger sample of known sex, ancestry and stature may allow more precise age range assignments and stature formulae to be calculated and tested on a Brazilian population.

Applicability gave a measure of whether or not the method could be applied when the bone is present. Frequently, this was due to poor preservation of the feature, which might be anticipated to differ from sample to sample. In some cases, however, it may have been due to damage during forensic autopsy or other post mortem effects relating more directly to the means of disposal of the body. Whilst applicability is not synonymous with preservation, it is associated with it and could be anticipated to correlate with reliability. In this study, assignments are typically based on the assessment of a number of features and the sub-samples of each feature of each relevant skeletal element is small, permitting only a general assessment of the influence of preservation. Statistical studies based on large sub-samples of elements with variably preserved features at each site—and where damage during formal autopsy, for example could be excluded—would allow such associations to be confidently demonstrated and may offer insight as to whether certain features should be abandoned in cases of poor preservation.

Although of considerable value in the context of methodological development in Brazil, the samples used were not entirely perfect in terms of preservation or stratification by age, sex, ancestry, handedness or stature—or wider socioeconomic factors which could skew development and wear on the skeleton, affecting the indicators observed. Obtaining access to large, stratified samples of fully intact skeletal material of known attribution that are representative of contemporary regional populations may be a fanciful proposition, however, and in any event—as time passes—socio-economic change will inevitably put the representativeness of any collection in doubt. Given that access to relatively small contemporary samples is most plausible, it is important that ante mortem biographic data is as complete and accurate as possible. One practical consequence of this research was the

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proposal to revise the administrative data collected prior to burial to include stature range and handedness (42).

5. Conclusions

The LAF/CEMEL forensic anthropology examination protocol is based on methods derived from North American and European populations and this study demonstrates it is comparably effective when applied to Brazilian samples of known attribution. Estimation of sex is generally reliable, and estimation of age, handedness and stature correspond with reported values for other regions. Estimation of ancestry is applicable to a Brazilian population, which has a very wide range of admixture. Reliability, however, is highly dependent on a careful choice of terminology that—with regard to both ante mortem and post mortem classifications—must be explained to investigators and the public using lay terminology applied broadly and with circumspection in order to avoid false exclusions.

As might be anticipated, for the protocol to be most effective, complete and uneroded skeletons are desirable in order that the methods can be applied optimally. The careful collection and curation of remains is therefore essential. The chances of identification will also be improved if ante mortem biographic data is complete and accurate, and improvements in civic burial recording are proposed.

Our approach to validation via the measurement of 'applicability' of the methods used and 'reliability' of the classifications obtained offer a model for application of standard forensic anthropology examination methods to other novel populations, and for the prospect of further distinguishing overtly between presence, and damage and preservation arising from diverse causes and their relationship with reliability.

The study provides a realistic assessment of the potential for forensic anthropological analysis to contribute to criminal and civil judicial investigations in specific conditions that are representative of these and other parts of Brazil, and other tropical and subtropical climates. It offers a basis from which practitioners may chose to focus their methods and for further methodological refinement.

6. Ethical approval

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The project was reviewed and approved by the Research Ethics Committee of the Hospital das Clínicas, FMRP/USP (HCRP 10613/2012), following authorization for the exhumations by the Companhia de Desenvolvimento Econômico de Ribeirão Preto (CODERP) (Ribeirão Preto Economic Development Corporation).

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Table 1.   Summary of standard methods used in age estimation in adults (1-7, 22-29)

Feature	Method of Assignment
Medial clavicle epiphysis	Stage of closing of the medial epiphysis of the clavicle (15-32 years) (22)
Pubic symphysis surface	Stage of change of the pubic symphysis surface: six stages by age group for males and females (23-25)
Auricular surface of the ilium	Stage of change of the auricular surface of the ilium (sacroiliac joint): eight stages by age group for males and females (26)
Fourth sternal rib end	Stage of change of the distal end of the fourth rib: eight stages by age group for males and females (27)
Cranial sutures	Extent of closure of the cranial sutures: analysis uses the external surface of the cranial sutures by assigning grades to each (28)
Developmental changes in the vertebrae	Extent of anatomical changes to the vertebrae, from the closure of the epiphyseal ring in the young to the formation of osteoarthritic changes in adults from 30 years (29)

Table 2a. **Summary of standard methods used in sex estimation from the pelvis (1-7, 30-33)**

Feature	Method of Assignment	
	Male	**Female**
Subpubic angle	Narrow	Wide
Ventral arc	Absent	Present
Medial ischio-pubic ridge	Absent	Present
Greater sciatic notch	Small and narrow	Large and wide
Sacral alae	Thick and robust	Thin and delicate
Curvature of sacrum	Very curved	Slightly curved
Sacral auricular surface	Large and long	Small and short
Projection of auricular surface	Shortly designed, tending to the flat surface of the hipbone	Very projected, stands out from the surface of the hipbone
Preauricular sulcus	Absent	Present

Table 2b. **Summary of standard methods used in sex estimation (skull and mandible) (1-7, 32-34)**

Feature	Method of Assignment	
	Male	**Female**
Supraorbital ridge	Prominent, standing out from the surface of the frontal bone	Smooth, tending to form a flat surface to the frontal bone
Occiptal protuberance	Clear and well marked	Slightly clear and marked
Mastoid process	Large and robust	Small and delicate
Supramastoid crest	Present and well marked	Absent or poorly marked
Zygomatic arch	Tall and robust	Small and delicate
Mentum	Large, protruding, typically with two eminences	Small, rounded, usually with a single central eminence
Mandibular angle	Open and protruding laterally (flaring)	Slightly open and non-protruding laterally (non-flaring)

Table 3.   Summary of standard methods used in ancestry estimation (1-7, 34, 35)

Feature	Method of Assignment		
	Caucasian	**Oriental**	**African**
Cranium (length)	Intermediate	Short	Long
Cranium (shape)	Rounded	Short and tall	Low and long
Nasal bridge	Tall and narrow	Intermediate	Low and wide
Nasal aperture (height)	Tall	Low	Low
Nasal aperture (width)	Pronounced	Small	Small
Nasal spine	Saddle	Flat	Grooved
Nasal base	Narrow	Mean	Wide
Facial profile (lateral)	Straight	Intermediate	Protruding
Facial profile (anterior)	Narrow	Wid	Narrow
Orbits	Angular	Rounded	Rectangular
Orbits (inferior margin)	Retracted	Protruding	Retracted
Orbits (superior margin)	Pronounced	Smooth	Smooth
Muscle attachments	Prominent	Smooth	Smooth
Form of sutures	Simple	Complex	Simple

Table 4.   Summary of standard methods used in handedness estimation (2, 3, 36, 37)

	Method of Assignment	
Feature	**Dexterous**	**Sinistrous**
Maximum length of clavicle	Right smaller	Left smaller
Attachment area of the costo-clavicular ligament	Right greater	Left greater
Maximum biepicondilar distance of the humerus	Right greater	Left greater
Width of the intertubercular groove of the humerus	Right greater	Left greater
Diameter of the nutrient foramen of the humerus	Right greater	Left greater
Sum of the lengths of the humerus and radius	Right greater	Left greater
Distance from dorsal tubercle to the styloid process of the radius	Right greater	Left greater
Area of the insertion site of the biceps (radius)	Right greater	Left greater

Table 5. **Summary of standard methods used in stature estimation (38, 39)—see Schwartz (4), p. 146.**

	Method of Assignment: Regression Equation (stature = maximum length ×)			
	Black		White	
Skeletal Element*	**Male**	**Female**	**Male**	**Female**
Femur	× 2.11 + 70.35 ± 3.94	× 2.28 + 59.76 ± 3.41	× 2.38 + 61.41 ± 3.27	× 2.47 + 54.10 ± 3.72
Humerus	× 3.26 + 62.10 ± 4.43	× 3.08 + 64.67 ± 4.25	× 3.08 + 71.78 ± 3.37	× 3.36 + 57.97 ± 4.45
Radius	× 3.42 + 81.56 ± 4.30	× 3.67 + 71.79 ± 4.59	× 3.78 + 79.01 ± 4.32	× 4.74 + 54.93 ± 4.24

*elements listed in order of preference

Table 6.   Age estimation: frequency with which each feature was present and the method could be applied

Feature	Exumed Sample (*n*=90)			Archive Sample (*n*=30)			Total Sample (*n*=120)		
	Present	**Applied**	**%**	**Present**	**Applied**	**%**	**Present**	**Applied**	**%**
Medial clavicle epiphysis	76	53	58.9	24	21	70.0	100	74	61.7
Pubic symphysis surface	51	21	23.3	29	26	86.7	80	47	39.2
Auricular surface of the ilium	68	55	61.1	29	27	90.0	97	82	68.3
Fourth sternal rib end	79	9	10.0	26	20	66.7	105	29	24.2
Cranial sutures	80	33	36.7	25	16	53.3	105	49	40.8
Developmental changes in the vertebrae	80	69	76.7	28	21	70.0	108	90	75.0

Table 7a.   **Sex estimation from the pelvis: frequency with each feature was present and the method could be applied**

Feature	Exhumed Sample (*n*=90)			Archive Sample (*n*=30)			Total (*n*=120)		
	Present	Applied	%	Present	Applied	%	Present	Applied	%
Subpubic angle	47	39	43.3	29	26	86.7	76	65	54.2
Ventral arc	45	35	38.9	29	28	93.3	74	63	52.5
Medial ischio-pubic ridge	45	38	42.2	29	28	93.3	74	66	55.0
Greater sciatic notch	81	80	88.9	29	28	93.3	110	108	90.0
Sacral alae	66	59	65.6	28	24	80.0	94	83	69.2
Curvature of sacrum	70	59	65.6	28	26	86.7	98	85	70.8
Sacral auricular surface	67	61	67.8	28	26	86.7	95	87	72.5
Projection of auricular surface	65	62	68.9	29	26	86.7	94	88	73.3
Preauricular sulcus	65	63	70.0	29	25	83.3	94	88	73.3

Table 7b. **Sex estimation from the skull and jaw: frequency with each feature was present and the method could be applied**

Feature	Exhumed Sample (n=90)			Archive Sample (n=30)			Total (n=120)		
	Present	Applied	%	Present	Applied	%	Present	Applied	%
Supraorbital ridge	76	73	81.1	24	20	66.7	100	93	77.5
Occiptal protuberance	79	73	81.1	24	21	70.0	103	94	78.3
Mastoid process	80	76	84.4	24	21	70.0	104	97	80.8
Supramastoid crest	75	61	67.8	24	22	73.3	99	83	69.2
Zygomatic arch	69	53	58.9	23	20	66.7	92	73	60.8
Mentum	77	77	85.6	24	22	73.3	101	99	82.5
Mandibular angle	76	76	84.4	24	19	63.3	100	95	79.2

Table 8.   Ancestry estimation: frequency with which each feature was present and the method could be applied

Feature	Exhumed Sample (*n*=90)			Archive Sample (*n*=30)			Total (*n*=120)		
	Present	Applied	%	Present	Applied	%	Present	Applied	%
Cranium (length)	58	58	64.4	23	23	76.7	81	81	67.5
Cranium (shape)	79	79	87.8	23	23	76.7	102	102	85.0
Nasal bridge	46	46	51.1	22	22	73.3	68	68	56.7
Nasal aperture (height)	46	46	51.1	20	20	66.7	66	66	55.0
Nasal aperture (width)	42	42	46.7	21	21	70.0	63	63	52.5
Nasal spine	16	16	17.8	14	14	46.7	30	30	25.0
Nasal base	42	42	46.7	21	21	70.0	63	63	52.5
Facial profile (lateral)	50	50	55.6	23	23	76.7	73	73	60.8
Facial profile (anterior)	42	42	46.7	22	22	73.3	64	64	53.3
Orbits	53	53	58.9	21	21	70.0	74	74	61.7
Orbits (inferior margin)	50	50	55.6	22	22	73.3	72	72	60.0

Orbits (superior margin)	56	56	62.2	23	23	76.7	79	79	65.8
Muscle attachments	34	34	37.8	22	22	73.3	56	56	46.7
Form of sutures	55	55	61.1	24	24	80.0	79	79	65.8

Table 9.   Handedness estimation: frequency with each feature was present and the method could be applied

Feature	Exhumed Sample (*n*=90)			Archive Sample (*n*=30)			Total (*n*=120)		
	Present	Applied	%	Present	Applied	%	Present	Applied	%
Maximum length of clavicle	76	30	33.3	23	19	63.3	99	49	40.8
Attachment area of the costo-clavicular ligament	76	41	45.6	23	18	60.0	99	59	49.2
Maximum biepicondilar distance of the humerus	82	36	40.0	24	20	66.7	106	56	46.7
Width of the intertubercular groove of the humerus	84	45	50.0	24	19	63.3	108	64	53.3
Diameter of the nutrient foramen of the humerus	84	62	68.9	24	17	56.7	108	79	65.8
Sum of the lengths of the humerus and radius	81	34	37.8	24	12	40.0	105	46	38.3
Distance from dorsal tubercle to the styloid process of the radius	83	44	48.9	24	12	40.0	107	56	46.7
Area of the insertion site of the biceps (radius)	83	57	63.3	24	19	63.3	107	76	63.3

Table 10. **Stature estimation: frequency with each feature was present and the method could be applied**

Feature*	Exhumed Sample (*n*=90)			Archive Sample (*n*=30)			Total (*n*=120)		
	Present	Applied	%	Present	Applied	%	Present	Applied	%
Calculation from length of femur	89	83	92.2	25	25	83.3	114	108	90.0
Calculation from length of humerus	84	49	54.4	24	21	70.0	108	70	58.3
Calculation from length of radius	83	49	54.4	24	21	70.0	107	70	58.3

*elements listed in order of preference (see Discussion)

Table 11.   Biographical parameters: frequency with which assignment is compatible with the known value

Parameter	Exhumed Sample (*n*=90)									Archive Sample (*n*=30)									Total (*n*=120)								
	Agree		Disagree		Not Det.					Agree		Disagree		Not Det.					Agree		Disagree		Not Det.				
	n	%	*n*	%	*n*	%				*n*	%	*n*	%	*n*	%				*n*	%	*n*	%	*n*	%			
Age	18	20.0	32	35.6	40	44.4				28	93.3	1	3.3	1	3.3				46	38.3	33	27.5	41	34.2			
Sex	76	84.4	5	5.6	9	10.0				29	96.7	0	0.0	1	3.3				105	87.5	5	4.2	10	8.3			
Ancestry†	21	23.3								22	73.3								43	35.8							
Ancestry‡	30	33.3								1	3.3								31	25.8							
Ancestry	51	56.7	4	4.4	35	38.9				23	76.7	0	0.0	7	23.3				74	61.7	4	3.3	42	35.0			
Handedness	-	-	-	-	-	-				14	46.7	4	13.3	12	40.0				-	-	-	-	-	-			
Stature†	-	-	-	-	-	-				12	40.0	10	33.3	8	26.7				-	-	-	-	-	-			
Stature‡	-	-	-	-	-	-				5	16.7								-	-	-	-	-	-			
Stature	-	-	-	-	-	-				17	56.7	5	16.7	8	26.7				-	-	-	-	-	-			

†   Exact agreement   ‡   Partial agreement (see Discussion)

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Figure S1.   Flow Chart illustrating the protocol followed in completing the LAF/CEMEL forensic anthropology examination report
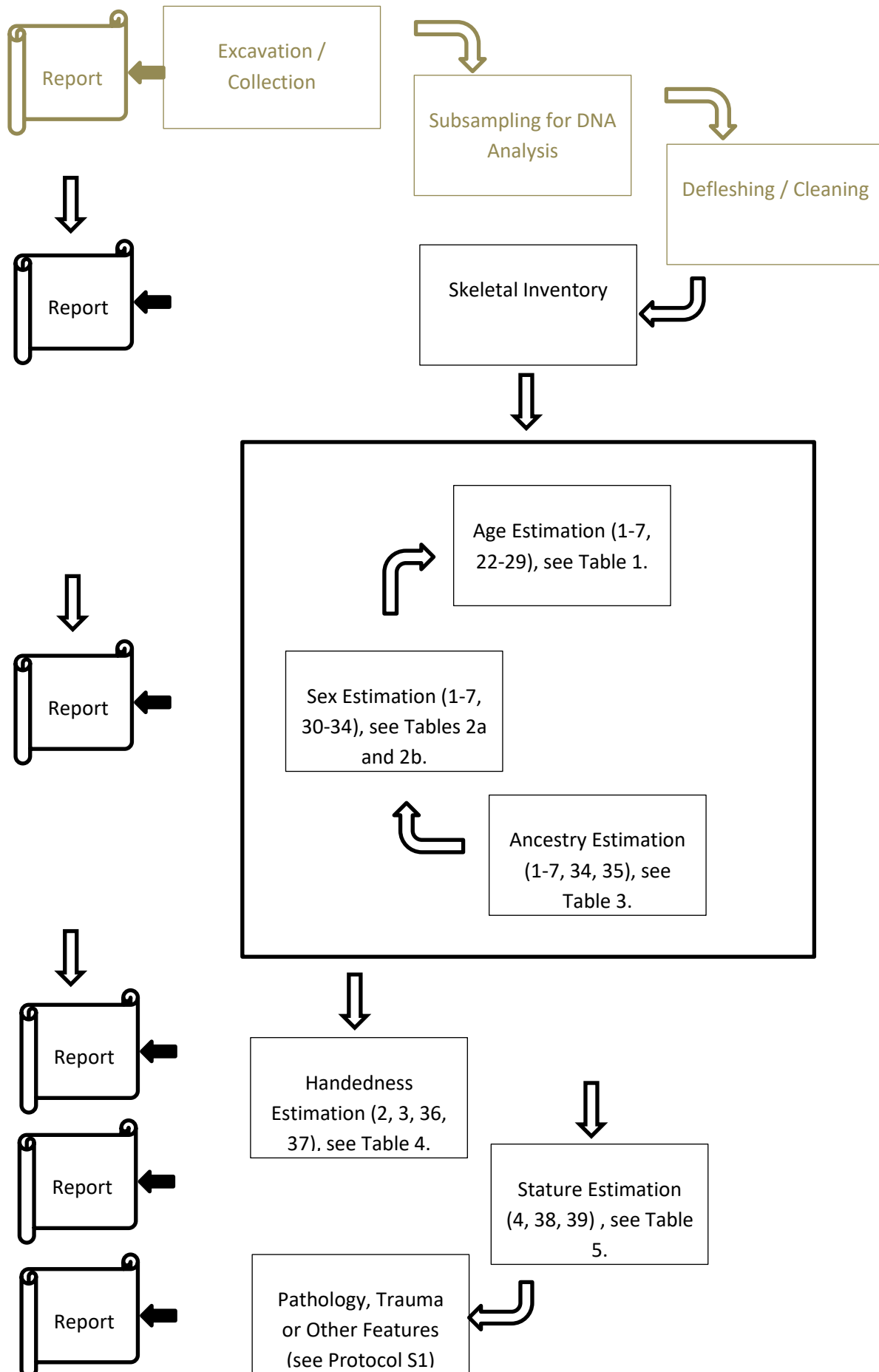
Figure S2.   Chart of Period of Burial versus Age at Death
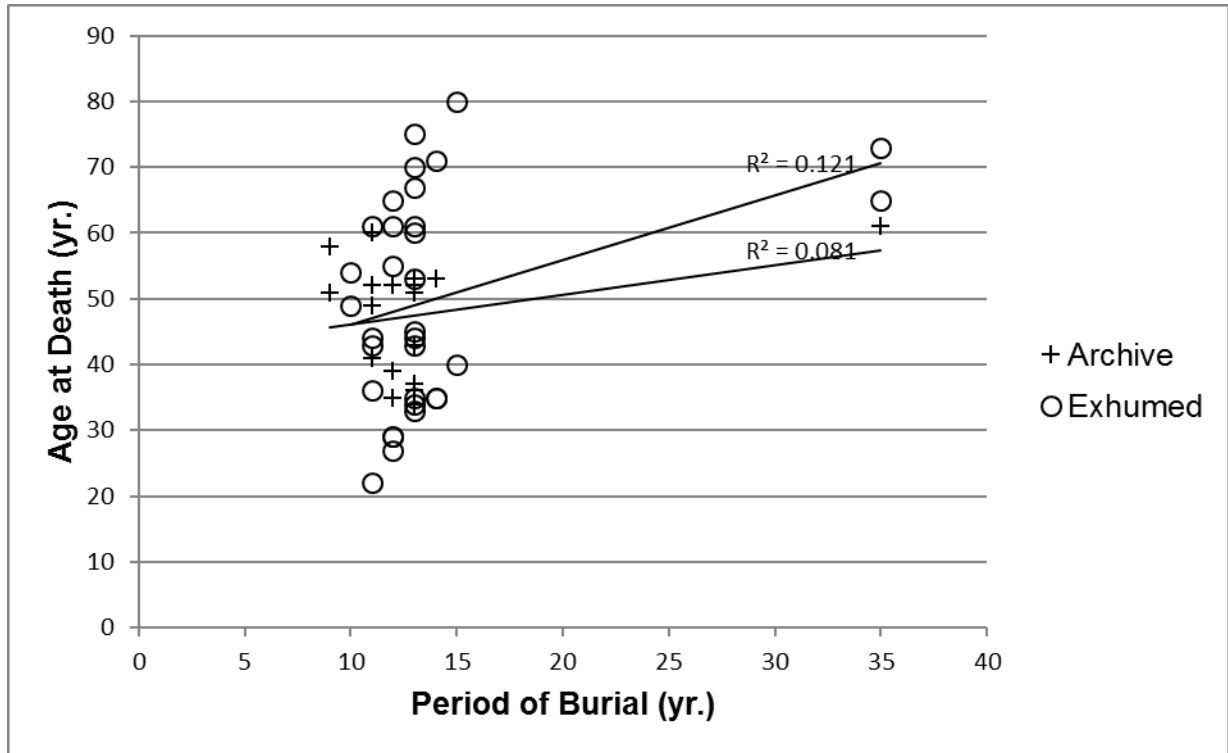
Table S1.   Known attributes of the exhumed sample (*n*=90)

Specimen	Period of Burial (yr.)	Age at Death (yr.)	Sex	Ancestry
001	13	36	Male	Unknown
002	13	34	Male	Unknown
003	13	67	Male	Mixed†
004	13	33	Male	Mixed†
005	11	75	Male	White
006	11	49	Male	Mixed†
007	11	27	Female	White
008	10	60	Male	Unknown
009	10	49	Male	Unknown
010	10	Unknown	Male	Mixed†
011	9	51	Male	Unknown
012	12	39	Female	White
013	11	43	Male	Unknown
014	11	60	Male	African
015	11	61	Female	White
016	11	52	Male	Mixed†
017	11	22	Male	White
018	10	54	Male	African

019	9	51	Female	White
020	9	58	Male	White
021	35	65	Male	African
022	35	73	Male	White
023	35	50	Male	White
024	35	61	Female	African
025	27	10	Male	Mixed†
026	27	51	Male	White
027	14	Unknown	Male	White
028	15	Unknown	Male	White
029	14	41	Male	White
030	13	43	Male	Mixed†
031	15	Unknown	Male	African
032	13	53	Male	African
033	12	Unknown	Male	White
034	14	Unknown	Male	White
035	13	86	Female	White
036	13	51	Male	African
037	13	61	Male	Unknown
038	14	Unknown	Male	Mixed†
039	13	Unknown	Male	Unknown

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040	14	Unknown	Male	African
041	14	53	Male	Mixed†
042	14	35	Male	White
043	13	52	Female	Mixed†
044	14	Unknown	Male	Mixed†
045	15	80	Male	Mixed†
046	14	55	Male	Mixed†
047	13	70	Male	Unknown
048	15	85	Male	White
049	14	Unknown	Male	White
050	13	45	Male	Unknown
051	14	35	Male	Mixed†
052	14	Unknown	Male	African
053	11	41	Male	White
054	11	Unknown	Male	Unknown
055	12	Unknown	Female	Unknown
056	12	27	Female	Unknown
057	12	26	Unknown	Unknown
058	12	61	Male	White
059	12	53	Male	Unknown
060	12	65	Male	White

061	14	71	Male	White
062	12	52	Male	White
063	12	29	Male	Unknown
064	12	Unknown	Male	White
065	11	36	Male	African
066	12	73	Female	Mixed†
067	15	40	Male	African
068	13	Unknown	Male	African
069	11	Unknown	Male	White
070	12	35	Male	African
071	12	55	Male	White
072	13	60	Female	White
073	13	37	Male	African
074	12	29	Male	White
075	11	44	Male	Mixed†
076	13	43	Male	Mixed†
077	Unknown	Unknown	Unknown	Unknown
078	13	Unknown	Male	Unknown
079	7	Unknown	Male	Unknown
080	13	44	Male	Unknown
081	13	53	Male	White

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082	13	34	Female	Mixed†
083	13	35	Male	Mixed†
084	13	70	Male	White
085	13	Unknown	Unknown	Unknown
086	13	35	Male	White
087	13	20	Male	Unknown
088	13	41	Male	Mixed†
089	13	44	Male	White
090	13	75	Female	African

Mixed†   Person of Mixed African and White ancestry

Table S2.   Known attributes of the archive sample (*n*=30)

Specimen	Age at Death (yr.)	Sex	Ancestry	Handedness	Stature (m.)
001	45	Male	Mixed†	Right	1.70-1.75
002	42	Male	White	Right	1.65
003	19	Male	White	Unknown	1.68
004	16	Male	White	Unknown	Unknown
005	14	Male	Mixed†	Unknown	1.55
006	42	Male	Mixed†	Left	1.94-1.96
007	54	Male	Unknown	Unknown	Unknown
008	34	Male	Unknown	Unknown	Unknown
009	25	Male	Unknown	Unknown	Unknown
010	39	Male	Mixed†	Unknown	Unknown
011	27	Male	White	Right	1.70
012	42	Male	White	Right	1.70
013	60	Male	White	Right	1.70
014	27	Female	Mixed†	Unknown	1.55
015	20	Male	White	Right	1.70
016	46	Male	Mixed†	Right	1.80

017	26	Male	White	Right	1.85-1.90
018	30	Male	Mixed†	Right	1.78-1.80
019	27	Male	Mixed†	Left	1.65-1.70
020	65	Male	Mixed†	Left	1.75-1.80
021	19	Female	White	Unknown	Unknown
022	54	Male	Unknown	Unknown	Unknown
023	39	Male	White	Left	1.70
024	20	Male	Mixed†	Right	1.90
025	51	Male	Mixed†	Right	1.80
026	23	Male	White	Right	Unknown
027	33	Male	Mixed†	Right	1.70
028	43	Male	Mixed†	Right	1.78
029	30	Male	White	Left	1.70
030	21	Male	White	Right	1.69

Mixed†   Person of Mixed African and White ancestry

Table S3. **Classifications of age, sex, ancestry, handedness and stature for the exhumed sample (n=90)**

Specimen	Age (yr.)	Sex	Ancestry	Handedness	Stature Range (m.)
001	>30	Indet.	Indet.	Indet.	1.50-1.66
002	38-62	Male	Mixed†	Right	1.65-1.77
003	41-60	Male	White	Right	1.69-1.78
004	40-57	Male	Mixed†	Right	1.70-1.83
005	Indet.	Male	Mixed†	Right	1.60-1.68
006	45-59	Male	White	Right	1.75-1.83
007	Indet.	Female	White	Right	1.54-1.62
008	Indet.	Male	White	Indet.	1.66-1.75
009	50-59	Male	Mixed†	Left	1.61-1.72
010	>60	Male	White	Indet.	1.59-1.68
011	41-59	Male	Mixed†	Right	1.69-1.81
012	31-41	Male	White	Right	1.42-1.50
013	25-38	Male	Mixed†	Left	1.69-1.82
014	>30	Male	African	Indet.	1.71-1.79
015	50-59	Female	Mixed†	Indet.	1.42-1.51
016	45-63	Male	White	Right	1.67-1.75
017	34-56	Male	Mixed†	Left	1.56-1.67

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018	38-53	Male	Mixed†	Right	1.65-1.77
019	Indet.	Female	Indet.	Indet.	Indet.
020	>30	Male	Mixed†	Indet.	1.57-1.68
021	43-60	Male	Mixed†	Left	1.61-1.72
022	43-62	Female	Mixed†	Right	1.37-1.46
023	Indet.	Male	Indet.	Indet.	1.56-1.67
024	36 -61	Male	Mixed†	Indet.	1.50-1.60
025	Indet.	Male	Indet.	Indet.	1.56-1.67
026	Indet.	Male	Indet.	Indet.	1.61-1.72
027	37-43	Male	White	Left	1.57-1.68
028	Indet.	Male	Indet.	Right	1.65-1.77
029	Indet.	Male	White	Indet.	1.71-1.79
030	30-34	Male	African	Right	1.57-1.66
031	Indet.	Indet.	Indet.	Indet.	Indet.
032	43 -61	Male	Mixed†	Left	1.65-1.77
033	Indet.	Male	Mixed†	Indet.	1.57-1.68
034	50-59	Male	Mixed†	Indet.	1.69-1.81
035	Indet.	Indet.	Indet.	Indet.	Indet.
036	45-62	Male	African	Left	1.71-1.80
037	42-56	Male	Mixed†	Indet.	1.60-1.71
038	27-34	Male	White	Indet.	1.58-1.66

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039	42-56	Male	Mixed†	Right	1.61-1.72
040	50-59	Male	White	Indet.	1.71-1.80
041	50-59	Male	Mixed†	Right	1.67-1.79
042	39-52	Male	Indet.	Right	1.62-1.73
043	49-66	Male	White	Right	1.64-1.72
044	34-56	Male	Mixed‡	Indet.	1.66-1.75
045	43-64	Male	Mixed‡	Indet.	1.69-1.82
046	Indet.	Indet.	Indet.	Indet.	Indet.
047	42-57	Male	Indet.	Indet.	1.59-1.70
048	Indet.	Male	Mixed†	Indet.	1.56-1.69
049	40-44	Indet.	Indet.	Indet.	Indet.
050	30-42	Male	Mixed†	Left	1.54-1.65
051	37-55	Male	White	Indet.	1.70-1.78
052	Indet.	Male	Mixed†	Indet.	1.62-1.73
053	41-57	Male	African	Right	1.60-1.68
054	Indet.	Male	African	Indet.	1.60-1.68
055	34-52	Female	Mixed†	Left	1.51-1.61
056	40-44	Female	Mixed†	Right	1.53-1.63
057	Indet.	Male	Mixed†	Right	1.57-1.68
058	30-34	Male	White	Amb.	1.69-1.77
059	Indet.	Male	Mixed†	Right	1.59-1.70

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060	45-63	Male	Mixed‡	Right	1.62-1.71
061	43-64	Male	White	Indet.	Indet.
062	45-61	Male	Mixed†	Left	1.65-1.76
063	32-44	Male	White	Right	1.60-1.69
064	Indet.	Male	White	Left	1.63-1.71
065	43-57	Male	Mixed†	Right	1.62-1.74
066	Indet.	Indet.	Indet.	Indet.	Indet.
067	35-39	Male	Mixed†	Right	1.61-1.73
068	15-32	Female	White	Right	1.49-1.57
069	40-56	Male	Mixed†	Right	1.63-1.74
070	31-47	Male	Mixed†	Right	1.70-1.82
071	38-54	Male	White	Right	1.63-1.72
072	42-58	Female	Mixed†	Right	1.51-1.61
073	34-39	Male	White	Right	1.72-1.80
074	39-56	Male	White	Left	1.61-1.69
075	30-42	Male	Mixed†	Right	1.73-1.85
076	42-58	Male	Mixed†	Right	1.68-1.80
077	Indet.	Indet.	Indet.	Indet.	Indet.
078	37-54	Male	Mixed†	Left	1.59-1.71
079	38-54	Male	Mixed†	Indet.	1.68-1.80
080	Indet.	Male	African	Indet.	1.63-1.72

081	37-50	Male	White	Indet.	1.67-1.75
082	29-39	Female	African	Right	1.56-1.64
083	39-58	Male	Mixed†	Indet.	1.64-1.76
084	Indet.	Male	Indet.	Indet.	1.61-1.72
085	50-59	Female	Indet.	Right	1.55-1.66
086	Indet.	Male	White	Indet.	1.67-1.75
087	Indet.	Male	Indet.	Indet.	1.71-1.80
088	Indet.	Male	Mixed†	Indet.	1.66-1.77
089	35-39	Male	Indet.	Indet.	1.58-1.69
090	39-64	Female	Mixed†	Indet.	1.54-1.64

Mixed†   Person of mixed African and White ancestry

Mixed‡   Person of mixed African, White and Oriental or Indigenous ancestry

Abbreviations: Amb.: Ambidextrous, Indet.: Indeterminate

Table S4. **Classifications of age, sex, ancestry, handedness and stature for the archive sample (*n*=30)**

Specimen	Age (yr.)	Sex	Ancestry	Handedness	Stature Range (m.)
001	35-45	Male	Mixed†	Right	1.60-1.71
002	35-55	Male	White	Right	1.64-1.73
003	17-23	Male	White	Right	1.66-1.74
004	11-17	Male	White	Right	1.75-1.83
005	<15	Male	Mixed†	Right	1.56-1.67
006	37-45	Male	Indet.	Indet.	1.76-1.89
007	42-57	Male	Indet.	Indet.	Indet.
008	33-44	Male	Indet.	Indet.	Indet.
009	21-29	Male	Indet.	Indet.	Indet.
010	31-48	Male	Mixed†	Indet.	1.71-1.79
011	19-28	Male	White	Right	1.67-1.76
012	40-44	Male	Indet.	Left	1.72-1.81
013	40-58	Male	White	Right	1.72-1.81
014	25-37	Female	Mixed†	Indet.	1.58-1.68
015	17-23	Male	White	Right	1.63-1.72
016	34-50	Male	Mixed†	Left	1.69-1.82
017	19-26	Male	White	Right	1.85-1.94

018	27-39	Male	Mixed†	Right	1.69-1.80
019	24-32	Male	Mixed†	Left	1.66-1.78
020	38-57	Male	Mixed†	Left	1.68-1.80
021	Indet.	Indet.	Indet.	Indet.	Indet.
022	33-50	Male	Indet.	Indet.	Indet.
023	35-47	Male	Mixed†	Amb.	1.63-1.75
024	18-22	Male	Mixed†	Right	1.75-1.84
025	35-51	Male	Mixed	Right	1.69-1.81
026	20-26	Male	White	Right	1.69-1.78
027	32-46	Male	Mixed†	Right	1.63-1.74
028	33-44	Male	Mixed†	Right	1.65-1.77
029	24-30	Male	White	Indet.	1.64-1.72
030	17-21	Male	White	Left	1.64-1.73

Mixed†   Person of mixed African and White ancestry

Abbreviations   Amb.: Amidextrous, Indet.: Indeterminate

Universidade de São Paulo
Faculdade de Medicina de Ribeirão Preto
Departamento de Patologia e Medicina Legal
Centro de Medicina Legal - CEMEL

Laboratório de Antropologia Forense
Forensic Anthropology Laboratory

Relatório de Exame de Antropologia Forense
Forensic Anthropology Examination Report

LAF R –

Data do exame:
Date of examination

A   Geral
General

A.1   Descrição geral dos restos e estado de preservação
General description of the remains and state of preservation

A.2   Resumo e conclusões
Summary and conclusions

Perfil Bioantropológico:

Responsáveis pelo Relatório

Profa. Ms. Raffaela Arrabaça Francisco
Biomédica, CRBM 9214
Doutoranda em Antropologia Forense

Prof. Dr. Marco Aurélio Guimarães
Médico, CRM 78.828
Diretor Técnico do CEMEL/FMRP-USP

Ribeirão Preto, XX de agosto de XXXX.

B   Características de identificação e achados patológicos do esqueleto
Identifying features and pathological findings of the skeleton

B.1a   Inventário do esqueleto
Inventory of skeleton

Elemento	Element	Número / *Number*	Comentário / *Comment*
Crânio	Cranium		
Frontal	Frontal		
Occipital	Occipital		
Esfenóide	Sphenoid		
Maxilar	Maxilla		
Palatino	Palatine		
Vômer	Vomer		
Parietal esquerdo	Parietal Left		
Temporal esquerdo	Temporal Left		
Concha nasal inferior esquerda	Inferior Nasal Concha Left		
Etmóide esquerdo	Ethmoid Left		
Lacrimal esquerdo	Lacrimal Left		
Nasal esquerdo	Nasal Left		
Zigomático esquerdo	Zygomatic Left		
Parietal direito	Parietal Right		
Temporal direito	Temporal Right		
Concha nasal inferior direita	Inferior Nasal Concha Right		
Etmóide direito	Ethmoid Right		
Lacrimal direito	Lacrimal Right		
Nasal direito	Nasal Right		
Zigomático direito	Zygomatic Right		
Hióide	Hyoid		
Cartilagem da tireóide	Thyroid cartilage		
Mandíbula	Mandible		

Elemento	Element	Número / Number	Comentário / Comment
Axial	***Axial***		
Manúbrio	*Manubrium*		
Mesosterno	*Mesosternum*		
C1 Atlas	*C1 Atlas*		
C2 Axis	*C2 Axis*		
C3-7	*C3-7*		
T1-12	*T1-12*		
L1-5	*L1-5*		
Sacro	*Sacrum*		
Cóccix	*Coccyx*		
Costelas e Pelve	***Ribs and Pelvis***		
Costela 1 esquerda	*Rib 1 Left*		
Costela 2-12 esquerdas	*Ribs 2-12 Left*		
Costela 1 direita	*Ribs 1 Right*		
Costela 2-12 direitas	*Ribs 2-12 Right*		
Pelve esquerda	*Pelvis Left*		
Pelve direita	*Pelvis Right*		

Elemento	Element	Número / Number	Comentário / Comment
Apêndice superior esquerdo	***Left superior appendicular***		
Clavícula esquerda	*Clavicle Left*		
Escápula esquerda	*Scapula Left*		
Úmero esquerdo	*Humerus Left*		
Rádio esquerdo	*Radius Left*		
Ulna esquerda	*Ulna Left*		
Escafóide esquerdo	*Scaphoid Left*		
Semi-lunar esquerdo	*Lunate Left*		
Piramidal esquerdo	*Triquetral Left*		
Pisiforme esquerdo	*Pisiform Left*		
Trapézio esquerdo	*Trapezium Left*		
Trapezóide esquerdo	*Trapezoid Left*		
Capitato esquerdo	*Capitate Left*		
Hamato esquerdo	*Hamate Left*		
Metacarpo 1 esquerdo	*Metacarpal 1 Left*		
Metacarpo 2 esquerdo	*Metacarpal 2 Left*		
Metacarpo 3 esquerdo	*Metacarpal 3 Left*		
Metacarpo 4 esquerdo	*Metacarpal 4 Left*		
Metacarpo 5 esquerdo	*Metacarpal 5 Left*		
Falange proximal 1 esquerda	*Proximal Phalanx 1 Left*		
Falange proximal 2-5 esquerdas	*Proximal Phalanx 2-5 Left*		
Falange intermediária 2-5 esquerdas	*Intermediate Phalanx 2-5 Left*		
Falange distal 1 esquerda	*Distal Phalanx 1 Left*		
Falange distal 2-5 esquerdas	*Distal Phalanx 2-5 Left*		

Elemento	Element	Número / Number	Comentário / Comment
Apêndice inferior esquerdo	*Left inferior appendicular*		
Fêmur esquerdo	*Femur Left*		
Patela esquerda	*Patella Left*		
Tíbia esquerda	*Tibia Left*		
Fíbula esquerda	*Fibula Left*		
Talus esquerdo	*Talus Left*		
Calcâneo esquerdo	*Calcaneus Left*		
Cubóide esquerdo	*Cuboid Left*		
Navicular esquerdo	*Navicular Left*		
Cuneiforme medial esquerdo	*Medial Cuneiform Left*		
Cuneiforme intermediário esquerdo	*Intermediate Cuneiform Left*		
Cuneiforme lateral esquerdo	*Lateral Cuneiform Left*		
Metatarso 1 esquerdo	*Metatarsal 1 Left*		
Metatarso 2 esquerdo	*Metatarsal 2 Left*		
Metatarso 3 esquerdo	*Metatarsal 3 Left*		
Metatarso 4 esquerdo	*Metatarsal 4 Left*		
Metatarso 5 esquerdo	*Metatarsal 5 Left*		
Falange proximal 1 esquerda	*Proximal Phalanx 1 Left*		
Falange proximal 2-5 esquerdas	*Proximal Phalanx 2-5 Left*		
Falange intermediária2-5 esquerdas	*Intermediate Phalanx 2-5 Left*		
Falange distal 1 esquerda	*Distal Phalanx 1 Left*		
Falange distal 2-5 esquerdas	*Distal Phalanx 2-5 Left*		

Elemento	Element	Número / Number	Comentário / Comment
Apêndice superior direito	*Rigth superior appendicular*		
Clavícula direita	Clavicle Right		
Escápula direita	Scapula Right		
Úmero direito	Humerus Right		
Radio direito	Radius Right		
Ulna direita	Ulna Right		
Escafóide direito	Scaphoid Right		
Semi-lunar direito	Lunate Right		
Piramidal direito	Triquetral Right		
Pisiforme direito	Pisiform Right		
Trapézio direito	Trapezium Right		
Trapezóide direito	Trapezoid Right		
Capitato direito	Capitate Right		
Hamato direito	Hamate Right		
Metacarpo 1 direito	Metacarpal 1 Right		
Metacarpo 2 direito	Metacarpal 2 Right		
Metacarpo 3 direito	Metacarpal 3 Right		
Metacarpo 4 direito	Metacarpal 4 Right		
Metacarpo 5 direito	Metacarpal 5 Right		
Falange proximal 1 direita	Proximal Phalanx 1 Right		
Falange proximal 2-5 direitas	Proximal Phalanx 2-5 Right		
Falange intermediária 2-5 direitas	Intermediate Phalanx 2-5 Right		
Falange distal 1 direita	Distal Phalanx 1 Right		
Falange distal 2-5 direitas	Distal Phalanx 2-5 Right		

Elemento	Element	Número / Number	Comentário / Comment
Apêndice inferior direito	**Rigth inferior appendicular**		
Fêmur direito	Femur Right		
Patela direita	Patella Right		
Tíbia direita	Tibia Right		
Fíbula direita	Fibula Right		
Talus direito	Talus Right		
Calcâneo direito	Calcaneus Right		
Cubóide direito	Cuboid Right		
Navicular direito	Navicular Right		
Cuneiforme medial direito	Medial Cuneiform Right		
Cuneiforme intermediário direito	Intermediate Cuneiform Right		
Cuneiforme lateral direito	Lateral Cuneiform Right		
Metatarso 1 direito	Metatarsal 1 Right		
Metatarso 2 direito	Metatarsal 2 Right		
Metatarso 3 direito	Metatarsal 3 Right		
Metatarso 4 direito	Metatarsal 4 Right		
Metatarso 5 direito	Metatarsal 5 Right		
Falange proximal 1 direita	Proximal Phalanx 1 Right		
Falange proximal 2-5 direitas	Proximal Phalanx 2-5 Right		
Falange intermediária 2-5 direitas	Intermediate Phalanx 2-5 Right		
Falange distal 1 direita	Distal Phalanx 1 Right		
Falange distal 2-5 direitas	Distal Phalanx 2-5 Right		

B.1b Fotografias dos restos do esqueleto
Photographs of the skeletal remains

B.1c Fotografia do crânio
Photographs of skul

B.1d Fotografia da pelve
Photographs of pelvis

B. 2 Resumo do determinação do sexo
Sexing estimation summary

B.2a Determinação do sexo pela pelve[1-7, 30-33]
Sexing of pélvis

Característica	Feature	Sexo / Sex	Comentário / Comment
Tamanho do ângulo sub-púbico	Size of subpubic angle		
Presença do arco ventral	Presence of ventral arc		
Presença da crista medial isquio-púbica	Presence of medial ischio-pubic ridge		
Tamanho do sulco isquiático maior	Size of greater sciatic notch		
Espessura da asa do sacro	Width of sacral alae		
Curvatura do sacro	Curvature of sacrum		
Tamanho da superfície auricular sacral	Extent of sacral auricular surface		
Projeção da superfície auricular	Projection of auricular surface		
Presença do sulco pré-auricular	Presence of preauricular sulcus		

B.3a Determinação do sexo pelo crânio[1-7, 32-34]
Sexing of skull

Característica	Feature	Sexo / Sex	Comentário / Comment
Forma da glabela / pontes supra-orbitais	Shape of glabella / supraorbital ridges		
Presença da protuberância occipital	Presence of occipital protruberance		
Tamanho do processo mastóide	Size of mastoid processes		
Presença da crista supramastoide	Presence of supramastoid crest		
Altura / robustez do zigomático	Height / robusticity of zygomatic		
Tamanho e forma do mento	Size and shape of mentum		
Abertura do ângulo mandibular	Flaring of mandibular angle		

B.4a Ancestralidade[1-7, 34, 35]
Ancestry

Característica	Feature	Ancestralidade / Ancestry*	Comentário / Comment
Comprimento / largura da face	Length / width of face		
Forma da abóbada craniana	Shape of cranial vault		
Inclinação / forma das órbitas	Slope / shape of orbits		
Proeminência dos ossos da bochecha	Prominence of cheek bones		
Espinha nasal	Nasal spine		
Abertura nasal	Nasal opening		
Margem nasal	Nasal margin		
Forma dos incisivos / molares	Shape of incisors / molars		

*Arfricano(a), African; Branco(a), White; Oriental; Indigeno(a), Indigenous; Mistura, Mixed

B.5a Idade em adultos[1-7, 22-29]
Age in adults

Característica	*Feature*	Estado de fusão **State of fusion**	Comentário **Comment**
Epífise medial da clavícula[22]	*Medial clavicle epiphysis*		

Característica	*Feature*	Citada em **Cited in**	Fase E **Phase L**	Faixa E **Range L**	Fase D **Phase R**	Faixa D **Range R**	Comentário **Comment**
Sínfise púbica[23-25]	*Pubic symphysis*						
Auricular do Ilíaco[26]	*Auricular ilium*						
Final (esternal) da 4ª costela[27]	*4th sternal rib end*						

Característica	*Feature*	Estado **State**	Comentário **Comment**
Suturas cranianas[28]	*Cranial sutures*		
Mudanças artríticas nas vértebras[29]	*Arthritic changes in vertebrae*		

B.6 Estatura
Stature

B.6a Estimativa de estatura para o provável sexo e ancestralidade
Stature estimation for probable sex and ancestry

Elemento do esqueleto:	*Skeletal element:*	Fêmur / *Femur* (Se ausente usar / *If absent use:*)
Lado:	*Side:*	Direito / *Right* (Se ausente usar / *If absent use:*)
Comprimento:	*Length:*	
Fórmula:	*Formula:*	
Citado em:	*Cited in:* (4, 38, 39)	
Faixa:	*Range:*	

B.6b Resumo da estimativa de estatura
Stature estimation summary

B.7   Destreza Manual
Handedness

B.7a   Estimativa da destreza manual[2, 3, 36, 37]
Handedness estimation

Elemento	*Element*	Característica pessoal	*Trait*	Mão- / *Handedness*
Clavícula	*Clavicle*	Comprimento máximo (**menor** no lado dominante)	*Max length (**shorter** on dominant side)*	
Clavícula	*Clavicle*	Área de ligação do ligamento costoclavicular	*Area of costoclavicular ligament attachment*	
Úmero	*Humerus*	Máxima distância biepicondilar	*Max biepicondylar breadth*	
Úmero	*Humerus*	Largura do sulco intertubercular	*Breadth of inter-tubercular grove*	
Úmero	*Humerus*	Diâmetro do forame nutriente	*Diameter of nutrient foramen*	
Úmero + rádio	*Humerus + radius*	Comprimentos máximos somados	*Summed maximum lengths*	
Rádio	*Radius*	Distância do tubérculo dorsal ao processo estilóide	*Breath from dorsal tubercle to styloid process*	
Rádio	*Radius*	Área de ligação do bíceps	*Area of biceps attachment*	

C   Identificação de características e achados patológicos na dentição
Identifying features and pathological findings of the dentition

Se não estiver presente, declare 'NADA'.
If none, state NONE.

C.1   Inventário da dentição

Dente	*Tooth*	UNS		Número / *Number*	Comentário / *Comment*
Superior direito	*Upper Right*				
Incisivo central	*Central incisor*	1	11		
Incisivo lateral	*Lateral incisor*	2	12		
Canino	*Canine*	3	13		
1º pré-molar	*1st premolar*	4	14		
2º pré-molar	*2nd premolar*	5	15		
1º molar	*1st molar*	6	16		
2º molar	*2nd molar*	7	17		
3º molar	*3rd molar*	8	18		
Superior esquerdo	*Upper Left*				
Incisivo central	*Central incisor*	9	21		
Incisivo lateral	*Lateral incisor*	10	22		
Canino	*Canine*	11	23		
1º pré-molar	*1st premolar*	12	24		
2º pré-molar	*2nd premolar*	13	25		
1º molar	*1st molar*	14	26		
2º molar	*2nd molar*	15	27		
3º molar	*3rd molar*	16	28		

Dente	*Tooth*	UNS		Número / *Number*	Comentário / *Comment*
Inferior esquerdo	*Lower Left*				
3º molar	*3rd molar*	17	38		
2º molar	*2nd molar*	18	37		
1º molar	*1st molar*	19	36		
2º pré-molar	*2nd premolar*	20	35		
1º pré-molar	*1st premolar*	21	34		
Canino	*Canine*	22	33		
Incisivo lateral	*Lateral incisor*	23	32		
Incisivo central	*Central incisor*	24	31		
Inferior direito	*Lower Right*				
Incisivo central	*Central incisor*	25	41		
Incisivo lateral	*Lateral incisor*	26	42		
Canino	*Canine*	27	43		
1º pré-molar	*1st premolar*	28	44		
2º pré-molar	*2nd premolar*	29	45		
1º molar	*1st molar*	30	46		
2º molar	*2nd molar*	31	47		
3º molar	*3rd molar*	32	48		

C.2a   Fotografia dental (superior)
Dental photograph (upper)

C.2b   Fotografia dental (inferior)
Dental photograph (lower)

As referências citadas são as dadas em Francisco et al. (2017).
The references cited are those given in Francisco et al. (2017).

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