

SAMPLE-SPECIFIC SEX ESTIMATION IN ARCHAEOLOGICAL CONTEXTS WITH COMMINGLED HUMAN REMAINS: A CASE STUDY FROM THE MIDDLE NEOLITHIC CAVE OF BOM SANTO IN PORTUGAL

David Gonçalves<sup>1-3\*</sup>, Raquel Granja<sup>4</sup>, Francisca Alves Cardoso<sup>5</sup>, António Faustino de Carvalho<sup>4,6</sup>

<sup>1</sup>Research Centre for Anthropology and Health (CIAS), Universidade de Coimbra, Rua do Arco da Traição, 3000-056 Coimbra, Portugal.

\*Corresponding author: [davidmiguelgoncalves@gmail.com](mailto:davidmiguelgoncalves@gmail.com)

<sup>2</sup>Forensic Sciences Centre (CENCIFOR), Largo da Sé Nova, 3000-213 Coimbra, Portugal.

<sup>3</sup>Laboratório de Arqueociências, Direcção Geral do Património Cultural and LARC/CIBIO/InBIO, Rua da Bica do Marquês 2, 1300-087 Lisboa, Portugal.

<sup>4</sup>Universidade do Algarve, FCHS, Campus de Gambelas, 8000-117 Faro, Portugal. E-mail: [ragranja@ualg.pt](mailto:ragranja@ualg.pt)

<sup>5</sup>CRIA – Centro em Rede de Investigação em Antropologia, Faculdade de Ciências Sociais e Humanas, Universidade Nova de Lisboa; Av. Berna, 26-C, 1069-061, Lisboa, Portugal. Email: [francicard@fcsh.unl.pt](mailto:francicard@fcsh.unl.pt)

<sup>6</sup>E-mail: [afcarva@ualg.pt](mailto:afcarva@ualg.pt)

ABSTRACT

Estimating sex on large assemblages of commingled skeletal human remains is challenging because it prevents the systemic observation of the skeleton and thus reduces the reliability of sex-ratio estimation. In order to tackle this problem, the applicability of sample-specific odontometric methods was assessed on the human skeletal remains from the Middle Neolithic cave necropolis of Bom Santo in Portugal. We present an approach that confirms some of the assumptions – the normal distribution of the data and the 1:1.5 sex ratio – indicated by Albanese et al. (2005) for the application of sample-specific methods. These assumptions are often difficult to assess in archaeological samples and thus prevent the use of sample-specific methods.

The mean bucco-lingual diameter of 51 lower right canine teeth was used as a cut-off point to discriminate between sexes within a sample from Bom Santo. Before that, Shapiro-Wilk statistics was used to confirm that the distribution of the data in a sample of 51 lower canine teeth was normal. In addition, the range and central tendency of the data were compared to other samples for which the sex of the individuals was known in order to confirm that those parameters were consistent with those of a sample presenting a balanced sex ratio. The canine sex estimations were then compared with the sex estimation obtained from mandibles where canine teeth were still *in situ* (n = 8). No clear disagreement between the two methods was found thus demonstrating good potential of this method for sex estimation and for the sex ratio estimation in commingled human skeletal remains. Results indicated that sex ratios in Room A and Room B at Bom Santo were quite different. This indicates that the two locations may have been used in a different way according to sex.

Keywords: biological anthropology; bioarchaeology; odontometry; sex ratio; biological profile.

## 1. INTRODUCTION

One contribution of biological anthropology to archaeology is the estimation of the biological profile of the individual humans by analysing their remains, generally their skeletons. This profiling is paramount in order to interpret certain socio-cultural aspects of these populations, for example linking specific burials to specific age groups or sex (Mays, 1998; Molleson, 2006; Milner et al., 2008). Even with the advent of archaeogenetics (e.g.: Haak et al., 2008), skeletal analysis is still the quickest, cheapest and most practical way of assessing some parameters of the biological profile, especially when the human assemblage comprises many individuals. However, the methods adopted by biological anthropology are seldom completely reliable (Milner et al., 2008), especially when the analysis focuses on incomplete skeletons or commingled assemblages. Examples include the determination of their sex, a parameter for which successful estimation depends on which bones are available for examination.

The pelvis is the most sexually dimorphic part of the human skeleton and therefore the best predictor of sex. In fact, some methods based on the pelvis, specifically on the hip bone, may be almost 100% accurate (Bruzek, 2002; Albanese, 2003; Murail et al., 2005). Unfortunately however, this bone is often poorly preserved thus impairing its examination. Other methods, such as the ones based on cranial morphology or measurements of post-cranial bones, are not as reliable either because sexual dimorphism is less pronounced or because those variables tend to be more population-specific. The application of a population-specific method across populations usually decreases its accuracy due to inter-population differences in shape, size and sexual dimorphism (Işcan et al., 1998; Albanese et al., 2005; Cardoso, 2008). Consequently, no adequate referential samples are available for comparison with archaeological remains. Those are some of the problems that need to be considered when attempting to sex a skeleton. The difficulty is even greater when the human remains are poorly preserved and/or commingled. In these cases, often the number of individuals allocated to a specific sex is much smaller than the minimum number of individuals of that very same context and therefore may not give a clear picture of the sex distribution. As a result, any inferences regarding sex-ratio and any subsequent discussion about sex-related correlations with funerary locations, specific mortuary practices or particular sets of artefacts may be unreliable.

Albanese et al. (2005) propose a sample-specific approach to help solve this problem. Briefly, they argue that the osteometric mean of certain skeletal standard measurements obtained from a set of unknown individuals, could be used to carry out sex estimation on that very same set. This approach was developed using the humeral joint measurements, and provided significant correct sex allocation of all individuals (88-100%). Cardoso (2008) further explored this approach by testing it on human teeth and concluded that odontometrics are also useful for the development of sample-specific univariate methods. His results indicate that the bucco-lingual diameter (BLD) of the permanent canine provides the best sex allocation (100% for the upper canine and 86% for the lower canine). Others also found a very significant sexual

dimorphism in the BLD of canines in samples of individuals of known-sex (Yamada and Sakai 1992; Işcan and Kedici, 2003; Cardoso 2008; Rai et al. 2008; Ruengdit et al. 2011; Zorba et al. 2011) and on an archaeological sample of individuals whose sex could be ascertained from their pelvis and long bones (Ditch and Rose, 1972). In contrast, Vodanovic et al. (2007) failed to do this in a sample of medieval Croatians of unknown sex. However, this may have been due to the fact that sex estimation had been carried out on cranial features rather than on the hip bone. In addition, Harris and Nweeia (1980) also did not find significant sexual dimorphism on a sample of individuals from the Amerindian Ticuna population in Colombia and suggested that indigenous South Americans may be characterized by reduced sexual dimorphism. Although no statistical testing was done by Ling and Wong (2007), sexual dimorphism was apparently small in their sample of canines from southern Chinese people. It therefore seems likely that sexual dimorphism of human canines may vary geographically and therefore this tooth may not be useful for sex estimation in certain populations.

The results of Cardoso (2008) study are encouraging and suggest that it may be relatively easy to sex commingled skeletons. First, his method avoids the use of metric references that are not specific to the context that is being examined. Second, his method is based on teeth which are often the most common and well preserved parts of the skeleton and thus usually provide the highest minimum number of individuals. Third, and taking into consideration the results from some authors like Garn et al., (1977) and Cardoso, (2008), it seems to allow for the quite successful sex estimation of subadult individuals because mineralization of the crown of the permanent canine is complete long before adulthood is attained (Smith, 1991). Unfortunately, sex estimations based on sample-specific univariate methods are usually less reliable than sex estimations based on the systemic examination of the skeleton. However, in the case of odontometrics, the use of several teeth and measurements did not improve the sex allocation estimates obtained on the canine tooth alone (Cardoso, 2008).

The aims of this article are to: 1) assess the applicability of the sample-specific method of Albanese et al. (2005) and Cardoso (2008) by using commingled human remains from the Middle Neolithic cave-necropolis of Bom Santo (Alenquer, Portugal); and 2) provide a more comprehensive portrait of the sex-ratio present in Bom Santo. The mean sex-pooled BLD of the lower canine obtained from this sample was used to allocate individuals according to sex. It is not possible to confirm sex estimation results by examining the pelvis of the respective individual when dealing with commingled remains, so the mandible – which does present some sexual dimorphism (Ferembach et al., 1980; Buikstra and Ubelaker, 1994) – was used as a measure of concordance. In addition, no specific burial practices or associated artefacts provided any corroborative information concerning the sex of the individuals. The results we present may contribute towards a discussion of the usefulness of the sample-specific method proposed by Albanese et al. (2005) and Cardoso (2008).

## 2. MATERIAL AND METHODS

The material used in this study was recovered from Bom Santo, a Middle Neolithic burial cave located on the eastern slope of the Montejunto Mountain, 350 meters a.s.l., some 50 km north of Lisbon, Portugal (Fig. 1). Discovered intact in 1993 during a speleological survey, it was

immediately recognized as an important archaeological site. Such a conclusion was based, among other things, on the vast cemetery complex it contains. The cave comprises several galleries and corridors subdivided into 11 distinct sectors, totalling 285 m<sup>2</sup>, in which a provisionally estimated minimum number of 121 individuals were present - both adults and sub-adults. They lie on the surface of the cave (Duarte 1998). Furthermore, the absence of multi-stratified archaeological deposits indicates a relatively brief occupation, between 3800 and 3400 cal BC, according to available radiocarbon determinations (Carvalho et al., 2012). This conclusion is also supported by a very homogeneous material culture, composed mostly of knapped flint blades and microliths, and polished axes and adzes made of metamorphic rocks. There are also some bone tools -mainly awls or points - and personal adornments such as beads, pendants and bracelets made of shell or stone. There are also four pottery vessels. Such a scant number of pots is also observed in other Middle Neolithic cemeteries in caves, hypogea, and megalithic tombs, which seems to indicate common funerary practices.

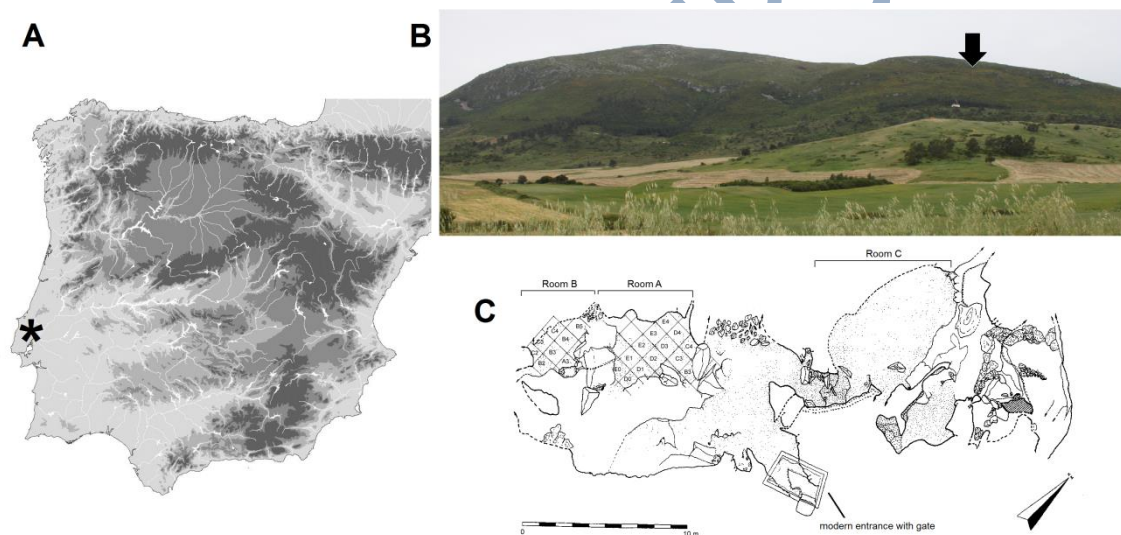


Figure 1. A - Location of Bom Santo Cave in the Iberian Peninsula (star); B - View of Montejunto Mountain, from the North-East, with approximate location of the cave (arrow); C - Topographic plan of Rooms A, B and C with their excavation units. [Two-column fitting image]

Four seasons of systematic survey and excavations were undertaken between 1994 and 2001, in so-called Rooms A and B (Fig. 1), under the direction of Cidália Duarte (in collaboration with J. Morais Arnaud in the 1994 season of excavations). Given the scientific and historical importance of this archaeological site and its contents, a research project is underway to assess and study the human remains and other material recovered during the excavations. The material is housed in the *Museu Nacional de Arqueologia* (Lisbon). Data on human biological profile, paleopathology as well as palaeoisotopes were analysed and are currently being published (see among others, Carvalho et al., 2012; Carvalho and Petchey, 2013; Carvalho, 2014).

The human bones uncovered at Bom Santo derived from both primary and secondary depositions (Carvalho et al., 2012). This pattern was consistent throughout the cave, although only Room B (Fig. 1) showed a clear distinction between both practices. Two skeletons displaying preserved anatomical connections, as well as cases of bone re-arrangements, particularly one composed of four skulls, were found in this room. A similar cranial arrangement, comprising six skulls and some other postcranial bones, was also found in Room A. However, although not completely excavated, no clear primary depositions were found in Room A. In this room, skeletal assemblages were possibly all derived from secondary depositions. Detailed description of the funerary anthropology and taphonomy are in Granja et al. (2014a; 2014b).

From the collection comprising 2039 teeth, a sample of 90 mandibular canines was selected for this study. Of these, 39 were from the left side (FDI 33 - the World Dental Federation notation identification of tooth will be used in this manuscript) and 51 were from the right side (FDI 43). Another 16 mandibular right canines were present but none was sufficiently well preserved to allow measurement of the BLD. This tooth was selected because it presented a sample large enough to statistically test tooth variation and its possible association with sexual dimorphism. In addition, the selection of a mandibular tooth allowed us to compare results of dental sex estimation with those of mandibular sex estimation. The canine mesio-distal diameter was not considered in this study because tooth wear may prevent measurements from being taken due to the obliteration of the required dental landmarks (Hillson et al., 2005) and this was true for several of the Bom Santo canines. Thus the sample size was considerably reduced. No other dental measurements were used because successful sex allocation was proven to be less reliable than the results based on canine measurements (Ditch and Rose, 1972; Işcan and Kedici, 2003; Cardoso, 2008). Furthermore, in the Bom Santo series, canines were numerically one of the better represented teeth.

Intra- and inter-observer errors associated with the measurement of BLD were tested on a sub-sample of 20 teeth by using both the absolute and the relative technical errors of measurement (TEM) (Ulijaszek and Lourie, 1994). As stated above, we selected FDI43 for the calculation of the sample-specific mean. Bilateral asymmetry between FDI33 and FDI43 was not statistically tested because the sample of paired left and right mandibular canines was too small ( $n = 5$ ).

In order to investigate the method proposed by Albanese et al. (2005), consideration was given to the three assumptions associated with their approach. The sample was composed of more than the required 40 individuals as the BLD was measurable in 51 FDI43 teeth; and the sample also included both sexes (Carvalho et al., 2012). The third assumption concerning the sex-ratio was more difficult to assess. The sex estimation based on measurements of postcranial bones revealed a wide variation of sex ratios depending on which bone is considered. This ranged from 1:1 (based on 8 tibiae) to 9:1 (based on 20 tali). The pelvis was not very informative as only two males and two females could be recognised (the sex of a fifth individual was undetermined). The third assumption was therefore investigated: by analyzing the distribution of the BLD values of the canines using the Shapiro-Wilk test to check for the normality; and by comparing the BLD descriptive statistics with data from other collections (Table 1) to confirm that they were consistent with samples presenting a somewhat balanced sex ratio.

After calculating the overall mean of the BLD, this value was used as a cut-off point (7.55 mm) to allocate the individuals of Bom Santo cave according to sex: teeth with BLD values larger than the cut-off point were classified as males; and teeth with BLD values smaller than the cut-off point were classified as females. Finally, the canine sex allocations were compared with the allocations based on the morphology of the mandibles (Ferembach et al., 1980), in cases where canines were still in situ. Mandibles with scores above +1 were allocated to males while mandibles with scores lower than -1 were attributed to females. All mandibles with scores between -1 and +1 were attributed to individuals of undetermined sex. Unfortunately, the comparison of the sex estimation from canines could only be done for 8 mandibles. The remaining mandibles were insufficiently preserved. Some of the comparisons were carried out with FDI33 teeth instead of FDI43 teeth. Since it was not possible to statistically test it, we had to assume that the sample-specific mean of the latter could be applied to the former.

### 3. RESULTS

The intra-observer error associated with the BLD was quite small. The technical error of measurement was 0.09 mm and the relative technical error of measurement was 1.21% with a coefficient of reliability of 0.97. Inter-observer error was also small (TEM = 0.14 mm). The relative technical error of measurement was 1.76% and the coefficient of reliability was 0.95 thus demonstrating good reliability of the BLD.

The BLD of FDI33 (median = 7.70; range = 0.61; mean = 7.62; sd = 0.44) was apparently similar to the BLD of FDI43 (median = 7.89; range = 0.36; mean = 7.55; sd = 0.59) and we therefore assumed that the mean of the latter could be applied to that of the former to estimate sex.

The variation of the canine BLD provided significant evidence indicating a balanced sex-ratio. The BLD had a normal distribution, as revealed by the Shapiro-Wilk statistics level of significance (S-W = 0.952, df = 51, p = 0.040), although only at the 0.01 level. The range of the diameter (6.30-8.67 mm; an outlier of 9.70 was also present in the sample) was quite similar to the one found by Cardoso (pers. comm.) in the Lisbon collection (6.42-9.15), as was also the overall mean and standard deviations of the samples used in several other studies – which, at the most, were just 0.18 mm and 0.05 mm, respectively, different from modern European samples (Table 1). The comparison found major differences with the results of Yamada and Sakai (1992) on a sample from a population in the Cook Islands and the one from Ling and Wong (2007) on a sample of southern Chinese people, which are actually also different from all other populations considered here. We therefore assumed that the third assumption regarding the sex ratio of the sample was also fulfilled. These parameters seem to be compatible with a balanced sex-ratio. For example, if the individuals were mostly males, they could eventually present a mean BLD similar to the sample from the Lisbon collection (Cardoso, 2008), but they would hardly present similar standard deviation and range while simultaneously having a normal distribution. Therefore, the pooling of several data seem to be helpful in detecting samples with a sex ratio compatible with the use of sample-specific methods.

The application of the mean of the Bom Santo collection as a sex-discriminating cut-off point identified 25 males (mean = 8.02 mm; sd = 0.42; median = 7.97 mm) and 26 females (mean = 7.09; sd = 0.30; median = 7.15 mm). This outcome indicated a sex ratio of 1:1. However, the adoption of a cut-off point may not be the safest approach since the chance of allocating individuals that are near the cut-off point to the wrong sex group is quite substantial. A more conservative procedure based on the implementation of an “undetermined sex” category may be more recommendable since, contrary to the former approach, it is better at avoiding the sex allocation of individuals close to the cut-off point – this is where the distribution curves of males and females usually overlap thus increasing the chance of error (Marini et al., 1999; Milner et al., 2008). Such a category was thus implemented by creating an interval based on the mean BLD. The upper and lower bounds of a 99% interval were built based on the standard error (0.08). Although a category for “undetermined sex” was used, the tendency (M?; F?) was nonetheless recorded. As a result, all individuals with BLD values between 7.34 mm and 7.76 mm: n = 13 (mean = 7.56 mm; sd = 0.15; median = 7.60 mm) were classified as undetermined. As for the remaining sample, we estimated the presence of 18 males (mean = 8.15 mm; SD = 0.43; median = 8.04 mm) and 20 females (mean = 6.99 mm; SD = 0.28; median = 7.07 mm). In terms of sex ratio (1.1:1), these results were only slightly different from the ones obtained by merely using the cut-off point.

A comparison of the sex allocation results based on the canines with results based on the respective mandibles was undertaken to check if they are consistent. For the 6 mandibles presenting *in situ* canines and allowing for more reliable sex estimations (297, 1243, 3527, 2921, 252 and 1313 = M), all were in agreement with canine sex allocations when merely using the sample-specific mean value as a discriminant cut-off point. For two mandibles with less reliable sex estimation (1244 = M?; 1250 = F?), the canine sex allocation confirmed those estimations. By using the “undetermined sex” category, all sex estimations (n = 5) confirmed the mandibular sex allocations. Details of these 8 cases are presented in Table 2. Note that for mandibles 297, 1243 and 3527, tooth 33 was used instead of tooth 43 because the latter was not present – such procedure was legitimized by t-testing which did not find any significant difference between the means of the left and right samples.

Table 1. Samples descriptive statistics for the bucco-lingual diameter of the mandibular canine (FDI43).

	Sex-pooled			Males			Females		
	n	Mean	sd	n	Mean	sd	n	Mean	sd
Bom Santo	51	7.55	.59	-	-	-	-	-	-
Modern Ticuna (Harris and Nweeia, 1980)	54	7.35*	-	27	7.45	-	27	7.24	-
Modern Pukapuka (Yamada and Sakai, 1992)	39	8.03*	.42*	21	8.33	.48	18	7.67	.34
Modern Turkish (Işcan and Kedici, 2003)	100	7.62*	.54*	50	8.04	.64	50	7.19	.44
Modern South Chinese (Ling and Wong, 2007)	325	7.13*	.66*	188	7.20	.70	137	7.04	.60
Medieval Croatians (Vodanovic et al., 2007)	43	7.60*	.65*	23	7.73	.71	20	7.44	.56
Modern Portuguese (Cardoso, 2008)	75	7.73	.56	40	-	-	35	-	-
Modern Thai (Ruengdit et al., 2011)	171	7.50*	.54*	62	7.76	.58	109	7.35	.52
Modern Greek (Zorba et al., 2011)	57	7.61*	.56*	32	7.94	.63	25	7.19	.46

\*Sex-pooled descriptive statistics were calculated based on the original data provided by the authors (according to published results). This was conducted so that Bom Santo results could be compared to those studies in which sex-pooled values had not been published.



Table 2: Comparison between the sex estimation based on the canine metrics and the sex estimation based on mandibular features.

Number	Tooth (FDI)	BLD (mm)	Canine Und	Canine COff	Mandible
297	33	8.07	M	M	M
1243	33	7.72	M?	M	M
3527	33	8.57	M	M	M
292	43	7.76	M?	M	M
1244	43	7.92	M	M	M?
1250	43	6.98	F	F	F?
1252	43	7.97	M	M	M
1313	43	7.61	M?	M	M

Key: BLD = Bucco-lingual diameter; Canine Und = canine sex estimation by using a category for individuals of undetermined sex; Canine COff = canine sex estimation by using the cut-off point of 7.55 mm; Mandible = mandible sex estimation by using the recommendations of Ferembach et al. (1980).

#### 4. DISCUSSION

Our attempt to determine the sex profile of the individuals present at Bom Santo by using sample-specific odontometric references provides encouraging results. This procedure allowed us to estimate the sex of 51 individuals, a figure which is considerably higher than the one obtained for the most represented non-dental feature at Bom Santo - humeral minimum circumference (n = 25). Therefore, 70% of the minimum number of individuals estimated for the skeletal assemblage of rooms A and B (N = 73, based on tooth 46) were sex estimated instead of a mere 33% permitting a more general and representative portrait of the sex profile. Also, agreement in 100% of the cases (n = 5) between canine sex estimation and mandibular sex estimation indicates that the method may well be reliable. However, results must be interpreted with caution since only a small portion of the canine teeth could be compared with their respective mandibles.

Apparently, the sex ratio was well balanced in Bom Santo cave thus demonstrating that funerary access was open equally to both sexes. This interpretation would have been hard or even impossible to achieve if based on other parts of the skeleton. This procedure would have had some problems too. First, it would have been based on small samples, as was the case for the humeral minimum circumference (n = 25) or the pelvis (n = 5). Second, for the sexually dimorphic osteometric features, sex estimation would not have been carried out based on sample-specific references because the sample size assumption was not met. Consequently, the application of other than sample-specific references would most probably have been biased (Ferembach et al., 1980; Işcan et al., 1998; Albanese et al., 2005; Cardoso, 2008). Therefore, the procedure proposed by Albanese et al. (2005) and Cardoso (2008) seems to be more appropriate, although not without its own problems. In particular, it will tend to impose a sex ratio of 1:1 because it assumes that a similar amount of males and females are present in the sample, as stated by Cardoso (2008). The lowest sex allocation accuracy obtained by this

author with the sample-specific mean of the canine BLD was of 80%. Taking this value into consideration, we may assume that about 41 (80.4%) of the individuals from Bom Santo were correctly classified and that the remaining 10 individuals were incorrectly classified. A sex ratio interval can thus be established if these 10 cases are in turn subtracted from the sample of estimated females ( $n=26$ ) and the sample of estimated males ( $n=25$ ). These two scenarios would be the ones presenting the largest possible difference between the amount of males and females. In the first case, a set of 16 females and 25 males would give a sex ratio of 1:1.7. In the second case, a set of 26 females and 15 males would give a sex ratio of 1:1.6. Therefore, the sex ratio at the Bom Santo cave probably ranged between 1:1 and 1:1.7, accepting that 80% of the individuals were allocated to the correct sex and that all assumptions recommended by Albanese et al. (2005) were met.

Our attempt to determine if the sex ratio of this archaeological population was compatible with the use of a sample-specific methodology was based upon the transposing of some data. First, we tested the normality of the distribution of the values using the Shapiro-Wilk statistic. Then, we compared the descriptive statistics of our sample with other samples given in Table 1. For Bom Santo, the mean and standard deviation appeared to be very similar to most of those samples. In addition, the range of the data was quite similar to that found for the Lisbon Collection (2.37 mm vs 2.73 mm) which suggests that the sample was composed of canines from both females and males. The descriptive statistics were compared with other references because the mere normality of the distribution was not considered sufficient as a criterion to assess sex ratio, since a sample exclusively composed of males could also have a normal distribution. It should be noted that the presence of outliers may interfere with the descriptive statistics of the sample under study, especially with the calculation of range.

Of course, if the samples are not normally distributed, the application of this method will lead to errors. For instance, if a given sample is skewed and comprises mainly smaller canines, the mean value used as cut-off point will be smaller as well and there will be a tendency to mistakenly allocate females with larger teeth to the male sex. The opposite also applies. In cases such as these, rather than using the mean value, the median should be considered as an alternative. Either way, caution must be exercised with the sex ratio assumption although the method seems to be reasonably robust. Albanese et al. (2005) writes that they had good results even with a sex ratio of 1.9:1.

Although the sex ratio at Bom Santo was apparently well balanced when applied to the whole assemblage, the scenario was quite different when rooms were considered individually. Spatial distribution of the canines in Rooms A and B suggests that the access to specific areas of the cave may have been somewhat different. Figure 2 presents the distribution of 48 of the 51 canine teeth used in this study – no square unit provenance information was recorded for the remaining 3 teeth but all were from Room B (two estimated males and one possible female). This figure includes the combined results of canine and mandible sex estimation. Estimated males were far fewer in Room A ( $n = 4/N = 35$ ) than in Room B ( $n = 14/N = 36$ ), although the minimum number of individuals was similar in both rooms. In addition, 8 probable males were also present in the latter while no probable males were identified in Room A. As for females, they were apparently more numerous than males in Room A which presented the remains of 9 females and three probable females. A different scenario was apparently present in Room B

where 11 females and two probable females were identified. Therefore, and if we consider only the more reliable sex estimations, sex ratio in Room A and Room B was 2.3 females for every male and 1.3 males for every female, respectively. Such a pattern suggests that funerary space was managed according to specific social and gender rules. In fact, bone assemblages were often delimited by stones thus indicating that the remains were not deposited on the surface randomly.

This is just a preliminary observation and no definitive interpretation about the sex distribution in Bom Santo may be outlined yet since Room A was not completely excavated. Hence our conclusions may change when excavations have been completed. In addition, not all canines have been allocated to sex so we do not have the full picture regarding this issue. The fact that the cave was used as a cemetery for about 400 years implies that, if the remains belonged to a single population, secular changes in tooth size may have occurred during that period because it is dependent on environmental conditions (Harris et al., 2001). Another problem may have interfered with our analysis of the sex ratio in Rooms A and B: ancient DNA analyses revealed that the individuals from Bom Santo belonged to a much diversified set of mitochondrial haplogroups (Fernández and Arroyo-Pardo, 2014) and therefore different populations. If this was indeed the case, those may have had different levels of sexual dimorphism. Given that individuals with possible different population affinities were present in the cave, we cannot know for sure what effect this had on our attempt to estimate sex via the teeth. As Harris and Nweeia (1980), and Ling and Wong (2007) all showed, not all populations seem to display sexually dimorphic canines. This means that the method may not be useable in all cases. This reinforces the need for a conservative approach to this method by avoiding sexing the individuals that are nearer to the overall mean value. Nonetheless, the comparison between canines and mandibles suggested that it may well prove useful.

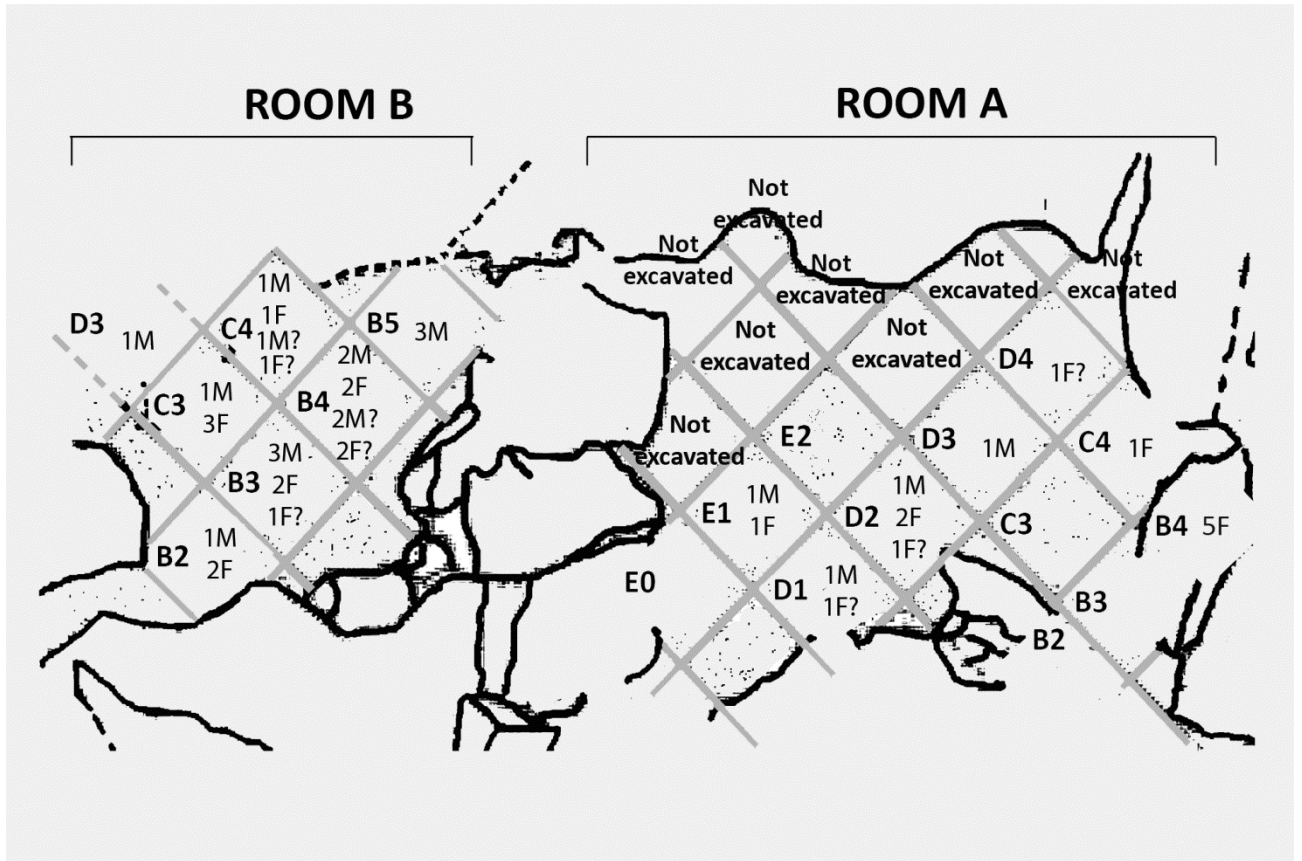


Figure 2. Spatial distribution of males and females in Rooms A and B of Bom Santo Cave according to sex allocation based on the lower canine bucco-lingual diameter and on the mandible. [Two-column fitting image]

## 5. CONCLUSION

The method proposed by Albanese et al. (2005) and Cardoso (2008) bodes well for the analysis of contexts comprising commingled remains, which constitutes the majority of Neolithic and Chalcolithic cemetery sites known in the Estremadura region of central Portugal. The assessment that was carried out in this study attempts to apply that method to an archaeological assemblage of unknown individuals, paying special attention to the identification of sample parameters that may be used to infer if the normal distribution and sex ratio assumptions are met or not. For this purpose, we first proposed to check the normality of the distribution using the Shapiro-Wilk statistic. Second, and after confirming that this previous condition was met, we compared the descriptive statistics (mean, standard deviation and range) of the studied collection with data from other studies to assess if those are consistent with a well balanced sex ratio. If both conditions are met, sample-specific methods can then be used reliably on a sample larger than 40 individuals, as was demonstrated by the comparison of canine sex estimation and mandible sex estimation. However, further investigation based on larger samples of mandibles is still required before a more definitive judgment can be made.

The usefulness of this method was well demonstrated in Bom Santo benefiting from the fact that the human remains were deposited in distinct sections of the cave. The spatial

distribution of individuals by sex, or by any other unknown reason which resulted in different distributions of males and females, indicated clear differences regarding the use of Room A and Room B. The latter apparently presented a more balanced sex ratio than the former. However, it is not possible at present to determine if that difference was the result of an intentional funerary practice or merely the outcome of a random practice.

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#### REFERENCES

- Albanese J. 2003. A metric method for sex determination using the hipbone and the femur. *Journal of Forensic Sciences* 48(2):263-273.
- Albanese J, Cardoso HFV, and Saunders SR. 2005. Universal methodology for developing univariate sample-specific sex determination methods: an example using the epicondylar breadth of the humerus. *Journal of Archaeological Science* 32:143-152. DOI: 10.1016/j.jas.2004.08.003
- Bruzek J. 2002. A method for visual determination of sex, using the human hip bone. *American Journal of Physical Anthropology* 117:157-168. DOI:10.1002/ajpa.10012
- Buikstra J, and Ubelaker D. 1994. Standards for data collection from human skeletal remains: Proceedings of a Seminar at the Field Museum of Natural History. *Arkansas Archaeological Survey Report* 44.
- Cardoso H. 2008. Sample-specific (universal) metric approaches for determining the sex of immature human skeletal remains using permanent tooth dimensions. *Journal of Archaeological Science* 35:158-168. DOI:10.1016/j.jas.2007.02.013
- Carvalho AF, editor. 2014. *Bom Santo Cave (Lisbon) and the Middle Neolithic societies of Southern Portugal*. Faro: University of Algarve..

Carvalho AF, Gonçalves D, Granja R, Petchey F. 2012. Algar do Bom Santo: a Middle Neolithic necropolis in Portuguese Estremadura. In: Gibaja JL, Carvalho AF and Chambon P, editors. *Funerary practices in the Iberian Peninsula from the Mesolithic to the Chalcolithic*. Oxford: Archaeopress (British Archaeological Reports - International Series; 2417): 77-90.

Carvalho AF, and Petchey F. 2013. Stable isotope evidence of Neolithic palaeodiets in the coastal regions of Southern Portugal. *Journal of Island & Coastal Archaeology*, 8: 361-383. DOI: 10.1080/15564894.2013.811447

Ditch LE, and Rose JC. 1972. A multivariate dental sexing technique. *American Journal of Physical Anthropology* 37:61-64. DOI: 10.1002/ajpa.1330370108

Duarte C. 1998. Necrópole neolítica do Algar do Bom Santo: contexto cronológico e espaço funerário. *Revista Portuguesa de Arqueologia* 1(2):107-118.

Ferembach D, Schwidetzky I, and Stloukal M. 1980. Recommendations for Age and Sex Diagnoses of Skeletons. *Journal of Human Evolution* 9:517-549.

Fernández E and Arroyo-Pardo E. 2014. Palaeogenetic study of the human remains. In: Carvalho, AF, editor. *Bom Santo Cave (Lisbon) and the Middle Neolithic societies of Southern Portugal*. Faro: Universidade do Algarve. p 133-142.

Garn SM, Cole PE, Wainwright RL, and Guire KE. 1977. Sex discriminatory effectiveness using combinations of permanent teeth. *Journal of Dental Research* 56(6):697.

Granja R, Cardoso FA, and Gonçalves D. 2014a. Taphonomy and funerary practices. In: Carvalho AF, editor. *Bom Santo Cave (Lisbon) and the Middle Neolithic societies of Southern Portugal*. Faro: Universidade do Algarve. p 79-100.

Granja R, Gonçalves D, and Cardoso FA. 2014b. Osteological Profile. In: Carvalho AF, editor. *Bom Santo Cave (Lisbon) and the Middle Neolithic societies of Southern Portugal*. Faro: Universidade do Algarve. p 101-120.

Haak W, Brandt G, Jung H, Meyer C, Ganslmeier R, Heyden V, Hawkesworth C, Pike AWG, Meller H, and Alt K. 2008. Ancient DNA, Strontium isotopes, and osteological analyses shed light on social and kinship organization of the Later Stone Age. *Proceedings of the National Academy of Sciences* 105(47):18226-18231. DOI: 10.1073/pnas.0807592105

Harris EF, and Neave MT. 1980. Tooth size of Ticuna Indians, with phenetic comparisons to other Amerindians. *American Journal of Physical Anthropology* 53:81-91. DOI: 10.1002/ajpa.1330530112

Harris EF, Potter RH, and Lin J. 2001. Secular trend in tooth size in urban Chinese assessed from two-generation family data. *American Journal of Physical Anthropology* 115(4):312-318. DOI: 10.1002/ajpa.1087

Hillson S, Fitzgerald C, and Flinn H. 2005. Alternative dental measurements: proposals and relationships with other measurements. *American Journal of Physical Anthropology* 126:413-426. DOI: 10.1002/ajpa.10430

Işcan MY, and Kedici PS. 2003. Sexual variation in bucco-lingual dimensions in Turkish dentition. *Forensic Science International* 1(2-3):160-164. DOI:10.1016/S0379-0738(03)00349-9

Işcan MY, Loth SR, King CA, Shihai D, and Yoshino M. 1998. Sexual dimorphism in the humerus: A comparative analysis of Chinese, Japanese and Thais. *Forensic Science International* 98(1):17-29. DOI: 10.1016/S0379-0738(98)00119-4

Ling JYK, and Wong RWK. 2007. Tooth dimensions of Southern Chinese. *Homo - Journal of Comparative Human Biology* 58:67-73. DOI:10.1016/j.jchb.2006.08.003

Marini E, Racugno W, and Tarli SMB. 1999. Univariate estimates of sexual dimorphism: the effects of intrasexual variability. *American Journal of Physical Anthropology* 109(4):501-508. DOI: 10.1002/(SICI)1096-8644(199908)109:4<501::AID-AJPA6>3.0.CO;2-7

Mays S. 1998. *The Archaeology of Human Bones*. New York: Routledge.

Milner GR, Wood JW, and Boldsen JL. 2008. Advances in Paleodemography. In: Katzenberg A, and Saunders SR, editors. *Biological Anthropology of the Human Skeleton*. 2nd edition ed. Hoboken, New Jersey: John Wiley & Sons, Inc.

Molleson T. 2006. Hunters of Nemrik. *Studies in Historical Anthropology* 3:5-18.

Murail P, Bruzek J, Houet F, and Cunha E. 2005. DSP: a tool for probabilistic sex diagnosis using worldwide variability in hip-bone measurements. *Bulletins et Mémoires de la Société d'Anthropologie de Paris* 17(3-4):167-176.

Rai B, Dhattarwal SK, Anand SC. 2008. Sex determination from tooth. *Medico-Legal Update* 8 (1): 3-5.

Ruengdit S, Riengrojpitak S, Tiensuwan M, and Santiwong P. 2011. Sex Determination from Teeth Size in Thais. Proceedings of the 6th CIFS Academic Day, 14-15th of September. Muang Thong Thani, Nonthaburi.

Silva AM. 1995. Sex assessment using the calcaneus and talus. *Antropologia Portuguesa* 13: 107-119.

Smith BH. 1991. Standards of human tooth formation and dental age assesment. In: Kelley MA, and Larsen CS, editors. *Advances in Dental Anthropology*. New York: Wiley-Liss. p 143-168.

Ulijaszek SJ, and Lourie JA. 1994. Intra- and inter-observer error in anthropometric measurement. In: Ulijaszek SJ, and Mascie-Taylor CGN, editors. *Anthropometry: the individual and the population*. Cambridge: Cambridge University Press. p 30-55.

Vodanovic M, Demo Z, Njemirovskij V, Keros J, Brkic H. 2007. Odontometrics: A useful method for sex determination in an archaeological skeletal population? *Journal of Archaeological Science*, 34: 905-913. DOI: 10.1016/j.jas.2006.09.004

Wasterlain SN. 2000. *Morphé: análise das proporções entre os membros, dimorfismo sexual e estatura de uma amostra da coleção de esqueletos identificados do Museu Antropológico da Universidade de Coimbra*. Master thesis. University of Coimbra.

Wasterlain SN, and Cunha E. 2000. Comparative performance of femur and humerus epiphysis for sex diagnosis. *Biométrie Humaine et Anthropologie* 18(1-2):9-13.

Yamada Y, and Sakai T. 1992. Sexual Dimorphism in Tooth Crown Diameter of the Cook Islanders. In: Smith P, and Tchernov E, editors. *Structure, Function and Evolution of Teeth*. London: Freund Publishing House. p 437-450.

Zorba E, Moraitis K, and Manolis SK. 2011. Sexual dimorphism in permanent teeth of modern Greeks. *Forensic Science International* 210:74-81. DOI:10.1016/j.forsciint.2011.02.001

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