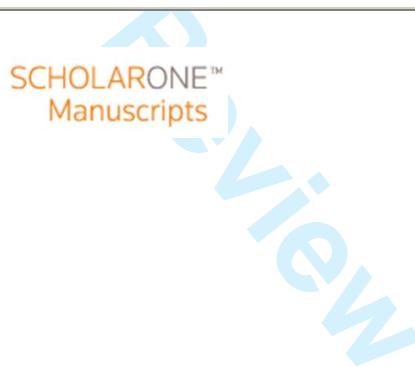


Ancestry estimation based on morphoscopic traits in a sample of African slaves from Lagos, Portugal (15th-17th centuries)

Journal:	<i>International Journal of Osteoarchaeology</i>
Manuscript ID	OA-16-0026.R1
Wiley - Manuscript type:	Short Report
Date Submitted by the Author:	n/a
Complete List of Authors:	Coelho, Catarina; University of Coimbra, Department of Life Sciences Navega, David; University of Coimbra, Department of Life Sciences Eugenia, Cunha; Universidade de Coïmbra, Departamento de Antropologia Ferreira, Maria Teresa; University of Coimbra, Department of Life Sciences; Wasterlain, Sofia; University of Coimbra, Department of Life Sciences
Keywords:	Ancestry, Morphoscopic traits, Enslaved Africans, 15th-17th centuries, Bioarchaeology



1
2 **Ancestry estimation based on morphoscopic traits in a sample of African slaves from Lagos,**
3
4 **Portugal (15th-17th centuries)**
5

6
7
8 **Catarina Coelho^{a,b*}, David Navega^{a,b}, Eugénia Cunha^{a,b}, Maria Teresa Ferreira^{a,b,c}, Sofia N.**
9
10 **Wasterlain^{b,c}**
11

12
13
14
15 ^aLaboratory of Forensic Anthropology, Department of Life Sciences, University of Coimbra,
16
17 Coimbra, Portugal

18
19 ^bCentre for Functional Ecology, University of Coimbra, Coimbra, Portugal

20
21 ^cResearch Centre for Anthropology and Health, Department of Life Sciences, University of
22
23 Coimbra, Coimbra, Portugal
24

25
26
27
28 **Running Title:** Ancestry estimation in a slave sample from Portugal

29
30 **Key words:** Ancestry; Morphoscopic traits; Enslaved Africans; 15th-17th centuries; Bioarchaeology.
31
32

33
34
35
36
37 *Corresponding author:

38
39 Catarina Coelho

40
41 Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456

42
43 Coimbra, Portugal

44
45 Telephone: +351 916519858

46
47 Fax: +351 239854129

48
49 E-mail address: coelho.catarina.rs@gmail.com
50
51
52
53
54
55
56
57
58
59
60

Abstract

In 2009, a skeletal collection of 158 individuals was excavated in Valle da Gafaria, Lagos, Portugal. These individuals were buried in an unusual way, having been discarded in an urban dump located outside the medieval city walls, dated from the 15th-17th centuries. Lagos was, at the time, an important slave trade harbour, and during the excavation, the morphological appearance of the skulls and the presence of intentionally modified teeth in some individuals raised suspicion that they were African slaves. Despite the extensive historical information about the Atlantic slave trade, so far skeletal remains identified as slaves were scarce, especially in Europe. The aim of the present study is to estimate the ancestry of a sample of 33 adult individuals (28 females and 5 males) recovered in the Valle da Gafaria applying the eleven morphological characteristics recommended by Hefner (2009) using the naïve Bayes classifier. When comparing the individuals with four groups of classification (European, African, American Indian, and Asian), 24 (72.7%) specimens were classified as Africans with a posterior probability greater than 0.90. When only two groups were considered (the African and the European), 31 (93.9%) individuals were classified as Africans with a posterior probability greater than 0.90. These results are in accordance with the historical record and previous genetic studies suggesting that this sample represents a rare archaeological sample of great interest to the history of the Atlantic slave trade, i.e., the Lagos individuals were probably of African ancestry. Although the ancestry is a parameter of the biological profile mainly estimated in forensic Anthropology, this study confirms the importance of its investigation in past populations.

Key words: Ancestry; Morphoscopic traits; Enslaved Africans; 15th-17th centuries; Bioarchaeology.

1. Introduction

Due to the construction of an underground car park in Valle da Gafaria (Lagos, Portugal), an archaeological excavation allowed the identification of two burial places: one related with a leprosarium (Ferreira et al., 2013) and another associated with a deposit of urban waste (Wasterlain et al., 2015). From this deposit of urban waste, dated from the 15th-17th centuries, the skeletons of 158 individuals were recovered.

The radiocarbon dating of one skeleton (individual 169) revealed a date of 450 +/- 40BP (2 sigma: Cal AD 1420 - 1480; cal BP 540 - 470; Beta - 276508). During that period, the 15th century, Lagos was the harbour where slaves arrived from Africa and from which they were redistributed to the Kingdom of Portugal, the Mediterranean Sea and Northern Europe (Fonseca, 2010; Caldeira, 2013). Additionally, several archaeological findings support the hypothesis that the 158 individuals recovered from the deposit waste were African slaves: the fact that their bodies were dropped mixed with waste in a large pit, disregarding the burial traditions; the recovery of several African ornaments associated with the skeletons; the apparent African facial morphology (Figure 1) (Coelho, 2012); and the presence of intentionally modified teeth in 63 individuals. For a more comprehensive contextualization of this collection please see Wasterlain et al. (2015).

The aim of the present study is to estimate the ancestry of a sample of individuals recovered in the Valle da Gafaria by applying the eleven morphological characteristics recommended by Hefner (2009) using the naïve Bayes classifier.

The ability to identify potential slaves will lead to several pertinent questions about the slave trade, such as: Were both sexes equally likely to be victims in the slave trade? What was the health status of these individuals? Is there any evidence of patterned degenerative lesions that indicate that the slaves were used for heavy manual labour? Is there any evidence of traumatic injuries that may suggest that these individuals were subjected to physical abuse? After confirming that these

1 individuals were African slaves future research on this collection could help address this kind of
2
3 questions of wider anthropological interest.
4
5
6
7

8 **2. Material and Methods**

9

10 The morphological analysis is based on two different types of nonmetric traits, the
11 anthroposcopic traits, and the binary nonmetric traits (Hughes et al., 2011). While the
12 anthroposcopic traits evaluate the different degrees of expression or shape of the trait (e.g. the malar
13 tubercle), the binary nonmetric traits are recorded as “present” or “absent” (e.g. the nasal
14 overgrowth) (Hughes et al., 2011). In the present study, the anthroposcopic traits recommended by
15 Hefner (2009) were applied using the naïve Bayes classifier to estimate ancestry of the individuals
16 recovered in Valle da Gafaria (Lagos, Portugal).
17
18
19
20
21
22
23
24

25 Of the 158 individuals of the Valle da Gafaria collection, only 33 were sufficiently
26 preserved to allow morphological analysis. The state of preservation and completeness of the
27 selected skeletons are presented in Table 1. Sex and age-at-death estimation, relied on metric and
28 morphological analyses of the coxal bone and the skull (Uyterschaut, 1986; Ferembach et al., 1990;
29 Buikstra and Ubelaker, 1994; Bruzek, 2002; Ozle et al., 2007; Shirley and Jantz, 2011). The ages
30 are comprised between 15 and 40 years. In all, the sample is composed of 28 females and 5 males
31 (Table 1). In the present study the sexes were combined together because the reference data
32 provided by Hefner (2009) also pool the sexes. Besides, there are no issues with the unequal
33 samples in the current study.
34
35
36
37
38
39
40
41
42
43
44
45

46 Eleven morphological characteristics were observed to estimate ancestry: anterior nasal
47 spine (ANS), inferior nasal aperture (INA), interorbital breadth (IOB), malar tubercle (MT), nasal
48 aperture width (NAW), nasal bone contour (NBC), nasal overgrowth (NO), postbregmatic
49 depression (PBD), supranasal suture (SPS), transverse palatine suture (TPS Shape),
50
51
52
53
54
55
56
57
58
59
60

1 zygomaticomaxillary suture (ZS Shape). All characteristics were evaluated following the
2 descriptions of Hefner (2009) and the images of Osteoware (Smithsonian Institution, 2011).
3
4

5
6 The first step of this investigation was to ascertain if there was agreement between two
7 observations performed by the same observer (intra-observer error) and by two different observers
8 (inter-observer error). To assess intra-observer error, observations were collected with one week
9 interval between each observation by the first author (CC). To investigate the inter-observer error
10 17 skulls were analysed by another author (MTF). Both errors were calculated through the Cohen's
11 Kappa coefficient, measured on a -1 to 1 scale (Viera and Garrett, 2005).
12
13
14
15
16
17
18

19 Ancestry estimation can be formulated and addressed from a mathematical point of view as
20 a statistical classification problem, that is, to allocate an object into predefined classes based on its
21 observed features. Statistical classification and pattern recognition tools are common in
22 anthropology because biological profiling from skeletal remains is in its essence an exercise of
23 statistical prediction and estimation. To be able to estimate sex, age-at-death, ancestry, and stature,
24 statistical information must be first extracted from identified reference samples. Such information
25 can be later used to reconstruct any biological parameter from unidentified skeletal remains based
26 on the characteristics of the osteological material. The accuracy and precision of such
27 reconstruction depends on the quality of the methods of osteological observation and the techniques
28 underlying statistical prediction and estimation.
29
30
31
32
33
34
35
36
37
38
39
40

41 Hefner and Ousley (2014) provided a significant contribution to statistical ancestry
42 estimation by morphoscopic analysis. The authors evaluated the utility of several statistical
43 algorithms in ancestry prediction, demonstrating that morphoscopic ancestry estimation can also be
44 framed within a robust mathematical approach. Their work, however, is incomplete in the sense that
45 the statistical models created and tested by the authors lack a practical and easy-to-use
46 implementation. In fact, some of the techniques employed are impossible to use without specialized
47 software (i.e., neural network, random forest, and support vector machine models).
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45

Among the techniques used is the naïve Bayes algorithm. The naïve Bayes classifier is a simple, yet effective, probabilistic model that makes use of Bayesian theory with strong independence assumption (Fielding, 2007). It assumes that the presence or absence of a characteristic is not related to the presence or absence of another trait given the class variable, that is, it takes no account for partial correlations. Such assumption is called conditional independence. This modelling assumption offers a dramatic simplification of model induction: individual class-conditional marginal density of features can be estimated separately using a one-dimensional kernel density estimator or any distribution model appropriate for the continuous data. For discrete predictor class-conditional probability tables can be obtained using a histogram estimator (Hastie et al., 2009).

46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

It is important to note that unless the data is projected into a subspace with forced orthogonality (i.e., PCA scores), it is common to observe some degree of dependency among predictors, which normally occurs in real data. However, even when there are deviations from this underlying independence assumption, this technique works well often outperforming more sophisticated algorithms that explicitly model dependencies. This occurs because model predictions will be accurate as long as probability for the true class (i.e., ancestry) is greater than the probability of any other class. To obtain correct predictions only an approximate solution is required to rank order class probabilities. Marginal class-conditional densities may be biased but that has little effect on the final posterior probabilities, especially in the decision regions (Fielding, 2007; Hastie et al., 2009).

Under the naïve Bayes classifier ancestry estimation is expressed as:

$$P(A_k|X_i) = \frac{P(A_k) \prod_{i=1}^p P(X_i|A_k)}{P(X_i)} = \frac{P(A_k) \prod_{i=1}^p P(X_i|A_k)}{\sum_{i=1}^r P(A_k) \prod_{i=1}^p P(X_i|A_k)}$$

1
2 Where A stands for ancestral group, X for the morphoscopic traits, r is the number of
3
4 ancestral groups involved in the analysis, and p the number of morphoscopic traits used to compute
5
6 the posterior probability.
7
8
9

10
11 To assess ancestry using this statistical approach, the posterior probability that a skull is
12
13 from a specific ancestry, given p observed traits, is computed for each ancestral group considered in
14
15 the analysis. The ancestral group that maximizes the posterior probability is considered as the most
16
17 likely. In this study, the prior probability for each ancestral group, $P(A_k)$, was assumed to be
18
19 uniform.
20

21
22 The main advantage of the naïve Bayes classifier is that it only requires knowledge of
23
24 probabilities to obtain ancestry estimates. In this case the most important one is $P(X_i|A_k)$, the
25
26 probability of observing a specific stage/state of a morphological trait given that ancestry is known.
27
28 Bayes' theorem is then used to invert these probabilities and obtain $P(A_k|X_i)$, i.e. the probability of
29
30 a skull being of a certain ancestry given the observed morphological trait(s). The required
31
32 probabilities $P(X_i|A_k)$ are available from Hefner (2009), and allowed us to construct a naïve Bayes
33
34 classifier. Also, detailed information regarding the reference samples, namely their size,
35
36 composition, chronology, and geographic origin, can be obtained from Hefner (2009). A web
37
38 application that implements the naïve Bayes classifier for morphoscopic ancestry estimation is
39
40 available at www.apps.osteomics.com/hefneR.
41
42
43

44
45 Two predictive analyses were conducted based on the number of biogeographic ancestral
46
47 groups. The first analysis included four ancestral groups (European, African, American Indian, and
48
49 Asian). The second analysis was restricted to European and African ancestral groups. As previously
50
51 mentioned, statistical classification was performed based on the maximization of posterior
52
53 probability principle, the ancestral group with the highest posterior probability was assigned to the
54
55
56
57
58
59
60

1 individual. It means that under a uniform prior, the assigned ancestral group is the one with a
2
3
4 posterior probability greater than the (prior) random allocation probability.
5
6
7

8 **3. Results**

9 *Intra- and inter-observer errors*

10
11 The results of the Cohen's Kappa coefficient for the intra- and inter-observer errors are
12
13 presented in Table 2. Following the interpretation of Kappa by Viera and Garrett (2005), the overall
14
15 results showed substantial agreement. More specifically, in the intra-observer analysis, the
16
17 zygomaticomaxillary suture ($K= 0.459$) showed a moderate agreement. The inferior nasal aperture
18
19 ($K= 0.629$), the malar tubercle ($K= 0.718$) the supranasal suture ($K= 0.770$), and the anterior nasal
20
21 spine ($K=0.798$) demonstrated a substantial agreement. The other five traits – nasal bone contour
22
23 ($K= 0.812$), transverse palatine suture ($K= 0.816$), nasal aperture width ($K= 0.829$), postbregmatic
24
25 depression ($K= 0.927$), interorbital breadth ($K= 0.933$) – showed an almost perfect agreement. The
26
27 nasal overgrowth ($K= 1$) presented a perfect agreement between the two observations.
28
29
30
31

32
33 Regarding the inter-observer error, the malar tubercle ($K= 0.186$) showed a slight
34
35 agreement. The zygomaticomaxillary suture ($K= 0.214$) and the supranasal suture ($K= 0.393$)
36
37 showed a fair agreement. Four traits – anterior nasal spine ($K= 0.500$), inferior nasal aperture
38
39 ($K=0.553$), nasal bone contour ($K= 0.553$), and the interorbital breadth ($K= 0.589$) – presented
40
41 moderate agreement. The transverse palatine suture ($K= 0.827$) had an almost perfect agreement.
42
43 The nasal aperture width ($K= 1$), the nasal overgrowth ($K= 1$), and the postbregmatic depression
44
45 ($K= 1$) showed a perfect agreement among the two observers.
46
47
48
49

50 *Four groups classification*

51
52 The results obtained by using the naïve Bayes classifier on the first analysis are presented in
53
54 Table 3. Twenty-four specimens (72.7%) were classified as Africans with posterior probability
55
56
57
58
59
60

1 greater than 0.90. Two individuals (6.1%) were assigned to the African group with posterior
2 probability greater than 0.80. Three specimens (9.1%) were classified as Africans with posterior
3 probability greater than 0.80. Three specimens (9.1%) were classified as Africans with posterior
4 probability greater than 0.80. Three specimens (9.1%) were classified as Africans with posterior
5 probability greater than 0.70. Two skulls (6.1%) were classified in the African group with posterior
6 probability greater than 0.70. Two skulls (6.1%) were classified in the African group with posterior
7 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
8 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
9 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
10 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
11 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
12 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
13 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
14 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
15 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
16 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
17 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
18 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
19 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
20 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
21 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
22 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
23 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
24 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
25 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
26 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
27 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
28 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
29 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
30 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
31 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
32 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
33 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
34 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
35 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
36 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
37 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
38 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
39 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
40 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
41 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
42 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
43 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
44 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
45 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
46 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
47 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
48 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
49 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
50 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
51 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
52 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
53 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
54 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
55 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
56 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
57 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
58 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
59 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior
60 probability greater than 0.50. One individual (3.0%) was classified as African with a 0.44 posterior

Two groups classifications

The results of the second analysis using only two groups, European and African, are presented in Table 4. Thirty-one individuals (93.9%) were classified as Africans with posterior probability greater than 0.90. Only two specimens (6.1%) were classified as Africans with posterior probability greater than 0.80.

4. Discussion

Generally, the estimation of ancestry is considered more relevant for Forensic Anthropology than for past population studies, since it is one of the big four generic factors of a biological profile that help to establish identity and strong contextual information is usually associated with bioarchaeological investigations (i.e., artefacts, chronology, location) making ancestry estimation and the bio-geographic origin of a particular population implicit. However, there are several situations where it is worth investigating ancestry in archaeological samples, namely when the history of colonization of a given region is being investigated, in studies of endogamy vs. exogamy, when different facial morphologies inside a close group suggests the presence of newcomer individuals, and to confirm the identity when investigating historical characters. Finally, ancestry is a crucial factor in the study of the history of slavery.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

As a parameter of paramount importance, the estimation of ancestry has attracted the attention of researchers in order to find more accurate methods of evaluation (Gill, 2009, Hefner, 2009; DiGangi and Hefner, 2013). The estimation of ancestry is preferably accomplished through analysis of the skull, particularly from the delicate bones of the mid-face, because this region is the most discriminant part of the skeleton (Gill, 2009). However, the most discriminative features are commonly reported only for three major population groups - the European, the African, and the Asian (Byers, 2011) - which makes it difficult to identify small-scale migrations in the past.

Morphological analysis to estimate ancestry has been largely criticized for being highly subjective and dependent on the experience of the observer. However, it has some advantages over metric analysis. For instance, the morphological traits are easily observed, not being required instruments (Hefner and Ousley, 2014). Moreover, along with adequate statistical approaches, the morphoscopic traits can be used to accurately assess the ancestry, without relying only on the experience of the observer (Isan and Steyn, 2013; Hefner and Ousley, 2014).

The skeletal collection recovered in the Valle da Gafaria (Lagos, Portugal) is a unique sample composed of the remains of 158 individuals who lived in the 15th-17th centuries, and who were dropped in a dump after death, disregarding the canonical burial traditions. The Lagos's historical context and the presence of many individuals with intentionally modified teeth (for more details, please see Wasterlain et al., 2015) are among the facts that led the authors to suspect that these skeletons were the remains of African slaves who arrived to the Lagos's harbour during that time period. Therefore, this study aimed to estimate the ancestry of 33 adult individuals (28 females and 5 males), applying the eleven morphological characteristics recommended by Hefner (2009) using the naïve Bayes classifier.

Overall, the results obtained in the present study confirmed the African ancestry of the Lagos's individuals. More specifically, the comparison of the 33 skulls with the four major ancestral groups (European, African, American Indian, and Asian) assigned 24 (72.7%) to an

1 African ancestry with a posterior probability greater than 0.90. Surprisingly, one individual (number
2
3
4 63) was assigned to the American Indian group. As it can be observed in Figure 2, the cranium 63
5
6 presents some traits that are shared by Africans and American Indians, such as the inferior nasal
7
8 aperture, and the interorbital breadth. It should not be ignored, however, that this skull was
9
10 incomplete, fragmented, and it was necessary to proceed to its reconstruction, which certainly
11
12 contributed and increased its post-mortem deformation. In fact, the nasal overgrowth and the
13
14 postbregmatic depression are two characteristics that are important to distinguish these two
15
16 ancestries and that were not possible to observe in this individual. These circumstances may have
17
18 biased the obtained result. On the other hand, the likelihood of encountering an American Indian
19
20 within this sample is quite low.
21
22
23

24 The results obtained when only two groups were considered (the African and the European)
25
26 were even more consistent with an African origin, since 31 (93.9%) individuals were classified as
27
28 Africans with a posterior probability greater than 0.90.
29

30 In 2014, Martiniano et al. obtained random short autosomal sequence reads from two
31
32 individuals (125 and 166) recovered in the discard deposit burials from Valle da Gafaria, from
33
34 which only one was also included in the present analysis. Interestingly, the next generation
35
36 sequencing (NGS) of historical DNA sampled from the metatarsal bone of the individual 125
37
38 showed affinity with Bantu-speaking groups and Western African Mandenka and Yoruba
39
40 populations, which is also consistent with African origins for these individuals.
41
42
43

44 Given the results obtained in the present study, which are consistent with historical records
45
46 and are in accordance with the genetic analysis performed previously in this osteological collection,
47
48 we can now state that the Lagos's individuals were probably of African ancestry. Moreover, this
49
50 study confirms the importance of investigating the ancestry in past populations. Future research on
51
52 this collection will help address many questions of wider anthropological interest, namely regarding
53
54
55
56
57
58
59
60

1
2 their overall health status, their labour conditions, and if they were subjected to physical abuse,
3
4 among others.
5
6
7

8 **Acknowledgments**

10 The authors thank Centro de Investigação em Antropologia e Saúde, Centro de Ecologia
11 Funcional, Dryas Arqueologia Lda., and Styx, Estudos de Antropologia Lda. The co-author
12 Catarina Coelho was financed by University of Coimbra. The co-author David Navega was
13 financed by national funds by FCT- Fundação para a Ciência e Tecnologia with the reference
14 SFRH/BD/99676/2014. The co-author Maria Teresa Ferreira was financed by Gerda Henkel
15 Foundation (AZ 09/F/15). The co-author Sofia N. Wasterlain was financed by national funds by
16 FCT – Fundação para a Ciência e Tecnologia, under the project with the reference
17 UID/ANT/00283/2013. The authors also acknowledge the anonymous reviewers whose valuable
18 comments and suggestions allowed us to improve the manuscript.
19
20
21
22
23
24
25
26
27
28
29

30 The authors state that they do not have any conflict of interest to declare.
31
32
33
34

35 **References**

- 36
37 Bruzek J. 2002. A method for visual determination of sex, using the human hip bone. *American*
38 *Journal of Physical Anthropology* **117**: 157-168. DOI: 10.1002/ajpa.10012
39
40
41 Buikstra JE, Ubelaker DH. 1994. Standards for data collection from human skeletal remains.
42 *Arkansas Archaeological Survey Research Series No 44*.
43
44
45 Byers SN. 2011. *Introduction to forensic anthropology* (4th Ed). Prentice Hall: Boston.
46
47
48 Caldeira AM. 2013. *Escravos e traficantes no Império Português: o comércio negreiro português*
49 *no Atlântico durante os séculos XV a XIX*. A Esfera dos Livros: Lisboa.
50
51
52
53
54
55
56
57
58
59
60

- 1
2 Coelho C. 2012. Uma Identidade perdida no mar e reencontrada nos ossos: avaliação das afinidades
3 populacionais de uma amostra de escravos dos séculos XV-XVI. Masters Dissertation on
4 Human Evolution and Biology. University of Coimbra: Coimbra.
5
6
7
8 DiGangi EA, Hefner JT. 2013. Ancestry estimation. In *Research methods in human skeletal biology*,
9 DiGangi A, Moore MK (eds.). Academic Press: Oxford; 117-149.
10
11
12 Fielding A. 2007. *Cluster and Classification Techniques for the Biosciences*. Cambridge University
13 Press: Cambridge.
14
15
16
17 Ferembach D, Schwidetzky I, Stloukal M. 1980. Recommendations for age and sex diagnoses of
18 skeletons. *Journal of Human Evolution* **9**: 517-49.
19
20
21 Ferreira MT, Neves MJ, Wasterlain SN. 2013. Lagos leprosarium (Portugal): evidences of disease.
22 *Journal of Archaeological Science* **40**: 2298-2307. DOI:10.1016/j.jas.2012.12.039
23
24
25
26 Fonseca J. 2010. *Escravos e Senhores na Lisboa quinhentista*. Edições Colibri: Lisboa.
27
28
29 Gill WG. 2009. Assessing Ancestry (Race) from the Skeleton. In *The use of forensic anthropology*,
30 Pickering R, Bachman D (eds.). CRC Press: New York; 103-111.
31
32
33 Hastie T, Tibshirani R, Friedman, J. 2009. *The Elements of Statistical Learning: Data Mining,*
34 *Inference, and Prediction* (2nd Ed). Springer: New York.
35
36
37 Hefner JT. 2009. Cranial Nonmetric Variation and Estimating Ancestry. *Journal of Forensic*
38 *Sciences* **54**: 985–995. DOI: 10.1111/j.1556-4029.2009.01118.x
39
40
41 Hefner JT, Ousley SD. 2014. Statistical Classification Methods for Estimating Ancestry Using
42 Morphoscopic Traits, *Journal of Forensic Sciences* **59**: 883–890. DOI: 10.1111/1556-
43 4029.12421
44
45
46
47
48 Hughes CE, Juarez CA, Hughes TL, Galloway A, Fowler G, Chacon S. 2011. A Simulation for
49 exploring the effects of the “trait list” method’s subjectivity on consistency and accuracy of
50 ancestry estimation. *Journal of Forensic Sciences* **56**: 1094-1106. DOI: 10.1111/j.1556-
51 4029.2011.01875.x
52
53
54
55
56
57
58
59
60

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- Iscan MY, Steyn M. 2013. *The Human Skeleton in Forensic Medicine* (3rd Ed). Charles C. Thomas
Publisher, Limited: Springfield.
- Martiniano R, Coelho C, Ferreira MT, Neves MJ, Pinhasi R, Bradley DG. 2014. Genetic Evidence
of African Slavery at the Beginning of the Trans-Atlantic Slave Trade. *Scientific Reports* **4**:
5994. DOI: 10.1038/srep05994
- Osteoware. 2011. *Standardized skeletal documentation software*. Washinton, DC: Smithsonian
Institution National Museum of Natural History. [computer program].
- Ozle A, Van Niekerk P, Schulz R, Schmelting A. 2007. Studies of the chronological course of
wisdom tooth eruption in a black African population. *Journal of Forensic Sciences* **52**:
1161-1163. DOI: 10.1111/j.1556-4029.2007.00534.x
- Shirley NR, Jantz RL. 2011. Spheno-Occipital synchondrosis fusion in modern Americans. *Journal*
of Forensic Sciences **56**: 580-585. DOI: 10.1111/j.1556-4029.2011.01705.x
- Uyterschaut HT. 1986. Sexual dimorphism in human skulls: a comparison of sexual dimorphism in
different populations. *Journal of Human Evolution* **1**: 243-50.
- Viera AJ, Garrett JM. 2005. Understanding interobserver agreement: the kappa statistic. *Family*
Medicine **37**: 360-363.
- Wasterlain SN, Neves MJ, Ferreira MT. 2015. Dental Modifications in a Skeletal Sample of
Enslaved Africans Found at Lagos (Portugal). *International Journal of Osteoarchaeology*.
DOI: 10.1002/oa.2453

Table 1. Sex diagnosis, age-at-death, preservation and completeness of the skeletal individuals analysed in the present study.

Individual no.	Age-at-death	Sex	Preservation	Completeness
20	20-30 years	Female	Fair	Almost complete
21	20-30 years	Female	Good	Almost complete
25	15-25 years	Male	Good	Almost complete
38	20-30 years	Female	Good	Almost complete
39	20-30 years	Female	Good	Almost complete
41	30-40 years	Female	Good	Almost complete
57	15-25 years	Female	Good	Almost complete
63	30-40 years	Male	Poor	Very incomplete
65	25-35 years	Male	Fair	Almost complete
66	30-40 years	Male	Good	Almost complete
67	30-40 years	Female	Good	Almost complete
68	30-40 years	Female	Good	Almost complete
69	15-25 years	Female	Fair	Relatively complete
75	30-40 years	Female	Fair	Almost complete
77	30-40 years	Male	Good	Almost complete
78	30-40 years	Female	Good	Almost complete
81	15-25 years	Female	Good	Almost complete
82	25-35 years	Female	Good	Almost complete
93	20-30 years	Female	Good	Almost complete
95	20-30 years	Female	Good	Almost complete
96	30-40 years	Female	Good	Almost complete
110	20-30 years	Female	Fair	Almost complete

114	20-30 years	Female	Good	Almost complete
123	20-30 years	Female	Poor	Relatively complete
125	20-30 years	Female	Good	Almost complete
133	15-25 years	Female	Good	Almost complete
136	25-35 years	Female	Fair	Relatively complete
150	30-40 years	Female	Fair	Almost complete
153	25-35 years	Female	Fair	Almost complete
162	20-30 years	Female	Good	Almost complete
163	20-30 years	Female	Fair	Almost complete
169	20-30 years	Female	Fair	Almost complete
170	20-30 years	Female	Fair	Relatively complete

Poor – Severe bone damage, metaphyseal loss with long bones and cancellous exposure of the vertebrae.

Fair – Slight bone damage, erosion of bone surface and/or bone fragmentation.

Good – No significant damage of the bone surface or bone fragmentation.

Very incomplete – More than 50% of the skeleton is absent.

Relatively complete – 25-50% of the skeleton is absent.

Almost complete – Less than 25% of the skeleton is absent.

Table 2. Intra- and inter-observer error analysis using the Cohen's Kappa coefficient.

Trait	Intra-observer Cohen's Kappa coefficient	Inter-observer Cohen's Kappa coefficient
Anterior nasal spine (ANS)	0.798	0.500
Inferior nasal aperture (INA)	0.629	0.553
Interorbital breadth (IOB)	0.933	0.589
Malar tubercle (MT)	0.718	0.186
Nasal aperture width (NAW)	0.829	1
Nasal bone contour (NBC)	0.812	0.553
Nasal overgrowth (NO)	1	1
Postbregmatic depression (PBD)	0.927	1
Supranasal suture (SPS)	0.770	0.393
Transverse palatine suture (TPS Shape)	0.816	0.827
Zygomaticomaxillary suture (ZS Shape)	0.459	0.214
Mean	0.790	0.620

Table 3. Summary of the results obtained by comparing the individuals from Valle da Gafaria to four groups of ancestry classification.

Individual no.	African	American Indian	Asian	European
20	0.550517	0.281084	0.155829	0.012571
21	0.885845	0.064897	0.045798	0.003459
25	0.993707	0.003025	0.002854	0.000414
38	0.437627	0.296149	0.172366	0.093858
39	0.754649	0.125582	0.073866	0.045902
41	0.970815	0.003785	0.023625	0.001775
57	0.994896	0.001698	0.003187	0.000219
63	0.145385	0.635085	0.201174	0.018356
65	0.998003	0.000274	0.001612	0.000110
66	0.995642	0.002308	0.001885	0.000165
67	0.993695	0.000229	0.005946	0.000130
68	0.997024	0.001694	0.001156	0.000127
69	0.998380	0.000000	0.001599	0.000000
75	0.969394	0.004984	0.025250	0.000371
77	0.982793	0.004169	0.011894	0.001144
78	0.911316	0.005408	0.082632	0.000644
81	0.995617	0.001484	0.001265	0.001635
82	0.980205	0.004061	0.014015	0.001719
93	0.956052	0.013309	0.025234	0.005406
95	0.785586	0.083371	0.094501	0.036542

1					
2					
3	96	0.976501	0.005867	0.016825	0.000808
4					
5					
6	110	0.995931	0.001214	0.002762	0.000000
7					
8	114	0.998301	0.000181	0.001425	0.000000
9					
10					
11	123	0.979222	0.005324	0.015074	0.000380
12					
13	125	0.579405	0.196752	0.213658	0.010185
14					
15	133	0.997265	0.000722	0.001931	0.000000
16					
17					
18	136	0.999610	0.000000	0.000262	0.000000
19					
20	150	0.983811	0.010960	0.002163	0.003066
21					
22	153	0.981274	0.005890	0.009274	0.003562
23					
24					
25	162	0.708830	0.224055	0.056727	0.010388
26					
27	163	0.886734	0.065532	0.042485	0.005249
28					
29	169	0.989513	0.004909	0.00526	0.000318
30					
31					
32	170	0.995931	0.001214	0.002762	0.000000
33					
34					
35					
36					
37					
38					
39					
40					
41					
42					
43					
44					
45					
46					
47					
48					
49					
50					
51					
52					
53					
54					
55					
56					
57					
58					
59					
60					

Table 4. Summary of the results obtained by comparing the individuals from Valle da Gafaria to two groups of ancestry classification.

Individual no.	African	European
20	0.977675	0.022325
21	0.996110	0.003890
25	0.999583	0.000417
38	0.823404	0.176596
39	0.942662	0.057338
41	0.998175	0.001825
57	0.999780	0.000220
63	0.887895	0.112105
65	0.999889	0.000111
66	0.999835	0.000165
67	0.999869	0.000131
68	0.999873	0.000127
69	0.999993	0.000000
75	0.999617	0.000383
77	0.998838	0.001162
78	0.999293	0.000707
81	0.998361	0.001639
82	0.998249	0.001751
93	0.994378	0.005622
95	0.955552	0.044448

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

96	0.999173	0.000827
110	0.999906	0.000000
114	0.999906	0.000000
123	0.999612	0.000388
125	0.982725	0.017275
133	0.999918	0.000000
136	0.999957	0.000000
150	0.996893	0.003107
153	0.996383	0.003617
162	0.985556	0.014444
163	0.994116	0.005884
169	0.999678	0.000322
170	0.999906	0.000000



Figure 1. Cranium of individual 65 from Valle da Gafaria sample in anterior view, with an apparent African facial morphology.

914x1365mm (72 x 72 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



Figure 2. Cranium of individual 63 from Valle da Gafaria sample in anterior view, which was assigned to the American Indian group when applying the four major ancestral groups (European, African, American Indian and Asian).
1930x1286mm (72 x 72 DPI)

Review