



Multidisciplinary investigation reveals an individual of West African origin buried in a Portuguese Mesolithic shell midden four centuries ago

Rita Peyroteo-Stjerna^{a,b,1,2,*}, Luciana G. Simões^{a,1,3}, Ricardo Fernandes^{c,d,e,4}, Gonçalo Lopes^f, Torsten Günther^{a,5}, Mattias Jakobsson^{a,6}

^a Human Evolution, Department of Organismal Biology, Uppsala University, Norbyvägen 18C, 752 36 Uppsala, Sweden

^b UNIARQ, Centro de Arqueologia da Universidade de Lisboa, Portugal

^c Department of Archaeology, Max Planck Institute for the Science of Human History, Jena, Germany

^d School of Archaeology, University of Oxford, Oxford, United Kingdom

^e Faculty of Arts, Masaryk University, Brno, Czech Republic

^f Independent Researcher, Portugal

ARTICLE INFO

Keywords:

Atlantic Slave Trade
Portugal
Shell midden
Biomolecular archaeology
Ancient DNA
Radiocarbon dating
Stable isotopes
Parish records

ABSTRACT

Cabeço da Amoreira is a well-studied shell midden with a robust chronology based on a large number of radiocarbon dates on Mesolithic human burials. Surprisingly, we discovered one individual that lived about 400 years ago buried in this site. We employed a multidisciplinary approach integrating archaeology, historical records, genetics, radiocarbon dating and stable isotope analysis to investigate the biogeographic origins of this individual and burial circumstances. We could determine that this was a man of West African origin, probably from Senegambia, arriving in Portugal via the Trans-Atlantic Slave Trade. Our study provides new insights into aspects of the life and death of a first-generation African individual in Portugal during the Trans-Atlantic Slave Trade period and highlights the power of multidisciplinary research to unravel unwritten history.

1. Introduction

On October 1st, 1930, archaeologists identified one remarkable grave at the shell midden of Cabeço da Amoreira in Muge, Tagus valley, Portugal. Shell middens in the Tagus and Sado valleys in Portugal were used as burial grounds by the last hunter-gatherers in the Late Mesolithic, ca. 6500 to 5000 cal BCE (Paço, 1938; Santos et al., 1990; Cunha and Umbelino, 1997; Cunha and Cardoso, 2001, 2003; Roksandic, 2006; Jackes and Meiklejohn, 2008; Meiklejohn et al., 2009; Bicho et al., 2013; Diniz et al., 2014). Although this grave did not stand out as intrusive, as it resembled the graves of Mesolithic hunter-gatherers in the region, it was noticeable because it contained the well-preserved remains of an unusually tall individual. As all other graves at Amoreira, it was

unmarked, and it did not contain artefacts (Cardoso and Rolão, 2000).

While studying the Mesolithic population buried at Cabeço da Amoreira, we came across outstanding genetic results for the 1930's burial that did not conform with what is known for Mesolithic hunter-gatherers in Europe (e.g., Sánchez-Quinto et al., 2012; Lazaridis et al., 2014; Villalba-Mouco et al., 2019). We employed a multidisciplinary approach, integrating archaeology, historical records, genetics, radiocarbon dating and stable isotope analysis to investigate the chronology, genetic ancestry, and place of origin of this individual. Our analysis shows that an adult of African descent was buried sometime between the sixteenth and eighteenth centuries at Cabeço da Amoreira. This find provided a unique opportunity to examine the unusual use of prehistoric shell middens in modern times and to explore the motivation for the

* Corresponding author.

E-mail addresses: rita.peyroteo.stjerna@ebc.uu.se (R. Peyroteo-Stjerna), luciana.simoese@ebc.uu.se (L.G. Simões), fernandes@shh.mpg.de (R. Fernandes), g.simoese@ebc.uu.se (G. Lopes), torsten.guenther@ebc.uu.se (T. Günther), mattias.jakobsson@ebc.uu.se (M. Jakobsson).

¹ These authors contributed equally to this work.

² <https://orcid.org/0000-0002-3309-474X>.

³ <https://orcid.org/0000-0002-6119-9776>.

⁴ <https://orcid.org/0000-0003-2258-3262>.

⁵ <https://orcid.org/0000-0001-9460-390X>.

⁶ <https://orcid.org/0000-0001-7840-7853>.

<https://doi.org/10.1016/j.jasrep.2022.103370>

Received 24 November 2021; Received in revised form 22 January 2022; Accepted 30 January 2022

Available online 21 February 2022

2352-409X/© 2022 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

recent use of a prehistoric shell midden for the burial of an African person.

The history of African communities in Early Modern European history is strongly connected with the Trans-Atlantic Slave Trade. Between the fifteenth and the nineteenth centuries, Portugal alone received two to three thousand slaves every year (Caldeira, 2013). Most Africans arriving in Portugal during this period were captured through slave trade, though some became free and relatively independent, under low social status and racial prejudice (Henriques, 2019). Research on the Atlantic Slave Trade and its consequences has relied on written sources but biomolecular methods such as stable isotopes and genetics are providing new insights on individual histories (e.g., Schroeder et al., 2015; Santana et al., 2016; Laffoon et al., 2018). In Portugal, the first genetic study of slave graves in a major trading post confirmed the West African ancestry of the captives in agreement with historical descriptions (Martiniano et al., 2014). However, for the most part, little is known about individual histories or the specific geographic and ethnographic origin of Africans in Portugal during this period since this is not always evident from the historical records. This study provides an example of how multidisciplinary analyses can help resolve historical questions, especially when dealing with poorly documented events such as those during the Atlantic Slave Trade.

2. Archaeological material

Archaeological shell middens can be found in coastal, lacustrine and river environments worldwide. These sites span the past 140,000 years and are highly variable in form, size, use and chronology. Their appearance results from local processing of shellfish foods, and many of these places have been settlement locations and/or funerary and ritual sites (Álvarez et al., 2011).

Cabeço da Amoreira is one of many shell middens with Mesolithic burials on the banks of the Muge River in the Tagus estuary (Roche, 1965). Located about 80 km north-east from Lisbon (Fig. 1), Amoreira is

situated on an historical farmland owned by the Portuguese royal family in the sixteenth century and by noble families since the early seventeenth century (Casa Cadaval, 2020). Archaeological investigations started in 1884–1885 (Paula e Oliveira, 1888), but the human remains of 29 individuals so far identified in the site were excavated some decades later, in the 1930 s, 1960 s, and early 2000 s (Roche, 1965; Cardoso and Rolão, 2000; Roksandic, 2006; Bicho et al., 2013; Peyroteo-Stjerna, 2021).

Our samples were collected in the scope of an ongoing Mesolithic project at Uppsala University (Peyroteo Stjerna, 2016, 2021), and respective permits were obtained from the Museum of Natural History and Science - University of Porto, where the 1930 s Amoreira archaeological collection is curated. We sampled the maxilla and the root of the left maxillary first premolar, and attributed mug019 as the identification number of our samples. Due to bone fragmentation status and the mixing of several skeletons from Amoreira within the same storage container, it was not possible to proceed with detailed osteological analyses. Fragmentation and mixing of the material also led to contradictions regarding the identification of the excavation number of the specimen (see Online Supplementary Material, S1).

3. Methods

3.1. Radiocarbon and stable isotope analyses of carbon, nitrogen, and oxygen

Radiocarbon (^{14}C), carbon (^{13}C), and nitrogen (^{15}N) stable isotope analyses of extracted bone collagen were conducted at the Tandem Laboratory, Uppsala University. Bone carbonate was cleaned of exogenous carbonate following the method described by (Fernandes et al., 2014a) and on this material carbon (^{13}C) and oxygen (^{18}O) stable isotope analyses were conducted at the Bloomsbury Environmental Isotope Facility (BEIF), University College London. See online supplementary material (S2) for additional information on sample treatment

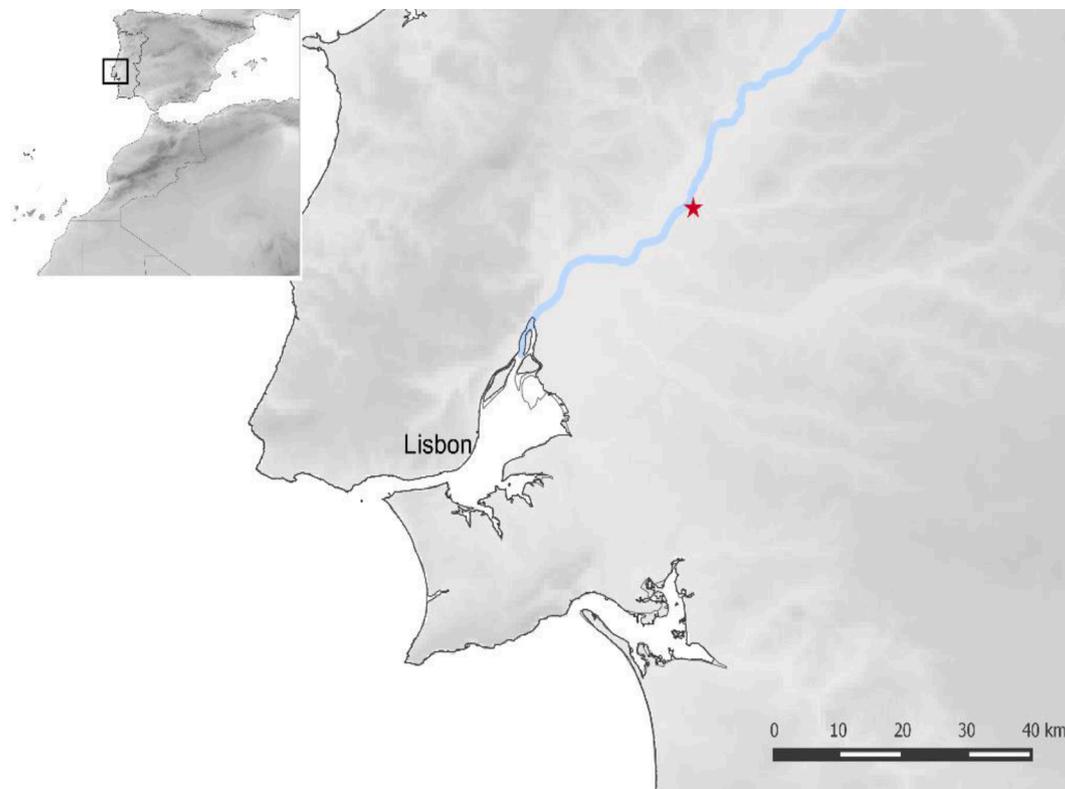


Fig. 1. Location of Cabeço da Amoreira shell midden (indicated by the star), Muge, Tagus valley, Portugal.

and measurement methodology.

3.2. Bayesian modelling of chronology, diet, and spatial mobility

We employed the models ReSources, AverageR and LocateR, developed within the Pandora and IsoMemo initiatives (see <https://isomem.oapp.com/>) to estimate dietary contributions from different foods, produce baseline maps for water $\delta^{18}\text{O}$ and marine radiocarbon reservoir effects, and to generate probability maps on the likely place of origin of mug019 (Fernandes et al., 2014b; Wilkin et al., 2020; Cubas et al., 2020; Wang et al., 2021). We employed a Bayesian chronological model implemented within the software package OxCal v. 4.4 to estimate the lifetime of the investigated individual which included a dietary correction for a marine radiocarbon reservoir effect (Bronk Ramsey, 2009; Fernandes, 2016). Detailed descriptions of Bayesian modelling are given in online [supplementary material](#) (S2).

3.3. Ancient DNA

Genomic data was generated in dedicated ancient DNA (aDNA) facilities at the Human Evolution Laboratory, Uppsala University. Tooth root samples were used for aDNA analysis, since teeth, particularly the outermost cementum layer, are known for the relatively good preservation of aDNA (Adler et al., 2011; Higgins et al., 2013). We used adapted versions of (Yang et al., 1998) and (Dabney et al., 2013) protocols to generate two DNA extracts which were then converted to double-stranded DNA libraries. Extract 1 was also used to build double-stranded libraries with USER enzyme to remove deaminations at the ends of post-mortem DNA fragments while extract 2 was used to build blunt-end libraries without USER enzyme. Libraries were amplified with a unique indexed primer (Meyer and Kircher, 2010). After quality control, libraries were pooled and sequenced on Illumina HiSeq X at the SNP & SEQ Technology Platform in Uppsala.

After sequence demultiplexing, forward and reverse paired-end reads were trimmed and merged when an overlap of at least 11 bp was found. Merged reads were mapped against the human reference genome using bwa aln 0.7.13 (Li and Durbin, 2009). Fragments with identical start and end positions were considered PCR duplicates and collapsed into consensus sequences. We filtered out reads shorter than 35 bp and more than 10% mismatches to the human reference genome. For each library, we merged bam files resulting from all resequencing rounds using samtools merge v1.5 (Li et al., 2009).

Data authenticity was confirmed by deamination patterns and short fragment size (Sawyer et al., 2012) (Fig. S3.1). We used the method described by (Skoglund et al., 2013) for biological sex determination. Contamination was estimated based on contradicting signals in the mitochondria (Green et al., 2008) and in the X chromosome (Rasmussen et al., 2011) using ANGSD v.0.902 (Korneliussen et al., 2014).

Mitochondrial haplogroup was assigned using HaploFind (Vianello et al., 2013). Y chromosome haplogroup was ascertained on informative SNP positions from Phylotree (version 9 Mar 2016) (van Oven et al., 2014).

Data from all libraries (USER treated and untreated) were merged to maximise genomic coverage to 0.8-fold. Analyses were restricted to SNP transversion sites. To compare the individual's genetic makeup to that of current-day people, we intersected our data with the Human Origins panel of worldwide modern populations (Lazaridis et al., 2014). We conducted exploratory population genetic analyses: Principal Component Analysis (PCA), unsupervised model-based genetic clustering (Alexander et al., 2009) and outgroup- f_3 statistics (Patterson et al., 2012). Finally, we investigated genes coding for traits previously recognised as relevant for African populations, such as resistance to malaria and African sleeping sickness, lactase persistence and skin pigmentation (Schlebusch et al., 2017). See online [supplementary material](#) for specific detail on aDNA data generation and population genomic analysis (S2-3).

3.4. Archaeological and historical record

Archaeological analysis was based on published field notes (Cardoso and Rolão, 2000) by the anthropologist and excavation director Mendes Corrêa providing background information on the stratigraphy and grave context.

We consulted Parish Registers for death records of individuals of African ancestry in the Muge Parish, because in Portugal, from the Middle Ages up to mid-nineteenth centuries, the dead were typically buried in religious grounds (Queiroz and Rugg, 2008). Documentation is available from 1628 onward and is accessible online (Tombo.pt, Portuguese parish records or genealogy, 2020). The Muge Parish Registers consist of the Church records of birth/baptisms, marriages, and death/burials. Registers may provide the name of the individual, the name of the owner (in the case of enslaved individuals), burial place and date, and place of death. Often, enslaved individuals were baptized and buried according to Christian tradition but frequently in collective graves. Yet, it was not unusual to use waste pits outside the churchyard or even burn the bodies (de Saunders, 1982). Exceptions such as burial of slaves on road sides, in wastelands or olive groves have been documented, suggesting strategies for the rapid disposal of cadavers based on fear of spreading diseases (Fonseca, 2010).

To identify potential places of origin of Africans arriving in Portugal through the slave trade, we used the online African Slave Trade database (Slave Voyages: The Trans-Atlantic Slave Trade Database, 2020). It comprises records of nearly 35,000 slaving voyages between 1514 and 1866, listing the country of origin, the voyage itinerary, number of slaves transported and other details. Unfortunately, the data on Portuguese and Spanish slave trade is only reasonably complete after 1750.

4. Results

4.1. Chronology

A large number of radiocarbon dates on bone, charcoal and shells have consistently dated Amoreira to the Late Mesolithic, ca. 6500–5000 cal BCE (Cunha and Cardoso, 2001; Roksandic, 2006; Meiklejohn et al., 2009; Bicho et al., 2013; Peyroteo-Stjerna, 2021).

To estimate the chronology of the lifetime of the individual we modelled the bone collagen uncalibrated ^{14}C age of 292 ± 32 years BP (Ua-58726) (See Online [Supplementary Material](#), S2). The posterior density estimate for the chronological model places the lifetime of mug019 between cal CE 1529 and 1763 (95.4% credible interval) although a 93.7% credible interval is constrained between 1631 and 1763 CE (Fig. 2).

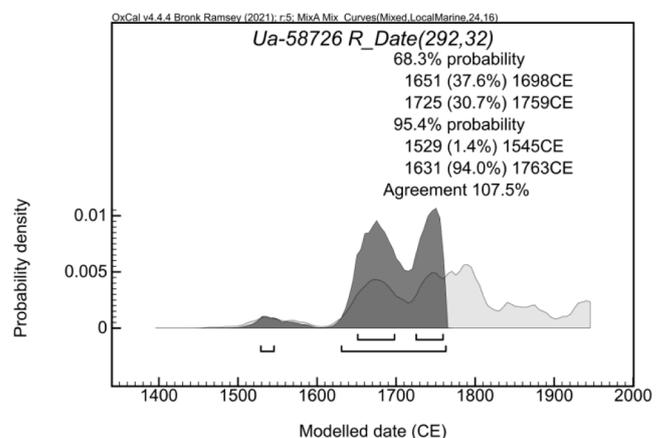


Fig. 2. Probability density function for the lifetime chronology of the analysed individual modelled using OxCal 4.4 (Bronk Ramsey, 2009).

4.2. Archaeological record

The remains of mug019 were excavated ca. 40 cm from the top layer of the shell midden on October 1st, 1930. It was described by M. Corrêa as an adult male, laid on the lateral side with the lower limbs in flexion close to the upper body. The cranium was fragmented, and bones of the forearm were found near the cranium, suggesting that at least one of the upper limbs laid near the head at the time of burial.

The excavators reported that these remains were better preserved than those of two other skeletons excavated that year, and that the individual's height was greater than the average for the individuals excavated in the Muge shell middens, suggesting an outlier to the series. According to Corrêa's description, the floor of the grave was sandy and sloping. Although sand is naturally available at the site, sandy grave floors were not observed in other burials excavated at the same depth, indicating possible preparation of the floor of the grave. As is the case with most burials in the Muge shell middens, the skeletal remains were not found in association with grave goods or other material culture.

4.3. Genetic analysis

The ratio of Y to X chromosome coverage revealed that mug019 was a male. His mitochondrial haplogroup, L3b1a, is widespread in Africa and is one of the most common L3 subclades in West and Central Africa (Soares et al., 2012), as well as one of the most common lineages in the Lake Chad Basin (Cerezo et al., 2021). His Y-chromosome lineage, E1b1a, is the most frequent in sub-Saharan Africa. It is commonly found in Nigeria, Congo, Cameroon, Gabon, (Berniell-Lee et al., 2009; Montano et al., 2011), Guinea-Bissau (Carvalho et al., 2011), and Bantu-speakers in Southern Africa (Naidoo et al., 2010). Hence, both uniparental genetic markers point to an African ancestry.

Principal Component Analysis shows that, from a panel of worldwide modern populations, this individual has higher genomic affinity with present-day West African populations and specifically with Bantu-speaking populations (Fig. 3A, Fig. S3.2). We formally tested which modern African populations are genetically closest to the studied individual using outgroup- f_3 statistics, obtaining higher scores for Mandenka and Gambian, followed by other North and West African populations (Fig. 3B).

Unsupervised model-based genetic clustering (using ADMIXTURE) based on individuals from African and European populations show well-known ancestry components (Schlebusch and Jakobsson, 2018). When assuming five clusters ($K = 5$), genetic components maximized in Europeans, South, West, East and North African populations are

differentiated. The genetic ancestry pattern of mug019 is similar to that found in individuals of the Mandenka and Gambian present-day West African populations (Fig. 4). In fact, his clustering patterns are consistent with Mandenka and Gambian individuals throughout all choices of the number of ancestry components (Fig. S3.3).

We investigated several genetic regions known to govern specific traits of interest. We identified the FY*B allele, which is often found in association with the Duffy null allele (McManus et al., 2017), indicating some level of resistance to malaria. Lactase persistence alleles were not found, suggesting the individual was lactose intolerant. Skin pigmentation variation in human populations are complex traits influenced by several genes (Feng et al., 2021). We ascertained that mug019 exhibits gene variants affecting skin pigmentation that are found in higher frequencies in African populations than in Europeans (or even absent in European populations) (e.g., MFSD12 rs10424065; DDB1 rs11230664; OCA2 rs1800404; SLC45A2 rs16891982; HERC2 rs6497271; see Table S3.2). However, these genes only account for a small fraction of the phenotypic variation (Crawford et al., 2017) which in addition to the limited sequencing coverage of mug019 hinders a more robust assessment of this phenotype.

4.4. Stable isotopes, diet, and place of origin

The results of stable isotope measurements on bone collagen were -7.3‰ and $+9.0\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Bayesian dietary estimates (Fig. 5) show that C_4 plants were the main caloric source ($60 \pm 17\%$) followed by marine contributions ($24 \pm 16\%$). The results clearly show that the contributions from C_3 plants ($9 \pm 7\%$) and C_3 terrestrial animal products ($8 \pm 7\%$) were comparatively minor. However, there is some overlap in estimates, particularly for 95% credible ranges, arising from model uncertainties and the overlap in food isotopic signals such as those for C_4 plants and low trophic level marine foods (e.g., bivalve molluscs).

Additional dietary information is given by $\delta^{13}\text{C}$ measurements on bone bioapatite, although this is susceptible to diagenetic overprint (Fernandes et al., 2013). The result of the $\delta^{13}\text{C}$ measurement on bone bioapatite (-4.3‰) is clearly C_4 /marine and is in accordance with the bone collagen results (Lee-Thorp et al., 1989).

Overall, the typical post-Medieval diet in Portugal and Spain throughout the Modern period was based on locally produced C_3 foods (Curto et al., 2019; MacKinnon et al., 2019; Sarkic et al., 2019), thus suggesting a foreign origin of this individual, to a region where C_4 crops were readily available. In West Africa, traditional plant food-producing systems have relied on three long-established crop zones (Harris, 1976):

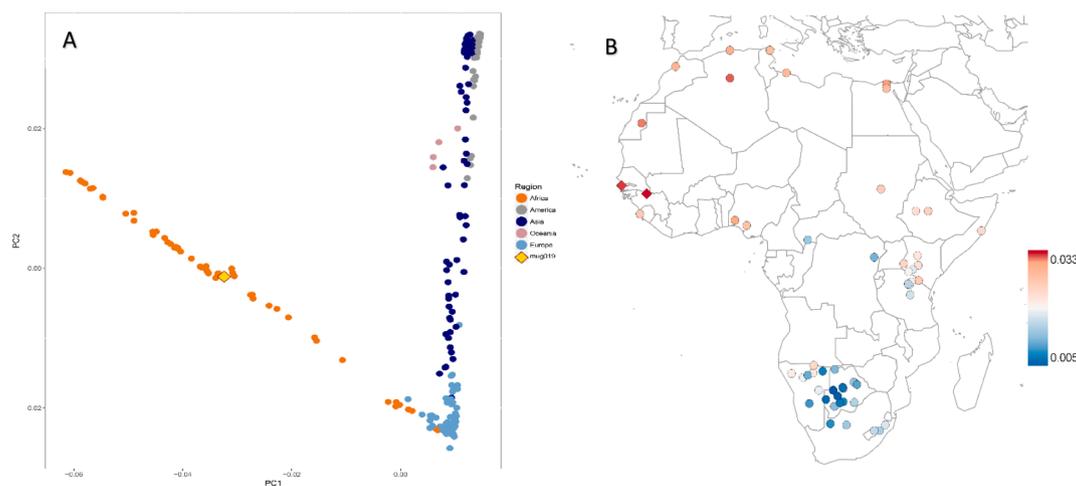


Fig. 3. A. Principal component analysis. Worldwide modern populations (circles coloured according to continent) and mug019 projected as a yellow, red outlined diamond. B. Geographic distribution of the genetic affinity of the studied individual with modern African populations, measured by outgroup- f_3 . The two highest f_3 scores are depicted with diamonds.

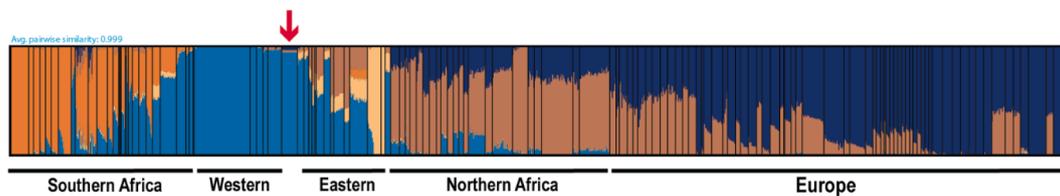


Fig. 4. Genetic clustering analysis at K = 5, the red arrow marks mug019.

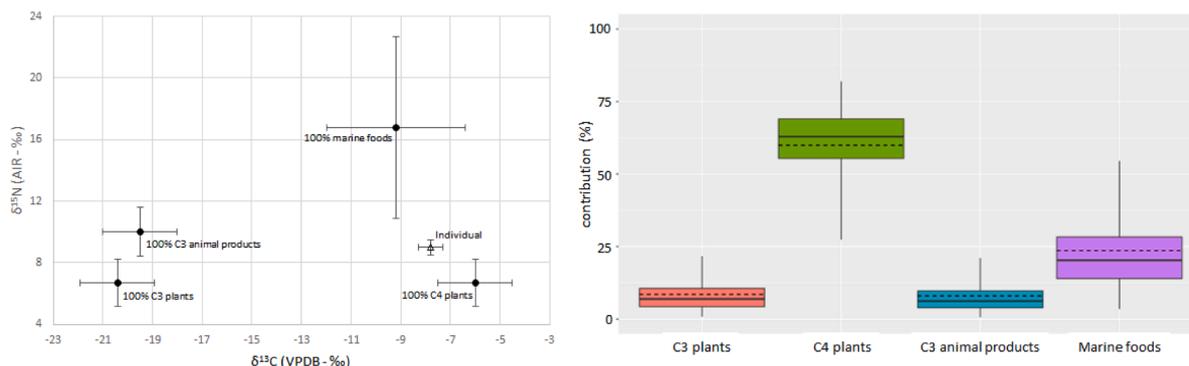


Fig. 5. Dietary estimates. (A) Stable isotope values for the analysed individual and endpoints assuming full reliance on a food source. (B) Bayesian estimates of food source contribution. Boxes represent a 68% credible interval while whiskers represent a 95% credible interval. The horizontal lines represent the mean estimate whereas the dotted horizontal lines represent the median estimates.

the sorghum-millet zone (C₄ plants) which extends over the interior West Africa from the southern limits of the Sahara to the northern margins of the forest zone, the rice zone (C₃ plants) in the humid forest zone west of the Bandama River in Ivory Coast, and the vegetural zone (C₃ plants) in the eastern forest zone. These results corroborate that mug019 lived in West Africa, directing the next steps of our analyses to this region.

The dietary agreement between bone collagen and bioapatite δ¹³C measurements suggests that isotopic signals in bioapatite have been well-preserved and that bone bioapatite δ¹⁸O still reflects the δ¹⁸O value of ingested water at the place of origin. Bayesian spatial estimates based on the dietary analysis and DNA results indicate a non-Iberian place of origin, which can be further narrowed to West Africa. Our measurement of δ¹⁸O_{carbonate} (−3.0‰ VPDB) when converted into values of ingested water (δ¹⁸O_{water} = −4.0‰ SMOW) and compared with the spatial distribution of δ¹⁸O for precipitation (Fig. 6, left) show that the highest probability densities for a place of origin are observed for the coastal areas of Senegal and Mauritania followed closely by Gambia (Fig. 6, right). A relatively high probability area is also observed for southeast of our study region.

In summary, the bone collagen and carbonate isotopic data suggest that the studied individual resided in Africa for several years before

arriving to Portugal, somewhere in the coastal areas of western Africa, in present-day Mauritania, Senegal and Gambia (Fig. 7).

4.5. Historical record

The Trans-Atlantic Slave Trade Database indicates West Africa, and in particular Guinea-Bissau and Gambia, as the principal region of slave purchase for slaves transported to Portugal between the sixteenth and eighteenth centuries. Other places of purchase include Cape Verde islands, Princes Island and São Tomé, as well as Bance Island (Sierra Leone), Gold Coast (Ghana), Senegambia and offshore Atlantic and Whydah (Benin) (Table S3.3).

We identified seven males documented as slaves, former slaves, or of African ancestry in the Parish burial records (Table S3.4). Most entries report slave burials in the churchyard, in accordance with Catholic practices. However, other methods that have been elsewhere documented for the disposal of slaves, or former slaves, were not typically registered by the church. Two entries required a closer inspection since the place of burial or circumstances of death could describe the burial of mug019. First, an entry dating to May 5th, 1633, lists the burial of a slave which, unlike in most accounts, the burial location is not specified. Second, an account from Nov 1st, 1676, mentions the murder of a young

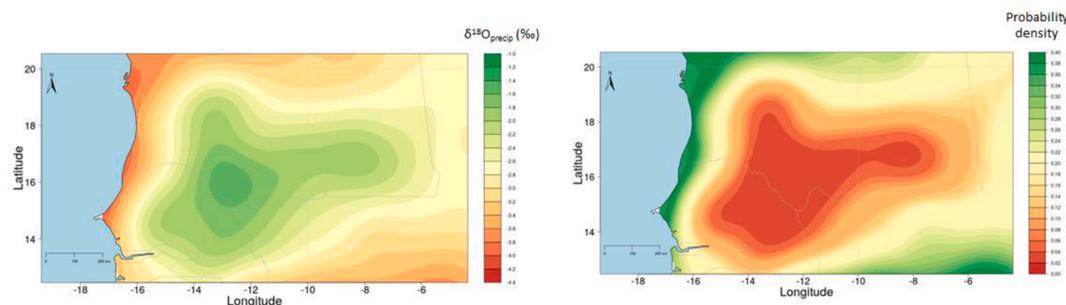


Fig. 6. Left panel shows the output of the model AverageR for the distribution of δ¹⁸O_{precip} values within the West African region. Right panel shows the probability density for place of origin generated by the model LocateR.

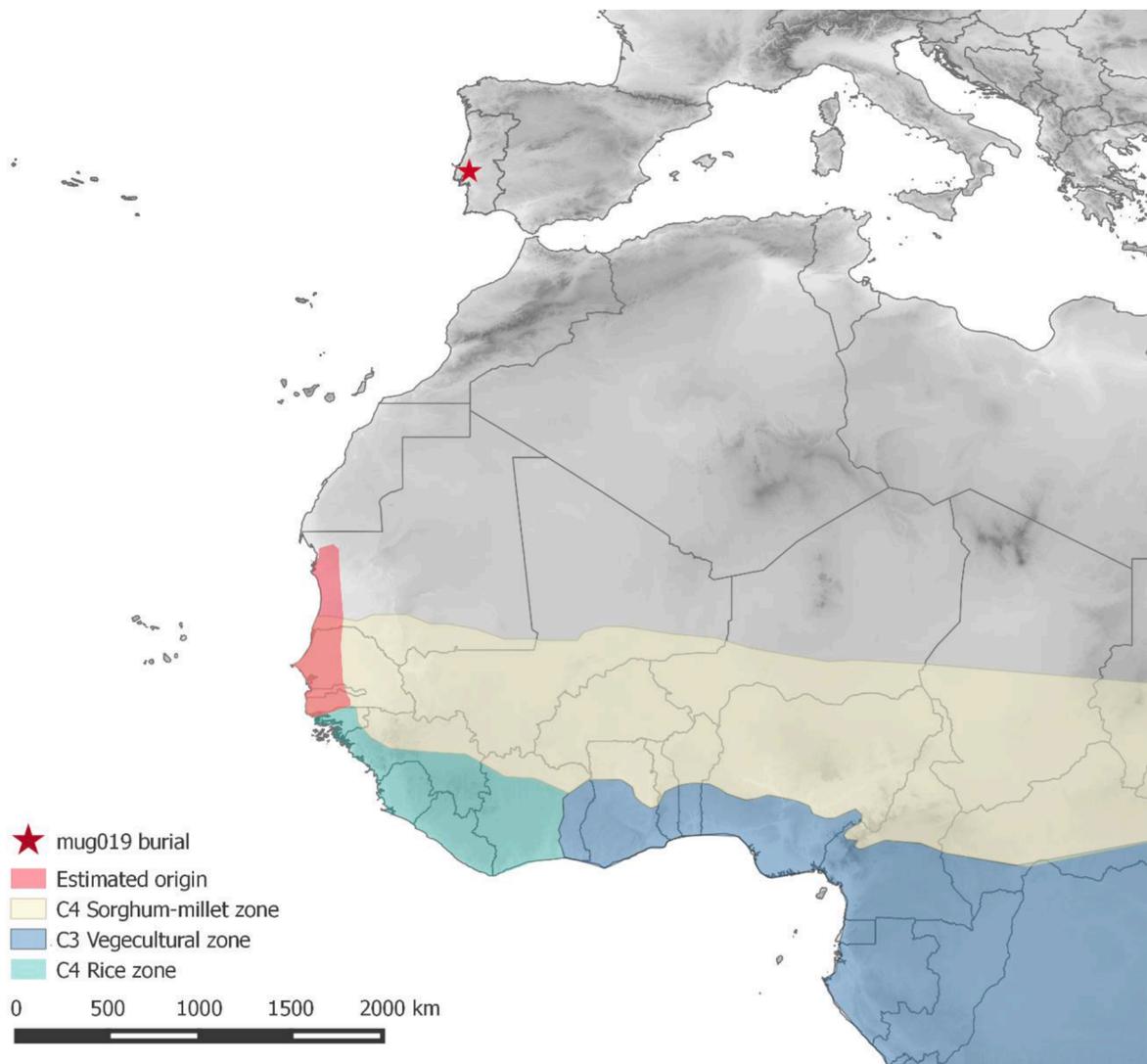


Fig. 7. Estimated area of origin of mug019 in West Africa and place of burial in Portugal. Traditional plant food-producing systems in West Africa adapted from Harris 1976, Fig. 4.

man named João at Arneiro da Amoreira, which is precisely the area where the shell midden is located, and where mug019 was found. The victim is described as dun or brown (Portuguese: *pardo*), and that he was buried in the churchyard. His social status is not mentioned (Fig. S3.3).

5. Discussion

Our combined biomolecular results present strong evidence supporting the discovery of the remains of a man of African ancestry buried sometime between the sixteenth and the eighteenth centuries in the shell midden of Cabeço da Amoreira, Muge, Portugal, who was originally from Senegambia. At this time, the slave trade was the primary force bringing Africans to Europe. We identify a strong genetic affinity with modern-day West African individuals. Even though historical records point to equatorial West Africa as the main place of slave purchase during this period (Lahon and Alves, 1999), our genome-wide analysis indicates lower affinity with populations from equatorial and sub-equatorial West Africa, such as present-day Nigerians or Angolans. Instead, his origin in Senegambia is in line with records of shipments of slaves arriving in Portugal during those centuries, departing from Guinea-Bissau and Gambia (Slave Voyages: The Trans-Atlantic Slave Trade Database, 2020). However, the trade centres may not always represent the locations where slaves were originally captured.

Additionally, the genetics-geography association is limited by the distribution of reference populations available in the comparative data set, and also assumes that current population distribution patterns still resemble those of four centuries ago.

Isotopic data are consistent with the genetic results. His diet was predominantly based on C₄ plants and marine foods to some extent, consistent with traditional diets on the coastal semi-arid region of Africa where he probably lived for several years before arriving to Portugal. A person born in Portugal would not be expected to show such a dietary pattern, since C₄ foods were not commonly consumed in Portugal at the time. On the other hand, C₃ plants are also dominant in West Africa's tropical, humid regions (such as at the vegeticultural belt or the Rice Coast), which could have been his region of origin according to historical records. We further circumscribe his native region using oxygen stable isotopes data, which shows higher probabilities for a place of origin located in coastal regions, in agreement with the indication of consumption of marine foods.

We could not unequivocally identify this individual in the Parish Registers documentation. This could be explained by the incompleteness, lack of detail or even accuracy of the registers. While the documented brown or dun male was murdered in the same location as the investigated burial, the registers refer that the victim was buried in the churchyard. Furthermore, the described skin colour of the murdered

man could have been used to describe an interracial individual, but our results show that mug019 had unadmixed African ancestry.

For more than three centuries, Africans were brutally dislocated to Portugal from their homeland and families while forced to adopt a new religion, a new name, and a new language. In 1761, Portugal initiated the process of abolishing slavery with a decree determining that all new captives landing in Portugal should be liberated upon arrival (National Archive Torre do Tombo, 2020), yet the number of slaves remained high until complete abolishment in 1836–1869 (Henriques, 2019). Portuguese written records, illustrations and paintings from the sixteenth century onwards provide evidence of strategies developed by African communities to preserve their socio-cultural identity and values (Henriques, 2019), as also documented in the Americas (Domingues da Silva and Misevich, 2018). Catholic festivities were celebrated with African music instruments, dance and traditional clothing, alongside the worship of ancestral spirits and deities. The reinvention of ancient practices in a new setting was particularly clear in urban neighbourhoods that provided the required concealment for the maintenance of African socio-cultural practices related to birth, marriage, and death (Henriques, 2019). The burial of this man in an 8000-years old site could be another example of the maintenance of African cultural beliefs and practices by African people translocated to Europe, even though this practice is not documented in the historical records. Despite its simplicity, the grave seems to have been arranged with a layer of sand, suggesting a level of preparation for a burial in a seemingly deviant place. It is noteworthy that up to present day, shell middens are actively used in western Africa. In Senegambia in particular, the usage of shell middens includes ancient and modern cemeteries (Hardy et al., 2016). Like many other archaeological sites, Amoreira was probably known by the local populations as an ancient burial ground, given the abundance of animal and human bones at the site. Other examples of non-Christian funerary practices have been identified in a cemetery of enslaved people in the Canary Islands, explained by a less strict control by the church and Spanish crown than in the mainland (Santana et al., 2016). This is not the case in Amoreira, which is relatively close to Lisbon and is owned by the nobility since at least the sixteenth century. However, the burial of this individual in a Portuguese shell midden could indicate the recognition of the site as a meaningful place by the African community of Amoreira, possibly according to West African socio-cultural traditions. Future investigations may clarify if this was an isolated event or part of a broader movement.

6. Conclusion

The identification and further investigation of a surprisingly recent burial event in a well-known Mesolithic shell midden was only possible with the application of a multidisciplinary approach integrating archaeology, history, and biomolecular methods. Despite the incompleteness of the human remains and the historical records, the intersection of several lines of investigation enabled the reconstruction of specific aspects of the life and death of a first-generation African individual in Portugal during the Trans-Atlantic Slave Trade period, which would not otherwise have been possible to scrutinise from the skeletal material in the archaeological context. Furthermore, the different lines of investigation converged to the same conclusion, conferring robustness and reproducibility to the results.

Overall, this study demonstrates the power of biomolecular archaeology to retrieve information from highly fragmentary evidence, but more importantly, it shows the value of multidisciplinary research to investigate individual African life-histories in Early Modern Europe which have been obscured in large-scale studies.

Authors statement

This manuscript or a very similar manuscript has not been published, nor is under consideration by any other journal.

The authors declare no conflict of interest.

Acknowledgements

We thank the Museum of Natural History and Science University of Porto for assistance and access to the collections and the Portuguese Infrastructure of Scientific Collections (PRISC.pt). The Bergman Foundation on Fårö for sponsoring writing retreat. Alice Toso for providing relevant references. Arielle R. Munters for the initial processing of the genetic data. Federico Sanchez-Quinto, Mario Vicente and Cesar Fortes-Lima for insight on genetic data analysis and discussions.

This work was supported by the Knut and Alice Wallenberg foundation and the Swedish Research Council (VR, grant number 2018-05537). The genetic sequencing was performed at the SNP&SEQ Technology platform from the National Genomic Infrastructure (NGI) Uppsala, and computations were conducted using Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jasrep.2022.103370>.

References

- Adler, C.J., Haak, W., Donlon, D., Cooper, A., 2011. Survival and recovery of DNA from ancient teeth and bones. *J. Archaeol. Sci.* 38 (5), 956–964. <https://doi.org/10.1016/j.jas.2010.11.010>.
- Alexander, D.H., Novembre, J., Lange, K., 2009. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* 19 (9), 1655–1664. <https://doi.org/10.1101/gr.094052.109>.
- Álvarez, M., Briz Godino, I., Balbo, A., Madella, M., 2011. Shell middens as archives of past environments, human dispersal and specialized resource management. *Quat. Int.* 239 (1–2), 1–7. <https://doi.org/10.1016/j.quaint.2010.10.025>.
- Berniell-Lee, G., Calafell, F., Bosch, E., Heyer, E., Sica, L., Mouguiama-Daouda, P., van der Veen, L., Hombert, J.-M., Quintana-Murci, L., Comas, D., 2009. Genetic and Demographic Implications of the Bantu Expansion: Insights from Human Paternal Lineages. *Mol. Biol. Evol.* 26 (7), 1581–1589.
- Bicho, N., Cascalheira, J., Marreiros, J., Gonçalves, C., Pereira, T., Dias, R., 2013. Chronology of the Mesolithic occupation of the Muge valley, central Portugal: The case of Cabeço da Amoreira. *Quat. Int.* 308–309, 130–139.
- Bronk Ramsey, C., 2009. Bayesian Analysis of Radiocarbon Dates. *Radiocarbon* 51 (1), 337–360. <https://doi.org/10.1017/S0033822200033865>.
- Caldeira, A.M., 2013. Escravos e traficantes no Império Português: o comércio negro português no Atlântico durante os séculos XV a XIX. *A Esfera dos Livros*, Lisboa.
- Cardoso, J.L., Rolão, J., 2000. Prospecções e escavações nos concheiros mesolíticos de Muge e Magos (Salvaterra de Magos): contribuição para a história dos trabalhos arqueológicos efectuados. *Estudos Arqueológicos de Oeiras* 8, 88–240.
- Carvalho, M., Brito, P., Bento, A.M., Gomes, V., Antunes, H., Costa, H.A., Lopes, V., Serra, A., Balsa, F., Andrade, L., Anjos, M.J., Corte-Real, F., Gusmão, L., 2011. Paternal and maternal lineages in Guinea-Bissau population. *Forensic Sci. Int. Genet.* 5 (2), 114–116. <https://doi.org/10.1016/j.fsigen.2010.10.007>.
- Casa Cadaval. 2020. Accessed July 24. <https://casacadaval.pt/historia>.
- Cerezo, M., V. Černý, A. Carracedo & A. Salas, 2011. New Insights into the Lake Chad Basin Population Structure Revealed by High-Throughput Genotyping of Mitochondrial DNA Coding SNPs. *PLOS ONE* 6. Public Library of Science: e18682. <https://doi.org/10.1371/journal.pone.0018682>.
- Crawford, N.G., Kelly, D.E., Hansen, M.E.B., Beltrame, M.H., Fan, S., Bowman, S.L., Jewett, E., Ranciaro, A., Thompson, S., Lo, Y., Pfeifer, S.P., Jensen, J.D., Campbell, M.C., Beggs, W., Hormozdiari, F., Mpoloka, S.W., Mokone, G.G., Nyambo, T., Meskel, D.W., Belay, G., Haut, J., Rothschild, H., Zon, L., Zhou, Y.i., Kovacs, M.A., Xu, M., Zhang, T., Bishop, K., Sinclair, J., Rivas, C., Elliot, E., Choi, J., Li, S.A., Hicks, B., Burgess, S., Abnet, C., Watkins-Chow, D.E., Oceana, E., Song, Y.S., Eskin, E., Brown, K.M., Marks, M.S., Loftus, S.K., Pavan, W.J., Yeager, M., Chanock, S., Tishkoff, S.A., 2017. Loci associated with skin pigmentation identified in African populations. *Science* 358 (6365). <https://doi.org/10.1126/science.aan8433>.
- Cubas, M., Lucquin, A., Robson, H.K., Colonese, A.C., Arias, P., Aubry, B., Billard, C., Jan, D., Diniz, M., Fernandes, R., Fábregas Valcarce, R., Germain-Vallée, C., Juhel, L., de Lombera-Hermida, A., Marcigny, C., Mazet, S., Marchand, G., Neves, C., Ontañón-Peredo, R., Rodríguez-Álvarez, X.P., Simões, T., Zilhão, J., Craig, O.E., 2020. Latitudinal gradient in dairy production with the introduction of farming in Atlantic Europe. *Nat. Commun.* 11 (1) <https://doi.org/10.1038/s41467-020-15907-4>.
- Cunha, E., Cardoso, F., 2001. The osteological series from Cabeço Da Amoreira (Muge, Portugal) La série ostéologique de Cabeço Da Amoreira (Muge, Portugal). *bmsap* 13 (3–4). <https://doi.org/10.4000/bmsap10.4000/bmsap.611210.4000/bmsap.6231>.

- Cunha, E., Cardoso, F., 2003. New data on Muge shell middens: a contribution to more accurate numbers and dates. *Estudos Arqueológicos de Muge I*, 171–184.
- Cunha, E., Umbelino, C., 1997. Abordagem antropológica das comunidades mesolíticas dos Concheiros do Sado. *O Arqueólogo Português* 13–15 (4), 161–179.
- Curto, A., Maurer, A.-F., Barrocas-Dias, C., Mahoney, P., Fernandes, T., Fahy, G.E., 2019. Did military orders influence the general population diet? Stable isotope analysis from Medieval Tomar, Portugal. *Archaeol. Anthropol. Sci.* 11 (8), 3797–3809. <https://doi.org/10.1007/s12520-018-0637-3>.
- Dabney, J., Knapp, M., Glocke, I., Gansauge, M.-T., Weihmann, A., Nickel, B., Valdiosera, C., Garcia, N., Paabo, S., Arsuaga, J.-L., Meyer, M., 2013. Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl. Acad. Sci.* 110 (39), 15758–15763. <https://doi.org/10.1073/pnas.1314445110>.
- Diniz, M., Arias, P., Teira, L., Araújo, A., Cubas, M., Neves, M., Nukushina, D., Rocha, L., Cerillo, E. & Fernández Sánchez, P., 2014. Relatório de progresso. Os últimos caçadores-recolectores e os primeiros grupos agro-pastoris no vale do Sado. Estudo arqueológico num contexto regional (SADO-MESO): Poças de S. Bento, Arapouco e Vale de Romeiras (Alcácer do Sal). Ano 4, 2013. Unpublished report, Lisboa: Direção-Geral do Património Cultural.
- Domingues da Silva, D.B., Misevich, P., 2018. Atlantic Slavery and the Slave Trade: History and Historiography, in Oxford Research Encyclopedia. African History Oxford University Press. <https://doi.org/10.1093/acrefore/9780190277734.013.371>.
- Feng, Y., McQuillan, M.A., Tishkoff, S.A., 2021. Evolutionary genetics of skin pigmentation in African populations. *Hum. Mol. Genet.* 30, R88–R97. <https://doi.org/10.1093/hmg/ddab007>.
- Fernandes, R., 2016. A Simple(R) Model to Predict the Source of Dietary Carbon in Individual Consumers. *Archaeometry* 58 (3), 500–512. <https://doi.org/10.1111/arc.12193>.
- Fernandes, R., Hüls, M., Nadeau, M.-J., Grootes, P.M., Garbe-Schönberg, C.-D., Hollund, H.L., Lotnyk, A., Kienle, L., 2013. Assessing screening criteria for the radiocarbon dating of bone mineral. *Nucl. Instrum. Methods Phys. Res., Sect. B* 294, 226–232. <https://doi.org/10.1016/j.nimb.2012.03.032>.
- Fernandes, R., M.-J. Nadeau & P.M. Grootes, 2014a. EDTA based protocols for the cleaning of ancient bone bioapatite., in *Proceedings of the 39th International Symposium for Archaeometry*: 73–78. Leuven.
- Fernandes, R., Millard, A.R., Brabec, M., Nadeau, M.-J., Grootes, P., Bondioli, L., 2014b. Food Reconstruction Using Isotopic Transferred Signals (FRUITS): A Bayesian Model for Diet Reconstruction. *PLoS ONE* 9 (2), e87436. <https://doi.org/10.1371/journal.pone.0087436>.
- Fonseca, J., 2010. *Escravos e Senhores na Lisboa Quinhentista*. Edições Colibri, Lisboa.
- Green, R.E., Malaspina, A.-S., Krause, J., Briggs, A.W., Johnson, P.L.F., Uhler, C., Meyer, M., Good, J.M., Maricic, T., Stenzel, U., Prüfer, K., Siebauer, M., Burbano, H. A., Ronan, M., Rothberg, J.M., Egholm, M., Rudan, P., Brajković, D., Kučan, Ž., Gusić, I., Wikström, M., Laakkonen, L., Kelso, J., Slatkin, M., Pääbo, S., 2008. A complete Neandertal mitochondrial genome sequence determined by high-throughput sequencing. *Cell* 134 (3), 416–426. <https://doi.org/10.1016/j.cell.2008.06.021>.
- Hardy, K., Camara, A., Piqué, R., Dioh, E., Guèye, M., Diadiou, H.D., Faye, M., Carré, M., 2016. Shellfishing and shell midden construction in the Saloum Delta, Senegal. *J. Anthropol. Archaeol.* 41, 19–32. <https://doi.org/10.1016/j.jaa.2015.11.001>.
- Harris, D.R., 1976. Traditional Systems of Plant Food Production and the Origins of Agriculture in West Africa, in J.R. Harlan (ed.) *Origins of African Plant Domestication*: 311–56. De Gruyter, Inc.; ProQuest Ebook Central. <http://ebookcentral.proquest.com/lib/uu/detail.action?docID=3040488>.
- Henriques, I.C., 2019. A presença Africana em Portugal, uma História secular: preconceito, integração, reconhecimento (séculos XV-XX). República Portuguesa. Secretária de Estado para a Cidadania e Igualdade, Lisboa.
- Higgins, D., Kaidonis, J., Townsend, G., Hughes, T., Austin, J.J., 2013. Targeted sampling of cementum for recovery of nuclear DNA from human teeth and the impact of common decontamination measures. *Invest. Genet.* 4 (1), 18. <https://doi.org/10.1186/2041-2223-4-18>.
- Jackes, M., Meiklejohn, C., 2008. The paleodemography of central Portugal and the Mesolithic-Neolithic transition. In: Bocquet-Appel, J.-P. (Ed.), *Recent Advances in Paleodemography: Data*. Springer Science+Business Media B.V, Techniques, Patterns, pp. 209–258.
- Korneliussen, T.S., Albrechtsen, A., Nielsen, R., 2014. ANGSD: Analysis of Next Generation Sequencing Data. *BMC Bioinf.* 15, 356. <https://doi.org/10.1186/s12859-014-0356-4>.
- Laffoon, J.E., Espersen, R., Mickleburgh, H.L., 2018. The Life History of an Enslaved African: Multiple Isotope Evidence for Forced Childhood Migration from Africa to the Caribbean and Associated Dietary Change. *Archaeometry* 60 (2), 350–365. <https://doi.org/10.1111/arc.12354>.
- Lahon, D., Alves, I., 1999. O negro no coração do império: uma memória a resgatar, séc. XV-XIX. Secretariado Entreculturas, Lisboa.
- Lazaridis, I., Patterson, N., Mittnik, A., Renaud, G., Mallick, S., Kirsanow, K., Sudmant, P. H., Schraiber, J.G., Castellano, S., Lipson, M., Berger, B., Economou, C., Bollongino, R., Fu, Q., Bos, K.I., Nordenfelt, S., Li, H., de Filippo, C., Prüfer, K., Sawyer, S., Posth, C., Haak, W., Hallgren, F., Fornander, E., Rohland, N., Delsate, D., Francken, M., Guinet, J.-M., Wahl, J., Ayodo, G., Babiker, H.A., Bailliet, G., Balanovska, E., Balanovsky, O., Barrantes, R., Bedoya, G., Ben-Ami, H., Bene, J., Berrada, F., Bravi, C.M., Brisighelli, F., Busby, G.B.J., Cali, F., Churnosov, M., Cole, D.E.C., Corach, D., Damba, L., van Driem, G., Dryomov, S., Dugoujon, J.-M., Fedorova, S.A., Gallego Romero, I., Gubina, M., Hammer, M., Henn, B.M., Hervig, T., Hodoiglul, U., Jha, A.R., Karachanak-Yankova, S., Khusainova, R., Khusnutdinova, E., Kittles, R., Kivisild, T., Klitz, W., Kucinskas, V., Kushniarevich, A., Laredj, L., Litvinov, S., Loukidis, T., Mahley, R.W., Melegh, B., Metspalu, E., Molina, J., Mountain, J., Näkkäläjärvi, K., Nesheva, D., Nyambo, T., Osipova, L., Parik, J., Platonov, F., Posukh, O., Romano, V., Rothhammer, F., Rudan, I., Ruizbakiev, R., Sahakyan, H., Sajantila, A., Salas, A., Starikovskaya, E.B., Tarekgn, A., Toncheva, D., Turdikulova, S., Uktveryte, I., Utevska, O., Vasquez, R., Villena, M., Voevoda, M., Winkler, C.A., Yepiskoposyan, L., Zalloua, P., Zemanek, T., Cooper, A., Capelli, C., Thomas, M.G., Ruiz-Linares, A., Tishkoff, S.A., Singh, L., Thangaraj, K., Vilems, R., Comas, D., Sukernik, R., Metspalu, M., Meyer, M., Eichler, E.E., Burger, J., Slatkin, M., Pääbo, S., Kelso, J., Reich, D., Krause, J., 2014. Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature* 513 (7518), 409–413.
- Lee-Thorp, J.A., Sealy, J.C., van der Merwe, N.J., 1989. Stable carbon isotope ratio differences between bone collagen and bone apatite, and their relationship to diet. *J. Archaeol. Sci.* 16 (6), 585–599. [https://doi.org/10.1016/0305-4403\(89\)90024-1](https://doi.org/10.1016/0305-4403(89)90024-1).
- Li, H., Durbin, R., 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25 (14), 1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>.
- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* (Oxford, England) 25 (16), 2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>.
- MacKinnon, A.T., Passalacqua, N.V., Bartelink, E.J., 2019. Exploring diet and status in the Medieval and Modern periods of Asturias, Spain, using stable isotopes from bone collagen. *Archaeol. Anthropol. Sci.* 11 (8), 3837–3855. <https://doi.org/10.1007/s12520-019-00819-2>.
- Martiniano, R., Coelho, C., Ferreira, M.T., Neves, M.J., Pinhasi, R., Bradley, D.G., 2014. Genetic Evidence of African Slavery at the Beginning of the Trans-Atlantic Slave Trade. *Sci. Rep.* 4, 5994. <https://doi.org/10.1038/srep05994>.
- McManus, K.F., A.M. Taravella, B.M. Henn, C.D. Bustamante, M. Sikora & O.E. Cornejo, 2017. Population genetic analysis of the DARC locus (Duffy) reveals adaptation from standing variation associated with malaria resistance in humans. *PLOS Genetics* 13. Public Library of Science: e1006560.
- Meiklejohn, C., Roksandic, M., Jackes, M., Lubell, D., 2009. Radiocarbon dating of Mesolithic human remains in Portugal. *Mesolithic Miscellany* 20, 4–16.
- Meyer, M., Kircher, M., 2010. Illumina Sequencing Library Preparation for Highly Multiplexed Target Capture and Sequencing. *Cold Spring Harbor Protocols* 2010, 1–11. <https://doi.org/10.1101/pdb.prot5448>.
- Montano, V., Ferri, G., Marcari, V., Batini, C., Anyaele, O., Destro-Bisol, G., Comas, D., 2011. The Bantu expansion revisited: a new analysis of Y chromosome variation in Central Western Africa. *Mol. Ecol.* 20, 2693–2708. <https://doi.org/10.1111/j.1365-294X.2011.05130.x>.
- Naidoo, T., Schlebusch, C.M., Makkan, H., Patel, P., Mahabeer, R., Erasmus, J.C., Soodyall, H., 2010. Development of a single base extension method to resolve Y chromosome haplogroups in sub-Saharan African populations. *Invest. Genet.* 1 (1), 6. <https://doi.org/10.1186/2041-2223-1-6>.
- National Archive Torre do Tombo, 2020 *Arquivo Nacional Torre do Tombo. DGLAB. Direção-Geral do Livro, dos Arquivos e das Bibliotecas*. Accessed June 3. <https://digitar.q.arquivos.pt/details?id=4662332>.
- Paço, A., 1938. *Novos concheiros do Vale do Tejo*. *Brotéria* 27, 66–75.
- Patterson, N., P. Moorjani, Y. Luo, S. Mallick, N. Rohland, Y. Zhan, T. Genschoreck, T. Webster & D. Reich, 2012. Ancient Admixture in Human History. *Genetics* 192. <https://doi.org/10.1534/genetics.112.145037>.
- Paula e Oliveira, F., 1888. *Nouvelles fouilles faites dans les kiokkenmoedings de la vallée du Tage* (posthumous publication). *Comunicações da Comissão dos Trabalhos Geológicos* 2, 57–81.
- Peyroteo Stjerna, R., 2016. On Death in the Mesolithic: Or the Mortuary Practices of the Last Hunter-Gatherers of the South-Western Iberian Peninsula, 7th–6th Millennium BCE. Uppsala University, Uppsala. PhD Thesis.
- Peyroteo Stjerna, R., 2021. Chronology of the burial activity of the last hunter-gatherers in the Southwestern Iberian Peninsula, Portugal. *Radiocarbon* 63 (1), 265–299.
- Queiroz, F., Rugg, J., 2003. The development of cemeteries in Portugal c.1755-c.1870. *Mortality* 8 (2), 113–128. <https://doi.org/10.1080/1357627031000087370>.
- Rasmussen, M., Guo, X., Wang, Y., Lohmueller, K.E., Rasmussen, S., Albrechtsen, A., Skotte, L., Lindgreen, S., Metspalu, M., Jombart, T., Kivisild, T., Zhai, W., Eriksson, A., Manica, A., Orlando, L., De La Vega, F.M., Tridico, S., Metspalu, E., Nielsen, K., Ávila-Arcos, M.C., Moreno-Mayar, J.V., Muller, C., Dortch, J., Gilbert, M. T.P., Lund, O., Wesolowska, A., Karmin, M., Weinert, L.A., Wang, B.O., Li, J., Tai, S., Xiao, F., Hanihara, T., van Driem, G., Jha, A.R., Ricaut, F.-X., de Knijff, P., Migliano, A.B., Gallego Romero, I., Kristiansen, K., Lambert, D.M., Brunak, S., Forster, P., Brinkmann, B., Nehlich, O., Bunce, M., Richards, M., Gupta, R., Bustamante, C.D., Krogh, A., Foley, R.A., Lahr, M.M., Balloux, F., Sicheiritz-Pontén, T., Vilems, R., Nielsen, R., Wang, J., Willerslev, E., 2011. An Aboriginal Australian Genome Reveals Separate Human Dispersals into Asia. *Science* (New York) 334 (6052), 94–98.
- Roche, J., 1965. Note sur la stratigraphie de l'amas coquillier mésolithique de Cabeço de Amoreira (Muge). *Comunicações dos Serviços Geológicos de Portugal* 48, 191–199.
- Roksandic, M., 2006. Analysis of Burials from the New Excavations of the Sites Cabeço da Amoreira and Cabeço da Arruda (Muge, Portugal). In: Bicho, N., Carvalho, A.F. (Eds.), *Actas do IV Congresso de Arqueologia Peninsular, Promontória Monográfica 4*. Universidade do Algarve, Faro, pp. 1–10.
- Sánchez-Quinto, F., Schroeder, H., Ramirez, O., Ávila-Arcos, María C., Pybus, M., Olalde, I., Velazquez, A.V., Marcos, M., Encinas, J., Bertranpetit, J., Orlando, L., Gilbert, M.T., Lalueza-Fox, C., 2012. Genomic Affinities of Two 7,000-Year-Old

- Iberian Hunter-Gatherers. *Curr. Biol.* 22 (16), 1494–1499. <https://doi.org/10.1016/j.cub.2012.06.005>.
- Santana, J., Fregel, R., Lightfoot, E., Morales, J., Alamón, M., Guillén, J., Moreno, M., Rodríguez, A., 2016. The early colonial atlantic world: New insights on the African Diaspora from isotopic and ancient DNA analyses of a multiethnic 15th–17th century burial population from the Canary Islands, Spain. *Am. J. Phys. Anthropol.* 159 (2), 300–312. <https://doi.org/10.1002/ajpa.22879>.
- Santos, M.F., J. Rolão & M. Marques. 1990. Duas novas jazidas Epipaleolíticas do Baixo Tejo: nºs 1 e 2 do Vale da Fonte da Moça (Almeirim), sua exploração arqueológica e salvaguarda., in *I Congresso do Tejo. Que Tejo, que futuro? Lisboa, 1987*: 33–38. Associação dos Amigos do Tejo.
- Sarkic, N., López, J.H., López-Costas, O., Grandal-d'Anglade, A., 2019. Eating in silence: isotopic approaches to nuns' diet at the convent of Santa Catalina de Siena (Belmonte, Spain) from the sixteenth to the twentieth century. *Archaeol. Anthropol. Sci.* 11, 3895–3911. <https://doi.org/10.1007/s12520-018-0734-3>.
- Saunders, A.C. de C.M. 1982. *A social history of black slaves and freedmen in Portugal, 1441-1555* (Cambridge Iberian and Latin American Studies). Cambridge: Cambridge University Press.
- Sawyer, S., J. Krause, K. Guschanski, V. Savolainen & S. Pääbo, 2012. Temporal Patterns of Nucleotide Misincorporations and DNA Fragmentation in Ancient DNA. *PLOS ONE* 7. Public Library of Science: e34131. <https://doi.org/10.1371/journal.pone.0034131>.
- Schlebusch, C.M., Jakobsson, M., 2018. Tales of Human Migration, Admixture, and Selection in Africa. *Annu. Rev. Genomics Hum. Genet.* 19, 405–428.
- Schlebusch, C.M., Malmström, H., Günther, T., Sjödin, P., Coutinho, A., Edlund, H., Munters, A.R., Vicente, M., Steyn, M., Soodyall, H., Lombard, M., Jakobsson, M., 2017. Southern African ancient genomes estimate modern human divergence to 350,000 to 260,000 years ago. *Science* 358 (6363), 652–655.
- Schroeder, H., Ávila-Arcos, M.C., Malaspina, A.-S., Poznik, G.D., Sandoval-Velasco, M., Carpenter, M.L., Moreno-Mayar, J.V., Sikora, M., Johnson, P.L.F., Allentoft, M.E., Samaniego, J.A., Havisser, J.B., Dee, M.W., Stafford, T.W., Salas, A., Orlando, L., Willerslev, E., Bustamante, C.D., Gilbert, M.T.P., 2015. Genome-wide ancestry of 17th-century enslaved Africans from the Caribbean. *Proc. Natl. Acad. Sci.* 112 (12), 3669–3673. <https://doi.org/10.1073/pnas.1421784112>.
- Skoglund, P., Storå, J., Götherström, A., Jakobsson, M., 2013. Accurate sex identification of ancient human remains using DNA shotgun sequencing. *J. Archaeol. Sci.* 40 (12), 4477–4482. <https://doi.org/10.1016/j.jas.2013.07.004>.
- Slave Voyages: The Trans-Atlantic Slave Trade Database. 2020. Database *Slave Voyages*. Accessed May 13. <https://www.slavevoyages.org/>.
- Soares, P., Alshamali, F., Pereira, J.B., Fernandes, V., Silva, N.M., Afonso, C., Costa, M.D., Musilova, E., Macaulay, V., Richards, M.B., Cerny, V., Pereira, L., 2012. The Expansion of mtDNA Haplogroup L3 within and out of Africa. *Mol. Biol. Evol.* 29 (3), 915–927. <https://doi.org/10.1093/molbev/msr245>.
- Tombo.pt, Portuguese parish records or genealogy. 2020. Database *Tombo.pt*. Accessed May 26. <https://tombo.pt/en/f/smg03>.
- van Oven, M., Van Geystelen, A., Kayser, M., Decorte, R., Larmuseau, M.H.D., 2014. Seeing the wood for the trees: a minimal reference phylogeny for the human Y chromosome. *Hum. Mutat.* 35 (2), 187–191. <https://doi.org/10.1002/humu.22468>.
- Vianello, D., Sevini, F., Castellani, G., Lomartire, L., Capri, M., Franceschi, C., 2013. HAPLOFIND: a new method for high-throughput mtDNA haplogroup assignment. *Hum. Mutat.* 34 (9), 1189–1194. <https://doi.org/10.1002/humu.22356>.
- Villalba-Mouco, V., van de Loosdrecht, M.S., Posth, C., Mora, R., Martínez-Moreno, J., Rojo-Guerra, M., Salazar-García, D.C., Royo-Guillén, J.I., Kunst, M., Rougier, H., Crevecoeur, I., Arcusa-Magallón, H., Tejedor-Rodríguez, C., García-Martínez de Lagrán, I., Garrido-Pena, R., Alt, K.W., Jeong, C., Schiffels, S., Utrilla, P., Krause, J., Haak, W., 2019. Survival of Late Pleistocene Hunter-Gatherer Ancestry in the Iberian Peninsula. *Curr. Biol.* 29 (7), 1169–1177.e7. <https://doi.org/10.1016/j.cub.2019.02.006>.
- Wang, X., Roberts, P., Tang, Z., Yang, S., Storozum, M., Groß, M., Fernandes, R., 2021. The Circulation of Ancient Animal Resources Across the Yellow River Basin: A Preliminary Bayesian Re-evaluation of Sr Isotope Data from the Early Neolithic to the Western Zhou Dynasty. *Front. Ecol. Evol.* 9 <https://doi.org/10.3389/fevo.2021.583301>.
- Wilkin, S., Ventresca Miller, A., Miller, B.K., Spengler, R.N., Taylor, W.T.T., Fernandes, R., Hagan, R.W., Bleasdale, M., Zech, J., Ulziibayar, S., Myagmar, E., Boivin, N., Roberts, P., 2020. Economic Diversification Supported the Growth of Mongolia's Nomadic Empires. *Sci. Rep.* 10 (1) <https://doi.org/10.1038/s41598-020-60194-0>.
- Yang, D.Y., Eng, B., Wayne, J.S., Dudar, J.C., Saunders, S.R., 1998. Technical note: improved DNA extraction from ancient bones using silica-based spin columns. *Am. J. Phys. Anthropol.* 105, 539–543. [https://doi.org/10.1002/\(SICI\)1096-8644\(199804\)105:4<539::AID-AJPA10>3.0.CO;2-1](https://doi.org/10.1002/(SICI)1096-8644(199804)105:4<539::AID-AJPA10>3.0.CO;2-1).