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Strengths and constraints in ivory provenance research: La Beleña (Córdoba, Spain) as a case study

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This study presents a multidisciplinary analysis of 119 osseous and ivory fragments from the necropolis of La Beleña (Córdoba, Spain) dating between the second half of the 4th and the beginnings of the 3rd Millennium BC, aiming to assess the effectiveness and limitations of current methods used for identifying ivory provenance in prehistoric contexts. Traditional macroscopic and microscopic techniques were supplemented with biomolecular analyses, including Zooarchaeology by Mass Spectrometry (ZooMS), Fourier Transform Infrared Spectroscopy (FTIR), and ancient DNA extraction (aDNA). Our results reveal significant challenges in raw material identification when based solely on morphological criteria, particularly in highly fragmented assemblages. FTIR analysis showed limited discriminatory capacity between ivory and bone, or between elephantid species. Ancient DNA yielded insufficient endogenous material for analysis. In contrast, ZooMS successfully identified all ivory samples as deriving from African elephants (*Loxodonta*) and corrected several prior misidentifications. Technological analysis indicates that both transverse and longitudinal tusk exploitation were used, with manufacturing techniques likely involving copper saws and bending. Ivory items were deposited as decorated finished objects, consistent with funerary practices at other Iberian Chalcolithic sites. These findings underscore the need to revise current protocols for ivory identification and provenance, and to expand open-access reference databases. The integration of biomolecular methods—especially proteomics—offers a more reliable path for tracing prehistoric exchange networks and understanding the cultural significance of ivory.

Keywords FTIR, ZooMS, aDNA, *Elephas*, *Loxodonta*, Late prehistory

The role of exchange and the circulation of objects, people, technologies and ideas during the later prehistory of the Iberian Peninsula remains a central theme in current archaeological discourse. These dynamics, particularly the networks formed through such interactions, are increasingly recognized as essential for understanding the development of prehistoric societies[1–3]. During the 3rd Millennium BC, there was a marked increase in interregional connectivity, shaping new social and economic structures across the Mediterranean. Interaction between communities in the western Maghreb and the Iberian Peninsula intensified significantly, far surpassing earlier periods[4]. Archaeological evidence attests to active exchange between both shores, mostly during the 3rd but also the 2nd Millennium BC, including ceramic and metallic objects of probable Iberian origin found along the North African coast—such as axes from Oued Akrech (Rabat) and an alabarda from the dolmen of Mers 5 in Tangier[5–7]. These artefacts have been recovered across the western Maghreb and into Algeria[8], underscoring sustained contact with the Iberian coast. In parallel, exotic materials such as ostrich eggshells and elephant ivory—traditionally linked to North African contacts—have been found in southern Iberia[6,9]. These items often co-occur with other exotic materials like amber, rock crystal, and jet, all of which held ideological significance and contributed to the construction of social and gender-based hierarchies[10]. Their co-presence suggests a complex web of exchange that extended beyond North Africa to other regions of the Iberian Peninsula and the

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wider Mediterranean. Some scholars have associated the use of such exotic materials with ceremonial practices akin to those documented in western Mediterranean contexts like Malta and Corsica[11].

Ivory and other exotic materials appear in the Iberian archaeological record as early as the Late Neolithic. This includes sites such as La Beleña—subject of the present study—as well as Portuguese sites like Leceia and Anta da Herdade da Capela, where multiple ivory objects have been documented[12–15]. Nevertheless, the presence of ivory in Neolithic contexts is much more limited than the exponential growth observed during the 3rd millennium BC, particularly in funerary contexts[16,17]. During the Chalcolithic (3100 – 2200 BC), the largest and most diverse ivory assemblages are concentrated in southern Iberia, particularly at sites such as Perdigões (Portugal), Valencina de la Concepción (Seville), Los Millares or Almizaraque (Almería), which—like some other contemporary sites— are located near the coast or major river systems. These sites may have functioned as gateways for the reception and redistribution of exotic goods inland. The ivory assemblages from these sites include highly specialised items such as carved vessels, dagger handles, decorated plaques, cylindrical boxes, bracelets, anthropomorphic and zoomorphic figurines, pins, beads, combs, buttons, and pendants[17–21]. In the Early and Middle Bronze Age (2250 – 1550 BC), ivory use declined both in geographic spread and typological diversity, being mostly confined to the eastern Iberian coast. Although more objects have been found numerically compared to the Chalcolithic[22], they are generally smaller in size, with buttons being the most common type, alongside occasional combs, beads, bracelets, and plaques[23–25].

The discovery of unworked ivory tusks at Perdigões and Valencina de la Concepción[17,18] suggests the arrival of raw material and the possible presence of local artisans. While some evidence points to local ivory craftsmanship[26,27], its extent remains unclear due to two key limitations: (1) the lack of chronological overlap between production debris and finished objects, and (2) the difficulty of identifying the chaîne opératoire(s) due to the absence of intermediate products, such as in-process elements[20]. However, some eastern Iberian sites, such as Illeta dels Banyets (Alicante), Fuente Álamo, El Argar (Almería), and La Mola d'Agres, provide evidence of workshop activity or semi-finished products[28–32]. These findings have long raised questions about the origin of Iberian ivory. Early hypotheses, based on stylistic comparisons and the presence of exotic imports, proposed North African or even Egyptian sources[5,33–36]. However, others contested these interpretations, noting the absence of elephant tusks in North African archaeological contexts[37]. The potential use of fossil ivory was also dismissed in early studies due to assumptions about its degradation and workability[5,38].

The term "ivory" encompasses a range of biological materials—including proboscidean dentine (e.g. *Elephas antiquus*, *Mammuthus*, *Loxodonta*) and other animal sources such as hippopotamus teeth[39–41]. This study will specify the zoological origin of each type rather than using the term generically. Moreover, other materials like bone and shell have historically been used as ivory substitutes, leading to frequent misidentifications[42,43]. The chemical composition of mammalian teeth is generally consistent[42–44], though morphological differences exist depending on species and tooth function[45,46]. Elephant tusks, for instance, are elongated incisors with a large pulp cavity and continuous cementum layer, characteristics that differentiate them from other teeth[44].

In response to global restrictions on the trade of endangered species, analytical techniques for ivory identification have evolved substantially. Initial identifications relied on Schreger line patterns[42,45,47], but recent studies have added new diagnostic features visible macroscopically or under low magnification, contributing to both proboscidean ivory identification and understanding of production techniques[20]. The ability to distinguish elephant species has become increasingly relevant in archaeological research as a proxy for reconstructing long-distance exchange networks. Recent advances in archaeometric techniques—including Zooarchaeology by Mass Spectrometry (ZooMS), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM-EDS), micro-CT, Raman spectroscopy, and ancient DNA—have significantly improved the resolution of these analyses[27,48,49].

Within this framework, we present the study of 119 osseous and ivory fragments from the necropolis of La Beleña (Córdoba, Spain), dating to the second half of the 4th and the beginnings of the 3rd Millennium BC, focusing on the limitations encountered when attempting to identify species—and thus provenance—through FTIR. These limitations are discussed in light of complementary methods such as ZooMS and aDNA, aiming to contribute to the refinement of analytical protocols for provenance studies and prehistoric exchange networks.

La Beleña Necropolis (Córdoba, Spain)

Hypogea in southern Iberia first emerged in the mid-4th millennium BC and continued, alongside other megalithic funerary expressions—such as dolmens, chamber and corridor tombs, gallery graves, and tholoi—throughout the 3rd millennium BC. Some researchers have hypothesized that this phenomenon may have reached the peninsula via the islands of the central Mediterranean, in a movement that arrived more or less simultaneously in Provence and the Iberian Peninsula[50].

La Beleña necropolis, located in southern Iberia (Cabra, Spain) (Fig. 1), was discovered accidentally in 1973 following the collapse of the dome of a hypogeum (Burial 1). Inside, human skeletal remains were recovered alongside a funerary assemblage comprising ceramics, knapped and polished stone tools, and personal ornaments. These materials were collected by the municipal archaeologist and deposited in the Archaeological Museum of Cabra[51–54]. In 2015, agricultural activity led to the discovery of a second hypogeum. Once again, the collapse of the central part of the dome exposed a remarkably well-preserved funerary context. This prompted the launch of a systematic research project aimed at identifying new structures and excavating five of them between 2015 and 2019. The project sought to conduct a comprehensive analysis of the tombs and their contents, including the diversity of burial practices, the composition of grave goods, a precise chronological framework for each structure, and osteo-biographical studies of the individuals inhumated[50,55].

With the exception of Tombs 2 and 6, the preservation state of the hypogeal structures is variable, primarily due to decades of agricultural modification and root activity from crops. Tombs 4 and 5 had partially lost their roofing and access corridors, while Tomb 3 preserved only the lower third of its construction. Despite these

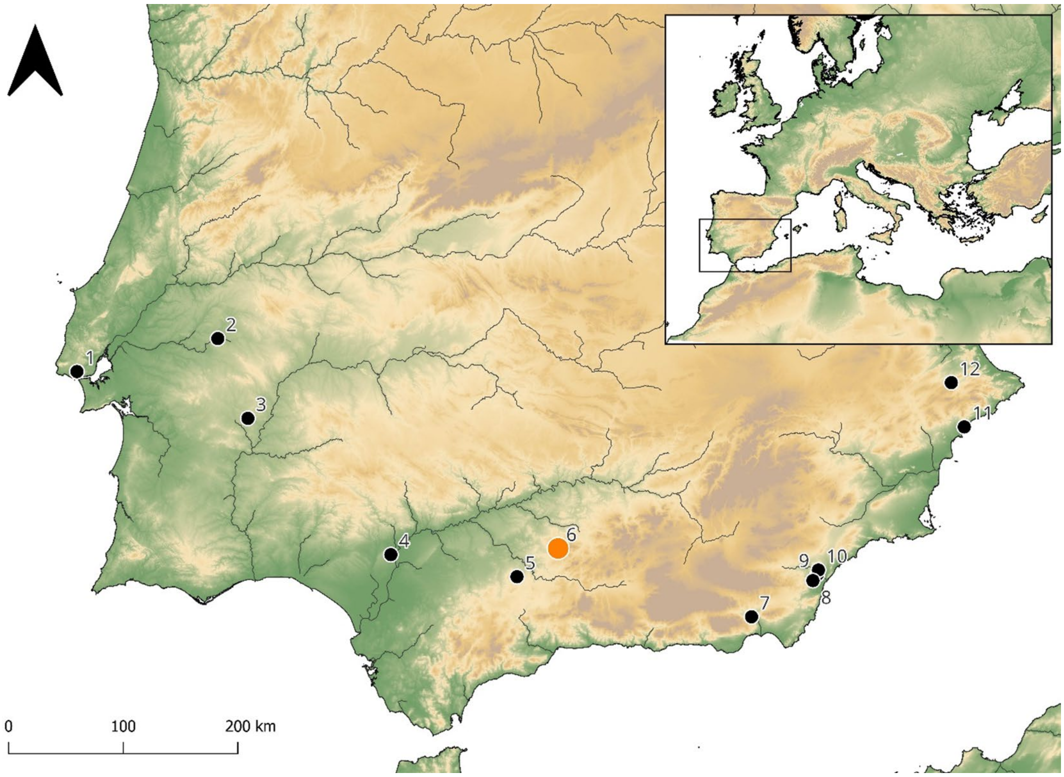

Fig. 1. Location of the archaeological sites mentioned in the text. (1) Leceia (2) Anta da Herdade (3) Perdigões (4) Valencina de la Concepción (5) La Molina (6) La Beleña (7) Los Millares (8) El Argar (9) Almizaraque (10) Fuente Álamo 11. Illeta del Banyets 12. La Mola d'Agres. The map was created by the authors using QGIS version 3.44.8 (https://www.qgis.org). Hypsometric representation was derived from digital elevation model data available from DIVA-GIS (https://diva-gis.org).

structural alterations, the integrity of the funerary depositional contexts remained intact. However, osteological preservation and certain grave goods were affected by root activity from successive vine and olive cultivation. Nonetheless, clear evidence of deliberate sealing events was identified, indicating the formal closure of these tombs and the cessation of their funerary use[55]. These features render La Beleña an exceptional archaeological site for examining the architectural and social complexity of hypogea and the communities that constructed and used them, within a clearly defined chronological framework spanning ca. 3400–2900 cal BC[50]. Each of the five excavated hypogea was cut into compact marl and shares a similar architectural layout: a circular or oval chamber with a corbelled dome and a short corridor oriented westward, providing access through a narrow entrance. This structural consistency contrasts with the internal spatial arrangements, which vary notably between tombs[55].

The funerary assemblages consist of multiple collective deposits, including primary inhumations—some of which exhibit deliberate alterations—and secondary manipulations such as cut marks, fragmentation, removal, and re-deposition of remains. These are accompanied by a diverse array of grave goods, including knapped and polished lithic tools, personal ornaments, ceramics, and items made from hard animal materials such as bone, shell, and ivory. The distribution of these materials varies across the tombs. Objects made from hard animal materials—particularly ivory, which is the focus of this study—are exclusively found in Tombs 2, 5, and 6, which also exhibit the most complex internal architectural designs. This same pattern applies to concave or slightly concave-based bifacially retouched arrowheads and the use of cinnabar. Aside from limited traces in Tomb 3, cinnabar is widely documented in Tombs 5 and 6, and to a lesser extent in Tomb 2 (Fig. 2).

Materials and methods

The assemblage under study comprises 119 osseous and ivory items, presenting varying degrees of fragmentation (Fig. 3; Supplementary Material 1). Initial classification was carried out through macroscopic observation following further sampling for molecular, proteomic and genomic analysis.

Optical microscopy characterisation

The study was conducted using a binocular magnifier and an optical microscope at the Antonio Arribas Palau Archaeometry Laboratory, Department of Prehistory and Archaeology, University of Granada. The aim of this initial macroscopic inspection was to classify the material according to raw material whenever possible, with particular emphasis on distinguishing proboscidean ivory from other materials.

The primary diagnostic feature used to identify proboscidean ivory (both extant and extinct taxa) is the Schreger pattern, the distinctive cross-hatched patterns visible in elephant, also referred to as Schreger lines or

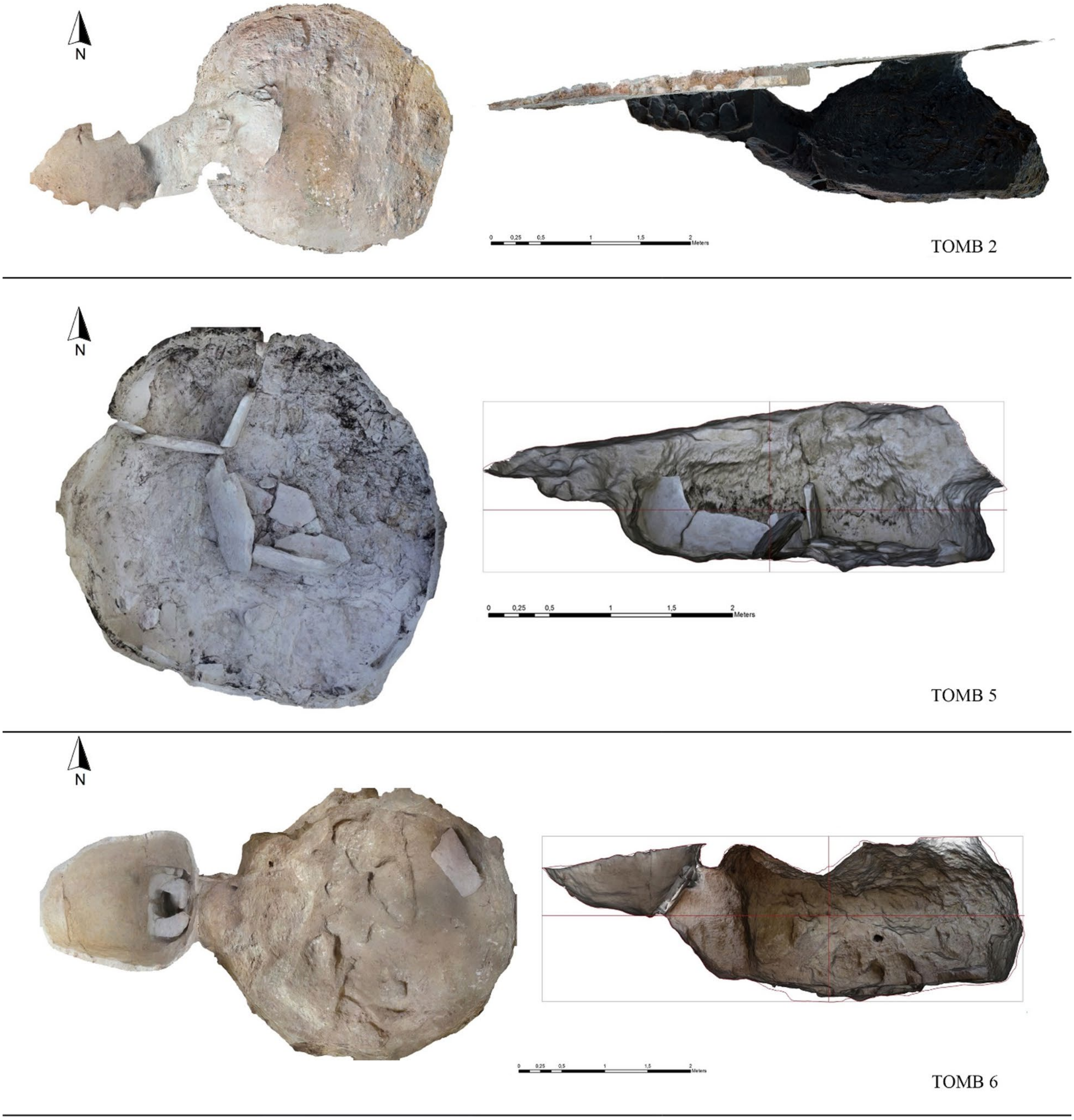

Fig. 2. Floor plan and section of Tombs 2, 5 and 6.

Schreger structure[46,47]. However, this feature is only visible in transverse sections of tusks and is therefore often absent in archaeological assemblages. Items were initially identified as ivory when a well-preserved Schreger structure was clearly visible. Pieces exhibiting an altered but still recognizable Schreger structure, expressed as an intertwined filaments crack-pattern or a column-like crack-pattern ("cracked"), were also classified as ivory. Conversely, items lacking any form of Schreger pattern but displaying a combination of other diagnostic features described by Luciañez-Triviño[20] were classified as probable ivory ("ivory?"). These features include: (a) a feather crack pattern combined with dark and light parallel lines formed by subtle grooves and parallel elevations; and (b) a feather crack pattern combined with a wavy surface pattern. Items exhibiting only dark and light parallel lines formed by subtle grooves and parallel elevations, or dark and light almost parallel longitudinal lines[19], were likewise provisionally classified as possible ivory ("ivory?"). Conversely, items displaying a porous trabecular microstructure characteristic of osseous tissue were excluded from the ivory category.

For the technological analysis, the material was first catalogued into five categories: blocks (primary block, secondary block, prepared block), blanks, preforms/roughouts, debris/production waste, and finished objects/end products. The description of surface technical traces and the general technological terminology follow

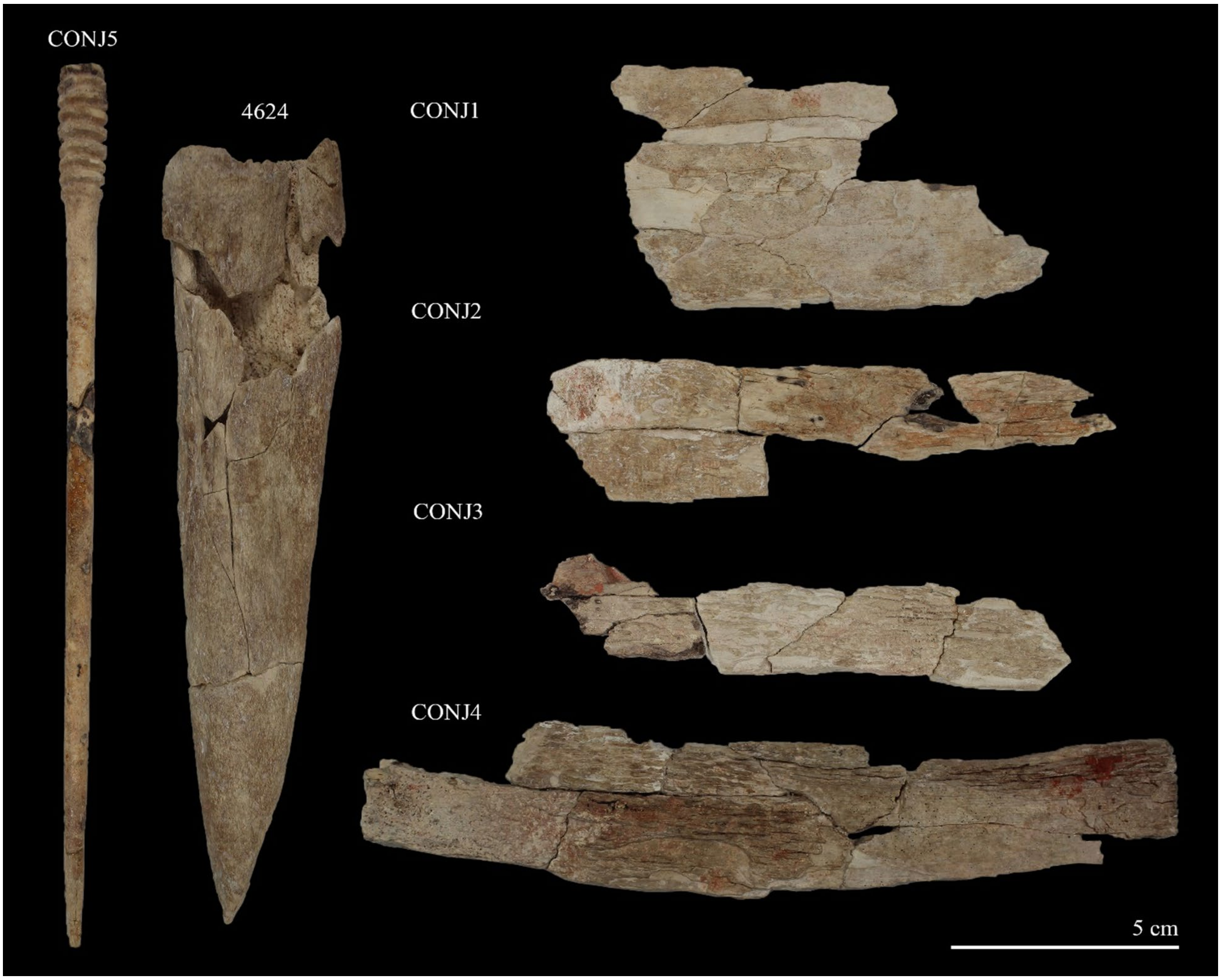

Fig. 3. Selection of analysed materials.

Averbouh and Provenzano[56] and the *Multilingual Lexicon of Bone Industries*[57]. Cataloguing and the definitions of finished objects and decorative elements are based on previous publications[19–21].

Zooarchaeology by mass spectrometry (ZooMS)

Due to the high degree of fragmentation, basic macroscopic identification of the raw material (bone vs. ivory) was not possible for all specimens. In uncertain cases, we applied ZooMS at the University of Tübingen to distinguish elephant from other taxa. We prioritized specimens previously identified as ivory or hippopotamus ivory that exhibited good preservation and provided sufficient material for sampling. Additionally, random samples identified as bone were included to verify the accuracy of the identifications. Nineteen samples were selected for ZooMS analysis (see Supplementary Material 1), employing collagen peptide markers—the most abundant protein in bone—to determine taxonomic origin. Collagen was extracted and purified following established ZooMS protocols[58]. In brief: ~20 mg subsample was demineralized in 300 µL 0.5 M HCl for 6 h at 4 °C. The supernatant was then removed and the pellets were rinsed 3 times using 0.5 M NH_4HCO_3 until a 7–8 pH was reached. The pellets were then incubated at 65 °C for 1 h, in 100 µL of 50 mM NH_4HCO_3. Following incubation, 50 µL supernatant was collected and was digested using 0.4 µg trypsin at 37 °C for 12 h. The digested samples were purified using C18 tips before spotting for the MALDI-TOF-MS analysis (Autoflex maX TOF, Bruker).

Mass range of 700–3500 m/z was collected.[58,59]. Each sample was spotted in triplicate with a-Cyano-4-hydroxycinnamic acid matrix onto a 384 MTP groundsteel plate and analysed using a Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometer (Bruker Autoflex). Analysis of the results was carried out on mMass (version 6.0.2[60,61]). The comparative reference library was compiled from previously published literature[58–65] following established nomenclature[66] and based on known species at the site and within the region.

Fourier transform infrared spectroscopy (FTIR)

Following the ZooMS results, 52 samples were selected for FTIR analysis, the most frequently applied technique in archaeological literature for distinguishing between elephantid species. FTIR is widely considered a valid proxy for determining the provenance of archaeological ivory artefacts[15,18,25,27,67–73]. The distinction between *Loxodonta* and *Elephas maximus* is based on the presence of a shoulder at ~1092 cm^{-1}[74]. In contrast, hippopotamus ivory is characterised by the absence of peaks at 1500 and 1700 cm^{-1} and by the presence of diagnostic peaks at 1020 and 870 cm^{-1}[71,72,73,75]. Samples previously identified as ivory were selected for analysis when preservation and sample mass were adequate. For control purposes, the dataset was supplemented with ten animal bone samples, one human canine and one human tibia. The human canine and tibia analysed in this study derive from the Panoria Necropolis (Granada, Spain). The site was excavated by the University of Granada and the remains are curated at the University of Granada (UGR). Consequently, no additional permits from other institutions were required to carry out the analyses. FTIR analyses were performed using a Jasco FTIR-4700 spectrometer with ATR module at the Antonio Arribas Palau Laboratory of the University of Granada. To avoid contamination from surrounding calcareous sediments, sampling was conducted on the internal section of each fragment. Samples were manually powdered using an agate mortar and analysed 50 times each with a spectral resolution of 4 cm^{-1} in the 4000–400 cm^{-1} range. Spectra were processed using Spectra Manager software.

To explore whether subtle variations could still be informative, we conducted a **Principal Component Analysis (PCA)** based on quantitative spectral indices derived from peak height ratios rather than absolute absorbance values. This approach minimised potential distortions due to sample mass, moisture content, or ambient CO_2 levels. We selected eight indices previously used in conservation and biomaterial studies[76–78]:

- **SF (Splitting Factor)**: $(A_{605}+A_{564})/A_{590}$.
- **C/C (Carbonyl/Carbonate ratio)**: A_{1455}/A_{1015}.
- **C/P (Carbonate/Phosphate ratio)**: A_{1415}/A_{1035}.
- **BPI (Type B carbonate/phosphate ratio)**: A_{1415}/A_{604}.
- **API (Type A carbonate/phosphate ratio)**: A_{1455}/A_{604}.
- **BC/AC (Type B/Type A carbonate ratio)**: A_{1415}/A_{1455}.
- **WAMPI (Water–Amide on Phosphate Index)**: A_{1640}/A_{605}.
- **Am/P (Amide/Phosphate ratio)**: A_{1645}/A_{1020}.

Ancient DNA sequencing

Twenty-four samples previously identified as ivory were collected for aDNA. These samples were selected because they provided sufficient material for analysis and exhibited superior preservation for genomic analysis to identify the elephant species. Analyses were conducted in a dedicated clean ancient DNA laboratory at the Lundbeck Foundation GeoGenetics Centre (Globe Institute, University of Copenhagen), following strict aDNA protocols: full-body protective suits, double gloves, and sterilisation with bleach, ethanol and UV exposure.

Surface decontamination of each sample involved a 10-minute bath in 3% bleach, followed by three washes in UV-sterilised distilled water. Samples were dried and pulverised with UV-treated mortars and pestles between uses. Between 50 and 150 mg of tooth ivory powder per sample was used for DNA extraction and library preparation, following the semi-automated protocol described in Barrie et al.[79]. In brief, the ivory powder was pre-digested for 30 min[80], and 150 µl of demineralized material was then combined with 1.5 ml binding buffer and 10 µl of paramagnetic beads for a total of 15 minutes[81]. Pelleted beads were washed twice in 80% ethanol + 20% 10mM Tris-HCl, respectively, and eluted in 10 mM Tris-HCl + 0.05% Tween-20. The DNA extract was incubated with USER enzyme for 3 h at 37 °C. Compared to the original protocol[79], we reduced the volume of USER enzyme from 10 µL to 2.5 µL, and substituted 7.5 µL with water. DNA shotgun sequencing libraries were prepared using the Meyer and Kircher[82] protocol in 50 µL total reaction volume, including 42.5 µl of DNA template. Clean-up procedures after end-repair and adapter-ligation were performed with 10 µl of paramagnetic beads in 10 volumes of the binding buffer. Indexing PCR was performed using 8-bp unique dual indexing (Illumina TruSeq UDI0001-0096) with KAPA HiFi HotStart Uracil+, and final purification was performed with HighPrep™ PCR beads.

Sequencing libraries were pooled in equimolar concentrations, including two negative controls, and sequenced on an Illumina NovaSeq6000 platform (S4 lane, 2 × 100 bp configuration) at the GeoGenetics Sequencing Core, Copenhagen. Sequence reads were mapped to the reference genome of the African savanna elephant (*Loxodonta africana*) including the nuclear LoxAfr4 genome[83] and a mitochondrial reference genome (GenBank accession NC_000934.1) using the GenErode pipeline as described in Kutschera et al.[84]. The quality of the samples (endogenous elephant DNA content) was assessed as the proportion of filtered reads mapping to the African savanna elephant reference with mapping quality of at least 30 out of the total sequenced reads.

Results

Optical microscopy and macroscopic identification of raw materials and technological traces

Optical microscopy allowed the identification of 25 ivory fragments and 6 additional fragments as potentially ivory. A total of 43 bone fragments and 7 probable bone pieces were also recorded, along with one red deer antler and one worked wild boar tusk. Additionally, 8 fragments were previously identified as hippopotamus (*Hippopotamus amphibius*) in an earlier report by A. Banerjee (Unpublished report requested in 2017 by the excavation directors (MDCM and DMS) and funded by the project. Avaliable upon request) (Fig. 4g, h). The remaining 36 fragments could not be identified due to severe deterioration and high fragmentation (ranging from 0.9 to 16 cm length), which resulted in the loss of key anatomical features necessary for classification.

As previously mentioned, following guidelines[42], and supported by other references[45,46], ivory fragments were identified primarily through the presence of Schreger lines[47]. In cases where Schreger structures were absent, the methodology[20] was applied to macro-identify proboscidean ivory and analyse manufacturing techniques (see Sect. 3.1.) (Fig. 4).

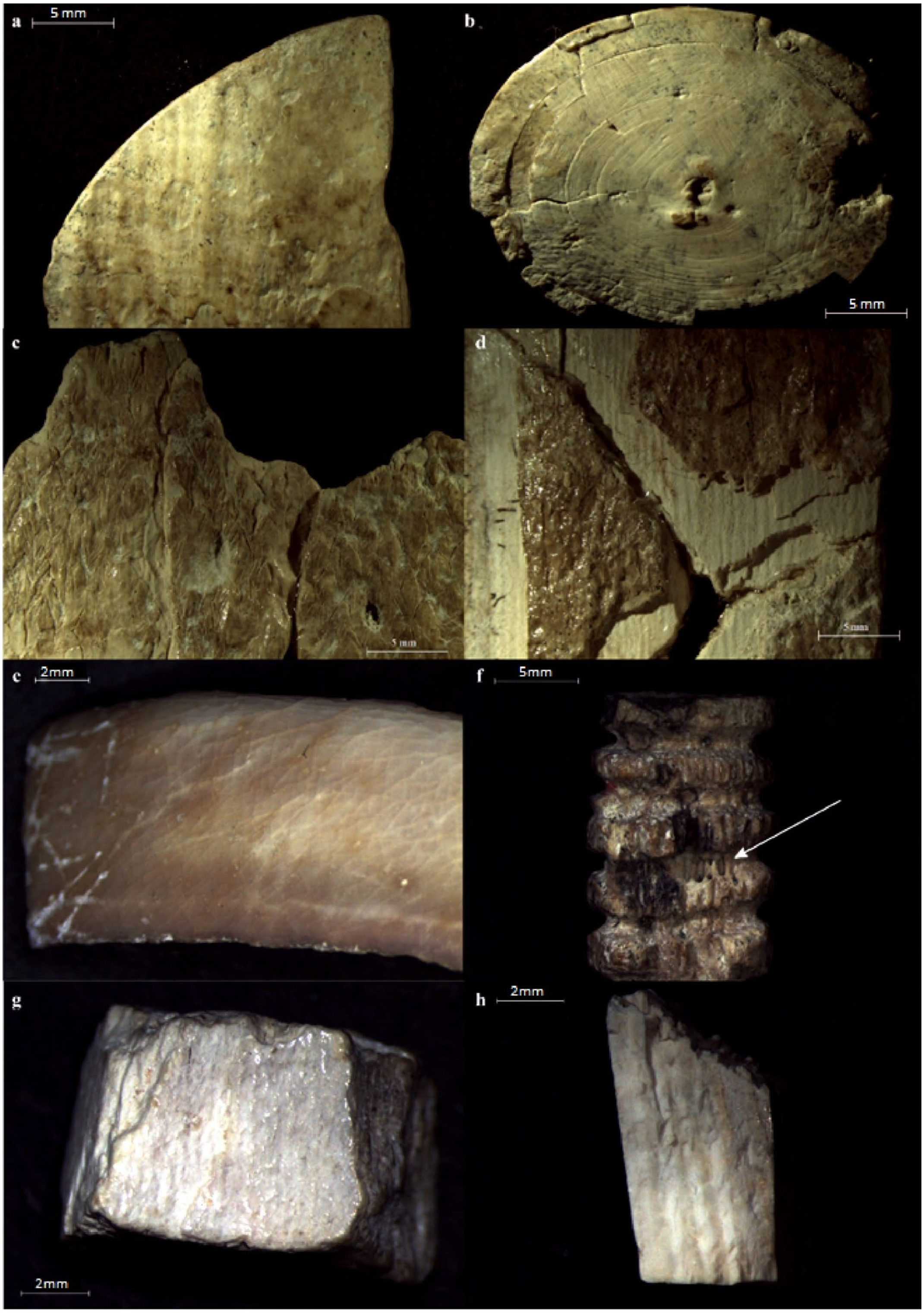

Fig. 4. Examples of fragments analysed under optical microscopy and the characteristic non-Schreger features observed in the La Beleña assemblage: (**a**) 8557, wavy surface pattern from the separation of outer dentine cones and the Cementum-Dentine Junction (CDJ)—natural tangential plane, (**b**) 8275, slightly separated growth cones—transverse section, (**c**) 1113, feather-like cracking pattern—tangential plane, (**d**) CONJ4, separation between dentine. See the dark and light parallel lines formed by subtle grooves and parallel elevations between them; —tangential plane, (**e**) 4673, wild boar tusk, crystalline and translucent appearance; (**f**) CONJ5, porous, trabecular structure indicative of bone tissue, (**g**) 1105, separation plane of two dentine cones—tangential plane, as in 4.d; (**h**) 0468-5, tangentially cut dentine cones, observed as dark and light almost parallel longitudinal lines—tangential or radial plane.

Parallel banding and undulating lamellae, resembling those described for hippopotamus tusk in reference images[42], suggested the potential presence of hippopotamus ivory in the assemblage. This interpretation had been previously proposed by Banerjee based on both visual features under microscopy and elemental composition results from SEM-EDS analysis. Overall, the ivory assemblage is in a poor state of preservation and highly fragmented, limiting the ability to determine the original number and types of artefacts. Technological analysis suggests that only finished objects were deposited at La Beleña, with a total estimated weight of 130 g (100 g confidently identified as ivory and 30 g as possibly ivory). No unworked blocks or large tusk segments were found. Several fragments appear to correspond to the curved walls of containers, although their precise form and typology cannot be determined. One piece (no. 8557) may represent a lid, similar to examples from the Montelirio tholos (Seville[21]. It is well documented that Chalcolithic ivory objects include a wide array of artefacts such as vessels, decorated plaques, handles, combs, and figurines[19,20,85,86].

Decorative and technical traces

Although preservation issues limit the extent of technological and decorative analysis, certain diagnostic features were observed. For example, piece no. 8275 (Fig. 5b) displays sawing striations consistent with peripheral cutting using a metal saw, evidenced by parallel, straight, and fine lines. Overlapping sets of striations reflect changes in sawing angle (Fig. 5b). This technique was likely employed to section a distal segment of a relatively thin tusk (approximately 3 cm in diameter).

Decorative motifs include parallel incised lines (e.g., pieces 8211, 8110, and Conjunto 2; Fig. 6a and b), as well as zigzag incisions on two fragments (8551a and 8551b; Fig. 6c and d). Thirteen fragments also exhibit cinnabar residues on their surface. These may be post-depositional in origin and not necessarily intentional decoration, as cinnabar was also documented on slabs, grave goods and human remains (Fig. 6e and f).

Technological traces provide insight into manufacturing techniques. Piece no. 8275 shows clear signs of transverse exploitation through peripheral sawing, followed by bending to complete the separation. This method, known as *tronçonnage*, involved cutting around the perimeter of the tusk with a metal blade, then breaking it off with a controlled flexion. Evidence of this is seen in a nearly circular pedicle at the centre of the cut surface (Fig. 5a, c). This indicates that both transverse and longitudinal exploitation strategies were employed at La Beleña[20]. However, due to the fragmented nature of the assemblage, many fragments likely belong to the same original items and are no longer recognisable. Morphological analysis suggests that most items were manufactured from flat, plaque-like blanks, implying a predominant use of longitudinal segmentation, as observed in other Chalcolithic sites.

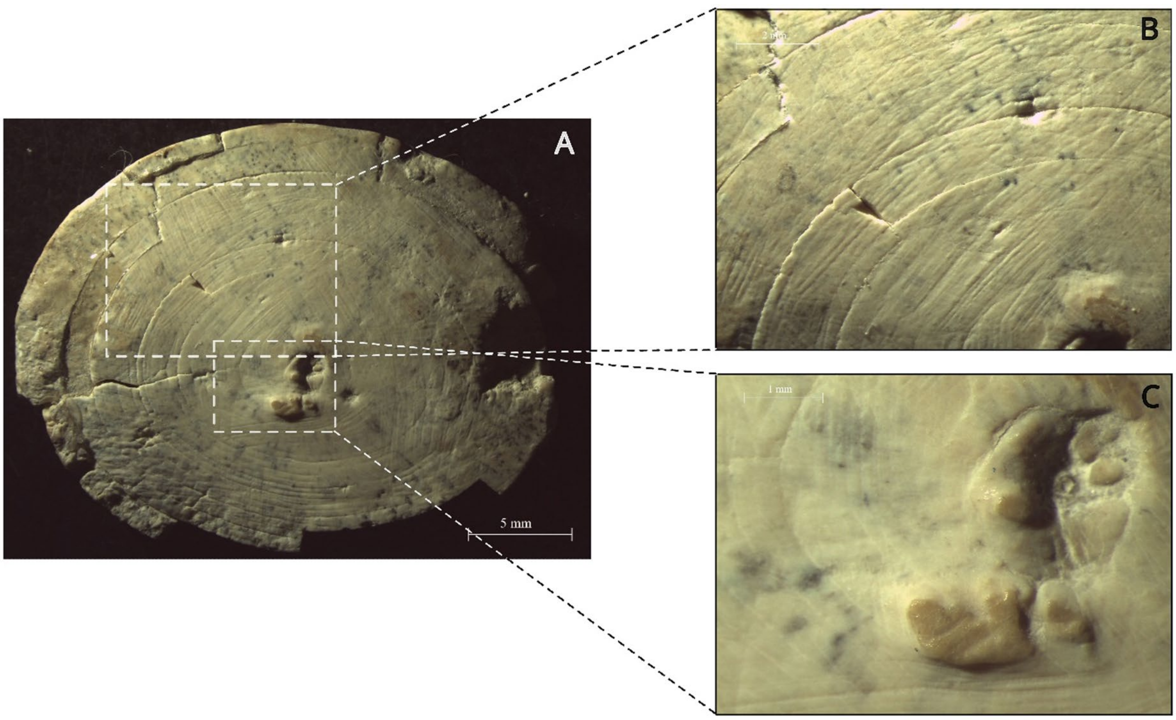

Fig. 5. (**a**) Piece no. 8275. (**a**) Sawing traces and pedicle resulting from transverse segmentation using a metal saw. (**b**) Detail of the parallel, straight, fine sawing striations. Overlapping striation sets indicate changes in the sawing angle. (**c**) Close-up view of the pedicle at the center of the cut surface, providing evidence of the final separation of the fragments through controlled flexion.

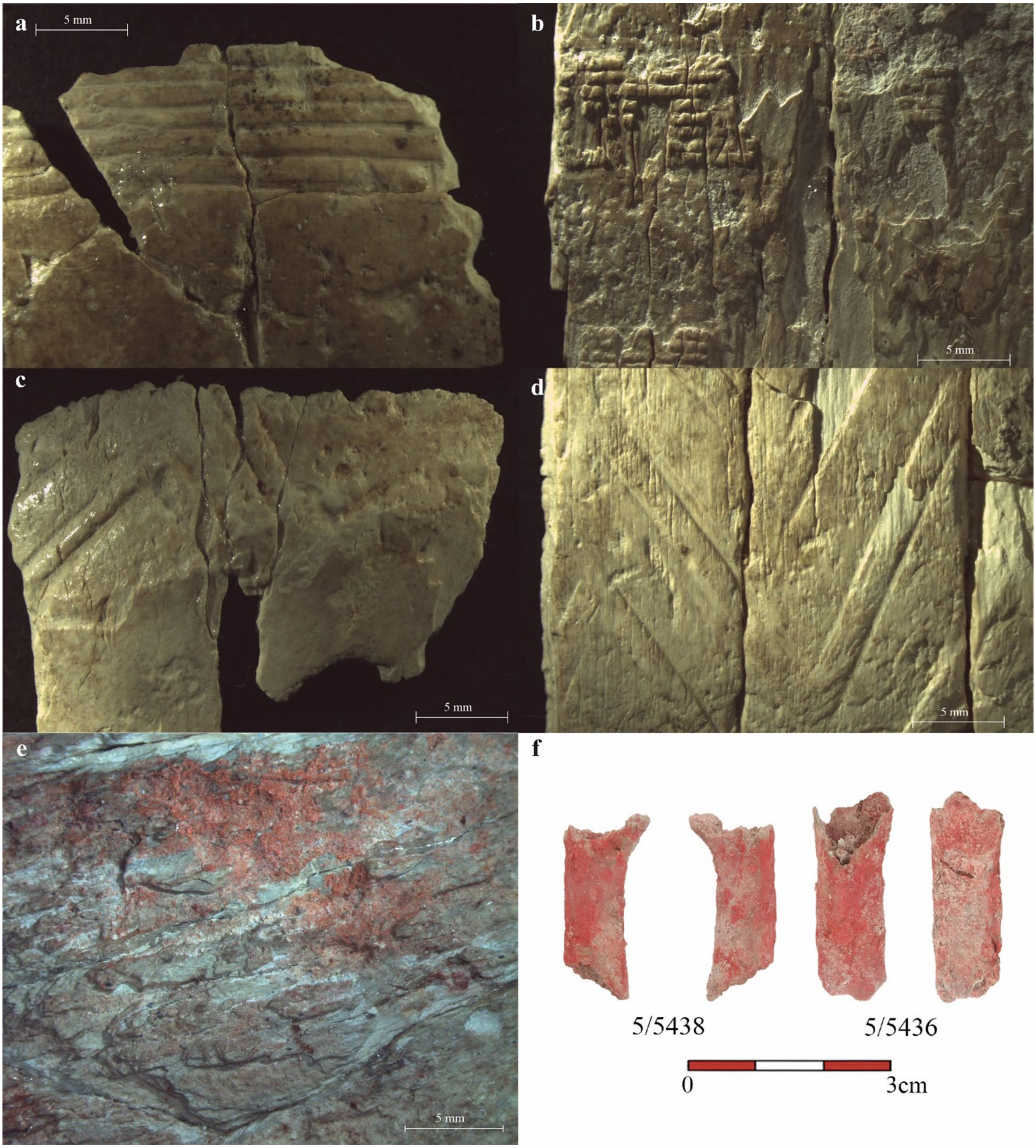

Fig. 6. Decoration observed on selected pieces. (**a**) 8110a features incised parallel lines combined with plain (undecorated) surfaces, typical of the decoration found on some rims of vessels., (**b**) CONJ2 features incised parallel lines combined with plain (undecorated) surfaces, a pattern that is repeated across the body of the object (lines-plain surface-lines-plain surface), (**c**,**d**) show incised zig-zag decorations on pieces 8551a and 8551b, (**e**) Cinnabar on the surface of CONJ1, (**f**) Cinnabar on human remains.

ZooMS analysis

Zooarchaeology by Mass Spectrometry (ZooMS) analysis successfully identified the raw material of previously uncertain samples. Nineteen specimens were analysed, and all were assigned to the family Elephantidae. This included five samples initially interpreted macroscopically as hippopotamus ivory and classified as such by A. Banerjee. The peptide markers used for species determination are listed in Supplementary Materials 2 and illustrated in Fig. 7. Elephantidae were identified by the presence of the peptide combination of COL1α2 484–498 (1453.7 *m/z*), COL1α2 793–816 (2115.1 *m/z*), and COL1α1 586–618 (2853.3 *m/z*, in seven samples).

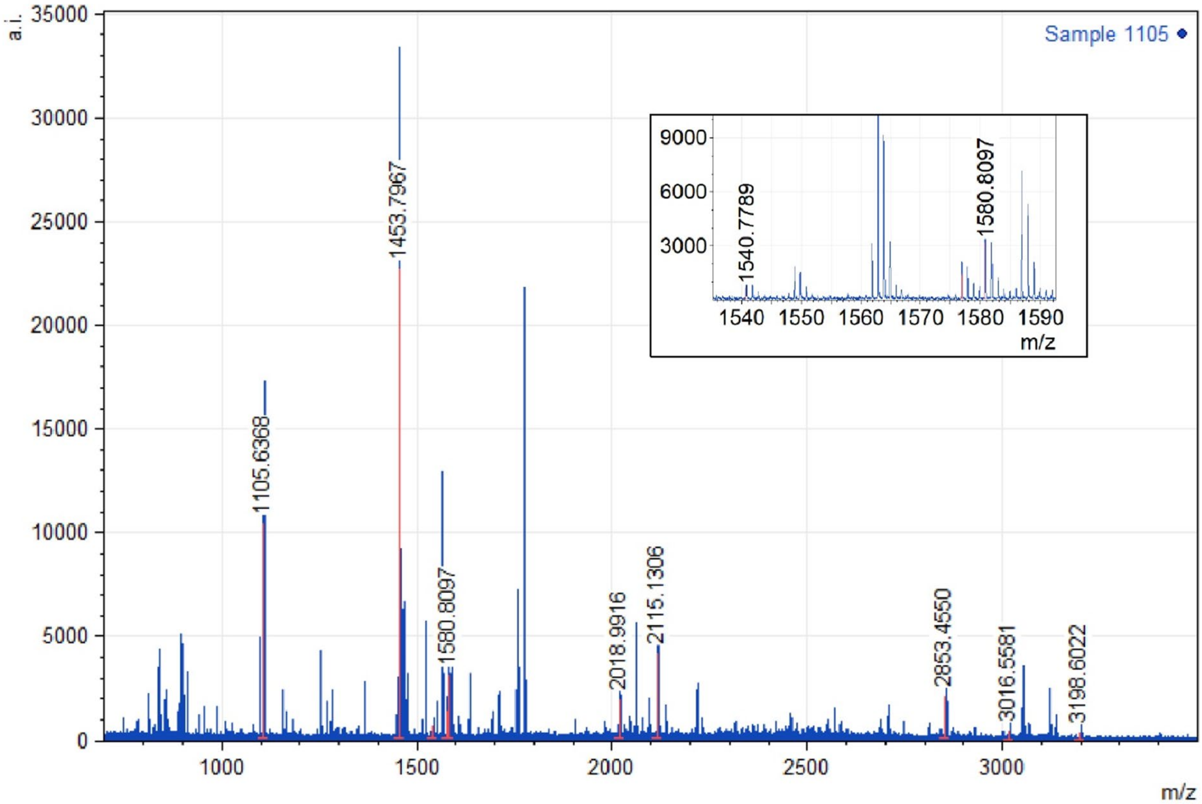

Fig. 7. ZooMS spectrum of sample 1105 as an example, highlighting peptide markers used for identifying Elephantidae. Mass range 1530–1600 is zoomed in for COL1α2 292–309 (1540.8 m/z) and COL1α2 889–906 (1576.8 m/z).

cm^{-1}	Carbonate asymmetric stretching $\nu_2(CO_3^{2-})$		Phosphate symmetric stretching $\nu_3(PO_4^{3-})$	Phosphate symmetric stretching $\nu_1(PO_4^{3-})$	Carbonate asymmetric bending $\nu_2(CO_3^{2-})$	Phosphate asymmetric bending $\nu_4(PO_4^{3-})$	
	1455 – 1446	1417 – 1403	1020	960	870	600	560
Deer antler (n. 1)		1416	1021	961	872	600	561
Wild Boar tusk (n. 2)		1417	1022	960	870 ± 5	599 ± 5	561
Human canine (n. 1)	1455	1415	1020	960	872	600	561
Bone (n. 10)	1451 ± 10	1416 ± 10	1022 ± 5	961 ± 5	872 ± 5	600 ± 5	561 ± 5
Human bone (n. 1)	1447	1413	1016	960	871	599	560
Ivory (n. 26)	1455 ± 10	1415 ± 10	1019 ± 10	961 ± 5	871 ± 5	600 ± 5	560 ± 5
Unknown (n. 10)	1455	1416 ± 10	1022 ± 10	961 ± 5	871 ± 5	599 ± 5	560 ± 5

Table 1. Main absorption bands observed in FTIR spectra of the analysed samples.

Previous studies[58,59,87] have associated the presence of COL1α2 292–309 and COL1α2 889–906 peptides at mass of 1540.8 (GSSGEAGSAGPAGPPGLR) and 1576.8 (GEPGPAGSVGPVGAVGPR) for *Loxodonta*, while mass of 1568.8 (GSNGEAGSAGPAGPPGLR) and 1590.8 (GEPGPAGSVGPVGAIGPR) are indicative of *Elephas maximus*[58]. In our dataset, all analysed samples from La Beleña displayed peak patterns consistent with African elephant (*Loxodonta*) (Fig. 7, zoom-in), confirming the absence of hippopotamus and *Elephas* ivory in the assemblage.

FTIR results

All analysed samples yielded highly similar FTIR spectra, with principal absorption peaks occurring in the 1500–400 cm^{-1} region (Table 1; Fig. 8). These spectra are characterised by bands associated with carbonate and phosphate vibrations. Specifically, carbonate (CO_3^{2-}) asymmetric stretching and bending peaks (ν_2, ν_3) were observed at 1452, 1412, and 870 cm^{-1}, while phosphate (PO_4^{3-}) asymmetric and symmetric stretching and bending peaks (ν_1, ν_3, ν_4) appeared at 1020, 960, 600, and 560 cm^{-1} (Table 2; Fig. 8; see also Supplementary

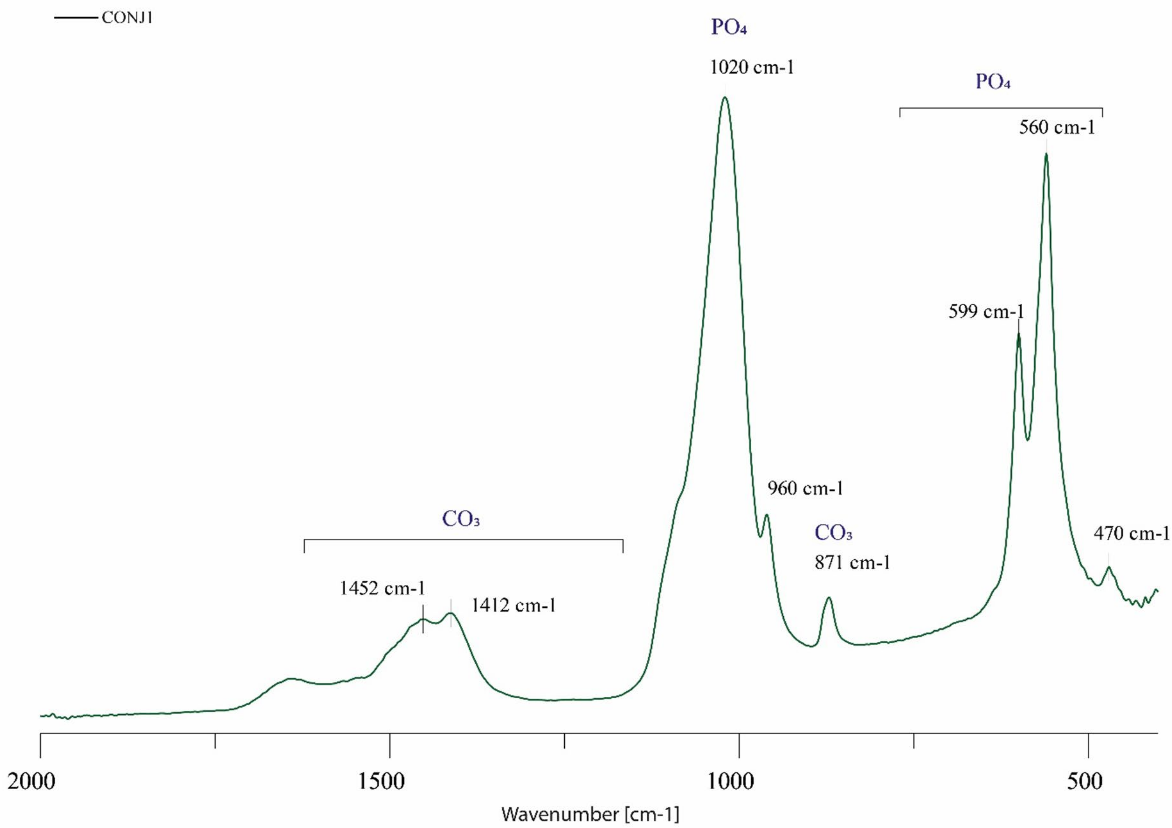

Fig. 8. Representative FTIR spectra for archaeological ivory, bone, and control samples.

Index	Component 1	Component 2	Component 3
Am/P	0.841	-0.536	0.004
C/P	0.572	0.796	0.103
C/C	0.700	-0.671	0.168
SF	-0.746	-0.302	0.591
BPI	0.573	0.799	0.161
API	0.686	0.703	0.173
BC/AC	-0.837	0.517	-0.094
WAMPI	0.944	-0.282	-0.030

Table 2. Component matrix of the eight FTIR-derived indices (PCA).

Materials 3 and 4). These bands are characteristic of the collagen and carbonated hydroxyapatite components found in both bone and ivory[88–91]. In some samples, a carbonate absorption band was observed at 710 cm^{-1}, typically associated with calcium carbonate ($CaCO_3$), and interpreted as potential contamination from calcareous sediments[76].

The control samples (including human tibia and canine, animal bone, deer antler, and boar tusk) displayed nearly identical spectral patterns to the ivory samples (Fig. 9). This uniformity reflects the common molecular composition of these materials. Dentine, like bone, consists of a mineralised connective tissue with a collagenous organic matrix and hydroxyapatite as the primary inorganic component. Spectral comparisons also revealed no qualitative differences between samples previously identified as hippopotamus ivory and those classified as elephant ivory, nor between ivory and bone (Fig. 10). Even the shoulder feature at 1085 ± 5 cm^{-1}—traditionally used to differentiate African from Asian elephant ivory—was inconsistently observed and, in some cases, appeared more pronounced in the human canine control sample than in ivory specimens (Fig. 10).

This spectral similarity calls into question the reliability of FTIR for both raw material discrimination and provenance determination. PCA using the eight indices explained 92.7% of the variance with two components, and 98.3% when a third component was included (Fig. 11a; Table 2). Although no clear clustering of materials

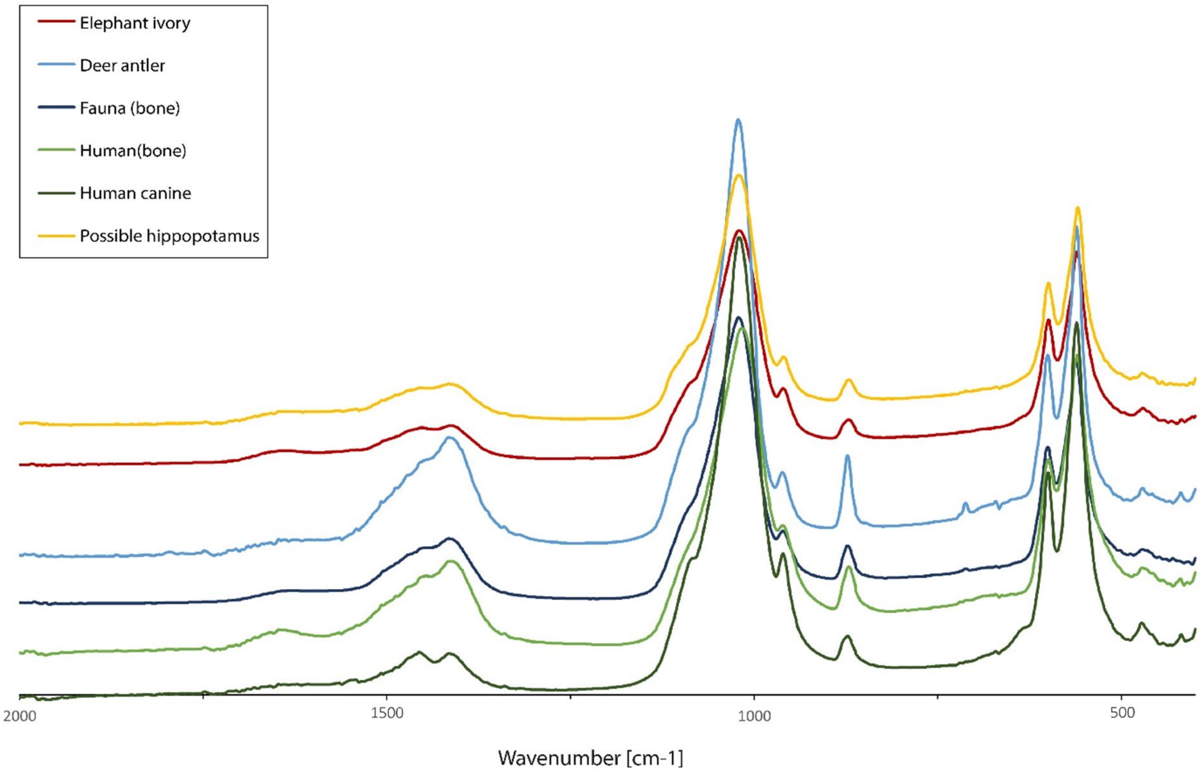

Fig. 9. FTIR spectra of different sample types: elephant ivory (red), faunal remains (blue), human remains (green) and one sample previously identified as hippo (yellow). Note that all samples display the same spectrum.

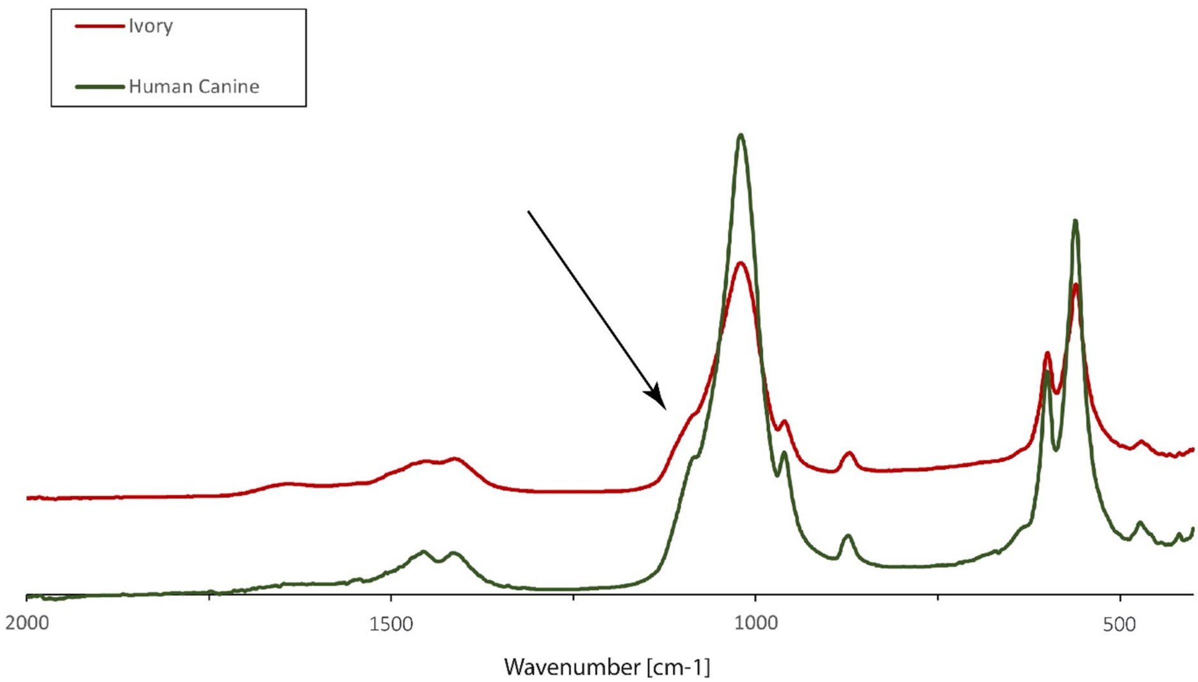

Fig. 10. Comparison of FTIR spectra from an ivory sample and the human canine control, both showing the 1085 cm^{-1} shoulder.

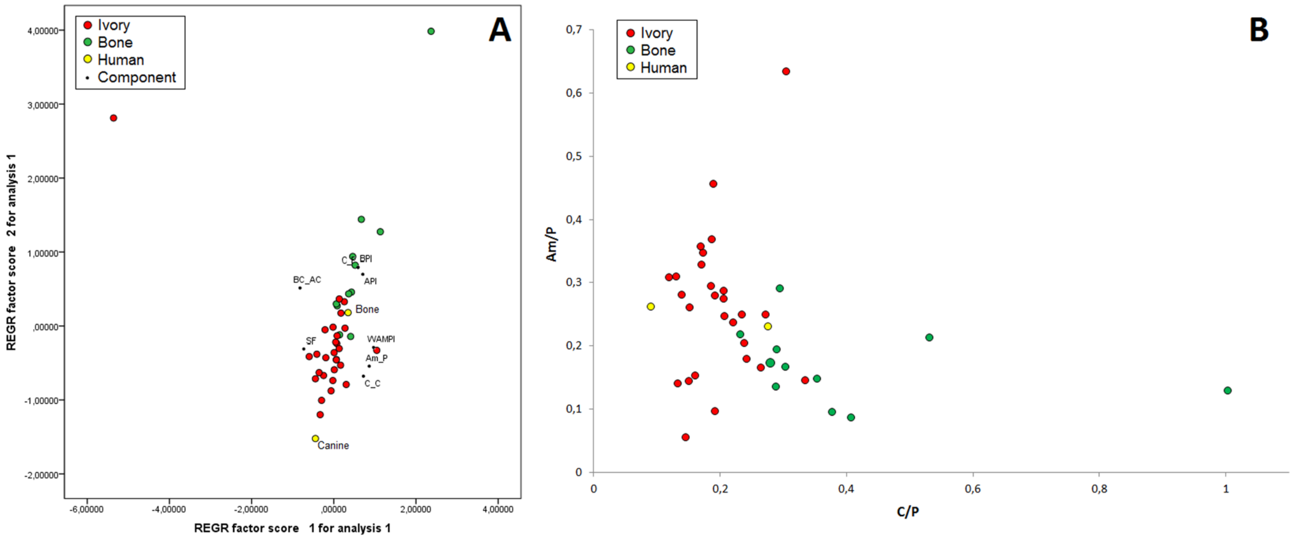

Fig. 11. (**a**) PCA plot of Am/P, C/P, C/C, SF, BPI, API, BC/AC, and WAMPI indices. (**b**) Plot of Am/P vs. C/P ratios, showing distribution of ivory, bone, and human control samples.

ID	Weight sampled (g)	Total sequenced reads	Mapped reads	Filtered MQ30 reads	Endogenous DNA
0001-1a	0.140	19,916,808	2491	746	0,00%
1069b	0.150	18,345,585	949	379	0,00%
1103	0.135	20,022,063	3902	1988	0,01%
0468-5	0.067	15,911,357	1125	463	0,00%
944	0.133	14,913,137	1126	474	0,00%
CONJ1	0.149	18,847,177	1430	573	0,00%
4678	0.112	13,244,922	1472	636	0,00%
CONJ2	0.133	10,956,148	1415	573	0,01%
CONJ3	0.073	13,357,326	1966	550	0,00%
CONJ4	0.095	17,474,563	1350	543	0,00%
8018-1	0.131	10,894,345	2136	718	0,01%
1069c	0.142	16,827,446	1173	415	0,00%
1110	0.077	20,381,230	2265	859	0,00%
8080a	0.117	18,170,309	2923	1104	0,01%
8153	0.140	13,104,174	1611	710	0,01%
8211	0.094	13,259,405	1434	647	0,00%
8275	na	11,698,942	1730	887	0,01%
8360b	0.088	12,342,762	2325	998	0,01%
8439-34	0.081	18,541,949	3135	1385	0,01%
8551a	0.068	12,403,634	967	481	0,00%
8551b	0.118	13,620,501	1244	444	0,00%
8557	0.128	21,359,005	1392	536	0,00%
8577-6a	0.144	18,347,707	2087	723	0,00%
8592	0.140	19,588,090	2693	1102	0,01%

Table 3. Ancient DNA analysis and pre numbers of sequenced and mapped elephant reads.

by species was observed, bone samples tended to show higher values in the C/P, BPI, and API ratios compared to ivory, including the two human control samples. This trend suggests a higher carbonate content in bone than in dentine, consistent with previous observations[67]. This distinction becomes more apparent when plotting Am/P against C/P ratios (Fig. 11b).

Ancient DNA results

Extraction and sequencing of aDNA yielded extremely low levels of endogenous elephant DNA—no higher than 0.01% of mapped reads—which made downstream analysis unfeasible (Table 3).

Discussion

This study addressed two key challenges commonly encountered in the analysis of animal hard matter, osseous and ivory archaeological materials: (1) the difficulty of accurately identifying the raw material (bone, hippopotamus ivory, or elephant ivory), especially in highly fragmented assemblages, and (2) the determination of provenance based on the differentiation of elephant species and their archaeological implications.

Methodological questions of raw material identification

The use of binocular and optical microscopy remains the standard approach for identifying osseous and ivory materials. When preservation conditions are favourable, diagnostic features—such as Schreger lines in ivory or the presence of vascular canals in bone—can indeed allow for accurate classification. However, in assemblages with a high degree of fragmentation and taphonomic degradation, these features may not be visible, leading to substantial uncertainty in material identification. Other morphological traits, such as the presence of parallel banding and undulating lamellae, have traditionally been used to identify hippopotamus ivory[73]. While these features are described in the reference manual by Espinoza and Mann[42] as typical of modern, well-preserved hippopotamus tusks, their reliability for archaeological specimens—especially those that are heavily degraded or fragmentary—is questionable. Importantly, this reference manual was originally designed for use in customs inspections to detect illegal ivory trade and counterfeit materials. It relies primarily on observations of complete, unaltered modern specimens. Its application to archaeological contexts, where most material is fragmentary, weathered, and lacks a clear anatomical context, is therefore limited.

Our analysis confirms this limitation: in several cases, ZooMS analysis conclusively identified Elephantidae markers in samples that had been classified under binocular microscopy as hippopotamus ivory. These results underscore the need to reassess previous identifications in Iberian and Mediterranean contexts that relied solely on macroscopic features. ZooMS analysis has been proven to be a powerful and minimally destructive method for raw material identification. For many mammalian taxa, ZooMS typically allows identification only to the family level. However, in the case of Elephantidae, genus-level identification is achievable through specific peptide markers such as COL1α2 292–309 and COL1α2 889–906, including several extinct genera within the Elephantidae. In addition, ZooMS is particularly advantageous in archaeological contexts where the material is highly fragmented and morphologically undiagnostic.

The FTIR analysis performed on La Beleña assemblage did not yield satisfactory results for the discrimination of either raw materials or elephant species. All samples, regardless of whether they had been previously classified as bone, ivory, or hippopotamus ivory, produced nearly identical spectra. Even the control samples (human tibia and canine) displayed indistinguishable spectral profiles from the archaeological materials (Table 1). This overall spectral similarity is due to the shared molecular composition of bone and dentine. Both are mineralized connective tissues composed of an organic collagen matrix and an inorganic phase dominated by hydroxyapatite. The FTIR absorption bands observed—representing carbonate (CO_3^{2-}), phosphate (PO_4^{3-}), and water—reflect this composition and offer limited discriminatory power for species or material differentiation[42]. While FTIR may retain utility for assessing the conservation state of samples, its current use as a provenance tool—particularly in the absence of open-access spectral reference databases—remains problematic.

Moreover, the characteristic shoulder at 1085 ± 5 cm^{-1}, which has been used to distinguish African elephant (*Loxodonta*) ivory from that of Asian elephants (*Elephas Maximus*) based on FTIR data[92,93], was observed in only one out of 27 ivory samples (BLN-6212). This suggests that either the shoulder is not a reliable marker in degraded archaeological contexts or that spectral differences are not as distinct as previously suggested. This 1085 cm^{-1} shoulder has been associated in some studies with a more ordered state of bioapatite and better material preservation[94–97]. These authors argue that FTIR is more effective for evaluating the conservation state of archaeological materials—serving as a prescreening tool prior to isotopic or genetic analyses—than for differentiating between ivory sources or species[95,97]. Other researchers have echoed these concerns, questioning the use of FTIR for proboscidean species identification and suggesting that its primary utility lies in distinguishing real ivory from modern fakes or substitutes[43,49].

A further limitation of FTIR as a tool for provenancing archaeological ivory is the lack of a robust and accessible reference database of spectra from modern African and Asian elephant ivory. The existing reference database most frequently cited in the literature (from INCENTIVS, University of Mainz) is not publicly available. Key information such as the number of reference samples, the analytical protocol, or preservation conditions of those samples is currently unknown[93,98]. In fact, the FTIR reference spectra used in most archaeological studies derive from previously analysed archaeological ivory, which—by definition—cannot serve as true modern references with confirmed taxonomic and taphonomic profiles. This situation underscores the need for a rigorous methodological reevaluation and for the development of open-access spectral reference libraries based on well-documented modern specimens.

Compared to peptide mass-based ZooMS identification, DNA analysis offers better resolution, allowing for the identification of different breeds within the same species. Ancient DNA (aDNA) analysis holds considerable promise for identifying elephant species, even enabling provenance studies at intra-continental scales. However, the success of this approach is strongly conditioned by the preservation state of the archaeological material. In the case of La Beleña assemblage, the extremely low DNA preservation is likely the result of advanced sample degradation due to environmental conditions, however higher resolution within African populations may be produced with aDNA analyses on better preserved samples.

In contrast, proteomic methods such as ZooMS offer a highly effective, minimally destructive. Its target, collagen, is a highly stable structural protein that typically survives longer than DNA under the same environmental conditions. Our results demonstrate that this technique allows for reliable taxonomic identification, even for small and poorly preserved fragments. The results obtained through ZooMS at La Beleña not only confirm the exclusive presence of *Loxodontaa*, but also call into question some previous macroscopic identifications of hippopotamus ivory, suggesting a need for revaluation of earlier attributions in Iberian and Mediterranean contexts. These findings highlight the potential of biomolecular approaches to refine our understanding of the circulation and use of ivory in prehistoric societies, as well as the limitations of traditional morpho-stylistic or spectroscopic methods when used in isolation.

Archaeological implications of ivory identification

The taxonomic results obtained in this study have direct implications for the interpretation of long-distance exchange networks and the circulation of prestige materials in the Iberian Peninsula. Previous attributions of some of La Beleña assemblage to hippopotamus ivory were used to support hypotheses involving early trade connections with the Near East[99]. The exclusive identification of African elephant (*Loxodonta*) through proteomic analysis challenges these interpretations and calls for a reassessment of such long-distance exchange models[71,100].

If ivory reached southern Iberia through established exchange networks, the present results suggest that routes involving North Africa are more parsimonious than those implying Near Eastern connections. This reinterpretation aligns with growing evidence for North African–Iberian interactions during the Neolithic-Chalcolithic[4–6,101,102] and underscores the importance of secure taxonomic identification as a prerequisite for reconstructing prehistoric exchange systems. The exclusive presence of African elephant ivory at La Beleña, and the methodological constraints raised by this study also bears on sites where higher taxonomic diversity has been reported, such as Valencina de la Concepción, where *Loxodonta*, *Elephas maximus*, and *Elephas antiquus* have been proposed[18]. This study highlights the need for a systematic re-evaluation of previously published datasets using comparable biomolecular methods and standardized diagnostic criteria[103]. Such an approach is essential for assessing the true extent of taxonomic variability and its implications for regional and supra-regional models of ivory circulation.

At a regional scale, the location of La Beleña in the Subbetica mountain range illustrates the integration of inland communities into broader exchange networks[50,55]. Although the site lacks the abundance or technical sophistication of ivory objects documented at major centres such as Valencina de la Concepción or Los Millares, its assemblage nevertheless indicates participation in supra-regional circuits for the acquisition and transformation of exotic raw materials[18]. This suggests that access to ivory was not restricted to primary aggregation centres but extended, albeit unevenly, to secondary or peripheral contexts[22,70].

The technological characterisation of the assemblage aligns closely with patterns observed at other Chalcolithic Iberian sites. As is common since the Late Neolithic and during the Chalcolithic, ivory objects appear primarily in large funerary monuments, while evidence of production contexts—such as debris or unfinished items—remains rare[20,27]. At La Beleña, the ivory was deposited in the form of finished artefacts, often decorated according to the stylistic preferences of specific individuals or groups[18]. However, these objects frequently share motifs and aesthetic conventions, suggesting some degree of standardisation or shared symbolic language.

Importantly, there is a general absence of intermediary production stages in the assemblage—no complete tusks, partially worked items, or manufacturing by-products were recovered. This pattern is consistent with what is observed at most contemporary sites, except for rare exceptions like Valencina de la Concepción, where complete tusks were deposited (structure 10042–10049 and Montelirio tholos[18], or Perdigões, where fragmented tusks have been found in cremation contexts[17]. The decorative repertoire, characterised by incised geometric motifs such as parallel lines and zigzags and no figurative, animal, or vegetal motifs, corresponds- according to the current state of knowledge – to typical ivory and bone decorative traditions of beginning of the Chalcolithic, end of the Neolithic period. Technological traces suggest the use of a diverse toolkit, possibly including copper-based saws (e.g., sample 8275), lithic tools (e.g., perforators, sample 8106), and possibly other instruments such as burins, awls, and abrasives—though preservation issues may obscure their traces.

Technological analysis further confirms that tusk segmentation through sawing and bending formed part of a shared technical repertoire across Chalcolithic Iberia. The likely use of copper saws for small-diameter tusks, as documented at La Beleña, and of cord abrasion techniques for larger elements, as observed at Valencina de la Concepción[20], indicates that Chalcolithic metal tools were effective for ivory working, albeit mainly for the production of thin blanks. La Beleña nevertheless differs from other sites in its apparently balanced use of transverse and longitudinal tusk exploitation, whereas longitudinal segmentation dominates at Valencina de la Concepción and La Molina (Seville)[20]. Although the high degree of fragmentation at La Beleña complicates refitting and full *chaîne opératoire* reconstruction, the predominance of flat blanks suggests a general preference for longitudinal exploitation. Transverse segments appear to have been preferentially used for containers, while longitudinal sections were employed for lids and decorated plaques.

The uneven distribution and high fragmentation of ivory across the funerary contexts at La Beleña further suggest that access to this material was intermittent and socially mediated. Rather than reflecting standardized funerary practices, the selective inclusion of ivory objects points to variable social or symbolic values attributed to this raw material within the community.

Finally, it is noteworthy that ivory —rather than other exotic materials such as amberor ostrich eggshell— is represented among the funerary offerings at La Beleña. This pattern should not be interpreted as evidence for a higher symbolic or economic value of ivory per se, but rather as reflecting differential access to raw materials within regional and supra-regional exchange networks[104]. In contrast to ivory, whose circulation pathways into southern Iberia appear to be well established during the Chalcolithic, other exotic materials such as amber may not have been equally accessible to the community at La Beleña. The presence of ivory in funerary contexts therefore likely reflects the specific connectivity of the site within existing exchange networks, as well as the social significance attributed to materials that were effectively available through those channels.

Conclusions

The analyses conducted on the osseous and ivory assemblage from La Beleña raise several issues of broad relevance for archaeological methodology and the interpretation of long-distance exchange networks.

First, at the site-specific level, this study confirms that no hippopotamus ivory was identified in the funerary assemblage of La Beleña. All ivory material securely analysed belongs to elephantid species, specifically to *Loxodonta* (African elephant), as demonstrated through ZooMS. From a methodological perspective, this study highlights the need to re-evaluate identification protocols for ivory in archaeological contexts. Morphological traits derived from complete, well-preserved modern specimens[42] have proven insufficient for the identification of degraded and fragmented archaeological samples. Their widespread use in archaeological contexts—including in studies across Iberia and the wider Mediterranean[71,73]—should be reconsidered in light of biomolecular data.

Methodologically, our findings highlight the limitations of traditional optical and spectroscopic approaches for distinguishing ivory from bone or for determining elephant species in highly fragmented archaeological contexts. FTIR analysis showed limited discriminatory capacity, while aDNA proved unfeasible due to poor preservation. In contrast, proteomic analysis (ZooMS) provided reliable genus-level identification with minimal sample destruction, demonstrating its particular suitability for degraded assemblages. La Beleña case study demonstrates its relevance for reviewing previous identifications and for reconstructing long-distance exchange networks with greater resolution.

Ultimately, the contextual and biological characterisation of ivory—its source, working techniques, and social meaning—remains essential for understanding prehistoric value systems. The selection of a particular ivory type—African, Asian, or fossil—may reflect distinct choices, reflecting specific economic routes and cultural connections. Thus, improved analytical protocols are necessary not only for accurate identification, but also for reconstructing the symbolic and relational landscapes of Chalcolithic societies.

Data availability

All data generated or analysed during this study are included in this published article (and its supplementary information files).The datasets generated in this article are available in the European Nucleotide Archive under accession code PRJEB95946 and in Zenodo repository (https://urldefense.com/v3/__https://zenodo.org/records/16885952__;!!D9dNQwwGXtA!X1GqjC4BleYt5YMs6MPd7ItIxFnpcC0Sedfbh6E3KkQbyqY_Bz3M_zlKWEYU1iF_0ygkhIfqpHVAoFp-UHWYQsXx-w_8gNIF$).

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Author contributions
MNR conducted the FTIR analysis, prepare the figures and wrote the main manuscript text, NW and SB conducted the ZooMS analyses, MLT conducted the technological analysis, PP conducted the aDNA analysis, JSC, FJRS, MDCM and DMS excavated the site and provided the samples, MMB designed and supervised the research, prepare the figures, conducted the statistical analysis, wrote the main manuscript text and got the funding. All authors revised the final version of the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

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