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Dental evidence of textile-related task activities in the Bronze Age site of Laderas del Castillo (Alicante, Spain)

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A B S T R A C T

The burial 7 from the Argaric site of Laderas del Castillo (Alicante, Spain), dating from the 2nd millennium BC, included a skeleton associated with a carinated vessel indicative of social recognition. Anthropological and DNA-based methods were combined to determine that the individual corresponds to a young female. Macro- and scanning microscopic analyses revealed morphologically uniform labial notches with smooth and polished enamel on maxillary central incisors, which suggest tooth-tool use involving craft tasks. Plant fibers entrapped in dental calculus suggest that the individual was involved in textile and craft production, most likely working with flax or hemp. These findings provide new and direct insights into the use of teeth as tools related to crafts involving threads and are valuable for assessing the social division of labor within the Argaric prehistoric populations from Southeastern Iberia.

1. Introduction

Since the Neolithic times, human societies have used extensively animal and plant fibers for thread production using different techniques such as splicing or draft spinning (Leuzinger and Rast-Eicher, 2011; Médard, 2012; Gleba and Harris, 2019). In this context, anthropological evidence from ethnographic and past human populations suggest that specific dental wear features are related to non-masticatory uses of the anterior dentition for craft activities (Milner and Larsen, 1991; Alt and Pichler, 1998; Prpić-Mehičić et al., 1998; Sperduti et al., 2018). Overall, tooth modifications in the form of incisal notches and grooves are mainly associated with cultural idiosyncratic behaviours, suggesting also that certain cultural activities are female-dominated (Erdal, 2008; Scott and Jolie, 2008; Sperduti et al., 2018). However, despite that, for ethnohistorical societies, it is possible to determine the use of dentition as a tool of females in the production of hand-crafted threads and metals (Fidalgo et al., 2019; Willman et al., 2021) for prehistoric societies, it is much more challenging to do so.

In this study, we provide new insights into extra-masticatory uses of the anterior dentition from an articulated skeleton found in the burial 7 of the Argaric site of Laderas del Castillo (Alicante, Spain), one of the most critical and socially developed Bronze Age societies in Western Europe, and from which a remarkable amount of textile remains are preserved (Jover and López, 2013; Basso et al., 2021; Basso, 2022, 2023). Early reports from other Chalcolithic (Díaz-Navarro et al., 2023) and Bronze Age (Romero, 2016; Lozano et al., 2021; Oliart, 2021; Rubio, 2021; Fregeiro et al., 2023) sites located in Southeastern Iberia have identified similar tooth-wear patterns mainly associated to femalés craft specialization. A multi-proxy approach to gathering evidence on non-food-related dental wear at the Iberian southeast Argaric sites was used. This approach includes physical anthropology, microscopic analysis of teeth, DNA-based sex identification, and a study of micro-remains

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found in dental calculus. The aim is to gain new insights into the social division of labor, related specifically to crafts involving threads.

1.1. The Argaric site of Laderas del Castillo

Laderas del Castillo site is located on one of the south-eastern foothills of the Callosa de Segura mountains (Alicante, Spain), on the edge of the urban area of the town of the same name, and at a short distance from the Segura River (Fig. 1a-b). Excavated at the beginning of the last century (Furgús, 1909; Colominas, 1936), the site extends along the southeastern slopes of the mountains. The size of the Argaric occupation may have reached a surface area of approximately 2 Ha. The fieldwork carried out between 2013 and 2021 was concentrated on the eastern slope and yielded valuable stratigraphic and chronological information, with more than 40 radiocarbon dates that place the occupation between 2250 and 1700 cal BC (Hernández et al., 2021).

Around 2050 cal BC, extensive urban remodelling is documented at various settlement areas, with the construction of new terraces and household areas. New dwellings were built with an elongated rectangular floor plan in which mainly masonry was used, with embedded posts and benches attached. The Argaric communities used to bury some of their dead inside the dwellings, either beneath the floors or within benches. The earliest known burials, specifically those found in pits covered with large blocks of stone and masonry, dated back to around 1900 cal BC.

One such burials, known as Burial 7, located in the so-called Structural Complex (SC) K, associated with the highest terrace level of the site excavated area (Fig. 1c-d). The burial exhibited a rectangular pit with rounded corners of approximately 0.5 m in wide (N-S direction) and 1.10 m in length (E-W direction). It was excavated to a depth of approximately 0.35 m from the ground level. The northern edge was in the form of a shallow step, on which the limestone blocks of the tomb roof were laid, the original appearance of which would have been slightly vaulted. This stone structure was then covered with a thick layer of irregularly kneaded clay.

The skeleton was found with the face facing north. The body was deposited on its right side with the legs bent. The left hand was placed near the knees, and the right hand on the chest (Fig. 1d). A small carinated vessel (Siret's Form 5) was found at the level of the right elbow, and near the right hand, the bones of the foreleg of an ovicaprine. The burial was radiocarbon dated (Beta-447234: 3530 ± 30 BP) from a fragment of right fibula bones and placed chronologically around 1850–1800 cal BC (López et al., 2018).

2. Material and methods

The skeleton was found in anatomical connection and optimal preservation (see Fig. 1d). However, the collapse of the wooden and stone cover of the pit caused several fractures mainly in the long bones of the upper limbs. Age-at-death was estimated according to the stage of dental development (Buikstra and Ubelaker, 1994), epiphyseal fusion (long bones of the limbs, iliac crest and sternum) and, the size of the diaphysis of the long bones (Scheuer and Black, 2000). Sex diagnosis followed the characteristics of the skull and pelvis morphology (Buikstra and Ubelaker, 1994). Cranio-dental and postcranial features indicated a juvenile individual (aged between 15 and 18 years), probably female. The cranial and postcranial skeleton showed clear signs of development and robust complexion, with upper and lower limb measurements somewhat reduced for a male. The maxilla and mandible showed *in situ* permanent anterior (I1 to C) and post-canine (P3 to M2) teeth except for non-erupted lower third permanent molars. The maxillary second molars (UM2s) were not recovered. A slight to medium quantity of supragingival calculus was found in the anterior dentition. No other dental pathologies were recorded. Occlusal wear for anterior and post-canine teeth was also slight (ranging between grades 2–3, according to Smith 1984), reflecting the individual young age. Of total preserved teeth, only the first upper left (ULI1) and right (URI1) incisors showed macroscopic signs of non-alimentary use in the form of U-shape grooves running across the incisal surfaces. Marks were first observed with a stereomicroscope Leica® EZ4 and micrographs were obtained under an Environmental Scanning Electron Microscope (ESEM) Hitachi S3000N at 20Kv and magnification range 25–100 × following previous procedures (Galbany et al., 2006).

2.1. DNA extraction

The external surface of the second right maxillary molar (URM2) was polished using a sterile tungsten tip placed into a micro drill to remove the outermost millimetres. Then, a 2–3 cm section of the root was cut using a tungsten sterile disk and pulverized with a hammer. After that, 100–150 mg of sample was used for DNA extraction.

DNA was extracted in the ancient DNA Laboratory of the Biological Anthropology Unit of the Universitat Autònoma de Barcelona (UAB), Spain, using the High Pure Viral Nucleic Acid Large Volume kit (Roche), and following the specifications of Vinueza-Espinosa et al. (2020). EDTA (1 ml, 0.5 M, pH 8 for 150–180 mg of bone powder) and proteinase K (25 μl) were added to the powdered sample for the lysis step. The lysis solution was incubated at 37 °C for 48 h. 400 μl of Sodium Acetate and

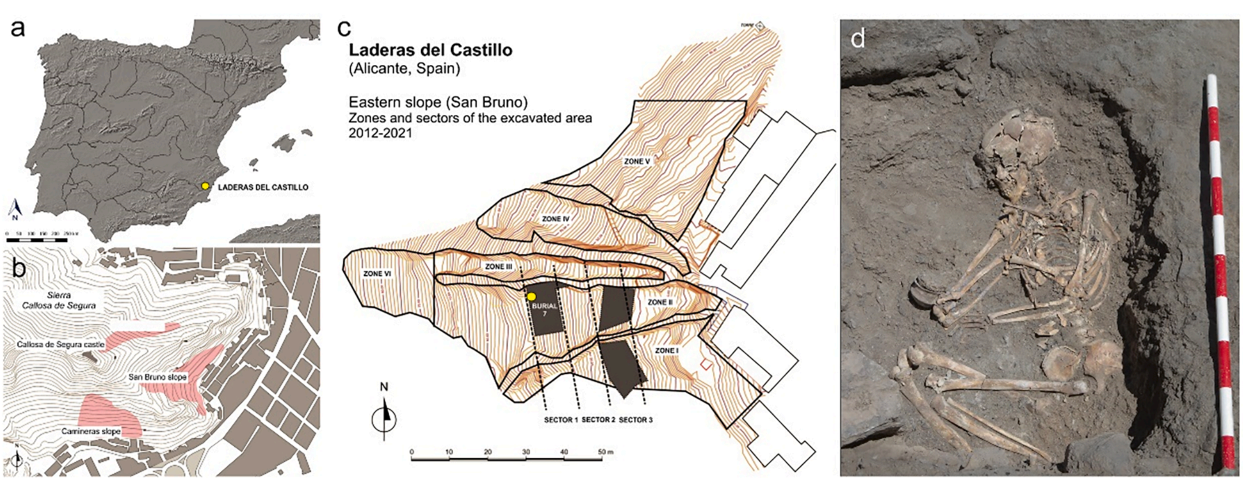

Fig. 1. (a) Iberian Peninsula map showing the geographic location of the Laderas del Castillo site in southern Spain (Callosa de Segura, Alicante). (b) Digital planimetry of the Callosa de Segura mountains showing the San Bruno slope where the site is located. (c) Floor plan of the site and burial 7 location. (d) Details of the burial and articulated skeleton analyzed in this study; note the carinated vessel next to the upper limbs.

10 ml of Binding Buffer were mixed with the extraction lysate and the Binding Buffer was eliminated after centrifugations. The flow-through was discarded. Two washing steps were performed by adding 450 μl of PE Buffer and centrifuging the solution in columns with a silica membrane. A third centrifugation was applied to eliminate the possible remaining Buffer. Finally, 55 μl of Elution Buffer was added and, after a 5-minute incubation at room temperature, centrifugated for 1 min at 13,000 rpm. The flow-through was then re-eluted at the same conditions as before (5 min of incubation and 1 min centrifugation at 13,000 rpm). The flow-through was stored in 1.5 LoBind microcentrifuge tubes at −20 °C. Blank was used to confirm the absence of contamination.

The quality of the sample was validated with the amplification of a 200 base pairs region from the mitochondrial DNA. To diagnose the chromosomic sex of the individual, the alphoid centromeric regions of the X and Y chromosomes (Lin et al., 1995) were amplified twice, to verify the results found.

2.2. Dental calculus sampling and processing

Supragingival fragments of calculus were extracted from the left maxillary lateral incisor (ULI2) and the right mandibular central incisor (LRI1). Teeth were described and photographed before and after picking the calculus up. Teeth were mechanically washed with distilled water using a new and soft toothbrush. Calculus deposits were sampled from every side of the tooth using a dental scaler and placed inside a box covered with aluminium foil. This procedure was performed to avoid material loss. Calculus fragments were removed separately depending on the part of the tooth they come from (lingual, buccal, distal, medial, or occlusal). Then, the samples were weighed and transferred into 1.5 ml plastic tubes. The weights ranged between 1.6 mg and 4.6 mg.

Decontamination procedures are essential in dental calculus analysis. Thus, the samples have been processed under the cleanest conditions possible. To avoid modern and cross-sample contamination, all the extraction and processing were undertaken in a laminar flow hood previously cleaned with detergents, 5 % sodium hypochlorite, and 90 % ethanol, allowing it to evaporate. Starch-free gloves, lab coats, and face masks were worn, and all the instruments were changed or cleaned with the detergents for each sample. In addition, control slides were placed inside and outside the hood to validate the decontamination processes and then observed at the microscope. This was performed to identify the possibility of contamination during the processing of the samples. The following steps were obtained by mixing several protocols guidelines adapted to our material (Cummings et al., 2016; Hardy et al., 2016; Weyrich et al., 2017; Cristiani et al., 2018; Gismondi et al., 2018).

Soil contamination was removed from the calculus, and the samples were immersed in 0.4 HCl for an hour. Then, the samples were moved into a new sterile 1.5 ml plastic tube, rinsed with distilled water, and centrifuged three times to eliminate the loose soil. Then, the samples were ready for the decalcification process. This step consisted of adding 1 ml 0.6 HCl to the clean samples (without exceeding 24 h) until they were dissolved. The samples were mixed manually and with the vortex several times during the first twelve hours to control the breaking velocity. The exposure time to the acid depended on each sample and its size. When the samples were dissolved, they were centrifuged at 5000 rpm for 5 min, and the supernatant was removed. The samples were rinsed with distilled water and centrifuged again to remove any remaining acid. Once the samples were cleaned, some drops of 70 % ethanol were added to prevent it from becoming statically charged. The samples were mounted on microscope slides with pure glycerine and sealed with transparent nail polish. The samples were examined using an Olympus® BX43 optical microscope with a polarizing filter coupled with an Olympus® DP26 camera linked to Olympus® cellSens software. The micro-remains were photographed and measured as needed using the ImageJ software and compared with modern reference materials. Overall, more attention has been paid to micro-remains embedded in the calculus matrix and those situated in the centre of the slides, which means and supports the absence of contamination. Sealing with nail polish can cause some remains to stick on the slides and be confusing. That is why the micro-remains found on the edges of the slides were discarded from the analysis.

3. Results

The comprehensive study of the human remains found in the Burial 7 of Laderas del Castillo has led to the identification of a young individual between 15 and 18 years old. Sex-related anthropological features were confirmed by DNA processing, which provided valid extracts for the analysis. The amplification of the alphoid centromeric fragments of sex chromosomes resulted in the exclusive presence of the female marker.

Maxillary right (URI1) and left (ULI1) central incisors show concave rounded indentations in the form of grooves involving incisal edges transversally in a labio-lingual direction (Fig. 2a). No similar alterations in opposite teeth were found. Although the depression on the URI1 is deeper and greater in size than in ULI1, both teeth exhibit morphologically uniform notches with smooth and polished enamel. The polished grooves suggest that some abrasive but flexible material passed transversely with a continuous and repetitive action across the incisal surfaces of both maxillary central incisors. ESEM analysis also reveals specific labial features affecting teeth (Fig. 2b-c). The URI1 shows labial enamel chippings associated with the incisal groove. However, the labial surface of the ULI1 revealed a pattern of mesiodistally oriented shallow scratches. Specific microscopic features found on the incisors's labial surfaces suggest that wearing was rubbing-produced habitually.

Several long plant fibers were detected in the dental calculus. However, we have focused only on those embedded in the calculus matrix to prove that they were *in situ*. One plant fiber was observed on the labial side of the maxillary left second incisor (ULI2; see Fig. 3a). This fiber presents a rounded section (Fig. 3c). Both edges are frayed, showing an internal striated texture. The whole fiber, which is colourless, is divided into smaller units. When polarizing the light (Fig. 3d), the fiber gets brownish, and transversal lines correspond to the cross-markings. No nodes are visible. These transversal lines are consistent with those that appear in the commonly known bast fibers, but they differ in some specific features. Analysing the fibrillar orientation and performing the modified Herzog test, it seems to present an S-twist which may be similar to those present in nettle or flax (*Linum usitatissimum*). The cross-section is not visible, although the mean diameter measure of 9.128 μm is compatible with the three typical textile fibers of the chronological context (Bergfjord and Holst, 2010; Sperduti et al., 2018), including flax, hemp (*Cannabis sativa*), and nettle (*Urtica dioica*). In addition, comparing the fiber with modern nettle and flax reference images and observing the dislocations and the cross-marks points out the possibility of being flax (Fig. 3e-f). Even though the lack of nodes and a high amount of cross-marking are not present only in flax, they are also common in hemp (Haugan and Holst, 2014). However, the fact that hemp presents a left or Z-twist reinforces the possibility that this fiber corresponds to flax.

Another fiber was found on the lingual surface of the mandibular right first incisors (LRI1; see Fig. 3b). It is immersed in a thick matrix of non-decalcified dental calculus (Fig. 3g-h). As the samples were mounted using glycerine, we tried to detach the fiber by pressing the cover. Unfortunately, isolating the fibre was impossible, and no other properties could be visible for its identification, nor by polarizing the light. The diameter measure of 5.543 μm is also compatible with the cross-section diameters of flax and nettle, but not with hemp (Bergfjord and Holst, 2010). Other non-identified plant elements were found. However, it was not possible to differentiate them from different plant species or anatomical parts.

36 starch grains embedded in dental calculus were also found. In both analysed teeth, 19 starch grains were identified with features compatible with those of the Paniceae tribe. These grains are characterized by a round to polyhedral 3D morphology, a central hilum,

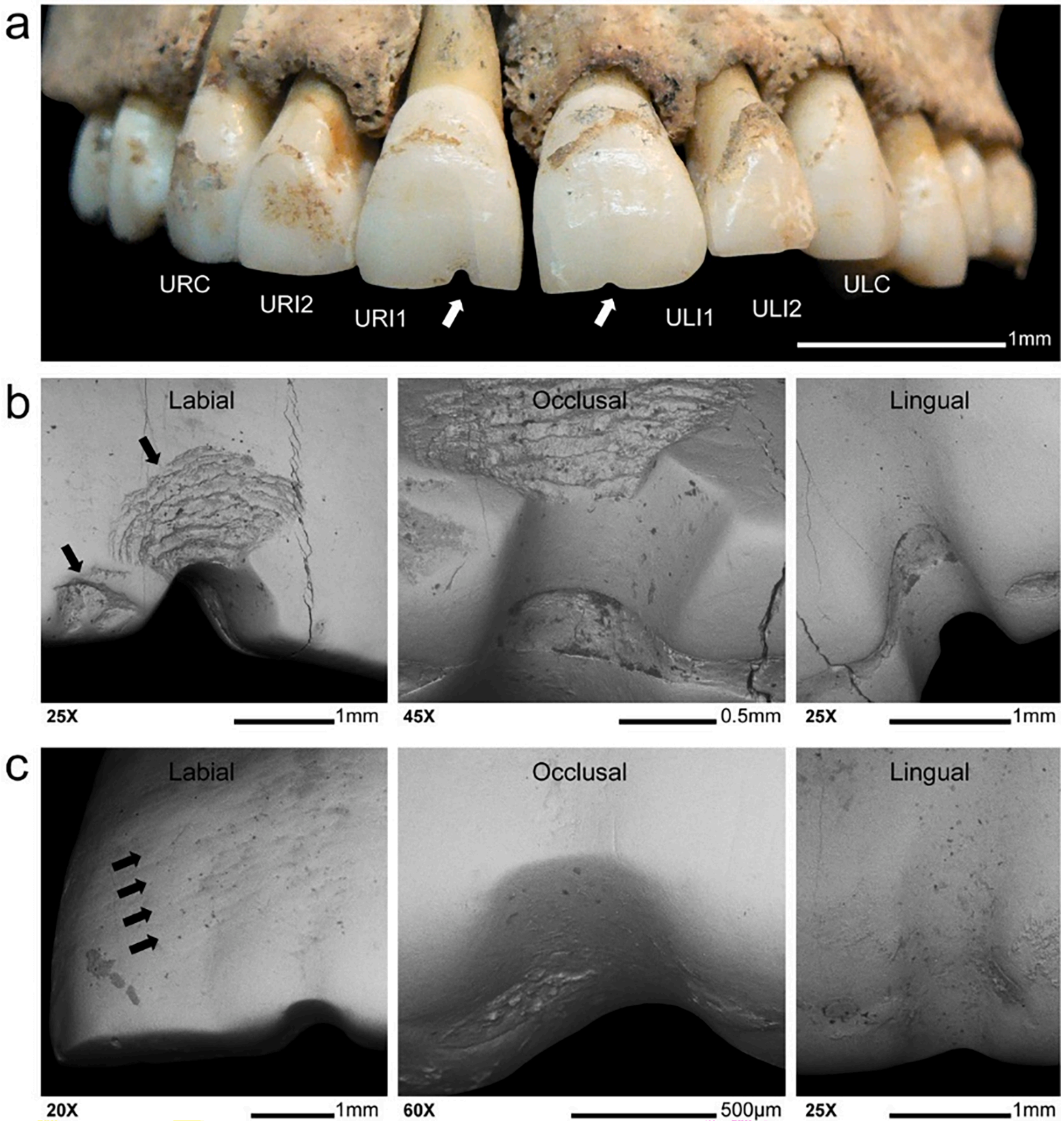

Fig. 2. (a) Non-alimentary tooth use in the maxillary right (URI1) and left (ULI1) central incisors of a female individual from the Laderas del Castillo site. Both incisors show rounded U-notches on labial surfaces (see arrows). (b-c) ESEM images reveal detailed aspects of wearing in the form of chipping (URI1) and abraded (ULI1) non-occlusal enamel surfaces (see arrows). The incisal surfaces of both incisors exhibit smooth and polished grooves. Microscopic magnification and scale bars are indicated.

sometimes with a radiate fissure, and maximum lengths under 20 μm. These finds could belong to *Panicum* spp., *Setaria* spp., and *Echinochloa* spp. (Cristiani et al., 2021; Nava et al., 2021; González-Rabanal et al., 2022). Only one partially damaged starch grain was larger than 20 μm; its dimension might be due to the cooking process, which can alter the grain's features (Henry et al., 2009; Lippi et al., 2015). In addition, two starch grains were assigned to the Triticeae tribe because of their morphology. Although a bimodal distribution was not found, the two grains had a 2D oval/circular shape, a 3D lenticular shape, a centric and closed hilum, and concentric and distinct lamellae. Among the non-diagnostic grains, five possess features compatible with those belonging to underground storage organs (e.g., eccentric hilum, elongated shape).

4. Discussion

Burial 7 recovered from the Laderas del Castillo Argaric site corresponds to a young female of about 15–18 years of age at death. According to the proposal made by Lull and Estévez (1986: 451), the presence of only one vessel of Siret form 5 would place her in category 4, so she would not be a person belonging to the dominant social groups, but she would be socially recognised. Furthermore, recent studies on the presence of meat portions as grave goods (Andúgar et al., 2021; Aranda et al., 2021) have suggested that this practice is only found in a small proportion of those burials, giving them greater importance and social status.

The particular wear patterns found within the anterior dentition of the female individual from the Laderas del Castillo site should be related to the non-masticatory use of teeth in processing vegetable fibers for material culture production. The use of teeth in extra-masticatory

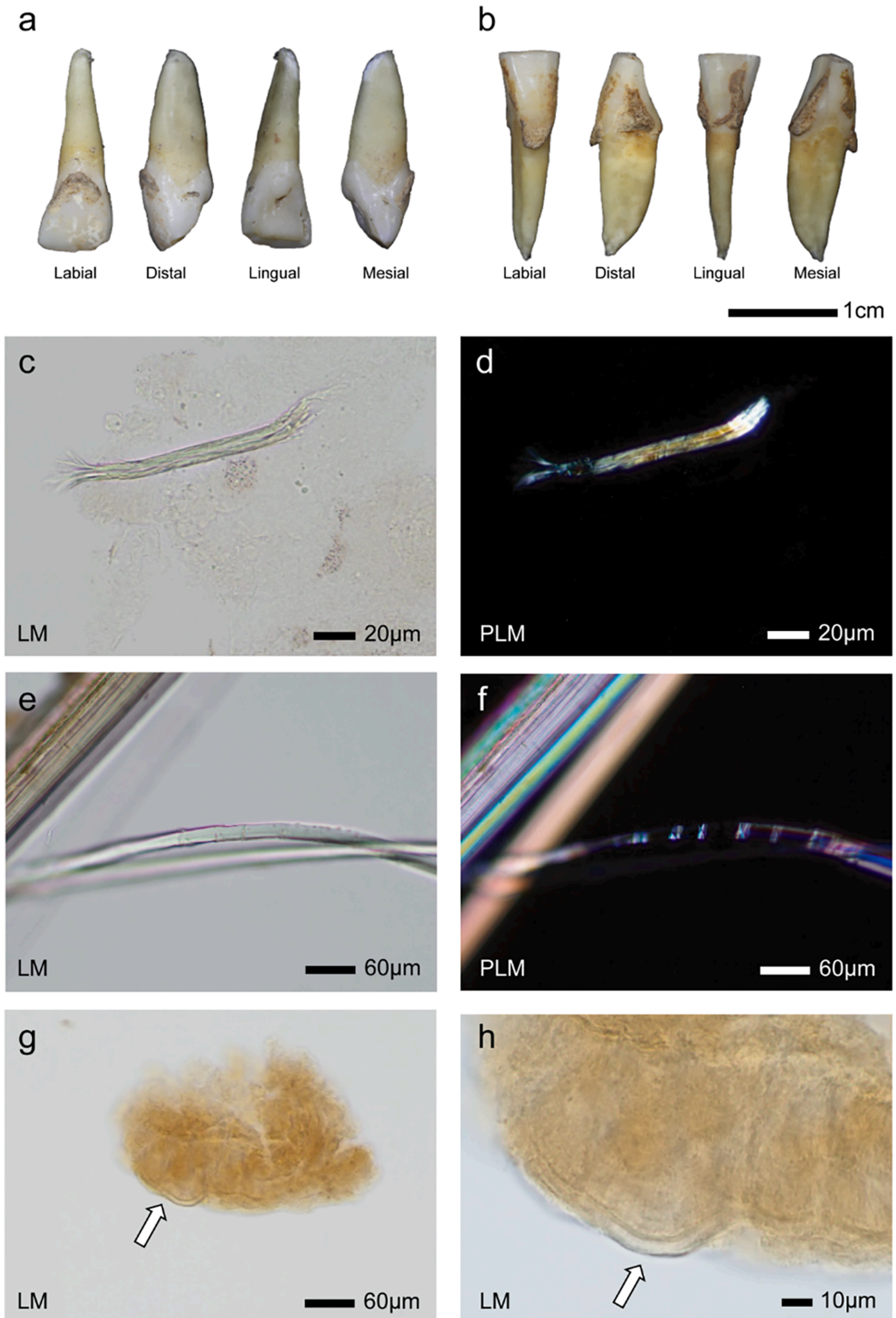

Fig. 3. (a) Isolated maxillary left second (ULI2) and (b) mandibular right first (LRI1) incisors used for calculus analyses. (c-d) Plant fibre embedded in the calculus matrix from the labial side of ULI2. (e-f) Flax reference collection images. (g) Plant fibre isolated from the lingual surface of LRI1 (see arrow); (h) more detailed fibre image. Light microscopy (LM); polarized light microscopy (PLM). Scale bars are indicated.

cultural activities has been widely described in human populations from ethnographic and archaeological contexts (Schulz, 1977; Milner and Larsen, 1991; Scott and Jolie, 2008; Lozano et al., 2008). These non-masticatory dental alterations reflect task-specific long-term wearing processes and are most frequently found on the front teeth, mainly upper incisors, bilateral or not (Alt and Pichler, 1998; Prpić-Mehičić et al., 1998; Erdal, 2008). Particularly, small-scale damages found on the maxillary central incisors were probably associated with continuous and repetitive actions, such as rubbing enamel surfaces with soft and flexible vegetable fibers. In addition, the pattern of oriented notches and shallow scratches suggest, in turn, an aetiology of these labial marks and incisal grooves related to the habit of processing vegetable fibers to produce textile elements (Prpić-Mehičić et al., 1998; Erdal, 2008; Sperduti et al., 2018). The lack of similar marks on the front teeth in the mandible suggests that plant fibers were probably strongly held with teeth and pulling upwards to tighten with different medio-lateral movements in compressive intensity. In addition, this case's importance lies in preserving vegetable fibers mainly compatible with flax in the dental calculus analysed. This evidence allows us to directly associate the dental wear found with using teeth as a tool and possibly, a direct production evidence of flax or hemp-based hand-crafted threads (Sperduti et al., 2018). Overall, non-alimentary wear features found could have been produced mainly by clamping and wetting the fiber to facilitate both splicing and plying (Erdal, 2008). These procedures would have been standard for thread production with plant fibers during recent prehistory in much of Europe and the Mediterranean (Gleba and Harris, 2019).

Regarding plant fibers, several species were used in the Argaric environment to produce textiles and basketry, including flax, esparto grass (*Stipa tenacissima*), bulrush (*Typha* sp.) and probably reed (Cyperaceae/Juncaceae) and hemp. However, flax was the primary fiber used to make fabrics (Jover and López, 2013; Basso et al., 2021). This fact is corroborated by the significant amount of cloth remains that have been preserved, mainly in Argaric funerary contexts, thanks to the impregnation of metal artefacts included as grave goods (Basso, 2023). Although more than 150 pieces of evidence corresponding to different textiles are known, only a third of them have been directly analysed by specialists, identifying flax as a fiber in all cases (Alfaro, 1984; 2012; Hundt, 1991). The Argaric textiles, as well as those found in neighbouring areas, display similar characteristics, especially in terms of the techniques used for their production (Basso et al., 2022). Thus, our findings allow us to establish a series of far-reaching inferences to the production of thread and the female social roles from the Iberian southeast early prehistoric societies. In addition, dental calculus analysis has also provided relevant information about the consumption of cereals and underground storage organs (e.g., bulb, rhizome, root tuber). Among the cereals, it is interesting to note the presence of starch grains belonging to the Paniceae tribe. Little evidence of these starch grains has been found in the dental calculus from the prehistoric Iberian Peninsula, and this study adds fresh data about the consumption of millet (Bucchi et al., 2019; González-Rabanal et al., 2022).

To date, extra-masticatory tooth uses associated with textile-related work have been documented at several Argaric sites and sites from neighboring and contemporary societies. These tasks were mainly carried out by anthropologically-determined female individuals. In this context, the first evidence was related to a young female from the non-Argaric Bronze Age site of Cabezo Redondo (Romero, 2016). More recently, new evidence of females showing similar wear marks on anterior dentition has been described by Lozano et al. (2021) in the Argaric site of Castellón Alto (Galera, Granada), together with other cases from the Granada province, such as Terrera del Reloj, Cuesta del Negro, Cerro de la Encina, Fuente Amarga and Cerro de la Virgen (Rubio, 2021) and, others from Cerro del Morrón in Murcia (Fregeiro et al., 2023). In all of them, dental wear features are linked to tasks that use threads and cords, probably vegetable fibers in origin, and they have been associated with females of different age cohorts, from youngest to adult individuals. This role probably was socially determined from the time when, in their childhood, individuals began to have the capacity to work. Overall, this would allow us to infer a technical and social division of labour beyond the sexual division already noted. However, although this possibility should not be ruled out, as Rubio (2021) has pointed out, the limited number of extra-masticatory wear could be linked to the greater degree of macroscopic occlusal wear observed in females than in males, so many of these wear marks would remain hidden without more detailed studies. In turn, it should be noted that, although sex-based DNA studies are not yet available, anthropological studies have pointed to some cases of adult males with similar tooth-wear, located both in the Argaric site of La Bastida de Totana (Murcia, Spain) (Oliart, 2021) and the multiple Chalcolithic burial of Camino del Molino (Caravaca, Murcia, Spain) (Díaz-Navarro et al., 2023). If their sexual attribution is confirmed, some males would also use their mouths in textile tasks, as would many other females. Otherwise, because the female cases with evidence of tooth tool use are still limited and subject to accurate sex-related diagnoses, we cannot claim that this kind of task was exclusively female-limited. However, specific technical processes involved in textile production, especially intensive ones such as thread production, seem to have been assigned to females from a very early age. Accordingly, we need further dental evidence based on accurate sexual diagnoses to discern aspects of the sexual division of labour during the Iberian Bronze Age.

To summarize, here, we present DNA-determined evidence that a young female from the Argaric site of the Laderas del Castillo exhibits non-alimentary dental wear on the anterior dentition associated with craft tasks, at least with plant fibers compatible with flax or hemp. Overall, this evidence represents a significant advance in the study of textile production and its significance in early prehistoric societies, specifically regarding El Argar culture.

CRediT authorship contribution statement

Alejandro Romero: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Ricardo E. Basso Rial:** Writing – review & editing, Validation, Resources, Methodology, Investigation. **Francisco Javier Jover Maestre:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Maria Herrero-Otal:** Writing – review & editing, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis. **Daniel R. Cuesta-Aguirre:** Writing – review & editing, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis. **Elena Fiorin:** Writing – review & editing, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis. **Assumpció Malgosa:** Writing – review & editing, Visualization, Validation, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis. **Patxuka de Miguel Ibáñez:** Writing – review & editing, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis. **Juan A. López Padilla:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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