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Species identification of osseous museum artefacts through peptide mass fingerprinting illustrated by a study on objects from Neolithic to Iron Age Armenia

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Identifying animal species used in osseous industry production is crucial for reconstructing human-animal interactions in ancient societies. However, bone artefact manufacture often involves intensive modifications to raw materials that hamper taxonomic identifications. Here, for the first time in central Eurasia, we taxonomically assess bone objects stored in museum collections, recovered from Late Neolithic to Iron Age contexts in Armenia, using a minimally invasive peptide mass fingerprinting technique, also known as Zooarchaeology by Mass Spectrometry (ZooMS). Our pilot study shows remarkable collagen preservation in the bone artefacts, demonstrating the rich potential of ZooMS for examining legacy collections. The successful ZooMS screening provided taxonomic identification for 86% of the artefacts, offering insights into species selection for bone manufacturing, as well as broader socioeconomic developments and interregional links. Our study underscores the utility of minimally invasive proteomic techniques, enabling the preservation of cultural and historical artefacts while addressing limitations of studying museum collections.

Osseous artefacts such as tools and personal ornaments can offer deep insights into past human behaviours, cultural expressions, and technological innovations^{1,2}. Since their earliest known presence in the Lower Palaeolithic in Eurasia³ bone artefacts have been found in archaeological contexts worldwide, highlighting the roles that animals played beyond subsistence in human societies throughout history. Artefacts made from osseous raw materials have provided insights into how early humans adapted to their environments, utilising available resources to create functional implements for hunting, processing food, crafting, and other daily tasks^{4–6}. Bone-made personal ornaments, including pendants, beads, and carvings, have also been used to point to complex social behaviours such as expressing identity, gender, status, and beliefs^{2,7,8}. Thus, these artefacts contribute to understanding cognitive and cultural development in human history, shedding light on early human material and social interaction with their environments and each other^{9–12}.

Currently, only a few studies have been carried out on bone artefacts to characterize human-animal interactions and socio-economic strategies in the South Caucasus. In the region, the first evidence of the technical and symbolic use of bone, antler, and teeth appears during the Middle Palaeolithic^{13–17}. However, it is only from the Late Neolithic (6000–5500/5200 BCE) onwards bone production increased in quantity, diversity, and functionality^{18–23}. The rich repertoire of bone artefacts includes polishers, pointed tools, cutting/cutting-edged tools, miscellaneous tools, and personal ornaments^{18–22}. However, most of the research of this nature in the region primarily focuses on typological and technological aspects, with little understanding of the selection of source materials and the role animals played in daily life^{15,23–34}. This is mostly due to the fact that the worked bones are often significantly modified and do not preserve diagnostic features sufficient for taxonomic or anatomical identification. In rare cases, where precise taxonomic affiliation is possible, distinct diagnostic elements, such as

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an antler or teeth, are used^{15,22,29}, while most commonly the identifications are typically broad, categorized by size class groups or restricted to the subfamily level. This limits our understanding of past human-animal relationships and the cultural, economic, ecological, and social criteria for bone material in tool production.

Recent advances in analytical techniques, particularly in proteomics, have enabled researchers to address this limitation and retrieve valuable taxonomic information when morphometric techniques fail. One such method, Zooarchaeology by Mass Spectrometry (ZooMS), has emerged as a powerful tool to identify the taxonomic origin of collagenous materials such as bone, antler, and leather^{35–37} for reviews. ZooMS utilises peptide mass fingerprinting to differentiate between taxa based on amino acid sequence variations of collagen type I protein³⁸. The method has been successfully applied to taxonomically identify bone specimens to family, genus, or species level, recovered from different temporal and spatial contexts (see³⁶ for review). The application of ZooMS in the South Caucasus region is still in its early stages. However, it has already yielded significant insights into past faunal dynamics and human-animal interactions. The method has been employed to identify the earliest evidence of domesticated water buffalo in the medieval Caucasus³⁹. It has also been successfully used to differentiate between sheep and goat in Chalcolithic contexts, providing insights into herd management and subsistence practices⁴⁰. Additionally, the method has contributed to reconstructing faunal dynamics during the Late Pleistocene, revealing past biodiversity and extinction processes and providing novel insights into environmental conditions⁴¹.

Conventional ZooMS protocols typically involve drilling or cutting a small sample (10–30 mg) from the bone, which can cause irreversible changes to fragile and often unique and therefore important artefacts. However, recent developments have shown that ZooMS can also be applied non-destructively by sampling the plastic bag or storage box that contained the artefact rather than the artefact itself^{42,43}. Further, several minimally invasive sampling techniques have been developed to study artefacts without substantial damage to the material. These include methods which analyse collagen collected through rubbing with an eraser^{43–45}, ultra-fine polishing films⁴⁶, and skin sampling strips⁴⁷. Several studies have compared the efficacy of some of these sampling methods highlighting the high success rate achieved with the use of polishing films^{48,49}. The application of minimally invasive techniques allows researchers to revisit culturally significant and rare materials and retrieve important taxonomic information. This ensures that the physical condition of artefacts is preserved, aligning with contemporary heritage conservation and management standards.

Here we present the first application of the minimally invasive ZooMS method, through the use of polishing films, to a selection of bone artefacts spanning from the Late Neolithic to the Iron Age of Armenia. The methodology was applied to a highly diverse group of objects recovered from 17 archaeological sites, housed in five museums across Armenia. Through this investigation, we seek to test the utility of the minimally invasive ZooMS technique on material stored in museums and contribute to the broader understanding of human-animal interactions and technological choices, highlighting the importance of novel analytical techniques applied to museum collections in archaeological research.

Methods

Bone artefacts

The artefacts were sampled from five museums in Armenia: The History Museum of Armenia (Yerevan), Erebuni Archaeological Museum (Yerevan), National Museum of Armenian Ethnography (Araks, Armavir Province), the Metsamor Historical-Archaeological Museum-Reserve (Taronik, Armavir Province), and the Yeghegnadzor Regional Museum (Yeghegnadzor, Vayots Dzor Province). This strategy enabled us to sample artefacts spanning from the Late Neolithic (6000–5500/5200 BCE) to the Late Iron period (700–600 BCE), without temporal gaps. In total, 17 archaeological sites are targeted for this study, represented by 93 artefacts (Fig. 1, Supplementary Table 1).

Site descriptions can be found in the Supplementary text. The studied material includes: arrowhead fragments ($n = 2$), arrowhead preforms ($n = 2$), awls ($n = 27$), awl/needle preform ($n = 1$), beads ($n = 8$), bead blanks/preforms ($n = 3$), blanks ($n = 3$), bridles ($n = 4$), button preform ($n = 1$), container ($n = 1$), handle ($n = 1$), handle preform ($n = 1$), needle ($n = 1$), pendants ($n = 3$), pendant preforms ($n = 10$), pierced horn ($n = 1$), pins ($n = 5$), plaques ($n = 2$), pointed fragment ($n = 1$), pressure flaker ($n = 1$), pyxis ($n = 2$), rings ($n = 2$), smoother ($n = 1$), spearhead preform ($n = 1$), spindle whorls ($n = 5$) and undetermined objects ($n = 4$) (See supplementary data for artefact pictures and descriptions). The classification of individual objects into their respective archaeological periods was determined by the archaeological context in which they were discovered.

Morphological identifications

Skeletal elements and broad taxonomic identifications were carried out for all sampled artefacts. Anatomical identification and morphological taxonomic affiliation of the remains were carried out based on osteological catalogues^{50–53} and comparative collections of recent specimens. Identifiable bones included articular ends and shafts of long bones, astragali, and horns/antlers. Finer taxonomic identifications of closely related species were not achieved. Skeletal elements that could not be assigned taxonomically were classified to size based on live weight following a modification of the criteria established by Thomas⁵⁴ and Grayson⁵⁵: small mammals (SM, 1–10 kg); intermediate mammals (IM, 10–50 kg); large mammal class 1 (LM1, 50–100 kg), and large mammal class 2 (LM2, >100 kg). Bone fragments that could not be assigned to a taxon but could be identified as a skeletal element were also assigned to a size class considering the relative size of the element (e.g. cortical bone thickness).

Sampling

In this study, a minimally invasive sampling technique, using polishing films, was applied to the worked bone material to avoid visible alterations to the artefact surface. The polishing film technique was initially developed to analyse organic surface coatings on photographs and artwork⁴⁶. The approach has recently been successfully adapted for archaeological bone analysis, demonstrating high identification rates^{49,56,57}. Microscopic images of representative samples were taken before and after sampling using a Keyence digital microscope (VHX-6000) to demonstrate the minimal invasiveness of the sampling method. Magnifications of 50x, 100x, and 200x were used to observe changes on the bone surface. The polishing film often left minor traces of polish or scratches, which were undetectable at 50x magnification but became more visible at 100x and 200x magnification (Fig. 2).

Only artefacts without adhesive treatment were selected for sampling, as glues, particularly collagen-based, can penetrate the porous structure of bone and interfere with analytical results. Measures were taken to minimize potential cross-contamination. This included nitrile gloves and face masks, equipment, and workspace cleaning with bleach and ethanol after each sampling. The samples were abraded (1 × 0.5 cm of the bone surface) with coarse polishing film (Fiber Optic Polishing Film by Precision Fiber Products, grit size: 30 μm) causing a small amount of organic material to adhere to the film. Each of these samples was visible as a small amount of powdered material on the surface of the film⁴⁶.

Collagen fingerprinting

The film containing bone powder was incubated in 100 μL 50 mM ammonium bicarbonate solution (AmBic) at 65°C for one hour. Samples were then spun briefly at 10,000 rpm, and 50 μL of supernatant was transferred to a fresh 1.5 mL microcentrifuge tube for subsequent enzyme digestion. The sample was incubated overnight with trypsin solution (0.4 μL/μg) after which 1 μL of 5% Trifluoroacetic acid (TFA) was added to stop enzymatic action. The peptides were purified using C18 ZipTip pipette tips. A total of 5 μL of collagen extract was mixed with 5 μL of α-cyano-hydroxycinnamic acid matrix. One μL of the sample was spotted in triplicate, each with a different calibrant/reference spot, on a 384-well Bruker MALDI ground steel target

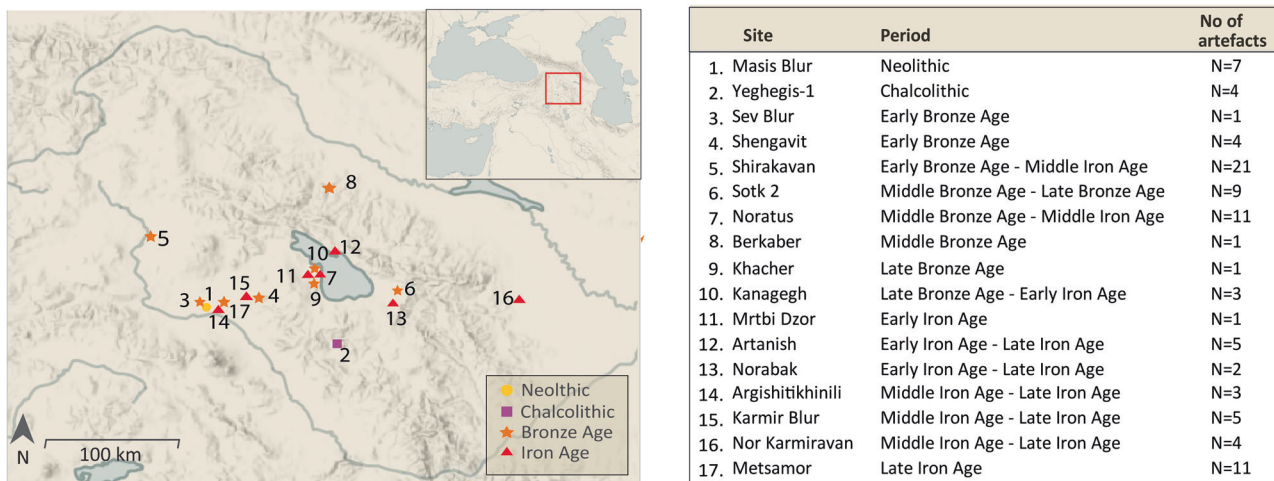


Fig. 1 | Spatial and temporal distribution of archaeological sites associated with the studied artefacts.

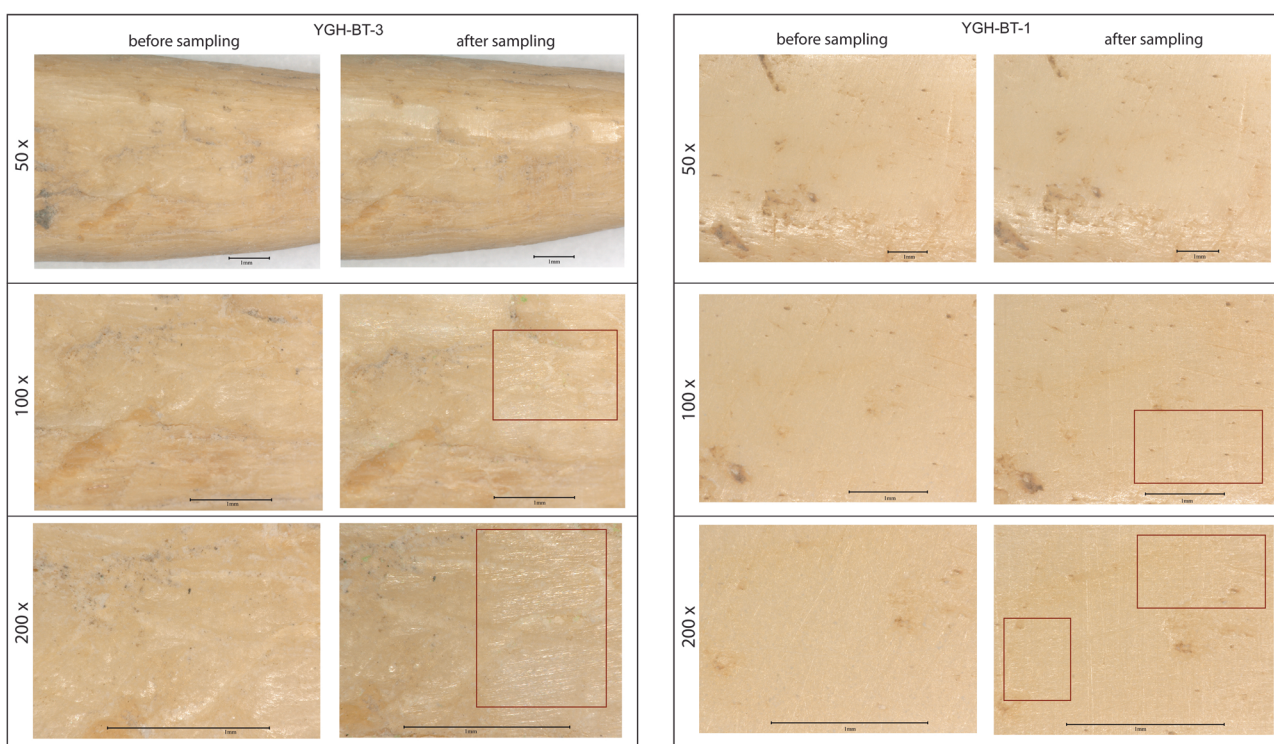


Fig. 2 | Photomicrographs illustrating changes in the surface of representative samples (YGH-BT-3 and YGH-BT-1) after minimally invasive sampling with coarse polishing film. Magnifications at 50x, 100x and 200x. Photo scale: 1 mm.

plate. The samples were run on a Bruker Autoflex Speed MALDI-TOF mass spectrometer (Bruker Daltonics) to produce spectra/fingerprints for taxonomic identification. ZooMS screening was carried out at the dedicated proteomics laboratory at the Max Planck Institute of Geoanthropology. The resulting peptide markers were identified via mMass software; v5.5.0⁵⁸. The registered collagen fingerprints of each specimen are presented in Supplementary Table 2.

Results

Recovered taxonomic composition

A total of 93 bone artefacts were analysed in this study. All of the specimens were first morphologically screened and identified to a taxon based on anatomical characteristics. Only in two cases was it possible to taxonomically identify artefacts to the genus level based on morphology: a

complete cattle phalange used as a pendant (BAA-89) and a cattle horn utilised as an awl (BAA-72). In rare cases, the identification was possible at the subfamily level (Caprines, i.e. sheep or goat, $n = 7$). The rest of the samples were classified into size groups (Large mammal 1, Large mammal 2, Intermediate mammal, and Small mammal), as more precise taxonomic identifications were not possible due to extensive modifications during artefact manufacture.

ZooMS was applied to all bone artefacts using a minimally invasive sampling method. Out of the 93 bone artefacts analysed, only 14 specimens failed to generate enough collagen due to poor molecular preservation, while 81 (87%) generated enough collagen for taxonomic identification, which is indicative of excellent protein preservation overall (Fig. 3). Extraction blanks were included throughout all stages to monitor the introduction of potential contamination. All blanks were empty of collagen type I, suggesting no

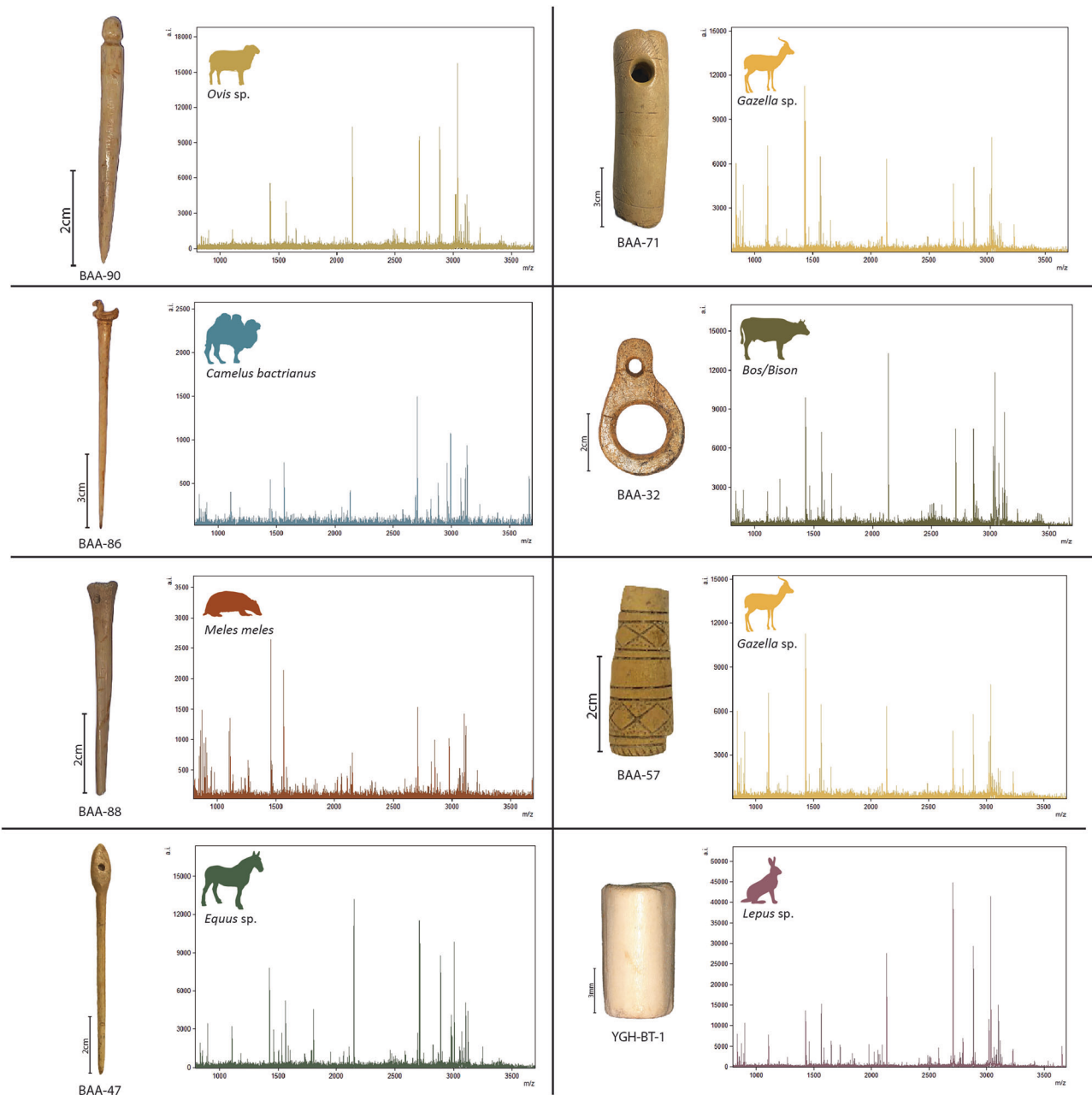


Fig. 3 | Example ZooMS spectra for selected osseous artefacts. BAA-90, a pin from Late Iron Age Metsamor manufactured from sheep bone; BAA-71, a container from Late Bronze Age Shirakavan made from gazelle bone; BAA-86, a decorated pin with avian motif from Late Iron Age Metsamor made of bone from Bactrian camel; BAA-32, a cattle bone pendant from Late Bronze Age Sotk 2; BAA-88, a pin from

Late Iron Age Metsamor manufactured from a European badger bone; BAA-57, fragment of a decorated bridle from Early Iron Age Mrtbi Dzor made of gazelle bone; BAA-47, a pin made of equine bone from Late Bronze Age Khacher and YGH-BT-1, a bead manufactured from hare/rabbit bone recovered from Chalcolithic Yeghegis-1.

protein contamination was introduced during the laboratory protocol. A total of seven regional taxa were identified using ZooMS, including regionally extinct gazelles (most likely *Gazella subgutturosa*). The results of the taxonomic identification through ZooMS are represented in Table 1, and the list of the recorded ZooMS markers is presented in Supplementary Table 2. The identified taxa include wild and domestic fauna revealing a wide spectrum of taxa targeted for osseous raw material manufacturing.

For most of the cases, identification was performed to the genus level (86% of the identified specimens). In some samples, however, insufficient preservation of collagen restricted identification to subfamily (8%) or family (5%). For instance, two specimens (a handle, BAA-01, and an awl BAA-72) could only be classified as “Bovidae/Cervidae” through ZooMS due to poor preservation resulting in a lack of diagnostic markers. Additionally, in some

cases (BAA-05, awl; BAA-28, burnisher; BAA-33 and BAA-87, pendants; BAA-93, pin), the discrimination between regionally extinct gazelle (*Gazella*) and deer (*Cervus*) was impossible due to the absence of diagnostic peptide markers COL1A2 375 and $\alpha 2$ 889 (m/z 1182, 2056, 2072 and 1532 for the members of the Antilipini tribe)^{56,59}.

Precise ZooMS identification is frequently impossible due to a shared genetic makeup between different genera, such as *Bos* and *Bison*. These Bovids can be distinguished from other artiodactyls based on the combination of COL1a2 978–990 (m/z 1192.6, 1208.6), COL1a2 484–498 (m/z 1427.7), COL1a1 586–618 (m/z 2853.4), and COL1a2 757–789 (m/z 3033.4) markers. However, the reference markers for *Bison* are identical to those for *Bos*, and identification, where possible, relies on consideration of the current range of the fauna and their past range as indicated by

Table 1 | Chronological distribution of artefacts identified through ZooMS.

Lab no	Museum	Site	Specimen	Morph. ID	ZooMS ID
Late Neolithic (6000-5200 BCE)					
BAA-08	History Museum of Armenia	Masis Blur	Awl	LM2	<i>Bos</i> sp.
BAA-09	History Museum of Armenia	Masis Blur	Awl	LM1	<i>Ovis</i> sp.
BAA-10	History Museum of Armenia	Masis Blur	Awl	LM1	<i>Ovis</i> sp.
BAA-11	History Museum of Armenia	Masis Blur	Smoother	LM2	<i>Bos</i> sp.
BAA-12	History Museum of Armenia	Masis Blur	Awl	LM1	<i>Ovis</i> sp.
BAA-15	History Museum of Armenia	Masis Blur	Awl	LM1	<i>Ovis</i> sp.
BAA-16	History Museum of Armenia	Masis Blur	Awl	LM1	<i>Ovis</i> sp.
Chalcolithic (5200-3500 BCE)					
YGH-1	Yeghegnadzor Regional Museum	Yeghegis-1	Bead	SM	<i>Lepus</i> sp.
YGH-3	Yeghegnadzor Regional Museum	Yeghegis-1	Awl	LM1	<i>Ovis</i> sp.
YGH-5	Yeghegnadzor Regional Museum	Yeghegis-1	Bead preform	LM1	<i>Ovis</i> sp.
YGH-10	Yeghegnadzor Regional Museum	Yeghegis-1	Bead preform	LM1	<i>Gazella</i> sp.
Early Bronze Age (3500-2400 BCE)					
BAA-05	History Museum of Armenia	Shengavit	Awl	LM1	<i>Gazella/Cervus</i>
BAA-06	History Museum of Armenia	Shengavit	Awl/fractured point	LM1	<i>Bos</i> sp.
BAA-07	History Museum of Armenia	Sev Blur	Bead blank	LM2	<i>Bos</i> sp.
BAA-13	History Museum of Armenia	Shengavit	Awl	Caprine	<i>Ovis</i> sp.
BAA-14	History Museum of Armenia	Shengavit	Awl	Caprine	<i>Ovis</i> sp.
BAA-71	National Museum of Armenian Ethnography	Shirakavan	Container	LM2	<i>Gazella</i> sp.
BAA-72	National Museum of Armenian Ethnography	Shirakavan	Awl	<i>Bos</i>	<i>Bovoid/Cervid</i>
BAA-73	National Museum of Armenian Ethnography	Shirakavan	Awl	LM1	<i>Ovis</i> sp.
Middle Bronze Age (2400-1500 BCE)					
BAA-24	Erebuni Archaeological Museum	Sotk 2	Awl	LM1	<i>Bos</i> sp.
BAA-26	Erebuni Archaeological Museum	Sotk 2	Awl	LM1	<i>Ovis</i> sp.
BAA-28	Erebuni Archaeological Museum	Sotk 2	Pressure flaker	LM1	<i>Gazella/Cervus</i>
BAA-29	Erebuni Archaeological Museum	Sotk 2	Awl	LM1	<i>Ovis</i> sp.
BAA-33	Erebuni Archaeological Museum	Noratus	Pendant preform	LM1	<i>Gazella/Cervus</i>
BAA-34	Erebuni Archaeological Museum	Noratus	Pendant preform	LM2	<i>Bos</i> sp.
BAA-81	National Museum of Armenian Ethnography	Berkaber	Handle preform	LM1	<i>Gazella</i> sp.
Late Bronze Age (1500-1200 BCE)					
BAA-25	Erebuni Archaeological Museum	Sotk 2	Arrowhead preform	LM1	<i>Ovis</i> sp.
BAA-27	Erebuni Archaeological Museum	Sotk 2	Awl	LM1	<i>Bos</i> sp.
BAA-30	Erebuni Archaeological Museum	Sotk 2	Secondary block / pierced blank	LM1	<i>Bos</i> sp.
BAA-31	Erebuni Archaeological Museum	Sotk 2	Ring/Personal ornament	LM1	<i>Bos</i> sp.
BAA-32	Erebuni Archaeological Museum	Sotk 2	Pendant/Personal ornament	LM1	<i>Bos</i> sp.
BAA-35	Erebuni Archaeological Museum	Noratus	Awl	LM1	<i>Gazella</i> sp.
BAA-44	Erebuni Archaeological Museum	Kanagegh	Ring/Personal ornament	LM1	<i>Bos</i> sp.
BAA-45	Erebuni Archaeological Museum	Kanagegh	Spindle whorl preform/ <i>Rouelle</i> preform	LM1	<i>Gazella</i> sp.
BAA-47	Erebuni Archaeological Museum	Khacher	Pin	LM1	<i>Equus</i> sp.
BAA-80	National Museum of Armenian Ethnography	Shirakavan	Awl	LM1	<i>Gazella</i> sp.
BAA-82	National Museum of Armenian Ethnography	Shirakavan	Pierced horn	<i>Capra/Gazella</i>	<i>Gazella</i> sp.
Early Iron Age (1200-900 BCE)					
BAA-18	Erebuni Archaeological Museum	Artanish 23	Button preform/ <i>Rouelle</i> preform	LM1	<i>fail</i>
BAA-22	Erebuni Archaeological Museum	Artanish 23	Blank	LM1	<i>Gazella</i> sp.
BAA-23	Erebuni Archaeological Museum	Artanish 23	Arrowhead preform	LM1	<i>Gazella</i> sp.
BAA-46	Erebuni Archaeological Museum	Kanagegh	Arrowhead preform/awl	LM1	<i>Bos</i> sp.
BAA-57	Erebuni Archaeological Museum	Mrtbi dzor	Bridle	LM1	<i>Gazella</i> sp.
BAA-76	National Museum of Armenian Ethnography	Shirakavan	Awl	LM1	<i>fail</i>
BAA-77	National Museum of Armenian Ethnography	Shirakavan	Arrowhead point	LM1	<i>Gazella</i> sp.
BAA-78	National Museum of Armenian Ethnography	Shirakavan	Arrowhead point fragment	LM1	<i>Gazella</i> sp.

Table 1 (continued) | Chronological distribution of artefacts identified through ZooMS.

Lab no	Museum	Site	Specimen	Morph. ID	ZooMS ID
BAA-61	National Museum of Armenian Ethnography	Shirakavan	Awl/needle preform	LM1	<i>Ovis</i> sp.
BAA-62	National Museum of Armenian Ethnography	Shirakavan	Awl	LM1	<i>Ovis</i> sp.
BAA-63	National Museum of Armenian Ethnography	Shirakavan	Awl	LM1	<i>Ovis</i> sp.
BAA-64	National Museum of Armenian Ethnography	Shirakavan	Awl	LM1	<i>Ovis</i> sp.
BAA-65	National Museum of Armenian Ethnography	Shirakavan	Awl	LM1	<i>Ovis</i> sp.
BAA-66	National Museum of Armenian Ethnography	Shirakavan	Awl fragment	LM1	<i>Ovis</i> sp.
BAA-67	National Museum of Armenian Ethnography	Shirakavan	Awl	LM1	<i>Ovis</i> sp.
BAA-68	National Museum of Armenian Ethnography	Shirakavan	Undetermined	LM1	<i>Gazella</i> sp.
BAA-69	National Museum of Armenian Ethnography	Shirakavan	Undetermined	LM1	fail
BAA-59	National Museum of Armenian Ethnography	Shirakavan	Pendant/pierced phalange	Caprine	<i>Ovis</i> sp.
BAA-60	National Museum of Armenian Ethnography	Shirakavan	Awl/needle preform	LM1	<i>Ovis</i> sp.
BAA-70	National Museum of Armenian Ethnography	Shirakavan	Spindle whorl/ <i>Rouelle</i>	LM2	<i>Bos</i> sp.
Middle Iron Age (900-700 BCE)					
BAA-36	Erebuni Archaeological Museum	Noratus	Bead	LM1	fail
BAA-37	Erebuni Archaeological Museum	Noratus	undetermined object	LM1	<i>Gazella</i> sp.
BAA-38	Erebuni Archaeological Museum	Noratus	Pendant preform	LM1	fail
BAA-39	Erebuni Archaeological Museum	Noratus	Bead preform	LM1	<i>Gazella</i> sp.
BAA-40	Erebuni Archaeological Museum	Noratus	Bead	LM1	fail
BAA-41	Erebuni Archaeological Museum	Noratus	Bead	LM1	fail
BAA-42	Erebuni Archaeological Museum	Noratus	Bead	LM1	<i>Bos</i> sp.
BAA-43	Erebuni Archaeological Museum	Noratus	Bead	LM1	fail
BAA-56	Erebuni Archaeological Museum	Karmir Blur	Spindle whorl/ <i>Rouelle</i>	LM2	<i>Bos</i> sp.
BAA-58	Erebuni Archaeological Museum	Argishti-Khinili	Pendant preform	LM1	<i>Bos</i> sp.
BAA-75	National Museum of Armenian Ethnography	Armavir	Pointed fragment	LM1	fail
BAA-79	National Museum of Armenian Ethnography	Shirakavan	Plaque	LM1	<i>Gazella</i> sp.
BAA-50	Erebuni Archaeological Museum	Norabak K1	Pendant	LM1	<i>Gazella</i> sp.
BAA-01	Institute of Archaeology and Ethnography	Nor Karmiravan	Bridle	LM1	<i>Bovid/Cervid</i>
BAA-02	Institute of Archaeology and Ethnography	Nor Karmiravan	Bridle	LM1	fail
BAA-03	National History Museum	Nor Karmiravan	Bridle	LM1	fail
BAA-04	National History Museum	Nor Karmiravan	Undetermined	LM1	fail
Late Iron Age (700-600 BCE)					
BAA-52	Erebuni Archaeological Museum	Karmir Blur	Spindle whorl/ <i>Rouelle</i>	LM2	<i>Equus</i> sp.
BAA-53	Erebuni Archaeological Museum	Karmir Blur	Plaque	LM1	<i>Camelus bactrianus</i>
BAA-54	Erebuni Archaeological Museum	Karmir Blur	Pyxis	LM1	fail
BAA-55	Erebuni Archaeological Museum	Karmir Blur	Pyxis	LM1	Mustelid
BAA-74	National Museum of Armenian Ethnography	Armavir	Handle	LM2	fail
BAA-83	Metsamor Historical-Archaeological Museum	Metsamor	Awl	LM1	<i>Ovis</i> sp.
BAA-84	Metsamor Historical-Archaeological Museum	Metsamor	Pendant preform/ pierced astragalus	Caprine	<i>Ovis</i> sp.
BAA-85	Metsamor Historical-Archaeological Museum	Metsamor	Pendant preform/ pierced phalange	Caprine	<i>Ovis</i> sp.
BAA-86	Metsamor Historical-Archaeological Museum	Metsamor	Pin	LM1	<i>Camelus bactrianus</i>
BAA-87	Metsamor Historical-Archaeological Museum	Metsamor	Pendant preform/ pierced astragalus	Caprine	<i>Gazella/Cervus</i>
BAA-88	Metsamor Historical-Archaeological Museum	Metsamor	Pin/Pierced needle preform	IM	<i>Meles meles</i>
BAA-89	Metsamor Historical-Archaeological Museum	Metsamor	Pendant preform/ pierced phalange	<i>Bos</i>	<i>Bos</i> sp.
BAA-90	Metsamor Historical-Archaeological Museum	Metsamor	Pin	LM1	<i>Ovis</i> sp.
BAA-91	Metsamor Historical-Archaeological Museum	Metsamor	Pierced blank	LM1	<i>Ovis</i> sp.
BAA-92	Metsamor Historical-Archaeological Museum	Metsamor	Pendant preform/ pierced phalange	Caprine	<i>Ovis</i> sp.
BAA-93	Metsamor Historical-Archaeological Museum	Metsamor	Pin	LM1	<i>Gazella/Cervus</i>
BAA-19	Erebuni Archaeological Museum	Artanish 29	Pendant preform	LM1	<i>Gazella</i> sp.
BAA-21	Erebuni Archaeological Museum	Artanish 29	Bead	LM1	<i>Gazella</i> sp.
BAA-51	Erebuni Archaeological Museum	Norabak K 1	Spindle whorl/ <i>Rouelle</i>	LM1	<i>Gazella</i> sp.

archaeological records from the study region. The attribution to *Bison*, in our case, can likely be excluded since this genus has not been identified in any archaeological and palaeontological faunal assemblages recovered from Middle-Late Holocene sites in the South Caucasus. On the other hand, the application of ZooMS allowed the discrimination between another group of closely related species, sheep and goat, that can be differentiated based on the presence of one diagnostic marker COL1a2 757–789, and its oxidised form (m/z 3077.4; 3093.4 for goat and m/z 3017.4; 3033.4 for sheep). Differentiating gazelles (*Gazella sp.*) from deer (*Cervus sp.*) is less straightforward, as both taxa are present in the region and display a similar set of diagnostic markers. However, a recent study introduced new markers to distinguish members of the Antilopini tribe, these are COL1A2 375 and a2 889 that, for Antilopini, display m/z 1182, 2056, 2072, and 1532, respectively⁵⁹. Additionally, m/z 3227 was identified as a potential gazelle-specific marker⁶⁰, while m/z 2216 was reported as characteristic for red deer^{59,61}. We applied these traits to guide our identifications, and the samples that had at least three of the above-mentioned markers were identified as gazelle or deer. In cases where these markers were absent, the identification was restricted to *Gazella/Cervus*. Additionally, ZooMS can identify two extant species in the genus *Camelus* at the species level: Bactrian camel (*C. bactrianus*) and Dromedary camel (*C. dromedarius*)⁶². While both species share most of the diagnostic markers, differences are recorded for marker COL1a2 484–498, with *C. bactrianus* displaying m/z 1443.7 and *C. dromedarius* - m/z 1453.7. Additionally, the Bactrian camel has two unique markers that are absent in the Dromedary camel, COL1a2 889–906 (m/z 1634.8) and COL1a2 454–483 (m/z 2820.4).

The majority of the analysed tools were manufactured from artiodactyl bones, with sheep (*Ovis*) being the most common taxon ($n = 28$) in the ZooMS-identified assemblage. It is impossible to differentiate between domestic and wild sheep using ZooMS due to the relatively early genetic isolation of these two groups. Considering that both wild and domestic sheep are present in the Neolithic to Iron Age contexts of Armenia, this restricts the understanding of strategies in selecting raw materials for bone tool production for these species. Sheep bones were used to manufacture different artefacts including awls, beads, pins, and pendants. The second abundant taxon in the studied collection is the regionally extinct gazelle ($n = 21$), with five further specimens being identified as *Gazella/Cervus*. In this case, we have clear evidence of wild game use in bone manufacture. Gazelle bones were used to manufacture weapons (or possibly hunting implements) such as arrowheads and spearheads as well as working tools (awls, pressure flaker, spindle whorl) and personal adornments (pendants and beads).

Another common group is *Bos* ($n = 17$) used to produce a wide array of artefacts (awls, spearheads, pendants, rings, beads). It is also not possible to differentiate between the domestic cattle (*Bos taurus*) and aurochs, its wild (*Bos primigenius*) counterpart. Although aurochs is a currently extinct taxon, it is known to have been present in the region throughout the late Neolithic and early Bronze Ages (ca 6000–3000 BCE)^{24,63}. Additionally, two artefacts were made from camel bones, and these were the rare cases when the identification was possible at the species level (*Camelus bactrianus*; $n = 2$). ZooMS markers are developed for two Asian camels—the Bactrian camel (*C. bactrianus*) and the Dromedary camel (*C. dromedarius*). These species share most of the ZooMS peptide markers except for a single diagnostic marker at COL1a2 484–498. This peptide for Bactrian camel is present at m/z 1443.7 and for Dromedary camel at 1453.7. A relatively small group is Perissodactyls, represented by a pin and a spearhead, manufactured from *Equus* bones. In rare cases, bones of small mammals were used in artefact manufacture; these include a bead made from rabbit bone (*Lepus sp.*), a pin made of badger bone (*Meles sp.*), and a decorated bone fragment made of a Mustelid bone.

Discussion

Bone artefact collections housed in museums and repositories worldwide provide valuable data for understanding patterns of resource use and the factors driving material selection in past societies. The use of osseous raw material for the manufacture of specific objects can be influenced by both its

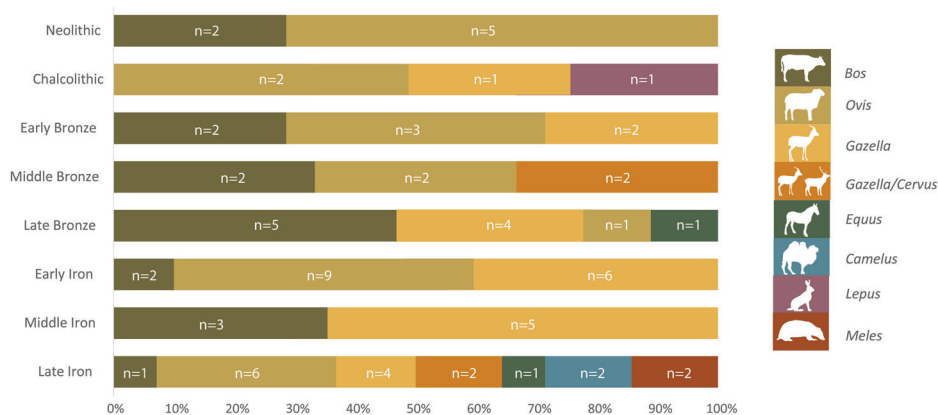
accessibility and specific mechanical properties, such as dimensions, durability, workability, etc.,^{64,65}. The physical characteristics of different anatomical parts would have guided early humans toward using osseous raw materials for tools, ornaments, and other artefacts, while cultural and symbolic values likely influenced the selection of particular species for these items. These choices can reflect cultural meanings attributed to certain animals, revealing how past societies assigned significance to the materials beyond functional use⁶⁶. In this way, analyses of museum artefacts can shed light on the socio-cultural, economic, and environmental contexts that shaped the production of tools and ornaments. Additionally, revealing animal taxa used for crafting artefacts can provide insights into the local fauna available at the time, contributing to our understanding of regional biodiversity and human-environment interactions⁶⁷. At the same time, the selection preferences for animal species not only reflect their availability and importance for subsistence activities but also their significance within cultural expressions⁶⁸. For instance, bones from non-local species within assemblages can serve as indicators of trade and communication networks, illustrating the movement of materials across different groups and locations^{69–71}. This highlights the importance of museum collections as critical archives for advancing interdisciplinary research in archaeology, biology, and heritage science.

This study represents the first application of Zooarchaeology by Mass Spectrometry (ZooMS) to bone artefacts from Armenia and further highlights the utility of advanced analytical techniques in facilitating the taxonomic identification of museum artefacts. The use of a minimally invasive sampling technique proved effective, as it enabled the identification of animal species while preserving the integrity of cultural heritage objects, many of which had previously been inaccessible for biomolecular research due to restrictions on destructive sampling. The high success rate of the analysis highlights the benefits of minimally invasive sampling without sacrificing the high hit rate. This approach enables the preservation of cultural and historical artefacts housed in museums for future research and demonstrates the potential of minimally invasive proteomics to support ethical and sustainable practices in archaeological research.

The initial morphological screening conducted to taxonomically identify the artefacts based on anatomical features was highly restricted due to extensive modifications during artefact production. For the majority of artefacts, the manufacturing process included substantial alterations to the bone such as breaking, polishing, and reshaping which resulted in the removal of the original anatomical structure. These modifications obscure taxonomically significant morphological features, hampering zooarchaeological identifications. Additionally, zooarchaeological screening proved to be limited in the identification of closely related species that share almost identical postcranial morphological features (e.g. sheep, goat, gazelle), limiting identifications to the subfamily level. This highlights the necessity of molecular screening of bone artefacts and wider zooarchaeological assemblages to precisely identify species allowing for more nuanced interpretations of human-animal relationships. While ZooMS cannot always differentiate between closely related species, as seen in the case of gazelle and deer, the method provides a level of taxonomic identification that is often unattainable through traditional approaches. Although it can often provide higher-resolution taxonomic identification, ZooMS presents a number of challenges in integrating its identifications with existing zooarchaeological datasets and quantification indices⁷².

Our ZooMS identifications provide insights into the diachronic patterns of bone tool production and use over time (Fig. 4, Supplementary Fig. 1). For instance, the ZooMS identified a portion of artefacts from the Neolithic site of Masis Blur, which are shown to be manufactured from sheep and cattle bones. These results are in agreement with the previously established zooarchaeological estimates at the site^{63,73}. The zooarchaeological evidence from Masis Blur suggested that animal husbandry, particularly of sheep and goats, was an important component of the subsistence economy⁶³. *Ovis* bones were predominantly used to craft tools such as awls. Notably, our research indicates that awls or awl preforms made from sheep bones continued to be used in Armenia at least until the Late Iron Age. Wild

Fig. 4 | Distribution of ZooMS identified taxa targeted for bone artefact manufacturing through time.



animals (including gazelles) are also present in the zooarchaeological assemblage of Masis Blur, but in very low numbers⁷³, suggesting that the early farmers in the region mostly utilized readily available resources for bone manufacture. The presence of bones of wild game, such as hare and gazelle, at the Chalcolithic Yeghegis site points to a greater reliance on hunted taxa for the production of artefacts. Gazelles and hares have been identified in Yeghegis-1 through the ZooMS screening of faunal assemblage, though in smaller numbers in comparison to the domesticated fauna⁴⁰. This may reflect the deliberate selection of wild game in bone manufacturing, particularly for adornments such as beads. Gazelle bones were also used in bone manufacturing in later periods, as seen at Early Bronze Age Shengavit and Shirakavan, and Middle to Late Bronze Sotk 2, Noratus, and Kanagegh sites, along with sheep and cattle bones. Even though gazelles are known to be present in the region at the time⁷⁴, gazelle bones have not been identified in these sites. This can be explained by the fact that gazelles bear similar postcranial morphological features to Caprines, making the differentiation of these animals difficult if not impossible when dealing with fragmented assemblages. This potentially highlights the importance and necessity of molecular screening of fossil bones that offers more precise taxonomic resolution, enabling identification between related species. The Late Bronze Khacher site reveals the first documented evidence of the use of Equine bones for tool manufacture in the region. Equine species present in the area during this period include both wild (*E. ferus*) and domestic (*E. caballus*) horses, wild ass (*E. hemionus*), and domestic donkey (*E. asinus*), as well as hybrids of these species. The applied method is insufficient to differentiate between horses and donkeys, however, recently a new extraction method was developed allowing to differentiation between these two species⁷⁵ and this avenue will be tested in our forthcoming research.

In the Early to Middle Iron Ages, a preference for bone tool manufacture was given to sheep, gazelle, and cattle, in concordance with zooarchaeological assemblages from that time period. Early Iron Age deposits also reveal symbolic or aesthetic expression in the selection of raw materials. For instance, the selection of hunted game such as gazelles to carve arrowheads and spearheads, evidenced in Artanish 23 and Kanagegh, may be driven by culture-specific meanings or symbolism attributed to a particular species. Some ethnographic accounts state that only weapons made from the remains of certain species could be used to hunt particular prey species⁷⁶. It is also possible that there was no species selection and the choice was rather opportunistic, reflecting the availability of this species.

The first half of the first millennium BCE, particularly during the Late Iron Age, reflects a diverse range of resources including unique and previously undocumented animal materials. During this period, bone carving of valuable items was widespread across the Near East. Notable examples include elaborately designed Assyrian and Urartian ivories, such as pyxides, various types of plaques, inlays for furniture and sculptures, horse harnesses, and other items⁷⁷⁻⁷⁹, which represent a continuation of a well-established ivory-carving tradition by this time. However, in the region corresponding to modern-day Armenia, ivory artefacts are relatively rare.

Instead, valuable items were predominantly crafted from other faunal bones. One such example is a pyxis recovered from the Urartian site of Karmir Blur (BAA-55). Our study shows that this particular object was made from the bone of a mustelid, an exceptional and rare choice of material for the period. This structure, interpreted as an elite residence, dates back to the 8th century BCE and has been hypothesized to belong to an Urartian medical practitioner or healer. Similarly, plaques discovered at several South Caucasian sites, such as Lori Berd⁸⁰ and the Urartian sites of Karmir Blur⁸¹ and Bastam⁸² as well as in Hasanlu⁸³ and Ziwiyeh⁸⁴ were fashioned from bone. These plaques, such as the example from Shirakavan (BAA-79) and Karmir Blur (BAA-53), likely served as inlays for wooden objects or furniture, illustrating the adaptability of bone as a material for decorative and functional purposes during this period.

Our demonstrated use of camel bones for the above-mentioned plaque from Karmir Blur (BAA-53) as well as for an elaborated pin from Metsamor (BAA-86) is exceptional, as these are, to date, the only indication of artefacts from Bactrian camel bones found at Late Iron Age sites in Armenia. This might reflect the exploitation of new trade routes and increased interactions with neighbouring regions, which were already established during the Middle Iron Age through the integration of Armenian sites into the Urartian influence zone. Urartu, established in the basin of Lake Van, spread its influence and territories in what is now the South Caucasus between the 9th and 7th centuries BCE. Camels were widely known in Urartu: a number of wall paintings were discovered in Erebuni, depicting both the Bactrian and the Dromedary camels⁸⁵. There is also textual evidence by Urartians mentioning camels, for example, King Sarduri 2nd left a report of 613 horses, 115 camels, 16,529 cattle, and 37,685 small domestic animals stolen from the country of Eriahe, localised in nowadays northwestern Armenia⁸⁶. Camel bones were also found during archaeological excavations from the 1st half of the 1st Millennium BC such as in the Urartian sites of Erebuni⁸⁷ and Metsamor⁸⁸, as well as at the Lori Berd⁸⁹ and Karabulagh sites (in the latter, with golden bits for camels)⁹⁰. In addition to this, our results from Shirakavan (BAA-53) and Metsamor (BAA-86) show that Armenia was not only part of the camel trade but also involved in the production or trade of objects made from camel bones, which were rare during the 1st millennium BCE.

As for the horse bones, we identified two objects made from *Equus* bones: a spindle whorl from Iron Age Karmir Blur (BAA-52) and a Late Bronze Age pin from Metsamor (BAA-47). During the Iron Age, horse breeding developed into a large-scale enterprise in the region. This is evidenced not only by the increased number of tombs containing horse bones⁹¹ but also by multiple inscriptions left by Urartian kings⁹². A significant factor here was the migration of Eurasian nomadic groups into the region, whose mobility heavily depended on horses. Their influence on the material culture of the Caucasus is evident⁹³. For example, horse bits in the so-called animal style, found in Nor Karmiravan, have parallels across the Eurasian steppes and Armenia^{80,81}. Interestingly, a large number of similar bone bits from Western Ukraine were predominantly crafted from roe deer antlers⁹⁴. In the

case of Nor Karmiravan, however, the material used for the bit is Bovidae or Cervidae.

Our identification of regionally extinct gazelles from Late Iron Age deposits at the Artanish site represents their last documented occurrence in the region. While gazelles were previously thought to have been hunted to extinction in the Near East by Neolithic hunter-gatherers, previous evidence suggested their persistence in Armenia until the Early Iron Age⁷⁴. Our analyses extend this timeline, demonstrating that gazelles survived in the region until at least the Late Iron Age. This highlights the potential of biomolecular methods in tracing extinct species and re-establishing chronological frameworks of specific taxa.

The absence of goats as a raw material source in the prehistoric assemblages is worth mentioning, given that goats are known to be an essential part of herds starting as early as the late Neolithic⁶³. The absence of any goat may be a sampling bias, or it may indicate that these animals were deliberately not used to make bone tools. If the latter, this reflects that people selected a narrower range of species for tool manufacture than for food and that certain species may have been specifically selected for tool making.

Overall, our results highlight the untapped potential of museum collections as dynamic resources for advancing research at the intersection of archaeology, palaeoproteomics, palaeoecology, and cultural heritage. We demonstrate the effectiveness of the ZooMS proteomic technique in the taxonomic identification of bone artefacts, providing important insights into human-animal interactions and species loss. By overcoming the limitations of morphological analysis, particularly for heavily modified or fragmented artefacts, ZooMS provides detailed taxonomic information that was previously inaccessible. The minimally invasive sampling approach further highlights its suitability for museum-held artefacts, ensuring that the physical integrity of rare and fragile objects is preserved while maximising their scientific potential.

Our pilot study has revealed that remarkable collagen preservation is exhibited by the worked bone collections recovered from Neolithic to Iron Age sites in Armenia and currently housed at various museums. Through successful ZooMS screening, we were able to obtain valuable taxonomic information from 87% of artefacts, reflecting broader patterns in the selection and use of animal resources. The successful identification of species used in artefact production reflects both the availability of raw materials and the cultural, symbolic, or economic considerations guiding their selection. Our analysis reveals a preference for regionally available ungulates, such as sheep and cattle, in artefact manufacturing. Additionally, ZooMS results point to a continued reliance on hunted taxa for the production of specific tools along with domestic fauna from the Neolithic period to the Iron Age.

Although our study's small sample size limits a comprehensive understanding of the complex dynamics of human-animal interactions and the specific roles of animals across different time periods, it demonstrates the potential of a novel approach to existing collections that could provide new insights into longstanding questions. Expanding such analyses to larger datasets and diverse assemblages will enhance our understanding of diachronic trends in socio-economic and cultural factors affecting material choice. Additionally, this will allow us to reveal fauna available at a time and trace patterns of species loss.

Data availability

All data generated or analysed during this study are included in this paper and its Supplementary Information files. All ZooMS spectra for identified samples are available on Mendeley Data open access repository; DOI: 10.17632/dbyyjhfb5z.1

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