



Complementarity between morphological and proteomic taxonomic identification methods of Late Upper Palaeolithic and Early Neolithic bone fragments from the NE Iberian Peninsula

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ABSTRACT

Zooarchaeology by Mass Spectrometry (ZooMS) has been widely used for taxonomic identification of fragmented bones, yet most direct comparisons with morphological identifications originate from European Palaeolithic contexts. This study provides a general assessment of the two taxonomic identification approaches of one Late Upper Palaeolithic and three Early Neolithic bone assemblages, all located in the northeastern Iberian Peninsula. The results confirm previously reported patterns where large-bodied taxa, especially *Bos* sp., were either more abundant in the ZooMS component or showed close similarity between identification methods regardless of local subsistence economy. These trends were broadly consistent across temporal, cultural, and environmental contexts, as well as in comparison with 27 other contexts from previously published studies comparing ZooMS and morphological data. However, variation among cervid taxa suggests that finer taxonomic subdivisions may prove important for further comparisons between identification methods. Although broad taxon- and body-size-related patterns are evident across bone assemblages, their expression within individual assemblages remains dependent on context specific taphonomic histories. Such trends should therefore not be interpreted as intrinsic properties of particular taxa, but as the outcome of taphonomic events that differentially influence taxonomic identifiability within fragment size classes.

1. Introduction

The taxonomic identification of large-scale archaeological bone assemblages through the palaeoproteomic method Zooarchaeology by Mass Spectrometry (ZooMS) (Buckley et al., 2009) has steadily been

increasing during the last approx. ten years (Brown et al., 2021b; Hol-loran et al., 2024; Lee-Michaelis et al., 2026; Oertle et al., 2025; Sinet-Mathiot et al., 2019). Such large-scale taxonomic identifications of osseous material have been enabled by the cost-effectiveness and rapidity of the ZooMS approach (Buckley et al., 2009). ZooMS is a

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method of peptide mass fingerprinting (PMF) that relies on collagen type I (COL1) to enable taxonomic identifications, typically at the family, genus, or even species level depending on the spatiotemporal context and peptide markers detected (Buckley et al., 2010, 2009). Thus far, research has focused, among other things, on highly fragmented bone assemblages dated to the Pleistocene exploring Denisovan (Brown et al., 2021b; Xia et al., 2024), Neanderthal (Ruebens et al., 2023, 2022), and *Homo sapiens* (Nel et al., 2023; Pothier Bouchard et al., 2020; Smith et al., 2024) subsistence strategies, while studies on Holocene sites have, especially, focused on the taxonomic identification of different bovids in addition to modes of subsistence (Bondetti et al., 2021; Culley et al., 2021; Janzen et al., 2021; McGrath and da Montes, 2026; Peters et al., 2021; Pilaar Birch et al., 2019). ZooMS has also been incredibly valuable in providing taxonomic identifications of specimens where diagnostic features have been modified, usually the more fragmented portion of bone assemblages, and discovering new taxa that were previously not recognised morphologically (Siné-Mathiot et al., 2019).

Studies focusing on these large Pleistocene bone assemblages have usually employed and compared two taxonomic identification methods: ZooMS and a morphology-based assessment of bone specimens, comparing diagnostic features with a reference collection in order to obtain taxonomic identifications, a well-known and commonly used approach in archaeology (Fagan, 2022; Peters et al., 1999; Saña Seguí, 2011). The identification methods usually also represent two different fragmentary states; ZooMS analysed bone specimens commonly reflecting a more advanced fragmentation. Moreover, these methods and fragmentary states may reflect substantial variations in their taxonomic compositions (Morin et al., 2023; Siné-Mathiot et al., 2023). Particular attention has been given to the proportional increase of larger ungulates within ZooMS identifications of Pleistocene bone assemblages, especially *Bos* sp./*Bison* sp. (Discamps et al., 2024). However, no studies have yet addressed this formation process issue in detail on Neolithic bone material, representing a contrasting subsistence economy.

To address this issue, we analyse highly fragmented bone remains from three Early Neolithic sites (Chaves, Fosca, and La Draga) and one Late Upper Palaeolithic site (Matutano) in the northeastern Iberian Peninsula using ZooMS and compare their taxonomic composition with previously published morphology-based identifications. The Early Neolithic sites encompass two year-round settlements characterised by complex husbandry and agricultural practices and one site, Fosca, that may have functioned primarily as a sheepfold (Castaños Ugarte, 2004; Estévez, 1988; Saña Seguí, 2011; Sierra, 2019; Watson, 1999), while Matutano functions as a temporal and subsistence economic outlier (Watson, 1999). Though several studies within the Iberian Peninsula analysing bone assemblages with the implementation of ZooMS exist (García-Vázquez et al., 2021; Sierra et al., 2024; Viñas-Caron et al., 2022), only two studies have so far compared the two taxonomic identification methods on Palaeolithic bone assemblages from the Iberian sites of La Viña (Torres-Iglesias et al., 2024) and Cova Eirós (Bal-García et al., 2026). Here, we compare the proportional taxonomic compositions obtained through the two identification methods across the four sites and assess whether differences in representation are associated with taxon, bone fragmentation, site-specific taphonomic histories, and subsistence practices. We further place these results within a broader comparative framework of previously published ZooMS and morphology-based datasets to evaluate whether patterns observed in northeastern Iberian Peninsula persist across wider temporal, geographic, and cultural contexts. Through these analyses, we aim to assess how bone fragmentation degrees and assemblage formation influence taxonomic representation between ZooMS and morphological identification methods and, consequently, the extent to which the two approaches provide complementary perspectives on archaeological faunal assemblages.

1.1. Archaeological sites

Four sites representing different spatiotemporal contexts were selected for this study. Each site included mammalian bone specimens that had previously been taxonomically identified based on morphological diagnostic features, as well as specimens that could not be morphologically identified, making the latter well suited for ZooMS analysis.

One Late Upper Palaeolithic (LUP) site, Matutano Cave, represents a temporal outlier (Fig. 1). Within relatively close proximity of Matutano Cave in the northern mountainous region of Valencia, lies the Early Neolithic (EN) site of Fosca Cave. The third cave site, Chaves, is located further north in the Aragonese pre-Pyrenees and is dated to the EN as well. Lastly, the EN site of La Draga, located in Catalonia, differs from the others as an open-air site. The three Neolithic sites each exhibit somewhat distinct subsistence economies. Detailed descriptions of the contexts included can be found in SI A.

2. Material and methods

2.1. Specimen selection and sampling

A total of 925 highly fragmented bone specimens, taxonomically unidentifiable based on morphology, were randomly selected for ZooMS analysis. Specifically, 278 bone fragments from Matutano Cave, Sector 3, Level 6/A, were selected, and 320 fragments from two levels originating from Chaves Cave. These were 267 specimens from Level Ia and 53 specimens from the older Level Ib. The smallest number of bone fragments was selected from Fosca Cave, 136 specimens in total. This was in part due to the amount of fully burned bone fragments that are generally not suitable for ZooMS (Hansen et al., 2024b; Yates, 2013), and the low quantity of specimens available from the Neolithic layers of Phase I-II, Sector B. Lastly, 191 bone fragments were selected from two of La Draga's four sectors, Sector A ($n = 141$) and Sector D ($n = 50$).

The specimens were sampled using a plier, obtaining approx. 5 mg per specimen, into either 1.5 mL microtubes (Protein LoBind, Eppendorf) or 400 μ L 96-well plates (Thermo Scientific), followed by HCl (hydrochloric acid) demineralisation for best proteomic output (Evans et al., 2023; Hansen et al., 2024a). Each 96-well plate had one cell functioning as a blank, while samples processed in tubes had one blank per batch consisting of various amounts. Instruments and surfaces were cleaned in between each sampling using 0.07 M NaOCl (bleach) and 1.19 M $\text{CH}_3\text{CH}_2\text{OH}$ (ethanol). Cortical bone was sampled if possible, as collagen is less deteriorated in cortical bone compared with trabecular bone (Ásmundsdóttir et al., 2024).

2.2. Protein extraction and MALDI-ToF analysis

All samples were left overnight in 100 μ L 0.05 M NH_4HCO_3 (ammonium bicarbonate, hereafter AmBic) at 4 °C. After centrifugation at 10,000 rpm for one minute and discarding the AmBic, the samples were demineralised in 150 μ L 0.6 M HCl for 24 h at 4 °C. The HCl was then removed and samples were rinsed three times with 100 μ L 0.05 M AmBic, vortexed shortly, and centrifuged between rinses. 100 μ L 0.05 M AmBic was then finally added for a one hour incubation at 65 °C. 50 μ L of the supernatant was transferred to a 1.5 mL microtube along with 0.4 μ g trypsin (Promega, #V115A) for digestion at 37 °C using a heating block (Thermal Shake lite, VWR) for 18 h. Following digestion, the protease was deactivated by adding 1 μ L of 0.13 M $\text{CF}_3\text{CO}_2\text{H}$ (Trifluoroacetic acid, hereafter TFA). Using a C18 Hypersep™ plate (Thermo Fisher) the samples were desalted and purified for subsequent spotting on an MTP 384 target ground steel MALDI plate (Bruker) in triplicates consisting of 1 μ L eluted peptides and 1 μ L of α -cyano-4-hydroxycinnamic acid (CHCA) matrix solution (Buckley et al., 2009; Welker et al., 2016).

141 samples with insufficient taxonomic identification were selected

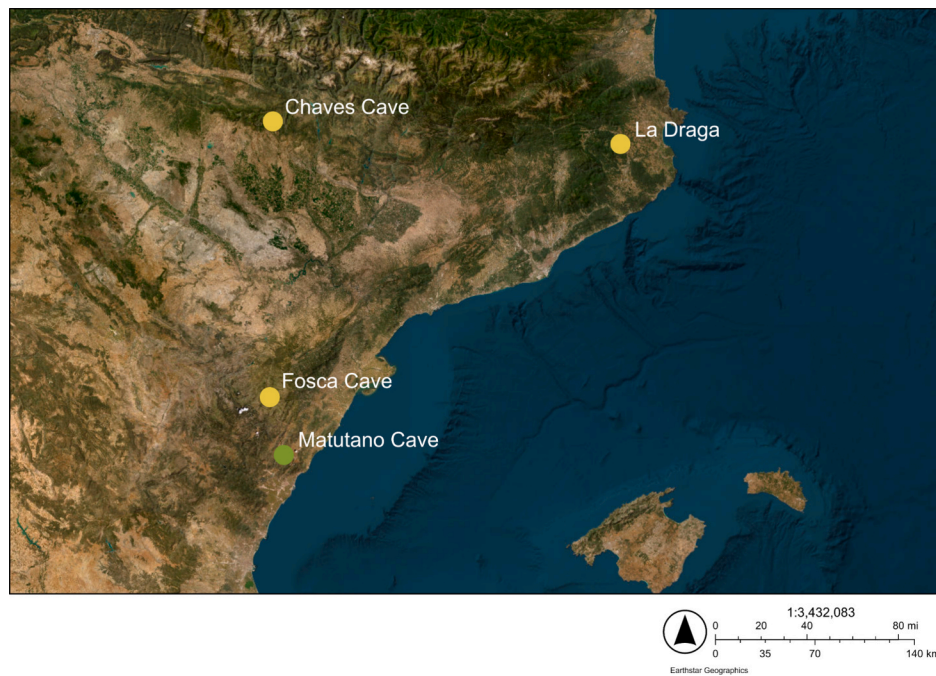


Fig. 1. Location of the four sites located within the eastern Iberian Peninsula. The yellow dots indicate bone material collected from EN sites and/or levels, while the green dot indicates material retrieved from an Upper Palaeolithic site. The map was created using USGS ArcGIS Online Map Viewer (U.S. Geological Survey, 2024).

for reprocessing with a sodium hydroxide (NaOH) washing step to remove humic contaminants (Szpak et al., 2017). 95 samples from La Draga were directly processed with the NaOH washing step incorporated into the initial extraction as humic contaminants were detected through a previously processed batch of 96 samples. The remaining 50 μL 0.05 M AmBic from the gelatinised samples processed previously was removed and 100 μL 0.1 M NaOH was added, followed by a brief vortex of no more than 30 s. After centrifuging at 10,000 rpm for one minute, the NaOH was discarded. The pellet's pH was then neutralised by rinsing three times with 100 μL of 0.05 M AmBic, briefly vortexing, centrifuging for one minute at 10,000 rpm, and discarding the AmBic after each wash. Next, 100 μL 0.05 M AmBic was added for subsequent incubation at 65 °C for one hour. Hereafter, the protocol was repeated as previously described.

Mass spectra were acquired using two different mass spectrometers. Initially, all samples and 61 reprocessed samples were run through a Bruker UltrafleXtreme MALDI-ToF MS in reflector mode, positive polarity, set to a laser intensity of 50–70 %, and a mass range of 799–4,000 m/z . Each sample was externally calibrated against adjacent spots containing a mixture of six peptides (des-Arg1 Bradykin m/z = 904.681, Angiotensin I m/z = 1295.685, Glu1-Fibrino-peptide B m/z = 1750.677, ACTH (1–17 clip) m/z = 2093.086, ACTH (18–39 clip) m/z = 2465.198 and ACTH (7–38 clip) m/z = 3657.929).

80 out of the 141 reprocessed samples were run on a Bruker timsTOF flex MALDI-tims-Q-ToF in reflector mode, positive polarity, set to a laser intensity of 40 %, and a mass range of 799–4,000 m/z . Because the laser impacts the calibration spot in an orthogonal geometry and the time-of-flight analyser is highly stable, only a single calibration spot is typically required. However, in this study, three calibration spots were used, with the latter two serving to verify the consistency of the first. The calibration mixture comprised the six peptides listed above. Samples were analysed on different mass spectrometers due to time constraints and availability.

Lastly, triplicate MALDI spectra were merged using MALDIquant v.1.22.2 (Gibb and Strimmer, 2012) and MALDIquantForeign v.0.14.1 (Gibb, 2023) and analysed using mMass (Niedermeyer and Strohm, 2012) as described previously (Le Meillour et al., 2024). mzXML files were converted into separate.txt files using mzXMLConverter v.480fe6e

using the software Python (Van Rossum and Drake, 1995). Deamidation values were calculated using the software Betacalc3 (Wilson et al., 2012). Proteomic data,.txt files, are available on [Zenodo.org](https://doi.org/10.5281/zenodo.17288657) (ID: <https://doi.org/10.5281/zenodo.17288657>).

2.3. Registration variables

The bone fragments were assigned to a skeletal element (France, 2009), and fraction thereof, sex, age, fusion stage (Dammers, 2015; Davis et al., 2012), and size class, as well as taphonomic characteristics if applicable and possible. Size classes were based on the work of Bunn (1986) and Torres-Iglesias et al. (2024) entailing small terrestrial mammals 1 (STM1), <2 kg; STM2, approx. 2–20 kg; STM3, approx. 20–35 kg; medium terrestrial mammals (MTM), 35–230 kg; large terrestrial mammals (LTM), >230 kg. Taphonomic factors included weathering stage (Behrensmeier, 1978), degree of thermal application (Stiner et al., 1995), anthropic alterations, and tissue type. Furthermore, the maximum length and maximum cortical thickness were measured. Bone elements were also assigned to a broader type of body categories, including cranium, axial, limb, forelimb, hindlimb, and non-determined bones; however, more specific assignments of anatomical elements were assigned when possible. All this information can be found in SI B.

Additionally, a subset of bones taxonomically identified morphologically from La Draga were subject to further measurements. 354 bones identified as *Bos* sp., *Caprinae*, and *Sus* sp., originating from various sectors and excavation years, had their maximum length and maximum cortical thickness measured (SI G).

2.4. Statistical analyses and tests

All visualisations and statistical analyses were produced and calculated via various packages using the software R (R Core Team, 2021) and Rstudio (Posit team, 2023).

More specifically, visualisations, resampling method, and significance tests, including two-way ANOVA, Mann-Whitney U, John Tukey's HSD, two-tailed one-sample *t*-test, Chi-square, and Welch Two-Sample *t*-test, were created using tidyverse v.2.0.0 (Wickham et al., 2019), ggplot2 v.3.4.4 (Wickham, 2016), paletteer v.1.6.0 (Hvitfeldt, 2021),

dplyr v.1.1.4 (Wickham et al., 2023), tidyr v.1.3.1 (Wickham et al., 2024), ggpubr v.0.6.0 (Kassambara, 2023), car v.3.1.2 (Fox and Weisberg, 2019), multcomp v.1.4.26 (Hothorn et al., 2008), cowplot v.1.1.3 (Wilke, 2024), boot v.1.3–28.1 (Canty and Ripley, 2022), and pheatmap v.1.0.12 (Kolde, 2019).

In order to investigate the potential differences between the two methods' taxonomic identifications across sites and taxa, a bootstrap simulation was implemented. After filtering out taxonomic groups covering diverse body-size groups, only *Bos* sp./*Bison* sp., Caprinae, Cervidae, Equidae, and Suidae were retained. The relative proportions of identifications were reshaped into a site-by-taxon format, establishing paired values for ZooMS and morphology-based datasets (R-script and SI D). The standard deviation based on the percentages was calculated separately for each identification method per taxon. These were then combined to extract a pooled measure of uncertainty. Using these standard deviations, 1000 bootstrap replicates were generated per site-taxon combination by sampling from a normal distribution centered on the observed difference, with the variance adjusted to the variability of each row. For code, search for Table 3 and Fig. 4, bootstrap analysis in the R-script.

Moreover, a script was developed to estimate the species composition within specimens identified within the sub-family of Caprinae. To determine the lowest taxonomic rank possible across both morphological and proteomic identification methods, the R-script developed was based on a defined taxonomic hierarchy within the Caprinae subfamily. The script recursively allocates percentages from higher-order taxa (e.g. Caprinae, Caprinae (not *Capra* sp.), or *O. aries*/*C. hircus*) to their most specific descendants, depending on which species were present at each context. Morphological data was first used to establish the most specific taxonomic level, and this resolution was then used as the basis for resolving ambiguous proteomic data. Once all taxa were assigned, the morphological and proteomic proportions were averaged to produce a combined composition estimate, with both data types weighted equally (Table 2).

2.5. Taxonomic reasoning: Morphology and ZooMS

The taxonomic identifications are based on a combination of the zooarchaeological record within the Iberian Peninsula and the peptide markers observed via the ZooMS analyses. In Table 1, an overview of the species covered by the various taxonomic categories are listed. The geographic context and chronological period further constrain the range of possible taxonomic identifications. The broadest taxonomic category will mostly be used throughout this paper.

Both *Bos primigenius* and *Bos taurus* have been morphologically identified at the EN sites of La Draga (Saña Seguí, 2011), Chaves (Castaños Ugarte, 2004; Sierra, 2019), and Fosca Estévez, 1988. *B. primigenius* and *B. taurus* cannot be distinguished from each other through ZooMS (Buckley et al., 2017, 2009; Buckley and Collins, 2011; Kirby et al., 2013; Welker et al., 2016). Consequently, the ZooMS analysed bone fragments from these sites are identified as *Bos* sp. For the LUP site of Matutano Cave, *B. primigenius* is the most likely candidate, as *Bison priscus* is believed to have gone extinct in the northern and central parts Iberian Peninsula sometime during the Late Pleistocene (MIS 3/2) (Castaños et al., 2016; López González et al., 1999). Moreover, *B. priscus* is entirely absent in the southwest from at least 23,000 to 17,000 cal BCE (de Carvalho et al., 2022; Pfeiffer, 1999; Sala et al., 2010).

Ovis aries and *Capra hircus* has been morphologically identified at the three EN sites, while the wild goat counterpart *Capra pyrenaica* was securely identified at Chaves (Castaños Ugarte, 2004; Sierra, 2019) and La Draga (Saña Seguí, 2011). *C. pyrenaica* and *Rupicapra rupicapra* were morphologically identified at Matutano (Watson, 1999). Though it is secure to relate ZooMS identifications of the bone fragments originating from Matutano to *C. pyrenaica* and *R. rupicapra*, the ZooMS analysed portion from the EN sites cannot be exclusively determined to *C. hircus* and *O. aries* (Buckley et al., 2017, 2010, 2009; Buckley and Collins, 2011; Kirby et al., 2013; Welker et al., 2016), though it is likely that these two domestic species represent the majority of the EN settlement identifications. Within the Iberian Peninsula *C. pyrenaica* (Alados, 1985; Gonzales, 1982) and *R. rupicapra* (or *Rupicapra r. pyrenaica*) (Cardoso and Antunes, 1989; de los Terreros, J.Y.S., 2001) are suggested to have been present during the EN and LUP. Therefore, the taxonomic

Table 1

Possible taxa existing during the Late Upper Palaeolithic and Early Neolithic within the regions of Aragon, Catalonia, and Valencia.

Taxonomic ID		Late Upper Palaeolithic Valencia	Early Neolithic Aragon, Catalonia, and Valencia	References
<i>Bos</i> sp.	<i>Bos primigenius</i>	Y	Y	Buckley et al., 2017, 2009; Buckley and Collins, 2011; Castaños Ugarte, 2004; Estévez, 1988; Kirby et al., 2013; Saña Seguí, 2011; Sierra, 2019; Van Vuure, 2005; Welker et al., 2016
	<i>Bos taurus</i>	N	Y	
Caprinae	<i>Capra</i> sp.			Alados, 1985; Buckley et al., 2017, 2010, 2009; Buckley and Collins, 2011; Cardoso and Antunes, 1989; Castaños Ugarte, 2004; de los Terreros, 2001; Estévez, 1988; Gonzales, 1982; Kirby et al., 2013; Saña Seguí, 2011; Sierra, 2019; Watson, 1999; Welker et al., 2016
	<i>Capra hircus</i>	N	Y	
	<i>Capra</i>	Y	Y	
	<i>pyrenaica</i>			
	Caprinae (not <i>Capra</i> sp.)			
	<i>Ovis aries</i>	N	Y	
	<i>Rupicapra</i>	Y	Y	
	<i>rupicapra</i>			
Carnivora	<i>Felis silvestris</i> / <i>Lynx pardinus</i>	Y	Y	Buckley et al., 2017, 2009; Buckley and Collins, 2011; Castaños Ugarte, 2004; Estévez, 1988; Kirby et al., 2013; Saña Seguí, 2011; Sierra, 2019; Welker et al., 2016
	Mustelidae	Y	Y	
	<i>Ursus arctos</i>	Y	Y	
	<i>Vulpes vulpes</i>	Y	Y	
Cervidae	<i>Capreolus capreolus</i>	Y	Y	Buckley et al., 2009; Buckley and Collins, 2011; Castaños Ugarte, 2004; Davis and MacKinnon, 2009; Estévez, 1988; Gómez-Olivencia et al., 2013; Jensen et al., 2020; Kirby et al., 2013; McGrath et al., 2019; Saña Seguí, 2011; Sierra, 2019; Watson, 1999; Welker et al., 2016
	<i>Cervus elaphus</i>	Y	Y	
<i>Equus</i> sp.	<i>Equus ferus</i>	Y	Y	Bernaldez-Sánchez et al., 2024; Buckley et al., 2017, 2009; Buckley and Collins, 2011; Castaños Ugarte, 2004; Desmond et al., 2018; Estévez, 1988; Kirby et al., 2013; Lira et al., 2010; Sierra, 2019; Strani and DeMiguel, 2023; Welker et al., 2016
	<i>Equus hydruntinus</i>	Y	N?	
<i>Homo sapiens</i>		Y	Y	Buckley and Collins, 2011; Buckley and Kansa, 2011; Welker et al., 2016
Leporidae	<i>Lepus</i> sp.	Y	Y	Alves et al., 2008; Buckley et al., 2017, 2009; Castaños Ugarte, 2004; Estévez, 1988; Llorente Rodríguez, 2010; Marques et al., 2017; Saña Seguí, 2011; Sierra, 2019; Welker et al., 2016
	<i>Oryctolagus cuniculus</i>	Y	Y	
<i>Sus</i> sp.	<i>Sus scrofa</i>	Y	Y	Buckley et al., 2017, 2009; Buckley and Collins, 2011; Castaños Ugarte, 2004; Saña Seguí, 2011; Watson, 1999; Welker et al., 2016
	<i>Sus scrofa domesticus</i>	N	Y	

specificity for Caprinae species in the EN sites is lower: Caprinae (not *Capra* sp.) covers *O. aries* or *R. rupicapra*, while *Capra* sp. entail *C. hircus* or *C. pyrenaica*. As ZooMS relies on COL1 peptide markers observed in MS1 spectra (Buckley et al., 2009), its taxonomic resolution is generally lower than that achieved with sequenced proteomic data, which involves fragmenting precursor ions (MS1) to generate fragment ion spectra (MS2) containing amino acid sequence information (Rüther et al., 2021). Additionally, COL1, and collagens in general, evolve at a slower rate than non-collagenous proteins. In particular, COL1 α 1, COL1 α 2, and COL2 α 1 exhibit relatively slow evolutionary rates (Zazueta et al., 2024). Because ZooMS is based exclusively on MS1 peptide markers and COL1, this partly explains the difficulty in distinguishing between closely related taxa such as *C. pyrenaica* and *C. hircus*, or *O. aries* and *R. rupicapra*.

Various Carnivora species have been identified amongst the EN sites morphologically, though none have been registered in the LUP layer included in this study (Watson, 1999). *Canis familiaris* (including *Canis* sp. at Chaves), *Felis silvestris*, and *Vulpes vulpes* were all found in the zooarchaeological material at Chaves and La Draga (Castaños Ugarte, 2004; Saña Seguí, 2011; Sierra, 2019). *Ursus arctos* and *Lynx pardinus* were exclusively uncovered at Fosca Cave (Estévez, 1988). *Meles meles* and *Martes foina*/*Martes* sp. were morphologically recorded across all EN sites (Estévez, 1988; Saña Seguí, 2011; Sierra, 2019). These species are not restricted to a single temporal period within the scope of this study. However, here ZooMS represents several taxonomic limitations as well. It is not possible to distinguish between *F. silvestris* and *L. pardinus* using ZooMS (Buckley et al., 2009; Buckley and Collins, 2011; Welker et al., 2016), and although other Ursidae species beyond *U. arctos* can be excluded based on their geographical distributions, ZooMS cannot differentiate among those taxa (Buckley et al., 2017; Buckley and Collins, 2011; Kirby et al., 2013; Welker et al., 2016).

Cervus elaphus and *Capreolus capreolus* are the only Cervidae species uncovered through the sites morphologically (Castaños Ugarte, 2004; Estévez, 1988; Saña Seguí, 2011; Sierra, 2019), except for at Matutano where *C. elaphus* was exclusively recorded (Watson, 1999). Though these two species can be distinguished through ZooMS they are mainly grouped together under the category Cervidae in this paper as very few *C. capreolus* have been identified in the ZooMS analysed bone fragments (Buckley et al., 2017, 2009; Buckley and Collins, 2011; Jensen et al., 2020; Kirby et al., 2013; McGrath et al., 2019; Welker et al., 2016). *Dama dama* has been excluded as it was first introduced during the Roman period (Davis and MacKinnon, 2009) as well as *Rangifer tarandus* since it was limited to the Cantabrian region and northeastern part of Catalonia during the Pleistocene (Gómez-Olivencia et al., 2013).

Morphologically, *Equus* sp. have been registered at Chaves (Castaños Ugarte, 2004; Sierra, 2019) and Fosca (Estévez, 1988). Both *Equus ferus* and *Equus hydruntinus* are well attested in the Iberian Peninsula during the Late Pleistocene, although *E. ferus* is consistently the dominant and more widely distributed taxon, while *E. hydruntinus* appears in lower frequencies and more patchily (Bernáldez-Sánchez et al., 2024; Lira et al., 2010; Strani and DeMiguel, 2023). However, their precise distribution remains difficult to reconstruct, which is an issue continuing into the EN. Although *E. hydruntinus* is well attested in the Late Pleistocene, its persistence into the EN is uncertain and it is generally considered to have disappeared by the end of the Pleistocene or early Holocene (Bernáldez-Sánchez et al., 2024; Strani and DeMiguel, 2023). ZooMS identifications based on trypsin digestion do not allow differentiation between these closely related equids (Buckley et al., 2017, 2009; Buckley and Collins, 2011; Desmond et al., 2018; Kirby et al., 2013; Welker et al., 2016). Therefore, most analysed specimens are assigned to *Equus* sp., although *E. ferus* is the most likely candidate for many of these identifications.

Oryctolagus cuniculus and *Lepus* sp. have been morphologically identified at all EN sites included in this study (Castaños Ugarte, 2004; Estévez, 1988; Saña Seguí, 2011; Sierra, 2019), with the majority of remains attributed to *O. cuniculus*. Although no Leporidae were

identified in the specific Magdalenian layer of Matutano analysed here (Watson, 1999), both *O. cuniculus* and *Lepus* sp. were present in the Iberian Peninsula during the LUP and EN. Within Iberia, *Lepus* sp. most likely comprised *Lepus europaeus* and *Lepus granatensis* (Llorente Rodríguez, 2010), however, mtDNA introgression from the now locally extinct *Lepus timidus* has been detected in modern populations of both species, particularly in northern Iberia (Alves et al., 2008; Marques et al., 2017). *L. timidus* was likely completely replaced sometime after the Last Glacial Maximum (Marques et al., 2017). As ZooMS cannot distinguish between the aforementioned *Lepus* species and *O. cuniculus* (Buckley et al., 2017, 2009; Welker et al., 2016), all identifications are referred to as Leporidae throughout this study.

Both *Sus scrofa* and *Sus scrofa domesticus* have been recorded at the EN sites of La Draga (Saña Seguí, 2011) and Chaves (Castaños Ugarte, 2004), however, at Fosca, identifications could not be specified beyond *Sus* sp. Estévez, 1988. In the Magdalenian layers of Matutano, only *S. scrofa* has been identified, as domestic forms had not yet been introduced during this period (Watson, 1999). As ZooMS cannot distinguish between *S. scrofa* and *S. s. domesticus* (Buckley et al., 2017, 2009; Buckley and Collins, 2011; Welker et al., 2016), all specimens are hereafter classified as *Sus* sp., although those from Matutano can be assigned to *S. scrofa*.

3. Results and discussion

3.1. General metric, taphonomic, and skeletal element characteristics of the ZooMS analysed material

For the LUP site of Matutano, the bone assemblage was generally in good condition with little to no weathering (0.26 ± 0.5 SD out of 5). There were some appearances of root marks and fissures or microcracks. Limb bones ($n = 135$) were the largest broader skeletal element group identified, of which 12 could be determined as forelimb and 29 as hindlimb (Fig. 2). Axial bones made up the second largest group ($n = 101$) (SI Tab. A.2). The bone fragments were on average $38.15 \text{ mm} \pm 15.16$ SD in maximum length and $2.97 \text{ mm} \pm 1.75$ SD in maximum cortical thickness. 11 specimens out of 278 had cut marks evenly split between limb and axial bones (excl. cranial elements) which derived from Cervidae ($n = 6$, *C. elaphus* being securely represented), Caprinae ($n = 4$, both *C. pyrenaica* and *R. rupicapra*), and one *Equus* sp. (SI B) indicating butchery activities. This interpretation is further supported by the morphologically analysed bone assemblage from contemporaneous levels in Sector 2, where burning, cut marks, and marrow extraction provide evidence of on-site carcass processing (Albiol et al., 1999).

Little weathering (0.89 ± 0.83 SD out of 5) was observed within the bone fragments from Chaves Cave ($n = 320$), levels Ia and Ib. Level Ia and Ib maximum lengths were of similar measurements, $42.24 \text{ mm} \pm 17.72$ SD and $41.97 \text{ mm} \pm 12.99$ SD, respectively. A significant difference in cortical thickness was found between the two layers (Mann-Whitney U, $p = 0.029$), Ia being $3.17 \text{ mm} \pm 3.07$ SD and Ib being $4.08 \text{ mm} \pm 3.30$ SD in maximum cortical thickness. This could be due to sample size differences, or a higher intensity of activities during the Level Ia occupation, e.g., marrow extraction, butchering, artefact production, and mechanical pressure (Bovy et al., 2019). One rib and three long bones were observed with cut marks, all from Level Ia, originating from two *Capra* sp., one *Sus* sp., and one indeterminate. Additionally, sawing traces were observed on one *Bos* sp. long bone fragment from Level Ia (SI B). In general, across both stratigraphic layers, limb bones were the largest group represented ($n = 156$). As seen at Matutano, hindlimbs ($n = 50$) were the more abundant group compared to forelimbs ($n = 32$). It is however noteworthy that the bone element composition, in general, is similar between the levels Ia and Ib, except for the forelimb and hindlimb distribution, where it is equal between them within Level Ib, while approx. 30 % more hindlimbs compared with forelimbs can be observed in Level Ia. Axial bones constituted the same relative fraction at Chaves as seen within the sites of Matutano and

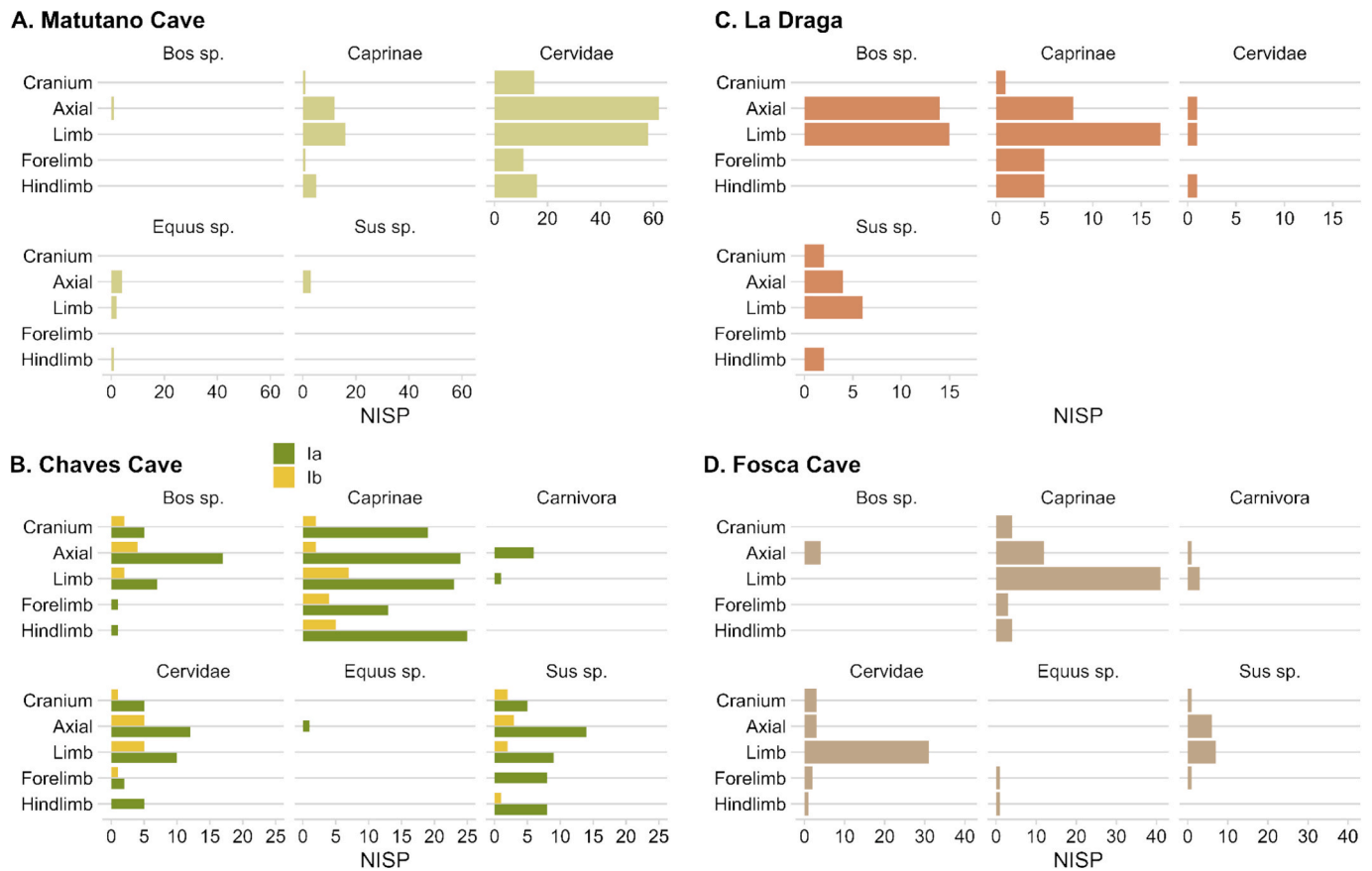


Fig. 2. Broad grouping of anatomical elements across taxonomic identifications and sites in NISP. Data is based on ZooMS identifications (SI B; SI Tab. A.2).

La Draga, however, approx. 12–16 percentage points fewer axial bone specimens were observed within Fosca (SI Tab. A.2). Paying exclusive attention to the EN sites, this could suggest that Chaves and La Draga had a higher degree of on-site butchery processing activities compared with Fosca, focusing on local domestic animals. For Chaves in particular, it is interesting that the skeletal element composition of Cervidae is similar to the local domestic animals. It may be that whole carcasses were transported back to the site rather than selectively targeting high meat-yield body parts. In general, the skeletal element composition of Chaves Cave seems to be similar between the ZooMS analysed assemblage and the morphologically assessed specimens (excl. teeth) (Sierra, 2019).

At Fosca Cave, little weathering (1.33 ± 0.99 SD out of 5) has been recorded in the studied bone fragments ($n = 136$), though corrosion, root marks, and manganese oxide traces were registered across 54 specimens. The bone fragments were on average $42.19 \text{ mm} \pm 19.47$ SD and $3.14 \text{ mm} \pm 1.56$ SD maximum length and cortical thickness, respectively, similar to those seen at the sites of Matutano and Chaves (Level Ia). Six fragments had been thermally altered, all originating from long bones except one rib. One *C. elaphus* specimen showed clear signs of cut marks (SI B). Limb bones constituted 74.27 % ($n = 101$), which is approx. 1.5 times more than observed at the sites of Matutano, Chaves, and La Draga. This correlates with what has been observed in the morphological taxonomically identified assemblage from Sector B, levels N-I and N-II (Estévez, 1988). Fosca Cave's lack of evidence of indirect markers of crop cultivation (Yll and Olària, 1988) and its likely primary use as a sheepfold (Llorente-Rodríguez et al., 2014), together with the pronounced amount of limb bones originating from Cervidae, implies a higher degree of off-site butchery practices compared with Chaves and La Draga as well as a stronger reliance on hunted meat for on-site consumption. Furthermore, a resemblance between the forelimb

and hindlimb bones studied here and the bones assessed morphologically by Estévez (1988) has been found in that there is no noticeable difference in the proportion between them (SI Tab. A.2).

At La Draga ($n = 191$), a significantly higher weathering stage was observed in Sector A (1.25 ± 1.09 SD out of 5) compared to Sector D (0.1 ± 1.09 SD out of 5) (John Tukey's HSD, $p = 0.001$). Microcracks in bone were especially found within Sector A. In fact, the weathering stages at Fosca, Chaves, and La Draga (Sector A) were all significantly higher in contrast to the site of Matutano and La Draga (Sector D) (John Tukey's HSD, $p = 0.001$) (SI Fig. A1). Explanations for the low weathering stage at Matutano can be difficult to assess, though at La Draga, the exposure to subaerial weathering and the dry environment of Sector A may explain its more advanced weathering to that of the anaerobic and saturated Sector D (Saña et al., 2013). Gnawing marks constituted 4.71 % ($n = 9$) and root marks 11.52 % ($n = 22$) of the specimens from La Draga, though exclusively found in specimens from Sector A, which is in agreement with a previous study (Saña et al., 2013). 38 specimens had been burned, mostly at the extremities of the bones, the majority belonging to Sector D, though this should not necessarily be considered a characteristic for Sector D (SI B). The skeletal composition at La Draga is somewhat similar to the sites of Chaves (Level Ia) and Matutano in regards to axial and limb fragments registered, though the forelimbs and hindlimbs recorded were equally abundant, similar to Fosca and Chaves (Level Ib) (SI Tab. A.2). Fewer cranium parts were found ($n = 6$, 3.14 %) in the ZooMS analysed portion compared with the morphologically assessed assemblage, though this is likely due to the inclusion of teeth in the latter assessment (Saña Seguí, 2011). In general, the distribution of skeletal elements corresponds to that observed at Chaves, suggesting a site characterised by complex husbandry practices and predominantly on-site butchery of domestic animals (Antolín et al., 2014; Revelles et al., 2016), complemented by a small contribution of hunted

mammals, although the latter appears more pronounced at Chaves. Lastly, the recorded maximum length and cortical thickness were respectively 24.89 ± 13.36 SD and 2.47 ± 1.05 SD. These measurements both differ from what has been reported at the sites of Matutano, Chaves, and Fosca. This could be due to sample size and, in general, the local formation history.

3.2. Taxonomic compositions

Sufficient taxonomic identifications, reaching family or genus level, through ZooMS were possible at various success rates across the four sites of Matutano ($n = 231/278$, 83.09 %), Chaves (level Ib, $n = 50/53$, 94.34 %; level Ia, $n = 232/267$, 86.89 %), Fosca ($n = 131/136$, 96.32 %), and La Draga ($n = 86/191$, 45.03 %). The identification rate and preservation is further discussed in Section 3.3. Meanwhile, the combined 730 out of 925 (78.92 %) successful taxonomic identifications retrieved through ZooMS were compared with species identified through bone morphology from previous studies (Castaños Ugarte, 2004; Estévez, 1988; Saña Seguí, 2011; Sierra, 2019; Watson, 1999) (SI E). The morphologically identified specimens were typically less

fragmented than those selected for ZooMS. While taxonomic data were available for comparison across all four sites, comparable metric data were only available for La Draga, where maximum bone length and cortical thickness were also recorded for a subset of morphologically identified specimens. Consequently, direct comparisons of these metrics between ZooMS analysed and morphologically identified specimens were restricted to La Draga.

As ZooMS does not allow for differentiation between domesticated and wild taxa and generally does not have the same taxonomic resolution a morphological assessment can provide, some taxa have been grouped together, following the logic from Section 2.5., to enable a comparison between the two taxonomic identification approaches (Fig. 3). Though some species have been grouped together, consequently reducing taxonomic specificity, an initial understanding of the domestic and wild taxa distribution present has been indicated in Fig. 3 (upper panel) via the morphologically assessed specimens. Here it is again obvious that amongst the EN sites, Fosca differs from Chaves and La Draga. This feeds into the narrative of there being a different subsistence economy present at Fosca compared with La Draga and Chaves, despite the two latter representing two different site forms, a cave and an open-

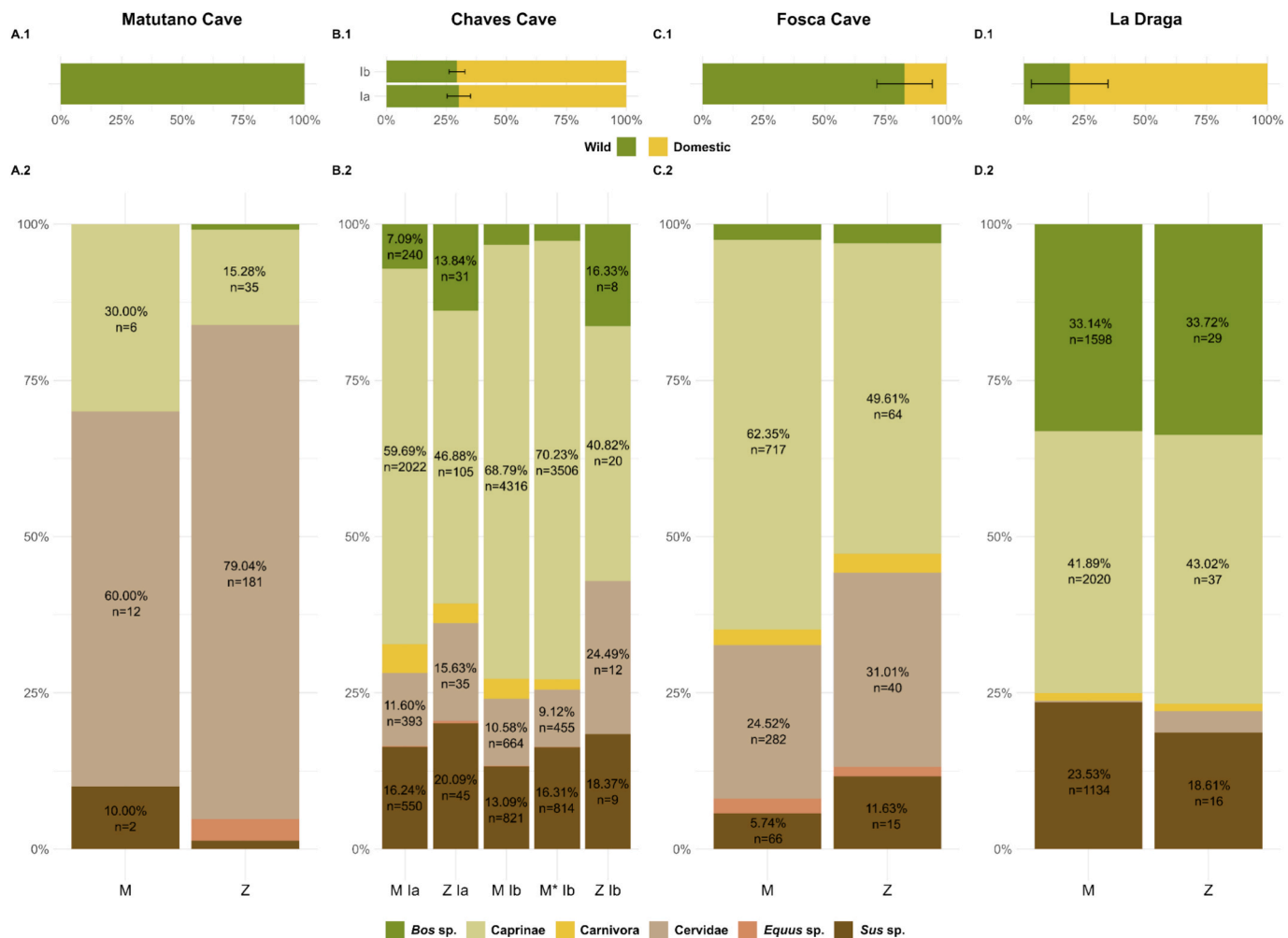


Fig. 3. The upper panel illustrates the ratio of domestic to wild taxa among the specimens from the four archaeological sites (A.1) Matutano Cave (Watson, 1999); B.1) Chaves Cave Level Ia (Castaños Ugarte, 2004) and Level Ib (Castaños Ugarte, 2004; Sierra, 2019); C.1) Fosca Cave (Estévez, 1988); D.1) La Draga (Saña Seguí, 2011)), whereas the lower panel shows the taxonomic distribution as determined by both morphological (M) and ZooMS (Z) identifications. The standard deviation bars for the upper panel's domestic-wild ratio plots were calculated using known morphologically assessed domestic and wild taxonomic identifications and often using genus-based classifications as deviation (SI C, explanations for symbols within SI C can be found in the "read me" file). The taxonomic compositions are A.2) Matutano Cave M ($n = 20$) (Watson, 1999) and Z ($n = 229$); B.2) Chaves Cave M Ia ($n = 3,367$) (Castaños Ugarte, 2004), Z Ia ($n = 224$), M Ib ($n = 6,216$) (Castaños Ugarte, 2004), M* Ib (* indicates another zooarchaeologist's assessment of the bone material from Chaves Ib; $n = 4,992$) (Sierra, 2019), and Z Ib ($n = 49$); C.2) Fosca Cave M ($n = 1,150$) (Estévez, 1988) and Z ($n = 129$); D.2) La Draga's taxonomic composition for M ($n = 4,822$) (Saña Seguí, 2011) and Z ($n = 86$) (SI D).

air site.

In general, the same families and genera were represented when comparing the ZooMS and morphology-based taxonomic identifications (Fig. 3, lower panel). Using ZooMS, Leporidae was identified at the sites of Matutano (n = 2), Chaves (n = 3), and Fosca (n = 3), while six *H. sapiens* fragments were identified at Chaves, Layer Ia. These two taxa have, however, been excluded from Fig. 3, both from the ZooMS and morphology parts. Leporidae have been omitted as human activities likely have little to do with the accumulation of Leporidae, but rather represent regurgitated pellets from birds of prey, probably mainly *Bubo bubo* (Llorente Rodríguez, 2010). In addition, the sheer amount of Leporidae registered within the morphologically assessed assemblages at the caves of Chaves, Matutano, and Fosca would skew the general taxonomic composition drastically. *H. sapiens* has been excluded from further analysis as well since our focus lies on non-human mammals.

Furthermore, accounting for ZooMS identifications, two new genera, *Bos* sp. and *Equus* sp., were identified at Matutano, though *Bos* sp. has been recorded morphologically within a younger stratigraphic level of Matutano (Watson, 1999).

In addition, focusing on major findings, Cervidae identified through ZooMS is mainly *C. elaphus*, though four *C. capreolus* have been identified across the EN sites. The absence of *C. capreolus* at Matutano correlates with the morphologically assessed bones (Watson, 1999). Nevertheless, 50 % of all cervid identification could only be identified as Cervidae mainly due to the lack of the peptide marker COL1a2 793–816 (Brown et al., 2021a; Buckley et al., 2009).

Focusing on the Caprinae identifications, the combination of a proteomic and morphological approach may help untangle two issues, one for each identification technique. In the case of Caprinae, ZooMS has a taxonomic specificity limitation which means that, in the spatiotemporal context of the EN Iberian sites, Caprinae (not *Capra* sp.) could be either *O. aries* or *R. rupicapra*, while *Capra* sp. could be *C. hircus* or *C. pyrenaica*. For the LUP site, Matutano Cave, however, we can assume that ZooMS-based identifications of Caprinae (not *Capra* sp.) and *Capra* sp. relate respectively to *R. rupicapra* and *C. pyrenaica*. Additionally, the sites of Chaves and La Draga deal with the issue of having large portions of *O. aries*/*C. hircus* classifications within the morphologically assessed component, between 60–80 %, a well-known issue amongst zooarchaeologists (Boessneck, 1970; Payne, 1969).

Using a hierarchical approach, suggestive ratio estimates of the Caprinae composition for all contexts have been provided in Table 2. Based on the output values, the three EN contexts of Chaves and La Draga seem to have mainly been composed of *C. hircus* and *O. aries*, though the majority consisted of the latter, especially for Chaves level Ib (approx. 77 %). The predominance of *O. aries* within the Caprinae assemblage suggests that sheep constituted the principal caprine resource within the broader mixed farming economy (Antolín et al., 2014), especially for Chaves (Sierra, 2019). The lower representation of *C. hircus* indicates a complementary but secondary role for goats (Navarrete et al., 2019), while the near absence of *C. pyrenaica* suggests that hunting of wild caprines was of minimal economic significance (Saña Seguí, 2011). Matutano's *C. pyrenaica* and *R. rupicapra*-ratio of approx. 6:1 appears somewhat unchanged.

In the case of Fosca Cave, the script could not apply its standard logic due to a specific limitation, namely the presence of both Caprinae and *Capra* sp. in the data, without any higher resolution species like *C. hircus* or *C. pyrenaica*. Since *Capra* sp. is the lowest assignable level in such a case, the Caprinae percentages were manually distributed between *Capra* sp. and *O. aries*, proportionally if both were present in the same modality, or fully assigned to the one present if the other was absent. Additionally, Caprinae (not *Capra* sp.) was allocated entirely to *O. aries*, as no evidence supported the presence of *R. rupicapra* in the morphological record. Meanwhile, a zooarchaeological assessment of the bone assemblage from the EN layers of Sector C suggests that the *Capra* sp. fraction in Sector B primarily consisted of *C. pyrenaica*, accounting for up to 98 % (Llorente Rodríguez, 2007).

Table 2

Initial ZooMS and morphological Caprinae identification ratios, based on the NISP, and the combined output. *Chaves has had Level Ib assessed by two different zooarchaeologists (for more information see SI F). For code, search for combining morph and ZooMS identifications of Caprinae species in R-script.

Site	Taxon	Identification approach		Output values (%)		
		Morph (%)	ZooMS (%)	Morph	ZooMS	Combined
Chaves Ia	Caprinae		11.43			
	Caprinae (not <i>Capra</i> sp.)		70.48			
	<i>Capra</i> sp.		18.09			
	<i>Ovis aries</i> / <i>Capra hircus</i>	86.49				
	<i>Capra hircus</i>	4.59		41.5	21.73	31.61 ± 9.89 SD
	<i>Capra pyrenaica</i>	2.72		2.72	1.42	2.07 ± 0.65 SD
	<i>Ovis aries</i>	6.18		55.78	76.85	66.32 ± 10.54 SD
	Caprinae		25			
	Caprinae (not <i>Capra</i> sp.)		65			
	<i>Capra</i> sp.		10			
Chaves Ib	<i>Ovis aries</i> / <i>Capra hircus</i>	86.08; 72.59*				
	<i>Capra hircus</i>	4.01; 3.99*		23.73	14.31	19.02 ± 4.71 SD
	<i>Capra pyrenaica</i>	2.85; 6.3*		4.58	2.76	3.67 ± 0.91 SD
	<i>Ovis aries</i>	7.07; 17.11*		71.7	82.92	77.31 ± 5.61 SD
	Caprinae	1.81	4.69			
	Caprinae (not <i>Capra</i> sp.)		9.375			
	<i>Capra</i> sp.	96.51	85.94	98.29	90.63	94.46 ± 3.83 SD
	<i>Ovis aries</i>	1.67		1.7	9.38	5.54 ± 5.43 SD
	Caprinae		59.46			
	Caprinae (not <i>Capra</i> sp.)		37.84			
La Draga	<i>Capra</i> sp.		2.7			
	<i>Ovis aries</i> / <i>Capra hircus</i>	60.05				
	<i>Capra hircus</i>	18.91		47.95	31.17	39.56 ± 8.39 SD
	<i>Capra pyrenaica</i>	0.84		0.84	0.55	0.69 ± 0.15 SD
	<i>Ovis aries</i>	20.19		51.21	68.29	59.75 ± 8.54 SD
	Caprinae		28.57			
	Caprinae (not <i>Capra</i> sp.)		5.71			
	<i>Capra</i> sp.		65.71			
	<i>Capra pyrenaica</i>	83.33		83.33	89.52	86.43 ± 3.1 SD
	<i>Rupicapra rupicapra</i>	16.67		16.67	10.48	13.57 ± 3.1 SD
Matutano	Caprinae		28.57			
	Caprinae (not <i>Capra</i> sp.)		5.71			
	<i>Capra</i> sp.		65.71			
	<i>Capra pyrenaica</i>	83.33		83.33	89.52	86.43 ± 3.1 SD

This approach has further limitations as it assumes that both morphological and proteomic data are equally reliable and representative, which may not hold across varying preservation conditions and formation process histories. The proportional redistribution of ambiguous taxa also introduces potential bias, especially when one species disproportionately dominates a category. Moreover, the current implementation does not incorporate sample size, confidence intervals, or

uncertainty modeling, which restricts its ability to reflect the full range of data variability. Ultimately, this workflow should be regarded as a preliminary attempt to reconcile two identification systems under a shared taxonomic framework. To evaluate its reliability, controlled experimental validation is essential. Such experiments would involve known faunal specimens that are deliberately fragmented and then reanalysed using both morphological and proteomic methods. Comparing the known taxonomic identities to those inferred by the workflow would offer crucial insights into the accuracy, limitations, and potential refinements of this approach.

3.2.1. Comparing NISP counts derived from ZooMS and morphology-based identifications

Elaborating on Section 3.2., a few challenges exist in attempting to compare the two taxonomic identifications methods and the results thereof. Besides having to create broader taxonomic classifications to compare datasets, it was difficult to conduct any meaningful intra- or inter-site-specific statistical tests of the taxonomic distribution, as only two to three data points exist for each context, at least within this study. Nevertheless, in order to combine both morphologically and ZooMS-identified bone specimens, and overcome the lack of data points, we used a simulation-based bootstrap for resampling data presented in this section and in Section 3.2.2, focusing on *Bos* sp., Caprinae, Cervidae, *Equus* sp., and *Sus* sp. As 'Carnivora' and 'Other' respectively cover mammal size classes from *M. meles* to *U. arctos* and Rodentia to Elephantidae, the categories were excluded from further analysis.

Based on the five archaeological contexts examined in this study, the relative proportions of *Bos* sp., Cervidae, and *Equus* sp. were significantly higher in the component identified using ZooMS, whereas Caprinae were significantly more abundant in the morphologically identified component (two-tailed one-sample *t*-test, $p = 0.001$ for all comparisons). In contrast, *Sus* sp. did not exhibit a significant bias toward either taxonomic identification method (two-tailed one-sample *t*-test, $p = 0.183$). *Equus* sp., which displays the lowest variation across sites (1.903 SD) (Table 3), likely achieved statistical significance because the bootstrap analysis consistently returned mean values distinct from zero. In contrast, *Sus* sp., despite a higher mean relative difference, exhibited greater variability (6.645 SD), resulting in a wider bootstrap distribution that frequently overlapped with zero. This suggests that fragment size-related identification biases may affect some taxa more than others, though in cases like La Draga, where overall variation between methods is minimal (3.482 SD), such biases seem limited.

Despite various subsistence economic realities, occupation types, environments, geographic location, and periods being represented

among the five archaeological contexts (see SI A for more information on sites), consistent differences in taxonomic representation were generally observed between the two identification methods. *Bos* sp., Cervidae, and *Equus* sp. were significantly more represented in the ZooMS analysed component, whereas Caprinae were significantly more represented in the morphologically assessed component. As these components generally represent more and less fragmented bone specimens, respectively, these differences may point towards taxon-specific patterns associated with bone fragmentation. However, the magnitude of these differences varied among contexts, likely reflecting their complex and distinct taphonomic histories. Although it remains difficult to determine which factors, or combinations of factors, drive these differences, the consistency observed for some taxa raises the possibility that they may be more frequently represented within particular fragment size groups. To investigate whether these patterns extend beyond the assemblages analysed here, we broadened the comparison in the following section using previously published ZooMS and morphology-based datasets.

3.2.2. An additional regional comparison of NISP counts from ZooMS and morphology-based identifications

As the sites included in this study represent four EN contexts, Fosca Cave possibly reflecting a more herder-based economy in the EN group, and one hunter-gatherer context, an additional 27 contexts (SI D) were incorporated to assess the identification methods' behaviour across a broader temporal and subsistence economic spectrum. Here, a simulation-based bootstrap was used to analyse data as well to accommodate limited data points. The contexts represent temporally approx. 300,000 years, several techno complexes and cultural periods, and a geographical area encompassing most of Europe and East Asia (Balgarcía et al., 2026; Boger et al., 2014; Brown et al., 2021b; Discamps et al., 2019; He, 2016; Holloran et al., 2024; Lehmann, 1954; Luo et al., 2009; Masini and Abbazzi, 1997; Morin et al., 2023; Morin and Soulier, 2017; Niven, 2006; Oertle et al., 2025; Raymond et al., 2024; Romandini et al., 2020; Ruebens et al., 2024; Scott, 2018; Sinet-Mathiot et al., 2023, 2019; Smith et al., 2024; Tagliacozzo et al., 2013; Torres-Iglesias et al., 2024; Vasiliev et al., 2018, 2017; Wang et al., 2024, 2023; Xia et al., 2024). To encompass the spatiotemporal faunal diversity, the following taxonomic categories will be used throughout the following paragraphs: *Bos* sp./*Bison* sp., Caprinae, Cervidae, Equidae, and Suidae.

Bos sp./*Bison* sp. continues to be significantly more abundantly represented in the ZooMS analysed component (two-tailed one-sample *t*-test, $p = 0.001$), displaying the highest mean difference (11.4 ± 21.07 SD) between the two identification methods across all taxa (Fig. 4). Likewise, Equidae is more abundantly represented in the ZooMS analysed component (two-tailed one-sample *t*-test, $p = 0.001$). Similarly to *Bos* sp./*Bison* sp., Equidae is generally characterised by either high similarity between identification methods or a consistent bias toward ZooMS identifications, with the exception of Vogelherd Cave, which explain some of Equidae's lower overall mean difference (4.07 ± 21.57 SD). Vogelherd Cave's morphologically assessed taxonomic composition may, however, be skewed by the work of Lehmann (1954) as he only included intact specimens, possibly overrepresenting less fragmented taxa (Wang et al., 2024).

It is worth noting the consistent inflation of *Bos* sp./*Bison* sp. in the ZooMS analysed component, or the close correspondence between identification methods, regardless of spatiotemporal context. This pattern has for some contexts been suggested as reflecting differential processing, whereby larger-bodied taxa were more likely to be selectively transported, processed, or preserved in ways that enhance their representation in ZooMS analysed components (Discamps et al., 2024; Sinet-Mathiot et al., 2023, 2019). This may also apply to the other LTM-sized taxon, Equidae. The overrepresentation of LTM-sized mammals in the ZooMS component may result from their higher proportion of trabecular bone, which is more prone to fragmentation, increasing their susceptibility to post-depositional breakage relative to smaller taxa (Bratlund, 1991; Morin et al., 2023).

Table 3

The relative difference between taxonomic identification approaches in percentages. Negative values indicate a higher relative abundance of a given taxon in the bone component identified through morphological means, whereas positive values indicate greater representation in the component analysed using ZooMS (SI J). Standard deviations have been indicated for both sites and taxa. Data is extracted before bootstrap analysis.

Site	Relative difference (%) between identification method					SD across taxa
	<i>Bos</i> sp.	Caprinae	Cervidae	<i>Equus</i> sp.	<i>Sus</i> sp.	
Chaves Cave, Ia	6.753	-12.824	4.022	0.299	3.851	7.75
Chaves Cave, Ib	13.027	-27.976	13.906	-0.112	5.282	17.104
Fosca Cave	0.579	-12.735	6.486	-0.797	5.889	7.743
La Draga	0.581	1.132	3.26		-4.913	3.482
Matutano Cave	0.873	-14.716	19.039	3.494	-8.689	12.918
SD across contexts	5.513	10.321	6.862	1.903	6.645	

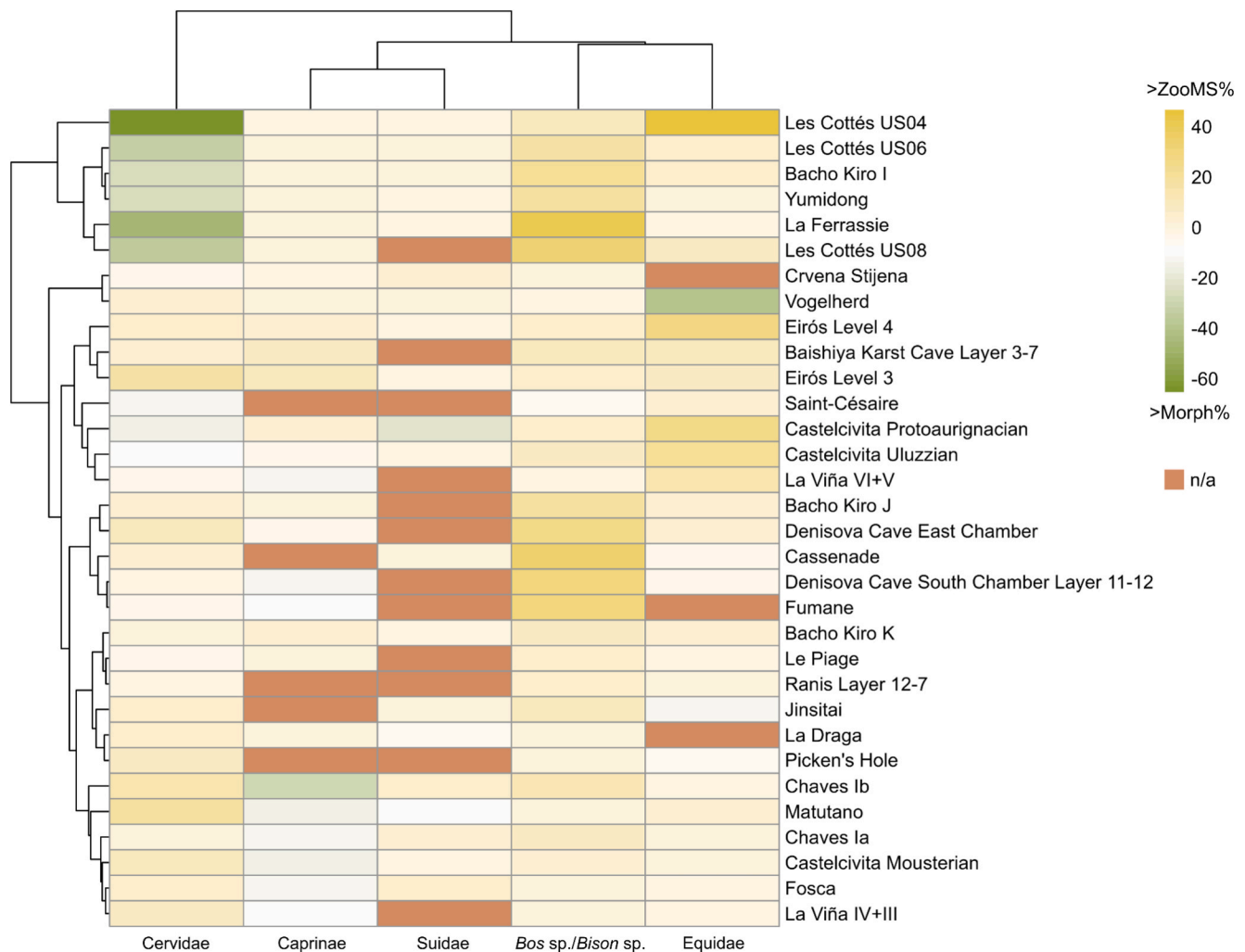


Fig. 4. Heatmap illustrating the relative proportional tendencies of ZooMS and morphology-based taxonomic identifications across sites and taxa. Negative values (green) indicate a higher proportion of morphology-based identifications, while positive values (yellow) indicate a higher proportion of ZooMS-based identifications for a given taxon. Ochre-red panels mark taxa not identified at a site.

In contrast, Caprinae, Cervidae, and Suidae, all associated with size classes MTM and STM3, are significantly more represented in the morphologically assessed component (two-tailed one-sample *t*-test, $p = 0.001$ for all comparisons). While this trend contrasts with the pattern observed for *Bos* sp./*Bison* sp. and Equidae (both classified as LTM), the Cervidae group exhibits a less uniform pattern across contexts (mean difference: -5.12 ± 35.33 SD), an inconsistency that is less apparent in Caprinae (-3.87 ± 26.69 SD) and Suidae (-1.02 ± 8.65 SD). Cervidae's skewed distribution is likely due to the relative larger proportion of particularly *Rangifer tarandus* and Cervidae/*Saiga tatarica* registered via morphological means at Les Cottés, Bacho Kiro (Layer I), and Les Ferrassie (Sinet-Mathiot et al., 2023), sometimes displaying a four-fold increase, and the lack of Cervidae uncovered via ZooMS at Yumidong Cave (Wang et al., 2023). These abundant differences between taxonomic identifications methods are not clearly understood yet, though Sinet-Mathiot et al. (2023) suggests sample size, taphonomy, and site formation practices may explain the phenomenon, while Wang et al. (2023) suggests, in regards to Yumidong Cave, that the challenges in differentiating between cervids and bovids through ZooMS among East Asian fauna may mask species diversity. This issue has been further highlighted in European contexts, particularly for *R. tarandus* and *Capra* sp. in ZooMS analyses (Morin et al., 2023). Additionally, *R. tarandus* is more easily distinguished morphologically from *Bison* sp. and Equidae, which may further contribute to discrepancies between identification methods

(Morin, 2012). However, at Le Piage (Raymond et al., 2024) and Saint-Césaire (Morin et al., 2023), *R. tarandus* is the dominant species, constituting respectively approx. 85 % and 45 %, and illustrates little dissonance between the identification methods. These disparities in Cervidae representation underscore the importance of contextual analyses of site formation processes to elucidate underlying fragmentation patterns. Another example highlighting the importance of such contextual analysis comes from bear remains from the Palaeolithic site of Geißenklösterle (Lee-Michaelis et al., 2026) showed a higher proportional representation in the ZooMS analysed bone assemblage than in the morphologically assessed assemblage in layers associated with greater hominin activity. In the older layer, however, the opposite pattern was observed. The authors suggested that this difference may reflect bears dying naturally during the formation of the older layer, with their remains undergoing relatively little taphonomic modification, whereas in the younger layers, hominin access to bone marrow may have resulted in more intensive fragmentation.

In general, Caprinae and Suidae appear to cluster more narrowly around zero, suggesting a relatively higher similarity between the identification methods. Chaves Cave, Level Ib, is especially noticeable in the Caprinae group with the highest relative difference between identification approaches (SI J). The larger proportion of Caprinae identified via morphological means may likely be explained by the relatively low number of specimens successfully determined ($n = 49$). The skeletal

element distribution of Level Ib probably does not explain the dissonance between identification methods, as the anatomical element composition between Chaves Cave's levels are indeed similar. Moreover, removing Chaves Cave, Level Ib, from the bootstrap analysis does not alter the overall significance of Caprinae's overrepresentation bias in the morphologically analysed component, it merely reduces the mean difference between identification methods to -2.85 ± 24.94 SD. Suidae differs from the other taxonomic categories, as its NISP count across morphologically and ZooMS assessed bone components for all pre-Neolithic sites are low. The low abundance of Suidae osseous specimens may not accurately reflect the true ZooMS-to-morphological identification ratio in Pre-Neolithic sites, potentially affecting the distribution or suggesting statistical significance where none exists. Caution is therefore advised when interpreting the comparability of Suidae representation across identification methods.

Lastly, the morphological component may also include burned bones and teeth, which are typically excluded from ZooMS analysis due to the poor collagen preservation in thermally altered osseous tissues (Yates, 2013). In teeth, collagen type I (COL1) is restricted to the cementum and dental pulp, and absent from enamel (Yamamoto et al., 2016). Moreover, teeth are often identifiable morphologically due to their distinctive features, further reducing chances of them being selected for ZooMS. This can influence the overall taxonomic composition of morphologically assessed bones, as exemplified at Cassenade, where dental elements dominate the identified remains of carnivores and Equidae (Discamps et al., 2024).

Similarly to what was observed in Section 3.2.1., *Bos* sp./*Bison* sp. and Equidae are generally identified to a higher degree in the ZooMS analysed bone specimens, representing smaller fragments, despite the great cultural, technological, environmental, and geographical variability between archaeological contexts. However, incorporating additional sites into the analysis increased the heterogeneity of the Cervidae component. It may be that the Cervidae group encompasses too many

species, and such analysis would benefit from separating *R. rangifer* from *C. elaphus* and *S. tatarica*. Caprinae was still generally overrepresented in the morphologically assessed bone assemblages or found clustering around zero, suggesting high similarity between identification methods, as was the case with Suidae, though the latter represented the least amount of data and sites available. Lastly, taphonomic histories constitute a key factor in interpreting such data and an important limitation of this analysis, having not been explored more in-depth. Rather than being reduced to straightforward taxon-specific explanations, the results should instead be understood as the product of multiple taphonomic and human behavioral trajectories.

3.2.3. Comparison of maximum length and cortical thickness between ZooMS analysed and morphologically assessed specimens from La Draga

Paying strict attention to the ZooMS analysed bone specimens of Chaves, Fosca, La Draga, and Matutano, and examining the taxonomic distribution across six intervals of maximum length (0–0.99, 1–1.99, 2–2.99, 3–3.99, 4–4.99, and > 5 cm) and cortical thickness (0–0.09, 0.1–0.19, 0.2–0.29, 0.3–0.39, 0.4–0.49, and > 0.5 cm), no immediate pronounced differences are evident between taxa or mammal body-size classes (Figs. 5 and 6; SI H and I). In regards to maximum length, this corresponds with observations made at the Upper Palaeolithic sites of Fumane (Sinet-Mathiot et al., 2019) and Picken's Hole (Holloran et al., 2024), at least for *Bos* sp./*Bison* sp., Caprinae, Cervidae, and Equidae, and corresponds with *Sus* sp. based on the EN sites from this study. Overall, neither maximum length nor cortical thickness appears to significantly influence the distribution of taxon or mammal body-size class across these categories.

Comparing the distribution of taxa identified via ZooMS ($n = 82$) with those identified morphologically ($n = 354$; SI G) at La Draga across the defined maximum length intervals, both identification methods appear to perform adequately across all size categories (Fig. 7.A). No metric data on the morphologically analysed specimens was available

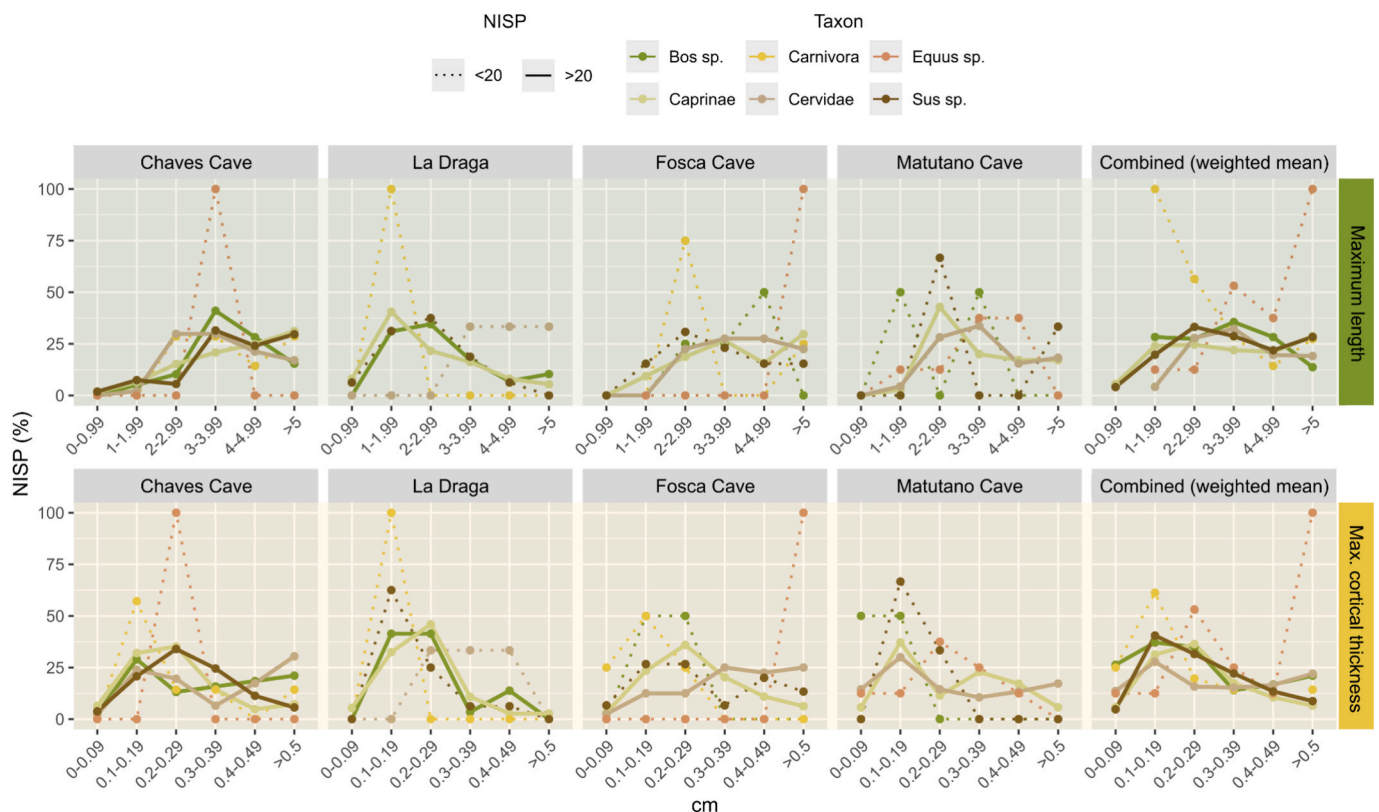


Fig. 5. The distribution of six different taxonomic groups across archaeological contexts and two measurements, maximum length and maximum cortical thickness. Taxa in contexts with less than 20 specimens are marked as dotted lines.

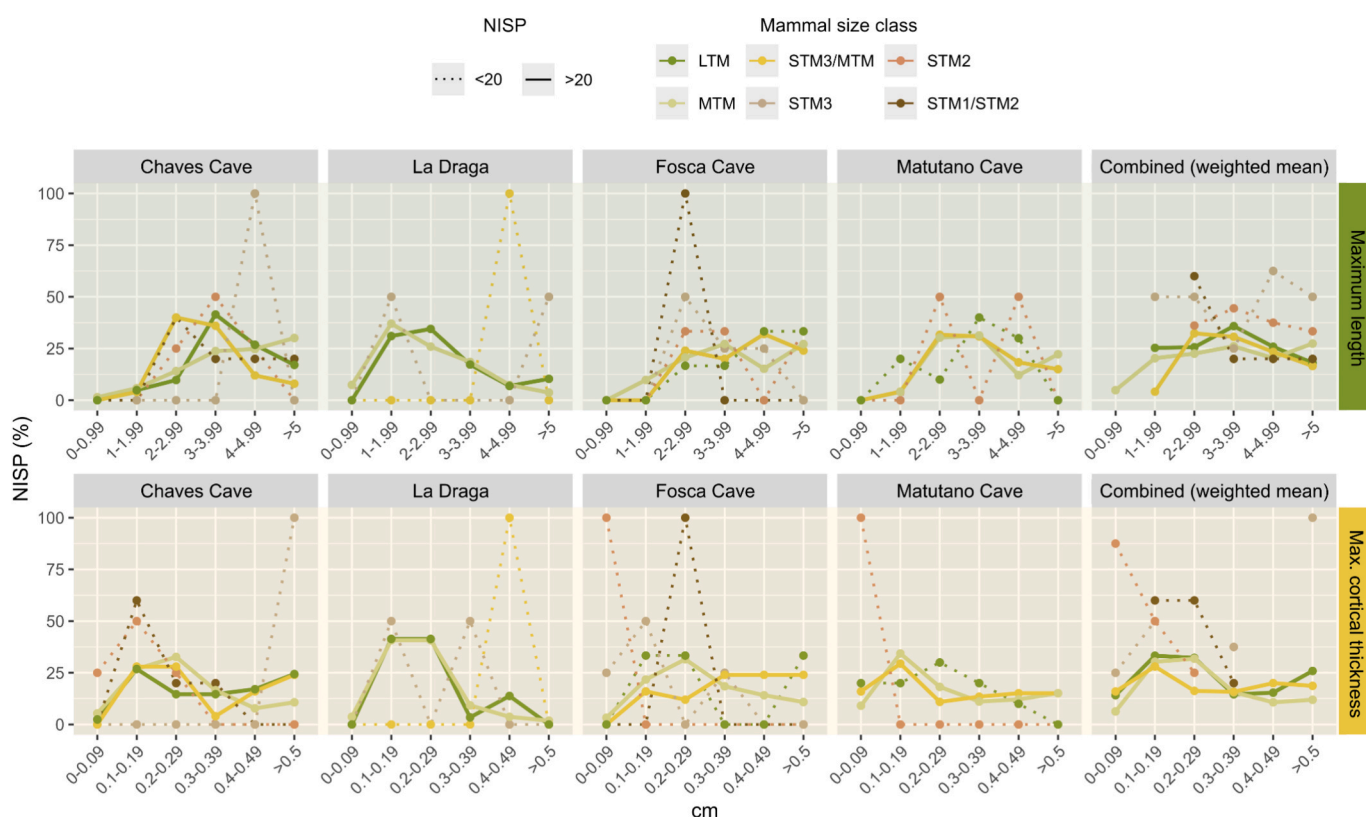


Fig. 6. The distribution of six different mammal size classes across archaeological contexts and two measurements, maximum length and maximum cortical thickness. Taxa in contexts with less than 20 specimens are marked as dotted lines.

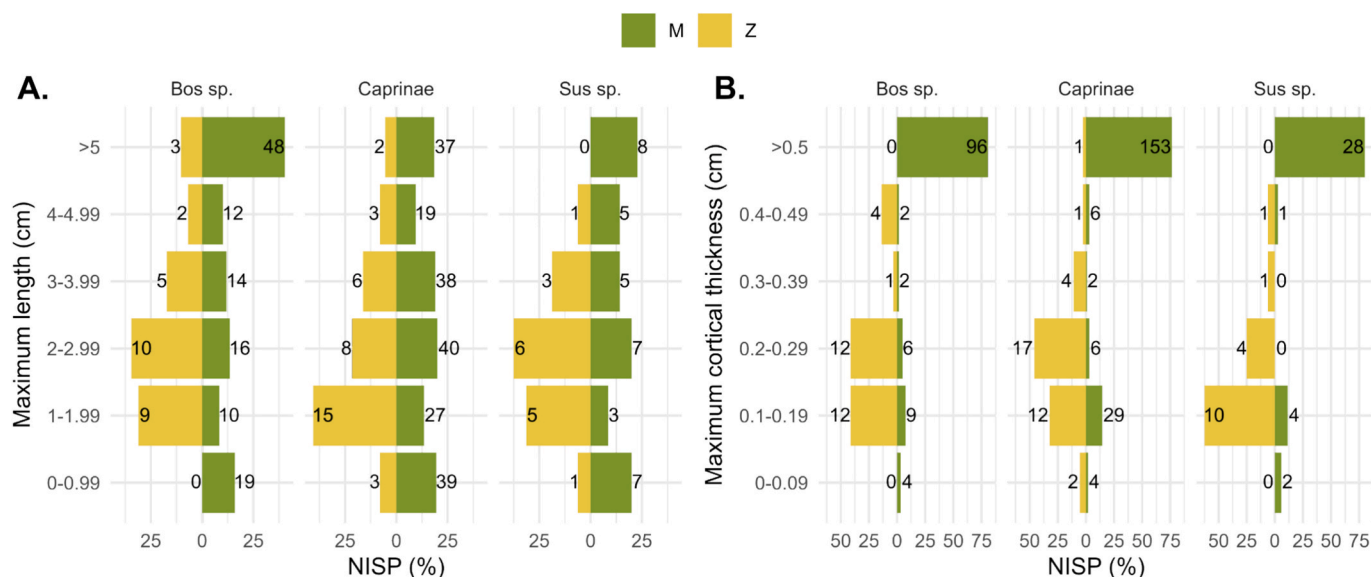


Fig. 7. Comparison of ZooMS (Z, n = 82) and morphological (M, n = 354) taxonomic identifications and their metric size class distribution in A) maximum length (cm), and B) maximum cortical thickness (cm) from La Draga (SI G).

from the other sites, which is why the comparison is focused exclusively on La Draga. 40.34 % of *Bos* sp. specimens identified morphologically were found in the > 5 cm maximum length interval, suggesting that at La Draga, morphological identification may rely on a higher size threshold. A similar pattern has been observed at the site of Bacho Kiro for *Bos* sp./*Bison* sp. (Siné-Mathiot et al., 2023), though it is not necessarily an exclusive pattern to that taxon, as demonstrated at Les Cottés where

such bipolar distribution is not evident (Siné-Mathiot et al., 2023). A more pronounced threshold around 0.5 cm for morphologically identified specimens in the maximum cortical thickness is evident for *Bos* sp., Caprinae, and *Sus* sp. (Fig. 7.B). Targeting bones with a cortical thickness of less than 0.5 cm for ZooMS may therefore be beneficial for capturing specimens that would otherwise remain unidentified through morphological analysis. However, employing a non-random selection

strategy within the non-determined bone component could introduce additional biases.

3.3. Preservation: Peptide markers and deamidation

As stated previously, the success rate for sufficient taxonomic identification varied between contexts. 730 out of 925 successful identifications were based on the combination of samples that were processed once, mostly following the HCl protocol, and 141 samples that underwent a re-extraction which included a NaOH cleaning step prior to gelatinisation (see SI A). No samples from Matutano were reprocessed.

In regards to peptide markers, accounting for samples that were not reprocessed and excluding the less successful twin of reprocessed samples, the sites of Matutano, Chaves, and Fosca performed somewhat equally well with 6.59 ± 2.46 SD out of 9 peptide markers observed (Fig. 8, upper panel). Comparably fewer peptide markers were observed within La Draga, namely 3.25 ± 3.18 SD. Cave sites may have had a preservation advantage over the open-air site, being less susceptible to temperature and water table fluctuations, both assisting in preserving collagen (Nielsen-Marsh and Hedges, 2000; Peters et al., 2023). In this case, time does not seem to have affected the collagen preservation within Chaves' layers significantly (Welch Two-Sample *t*-test, $p = 0.611$), or, at least the approx. 5,000 year gap between the EN cave sites and the LUP site of Matutano. However, the bone collagen preservation between La Draga's sectors, representing a dry/terrestrial (Sector A) and a phreatic/waterlogged (Sector D) environment appears to have significantly impacted the collagen preservation (Welch Two-Sample *t*-test, $p = 0.001$). This proteomic preservation difference between dry and phreatic sectors has been documented previously at La Draga (Ásmundsdóttir et al., 2024; Hansen et al., 2024a; Le Meillour et al., 2024). The greater degree of preservation observed in the phreatic sector may be attributed to anaerobic conditions, whereas the poor collagen preservation in the terrestrial Sector A could result from a more advanced stage of weathering, although similar weathering conditions have also been observed at Fosca (SI Fig. A1). Additionally, dry sediment has a lower heat capacity than saturated sediment (Guo and Ma, 2018), which could have caused greater temperature fluctuations in Sector A

than within Sector D, possibly advancing the loss of bone collagen in Sector A. However, it is difficult to temporally assess how long Sector A has been dry, and the amount and extent of water fluctuations which have occurred throughout time. Therefore, though explanations for the preservation difference between sectors can be observed both visually (Saña et al., 2013) and proteomically, the exact taphonomic processes and their share in collagen deterioration is difficult to assess.

In contrast, time did have a significant impact on deamidation rates. In this study, we focused on the glutamine (Q) deamidation calculated from the peptide COL1 α 1 508–519. This is because glutamine is more stable and thus has a slower deamidation rate over asparagine (N), while the COL1 α 1 508–519 peptide has a slow deamidation rate in contrast to other peptides (Nair et al., 2023; Simpson et al., 2016; Wilson et al., 2012). Furthermore, the peptide has an identical amino acid sequence between the terrestrial mammals identified in this study, implying that primary structure cannot have affected the observed deamidation values. Deamidation is a post-translational modification (PTM) that can be affected by temperature, pH, batch effect, direct side chain hydrolysis and ionic strength, among others, during diagenesis, and *in vivo* (Schroeter and Cleland, 2016; Wilson et al., 2012; Yang et al., 2025). However, due to the relatively strong stability of glutamine deamidation, it has proven useful as an indicator of collagen authenticity (Welker et al., 2017; Xia et al., 2024).

Unsurprisingly, the LUP site of Matutano has a significantly advanced deamidation rate, or α -value, compared with the EN sites (John Tukey's HSD, $p = 0.001$; Fig. 8, lower panel). Even within the two stratigraphic layers of Chaves, a significant difference could be detected (Mann-Whitney U, $p = 0.034$), the older Level Ib having more advanced deamidation. Likewise, a significant difference was detected between La Draga's two sectors (Mann-Whitney U, $p = 0.024$), with the phreatic sector trending towards a more progressed deamidation rate. When comparing deamidation rates between sites and the broad categorisation of skeletal elements (cranium, axial, and limb bones), maximum length, maximum cortical thickness, as well as weathering stage, using a two-way ANOVA, no significant differences were found. This is in accordance with previous studies on Palaeolithic bone assemblages (Smith et al., 2024; Welker et al., 2017; Xia et al., 2024). A significant difference

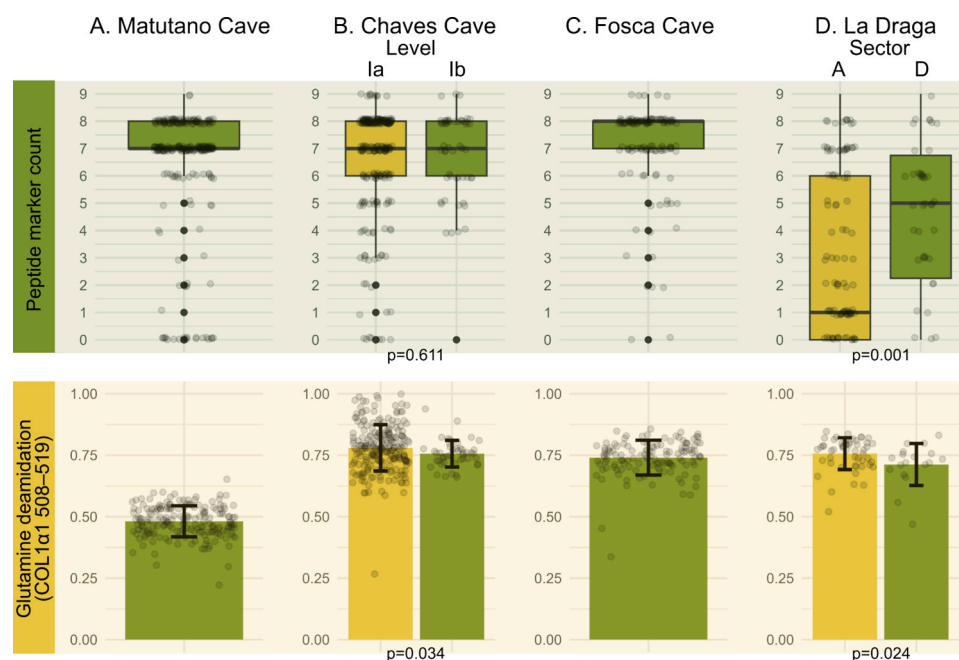


Fig. 8. Upper panel) Boxplot indicating peptide marker distribution across contexts (SI B). Both *p*-values are based on Welch Two-Sample *t*-test, one comparing the Cave of Chaves' stratigraphic layers, and another comparing La Draga's sectors. Dots indicate individual data points and are jittered for visual purposes. **Lower panel)** Deamidation rate of COL1 α 1 508–519. An α -value of 1 indicates no deamidation, while 0 suggests full deamidation. Both *p*-values are based on Mann-Whitney U test, one comparing the Cave of Chaves' stratigraphic layers, and another comparing La Draga's sectors. Dots are individual data points.

was found, however, between the taxonomic origin (two-way ANOVA, $p = 0.023$), though, after a closer inspection using a John Tukey's HSD test, most differences seemed to be related to the taxonomic specification of "No ID", which usually have more advanced deamidation rates, perhaps related to the more progressed collagen deterioration of those specific specimens.

All samples were demineralised with HCl, which is known to increase the α -value, imitating a less advanced deamidation (Simpson et al., 2016) compared with samples treated with the AmBic protocol (van Doorn et al., 2011). Furthermore, the pH-values could be recovered from the sites of Fosca and La Draga which respectively were 7.8 to 8.1 (Estévez, 1988) and 8.4 (level VII) to 8.9 (lacustrine layers). Though it is nearly impossible to recreate the pH-values for each site at a temporal level, the slightly alkaline pH of La Draga may have caused the accelerated deamidation rates observed within Sector D, however, the pH-value from Sector A is needed to confirm this. The continuously waterlogged nature of the La Draga, Sector D, bone specimens could also have caused the more advanced deamidation compared to those of Sector A. The specimens being in "solution" may have precipitated further side-chain hydrolysis, though this is speculative. Additionally, the batch effect may also have significantly affected deamidation values as well as an accumulation of phosphates in the surrounding soil, e.g. originating from manure, as suggested by Yang et al. (2025).

4. Conclusions

This study assessed how ZooMS and morphology-based taxonomic identifications differ across Late Upper Palaeolithic and Early Neolithic bone assemblages from the northeastern Iberian Peninsula, and whether patterns observed within these assemblages persist across broader temporal, geographic, and cultural contexts. Across both the Iberian dataset and the expanded comparative framework, LTM taxa, particularly *Bos* sp./*Bison* sp. and Equidae, were generally more abundant in the ZooMS analysed component, whereas MTM- and STM-sized taxa, including Caprinae, Cervidae, and Suidae, tended to be more represented in the morphologically assessed component. However, the magnitude and consistency of these differences varied among taxa and archaeological contexts, with some sites, such as La Draga, showing comparatively little divergence between identification methods. Variability was particularly pronounced among Cervidae, suggesting that broader taxonomic groupings may obscure species-specific patterns and that, where data permit, separating individual cervid taxa may provide a clearer understanding of differences in taxonomic composition between identification methods. Together, these variations indicate that the observed patterns are neither universal nor adequately explained by body-size or taxonomic affiliation alone.

The underlying variation of these broader patterns highlights the importance of bone assemblage formation processes when interpreting differences between ZooMS analysed and morphologically assessed specimens. The two approaches generally operate on differently fragmented portions of archaeological bone assemblages, with ZooMS enabling taxonomic identification of highly fragmented specimens that may not retain sufficient diagnostic features for morphological identification. At La Draga, comparisons of maximum length and cortical thickness provided further insight into these differences. In particular, the representation of ZooMS analysed specimens below approximately 0.5 cm in maximum cortical thickness suggests that ZooMS may facilitate taxonomic identification of specimens that are less likely to be resolved morphologically. However, neither maximum length nor cortical thickness alone appeared to explain inter-taxonomic variation within the ZooMS analysed assemblages. The observed threshold should therefore not be regarded as a general boundary between the two identification approaches, but rather as a pattern specific to the material examined here that warrants further investigation. More broadly, taxonomic and body-size-related differences between identification methods should not be interpreted as intrinsic properties of particular taxa, as

their expression within individual assemblages may be shaped by context-specific taphonomic histories that influence fragmentation and, consequently, taxonomic identifiability.

For the archaeology of the Iberian Peninsula, and in general, these findings are particularly relevant as ZooMS is increasingly applied to highly fragmented faunal material. The comparison of Early Neolithic assemblages characterised by different subsistence and occupation systems with a Late Upper Palaeolithic context suggests that differences between ZooMS and morphological taxonomic compositions are not restricted to Palaeolithic assemblages. Furthermore, the preliminary integration of ambiguous Caprinae identifications further illustrates the potential value of combining information from both approaches rather than treating their taxonomic outputs as independent assemblages. Although further validation is required, such integrative approaches may be particularly useful in contexts where the taxonomic resolution of either identification method alone is limited.

More broadly, the findings support previous studies indicating that morphology-based and ZooMS identifications should be regarded as complementary rather than interchangeable sources of zooarchaeological information. Further investigation is needed to determine whether the patterns observed here extend to a wider range of taxonomic groups and body-sizes. This includes disentangling patterns among carnivores of different sizes, small mammals such as rodents, and very large-bodied taxa such as Rhinocerotidae and Elephantidae, as well as extending comparative approaches beyond mammals to other vertebrate groups, including birds and fish. Expanding such comparisons across taxonomic groups, archaeological contexts, and geographic regions could provide a stronger basis for understanding how ZooMS and morphology-based assessments access different components of archaeological bone assemblages and how their integration may contribute to more comprehensive reconstructions of past faunal communities and subsistence practices.

CRedit authorship contribution statement

Jakob Hansen: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Krista McGrath:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation. **Mar Martínez Miralles:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Vanessa Navarrete:** Writing – review & editing, Methodology, Investigation. **Alejandro Sierra:** Writing – review & editing, Methodology, Investigation. **Ingrid Bertin:** Writing – review & editing, Investigation. **Martha Muñoz Alegre:** Writing – review & editing, Investigation. **Tina Ravensborg:** Writing – review & editing, Resources, Investigation. **Ole N. Jensen:** Writing – review & editing, Resources. **Xavier Terradas:** Writing – review & editing, Resources. **Antoni Palomo:** Writing – review & editing, Resources. **Raquel Piqué:** Writing – review & editing, Resources. **Pilar Utrilla:** Writing – review & editing, Resources. **José María Rodanés Vicente:** Writing – review & editing, Resources. **Gustau Aguilera:** Writing – review & editing, Resources. **Dídac Román:** Writing – review & editing, Resources. **Ignacio Clemente Conte:** Writing – review & editing, Supervision. **Frido Welker:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Conceptualization. **María Saña Seguí:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jasrep.2026.106017>.

Data availability

Proteomic data is available on Zenodo.org (<https://doi.org/10.5281/zenodo.17288657>).

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